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Prevalence and Mycotoxin Production of *Fusarium* Species Isolated from Wheat Grains in Hungary

A. TÓTH[1], ILDIKÓ BARNA-VETRÓ[2], ÁGNES GYÖNGYÖSI[2], ANDREA POMÁZI[2], Á. SZÉCSI[3] and L. HORNOK[2]

[1]Department of Plant Protection, Agricultural University, Gödöllő; [2]Agricultural Biotechnology Center, Gödöllő; [3]Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

Fifteen *Fusarium* species were identified in 204 isolates obtained from wheat grain samples. *F. equiseti* and *F. poae* were the most frequent species, followed by *F. graminearum* and *F. sporotrichioides*. All strains were tested by thin-layer chromatography for production of trichothecenes and zearalenone on rice meal agar. Forty-eight isolates were able to produce deoxynivalenol, nivalenol, T-2 toxin or zearalenone. Zearalenone was the most frequently identified toxin, whereas diacetoxyscirpenol could not be detected in any culture. Monoclonal antibody was prepared against T-2 and used for quantitative determination of the toxin in strains of *F. sporotrichioides*, the only species found to synthesize this compound. Crossproduction of trichothecene B and zearalenone was observed in several isolates of *F. graminearum*, *F. equiseti*, and *F. semitectum*. This is the first report on the occurrence of such crossproducing strains in the latter two species.

In Hungary, head blight of wheat is caused by *Fusarium culmorum* (W. G. Smith) Sacc. and *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch] therefore, a breeding program against these pathogens was started in the late seventies (Mesterházy, 1983). This program resulted in the introduction of tolerant cultivars and the infection level decreased throughout the country. However, many other *Fusarium* species have been reported to occur in naturally infected grains either from Hungary (Mesterházy, 1984) or from other parts of the world (Scott et al., 1980; Chelkowski et al., 1984; Wilcoxson et al., 1988). These fusaria are especially worthy of further study because of their ability to produce toxic secondary metabolites. In a recent review (Thrane, 1989) 19 species of the genus were mentioned as well-documented toxigenic ones while a further six taxa were regarded as possible mycotoxin producers. The uncertainties that still exist in the mycotoxin profiles of one or other species are mainly due to morphological misidentification or insufficient toxin characterization. Although the presence or absence of a particular secondary metabolite may be a useful additional taxonomical trait (Thrane and Frisvad, 1988), a radical revision in the classification of fusaria is not warranted on this basis. At present the only member of the genus that contains distinct chemotaxonomic subgroups of nivalenol and deoxynivalenol producing strains is *F. graminearum* (Ichinoe et al., 1983), other toxigenic species exhibit a rather controversial picture in this respect.

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This study was carried out in order to reveal changes incited by tolerant cultivars in the species composition of *Fusarium* isolations from wheat grain samples. All strains were screened for the presence of the major trichothecenes, namely diacetoxyscirpenol (DAS), deoxynivalenol (DON), nivalenol (NIV), and T-2 toxin (T-2), as well as for a non-trichothecene toxin, zearalenone (ZEA), with the aim to provide further information concerning secondary metabolite profiles of some lesser known and several well known species. This survey afforded a good opportunity to test a monoclonal antibody preparation developed recently at the Institute for Animal Sciences, Agricultural Biotechnology Center, Gödöllő for detecting T-2.

Materials and Methods

Wheat grain samples collected in 1990/91 from the central region of the country were surface disinfected 1% (w/v) sodium hypochlorite, rinsed with sterile water and placed on peptone–PCNB medium (Papavizas, 1967). After seven days *Fusarium*-like colonies were subcultured on potato–sucrose agar and carnation leaf agar (Fischer et al., 1982), single spored, if needed, incubated for two weeks at room temperature under normal daily light-dark cycles, and identified according to the criteria of Booth (1971) and Nelson et al. (1983). All strains are deposited at the Department of Plant Protection, Agricultural University, Gödöllő.

Fusarium isolates were three-point inoculated on rice meal agar plates (Thrane, 1986) and incubated for two weeks at 25 °C in the dark. The whole cultures were then minced, extracted by shaking with methanol for 1 h, concentrated, defatted, dried and finally redissolved in 1 ml dichloromethane as described by Nowotny et al. (1983).

T-2 (Sigma Chemical Co., St. Louis, USA) was converted to T-2-hemisuccinate (T-2HS) according to the method of Chu et al. (1979). Ten mg T-2 in 0.4 ml pyridine was reacted with 210 mg succinic anhydride in a steam bath for 4 h. The reaction mixture was evaporated to dryness under nitrogen, redissolved in chloroform and washed with distilled water. The conversion of T-2 to T-2HS was detected by thin-layer chromatography.

T-2HS was then coupled to bovine serum albumin (T-2HS-BSA) and used for immunization of Balb/c mice (LATI, Gödöllő, Hungary). Mice received one subcutaneous injection of 100 μg T-2HS-BSA in 0.1 ml physiological saline emulsified in the same volume of Freund's complete adjuvant (Sigma) followed, after one month by an intraperitoneal injection of the same antigen volume but mixed in Freund's incomplete adjuvant (Sigma). A final booster injection without adjuvant was performed again after one month into the tall vein. Three days later spleen cells of the immunized animals were aseptically removed and fused with Sp2/0 mouse myeloma cell line. Hybridomas developed after this fusion were selected in an indirect competitive enzyme immunoassay and cloned. Ascites was induced in mice injected intraperitoneally with 10^7 cloned hybridoma cells in 0.5

ml saline. Globulins were purified from the ascites fluid collected after 12–14 days by precipitation with ammonium sulphate. This monoclonal antibody preparation was IgG_1 with kappa type light chain. As determined by direct competitive ELISA the sensitivity of the anti-T-2 antibody towards pure T-2 toxin was 1.4 ng ml^{-1} with limited crossreaction to acetyl T-2 (12.8%), HT-2 toxin (3.4%) and *iso*-T-2 toxin (2.5%).

T-2HS was conjugated with horse-radish peroxidase (HRPO, Reanal, Budapest, Hungary) by the activated ester method (Esgin et al., 1989). Standard crystalline T-2 (Sigma) or culture extracts dissolved in 10 % (v/v) ethanol (in phosphate buffered saline, 0.01 M PO_4, pH 7.2) were subjected to direct competitive enzyme-linked immunosorbent assay (ELISA) using this T-2HS-HRPO conjugate. Absorbance was measured by a Multiscan Plus microplate reader (Labsystems Oy, Helsinki, Finland) at 450 nm. Toxin concentrations of the samples were calculated from a calibration curve in ng ml^{-1} unit.

For chromatographic detection of the toxins, culture extracts dissolved in dichloromethane were spotted on TLC plates (Kieselgel 60 F254, art. 5554, Merck AG, Darmstadt, Germany) together with griseofulvin (Aldrich-Europe, Beerse, Belgium) as external standard. Internal standards were DAS, DON (Sigma), NIV (Serva, Heidelberg, Germany), T-2 and ZEA. Two developing mixtures were used, toluene/acetone/methanol (5/3/2) (Kamimura et al., 1981) and toluene/ethyl acetate/90% formic acid (5/4/1) (Filtenborg et al., 1983). Visualization treatments were spraying with 20% (w/v) aluminium chloride in 60% (v/v) ethanol and with 20% (v/v) sulphuric acid, then heated for 10 min at 110 and 120 °C, respectively. Before and after visualization treatments the plates were examined under normal light, as well as at 254 and 312 nm. Colour reactions were recorded according to the references (Filtenborg et al., 1983; Kamimura et al., 1981) and all retardation factors (R_f) were calculated relative to griseofulvin as recommended by Thrane (1983).

Results and Discussion

Table 1 is a summary of the *Fusarium* species and their toxin production as determined in this survey. The relative proportion of the species, as well as the ratio between toxin producing and nontoxogenic strains of each species are presented in Fig. 1.

Over 200 isolates that belonged to 15 species were identified. *Fusarium equiseti* (Corda) Sacc. (teleomorph: *Gibberella intricans* Wollenw.) and *Fusarium poae* (Peck) Wollenw. were the two most prevalent species comprising together some 35 per cent of the whole collection. The next most frequently found species were *F. graminearum* and *Fusarium sporotrichioides* Sherb., each constituted 11.71% of the isolates. They were followed by *Fusarium semitectum* Berk. et Rav. (5.85%) and *F. culmorum* (5.36%). Each of the other nine species – *Fusarium acuminatum* Ell. et Ev. (*Gibberella acuminata* Wollenw.), *Fusarium avenaceum*

Table 1

Distribution of trichothecene and zearalenone producing isolates in 15 *Fusarium* species

Species	Number of toxigenic isolates							Number of nontoxigenic isolates	Total number of isolates	Percentage of toxigenic isolates
	DAS	DON	NIV	T-2	ZEA	DON+ZEA	NIV+ZEA			
F. acuminatum	0	1	0	0	0	0	0	9	10	10.00
F. avenaceum	0	2	0	0	0	0	0	8	10	20.00
F. chlamydosporum	0	0	0	0	0	0	0	5	5	0
F. culmorum	0	5	0	0	0	0	0	6	11	45.45
F. equiseti	0	2	2	0	14	1	2	15	36	58.33
F. graminearum	0	1	1	0	2	1	1	18	24	25.00
F. heterosporum	0	0	0	0	0	0	0	2	2	0
F. moniliforme	0	0	0	0	0	0	0	8	8	0
F. oxysporum	0	0	0	0	0	0	0	10	10	0
F. poae	0	0	0	0	0	0	0	36	36	0
F. semitectum	0	0	0	0	7	1	0	3	11	72.73
F. sporotrichioides	0	0	0	5	0	0	0	19	24	20.83
F. subglutinans	0	0	0	0	0	0	0	7	7	0
F. tricinctum	0	0	0	0	0	0	0	6	6	0
F. ventricosum	0	0	0	0	0	0	0	4	4	0
Total	0	11	3	5	23	3	3	156	204	23.53

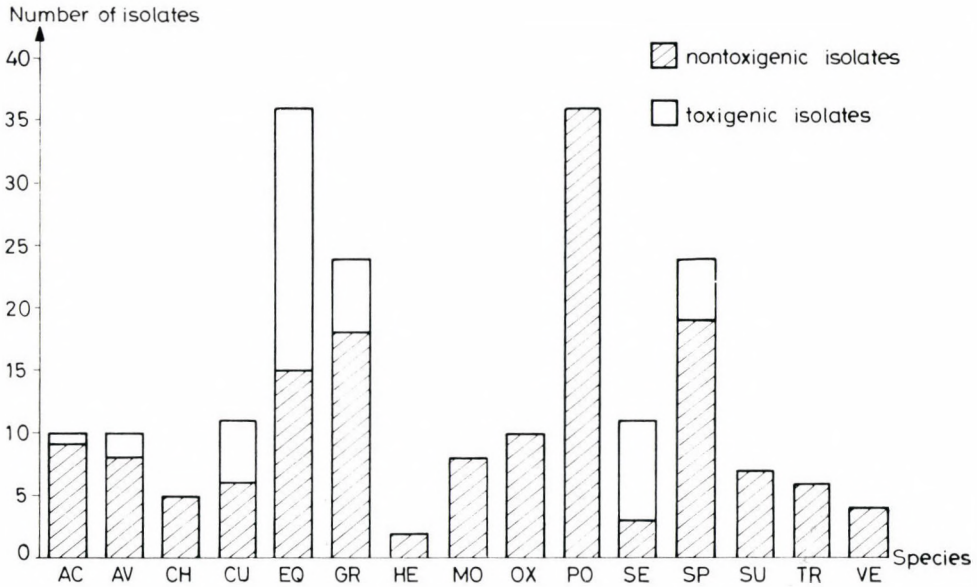

Fig. 1. Frequency of different *Fusarium* species. Abbreviations: AC: *F. acuminatum;* AV: *F. avenaceum;* CH: *F. chlamydosporum;* CU: *F. culmorum;* EQ: *F. equiseti;* GR: *F. graminearum;* HE: *F. heterosporum;* MO: *F. moniliforme;* OX: *F. oxysporum;* PO: *F. poae;* SE: *F. semitectum;* SP: *F. sporotrichioides;* SU: *F. subglutinans;* TR: *F. tricinctum;* VE: *F. ventricosum*

(Fr.) Sacc. (*Gibberella avenacea* Cook), *Fusarium chlamydosporum* Wollenw. et Reinking, *Fusarium heterosporum* Nees (*Gibberella gordonii* Booth), *Fusarium moniliforme* Sheldon [*Gibberella fujikuroi* (Sawada) Wollenw.], *Fusarium oxysporum* Schlecht, *Fusarium subglutinans* (Wollenw. et Reinking) Nelson, Toussoun et Marasas [*Gibberella subglutinans* (Edwards) Nelson, Toussoun et Marasas], *Fusarium tricinctum* (Corda) Sacc. (*Gibberella tricincta* El-Gholl, McRitchie, Schoulties et Ridings), and *Fusarium ventricosum* Appel et Wollenw. (*Nectria ventricosa* Booth) – amounted to less than 5%.

In an earlier Hungarian survey (Mesterházy, 1984) *F. graminearum* and *F. culmorum* proved to be strongly dominant species on wheat ears and grains, comprising nearly 85% of the isolates, while *F. equiseti* and *F. poae* showed only sporadic occurrence. Reports from other countries also confirmed the prevalence of *F. culmorum* (Chełkowski et al., 1984) or *F. graminearum* (Wilcoxson et al., 1988) depending on climatic conditions. *F. equiseti* has not previously been isolated from cereal grains in such high numbers and only Wilcoxson et al. (1988) mentioned *F. poae* as a noteworthy inhabitant of scabby wheat in Minnesota, USA. Although repeated attempts were made to isolate *Fusarium crookwellense* Burgess, Nelson et Toussoun, a well-known toxigenic incitant of head blight (Miller et al., 1990; Vesonder et al., 1991) this fungus was not found in Hungarian wheat grains samples.

Four trichothecenes (DAS, DON, NIV, T-2) and ZEA were selected for screening because they are regarded as the most frequent and harmful *Fusarium* toxins (Joffe, 1986). Of the five metabolites tested four were identified in our samples, DAS being the only one not detected in any of the 204 isolates. The minimum concentrations of DON, NIV, and ZEA in the fungal cultures ranged between 0.02 and 0.5 $\mu g\ ml^{-1}$ as calculated from the known sensitivity of TLC (Filtenborg and Frisvad, 1980) and from the purification and concentration procedures used in this experiment. In certain isolates the amount of extract spotted on TLC plate was doubled in order to confirm questionable toxin responses. The concentration of T-2 was more precisely determined using a calibration curve prepared from dilutions of the standard toxin. Cultures of T-2 producing strains contained this secondary metabolite at levels from 24.6 to 47.0 ng per Petri dish.

Seven of the 15 *Fusarium* species identified in the survey contained strains that produced mycotoxins under our test conditions. The largest number of toxigenic isolates occurred in *F. semitectum* (72.73%) followed by *F. equiseti* (58.33%), *F. culmorum* (45.45%), *F. graminearum* (25.0%), and *F. sporotrichioides* (20.83%). *F. acuminatum* and *F. avenaceum* had only one and two toxic strains, respectively. The percentage of toxin positive isolates in the whole collection was 23.53% and appeared to be lower than percentages reported elsewhere (Ichinoe et al., 1983; Mańka et al., 1985; Thrane, 1986; Faifer et al., 1990.) Eight *Fusarium* species produced neither trichothecenes, nor zearalenone; many of these species, namely *F. heterosporum*, *F. moniliforme*, *F. oxysporum*, *F. poae*, and *F. tricinctum* were also found to be nontoxigenic by Chełkowski et al. (1984). A noteworthy peculiarity of the present study was the nontoxigenic nature of *F. poae*, one of our most frequently occurring species. Review articles (Ichinoe and Kurata, 1983; Marasas et al., 1984; Thrane, 1989) refer to this fungus as a certified trichothecene A producer, while other, more recent surveys did not reveal any toxic strain in this species (Chełkowski, 1989) or found only a single DAS producing isolate in a world-wide collection (1990).

Figure 2, a circular diagram constructed from the data of Table 1 illustrates the percentage distribution of toxin chemotypes identified in this study. Altogether, 48 isolates produced one or more of the four mycotoxins mentioned above. The largest group was composed of ZEA producing strains which occurred in *F. equiseti*, *F. graminearum*, and *F. semitectum* in complete accordance with literature data (Marasas et al., 1984; Thrane, 1989). The second most frequent chemotype involved DON producers. Several *F. culmorum* isolates synthesized this toxin as previously reported for other European strains of this species (Chełkowski et al., 1984; Mańka et al., 1985; Miller et al., 1990; Snijders and Perkowski, 1990). Two isolates each of *F. avenaceum* and *F. equiseti* were also found to produce this metabolite, although these species are classified by Thrane (1989) as doubtful trichothecene B producers. In spite of the limited resolution power of TCL we are convinced that DON was present in our samples, since this compound produces a very characteristic spot on the plates developed by any of the two systems; also these fungi are difficult to misidentify because of their distinctive

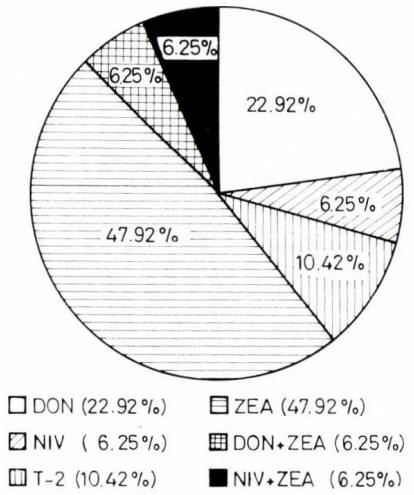

Fig. 2. Percentage of toxin chemotypes as compared to the total unuber of trichothecene and zearalenone producing *Fusarium* isolates

macroconidia. The apparent controversy between researchers, who did not identify DON in *F. avenaceum* and *F. equiseti* (Chełkowski et al., 1984; Mańka et al., 1985) and those, who did (Abbas et al., 1984) cannot be definitively resolved by our data. However, they support the latter. T-2 was found only in *F. sporotrichioides*. Many conflicting reports have been published on the T-2 production by other members *(F. chlamydosporum, F. poae, F. tricinctum)* of the section Sporotrichiella. In the present survey nearly 50 strains of these three species were found by TLC to be T-2 negative and this finding was also confirmed by a highly sensitive ELISA. The detection limit of this immunoassay for T-2 was as low as 1 ng ml^{-1}, comparable to other similar tests (Gendloff et al., 1987).

There were three NIV producing isolates in the collection; two of them were identified as *F. equiseti*, the third as *F. graminearum*. The former finding can be regarded as a confirmation of trichothecene B production by members of the Gibbosum section, the latter needs some more explanation. Distinct chemotaxonomic groups, namely DON or NIV producing subpopulations, were first observed in *F. graminearum* (Ichinoe et al., 1983). Further studies (Lee et al., 1986; Logrieco et al., 1988; Faifer et al., 1990; Miller et al., 1990; Sugiura et al., 1990) revealed that while DON-type strains of this fungus are distributed worldwide, the occurrence of NIV-types is restricted to Japan, Korea, Taiwan and Italy. NIV-contaminated grains reported from North America and South Africa (Tanaka et al., 1988) might be associated with *F. crookwellense*. According to our survey NIV producing *F. graminearum* isolates do occur in Hungary, nevertheless cereal grains contaminated with this toxin have not been found yet (Szécsi, 1990). This seeming contradiction can be resolved, if we consider that the Hungarian

climate is rather continental-like, not favourable to the spread of NIV-strains, but these strains may survive and express their toxin producing ability under optimal laboratory conditions.

Finally, some isolates synthesized both trichothecenes and ZEA. Such cross-production of these mycotoxins is well-established in *F. graminearum* (Ichinoe et al., 1990; Chełkowski et al., 1984; Mańka et al., 1985; Mirocha et al., 1989; Faifer et al., 1990; Miller et al., 1990), doubtful in *F. equiseti* (Thrane, 1989) and has never been observed in *F. semitectum*.

This study clearly demonstrated trichothecene B production in species like *F. avenaceum*, *F. equiseti*, and *F. semitectum* and this can be a noteworthy contribution to our knowledge of the mycotoxin chemotypes in *Fusarium*. However, as we are aware of the limits of TLC, preparations have already been made for the confirmation of these data by GC/MS analysis.

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Literature

Abbas H. K., Mirocha, C. J. and Shier, W. T. (1984): Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. Appl. Environ. Microbiol. 48, 654–661.

Booth, C. (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Kew. 237 p.

Chełkowski, J., Mańka, M., Kwaśna, H., Visconti, A. and Goliński, P. (1989): *Fusarium sporotrichioides* Sherb., *Fusarium tricinctum* (Corda) Sacc. and *Fusarium poae* (Peck) Wollenw. — Cultural characteristics, toxinogenicity and pathogenicity towards cereals. J. Phytopath. 124, 155–161.

Chełkowski, J., Visconti, A., Solfrizzo, M. and Bottalico, A. (1984): Formation of mycotoxins by *Fusarium* species from cereals in Poland. Phytopathol. Medit. 23, 43–46.

Chu, F. S., Grossman, S., Wei, Ru-Dong and Mirocha, C. J. (1979): Production of antibody against T-2 toxin. Appl. Environ. Microbiol. 37, 104–108.

Esgin, von S., Märtbauer, E. and Terplan, G. (1989): Entwicklung und Anwendung eines enzymimmunologischen Verfahrens zum Nachweis von T-2 toxin in Milch. Arch. Lebensmittelhyg. 40, 97–120.

Faifer, G. C., De Miguel, M. S. and Godoy, H. M. (1990): Patterns of mycotoxin production by *Fusarium graminearum* isolated from Argentine wheat. Mycopathologia 109, 165–170.

Filtenborg, O. and Frisvad, J. C. (1980): A simple screening-method for toxigenic moulds in pure cultures. Lebensm-Wiss. Technol. 13, 128–130.

Filtenborg, O., Frisvad, J. C. and Svendsen, J. A. (1983): Simple screening method for moulds producing intracellular mycotoxins in pure cultures. Appl. Environ. Microbiol. 45, 581–585.

Fischer, N. L., Burgess, L. W., Toussoun, T. A. and Nelson, P. E. (1982): Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology, 72, 151–153.

Gendloff, E. H., Pestka, J. J., Dixon, D. E. and Hart, L. P. (1987): Production of a monoclonal antibody to T-2 toxin with strong cross-reactivity to T-2 metabolites. Phytopathology 77, 57–59.

Ichinoe, M. and Kurata H. (1983): Trichothecene-producing fungi. In: Y. Ueno (ed.): Trichothecenes — chemical, biological and toxicological aspects. Elsevier, Amsterdam. pp. 73–82.

Ichinoe, M., Kurata, H., Sugiura, Y. and Ueno, Y. (1983): Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. 46, 1364–1369.

Joffe, A. Z. (1986): *Fusarium* species: Their biology and toxicology. John Wiley and Sons, Inc., New York. 588 p.

Kamimura, H., Nishijama, M., Yasuda, K., Saito, K., Ibe, A., Nagayama, T., Ushiyama H. and Naoi Y. (1981): Simultaneous detection of several *Fusarium* mycotoxins in cereals, grains, and foodstuffs. J. Ass. Off. Anal. Chem. 64, 1067–1073.

Lee, U. S., Jang, H. S., Tanaka, T., Toyasaki, N., Sugiura, Y., Oh, Y. J. and Ueno, Y. (1986): Mycological survey of Korean cereals and production of mycotoxins by *Fusarium* isolates. Appl. Environ. Microbiol. 52, 1258–1260.

Logrieco, A., Bottalico, A. and Altomare, C. (1988): Chemotaxonomic observations on zearalenone and trichothecene production by *Gibberella zeae* from cereals in Southern Italy. Mycologia 80, 892–895.

Logrieco, A., Chełkowski, J., Bottalico, A. and Visconti, A. (1990): Further data on specific trichothecene production by *Fusarium* sect. *Sporotrichiella* strains. Mycol. Res. 94, 587–589.

Mańka, M., Visconti, A., Chełkowski, J. and Bottalico, A. (1985): Pathogenicity of *Fusarium* isolates from wheat, rye and triticale towards seedlings and their ability to produce trichothecenes and zearalenone. Phytopath. Z. 113, 24–29.

Marasas, W. F. O., Nelson, P. E. and Toussoun, T. A. (1984): Toxigenic *Fusarium* species. Identity and mycotoxicology. The Pennsylvania State University Press, University Park. 328 p.

Mesterházy, Á. (1983): Breeding wheat for resistance to *Fusarium graminearum* and *Fusarium culmorum*. Z. Pflanzenzüchtg. 91, 295–311.

Mesterházy, Á. (1984): *Fusarium* species of wheat in South Hungary, 1970–1983. Cereal. Res. Comm. 12, 167–170.

Miller, J. D., Greenhalgh, R., Wang, Y. Z. and Lu, M. (1990): Trichothecene chemotypes of three *Fusarium* species. Mycologia 83, 121–130.

Mirocha, C. J., Abbas, H. K., Windels, C. E. and Xie, W. (1989): Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyl-deoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. Appl. Environ. Microbiol. 55, 1315–1316.

Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. (1983): *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193 p.

Nowotny, P., Baltes, W., Krönert, W. und Weber, R. (1983): Dünnschichtschromatographische Methode zur Bestimmung von 22 Mykotoxinen in verschimmelten Lebensmitteln. Chem. Mikrobiol. Technol. Lebensm. 8, 24–28.

Papavizas, G. (1967): Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. Phytopathology 57, 848–852.

Scott, P. M., Harwig, J. and Blanchfield, B. J. (1980): Screening of *Fusarium* strains isolated from overwintered Canadian grains for trichothecenes. Mycopathologia 72, 175–180.

Snijders C. H. A. and Perkowski, J. (1990): Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology 80, 566–570.

Sugiura, Y., Watanabe, Y., Tanaka, T., Yamamoto, S. and Ueno, Y. (1990): Occurrence of

Gibberella zeae strains that produce both nivalenol and deoxynivalenol. Appl. Environ. Microbiol. 56, 3047–3051.

Szécsi Á. (1990): Fuzáriotoxinok. Növénytermelés 39, 369–379.

Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U. S., Sugiura, Y. and Ueno, Y. (1988): Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone. I. Survey of 19 countries. J. Agric. Food Chem. 36, 979–983.

Thrane, U. (1986): Detection of toxigenic *Fusarium* isolates by thin layer chromatography. Lett. Appl. Microbiol. 3, 93–96.

Thrane, U. (1989): *Fusarium* species and their specific profiles of secondary metabolites. In: J. Chełkowski (ed.): *Fusarium* mycotoxins, taxonomy and pathogenicity. Topics in secondary metabolism — Volume 2. Elsevier, Amsterdam. pp. 199–225.

Thrane, U. and Frisvad, J. C. (1988): Species specific profiles of secondary metabolites within the genus *Fusarium*, obtained by reversed phase high-performance liquid chromatography. Mycotoxin. Res. 4, 21–24.

Vesonder R. F., Goliński, P., Plattner, R. and Zietkiewicz, D. L. (1991): Mycotoxin formation by different geographic isolates of *Fusarium crookwellense*. Mycopathologia 113, 11–14.

Wilcoxson R. D., Kommedahl, T., Ozmon E. A. and Windels C. E. (1988): Occurrence of *Fusarium* species in scabby wheat from Minnesota and their pathogenicity to wheat. Phytopathology 78, 586–589.

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Toxin Production by *Alternaria helianthi*, the Leaf-spot and Blight Pathogen of Sunflower

S. C. SHARMA, MAHIPAL S. GHEMAWAT[1] and J. M. AGRAWAT[2]

Department of Botany, Government College, Kota RAJ. 324001, India

Each of the three isolates of *Alternaria helianthi* (Akola, Jalgaon and Kota) produces two toxins both in vitro and in vivo. In vitro, the crude toxin-production is the highest in the moderately virulent Jalgaon isolate while in vivo, its maximum amount is produced with the most virulent Kota isolate. Even a 20 ppm concentration of the crude toxin from the Kota isolate is able to produce both of the typical symptoms of the disease, namely dark-brown spots, surrounded by halos. The two toxins have R_f values of 0.88 and 0.08, respectively, and the amounts of one fraction ($R_f = 0.88$) are much more than those of the other fraction ($R_f = 0.08$) for each isolate. These toxins are host-specific and thermolabile and reduce seed germination as well as root/shoot ratio of the host. The toxin with the R_f value of 0.88 is possibly a pathotoxin as it is host-specific as well as capable of producing both the typical symptoms of the disease.

Alternaria helianthi (Hansf.) Tubaki and Nishihara causes a severe leaf-spot and blight disease of sunflower (*Helianthus annuus* L.) in many countries. Bhaskaran and Kandaswamy (1978) reported the production of a toxin both in vitro by *A. helianthi* and in vivo by *A. helianthi*-sunflower tissue interaction. Tal et al. (1985) have isolated five phytotoxic metabolites from culture filtrate of *A. helianthi*. Hence toxin-production by *A. helianthi* both in vitro and in vivo was studied.

Materials and Methods

Three isolates of *A. helianthi* from sunflower, out of seven collected from all over India, were selected on the basis of different degrees of virulence, the Akola isolate being the least, the Jalgaon isolate being moderate and the Kota isolate being the most virulent (Ghemawat et al., 1986). Sunflower used was cv. EC 68414. The inoculated and the control plants/leaves were always incubated for the first two days under >95% relative humidity and then under 50–65% relative humidity.

[1] Present address (and address for reprints) 235 Newton Avenue, Riverhead NY 11901 U.S.A.

[2] Agric. Research Station, Sukhadia University, KOTA, RAJ., INDIA.

Akadémiai Kiadó, Budapest

For in vitro production of toxin, three flasks, each containing 125 ml Czapek's medium, were seeded each with 2 ml of spore-suspension (3×10^3 spores/ml) of each of the three isolates. This experiment had three replicates. Unseeded Czapek's medium was used as the control. Both the seeded and the control flasks were incubated at 27 ± 2 °C and 1.65×10^4 ergs/mm²/sec light intensity for 15 days. The cultures were then filtered through a Buchner funnel. All the filtrates were pooled separately for each isolate and then centrifuged at 2000 rpm for 20 min. The supernatant was collected and the toxin was extracted as per the method described by Purushothaman and Prasad (1972). The extracted toxin was evaporated and the residue was redissolved in methanol and stored at 0–4 °C.

For in vivo production of toxin, leaves of twenty 30-day-old sunflower plants were inoculated with 100 ml of spore-suspension (3×10^3 spores/ml) of each of the three isolates. This experiment had three replicates. The control plants were sprayed with distilled water. Fifteen days after inoculation, 25 g leaf sample was collected separately for each isolate and the toxin was extracted as per the method described by Bhaskaran (1976). It was stored at 0–4 °C.

Fifty µl of crude toxin from each isolate was chromatographed on Whatman filter paper No. 1 and n-butanol : acetic acid : water (4 : 1 : 1 v/v/v) solvent was used for ascending chromatography. Different chromatograms were developed by five different developers (2% $FeCl_3$ in 80% ethanol; 0.1% bromophenol blue in 80% ethanol; 50% commercial Folin-Ciocalteu reagent, followed by 20% sodium carbonate solution while the chromatograms were still damp; alkaline diazotized sulfanilic acid (DSA) on dried chromatograms, followed by 20% sodium carbonate solution while the chromatograms were still damp; and 0.1% ninhydrin in acetone). Colour and R_f values of the spots were recorded for elucidating the nature of the toxin.

The undeveloped chromatograms were observed under UV light directly as well as in the presence of ammonia vapour (Bhaskaran 1976) and the fluorescent spots, if any, were marked.

Further studies with the toxin were conducted using only the Kota isolate. Culture filtrate of the Kota isolate and the crude toxin extracted both from the culture filtrate and from the leaves inoculated with the Kota isolate were separately applied to sunflower leaves. Suitable controls were also included.

Dilutions of the extracted crude toxin were made so as to get 10,000, 5000, 2000, 1000, 500, 200, 100, 50, 20, 10, and 5 ppm concentrations and sprayed separately on detached sunflower leaves. The control leaves were sprayed with distilled water. Observations were recorded up to seven days.

Detached leaves of 17 weed and ornamental plant species (*Ageratum conyzoides*, *Blumea laciniata*, *Calendula arvensis*, *Carthamus tinctorius*, *Chrysanthemum indicum*, *Cosmos sulfureus*, *Dahlia rosea*, *Echinops echinatus*, *Eclipta alba*, *Gnaphalium pulvinatum*, *Launaea resedifolia*, *Sonchus oleraceus*, *Tagetes erecta*, *Tridex procumbens*, *Vernonia cinerascens*, *Xanthium strumarium* and *Zinnia elegans*), belonging to the same family Asteraceae (Compositae) as sunflower, were

sprayed with the crude toxin produced in vivo. Leaves of sunflower plants, sprayed with the crude toxin, served as controls. Observations were recorded up to seven days.

To find the thermostability of the toxin, the crude toxin in distilled water was heated at 180 °C to dryness. It was redissolved in distilled water and sprayed on detached sunflower leaves. The control leaves were sprayed with the unheated crude toxin. Observations were recorded up to seven days.

Sunflower seeds were soaked in culture filtrate for 24 h. For controls, seeds were soaked in sterile distilled water. Later, both the treated and the control seeds were kept in dark on damp sterile filter paper in sterilized Petri plates. Seed germination was recorded 15 days later.

Culture filtrate was applied to the germinated sunflower seeds in place of sterile distilled water which was used for the controls. Root and shoot lengths were measured 15 days later and the root/shoot ratios were calculated.

Areas were cut from the undeveloped chromatograms at comparable spots as those on the developed ones and were eluted separately in 50 ml of methanol. After the eluted solutions were evaporated to dryness and the residues redissolved separately in one ml of distilled water, they were applied to the detached sunflower leaves. Sterile distilled water was applied to the control leaves.

Results

Each of the six extracts (three from culture filtrates from the three isolates of *A. helianthi* and three from sunflower leaves inoculated with the three isolates) yielded two spots with each of the developers used except that no spot developed with ninhydrin. Both the spots from each of the six sources developed (i) as reddish-violet spots on yellow background with 2% $FeCl_3$, (ii) as yellow spots on blue background with bromophenol blue, (iii) as blue spots on white background with Folin-Ciocalteu reagent, and (iv) as yellow spots on white background with DSA. The more-pronounced spot had R_f value of 0.88 and the other had R_f value of 0.08. Both under direct UV light and under UV light with ammonia, the undeveloped chromatograms showed fluorescent spots at the same places as in case of the developed chromatograms.

Large, dark-brown spots developed on sunflower leaves within two days of applying either the culture filtrate from the Kota isolate of *A. helianthi* grown on Czapek's medium or the crude toxin extracted either from the culture filtrate of the Kota isolate or from the sunflower leaves inoculated with the Kota isolate. Halos were also present around these spots. On the other hand, the controls never showed any symptom. On comparing the three isolates of *A. helianthi*, the highest amount of the toxin was extracted from the culture filtrate of the moderately virulent Jalgaon isolate while the least amount was from that of the least virulent Akola isolate (Table 1). In vivo, however, the toxin production was the highest

Table 1

Toxin-production in vitro and in vivo (in sunflower leaves) by three isolates of *Alternaria helianthi*

	Toxin production (g)		
	Akola isolate (least virulent)	Jalgaon isolate (moderately virulent)	Kota isolate (most virulent)
In vitro (per 450 ml Czapek's medium)	0.0175	0.025	0.0198
In vivo (per 25 g leaves)	0.057	0.0316	0.0646

with the most virulent Kota isolate while it was the least with the moderately virulent Jalgaon isolate (Table 1).

Typical symptoms (dark-brown spots with halos) developed with up to 20 ppm concentration of the crude toxin obtained from the Kota isolate below which no symptom developed. Inspite of uniform drop-size, the size of the developed spots and the halos were commensurate with the concentration of the crude toxin. With 20 ppm concentration, the spots were just small specks surrounded by hardly visible halos.

Neither dark-brown spot nor halo was observed even by seven days on any of the 17 weed and ornamental plant species belonging to the same family Asteraceae as sunflower, inoculated with the crude toxin. On the other hand, within two days, dark-brown spots and halos developed on sunflower leaves used as controls.

The heat-dried toxin produced none of the symptoms on sunflower leaves even seven days after application while the non-heated toxin, used as the control, produced both dark-brown spots as well as halos within two days.

The culture filtrate, when used in place of distilled water for germinating sunflower seeds, reduced germination by 30% from that of the control seeds (Table 2). The culture filtrate also reduced the root/shoot ratio of sunflower seedlings by ca. 5.7% (Table 2).

Table 2

Effect of *Alternaria helianthi* toxin on sunflower seed germination and root/shoot ratio

Replicates	Seed germination (%)		Root/shoot ratio	
	Toxin-treated	Control	Toxin-treated	Control
1	44	92	1.27	1.38
2	72	96	1.44	1.41
3	80	92	1.27	1.44
Average	65.33	93.33	1.33	1.41

The toxin fraction with the R_f value 0.08 from the Kota isolate produced neither a brown spot nor any halo on sunflower leaves. On the other hand, the toxin fraction with the R_f value 0.88 from the same source produced a dark-brown spot with a very faint halo. The two fractions when applied in combination, produced dark-brown spots with slightly more pronounced halos.

The toxin recovered from undeveloped chromatograms produced typical symptoms in the form of dark-brown spots surrounded by halos while no such symptom developed in the control leaves.

Discussion

Partially-purified ether-extracted toxins, produced both in vitro as well as in vivo by each of the three *A. helianthi* isolates, gave similar reactions with all the five detection reagents but they differed from the one isolated by Bhaskaran and Kandaswamy (1978) in giving a reddish-violet colour with $FeCl_3$ and a yellow colour with DSA and in having two fractions with the R_f values of 0.88 and 0.08, respectively. The toxin obtained by Bhaskaran and Kandaswamy (1978), on the other hand, gave no colour reaction with $FeCl_3$, a brick-red colour with DSA and had only one fraction (R_f value 0.968). It may be that both the toxins obtained by us are different from the one obtained by Bhaskaran and Kandaswamy (1978) and this may be due to different pathogen isolates and/or host varieties used in these two studies. Alternatively, some of these differences may be explained by assuming that our toxins were obtained in higher concentrations so as to give a positive colour test with $FeCl_3$ and the appearance of the second fraction (R_f value 0.08) as a small and faint spot. The difference in the R_f values (0.968 and 0.88) may be due to some physical factor(s), such as temperature, etc. However, the different colours with DSA test can not be explained except by assuming different toxins. Considering all the differences, it is quite likely that both the present toxins are different from the one reported by Bhaskaran and Kandaswamy (1978).

Colour reactions with $FeCl_3$, Folin-Ciocalteu and DSA, and fluorescence with UV light (both with and without ammonia) indicate that *A. helianthi* toxins are phenolic in nature as also reported by Bhaskaran and Kandaswamy (1978). Tal et al. (1985) have listed five toxic compounds from culture filtrate of *A. helianthi* from sunflower, the major metabolite being deoxyradicinin, followed by 3-epideoxyradicinol, and three minor ones, namely radicinin, deoxyradicinol and radianthin. It may be that our toxin with the R_f value of 0.88 is deoxyradicinin and the other toxin (R_f value 0.08) is one of the minor toxins (radicinin, deoxyradicinol or radianthin) but the exact nature of our toxins could not be determined because of lack of facilities. The same reason may also be responsible for not detecting any of the remaining toxins of Tal et al. (1985). Neither of our toxins is likely to be 3-epideoxyradicinol as it has not been isolated in vivo (Robeson and Strobel 1985). It was also observed that when both the fractions were applied

separately to sunflower leaves, only the fraction with the higher R_f value produced necrotic spots with halos. Robeson and Strobel (1985) also obtained both necrosis and chlorosis with deoxyradicinin. Though the other fraction (R_f = = 0.08) alone did not produce any symptom, when it was applied jointly with the fraction with the R_f value 0.88, the halo was slightly more pronounced. It indicates this fraction (R_f = 0.08) may help in producing only halos. However, Tal et al. (1985) obtained dark-brown necrotic spots with one of the minor fractions (radianthin) also. It has also been reported that when *A. helianthi* toxin is sprayed on sunflower leaves, no halo is produced (Kolte 1985). However, our toxin fraction with the R_f value 0.88 does produce halos also as reported by Robeson and Strobel (1985).

The culture filtrate and the crude toxin isolated both from the culture filtrate and from the sunflower leaves inoculated with the Kota isolate, produced necrotic spots with halos and hence this toxin may be involved in pathogenesis. The maximum toxin production in vivo by the most virulent Kota isolate may be involved in the faster disease development and in more severe disease on both leaves and stems. Further, the size of both the spot and the halo was directly proportional to the toxin concentration.

It was observed that when *A. helianthi* crude toxin was applied to several non-host plants of the pathogen (belonging to the same family Asteraceae as sunflower), it produced neither any necrotic spot nor any halo, indicating that *A. helianthi* toxins are host-specific. However, with deoxyradicinin, Robeson and Strobel (1982) obtained necrosis on a non-host plant *(Cirsium arvense)* also.

On the basis of production of all the symptoms of *A. helianthi* on the host plant by the toxin fraction (R_f 0.88) and because of its host-specific nature, this toxin fraction can be tentatively classified as a pathotoxin (Wheeler, 1976).

The present toxins are thermolabile. They reduced the host seed germination as well as the root/shoot ratio of the host seedlings as also reported by Bhaskaran and Kandaswamy (1978) and Islam and Maric (1980). Robeson and Strobel (1982) also observed necrosis and loss of turgor due to deoxyradicinin. These observations indicate that our toxins may be disturbing one or more metabolic activities of the host plant.

This disease is both seed-borne as well as soil-borne (Bhaskaran, 1976) and hence the importance of toxin-production is further increased as the host seed is consumed (both directly, and indirectly in the form of oil) by human beings. Occurrence of toxins in the *A. helianthi*-infected sunflower leaves raises the possibility that they may also occur in sunflower seeds when *A. helianthi* infects sunflower heads and this aspect should be investigated.

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Literature

Bhaskaran, R. (1976): Studies on the leaf spot disease of sunflower caused by *Alternaria helianthi*. Ph. D. Thesis submitted to the Tamil Nadu Agricultural University, Coimbatore.

Bhaskaran, R. and Kandaswamy, T. K. (1978): Production of a toxic metabolite by *Alternaria helianthi* in vitro and in vivo. Madras Agriculture Journal 65, 801–804.

Ghemawat, M. S., Sharma, S. C. and Agrawat, J. M. (1986): Production of IAA in the sunflower leaf-spot and blight disease caused by *Alternaria helianthi*. Acta Phytopathologica et Entomologica Hungarica 21, 267–271.

Islam, U. and Maric, A. (1980): Contribution to the studies on biology, epidemiology and resistance of sunflower to *Alternaria helianthi* (Hansf.) Taub. Nish. Zastita Bilja 31, 35–49.

Kolte, S. J. (1985): Diseases of Annual Edible Oilseed Crops. Vol. III: Sunflower, Safflower and Nigerseed Diseases, CRC Press, Boca Raton.

Purushothaman, D. and Prasad, N. N. (1972): Isolation of a toxin from *Xanthomonas oryzae*. Phytopathologische Zeitschrift 75, 178–180.

Robeson, D. J. and Strobel, G. A. (1982): Deoxyradicinin, a novel phytotoxin from *Alternaria helianthi*. Phytochemistry 21, 1821–1823.

Robeson, D. J. and Strobel, G. A. (1985): Deoxyradicinin and 3-epideoxyradicinol production by the sunflower pathogen *Alternaria helianthi*. Annals of Applied Biology 107, 409–415.

Tal, B., Robeson, D. J., Burke, B. A. and Aasen, A. J. (1985): Phytotoxins from *Alternaria helianthi:* radicinin, and the structures of deoxyradicinol and radianthin. Phytochemistry 729–731.

Wheeler, H. (1976): The role of phytotoxins in specificity. *In* Specificity in Plant Disease (Ed. R. K. S. Wood and A. Graniti), Plenum Publishing Corp., New York, p. 217–235.

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A List of Proposed Letter Codes for Hosts and Non-Hosts of Plant Viruses[1]

J. HORVÁTH

Pannonian University of Agricultural Sciences, Institute for Plant Protection, Department of Plant Pathology, H-8361 Keszthely, P.O. Box 71, Hungary

The author makes a proposal on the use of a code system elaborated for the major test plants (hosts, non-hosts) of plant viruses as well as for diagnostically important cultivars and hybrids by adapting and using the Weed Science Society of America (WSSA) and Weed Science Society of Japan (WSSJ) approved computer codes; and also suggests the establishment of a data base. The code system covers the virus acronyms (e.g., TMV for tobacco mosaic virus), the virus family/groups and subgroups (e.g., TOBAMO for tobacco mosaic virus group; or BROMO for brome mosaic virus group, etc.), the hosts and non-hosts (five letter abbreviations; e.g., AGRIT for *Agropyron intermedium*), the cultivars and hybrids (three letter codes; e.g., SAM for cultivar Samsun of *Nicotiana tabacum*, or GTC for hybrid of *Nicotiana glutinosa* × *Nicotiana clevelandii*), the plant families (three letter codes; e.g., AIZ for Aizoaceae) and the host characters (2–4 letter abbreviations; e.g., AH for assay host, DH for diagnostic host, PH for propagation host). With these abbreviations used a code system can be written down: e.g., AILV — NEPO — NIOTA — WHB — DH — SOL, which expresses that the artichoke Italian latent Nepovirus (AILV — NEPO) has the *Nicotiana tabacum* (NIOTA) White Burley cultivar (WHB) as host, which is a diagnostic host (DH) for the virus, and belongs to the family Solanaceae (SOL).

The computer codes approved by the Weed Science Society of America (WSSA) and Weed Science Society of Japan (WSSJ) cover those plant and plant groups "that are important to man and animal as well as those which compete with economically important crops" (Anonymous, 1992). The approved computer codes are, however, important not only in herbology but also in plant virology. Considering that the role of plants is important in the era of molecular virology too, it is reasonable to introduce computer codes that contain the names of test plants (hosts and non-hosts) and viruses. The names of hosts, non-hosts and viruses are part of a data base, highly important in virology. Since to our best knowledge the use of computer codes for plants has not spread so far in plant virology, suggestion is made here to develop and use such a system. This is all the more justified, as a publication has recently appeared on proposed acronyms of plant viruses and viroids (Hull et al., 1991), and highly important is the information on viruses and host plants that has been stored and manipulated using the DELTA (*De*scription *L*anguage for *Ta*xonomy) system in the WAX 11/750 com-

[1] Dedicated to the late Dr. L. Beczner (1938–1988) on the occasion of his 55th birthday.

Akadémiai Kiadó, Budapest

puter of the Research School of Biological Sciences, Australian National University, Canberra (Boswell and Gibbs, 1983, 1986; Brunt et al., 1990).

The joint use of virus acronyms and letter codes of hosts and non-hosts would greatly facilitate the storage of information in a data base.

Basic Principles of Codes

1. *Virus acronyms*

Hull et al. (1991) published a list of proposed standard acronyms for plant viruses (and viroids). For example, TMV for tobacco mosaic virus, or MYFV for Melandrium yellow fleck virus. The last "V" letter of the virus acronyms (e.g., in TMV for tobacco mosaic virus) refers to the word "virus", which has recently been replaced by the name of the virus family/group or subgroup (e.g., tobacco mosaic TOBAMOvirus, or Melandrium yellow fleck BROMOvirus; see Francki et al., 1991; Matthews, 1991; Horváth, 1992). Detailed information concerning the acronyms is to be found in Hull et al. (1991).

2. *Hosts, non-hosts and family codes*

Five-letter abbreviations are based on the scientific (Latin) names of plants (subspecies, varieties or forms). The first three letters refer to the genus (e.g., AGR for *Agropyron*), while the last two (e.g., IT for *intermedium*) denote the species, subspecies or forms in abbreviated form (Anonymous, 1992). If the species are not designated, it is symbolized by the letters "SS" (e.g., AMASS for *Amaranthus* species). The different cultivars and/or hybrids may react differently to the same virus, therefore the names of cultivars and/or hybrids must be also given in abbreviated form. In 1976 the BAYER AG (Leverkusen, Germany) began to develop a code system for names of cultivars and hybrids, but the attempt remained at that (Faust, 1992). Neither do the computer codes approved by the WSSA/WSSJ contain abbreviations for cultivars and hybrids (Anonymous, 1992). We therefore suggest to use three letters for the abbreviation of cultivar- and hybrid names: e.g., MOB for cultivar Monobush of *Beta vulgaris*.

For the family status of plants the WSSA/WSSJ code system suggests the use of a three-letter code; e.g., CHE for Chenopodiaceae. Accordingly, the approved computer code for *Beta vulgaris* cv. Monobush from the family Chenopodiaceae is: BEAVA – MOB – CHE.

3. *Codes of the host characters*

The virus hosts can be placed by their characteristics in three major groups: assay hosts, diagnostic hosts and propagation hosts. In the CMI/AAB Descriptions of Plant Virus Diseases (now AAB) series (see Murant and Harrison, 1970–1988), or in the latest book of Brunt et al. (1990) the hosts of the various viruses are also discussed in the above way. In some host-virus relations e.g. well defined,

typical local assay hosts are known. Such is *Nicotiana glutinosa* (NIOGT) for tobacco mosaic TOBAMOvirus (TMV) (Holmes, 1929). In other cases so-called systemic assay hosts are known too (e.g., *Nicotiana tabacum* cv. Kentucky 14 for tobacco vein mottling POTYvirus; Pirone and Shaw, 1988). In the above book of Brunt et al. (1991) "species for local (L) lesion or whole (W) plant assay" are mentioned. And there are assay species (e.g., *Datura stramonium* for African cassava mosaic GEMINIvirus; Bock and Harrison, 1985), which at the same time are local and systemic assay hosts. Moreover, we know of species (e.g., *Chenopodium amaranticolor* and *C. quinoa* for lucerne transient streak SOBEMOvirus; Forster and Jones, 1980) which are local assay hosts for some strain of a virus and systemic assay hosts for another strain of the same virus. The reaction to viruses of a given host plant is a very important criterion, we therefore make a proposal on the code system of the above basic characters and on the use of that code system. We suggest to use the following 2–4 letter codes: AH for assay host, AHL for local assay host, AHLS for local and systemic assay host, AHL(S) for local assay host, when some virus strains produce also systemic symptoms, AHS for systemic assay host, DH for diagnostic host and PH for propagation host; (see Murant and Harrison, 1970–1988; Horváth, 1993). If a virus has no assay host, or diagnostic host or propagation host known, we suggest to use a code marked with five Xs (XXXXX) for denoting the genus and species, and a code marked with three Xs (XXX) for the family, before the code of the corresponding plant character (see Horváth, 1993). For example, XXXXX–XXX AH, or XXXXX–XXX DH, or XXXXX–XXX PH.

Application of the Codes

On the basis of the above we give the codes (virus acronyms as well as host, non-host and family abbreviations) which refer to mechanically transmissible viruses to be found in the series CMI/AAB Descriptions of Plant Virus Diseases (now AAB) (see Murant and Harrison, 1970–1988) and to plants listed in our recent papers (see Horváth, 1992, 1993). For the virus acronyms the suggestions of Hull et al. (1991), while for the hosts, non-hosts and their families the computer codes approved by the WSSA/WSSJ (Anonymous, 1992) were taken into consideration. Since codes for many virus hosts and non-hosts, including cultivars and hybrids cannot be found in the above publication, we offer our own proposal for consideration.

1. *Proposed standard acronyms for mechanically transmissible plant viruses*

In the series CMI/AAB Descriptions of Plant Virus Diseases (see Murant and Harrison, 1970–1988) some 232 mechanically transmissible viruses are described. The acronyms proposed by Hull et al. (1991) for these viruses are contained in Table 1.

Table 1

Proposed standard acronyms for mechanically transmissible plant viruses[1]

Virus name	Acronym	Family/group and subgroup[2]
African cassava mosaic	ACMV	GEMINI II
Agropyron mosaic	AgMV	POTY (mite)
Alfalfa latent (= pea streak)	ALV	CARLA
Alfalfa mosaic	AMV	AMV group
American hop latent	AHLV	CARLA
American plum line pattern	APLPV	ILAR V
Andean potato mottle	APMV	COMO
Apple chlorotic leaf spot	ACLSY	? CLOSTERO
Apple mosaic (= some isolates of rose mosaic)	ApMV	ILAR
Arabis mosaic	ArMV	NEPO
Arracacha A	AVA	NEPO
Arracacha B	AVB	? NEPO
Artichoke Italian latent	AILV	NEPO
Artichoke vein banding	AVBV	? NEPO
Artichoke yellow ringspot	AYRSV	NEPO
Asparagus 2	AV2	ILAR II
Barley yellow mosaic	BaYMV	POTY (fungus)
Bean common mosaic	BCMV	POTY (aphid)
Bean golden mosaic	BGMV	GEMINI II
Bean mild mosaic	BMMV	? CARMO
Bean pod mottle	BPMV	COMO
Bean rugose mosaic	BRMV	COMO
Bean yellow mosaic	BYMV	POTY (aphid)
Bearded iris mosaic	BIMV	POTY (aphid)
Beet mosaic	BtMV	POTY (aphid)
Beet necrotic yellow vein	BNYVV	? FURO
Beet yellows (= sugarbeet yellows)	BYV	CLOSTERO
Belladonna mottle	BeMV	TYMO
Bidens mottle	BiMoV	POTY (aphid)
Blackeye cowpea mosaic	BlCMV	POTY (aphid)
Blackgram mottle	BMoV	? CARMO
Black raspberry latent	BRLV	ILAR
Blueberry leaf mottle	BLMV	NEPO
Blueberry red ringspot	BRRV	CAULIMO
Broad bean mottle	BBMV	BROMO
Broad bean necrosis	BBNV	? FURO
Broad bean stain	BBSV	COMO
Broad bean true mosaic	BBTMV	COMO
Brome mosaic	BMV	BROMO
Cacao necrosis	CNV	NEPO
Cacao yellow mosaic	CYMV	TYMO
Cactus X	CVX	POTEX
Carnation etched ring	CERV	CAULIMO
Carnation latent	CLV	CARLA
Carnation mottle	CarMV	CARMO
Carnation necrotic fleck	CNFV	CLOSTERO
Carnation ringspot	CRSV	DIANTHO

Table 1 (cont.)

Virus name	Acronym	Family/group and subgroup[z]
Carnation vein mottle	CVMV	POTY (aphid)
Carrot thin leaf	CTLV	POTY (aphid)
Cassava common mosaic	CsCMV	POTEX
Cassia yellow blotch	CYBV	BROMO
Cauliflower mosaic	CaMV	CAULIMO
Celery mosaic	CeMV	POTY (aphid)
Cherry leaf roll	CLRV	NEPO
Cherry rasp leaf	CRLV	? NEPO
Chicory yellow mottle	ChYMV	NEPO
Chrysanthemum B	CVB	CARLA
Citrus leaf rugose	CiLRV	ILAR II
Clitoria yellow vein	CYVV	TYMO
Clover yellow mosaic	ClYMV	POTEX
Clover yellow vein	ClYVV	POTY (aphid)
Cocksfoot mild mosaic	CMMV	? SOBEMO
Cocksfoot mottle	CoMV	SOBEMO
Cocksfoot streak	CSV	POTY (aphid)
Cowpea aphid-borne mosaic (= azuki bean mosaic)	CABMV	POTY (aphid)
Cowpea chlorotic mottle	CCMV	BROMO
Cowpea mild mottle	CPMMV	CARLA
Cowpea mosaic	CPMV	COMO
Cowpea mottle	CPMoV	? CARMO
Cowpea severe mosaic	CPSMV	COMO
Cucumber green mottle mosaic	CGMMV	TOBAMO
Cucumber leaf spot	CLSV	? CARMO
Cucumber mosaic	CMV	CUCUMO
Cucumber necrosis	CuNV	TOMBUS
Cymbidium mosaic	CyMV	POTEX
Cymbidium ringspot	CyRSV	TOMBUS
Dahlia mosaic	DMV	CAULIMO
Daphne X	DVX	POTEX
Dasheen mosaic	DsMV	POTY (aphid)
Desmodium yellow mottle	DYMV	TYMO
Dioscorea latent	DLV	POTEX
Eggplant mosaic	EMV	TYMO
Elderberry carla (= elderberry A)	ECV	CARLA
Elderberry latent	ELV	? CARMO
Elm mottle	EMoV	ILAR II
Erysimum latent	ErLV	TYMO
Foxtail mosaic	FoMV	POTEX
Frangipani mosaic	FrMV	TOBAMO
Galinsoga mosaic	GaMV	CARMO
Ginger chlorotic fleck	GCFV	? SOBEMO
Grapevine Bulgarian latent	GBLV	NEPO
Grapevine chrome mosaic	GCMV	NEPO
Grapevine fanleaf	GFLV	NEPO
Guinea grass mosaic	GGMV	POTY (aphid)
Helenium S	HVS	CARLA
Henbane mosaic	HMV	POTY (aphid)

Table 1 (cont.)

Virus name	Acronym	Family/group and subgroup[2]
Heracleum latent	HLV	? CLOSTERO
Hibiscus chlorotic ringspot	HCRSV	CARMO
Hibiscus latent ringspot	HLRSV	NEPO
Hippeastrum mosaic	HiMV	POTY (aphid)
Honeysuckle latent	HnLV	CARLA
Hop latent	HpLV	CARLA
Hop mosaic	HpMV	CARLA
Hydrangea ringspot	HRSV	POTEX
Hypochoeris mosaic	HyMV	? FURO/? TOBAMO
Iris fulva mosaic	IFMV	POTY (aphid)
Iris mild mosaic	IMMV	POTY (aphid)
Iris severe mosaic	ISMV	POTY (aphid)
Kennedya yellow mosaic	KYMV	TYMO
Leek yellow stripe	LYSV	POTY (aphid)
Lettuce mosaic	LMV	POTY (aphid)
Lilac chlorotic leafspot	LCLV	CLOSTERO
Lilac ring mottle	LRMV	ILAR VII
Lily symptomless	LSV	CARLA
Lucerne Australian latent	LALV	NEPO
Lucerne transient streak	LTSV	SOBEMO
Maclura mosaic	MacMV	? POTY (aphid)
Maize chlorotic mottle	MCMV	? SOBEMO
Melandrium yellow fleck	MYFV	BROMO
Melon necrotic spot	MNSV	CARMO
Mulberry ringspot	MRSV	NEPO
Mungbean yellow mosaic	MYMV	GEMINI II
Myrobalan latent ringspot	MLRSV	NEPO
Narcissus latent (= gladiolus ringspot)	NLV	CARLA
Narcissus mosaic	NMV	POTEX
Narcissus tip necrosis	NTNV	? CARMO
Narcissus yellow stripe	NYSV	POTY (aphid)
Nerine X	NVX	POTEX
Nicotiana velutina mosaic	NVMV	? FURO
Oat mosaic	OMV	POTY (fungus)
Oat necrotic mottle	ONMV	POTY (? mite)
Odontoglossum ringspot	ORSV	TOBAMO
Okra mosaic	OkMV	TYMO
Olive latent ringspot	OLRSV	NEPO
Onion yellow dwarf	OYDV	POTY (aphid)
Panicum mosaic	PMV	? SOBEMO
Papaya mosaic	PapMV	POTEX
Papaya ringspot (= watermelon mosaic 1)	PRSV	POTY (aphid)
Parsnip mosaic	ParMV	POTY (aphid)
Passionfruit woodiness	PWV	POTY (aphid)
Pea early-browning	PEBV	TOBRA
Pea enation mosaic	PEMV	PEMV group
Pea seed-borne mosaic	PSbMV	POTY (aphid)
Pea streak (= alfalfa latent)	PeSV	CARLA

Table 1 (cont.)

Virus name	Acronym	Family/group and subgroup[2]
Peach rosette mosaic	PRMV	NEPO
Peanut clump	PCV	FURO
Peanut mottle	PeMoV	POTY (aphid)
Peanut stunt (= Robinia mosaic)	PSV	CUCUMO
Pelargonium flower-break	PFBV	CARMO
Pepper mild mottle	PMMV	TOBAMO
Pepper mottle	PepMoV	POTY (aphid)
Pepper veinal mottle	PVMV	POTY (aphid)
Peru tomato	PTV	POTY (aphid)
Plantain X	PlVX	POTEX
Plum pox	PPV	POTY (aphid)
Poinsettia mosaic	PnMV	? TYMO
Pokeweed mosaic	PkMV	POTY (aphid)
Poplar mosaic	PopMV	CARLA
Potato A	PVA	POTY (aphid)
Potato aucuba mosaic	PAMV	? POTEX
Potato black ringspot	PBRSV	NEPO
Potato M	PVM	CARLA
Potato mop-top	PMTV	FURO
Potato S (= pepino latent)	PVS	CARLA
Potato V	PVV	POTY (aphid)
Potato X	PVX	POTEX
Potato Y	PVY	POTY (aphid)
Prune dwarf	PDV	ILAR III
Prunus necrotic ringspot (= some isolates of rose mosaic)	PNRSV	ILAR III
Quail pea mosaic	QPMV	COMO
Radish mosaic	RaMV	COMO
Raspberry bushy dwarf	RBDV	"Idaeovirus group"
Raspberry ringspot	RRSV	NEPO
Red clover mottle	RCMV	COMO
Red clover necrotic mosaic	RCNMV	DIANTHO
Red clover vein mosaic	RCVMV	CARLA
Ribgrass mosaic	RMV	TOBAMO
Rice necrosis mosaic	RNMV	POTY (fungus)
Rice yellow mottle	RYMV	SOBEMO
Robinia mosaic	RbMV	CUCUMO
Ryegrass mosaic	RGMV	POTY (mite)
Saguaro cactus	SCV	CARMO
Satsuma dwarf	SDV	? NEPO
Scrophularia mottle	ScrMV	TYMO
Shallot latent	SLV	CARLA
Soil-borne wheat mosaic	SBWMV	FURO
Solanum nodiflorum mottle	SNMV	SOBEMO
Southern bean mosaic	SBMV	SOBEMO
Sowbane mosaic	SoMV	SOBEMO
Soybean chlorotic mottle	SbCMV	CAULIMO
Soybean mosaic	SbMV	POTY (aphid)
Spinach latent	SpLV	ILAR

Table 1 (cont.)

Virus name	Acronym	Family/group and subgroup[2]
Squash mosaic	SMV	COMO
Strawberry latent ringspot	SLRSV	? NEPO
Subterranean clover mottle	SCMoV	SOBEMO
Sugarcane mosaic	SCMV	POTY (aphid)
Sunn-hemp mosaic	SHMV	TOBAMO
Sweet clover necrotic mosaic	SCNMV	DIANTHO
Sweet potato mild mottle	SPMMV	POTY (whitefly)
Tephrosia symptomless	TeSV	? CARMO
Tobacco etch	TEV	POTY (aphid)
Tobacco mosaic	TMV	TOBAMO
Tobacco necrosis	TNV	NECRO
Tobacco rattle	TRV	TOBRA
Tobacco ringspot	TRSV	NEPO
Tobacco streak	TSV	ILAR I
Tobacco vein mottling	TVMV	POTY (aphid)
Tomato aspermy	TAV	CUCUMO
Tomato black ring	TBRV	NEPO
Tomato bushy stunt	TBSV	TOMBUS
Tomato golden mosaic	TGMV	GEMINI II
Tomato mosaic	ToMV	TOBAMO
Tomato ringspot	ToRSV	NEPO
Tomato spotted wilt	TSWV	TOSPO[3]
Tulare apple mosaic	TAMV	ILAR II
Tulip breaking	TBV	POTY (aphid)
Tulip X	TVX	POTEX
Turnip crinkle	TCV	CARMO
Turnip mosaic	TuMV	POTY (aphid)
Turnip rosette	TRoV	SOBEMO
Turnip yellow mosaic	TYMV	TYMO
Ullucus C	UVC	COMO
Velvet tobacco mottle	VTMoV	SOBEMO
Viola mottle	VMV	POTEX
Voandzeia necrotic mosaic	VNMV	TYMO
Watermelon mosaic virus 2	WMV2	POTY (aphid)
Wheat spindle streak mosaic	WSSMV	POTY (fungus)
Wheat streak mosaic	WSMV	POTY (mite)
White clover mosaic	WClMV	POTEX
Wild cucumber mosaic	WCMV	TYMO
Wineberry latent	WLV	POTEX
Yam mosaic	YMV	POTY (aphid)
Zucchini yellow mosaic	ZYMV	POTY (aphid)

[1] From Hull et al. (1991).

[2] Roman numerals (I, II, etc.) indicate recognized subgroups. The vector (e.g., mite, aphid, leafhopper, etc.) is indicated where of taxonomic importance. Question mark indicates uncertain taxonomic position, or other doubt (e.g., ? CLOSTERO, ? NEPO, etc.).

[3] German et al. (1992).

2. *Five- and three-letter abbreviations for virus hosts, non-hosts, families, including cultivars and hybrids*

In the series CMI/AAB Descriptions of Plant Virus Diseases (see Murant and Harrison, 1970–1988) the treatment of virus hosts (assay hosts, diagnostic hosts, propagation hosts) covers 421 plants of 151 genera from 39 plant families. The approved computer codes of these plants (see Anonymous, 1992) and the codes proposed by us for them can be found in Tables 2 and 3.

Table 2

Five and three-letter abbreviations for virus hosts, non-hosts, families including cultivars and hybrids[1]

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Ageratum conyzoides cv. Blue Ball			AGECO	BLB	COM
Agropyron elongatum	AGREL	GRA			
Agropyron intermedium	AGRIT	GRA			
Agropyron repens	AGRRE	GRA			
Agrostis alba	AGSGI	GRA			
Agrostis palustris	AGSPL	GRA			
Agrostis tenuis	AGSTE	GRA			
Allium ascalonicum	ALLAS	LIL			
Allium cepa	ALLCE	LIL			
Allium fistulosum	ALLFI	LIL			
Allium porrum	ALLPO	LIL			
Althaea spp.	ALGSS	MAV			
Amaranthus caudatus	AMACA	AMA			
Amaranthus retroflexus	AMARE	AMA			
Anthoxanthum aristatum	AOXPU	GRA			
Anthriscus sylvestris (= A. silvestris)	ANRSY	UMB			
Antirrhinum majus[a]	ATHMM	SCP			
Apium graveolens cv. Utah 10B			APUGV	UTH	UMB
Apium graveolens var. dulce	APUGD	UMB			
Arachis hypogaea	ARHHY	LEG			
Atriplex hortensis	ATXHO	CHE			
Avena sativa	AVESA	GRA			
Avena sativa cv. Blenda			AVESA	BLD	GRA
Avena strigosa	AVESG	GRA			
Bauhinia purpurea	BAUPU	LEG			
Belamcanda chinensis	BMCCH	IRI			
Beta macrocarpa	BEAMA	CHE			
Beta patellaris			BEAPA		CHE
Beta vulgaris	BEAVA	CHE			
Beta vulgaris cv. Detroit			BEAVA	DET	CHE
Beta vulgaris cv. Greentop			BEAVA	GRT	CHE
Brassica campestris	BRSRA	CRU			
Brassica campestris cv. Just Right			BRSRA	JUR	CRU

Table 2 (cont.)

Hosts[a] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[a]	Code of botanical name	Cultivar or hybrid code	Family code[a]
Brassica chinensis	BRSCH	CRU			
Brassica juncea	BRSJU	CRU			
Brassica napus	BRSNN	CRU			
Brassica napus var. napobrassica	BRSNA	CRU			
Brassica oleracea	BRSOX	CRU			
Brassica oleracea var. botrytis	BRSOB	CRU			
Brassica oleracea var. capitata	BRSOR	CRU			
Brassica pekinensis	BRSPK	CRU			
Brassica perviridis	BRSPE	CRU			
Brassica rapa	BRSRA	CRU			
Bromus inermis	BROIN	GRA			
Bromus mollis	BROMO	GRA			
Bromus racemosus	BRORA	GRA			
Bromus secalinus	BROSE	GRA			
Bromus tectorum	BROTE	GRA			
Cajanus cajan	CAJCA	LEG			
Caladium hortulanum			CLFHO		ARA
Canavalia ensiformis	CNAEN	LEG			
Capsicum annuum	CPSAN	SOL			
Capsicum annuum cv. Long Red			CPSAN	LOR	SOL
Capsicum chacoense			CPSCH		SOL
Capsicum frutescens	CPSFR	SOL			
Capsicum frutescens cv. Early Calwonder			CPSFR	EAC	SOL
Capsicum frutescens cv. Tabasco			CPSFR	TAB	SOL
Capsicum praetermissum			CPSPR		SOL
Capsicum spp.	CPSSS	SOL			
Carica papaya	CIAPA	CAA			
Carnegiea gigantea			CANGI		CAC
Carthamus tinctorius	CAUTI	COM			
Cassia bicapsularis			CASBI		LEG
Cassia obtusifolia	CASOB	LEG			
Cassia occidentalis	CASOC	LEG			
Cassia tora	CASTO	LEG			
Celosia argentea	CEOAR	AMA			
Celosia cristata	CEOAR	AMA			
Chaenomeles japonica	CNMJA	ROS			
Cheiranthus cheiri	CHUCH	CRU			
Chenopodium album	CHEAL	CHE			
Chenopodium amaranticolor[b]	CHEGI	CHE			
Chenopodium ambrosioides	CHEAM	CHE			
Chenopodium capitatum	CHECA	CHE			
Chenopodium foetidum	CHEVU	CHE			
Chenopodium foliosum			CHEFO		CHE
Chenopodium hybridum	CHEHY	CHE			
Chenopodium murale	CHEMU	CHE			
Chenopodium spp.	CHESS	CHE			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Chenopodium quinoa	CHEQU	CHE			
Cichorium endivia	CICED	COM			
Citrullus lanatus	CITLA	CUC			
Citrullus vulgaris	CITLA	CUC			
Citrus aurantiifolia	CIDAF	RUB			
Citrus limon	CIDLI	RUB			
Citrus medica	CIDME	RUB			
Citrus paradisi	CIDPA	RUB			
Claytonia perfoliata	CLAPE	POR			
Clitoria ternatea	CXCTE	LEG			
Colocasia esculenta	CXSES	ARA			
Conium maculatum	COIMA	UMB			
Coriandrum sativum	CORSA	UMB			
Crocus vermus				CVOVE	IRI
Crotalaria spectabilis	CVTSP	LEG			
Cucumis melo	CUMME	CUC			
Cucumis metuliferus			CUMMU		CUC
Cucumis sativus	CUMSA	CUC			
Cucumis sativus cv. Butcher's Disease Res.			CUMSA	BDS	CUC
Cucumis sativus cv. Lange Gele Tros			CUMSA	LGT	CUC
Cucumis sativus cv. National Pickling			CUMSA	NAP	CUC
Cucumis sativus cv. Windermoor Wonder			CUMSA	WIW	CUC
Cucurbita maxima cv. Buttercup			CUUMA	BUT	CUC
Cucurbita moschata	CUUMO	CUC			
Cucurbita okeechobeensis			CUUOK		CUC
Cucurbita pepo	CUUPE	CUC			
Cucurbita pepo cv. Early Prolific Straight	CUUPE	CUC	CUUPE	EPS	CUC
Cyamopsis tetragonoloba	CMOTE	LEG			
Cyphomandra betacea	CYJBE	SOL			
Dactylis glomerata	DACGL	GRA			
Dactylis spp.	DACSS	GRA			
Dahlia pinnata	DAHPI	COM			
Datura metel	DATME	SOL			
Datura meteloides	DATIN	SOL			
Datura stramonium	DATST	SOL			
Daucus carota var. sativa	DAUCS	UMB			
Desmodium tortuosum	DEDTO	LEG			
Dianthus barbatus	DINBA	CAF			
Dianthus caryophyllus	DINCA	CAF			
Dianthus caryophyllus cv. Joker			DINCA	JOK	CAF
Dieffenbachia picta	DIFMA	ARA			
Dinebra retroflexa	DIMRE	GRA			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Dioscorea cayenensis			DIUCA		DIO
Dioscorea composita			DIUCO		DIO
Dioscorea esculenta			DIUES		DIO
Dioscorea floribunda	DIUFL	DIO			
Dioscorea preussii			DIUPR		DIO
Dolichos biflorus	MTMUN	LEG			
Dolichos lablab[c]	DOLLA	LEG			
Elymus junceus	ELYJU	GRA			
Emilia sagittata	EMIFO	COM			
Euphorbia cyatophora	EPHCT	EUP			
Euphorbia prunifolia	EPHPR	EUP			
Festuca capillata	FESTE	GRA			
Festuca elatior	FESPR	GRA			
Festuca rubra	FESRU	GRA			
Festuca rubra var. commutata	FESNI	GRA			
Galinsoga parviflora	GASPA	COM			
Glycine max	GLXMA	LEG			
Glycine max cv. Biloxi			GLXMA	BIL	LEG
Glycine max cv. Chippewa			GLXMA	CHI	LEG
Glycine max cv. Gibson			GLXMA	GIB	LEG
Glycine max cv. Kanrich			GLXMA	KAN	LEG
Glycine max cv. Lee			GLXMA	LEE	LEG
Gomphrena globosa	GOMGL	AMA			
Gossypium hirsutum	GOSHI	MAV			
Helianthus annuus	HELAN	COM			
Hibiscus cannabinus	HIBCA	MAV			
Hibiscus esculentus cv. Clemson Spineless			ABMES	CES	MAV
Hibiscus rosa-sinensis	HIBRS	MAV			
Hippeastrum hybridum	HPSHY	AMY			
Hordeum jubatum	HORJU	GRA			
Hordeum murinum	HORMA	GRA			
Hordeum vulgare	HORVX	GRA			
Humulus lupulus	HUMLU	MOR			
Hyoscyamus niger	HSYNI	SOL			
Ipomoea nil cv. Scarlett O'Hara			IPONI	SOH	COV
Ipomoea setosa			IPOSE		COV
Iris fulva			IRIFU		IRI
Iris× *Hollandica* cv. Professor Blaauw			IRIHO	PRB	IRI
Iris× *Hollandica* cv. Wedgwood			IRIHO	WED	IRI
Iris reticulata			IRIRE		IRI
Iris sibirica			IRISI		IRI
Iris spp.	IRISS	IRI			
Iris tectorum			IRITE		IRI
Kennedya rubicunda (= *Kennedia*)			KENRU		LEG
Lactuca sativa	LACSA	COM			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Lagurus ovatus	LASOV	GRA			
Lamarckia aurea	LAAAU	COM			
Lathyrus odoratus	LTHOD	LEG			
Lavatera trimestris	LVATR	MAV			
Lilium formasanum	LILFO	LIL			
Lilium longiflorum	LILLO	LIL			
Lolium multiflorum	LOLMU	GRA			
Lolium multiflorum cv. S22			LOLMU	STT	GRA
Lolium perenne	LOLPE	GRA			
Lolium persicum	LOLPS	GRA			
Lolium temulentum	LOLTE	GRA			
Luffa acutangula	LUFAC	CUC			
Lunaria annua			LUNAN		CRU
Lycium ssp.	LYUSS	SOL			
Lycopersicon chilense			LYPCI		SOL
Lycopersicon esculentum	LYPES	SOL			
Lycopersicon esculentum cv. Kondine Red			LYPES	KOR	SOL
Lycopersicon esculentum cv. Moneymaker			LYPES	MYK	SOL
Lycopersicon pimpinellifolium	LYPPI	SOL			
Maclura pomifera	MACPO	MOR			
Malus platycarpa			MABPL		ROS
Malus sylvestris	MABSY	ROS			
Malus sylvestris cv. R12740–7A			MABSY	ROA	ROS
Manihot esculenta	MANES	EUP			
Matthiola incana	MTLIN	CRU			
Matthiola incana var. annua			MTLIA		CRU
Medicago truncatula	MEDTR	LEG			
Melandrium album	MELAL	CAF			
Melilotus alba (= *M. albus*)	MEUAL	LEG			
Momordica balsamina	MOMBA	CUC			
Montia perfoliata	CLAPE	POR			
Morus alba	MORAL	MOR			
Narcissus cv. Boswin			NARPS	BOS	AMY
Narcissus cv. Duch Muster			NARPS	DUM	AMY
Narcissus jonquilla	NARJO	AMY			
Narcissus pseudonarcissus	NARPS	AMY			
Nerine bowdenii	NRIBO	AMY			
Nicandra bigelovii			NICBI		SOL
Nicandra physaloides	NICPH	SOL			
Nicotiana benthamiana			NIOBE		SOL
Nicotiana bigelovii			NIOBI		SOL
Nicotiana clevelandii			NIOCL		SOL
Nicotiana clevelandii × *Nicotiana glutinosa*			NIOHY	CLG	SOL
Nicotiana debneyi			NIODE		SOL

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Nicotiana edwardsonii			NIOED		SOL
Nicotiana× *edwardsonii*			NIOHY	EDW	SOL
Nicotiana glutinosa	NIOGT	SOL			
Nicotiana glutinosa× *Nicotiana clevelandii*			NIOHY	GTC	SOL
Nicotiana megalosiphon[d]	NIOSU	SOL			
Nicotiana occidentalis			NIOOC		SOL
Nicotiana repanda			NIORE		SOL
Nicotiana rustica	NIORU	SOL			
Nicotiana spp.	NIOSS	SOL			
Nicotiana sylvestris (= *silvestris*)	NIOSI	SOL			
Nicotiana tabacum	NIOTA	SOL			
Nicotiana tabacum cv. Brigh Yellow			NIOTA	BRY	SOL
Nicotiana tabacum cv. Burley 21			NIOTA	BUR	SOL
Nicotiana tabacum cv. Burley Xanthi			NIOTA	BUX	SOL
Nicotiana tabacum cv. Dutch A			NIOTA	DTA	SOL
Nicotiana tabacum cv. Harrow Velvet			NIOTA	HAR	SOL
Nicotiana tabacum cv. Havana 423			NIOTA	HFT	SOL
Nicotiana tabacum cv. Havana 425			NIOTA	HFF	SOL
Nicotiana tabacum cv. Java			NIOTA	JAV	SOL
Nicotiana tabacum cv. Judy's Pride			NIOTA	JYP	SOL
Nicotiana tabacum cv. Samsun			NIOTA	SAM	SOL
Nicotiana tabacum cv. Samsun NN			NIOTA	SNN	SOL
Nicotiana tabacum cv. Smal			NIOTA	SMA	SOL
Nicotiana tabacum cv. Turkish			NIOTA	TUR	SOL
Nicotiana tabacum cv. Turkish Samsun			NIOTA	TUS	SOL
Nicotiana tabacum cv. Virginia Gold			NIOTA	VIG	SOL
Nicotiana tabacum cv. White Burley			NIOTA	WHB	SOL
Nicotiana tabacum cv. Wisconsin Havana 425			NIOTA	WSH	SOL
Nicotiana tabacum cv. Xanthi			NIOTA	XAN	SOL
Nicotiana tabacum cv. Xanthi-nc			NIOTA	XNC	SOL
Nicotiana velutina			NIOVE		SOL
Ocimum basilicum	OCIBA	LAB			
Oryza punctata	ORYPU	GRA			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Oryza sativa	ORYSA	GRA			
Oryza sativa cv. IR 20			ORYSA	IRT	GRA
Oryza sativa cv. Sindano			ORYSA	SIN	GRA
Panicum dichtomiflorum	PANDI	GRA			
Panicum maximum	PANMA	GRA			
Panicum maximum cv. K 189			PANMA	KOE	GRA
Panicum miliaceum	PANMI	GRA			
Paspalum membranaceum[e]	PASDJ	GRA			
Pastinaca sativa	PAVSA	UMB			
Passiflora edulis	PAQED	PAS			
Passiflora edulis f. flavicarpa	PAQEF	PAS			
Passiflora edulis× *Passiflora edulis* f. flavicarpa			PAQHY	FLA	PAS
Passiflora suberosa	PAQSU	PAS			
Petunia hybrida	PEUHY	SOL			
Petunia hybrida cv. Fire Chief			PEUHY	FIC	SOL
Petunia hybrida cv. Minstrel			PEUHY	MIN	SOL
Petunia hybrida cv. Pink Beauty			PEUHY	PIB	SOL
Petunia hybrida cv. Rosy Morn			PEUHY	ROM	SOL
Petunia spp.	PEUSS	SOL			
Phalaris arundinacea	TYPAR	GRA			
Phalaris paradoxa	PHAPA	GRA			
Phaseolus acutifolium	PHSAF	LEG			
Phaseolus aureus	PHSAU	LEG			
Phaseolus lathyroides	PHSLY	LEG			
Phaseolus lunatus	PHSLU	LEG			
Phaseolus mungo	PHSMU	LEG			
Phaseolus vulgaris	PHSVX	LEG			
Phaseolus vulgaris cv. Bataaf			PHSVX	BAT	LEG
Phaseolus vulgaris cv. Beka			PHSVX	BEK	LEG
Phaseolus vulgaris cv. Black Valentine			PHSVX	BLV	LEG
Phaseolus vulgaris cv. Bountiful			PHSVX	BTF	LEG
Phaseolus vulgaris cv. Canadian Wonder			PHSVX	CAW	LEG
Phaseolus vulgaris cv. Cherokee Wax			PHSVX	CRW	LEG
Phaseolus vulgaris cv. Col. 109-R			PHSVX	COL	LEG
Phaseolus vulgaris cv. Great Northern	PHSVN	LEG	PHSVN	GRN	LEG
Phaseolus vulgaris cv. Hawkesbury Wonder			PHSVX	HAW	LEG
Phaseolus vulgaris cv. Jamapa			PHSVX	JAM	LEG
Phaseolus vulgaris cv. Kinghorn Wax			PHSVX	KIW	LEG
Phaseolus vulgaris cv. Kintoki			PHSVX	KIN	LEG
Phaseolus vulgaris cv. La Victoire			PHSVX	LAV	LEG

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Phaseolus vulgaris cv. Long Tom			PHSVX	LOT	LEG
Phaseolus vulgaris cv. Manteiga			PHSVX	MAN	LEG
Phaseolus vulgaris cv. Masterpiece			PHSVX	MAS	LEG
Phaseolus vulgaris cv. Mex. 27-R			PHSVX	MEX	LEG
Phaseolus vulgaris cv. Pencil Pod			PHSVX	PEP	LEG
Phaseolus vulgaris cv. Pinto	PHSVN	LEG	PHSVN	PIN	LEG
Phaseolus vulgaris cv. Pinto III.			PHSVN	PIT	LEG
Phaseolus vulgaris cv. Plentiful			PHSVX	PLT	LEG
Phaseolus vulgaris cv. Premier			PHSVX	PRE	LEG
Phaseolus vulgaris cv. Purple King			PHSVX	PPK	LEG
Phaseolus vulgaris cv. Red Kidney			PHSVX	RKY	LEG
Phaseolus vulgaris cv. Redland Pioneer			PHSVX	RDP	LEG
Phaseolus vulgaris cv. Royal Windsor			PHSVX	ROW	LEG
Phaseolus vulgaris cv. Scotia			PHSVX	SCO	LEG
Phaseolus vulgaris cv. Tendercrop			PHSVX	TDC	LEG
Phaseolus vulgaris cv. Tendergreen			PHSVV	TDG	LEG
Phaseolus vulgaris cv. The Prince			PHSVV	TPR	LEG
Phaseolus vulgaris cv. Topcrop			PHSVV	TCR	LEG
Phaseolus vulgaris cv. Yamahiro Kurosando			PHSVX	YAK	LEG
Philodendron selloum	PIOSE	ARA			
Philodendron verrucosum			PIOVE		ARA
Phleum arenarium			PHLAR		GRA
Phleum pratense	PHLPR	GRA			
Physalis floridana			PHYFL		SOL
Physalis peruviana	PHYPE	SOL			
Phytolacca americana	PHTAM	PHY			
Pisum sativum	PIBST	LEG			
Pisum sativum cv. Alaska			PIBST	ALA	LEG
Pisum sativum cv. Early Massey Melbourne Market			PIBST	MMM	LEG
Pisum sativum cv. Greenfeast			PIBST	GRF	LEG
Pisum sativum cv. Meteor			PIBST	MET	LEG
Pisum sativum cv. Onward			PIBST	ONW	LEG
Pisum sativum cv. Ranger			PIBST	RAN	LEG
Plantago rugelii	PLARU	PLA			
Poa annua	POAAN	GRA			
Poa compressa	POACO	GRA			
Poa pratensis	POAPR	GRA			
Poa trivialis	POATR	GRA			
Primula malacoides	PRIMA	PRI			
Prunus americana	PRNAM	ROS			
Prunus armeniaca	PRNAR	ROS			
Prunus avium	PRNAV	ROS			
Prunus cerasifera	PRNCF	ROS			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Prunus domestica cv. Grosse Grüne Reneklode			PRNDO	GGR	ROS
Prunus domestica cv. Hauszwetsche			PRNDO	HWE	ROS
Prunus domestica cv. Pozegaca			PRNDO	POZ	ROS
Prunus mahaleb	PRNMH	ROS			
Prunus persica	PRNPS	ROS			
Prunus salicina	PRNSC	ROS			
Prunus serrulata cv. Shirofugen			PRNSL	SHI	ROS
Prunus spp.	PRNSS	ROS			
Ranunculus arvensis	RANAR	RAN			
Ranunculus sardous	RANSA	RAN			
Raphanus sativus cv. Chinese White Winter			RAPSV	CWW	CRU
Raphanus sativus cv. White Icicle			RAPSV	WHI	CRU
Ricinus communis	RIICO	EUP			
Rubus spp.	RUBSS	ROS			
Saccharum officinarum	SACOF	GRA			
Saccharum spp.	SACSS	GRA			
Sambucus nigra	SAMNI	CPF			
Sambucus racemosa	SAMRA	CPF			
Saponaria vaccaria cv. Pink Beauty			VAAPY	PIB	CAF
Secale cereale	SECCE	GRA			
Sesamum indicum	SEGIN	PED			
Sesbania exaltata	SEBEX	LEG			
Setaria italica	SETIT	GRA			
Setaria macrostachia			SETMA		GRA
Setaria viridis	SETVI	GRA			
Silene armeria	SILAR	CAF			
Sinapis alba	SINAL	CRU			
Solanum chacoense (TE_1)			SOLCH	TEO	SOL
Solanum demissum[f] (SdA)			SOLHY	SDA	SOL
Solanum demissum Y			SOLHY	TUY	SOL
Solanum demissum× *Solanum tuberosum* A6			SOLHY	TUA	SOL
Solanum nodiflorum	SOLAM	SOL			
Solanum rostratum	SOLCU	SOL			
Solanum tuberosum cv. Desirée			SOLTU	DES	SOL
Solanum tuberosum cv. Duke of York			SOLTU	DOY	SOL
Solanum tuberosum cv. Saco			SOLTU	SAC	SOL
Solanum tuberosum cv. U.S.D.A.			SOLTU	USD	SOL
Solanum tuberosum ssp. tuberosum cv. Arran Pilot			SOLTU	API	SOL
Solanum tuberosum ssp. tuberosum cv. Maris Bard			SOLTU	MAB	SOL

Table 2 (cont.)

Hosts[a] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Sorghum arundinaceum (= *Sorgum*)	SORVE	GRA			
Sorghum bicolor	SORVU	GRA			
Sorghum bicolor cv. Atlas			SORVU	ALS	GRA
Sorghum bicolor cv. Rio			SORVU	RIO	GRA
Sorghum bicolor cv. Sart			SORVU	SRT	GRA
Spinacia oleracea	SPQOL	CHE			
Stenotaphrum secundatum	STPSE	GRA			
Tephrosia villosa			TEPVI		LEG
Tetragonia expansa[g]	TEATE	AIZ			
Theobroma cacao	THOCA	STE			
Theobroma cacao cv. West African Amelonado			THOCA	WAA	STE
Tithonia speciosa			TITSP		COM
Torenia fournieri	TONFO	SCP			
Trifolium dubium	TRFDU	LEG			
Trifolium hybridum	TRFHY	LEG			
Trifolium incarnatum	TRFIN	LEG			
Trifolium pratense	TRFPR	LEG			
Trifolium repens	TRFRE	LEG			
Trifolium spp.	TRFSS	LEG			
Trifolium subterraneum	TRFSU	LEG			
Triticum aestivum	TRZAX	GRA			
Triticum aestivum cv. Parker			TRZAX	PKR	GRA
Triticum durum	TRZDU	GRA			
Tropaeolum majus	TOPMA	TRP			
Tulipa gesneriana cv. Clara Butt			TULGE	CAB	LIL
Tulipa gesneriana cv. Rose Copland			TULGE	ROC	LIL
Verbesina encelioides	VEEEN	COM			
Vicia faba	VICFX	LEG			
Vicia faba cv. Broad Windsor			VICFX	BRW	LEG
Vicia faba cv. The Sutton			VICFX	SUT	LEG
Vicia sativa	VICSA	LEG			
Vigna cylindrica	VIGSC	LEG			
Vigna radiata	PHSAU	LEG			
Vigna sesquipedalis	VIGSQ	LEG			
Vigna sinensis	VIGSI	LEG			
Vigna sinensis cv. Blackeye			VIGSI	BLE	LEG
Vigna sinensis cv. Monarch[h]			VIGSI	MON	LEG
Vigna unguiculata	VIGSI	LEG			
Vigna unguiculata cv. Blackeys Early Ramsh.			VIGSI	BER	LEG
Vigna unguiculata cv. Early Ramshorn			VIGSI	EAR	LEG
Vigna unguiculata cv. Monarch[i]			VIGSI	MON	LEG
Vigna unguiculata ssp. cylindrica	VIGSC	LEG			
Vigna unguiculata ssp. sesquipedalis	VIGSQ	LEG			

Table 2 (cont.)

Hosts[2] of viruses	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Vigna unguiculata ssp. sinensis			VIGSI		LEG
Vigna unguiculata subsp. unguiculata	VIGSI	LEG			
Vinca rosea	CTURO	APO			
Vitis labrusca cv. Concord			VITLA	CON	VIT
Vitis rupestris	VITRU	VIT			
Vitis spp.	VITSS	VIT			
Vitis vinifera	VITVI	VIT			
Voandzeia subterranea	VOASU	LEG			
Xanthosoma caracu			XATCA		ARA
Zantedeschia sp.	ZNTSS	ARA			
Zea mays	ZEAMX	GRA			
Zinnia elegans	ZIIEL	COM			
Zingiber officinale	ZINOF	ZIN			

[1] Five-letter abbreviations used for botanical name (ssp., var. oder f.) of plants. Three-letter abbreviations used for cultivar, hybrid and family names.

[2] a) Syn.: *Antirrhinum majus* ssp. majus; b) Syn.: *Chenopodium giganteum;* c) Syn.: *Lablab purpureus;* d) Syn.: *Nicotiana suaveolens;* e) Syn.: *Paspalum dissectum;* f) Syn.: *Solanum tuberosum;* g) Syn.: *Tetragonia tetragonoides;* h) Syn.: *Vigna unguiculata* cv. Monarch; i) Syn.: *Vigna sinensis* cv. Monarch.

[3] AIZ, Aizoaceae; AMA, Amaranthaceae; AMY, Amarylidaceae; APO, Apocynaceae; ARA, Araceae; CAA, Caricaceae; CAC, Cactaceae; CAF, Caryophyllaceae; CHE, Chenopodiaceae; COM, Compositae; COV, Convolvulaceae; CPF, Caprifoliaceae; CRU, Cruciferae; CUC, Cucurbitaceae; DIO, Dioscoreaceae; EUP, Euphorbiaceae; GRA, Gramineae; IRI, Iridaceae; LAB, Labiatae; LEG, Leguminosae; LIL, Liliaceae; MAV, Malvaceae; MOR, Moraceae; PAS, Passiflora (=Passifloraceae); PED, Pedaliaceae; PHY, Phytolaccaceae; PLA, Plantaginaceae; POR, Portulaceae; PRI, Primulaceae; RAN, Ranunculaceae; ROS, Rosaceae; RUB, Rubiaceae; SCP, Scrophulariaceae; SOL, Solanaceae; STE, Sterculiaceae; TRP, Tropaeolaceae; UMB, Umbelliferae; VIT, Vitaceae (=Vitidaceae); ZIN, Zingiberaceae.

Table 3

Abbreviations of virus hosts and non-hosts in alphabetical order

Abbreviations of plants[1,2]	Name of plants	Abbreviations of plants[1,2]	Name of plants
ABMES-CES	*Hibiscus esculentus* cv. Clemson Spineless	*BEAVA-DET*	*Beta vulgaris* cv. Detroit
AGECO-BLB	*Ageratum conyzoides* cv. Blue Ball	*BEAVA-GRT*	*Beta vulgaris* cv. Greentop
AGREL	*Agropyron elongatum*	BMCCH	*Belamcanda chinensis*
AGRIT	*Agropyron intermedium*	BROIN	*Bromus inermis*
AGRRE	*Agropyron repens*	BROMO	*Bromus mollis*
AGSGI	*Agrostis alba*	BRORA	*Bromus racemosus*
AGSPL	*Agrostis palustris*	BROSE	*Bromus secalinus*
AGSTE	*Agrostis tenuis*	BROTE	*Bromus tectorum*
ALGSS	*Althaea spp.*	BRSCH	*Brassica chinensis*
ALLAS	*Allium ascalonicum*	BRSJU	*Brassica juncea*
ALLCE	*Allium cepa*	BRSNA	*Brassica napus* var. napobrassica
ALLFI	*Allium fistulosum*	BRSNN	*Brassica napus*
ALLPO	*Allium porrum*	BRSOB	*Brassica oleracea* var. botrytis
AMACA	*Amaranthus caudatus*	BRSOR	*Brassica oleracea* var. capitata
AMARE	*Amaranthus retroflexus*	BRSOX	*Brassica oleracea*
ANRSY	*Anthriscus sylvestris* (= *A. silvestris*)	BRSPE	*Brassica perviridis*
AOXPU	*Anthoxanthum aristatum*	BRSPK	*Brassica pekinensis*
APUGD	*Apium graveolens* var. dulce	BRSRA	*Brassica campestris* (syn.: *B. rapa*)
APUGV-UTH	*Apium graveolens* cv. Utah 10B	BRSRA	*Brassica campestris* (syn.: *B. campestris*)
ARHHY	*Arachis hypogaea*	*BRSRA-JUR*	*Brassica campestris* cv. Just Right
ATHMM	*Antirrhinum majus*	CAJCA	*Cajanus cajan*
ATXHO	*Atriplex hortensis*	*CANGI*	*Carnegiea gigantea*
AVESA-BLD	*Avena sativa* cv. Blenda	*CASBI*	*Cassia bicapsularis*
AVESA	*Avena sativa*	CASOB	*Cassia obtusifolia*
AVESG	*Avena strigosa*	CASOC	*Cassia occidentalis*
BAUPU	*Bauhinia purpurea*	CASTO	*Cassia tora*
BEAMA	*Beta macrocarpa*	CAUTI	*Carthamus tinctorius*
BEAPA	*Beta patellaris*	CEOAR	*Celosia argentea* (syn.: *C. argentea* var. cristata)
BEAVA	*Beta vulgaris*	CEOAR	*Celosia cristata* (syn.: *C. argentea*)

CHEAL	*Chenopodium album*
CHEAM	*Chenopodium ambrosioides*
CHECA	*Chenopodium capitatum*
CHEFO	*Chenopodium foliosum*
CHEGI	*Chenopodium amaranticolor* (syn.: *C. giganteum*)
CHEHY	*Chenopodium hybridum*
CHEMU	*Chenopodium murale*
CHEQU	*Chenopodium quinoa*
CHESS	*Chenopodium* spp.
CHEVU	*Chenopodium foetidum*
CHUCH	*Cheiranthus cheiri*
CIAPA	*Carica papaya*
CICED	*Cichorium endivia*
CIDAF	*Citrus aurantiifolia*
CIDLI	*Citrus limon*
CIDME	*Citrus medica*
CIDPA	*Citrus paradisi*
CITLA	*Citrullus lanatus* (syn.: *C. vulgaris*)
CITLA	*Citrullus vulgaris* (syn.: *C. lanatus*)
CLAPE	*Claytonia perfoliata* (syn.: *Montia perfoliata*)
CLAPE	*Montia perfoliata* (syn.: *Claytonia perfoliata)*
CLFHO	*Caladium hortulanum*
CMOTE	*Cyamopsis tetragonoloba*
CNAEN	*Canavalia ensiformis*
CNMJA	*Chaenomeles japonica*
COIMA	*Conium maculatum*
CORSA	*Coriandrum sativum*
CPSAN-LOR	*Capsicum annuum* cv. Long Red
CPSAN	*Capsicum annuum*
CPSCH	*Capsicum chacoense*
CPSFR	*Capsicum frutescens*
CPSFR-EAC	*Capsicum frutescens* cv. Early Calwonder
CPSFR-TAB	*Capsicum frutescens* cv. Tabasco
CPSPR	*Capsicum praetermissum*
CPSSS	*Capsicum* spp.
CTURO	*Vinca rosea*
CUMME	*Cucumis melo*
CUMMU	*Cucumis metuliferus*
CUMSA	*Cucumis sativus*
CUMSA-BDS	*Cucumis sativus* cv. Butcher's Disease Resistant
CUMSA-LGT	*Cucumis sativus* cv. Lange Gele Tros
CUMSA-NAP	*Cucumis sativus* cv. National Pickling
CUMSA-WIW	*Cucumis sativus* cv. Windermoor Wonder
CUUMA-BUT	*Cucurbita maxima* cv. Buttercup
CUUMO	*Cucurbita moschata*
CUUOK	*Cucurbita okeechobeensis*
CUUPE	*Cucurbita pepo*
CUUPE-EPS	*Cucurbita pepo* cv. Early Prolific Straight
CVOVE	*Crocus vermus*
CVTSP	*Crotalaria spectabilis*
CXCTE	*Clitoria ternatea*
CXSES	*Colocasia esculenta*
CYJBE	*Cyphomandra betacea*
DACGL	*Dactylis glomerata*
DACSS	*Dactylis* spp.
DAHPI	*Dahlia pinnata*
DATIN	*Datura meteloides*
DATME	*Datura metel*
DATST	*Datura stramonium*
DAUCS	*Daucus carota* var. sativa
DEDTO	*Desmodium tortuosum*
DIFMA	*Dieffenbachia picta*
DIMRE	*Dinebra retroflexa*
DINBA	*Dianthus barbatus*
DINCA	*Dianthus caryophyllus*
DINCA-JOK	*Dianthus caryophyllus* cv. Joker
DIUCA	*Dioscorea cayenensis*
DIUCO	*Dioscorea composita*
DIUES	*Dioscorea esculenta*
DIUFL	*Dioscorea floribunda*

Table 3 (cont.)

Abbreviations of plants[1,2]	Name of plants	Abbreviations of plants[1,2]	Name of plants
DIUPR	*Dioscorea preussii*	*IRIFU*	*Iris fulva*
DOLLA	*Dolichos lablab* (syn.: *Lablab purpureus*)	*IRIHO-PRB*	*Iris* × *Hollandica* cv. Professor Blaauw
ELYJU	*Elymus junceus*	*IRIHO-WED*	*Iris*× *Hollandica* cv. Wedgwood
EMIFO	*Emilia sagittata*	*IRIRE*	*Iris reticulata*
EPHCT	*Euphorbia cyatophora*	*IRISI*	*Iris sibirica*
EPHPR	*Euphorbia prunifolia*	*IRISS*	*Iris* spp.
FESNI	*Festuca rubra* var. commutata	*IRITE*	*Iris tectorum*
FESPR	*Festuca elatior*	*KENRU*	*Kennedya rubicunda* (= *Kennedia*)
FESRU	*Festuca rubra*	LAAAU	*Lamarckia aurea*
FESTE	*Festuca capillata*	LACSA	*Lactuca sativa*
GASPA	*Galinsoga parviflora*	LASOV	*Lagurus ovatus*
GLXMA	*Glycine max*	LILFO	*Lilium formasanum*
GLXMA-BIL	*Glycine max* cv. Biloxi	LILLO	*Lilium longiflorum*
GLXMA-CHI	*Glycine max* cv. Chippewa	LOLMU	*Lolium multiflorum*
GLXMA-GIB	*Glycine max* cv. Gibson	*LOLMU-STT*	*Lolium multiflorum* cv. S22
GLXMA-KAN	*Glycine max* cv. Kanrich	LOLPE	*Lolium perenne*
GLXMA-LEE	*Glycine max* cv. Lee	LOLPS	*Lolium persicum*
GOMGL	*Gomphrena globosa*	LOLTE	*Lolium temulentum*
GOSHI	*Gossypium hirsutum*	LTHOD	*Lathyrus odoratus*
HELAN	*Helianthus annuus*	LUFAC	*Luffa acutangula*
HIBCA	*Hibiscus cannabinus*	*LUNAN*	*Lunaria annua*
HIBRS	*Hibiscus rosa-sinensis*	LVATR	*Lavatera trimestris*
HORJU	*Hordeum jubatum*	*LYPCI*	*Lycopersicon chilense*
HORMA	*Hordeum murinum*	LYPES	*Lycopersicon esculentum*
HORVX	*Hordeum vulgare*	*LYPES-KOR*	*Lycopersicon esculentum* cv. Kondine Red
HPSHY	*Hippeastrum hybridum*	*LYPES-MYK*	*Lycopersicon esculentum* cv. Moneymaker
HSYNI	*Hyoscyamus niger*	LYPPI	*Lycopersicon pimpinellifolium*
HUMLU	*Humulus lupulus*	LYUSS	*Lycium* spp.
IPONI-SOH	*Ipomoea nil* cv. Scarlett O'Hara	*MABPL*	*Malus platycarpa*
IPOSE	*Ipomoea setosa*	*MABSY-ROA*	*Malus sylvestris* cv. R12740-7A

MABSY	*Malus sylvestris*
MACPO	*Maclura pomifera*
MANES	*Manihot esculenta*
MEDTR	*Medicago truncatula*
MELAL	*Melandrium album*
MEUAL	*Melilotus alba* (= *M. albus*)
MOMBA	*Momordica balsamina*
MORAL	*Morus alba*
MILIA	*Matthiola incana* var. annua
MTLIN	*Matthiola incana*
MTMUN	*Dolichos biflorus*
NARJO	*Narcissus jonquilla*
NARPS	*Narcissus pseudonarcissus*
NARPS-BOS	*Narcissus* cv. Boswin
NARPS-DUM	*Narcissus* cv. Duch Muster
NICBI	*Nicandra bigelovii*
NICPH	*Nicandra physaloides*
NIOBE	*Nicotiana benthamiana*
NIOBI	*Nicotiana bigelovii*
NIOCL	*Nicotiana clevelandii*
NIODE	*Nicotiana debneyi*
NIOED	*Nicotiana edwardsonii*
NIOGT	*Nicotiana glutinosa*
NIOHY-CLG	*Nicotiana clevelandii*× *Nicotiana glutinosa*
NIOHY-EDW	*Nicotiana*× *edwardsonii*
NIOHY-GTC	*Nicotiana glutinosa*× *Nicotiana clevelandii*
NIOOC	*Nicotiana occidentalis*
NIORE	*Nicotiana repanda*
NIORU	*Nicotiana rustica*
NIOSI	*Nicotiana sylvestris* (= *silvestris*)
NIOSS	*Nicotiana* spp.
NIOSU	*Nicotiana megalosiphon* (syn.: *N. suaveolens*)
NIOTA-BRY	*Nicotiana tabacum* cv. Brigh Yellow
NIOTA-BUR	*Nicotiana tabacum* cv. Burley 21
NIOTA-BUX	*Nicotiana tabacum* cv. Burley Xanthi
NIOTA-DTA	*Nicotiana tabacum* cv. Dutch A
NIOTA-HAR	*Nicotiana tabacum* cv. Harrow Velvet
NIOTA-HFF	*Nicotiana tabacum* cv. Havana 425
NIOTA-HFT	*Nicotiana tabacum* cv. Havana 423
NIOTA-JAV	*Nicotiana tabacum* cv. Java
NIOTA-JYP	*Nicotiana tabacum* cv. Judy's Pride
NIOTA-SAM	*Nicotiana tabacum* cv. Samsun
NIOTA-SMA	*Nicotiana tabacum* cv. Smal
NIOTA-SNN	*Nicotiana tabacum* cv. Samsun NN
NIOTA	*Nicotiana tabacum*
NIOTA-TUR	*Nicotiana tabacum* cv. Turkish
NIOTA-TUS	*Nicotiana tabacum* cv. Turkish Samsun
NIOTA-VIG	*Nicotiana tabacum* cv. Virginia Gold
NIOTA-WHB	*Nicotiana tabacum* cv. White Burley
NIOTA-WSH	*Nicotiana tabacum* cv. Wisconsin Havana 425
NIOTA-XAN	*Nicotiana tabacum* cv. Xanthi
NIOTA-XNC	*Nicotiana tabacum* cv. Xanthi-nc
NIOVE	*Nicotiana velutina*
NRIBO	*Nerine bowdenii*
OCIBA	*Ocimum basilicum*
ORYPU	*Oryza punctata*
ORYSA	*Oryza sativa*
ORYSA-IRT	*Oryza sativa* cv. IR 20
ORYSA-SIN	*Oryza sativa* cv. Sindano
PANDI	*Panicum dichtomiflorum*
PANMA	*Panicum maximum*
PANMI	*Panicum miliaceum*
PANMA-KOE	*Panicum maximum* cv. K 189
PAQED	*Passiflora edulis*
PAQEF	*Passiflora edulis* f. flavicarpa
PAQHY-FLA	*Passiflora edulis*× *Passiflora edulis* f. flavicarpa
PAQSU	*Passiflora suberosa*
PASDJ	*Paspalum membranaceum* (syn.: *P. dissectum*)
PAVSA	*Pastinaca sativa*
PEUHY-FIC	*Petunia hybrida* cv. Fire Chief
PEUHY-MIN	*Petunia hybrida* cv. Minstrel
PEUHY-PIB	*Petunia hybrida* cv. Pink Beauty

Table 3 (cont.)

Abbreviations of plants[1, 2]	Name of plants	Abbreviations of plants[1, 2]	Name of plants
PEUHY-ROM	*Petunia hybrida* cv. Rosy Morn	*PHSVX-PEP*	*Phaseolus vulgaris* cv. Pencil Pod
PEUHY	*Petunia hybrida*	*PHSVN-PIN*	*Phaseolus vulgaris* cv. Pinto
PEUSS	*Petunia* spp.	*PHSVN-PIT*	*Phaseolus vulgaris* cv. Pinto III.
PHAPA	*Phalaris paradoxa*	*PHSVX-PLT*	*Phaseolus vulgaris* cv. Plentiful
PHLAR	*Phleum arenarium*	*PHSVX-PPK*	*Phaseolus vulgaris* cv. Purple King
PHLPR	*Phleum pratense*	*PHSVX-PRE*	*Phaseolus vulgaris* cv. Premier
PHSAF	*Phaseolus acutifolium*	*PHSVX-RDP*	*Phaseolus vulgaris* cv. Redland Pioneer
PHSAU	*Phaseolus aureus* (syn.: *Vigna radiata*)	*PHSVX-RKY*	*Phaseolus vulgaris* cv. Red Kidney
PHSAU	*Vigna radiata* (syn.: *Phaseolus aureus*)	*PHSVX-ROW*	*Phaseolus vulgaris* cv. Royal Windsor
PHSLU	*Phaseolus lunatus*	*PHSVV-TCR*	*Phaseolus vulgaris* cv. Topcrop
PHSLY	*Phaseolus lathyroides*	*PHSVV-TDG*	*Phaseolus vulgaris* cv. Tendergreen
PHSMU	*Phaseolus mungo*	*PHSVV-TPR*	*Phaseolus vulgaris* cv. The Prince
PHSVX-BAT	*Phaseolus vulgaris* cv. Bataaf	*PHSVX-SCO*	*Phaseolus vulgaris* cv. Scotia
PHSVX-BEK	*Phaseolus vulgaris* cv. Beka	*PHSVX-TDC*	*Phaseolus vulgaris* cv. Tendercrop
PHSVX-BLV	*Phaseolus vulgaris* cv. Black Valentine	*PHSVX-YAK*	*Phaseolus vulgaris* cv. Yamahiro Kurosando
PHSVX-BTF	*Phaseolus vulgaris* cv. Bountiful	PHTAM	*Phytolacca americana*
PHSVX-CAW	*Phaseolus vulgaris* cv. Canadian Wonder	*PHYFL*	*Physalis floridana*
PHSVX-COL	*Phaseolus vulgaris* cv. Col. 109-R	PHYPE	*Physalis peruviana*
PHSVX-CRW	*Phaseolus vulgaris* cv. Cherokee Wax	*PIBST-ALA*	*Pisum sativum* cv. Alaska
PHSVN-GRN	*Phaseolus vulgaris* cv. Great Northern	*PIBST-GRF*	*Pisum sativum* cv. Greenfeast
PHSVX-HAW	*Phaseolus vulgaris* cv. Hawkesbury Wonder	PIBST	*Pisum sativum*
PHSVX-JAM	*Phaseolus vulgaris* cv. Jamapa	*PIBST-MET*	*Pisum sativum* cv. Meteor
PHSVX-KIN	*Phaseolus vulgaris* cv. Kintoki	*PIBST-MMM*	*Pisum sativum* cv. Early Massey Melbourne Market
PHSVX-KIW	*Phaseolus vulgaris* cv. Kinghorn Wax		
PHSVX-LAV	*Phaseolus vulgaris* cv. La Victoire	*PIBST-ONW*	*Pisum sativum* cv. Onward
PHSVX	*Phaseolus vulgaris*	*PIBST-RAN*	*Pisum sativum* cv. Ranger
PHSVX-LOT	*Phaseolus vulgaris* cv. Long Tom	PIOSE	*Philodendron selloum*
PHSVX-MAN	*Phaseolus vulgaris* cv. Manteiga	*PIOVE*	*Philodendron verrucosum*
PHSVX-MAS	*Phaseolus vulgaris* cv. Masterpiece	PLARU	*Plantago rugelii*
PHSVX-MEX	*Phaseolus vulgaris* cv. Mex. 27-R	POAAN	*Poa annua*

POACO	*Poa compressa*
POAPR	*Poa pratensis*
POATR	*Poa trivialis*
PRIMA	*Primula malacoides*
PRNAM	*Prunus americana*
PRNAR	*Prunus americana*
PRNAV	*Prunus avium*
PRNCF	*Prunus cerasifera*
PRNDO-GGR	*Prunus domestica* cv. Grosse Grüne Reneklode
PRNDO-HWE	*Prunus domestica* cv. Hauszwetsche
PRNDO-POZ	*Prunus domestica* cv. Pozegaca
PRNMH	*Prunus mahaleb*
PRNPS	*Prunus persica*
PRNSC	*Prunus salicina*
PRNSL-SHI	*Prunus serrulata* cv. Shirofugen
PRNSS	*Prunus* spp.
RANAR	*Ranunculus arvensis*
RANSA	*Ranunculus sardous*
RAPSV-CWW	*Raphanus sativus* cv. Chinese White Winter
RAPSV-WHI	*Raphanus sativus* cv. White Icicle
RIICO	*Ricinus communis*
RUBSS	*Rubus* spp.
SACOF	*Saccharum officinarum*
SACSS	*Saccharum* spp.
SAMNI	*Sambucus nigra*
SAMRA	*Sambucus racemosa*
SEBEX	*Sesbania exaltata*
SECCE	*Secale cereale*
SEGIN	*Sesamum indicum*
SETIT	*Setaria italica*
SETMA	*Setaria macrostachia*
SETVI	*Setaria viridis*
SILAR	*Silene armeria*
SINAL	*Sinapis alba*
SOLCH-TEO	*Solanum chacoense* (TE_1)
SOLHY-SDA	*Solanum demissum* (SdA) (*Solanum tuberosum*)
SOLHY-TUA	*Solanum demissum*× *Solanum tuberosum* A6
SOLHY-TUY	*Solanum demissum* Y
SOLAM	*Solanum nodiflorum*
SOLCU	*Solanum rostratum*
SOLTU-DES	*Solanum tuberosum* cv. Desiree
SOLTU-DOY	*Solanum tuberosum* cv. Duke of York
SOLTU-SAC	*Solanum tuberosum* cv. Saco
SOLTU-API	*Solanum tuberosum* ssp. tuberosum cv. Arran Pilot
SOLTU-MAB	*Solanum tuberosum* ssp. tuberosum cv. Maris Bad
SOLTU-USD	*Solanum tuberosum* cv. U.S.D.A.
SORVE	*Sorghum arundinaceum* (= *Sorgum*)
SORVU-ALS	*Sorghum bicolor* cv. Atlas
SORVU	*Sorghum bicolor*
SORVU-RIO	*Sorghum bicolor* cv. Rio
SORVU-SRT	*Sorghum bicolor* cv. Sart
SPQOL	*Spinaca oleracea*
STPSE	*Stenotaphrum secundatum*
TEATE	*Tetragonia expansa* (syn.: *T. tetragonoides*)
TEPVI	*Tephrosia villosa*
THOCA	*Theobroma cacao*
THOCA-WAA	*Theobroma cacao* cv. West African Amelonado
TITSP	*Tithonia speciosa*
TONFO	*Torenia fournieri*
TOPMA	*Tropaeolum majus*
TRFDU	*Trifolium dubium*
TRFHY	*Trifolium hybridum*
TRFIN	*Trifolium incarnatum*
TRFPR	*Trifolium pratense*
TRFRE	*Trifolium repens*
TRFSS	*Trifolium* spp.
TRFSU	*Trifolium subterraneum*
TRZAX	*Triticum aestivum*
TRZAX-PKR	*Triticum aestivum* cv. Parker
TRZDU	*Triticum durum*

Table 3 (cont.)

Abbreviations of plants[1, 2]	Name of plants	Abbreviations of plants[1, 2]	Name of plants
TULGE-CAB	*Tulipa gesneriana* cv. Clara Butt	VIGSI	*Vigna unguiculata* subsp. unguiculata (syn.: *V. sinensis*)
TULGE-ROC	*Tulipa gesneriana* cv. Rose Copland		
TYPAR	*Phalaris arundinacea*	*VIGSI-MON*	*Vigna sinensis* cv. Monarch (syn.: *V. unguiculata* cv. Monarch)
VAAPY-PIB	*Saponaria vaccaria* cv. Pink Beauty		
VEEEN	*Verbesina encelioides*	*VIGSI-MON*	*Vigna unguiculata* cv. Monarch (syn.: *V. sinensis* cv. Monarch)
VICFX-BRW	*Vicia faba* cv. Broad Windsor		
VICFX	*Vicia faba*	VIGSQ	*Vigna sesquipedalis* (syn.: *V. unguiculata*)
VICFX-SUT	*Vicia faba* cv. The Sutton	VIGSQ	*Vigna unguiculata* ssp. sesquipedalis (syn.: *V. sesquipedalis*)
VICSA	*Vicia sativa*		
VIGSC	*Vigna cylindrica* (syn.: *V. unguiculata* ssp. cylindrica)	*VITLA-CON*	*Vitis labrusca* cv. Concord
		VITRU	*Vitis rupestris*
VIGSC	*Vigna unguiculata* ssp. cylindrica (syn.: *V. cylindrica*)	VITSS	*Vitis* spp.
		VITVI	*Vitis vinifera*
VIGSI-BER	*Vigna unguiculata* cv. Blackeys Early Ramsh.	VOASU	*Voandzeia subterranea*
VIGSI-BLE	*Vigna sinensis* cv. Blackeye	*XATCA*	*Xanthosoma caracu*
VIGSI-EAR	*Vigna unguiculata* cv. Early Ramshorn	ZEAMX	*Zea mays*
VIGSI	*Vigna sinensis* (syn.: *V. unguiculata*)	*ZINOF*	*Zingiber officinale*
VIGSI	*Vigna unguiculata* (syn.: *V. sinensis*)	ZIIEL	*Zinnia elegans*
VIGSI	*Vigna unguiculata* ssp. sinensis (syn.: *V. sinensis*)	ZNTSS	*Zantedeschia* sp.

[1] The first three letters refer to the genus, the next two denote the species, and the last three after the rule symbolized the cultivar or hybrid. If the species is not designated, it is symbolized by the letters "SS" (e.g. ALGSS for *Althaea* spp.).

[2] Abbreviations printed in italics are the proposed codes, all another codes approved by WSSA/WSSJ (see Anonymous, 1991).

3. *Selected and proposed hosts and non-hosts of viruses and their approved and proposed codes*

In an earlier paper and in its enlarged and revised edition (see Horváth, 1992, 1993) we listed selected and proposed hosts and non-hosts of 232 mechanically transmissible viruses as well as plants suitable for diagnosing 24 virus groups. The 234 plants to be found in the list – which belong to 105 genera and 35 families – make it possible to diagnose the 232 mechanically transmissible viruses and the 24 virus groups. The number of selected and proposed hosts and non-hosts could be reduced by some 187, that of the genera by 46, and the number of plant families by 4 compared to the plants discussed in the series CMI/AAB Descriptions of Plant Virus Diseases (see Murant and Harrison, 1970–1988). The approved and proposed codes for the selected hosts and non-hosts can be found in Tables 4 and 5.

Conclusions

We hope that the computer-friendly abbreviations suggested to denote hosts, non-hosts (including cultivars and hybrids), plant families and host characters (assay-, diagnostic- and propagation hosts) will be suitable for use, but we should like to emphasize that they will need continuous completion by new ones as well as revision from time to time.

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Table 4

Five and three-letter abbreviations for virus hosts, non-hosts, families including cultivars and hybrids[1]

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Agropyron intermedium	AGRIT	GRA			
Agropyron repens	AGRRE	GRA			
Agropyron smithii	AGRSM	GRA			
Allium cepa	ALLCE	LIL			
Allium fistulosum	ALLFI	LIL			
Allium neapolitanum	ALLNE	LIL			
Allium porrum	ALLPO	LIL			
Amaranthus caudatus	AMACA	AMA			
Amaranthus retroflexus	AMARE	AMA			
Amaranthus spp.	AMASS	AMA			
Ammi majus	AMIMA	UMB			
Antirrhinum majus[a]	ATHMM	SCP			
Apium graveolens var. dulce	APUGD	UMB			
Avena sativa	AVESA	GRA			
Belamcanda chinensis	BMCCH	IRI			
Beta macrocarpa	BEAMA	CHE			
Beta vulgaris	BEAVA	CHE			
Beta vulgaris cv. Monobusch			BEAVA	MOB	CHE
Brassica campestris[b]	BRSRA	CRU			
Brassica campestris cv. Just Right			BRSCA	JTR	CRU
Brassica chinensis	BRSCH	CRU			
Brassica oleracea	BRSOX	CRU			
Brassica oleracea var. botrytis	BRSOB	CRU			
Brassica pekinensis	BRSPK	CRU			
Brassica rapa[c]	BRSRA	CRU			
Bromus inermis	BROIN	GRA			
Bromus mollis	BROMO	GRA			
Capsicum annuum	CPSAN	SOL			
Capsicum frutescens	CPSFR	SOL			
Capsicum frutescens cv. Tabasco			CPSFR	TAB	SOL
Capsicum spp.	CPSSS	SOL			
Carica papaya	CIAPA	CAA			
Carthamus tinctorius	CAUTI	COM			
Cassia occidentalis	CASOC	LEG			
Cassia tora	CASTO	LEG			
Chenopodium album	CHEAL	CHE			
Chenopodium amaranticolor[d]	CHEGI	CHE			
Chenopodium capitatum	CHECA	CHE			
Chenopodium foetidum	CHEVU	CHE			
Chenopodium foliosum			CHEFO		CHE
Chenopodium hybridum	CHEHY	CHE			
Chenopodium murale	CHEMU	CHE			
Chenopodium quinoa	CHEQU	CHE			

Table 4 (cont.)

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Chenopodium spp.	CHESS	CHE			
Cicer arietinum	CIEAR	LEG			
Citrullus lanatus	CITLA	CUC			
Citrus unshiu cv. Mexican Lime			CIDUN	MXL	RUB
Citrus unshiu cv. Rough lemon			CIDUN	RGL	RUB
Commelina spp.	COMSS	CMM			
Crambe abyssinica	CRMAB	CRU			
Crocus spp.	CVOSS	IRI			
Crotaria spectabilis	CVTSP	LEG			
Cucumis anguria var. longipes			CUMAN	LGI	CUC
Cucumis melo	CUMME	CUC			
Cucumis myriocarpus	CUMMY	CUC			
Cucumis sativus	CUMSA	CUC			
Cucumis sativus cv. Delicatess			CUMSA	DEL	CUC
Cucumis sativus cv. Lange Gele Tros			CUMSA	LGT	CUC
Cucumis sativus cv. Sperlings Mervita			CUMSA	SPM	CUC
Cucumis spp.	CUMSS	CUC			
Cucurbita ficifolia	CUUFI	CUC			
Cucurbita maxima	CUUMA	CUC			
Cucurbita maxima cv. Buttercrop			CUUMA	BUT	CUC
Cucurbita pepo	CUUPE	CUC			
Cucurbita pepo cv. capitatum			CUUPE	CAP	CUC
Cucurbita pepo convar patissonina f. radiata	CUUPM	CUC			
Cucurbita pepo cv. Small Sugar			CUUPE	SMS	CUC
Cyamopsis tetragonoloba	CMOTE	LEG			
Cymbidium spp.			CMFSS		ORC
Cyperus esculentus	CYPES	CYP			
Dactylis glomerata	DACGL	GRA			
Datura metel	DATME	SOL			
Datura spp.	DATSS	SOL			
Datura stramonium	DATST	SOL			
Daucus carota var. sativa	DAUCS	UMB			
Desmodium tortuosum	DEDTO	LEG			
Dianthus barbatus	DINBA	CAF			
Dianthus caryophyllus	DINCA	CAF			
Dianthus caryophyllus cv. William Sim			DINCA	WLS	CAF
Dioscorea cayenensis			DIUCA		DIO
Dioscorea composita			DIUCO		DIO
Dioscorea floribunda	DIUFL	DIO			
Dolichos lablab	DOLLA	LEG			
Festuca pratensis	FESPR	GRA			
Glycine max	GLXMA	LEG			
Gomphrena globosa	GOMGL	AMA			

Table 4 (cont.)

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Hibiscus cannabinus	HIBCA	MAV			
Hibiscus rosa-sinensis	HIBRS	MAV			
Hippeastrum hybridum	HPSHY	AMY			
Hordeum vulgare	HORVX	GRA			
Humulus lupulus	HUMLU	MOR			
Ipomoea nil cv. Scarlet O'Hara			IPONI	SOM	COV
Iris× hollandica cv. Wedgwood			IRIHO	WED	IRI
Iris spp.	IRISS	IRI			
Kennedya rubicunda (= *Kennedia)*			KENRU		LEG
Lablab purpureus[e]	DOLLA	LEG			
Lactuca sativa	LACSA	COM			
Lactuca sativa cv. Valmaine			LACSA	VAL	COM
Lagenaria siceraria	LGNSI	CUC			
Lathyrus odoratus	LTHOD	LEG			
Lavatera trimestris	LVATR	MAV			
Lilium formosanum	LILFO	LIL			
Lilium longiflorum	LILLO	LIL			
Lolium multiflorum	LOLMU	GRA			
Lolium multiflorum cv. S22			LOLMU	STT	GRA
Lolium perenne	LOLPE	GRA			
Lolium persicum	LOLPS	GRA			
Luffa acutangula	LUFAC	CUC			
Lupinus albus	LUPAL	LEG			
Lycium spp.	LYUSS	SOL			
Lycopersicon esculentum	LYPES	SOL			
Lycopersicon pimpinellifolium	LYPPI	SOL			
Manihot esculenta	MANES	EUP			
Matthiola incana	MTLIN	CRU			
Medicago sativa	MEDSA	LEG			
Melilotus alba (= *M. albus)*	MEUAL	LEG			
Narcissus cv. Barett Browning			NARXX	BAB	AMY
Narcissus pseudonarcissus	NARPS	AMY			
Narcissus spp.	NARSS	AMY			
Nicandra physaloides	NICPH	SOL			
Nicotiana benthamiana			NIOBE		SOL
Nicotiana clevelandii			NIOCL		SOL
Nicotiana debneyi			NIODE		SOL
Nicotiana glauca	NIOGL	SOL			
Nicotiana glutinosa	NIOGT	SOL			
Nicotiana glutinosa× Nicotiana clevelandii			NIOHY	GTC	SOL
Nicotiana megalosiphon[f]	NIOSU	SOL			
Nicotiana occidentalis			NIOOC		SOL
Nicotiana rustica	NIORU	SOL			
Nicotiana spp.	NIOSS	SOL			
Nicotiana sylvestris (= *silvestris)*	NIOSI	SOL			
Nicotiana tabacum	NIOTA	SOL			

Table 4 (cont.)

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Nicotiana tabacum cv. Burley 21			NIOTA	BUR	SOL
Nicotiana tabacum cv. Havana 423			NIOTA	HFT	SOL
Nicotiana tabacum cv. Havana 425			NIOTA	HFF	SOL
Nicotiana tabacum cv. Kentucky 14			NIOTA	KEN	SOL
Nicotiana tabacum cv. Samsun			NIOTA	SAM	SOL
Nicotiana tabacum cv. Turkish			NIOTA	TUR	SOL
Nicotiana tabacum cv. V. 20			NIOTA	VTO	SOL
Nicotiana tabacum cv. White Burley			NIOTA	WHB	SOL
Nicotiana tabacum cv. Xanthi-nc			NIOTA	XNC	SOL
Nicotiana velutina			NIOVE		SOL
Nicotiana× *edwardsonii*			NIOHY	EDW	SOL
Ocimum basilicum	OCIBA	LAB			
Oryza sativa	ORYSA	GRA			
Panicum maximum cv. K 189			PANMA	KOE	GRA
Panicum miliaceum	PANMI	GRA			
Paspalum membranaceum[g]	PASDJ	GRA			
Passiflora edulis	PAQED	PAS			
Pastinaca sativa	PAVSA	UMB			
Paulownia fargesii	PAZFA	SCP			
Pelargonium domesticum cv. Nittany Lion			PELGR	NIL	GER
Petunia hybrida	PEUHY	SOL			
Phaseolus aureus	PHSAU	LEG			
Phaseolus lunatus	PHSLU	LEG			
Phaseolus lunatus cv. Henderson			PHSLU	HEN	LEG
Phaseolus mungo	PHSMU	LEG			
Phaseolus vulgaris	PHSVX	LEG			
Phaseolus vulgaris cv. Bataaf			PHSVX	BAT	LEG
Phaseolus vulgaris cv. Black Valentine			PHSVX	BLV	LEG
Phaseolus vulgaris cv. Bountiful			PHSVX	BTF	LEG
Phaseolus vulgaris cv. Greet Northern	PHSVN	LEG	PHSVN	GRN	LEG
Phaseolus vulgaris cv. Kinghorn Wax			PHSVX	KIW	LEG
Phaseolus vulgaris cv. Kintoki			PHSVX	KIN	LEG
Phaseolus vulgaris cv. La Victorie			PHSVX	LAV	LEG
Phaseolus vulgaris cv. Long Tom			PHSVX	LOT	LEG
Phaseolus vulgaris cv. Pinto	PHSVN	LEG	PHSVN	PIN	LEG
Phaseolus vulgaris cv. Plentiful			PHSVX	PLT	LEG
Phaseolus vulgaris cv. Red Kidney			PHSVX	RKY	LEG
Phaseolus vulgaris cv. Tendergreen			PHSVV	TDG	LEG
Phaseolus vulgaris cv. The Prince			PHSVV	TPR	LEG
Phaseolus vulgaris cv. Topcrop			PHSVV	TCR	LEG

Table 4 (cont.)

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Philodendron selloum	PIOSE	ARA			
Physalis floridana			PHYFL		SOL
Phytolacca americana	PHTAM	PHY			
Pisum sativum	PIBST	LEG			
Pisum sativum cv. Perfection			PIBST	PFC	LEG
Primula malacoides	PRIMA	PRI			
Ranunculus sardous	RANSA	RAN			
Rhynchosia minima	RHNMI	LEG			
Ricinus communis	RIICO	EUP			
Saponaria vaccaria var. Pink Beauty			VAAPY	PIB	CAF
Secale cereale	SECCE	GRA			
Setaria italica	SETIT	GRA			
Silene armeria	SILAR	CAF			
Sinapis alba	SINAL	CRU			
Solanum demissum[h] (SdA)			SOLHY	SDA	SOL
Solanum demissum× *Solanum tuberosum* A6			SOLHY	TUA	SOL
Solanum melongena	SOLME	SOL			
Solanum nigrum	SOLNI	SOL			
Solanum nodiflorum	SOLAM	SOL			
Solanum phureja CPC 4110			SOLPJ	CPC	SOL
Solanum rostratum	SOLCU	SOL			
Solanum sysimbrifolium	SOLSY	SOL			
Solanum tuberosum cv. Maris Piper			SOLTU	MAP	SOL
Solanum tuberosum ssp. andigena CPC 1801[i]			SOLAD	CPC	SOL
Solanum tuberosum ssp. tuberosum cv. Maris Bard			SOLTU	MAB	SOL
Sorghum bicolor (= *Sorgum)*	SORVU	GRA			
Sorghum bicolor cv. Asgrow Bugoff			SORVU	AGB	GRA
Sorghum bicolor cv. Rio			SORVU	RIO	GRA
Sorghum halapense (= *Sorgum halepense)*	SORHA	GRA			
Spinacia oleracea	SPQOL	CHE			
Tetragonia crystallina			TEACR		AIZ
Tetragonia expansa[j]	TEATE	AIZ			
Tetragonia tetragonoides[k]	TEATE	AIZ			
Tinantia erecta	TINER	CMM			
Trifolium incarnatum	TRFIN	LEG			
Trifolium spp.	TRFSS	LEG			
Trifolium subterraneum	TRFSU	LEG			
Triticum aestivum	TRZAX	GRA			
Triticum aestivum cv. Kent			TRZAX	KEN	GRA
Triticum aestivum cv. Michigan Amber			TRZAX	MIA	GRA
Triticum aestivum cv. Parker			TRZAX	PAR	GRA

Table 4 (cont.)

Selected and proposed hosts and non-hosts of viruses[2]	WSSA/WSSJ code		Proposed abbreviations		
	Code of botanical name	Family code[3]	Code of botanical name	Cultivar or hybrid code	Family code[3]
Triticum durum	TRZDU	GRA			
Tropaeolum majus	TOPMA	TRP			
Vaccinium spp.	VACSS	EGI			
Verbascum thapsus	VESTH	SCP			
Verbesina encelioides	VEEEN	COM			
Vicia faba	VICFX	LEG			
Vicia spp.	VICSS	LEG			
Vigna radiata	PHSAU	LEG			
Vigna sinensis[1]	VIGSI	LEG			
Vigna sinensis cv. Blackeye			VIGSI	BLE	LEG
Vigna sesquipedalis	VIGSQ	LEG			
Vigna unguiculata[m]	VIGSI	LEG			
Vigna unguiculata cv. Blackeye			VIGSI	BLE	LEG
Vigna unguiculata ssp. cylindrica	VIGSC	LEG			
Vigna unguiculata subsp. unguiculata	VIGSI	LEG			
Vitis labrusca cv. Delaware			VITLA	DEL	VIT
Vitis vinifera ssp. vinifera	VITVI	VIT			
Voandzeia subterranea	VOASU	LEG			
Zea mays	ZEAMX	GRA			
Zingiber officinale	ZINOF	ZIN			
Zinnia elegans	ZIIEL	COM			

[1] Five-letter abbreviations used for botanical name (ssp., var. oder f.) of planst. Three-letter abbreviations used for cultivar, hybrid and family names.

[2] a, Syn.: *Antirrhinum majus* ssp. majus; b, Syn.: *Brassica rapa;* c, Syn.: *Brassica campestris;* d, Syn.: *Chenopodium giganteum;* e, Syn.: *Dolichos lablab;* f, Syn.: *Nicotiana suaveolens;* g, Syn.: *Paspalum dissectum;* h, Syn.: *Solanum tuberosum,* i, Syn.: *Solanum andigenum;* j, Syn.: *Tetragonia tetragonoides;* k, Syn.: *Tetragonia expansa;* l, Syn.: *Vigna unguiculata;* m, Syn.: *Vigna sinensis.*

[3] AIZ, Aizoaceae; AMA, Amaranthaceae; AMY, Amarylidaceae; ARA, Araceae; CAA, Caricaceae; CAF, Caryophyllaceae; CHE, Chenopodiaceae; CMM, Commelinaceae; COM, Compositae; CRU, Cruciferae; CUC, Cucurbitaceae; CYP, Cyperaceae; DIO, Dioscoreaceae; ERI, Ericaceae; EUP, Euphorbiaceae; GER, Geraniaceae; GRA, Gramineae; IRI, Iridaceae; LAB, Labiatae; LEG, Leguminosae; LIL, Liliaceae; MAV, Malvaceae; MOR, Moraceae; ORC, Orchidaceae; PAS, Passiflorae (= Passifloraceae); PHY, Phytolaccaceae; PRI, Primulaceae; RAN, Ranunculaceae; RUB, Rubiaceae; SCP, Scrophulariaceae; SOL, Solanaceae; TRP, Tropaeolaceae; UMB, Umbelliferae; VIT, Vitaceae (= Vitidiaceae); ZIN, Zingiberaceae.

Table 5

Abbreviations of selected and proposed hosts and non-hosts of viruses in alphabetical order

Abbreviations of plants[1,2]	Name of plants	Abbreviations of plants[1,2]	Name of plants
AGRIT	*Agropyron intermedium*	CAUTI	*Carthamus tinctorius*
AGRRE	*Agropyron repens*	CHEAL	*Chenopodium album*
AGRSM	*Agropyron smithii*	CHECA	*Chenopodium capitatum*
ALLCE	*Allium cepa*	CHEFO	*Chenopodium foliosum*
ALLFI	*Allium fistulosum*	CHEGI	*Chenopodium amaranticolor* (syn.: *C. giganteum*)
ALLNE	*Allium neapolitanum*	CHEHY	*Chenopodium hybridum*
ALLPO	*Allium porrum*	CHEMU	*Chenopodium murale*
AMACA	*Amaranthus caudatus*	CHEQU	*Chenopodium quinoa*
AMARE	*Amaranthus retroflexus*	CHESS	*Chenopodium* spp.
AMASS	*Amaranthus* spp.	CHEVU	*Chenopodium foetidum*
AMIMA	*Ammi majus*	CIAPA	*Carica papaya*
APUGD	*Apium graveolens* var. dulce	CIEAR	*Cicer arietinum*
ATHMM	*Antirrhinum majus* (syn.: *A. majus* ssp. majus)	*CIDUN-MXL*	*Citrus unshiu* cv. Mexican Lime
AVESA	*Avena sativa*	*CIDUN-RGL*	*Citrus unshiu* cv. Rough lemon
BEAMA	*Beta macrocarpa*	CITLA	*Citrullus lanatus*
BEAVA	*Beta vulgaris*	*CMFSS*	*Cymbidium* spp.
BEAVA-MOB	*Beta vulgaris* cv. Monobusch	CMOTE	*Cyamopsis tetragonoloba*
BMCCH	*Belamcanda chinensis*	COMSS	*Commelina* spp.
BROIN	*Bromus inermis*	CPSAN	*Capsicum annuum*
BROMO	*Bromus mollis*	CPSFR	*Capsicum frutescens*
BRSCA-JTR	*Brassica campestris* cv. Just Right	*CPSFR-TAB*	*Capsicum frutescens* cv. Tabasco
BRSCH	*Brassica chiensis*	CPSSS	*Capsicum* spp.
BRSOB	*Brassica oleracea* var. botrytis	CRMAB	*Crambe abyssinica*
BRSOX	*Brassica oleracea*	*CUMAN-LGI*	*Cucumis anguria* var. longipes
BRSPK	*Brassica pekinensis*	CUMMY	*Cucumis myriocarpus*
BRSRA	*Brassica rapa* (syn.: *B. campestris*)	CUMSA	*Cucumis sativus*
BRSRA	*Brassica campestris* (syn.: *B. rapa*)	*CUMSA-DEL*	*Cucumis sativus* cv. Delicatess
CASOC	*Cassia occidentalis*	*CUMSA-LGT*	*Cucumis sativus* cv. Lange Gele Tros
CASTO	*Cassia tora*	*CUMSA-SPM*	*Cucumis sativus* cv. Sperlings Mervita

CUMSS	*Cucumis* spp.
CUUFI	*Cucurbita ficifolia*
CUUMA-BUT	*Cucurbita maxima* cv. Buttercrop
CUUMA	*Cucurbita maxima*
CUUPE-CAP	*Cucurbita pepo* cv. capitatum
CUUPE	*Cucurbita pepo*
CUUPE-SMS	*Cucurbita pepo* cv. Small Sugar
CUUPM	*Cucurbita pepo* convar. patissonia f. radiata
CVOSS	*Crocus* spp.
CVTSP	*Crotaria spectabilis*
CYPES	*Cyperus esculentus*
DACGL	*Dactylis glomerata*
DATME	*Datura metel*
DATSS	*Datura* spp.
DATST	*Datura stramonium*
DAUCS	*Daucus carota* var. sativa
DEDTO	*Desmodium tortuosum*
DINBA	*Dianthus barbatus*
DINCA	*Dianthus caryophyllus*
DINCA-WLS	*Dianthus caryophyllus* cv. William Sim
DIUCA	*Dioscorea cayenensis*
DIUCO	*Dioscorea composita*
DIUFL	*Dioscorea floribunda*
DOLLA	*Dolichos lablab* (syn.: *Lablab purpurens*)
DOLLA	*Lablab purpureus* (syn.: *Dolichos* lablab)
FESPR	*Festuca pratensis*
GLXMA	*Glycine max*
GOMGL	*Gomphrena globosa*
HIBCA	*Hibiscus cannabinus*
HIBRS	*Hibiscus rosa-sinensis*
HORVX	*Hordeum vulgare*
HPSHY	*Hippeastrum hybridum*
HUMLU	*Humulus lupulus*
IPONI-SOM	*Ipomoea nil* cv. Scarlet O'Hara
IRIHO-WED	*Iris*× hollandica cv. Wedgwood
IRISS	*Iris* spp.
KENRU	*Kennedya rubicunda* (= *Kennedia*)
LACSA	*Lactuca sativa*
LACSA-VAL	*Lactuca sativa* cv. Valmaine
LGNSI	*Lagenaria siceraria*
LILFO	*Lilium formosanum*
LILLO	*Lilium longiflorum*
LOLMU	*Lolium multiflorum*
LOLMU	*Lolium multiflorum* cv. S22
LOLPE	*Lolium perenne*
LOLPS	*Lolium persicum*
LTHOD	*Lathyrus odoratus*
LUFAC	*Luffa acutangula*
LUPAL	*Lupinus albus*
LVATR	*Lavatera trimestris*
LYPES	*Lycopersicon esculentum*
LYPPI	*Lycopersicon pimpinellifolium*
LYUSS	*Lycium* spp.
MANES	*Manihot esculenta*
MEDSA	*Medicago sativa*
MEUAL	*Melilotus alba* (= *M. albus*)
MTLIN	*Matthiola incana*
NARPS	*Narcissus pseudonarcissus*
NARSS	*Narcissus* spp.
NARXX-BAB	*Narcissus* cv. Barett Browning
NICPH	*Nicandra physaloides*
NIOBE	*Nicotiana benthamiana*
NIOCL	*Nicotiana clevelandii*
NIODE	*Nicotiana debneyi*
NIOGL	*Nicotiana glauca*
NIOGT	*Nicotiana glutinosa*
NIOHY-EDW	*Nicotiana*× *edwardsonii*
NIOHY-GTC	*Nicotiana glutinosa*× *Nicotiana clevelandii*
NIOOC	*Nicotiana occidentalis*
NIORU	*Nicotiana rustica*
NIOSI	*Nicotiana sylvestris* (= *N. silvestris*)
NIOSS	*Nicotiana* spp.

Table 5 (cont.)

Abbreviations of plants[1,2]	Name of plants	Abbreviations of plants[1,2]	Name of plants
NIOSU	*Nicotiana megalosiphon* (syn.: *N. suaveolens*)	*PHSVV-TDG*	*Phaseolus vulgaris* cv. Tendergreen
NIOTA	*Nicotiana tabacum*	*PHSVV-TPR*	*Phaseolus vulgaris* cv. The Prince
NIOTA-BUR	*Nicotiana tabacum* cv. Burley 21	PHSVX	*Phaseolus vulgaris*
NIOTA-HFF	*Nicotiana tabacum* cv. Havana 425	*PHSVX-BAT*	*Phaseolus vulgaris* cv. Bataaf
NIOTA-HFT	*Nicotiana tabacum* cv. Havana 423	*PHSVX-BLV*	*Phaseolus vulgaris* cv. Black Valentine
NIOTA-KEN	*Nicotiana tabacum* cv. Kentucky 14	*PHSVX-BTF*	*Phaseolus vulgaris* cv. Bountiful
NIOTA-SAM	*Nicotiana tabacum* cv. Samsun	*PHSVX-KIN*	*Phaseolus vulgaris* cv. Kintoki
NIOTA-TUR	*Nicotiana tabacum* cv. Turkish	*PHSVX-KIW*	*Phaseolus vulgaris* cv. Kinghorn Wax
NIOTA-VTO	*Nicotiana tabacum* cv. V. 20	*PHSVX-LAV*	*Phaseolus vulgaris* cv. La Victorie
NIOTA-WHB	*Nicotiana tabacum* cv. White Burley	*PHSVX-LOT*	*Phaseolus vulgaris* cv. Long Tom
NIOTA-XNC	*Nicotiana tabacum* cv. Xanthi-nc	*PHSVX-PLT*	*Phaseolus vulgaris* cv. Plentiful
NIOVE	*Nicotiana velutina*	*PHSVX-RKY*	*Phaseolus vulgaris* cv. Red Kidney
OCIBA	*Ocimum basilicum*	PHTAM	*Phytolacca americana*
ORYSA	*Oryza sativa*	*PHYFL*	*Physalis floridana*
PANMA-KOE	*Panicum maximum* cv. K 189	PIBST	*Pisum sativum*
PANMI	*Panicum miliaceum*	*PIBST-PFC*	*Pisum sativum* cv. Perfection
PAQED	*Passiflora edulis*	PIOSE	*Philodendron selloum*
PASDJ	*Paspalum membranaceum* (syn.: *P. dissectum*)	PRIMA	*Primula malacoides*
PAVSA	*Pastinaca sativa*	RANSA	*Ranunculus sardous*
PAZFA	*Paulownia fargesii*	RHNMI	*Rhynchosia minima*
PELGR-NIL	*Pelargonium domesticum* cv. Nittany Lion	RIICO	*Ricinus communis*
PEUHY	*Petunia hybrida*	SECCE	*Secale cereale*
PHSAU	*Phaseolus aureus*	SETIT	*Setaria italica*
PHSAU	*Vigna radiata*	SILAR	*Silene armeria*
PHSLU	*Phaseolus lunatus*	SINAL	*Sinapis alba*
PHSLU-HEN	*Phaseolus lunatus* cv. Henderson	*SOLAD-CPC*	*Solanum tuberosum* ssp. andigena CPC 1801 (syn.: *S. andigenum*)
PHSMU	*Phaseolus mungo*		
PHSVN-GRN	*Phaseolus vulgaris* cv. Greet Northern	SOLAM	*Solanum nodiflorum*
PHSVN-PIN	*Phaseolus vulgaris* cv. Pinto	SOLCU	*Solanum rostratum*
PHSVV-TCR	*Phaseolus vulgaris* cv. Topcrop	*SOLHY-SDA*	*Solanum demissum* (SdA) (syn.: *S. tuberosum*)

SOLHY-TUA	*Solanum demissum*× *Solanum tuberosum* A6
SOLME	*Solanum melongena*
SOLNI	*Solanum nigrum*
SOLPJ-CPC	*Solanum phureja* CPC 4110
SOLSY	*Solanum sysimbrifolium*
SOLTU-MAB	*Solanum tuberosum* ssp. tuberosum cv. Maris Bard
SOLTU-MAP	*Solanum tuberosum* cv. Maris Piper
SORHA	*Sorghum halapense* (= *Sorgum halepense*)
SORVU-AGB	*Sorghum bicolor* cv. Asgrow Bugoff
SORVU	*Sorghum bicolor*
SORVU-RIO	*Sorghum bicolor* cv. Rio
SPQOL	*Spinacia oleracea*
TEACR	*Tetragonia crystallina*
TEATE	*Tetragonia expansa* (syn.: *T. tetragonoides*)
TEATE	*Tetragonia tetragonoides* (syn.: *T. expansa*)
TINER	*Tinantia erecta*
TOPMA	*Tropaeolum majus*
TRFIN	*Trifolium incarnatum*
TRFSS	*Trifolium* spp.
TRFSU	*Trifolium subterraneum*
TRZAX	*Triticum aestivum*
TRZAX-KEN	*Triticum aestivum* cv. Kent
TRZAX-MIA	*Triticum aestivum* cv. Michigan Amber
TRZAX-PAR	*Triticum aestivum* cv. Parker
TRZDU	*Triticum durum*
VAAPY-PIB	*Saponaria vaccaria* var. Pink Beauty
VACSS	*Vaccinium* spp.
VEEEN	*Verbesina encelioides*
VESTH	*Verbascum thapsus*
VICFX	*Vicia faba*
VICSS	*Vicia* spp.
VIGSC	*Vigna unguiculata* ssp. cylindrica
VIGSI	*Vigna sinensis* (syn.: *V. unguiculata*)
VIGSI	*Vigna unguiculata* (syn.: *V. sinensis*)
VIGSI	*Vigna unguiculata* subsp. unguiculata
VIGSI-BLE	*Vigna sinensis* cv. Blackeye
VIGSI-BLE	*Vigna unguiculata* cv. Blackeye
VIGSQ	*Vigna sesquipedalis*
VITLA-DEL	*Vitis labrusca* cv. Delaware
VITVI	*Vitis vinifera* ssp. vinifera
VOASU	*Voandzeia subterranea*
ZEAMX	*Zea mays*
ZIIEL	*Zinnia elegans*
ZINOF	*Zingiber officinale*

[1] The first three letters refer to the genus, the next two denote the species, and the last three after the rule symbolized the cultivar or hybrid. If the species is not designated, it is symbolized by the letters "SS" (e.g. AMASS for *Amaranthus* spp.).

[2] Abbreviations printed in italics are the proposed codes, all another codes approved by WSSA/WSSJ (see Anonymous, 1991).

Literature

Anonymous (1992): Important Crops of the World and their Weeds. Scientific and Common Names, Synonyms, and WSSA/WSSJ Approved Computer Codes. 2nd Ed., Business Group Crop. Prot., Bayer AG, Leverkusen, Germany 1992., 1682 pp.

Bock, K. R. and Harrison, B. D. (1985): African cassava mosaic virus. AAB Descriptions of Plant Viruses 297, 1–6.

Boswell, K. F. and Gibbs, A. J. (1983): Viruses of Legumes 1983. Descriptions and Keys from VIDE. Australian National Univ., Research School of Biol. Sci., Canberra, 1983., 139 pp.

Boswell, K. and Gibbs, A. (1986): The VIDE data bank for plant viruses. In: Jones, R. A. C. and Torrance, L. (Eds.). Developments in Applied Biology. I. Developments in Application in Virus Testing. Assoc. Appl. Biologists, Wellesbourne, U. K., 1986. pp. 283–287.

Brunt, A., Crabtree, K. and Gibbs, A. (1990): Viruses of Tropical Plants. Descriptions and List from the VIDE Database. Redwood Press Ltd., Melkshom, Wiltshire, UK. 1990., 707 pp.

Faust, W. (1992): Personal communication.

Forster, R. L. S. and Jones, A. T. (1980): Lucerne transient streak virus. CMI/AAB Descriptions of Plant Viruses 224, 1–4.

Francki, R. I. B., Fauquet, C. M., Knudson, D. L. and Brown, F. (1991): Classification and Nomenclature of Viruses. 1st Rep. Internat. Comm. Taxonomy of Viruses. Arch. Virology, Suppl. 2., Springer-Verlag, Wien 1991., 450 pp.

German, T. L., Ullman D. E. and Moyer, J. W. (1992): Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Ann. Rev. Phytopathol. 30, 315—348.

Holmes, F. O. (1929): Inoculating methods in tobacco mosaic studies. Bot Gaz. 87, 56–63.

Horváth, J. (1992): Host plants in diagnosis. In: Matthews, R. E. F. (Eds.), Diagnosis of Plant Virus Diseases. CRC Press, Boca Raton, Florida 1992 (in press).

Horváth, J. (1993): Hosts and non-hosts in the diagnostical strategy of plant viruses: A review. Acta Phytopath. et Entomol. 28, in press.

Hull, R., Milne, R. G. and Van Regenmortel, M. H. V. (1991): A list of proposed standard acronyms for plant viruses and viroids. Archs. Virol. 120, 151–164.

Matthews, R. E. F. (1991): Plant Virology. 3rd ed., Academic Press, New York, 1991., 835 pp.

Murant, A. F. and Harrison, B. D. (1970–1988): CMI/AAB (now AAB) Descriptions of Plant Viruses. Assoc. Appl. Biol., Wellesbourne, Warwick.

Pirone, T. P. and Shaw, J. G. (1988): Tobacco vein mottling virus. AAB Descriptions of Plant Viruses 325, 1–4.

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Phytoalexins in Rice—*Pyricularia oryzae* Interaction: Factors Affecting Phytoalexin Production

S. KUMAR and R. SRIDHAR[1]

Division of Plant Pathology, Central Rice Research Institute, Cuttack 753006, India

Phytoalexin production in *Oryza sativa-Pyricularia oryzae* relationship as influenced by nitrogen fertilization and chemicals including growth regulators, the fungal toxin, α-picolinic acid and a systemic non-fungicidal blast controlling agent, probenazole was examined on detached leaf blade segments of blast susceptible (cv. Karuna) and resistant (cv. IR 8) rice cultivars with drop-diffusate technique. Nitrogen fertilization did not influence the production of phytoalexin. Gibberellic acid and indoleacetic acid accelerated senescence of detached leaf blade segments to a lesser extent than abscisic acid. However, these growth regulators did not affect the phytoalexin production. α-Picolinic acid induced the accumulation of phytoalexin in the leaf blade segments of resistant cultivar, but not in those of susceptible cultivar. Probenazole markedly enhanced the accumulation of phytoalexin in both cultivars.

Considerable evidence supports the view that accumulation of phytoalexin at the site of attempted infection is one mechanism by which plants resist disease. Ever since Cartwright et al. (1977) claimed that rice plants treated with the systemic fungicide 2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid accumulate the diterpene phytoalexins, momilactone A and B in response to infection by *Pyricularia oryzae*, interest to examine the role of phytoalexins in blast resistance of rice has intensified. Subsequently, four diterpenoids, oryzalexin A, B and C (Akatsuka et al., 1983) and oryzalexin D (Akatsuka et al., 1985; Matsuyama and Wakimoto, 1985) possessing antiblast properties have been characterized from blast-diseased rice leaves. Precise evidence to ascertain the significance of these substances in blast resistance is lacking. In an earlier communication from this laboratory, we (Kumar and Sridhar, 1984) reported the production of phytoalexin by a blast-resistant cultivar in response to injury. We have also shown that the formation of phytoalexin was augmented by the presence of the pathogen, *P. oryzae* at the wounded sites in incompatible host-parasite interaction, whereas the absence of injury failed to trigger the phytoalexin formation. One way of determining the importance of phytoalexin in host resistance is to examine its production under different situations. We report in this communication the

[1] To whom correspondence should be addressed

Akadémiai Kiadó, Budapest

influence of some of the factors like nitrogen fertilization and chemicals capable of altering host reaction and/or important in the metabolism of diseased plants on the phytoalexin production of rice as estimated indirectly, i.e. on the basis of antifungal activity of the extract from the infection drop-diffusate.

Materials and Methods

Plant material. Rice (*Oryza sativa* L.) cultivars, Karuna susceptible and IR 8 resistant to blast, were grown from seeds in 30-cm diameter shallow earthen pots filled with 5 kg of alluvial soil, under natural photoperiodic conditions inside a galvanized iron wire-nethouse. The pots were watered daily so as to maintain the plants under flooded conditions with 3 to 4 cm of standing water above the soil surface.

Phytoalexin production. Phytoalexin production was assessed by drop-diffusate technique (Cruickshank, 1980) by the use of 10-cm apical segments of fully emerged fifth leaf blades harvested from 30-day-old plants. The leaf blade segments were placed with their adaxial side up in groups of 10 in 18-cm-diameter Petri dishes lined with filter paper moistened with distilled water. They were wounded at various points, approximately 1 cm apart, by gently pressing their adaxial surface with the cut end of a 2-mm-diameter glass rod. Infection drops (5 to 8 drops/segment) containing *P. oryzae* (isolate P 258, compatible to cv. Karuna and incompatible to cv. IR 8) spores (ca. 3×10^4 spores ml^{-1}, obtained from 10-day-old cultures maintained on oat-meal agar slopes) were placed just above the injuries on the leaf blade segments with a 1-ml hypodermic syringe. The Petri dishes were incubated on a laboratory bench for 48 h at 12 h alternating light (Philips cool day light fluorescent tubes TL-40 W/54, intensity 8 $W \cdot m^{-2}$) and dark.

Phytoalexin extraction. At the end of the incubation, the infection drops were collected by the syringe and centrifuged at 3000 g for 30 min. Five-ml portions of the clear diffusates were extracted thrice with equal volumes of distilled ethyl acetate. The solvent fractions were pooled and evaporated to dryness at room temperature (28 $\pm$ 2 °C). The residue was dissolved in 1 ml of ethyl acetate (Mahadevan and Sridhar, 1986).

Phytoalexin bioassay. The antifungal activity of the extracts was bioassayed by slide germination method (Horsfall, 1956). Aliquots of 100 μl of the extracts were dispensed in three replicate cavity slide wells, and the solvent was allowed to evaporate at room temperature. Control slides contained an equal quantity of the solvent only in place of the extract. Into each well 50 μl of aqueous spore suspension (ca. 3×10^4 spores ml^{-1}) of *P. oryzae* was pipetted. After incubation of the slides at 28 $\pm$ 2 °C for 6 h in moist Petri dishes, a drop of lactophenol-cotton blue was added to each well. The proportion of germinated spores was evaluated from fifteen microscopic fields and the germ-tube length of sporelings was measured.

Effect of nitrogen fertilization. The plants were top dressed with different doses of ammonium sulphate (1.25, 2.5, 5 and 10 g/pot) when they were 20 days old (4-leaf stage). Control plants did not receive any fertilizer. Phytoalexin production was assessed by drop-diffusate technique as described above. Total nitrogen content of identical samples of oven-dried leaf blades was estimated by micro-Kjeldahl method (Bremner, 1960).

Effect of chemicals. The influence of different growth regulators on phytoalexin production by leaf blades was examined. Ten-centimeter apical segments of fully emerged fifth leaf blades harvested from 30-day-old plants were placed with their adaxial side up in groups of 10 in 18-cm-diameter Petri dishes lined with filter paper. The filter papers were previously moistened with 10 ml of either distilled water or 2×10^{-5} M solution of either abscisic acid (Sigma Chemical Co., USA), gibberellic acid (GA_3, Polfa-Kunto, Poland), indoleacetic acid (E. Merck, Germany) or kinetin (Loba Chemic, Austria). In a separate experiment, the leaf blade segments were treated with a systemic, non-fungicidal blast controlling agent, probenazole (100 $\mu g \cdot ml^{-1}$, trade name: Oryzemate, chemical name: 3-allyloxy-1,2-benzisothiazole-1,1-dioxide; Keiji Seika Kaisha Ltd., Japan) or the *P. oryzae* toxin, α-picolinic acid (2×10^{-5} M, Sigma Chemical Co., USA). All these chemicals at the concentration tested did not show any antifungal activity *in vitro* to *P. oryzae*. Production of phytoalexins by these leaf blade segments was assessed by drop-diffusate technique as described above.

To avoid variations among the assays, all the bioassays reported in this paper were performed simultaneously. As a measure of leaf senescence, the total chlorophyll content of the leaf blade segments, treated with different growth regulators at zero hour and at the end of 48 h of incubation was determined spectrophotometrically after extraction of the pigments in ethanol (Mahadevan and Sridhar, 1986).

Results

Effect of nitrogen fertilization. The extract of infection droplets collected from the leaf blade segments of susceptible cultivar grown without added nitrogen was only weakly toxic to the germination of *P. oryzae* spores and their germ-tube growth (Table 1). Although supply of a very low level (1.25 g of $(NH_4)_2SO_4$/pot) of nitrogen to the susceptible cultivar reduced the toxicity of the extract of infection droplets, further increase of nitrogen supply did not have any marked effect on decreasing the toxicity. In contrast, the extract of infection droplets collected from the injured leaf blades of resistant cultivar grown without any additional supply of nitrogen was highly toxic to *P. oryzae*. Nitrogen fertilization had no influence on the toxocity of the extracts of infection droplets to the blast pathogen. Total nitrogen content of the leaf blade tissues increased with an increase in the amount of nitrogen applied to both cultivars (Table 2).

Effect of growth regulators. The extracts of infection droplets collected from the untreated leaf blade segments of susceptible cultivar showed weak toxicity

Table 1

Effect of nitrogen fertilization on the toxicity of extract of infection droplets from the leaf blades of blast susceptible Karuna and resistant IR 8 cultivars

Cultivar	Control	$(NH_4)_2SO_4$ applied(g/pot)				
		0	1.25	2.5	5	10
		Spore germination (%)				
Karuna	99	85	93	95	96	96
		(14)	(6)	(4)	(3)	(3)
IR 8	99	12	13	14	11	9
		(88)	(87)	(86)	(89)	(91)
		Germ-tube growth (μm)				
Karuna	60	51	56	52	56	57
		(15)	(7)	(13)	(7)	(5)
IR 8	60	39	41	36	36	34
		(35)	(32)	(40)	(40)	(43)

Data on spore germination and germ-tube growth are based on the observation of 450 spores and 60 sporelings, respectively. Numbers in parentheses represent per cent inhibition over solvent control.

For spore germination LSD 0.05 = 3, LSD 0.01 = 4

For germ-tube growth LSD 0.05 = 6, LSD 0.01 = 8

Table 2

Effect of nitrogen fertilization on total nitrogen content of leaf blades of blast susceptible Karuna and resistant IR 8 cultivars

Cultivar	Total nitrogen (%)				
	$(NH_4)_2SO_4$ applied (g/pot)				
	0	1.25	2.5	5	10
Karuna	1.96±0.3	3.27±0.1	4.15±0.2	3.92±0.1	4.45±0.2
IR 8	1.78±0.5	3.19±0.2	3.97±0.1	3.96±0.2	4.12±0.3

Data represent the mean of three determinations and are per cent nitrogen contents ± S. E.

to germination of *P. oryzae* spores and to germ-tube growth (Table 3). In contrast, similar extract from the untreated leaf blade segments of the resistant cultivar exhibited a high level of toxicity to the pathogen. However, treatment of the leaf blade segments with the growth regulators did not cause any marked change in the toxicity of the extract of infection droplets.

The injured leaf blade segments incubated with the infection droplets on moist filter paper (water treatment) in Petri dishes for 48 h exhibited some signs of senescence. During this period, the leaf blade segments of susceptible and

Table 3

Effect of growth regulators on the toxicity of extract of infection droplets from leaf blades of blast susceptible Karuna and resistant IR 8 cultivars to spores of *P. oryzae*

Cultivar	Control	Growth regulators[1]				
		H_2O	K	GA	ABA	IAA
		Spore germination (%)				
Karuna	99	78	88	85	89	81
		(21)	(11)	(14)	(10)	(18)
IR 8	99	12	17	16	17	21
		(88)	(83)	(84)	(83)	(79)
		Germ-tube growth (μm)				
Karuna	60	48	43	35	41	40
		(20)	(28)	(42)	(32)	(33)
IR 8	60	30	39	36	29	33
		(50)	(35)	(40)	(52)	(45)

[1] K, Kinetin; GA, Gibberellic acid (GA_3); ABA, Abscisic acid; IAA, Indole-acetic acid.

Data on spore germination and germ-tube growth are based on the observation of 450 spores and 60 sporelings, respectively. Numbers in parentheses represent per cent inhibition over solvent control.

For spore germination LSD 0.05 = 4, LSD 0.01 = 4

For germ-tube growth LSD 0.05 = 7, LSD 0.01 = 8

Table 4

Total chlorophyll content of detached and injured leaf blade segments of blast susceptible Karuna and resistent IR 8 cultivars, incubated in the presence of infection droplets for 48 h. as affected by growth regulators

Cultivar	Initial	Growth regulators[1]				
		H_2O	K	GA	ABA	IAA
Karuna	35.3±1.2	26.8±1.3	27.8±0.7	22.2±0.1	7.3±0.5	22.3±0.9
		(76)	(79)	(63)	(21)	(63)
IR 8	35.3±1.2	32.5±0.4	31.8±0.8	25.3±1.4	12.5±0.1	20.5±0.6
		(92)	(90)	(72)	(35)	(58)

[1] K, Kinetin; GA, Gibberellic acid (GA_3); ABA, Abscisic acid; IAA, Indole-acetic acid.

Data represent the mean of four determinations and are in mg chlorophyll g^{-1} fresh leaf blades ± S. E. Numbers in parentheses represent per cent of initial value.

Table 5

Effect of probenazole on the toxicity of extract of infection droplets from leaf blades of blast susceptible Karuna and resistant IR 8 cultivars to spores of *P. oryzae*

Cultivar	Control	Treatment	
		H_2O	Probenazole
		Spore germination (%)	
Karuna	99	93	25
		(6)	(75)
IR 8	99	14	6
		(86)	(94)
		Germ-tube growth (μm)	
Karuna	60	52	34
		(13)	(43)
IR 8	60	26	26
		(57)	(57)

Data on spore germination and germ-tube growth are based on the observation of 450 spores and 60 sporelings, respectively. Numbers in parentheses represent per cent inhibition over solvent control.

For spore germination LSD 0.05 = 3, LSD 0.01 = 4
For germ-tube growth LSD 0.05 = 5, LSD 0.01 = 7

Table 6

Effect of α-picolinic acid on the toxicity of extract of infection droplets from the leaf blades of blast susceptible Karuna and resistant IR 8 cultivars to spores of *P. oryzae*

Cultivar	Control	Treatment	
		H_2O	α-Picolinic acid
		Spore germination (%)	
Karuna	99	93	94
		(6)	(5)
IR 8	99	14	5
		(86)	(95)
		Germ-tube growth (μm)	
Karuna	60	52	48
		(13)	(20)
IR 8	60	26	24
		(57)	(60)

Data on spore germination and germ-tube growth are based on the observation of 450 spores and 60 sporelings, respectively. Numbers in parentheses represent per cent inhibition over solvent control.

For spore germination LSD 0.05 = 3, LSD 0.01 = 4
For germ-tube growth LSD 0.05 = 5, LSD 0.01 = 4

resistant cultivars lost 24 and 8 per cent of their initial total chlorophyll contents respectively (Table 4). Kinetin treatment of the leaf blade segments did not have any marked effect on leaf senescence during 48 h of incubation. For, kinetin-treated leaf blade segments of susceptible and resistant cultivars lost 21 and 10 per cent of their initial total chlorophyll contents, respectively. Gibberellic acid and indoleacetic acid accelerated leaf senescence as measured by chlorophyll loss during 48 h of incubation to a less extent when compared to abscisic acid.

Effect of probenazole. The extract of infection droplets collected from the leaf blade segments of resistant cultivar non-treated with probenazole was highly toxic to both the germination of *P. oryzae* spores and their germ-tube growth (Table 5). Similar extract from the susceptible leaf blade segments was weakly toxic to the pathogen. Probenazole treatment remarkably enhanced the toxicity of the extracts of infection droplets collected from the susceptible leaf blades to *P. oryzae*.

Effect of fungal toxin. Treating the leaf blades with α-picolinic acid did not influence the toxicity of the extract of infection droplets collected from the injured leaf blades of the susceptible cultivar (Table 6). However, it enhanced the toxicity of the infection droplets collected from the resistant cultivar.

Discussion

Environmental variables and chemical treatments that alter resistance often result in corresponding change in the concentration of phytoalexins produced (Bell, 1981). Since significant amount of phytoalexin production is triggered by the presence of the blast pathogen, *P. oryzae* on the injured rice leaf blade tissues (Kumar and Sridhar, 1984), the influence of various factors like nitrogen fertilization; chemicals such as growth regulators, a systemic non-fungicidal blast controlling agent (probenazole) and the fungal toxin (α-picolinic acid) on phytoalexin production was experimented using this model.

That the susceptibility of rice plants to blast is augmented by nitrogen fertilization is well documented (Ou, 1985). It has been reported that high levels of nitrogen as compared to low levels reduce quantities of phytoalexin (medicarpin) accumulating in the infection droplets on white clover, and this corresponds with the change in host resistance (Cruickshank et al., 1979). However, the present study showed that nitrogen application did not have any marked effect on the phytoalexin produced by rice leaf blades. Presumably, resistance of susceptible plants expressed under low nitrogen level operates through different mechanisms, for the augmented susceptibility at high nitrogen level is believed to be due to a reduction in the amount or activity of prohibition level (Sridhar, 1972).

Growth regulators have been shown to affect the host susceptibility to plant pathogens, and there are reports that growth regulators influence phytoalexin production (Mahadevan, 1984). Indoleacetic acid and ethrel effectively inhibits the occurrence of rice blast, whereas kinetin, gibberellin and abscisic acid favours

the occurrence and development of the disease (Matsumoto et al., 1980). Oku and Nakanishi (1962) reported that indoleacetic acid when sprayed on rice plants stimulates phytoalexin synthesis which is believed to confer resistance against *Drechslera oryzae*. Mukhopadhaya and Purkayastha (1981) demonstrated that diffusates collected from rice leaf blades having treated with kinetin, benzyladenine or nickel sulphate capable of retarding senescence markedly inhibit the spore germination and germ-tube growth of *D. oryzae*. These workers believe that retardation of leaf senescence helps to maintain the level of antifungal substance in leaf diffusates and consequently the resistance of rice to *D. oryzae*. Cytokinins retard while abscisic acid accelerates senescence. Gibberellins have been shown to delay senescence in a few plant species. Although this effect is common in rice (Mohanty, 1984), the effects of kinetin and gibberellin were not well pronounced in this study, presumably due to short incubation period (48 h).

Matsuyama and Wakimoto (1984) reported the formation of oryzalexin D, toxic to blast fungus in the lower senescing leaf blades of a blast susceptible cultivar, which are resistant to *P. oryzae*. Abscisic acid is known to accumulate in senescing leaf tissues (Thomas and Stoddart, 1980). In the event, treatment of rice seedlings with abscisic acid enhances their susceptibility to blast as reported by Matsumoto et al. (1980), it is expected that treatment with abscisic acid should inhibit the phytoalexin formation by the senescing leaves. However, with the augmented senescence induced by abscisic acid in excised leaves, the toxicity of the extracts of the infection droplets from the leaf blades of either susceptible or resistant cultivar did not differ markedly. Senescence is considered as an essentially degradative process (Thimann, 1980). Conceivably, the formation of phytoalexin detected by us in rice is not associated with the degradative process of the host metabolism in contrast to oryzalexin D which accumulates in senescing rice leaves (Matsuyama and Wakimoto, 1985). Evidently, the phytoalexin detected by us might be different from oryzalexin D.

Probenazole, a systemic non-fungicidal blast controlling agent effectively controlled blast in the susceptible cultivar used in this study (data not presented). It is believed that the compound protects rice plants against blast through the activation of enzymic activities related to lignin formation in the zone surrounding cells infected with rice blast fungus (Sekizawa and Mase, 1980). However, no attempt has been made to examine the lignin formation of probenazole-treated and *P. oryzae*-inoculated plants. On the other hand, Langcake and Wickins (1975) reported that lignin formation does not take place in a systemic fungicide (2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid)-induced resistance of rice against blast. Further, rice plants treated with probenazole has been shown to accumulate four antifungal compounds in response to inoculation with *P. oryzae* (Shimura et al., 1981). Likewise, the production of two diterpene phytoalexins, momilactone A and B at the wounded sites of leaf blades of rice plants treated with the cyclopropane derivative and inoculated with *P. oryzae* has been demonstrated (Cartwright et al., 1977; Langcake et al., 1978; Cartwright and Langcake,

1980). However, no attempt has been made to identify the phytoalexin detected by us at this stage.

Blast fungus spores contain α-picolinic acid and the spores exude this toxin during germination (Ogasawara et al., 1961; Tamari et al., 1965). The presence of a substance of fungal origin in the spore germination fluid of mildly virulent isolates of *D. oryzae* has been thought to be the cause for induction of resistance in rice (susceptible) through the production of phytoalexin-like antifungal substance (Sinha and Trivedi, 1978). The capacity of the plants to detoxify or inactivate the pathogen-produced toxins is an important defense mechanism. Rice plants metabolize picolinic acid to picolinic acid methyl ester and N-methyl picolinic acid, and these are not toxic to tissues (Tamari et al., 1967). It is probable that rice plants differing in their resistance to blast fungus also differ in their ability to convert picolinic acid to inactive forms, and this might explain the ability of the toxin to augment production of phytoalexin in the resistant cultivar, but not in the susceptible cultivar.

The results of this study offer evidence that the phytoalexin detected by us in rice is not associated with the degradative process of the host metabolism common in senescing leaves. The probenazole-induced resistance of susceptible plants is related with the accumulation of phytoalexin and is similar to the mechanism operating in a resistant (untreated) plant. Since no evidence was obtained by us to demonstrate the relationship between the phytoalexin production and the changes in host susceptibility caused by nitrogen fertilization, resistance in rice against blast is not exclusively governed by the phytoalexin.

Literature

Akatsuka, T., Kodama, O., Kato, H., Kono, Y. and Takeuchi, S. (1983): 3-Hydroxy-7-oxo-sandaracopimaradiene (oryzalexin A). A new phytoalexin isolated from rice blast leaves. Agric. Biol. Chem. 47, 445–447.

Akatsuka, T., Kodama, O., Sekido, H., Kono, Y. and Takeuchi, S. (1985): Novel phytoalexins (oryzalexins A, B and C) isolated from rice blast leaves infected with *Pyricularia oryzae*. I. Isolation, characterization and biological activities of oryzalexins. Agric. Biol. Chem. 49, 1689–1694.

Bell, A. A. (1981): Biochemical mechanisms of disease resistance, Annu. Rev. Plant Physiol. 32, 21–81.

Bremner, J. M. (1960): Determination of nitrogen in soil by the Kjeldahl method. J. Agric. Sci. 55, 11–33.

Cartwright, D. and Langcake, P. (1980): Phytoalexin production in rice and its enhancement by a dichlorocyclopropane fungicide. Physiol. Plant Pathol. 17, 259–267.

Cartwright, D., Langcake, P., Pryce, R. J., Leworthy, D. P. and Ride, J. P. (1977): Chemical activation of host defense mechanisms as a basis for crop protection. Nature (London) 267, 511–513.

Cruickshank, I. A. M. (1980): Defenses triggered by the invader: Chemical defenses. In: J. G. Horsfall and E. B. Cowling (eds): Plant Disease, An advanced treatise. How Plants Defend themselves. Academic Press, New York. 4, 247–267.

Cruickshank, I. A. M., Spencer, K. and Mandryk, M. (1979): Nitrogen nutrition and the net

accumulation of medicarpin in infection-droplets on excised leaflets of white clover. Physiol. Plant Pathol. 14, 71–76.

Horsfall, J. G. (1956): Principles of Fungicidal Action. Chronica Botanica Co., U.S.A.

Kumar, S. and Sridhar, R. (1984): Phytoalexin production in rice-*Pyricularia oryzae* interaction: Induction by wounding. Acta Phytopathol. Hung. 19, 237–243.

Langcake, P. and Wickins, S. G. A. (1975): Studies on the action of the dichlorocyclopropanes on the host parasite relationship in the rice blast disease. Physiol. Plant Pathol. 7, 113–126.

Langcake, P., Cartwright, D., Leworthy, D. P., Pryce, R. J. and Ride, J. P. (1978): The dichlorocyclopropanes–fungicides with an indirect mode of action. Neth. J. Plant Pathol. (Suppl. 1) 83, 153–155.

Mahadevan, A. (1984): Growth Regulators, Microorganisms and Diseased Plants. Oxford and IBH Publishing Co., New Delhi.

Mahadevan, A. and Sridhar, R. (1986): Methods in Physiological Plant Pathology, 3rd edn. Sivakami Publications, Madras.

Matsumoto, K., Suzuki, Y., Mase, S., Watanabe, T. and Sekizawa, Y. (1980): On the relationship between plant hormones and rice blast resistance. Ann. Phytopathol. Soc. Japan 46, 307–314.

Matsuyama, N. and Wakimoto, S. (1984): On an antifungal substance mainly accumulated in lower rice leaves infected with rice blast fungus *Pyricularia oryzae* Cav. Ann. Phytopathol. Soc. Japan 50, 379–382.

Matsuyama, N. and Wakimoto, S. (1985): Purification and characterization of anti-blast substance, S-1, formed mainly in blast-resistant lower rice leaves. Ann. Phytopathol. Soc. Japan 51, 498–500.

Mohanty, S. K. (1984): Physiology of Rice Tungro Virus Disease. Doctoral thesis, Utkal Univ., Bhubaneswar, India.

Mukhopadhaya, R. and Purkayastha, R. P. (1981): Effect of age and senescence retarding compounds on biological activities of leaf diffusates and disease susceptibility of rice. Indian J. Exptl. Biol. 19, 267–270.

Ogasawara, N., Tamari, K. and Suga, M. (1961): Biochemical studies on the blast disease of rice plants. Part 20. Mechanism of spore germination of *Pyricularia oryzae* (2). Presence in spores of picolinic acid and the changes occurring during spore germination. J. Agric. Chem. Soc. Japan 35, 1412–1416.

Oku, H. and Nakanishi, T. (1962): Relation of phytoalexin like antifungal substance to resistance of the rice plant against *Helminthosporium* leaf spot disease. Takamine Kenkyusho Nempo 14, 120–128.

Ou, S. H. (1985): Rice Diseases. 2nd edn. Commonw. Mycol. Inst., Kew, Surrey, England.

Sekizawa, Y. and Mase, S. (1980): Recent progress in studies on non-fungicidal controlling agents, probenazole, with reference to the induced resistance mechanism of rice plant. Rev. Plant Protect. Res. 13, 114–121.

Shimura, M., Iwata, M., Tashiro, N., Sekizawa, Y., Suzuki, Y., Mase, S. and Watanabe, T. (1981): Anti-conidial germination factors induced in the presence of probenazole in infected host leaves. I. Isolation and properties of 4 active substances. Agric. Biol. Chem. 45, 1431–1436.

Sinha, A. K. and Trivedi, N. (1978): Fungitoxicity induced by infection in rice plants as a factor in host resistance to *Helminthosporium* infection. Z. Pflanzenkr. Pflanzenschutz 85, 22–29.

Sridhar, R. (1972): Influence of nitrogen fertilization and *Pyricularia oryzae* development on some oxidases, their substrates and respiration of rice plants. Acta Phytopathol. Hung. 7, 57–70.

Tamari, K., Ogasawara, N. and Kaji, J. (1965): Biochemical products of the metabolism of *Pyricularia oryzae*. In: Rice Blast Disease. Proc. Symp. Int. Rice Res. Inst Los Banos, Philippines, July 1963. The Johns Hopkins Press, Baltimore, Maryland, 35–68.

Tamari, K., Ogasawara, N. and Kaji, J. (1967): Biochemical response of plants to toxins produced by the rice blast fungus. In: The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction. Am. Phytopathol. Soc., Minneapolis, Minn., 203–216.

Thimann, K. V. (1980): The senescence of leaves. In: K. V. Thimann (ed): Senescence in Plants. The Chemical Rubber Co. Press, Boca, Raton, Fl., 85–115.

Thomas, H. and Stoddart, J. L. (1980): Leaf senescence. Annu. Rev, Plant Physiol. 31, 83–111.

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Identification Key for Alate Aphids Caught in Yellow Pan Traps

ZSUZSA BASKY

Vegetable Crop Research Institute, 6001 Kecskemét, P.O.B. 116, Hungary

Alate aphids were collected by Moericke-type yellow pan traps in cucumber and pepper fields. In the present paper the determination key of 95 species is presented. based on morphological characteristics visible with normal stereoscopic microscope. The following genera are included into the key: *Pemphigus*, *Forda*, *Tetraneura*, *Eulachnus*, *Eriosoma*, *Schizaphis*, *Anoecia*, *Diuraphis*, *Phyllaphis*, *Cromaphis*, *Pterocallis*, *Tuberculatus*, *Euceraphis*, *Eucallipterus*, *Myzocallis*, *Therioaphis*, *Callaphis*, *Periphyllus*, *Chaitophorus*, *Iziphia*, *Sipha*, *Rhopalosiphum*, *Semiaphis*, *Protaphis*, *Aphis*, *Longiunguis Brevicoryne*, *Hayhurstia*, *Hyalopterus*, *Lipaphis*, *Myzus*, *Cryptomyzus*, *Capitophorus*, *Megourella*, *Hyadaphis*, *Cavariella*, *Amphorophora*, *Hyperomyzus*, *Phorodon*, *Nasonovia*, *Ovatus*, *Pleotrichoporus*, *Aulacorthum*, *Metopolophium*, *Sitobion*, *Macrosiphum*, *Dactynotus*, *Microlophium*, *Macrosiphonella*, *Acyrthosiphon*, *Macrosiphum*, *Brachycaudus*, *Dysaphis* and *Anuraphis*.

With few exceptions aphids as virus vectors are much more important than aphids as sucking insects. 300 aphid species have been tested in relation to 300 viruses and 300 host plants. 193 aphid species from the tested 300 are able to transmit, at least, 1 virus (Harris, 1981). In virus epidemiology identification of vector species is essential.

This key includes 95 frequent and less frequent aphid species caught by yellow traps.

Identification of aphid species

Aphids were collected by Moericke-type yellow traps in cucumber and pepper fields in the Great Hungarian Plain. For identification of alate aphids characters visible with normal stereoscopic microscope were used to distinguish different species. These characters are: number of antennal segments, shape of sensoria, length of processus terminalis, presence or lack of antennal tubercles, length of rostrum, wing venation, shape of cornicles, shape of cauda, pigmentation of abdomen, pigmented lateral sclerites are referred to as "marginals" abdominal hairs, caudal hairs. Works of Theobald (1926), Börner et al. (1957), Szalay-Marzsó (1969), Eastop (1966), Müller (1975), Stroyan (1977), Szelegiewicz (1977), Taylor (1980), Stroyan (1984), Blackman and Eastop (1985) were used for identifying the species. Identifications were controlled partly by Prof. F. P.

Present address: Crop Protection and Soil Conservation Station 5001 Szolnok P.O.B. 192, Hungary

Akadémiai Kiadó, Budapest

Müller (Rostock) and Prof. F. Leclant (Montpellier) partly Dr. L. Szalay-Marzsó (Gödöllő) and P. Andrásfalvy (Budapest).

1 (6) Media unbranched.
2 (5) No cornicles.
3 (4) Abdomen pale, no pigmentation.
Antennae 5 or 6 segmented if 6 segmented IV. antennal segment shorter than VI. If antenna 5 segmented III. antennal segment shorter than V.

Fig. 1. *Pemphigus* sp.

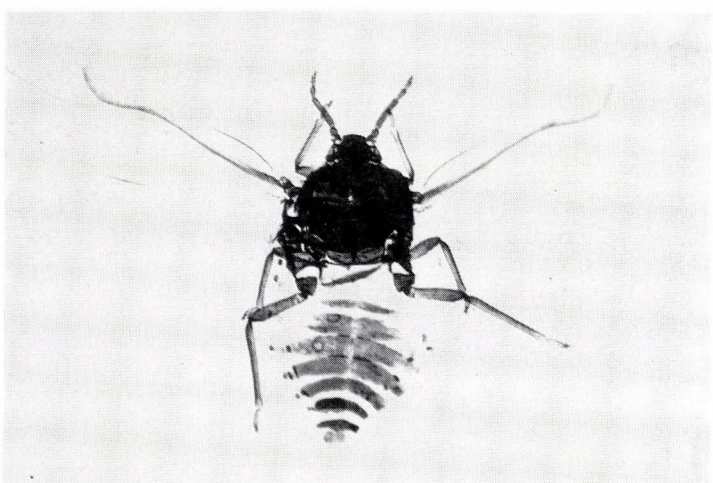

Fig. 2. *Forda marginata*

Transverse rhinaria on the antennal segments. Processus terminalis shorter than the diameter of the last antennal segment (Fig. 1).

4 (3) Pigmentation on abdomen.
Antenna short, 5 segmented, oval rhinaria on III., IV., V. Dark marginal spots and dark stripes on abdomen. Processus terminalis shorter than the diameter of the last antennal segment (Fig. 2).

5 (2) Cornicles present.
Antenna six segmented. IV. segment very short, just a little longer than VI. Transverse rhinaria. Processus terminalis shorter than diameter

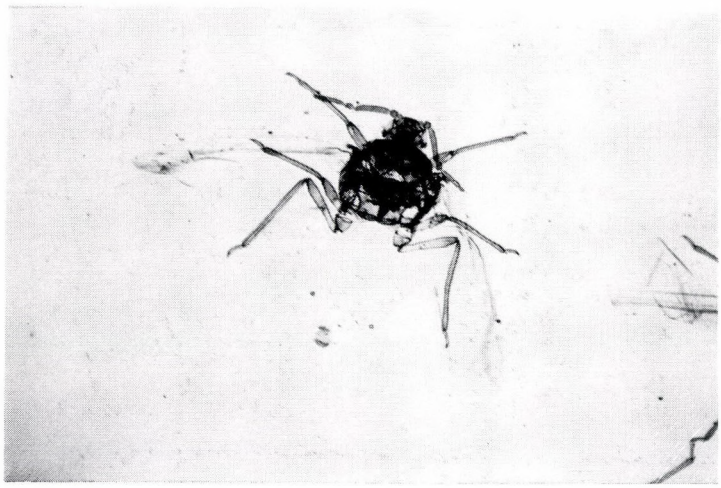

Fig. 3. *Tetraneura ulmi*

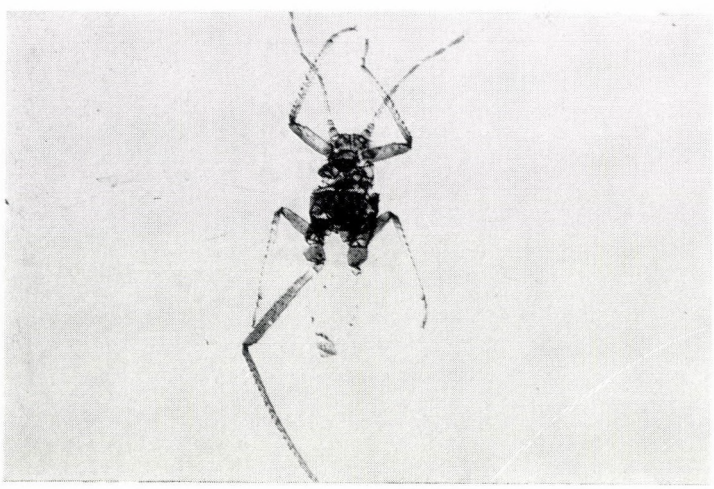

Fig. 4. *Eulachnus agilis*

of last antennal segment. Pore-like cornicles. Only marginals on abdomen (Fig. 3).

6 (1) Media branched.

7 (14) Media once-branched.

8 (13) Cornicles pore-like, cauda broadly rounded, processus terminalis shorter than base of VI.

9 (10) First tarsal segments elongate, longer than second antennal segment. Distal part of antennal segments III-V dark. Long thick hairs on tibiae, antennae and abdomen (Fig. 4).

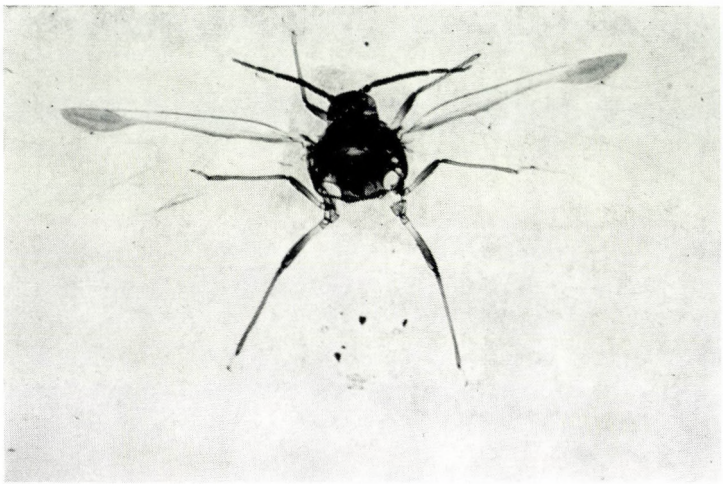

Fig. 5. *Eriosoma lanuginosum*

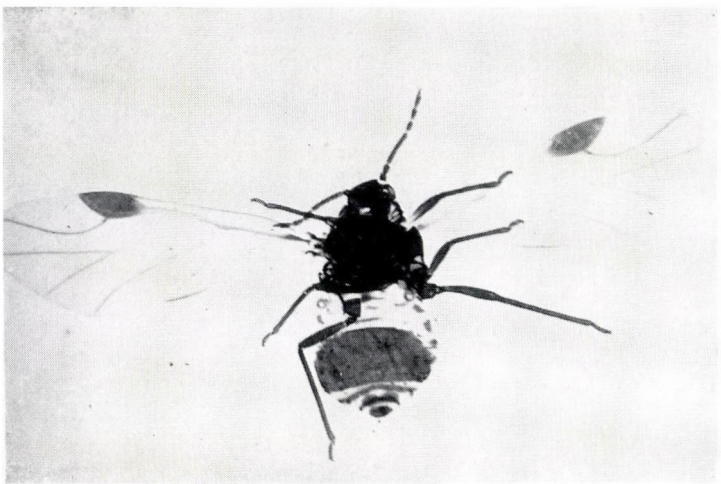

Fig. 6. *Anoecia corni*

10 (9) First tarsal segments normal, less than half as the second antennal segment. Body hairs less conspicuous.

11 (12) Little pigmentation on abdomen. Ring like rhinaria on III, IV, V (Fig. 5).

12 (11) Abdomen bearing a broad dark patch. Oval rhinaria on III, IV, V (Fig. 6).

13 (8) Cornicles cylindrical, as long as or longer than the elongate cauda. Abdomen pale. Processus terminalis 3 times as long as base VI (Fig 7).

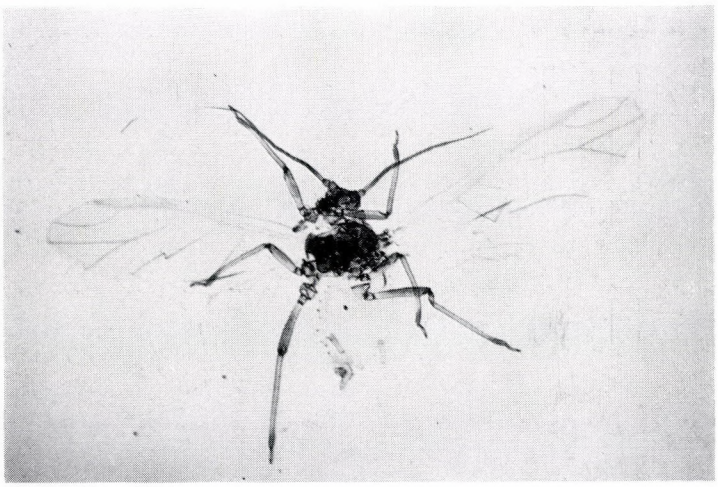

Fig. 7. *Schizaphis graminum*

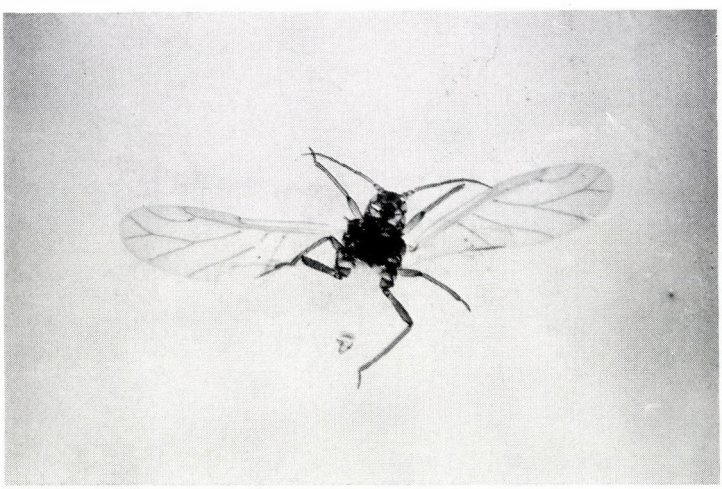

Fig. 8. *Diuraphis sp.*

14 (7) Media twice-branched.

15 (16) Cornicles shorter than wide, mammariform.
Antennae, 6 segmented. Processus terminalis just longer than base of VI. Head, thorax antennae, legs dark. Light marginals on abdomen only. No supracauda, no antennal tubercles (Fig. 8).

16 (15) Cornicles pore-like or longer than wide.

17 (18) Cornicles pore-like.
Antennae 6 segmented, processus terminalis shorter than diameter

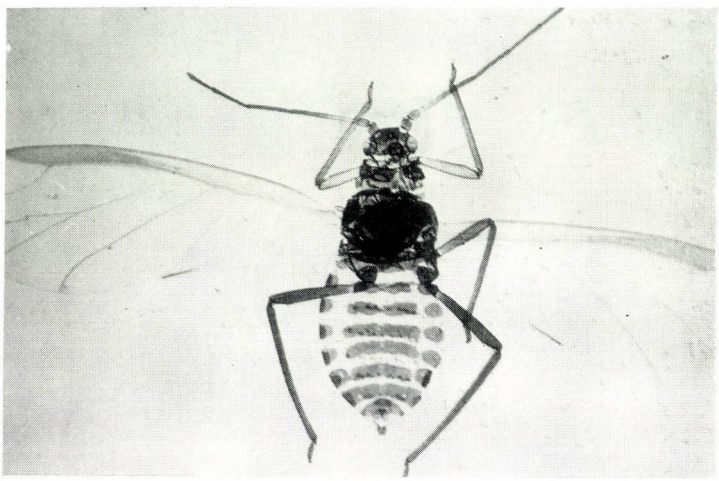

Fig. 9. *Phyllaphis fagi*

Fig. 10. *Cromaphis juglandicola*

of distal end of VI. oval rhinaria on III. Knobbed cauda with bilobed anal plate. Dark stripes and marginals on abdomen (Fig. 9).

18 (17) Cornicles truncate or elongate, tubular or swollen.

19 (50) Cornicles truncate.

20 (27) No pigmentation on abdomen.

21 (24) Radius absent or weakly developed.

22 (23) Anal plate slightly indented.
6 segmented antennae. Oval rhinaria only at base of III. Base of VI. 6 times as long as processus terminalis. Dark distal end of antennal

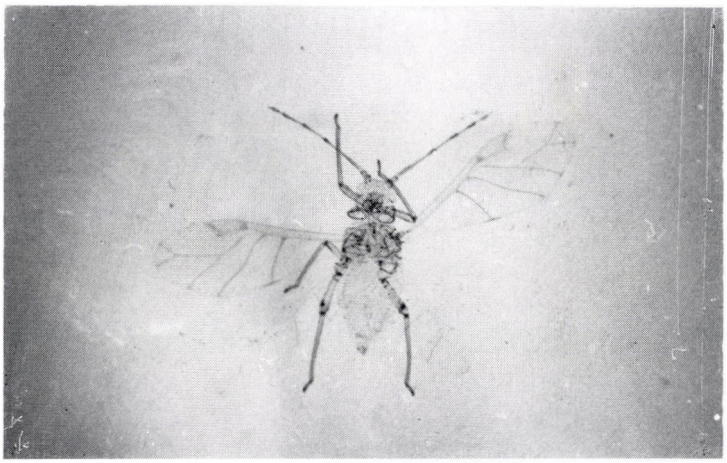

Fig. 11. *Pterocallis alni*

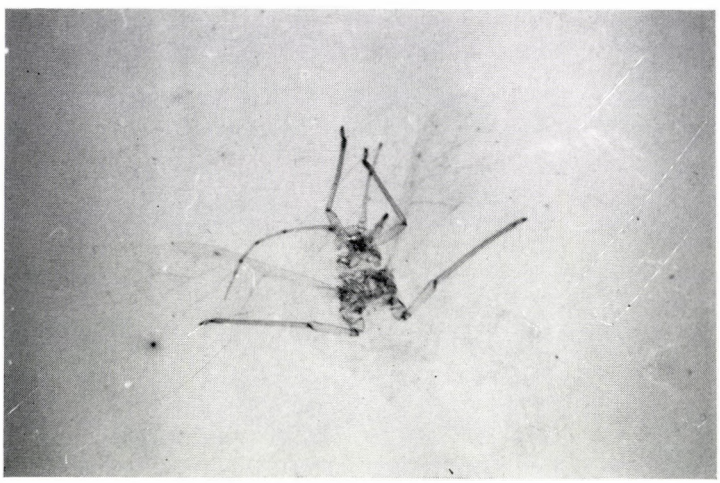

Fig. 12. *Tuberculatus annulatus*

segments. Cornicles pale, knobbed cauda. Hairs on abdomen in row. Dark spot on distal end of hind femur (Fig. 10).

23 (22) Bilobed anal plate.
Antennae 6 segmented. Processus terminalis shorter than base of VI. 1–2 long hairs on III. First cubitus of hind wing at right angles to radius. Knobbed cauda. Dark spot on distal end of hind femur (Fig. 11).

24 (21) Radius well developed.

25 (26) Legs pale, distal part of antennal segments may be dark. Antennae 6 segmented, processus terminalis longer than base of VI. Knobbed

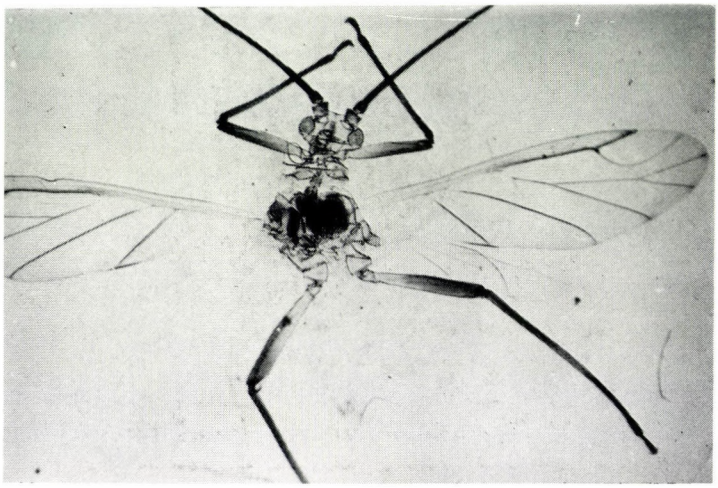

Fig. 13. *Euceraphis betulae*

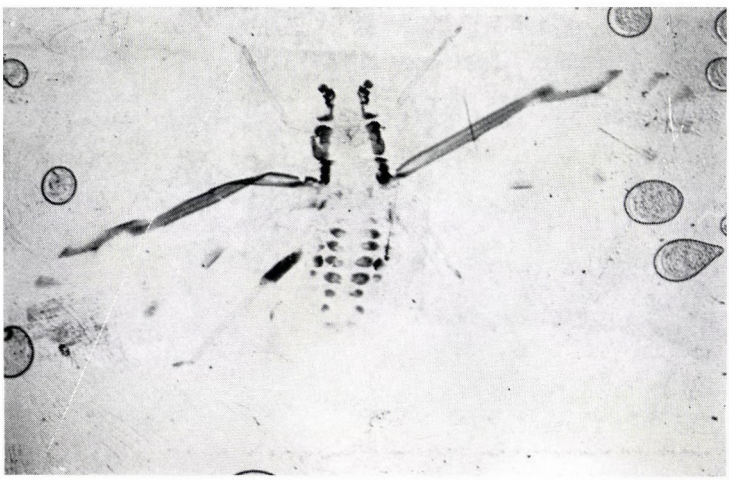

Fig. 14. *Eucallipterus tiliae*

cauda with bilobed anal plate. Transparent abdomen with two small darker tubercles in centre (Fig. 12).

26 (25) Distal end of femur and tibia dark, antennae black.
Antennae 6 segmented, oval rhinaria at base of III. Processus terminalis shorter than base of VI. Cauda helmet shaped (Fig. 13).

27 (20) Pigmentation on abdomen, dark cornicles.

28 (39) Small dark spots, flecks on abdomen, no stripes and patch.

29 (32) No dark spots on middle of abdomen.

30 (31) Head and thorax dark laterally, pale stripe down middle.
Antennae 6 segmented. Processus terminalis shorter than base of VI.

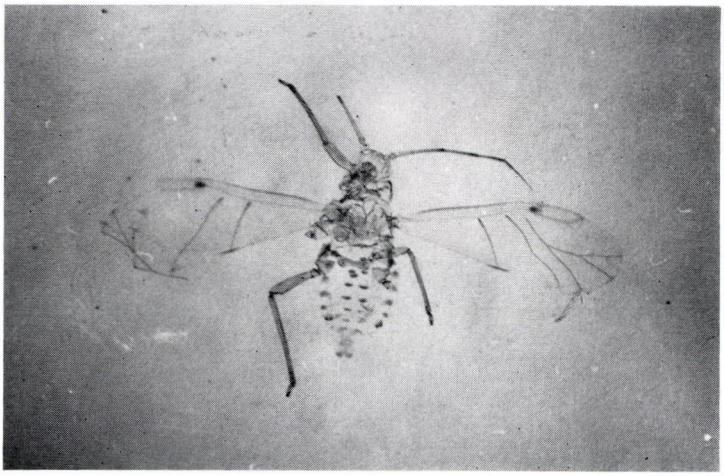

Fig. 15. *Myzocallis boerneri*

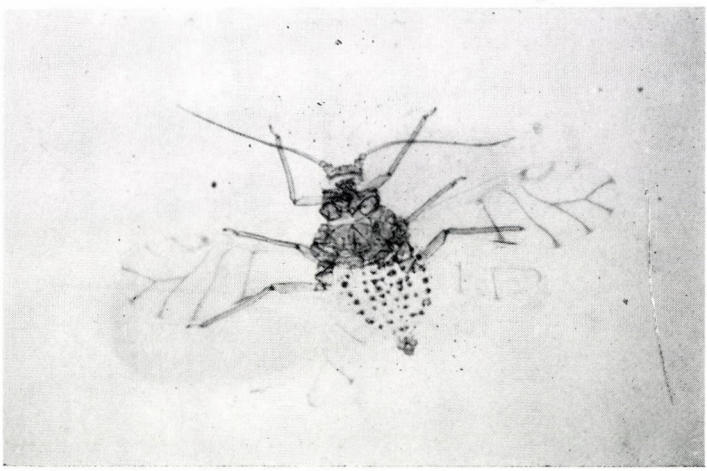

Fig. 16. *Therioaphis trifolii*

Oval rhinaria on III. Knobbed cauda with bilobed anal plate. Two rows of large dark spots down abdomen (Fig. 14).

31 (30) Head and thorax not laterally dark, no pale stripe down middle. Antennae 6 segmented, processus terminalis as long as base of VI. Round rhinaria on III. Knobbed cauda with bilobed anal plate. Two rows of dark oval spots down abdomen (Fig. 15).

32 (29) Dark spots down middle of abdomen.
Antennae 6 segmented, processus terminalis as long as base of VI. Knobbed cauda with bilobed anal plate.

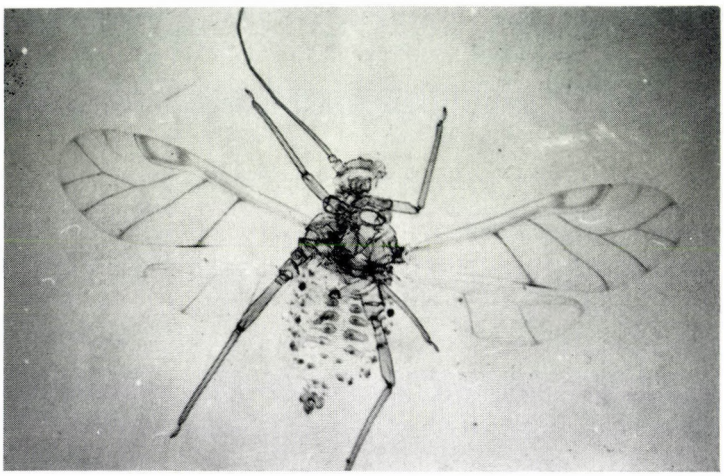

Fig. 17. *Therioaphis riehmi*

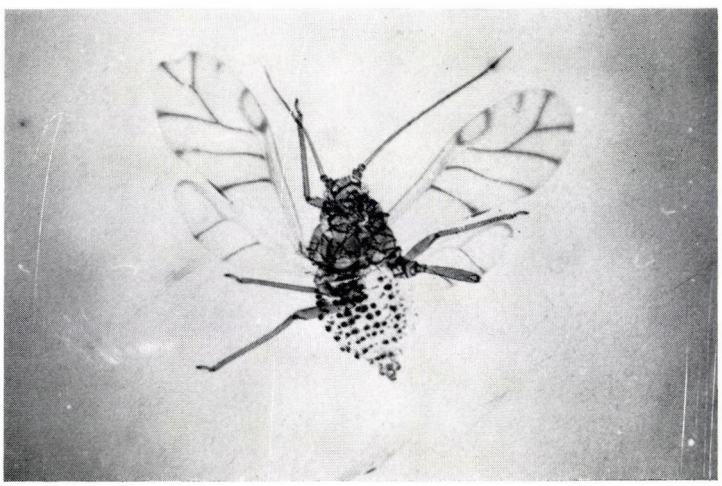

Fig. 18. *Therioaphis brachytricha*

33 (34) Body hairs capitate, long, arising from pigmented tubercles. Hair-bearing tubercles pigmented forming 4–6 rows alongside abdomen (Fig. 16).

34 (33) Short body hairs arising from pigmented tubercles.

35 (36) Big oval pigmented spots forming 2 rows alongside abdomen (Fig. 17).

36 (35) Dark hair-bearing pigmented tubercles forming 6–8 rows alongside abdomen.

37 (38) Size of hair-bearing pigmented tubercles different.
Fused spots on the middle of abdomen form big oval spots (Fig. 18).

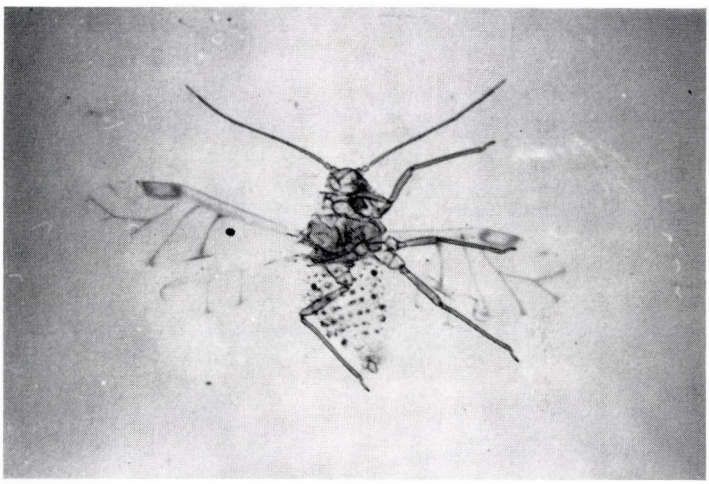

Fig. 19. *Therioaphis subalba*

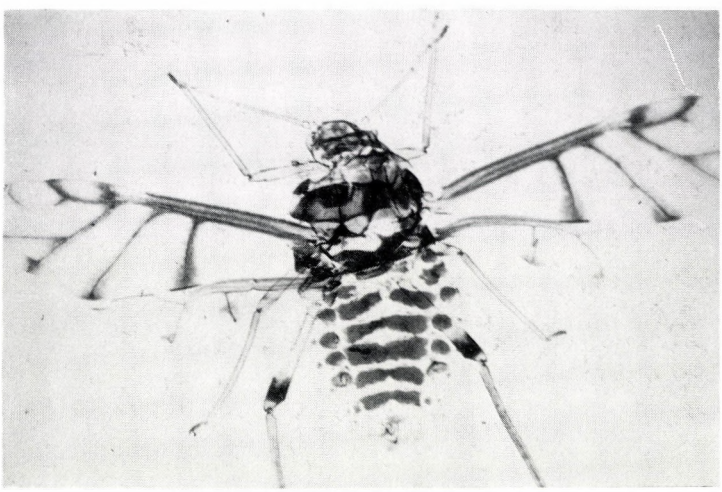

Fig. 20. *Callaphis juglandis*

38 (37) Uniform hair bearing pigmented tubercles.
Hair bearing pigmented tubercles mostly round (Fig. 19).
39 (28) Stripes or solid pach on abdomen.
40 (47) Dark transverse bars on each abdominal segment.
41 (44) Dark transverse bars not fused.
42 (43) Wings with brown-bordered veins.
Antennae short 6 segmented. Processus terminalis shorter than base of VI. Oval rhinaria on III. Dark marginals not fused with transverse

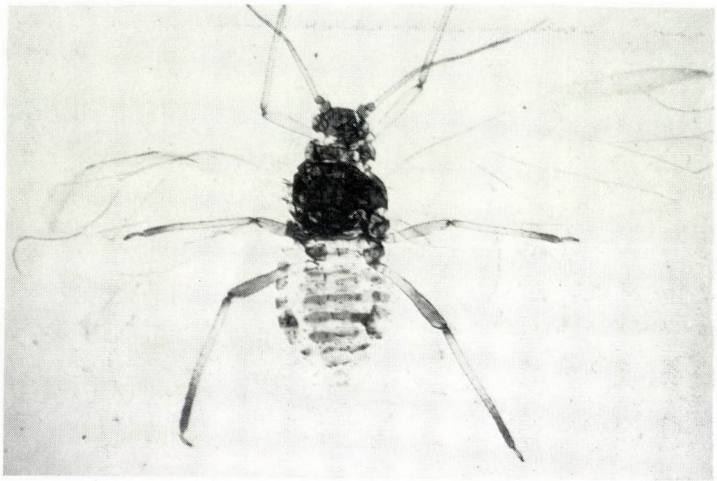

Fig. 21. *Periphyllus testudinaceaus*

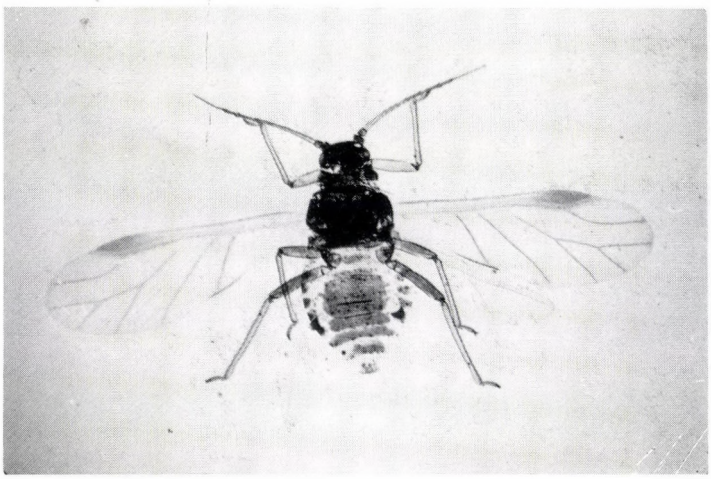

Fig. 22. *Chaitophorus saljaponicus niger*

bars. Dark spot on distal part of hind femur. Knobbed cauda, with bilobed anal plate (Fig. 20).

43 (42) Wing veins not brown-bordered, wing transparent.
Antennae 6 segmented, processus terminalis 3 times as long as base of VI. Round rhinaria on III. and IV. Broadly rounded cauda. 7 transverse bars on abdomen (Fig. 21).

44 (41) Dark transverse bars fused on some places.
Antennae 6 segmented, processus terminalis 3 times as long as base of VI. No sensoria on hind tibia. Transverse bars of abdomen very

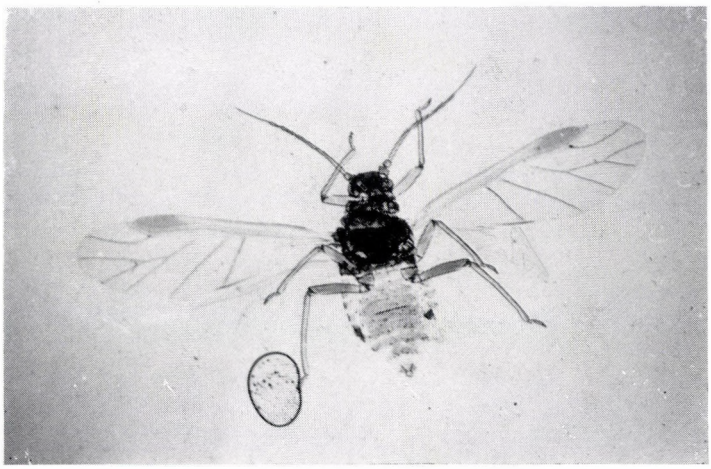

Fig. 23. *Chaitophorus leucomelas*

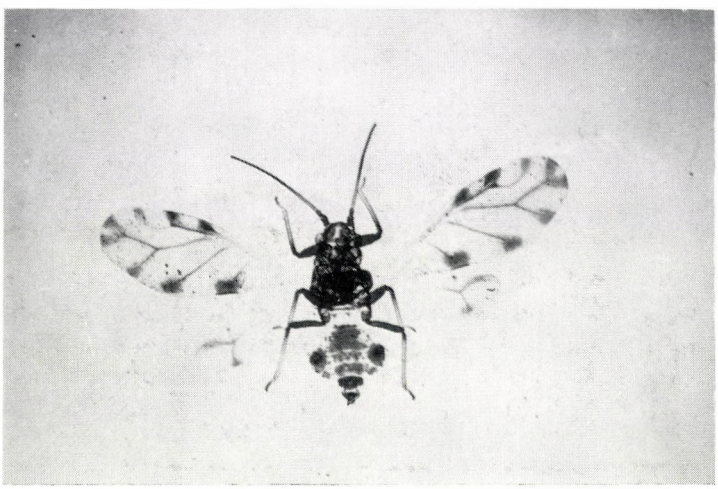

Fig. 24. *Iziphia bufo*

wide, 3–6 mostly fused. Transverse bars and dark marginals never fused. Knobbed cauda.

45 (46) 7–10 round rhinaria on III not in row (Fig. 22).

46 (45) 8–14 round rhinaria on III in row (Fig. 23).

47 (40) Dark transverse bars on 4, 5, 6. abdominal segments or dark solid patch on hind 2/3 of abdomen.

48 (49) Dark transverse bars on abdominal segments 4, 5, 6. not fused with spots surrounding cornicles.

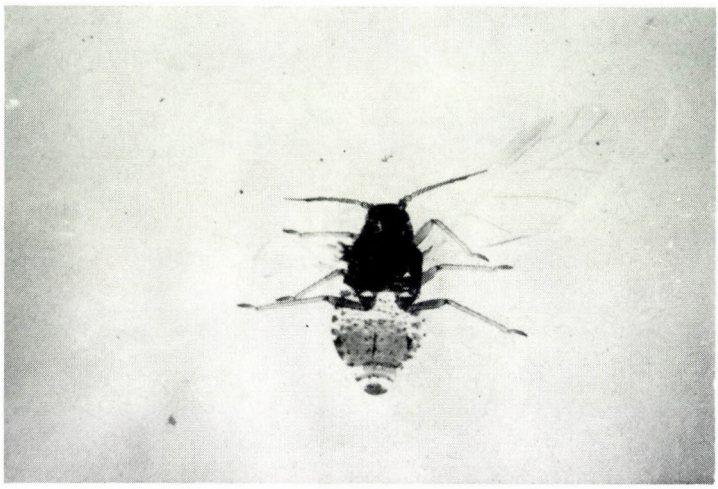

Fig. 25. *Sipha maydis*

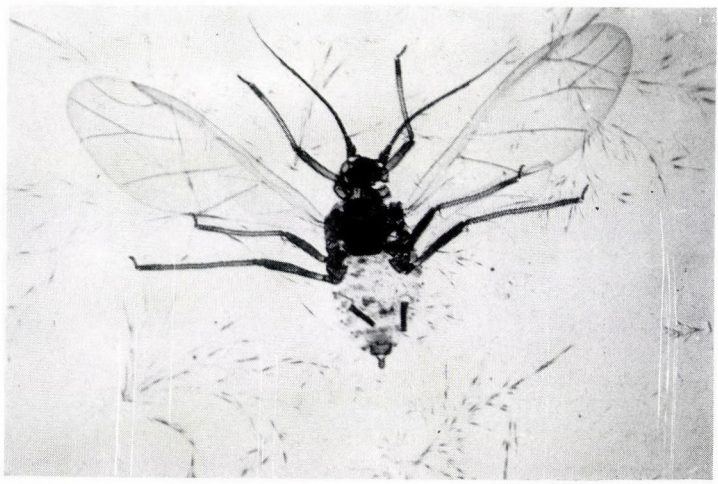

Fig. 26. *Rhopalosiphum rufiabdominale*

End of wing veins darkly bordered. The cubitus of hind wing, at right angles to radius. Antennae 6 segmented. Processus terminalis longer than base of VI. Knobbed cauda with bilobed anal plate (Fig. 24).

49 (48) Dark solid patch on hind 2/3 of abdomen.
Antennae 5 segmented. Processus terminalis longer than base of V. 4 dark marginals in front of solid patch. Cauda broadly rounded (Fig. 25).

50 (19) Cornicles cylindrical or tapering or swollen.

51 (88) Cornicles cylindrical short.

52 (59) Cornicles cylindrical, constricted at apex.
Antennae 6 segmented. Processus terminalis at least 2 times as long as base of VI. Round, rhinaria on III and IV. Second branch of media near to edge of wing.
Dark marginals on abdomen. Head, thorax legs, antennae, cornicles, cauda dark.

53 (54) Long abdominal hairs, some of them as long as diameter of coxae. Flagellum 5 times as long as base of VI. (Fig. 26).

54 (53) Short abdominal hairs, shorter or hardly longer than diameter of III.

55 (56) Processus terminalis 2 times as long as base of VI. Round rhinaria III, IV, V. Cornicles short, not longer than cauda (Fig. 27).

56 (55) Processus terminalis 4–5 times as long as base of VI. Round rhinaria on III, IV.

57 (58) Round-bodied aphids. Slender antenna. Processus terminalis 5 times as long as base of VI. Fourth marginal spot forms arch above cornicles. Irregular separation between antennal segments.

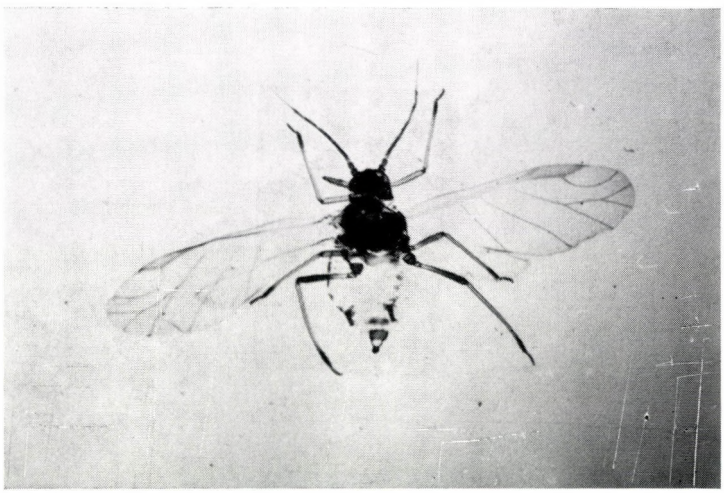

Fig. 27. *Rhopalosiphum maidis*

58 (57) Long-bodied aphids. Slender antenna. Processus terminalis 4 times, as long as base of VI. Fourth marginal spot behind cornicles (Fig. 29).

59 (52) Cylindrical cornicles not constricted at apex, or cornicles other than cylindrical.

60 (61) Cornicles cylindrical not longer than wide.
Head, thorax, legs, antennae, cornicle dark. Thick third antennal segment with more than 20 round rhinaria. Round rhinaria on IV. Processus terminalis at least 3 times as long as base of VI. Cauda long triangular (Fig. 30).

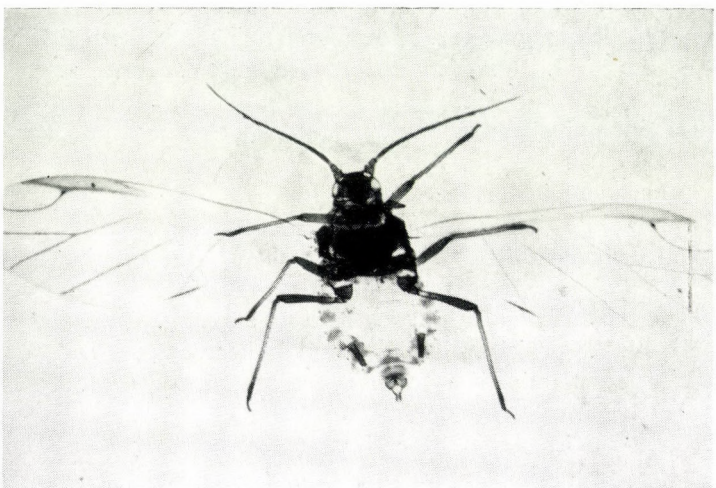

Fig. 28. *Rhopalosiphum padi*

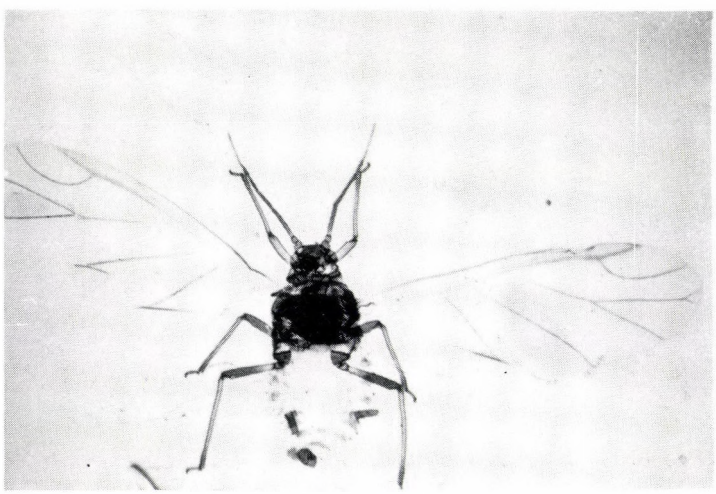

Fig. 29. *Rhopalosiphum insertum*

61 (60) Cylindrical cornicles longer than wide, tapering at apex.
62 (63) Processus terminalis as long as base of VI.
IV, V, VI. about same length. Cauda longer than wide, as long as tapering cornicles. Dark marginals and spot-like broken stripes on abdomen (Fig. 31).
63 (62) Processus terminalis 2–3 times as long as base of VI.
64 (79) Processus terminalis 2–2.5 times as long as base of VI.
65 (66) Light marginals. Dark but not black head, thorax, cornicles, knees.

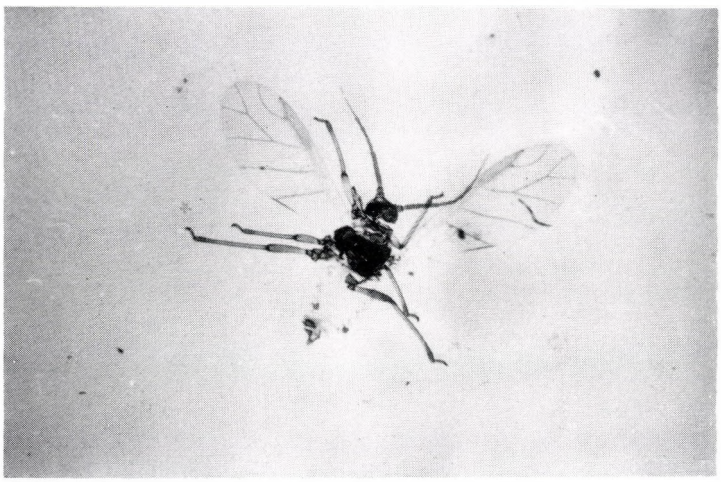

Fig. 30. *Semiaphis dauci*

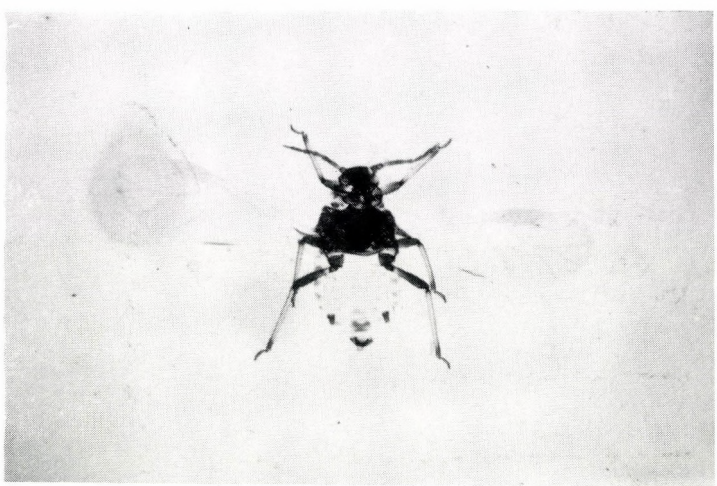

Fig. 31. *Protaphis* sp.

Round rhinaria on III, IV, V. Cornicles slightly curved away from body, short but longer than cauda (Fig. 32).

66 (65) Dark marginals.

67 (70) Dark transverse bars on abdomen.

68 (69) Transverse bars not fused.

Head, thorax, cornicles, cauda distal part of femur dark. First leg, antennae and tibia pale, distal part of tibia dark. More than 13 hairs on cauda. Cornicles longer than cauda (Fig. 33).

Fig. 32. *Aphis nasturtii*

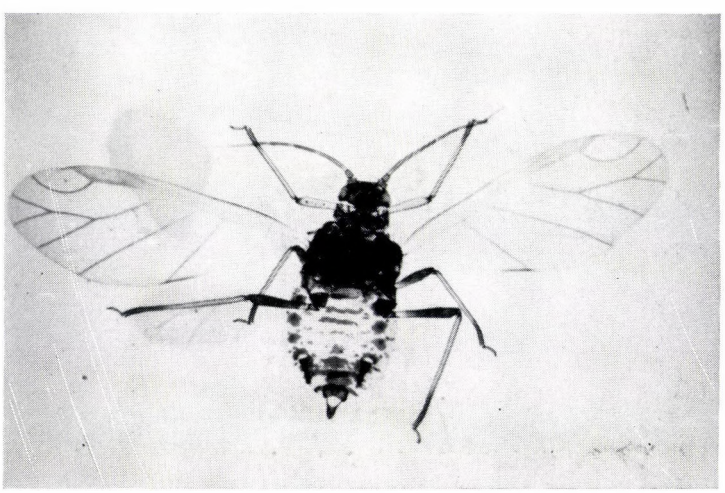

Fig. 33. *Aphis fabae*

69 (68) Transverse bars fused and form oval pattern at distal part of abdomen. Dark head, thorax, cornicles, cauda and knees. Cornicles longer than cauda. Less than 8 hairs on cauda (Fig. 34).

70 (67) No transverse bars on abdomen. Head, thorax cornicles and knees dark.

71 (76) Cornicles as long or longer than III.

72 (73) Cornicles thick, diameter at base thicker than hind femur. Cornicles 3 times as long as cauda. Legs evenly dark. 9–12 hairs on cauda (Fig. 35).

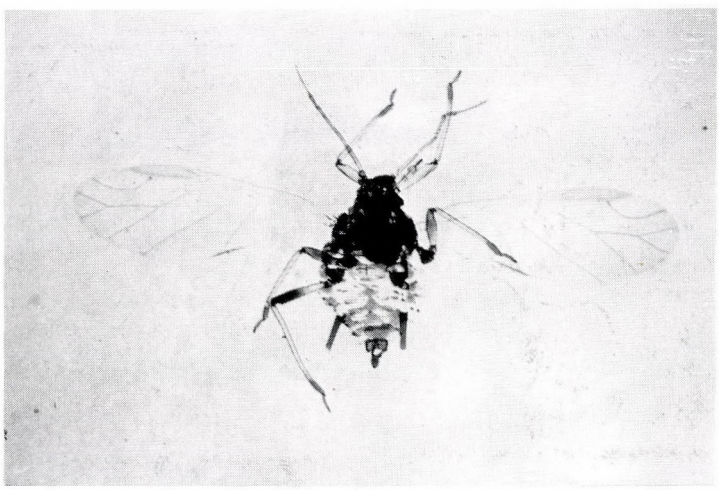

Fig. 34. *Aphis craccivora*

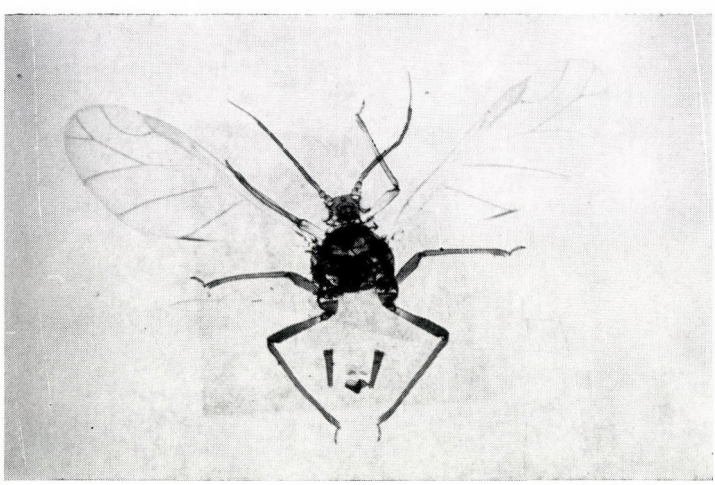

Fig. 35. *Aphis sambuci*

73 (72) Thin cornicles, thinner than diameter of hind tibia.
74 (75) Cornicles 2 times as long as cauda. Uniform marginal spots. 4–6 rhinaria on III (Fig. 36).
75 (74) Cornicles 1.5 times as long as cauda.
Secondary rhinaria on III, IV. More than 11 hairs on cauda (Fig. 37).
76 (71) Cornicles shorter than III.
77 (78) Thick cornicles two times as long as cauda.
5–8 rhinaria on III. in row. 5 hairs on cauda (Fig. 38).

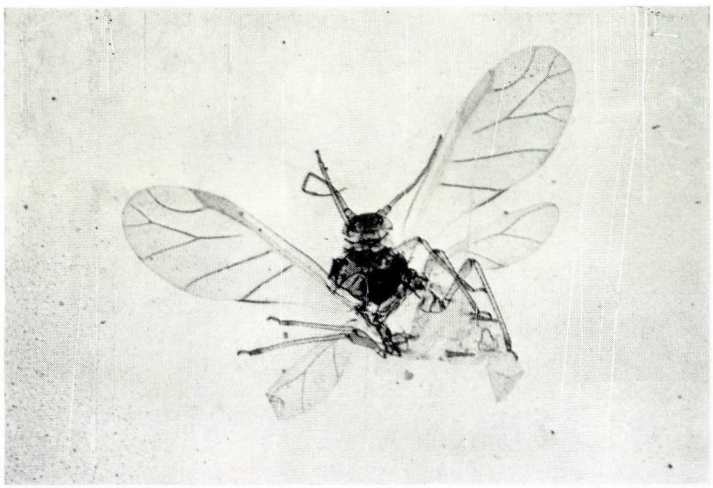

Fig. 36. *Aphis idaei*

Fig. 37. *Aphis pomi*

78 (77) Cornicles not thick, 1.5 times as long as cauda.
5–13 rhinaria on III. Length of III, IV and V about the same. 7–12 hairs on cauda. Processus terminalis 2 times as long as base of VI (Fig. 39).

79 (64) Processus terminalis 3 times as long as base of VI.
Dark head, thorax, cornicles and knees.

80 (81) Dark transverse bars on abdomen.
Cornicles shorter than cauda. 7 transverse bars on abdomen, round marginals III, IV about the same length with round rhinaria (Fig. 40).

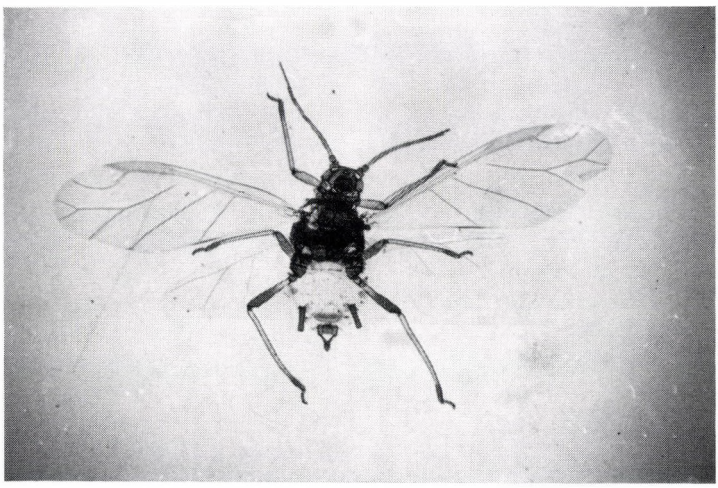

Fig. 38. *Aphis cloris*

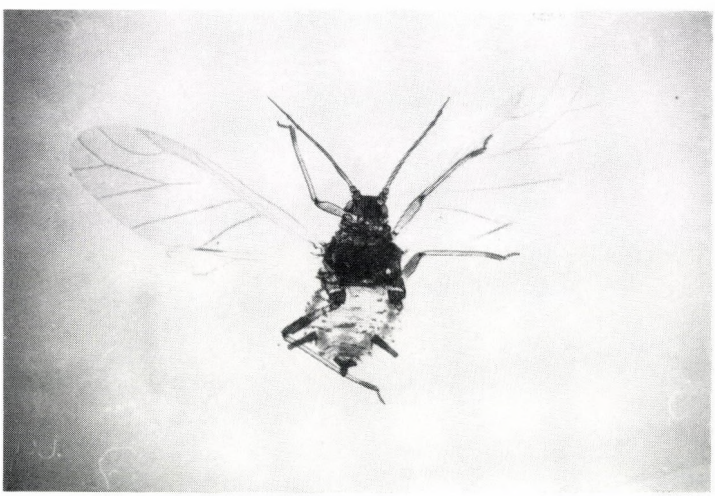

Fig. 39. *Aphis umbrella*

81 (80) No transverse bars on abdomen, marginals only.
82 (83) Cornicles longer than third antennal segment.
Big dark marginal tubercles. 16 rhinaria on III, IV, V same length (Fig. 41).
83 (82) Cornicles shorter than third antennal segment.
84 (85) III, IV, V about the same length. Cornicles 1.5 times as long as cauda. 4–8 rhinaria on III (Fig. 42).
85 (84) III far longer than IV, V.

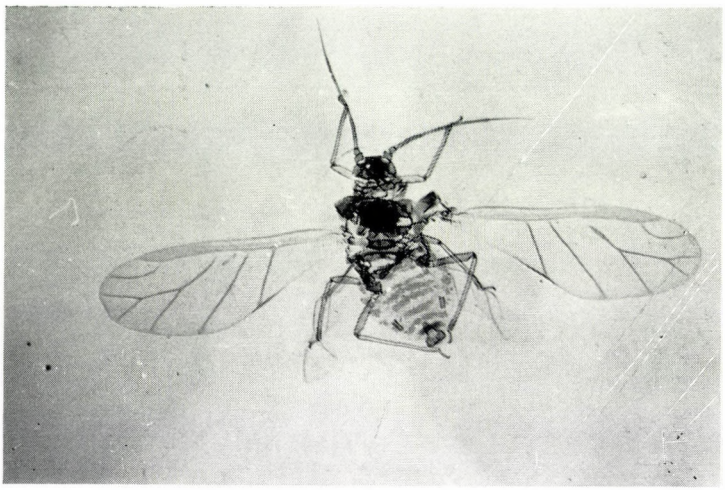

Fig. 40. *Longiunguis pyraria*

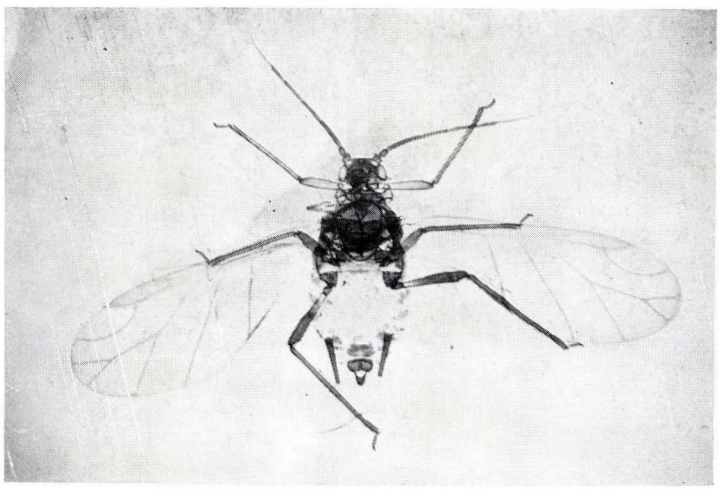

Fig. 41. *Aphis sambuci*

86 (87) IV, V. about the same length, rhinaria usually only on III. Cauda shorter than cornicles. Less than 10 hairs on cauda. Short hairs on femur (Fig. 43).

87 (86) V. shorter than IV.
Rhinaria on III, IV, V. Cornicles wider than diameter of hind tibia. Marginals behind cornicles fused with transverse bars. Hairs on abdomen (Fig. 44).

88 (51) Cornicles swollen, long cylindrical or short tapering.

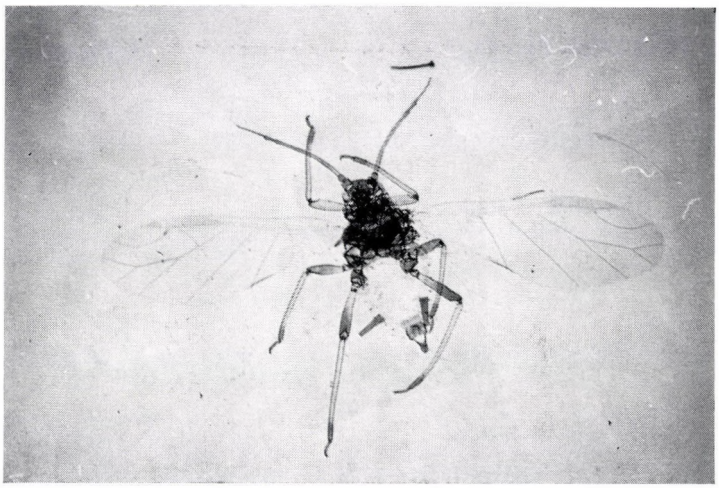

Fig. 42. *Aphis ruborum*

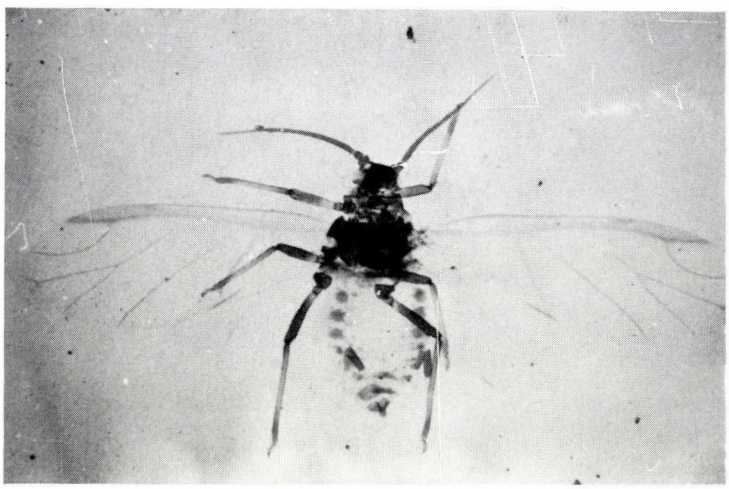

Fig. 43. *Aphis gossypii*

89 (120) Swollen cornicles.
90 (97) Cornicles short, barrel shaped or longer.
91 (96) Cornicles as long as base of VI.
92 (92) Dark transverse bars and dark marginals on abdomen
Dark head, thorax, antennae and legs. Barrel shaped cornicles as long as broadly triangular-shaped cauda. III. thick with more than 20 rhinaria (Fig. 45).
93 (92) Dark transverse bars only behind cornicles.

Fig. 44. *Aphis grossulariae*

Fig. 45. *Brevicoryne brassicae*

94 (95) Dark marginals, two transverse bars only behind cornicles. Digitate cauda 1.5 times as long as cornicles (Fig. 46).

95 (94) No dark marginals. No transverse bars behind cornicles. Only fragments. Cauda dark, two times as long as cornicles. Cornicles thinnest at base and widest at distal third (Fig. 47).

96 (91) Cornicles longer than base of VI. The end of cornicles barrel shaped. Pale abdomen, dark marginals, two transverse bars behind cornicles. More than 25 rhinaria on III. (Fig. 48).

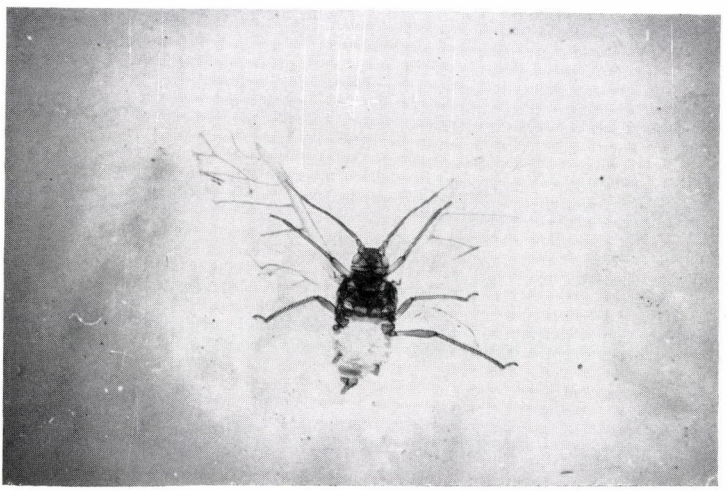

Fig. 46. *Hayhurstia atriplicis*

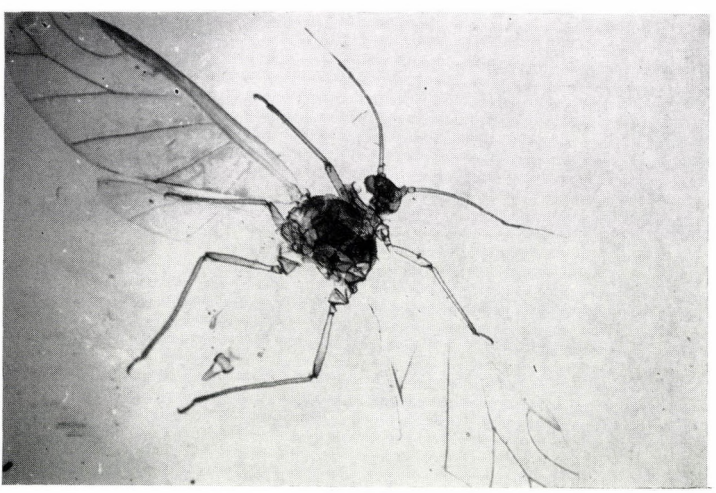

Fig. 47. *Hyalopterus pruni*

97 (90) Cornicles swollen.
98 (113) Small and medium-sized aphids.
99 (106) Antennal tubercles present.
100 (105) Patch on the abdomen.
101 (104) Wavy edge of dorsal abdominal patch.
102 (103) Transverse bars forming patch completely fused, not separated. The patch widest at middle. Few round rhinaria on III and IV. Head thorax, legs, cornicles, cauda dark. Cornicles two times as long as cauda (Fig. 49).

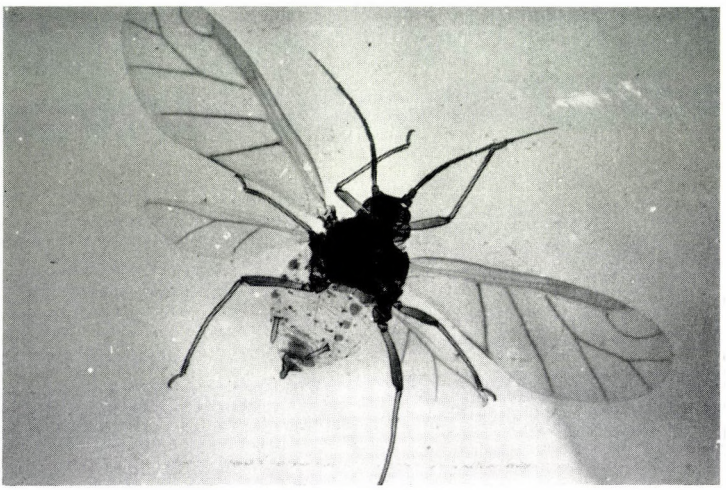

Fig. 48. *Lipaphis erysimi*

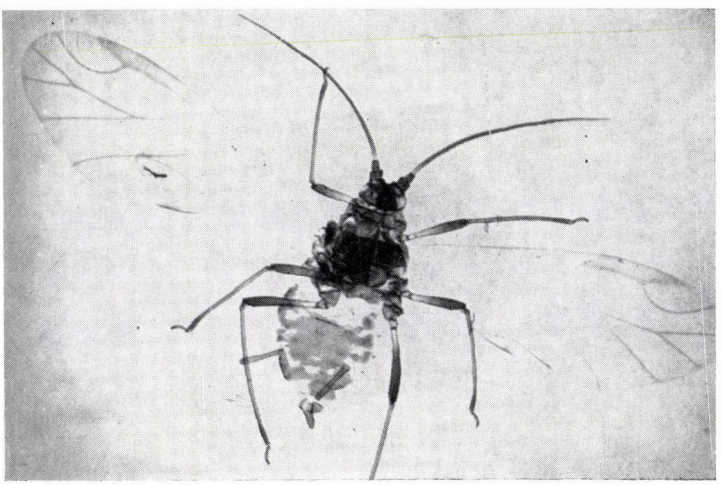

Fig. 49. *Myzus persicae*

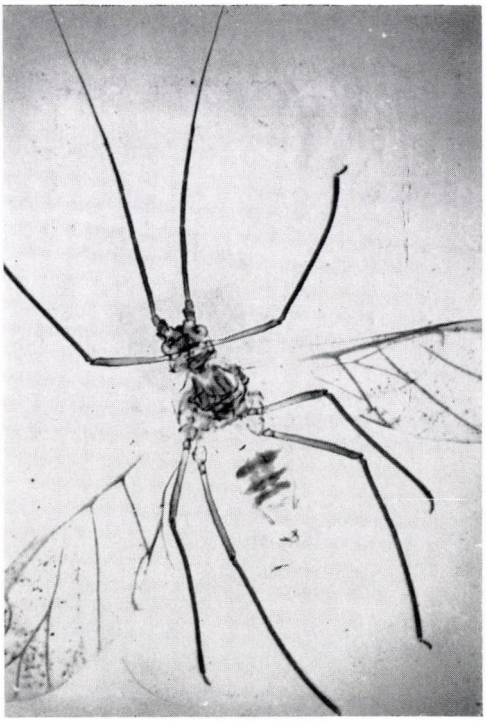

Fig. 50. *Cryptomyzus galeopsidis*

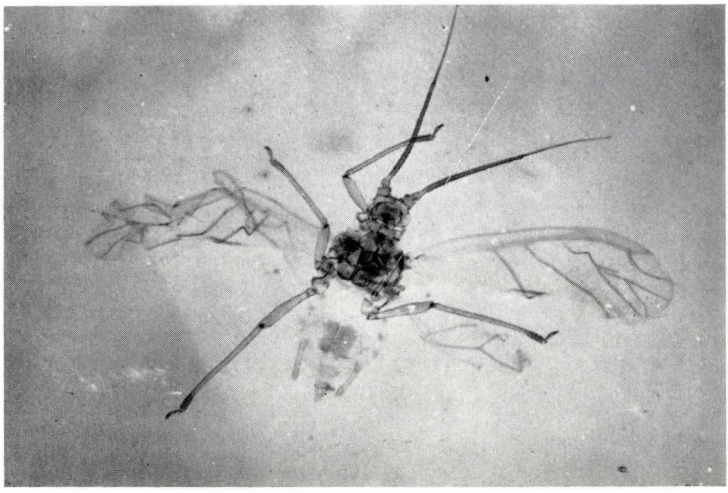

Fig. 51. *Capitophorus hippophaes*

103 (102) Transverse bars forming patch separated. The patch widest at beginning. Many round rhinaria on III, IV, V. Cornicles 2.5 times as long as base of VI (Fig. 50).

104 (101) Straight edge of dorsal abdominal patch. Rectangular solid black abdominal patch. Triangular-shaped cauda (Fig. 51).

105 (100) Transverse spots or broken transverse bars on the dorsal abdomen. Head, thorax, legs, cornicles, antennae dark. Long triangular-shaped cauda. Round rhinaria on III, IV. Cornicles 1.2 times as long as cauda (Fig. 52).

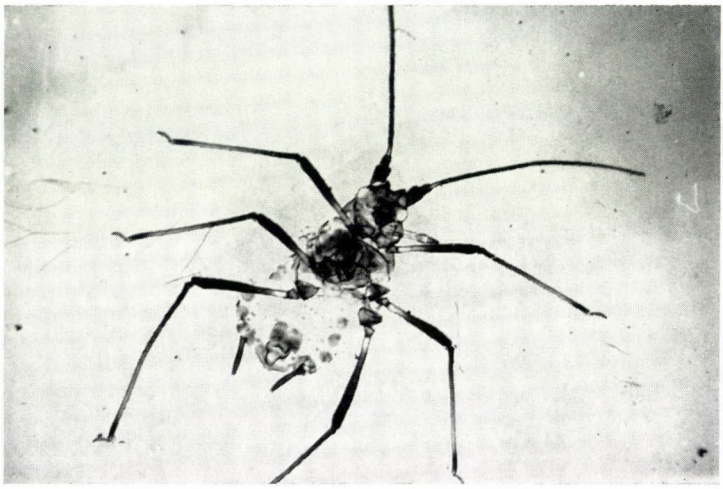

Fig. 52. *Megourella tribulis*

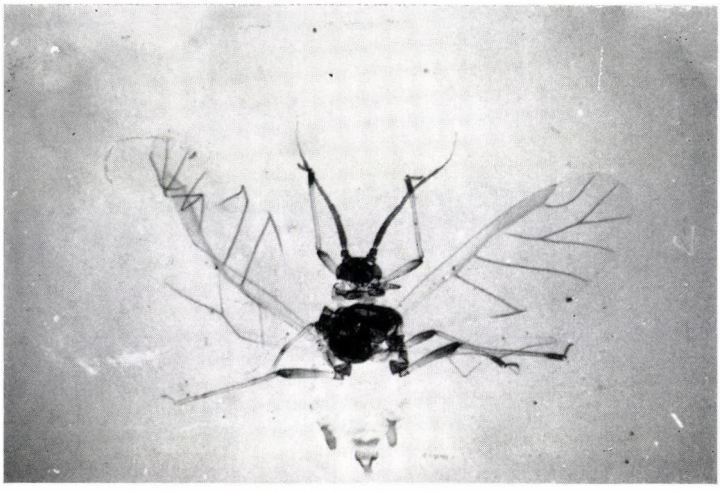

Fig. 53. *Hyadaphis foeniculi*

106 (99) No antennal tubercles.

107 (108) No supracauda.
Head, thorax, knees antennae, cornicles dark. Smudged wing veins. Swollen cornicles thinnest at base 1/4. Cornicles as long as cauda, darker than cauda. Small light marginals on abdomen, light transverse bar behind cornicles (Fig. 53).

108 (107) Supracauda present.
Head, thorax, legs, antennae, cornicles, cauda dark.

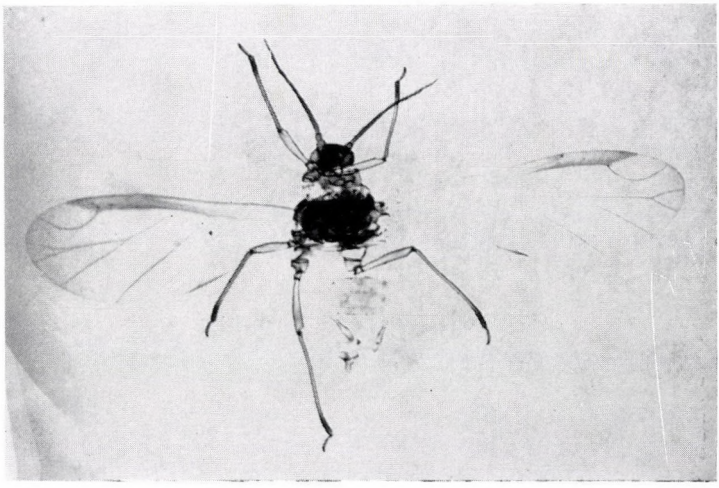

Fig. 54. *Cavariella aegopodi*

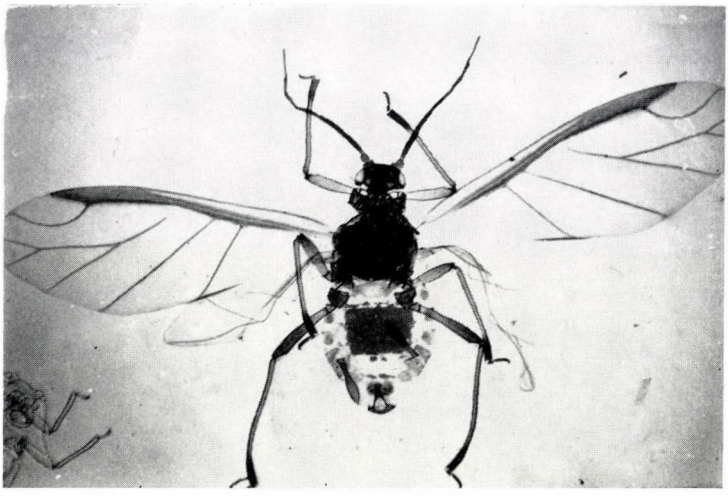

Fig. 55. *Cavariella konoi*

109 (110) Processus terminalis as long as base of VI.
Fused marginals and transverse bars form more or less solid dark patch. Cornicles two times as long as cauda (Fig. 54).
110 (109) Processus terminalis two times as long as base of VI.
111 (112) Straight sharp edge of the rectangular dark solid patch (Fig. 55).
112 (111) Wavy indistinct edge of the rectangular solid dark patch (Fig. 56).
113 (98) Large aphids with antennal tubercles.

Fig. 56. *Cavariella archangelicae*

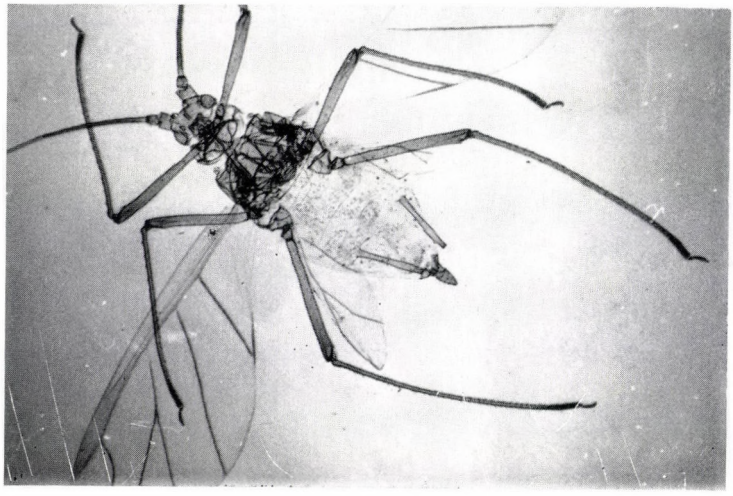

Fig. 57. *Amphorophora rubi*

114 (117) No pigmentation on abdomen.
Head, thorax, legs, cornicles lightly pigmented. Digitate cauda.
115 (116) Long hairs on abdomen in row.
7 pairs of hairs on cauda. More than 50 rhinaria on III. No rhinaria on IV. Cornicles as long as IV (Fig. 57).
116 (115) Scattered few long hairs on abdomen.
Five pairs of hairs on cauda (Fig. 58).

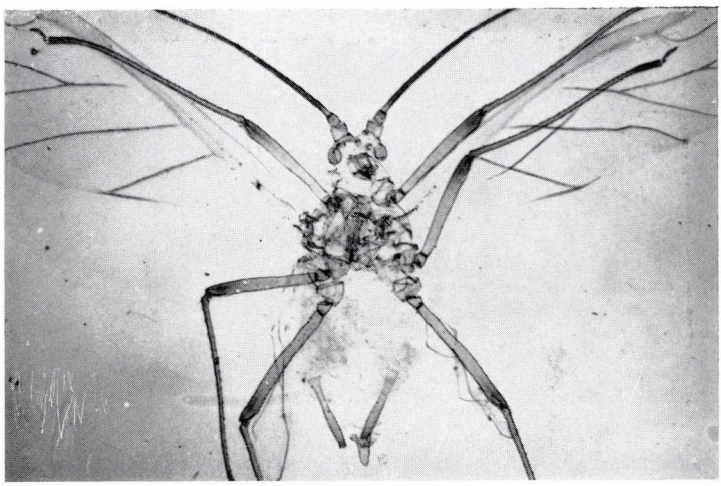

Fig. 58. *Amphorophora idaei*

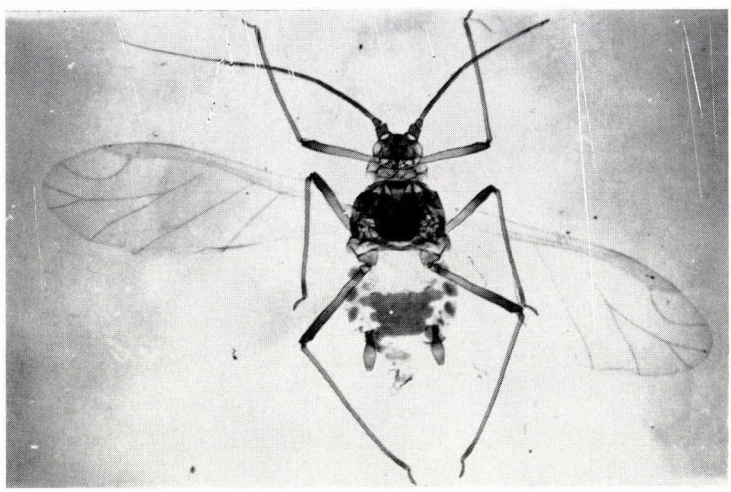

Fig. 59. *Hyperomyzus pallidus*

117 (114) Pigmentation on abdomen.
Head thorax, legs dark. Dark marginals on abdomen.
118 (119) Solid black dorsal abdominal patch with wavy edge.
Black cornicles (Fig. 59).
119 (118) Rather broken central dark patch with wavy edge on the dorsal abdomen. Slightly pigmented cornicles (Fig. 60).
120 (89) Cornicles not swollen, long cylindrical or short, tapering.
121 (170) Cornicles long, cylindrical.

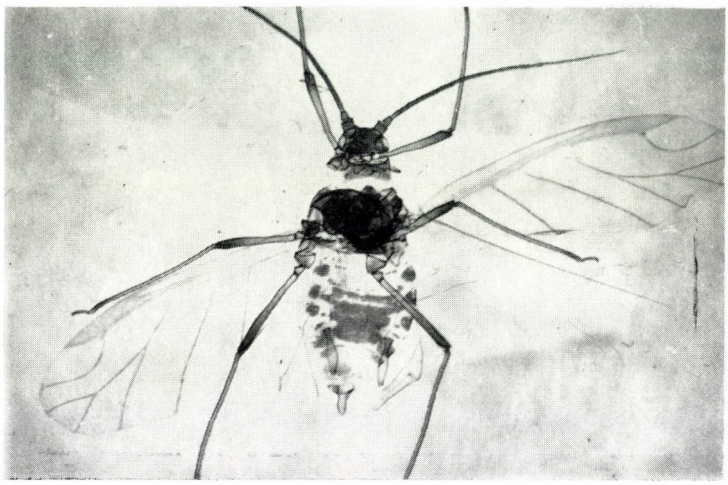

Fig. 60. *Hyperomyzus lactucae*

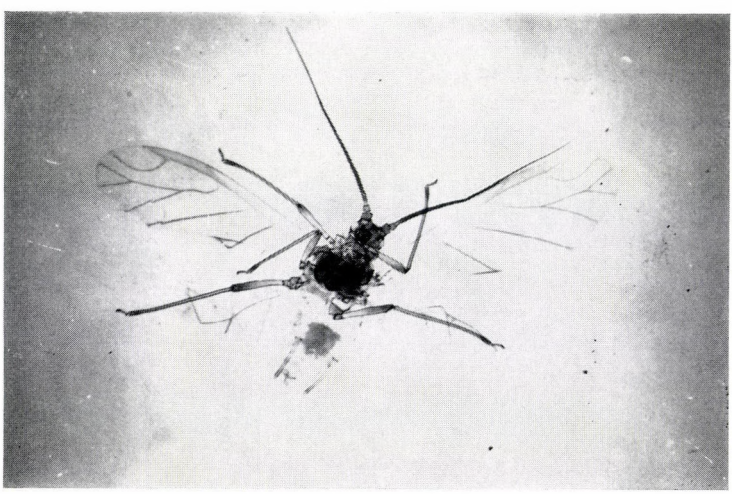

Fig. 61. *Capitophorus eleagni*

122 (145) Small to medium-sized aphids.
123 (144) Antennal tubercles present.
124 (139) Dark patch on the dorsal abdomen.
125 (130) Dorsal abdominal patch straight-sided.
126 (129) Solid dorsal abdominal patch.
127 (128) Distal end of cornicles dark.
Rectangular dorsal abdominal patch. Cornicles as long as III. Triangular shaped cauda slightly pigmented (Fig. 61).

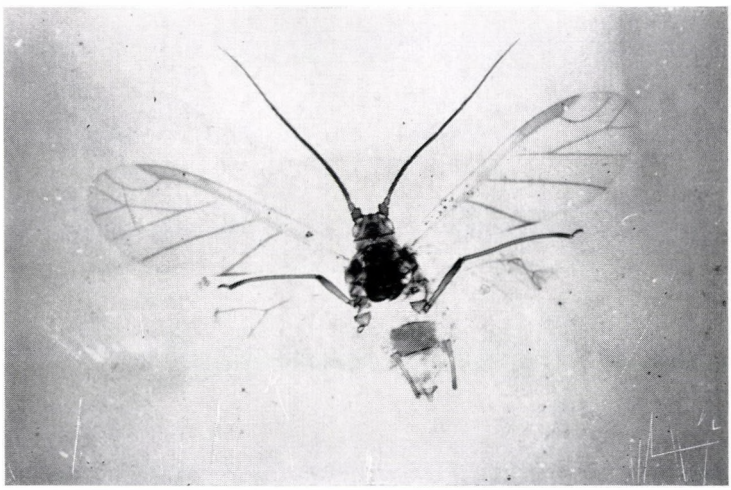

Fig. 62. *Capitophorus carduinus*

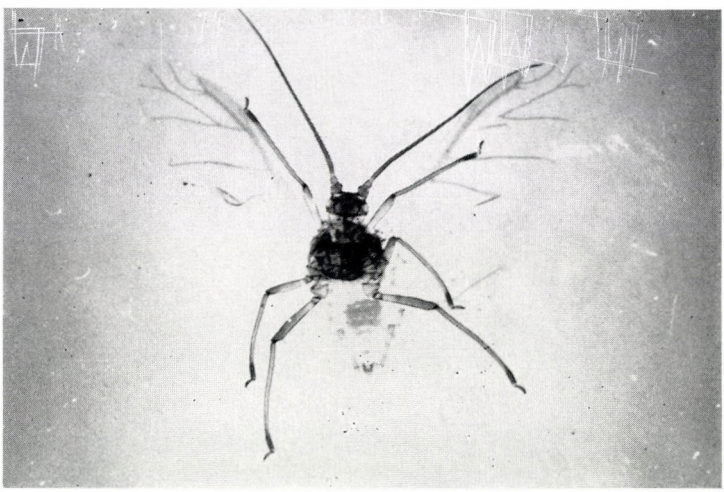

Fig. 63. *Capitophorus similis*

128 (127) Evenly pigmented dark cornicles.
Rectangular dorsal abdominal patch wider than long (Fig. 62).
129 (126) Dorsal abdominal patch divided into transverse bars. Light evenly pigmented cornicles (Fig. 63).
130 (125) Dorsal abdominal patch not straight-sided.
131 (136) Solid dorsal abdominal patch.
132 (135) Dorsal abdominal patch widest at middle.
133 (134) Transverse bar in front of the patch separated from the patch.
Thick tapering cornicles 1.5 times as long as cauda (Fig. 64).

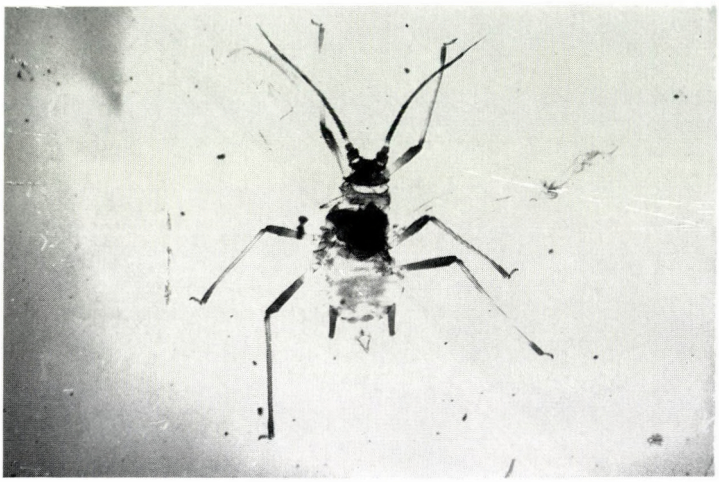

Fig. 64. *Myzus varians*

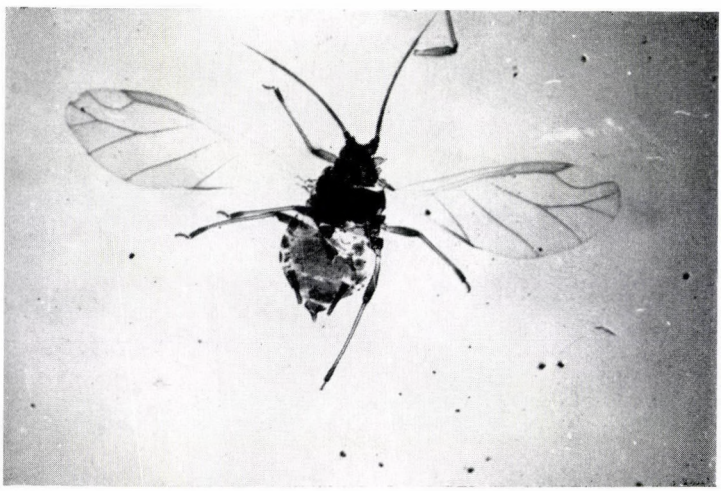

Fig. 65. *Myzus cerasi*

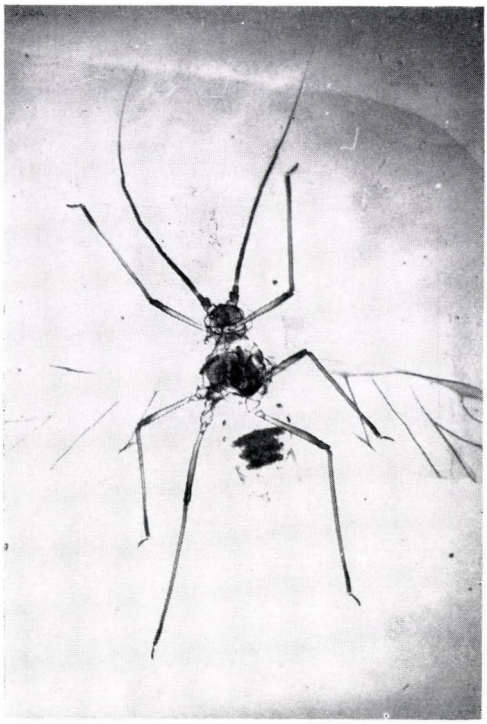

Fig. 66. *Cryptomyzus ribis*

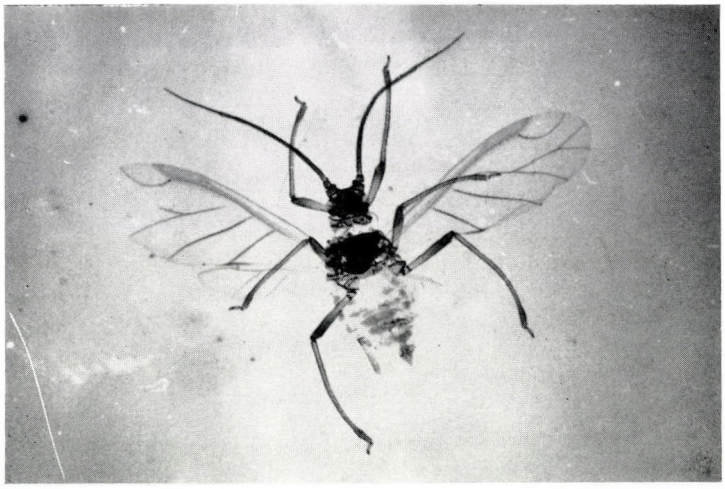

Fig. 67. *Myzus lythri*

134 (133) Transverse bar not separated from the patch.
Thin tapering cornicles 2 times as loug as cauda (Fig. 65).

135 (132) Dorsal abdominal patch widest at beginning.
Black antennae, many round rhinaria on III, IV, V, thick antennal segments. Processus terminalis 10 times as long as base of VI. Dark head, thorax, knees, ankles and wing veins (Fig. 66).

136 (131) Patch not solid, formed by transverse bars.

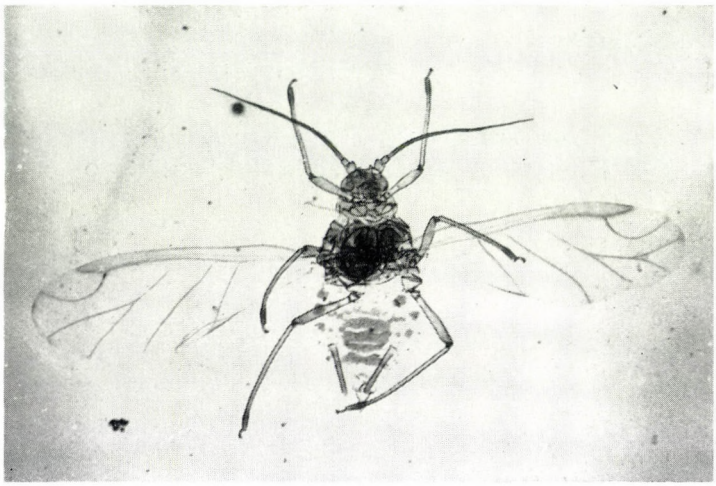

Fig. 68. *Phorodon cannabis*

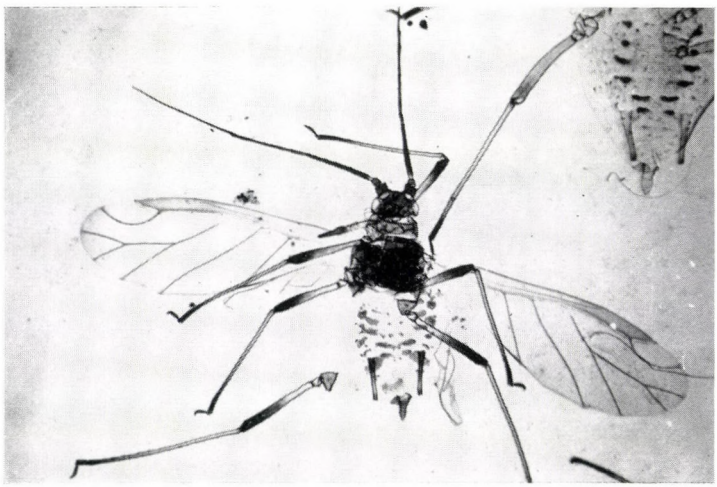

Fig. 69. *Nasonovia ribisnigri*

137 (138) First transverse bar broken at middle of the abdomen.
Cornicles shorter than III, thicker than base of hind femur. Dark head, thorax, legs, cornicles, cauda (Fig. 67).

138 (137) First transverse bar not broken.
Cornicles longer than III, thinner than base of hind femur. Dark head, thorax, legs, cornicles, cauda (Fig. 68).

139 (124) No dark patch on the dorsal abdomen.

140 (141) Dark marginals and dark broken transverse bars on dorsal abdomen. Dark round and oval spots, transverse bars in front of and behind

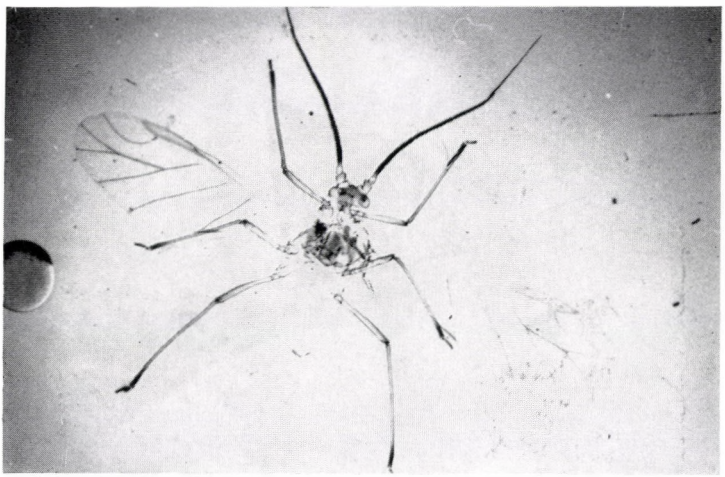

Fig. 70. *Ovatus insitus*

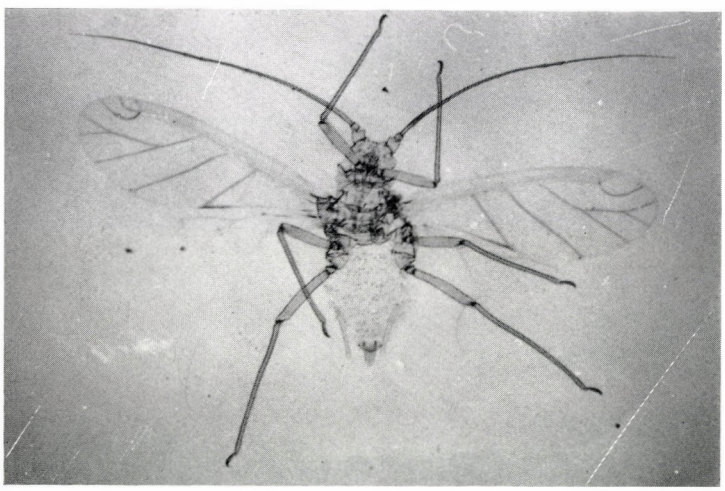

Fig 71. *Pleotrichoporus glandulosus*

cornicles form X-shaped pigmentation. III, thick with less than 40 round rhinaria. Digitate cauda (Fig. 69).

141 (140) No pigmentation or light pigmentation on abdomen.

142 (143) Dark head, thorax, femur, ankles. Black antennae, many round rhinaria on III, IV, V, III, thick. Cauda tongue-like.
Processus terminalis 6 times as long as base of VI. Pale abdomen (Fig. 70).

143 (142) Tarsus dark only. Pale head, thorax, legs, antennae. III. thin, with 6–12 rhinaria. No rhinaria on IV and V.

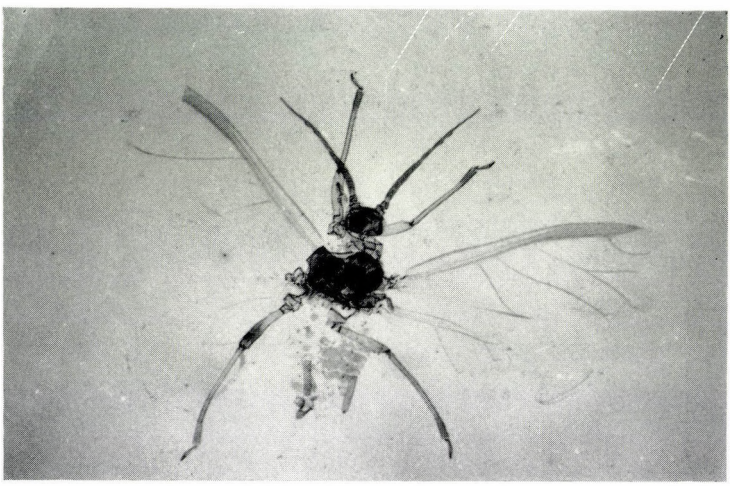

Fig. 72. *Cavariella theobaldi*

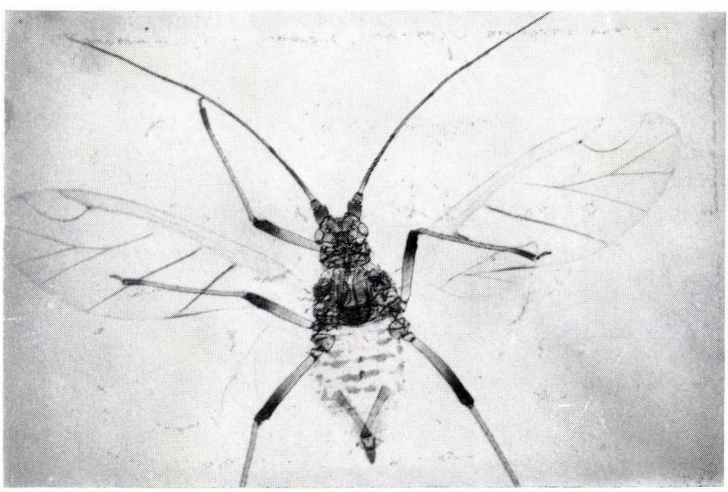

Fig. 73. *Aulacorthum solani*

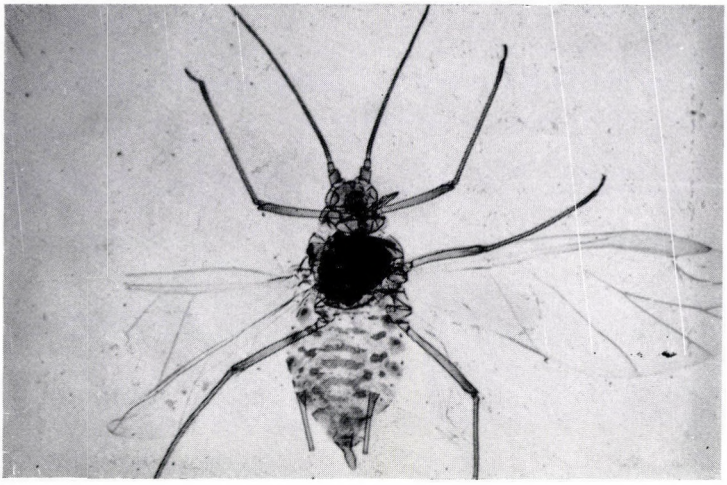

Fig. 74. *Metopolophium festucae*

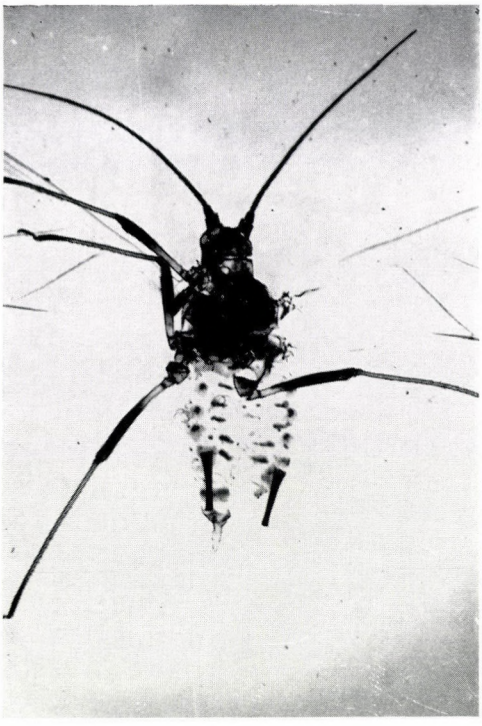

Fig. 75. *Sitobion avenae*

Light marginals and light oval pieces of broken transverse bars. Hairs in row on abdomen. Digitate cauda (Fig. 71).

144 (123) No antennal tubercles.
Supracauda present. Dark marginals and almost fused transverse bars on dorsal abdomen. Dark cornicles two times as long as cauda. Dark antennae, thick III, with many rhinaria (Fig. 72).

145 (122) Large aphids. With antennal tubercles.

146 (149) Unbroken transverse bars and dark marginals on abdomen.

147 (148) 3 unbroken transverse bars on dorsal abdomen.
Cornicles darkened to distal end, longer than the distance between their base. Large flange at apices, no polygonal reticulation (Fig. 73).

148 (147) 5 unbroken transverse bars on dorsal abdomen.
Cornicles with flange, as long as the distance between their base. No polygonal reticulation. Dark knees and ankles (Fig. 74).

149 (146) Broken transverse bars and marginals on abdomen.

150 (155) Broken transverse bars, small dark sclerites and dark marginals on abdomen.

151 (152) Broken transverse bars and dark marginals on abdomen.
Dark head, thorax, cornicles, antennae legs. Processus terminalis as

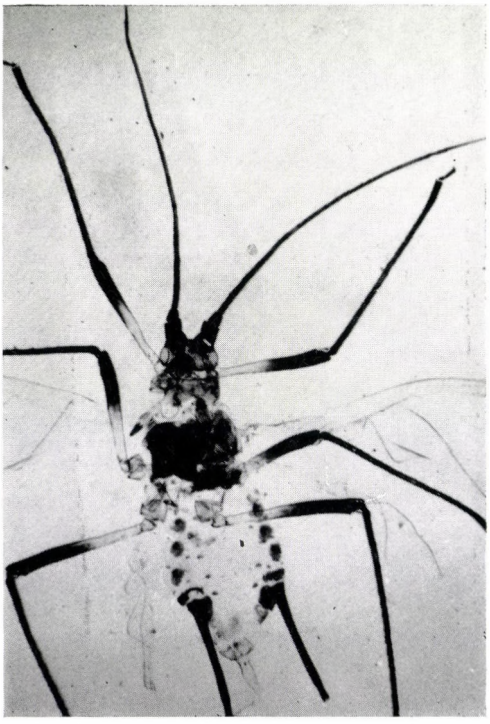

Fig. 76. *Macrosiphum rosae*

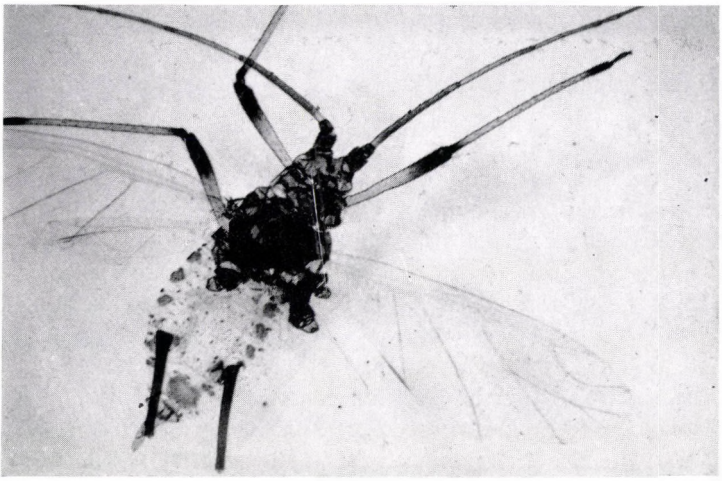

Fig. 77. *Dactynotus* sp.

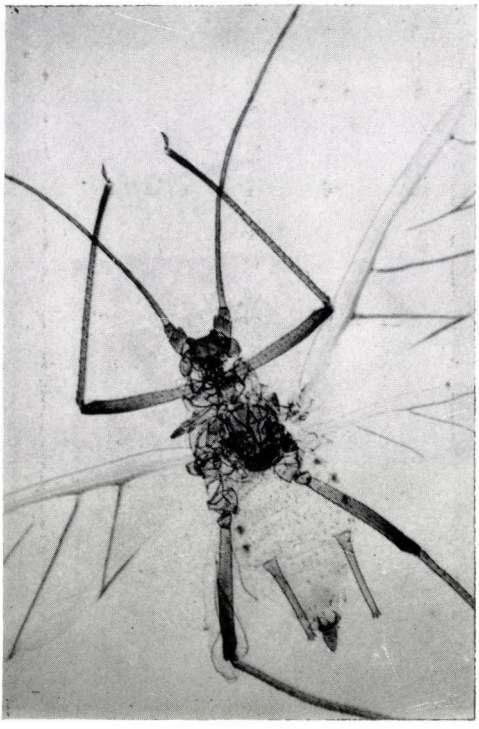

Fig. 78. *Microlophium evansi*

long as V. Cornicles as long as V. Cornicles with or subapical zone of polygonal reticulation extending over about distal one-third. Cauda lightly pigmented (Fig. 75).

152 (151) No broken transverse bars only dark sclerits and marginals on abdomen.

153 (154) Dark sclerites in row by marginals.
Frontal part of head black. Dark knees and ankles. Dark cornicles with a subapical zone of polygonal reticulation extending over about distal one-fifth. Black markings above and below cornicles (Fig. 76).

154 (153) Dark sclerites on dorsal abdomen in transverse rows.
Dark head, thorax, knees and ankles. Hairs on abdomen in row (Fig. 77).

155 (150) No pigmentation on dorsal abdomen.

156 (157) Small dark marginals. Dark knees and ankles.
Cornicles as long as processus terminalis. Cornicles pale flared into trumpet shaped. Rhinaria on III in row (Fig. 78).

157 (156) No dark marginals.

158 (163) Long digitate cauda.

159 (160) Cornicles as long as cauda. Cauda slightly wider than cornicles.
Dark head, thorax, cornicles, legs and cauda. Hairs on abdomen in row (Fig. 79).

160 (159) Cornicles 1.5 times as long as cauda. Cauda 2–3 times as wide as cornicles. Dark head, thorax, legs, cornicles.

161 (162) Cauda 2 times as wide as diameter of cornicles on middle. Hairs on abdomen in row (Fig. 80).

162 (161) Cauda 3 times as wide as diameter of cornicles in middle. Hairs on abdomen inconspicuous (Fig. 81).

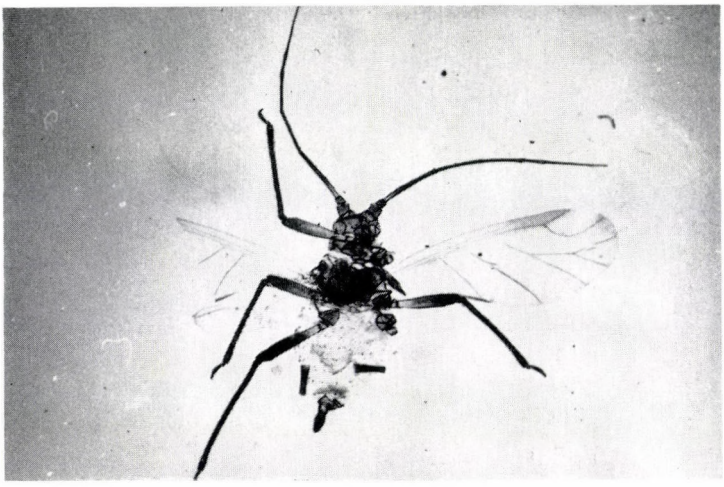

Fig. 79. *Macrosiphonella tanacetaria*

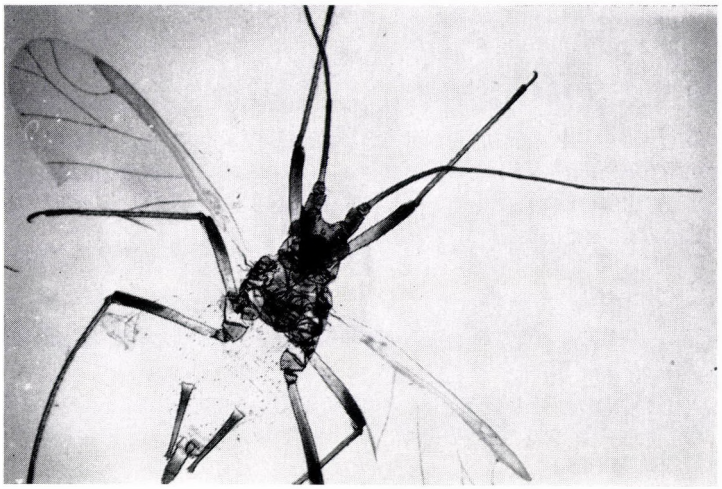

Fig. 80. *Macrosiphonella tapuskae*

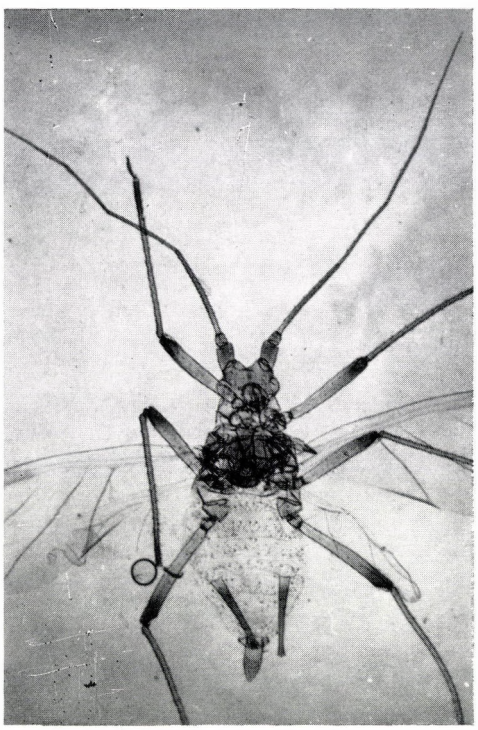

Fig. 81. *Macrosiphonella persequens*

163 (158) Cauda pointed, or tongue-like.

164 (165) Cauda tongue-like.
Antennae gradually darkened from base of III to apex. Cornicles as wide as diameter of hind tibiae. Cornicles longer than distance between their base. No polygonal reticulation on cornicles. Cornicles pale with slightly darker apices (Fig. 82).

165 (164) Long pointed cauda.

166 (167) Dark antennal joints.

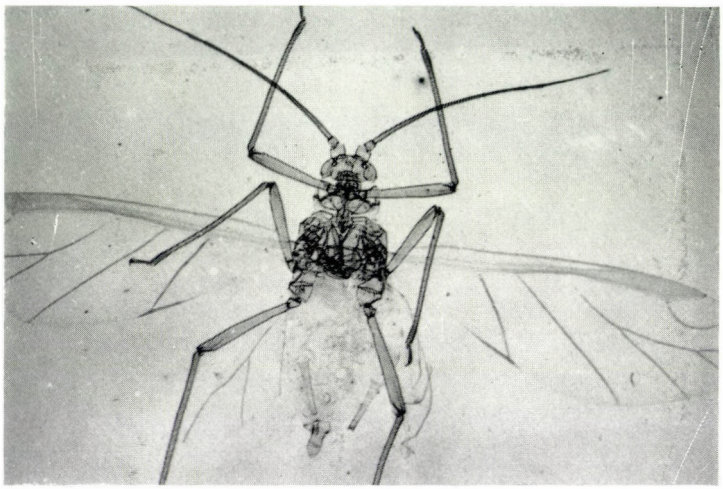

Fig. 82. *Metopolophium dirhodum*

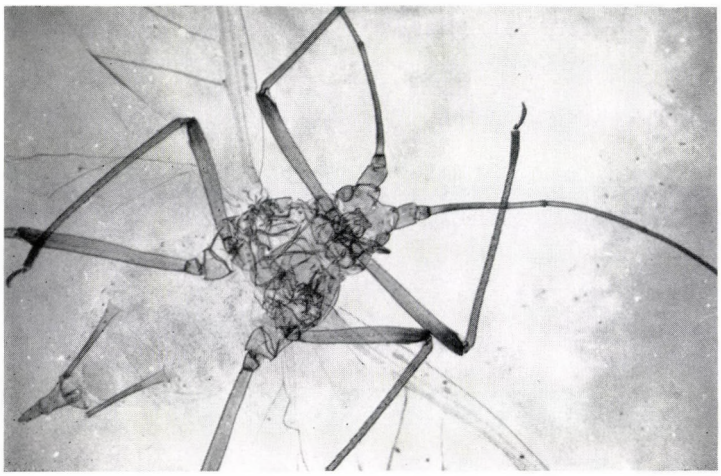

Fig. 83. *Acyrthosiphon pisum*

Cornicles thinner than diameter of of hind tibiae. Cornicles gradually darkened to the apex. Cauda almost as long as cornicles (Fig. 83).

167 (166) Antennal joints not dark

168 (169) Distal constricted part of he cornicles not wider than diameter of hind tibia at middle. Inner part of first antennal segment netlike. Dark cornicles with a subapical zone of polygonal reticulation extending over about distal one-fifth. Cornicles longer than distance between their base. Light marginals on abdomen (Fig. 84).

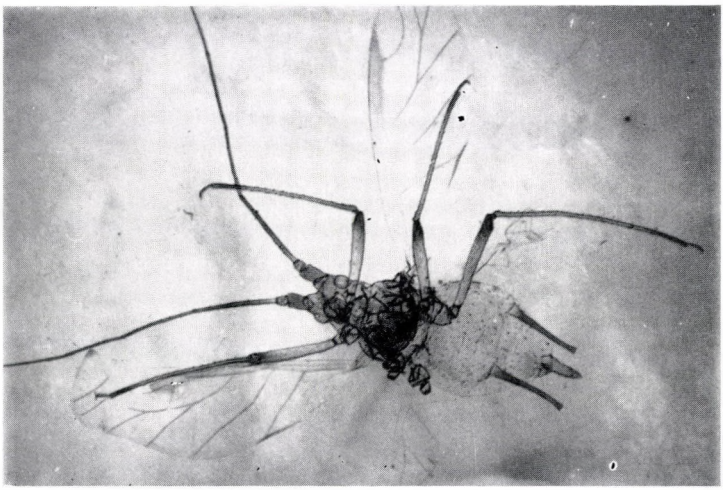

Fig. 84. *Macrosiphum euphorbiae*

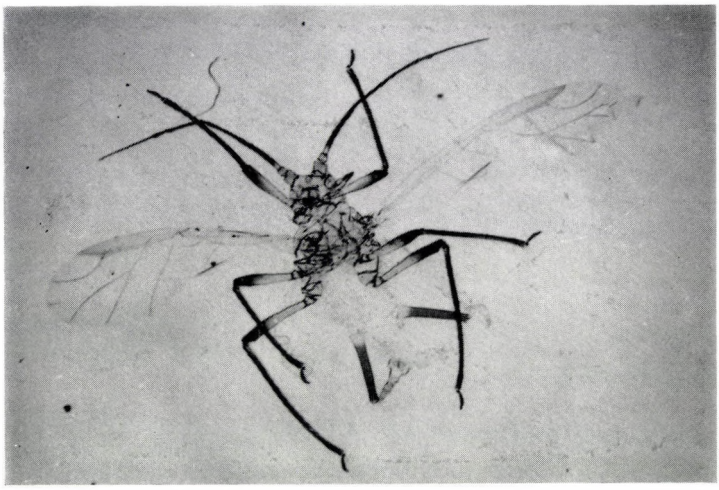

Fig. 85. *Dactynotus erigeronensis*

169 (168) Distal part of cornicles 1.5 times as wide as diameter of hind tibia at middle. Head, thorax, knees dark, cauda paler. Dark cornicles, pale at base with a subapical zone of polygonal reticulation extending over about distal one-third. No marginal pots (Fig. 85).

170 (121) Short, cylindrical, tapering or truncated cone like cornicles.

171 (184) Rostrum short, last rostral segment not reaching third coxae.

172 (179) No spinal tubercles on abdomen.
Dark head, thorax, knees and ankles. Dark edge of spiracles.

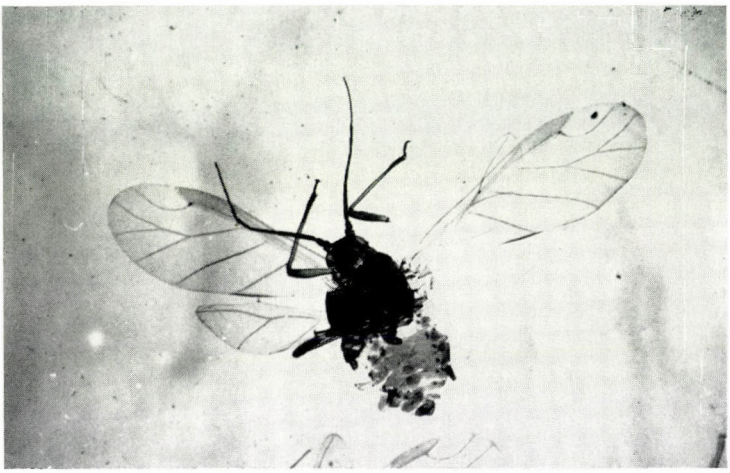

Fig. 86. *Brachycaudus amygdali*

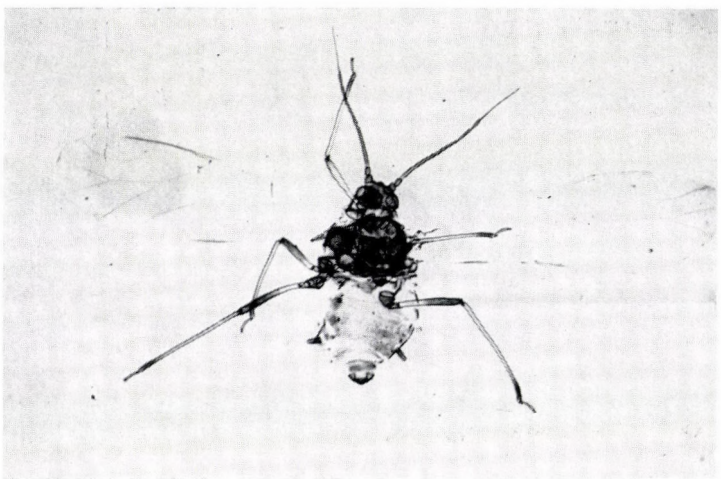

Fig. 87. *Brachycaudus prunicola* sp. *schwartzi*

173 (176) Cauda as long as wide.

174 (175) Cornicles at base 1.5 times as wide as diameter of hind tibia at base. Big dark marginals, extensive black patch on dorsal abdomen. Cornicles truncated cones, proximal to flange (Fig. 86).

175 (174) Cornicles at base as wide as diameter of hind tibia at base. Dark marginals solid black patch on dorsal abdomen with triangular-shaped window between cornicles (Fig. 87).

176 (173) Cauda longer than wide.

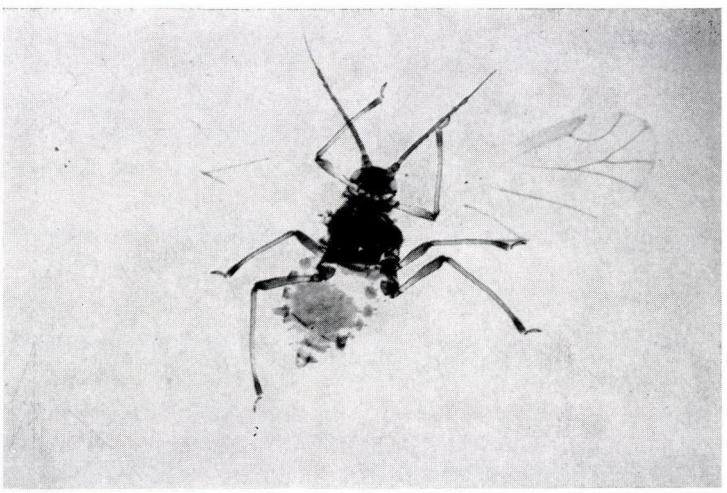

Fig. 88. *Brachycaudus helichrisi*

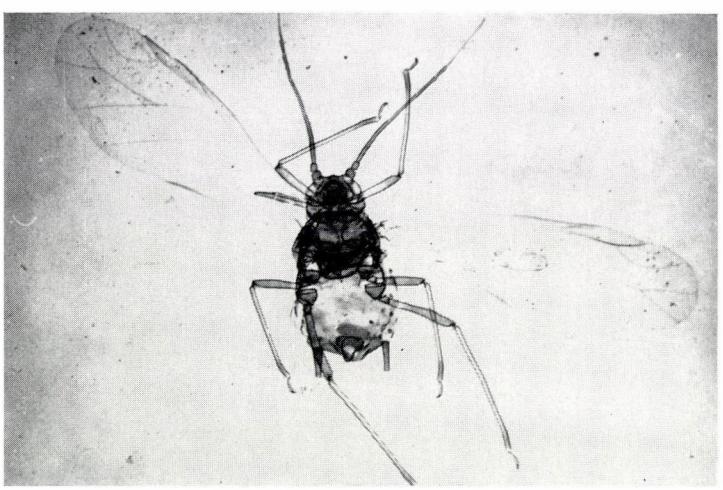

Fig. 89. *Brachycaudus cardui*

177 (178) Patch and marginals not fused.
Cornicles tapering, little longer than cauda. Patch mainly solid sometimes with window (Fig. 88).

178 (177) Patch and marginals fused.
Abdomen evenly black. Cylindrical cornicles 2–3 times as long as cauda. Cornicles dark, proximal to flange (Fig. 89).

179 (172) Spinal tubercles on abdomen.
Big bright glands in dark marginals. Head, thorax, legs, cornicles dark. Bright spinal glands in dark dorsal abdominal patch.

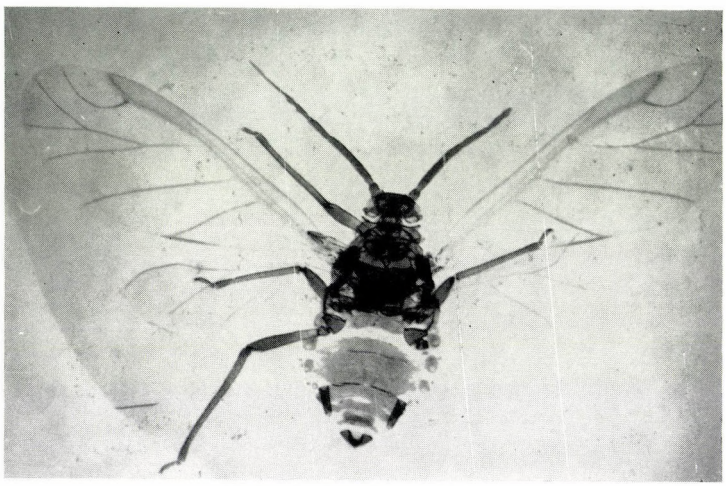

Fig. 90. *Dysaphis radicola*

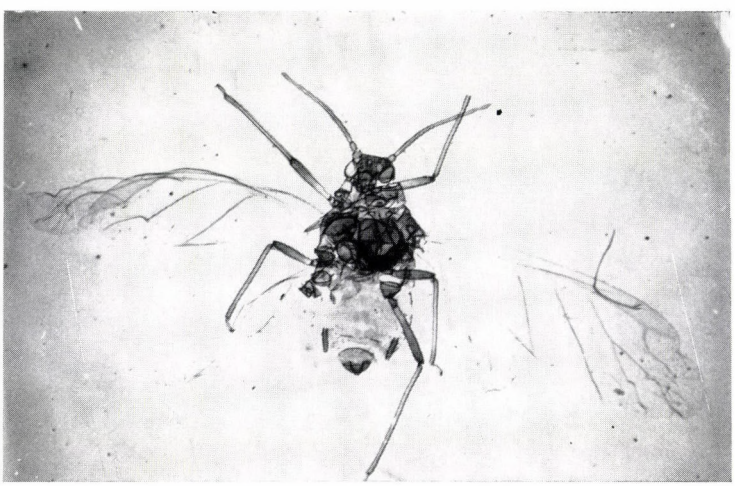

Fig. 91. *Dysaphis devecta*

180 (181) One pair of spinal tubercles behind cornicles.
Cauda as long as wide. Cornicles tapering 2 times as long as cauda. Patch with wavy edge not fused with marginals (Fig. 90).
181 (180) Two pairs of spinal tubercles behind cornicles.
182 (183) Patch with wavy edge not fused with marginals.
Cornicles 1.5 times as long as cauda. Cornicles at base as wide as base of hind tibia (Fig. 91).

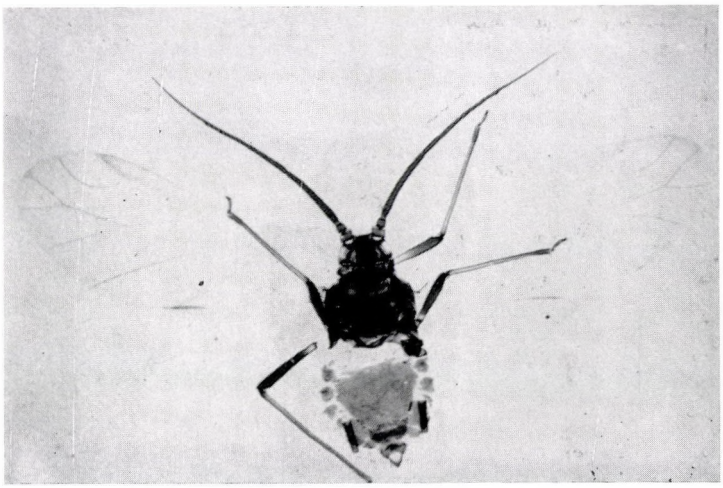

Fig. 92. *Dysaphis plantaginea*

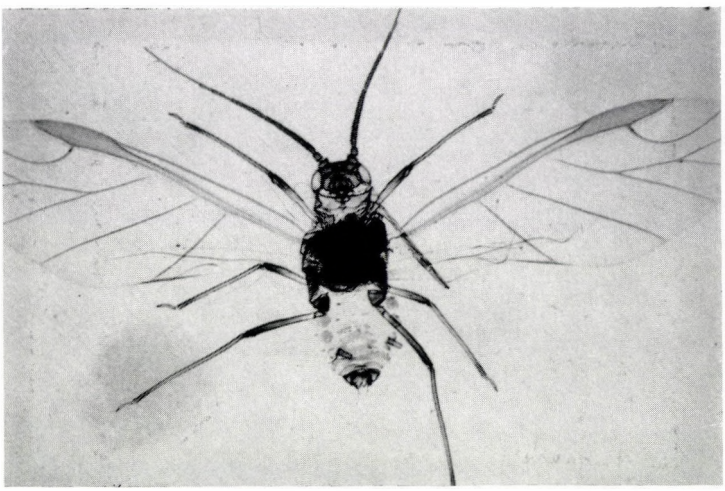

Fig. 93. *Anuraphis farfarae*

183 (182) Patch with straight edge fused with marginals behind cornicles. Cylindrical cornicles 2 times as long as cauda. Cornicles at base wider as base of hind tibia (Fig. 92).
184 (171) Rostrum long, last rostral segment reaching third coxae.
185 (188) Short dark transverse bars at anal part of dorsal abdomen.
186 (187) Cylindrical cornicles little longer than cauda.
Cornicles dark proximal to flange. Dark transverse bars on 4, 5, 6, 7, 8 abdominal segments (Fig. 93).

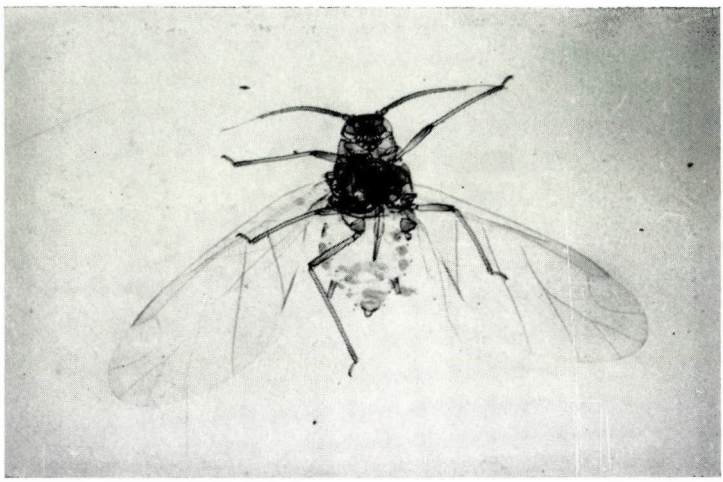

Fig. 94. *Anuraphis pyrilaseri*

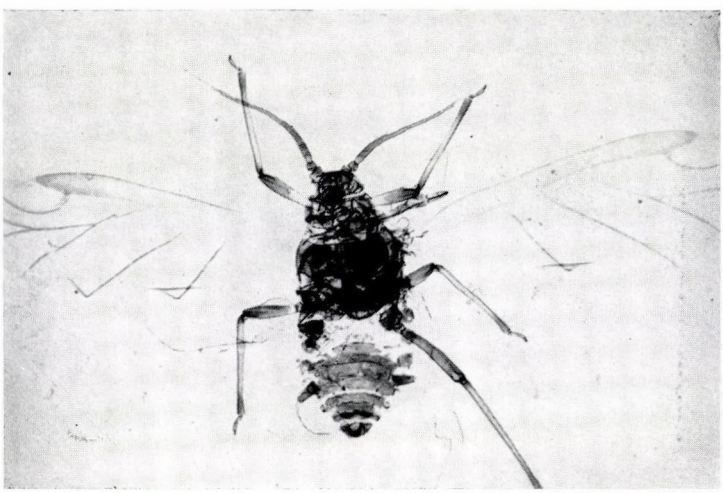

Fig. 95. *Anuraphis subterranea*

187 (186) Cylindrical cornicles two times as long as cauda.
Cornicles dark proximal to flange, 4 transverse bars at anal part of dorsal abdomen (Fig. 94).

188 (185) Dark patch at anal part of dorsal abdomen.
Bright thin stripe at anal two-third of dorsal abdominal patch. Cornicles truncated cones, dark proximal to flange (Fig. 95).

Acknowledgements

The author wishes to thank the late Prof. F. P. Müller, Prof. F. Leclant, Dr. L. Szalay-Marzsó and Mr. P. Andrásfalvy for controlling the identifications. Further thanks are due to Dr. R. Blackman, Prof. V. F. Eastop, Dr. J. Prinsen and Dr. G. Jenser for controlling the key, Dr. M. A. K. Nasser for collecting the aphids, Miss Á. Német for mounting the slides and Mr. E. Petró, Mr. L. Papp and Mrs. J. Herczeg for taking the photomicrographs.

Literature

Blackman, R. L., Eastop, V. F. (1985): Aphids on the world's crops An Identification Guide. John Wiley and Sons. Chichester. New York. Brisbane. Toronto. Singapore 466 p.

Börner, C., Heinze, K., Kloff, W., Lüdicke, M., Schmutterer, H. (1957): Handbuch der Pflanzenkrankheiten. Tierische Schädlinge an Nutzpflanzen 2. Teil Homoptera II. Teil. Berlin, Hamburg. Parey, 578 p.

Eastop, V. F. (1966): A taxonomic study of Australian Aphidoidea (Homoptera). Australian Journal of Zoology. 14, 399–592.

Harris, K. F. (1981): Arthropod and nematode vectors of plant viruses. Annual Review of Phytopathology. 19, 391–426.

Müller, F. P. (1975): Bestimmungsschlüssel für geflügelte Blattläuse in Gelbschalen. Archiv für Phytopathologie und Pflanzenschutz. 11, 49–77.

Stroyan, H. L. G. (1977): Homoptera Aphidoidea (Part): Chaitophoridae and Callaphididae. 130 p.

Stroyan, H. L. G. (1984): Aphids — Pterocommatinae and Aphidinae (Aphidini) Homoptera, Aphididae. 232 p.

Szalay-Marzsó, L. (1969): Levéltetvek a kertészetben. Budapest. Mezőgazd. K. 186 p.

Szelegiewicz, H. (1977): Levéltetvek I. Aphidinea I. Magyarország állatvilága. Fauna Hungariae. XVII. Heteroptera, Homoptera. 18. Füzet. 175 p.

Taylor, L. R. (1980): A handbook for aphid identification. Harpenden. Rothamsted Experimental Station. 171 p.

Theobald, F. U. (1926): The plant lice or Aphididae of Great Britain I–III. London. I. pp. 372, II. pp. 411, III. pp. 364.

Acta Phytopathologica et Entomologica Hungarica, 28 (1), pp. 123—127 (1993)

Seed Treatment Chemicals in Relation to Sesame Bacterial Blight Control

A. D. AKPA*, I. D. ERINLE and S. M. MISARI

Department of Crop Protection,
Ahmadu Bello University, P. M. B. 1044, Zaria, Nigeria

Six seed-applied chemicals were evaluated for the control of bacterial blight of sesame, in pot and field experiments conducted during the 1983 and 1984 wet seasons (June–September). Seeds *treated* with tetramethylthiuram disulphide and NaOCl had significantly lower seedling blight severity compared to seeds treated with aldrin + thiram, methylbenzimidazolcarbamate or unprotected as in the control. However, soaking seeds in streptomycin and NaOCl resulted in a lowered seedling emergence in the field.

Xanthomonas campestris pv. *sesami* (Sabet and Dowson) Dye which induces the bacterial blight of sesame (*Sesamum indicum* L.) is responsible for severe damage and often complete loss of crop under rain-fed conditions in the Sudan and India (Rao and Durgapal, 1966; Tarr, 1949). In Nigeria, bacterial blight is the major foliar disease of sesame and quite often cause severe defoliation of the crop (Erinle, 1981).

The phase of seedling infection has been described and factors affecting its occurrence in the field were reported (Bunting, 1954; Habish and Hammad, 1969). It has been reported that the disease is seed-borne and using seeds treated with chemicals had been found to totally eradicate the disease in pot experiments (Habish and Hammad, 1971; Rao and Durgapal, 1966). Similar reports of eradication of seed-borne bacterial pathogens following chemical seed treatments have been made on tomato. Devash *et al.* (1980) reported that washing tomato seeds with 1% sodium hypochlorite (NaOCl) completely eliminated *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye and Wilkie.

During the 1983 and 1984 wet season (June-September), pot experiments were carried out using aldrin + thiram, 75% (Aldrex T), tetramethylthiuram disulphide, 80% (Fernasan D), methylbenzimidazolcarbamate, 50%, (Delsene M) and NaOCl as seed dressing while in the 1984 wet season, field experiments were conducted using bromonitropropanediol, 12% (Bronocot), streptomycin and NaOCl as seed dressings, to determine the possibility of controlling the disease by chemical seed treatment.

* Present Address: Department of Pure and Applied Biology, Imperial College, London SW7 2BB.

Akadémiai Kiadó, Budapest

Materials and Methods

Pot experiments

Pot experiments were conducted in the 1983 and 1984 wet seasons using the variety 73A-58. Clay pots were filled with heat-sterilized soil and arranged in a randomized complete block design (RCBD) with three replications. Three sets of seeds were treated with aldrin + thiram, tetramethylthiuram disulphide, and methylbenzimidazolcarbamate at the rate of 2g dust kg^{-1} seed. Another set of seeds were soaked in a 1% solution of NaOCl for 30 min and then air-dried in the laboratory. The last set of seeds which received no chemical treatment was treated as control.

Ten seeds from each treatment were sown per pot and were later thinned down to three seedlings per pot two weeks after emergence. The level of seedling infection was assessed thirty days after sowing using a scale of 1–6 as outlined below:

1 = no disease;
2 = small pin-point lesions on cotyledonary leaves;
3 = small lesions on cotyledonary leaves, with infection extending to the true leaves;
4 = large lesions scattered on cotyledonary and true leaves;
5 = cotyledonary leaves completely dead with lesions coalescing and covering large areas of tue leaves; and
6 = seedling completely killed.

Field experiments

During the 1984 wet season, field trials were conducted using seeds of a local sesame variety obtained from Samaru market. Four sets of seeds were subjected to four treatments as follows: the first set was treated with bromonitropropanediol at the rate of 2 g dust kg^{-1} seed; the second and third sets were soaked in a 0.05% solution of streptomycin and a 1% solution of NaOCl respectively for 30 min and then air-dried in the laboratory; while the fourth set which received no chemical treatment was treated as control.

The four categories of seeds were sown in the field in a RCBD with four replications. Plot sizes were 4 m^2. The seeds were sown by broadcast method and the seedlings were thinned down two weeks after emergence. The level of seedling bacterial blight was assessed thirty days after sowing using the scale outlined above.

In order to determine the possible phytotoxicity of the chemicals used, four hundred seeds from each treatment were sown along one metre drills spaced 50 cm apart. A RCBD with four replications was used. Records of seedling emergence were taken twelve days after sowing.

Table 1

Effect of chemical seed treatment on the severity of seedling bacterial blight of sesami

Treatment	1983*	1984*	Combined Analysis*
Aldrin + Thiram	3.0[a]	3.3[a]	3.2[a]
Tetramethylthiuram disulphide	2.3[b]	2.0[c]	2.2[b]
Methylbenzimidazolcarbamate	3.0[a]	3.3[ab]	3.2[a]
NaOCl	2.3[b]	2.3[bc]	2.3[b]
Control	3.0[a]	3.7[a]	3.3[a]
$LSD_{0.05}$	0.54	1.06	0.56

* Figures followed by the same letter(s) are not significantly different at P = 0.05.

Table 2

Effect of chemical seed treatment on emergence of sesame seedlings

Treatment	Seedling emergence	% emergence over control
Control	46.0	0.0
NaOCl	31.0	−32.6
Streptomycin	46.0	0.0
Bromonitropropanediol	52.5	14.5
$LSD_{0.05}$	ns*	

* ns = not significant

Table 3

Effect of chemical seed treatment on the severity of seedling bacterial blight of sesami under field conditions

Treatment	Disease severity score	Number of leaves infected	Number of leaves shed
Bromonitropropanediol	3.30	6.05	0.20
Streptomycin	3.80	6.15	0.25
NaOCl	3.80	6.70	0.10
Control	3.75	5.70	0.50
$LSD_{0.05}$	ns	ns	ns

* ns = not significant

Results

The results obtained from pot experiments are shown in Table 1. Seed treatment with tetramethylthiuram disulphide and NaOCl significantly lowered the severity of seedling blight compared to seeds treated with aldrin + thiram, methylbenzimidazolcarbamate or unprotected as in the control.

The results of the 1984 field experiments are shown in Tables 2 and 3. There was no significant difference between the means of seedling blight severity, number of leaves infected, number of leaves shed and the level of seedling emergence of the various treatments. The level of seedling emergence proved rather low generally.

Discussion

From the results of the pot and field experiments, it is apparent that complete eradication of the pathogen was not achieved with any of the chemicals contrary to earlier reports (Habish and Hammad, 1971; Rao and Durgapal 1966).

Pyke *et al.* (1984) similarly observed that tomato sed treatment with NaOCl against the bacterial speck pathogen did not achieve complete eradication of the pathogen contrary to earlier reports (Devash *et al.*, 1980). While complete eradication of seedling bacterial blight of sesame was not possible, reduction of blight severity was achieved with some of the chemicals. In pot experiments, treatments with tetramethylthiuram disulphide and NaOCl resulted in a significantly greater lowering of seedling blight severity, compared to other chemicals used. The reduction in blight severity achieved with some of the chemicals will be of great significance if the level corresponds to such that can effectively reduce rapid secondary spread of the disease in the field.

Out of the three chemicals used in the field, only bromonitropropanediol gave an improved seedling emergence over control. Seeds given a streptomycin soak had the same level of seedling emergence as the control. The emergence of seeds given a NaOCl soak was significantly reduced, compared to the control. In the case of streptomycin and NaOCl, two factors could have been involved in the lower percentages of seedling emergence. First, the rate of application of 0.05% and 1% for streptomycin and NaOCl respectively, might have been too high; and secondly, the 30 min soaking period might have been too long for the chemicals not to have had phytotoxic effects on the seeds. Although the phytotoxicity of NaOCl has hitherto not been reported on any crop, that of streptomycin had been reported on phaseolus beans and peas (Taylor, 1970; Taylor and Dye, 1975). Using streptomycin as a chemical seed treatment against *P. syringae* pv. *tomato* (Okabe) Young, Dye and Wilkie which induces bacterial speck of tomato, Pyke *et al.* (1984) found the rate of seedling development to be retarded when seeds were treated with this antibiotic, although it did not reduce percentage germination in their study.

For the rather low level of seedling emergence recorded in the field, a seed

factor could have been involved. Since the seeds were obtained from the open market, it is possible that they had been stored for a long period of time. Viability of seeds is known to be reduced by long periods of storage (Gelmond, 1978).

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Literature

Bunting, A. H. (1954): Annual Report of the Research Division, Ministry of Agriculture, Sudan, pp. 125.

Devash, Y., Basham, Y., Okon, Y., and Henis, Y. (1980): Survival of Pseudomonas tomato in soil and seeds. Hassedeh, 60, 597–601.

Erinle, I. D. (1981): A Short Guide to Bacterial Diseases of Nigerian Plants, Hamdam Press, Zaria, p. 12.

Gelmond, H. (1978): Problems in crop seed germination. In: Crop Physiology, Gupta, U.S.ed. Oxford and IBH Publ. Co., New Delhi, pp. 1–62.

Habish, H. A. and Hammad, A. H. (1969): Seedling infection of sesame by Xanthomonas sesami Sabet and Dowson and the assessment of resistance to bacterial leaf spot diseases. Phytopath. Z., 64, 32–38.

Habish, H. A. and Hammad, A. H. (1971): Survival and chemical control of Xanthomonas sesami. FAO PL. Prot. Bull., 19, 36–40.

Pyke, N. B., Milne, K. S. and Neilson, H. F. (1984): Tomato seed treatments for the control of bacterial speck. New Zealand J. of Expt Agric., 12, 161–164.

Rao, Y. P. and Durgapal, J. C. (1966): Seed transmission of bacterial blight of sesame (Sesamun orientale L.) and eradication of seed infection. Indian Phytopath., 19, 402–403.

Tarr, S. A. J. (1949): Annual Report of the Research Division, Ministry of Agriculture, Sudan, pp. 125.

Taylor, J. D. (1970): Studies on Halo Blight Disease of Beans Caused by Pseudomonas phaseolicola (Burkh) Dowson. Ph. D. Thesis, University of Bath, England.

Taylor, J. D. and Dye, D. W. (1975): Evaluation of streptomycin seed treatments for the control of bacterial blight of peas caused by Pseudomonas pisi. N. Z. Journal of Agric. Research, 19, 91–95.

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Adsorption of Some Nonionic Tensides on Different Carriers II. Adsorption Capacity*

T. CSERHÁTI

Plant Protection Institute, Hungarian Academy of Sciences,
1525 Budapest, Box 102. Herman O. 15., Hungary

Adsorption capacity of kaolin, diatomaceous earth, Sillilin N8 and Ultrasil carriers for 19 nonionic tensides was determined. The adsorption capacity of carriers changed in the order: Ultrasil > diatomaceous earth > kaolin > Sillilin N8. The quantity of adsorbed tensides decreased considerably with growing length of hydrophilic ethyleneoxide chain. Sodium chloride increased the quantity of adsorbed tensides. Adsorption capacity of carriers increased with growing specific surface area. No correlation was found between the adsorption capacity and adsorption strength of carriers.

Nonionic tensides show marked biological activity as cytolytic effect on *Poteriochromas malhamensis* (Röderer, 1987), increase of permeation rate of hydrocortisone through skin (Sarpotdar and Latz, 1986), inhibition of adhesion of bacteria to solid surfaces (Humphries et al., 1986, Humphries et al., 1987), stimulation of protein secretion in *Trichoderma reersei* (Panda et al., 1987), irritation of skin (Blake-Haskins et al., 1986), fish toxicity (Yoshimura, 1986), enhancement of naloxone permeation through human skin (Aungst et al., 1986), influence on nonproton ion movements in purple membrane (Marinetti and Mauzerall, 1986) etc.

As some pesticide formulations simultaneously contain nonionic tensides and carriers and the carriers adsorb tensides with different strength (Cserháti, 1986) the determination of adsorption capacity of carriers for tensides is of considerable practical importance. The objectives of our investigations were to determine the adsorption capacity of some carriers and to study the influence of ethyleneoxide chain length of tensides and that of specific surface area of carriers on the adsorption.

Experimental

The nonionic tensides were the polyethoxylated derivatives of nonylphenol (average ethyleneoxide number per molecule 4, 5, 6, 8, 9, 10, 11, 15, 23 and 30) and those of tributylphenol (average ethyleneoxide number per molecule 4, 6,

* The first part was published in Acta Phytopath. Entom. Hung. 21 (1986) 151–156.

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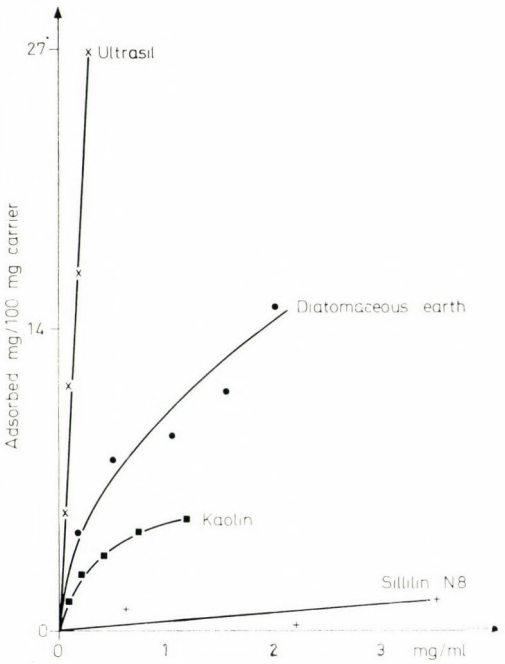

Fig. 1. Adsorption of nonylphenyl-hexaethoxylate on different carriers

8, 10, 13, 18, 30 and 50).The carriers were natural (kaolin, diatomaceous earth) and synthetic products (Sillilin N8, Ultrasil).

The determination of adsorption capacity of carriers was carried out by the traditional equilibration method: 8 cm^3 of aqueous tenside solution was added to 0.1–0.4 g carrier. The tenside concentration of the aqueous solution was in the range of 5–50 mg tenside/cm^3 water corresponding to the adsorption capacity of carriers.

To study the effect of ion environment on the adsorption of tensides some experiments were carried out with 1.5 M NaCl solution instead of distilled water.

The suspensions were shaken for 4 hours at room temperature, then centrifuged at 6.000 g for 15 minutes. The extinction of supernatant was measured at 220 nm wavelength, the concentration of non-adsorbed tensides was calculated with the help of calibration curves. To study the desorption of tensides the carrier pellets were resuspended in 8 cm^3 distilled water, shaken again for 4 hours, and repeating the procedure described above.

Each experiment was run in triplicate in two independent repetitions. The B. E. T. surface area of carriers was determined by Sorptometer 212 D (Perkin Elmer) and the surface area was calculated by the B. E. T. "one point" method.

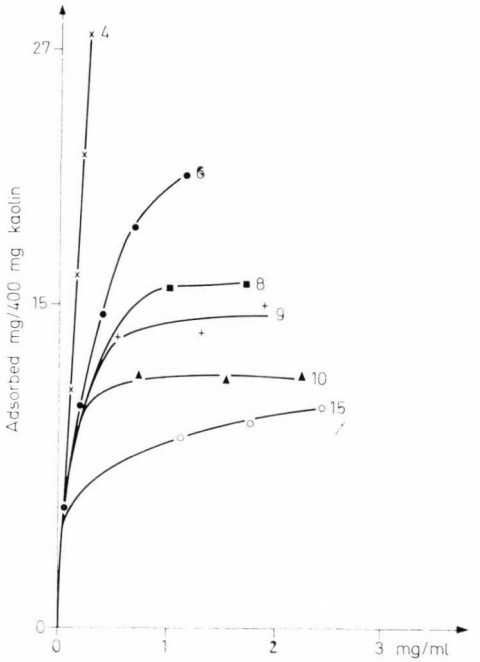

Fig. 2. Adsorption of nonylphenyl-ethyleneoxide polymers on kaolin. Numbers indicate the average ethyleneoxide number per molecule

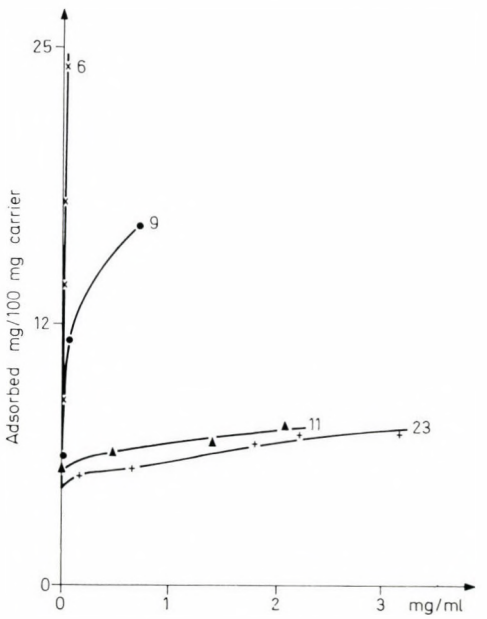

Fig. 3. Adsorption of nonylphenyl-ethyleneoxide polymers on diatomaceous earth. Numbers indicate the average ethyleneoxide number per molecule

Results and Discussion

The adsorption capacity of carriers differed considerably from each other (Fig. 1). Ultrasil showed the highest adsorption capacity followed by diatomaceous earth, kaolin and Sillilin N8. As it was previously established that the adsorption strength of tensides is the highest on kaolin and on diatomaceous earth and the lowest on Ultrasil it can be concluded that the adsorption strength and adsorption capacity of carriers do not show any correlation. As the degree of activity loss of tensides depends equally on the adsorbed quantity and on the strength of adsorption and these two physico-chemical parameters of carriers are not correlated, it makes difficult to predict the biological performance of carrier : tenside mixtures. According to our knowledge the biological efficiency of tensides adsorbed with different strength has never been evaluated experimentally or theoretically.

The adsorbed quantity of tensides decreased markedly with growing number of ethyleneoxide groups per molecule (Figs 2 and 3). This finding cannot be explained by the size increase of tenside molecules, the differences are too great (Fig. 4). We assume that the tensides with lower critical micelle concentration (shorter hydrophilic ethyleneoxide chain) are adsorbed as micelles on one adsorption centre while the more water-soluble longer tenside molecules occupy individually the adsorption sites. The adsorption isotherms seem to be of Langmuir character. Due to the lower water solubility of tensides with shorter hydrophilic ethyleneoxide chain their saturation concentration cannot be determined under the experimental conditions applied.

Sodium chloride increased the adsorbed quantity of tensides (Fig. 5). This somewhat surprising observation indicates that the adsorption sites on the carrier surface are different for the nonionic tensides and for sodium chloride, the coad-

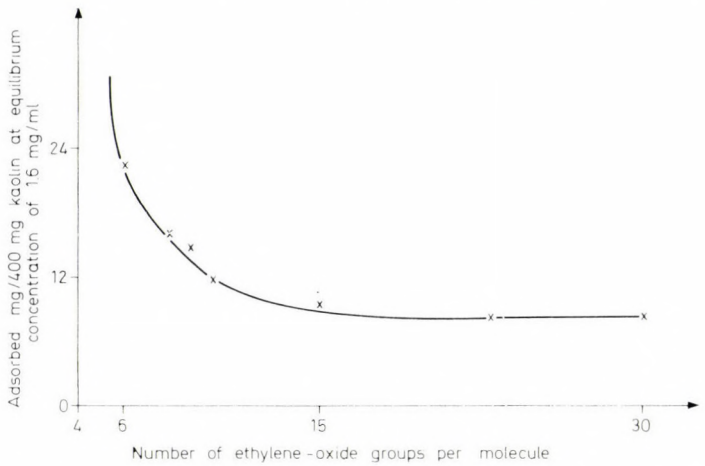

Fig. 4. Effect of the number of ethyleneoxide groups per molecule on the adsorbed quantity of nonylphenyl-ethyleneoxide polymers

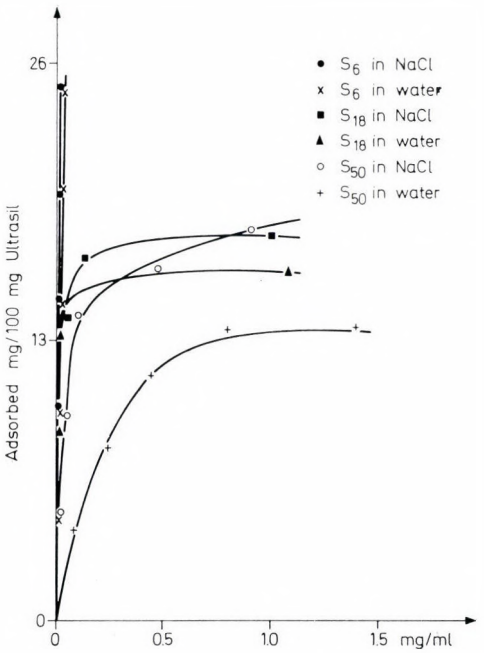

Fig. 5. Effect of sodium chloride on the adsorption of tributylphenyl-ethyleneoxide polymers. Numbers indicate the ethyleneoxide number per molecule

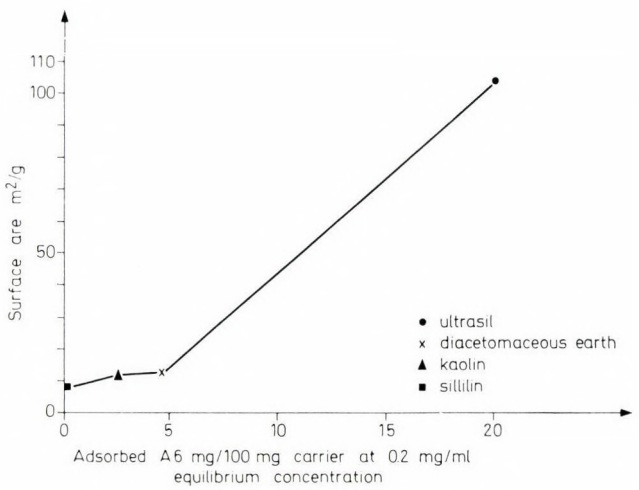

Fig. 6. Correlation between the specific surface area and adsorption capacity of carriers. A6 = nonylphenyl-hexaethoxylate

sorption is not competitive. The adsorption enhancing effect of sodium chloride can be explained by the assumption that the dissociated ions lessen the solubility of tensides in water (salting out effect). Sodium chloride exerted a higher influence on the adsorption of tensides with longer ethyleneoxide chain than on that with shorter chain suggesting that the chloride or sodium ions (or both of them) preferably interact with the hydrophilic ethyleneoxide moiety of tensides. However, the character of interaction is not clear and its elucidation needs further investigations.

The quantity of adsorbed tensides increased with the growing surface area of carriers (Fig. 6). This result suggests that the adsorption sites are quasi equidistantly distributed on the surface of carriers independently of their chemical character or the tensides cover evenly the carrier surface without requiring special sites for their adsorption.

When adsorbed from distilled water the quantity of desorbed tensides never exceeded the standard deviation of the determination (0.1 mg tenside/0.1 g carrier) that is we were not able to detect any significant desorption. This phenomenon implies chemisorption, however, in our cases it is highly improbable. Recently we do not have any valid hypothesis to explain this observation.

When the adsorption was carried out in sodium chloride solutions, distilled water resolved the quantity of tensides which was adsorbed under the influence of sodium chloride and no more. This finding makes it probable that adsorption from water and from sodium chloride solution differs in character.

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Literature

Aungst, B. J., Rogers, N. J. and Shefter, E. (1986): Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–237.

Blake-Haskins, J. C., Scala, D., Rhein, L. D. and Robbins, C. R. (1986): Predicting surfactant irritation from the swelling response of a collagen film. J. Soc. Cosmet. Chem. 37, 199–210.

Cserháti, T. (1986): Adsorption of some nonionic tensides on different carriers. I. Adsorption strength. Acta Phytopath. Entom. Hung. 21, 151–156

Humphries, M., Jaworzyn, J. F. and Cantwell, J. B. (1986): The effect of a range of biological polymers and synthetic surfactants on the adhesion of a marine Pseudomonas sp. strain NCMB 2021 to hydrophilic and hydrophobic surfaces. FEMS Microbiol. Ecol. 38, 299–308.

Humphries, M., Jaworzyn, J. F., Cantwell, J. B. and Eakin, A. (1987): The use of non-ionic ethoxylated and propoxylated surfactants to prevent the adhesion of bacteria to solid surfaces. FEMS Microbiol. Letters 42, 91–101.

Marinetti, T. and Mauzerall, D. (1986): Large transient nonproton ion movements in purple membrane suspensions are abolished by solubilization in Triton X-100. Biophys. J. 50, 405–415.

Panda, T., Gruber, H. and Kubicek, C. P. (1987): Stimulation of protein secretion in Trichoderma reersei by Tween surfactants is not correlated with changes in enzyme localization or membrane fatty acid composition. FEMS Microbiol. Letters 41, 85–90.

Röderer, G. (1987): Toxic effects of tetraethyl lead and its derivatives on the chrysophyte Poteriochromas malhamensis. VIII. Comparative studies with surfactants. Arch. Environ. Contam. Toxic. 16, 291–301.

Sarpotdar, P. P. and Latz, J. L. (1986): Percutaneous adsorption enhancement by nonionic surfactants. Drug Dev. Industr. Pharm. 12, 1625–1647.

Yoshimura, K. (1986): Biodegradation and fish toxicity of nonionic surfactants. JAOCS 63, 1590–1596.

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Book Reviews

Mahadevan, A. and Sridar, R.: *Methods in Physiological Plant Pathology*. Third edition, Sivakami Publications, Indira Nagar, Madras, 1986, pp. 328.

The authors are internationally acknowledged and popular figures in the circles of plant pathologists. So is their work. The third edition of this book met a nation-wide demand of students and researchers in India. After having perused the complimentary copy I understood why the book is so popular. First of all, because it is fully physiological plant pathologically oriented. It compiles the basic techniques used in contemporary physiological plant pathology studies. The techniques are introduced in the chapters as follows in order. Alteration in pigment / Photosynthesis and respiration / Estimation of fungal pathogen in diseased plant tissue and liquid cultures / Enzymes of infected plants and parasites / Toxins / Aflatoxins / Cell permeability / Impedin / Plasmids / Analysis of carbohydrates, amino acids and of organic acids from diseased plants / Nucleic acid in infected plants / Disease resistance / Bioassay of antimicrobial substances / Induction of resistance by exogenously applied substances / Growth regulators. Each of the chapters begins with the explanation of basic principles. The experimental sections list the materials and reagents to be used in the precisely detailed method that is supplemented with comments and reference. Thus one can find at one's fingertips everything required to perform the experiment. Last but not least, the authors' purpose, i.e. to select methods that are "simple and can be conducted in laboratories with minimum facilities" should also be appreciated.

Positive as it is, the first impression may not cover up the shortcomings of the book such as (i) the didactically improper order of chapters, (ii) the unaccountable briefness of the subjects of toxins and (iii) of plasmids. As to the latter, either it should not be implied or it should be detailed, even if at the expense of other chapters, by the implication of several up-to-date techniques that are now routinely used in gene manipulations.

Apart from the outlined shortcomings, I would have been glad to have such a manual in the course of my graduate years.

(T. É.)

Robert N. Goodman, Zoltán Király and K. R. Wood: *A beteg növény biokémiája és élettana* (The Biochemistry and Physiology of Plant Disease). Akadémiai Kiadó, Budapest 1991, pp. 828.

This is the Hungarian version of a book published eighteen years ago in the USA, then revised five years ago and published again in the USA, which is completed with the most recent achievements. A great merit of the book is that, in comparison with the latest American edition of 1986, it contains the most recent discoveries and results of molecular biology and biotechnology of the last 5–6 years. Reference to this is made in the 11th chapter of the book "Molecular and biotechnological aspects of plant diseases".

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Apart from a few chapters (e.g. "The process of infection", "The toxins", "Resistance to infection"), the book discusses the physiology and biochemistry of the healthy plant in a logical succession, then deals in detail with the pathological and biochemical processes induced by viral-, bacterial- and fungal infections. The authors — very cleverly — completed each chapter with a section "Further remarks and citations", rendering it possible to introduce the latest results that became known while the book was written. This construction has made this excellent work really up-to-date. The authenticity of what the work of the internationally recognized authors represents in professional line is hallmarked by 23 readers known all over the world.

The book contains the following 11 chapters: 1. The process of infection, 2. Photosynthesis, 3. Respiration, 4. The composition and metabolism of the cell-wall, 5. Nucleic acid- and protein metabolism, 6. Secondary metabolites, 7. Metabolism of growth regulators, 8. Nutrient transport through the cell-wall and in the vascular system, 9. Toxins, 10. Resistance to infection, molecular biology- and biotechnology aspects of plant diseases.

The 3038 references are listed at the end of each chapters. The text is made easier to understand by 310 electronmicroscopic- and microscopic pictures and drawings and 97 well-constructed tables. The "Subject index" at the end of the book includes some 2500 entries.

The book, which contains the latest results of molecular biology and biotechnology, is indispensable for those engaged in studies on viral-, bacterial- and fungal diseases of plants, on pathological processes and mechanisms of plant resistance, on the relations of cause and effect and on the biological and biochemical bases of an environment-sparing integrated plant protection.

The high level up-to-date content of the book does credit to the American, Hungarian and English authors. The authors also deserve praise for dedicating their work to the memory of Sir Frederic Bawden, Ernst Gäumann and David Gottlieb who have accomplished the most in the last five decades in widening the biochemical and physiological knowledge of phytopathology. The Hungarian translation of the English text does credit to co-author Zoltán Király. Further, thanks are due to the Rockefeller Foundation for creating opportunity for the authors to exchange views and have consultations, and to the Publishing House of the Hungarian Academy of Sciences (Budapest) for editing the book, promoting thereby the quick orientation of the new Hungarian scientists' generation in the first years of their career.

J. Horváth

Horst Börner and Ulrich Zunke: *Praktikum der Phytopathologie: Ein Farbatlas für Studium und Praxis.* Verlag Paul Parey, Berlin and Hamburg 1992, pp. 66.

With their book "Phytopathological Practice" the authors undertook the task of writing a highly important gap-bridging work. As it is known, data on the macroscopic symptoms of plant diseases caused by various pathogens (viruses, bacteria, fungi) can be found in many works of the relevant literature, but hardly any source is available concerning the microscopic preparations of pathogens and the histological changes. The "Phytopathological Practice" attempts to meet this need, beside describing the essential characteristics of the most important pathogens and showing the different methods of control as well as the macroscopic symptoms, by placing an emphasis on the microscopic preparations of the pathogens. To facilitate the use of the book the descriptions of the diseases (pathogens) and the respective coloured figures are to be found on opposite pages.

The book consists of three main parts: 1. Diseases of cultivated plants caused by viruses, 2. Diseases of cultivated plants caused by bacteria, 3. Diseases of cultivated plants caused by fungi. Of the three kinds of pathogen, fungi are discussed in the fullest detail. The book provides information, explanatory drawings and figures on the major classes, orders and 41 species belonging to the *Myxomycota* and *Eumycota* phyla and the *Mastigomycotina*, *Zygomycotina*, *Ascomycotina*, *Basidiomycotina* and *Deuteromycotina* sub-phyla, respectively.

At the end of the book a list of the major — mostly German — works of reference and the subject index can be found.

The only objection to this long needed work is that relatively little attention is paid to viruses and bacteria of high agricultural and horticultural importance. The authors deal with the two groups of pathogen on 6 pages (on 4 pages with viruses and on 2 pages with bacteria) while the fungi are discussed on 44 pages. Mycoplasms being not negligible due to their importance, are missing from the book. Furthermore, it would have been reasonable to treat major genera *(Leveillula, Microsphaera, Phyllactinia)* of the sub-phylum *Ascomycotina*, and some economically important genera (e.g. *Cytospora, Cylindrosporium, Stemphylium, Rhizoctonia*) of the sub-phylum *Deuteromycotina*. In a new edition of the book these deficiencies will certainly be easily made up for.

To be accessible to a larger readership, this important work of the phytopathological literature would deserve to be published in English, too.

H. Börner and U. Zunke's colour map, with the high level of the "Phytopathological Practice", the about 124 excellent-quality colour pictures and illustrative drawings, the fine typography of the Verlag Paul Parey, Berlin and Hamburg is an indispensable aid for university students interested in agriculture, horticulture and biology, as well as for professionals and teachers engaged in plant protection and nature conservation.

J. Horváth and *Cs. Pintér*

R. E. F. Matthews: *Plant Virology*. 3rd Edition. Academic Press, San Diego, California 1991, pp. 835.

Since the 2nd edition of "Plant Virology" in 1981 highly important achievements have made in plant virology, rendering it necessary to publish the 3rd edition of "Plant Virology". In this book all new results coming from the application of gene manipulation technology, have been included. A significant development in procedures was made in the 1980s, whereby for making DNA copies of RNA genomes and applying DNA sequencing techniques. In more than half of the virus groups with RNA genome the complete nucleotide sequences have become known. Owing to the highly important scientific achievements of the recent 10 years most chapters had to be totally rewritten and several chapters appeared with a new content. Due to the important new results obtained in plant virology more than 50% of the text and illustrations, 95% of the tables and 60% of the references are new in the 3rd edition.

R. E. F. Matthews' new book consists of 17 chapters, 2 appendices, bibliography and an index. Each chapter is made completed by a section of "Discussion and Summary". The book of 835 printed pages contains 251 figures, 21 tables, over 3000 references. The index includes some 2000 entries.

The 1st chapter, the "Introduction", deals with the discovery of viruses and gives a survey of the major results of research and a definition of virus.

The 2nd chapter, "Methods for assay, detection and diagnosis", discusses subjects such as methods involving biological activities of the virus and properties of the viral nucleic acid or those depending on physical properties of the virus particle and viral proteins.

The 3rd chapter, "Isolation", deals with the choice of plant material, extraction medium and procedure, preliminary isolation, purification and storage of the viruses, identification of the infective particles and criteria of purity as well as with the concentration in plants and yields of the purified virus.

The 4th chapter, "Components", discusses the nucleic acids, proteins and other components of viruses.

In the 5th chapter, "Architecture", the Author presents various methods (e.g. chemical and biochemical studies, sizes of viruses, electron microscopy, X-ray crystallographic analysis, serological methods etc.), then treats the problems of physical principles in the architecture of viruses, geometric viruses (ssRNA, dsRNA, DNA), enveloped viruses, and the arrangement of nucleic acid within icosahedral viruses.

The most detailed 6th to 8th chapters, "Replication I, II, III", — deal with the subjects of "Introduction to the study of virus replication", "Viruses with single-stranded positive sense RNA genomes" and "Other virus groups and families".

The 9th chapter, "Viroids, satellite viruses and satellite RNAs", discusses the structure and replication of viroids, the biological aspects, the molecular bases of biological activity and diagnostic procedures and deals with issues related with the satellite plant viruses and satellite RNAs.

The 10th chapter, "Transmission, movement and host range", is concerned with the possibilities of virus transmission (e.g. through seed and by vegetative propagation, grafting, dodders and invertebrates, fungi, *Agrobacterium tumefaciens* as well as mechanically) and discusses the virus movement in the plant cell and -tissues, the host-parasite relation (host range studies) and the molecular basis for host range.

The 11th chapter, "Disease symptoms and effects on metabolism", treats the questions of macroscopic symptoms, histological changes, cytopathological effects and effects on plant metabolism.

The 12th chapter, "Induction of disease", deals with the host response, the role of viral genes, the host proteins induced by virus infection, the process involved in disease induction and the factors influencing the course of infection and disease.

In the 13th chapter, "Variability", information on virus strains (isolation, strain criteria, characterization, host-strain relationships) is given.

The 14th chapter, "Relationships between plant viruses and invertebrates", discusses the vectors playing a part in virus transmission (nematoda, arthropoda, aphids, leafhoppers and planthoppers, beetles, mealybugs, whiteflies, bugs, thrips, mites), and treats the problems of host-virus-vector relations.

In the 15th chapter, "Ecology", the biological and physical factors as well as the survival through the seasonal cycle are dealt with.

The 16th chapter, "Economic importance and control", is concerned with the losses caused by viruses and with the biological and physical factors influencing them, as well as with the possibilities of control.

In the final 17th chapter, "Nomenclature, classification, origins and evolution", the systems for classifications and the concepts of families, groups, genera and virus species are discussed, and the criteria available for classifying viruses (e.g. nucleic acid, viral proteins, structure of the virus particles, serological relationships, activities in the plant, methods for transmission, etc.) are presented. In conclusion the problems of speculation on origin and evolution, and genome and amino acid sequence similarities between viruses infecting plants and animals are treated.

"Appendix 1" presents the major periodicals and review journals. "Appendix 2" lists in alphabetical order the viruses and viroids as well as their acronyms, furthermore those numbers of the series CMI/AAB (now AAB) Descriptions of Plant Viruses in which the different viruses and viroids are described. Considering the repeated revision of the names of virus groups, it should be noted that, e.g. the tomato-spotted wilt virus to be found on page 693 does not belong to the family/group Tospovirus, but it is a member of the family/group Bunyavirus (tomato-spotted wilt Bunyavirus). "Appendix 2", included in the book on the basis of the work of Hull, R., Milne, R. G. and Van Regensmortel, M. H. V.: "A list of proposed standard acronyms for plant viruses and viroids" in press (see *Archs. Virol.* **120,** 151–164, 1991), today can be used only in comparison to the cited work of Hull et al. (1991).

R. E. F. Matthews' excellent work is an indispensable plant virological handbook containing not only the latest achievements but also the most up-to-date knowledge; it is equally recommended to virologists and undergraduate and graduate students in the field of biology, plant biochemistry, physiology, biotechnology, agronomy, plant breeding and plant pathology.

J. Horváth

Alan Brunt, Karen Crabtree and Adrian Gibbs: *Viruses of Tropical Plants*. C. A. B. International, Wallingford 1990. pp. 707.

The book indispensable for plant virologists contains basic information concerning the viruses isolated from plants growing in the tropics not just at or near sea level but also on tropical hills and mountains. This book is a product of the international VIDE (Virus Identification Data Exchange) project. This project uses the DELTA (Description Language for Taxonomy) database systems to collect diagnostic information on all plant viruses.

It consists of five chapters following the "Preface" and the "List of contributors" (names of 171 participants supplying the data concerning the viruses): 1. *Introduction* (The VIDE database, Layout and use of the book, Plant virus systemics, Literature cited); 2. *Known natural hosts of plant viruses in tropical plants* (Alphabetical listing of infected plant species, Alphabetical listing by families); 3. *Virus descriptions* (Individual viruses); 4. *Virus group descriptions* (Virus groups, Table for virus group identification); 5. *Appendix* (Virus questionnaire, Sources of commercially available diagnostic aids).

The alphabetical listing of the botanical and common names as well as the family status of all the 525 plants infected by the various viruses does special credit to the book. The same applies the 69 plant families.

The description of more than 330 well-defined viruses and of their characteristics is invaluable. The viruses placed in alphabetical order are described according to the following aspects: Introduction, Natural host range and symptoms, Transmission and ecology, Geographical distribution, Experimental host range (diagnostic host species and symptoms, maintenance and propagation host species, species for local lesion or whole plant assay, host plants), Stability and concentration of particles in sap, Purification, Morphology, Physical properties, Chemical composition, Replication, Cytopathology, Serology, Relationships, References, Comments. After the description of the individual viruses by the above aspects the name of the author supplying the data and the year of compilation can be found (e.g. Data collected by H. Jeske, 1987). It is remarkable that in the lists of host plants not only the susceptible (S) but the insusceptible (I) species too are included. Furthermore, among the host species distinction is quite rightly made between species for local (L) lesion and whole (W) plant assay hosts, and special mention is made of the diagnostic host species — if they are latently susceptible. Very useful information is available in the descriptions of virus groups (43 groups or subgroups) too. The identification table for virus groups (see pp. 687–689) — giving the particle shape, particle dimensions (nm), particle details, sap concentrations, sedimentation coefficient, nucleic acid, coat protein into consideration — offers help for grouping the viruses according to the above characteristics.

The work of A. Brunt, K. Crabtree and A. Gibbs: "Viruses of Tropical Plants" represents a unique compilation of data and will be an indispensable work of reference for plant virologists.

J. Horváth

Á. Szentesi and T. Jermy (eds): *Insects — Plants '89*. Proceedings of the 7th International Symposium on Insect — Plant Relationships held in Budapest, Hungary, July 3–8, 1989. Symposia Biologica Hungarica 39. Akadémiai Kiadó, Budapest 1991, 576 pp. Distributors: "KULTURA" Hungarian Foreign Trading Company, P.O. Box 149. H-1389 Budapest. Price: 85,00 Forint.

The reason for having organized the symposium in Budapest is that academician Tibor Jermy and his pupils have studied the system of relations between insects and plants for more than 30 years so successfully that it earned the group great international reputation. The Hungarian organizing committee of the symposium included: Jermy, T. (chairman), Szentesi, Á. (secretary), Demeter, Á., Deseö, G., Horváth, J. and Kozár, F. The International Advisory Board consisted of: Bernays, E. A. (USA), Blaney, W. H. (UK), Hanson, F. E.

(USA), Herrebout, W. M. (Holland), Labeyrie, V. (France) and Schoonhoven, L. M. (Holland). The symposium had 129 participants from 23 countries.

On the insect — plant relationships 47 lectures were delivered in 4 days in 5 sections. The lectures were completed by 54 posters. Then an all-day excursion was made.

The volume contains the lectures in full extension while the posters in abstracts.

The first section was physiology in which 7 lectures discussed the various subjects. The introductory lecture "Gustatory codes in lepidopterous larvae" was held by Simmonds, M. S. J. and Blaney, W. M. (UK). The second section dealt with behavioural questions; Papaj, D. R. (USA) delivered the introductory lecture "Effects of adult experience on host selection behaviour: Consequences for reproductive success". This was followed by further 7 lectures. The 15 lectures of the third section approached the subject from ecological point of view. Here the introductory lecture "Theories of plant chemical defense: A brief historical survey" was held by Feeny, P. P. (USA). In the fourth section the evolution of insect-plant relationships was treated in 10 lectures. The introductory lecture "Phylogeny and host plant specialization: Small ermine moths (Yponomeuta) as an example" was delivered by the Dutch Herrebout, W. M. The 6 lectures of the fifth section dealt with questions of application. The introducer was Lenteren, J. C., a Dutch specialist, the title of his lecture was: Relationship between plant structure, host-plant resistance and biological control.

The subjects of the 4 Workshops were: Adaptations in insect-plant relationships; Evolutionary genetics; Ecology; Host recognition.

The above-listed introductions as well as the subsequent lectures show how intensive study is going on all over the world to explore the relationship between insects and plants. Any information concerning this relationship is of special importance in breeding plants for resistance to insects and in elaborating new, environment sparing methods of control (repellents, attractants).

At the end of the volume the American E. A. Bernays summarizes the essential points of the symposium under the title: General conclusions. It is followed by a list of the participants and closed by an index of keywords. The latter is of great help in getting directions concerning the subjects contained in the volume.

The high quality layout of the volume does great credit to Akadémiai Kiadó (the Publishing House of the Hungarian Academy of Sciences).

Gy. Sáringer

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Acta Phytopathologica et Entomologica Hungarica 28 (2–4), pp. 143–159 (1993)

Use of Somaclonal Variation and *in vitro* Selection for Induction of Plant Disease-Resistance: Prospects and Limitations

KASEM Z. AHMED[1] and FERENC SÁGI[2]

[1]Department of Genetics, Faculty of Agriculture, Minia University, Minia, Egypt
[2]Cereal Research Institute, Szeged, P.O. Box 391, H-6701 Hungary
(Corresponding author)

Somaclonal variation and *in vitro* selection techniques have already been used for obtaining potentially disease-resistant plants in a number of economically important crops. In spite of some encouraging results, the *in vitro* selection for disease-resistance is still in its early stage, due to various unsolved problems. The fundamental knowledges about the role of toxic compounds in pathogenesis, their mode of action and/or their interactions with host cells are still lacking for many host-pathogen systems. Furthermore, the induced resistance at cell level may be lost at intact plant level. Conventional resistance testing of the obtained somaclones and their progeny is very important to confirm the transmission of disease resistance. The related experiences show that not the applied *in vitro* techniques, but the fundamental approaches should be reconsidered to make the *in vitro* selection more efficient for obtaining disease-resistant plants.

The successful crop improvement via plant breeding has two essential prerequisites: sufficient genetic variation and availability of efficient selection procedures (Van den Bulk, 1991). Traditional breeding methods (mutation and wide hybridization) have been used with positive results to induce genetic variation in increased yield, protein quality, disease resistance and other features resulting in a more wide-spread cultivation and higher productivity of many crop plants. However, these classical methods have certain limitations, e.g. narrow natural variability, sexual incompatibility, or too slow breeding advance (Wenzel, 1985; Morrish et al., 1987). Nevertheless, recent developments of the *in vitro* culture may offer a solution to these problems. Variation induced by cell and tissue culture ("somaclonal variation", Larkin and Scowcroft, 1981), as well as *in vitro* selection allows production and identification of individuals having improved or specific traits, utilizable in breeding programs.

The *in vitro* selection techniques have some advantages over *in vivo* selection applied in traditional plant breeding as follows:

i. Screening greater number of individuals with saving space and time.
ii. Increased speed or convenience of screening and earlier production of a new variety.

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iii. Screening under closely controlled environmental conditions.

iv. Testing small amounts of tissue or even single cells instead of whole plants.

v. Increasing of genetic variability (Bright, 1985) and correcting some weaknesses of otherwise valuable genotypes.

However, *in vitro* selection as an *in vitro* culture technique is not free from drawbacks and disadvantages:

i. It is difficult to direct the somaclonal variation and regulate its frequency. Moreover, efficient plant regeneration is often restricted to a few genotypes (Bright, 1985; Daub, 1986; Evans, 1989; Ahmed, 1992).

ii. Genetic instability can be induced and plant regeneration ability can be lost during the *in vitro* culture or selective toxin treatment can reduce regenerative capacity of the calli (Pauly et al., 1987; Latunde-Dada and Lucas, 1988; Yan et al., 1990; Ahmed et al., 1991).

iii. The resistance and other acquired traits present at cell culture level may not be expressed at the regenerated plant level (see later).

iv. The selected resistant phenotype may possess unwanted traits, e.g. morphological or physiological abnormalities (Shahin and Spivey, 1987; Ahmed et al., 1991).

Consequently, production of somaclonal variation and the *in vitro* selection technology must be combined with traditional plant breeding methods. The unconventional *in vitro* techniques never can replace the classical breeding or be independent of it, but they can increase the overall efficiency of the breeding process (Wenzel, 1985; Daub, 1986; Van den Bulk, 1991).

The application of somaclonal variation and *in vitro* selection in crop improvement became more frequent in the last decade including selection for herbicide resistance (Chaleff, 1980; Thomas and Pratt, 1982; Taylor et al., 1989), salt and drought tolerance (Nabors et al., 1982; Nabors and Dykes, 1985), metal tolerance (Smith et al., 1983; Parrot and Bouton, 1990), frost resistance (Galiba and Sutka, 1989), amino acid overproduction (Ságí and Ságí, 1977; Bright, 1985; Kumpaisl et al., 1988) and disease resistance.

In this review, recent results of *in vitro* selection for disease resistance will be discussed more in detail.

In vitro Selection for Disease Resistance

Plant diseases continue to represent major limitations to realize the yield potential in most crops. Application of somaclonal variation and *in vitro* selection techniques for obtaining potentially disease-resistant plants has

been demonstrated in a number of economically important crop plants (see reviews of Wenzel, 1985; Daub, 1986; Widholm, 1988; Said, 1989; Van den Bulk, 1991). When selecting for disease resistance, some agents of known importance in the disease reaction should be used.

1. Selective agents utilized in the in vitro *selection for disease-resistance*

Successful selection for disease resistance can be possible using the pathogens or the toxin(s) produced by the pathogen causing disease symptoms (Widholm, 1988), as selective agents (Table 1).

1.1. Pathogen as the selective agent

This is a rarely approach in *in vitro* selection for disease-resistance, because the pathogen may overgrow the host cells and the culture medium rapidly, hampering the observations (Van den Bulk, 1991). Moreover, uniformity of infection is hard to achieve in a cell culture system (Daub, 1986).

In the majority of these selections, protoplasts and viruses have been involved and then for virus-free regenerated plants were screened (Harrison and Mayo, 1983). Toyoda et al. (1989a) used tobacco axillary buds infected with tobacco mosaic virus (TMV) to induce callus, and after 6 months subculture, healthy shoots were regenerated, from which 3% were highly resistant and 33% moderately resistant to TMV. Fungal pathogens are also used in *in vitro* selection. Pullman and Rappaport (1983) increased the frequency of celery plants resistant to *Fusarium* from tissue cultures by plating small regenerated shoots on a medium precolonized with *F. oxysporum* f. sp. *apii*. Möllers and Sarkar (1989) induced calli from internal stem sections of *Catharanthus roseus* heavily infected with 3 different mycoplasma-like organisms (MLOs). From obtained calli, MLO-free plants could be regenerated which remained healthy for one year in the greenhouse.

1.2. Pathogen metabolites as selective agents

This is a common method to select for disease-resistance *in vitro*. Cultured cells can be exposed easily and uniformly to the toxin by dispersing the cells in a toxin solution or plating them on toxin-containing media (Daub, 1986). Crude culture filtrates or purified toxins have been widely used to select resistant cells in culture. *In vitro* selection via culture filtrates: culture filtrates represent an easy and cheap way to produce pathotoxins which can be incorporated into cell culture media at appropriate concentrations for

Table 1

Examples of disease resistant-plants selsected by *in vitro* methods

Species	Selective agent	Pathogen	Selective target	Result of selection	Transmission to progeny	Reference
Wheat	CF, Crude toxin	Fusarium spp.	C	Resistant plants	Transmitted	Ahmed et al. (1991); Ahmed (1992)
Wheat	CF	Fusarium graminearum	C	Resistant plants	Transmitted	Yan et al. (1990)
Wheat	Syringomcin	Pseudomonas syringae	C	No resistance	–	Pauly et al. (1987)
Wheat	Deoxynivalenol (DON)	Fusarium graminearum	C	No resistance	–	Liu et al. (1991)
Wheat	CF	Helminthosporium sativum	C	Decreased sensitivity	Lost	Chawla and Wenzel (1987a); Wenzel and Foroughi-Weehr (1990)
Barley	CF	Helminthosporium sativum	C	Decreased sensitivity	Lost	Chawla and Wenzel (1987a)
Barley	FA	Fusarium spp.	C	Resistant plants to FA	Lost	Chawla and Wenzel (1987b); Wenzel and Foroughi-Wehr (1990)
Maize	Hm T toxin	Helminthosporium maydis	C	Resistant plants	Transmitted	Gengenbach and Green (1975); Gengenbach et al. (1977); Brettell et al. (1980
Maize	HC toxin	Helminthosporium carbonum	C	No resistance	–	Wolf and Earle (1990)
Rice	Crude toxin	Helminthosporium spp.	C	Increased resistance	Transmitted	Ling et al. (1985); Vidhyasekaran et al. (1990
Oats	Victorin	Helminthosporium victoriae	C	Resistant plants	Transmitted	Rines and Luke (1985)
Sugarcane	Phytotoxin	Helminthosporium sacchari	C	Resistant plants	Transmitted	Heinz et al. (1977); Larkin and Scowcroft (1983)
Tobacco	Pathogen	Tobacco mosaic virus	C	Virus-free plants	Transmitted	Toyoda et al. (1989a)
Tobacco	CF	Fusarium oxysporum	CS	Resistant plants	Nt	Selvapandiyan et al. (1988)
Celery	Pathogen	Fusarium oxysporum	C	Resistant plants	Nt	Pullman and Rappaport (1983)
Potato	CF	Phytophthora infestans	C	Increased resistance	Nt	Behnke (1979)

Eggplant	CF	Verticillium dahliae	CS	Resistant plants	Nt	Rotino et al. (1987)
Hop	CF	Verticillium alboatrum	C	Resistant plants	Nt	Connell and Heale (1987)
Tomato	CF	Pseudomonas solanacearum	C	Delayed symptom expression	Nt	Toyoda et al. (1989b)
Tomato	CF	Fusarium oxysporum	C	Tolerance to CF	Transmitted	Scala et al. (1984)
Tomato	FA	Fusarium spp.	PP	Resistant plants	Transmitted	Shahin and Spivey (1986)
Alfalfa	CF	Fusarium oxysporum	C	Increased resistance	Transmitted	Hartman et al. (1984); McCoy (1988)
Alfalfa	CF	Fusarium oxysporum	CS	Increased resistance	Nt	Binarová et al. (1990)
Alfalfa	CF	Fusarium oxysporum	C	Increased resistance	Nt	Arcioni et al. (1987)
Muskmelon	CF	Fusarium oxysporum	C	No resistance		Mégnégneau and Branchard (1991)
Peach	CF	Xanthomonas campestris	C	Increased resistance	Nt	Hammerschlag (1988)
Catharanthus roseus	Pathogen	MLO	C	MLO-free plants	Nt	Möllers and Sarkar (1989)

Abbreviations: C = Callus, CF = Culture filtrate, CS = Cell suspensions, FA = Fusaric acid, MLO = Mycoplasma-like organisms, Nt = Not tested, PP = Protoplasts.

selection. Culture filtrates of *Phytophthora infestans*, *Phoma lingam*, *Verticillium dahliae*, *V. alboatrum*, *Pseudomonas solanacearum* were successfully used for obtaining resistant calli and regenerated resistant plants in potato (Behnke, 1979), rape (Sacristán, 1982, 1985), eggplant (Rotino et al., 1987), hop (Connell and Heale, 1987), and tomato (Toyoda et al., 1989b), respectively, *Fusarium* culture filtrates have been used to select resistant plants in alfalfa (Hartman et al., 1984; Arcioni et al., 1987; McCoy, 1988; Binarová et al., 1990), potato (Behnke, 1980), tomato (Scala et al., 1984) and tobacco (Selvapandiyan et al., 1988). Furthermore, the resistance has been transmitted to the progeny of resistant plants in all investigated cases (Table 1).

In cereals, barley and wheat calli were screened for resistance to purified culture filtrates of *Helminthosporium sativum* P. K. and B. (Chawla and Wenzel, 1987a). The selection resulted in 6% to 17% surviving calli, from which less sensitive barley and wheat plants were regenerated. In wheat (*Triticum aestivum* L.), Yan et al. (1990) selected scab-resistant lines via culture filtrate technique, and found the resistance being transmitted into the progenies. Some lines were resistant not only to scab, but also to powdery mildew, matured earlier, were shorter and had a better grain quality than the standard variety "Sumai3". The double-layer culture and culture filtrate techniques have been used to select wheat calli for *Fusarium* metabolite-resistance (Ahmed, 1992). Among 79 selected and unselected lines obtained, 20.3% was found to be significantly more resistant than the original cultivars, 50.6% had similar reaction as the source materials and 29.1% was significantly more susceptible than the donors.

Vardi et al. (1986) investigated the suitability of *Phytophthora citrophthora* culture filtrates as *in vitro* selection agents. They found that culture filtrate effects on cells may be due to the action of secondary metabolites, possibly auxin-like substances, although culture filtrate contained toxic components as well. Unfortunately, the *in vitro* selection via culture filtrates or toxins does not assure that plants regenerated from the resistant calli will also be resistant to the pathogen (Binarová et al., 1990).

However, in culture filtrates beside the partially characterized toxins, other metabolites can also be present. Therefore, according to Daub (1986) and Vardi et al. (1986), utilization of culture filtrates is not the best approach for selecting to resistance *in vitro*, but, if no other material is available, culture filtrates can be used providing that some precautions are taken against selecting for resistance to non-specific substances present in the filtrates. *In vitro* selection via purified pathogen toxin(s): the first case of such a selection was reported by Gengenbach and Green (1975). Maize callus with T-cyto-

plasm was treated with partially purified toxin from *Helminthosporium maydis* race T (pathogen of southern corn leaf blight). All plants regenerated were resistant, and after 5 or more cycles of recurrent selection to the toxin and to the fungus, the resistance was inherited maternally (Gengenbach et al., 1977). Hm T toxin was also used by Brettell et al. (1980) to select resistant maize calli derived from another genotypes, and resistant regenerated plants were obtained, which transmitted the resistance into their progenies. However, Wolf and Earle (1990) failed to select or regenerate any resistant plants from calli of susceptible maize genotypes by using the HC toxin of *H. carbonum* race 1.

In rice, Ling et al. (1985) and Vidhyasekaran et al. (1990) selected calli resistant to the crude toxin of *Helminthosporium* spp., and showed an increased resistance to *H. oryzae* in the regenerated plants and their progeny.

For obtaining oats resistant to *H. victoriae*, Rines and Luke (1985) used victorin to select resistant calli, from wich resistant plants were recovered. The resistance has been transmitted to the following generations.

Increased resistance to *H. sacchari* in sugarcane has been obtained by using its phytotoxin for selecting calli. Resistance could be detected in regenerated plants and their progenies after vegetative propagation (Heinz et al., 1977; Larkin and Scowcroft, 1983).

In case of wheat, Pauly et al. (1987) subjected calli to syringomycin as selective agent for induction of *Pseudomonas syringae* pv. *syringae*-resistance. The inhibition of callus growth provided a means to select for resistance. No stable resistant cultures were obtained during the period, in which regenerable cultures could be maintained. Shahin and Spivey (1986) used fusaric acid, a pathotoxin of *Fusarium* spp., to select resistant tomato protoplasts and observed *Fusarium*-resistance in the regenerated plants. In contrast, Chawla and Wenzel (1987b) obtained resistant barley plants after 4 callus selection cycles in culture medium containing fusaric acid, but resistance in the progenies of these plants did not vary in that extent, as in the first *in vitro* generation, and *Fusarium*-resistance of the selected, regenerated plants was not tested. Lui et al. (1991) used pure or crude deoxynivalenol (DON), a toxin from *Fusarium graminearum*, and found that DON did not have any effect in selecting resistant mutants, but it acted as a growth regulator.

2. *Procedural problems encountered in the* in vitro *selection of disease-resistance*

There is a number of technical problems which must be overcome when pathogen toxins will be used as selective agents.

Pathogen metabolites may be atoxic, toxins can be present, but not

characterized or there is no information about the role of a toxin in pathogenesis (Wenzel, 1985; Daub, 1986; Vardi et al., 1986; Van den Bulk, 1991).

2.1. *Role of pathogen toxin in the* in vitro *selection*

It should be emphasized that fundamental knowledges about the role of toxic components in pathogenesis, their mode of action, properties of toxins and/or their interactions with host cells are still lacking for many host-pathogen systems (Behnke, 1980; Sacristán, 1982; Binarová et al., 1990; Van den Bulk, 1991, Ahmed 1992). Mégnégneau and Branchard (1991) studied the possibility of selecting muskmelon plants resistant to *Fusarium* wilt via the culture filtrate technique. They concluded that some toxic components of the filtrates play a limited role in pathogenesis and these toxic agents cannot be used to distinguish resistant from susceptible plant material *in vitro*. On the other hand, toxins present in the culture filtrates may not be identical with *Fusarium* toxins secreted *in vivo*.

The *in vitro* selection with host-specific toxins is an elegant method that can result in plants possessing qualitative, usually monogenic disease resistance. Plants with this type of resistance generally show complete resistance to a specific pathogen. However, mutations of the pathogen that can break down this resistance may occur. Although quantitative, polygenic resistance in plants is usually more durable, however, selection of plants exhibiting only partial resistance still might be of interest for resistance breeding. Therefore, non-specific toxins can particularly be valuable as selective agents (Wenzel, 1985; Daub, 1986; Van den Bulk, 1991).

2.2. *Relationship between resistance at cell level and intact plant level*

Resistance to a toxin may be or may not be expressed at cell level as a positive or negative relationship with resistance to the pathogen in both source material and regenerated plants.

Excellent correlation has been found between resistance to the pathogen at plant level and insensitivity to the toxin at cellular level in different plant species/pathogen systems, e.g. maize/*Helminthosporium maydis* race T (Pelcher et al., 1975; Wolf and Earle, 1990), hop/*Verticillium albo-atrum* (Connell et al., 1990), alfalfa/*Fusarium* spp. (Arcioni et al., 1987; Binarová et al., 1990), alfalfa/*Verticillium albo-atrum* (Frame et al., 1991), elm/*Ceratocystis ulmi* (Pijut et al., 1990), tomato/*Alternaria alternata* f. sp. *lycopersici* (Barsel, 1981), sunflower/*Phomopsis* spp. (Masirevec et al., 1988), barley/*Fusarium* spp., barley and wheat/*Helminthosporium sativum*

(Chawla and Wenzel, 1987a, b), and bread wheat/*Fusarium graminearum* (Yan et al., 1990; Lui et al., 1991; Ahmed et al., unpublished).

In contrast, Kumashiro (1983) selected tobacco cell cultures for resistance to tenuazonic acid, a non specific toxin produced by *Alternaria alternata* thought to play a role in the tobacco brown spot disease. Plants regenerated from the resistant cells, however, were susceptible to the toxin. When cells were isolated from these plants and put back in culture, they expressed toxin resistance again. In another example, Mégnégneau and Branchard (1991) failed to observe a correlation between the resistance genes to *Fusarium oxysporum* f. sp. *melonis* and the susceptibility of explants to the fungal culture filtrate in muskmelon (*Cucumis melo* L.).

In the barley/rhynchosporoside (Branchard, 1982) and sorghum/*Periconia circinata* (Earle, 1983) systems, no relationship has been found between reaction of the plants to the pathogens under tissue culture conditions and the reaction of the intact plants *in vivo* or vice versa.

3. *Factors affecting the success of* in vitro *selection for disease-resistance*

There are several factors which affect the efficiency of *in vitro* selection, as well as the nature of the variants induced by tissue culture (Karp and Bright, 1985).

3.1. *Plant genotype and disease*

Daub and Jenns (1989) reported that disease resistance in the *in vitro* culture of tobacco depended on the pathogen as well as on the genotype. Wright and Lacy (1988) obtained plants highly resistant to *Fusarium* yellows with much higher frequency from moderately resistant celery cultivars than from highly susceptible ones. Dependence of *in vitro* selection results on the genotype has been mentioned by Chawla and Wenzel (1987a, b), Mégnégneau and Branchard (1988), Wolf and Earle (1990), Yan et al. (1990) and Ahmed et al. (1991), too.

3.2. *Selection target and regeneration*

For selection and screening, different plant parts can be used, such as leaf slices, root pieces, developing inflorescences, florets, caryopses, mature or immature embryos, seeds or callus cultures of various somatic parts, e.g. anthers and immature leaves, as well as single cells in cell suspensions, protoplasts and pollen grains (Table 1). The physiological status, age and

form of the organs used in the *in vitro* selection strongly affect the efficiency of selection.

Nachmias et al. (1990) observed that protoplasts of a susceptible potato genotype were unaffected by the Vd toxin of *Verticillium dahliae*, whereas suspension cells were affected.

Wolf and Earle (1990) failed to recover resistant tissues and plants from susceptible maize callus selected by HC toxin. They explained the failure by use of a highly organized callus type not suitable for *in vitro* selection. Not only the type of callus was important in the study of Wolf and Earle (1990), but the callus size also, as the smaller calli were more accessible to toxin penetration influencing the efficiency of selection. Regenerated plants can otherwise be raised from susceptible cells of putative resistant calli, which escaped from toxin effects by partial exposure or protected by proximity of the resistant cells (Hammerschlag, 1988).

3.3. Selective medium and culture condition

The expression of resistance at cell and tissue level may be influenced strongly by medium composition, e.g. hormones (Briggs et al., 1984; Meulemans et al., 1986) and culture conditions, e.g. temperature (Haberlach et al., 1978; Hollidy and Klarman, 1979; Briggs et al., 1984).

3.4. In vitro *selection protocol*

The duration of the selection pressure, i.e. number of the selection cycles can considerably affect the outcome of *in vitro* selection experiments with culture filtrates of toxins (Van den Bulk, 1991). Chawla and Wenzel (1987b) found with barley and wheat that the discontinuous method with an additional subculture on toxin-free medium was superior and allowed the calli to regain their regeneration ability compared to the continuous selection. However, a longer selection period may induce more chromosomal abnormalities and a decreased regeneration ability (Thanutong et al., 1983; Hartman et al., 1984; Wolf and Earle, 1990).

4. Natural somaclonal variation vs. in vitro *somaclonal variation induced by selection*

Shahin and Spivey (1986) found that number of *Fusarium*-resistant plants obtained from non-selected tomato calli was of the same order as in the regenerants originated from fusaric acid-selected calli. In other *in vitro*

selection studies, resistant plants have also been obtained from control calli, to which no selection was applied, e.g. in sugarcane (Heinz et al., 1977), maize (Brettell et al., 1980), rape (Sacristán, 1982, 1985, MacDonald and Ingram, 1986), rice (Ling et al., 1985), celery (Heath-Pagliuso et al., 1988, 1989, Heath-Pagliuso and Rappaport, 1990), tomato (Toyoda et al., 1989b), and alfalfa (Binarová et al., 1990). *Fusarium* resistant plants have been obtained by screening somaclones and their progenies at the plant level (without *in vitro* selection), e.g. in alfalfa against *F. solani* and *F. oxysporum* f. sp. *medicaginis* (Binarová et al., 1990), in banana/*F. oxysporum* (Hwang and Ko, 1988), tomato/*F. oxysporum* f. sp. *lycopersici* (Shahin and Spivey, 1986, 1987), and in asparagus/*Fusarium* spp. combinations (Jullien, 1988).

Toyoda et al. (1991) also obtained two resistant strawberry lines from 1,225 unselected regenerants tested in a field heavily infested with *F. oxysporum* f. sp. *fragariae*.

In the study of Ahmed (1992), somaclones unselected *in vitro* produced higher number of resistant lines (28.3%) than the selected somaclones (16.7%). Some of the *Fusarium* metabolites or the interference between them may play a role in inhibition of the efficient *in vitro* selection for *Fusarium* metabolite-resistance causing lower regeneration frequency in the selected than in the unselected populations.

In a number of studies, selection for disease-resistance via application of toxic compounds was not successful. Pauly et al. (1987) failed to obtain syringomycin-resistant wheat calli, although bacterial syringomycin toxin is an effective selecting agent for resistance at cellular level. Similar findings have been reported in tomato (Gavazzi et al., 1987) and tobacco (Daub and Jenns, 1989), where selected somaclones resistant to TMV, *Verticillium* wilt or the nematode *Meloidogyne incognita* could not be obtained. Van den Bulk et al. (1991) also failed to produce tomato somaclones resistant to TMV or bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) by *in vitro* selection.

On the other hand, results of Ling et al. (1985), Toyoda et al. (1989a, b) and Binarová et al. (1990) show that after an *in vitro* preselection, increase in the percentage of resistant regenerants can be expected. In the studies of Sacristán (1982, 1985) with the rape/*Phoma lingam* system, 22% of the regenerants from selected cultures exhibited resistance or tolerance, while from the control cultures only 4% of regenerated plants became more tolerant.

Looking at the *in vitro* selection studies which failed to increase the frequency of resistant regenerants, it can be supposed that the toxic compounds used either did not play an important role in pathogenesis (Lui et al., 1991) or the protocol used for selection was inappropriate, e.g. applied at the wrong developmental stage (Van den Bulk, 1991).

When we make comparison between somaclonal variants without (Toyoda et al., 1991) and with (Chawla and Wenzel, 1987a, b; Toyoda et al., 1989b) *in vitro* selection as sources for disease-resistant materials, the screening procedure for putative resistant plants should be considered also from economical point of view. *In vitro* selection significantly reduce the total number of regenerated plants.

5. *Heritability of resistance obtained through somaclonal variation and* in vitro *selection techniques*

Somaclonal variation may be induced by gross changes in the chromosomal number and structure (Karp and Bright, 1985; Phillips et al., 1990). These chromosomal abnormalities may result in stable alterations, which can be transmitted sexually to the progeny. However, the selective growth of certain cell lines *in vitro* may not always be due to genetic change, as enhanced growth may equally be the consequence of physiological/epigenetic adaptation (Meins, 1983). Furthermore, the plant regeneration protocols may lead to the production of chimeras, if the plants are of multicellular origin (Morrish et al., 1987). Therefore, production of true mutants via *in vitro* technology must involve progeny testing through a number of generations to confirm the heritability of the variation.

Dominant and homozygous recessive traits can directly be selected. If the regenerants are heterozygous for disease-resistance character, resistant plants can only detected in the progeny (Van den Bulk, 1991).

Majority of publications dealing with *in vitro* selection and applying the inheritance test showed that in most cases the induced resistance is inherited in a stable manner (Wenzel, 1985; Daub, 1986; Van den Bulk, 1991; Ahmed, 1992, see Table 1). However, Wenzel and Foroughi-Wehr (1990) found that some progenies of several barley, wheat and potato regenerants selected for resistance to *Helminthosporium*, *Fusarium* or *Phytophthora* toxins did not express detectable resistance differences, as observed in the first *in vitro* generation. The regenerants did not differ significantly in their level of susceptibility from the susceptible starting material, and most probably, the first *in vitro*-grown somaclones tested were not uniform enough and the selection or the screening protocol were inadequate. Unfortunately, many *in vitro* selection studies do not give proof of stability and heritability of the obtained resistance (Table 1).

In vitro selection for disease resistance has resulted in the release of a few new cultivars or germplasms only. A new sugarcane cultivar with resistance to Fiji disease (Heinz et al., 1977), and the tomato variety "DNAP-

17" from a somaclonal variant with monogenic *Fusarium* wilt race 2 resistance (Evans, 1989) have been developed *in vitro*. Heath-Pagliuso et al. (1988, 1989), Heath-Pagliuso and Rappaport (1990) described the celery line "UT-T3 somaclone" with resistance to *Fusarium oxysporum* f. sp. *apii* for use by celery breeders.

Plant genetic transformation has already led and will probably lead to further development of plant breeding and a better understanding of basic mechanism involved in plant gene regulation (Weising et al., 1988). Gene transfer enables to introduce foreign genes, or specifically designed hybrid genes into host plant genomes, thus creating novel varieties with specifically designed characters. Recently, it has been demonstrated that plants can be genetically engineered for herbicide tolerance, virus resistance, and tolerance against insects and fungi by genetic manipulation (Gasser and Fraley, 1992). In the future the transfer of other, agronomically important, multigenic traits, e.g. yield and food quality (Fischhoff, 1989) can be expected, if gene isolation methods will be improved and regulation of foreign gene expression will be better understood. Until that, however, *in vitro* selection and somaclonal variation can create new, useful plant materials, supporting the efforts of and furnishing suitable variants for resistance breeding (Evans, 1989).

Literature

Ahmed, K. Z. (1992): *In vitro* selection and genetic transformation of wheat (*Triticum aestivum* L.). Ph. D. Thesis, Hungarian Acad. Sci. Budapest, Hungary, 127 pp.

Ahmed, K. Z., Mesterházy, Á. and Sági, F. (1991): *In vitro* techniques for selecting wheat (*Triticum aestivum* L.) for *Fusarium*-resistance I. Double-layer culture technique. Euphytica, 57, 251–257.

Arcioni, S., Pezzotti, M. and Damiani, F. (1987): *In vitro* selection of alfalfa plants resistant to *Fusarium oxysporum* f. sp *medicaginis*. Theor. Appl. Genet. 74, 700–705.

Barsel, S. (1981): Effects of host-specific toxin from *Alternaria alternata* f. sp. *lycopersici* on suspension cultures of *Lycopersicon esculentum*. MS Thesis, Michigan State Univ., 90 pp. (Cited after Daub 1986).

Behnke, M. (1979): Selection of potato callus resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. Theor. Appl. Genet. 55, 69–71.

Behnke, M. (1980): Selection of dihaploid potato callus for resistance to the culture filtrate of *Fusarium oxysporum*. Z. Pflanzenzüchtg. 85, 254–258.

Binarová, P., Nedelnik, J., Fellner, M. and Nedbálková, B. (1990): Selection for resistance to filtrates of *Fusarium* spp. in embryogenic cell suspension culture of *Medicago sativa* L. Plant Cell, Tissue and Organ Culture 22, 191–196.

Branchard, M. (1982): *In vitro* culture of barley: A method to study *Rhynchosporium* scald disease and select plants resistant to the toxin rhynchosporoside. In: Earle, E. D. and Demarly, Y. (eds): Variability in Plants Regenerated from Tissue Culture, Praeger, New York, 343–350.

Brettell, R. I. S., Thomas, E. and Ingram, D. S. (1980): Reversion of Texas male-sterile cytoplasm maize in culture to give fertile T-toxin resistant plants. Theor. Appl. Genet. 58, 55–58.

Briggs, S. P., Scheffer, R. P. and Haug, A. R. (1984): Selective toxin of *Helminthosporium victoriae:* Thermal relationships in effects on oat tissues and protoplasts. Phytopathol. 74, 768–773.

Bright, S. W. J. (1985): Selection *in vitro*. In: Bright, S. W. J. and Jones M. G. K. (eds): Cereal Tissue and Cell Culture, Nijhoff/Junk Publishers, Dordrecht. 231–260.

Chaleff, R. S. (1980): Further characterization of picloram tolerant mutants of *Nicotiana tabacum*. Theor. Appl. Genet. 58, 91–95.

Chawla, H. S. and Wenzel, G. (1987a): *In vitro* selection of barley and wheat for resistance against *Helminthosporium sativum*. Theor. Appl. Genet. 74, 841–845.

Chawla, H. S. and Wenzel, G. (1987b): *In vitro* selection for fusaric acid resistant barley plants. Plant Breeding 99, 155–163.

Connell, S. A. and Heale, J. B. (1987): Use of tissue culture to produce novel source of resistance to *Verticillium albo-atrum* attacking hop. Can. J. Plant Pathol. 9, 81 (Abstr.).

Connell, S. A., Legg, T. and Heale, J. B. (1990): Sensitivity of cells and protoplasts of hop cultivars to cytotoxic components of culture filtrates of *Verticillium albo-atrum* isolates from hop. Plant Pathol. 39, 92–101.

Daub, E. M. (1986): Tissue culture and the selection of resistance to pathogens. Ann. Rev. Phytopathol. 24, 159–186.

Daub, E. M. and Jenns, A. E. (1989): Field and greenhouse analysis of variation for disease resistance in tobacco somaclones. Phytopathol. 79, 600–605.

Earle, E. D. (1983): The use of phytotoxins for *in vitro* selection of disease resistant plants. In: Cell and Tissue Culture Techniques for Cereal Crop Improvement. Science, Beijing, 265–278.

Evans, D. A. (1989): Somaclonal variation – genetic basis and breeding application. Trends in Genet. 5, 46–50.

Fischhoff, D. A. (1989): Applications of plant genetic engineering to crop protection. Phytopathol. 79, 38–40.

Frame, B., Yu, K.-F., Christie, B. R. and Pauls, K. P. (1991): *In vitro* selection for resistance to *Verticillium* wilt in alfalfa (*Medicago sativa* L.) using a fungal culture filtrate. Physiol. Mol. Plant Pathol. 39, 325–340.

Galiba, G. and Sutka, J. (1989): Frost resistance of somaclones derived from *Triticum aestivum* L. winter wheat calli. Plant Breeding 102, 101–104.

Gasser, C. S. and Fraley, R. T. (1992): Transgenetic crops. Scientific American 266, 62–69.

Gavazzi, G., Tonelli, C., Todesco, G., Arreghini, E., Raffaldi, F., Vecchio, F., Barbuzzi, G., Biasini, M. G. and Sala, F. (1987): Somaclonal variation versus chemically induced mutagenesis in tomato (*Lycopersicon esculentum* L.). Theor. Appl. Genet. 74, 733–738.

Gengenbach, B. G. and Green, C. E. (1975): Selection of T-cytoplasm maize callus cultures resistant to *Helminthosporium maydis* race T pathotoxin. Crop Sci. 15, 645–649.

Gengenbach, B. G., Green, C. E. and Donovan, C. M. (1977): Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. USA 74, 5113–5117.

Haberlach, G. T., Budde, A. D., Sequeira, L. and Helgeson, J. P. (1978): Modification of disease resistance of tobacco callus tissues by cytokinins. Phytopathol. 62, 522–525.

Hammerschlag, F. A. (1988): Selection of peach cells for insensivity to culture filtrates of *Xanthomonas campestris* pv. *pruni* and regeneration of resistant plants. Theor. Appl. Genet. 76, 865–869.

Harrison, G. D. and Mayo, M.A. (1983): The use of protoplasts in plant virus research. In: Helgeson, J. P. and Deverall, B. J. (eds): Use of Tissue Culture and Protoplasts in Plant Pathology; Academic Press, New York, 67–137.

Hartman, C. L., McCoy, T. J. and Knous, T. R. (1984): Selection of alfalfa (*Medicago sativa*) cell lines and regeneration of plants resistant to the toxin(s) produced by *Fusarium oxysporum* f. sp. *medicaginis*. Plant Sci. Lett. 34, 183–194.

Heath-Pagliuso, S., Pullman, J. and Rappaport, L. (1988): Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f. sp. *apii*. Theor. Appl. Genet. 75, 446–451.

Heath-Pagliuso, S., Pullman, J. and Rappaport, L. (1989): "UC-T3 somaclone": Celery germplasm resistant to *Fusarium oxysporum* f. sp. *apii*, race 2. Hort. Sci. 24, 711–712.

Heath-Pagliuso, S. and Rappaport, L. (1990): Somaclonal variant UC-T3: the expression of *Fusarium* wilt in progeny arrays of celery, *Apium graveolens* L. Theor. Appl. Genet. 80, 390–394.

Heinz, D. J., Krishnamurthi, M., Nickell, L. G. and Maretzki, A. (1977): Cell, tissue, and organ culture in sugarcane improvement. In: Reinert, J. and Bajaj, Y. P. S. (eds): Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture, Springer-Verlag, Berlin, 3–17.

Hollidy, M. J. and Klarman, W. L. (1979): Expression of disease reaction types in soybean callus from resistant and susceptible plants. Phytopathol. 69, 576–578.

Hwang, S. C. and Ko, W. H. (1988): *In vitro* somaclonal variation in banana and its application for screening for resistance to fusarial wilt. Tech. Bull. Food and Fertilizer Technology Center for the Asian and Pacific Region No. 107, 8 pp.

Jullien, M. (1988): Effects of the *Fusarium* sp. toxins and selection of crude toxin resistant strains in mesophyll cell cultures of *Asparagus officinalis*. Plant Physiol. Biochem. 26, 713–721.

Karp, A. and Bright, S. W. J. (1985): On the causes and origins of somaclonal variation. In: Miflin, B. J. (ed.): Oxford Surveys of Plant Molecular and Cell Biology; Oxford Univ. Press, Oxford, Vol. 2, 199–234.

Kumashiro, T. (1983): Selection for tenuazonic acid tolerant cells of tobacco and characteristics of the regenerates. Japan J. Breeding 33 (Suppl. 1), 194–198.

Kumpaisl, R., Hoshimoto, T. and Yamada, Y. (1988): Selection and characterization of S–(2-aminoethyl)-L-cysteine-resistant wheat cell cultures. J. Plant Physiol. 133, 608–614.

Larkin, P. J. and Scowcroft, W. R. (1981): Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60, 197–212.

Larkin, P. J. and Scowcroft, W. R. (1983): Somaclonal variation and eyespot toxin tolerance in sugarcane. Plant Cell, Tissue and Organ Culture 2, 111–121.

Latunde-Dada, A. O. and Lucas, J. A. (1988): Somaclonal variation and resistance to *Verticillium* wilt in lucerne, *Medicago sativa* L., plants regenerated from callus. Plant Sci. 58, 111–119.

Ling, D. H., Vidhyasekharan, P., Borromeo, E. S., Zapata, F. J. and Mew, T. W. (1985): *In vitro* screening of rice germplasm for resistance to brown spot disease using phytotoxin. Theor. Appl. Genet. 7, 133–135.

Lui, Z. Z., Chen, Q. Q., Yao, Q. H., Huang, X. M., Lu, S. H., Wei, C. M., Sun, X. J., Zhang, F. Q. and Tang, Y. L. (1991): Growth hormone-like action of deoxynivalenol toxin on induction and differentiation of wheat calluses [in Chinese]. Acta Agriculturae Shanghai 7 (Suppl.), 1–7.

MacDonald, M. V. and Ingram, D. S. (1986): Towards the selection *in vitro* for resistance to *Alternaria brassicicola* (Schw.) wilts, in *Brassica napus* spp. *oleifera* (Metzg.) Sinks., winter oilseed rape. New Phytol. 104, 621–629.

Masirevec, S. N., Secor, G. A. and Gulya, T. J. (1988): Use of cell culture to screen sunflower germplasm for resistance to *Phomopsis* brown/gray stem spot. Plant Cell Rep. 7, 528–530.

McCoy, T. J. (1988): Tissue culture selection for disease resistant plants. Iowa State J. Res. 62, 503–521.

Mégnégneau, B. and Branchard, M. (1988): Toxicity of fusaric acid observed on callus cultures of various *Cucumis melo* genotypes. Plant Physiol. Biochem. 26, 585–588.

Mégnégneau, B. and Branchard, M. (1991): Effects of fungal culture filtrates on tissue from susceptible and resistant genotypes of muskmelon to *Fusarium oxysporum* f. sp. *melonis*. Plant Sci. 79, 105–110.

Meins, F. (1983): Heritable variation in plant cell culture. Ann. Rev. Plant Physiol. 34, 327–346.

Meulemans, M., Duchene, D., Tegera, P. and Fouarge, G. (1986): Selection of variants by dual cultures of potato and *Phytophthora infestans*. In: Semal, J. (ed.): Somaclonal Variations and Crop Improvement, Nijhoff, Dordrecht, 219–223.

Möllers, C. and Sarkar, S. (1989): Regeneration of healthy plants from *Catharanthus roseus* infected with mycoplasma-like organisms through callus culture. Plant Sci. 60, 83–89.

Morrish, F., Vasil, V. and Vasil, I. K. (1987): Developmental morphogenesis and genetic manipulation in tissue and cell cultures of the *Gramineae*. Adv. Genet. 24, 431–499.

Nabors, M. W. and Dykes, T. A. (1985): Tissue culture of cereal cultivars with increased salt, drought, and acid tolerance. In: Biotechnology in International Agricultural Research. Intl. Rice Res. Inst., Manila, Philippines, 121–138.

Nabors, M. W., Kroskey, C. S., McHugh, D. M. (1982): Green spots are predictors of high callus growth rates and shoot formation in normal and in salt stressed tissue cultures of oat (*Avena sativa* L.). Z. Pflanzenphysiol. 105, 341–349.

Nachmias, A. J., Orenstein, M. T. and Goren, M. (1990): Reactions to *Verticillium dahliae* phytotoxin in tissue cultures derived from susceptible and tolerant potato. Plant Sci. 68, 123–130.

Parrot, W. A. and Bouton, J. H. (1990): Aluminum tolerance in alfalfa as expressed in tissue culture. Crop Sci. 30, 387–389.

Pauly, M. H., Shane, W. W. and Gengenbach B. G. (1987): Selection for bacterial blight phytotoxin resistance in wheat tissue culture. Crop Sci. 27, 340–344.

Pelcher, L., Kao, K. N., Gamborg, O. L., Yoder, O. C. and Gracen, V. E. (1975): Effects of *Helminthosporium maydis* race T toxin of protoplasts of resistant and susceptible corn (*Zea mays*). Can. J. Bot. 53, 427–431.

Phillips, R. L., Kaeppler, S. M. and Peschke, V. M. (1990): Do we understand somaclonal variation ? In: Nijkamp, H. J. J., Van der Plas, L. H. W. and Van Aartrijk, J. (eds): Progress in Plant Cellular and Molecular Biology, Kluwer Academic Publ., Dordrecht, 131–141.

Pijut, P. M., Domir, S. C., Lineberger, R. D. and Schreiber, L. R. (1990): Use of culture filtrate of *Ceratocystis ulmi* as a bioassay to screen for disease tolerant *Ulmus americana*. Plant Sci. 70, 191–196.

Pullman, G. S. and Rappaport, L. (1983): Tissue culture-induced variation in celery for *Fusarium* yellows. Phytopathol. 73, 818 (Abstr.).

Rines, H. W. and Luke, H. H. (1985): Selection and regeneration of toxin-insensitive plants from tissue cultures of oat (*Avena sativa*) susceptible to *Helminthosporium victoriae*. Theor. Appl. Genet. 71, 16–21.

Rotino, G. L., Falavigna, A. and Restaino, F. (1987): *In vitro* selection of eggplant cells resistant to culture filtrate of *Verticillium dahliae* Kleb. and regeneration of plants. Capsicum Newslett. 6, 94–95.

Sacristán, M. D. (1982): Resistance responses to *Phoma lingam* of plants regenerated from selected cell and embryogenetic cultures of haploid *Brassica napus*. Theor. Appl. Genet. 61, 193–200.

Sacristán, M. D. (1985): Selection for disease resistance in *Brassica* cultures. Hereditas (Suppl.) 3, 57–63.

Sági, F. and Sági, H. (1977): Comparison of some methods of testing cereal plants for resistance to lysine-feedback. In: Novák, F. J. (ed.): Use of Tissue Cultures in Plant Breeding, Proc. Intl. Symp. 6–11 September, 1976, Olomouc, Czechoslovakia, 265–272.

Said, A. G. E. (1989): The role of tissue culture in selecting plants resistant to and plants free from diseases (in Arabic). J. King Saud Univ., Agric. Sci. 1, 101–118.

Scala, A., Bettini, P., Buiatti, M., Bogani, P., Pellegrini, G. and Tognoni, F. (1984): *In vitro* analysis of the tomato-*Fusarium oxysporum* system and selection experiments. In: Novák, F. J., Havel, L. and Dolezel, J. (eds): Plant Tissue Culture Applications to Crop Improvement, Czechosl. Acad. Sci. Prague, 361–362.

Selvapandiyan, A., Mehta, A. R. and Bhatt, P. N. (1988): Cellular breeding approach for development of *Fusarium* wilt resistant tobacco. Proc. Ind. Natl. Sci. Acad. B 54, 391–394.

Shahin, E. A. and Spivey, R. (1986): A single dominant gene for *Fusarium* wilt resistance in protoplast-derived tomato plants. Theor. Appl. Genet. 73, 164–169.

Shahin, E. A. and Spivey, R. (1987): *In vitro* breeding for disease resistance in tomato. In: Nevins, D. J. and Jones, R. A. (eds): Tomato Biotechnology, Alan R. Liss, New York, 89–97.

Smith, R. H., Bhaskaran, S. and Schertz, K. (1983): Sorghum plant regeneration from aluminum selection media. Plant Cell Rep. 2, 129–132.

Taylor, S. G., Shilling, D. G. and Quesenberry, K. H. (1989): *In vitro* selection for 2,4-D tolerance in red clover. Theor. Appl. Genet. 78, 265–270.

Thanutong, P., Furusawa, I. and Yamamoto, M. (1983): Resistant tobacco plants from protoplast-derived calluses selected for their resistance to *Pseudomonas* and *Alternaria* toxins. Theor. Appl. Genet. 66, 209–215.

Thomas, B. R. and Pratt, D. (1982): Isolation of paraquat-tolerant mutants from tomato cell cultures. Theor. Appl. Genet. 63, 169–176.

Toyoda, H., Chatani, K., Matsuda, Y. and Ouchi, S. (1989a): Multiplication of tobacco mosaic virus in tobacco callus tissues and *in vitro* selection for viral disease resistance. Plant Cell Rep. 8, 433–436.

Toyoda, H., Horikoshi, K., Yamano, Y. and Ouchi, S. (1991): Selection for *Fusarium* wilt disease resistance from regenerants derived form leaf callus of strawberry. Plant Cell Rep. 10, 167–170.

Toyoda, H., Shimizu, K., Chatani, K., Kita, N., Matsuda, Y. and Ouchi, S. (1989b): Selection of bacterial wilt–resistant tomato through tissue culture. Plant Cell Rep. 8, 317–320.

Van den Bulk, R. W. (1991): Application of cell and tissue culture and *in vitro* selection for disease resistance breeding – a review. Euphytica 65, 269–285.

Van den Bulk, R. W., Jansen, J., Lindhout, W. H. and Löffler, H. J. M. (1991): Screening of tomato somaclones for resistance to bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*). Plant Breeding 107, 190–196.

Vardi, A., Epstein, E. and Breiman, A. (1986): Is the *Phytophthora citrophthora* culture filtrate a reliable tool for the *in vitro* selection of resistant citrus variants? Theor. Appl. Genet. 72, 569–574.

Vidhyasekaran, P., Ling, D. H., Borromeo, E. S., Zapata, F. J. and Mew, T. M. (1990): Selection of brown spot-resistant rice plants from *Helminthosporium oryzae* toxin-resistant calluses. Ann. Appl. Biol. 117, 515–523.

Weising, K., Schell, J. and Kahl, G. (1988): Foreign genes in plants: transfer, structure, expression, and applications. Ann. Rev. Genet. 22, 421–477.

Wenzel, G. (1985): Strategies in unconventional breeding for disease resistance. Ann. Rev. Phytopathol. 23, 149–172.

Wenzel, G. and. Foroughi-Wehr, B. (1990): Progeny tests of barley, wheat, and potato regenerated from cell cultures after *in vitro* selection for disease resistance. Theor. Appl. Genet. 80, 359–369.

Widholm, J. M. (1988): *In vitro* selection with plant cell and tissue cultures: an overview. Iowa State J. Res. 62, 587–597.

Wolf, S. J. and Earle, E. D. (1990): Inhibition of corn callus growth by *Helminthosporium carbonum* race 1 toxin. Crop Sci. 30, 728–734.

Wright, J. C. and Lacy, M. L. (1988): Increase of disease resistance in celery cultivars by regeneration of whole plants from cell suspension cultures. Plant Dis. 72, 256–259.

Yan, Z., Cuilan, Z., Yuwen, W. and Shuxin, R. (1990): Scab-resistant wheat breeding by cell engineering. Proc. Intl. Symp., Wheat Breeding-Prospects and Future Approaches, June 4–8, 1990, Albena, Bulgaria, 136–138.

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Barley Responses to *Erysiphe graminis* f. sp. *hordei* (Marchal) Attack in the Preparasitic Stage of their Interaction

FRIDRICH FRIČ and LADISLAV TAMÁS

Institute of Botany, Slovak Academy of Sciences, 842 23 Bratislava, Dúbravská 14, Slovakia

This review presents the main biochemical and physiological symptoms of barley cells in the presence of *Erysiphe graminis, hordei* infection structures. Several mechanisms may operate within barley cells affecting growth and spread of the fungus. There is a marked need to focus attention on the prepenetration and preparastitic phase of their interaction to get insights into induced metabolical and physiological processes in the host plant. It is likely that some biochemical reactions or physiological processes in the preparasitic phase of barley – *E. graminis* f. sp. *hordei* interaction may induce resistance mechanisms, which are still poorly understood. Papers accumulated in the last decade show that there is a new search for "early" metabolical and physiological events to characterize biochemical and physiological processes determining or controlling the degree of host-parasite compatibility or incompatibility.

In the last decades much efford has been devoted to physiological and biochemical processes in plants infected by obligate parasitic fungi. A lot of papers accumulated in this topics. Although much is known about some metabolical responses of attacked plants or cells but the mechanisms responsible for host-parasite compatibility or incompatibility are still poorly understood.

Physiological and biochemical processes in host plants or cells leading to host-parasite compatibility or incompatibility can be advantageously studied in plants attacked by true obligate parasitic fungi.

In this review we wish to focus attention to an obligate parasitic fungus *Erysiphe graminis* f. sp. *hordei*, Marchal (powdery mildew) and to its host (barley). This article is not a detailed review about barley – *E. graminis* f. sp. *hordei* interaction, but rather an attempt to point out an array of physiological and metabolical processes in host plants i.e. cells induced by the fungus in the preparasitic phase of the interaction. One can suppose that the rate of activation, the capacity and the intensity of some of these metabolical events induced in barley cells can lastly influence the degree of host-parasite incompatibility.

Host-parasite Interaction

It is known that plants respond actively to a wide range of biotic and abiotic factors by triggering host cell metabolism. In barley this metabolical response to fungus *E. graminis* f. sp. *hordei* occurs soon, shortly after their physical contact

Akadémiai Kiadó, Budapest

i.e. in the preparasitic stage of the interaction. This phenomenon is a prove that as early as in this phase of their interaction a flow of "information" occurs between host and pathogen. The existence and importance of such "information" or recognition signals in host-parasite relations is now generally accepted and intensively studied (Sequeira, 1983; Daly, 1984; Callow, 1984; Frič, 1988; Templeton and Lamb, 1988; Kunoh et al., 1991). It is presumed that plant receptors localized mostly on cell surface are able to recognize pathogens as "self" or as "non self" and inducing an array of events based on a chain of processes in which genes of both partners are activated of repressed by specific signals. It was shown by many authors that e.g. barley cells are conditioned toward susceptibility by a prior pathogenic fungus attack (induced susceptibility), while resistance of the attacked cells is enhanced through prior attack by a nonpathogenic fungus (induced resistance) Ouchi, et al., 1976; Hwang and Heitefuss, 1982; Ouchi, 1983; Wolfgang and Pelcz, 1986; Kunoh, 1987; von Alten et al., 1988; Thordal-Christensen and Smedegaard-Petersen, 1988a; Kunoh et al., 1990). While progress is being made in understanding the activation of plant "defence" genes by pathogen metabolites, the molecular mechanism of this activation process i.e. the recognition of pathogen signals by plant cells and their transport to nucleus or other subcellular organelles is less known (Poovaiah et al., 1987; Morse et al., 1989).

The pathogen is capable of detecting and responding to signals from external environment. Conidia of *Erysiphe graminis* f. sp. *hordei* e.g. can discriminate between host and non-host leaf surface. It was demonstrated that only 40% of conidia germinated on non-host cucumber leaves, whereas 80% germination was noticed on barley leaf surface (Staub et al., 1974). The same phenomenon was noticed when conidia were germinating on host-plant leaves (barley) and inert glass-surface (Paulech, 1967). It is suggested that prior to penetration of *E. graminis* into epidermal host cell molecular communication signals pass from host to fungus and vice versa. Kunoh and Ishizaki (1981) demonstrated that germinated conidia of *E. graminis*, *hordei* having both a primary non-appressorial and an appressorial germ tube (5-6 h after inoculation) are able to absorb a fluorescent dye (acridine orange) from living epidermal cells of barley. The dye was translocated to appressorial apices and finally infiltrated into papillae and in epidermal cell walls corresponding to fluorescent "halos". Recently in compatible barley – *E. graminis*, *hordei* genotype interaction (4-24 h after the inoculation) Roestel et al. (1991) have established antigens to those on the surface of germinating conidia in mesophyll cells of the host i.e. in considerable distances from pathogen infection structures. This finding may indicate that fungal antigenes (signal substances) enter the mesophyll cells as early as in the preparasitic stage (4-24 h) of host-parasite interaction.

Metabolites or infection structures of virulent *E. graminis*, *hordei* conidia are not only inducing host metabolical responses leading to a distant degrees of local host resistance (Smedegaard-Petersen and Stolen, 1981; Thordal-Christensen and Smedegaard, 1988a; Gregersen and Smedegaard, 1989) but it have been shown also that the appressorium of *E. graminis*, *hordei* release suppressors which inhibit or delay the defence mechanism in barley (Komura et al., 1990).

Barley response to *Erysiphe graminis*, *hordei* conidia in the preparasitic phase

Metabolical response of barley cells to germinating conidia of *E. graminis*, *hordei* can be noticed few hours after the inoculation of barley leaves. The timing of physiological and biochemical responses of host cells are connected with distant developmental and growing stages of the pathogen, which are considerable dependent on environmental i.e. cultivation conditions. Owing to the fact that in the published papers the cultivation conditions are not always the same, the elapsed time (h) noticed from inoculation to host response is also different.

Conidia of *E. graminis*, *hordei* are able to germinate within 1 to 2 h under suitable conditions. Unicellular conidia produce one or more germ tubes. Primary (non-appressorial) germ tubes always precede secondary (apressorial) germ tubes by about 2 h (Kunoh et al., 1977, 1978; Kunoh, 1982). Primary germ tubes are able to breach the barley epidermal wall and induce host responses (halo, cytoplasmatic aggregates, papilla formation) few hours earlier than the secondary germ tubes do (Kunoh, 1982). Primary (non-appressorial) germ tubes do not form appressoria and never produce haustoria. Owing to the fact that they induce metabolical responses in host they are supposed to be associated with induced local "resistance" or "susceptibility" of attacked barley cells.

Up till now the earliest metabolic response of barley cells is the increasing of phenylalanine ammonia-lyase activity in barley leaves, which can be observed as early as 2 h after the inoculation. Consequently (3-4 h) after the inoculation an increased synthesis of cinnamic acid was noticed (Shiraishi et al., 1989). This phenomenon can be the cause of localized auto-fluorescence of cell walls round the germinating conidia (fluorescent halo) which was observed by u.v. fluorescence microscopy by many authors (Kunoh et al., 1982; 1985; Wright and Heale, 1988). Mayama and Shishiyama (1978) have already pointed out that autofluorescence at areas of fungal appressoria might be associated with phenolic compounds. It is to mention that fluorescent haloes are induced so by virulent as by avirulent *E. graminis*, *hordei* races (Thordal–Christensen and Smedegaard-

Petersen, 1988b). The appearance of localized fluorescence is followed by host cytoplasmatic aggregation at the contact spots of primary (5 h after inoculation), (Kunoh et al., 1978) and secondary appressorial germ tubes (10 h after the inoculation, Kunoh et al., 1982). As reported by Kunoh et al. (1985) fluorescence preceed the appearance of cytoplasmatic aggregates by 1 to 10 minutes and occurs generally prior to penetration of the infection peg from the mature appressorium.

Prior to appearance of cytoplasmatic aggregates in epidermal cells, in the stage of appressorium maturation a dramatic increase of host cell cytoplasmatic streaming can be observed (Bushnell, 1981; Kobayashi et al., 1990; Kunoh et al., 1991). This phenomenon proves that mature appressoria 4-5 h prior to penetration release signals to host cells.

Penetration phase

Generally on the contact surface of both primary (non-appressorial) and secondary (appressorial) germ tubes of virulent or avirulent races of *E. graminis*, *hordei*, the cuticula surfaces concentrical stainable areas known as "haloes", as well as wall appositions called "papillae" appear as host cell responses to the penetration activity of the fungus (Sargent and Gay, 1977; Tsuzuki et al., 1977; Kunoh et al., 1978; 1985; Frič, 1984).

Halo

The halo round the appressorium can be detected easily by an appropriate dye after extraction of leaf pigments by ethanol or ethanol-chloroform (Wolf and Frič, 1981; Kunoh et al., 1985). The stainable areas (haloes) around the infection pegs, reveals from changes in the primary cell wall. It was suggested that halo is a chemical response to metabolites and enzymes of the fungus i.e. that epidermal cell wall constituents are degraded by fungal enzymes (McKeen et al., 1969; Edwards and Allen, 1970; McKeen and Rimmer, 1973; Aist, 1976a, 1976b). According to detailed investigations of Sargent and Gay (1977), it is suggested that halo formation may be elicited by fungal enzymes prior to penetration, but halo is not a site of cuticle or cell wall degradation. The mentioned authors suggested that cuticular lipid secretion is necessary for halo formation. This view is supported also by the fact that Tween 20 or glycerine are also able to induce the halo without degrading cell wall constituents (Tsuzuki et al., 1977). On the other hand calcium ions i.e. $CaCl_2$ treatment of host tissue partially suppress the occurrence of haloes (Kunoh et al., 1985). Accumulation of some ions (calcium,

manganese, silicon) and other substances (lipides, protein) was also as certained in the area of halo (Kunoh and Ishizaki, 1975; Kunoh et al., 1975; Spencer, 1978). Silicium accumulation in halo region could be a part of a complex series of events which can influence the penetration process (Carver et al., 1987). The mechanism and the physiological function of such an accumulation of substances is not fully understood yet.

Papilla

Papillae are one of the most extensively studied structural barriers of the host plants against fungal penetration. Papillae at the sities of fungal penetrations represent a local cell-wall tickening on the inner surface of cell walls (appositions of cell wall material between the cell and the plasmalema).

These structures are with high probability formed by cytoplasmic aggregates which function could be seen on deposition of cell-wall like material on the cell wall affected by injury (Aist, 1976a; Ebrahim-Nesbat and Schönbeck, 1988). This suggestion is supported by the facts that cytoplasmatic aggregates contain Golgi apparatus, endoplasmic reticulum and secretory vesicles, and occur always earlier than papillae. Papilla differs chemically from the original host cell wall as was shown by many investigators. It is to mention that a chemical stimulus (metabolites of fungi) can also elicit papilla formation, therefore papilla cannot be regarded only as a generalized wound host response (Aist, 1976a; Wright and Heale, 1988).

Papilla has been implicated in resistance of both compatible and incompatible host-parasite interactions (Skou et al., 1984; Heitefuss and Ebrahim-Nesbat, 1986; Ebrahim-Nesbat et al., 1986; Aist, 1988). The effectiveness of papilla is dependent on the relative timing of fungal penetration and deposition processes, its size and density. Rate of growth and maturation of papilla play an important role in host cell resistance. This was clearly demonstrated in barley mutants with resistance genes in the m1-0 locus (Stolzenberg et al., 1984a, 1984b; Bayles et al., 1990). Maturation of papilla i.e. its incrustation with various substances (lignin, phenolics 1,3-beta glucan, proteins, silica, etc.) was correlated with the success or failure of cell-wall penetration of *E. graminis*, *hordei* (Aist, 1976a, 1976b; Zeyen et al., 1983; Bayles and Aist, 1987; Aist et al., 1988). According to Kita et al. (1981) accumulation of certain compounds in papillae may be more critical point in resistance than papilla formation itself. At present compact and mature papillae as penetration barriers cannot be ruled out as the mechanism of m1-0 resistance in barley. On the other hand papillae are not the only factors which determine the degree of barley – *E. graminis*, *hordei* incompatibility i.e. resistance.

Hypersensitive reactions

In certain barley cultivars carrying the M1-a resistance gene, the growth of *E. graminis*, *hordei* is mostly not stopped by the papilla, but subsequently, by a rapid collapse and cell death (necrosis) of the attacked epidermal cells. Necrosis is not limited only the attacked cells, frequently some neighbouring cells die even earlier than the attacked ones. This rapid plant reaction called "hypersensitivity" is a well-known phenomenon is non-hosts i.e. species which are immune to attacking fungal species. In hosts this hypersensitive reaction (HR) is accompanied by resistance i.e. reduced growth or death of the pathogen. In spring barley (cv. Algerian) carring the M1-a gene, papilla formation is completed (13.5-15 h) before the HR begins (Zeyen and Buschnell, 1979). There is always a low percentage of attacked cells that do not undergo HR.

The term HR includes all observable physiological or metabolical events associated with host death. The cause of cell death i.e. activation and mechanism of biochemical events leading to HR-response have not been established yet.

It is to mention that in non-hosts the infection structures of *E. graminis* DC frequently stop growing before HR is induced (Buschnell, 1982). On the other hand in host plants cell necroses are not always closely correlated with growth inhibition or death of the pathogen, thereby casting doubt is emerging whether the host cell death is determinative for resistance. It is known e.g. that *E. graminis*, *hordei* can continue to grow slowly as host cells die (Koga et al., 1978).

Biochemical symptoms

Biochemical and metabolical changes in the preparasitic phase of barley – *E. graminis*, *hordei* interaction have been well documented. In compatible as well as in incompatible barley – *E. graminis*, *hordei* genotype combinations all physiological and morphological events in attacked host cells and cells around invasion sities are the consequencies of significant biochemical reactions and processes. In various host – parasite genotype combinations a positive correlation between some host – enzyme activities and host resistance was reported. These findings has led to the hypothesis that some enzymes may be an integral part of plants defence mechanism. The interpretation of the mentioned correlations must be handled very carefully because changes in enzyme activities are mostly a consequence, rather than a determinant of resistance.

In barley attacked by *E. graminis* increased proteosynthesis, activition of enzymes and changes in cell membrane permeability were demonstrated as early as in the preparasitic phase of their interaction (Frič and Wolf, 1979, 1992; Frič,

1984; Frič and Speváková, 1985; Takahashi et al., 1985; Shiraishi et al., 1989; Kerby and Somerville, 1989). Increased proteosynthesis is always connected with increased mRNA and RNA synthesis (Shishiyama et al., 1976; Heitefuss and Wolf, 1976; Davidson et al., 1988). Inoculation, or other stress conditions induce in barley and other plants a strong accumulation of mRNA, encoding e.g. leaf-specific thionenins which represent a cell-wall protein toxic to fungi i.e. also to *E. graminis* DC (Fischer et al., 1989). Thionenins are closely related to pathogenesis related proteins and are possibly involved in host defence mechanism (Bohlmann et al., 1988; Ebrahim-Nesbat et al., 1989).

The activity of oxidative and hydrolytic enzymes isolated from attacked plant organs (leaves, epidermis, intercellular spaces) were significantly changed within few hours after the inoculation of compatible or incompatible barley cultivars. The most marked increase was revealed for peroxidase (Frič and Wolf, 1979; Frič, 1984). Peroxidases appear to be involved in great array of physiological processes (Gaspar et al., 1982; van Loon, 1986). It is well documented that pathogens as well as various stress conditions stimulate host peroxidase activity to different extent. The idea that the increased host peroxidase activity in the preparasitic phase of barley – *E. graminis* interaction lead to a higher host resistance is very attractive. Peroxidases in the preparasitic phase are thought to act in host resistance mainly through their role in lignification processes. Induced lignification of epidermal cell-walls and of papillae in attacked cells could be regarded as a host defence reaction. On the other hand it was revealed that increased peroxidase activity in barley is not confined only to incompatible barley – *E. graminis* interaction (Frič, 1984; Kerby and Somerville, 1989). Therefore more detailed and complex studies are necessary for understanding the specific functions of peroxidases in individual host cell compartments with regard to host-cell resistance.

Plant hydrolases has been proposed to play also an important role in plant resistance (Takahashi et al., 1985; Boller, 1987; Mauch and Staehelin, 1989).

Cytochemical studies revealed that some hydrolytic enzymes (acid phosphatase, non-specific esterase) were accumulated at the penetration sities, penetration pegs and papillae. A close relationship between hydrolytic enzyme activities and penetration efficiency of *E. graminis*, *hordei* into barley cells was observed (Takahashi et al., 1985).

Plant 1,3-beta-glucanases and chitinases have been shown to attack cell walls of pathogenic fungi. The degradation of fungal cell walls by glucanases is enhanced in the presence of chitinases (Young and Pegg, 1982). In incompatible and compatible barley – *E. graminis*, *hordei* interactions a derminate correlation was found between the 1,3-beta-glucansynthase, 1,3-beta-glucanase activities in whole leaf extracts and the degree of barley resistance. In epidermal cell extracts

such a correlation was not demonstrated. The role of both enzymes in barley resistance is therefore not unambiguous (Frič and Huttová, 1993).

One of important symptoms of barley attacked by *E. graminis, hordei* is the increased exocytosis of solutes from the host cells. This phenomenon can be noticed as early as in preparasitic phase, starting with the fungal penetration into epidermal cells (Frič, 1984, 1988). Changes of cell membrane permeability for solutes in pathogenesis is accompanied by several physiological and biochemical symptoms i.e. quantitative changes of membrans fatty acids, increased concentration of Ca^{2+} in the intercellular spaces, increased activity of lipoxigenase, superoxid dismutase (SOD), intra- and extracellular peroxidase etc. An important role is attributed to enzymes which could influence or disturb the integrity of the cell membranes e.g. lipoxidase and SOD. In the preparasitic phase the increased exocytose of solutes was always accompanied with enhanced lipoxigenase activity. This correlation was for SOD not so unambiguous (Frič, 1988, 1993). The role of lipoxigenase in cell membrane permeability is according to our recent knowledge not quite unambiguously proven.

High molecular substances have been found in *E. graminis, hordei* conidia, which have a significant enhancing effect on exocytosis of barley cells (Frič et al., 1992). It is assumed that these fungal metabolites play a substantial role in conditioning plant cells for enhanced transfer of nutritives from host to obligate parasitic fungus.

Literature

Aist, J. R. (1976a): Papillae and related wound plugs of plant cells. Ann. Rev. Plants Phytopathology 14, 145–163.

Aist, J. R. (1976): Cytology of penetration and infection – Fungi. In: Heitefuss, R. and Williams, P. H. (eds): Encyclopedia of Plant Phytopathology, New series, Vol. 4, Physiological Plant Pathology, 197–221. Springer Verlag, Berlin, Heildelberg, New York

Aist, J. R. (1988): Evidence that molecular compounds of papillae may be involved in ml-0 resistance to barley powdery mildews. Physiol. Mol. Plant Pathol. 33, 17–32.

McAinsh, M. R., Ayres, P. G. and Hetherington, A. M. (1989): The respiration of protoplasts from leaves of barley infected by powdery mildew (*Erysiphe graminis* f. sp. *hordei*). Plant Science (Limerick) 64 (2), 221–230.

Von Alten, H., Steiner, V., Oerke, E. C. and Schönbeck, F. (1988): 5th Int. Congress of Plant Pathol. Abst. papers Kyoto, Japan p. 244.

Bayles, C. J. and Aist, J. R. (1987): Apparent calcium mediation of resistance of an ml-0 barley mutant to powdery mildew. Physiol. Mol. Plant Pathology 30, 337–345.

Bayles, C. J., Ghemawat, M. S. and Aist, J. R. (1990): Inhibition by 2-deoxy-D-glucose of callose formation, papilla deposition, and resistance powdery mildew in an ml-0 barley mutant. Physiol. Mol. Plant Pathology 36, 63–72.

Boller, Th. (1987): Hydrolytic enzymes in plant disease resistance. In: Kosuge, T., Nester, E. W. (eds): Plant-Microbe Interactions, Molecular and Genetic Perspectives Vol. II. 385–413, Macmillan, New York

Bohlmann, H., Clausen, S., Behnke, S., Giese, H., Hiller, C., Reimann-Philipp, U., Schrader, G., Bakholt, V. and Apel, K. (1988): Leaf-specific thionins of barley – a novel class of cell wall proteins toxic to plant-pathogenic fungi and possibly involved in defence mechanism of plants. The Embo J., 7, 1559–1565.

Buschnell, W. R. (1981): Incompatibility conditioned by *Mla* gene in powdery mildew of barley. The halt in cytoplasmatic streaming. Phytopathology 71, 1062–1066.

Buschnell, W. R. (1982): Hypersensitivity in rust and powdery mildews. In: Asada, Y. et al. (eds): Plant Infection: The Physiological and Biochemical Basis. Japan Sci. Soc. Press, Tokyo/Springer Verlag, Berlin, 97–116.

Callow, J. A. (1984): Cellular and molecular recognition between higher plants and fungal pathogens. In: Linskens, H. F. and Heslop-Harrison, K. (eds): Encyclopedia of Plant Physiology, New series Vol. 17, 212–237.

Carver, T. L. W., Zeyen, R. J. and Ahlstrand, G. G. (1987): The relationship between insoluble silicon and success or failure of attempted primary penetration by powdery mildew (*Erysiphe graminis*) germinating on barley. Physiol. Mol. Plant Pathol. 31, 133–148.

Daly, J. M. (1984): The role of recognition in plant disease. Ann. Rev. Phytopathol. 22, 273–307.

Davidson, A. D., Manners, J. M., Simpson, R. S. and Scott, K. J. (1988): Altered host gene expression in near-isogenic barley conditioned by different genes for resistance during infection by *Erysiphe graminis* f. sp. *hordei*. Physiol. Mol. Plant Pathol. 32, 127–139.

Ebrahim-Nesbat, F., Heitefuss, R. and Rohringer, R. (1986): Ultrastructural and histochemical studies on mildew of barley (*Erysiphe graminis* DC. f. sp. *hordei* Marchal). IV. Characterization of papillae in fifth leaves exhibiting adult plant resistance. J. Phytopathology 117, 289–300.

Ebrahim-Nesbat, F. and Schönbeck, F. (1988): Further electron microscopical studies of induced resistance of barley against *Erysiphe graminis* f. sp. *hordei*. Phytopath. z. 113, 219–230.

Ebrahim-Nesbat, F., Behnke, S., Kleinhofs, A. and Apel, K. (1989): Cultivar-related differences in the distribution of cell wall bound thionins in compatible and incompatible interactions between barley and powdery mildew. Planta 179, 203–210.

Edwards, H. H. and Allen, P. J. (1970): A fine structure study of primary infection process during infection of barley by *Erysiphe graminis* f. sp. *hordei*. Phytopathology 60, 1504–1509.

Fischer, R., Behnke, S. and Apel, K.(1989): The effect of chemical stress on the polypeptide composition of the intercellular fluid of barley leaves. Planta 178, 61–68.

Frič, F. (1984): Biochemical changes in barley plants in the preparasitic stage of powdery mildew-barley infection. Acta Phytopathol. Acad. Sci. Hungaricae 19, 183–191.

Frič, F. (1988): Physiological and biochemical reactions of powdery mildew (*Erysiphe graminis* f. sp. *hordei* Marchal) infected barley plants. (in Slovak), Biológia (Bratislava) 43, 427–439.

Frič, F (1988): Biochemical principles of pathogen recognition by host plants. Proceedings of the fourth conference "On results of study in the field of pathological plant physiology". 125–142, Pieštany

Frič, F. and Wolf, G. (1979): Protein changes in the intercellular spaces of barley in the early stage of mildew pathogenesis. ESNA-Newsletter (Beograd) 47–57.

Frič, F. and Spevákova, A. (1985): Physiological and biochemical processes in barley plants in the preparasitic stage of host – parasite interaction (*Erysiphe graminis* f. sp. *hordei*). (in Slovak) Proceedings of Plant Pathology Conference, Inst. of Exp. Botany, Bratislava, 69–80.

Frič, F. and Wolf, G. (1992): Protease activity in powdery mildewed barley leaves. Biológia (Bratislava) 47, 307–315.

Frič, F, Wolf, G., Viskupová, A. (1992): Physiologically active metabolites of fungus *Erysiphe graminis* f. sp. *hordei* Marchal. Biol. Plantarum Abs. 34 (Suppl.) 491.

Frič, F. and Huttová, J. (1993): Host plant glucanase, glucan synthase and chitinase activities at compatible and incompatible barley – *Erysiphe graminis*, *hordei* interactions. Biologia Plantarum 35, 95–101.

Frič, F. (1993): Lipoxygenase and superoxid dismutase activities in powdery mildewed barley leaves. Acta Phytopathol. et Entomol. Hung. 28, 173–183.

Gaspar, Th., Penel, C., Thospe, T., Greppin, H. (1982): Peroxidases 1970–1980. A survey of their biochemical roles in higher plants. Geneva: Université de Genéve pp. 89–121.

Gregersen, Per. L. and Smedegaard, V. (1989): Induction of resistance in barley against *Erysiphe graminis* f. sp. *hordei* after preinoculation with a saprophytic fungus *Cladosporium macrocarpum*. J. Phytopathol. 124, 128–136.

Heitefuss, R. and Wolf, G. (1976): Nucleic Acids in Host-Parasite Interactions. In: Heitefuss, R., Williams, P. H. (eds): Encyclopedia of Plant Physiology New Series, Vol. 4, Physiological Plant Pathology. Springer Verlag Berlin, Heidelberg, New York, pp. 480–508.

Heitefuss, R. and Ebrahim-Nesbat, F. (1986): Ultrastructural and histochemical studies on mildew of barley (*Erysiphe graminis* DC. f. sp. *hordei* Marchal). J. Phytopathol. 116, 358–373.

Hwang, B. K. and Heitefuss, R. (1982): Induced resistance of spring barley to *Erysiphe graminis* f. sp. *hordei*. Phytopath. Z. 103, 41–47.

McKeen, W. E., Smith, R. and Bhattacharya, P. K. (1969): Alternation of the host wall surrounding the infecton peg of powdery mildew fungi. Can. J. Bot. 47, 701–706.

McKeen, W. E. and Rimmer, S. R. (1973): Inicial penetration process in powdery mildew infection of susceptible barley leaves. Phytopathology 63, 1049–1053.

Kerby, K. and Somerville, S. (1989): Enhancement of specific intercellular peroxidases following inoculation of barley with *Erysiphe graminis* f. sp. *hordei*. Physiol. Mol. Plant Pathol. 35, 323–337.

Kita, N., Toyoda, H. and Shishiyama, J. (1981): Chronological analysis of cytological responses in powdery mildewed barley leaves. Can. J. Bot. 59, 1761–1768.

Kobayashi, J., Komura, T., Sakamoto, Y. and Yamaoka, N. (1990): Recognition of a pathogen and a nonpathogen by barley coleoptile cells. I. Cytoplasmic responses to the nonpathogen, *Erysiphe pisi*, prior to its penetration. Physiol. Mol. Plant Pathol. 37, 479–490.

Koga, H., Mayama, S. and Shishiyama, J. (1978): Microscopic specification of compatible and incompatible interactions in barley leaves inoculated with *Erysiphe graminis*, *hordei*. Ann. Phytopathol. Soc. Japan 44, 111–119.

Komura, T., Kobayashi, I., Yamaoka, N. and Kunoh, H. (1990): Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. VII. Cytological evidence for suppressor(s) of host inaccessibility from *Erysiphe graminis*. Physiol. Mol. Plant Pathol. 37, 409–416.

Kunoh, H., Ishizaki, H. and Kondo F. (1975): Composition analysis of "halo" area of barley leaf epidermis incited by powdery mildew infection. Ann. Phytopathol. Soc. Japan 41, 33–39.

Kunoh, H. and Ishizaki, H. (1975): Silicone levels near penetration sities of fungi on wheat, barley, cucumber and morning glory leaves. Physiol. Plant Pathol. 5, 283–287.

Kunoh, H., Ishizaki, H. and Nakaya, K. (1977): Cytological studies of early stages of powdery mildew in barley and wheat leaves. II. Significance of the primary germ tube of *Erysiphe graminis* on barley leaves. Physiol. Plant Pathol. 10, 191–199.

Kunoh, H., Tsuzuki, T. and Ishizaki, M. (1978): Cytological studies of early stages of powdery mildew in barley and wheat. IV. Direct ingress from superficial primary germ tubes and appressoria of *Erysiphe graminis* f. sp. *hordei* on barley leaves. Physiol. Plant Pathol. 13, 327–333.

Kunoh, H. and Ishizaki, H. (1981): Cytological studies of early stages of powdery mildew in barley and wheat. VII. Reciprocal translocation of a fluorescent dye between barley coleoptile cells and conidia. Physiol. Plant Pathol. 18, 207–211.

Kunoh, H. (1982): Primary germ tubes of *Erysiphe graminis* conidia. In: Asada, Y. et al. (eds): Plant Infection: The Physiological and Biochemical Bases. Japan Sci. Soc. Press Tokyo/Springer Verlag, Berlin, pp. 45–59.

Kunoh, H., Yamamori, K. and Ishizaki, H. (1982): Cytological studies of early stages of powdery mildew in barley and wheat. VIII. Autofluorescence at penetration sities of *E. graminis* f. sp. *hordei* on living barley coleoptiles. Physiol. Plant Pathol. 21, 373–379.

Kunoh, H., Kuno, K. and Ishizaki, H. (1985): Cytological studies of the early stage of powdery mildew in barley and wheat. XI. Autofluorescence and haloes at penetration sities of appressoria of *Erysiphe graminis hordei* and *Erysiphe pisi* on barley coleoptiles. Can. J. Bot. 63, 1535–1538.

Kunoh, H. (1987): Induced susceptibility and enhanced resistance at the cellular level in barley coleoptiles. In: Nishimura, S. et al. (eds): Molecular Determinants of Plant Diseases. Japan Sci. Soc. Press, Tokyo Springer Verlag, pp. 59–73.

Kunoh, H., Komura, T., Kobayashi, J. and Yamaoka, N. (1990): Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. VII. Enhancement of inaccessibility at the prepenetration stage of a nonpathogen. Physiol. Mol. Plant Pathol. 37, 399–407.

Kunoh, H., Kobayashi, J. and Yamaoka, N. (1991): Recognition of fungal nonpathogens by plant cells at the prepenetration stage. In: Patil, S. S., Ouchi, S., Mills, D. and Vance, C. (eds): Molecular strategies of pathogens and host plants. Springer Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong, Barcelona, pp. 189–201.

Van Loon, L. C. (1986): The significance of changes in peroxidase in diseased plants. In: Greppin, H., Penel, C. and Gaspar, Th. (eds): Molecular and Physiological Aspects of Plant Peroxidase. Univ. de Genéve – Centre de Botanique. 405–417.

Mauch, F., Stachelin, L. A. (1989): Functional implications of the subcellular localization of ethylene-induced chitinase and β–1,3-glucanase in bean leaves. Plant Cell 1, 447–457.

Mayama, S. and Shishiyama, J. (1978): Localized accumulation of fluorescent and u.v. – absorbing compounds at penetration sities in barley leaves infected with *Erysiphe graminis hordei*. Physiol. Plant Pathol. 13, 347–354.

Morse, M. J., Satter, R. L., Crain, R. C. and Coté, G. G. (1989): Signal transduction and phosphatidylinositol turnover in plants. Physiol. Plantarum 76, 118–121.

Oku, H., Ouchi, S., Shiraishi, T., Komoto, Y. and Oki, K. (1975): Phytoalexin activity in barley powdery mildew. Ann. Phytopathol. Soc. Japan. 41, 185–191.

Ouchi, S., Hibino, Ch. and Oku, H. (1976): Effect of earlier inoculation on the establishment of a subsequent fungus as demonstrated in powdery mildew of barley by a triple inoculation procedure. Physiol. Plant Pathol. 9, 25–32.

Ouchi, S. (1983): Induction of resistance or susceptibility. Ann. Rev. Phytopathol. 21, 289–315.

Ouchi, S. (1991): Molecular biology of fungal host-parasite interactions. In: Patil, S., Ouchi, S., Mills, D., Vance, C. (eds): Molecular Strategies of Pathogens and Host Plants. Springer Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong, Barcelona, 15–27.

Paulech, C. (1967): Studies of the development of powdery mildew (*Erysiphe graminis* f. sp. *hordei* Marchal) on barley. I. Conidia germination. Biológia (Bratislava) 22, 721–730.

Poovaiah, B. W., Fadden, J. J. and Reddy, A. S. N. (1987): The role of calcium ions in gravity signal perception and transduction. Physiol. Plantarum 71, p. 401–407.

Roestel, C. Van., Smith, R., McKeen, W. E. and Day, A. W. (1991): Detection of antigens of powdery mildew, *Erysiphe graminis* f. sp. *hordei* in susceptible plant host cells, shortly after inoculation and during the early stages of infection. Bot. Gaz. 152, 460–467.

Rohringer, R., Ebrahim-Nesbat, F., Wolf, G. (1983): Proteins in intracellular washing fluids from leaves of barley (*Hordeum vulgare* L.). J. Exp. Bot. 34, 149, 1589–1605.

Sargent, C. and Gay, J. L. (1977): Barley epidermal apoplast structure and modification by powdery mildew contact. Physiol. Plant Pathol. 11, 195–205.

Sequeira, L. (1983): Mechanism of induced resistance in plants. Ann. Rev. Microbiol. 37, 51–79.

Shishiyama, J., Suwa, Y. and Nakamura, C. (1976): Early events in RNA and protein metabolism in compatible barley – powdery mildew interaction. In: Tomiyama, K., Daly, J. M., Uritani, I., Oku, H., Ouchi, S. (eds): Biochemistry and Cytology of Plant Parasite Interaction. Kodansha Ltd. Tokyo, Elsevier Scientific Publishing Company Amsterdam, Oxford, New York. 233–235.

Shiraishi, T., Yamaoka, N. and Kunoh, H. (1989): Association between increased phenylalanine ammonialyse activity and cinnamic acid synthesis and the induction of temporary inaccessibility, caused by *Erysiphe graminis* primary germ tube penetration of the barley leaf. Physiol. and Mol. Plant Pathol. 34, 75–83.

Skou, J. P., Jorgensen, J. H. and Lilholt, U. (1984): Comparative studies on cellose formation in powdery mildew compatible and incompatible barley. Phytopathol. Z. 109, 147–168.

Smedegaard-Petersen, V. and Stolen, O. (1981): Effect of energy-requiring defence reactions on yield and grain quality in a powdery mildew barley cultivar. Phytopathology 71, 396–399.

Spencer, D. M. (ed., 1978): The powdery mildews. Acad. Press London, New York, San Francisco.

Staub, F. H., Dahmen, H., Shwinn, F. Y. (1974): Light and scanning electron microscopy of cucumber and barley powdery mildew on host and non-host plants. Phytopathology 64, 364–372.

Stolzenberg, M. C., Aist, J. R. and Israel, H. W. (1984a): The role of papillae in resistance to powdery mildew conditioned by the ml-0 gene in barley. I. Correlative evidence. Physiol. Plant Pathol. 25, 337–346.

Stolzenberg, M. C., Aist, J. R. and Israel, H. W. (1984b): The role of papillae in resistance to powdery mildew conditioned by the ml-0 gene in barley. II. Experimental evidence. Physiol. Plant Pathol. 25, 347–361.

Takahashi, K., Aist, J. R. and Israel H. W. (1985): Distribution of hydrolytic enzymes at barley powdery mildew encounter sities implication for resistance associated with papilla formation in a compatible system. Physiol. Plant Pathol. 27, 167–184.

Templeton, M. D. and Lamb, C. J. (1988): Elicitors and defence gene activation. Plant, Cell and Environment 11, 395–401.

Thordal-Christensen, H. and Smedegaard-Petersen, V. (1988a): Comparison of resistance-inducing abilities of virulent and avirulent races of *Erysiphe graminis* f. sp. *hordei* and a race of *Erysiphe graminis* f. sp. *tritici* in barley. Plant Pathology 37, 20–27.

Thordal-Christensen, H. and Smedegaard-Petersen, V. (1988b): Correlation between induced resistance and host fluorescence in barley inoculated with *Erysiphe graminis*. J. Phytopathol. 123, 34–46.

Tsuzuki, T., Ishizaki, H. and Kunoh, F. (1977): Cytological studies of early stages of powdery mildew in barley and wheat leaves. I. Comparison of papillae and halos induced by *Erysiphe graminis* infection to wound plugs and halos induced by Tween 20 and/or glycine sprays. Ann. Phytopath. Soc. Japan 43, 202–206.

Wolf, G., Frič, F. (1981): A rapid staining method for *Erysiphe graminis* f. sp. *hordei* in and on whole barley leaves with a protein-specific dye. Phytopathology 71, 596–598.

Wolfgang, H., Pelcz, J. (1986): Induktion von Anfälligkeit für avirulente durch virulente und von Resistenz gegen virulente durch avirulente Mehltaupathotypen. Arch. Phytopathol. Pflanzenschutz (Berlin) 22, 25–31.

Wright, A. J. and Heale, J. B. (1988): Host responses to fungal penetration in *Erysiphe graminis* f. sp. *hordei* infections in barley. Plant Pathology 37, 131–140.

Young, D. H., Pegg, G. F. (1982): The action of tomato and *Verticillium albo-atrum* glycosidases on the hyphal wall of V. *albo-atrum*. Physiol. Plant Pathol. 21, 411–423.

Zeyen, R. J. and Buschnell, W. R. (1979): Papilla response of barley epidermal cells caused by *Erysiphe graminis*: rate and method of deposition determined by microcinematography and transmission electron microscopy. Can. J. Botany 57, 898–913.

Zeyen, R. J., Carver, T. L. W. and Ahlstrand, G. G. (1983): Relating cytoplasmic detail of powdery mildew infection to presence of insoluble silicon by sequential use of light microscopy, SEM and X-ray microanalysis. Physiol. Plant Pathol. 22, 101–108.

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Lipoxygenase and Superoxide Dismutase Activities in Powdery Mildewed Barley Leaves

FRIDRICH FRIČ

Institute of Botany, Dúbravská 14, 842 23 Bratislava, Slovakia

Alterations of host cell permeability in infected tissues seems to be important in pathogenesis of powdery mildew disease. As a consequence, a significant increase of oxidative enzyme activities occurs. There is a correlation between the increased lipoxygenase activity and the increased protein exocytosis. A good correlation was also found between the protein exocytosis, the increased free calcium ion level and the activity of lipoxygenase. Superoxide dismutase (SOD) activity increased in the preparasitic phase but in the parasitic phase only the resistant genotypes of barley exhibited higher SOD activity.

Physiological and biochemical processes in barley leaves of both susceptible and resistant barley – *Erysiphe graminis*, f. sp. *hordei* genotype combinations are significantly influenced after inoculation. Increased exocytosis of proteins, increased proteosynthesis and enzyme activities in attacked organs were already reported (Frič, 1984, 1988). These symptoms in attacked barley leaves can be observed as early as in the preparasitic stage of host-parasite interaction.

Generally the metabolic response of the plant in barley – *Erysiphe graminis*, f. sp. *hordei* incompatible interaction starts earlier and is more intensive than in susceptible host-parasite combinations. It seems that these early metabolic events in attacked barley leaves are unspecific and are induced by substances produced by the germinating conidia or by the penetrating infection hyphae of *E. graminis*, f. sp. *hordei* or other fungi.

One of the very important symptoms in the early phase of host-parasite interaction is the increased cell membrane permeability in of the attacked plant tissue.

The present study was conducted to determine the role of some enzymes in the cells of attacked barley leaves which could be responsible for this phenomenon.

Material and Methods

Four barley cultivars (*Hordeum distichon* L.) and one race of *Erysiphe graminis*, f. sp. *hordei* (C_6) were used for investigations. Cultivar Rupee and Koral were resistant to race C_6 (infection types 0–2), while cultivars "Dvoran"

and "Slovenský dunajský trh" were susceptible (infection types 3–4). The infection type in the host-parasite combination was judged according to Nover (1972).

Seedlings of barley were grown in clay pot in a phytotron under controlled conditions.

Light period: 16 h, 37.5 W m^{-2} FAR, temperature 20 °C + 1 °C (dark period 16 °C), relative humidity 70%.

Leaves of 7 day old seedlings were inoculated with freshly harvesed conidia of *E. graminis*, f. sp. *hordei*. The inoculated primary leaves were taken for analysis in appropriate time intervals.

Determination of lipoxygenase (EC. 1.13.11.12) *activity*. Barley leaves used for analysis was quickly frozen and stored at –20 °C until used. The frozen leaves (1 g) after thawing were at 4 °C homogenized in 2 ml 0.2 ***M*** phosphate buffer pH 6.8 containing 5.10^{-3} ***M*** cysteine. The homogenate was centrifuged at 15.000 g for 20 min. The supernatant was used for the enzyme assay.

Lipoxygenase activity was determined by oxygraphic method by a Clark O_2^- electrode (YSJ 5331, Yellow Springs Instr. Co. USA) at 25 °C in a 1.7 ml reaction medium. The substrate, an aqueous linoleinate solution was prepared in Tween 80 by a modification of Surrey (1964) method. (0.1 ml Tween 80 + 2 ml 0.1 ***M*** borate buffer pH=9 + 0.1 ml linoleic acid p. a. (dropwise) + cca 0.3 ml 1 ***M*** NaOH until a clear solution is obtained. To the solution, 18 ml 0.1 M borate buffer (pH=9) and 2.6 mg KCN, was added and the solution was adjusted with HCl to pH 6.8 and filled up to 40 ml by distilled water.

The standard assay mixture contains 1,5 ml substrate and 0.2 ml of enzyme extract. The O_2 concentration in air-saturated medium at 25 °C was taken as 273 μ mol l^{-1}. The enzyme activity was expressed as nmol O_2 consummed by 100 μg protein in 1 min.

Determination of the superoxide dismutase (E.C.1.15.11) activity. The enzyme activity was determined according to the protocol of Giannopolitis and Ries (1977).

Frozen plant material was homogenized in 0.1 ***M*** K-phosphate and 0.1 mM EDTA (pH 7.8) buffer at 0 °C. The homogenate was centrifuged at 14.000 g (0–4 °C) for 10 min. The supernatant was used for enzyme assay.

Enzyme assay: SOD – activity was monitored spectrophotometrically at 560 nm measuring the inhibition of p-nitroblue tetrazolium (NBT) reduction by O_2^-, generated photochemically in a system consisting methionine, riboflavin and NBT. (1 ml 0.15 ***M*** Na_2CO_3 pH 10.8, 30 μl 0.13 m***M*** riboflavin, 300 μl 0.13 ***M*** methionine, 300 μl 0.63 m***M*** NBT, enzyme extract (20–30 μg protein) and H_2O up to 3 ml.)

As blanks, identical reaction mixtures were used with boiled (10 min) enzyme extracts.

The samples were illuminated (Solux lamp) 30 min in glass tubes immersed in water bath at 25 ^{0}C, and placed on a rotating test tube holder.

SOD activity was determined and calculated as proposed by Asada et al. (1974) because a linear relationship up to 55% inhibition was found between SOD concentration and the V/v ratio

$V/v = K.\ SOD + 1$ V – reaction rate with boiled extract

v – reaction rate with crude extract

A unit of SOD activity was defined as that amount which inhibits the NBT – photoreduction by 50%.

At 50% inhibition of the reaction the product "K SOD" equals unity i.e. 1 and so SOD activity can be determined from the V/v ratio.

$$\text{SOD units in the reaction mixture (3 ml)} = \left[\frac{V}{v}\right] - 1 \text{ dilution factor}$$

Peroxidase (E.C. 1.11.1.7) *and catalase* (E.C. 1.11.1.6). The mentioned enzyme activities were after extraction determined spectrophotometrically by the methods distribed earlier (Frič and Fuchs, 1970). The extraction of both enzymes from desintegrated plant material was provided by 0.1 ***M*** acetate buffer pH=5.2. The supernatant after centrifugation (105 000 g, 20 min) was used for the determination of the enzyme activities.

Proteins in substomatal spaces. Proteins of substomatal spaces were obtained by vacuum infiltration of water into the leaves and subsequent centrifugation by a technique described by Klement (1965).

Protein content. Protein content of the extracts was determined according to the method of Bradford (1976).

Fatty acids. Bound and free fatty acids in leaves and epidermal cells were isolated and quantified by gas chromatography according to Mistrík et al. (1981).

Determination of Ca-ions. Free Ca^{+2} in substomatal spaces were determined by absorption flame spectrophotometry according to the protocol given by the manufacturer.

Mean values of three independent experiments with three replications each were calculated and for a better survey the results were expressed as a percentage of values determined for healthy plants.

Results

In our earlier papers we have shown that as early as in the preparasitic phase of compatible and incompatible barley – *E. graminis* interaction an increased exocytosis of some proteins and ions into substomatal spaces of leaves can be observed (Frič, 1984,1988). This phenomenon reflects changes in plasmalemma permeability. The cell membrane permeability changes for solutes in powdery mildew pathogenesis is accompanied by several physiological and biochemical symptoms i.e. quantitative changes of membrane fatty acids, increased activity of lipoxygenase, superoxide dismutase (SOD), peroxidase, catalase, increased Ca^{+2} concentration in substomatal spaces etc.

Quantification of bound fatty acids, the main components of the cell membrane apparatus in the inoculated leaves or epidermis revealed that linolenic acid concentration in the early phase of pathogenesis was significantly lowered in both resistant and susceptible barley cultivars. Later, at host-parasite genotype compatibility this tendency was maintained, while at incompatibility an increasing up to the normal values of control plants was noticed. Peroxidative degradation of unsaturated fatty acids is catalysed by lipoxygenase.

Lipoxygenase activity in the inoculated leaves was significantly increased very early, especially in resistant barley-powdery mildew genotype combinations. Generally lipoxygenase is activated as early as in the begining of pathogenesis. In resistant barley cultivars the enzyme activity is after a transient increase in the preparasitic phase decreasing under the values of control (healthy) plants.

In susceptible host-pathogen combinations in contrast to resistant ones, the enzyme activity is in the parasitic phase continually increasing (Fig. 1).

According to expectations in inoculated leaf or epidermis extracts significant SOD-activity changes can be determined (Fig. 2). Striking difference in NBT-reduction (SOD-activity) between compatible and incompatible barley-powdery mildew genotype combinations can be seen in parasitic phase (24–120 h after inoculation) of the host-parasite interaction only. In the preparasitic phase (0–24 h after inoculation) the differences were not unambiguous.

The activation of host peroxidase and catalase at compatible and incompatible host-parasite genotype combinations is also different, especially in the parasitic phase (Figs 3, 4). This phenomenon can be expected because both enzymes contribute to H_2O_2 degradation, which generation is in attacked or injured host cells stimulated.

The amount of free calcium ions in substomatal (intercellular) spaces of both susceptible and resistant barley cultivars is after the inoculation in preparasitic phase increased. Later in the parasitic phase the free calcium ion concentration

is significantly increased incompatible cultivars only (Fig. 5). At the incompatibility, the free calcium ion levels in substomatal spaces gradually reach the values of the control (healthy) plants.

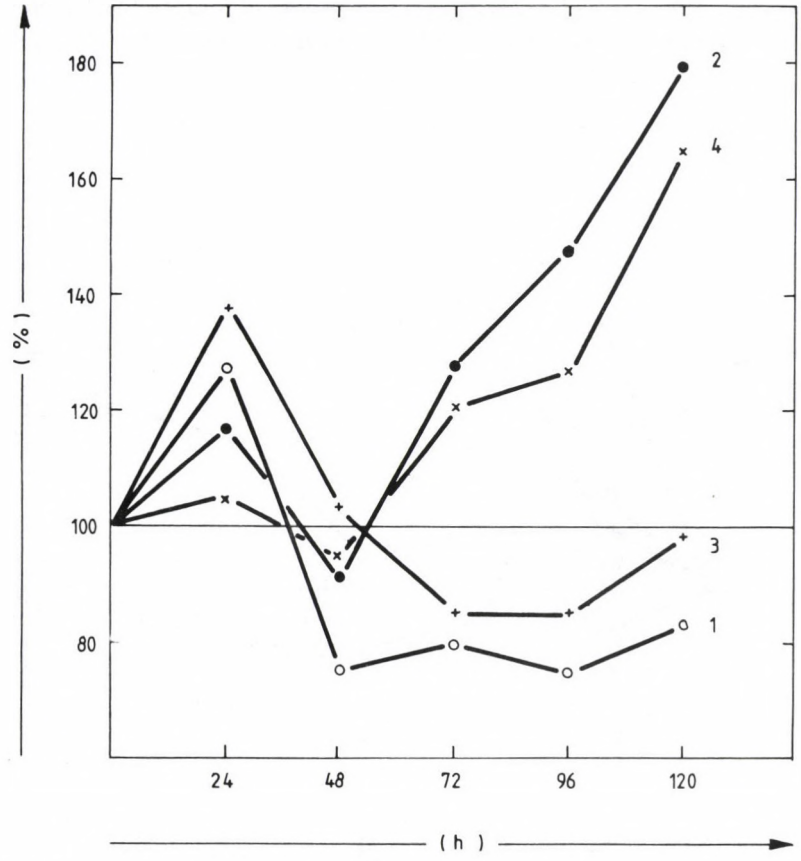

Fig. 1. Lipoxygenase activity in the leaves of resistant (1,3) and susceptible (2,4) barley cultivars after the inoculation with *E. graminis*, *hordei* conidia, race C_6. 1-cv Ruppe, 2-cv Slovensky dunajsky trh (SDT), 3-cv Koral, 4-cv Dvoran. Abscissa: hours after inoculation; Ordinate: activity in percent of uninoculated healthy leaves (100%)

Discussion

Earlier data show that the increase of newly synthetized ^{14}C-labelled protein concentration in substomatal (intercellular) spaces of powdery mildewed barley leaves is a consequence of increased exocytosis caused by the metabolites of the fungus (Frič, 1984, 1988). Increased exocytosis may reflect higher

permeability of the biological membranes. Their function as diffusion barriers is decreased by infection. This disease symptom i.e. alternation of host cell permeability seems to be important in pathogenesis of powdery mildew diseases. The infected epidermal cells of barley are not able to supply the fungus with nutrients. Increased transport of nutrients from neighbouring, especially of mesophyll, cells seems therefore to be critical.

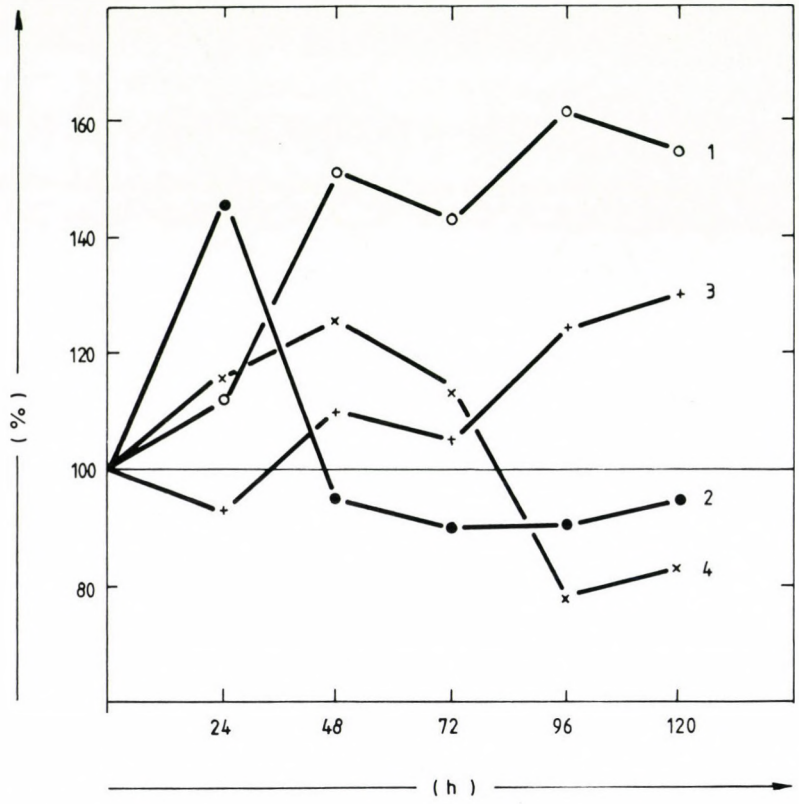

Fig. 2. Superoxide dismutase activity in the leaves of resistant (1,3) and susceptible (2,4) barley cultivars after the inoculation. Legend as in Fig. 1

The plasmalemma permeability for solutes i.e. proteins in both susceptible and resistant barley cultivars after inoculation can be influenced by many factors. An important role in cell membrane permeability is attributed to some enzymes regulating the concentration of free radicals and the integrity of membrane phospholipids.

The results presented here show that after inoculation of barley leaves with powdery mildew conidia a significant increase of oxidative enzyme activities

occur. The date in Fig. 1 show an enhanced activity of lipoxygenase in inoculated leaves of both susceptible and resistant barley cultivars. In preparasitic as well as in the parasitic phase of host-parasite interaction a good correlation can be found between increased lipoxygenase activity (Fig. 1) and increased protein exocytosis (Frič, 1984, 1988). The level of total linolenic acid in inoculated leaves was also lower than in healthy ones. This could be caused by the increased activity of lipoxygenase which specifically catalyses the oxidation of unsaturated fatty acids, especially of linolenic and linoleic acids, which are the most abundant constituents of plant membrane components.

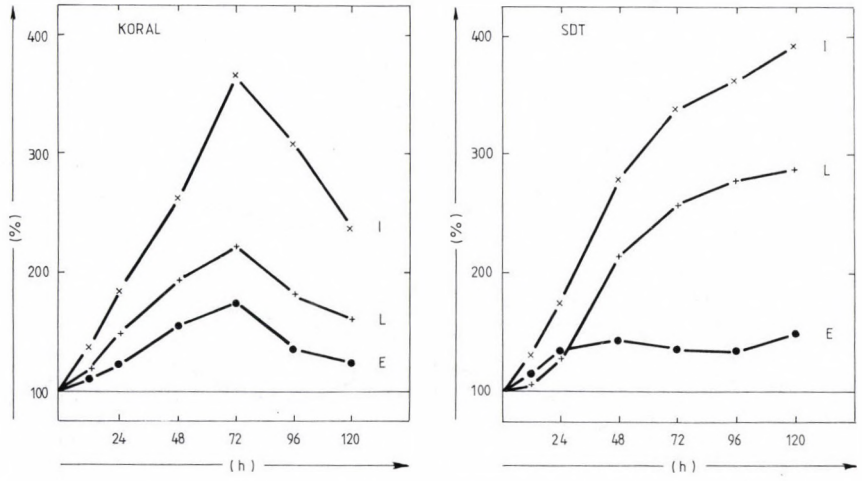

Fig. 3. Peroxidase activity in substomatal spaces (1), leaf extracts (L), and epidermis extracts (E) of resistant (Koral) and susceptible (SDT) barley cultivars. Abscissa: hours after inoculation; Ordinate: activity in percent of uninoculated healthy plants (100%)

At host-parasite compatibility the leaf lipoxygenase activity was significantly higher than at incompatibility. Generally, a good correlation was found between protein exocytosis, increased free calcium ion level in substomatal spaces of barley leaves and the activity of lipoxygenase.

Recent results support the view that the higher level of oxidation products of lipoxygenase e.g. lipid hydroperoxide radicals and other oxidation products could influence the cell membrane permeability. It has been hypothesized that lipoxygenase might be involved in plant growth, senescence, wound responses and resistance to pathogens (Hildebrand, 1989; Yamamoto and Tani, 1986; Lupu et al., 1980).

SOD activity in the barley leaves is significantly changed after their inoculation with *E. graminis*, f. sp. *hordei* conidia. It is known that the enhanced

activity of SOD is induced by the increased generation of the superoxide radicals in the infected or injured plants (Olsen and Cook, 1987; Doke, 1983a). Doke (1983b) reported that superoxide anions are generated in response to certain hyphall wall components.

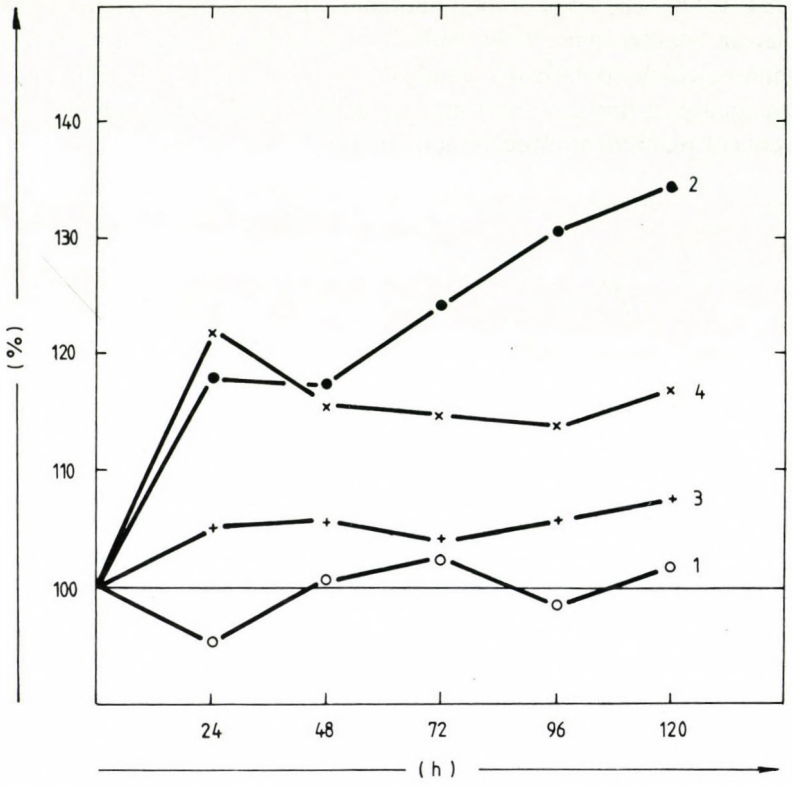

Fig. 4. Catalase activity in the leaves of resistant (1,3) and susceptible (3,4) barley cultivars after the inoculation with *E. graminis*, *hordei* conidia, race C_6. Legend as in Fig. 1

The ability of the free superoxide anion $^{-}O_2$ to iniciate lipid peroxidation has been demonstrated by Kellogg and Fridovich (1975). This process is considered to be an important mechanism of membrane deterioration. The results presented in this paper show that in the preparasitic phase the SOD activity was increased in the most cases, regardless the host-parasite compatibility or incompatibility (Fig. 2). Contribution of fungal SOD was negligible. In the parasitic phase, only resistant barley genotypes exhibit a high SOD activity. It can be supposed that the higher SOD activity in the resistant cultivars removes superoxide free radicals and thus avoids an extensive deterioration of cell membranes which leads to a gradual "normalisation" of the cell permeability. In the susceptible

cultivars because of the low SOD activity the free radicals are not so effectively scavenged and so the increased plasmalemma permeability was maintained. Little research has been done on the response of SOD during resistant and susceptible host-parasite interaction (Doke, 1983a, 1983b; Bounaurio et al., 1987). The results were often contradictory. Some authors found a rapid decline in SOD activity in response to wounding and infection at host-pathogen incompatibility (Olsen and Cook, 1987; Doke, 1983a, 1983b; Zacheo and Bleve-Zacheo, 1988). According to their opinion this phenomenon can be understood as a host defence reaction allowing the formation of superoxide radicals which are an antifugal agent. Bounaurio et al. (1987) found an enhanced levels of total SOD activity in leaves of both susceptible and resistant bean cultivars following Uromyces phaseoli infection.

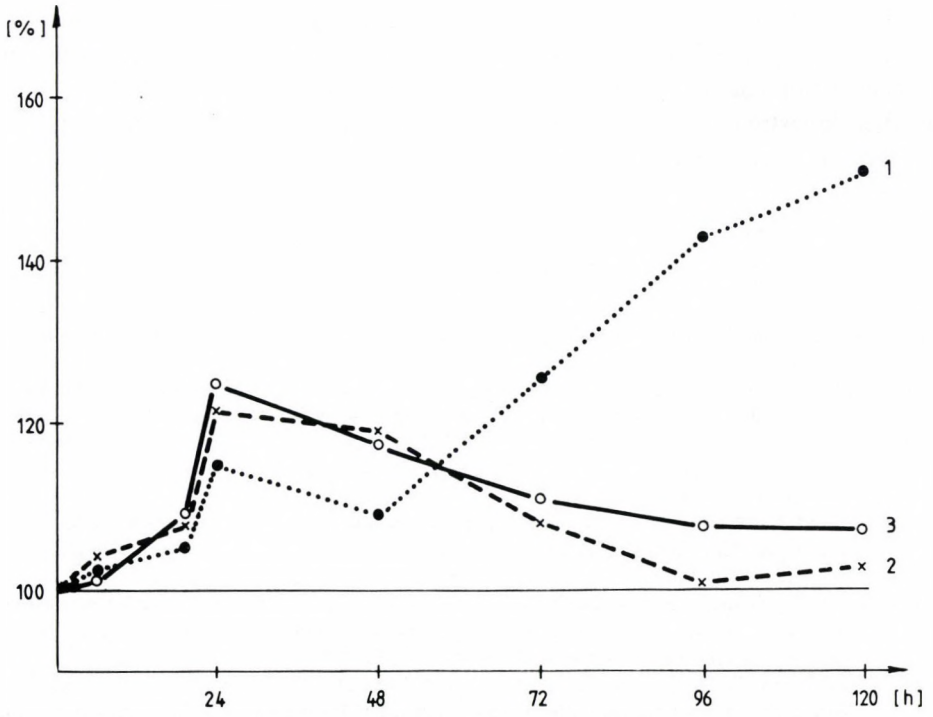

Fig. 5. Contents of free calcium ions in the substomatal spaces of barley plants at host-parasite compatibility (1-cv SDT-*E. graminis*, *hordei*, race, C_6), and at incompatibility (2-cv Rupee-*E. graminis*, *hordei*, race C_6 and 3-cv SDT-*E. graminis*, *tritici*, race 2). Abscissa: hours after inoculation; Ordinate: contents of free calcium ions in percent of uninoculated healthy plants (100%)

With regard to barley plant resistance to *E. graminis*, f. sp. *hordei*, we found a positive correlation between plant resistance and SOD activity, which contrasts with the results of Doke (1983a) studying potato-*Phytophthora infestans* interaction. Different results could be a question of specific metabolic regulation of free superoxide radical generation and quenching by SOD and other antioxidants in specific cell compartments in various host-parasite complexes. A balanced breakdown between production of free radicals and their quenching leads always to physiological and biochemical disturbances.

Increased protein exocytosis in *E. graminis*, f. sp. *hordei* inoculated barley leaves is accompanied with a higher level of free calcium ion in the substomatal spaces. It is to mention that the strong increase of peroxidase activity in the substomatal spaces (Frič, 1984, 1988) of barley leaves after inoculation with conidia of *E. graminis* could also be influenced by the increased free calcium ion concentration in substomatal spaces. It is well documented by many authors that exocytosis of peroxidase from plant cells is controlled by external calcium ion concentration (Sticher et al., 1981, 1982; Penel, 1986; Steer, 1988). However, further investigations are necessary to clarify the role of calcium ions on exocytosis of peroxidase or other proteins in infected barley leaves.

Literature

Asada, K., Takahashi, M. and Nagate, M. (1974): Assay and inhibitors of spinach superoxide dismutase. Agric. Biol. Chem. 38, 471–473.

Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein – dye binding. Anal. Biochem. 72, 248–254.

Buonaurio, R., Torre, G. D. and Montalbini, P. (1987): Soluble superoxide dismutase (SOD) in susceptible and resistant host-parasite complexes of *Phaseolus vulgaris* and *Uromyces phaseoli*. Physiol. and Molecular Plant Pathology, 31, 173–184.

Doke, N. (1983a): Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissue to infection with an incompatible race of *Phytophthora infestans* and to hyphal wall components. Physiol. Plant Pathol. 23, 345–357.

Doke, N. (1983b): Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal wall components of *Phytophthora infestans* and specific inhibition of the reaction by suppressors of hypersensitivity. Physiol. Plant Pathology 23, 350–367.

Frič, F. and Fuchs, W. H. (1970): Veränderungen der Aktivität einiger Enzyme im Weizenblatt in Abhängigkeit von der temperaturlabilen Verträglichkeit für *Puccinia graminis* tritici. Phytopath. Z. 67, 161–174.

Frič, F. (1984): Biochemical changes in barley plants in the preparasitic stage of powdery mildew-barley interaction. Acta Phytopathol. Acad. Sci. Hungaricae 19, 183–191.

Frič, F. (1988): Physiological and biochemical reactions of powdery mildewed (*Erysiphe graminis*, f. sp. *hordei* Marchal) infected barley plants (In Slovak). Biológia (Bratislava) 43, 427–439.

Giannopolitis, C. N. and Ries, St. K. (1977): Superoxide dismutases I. Occurrence in higher plants. Plant Physiol. 59, 309–314.

Hildebrand, D. F. (1989): Lipoxygenases. Physiol. Plantarum 76, 249–253.

Kellogg, E. W. and Fridovich, J. (1975): Superoxide, hydrogen peroxide and singlet oxigen in lipid peroxidation by a xanthine oxidase system. J. Biol. Chem. 250, 8812–8817.

Kenneth, Surrey (1964): Spectrophotometric method for determination of lipoxydase activity. Plant Physiol. 39, 67–70.

Klement, Z. (1965): Method of obtaining fluid from the intercellular spaces of foliage and the fluid merit as substrate for phytobacterial pathogens. Phytopathology 55, 1033–1034.

Lupu, R., Grossman, S. and Cohen, Y. (1980): The involvement of lipoxygenase and antioxidants in pathogenesis of powdery mildew on tobacco plants. Physiol. Plant Pathology 16, 241–248.

Mistrík, I., Kolek, J. and Vozár, J. (1981): Influence of chilling temperature on the composition of lipids and acids in mais roots (*Zea mays* L.). (in Slovak). Biológia (Bratislava) 36, 685–691.

Nover, J. (1972): Untersuchungen mit einer für den Rezistenzträger "Lyallpur 3645" virulenten Rasse von *Erysiphe graminis* DC f. sp. *hordei* Marchal. Arch. Pflanzenschutz 8, 436–445.

Olsen, J. A. and Cook, C. R. (1987): Superoxide dismutase in wounded etiolated pea seadlings. Phytochemistry 26, 71–73.

Penel, C. (1986): The role of calcium in the control of peroxidase activity. In: Greppin, H., Penel, C. and Gaspar, Th. (eds): Molecular and Physiological Aspects of Plant Peroxidase. Univ. de Genéve-Centre de Botanique, p. 155–164.

Steer, M. W. (1988): The role of calcium in exocytosis and endocytosis in plant cells. Physiol. Plantarum 72, pp. 213–220.

Sticher, L., Penel., C. and Greppin, H. (1981): Calcium requirement for the secretion of peroxidases by plant cell suspensions. J. Cell Sci. 48, 345–353.

Sticher, L., Penel., C. and Greppin, H. (1982): Calcium and light controlled peroxidase secretion in spinach cell suspension cultures. Plant Tissue Culture, 223–224.

Yamamoto, H. and Tani, T. (1986): Possible involvement of lipoxygenase in the mechanism of oats for *Puccinia coronata avenae*. J. Phytopathol. 116, 329–337.

Zacheo, G. and Bleve-Zacheo, T. (1988): Involvement of superoxide dismutases and superoxide radicals in the susceptibility and resistance of tomato plants to *Meloidogyne incognita* attack. Physiol. Mol. Plant Pathol. 32, 313–322.

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Inhibitory Effect of O_2^- Anion Generated *in vitro* on *Phytophthora infestans* (Mont.) de Bary

A. A. GALAL, A. L. ÁDÁM, T. ÉRSEK and Z. KIRÁLY

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 102, Hungary

Zoospores, cystospores and mycelia of *Phytophthora infestans* race 2.3.4 have been affected when exposed *in vitro* to a superoxide anion (O_2^-) generating system. Zoospores showed immobilization and then bursting. Damage to the zoospores increased by increasing the period of treatment in the superoxide generating system. However, this effect was prevented by adding active superoxide dismutase (SOD) enzyme to the reaction mixture. In the case of cystospores no bursting occurred but the germination of spores was delayed and the length of germ tubes decreased in dependence of the light exposition time. The radial growth of the fungus was also restricted by the action of the superoxide anion. This restriction depended on the time of illumination and on the concentration of the superoxide-producing substances (i.e. riboflavin/methionine system in the presence of light).

In response to pathogenic attack plants undergo a number of metabolic changes. Early events are likely to play a key role in the rapid induction of defense reactions which typifies incompatible interactions as compared to compatible ones. Membrane related signals might be significant in this respect.

The generation of reactive oxygen species, such as superoxide anion, and some antioxidant systems, have received increased attention in the last few years and are likely to be important factors in the deterioration of membranes following the induction of hypersensitive necrotic symptom expression (Doke, 1983 and 1985; Frank, 1985; Keppler and Novacky, 1987; Keppler and Baker, 1989; Ádám et al., 1989; Croft et al., 1990 and Sutherland, 1991). Moreover, the ability of plant cells to generate hydrogen peroxide H_2O_2, superoxide anion O_2^- and other active oxigen species in the cell wall and in the plasma membrane is often considered to be a defensive oxidative barrier to pathogenic microflora (Elstner, 1984; Aver'yanov et al., 1987 and Merzlyak, 1989). Recent findings indicate a considerable enhancement of superoxide production in potato tissues reacting hypersensitively to incompatible races of *P. infestans* (Doke et al., 1987; Merzlyak, 1989 and Király et al., 1990). This finding suggests that oxygen radicals may provide a specific response in infected plants.

The present study tries to elucidate the action of free radicals (especially the superoxide anion, O_2^-) generated in a photochemical system on the *P. infestans* zoospores, cystospores, and the mycelial growth of the fungus.

Akadémiai Kiadó, Budapest

Materials and Methods

Fungal materials

Zoospores of *P. infestans* (Mont.) de Bary race 2.3.4 were obtained as follows: 5-day-old fungal cultures grown on potato tuber slices were transferred by needle into glass-distilled water, agitated with glass rod then filtered through double layer cheesecloth and sporangia were collected and placed on Whatman No. 4 filter paper. Then, sporangia were suspended in distilled water and kept at 5–10 °C for 4–6 h to induce zoospore formation (Érsek, 1975). After the incubation period, the suspension was filtered through No. 4 filter paper to remove the empty sporangia and obtain pure zoospore suspension.

Zoospores were vigorously agitated for one minute that resulting in synchronous encystment (Oros et al., 1988).

Seven-day-old culture of fungus maintained on pea-broth agar plates was used to study the role of O_2^- generated *in vitro* on the radial growth of the fungus.

Photochemical system for O_2^- generation

The method of Beauchamp and Fridovich (1971) was used to produce superoxide anion photochemically. The complete system contained as final concentration per ml, 8 μM riboflavin and 130 μM L-methionine (as scavenger of hydroxyl radicals, Epperlein et al., 1986) in glass test tubes (1.5 · 7.5 cm) using Shandon lamp (Shandon Scientific Co. Ltd. London, N. W. 10) as a source of light (light intensity $7.3 \cdot 10^3$ Lux). Zoospores or cystospores (10 min-cyst or 50 min-cyst) were added to the reaction mixture obtaining final concentration ($2 \cdot 10^5$ cells/ml reaction mixture). Total volume of the reaction mixture was 3 ml. Under these conditions incubation of the suspensions was carried out for 0 (control), 15, 30 and 60 min at 12 and 16 °C for zoospores and cystospores, respectively. Micrographs (microscopic photos) were taken at intervals from the longest illumination (two micrographs per examination), parallelly with visual observations, on the motility of zoospores.

In case of test plates, pea-broth agar medium was used and supplemented with various concentrations of riboflavin i.e. 0, 3, 6, 12, 25 and 50 ppm with rational concentrations of L-methionine (1 riboflavin: 71 L-methionine). After sterilization, the media were poured into plates (20 ml/plate). Test plates were inoculated with 8-mm-diameter discs from the edge of a 7-day-old culture of *P. infestans* grown on pea-broth agar medium. Inoculated plates were incubated in the dark at 18 °C for 24 h before illumination, to allow mycelial growth to become established. Light treatment (light intensity $3.2 \cdot 10^3$ Lux) was carried out at

intervals, e.g. 0, 15, 30, 60 and 120 min. Radial growth of the fungus was measured after 7 days incubation at 18 °C in the dark (plates were incubated in the dark after the light exposition).

Results and Discussion

High degree of lysis was induced when zoospores were exposed to the light in the presence of O_2^- -producing substances (Figs 1a, b and Fig. 2a). Lysis of zoospores increased with increasing the exposure to light. In addition, the O_2^- treated zoospores which not lysed, lost their motility faster than the non-illumi-

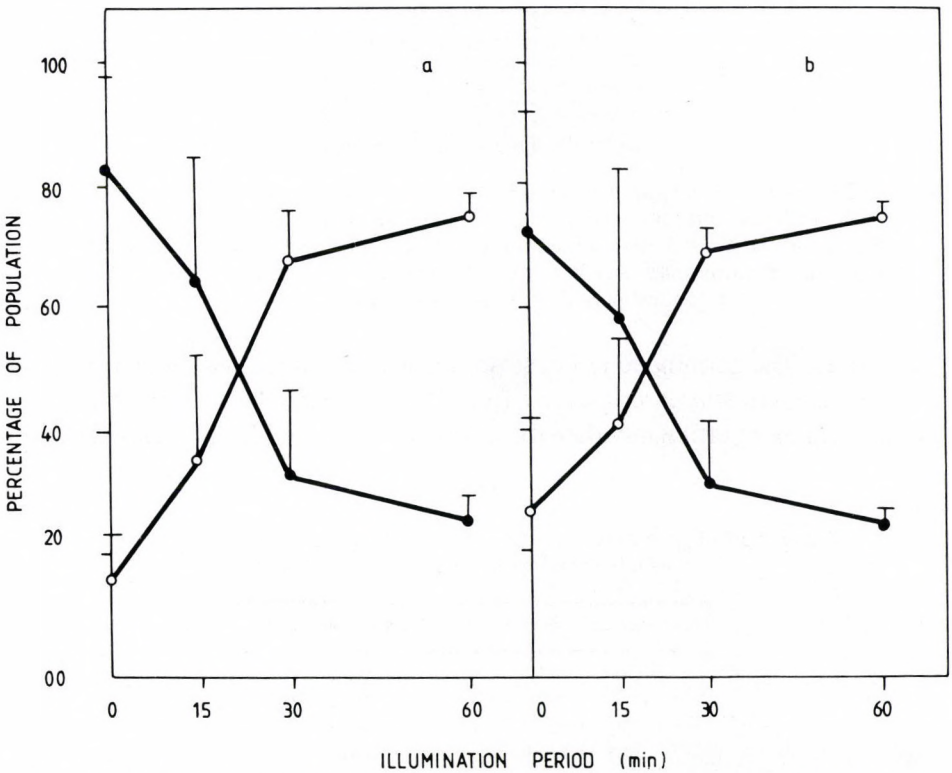

Fig. 1. Induction of lysis in *Phytophthora infestans* zoospore populations by O_2^- -producing substances. Zoospores were exposed to light at various periods, i.e. 0, 15, 30 and 60 min, in the presence of O_2^- -producing substances (riboflavin/L-methionine). Examinations of zoospore populations were conducted 10 min (a) and 60 min (b) after the longest illumination period (60 min). Bars are standard errors of mean of three separate experiments (at least 200 cells were counted per treatment). Percentage of the population (○—○) lysed and (●—●) intact zoo- and cystospores

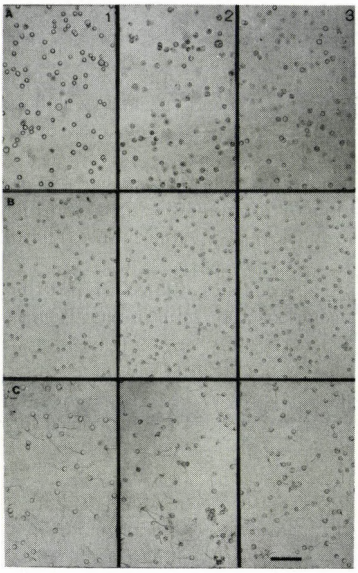

Fig. 2. Zoospores (A), 10 min-cystospores (B) and 50 min-cystospores (C) of *P. infestans* race 2. 3. 4 illuminated for 0 (control) (1), 30 (2), and 60 min (3) in the presence of O_2^- -producing substances (riboflavin/methionine). Micrographs were taken 10 min after the longest illumination for A and B while for C the photos were taken 30 min after the longest period of illumination. Scale 50 μm.

nated ones. The germination of cystospores was also inhibited by O_2^- treatment (Table 1). Cystospores originated from the non-treated (control) zoospores germinated more efficiently than the cystospores derived from zoospores that

Table 1

Percentage* of germinated cystospores formed from *P. infestans* zoospores as influenced by *in vitro* generated** O_2^-

Illumination period (min)	% of germination (± SE)***
0	16±3
15	7±1
30	5±2
60	0

*Data obtained at 60 min after longest period illumination in the presence of O_2^- -producing substances (riboflavin/methionine). **O_2^- treatment was applied to zoospores *** % of germinated cysts was counted on the basis of the number of zoo- and cystospores (cf. Fig. 1b). Data represent means (± SE) of three separate experiments.

have been illuminated 15–30 min (Table 1). Adding an active SOD enzyme to the reaction mixture under light condition (30 min illumination), resulted in complete protection from inhibition. In this case complete encystment was experienced (Fig. 3). The thermoinactivated SOD did not cause any protection against the inhibition. These data strongly suggest that O_2^- is involved in the inhibitory action of riboflavin/L-methionine system.

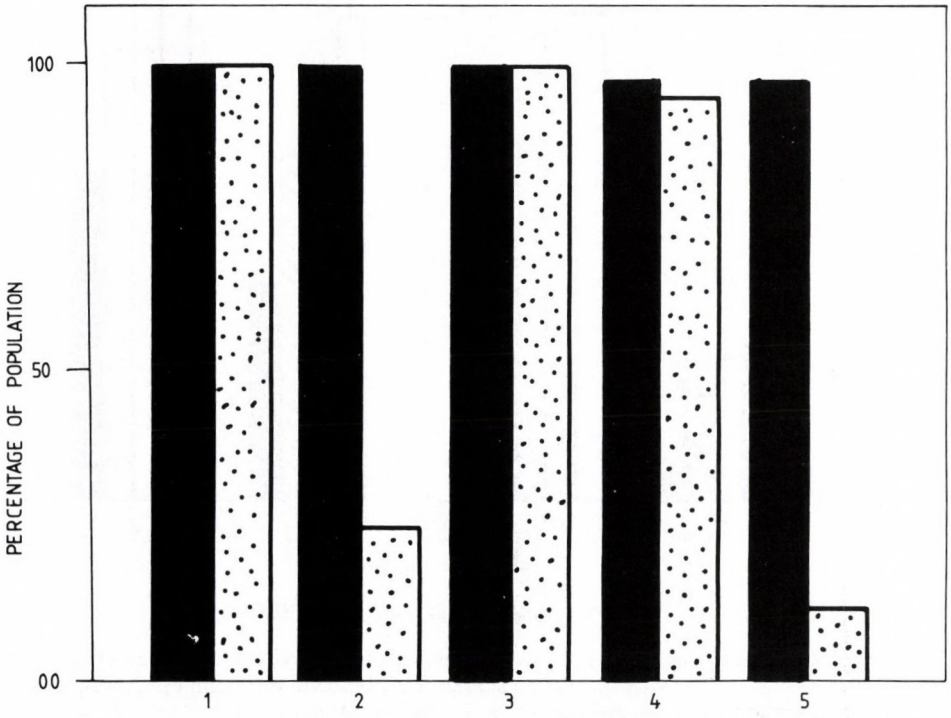

Fig. 3. Populations of zoo- and cystospores of *P. infestans* (■) in dark (control) or (▒) after a 30-min-illumination of the zoospores. Cells were counted at 90 min from the end of illumination in the presence of (1) only riboflavin, (2) riboflavin + methionine, (3) riboflavin + active SOD (50 μg/ml), horseradish SOD enzyme (Sigma, USA) was used, (4) riboflavin + methionine + active SOD, and (5) riboflavin + methionine + thermoinactived SOD. At least 200 cells were counted in each treatment and the experiment was repeated twice

Also the germ tube lenghts of both young and old cystospores were inhibited in this system in the presence of the photochemical system (Figs 2b,c and 4a,b), whereas the germination itself was not affected (Table 2), except that the germination of young cysts was delayed at first then it recovered. On the basis of these results, one can suggest that the presence of cell wall seems to hamped the inhibitory effect of O_2^-.

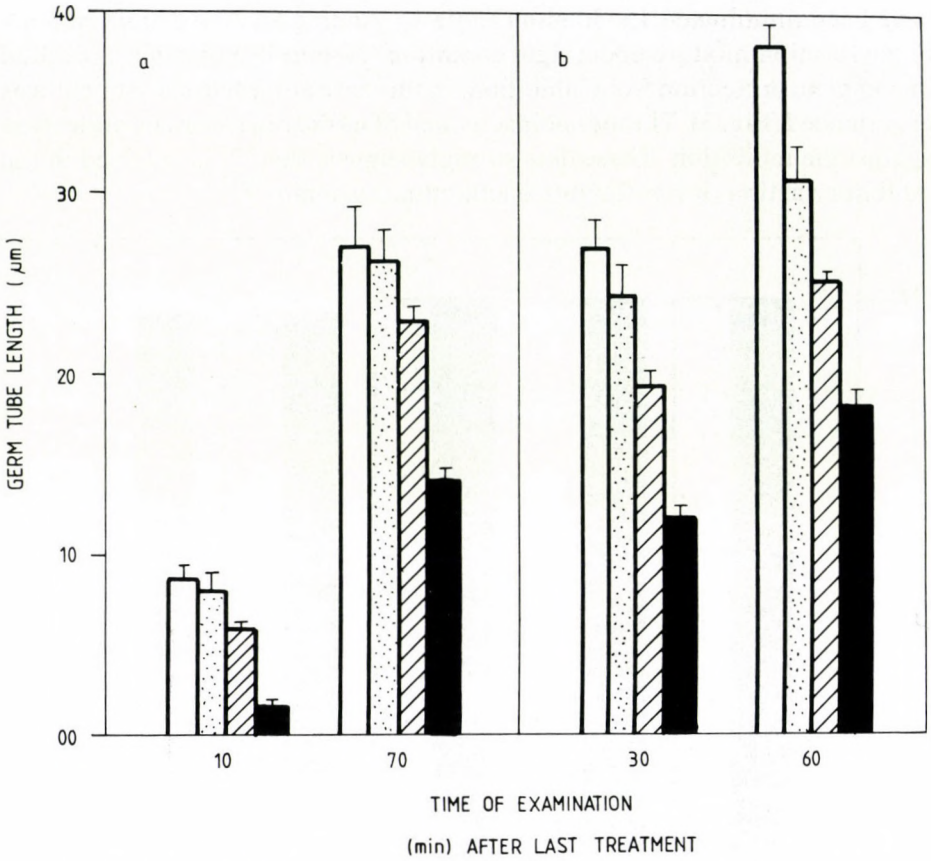

Fig. 4. Germ tube length (µm) of young (10 min-cysts) a), and b) old (50 min-cysts) cystospores as affected by O_2^--producing substances (riboflavin/methionine). Cystospores were exposed to light in the presence of O_2^--producing substances for (▭) 0 (control), (▭) 15, (▨) 30, and (■) 60 min. Then, germ tube lengths were measured at different examination times as indicated above. Bars are standard errors of mean of two separate experiments (at least 200 cyst/treatment were subjected to measure the germ tube lengths)

The mycelial growth of *P. infestans* was also inhibited by this system. The inhibition increased with increasing the concentrations of O_2^--producing substances (Figs 5a, 6a). This restriction in the fungal growth was also time-dependent (Figs 5b and 6b); in the presence of the riboflavin/methionine system it increased by the increase of the duration of illumination. A 24 h illumination exhibited complete inhibition of the fungal growth.

Table 2

Germination of *P. infestans* cystospores (% control) as affected by O_2^- *in vitro* generated

Illumination	10 min-cyst Evaluation time (min) after last treatment		50 min-cyst period (min) Evaluation time (min) after last treatment	
	10	70	30	60
0 (control)	100	100	100	100
15	98±2	96±0.5	103±5	95±1
30	90±3	98±5.5	98±2	96±3.5
60	49±2	100±1.5	94±3.5	91±4.5

Data represent means±SE of two separate experiments (at least 200 cystospores were evaluated per experiment).

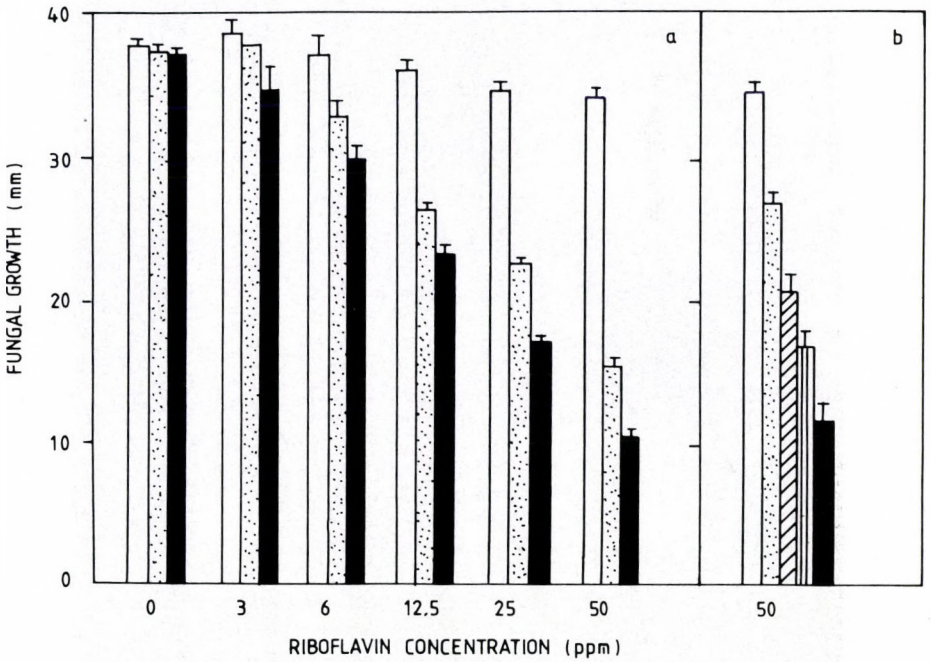

Fig. 5. Radial growth (mm) of 7-day-old *P. infestans* race 2.3.4 grown on pea-broth agar medium supplemented by different concentrations (ppm) of O_2^--producing substances (1 riboflavin : 71 methionine) a), and illuminated for (▭) 0, (▨) 60, or (■) 120 min, while b) explored the time-dependent effect of the photochemical system for O_2^- generation *in vitro* on the fungal growth at (▭) 0, (▨) 15, (▨) 30, (▨) 60, and (■) 120 min illumination using 50 ppm riboflavin with the rational concentration of methionine. Symbols (± SE) represent measures of four replicates/experiment, for three separate experiments

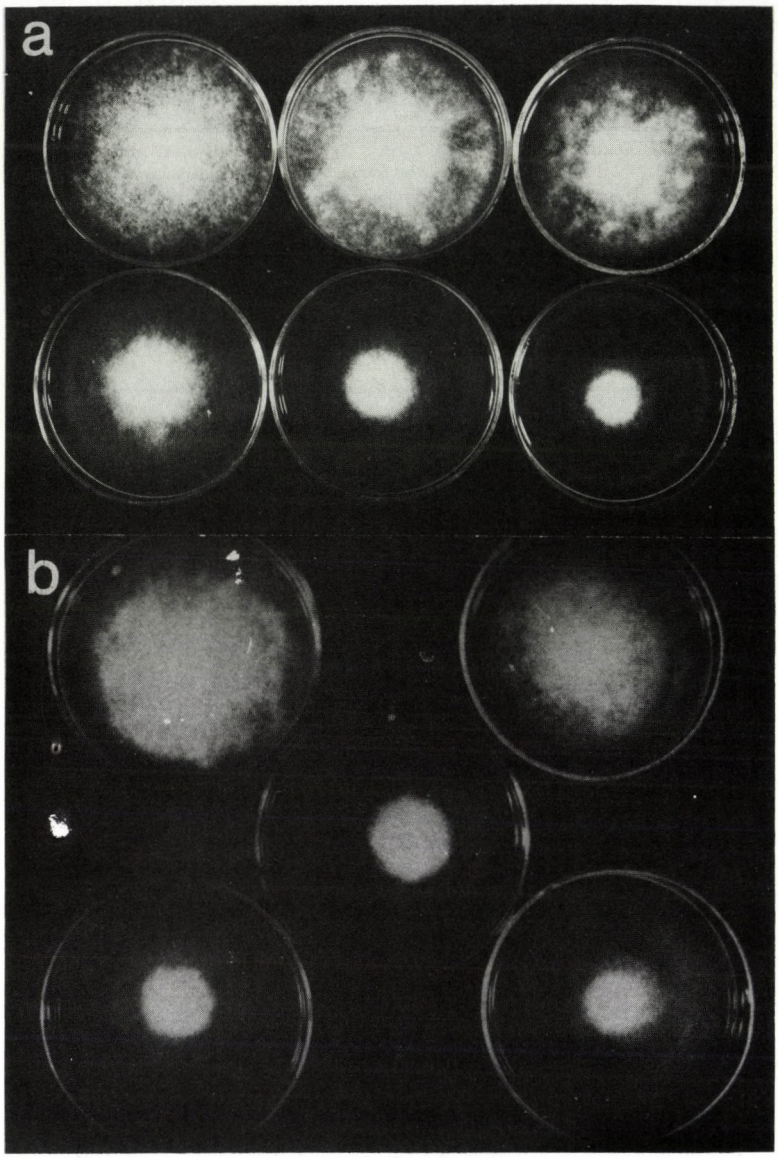

Fig. 6. a) 2h-illuminated culture of 7-day-old *P. infestans* race 2.3.4 grown on pea-broth agar medium supplemented with various concentrations i.e. 0, 3, 6 (upper row from left to right respectively), 12.5, 25 and 50 ppm (lower row from left to right, respectively) of riboflavin with rational concentrations of L-methionine. b) 7-day-old culture of *P. infestants* race 2.3.4 grown on pea-broth agar medium supplemented with 50 ppm riboflavin and rational concentrations of methionine, then illuminated at 0, 15 (upper row from left to right), 30, 60 (lower row from left to right) and 120 min (in the centre)

Our results suggest that zoospores of *P. infestans* are more sensitive to O_2^- than the cystospores (either young or old cysts) or the mycelium of the fungus. It would seem that one of the possible targets of O_2^- action is the plasma membrane (Monk et al., 1989; Merzlyak et al., 1990 and Vianello and Macri, 1991), whereas cell wall of cystospores or the mycelium provide some protection against O_2^-.

In conclusion, O_2^- could disrupt zoospores, inhibit cystospore germ tube lengths and restrict the mycelial growth of *P. infestans*. These events may take place during the incompatible interaction in potato- *P. infestans* system where a considerable increase in O_2^- production has been reported (Doke, 1983 and 1985; Doke et al., 1987; Merzlyak, 1989; Ádám et al., 1990 and Király et al. 1990). This means that the generation of O_2^- and other active oxygen species during the hypersensitive reaction can induce not only membrane damages and necrosis in the host plant cells however, damages to and inhibition of the pathogen itself. Riboflavin alone appeared to have fungicidal effect on the mycelial growth of the fungus; thus it might be possible to use riboflavin for the control of late blight disease as recommended previously by Jordan and DeVay (1989).

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Literature

Ádám, A., Farkas, T., Somlyai, G., Hevesi, M. and Király, Z. (1989): Consequence of O_2^- generation during a bacterially induced hypersensitive reaction in tobacco: Deterioration of membrane lipids. Physiol. and Mol. Plant Pathol. 34, 13–26.

Ádám, A., Érsek, T., Barna, B. and Király,. Z. (1990): Role of oxidative stress in plants on the development of necrosis induced by pathogens. In: B. Matkovics, L. Karmazsin and H. Kalász (eds.): Radicals, Ions and Tissue Damage, Akadémiaii Kiadó, Budapest, pp. 1–18.

Aver'yanov, A. A., Lapikova, V. P., Ummov, A. M. and Dzhhakhiya, V. G. (1987): Generation of superoxide radical by rice leaves in relation to plant tolerance to blast infection. (Sov) Plant Physiol. 34, 373–379.

Beauchamp, C. and Fridovich, I. (1971): Superoxide dismutase: improved assays and assay applicable to acrylamide gels. Anal. Biochem. 44, 276–287.

Croft, K. P. C., Voisey, C. R. and Slusarenko, A. J. (1990): Mechanism of hypersensitive cell collapse: Correlation of increased lipoxygenase activity with membrane damage in leaves of *Phaseolus vulgaris* (L.) inoculated with an avirulent strain of *Pseudomonas syringae* pv. *phaseolicola*. Physiol. and Mol. Plant Pathol. 36, 49–62.

Doke, N. (1983): Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiol. Plant Pathol. 23, 345–357.

Doke, N. (1985): NADPH-dependent O_2^- generation in membrane fractions isolated from wounded tubers inoculated with *Phytophthora infestans*. Physiol. Plant Pathol. 27, 311–322.

Doke, N., Chai, H. B. and Kawaguchi, A. (1987): Biochemical basis of triggering and suppression of hypersensitive cell response. In: S. Nishimura (ed.): Molecular Determinants of Plant Disease. Japan Sci. Phytopathol. Soc., Tokyo Press and Springer Verlag, Berlin, pp. 235–251.

Elstner, E. F. (1984): Comparison of inflammation in pine tree and in humans. In: W. Bors, M. Saran and D. Wait (eds): Oxygen Radicals in Chemistry and Biology. Walter de Grunter, Berlin, New York, pp. 369–381.

Epperlein, M. M., Noronha-Dutra, A. A. and Strange, R. N. (1986): Involvement of the hydroxyl radical in the abiotic elicitation of phytoalexins in legumes. Physiol. and Mol. Plant Pathol. 28, 67–77.

Érsek, T. (1975): The sensitivity of *Phytophthora infestans* to several antibiotics. Z. Pfl. Krankh. Pfl. Schutz. 82, 614–617.

Frank, L. (1985): Oxygen toxicity in eukaryotes. In: L. W. Oberley (ed.): Superoxide Dismutase. CRC Press, Vol. III, 1–43.

Jordan, C. M. and DeVay, J. E. (1989): Sensitivity of *Phytophthora infestans* to riboflavin and its possible use for control of late blight disease of potato and tomato. In: International *Phytophthora* Symposium (Abstr.). Dublin, Sept. 1989. pp. 37–38.

Keppler, L. D. and Baker, C. J. (1989): O_2^- initiated lipid peroxidation in a bacteria-induced hypersensitive reaction in tobacco cell suspensions. Phytopathology 79, 555–561.

Keppler, L. D. and Novacky, A. (1987): The initiation of membrane lipid peroxidation during bacteria induced hypersensitive reaction. Physiol. and Mol. Plant Pathol. 30, 233–245.

Király, Z., Ádám, A., Érsek, T. and Gullner, G. (1990): Relation of oxidative stress to lipid peroxidation and necrotic symptom expression in plants caused by pathogens and chemical damages. In: D. D. Randall and D. G. Blevins (eds): Current Topics in Plant Biochemistry and Physiology, Vol. 9. Univ. Missouri, p. 411.

Merzlyak, M. N. (1989): Activated oxygen and oxidative processes in plant cell membranes. Itogi nauki i tekhniki, ser. Plant Physiology Vol. 6, Moscow, VINITI (in Russian).

Merzlyak, M. N., Reshetnikova, I. V., Chivkunova, O. B., Ivanova, D. G. and Maximova, N. I. (1990): Hydrogen peroxide- and superoxide-dependent fatty acid breakdown in *Phytophthora infestans* zoospores. Plant Science 72, 207–212.

Monk, L. S., Kurt, V. F. and Robert, M. M. C. (1989): Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. Physiol. Plant 76, 456–459.

Oros, G., Érsek, T. and Virányi, F. (1988): Effect of tridemorph on *Phytophthora infestans* and *Plasmopara halstedii*. Acta Phytopathol. Entomol. Hung. 23, 11–19.

Sutherland, M. W. (1991): The generation of oxygen radicals during host plant responses to infection. Physiol. and Mol. Plant Pathol. 39, 79–93.

Vianello, A. and Macri, F. (1991): Generation of superoxide anion and hydrogen peroxide at the surface of plant cells. J. Bioenerg. Biomemb. 23, 409–423.

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Measurement of Exothermic Heat Flow and Leaf Temperature of TMV- and CMV-infected Plants by Microcalorimetry and Infrared Detection Technique

A. L. ÁDÁM[1], F. DUBERT[2], A. GILLY[3], B. BARNA[1] and A. SKOCZOWSKI[2]

[1]Department of Plant Pathology, Plant Protection Institute, Hungarian Academy of Sciences, P.O. Box 102. 1525 Budapest, Hungary [2]Department of Plant Physiology, Polish Academy of Sciences, Podluzna 3, 30239 Cracow, Poland [3]Department of Plant Protection, Agricultural University of Gödöllő, Páter K. 1, 2100 Gödöllő, Hungary

In TMV-infected *N. tabacum* cv. Xanthi nc. leaves considerably higher heat production could be detected only during lesion formation. Similarly, 14 h after temperature shift from 33 °C to 25 °C, when a very quick confluent necrotization of tissue takes place, we found higher heat flow from infected leaf samples as compared to control ones.

In *N. tabacum* cv. Samsun plants (compatible interaction) only upper leaves (bearing mosaic symptoms) showed extra heat production. Similar results were found in cucumber mosaic virus (CMV) infected soybean leaves: after the development of symptoms we detected increased leaf temperature.

These data suggest that both hypersensitive necrotization and systemic virus infection may lead to energy dissipation of plant cells as heat. However, this phenomenon does not seem to be useful tool for the prediction of infection. The possible source(s) of heat overproduction will be hypothesized.

Endo- and exothermic heat (thermal energy) production is a characteristic feature of general metabolic energy transformation processes of cells as well as of some phase transition of biomembranes (Atkinson, 1977; Cullis and Hope, 1985). Transformation of energy from chemically bound energy to ATP (respiration-induced phosphorylation) or from light energy to ATP (light-induced phosphorylation, photophosphorylation) necessarily lead to energy dissipation as heat: decrease in free energy (ΔG), which could be used for chemical work under the conditions of cells is not equal to total energy change (ΔE) of biochemical reactions ($\Delta G = \Delta E - T\Delta S$).

It is well known, that plant diseases of different origin considerably influence the energy transformation of cells (Goodman et al., 1986). Consequently, a study on heat production may give some information about these processes.

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Materials and Methods

Plant and virus

Plants (*N. tabacum* cv. Xanthi nc and *N. tabacum* cv. Samsun) after sowing were cultivated under ordinary greenhouse conditions. After the expansion of the second true leaf, the pots were transferred to phytotrone chambers (25 °C, 130 $\mu Em^{-2}\ s^{-1}$ illumination with 12/12 h day/night period). One group of plants after TMV-infection was placed at 33 °C, for six days.

The infection with tobacco mosaic virus (TMV) was carried out as described earlier (Ádám et al., 1990). At different intervals after infection the plants were sampled for microcalorimetric measurements. One to four leaf disks (0.8 cm in diameter) were taken from leaves, placed into a glass vial, hermetically sealed and were used immediately for the comparison of thermal energy production.

Measurement of heat production and leaf temperature

Heat flow (thermal power output) from tobacco leaf discs was measured in darkness at 25 °C by BioActivity Monitor 2277 (LKB, Sweden). Thermal energy (heat) is usually determined in units of joule (J). Thermal energy produced per unit of time represents a thermal power, usually expressed in units of watt ($1W = 1J\ sec^{-1}$). The BioActivity Monitor makes possible to detect temperature difference across the detector less than 10^{-6} °C, thus we could measure the thermal energy production in the range of 0 to 100 $\mu J\ s^{-1}$. Before measurement the system was calibrated using internal heat source, because the output from the detector is a voltage which is proportional to heat power output from the sample. Heat production was recorded as a function of time and the change in power was red after 16 min, indicated by "p" in the thermogram in Fig. 1. The results finally were expressed in $\mu J\ s^{-1}\ mg^{-1}$ dry weight (Tables 1 and 2). Each data are average of 4–8 repetitions in one or two experiments.

The other detection methods (infrared photography, infrared television) use different physical approaches. Infrared television camera at middle infrared wavelength (=3.5–5.6 μm) detects the emitted light from the leaf surface (Papp, 1989). On thermograms the regions of different leaf temperature could be distinguished (Fig. 3).

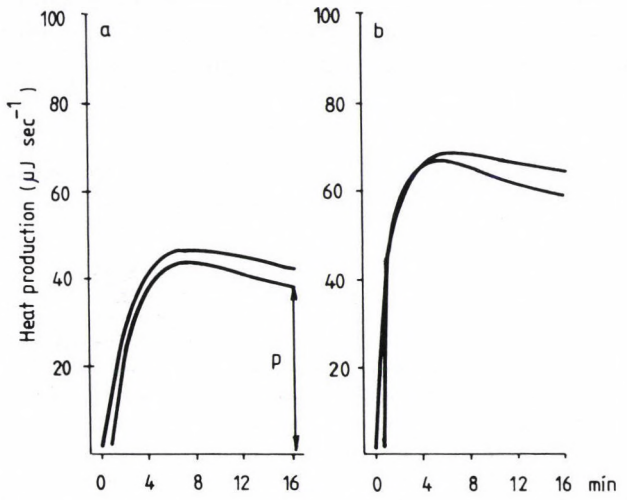

Fig. 1. Effect of temperature shift (from 33 to 25 °C) of TMV infected *N. tabacum* cv. Xanthi nc leaves on heat production (μJ s^{-1}). Sample disks (2 per measurement) were cut from the same leaf stage in control (a) and infected (b) plants. Thermograms on (a) and (b) represent two simultaneous measurements

Table 1

Effect of TMV-infection on heat production (μJ s^{-1} mg^{-1} dry wt) of *N. tabacum* cv. Xanthi nc leaves[1]

	20 h after infection	48 h	14 h after temperature shift[2]
Control	20.1±1.5	17.5±1.1	22.1±3.9
Infected	19.7±2.3	23.3±1.3	29.8±2.8

[1]Data are taken from thermograms similar to those given in Figs 1 and 2. To normalize data, values are corrected by the dry weight of the sample. Data are means ±SD (standard deviation) of 4–8 repetitions. [2]Plants after infection were kept at 33 °C for 6 days, then transferred to 25 °C.

Results and Discussion

TMV-infection both in compatible and incompatible host plants caused an increase in heat production of leaf discs (Tables 1 and 2).

In *N. tabacum* cv. Xanthi leaves the development of hypersensitive necrosis led to heat overproduction (Table 1), simultaneously with symptom development. In plants kept at 33 °C for 6 days after infection, the increased heat

production was also found during necrotization, 14 h after temperature shift to 25 °C (Table 1 and Fig. 1). During the incubation at 33°C we could not detect any differences in heat production (data not shown).

Table 2

Effect of TMV-infection on heat production (μJ s^{-1} mg^{-1} dry wt) of *N. tabacum* cv. Samsun leaves[1]

	Infected lower symptomless leaves		Upper mosaic leaves			
	20 h after infection	72 h	7 days[2] after infection	11 days		
Control	24.4±2.4	19.1±2.7	21.2±2.1	–	–	23.5
Infected	23.8±1.0	20.3±2.4	28.7±1.6	32.8[3]	31.8[4]	37.1[5]

[1]Data are taken from thermograms similar to those given in Figs 1 and 2. To normalize data, values are corrected by the dry weight of the sample. [2]Seven days after infection the mosaic symptoms were weakly developed. [3,4,5] Pale green (3), dark green (4) and intermediate (5) (consisting pale green and dark green areas) parts of mosaic leaves. $LSD_{1\%}$=3.24.

In *N. tabacum* cv. Samsun plants (compatible host-parasite complex) the inoculated lower leaves (which remained symptomless) did not show enhanced heat production (Table 2). In upper leaves showing systemic mosaic symptoms, a pronounced heat flow (Table 2 and Fig. 2) was found. The highest rate of heat production (37.1 μJ s^{-1} mg^{-1} dry wt) was detected on the border of yellowish-green and dark green zones of mosaic area.

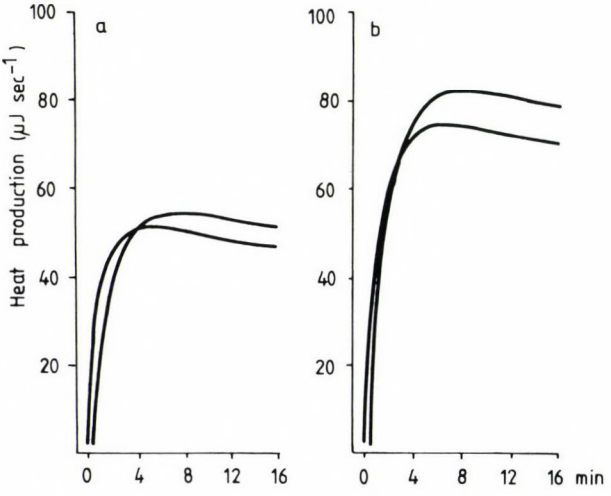

Fig. 2. Effect of systemic TMV infection (b) on heat production (μJ s^{-1}) of *N. tabacum* cv. Samsun leaves. Sample disks (2 per measurement) were taken from the intermediate part of mosaic leaves where both dark green and pale green areas were present. Thermograms on (a) and (b) represent two simultaneous measurements. Control disks (a) were cut from the same leaf stage

The increased heat production could be the consequence of general increase in the rate of cellular metabolism involving photosynthesis and respiration. Doke and Hirai (1970) found for example, that in the early stages of TMV-infection in tobacco producing systemic infection, there was a stimulation of $^{14}CO_2$ incorporation in cells in and around the site of infection. In hypersensitive hosts of viruses the stimulation of respiration is a characteristic feature, sometimes before, sometimes coincidently with local lesion appearance (Goodman et al., 1986). Since in TMV-infected *N. glutinosa* leaves higher rate of virus multiplication could be detected before lesions appeared, while increase in respiratory activity could not, Yamaguchi and Hirai (1970) concluded the rise to be directly related to necrosis, rather than virus multiplication. The same phenomenon is suggested in connection with heat overproduction of hypersensitive host (Table 1). Increased number of mitochondria was also reported in TMV-infected *N. glutinosa* leaves (Weintraub et al., 1972).

In systemically infected plants heat overproduction could be also related to development of symptoms (Table 2). In upper mosaic leaves the heat overproduction showed some connection with the strenght and/or mode of symptom development (Table 2). It is possible that above findings are related to the hormonal changes (i.e. accumulation of extrachloroplastic ABA and cytokinin-glucosides in dark green areas of mosaic) as well as to virus multiplication which takes place only in pale green (later yellowish) parts of mosaic (Whenham et al., 1986; 1989).

Similar results were obtained on cucumber mosaic virus (CMV) infected soybean leaves by infrared television technique. Increase in leaf temperature of infected leaves was 2–3°C. It was probably, at least in part, a consequence of higher heat production of CMV-infected leaves (Fig. 3).

In other host-parasite interactions (wheat-*Puccinia recognita*, tomato-*Septoria lycopersici*, sunflower-*Sclerotinia sclerotiorum*, sunflower-*Macrophomina phaseolina*, soybean-*Pseudomonas glycinea*) an increased leaf temperature were also found (Gilly, 1989, unpublished). The increased temperature in these host-parasite interactions, similarly to heat overproduction of TMV-infected leaves, was detectable only after the development of eye-visible symptoms. Therefore this phenomenon does not seem to be useful tool for the detection and/or prediction of infection before the appearance of macroscopic symptoms.

Acknowledgement

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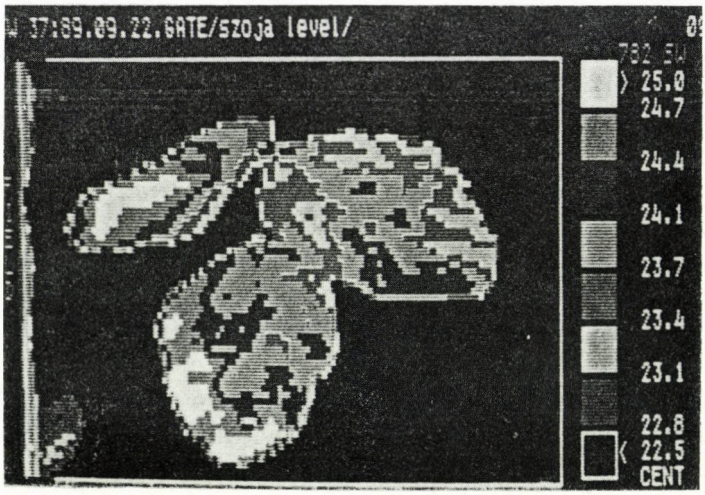

Fig. 3. Thermogram of cucumber mosaic virus (CMV) infected trifoliate part of soybean. The originally colour print on a black and white picture is less distinguishable. The red areas (24.1 – 24.4 °C on the scale) could be seen as dark spots. White areas (over 25.0 °C) appeared only in leaves with well developed mosaic symptoms (the photo was taken by 782 SW infracamera)

Literature

Ádám, A., Barna, B., Farkas, T. and Király, Z. (1990): Effect of TMV-induced systemic acquired resistance and removal of the terminal bud on membrane lipids of tobacco leaves. Plant Sci. 66, 173–179.

Atkinson, D. E. (1977): Cellular energy metabolism and its regulation. Academic Press, New York, p. 283.

Cullis, P. R. and Hope, M. J. (1985): Physical properties and functional roles of lipids in membranes. In: Biochemistry of Lipids and Membranes (Eds: D. E. Vance and J. E. Vance), The Benjamin/ Cummings Publishing Comp., Menlo Park, California, pp. 25–72.

Doke, N. and Hirai, T. (1970): Effects of tobacco mosaic virus infection on photosynthetic CO_2 fixation and $^{14}CO_2$ incorporation into protein in tobacco leaves. Virology 42, 68–77.

Goodmann, R. N., Király, Z. and Wood K. R. (1986): The Biochemistry and Physiology of Plant Disease. University of Missouri Press, Columbia, p. 433.

Papp Z. (1990): Távérzékelési eljárások körének bövítése, technológiák kialakítása mezőgazdasági hasznosításhoz. (Összefoglaló jelentés) (In Hungarian) MÉM Műszaki Intézet, Gödöllő, p. 93.

Weintraub, M., Ragetli, H. W. J. and Lo, E. (1972): Mitochondrial content and respiration in leaves with localized virus infections. Virology 50, 841–850.

Whenham, R. J., Fraser, R. S. S., Brown, L. P. and Payne, J. A. (1986): Tobacco mosaic virus-induced increase in abscisic acid concentration in tobacco leaves: intracellular location in light and relationship to symptom development. Planta 186, 592–598.

Whenham, R. J. (1989): Effect of systemic tobacco mosaic virus infection on endogenous cytokinin concentration in tobacco (*Nicotiana tabacum* L.) leaves: consequences for the control of resistance and symptom development. Physiol. Mol. Plant Pathol. 35, 85–95.

Yamaguchi, A. and Hirai, T. (1959): The effect of local infection with tobacco mosaic virus on respiration in leaves of *N. glutinosa*. Phytopathology 49, 447–499.

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Ascochyta fabae f. sp. *lentis* in Seeds of Lentil, its Location and Detection

KULWANT SINGH, M. N. KHARE and S. B. MATHUR

Danish Government Institute of Seed Pathology for Developing Countries, Ryvangs Allé 78, DK–2900 Hellerup, Denmark

Component plating, wholemount preparations and microtome sectioning showed inter- and intracellular mycelium of *Ascochyta fabae* f. sp. *lentis* in the seed coat, cotyledons and the embryonal axis of infected seed of lentil. Heavily infected seeds were usually small, discoloured with dark brown lesions. Using different media, maximum number of seeds infected with *A. fabae* f. sp. *lentis*. were detected in a seed sample when seeds, pre-treated with 2% available chlorine in an aqueous solution of sodium hypochlorite for 2–3 minutes, were plated on potato dextrose agar (PDA) and incubated for 7 days under 12 hours alternating cycles of NUV light and darkness at 22±2 °C. Colonies of *A. fabae* f. sp. *lentis* were rusty brown with abundant pycnidia.

Blight of lentil (*Lens culinaris* Medik.) caused by *Ascochyta fabae* f. sp. *lentis* Speg. is widespread in lentil growing areas of the world (Bondartzeva-Monteverde and Vassilievsky, 1940; Veiga, Manara, Manara and Tarrago, 1974; Mitidieri, 1975; Khare, Agrawal and Jain, 1979; Morrall and Sheppard, 1981; Kaiser and Hannan, 1982; Khan, Haq, Rehman and Aslam, 1983). Losses upto 40% have been reported from Canada, (Gossen and Morrall, 1983). The pathogen is seed-borne and seed transmitted. Gossen and Morrall (1986) have shown that transmission frequency from seed to plant depends on the degree of seed infection.

Seed-borne infection has been detected by a number of previous workers using different media (Morrall et al., 1981; Gossen et al., 1983; Sumar and Howard, 1983; Beauchamp, Morrall and Slinkard, 1986; Kaiser and Hannan, 1986). Recently, Morrall and Beauchamp (1988) concluded that plating lentil seeds on a nutrient-rich media such as PDA, MEA or V8 is most appropriate for detecting *Ascochyta fabae*. In this paper, results are presented on the location of the fungus in seed components and on comparative seed health testing conducted on PDA, water agar and blotter.

Materials and Methods

Six seed samples used in this study belonging to two varieties were received through the courtesy of Dr. Walter J. Kaiser, Western Regional Plant Introduction Center, USDA, Washington State Univ., Pullman, WA. All the

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samples were examined visually as well as under stereomicroscope for any morphological abnormality and for indication of infection. Seeds with lesions of *Ascochyta fabae* were used for studying the location of the pathogen in different seed components. This was done by component plating, whole mount preparations and microtome sectioning.

Thirty apparently diseased lentil seeds from a sample (acc. no 438517; Fig. 1) were soaked in distilled water for about 5 hours. Seed coat, cotyledons and embryonal axis were separated, surface sterilized with 2% available chlorine (aqueous solution of sodium hypochlorite) for 2–3 minutes and plated on PDA, components of two seeds per dish, and incubated under alternating cycles of 12 h NUV light and darkness for seven days at 22±2 °C.

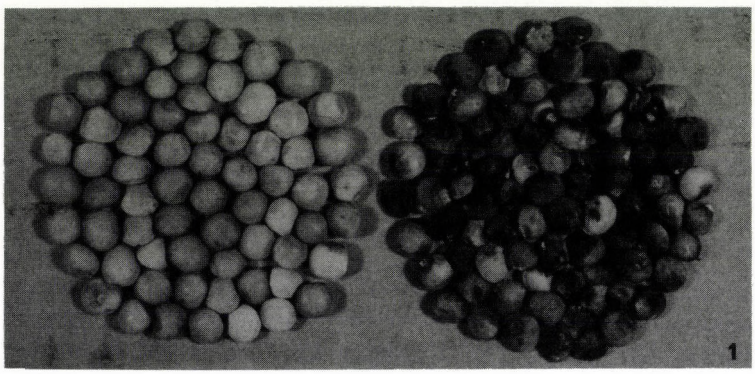

Fig. 1. Healthy looking seeds of lentil (left) and discoloured seeds infected with *Ascochyta fabae* f. sp. *lentis* (right)

For wholemount preparations, twenty discoloured seeds of sample no 438517, suspected to be infected with *Ascochyta fabae*, were boiled in water for about 10–15 minutes. The seed components were separated and boiled in lactophenol till softening. The components were stained in cotton blue and mounted on glass slides in polyvinyl alcohol (Omar, Bolland and Heather, 1979). The slides were dried in an oven at 60 °C overnight.

For making microtome sections, the discoloured seeds (acc. no 438517, Fig. 1) were softened by boiling in water for 10–15 min, dehydrated through tertiary butyl alcohol series, infiltrated and embedded in wax. Serial sections were cut, 12 microns thick, stained in saffranin and light green and mounted in Canada balsam.

Four hundred seeds, drawn at random, from each of the six samples were tested by blotter, 2% water agar and PDA methods. In the blotter method, 25 seeds were plated in each 9 cm petri dish containing three well water-soaked blotters while in water agar and PDA methods 10 seeds were plated in each dish. The seeds used in the PDA method were surface sterilized for 2–3 minutes in 2% available chlorine (aqueous solution of sodium hypochlorite). All dishes were incubated at 22±2 °C under 12 hours alternating cycle of light from NUV and darkness.

In the blotter method, each seed was examined under stereomicroscope for the pycnidia of the fungus. Confirmation of the identification was always done by observing pycniospores under higher magnifications of a compound microscope. On agar, only those seeds were counted as infected by *Ascochyta fabae* where rusty brown colonies with pycnidia were formed. Counting of seed infection in agar dishes was based on visual examination of the colonies. In all the three methods recording of infected seeds by *A. fabae* was done after 7. 9 and 11 days of incubation. Each experiment was repeated three times.

Results

Seeds heavily infected with *Ascochyta* were smaller, discoloured, brown to purplish black without any lustre (Fig. 1). The lesions were dark brown, small to very large. In large lesions, whitish mycelium and sometimes yellowish-brown pycnidia were seen. The discoloured infected seeds on incubation yielded *A. fabae* f. sp. *lentis*.

Histopathology

Out of the 30 selected seeds examined by the component plating method, seed coat of 22 seeds, 24 cotyledons and three embryonal axis showed the presence of the pathogen. All combinations were noticed, infection of one or both the cotyledons, infection of cotyledons but not the embryonal axis and infection of embryonal axis alone (Fig. 2).

The wholemount preparations of seed coat, cotyledons and embryonal axis showed the presence of thick, yellowish brown ramyfying mycelium. The mycelium was more brown and thicker in seed coat (Fig. 3a) as compared to cotyledons (Fig. 3b) and embryonal axis.

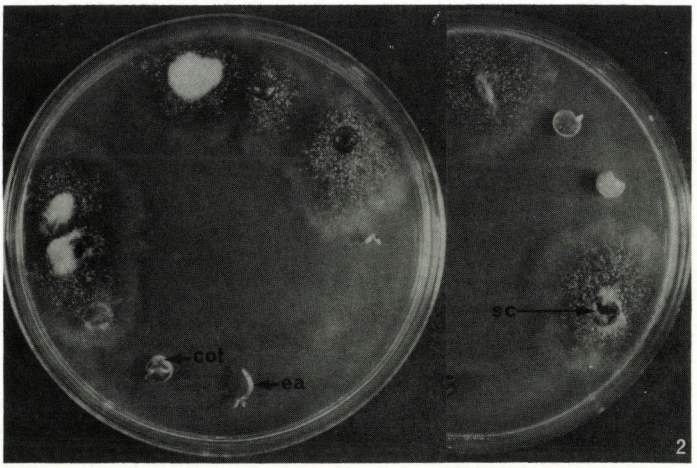

Fig. 2. Colonies of *A. fabae* f. sp. *lentis* developing from seed coat (sc), cotyledons (cot) and embryonal axis (ea) in component plating study

The microtome sections of infected seeds provided the exact picture of spread and location of the pathogen in different parts.

In mild to moderately infected seeds, the pathogen travelled horizontally and vertically in the pallisade cells, hour glass cells and inter- and intracellular in parenchymatous tissues (Fig. 3c). However, in heavily infected seeds, apart from seed coat, the pathogen covered the adaxial (outer) surface of the cotyledons and spread between the seed coat and cotyledons. The abaxial (inner) surface was also colonized by the fungus in some cases. Mycelial aggregations were seen in the hilar region below the seed coat (Fig. 3d). They appeared like microsclerotia. The mycelium was found spreading throughout the tissue in the hilar region (Fig. 3e, f). Cotyledonary cells were not affected in mild and moderate infections. However, they were loosened and vacoulated in moderate to heavy infections. It appears that the fungus depletes the protein contents making the cells empty. Fungal hyphae were seen in the trachieds.

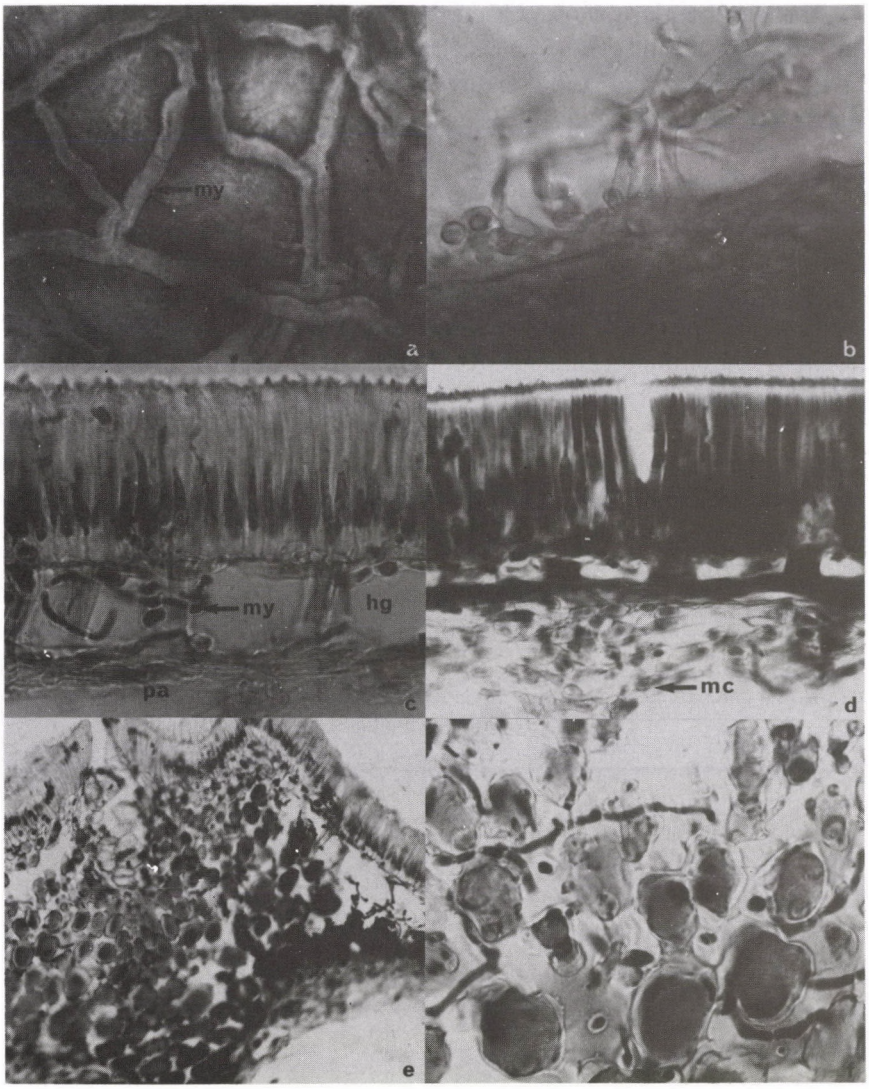

Fig. 3. Histopathology of lentil seed infected with *Ascochyta fabae* f. sp. *lentis*.
a) Wholemount preparation of seed coat showing thick mycelium (my) (×750);
b) Wholemount preparation of cotyledons. Note the emerging mycelium from a part of a cotyledon (×750); c) A part of longitudinal section of seed coat showing mycelium (my) in hour glass cells (hg) and parenchyma (pa) (×750); d) Same as in c). Note the aggregation of mycelium simulating microsclerotia (mc) below hour glass cells (×750); e) Longitudinal section passing through the hilar region showing spread of mycelium (×200);
f) A part of e magnified. Note the thick, septate mycelium (×750)

The seed sections showed accumulation of yellowish-brown pigment in the infected tissues and death of cells. The mycelial colour also became slightly yellowish. It appears that the fungus produces some metabolite(s) which result in the formation of lesions on the seed tissue.

Comparative seed health testing

The growth of *A. fabae* was observed on infected seeds in all the three methods. Detection was easiest, however, on PDA as more pycnidia were observed within the colonies as compared to more mycelium and less pycnidia on water agar and blotters. Colonies on PDA were very distinct, rusty brown in colour (Fig. 2).

The number of seeds counted infected by *A. fabae* were larger in the PDA method (Table 1). Prolonging the incubation period from 7 to 9 or 11 days did not contribute in increasing the counts. On the contrary, prolonged incubation resulted in increase of saprophytes which interfered with the recording of infection of *A. fabae*.

Table 1

Infection percentage of *Ascochyta fabae* f. sp. *lentis* in six lentil seed samples tested by different methods

Testing method	Days of incubation	Sample number with per cent seed infection					
		438516	471917	LSS-83129	438515	438518	438517
PDA	7	0.3	2.7	5.0	7.7	20.0	66.0
	9	0.3	2.7	5.0	7.7	20.0	66.0
	11	0.3	2.7	5.0	7.7	20.0	66.0
Water Agar	7	0.3	1.3	0.5	8.3	11.3	57.7
	9	0.3	1.3	0.5	8.3	11.3	57.7
	11	0.3	1.3	0.5	8.3	12.3	57.7
Blotter	7	0.3	1.3	2.0	6.3	8.0	61.7
	9	0.3	1.3	2.0	6.3	8.0	61.7
	11	0.3	1.3	2.0	6.3	8.0	61.7

Discussion

In 1986, Gossen and Morrall suggested that the mycelium of *A. fabae* enters the seed from lesions produced on the pod wall. They did not indicate which part of the seed is infected. However, from histopathological studies reported in this paper, it is clear that the hilar region of the seed is the first to receive infection and from there it spreads to the cotyledons and the embryonal axis. This conclusion is based on the fact that it is always the hilum region which has the mycelium of *A. fabae* whether the seed is mildly or heavily infected. This observation is further substantiated by the aggregation of the mycelium and formation of microsclerotia-like structures in the hilar region.

Gossen and Morrall (1986) further suggested that in seed with large or numerous areas of surface discolouration, the pathogen penetrates the embryo prior to germination, or is in close proximity to it, spreading, eventually, to the epicotyl during germination. Maden, Singh, Mathur and Neergaard (1975) showed that in chickpea infection of *A. rabiei* present on the dorsal surface of cotyledons in the micropyle region contributes to the initiation of the disease in seedlings. We assume, on the basis of the inoculum of *A. fabae* present in the hilar region of lentil seed that the inoculum may also be transferred to seedlings in a similar manner.

In a recent investigation, Morrall and Beauchamp (1988) showed that if an infected seed sample of lentil is surface sterilized for 1, 5, 10, 20 and 30 minutes in 1% available chlorine, rinsed with water and plated on PDA, there is a false increase in the number of seeds found infected by the *Ascochyta*. This increase has been attributed to contamination of healthy seeds by viable spores released from pycnidia present on the infected seed. Although no attempt was made in our studies to check this observation, we feel that false increase must have been due to the removal of sterilant by rinsing. In our investigations, the seeds were surface sterilized for 2–3 minutes in 2% available chlorine and were directly plated on PDA and it is evident from the results reported in this paper that PDA was comparatively better for recording *A. fabae* f. sp. *lentis* in lentil seed samples.

Literature

Beauchamp, C. J., Morrall, R. A. A. and Slinkard, A. E. (1986): The potential for control of Ascochyta blight of lentil with foliar-applied fungicides. Canadian Journal of Plant Pathology 8, 254–259.

Bondartzeva-Monteverde, V. N. and Vassilievsky, N. I. (1940): A contribution to the biology and morphology of some species of *Ascochyta* on Leguminosae. Pages 345–376. In: Acta Inst. Bot. Acad. Sci. U.R.S.S. 1938. Ser. II (Pl. Crypt.) (In Russian) (In: Rev. Appl. Mycol. 20, 232–233, 1941).

Gossen, B. D. and Morrall, R. A. A. (1983): Effect of Ascochyta blight on seed yield and quality of lentil. Canadian Journal of Plant Pathology 5, 168–173.

Gossen, B. D. and Morrall, R. A. A. (1986): Transmission of Ascochyta blight of lentil from infected seed and plant residue. Canadian Journal of Plant Pathology 8, 28–32.

Kaiser, W. J. and Hannan, R. M. (1982): *Ascochyta lentis*: Incidence and transmission in imported lentil seed. (Abstr.) Phytopathology 72, 944.

Kaiser, W. J. and Hannan, R. M. (1986): Indicence of seedborne *Ascochyta lentis* in lentil germ plasm. Phytopathology 76, 355–360.

Khan, B. A., Haq, I. U., Rehman, F. U. and Aslam, M. (1983): *Ascochyta* blight of lentil – A new disease in Pakistan. Pak. J. Bot. 15, 121.

Khare, M. N., Agrawal, S. C. and Jain, A. C. (1979): Diseases of lentil and their control. Tech. Bull., Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India, 29 pp.

Maden, S., Singh, D., Mathur, S. B. and Neergaard, P. (1975): Detection and location of seed-borne inoculum of *Ascochyta rabiei* and its transmission in chickpea (*Cicer arietinum*). Seed Science and Technology 3, 667–681.

Mitidieri, I. Z. M. de (1975): La mancha de la lenteja (*Lens culinaris* Medik.) causada por *Ascochyta lentis* Bond. y Vassil., en la Argentina, Rev. Invest. Agropecu., Ser. Cinco. Patol. Veg. Buenos Aires 11, 43–55.

Morrall, R. A. A. and Sheppard, J. W. (1981): *Ascochyta* blight of lentils in western Canada: 1978 to 1980. Can. Plant Dis. Surv. 61, 7–13.

Morrall, R. A. A. and Beauchamp, C. J. (1988): Detection of *Ascochyta fabae* f. sp. *lentis* in lentil seed. Seed. Sci. and Technol. 16, 383–390.

Omar, M. B., Bolland, L. and Heather, W. A. (1979): A permanent mounting medium for fungi. Bull. Brit. Mycological Soc. 13, 31–32.

Sumar, S. P. and Howard, R. J. (1983): Seed microflora of pulses grown in Alberta, Canada. Seed Science and Technology 11, 363–369.

Veiga, P., Manara, W., Manara, N. T. F. and Tarragó, M. F. S. (1974): Ocorrencia de *Ascochyta lentis* Bond. et Vassil. em lentilha (*Lens culinaris* Medik.) no Rio Grande do Sul. Rev. Cent. Cienc. Rurais 4, 239–242.

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Modification of Biocontrol Potential of *Trichoderma viride*

IDA SINHA and R. S. UPADHYAY

Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi – 221005, India

Modification of biocontrol agents can play a great role in improving the biocontrol potential of antagonists. It has been visualised that the next decade will see wide exploitation of genetic engineering and biotechnology in the service of biological control. With this idea the modification of *Trichoderma viride* was done by a mutagenic chemical N-methyl N'-nitro N-nitrosoguanidine (NTG). NTG was found a promosing agent for induction of mutation in *T. viride*. At 1500 μg/ml concentration of NTG a mutant (TVW–1) with pure white colonies was obtained. The mutant sporulated heavily and had shown a better biocontrol ability than the parent strain (TVP) when used against the wilt disease of pigeon-pea caused of *Fusarium udum*.

Modification of biocontrol agents offers a possible approach to further improve their biocontrol potential for plant disease control (Ball, 1980). The improvement of strains of microorganisms is usually done by mutation and selection. By this technique Backus and Stauffer (1955) increased the output of penicillin in *Penicillium chrysogenum* from 250 to 2500 μg/ml. Papavizas and Lewis (1981) initiated work on modifying biocontrol potential of antagonists for biological control of several plant pathogens. They induced mutations by UV-irradiation in *Trichoderma harzianum* and by implification of its biocontrol potential, *Rhizoctonia* damping-off of cotton and radish and white rot of onion caused by *Sclerotium copivorum*, damping-off and blight of bean caused by *Sclerotium rolfsii* and damping-off of peas caused by *Pythium ultimum* were more effectively controlled (Papavizas, 1985).

In the present investigation, an attempt has been made to produce a strain of *Trichoderma viride* – one of the effective antagonists of *Fusarium udum* (Upadhyay and Rai, 1988) for better biocontrol potential against *Fusarium udum* which causes wilt disease of pigeon-pea.

Materials and Methods

Production of mutant strain of T. viride

For this purpose, *Trichoderma viride* parent strain (TVP) was treated with N-methyl N'-nitro N-nitrosoguanidine (NTG) in phosphate buffer at pH 6.8. The spores were harvested by a hockeystick shaped glass rod from a 6-day old culture.

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The spore suspension was preparead in 100 ml Czapex-Dox Broth and the spore concentration was adjusted to 10^7 spores/ml. The suspension was incubated at 25±2 °C for 6 hours allowing the spores to germinate. The spores were then washed thrice with sterile phosphate buffer by centrifugation at 3,000 rpm. Meanwhile NTG solution was prepared by dissolving 0.5 g NTG in 50 ml phosphate buffer and it was added to spore suspension in test tubes in desired concentration as given in Table 1.

Table 1

NTG treatment in different concentrations to spore suspension

NTG solution (ml)	Spore suspension (ml)	Total desired concentration of NTG (µg/ml)
0.5	9.5	500
1.0	9.0	1000
1.5	8.5	1500
2.0	8.0	2000
3.0	7.0	3000
4.0	6.0	4000
4.5	5.5	4500
5.0	5.0	5000

The treatment and incubation were done in dark. Then the spores of all tubes were washed twice with sterilized distilled water. The spores were spread in Petri plates containing Czapek-Dox Agar medium. Three replicates were taken for each concentration of NTG. The plates were incubated at 25±2 °C for 6 days and then observation was made.

At 1500 µg/ml concentration of NTG, a distinct white coloured mutant (TVW–1) was observed which was isolated and characterized.

Radial growth

Five mm blocks of *T. viride* parent and mutant strains were inoculated in the centre over solid Czapek-Dox Agar medium in replicate Petri plates separately. Radial growth of both the strains was measured after 3, 5 and 7 days.

Colony interaction

Colony interaction between *F. udum* and parent and mutant strains of *T. viride* was studied in dual culture by placing 5 mm blocks of the test pathogen and

the *T. viride* over Czapek-Dox medium approximately 2.5–3.0 cm apart in paired combinations in replicate Petri dishes. The area covered by both of these microorganisms was measured by graph paper method. Hyphal interaction between *F. udum* and TVP and TVW–1 was observed by microscope. The per cent inhibition of growth of all the microorganisms was calculated with the help of the formula

$$\frac{a_c - a_t}{a_c} \times 100,$$

where a_c is the total area covered by the microorganism in the control plate and a_t is the total area covered by the microorganism in the treatment.

Morphological studies

Morphological variation between TVP and TVW–1 was observed by mounting a bit of mycelium with cotton blue and observed under light microscope.

Efficacy of parent and mutant strain in disease control in vivo

Pure soil inocula of *F. udum* as well as TVP and TVW–1 were prepared by the following method. Sandy loam soil consisting of 82.3% sand, 2.3% silt, 15% clay, 0.3% organic matter, 0.02% N, 0.06% K, 0.01% P and 0.003% extractable Fe and having moisture of 12.2%, pH 7.4 was well mixed with 20% acid washed sand + 3% maize meal before sterilization. 100 g such sterilized soil sample was taken into each 250 ml conical flask and was inoculated with the individual test organism separately by transferring into it 3 blocks (each of 10 mm diam) of their cultures. Three replicates were taken for each organism and thee flasks were incubated at 25±2 °C for 15 days for fungal growth. The inoculum-sand mixture was taken out from the flasks and was air dried under aseptic condition. The population of the test organism was adjusted to approximately 10×10^3 g^{-1} soil by mixing in it, appropriate weight of acid washed sterilized sand. The soil-sand inoculum of the test pathogen was amended with that of TVP and TVW–1 separately in the ratio of 1:1. The mixed soil inocula were put in earthenware pots (10 cm height and 8 cm diameter) in three replicates separately and the moisture of each pot was maintained at 20%. Eight surface sterilized seeds of "BAHAR" cultivar of pigeon-pea were sown in each pot. The pots were incubated in growth chamber at 25±2 °C for one month and then observation for per cent incidence of the wilt disease was made.

Results and Discussion

Production of mutant strain of T. viride

At 1500 µgNTG/ml, a distinct white colony of *T. viride* was developed in the plate. It was transferred for several generations on Czapek-Dox Agar medium. It did not revert. It was found to be the mutant of *T. viride* which has been designated as TVW–1.

Radial growth

The mutant grew faster than TVP on Czapek-Dox Agar medium. TVW–1 covered the whole plate after 5 days only while the parent took 7 days for the same (Table 2). Furthermore, sporulation also started earlier in TVW–1 than TVP.

Table 2

Effect of incubation period on area of *Trichoderma viride* parent (TVP) and mutant (TVW–1) strains *in vitro*

Test organisms	Incubation period (days)/mm²		
	3	5	7
TVP	225.0 ±2.8[a]	2009.0 ±3.6	6350.0 ±0.0
TVW –1	1242.0 ±0.0	6350.0 ±0.0	6350.0 ±0.0

a = Mean of triplicate; ± = S. E.

Colony interaction

The result of the colony interaction has been summarized in Table 3. TVW–1 more vigorously inhibited *F. udum* than TVP. The inhibition of *F. udum* was 74.0 and 85.0% by TVP and TVW–1 respectively. No hyphal interaction was observed between *F. udum,* and TVP and TVW–1.

Morphological studies

Microscopic observation revealed that the diameter of the mycelium of the mutant strain was less and its phialides were longer than the parent strain. The phialides of TVP were 8 × 2.4 µ while that of TVW–1 were 15 × 1.5 µ.

Table 3

In vitro colony interaction between *F. udum* vs *T. viride* parent (TVP) and mutant (TVW-1) strains and in vivo biocontrol of the wilt disease in soil inoculated with TVW-1 and TVP

Test organisms	% inhibition in area of the colony of *F. udum**	Height of plants* (cm)	% incidence of wilt*
TVP	74.0	–	–
TVW-1	85.0	–	–
Seeds + *F. udum*	–	27.5	98.0
Seeds + *F. udum* + TVP	–	35.0	37.5
Seeds + *F. udum* + TVW-1	–	40.0	12.5
Control (without any inoculum)	–	40.0	0.0

*Mean of three replicates

Efficacy of TVP and TVW–1 in disease control in vivo

After one month of sowing the plants in control pot (without inoculum) were found growing vigorously attaining an approximate height of 40 cm. The incidence of wilt in pigeon-pea plants was 98.0% in the pots containing only *F. udum* inoculum and their average height was 27.5 cm. The plants of the pot containing *F. udum* + TVP were in better condition than that of without TVP, their average height was 35 cm and the incidence of wilt was 37.5%. The plants of the pot containing *F. udum* + TVW–1 were in further better condition since the incidence of wilt was only 12.5% and the average height of plants was 40 cm as in the plants of the control pot.

The biocontrol potential of *Trichoderma* spp. against soil-borne plant pathogens is well known (Chet et al., 1979; Sivan and Chet, 1986; Sivan et al., 1984). Mutagenesis induced enzyme production has been successful with strains of *Trichoderma* used in industrial biological processes (Mandels et al., 1971; Montenecourt and Eveleigh, 1979). Induced mutagenesis has also been used successfully with strains of *T. harzianum* and *T. viride* to develop new biotypes (Papavizas and Lewis, 1982, 1983) with a better biocontrol potential against many soil-borne plant pathogens.

From the green coloured parent strain of *T. viride*, a white coloured mutant was obtained. It can be attributed to the loss of pigmentation in the parent strain due to mutagenesis. The new strain possessed better antagonistic as well as biocontrol ability against *F. udum* (Table 3). In dual cultures, the overgrowth is achieved when one fungal species exhibits higher growth rate, higher capacity of antibiotic production and good tolerance capacity of antibiotics produced than the other fungus (Upadhyay and Rai, 1987). In absence of hyphal interference

(either external or internal) *in vitro* dual culture experiment it is evident that antibiotic produced by the mutant strain has better above said qualities than the parent one against *F. udum*.

Pot experiments also provided better biocontrol potential of TVW–1 against wilt disease of pigeon-pea over TVP.

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Literature

Backus, M. P. and Stauffer, J. E. (1955): Mycologia, 47, 429–462.
Ball, C. (1980). In: Fungal Biotechnology. Smith, J. E. et al. (eds). Academic Press, London, pp. 43–54.
Chet, I., Hadar, Y., Elad, Y., Katan, J. and Hennis, Y. (1979). In: Soil-borne Plant Pathogens. Schippers, B. and Gams, W. (eds). Academic Press, London, pp. 585–591.
Fokkemma, N. J. (1976). In: Microbiology of Aerial Plant Surfaces. Dickinson, C. H. and Preece, T. F. (eds). Academic Press, London, pp. 487–506.
Mandels, N., Weber, J. and Parizek, R. (1971): Appl. Microbiol., 21, 152–154.
Montenecourt, B. S. and Eveleigh, D. E. (1979): Tech. Assoc. Pulp. Pap. Ind. Ann. Meet, pp. 101–118.
Papavizas, G. C. (1985): Ann. Rev. Phytopath., 23, 23–54.
Papavizas, G. C. and Lewis, J. A. (1981): Phytopathology, 71, 247–281.
Papavizas, G. C., Lewis, J. A. and Abd-El-Moity (1982): Phytopath., 72, 126–132.
Papavizas, G. C. and Lewis, J. A. (1983): Phytopathology, 73, 407–411.
Upadhyay, R. S. and Rai, B. (1987): Plant and Soil, 101, 79–93.
Upadhyay, R. S. and Rai, B. (1988). In: Biocontrol of Plant Diseases. Vol. 1, Mukerji, K. G. and Garg, K. L. (eds). CRC Press, Florida, pp. 15–36.
Sivan, A. and Chet, I. (1986): Phytopathol. Z., 116, 39–47.
Sivan, A., Elad, Y. and Chet, I. (1984): Phytopathology, 74, 498–501.

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Epidemiological Studies on the *Alternaria helianthi* Leaf-spot and Blight Disease of Sunflower at Kota, India

BRIJESH K. SAHU, MAHIPAL S. GHEMAWAT* and J. M. AGRAWAT

Government College, Kota, Raj. 324001, India

Humidity plays a major role in the development of *Alternaria helianthi* on sunflower. Leaf infection is favoured by >95% relative humidity for a period between 12–24 h. It appears to be the first report showing importance of humidity in stem infection (between 2–4 days of >95% relative humidity). In field experiments also, very high Percent Disease Indices (PDIs) develop only when very high relative humidities prevail, and with the fall in relative humidities, PDIs fall rapidly. Temperature also plays a role in the disease development. Minimum temperatures below 18 °C and maximum temperatures above 32 °C appear to reduce PDIs considerably. Present studies suggest planting of sunflower crop in Rajasthan, India during December/January so that the crop encounters only a dry season and consequently escapes any significant *A. helianthi* attack.

Alternaria helianthi (Hansf.) Tubaki and Nishihara causes a severe leaf-spot and blight disease of sunflower (*Helianthus annuus* L.) in many countries, including India. Sackston (1981) expressed severe reservations about the crop's future due to this disease. Studies on epidemiological conditions, which are favourable for the initial establishment of the pathogen and for the subsequent development of the disease, are helpful in planning out any chemical treatment schedule. Only a few epidemiological studies have been carried out on this disease. While Islam and Maric (1980) and Allen, Brown and Kochman (1983) found a correlation between this disease and both relative humidity and temperature, Bhaskaran and Kandaswamy (1977) found a correlation only with the latter.

Materials and Methods

Sunflower cultivar EC 68414, which has been found adaptable to various agro-climatic conditions and different cropping systems in India (Sindagi, 1985), was used throughout these studies.

Effect of relative humidity on infection and disease development

Fifteen pots (5 plants/pot) with one-and-a-half-month-old sunflower plants were inoculated in the last week of September 1978 with a spore-suspension of *A. helianthi* ($3 \cdot 10^3$ spores/ml) and kept covered with moist thick cloth-chambers

* Address for correspondence: Mahipal S. Ghemawat, 235 Newton Avenue, Riverhead, NY 11901 U.S.A.

Akadémiai Kiadó, Budapest

(relative humidity >95%). Three pots were removed after 12, 24, 48, 96 and 168 h each to a greenhouse (relative humidity 59–67%). Controls consisted of plants kept in the greenhouse since inoculations. Fifteen days after inoculations, Percent Disease Indices (PDIs) on 15 plants in each treatment were estimated as follows (modified from Bhaskaran, 1976 and Agrawat, Mathur and Chippa, 1979):

Leaf area covered by the disease	*Disease Rating*
No visible symptom/sign of the disease	0
Between >0% and 20%	1
Between >20% and 40%	2
Between >40% and 60%	3
Between >60% and 80%	4
>80%	5

The Percent Disease Index (PDI) was then calculated by the formula:

$$\frac{\text{Sum of all the Disease Ratings of a plant}}{\text{Total no. of leaves rated x maximum rating}} \cdot 100$$

This experiment was repeated in September 1979.

Effect of weather on infection and disease development

In order to find the relationship, if any, of weather (minimum and maximum temperatures and relative humidity) with infection and disease development, sunflower was sown in widely-separated plots ($5 \cdot 3$ m^2 each). Sowings were done on the first day of every month beginning from June 1978 to May 1980. Crop of each sowing date was inoculated with *A. helianthi* at the age of one-and-a-half-month. The disease incidence of five randomly selected plants in each plot was recorded every 3 days from the date of inoculation till the 15th day.

Meteorological data were obtained from the Agricultural Research Station, Sukhadia University, Borkheda, Kota.

Results

Effect of relative humidity of infection and disease development

Observations (Table 1) reveal that relative humidity plays a major role in the establishment of the pathogen. The disease develops much more severely on leaves with an increase in time of >95% relative humidity from 12 to 24 h. This dependence on >95% relative humidity for stem infections is for a still greater duration, where this critical period lies between 48 and 96 h. The PDI was low on the control leaves and absent on the control stems.

Table 1

Effect of duration of >95% artificial relative humidity on infection/development of *Alternaria helianthi* on sunflower (cultivar EC 68414)

Duration of >95% artificial relative humidity (h)	PDI of plants due to leaf (%)	Stem lesions
0 (Control)	2.0	Absent
12	6.1	Absent
24	56.0	Very sparse and very small
48	66.3	Medium-sized and scattered
96	73.5	Many and large, but generally isolated
168	80.9	Coalesced, covering almost the entire surface

Effect of weather on infection and disease development

Fortnightly progress of the disease (Fig. 1) indicates that humidity is the major factor which limits the progress of the disease. During July and August 1978, when the av. fortnightly relative humidities ranged between 89–94%, PDIs recorded were >70%. The av. fortnightly minimum and maximum temperatures during this period ranged from 24–26 °C and from 31–32 °C, respectively.

In July 1979 also, the PDI was very high (58%) but it was lower than those of July and August, 1978. During this fortnight, the av. fortnightly relative humidity was very high (91%). However, av. fortnightly minimum and maximum temperatures were slightly higher (26 °C and 33 °C, respectively) than those in July and August, 1978.

During cooler months (December, January and February) of the years 1978–79 and 1979–80, it appears that the disease could progress provided there was enough atmospheric relative humidity. The av. fortnightly minimum and

maximum temperatures of December 1978 and January and February 1979 were almost similar and the only apparent difference was the av. fortnightly higher relative humidity during January 1979 when PDI recorded was 47% as compared to 24.6% and 18.4% in December 1978 and February 1979, respectively. Similarly, av. fortnightly minimum and maximum temperatures of December 1979 and January 1980 were almost same and the difference in av. fortnightly relative humidities (82.5% and 66%, respectively) apparently resulted in 24.1% and 5.1% PDIs, respectively.

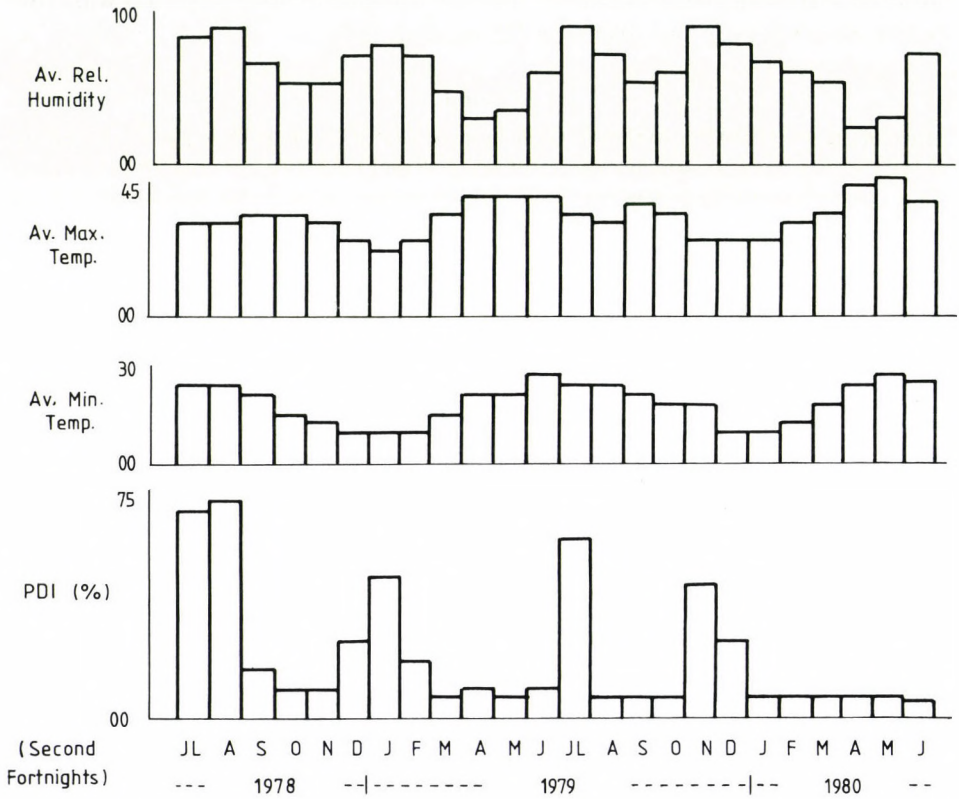

Fig. 1. Development of *Alternaria helianthi* leaf-spot and blight disease of sunflower under natural conditions at Kota, India, as related to relative humidity and minimum and maximum temperatures

More disease (PDI = 43.6%) was recorded during November 1979 (av. fortnightly relative humidity = 95%) which is higher than that (8.5%) of November 1978 (av. fortnightly relative humidity = 59.2%).

The January 1979 PDI was almost double that of December 1979.

The role of relative humidity in the disease development is further confirmed by low PDIs from September to November 1978, from March to June 1979, from August to October 1979, and from January to June 1980. Further, these low PDIs reached their maximums within 6 days.

Discussion

The results (Table 1 and Fig. 1) indicate a positive and profound effect of relative humidity (both artificial and natural) on disease development. A minimum period of >95% relative humidity is needed for good establishment of the pathogen. For leaves, this probably lies between 12 and 24 h because only after 24 h, a 56% PDI could be obtained (Table 1) as also found by Islam and Maric (1980). Though Allen et al. (1983) found a 12-h dew period to be enough for leaf lesions to develop, they also recorded that the disease covered a much more leaf area when dew was provided beyond the 12-h period. Similarly, this minimum critical period of very high relative humidity for infection/disease development on stems probably lies between 48 and 96 h because many and large stem lesions developed only after >95% relative humidity for 96 h (Table 1). This appears to be the first report regarding the humidity requirement of the stem lesions.

With almost similar av. fortnightly minimum and maximum temperatures (24 ±2 and 32.5 ±2 °C, respectively), very high PDIs (>50%) developed when the av. fortnightly relative humidities were very high (>85%) (July and August 1978 and July 1979) and a moderate PDI (10 –25%) with a moderate av. fortnightly relative humidity (>65–75%) (September 1978). Again, with almost similar av. fortnightly minimum and maximum temperatures (10±2 and 25± 2 °C, respectively), moderate PDIs developed with high av. fortnightly relative humidities (>75–85%) (February and December 1979) while only a low PDI (>1–10%) developed with a moderate av. fortnightly relative humidity (January 1980). Only low PDIs developed with low av. fortnightly relative humidities (45–65%) (October 1978, March and October 1979 and March 1980) when the av. fortnightly minimum and maximum temperatures (17±2 and 34±2 °C) were almost similar. These results show that the disease is favoured by high humidites, confirming Islam and Maric's (1980) results.

Bhaskaran and Kandaswamy (1977), however, found no correlation between Disease Indices and relative humidity which may possibly be due to different sunflower cultivar and/or pathogen isolate and/or due to natural infection used for the studies, but more probably due to the small range of relative humidity (78.6 to 90.6% at Coimbatore and 69.5 to 86.7 at Bhavanisagar) naturally occurring at the experimental sites.

An increase of 2 °C in the av. fortnightly minimum and maximum temperatures does not appear to have any influence on the PDIs (July and August 1978). However, when the av. fortnightly maximum temperature rises beyond 32 °C then it does lower the PDIs (e.g. July 1979 PDI as compared to July and August 1978 PDIs) (Fig. 1).

Similarly, if the av. fortnightly minimum temperature falls down to 18 °C then it does lower the PDIs. For example, November 1979 had a PDI of 43.6% while kharif month (July and August 1978 and July 1979), having more or less the same very high av. fortnightly relative humidities, showed much higher PDIs which is apparently due to av. fortnightly minimum temperature of 18 °C in the former as compared to 25±1 °C in the latter (Fig. 1). However, Bhaskaran and Kandaswamy (1977) found a highly significant positive correlation between the Disease Indices of *A. helianthi* on sunflower plants and the number of days when the minimum temperature was less than 20 °C. This adverse effect of 18 °C temperature under present studies on the PDI, in contrast to Bhaskaran and Kandaswamy's (1977) findings, may be due to differences in other environmental conditions and/or due to different cultivars and/or due to different fungal isolates. Our results confirm Islam and Maric's (1980) findings that much more infection develops at 24 or 27 °C as compared to that (much less) at 17 °C.

The almost double PDI in January 1979 as compared to that of December 1979 (Fig. 1) can, however, be explained neither on the basis of temperature nor on the basis of relative humidity.

Sunflower plant is day-neutral and hence this crop can be planted any time of the year. Our results suggest that in Rajasthan, India, it may be planted only during late rabi season (December/January) because that season is quite dry and consequently *A. helianthi* epidemics may be avoided which are common on kharif crop (planted in June/July).

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Literature

Agrawat, J. M., Mathur, S. J. and Chippa, H. P. (1979): Chemical control of *Alternaria* leaf spot of sunflower. Indian Journal of Mycology and Plant Pathology 9, 79–80.

Allen, S. J., Brown, J. F. and Kochman, J. K. (1983): The infection process, sporulation and survival of *Alternaria helianthi* on sunflower. Annals of Applied Biology 102, 413–419.

Bhaskaran, R. (1976): Studies on the leaf spot disease of sunflower caused by *Alternaria helianthi.* Ph. D. Thesis, Tamil Nadu Agricultural University, Coimbatore, 143. pp.

Bhaskaran, R. and Kandaswamy, T. K. (1977): Epidemiology of the leaf spot disease of sunflower. East African Agricultural and Forestry Journal 43, 5–8.

Islam, U. and Maric, A. (1980): Contribution to the studies on biology, epidemiology and resistance of sunflower to *Alternaria helianthi* (Hansf.). Taub. Nish. Zastita Bilja 31, 35–49.

Sackston, W. E. (1981): The sunflower crop and disease: progress, problems and prospects. Plant Disease 65, 643–648.

Sindagi, S. S. (1985): The current situation, constraints and prospects of sunflower crop (*Helianthus annuus* L.) in India. In: H. C. Srivastava, S. Bhaskaran, B. Vatsya and K. K. G. Menon (eds): Oilseed production: Constraints and Opportunities, pp. 141–145, Oxford & IBH Publishing Co., New Delhi.

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Ionophoretic Effect of Some Mycotoxins on Lipid Bilayers

W. ZIEGLER*, A. M. ZAJCHENKO**, J. POKORNÝ* and J. PAVLOVKIN***

*Institute of Ecobiology, Slovak Academy of Sciences, Dubravská cesta 14, S-814 34 Bratislava, Slovakia **Institute of Microbiology and Virology, Ukrainian Academy of Sciences, SU-252 143 Kiev, Ukr SSR ***Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, S-900 28 Ivanka pri Dunaji, Slovakia

Planar lipid bilayer made of asolectin were used to investigate whether or not mycotoxins (verrucarine A, roridin H, DON, F–2, and zearalenone) can influence the permeability of the lipid moiety of biomembranes. All toxins but DON are found to be able to induce ion channels in the lipid bilayer when added in concentration between 1 to 6 μg/ml to the aqueous solution on one side of the membrane. This was taken as evidence that, at least certain mycotoxins might express their toxic activity by depolarizing affected biomembranes.

The worldwide threat of mycotoxic fungi and their metabolites (mycotoxins) to food and feedstuffs continues to have a serious impact on the welfare of human and animal populations. Diseases caused by mycotoxins to animal and man have been extensively studied for years (Bilai and Pidoplichko, 1971). Later the mycotoxic influence on cultivated plants was given more attention (Scott, 1983; Szatmary, 1983; Paterson, 1983).

Fungi of the genera *Fusarium* and *Dendrodochium* produce a complex of non-host specific toxins. The thermostable *dendrodochines* are considered to be the most toxic material from any fungus known. *Fusarium* belongs to fungi that produce yellow wilt of turf grass. A number of species and subspecies of *Fusarium* have been found to produce estrogenic metabolites trivially named zearelone and F–2 toxin. These substances are assumed to be involved in the development of *Fusarium tricinctum* caused cornstalk rot and ear damage (Bamburg and Strong, 1971). Vianello and Macri (1978, 1981) have found that *zearalenone* inhibits oxidative phosphorylation, causes depolarisation of transmembrane potentials in cells of higher plants and stimulates leakage of electrolytes and organic compounds.

Trichothecenes such as *verrucarines* and *roridines* have been reported to affect cell membranes of higher plants causing their depolarisation and the leakage of affected cells (Pavlovkin et al., 1986a, 1986b). Pathogenic events of the kind mentioned above are not always linked with the toxin/membrane protein interactions; changes in the permeability status of the treated biomembranes often show up responsible for the initiation of the toxin-associated diseases.

Dedicated to the 60th anniversary of academician D. M. Grodzinski

Akadémiai Kiadó, Budapest

The purpose of our work was to elaborate whether or not certain mycotoxins can change the permeability behaviour of the lipid moiety of host cell membranes and what mechanism such changes might be based on.

Planar lipid bilayer membranes have been used as very useful models of biomembranes for decades. For example, by virtue of these membranes Ziegler and Pavlovkin (1986) and Ziegler et al. (1986) attributed the phytotoxic activity of syringotoxin to its effect on the lipid bilayer. For the same reason lipid bilayers have been chosen to study the possible influence of mycotoxins on the lipid moiety of affected biological membranes.

Material and Methods

Fig. 1 shows the experimental set-up used. Lipid bilayers of the Mueller–Rudin type (Mueller et al., 1962) were formed from asolectin (SERVA) in n-decane (50 mg/ml). A small amount of this solution was applied to a hole (0.4 mm in diameter) in a teflon septum dividing cis- and trans-compartment. Unless stated otherwise, both compartments of the bilayer chamber were previously filled with 0.1 ***M*** KCl, 10 m***M*** HEPES–KOH, pH 6.1. The membrane voltage (constant voltage pulses) was applied to the cis-compartment. The trans-compartment was via the electrometer (KEITHLEY, model 619) virtually grounded. Voltage generator and electrometer were connected to the aqueous solutions of the respective compartments via calomel electrodes and agar-bridges. A positive membrane current was defined as a cation flow from the cis- into the trans-compartment and/or an anion flow in the opposite direction. Membrane currents were recorded by a chart-recorder (TZ 4200) and an XY-recorder (XY 4103; both manufactured by LABORATORNE PRISTROJE, Prague, Czechoslovakia).

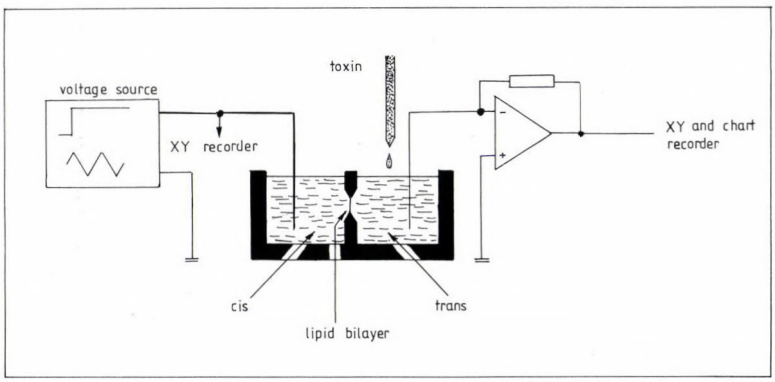

Fig. 1. Schematic diagram of the bilayer chamber

Appropriate aliquots of the respective mycotoxins (***DON***–2-deoxynivalenol, *roridin H*, *verrucarine A*, *F–2*, and *zearalenone*, were dissolved in UV-grade EtOH (1 mg/ml) and added to the aqueous solution in the trans-compartment. Since the membrane current contains valuable information related to the ionic permeability of the lipid membrane, it can thus reflect any toxin induced changes in the membrane conductivity.

Results and Discussion

All toxins, but *DON*, affected the conductance behaviour of the lipid bilayer membranes.

At a specific threshold concentration of the toxin in the trans-compartment often a dramatic increase of the membrane conductance was observed (Fig. 2). These thresholds are summarized in Table 1.

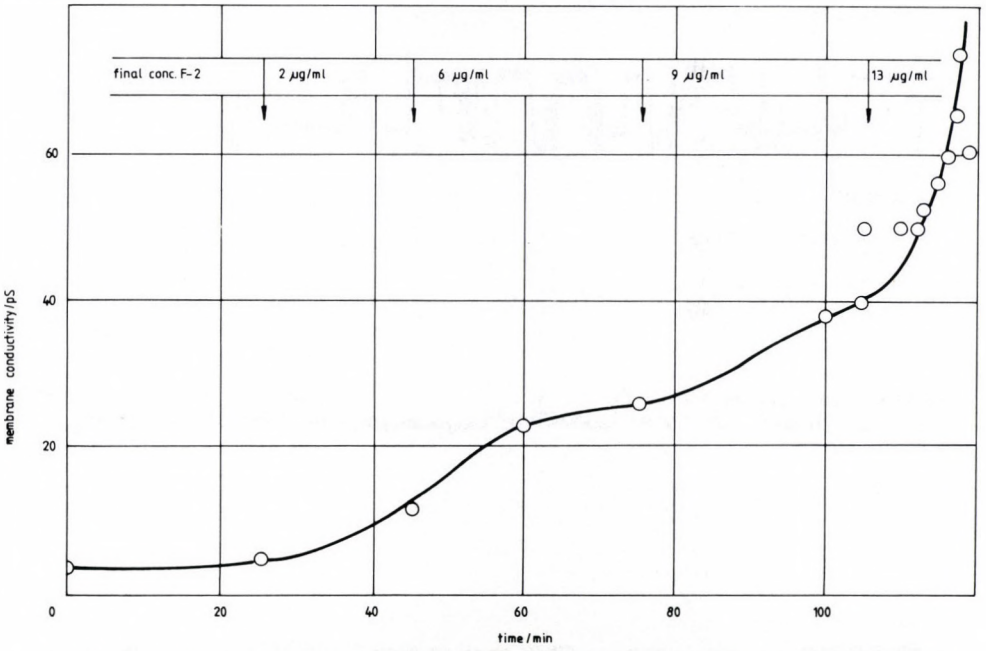

Fig. 2. Increasing bilayer conductance caused by subsequent supply of F–2 into the trans-compartment of the bilayer chamber. Aqueous solution: 0.1 ***M*** KCl, 10 m***M*** HEPES–KOH, pH 6.1

Table 1

Threshold concentrations of some mycotoxins

Toxin	Concentration (final)
Verrucarine A	4–6 µg/ml
Roridin H	6 µg/ml
DON	no effect detected
F–2	1–2 µg/ml
Zearalenone	1–2 µg/ml

In most cases, the effect of toxins on the membrane consisted in the initiation of more or less well-defined ion channels in the lipid bilayer. Such ion channels show up as stepwise jumps of the membrane current at a constant membrane voltage (Figs 3 to 6) or as different slopes in the I/V-curves of the bilayer (Fig. 7).

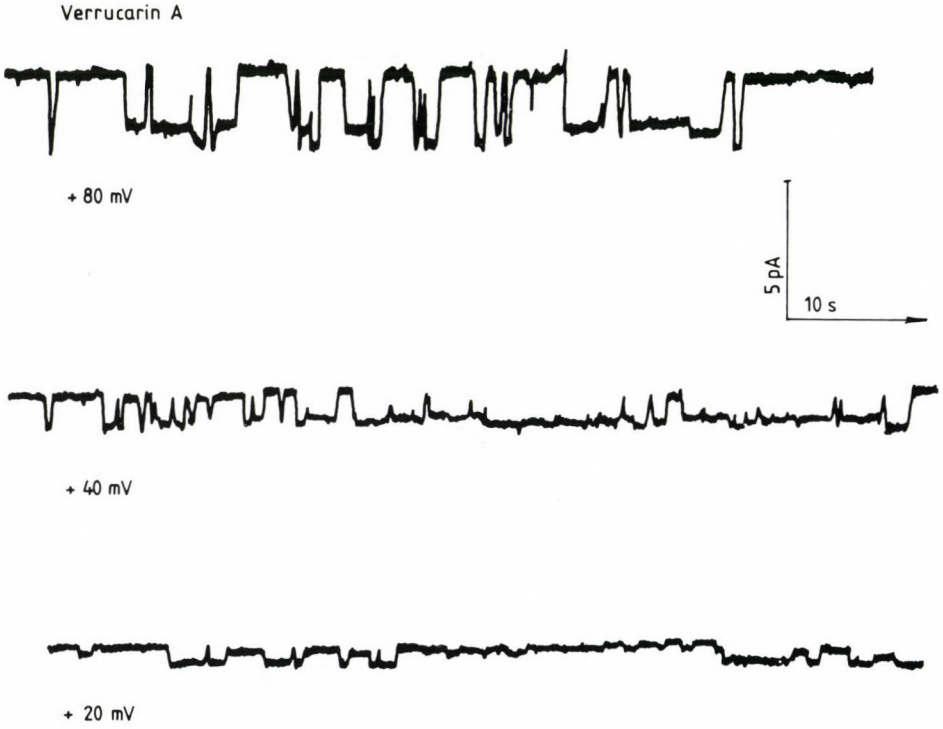

Fig. 3. Ion channels, induced by *verrucarin* A. Toxin concentration: 6 µg/ml. Aqueous solution: 0.1 ***M*** KCl, 0.1 ***M*** NaCl, pH 5.0

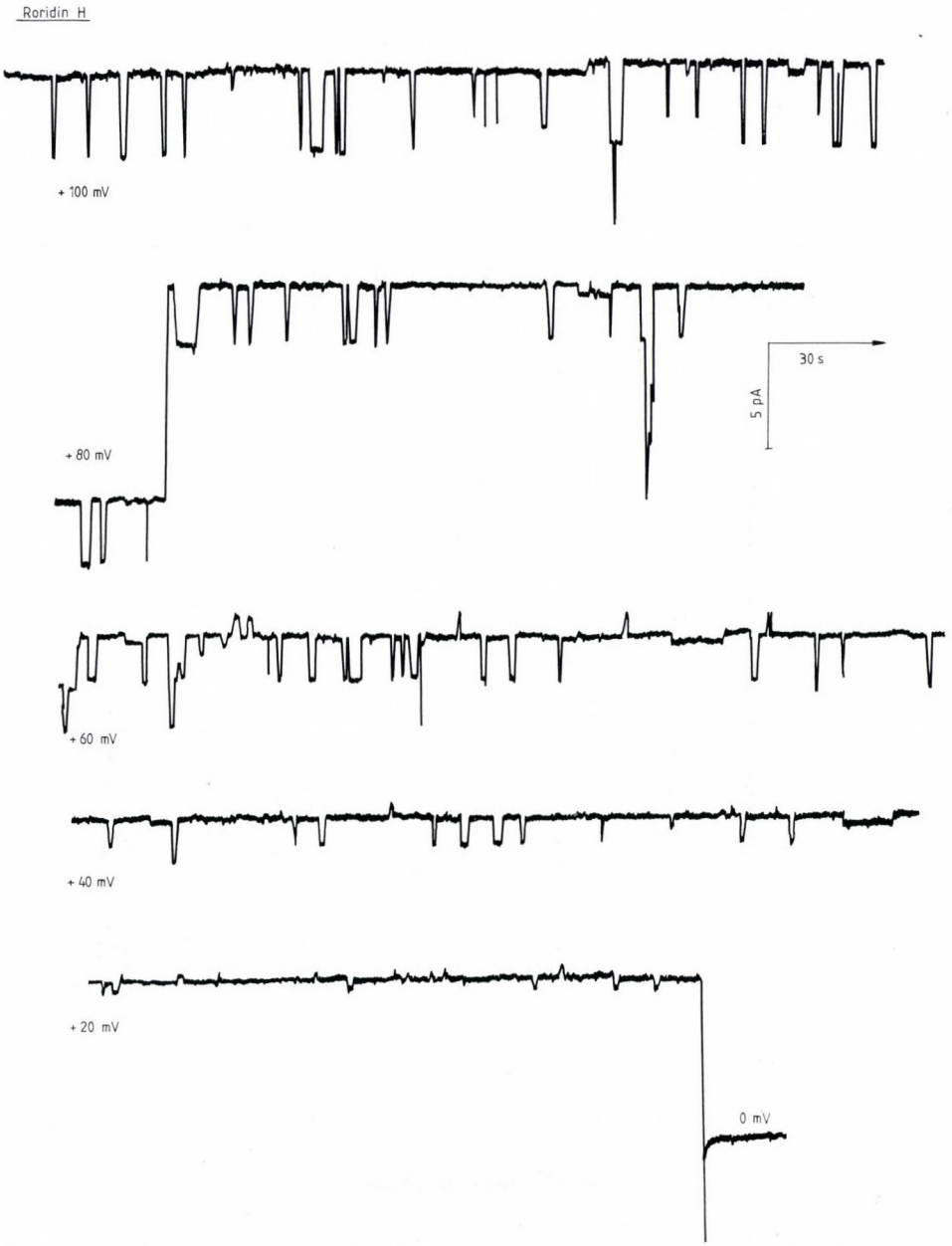

Fig. 4. Membrane voltage dependent open/close kinetics of ion channels induced by *roridin H* in the lipid bilayer. Toxin concentration: 6 µg/ml. Aqueous solution: 0.1 ***M*** KCl, 10 m***M*** HEPES–KOH, pH 6.1

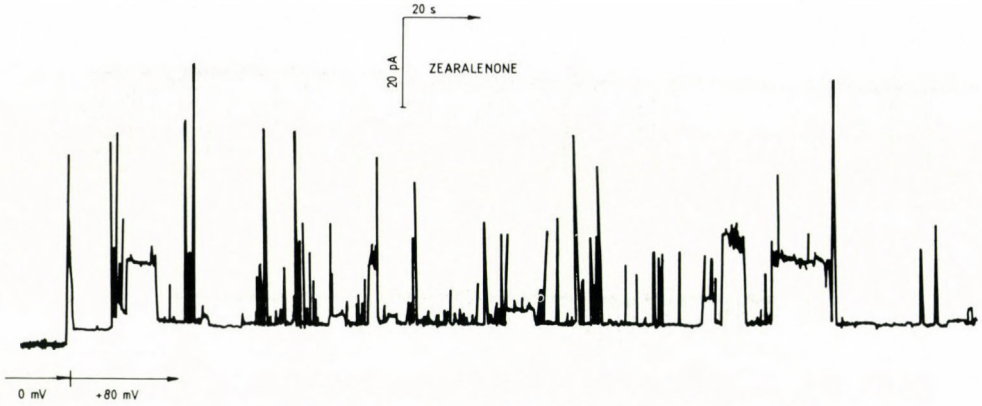

Fig. 5. Example of ion channels caused by *zearalenone*

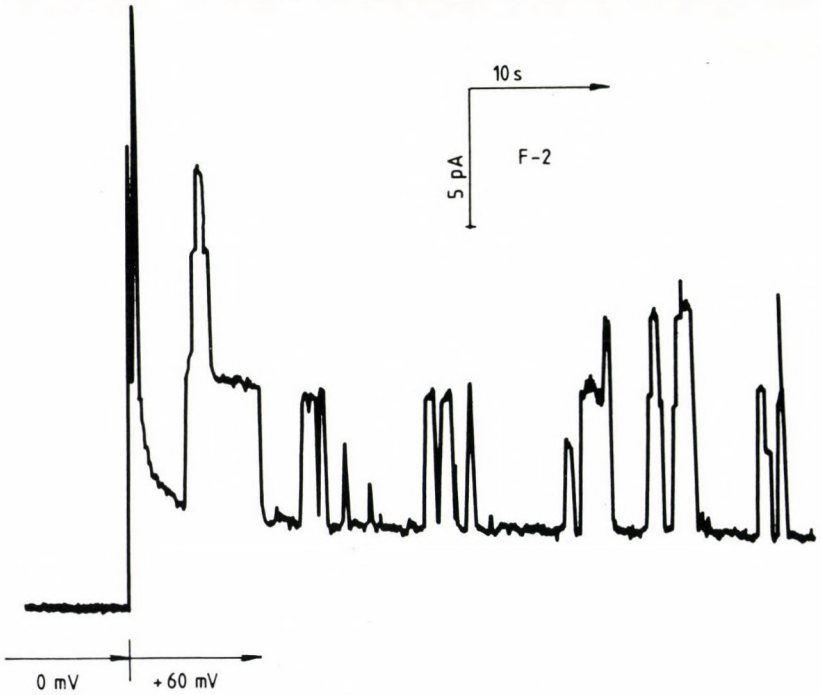

Fig. 6. *F–2* induced ion channels

Figures 3 to 6 are examples of ion channels induced by the respective mycotoxins in the lipid bilayer. Downward deflections of the membrane current are caused by channel closing. In some cases (e.g. *roridin H*) membrane voltage dependent open/close kinetics are evident.

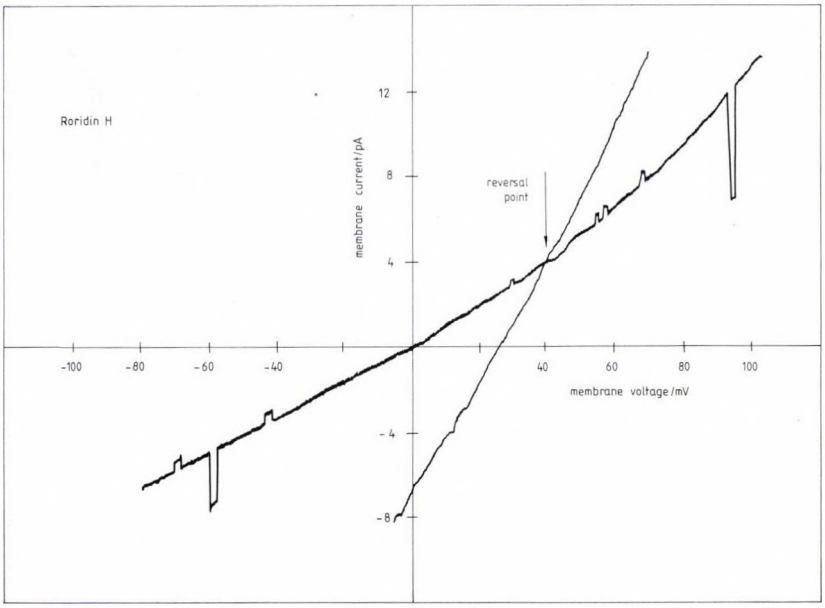

Fig. 7. I/V-curve of a lipid bilayer in the presence of *F–2* mycotoxin. Different slopes correspond to different numbers of open channels in the membrane. Toxin concentration: 6 µg/ml; Aqueous solution: 0.1 ***M*** KCl, 10 m***M*** HEPES–KOH, pH 6.1

By the use of a cis/trans salt gradient the cation/anion selectivity of ion channels can be determined. In this case I/V-curves corresponding to different numbers of open channels will not cross at the zero point as in Fig. 7, but they would do so at the so-called reverse potential which depends only on the salt gradient and on the selectivity properties of the particular channels. The relation between the respective Nernst potential for the given gradient and the reverse potential yields the cation/anion selectivity of the channel. A positive reversal potential of about 40 mV, as shown in Fig. 8 qualifies the channel as a cation-selective one. Based on the used salt gradient, a cation/anion selectivity ratio of about 14 was estimated.

Although toxin/protein interactions certainly account for numerous pathogenic events in impaired biological objects, the influence of mycotoxins on the permeability of the lipid moiety of affected biomembranes should not be ruled out.

The occurrence of ion channels observed in the lipid part of biomembranes in the bilayer experiments leads very likely to the loss of permselective properties and finally to the damage and death of the cells. On the basis of previous observations (Pavlovkin et al., 1986a, 1986b) such type of pathogenic process may be anticipated, especially in plant materials.

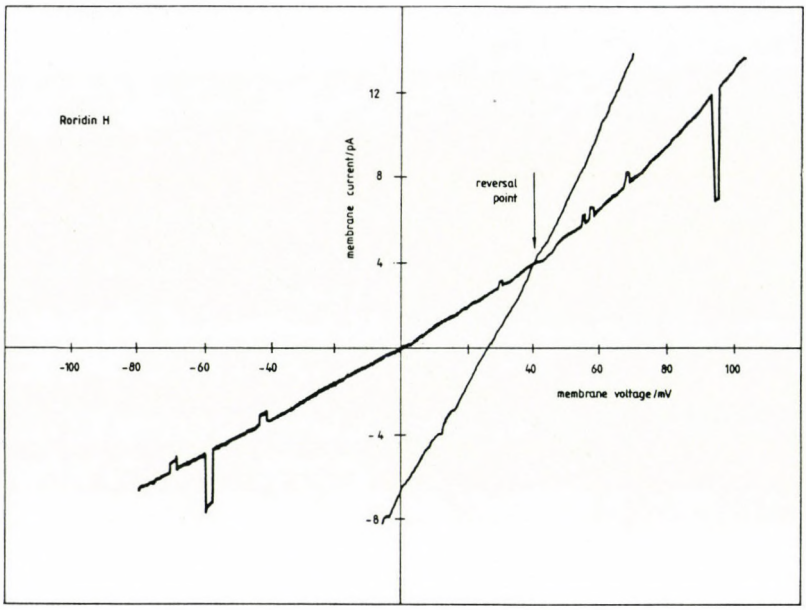

Fig. 8. Estimation of the reversal potential of the *roridin H* channel. Toxin concentration: 6 µg/ml; Aqueous solution, cis: 0.1 ***M*** KCl, Aqueous solution, trans: 0.6 ***M*** KCl; 10 m***M*** HEPES–KOH, pH 6.1

Literature

Bilai, V. J., Pidoplichko, N. I. (1971): Toxinobrazuyuschie mikroskopicheskije griby. Kyjev, Naukova Dumka, 289

Scott, P. M. (1983): Trichothecene problems in Canada. Dev. Food Sci. 4, 218–220.

Szatmary, C. I. (1983): Trichothecenes toxicoses and natural occurrence in Hungary. Dev. Food Sci. 4, 273–284.

Paterson, D. S. P. (1983): Trichothecenes: Toxicoses and natural occurrences in Britain. Dev. Food Sci. 4, 259–264.

Bamburg, J. R., Strong, F. M. (1971): 12,13-Epoxytrichothecenes. In: S. Kadis, A. Ciegler, S. J. Ajl (eds): Microbiological Toxins, Vol. 6, Academic Press, N. York, Chapter 7, 207–291.

Pavlovkin, J., Mistrík, I., Zajchenko, A. M., Mraz, J. (1986a): Effects of mycotoxins on cell membranes of higher plant roots. Biológia (Bratislava) 41, 681–686.

Pavlovkin, J., Mistrík, I., Zajchenko, A. M. (1986b): Effects of mycotoxins on the ultrastructure and permeability of primary maize rot cell membranes. Biológia (Bratislava), 41, 1085–1090.

Vianello, A., Macri, F. (1978): Inhibition of plant cell membrane transport phenomena induced by zearalenone (F–2) Planta 143, 51–57.

Vianello, A., Macri, F. (1981): Effect of zearalenone (F–2) on pea stem, maize root and rat liver mitochondria. Planta 153, 443–446.

Ziegler, W., Pavlovkin, J. (1986): Syringotoxin, ein Phytotoxin von *Pseudomonas syringae pv. syringae*, erzeugt Ionenkanäle in bimolekularen Lipidmembranen. Acta Phytopath. Acad. Sci. Hung. 20, 35–45.

Ziegler, W., Pavlovkin, J., Remiš, D., Pokorný, J. (1986): The anion/cation selectivity of the syringotoxin channel. Biológia (Bratislava) 41, 1091–1096.

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Effect of Syringotoxin on Structural and Functional Properties of Host Cell Membranes

I. MISTRÍK*, J. PAVLOVKIN** and W. ZIEGLER*

*Institute of Exptl. Biology and Ecology, CBES, Slovak Acad. Sci., Dubravská cesta 14, 814 34 Bratislava, Slovakia ** Institute of Exptl. Phytopathology and Entomology, CBES, Slovak Acad. Sci. 900 28 Ivanka p. Dunaji, Slovakia

Syringotoxin produced by *Pseudomonas syringae* pv. *syringae* bacteria caused a depolarisation of the membrane potentials (E_m) in the *Lemna gibba* cells, release of electrolytes from maize roots, the changes of the oxidative metabolism of maize root mitochondria their swelling in the presence of NADH and KCl and structural changes as well. The results strongly suggest that ST acts in different ways on the lipid and the protein part of biomembranes in host plant cells.

Pseudomonas syringae pv. *syringae* bacteria produce a nonspecific toxin, syringotoxin (ST), causing diseases in stone and citrus fruit trees in almost all growing areas of the world (Gross and DeVay, 1977). The chemical structure of the toxin molecule however is mostly unknown. According to Gross et al. (1977) the polypeptide chain of ST tentatively contains threonine, serine, glycine, ornithine, and a further unidentified basic amino acid at an equimolar ratio.

Surico and DeVay (1982) found that ST causes an uncoupling of the oxidative phosphorylation, inhibites ATP-synthesis, stimulates ATP-hydrolysis and NADH oxidation, reduces the membrane potential of maize root mitochondria and induces their swelling. They assumed that swelling of mitochondria in the presence of ST might be due to an accumulation of potassium ions. Therefore they suggested that the effect of ST resembles that of gramicidin and valinomycin. Sinden et al. (1971) found that ST is a surface active agent causing an increase of the permeability of the mitochondrial membrane to H^+ and OH^- ions what might affect ion gradients across these membrane. These effects indicate that the action of ST could be connected with disturbances in the permeability of host cell membranes abolishing transmembraneous ion gradients. Experiments with planar lipid bilayers (BLM) have shown that ST forms anion-selective ion channels in these model membranes which possess a well defined conductance increment (Ziegler et al., 1984, 1985, 1986).

In this paper we present the results of the ST influence on the membrane potential (E_m) of duckweed *Lemna gibba* L. cells, on the permeability properties of corn root cell membranes as well as changes in the functional and structural properties of corn root mitochondria.

Akadémiai Kiadó, Budapest

Key words: *Lemna gibba* L., primary corn root, electrical membrane potential (E_m), oxidative phosphorylation, swelling of mitochondria, ion transport in mitochondria, structural changes in mitochondria, syringotoxin (ST), fusicoccin (FC).

Material and Methods

Plant material: *Lemna gibba* L., strain Gl (obtained from the Lemna collection of Professor R. Kandler, Vienna, Austria) was grown axenically under short-day conditions (28 °C/8 h day to 23 °C/16 h night) at a light intensity of 25 W m^{-2}. The nutrient solution contained 3.96 mmol.l^{-1} KNO_3, 5.47 mmol.l^{-1} $CaCl_2$, 1.22 mmol.l^{-1} $MgSO_4$, 1.47 mmol.l^{-1} KH_2PO_4, 29 mmol.l^{-1} sucrose. The initial pH was 4.8.

Maize seeds (hybrid CE 330) were germinated on filter paper wetted with 0.2 mmol.l^{-1} $CaCl_2$ in a thermostat at a temperature setting of 28 °C. 3 days old seedlings were used for the experiments. Their roots were incubated in distilled water (control), or in distilled water supplied with 10 μg.ml^{-1} ST, respectively.

Electrophysiological measurements: The membrane potential of *Lemna gibba* L. cells was measured by help of glass microelectrodes (with fiber) supplied by WP Instruments (USA).

The electrodes were prepared on a vertical pipette puller (D. Kopf, California, USA) and filled with 3 mol.l^{-1} KCl. The tip diameter did not exceed 5 μm, the electrode potential was in a range of –5 to –15 mV, the electrode resistance between 5 to 15 MOhm. A polyethylene tube filled with 3 mol.l^{-1} KCl in 2% agar was used as a reference electrode. Both electrodes were connected via Ag/AgCl half cell to an electrometric amplifier (WPI 701,USA). Its output was equipped with a chart recorder and a double beam CRD (Tectronix, USA). The cell was illuminated by a halogene lamp. The light intensity in the object area was 118 W m^{-2}. The object was placed in a perfusion chamber of 4 ml internal volume. The microelectrode was mounted on a micromanipulator and its insertion into the cell observed through a microscope. A continuous stream of 10 ml.min^{-1} of the experimental solution was maintained through the chamber. The pH of the solution during the measurements was 5.7.

Determination of the specific conductivity of aqueous solutions and their K^+ content: Primary roots of 3 days old maize seedlings were incubated in distilled water with/without ST (10 μg.ml^{-1}). The overall amount of electrolytes released by the root cells over the time was determined by monitoring the changes of the specific conductivity of the incubation medium. A conductivity meter (OK–109–1) equipped with a bell-shaped measuring electrode (OK-902, both manufactured in Hungary) was used for this purpose.

The K^+ content of the electrolytes released from the cells into the incubation medium was determined by AA-spectrophotometry (Unicam SP 90A, England).

Isolation of mitochondria and measurements of O_2 consumption: Mitochondria were isolated according to Day and Hanson (1977) from apical segments (3 cm long) of primary roots. The integrity of the isolated mitochondria was checked by the oxygen consumption control. A YSI 5331 (USA) oxygenmeter was used. The absolute amount of oxygen used up for the substrate oxidation, the value of the respiratory control (RC), and the P/O ratio, respectively were computed from the graphic records as described by Estabrook (1967). The morphological and functional integrity of the mitochondria was further checked by electron microscopy and the determination of the succinate: cytochrome c-oxireductase activity as well as the NADH: Cytochrome c-oxireductase activity (Doucea et al., 1973). The protein content was determined according the method of Sedmak and Grossberg (1977).

Isolated mitochondria were further used for measurements of the mitochondrial ion transport.

Spectrophotometric measurements: Spectrophotometric studies of swelling and/or shrinking of mitochondria were performed spectrophotometrically at 540 nm (UV–VIS Spectrophotometer, GDR). The reaction medium in the 2 ml cuvette was composed of 0.1 $mol.l^{-1}$ KCl (or NaCl, respectively), 2 $mmol.l^{-1}$ $MgCl_2$, 10 $mmol.l^{-1}$ Tris-HCl, 1 $mmol.l^{-1}$ NADH, pH 7.2 and different toxin concentrations as specified later in this paper. The amount of mitochondrial protein in the cuvette was 0.5 $mg.l^{-1}$.

Electron microscopy: Samples for electron microscopy were prepared in the following manner: mitochondria were resuspended in the same aqueous media as used for the spectrophotometric studies. The fixation of the mitochondria was performed in 2.5% glutaralaldehyde (pH 7.2) for 60 min. After a washing step a second fixation in 1% OsO_4 (2 h) was carried out. After washing and dehydration the mitochondria were embedded in Spurr resin (Spurr, 1969). Sections were cut on an Ultratome III (Sweden) ultramicrotome. Electron micrographs were prepared on a TESLA BS 500 (Czechoslovakia) electron microscope.

Stock solutions of syringotoxin (ST) and fusicoccin (FC): Stocks of ST and FC were prepared by dissolving toxin crystals in distilled water (ST), and 98% ethanol (FC) respectively. Both solutions were adjusted to 1 $mg.ml^{-1}$. The desired working solutions were prepared by dilution.

Results

Figure 1 shows changes of the membrane potential (E_m) of a *Lemna gibba* cells caused by 10 μg.ml^{-1} ST (the highest concentration used), light/dark transitions and the fungal toxin fusicoccin (FC). At a concentration of 1 mmol.l^{-1} K^+ the values E_m of *Lemna gibba* cells were in a range of –240 to –280 mV. The light/dark transition caused a temporary depolarisation after which the value of E_m returned to its previously level. A state of anoxy was induced by replacing all oxygen in the bathing solution by nitrogen. In this way we were able to separate active (E_p) and passive part (E_d) of the membrane potential (E_m). Since the leaves of *Lemna gibba* possess considerable air pockets, allowing them to float on the water surface, this experiments took long times (more than 1 h). In the dark the level of the monitored diffusion potential (E_d) was in a range of –85 to –95 mV. After the bathing solution was again supplied with oxygen E_m returned to its original value. The addition of ST to the bathing solution caused a strong depolarization. E_m continued to decline as long as the cell was exposed to the influence of ST (see Fig. 1). To address the question if and how far ST can influence the plasma membrane proton pump we used FC which as known stimulates this enzyme (Marre, 1979). As we can see on Fig. 1 FC causes a moderate hyperpolarization of the ST-depolarised membrane potential. That indicates that ST not only acts on energy independent transport but on its active part as well.

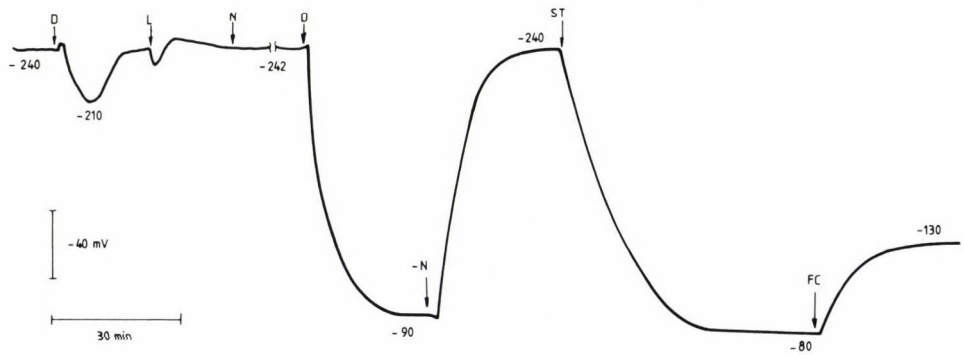

Fig. 1. Dynamic changes of the membrane potential (E_m) of *Lemna gibba* cells under different conditions. (D) in the dark; (L) in the light; (N) under anoxy condition; (ST) in the presence of syringotoxin (10 μg.ml^{-1}); (FC) under the influence of fusicoccin

Figure 2 shows depolarization levels caused by different ST-concentrations both in the light and in the dark phases. According to Surico and DeVay (1982) ST should influence the E_m only in the dark. But our experiments

demonstrate that the higher concentrations of ST can cause a transient depolarization even during the light phase. It has to be noted however, that the speed as well as the extent of this depolarization is considerable lower than in the dark (see Fig. 2).

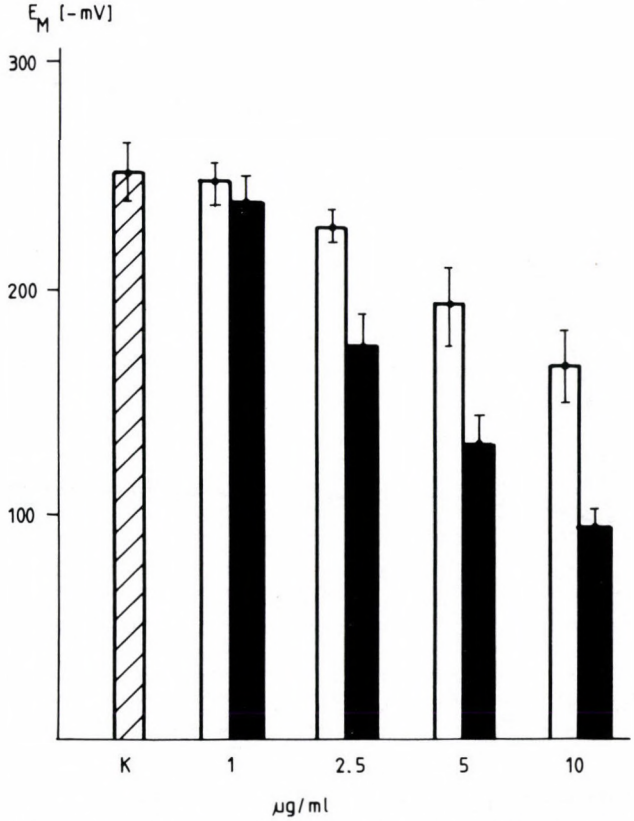

Fig. 2. Effect of different concentrations of syringotoxin on the membrane potential of *Lemna gibba* cells. K: control, open bars: in the light, filled bars: in the dark. The standard deviation is indicated for each bar

An extended incubation of the roots in distilled water with and without 10 $\mu g.ml^{-1}$ ST caused a release of electrolytes from the cells as monitored by an increasing specific conductivity of the incubation solution (see Fig. 3). The fastest increase was found during the first hour of incubation. The time dependence of the specific conductance is in good correlation with the K^+ content in this incubation medium (Fig. 4). After 24 h of incubation the K^+-content of the roots was determinated. While the root incubated in distilled water contained 29.70 $\mu mol.K^+.g^{-1}$ fresh weight, the roots incubated at presence of 10 $\mu g.ml.^{-1}$ ST contained 19.28 $\mu mol.K^+.g^{-1}$ fresh weight only (remained in the roots).

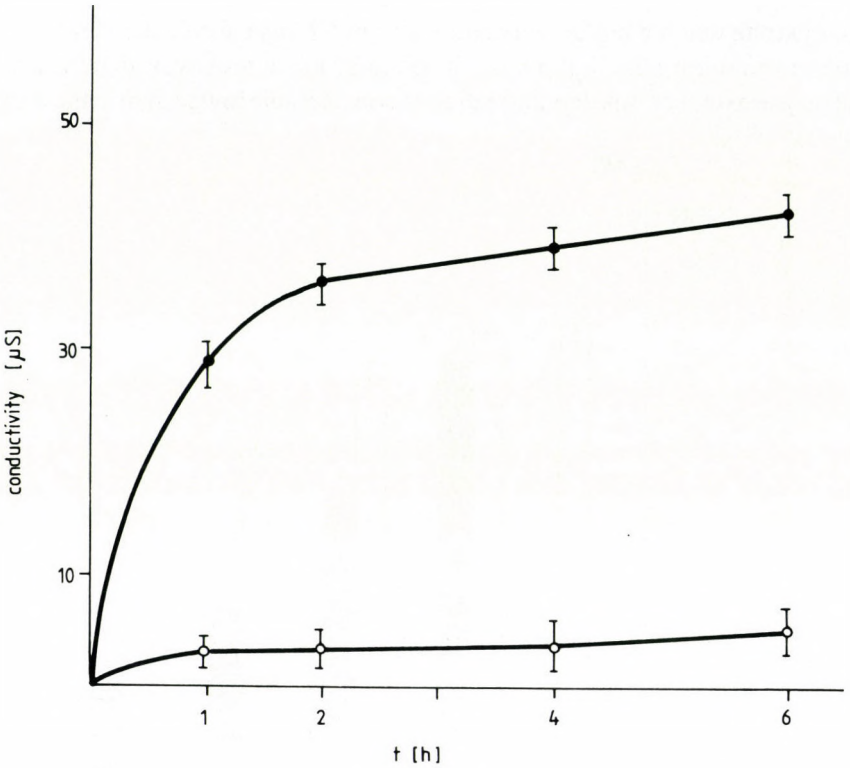

Fig. 3. Dynamic changes of the specific conductivity of the incubation medium in the absence (open circles) and the presence (filled circles) of ST (10 $\mu g.ml^{-1}$). The roots of 3 days old maize seedlings were bathing in the medium for the time indicated

To prove the supposed effect of ST on membrane systems we have studied the influence of the toxin on mitochondria as well. Mitochondria isolated from maise roots oxidize exogenous NADH (Fig. 5A) as well as succinate (Fig. 5B) relatively fast. The presence of ST in the mitochondrial suspension caused a concentration dependent stimulation of the oxygen consumption in state 4. While toxin concentrations from 5 to 10 $\mu g.ml^{-1}$ mitochondrial protein increased the oxygen consumption at the higher concentrations (over 10 $\mu g.ml^{-1}$) ST acted as an uncoupler of the oxidative phosphorylation (Figs 5C and D).

Since several toxins of phytopathogenic microorganisms are also acting on permeability properties of membrane structures of host plant cells (Hanchey and Wheeler, 1979) we have studied the influence of ST on transport processes of mitochondrial membranes. As can be seen in Fig. 6 addition of ST to a mitochondrial suspension without the respiration substrate (NADH) did not

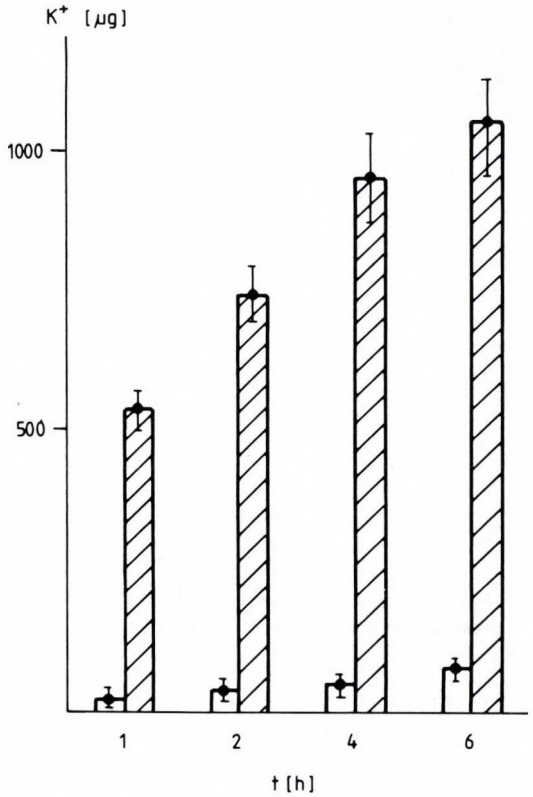

Fig. 4. Amounts of K^+ released by maize roots into the incubation medium (100 ml). Open bars refer to conditions without ST, shadowed bars to conditions when the incubation medium was supplied with 10 $\mu g.ml^{-1}$ ST

affect the size of the mitochondria (D). In the presence of NADH however all tested ST concentrations caused swelling of the mitochondria (Figs 6B and 7). Figure 7 reflects the effect of different ST-concentrations on the maximum swelling level reached. Swelling was monitored spectrophotometrically and confirmed by electron microscopy (Fig. 8B).

Contraction of mitochondria was observed in the presence of exogenous NADH both in the presence and absence of FC (Figs 6A and C) indicating that FC acts only on the plasma membrane H^+-ATPase and does not have any effect on the mitochondria.

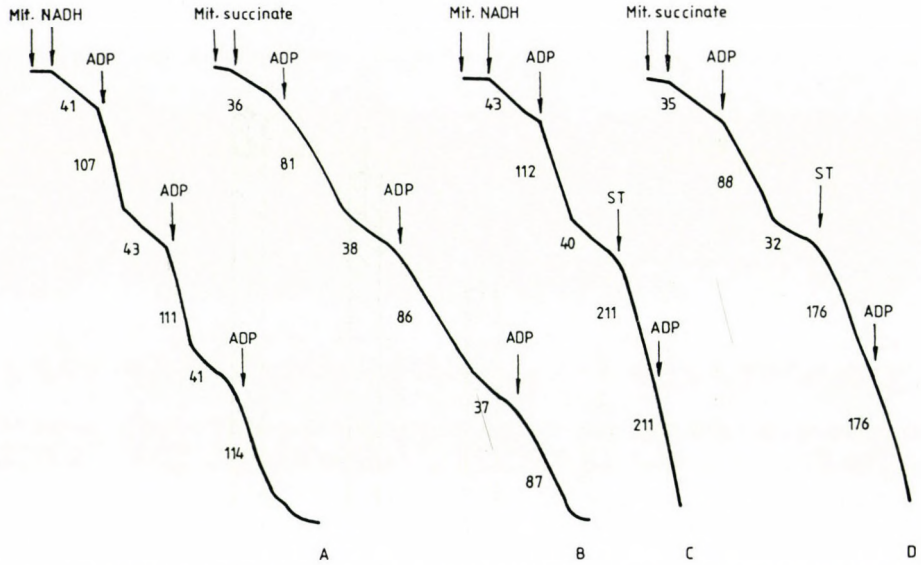

Fig. 5. Oxidation of NADH (A and C) and succinate (B and D) by maize root mitochondria. Mitochondria were suspended at 0.2 mg.ml^{-1} in medium contained 0,4 mol.l^{-1} sucrose 100 mmol.ml^{-1} phosphate buffer, pH 7.2. 5 mmol.l^{-1} $MgCl_2$, 0.1% BSA and 1 mmol.l^{-1} NADH (A and C) or 10 mmol.l^{-1} succinate (B and D). Concentration of ST was 24 μg.mg^{-1} of mitochondrial protein, (C and D) and ATP 140 μmol.l^{-1} . Numbers by each trace represent the rate of oxygen consumption in nmols O_2.min^{-1}. mg^{-1} protein

Discussion

The aim of the presented paper was to study critical events taking place in the membranes of host cells exposed to the action of ST. Modern electrophysiological methods of membrane potential measurements cause only a very low perturbation of the functional state of the cell but can give a highly relevant insight into the membrane transport processes *in situ*. Figure 1 shows that the membrane potential of *Lemna gibba* cells possesses an energy dependent component (E_p) as well as an energy independent one (E_d). Spanswick (1982) as well as Tazawa and Shimmen (1982) have pointed out that the energy dependent component, i.e. the pump, is voltage-dependent and must have a conductance similar to that of the diffusion component. The electrical membrane properties are generally by an equivalent circuit.

Results presented in Fig. 1 also suggest that during the action of ST (10 μg.ml$^{-1)}$ the value of E_m gradually decreases. Comparing these results with those in Figs 3 and 4 we deduce that ST simultaneously disturbs the active (E_p)

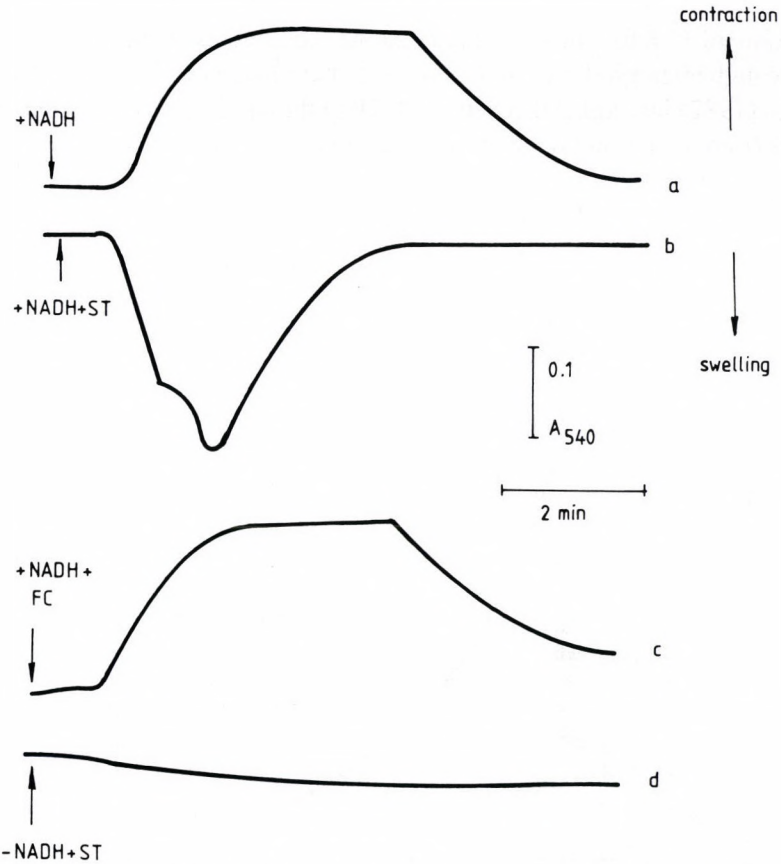

Fig. 6. Transient volume changes in mitochondria suspended in different media. Besides of 0.1 mol.l^{-1} KCl the individual contained: 1 mmol.l^{-1} NADH (a), 1 mmol.l^{-1} NADH and 14 µg/mg syringotoxin (b), 1 mmol.l^{-1} NADH and 10 µmol.ml^{-1} fusicoccin (c), 24 µg.mg^{-1} syringotoxin (d). The ST-concentration refers to micrograms per mg of mitochondrial protein

as well as the passive (E_d) component of the membrane potential (E_m). A similar effect was observed by Pavlovkin et al. (1986) on cotton cotyledons inoculated with *Pseudomonas syringae* pv. *tabaci* bacteria what led to a hypersensitive reaction. The fungal toxin fusicoccin (FC) caused only a partially increase of E_p. The results show that in the early state ST affects more the active part (E_p) than the passive (E_d) one. The reduction of the K^+-content in the roots and its release into the bathing solution (Figs 3 and 4) let assume that ST employs several action sites. Ziegler and Pavlovkin (1985) and Ziegler et al. (1986) demonstrated that ST induces ion channels in articial lipid bilayer which although partially anion-selective allow considerable amounts of cations to cross the lipid membrane. The selectivity of the channel caused by a positive netcharge in the channel structure

has a ratio of 1 : 8 for single charged cations. The selectivity ratio is higher in the case of higher charged cations. Comparing these results with those of Surico and DeVay (1982) let expect that action of ST in the lipid moiety of biomembranes differs from its action on membrane proteins.

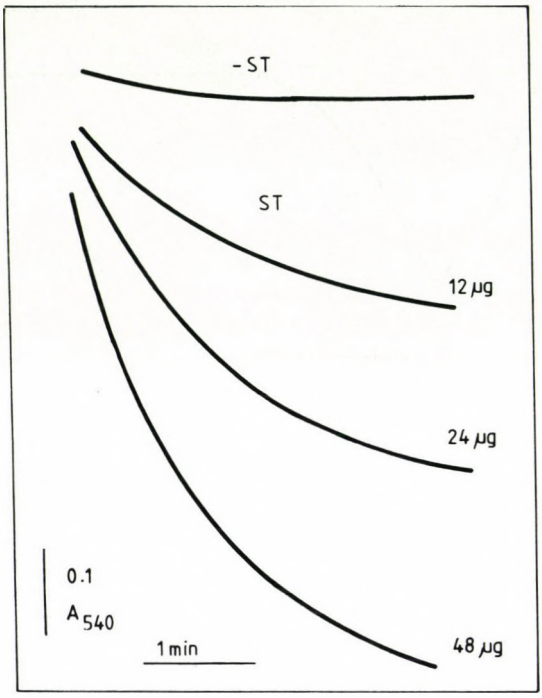

Fig. 7. Mitochondrial volume changes of suspended in 0.1 mol.l^{-1} KCl and 1 mmol.l^{-1} NADH at the absence and at the presence of different concentrations of syringotoxin (the ST-concentrations in the figure refer to micrograms per mg of mitochondrial protein). Difference from Fig. 6: only that part of volume changes is shown in which swelling reaches its maximum

Since biomembranes and especially the inner mitochondrial membrane possess considerable amounts of proteins we can expect a prevailing effect of the ST action on the membrane proteins. The kinetics of the ST caused changes in the stimulated oxygen consumption in the state 4 (see Fig. 5) and the effect of toxin concentrations higher than 10 µg.mg^{-1} mitochondrial protein shows that ST acts as an uncoupler of the oxidative phosphorylation. Similar increasing on oxygen consumption of mitochondria in the state 4 caused by bacterial toxins were observed by other authors as well (Bednarski et al., 1977; Surico and De Vay, 1982).

Gengenbach et al. (1973) proposes two possible ways of stimulation of exogenous NADH by the toxin. This could result either from an increased

accessibility of substrate to the NADH-specific flavoprotein, caused by the conformational changes in the enzyme, or from enzyme activation by the toxin.

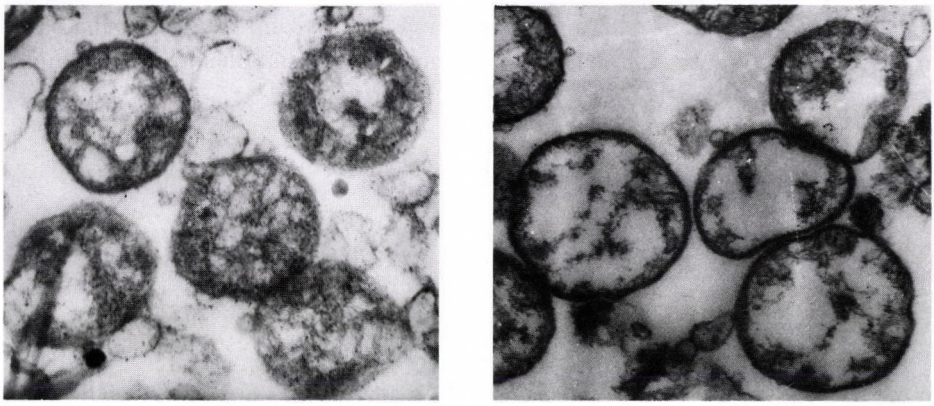

Fig. 8. Electron micrographs of mitochondria suspended in 0.1 mol.l^{-1} KCl, 2 mmol.l^{-1} $MgCl_2$ and 1 mmol.l^{-1} NADH whithout (a) and with (b) 24 μg ST.mg^{-1} mitochondrial protein (3 min after adding of ST)

The experimental results of Mistrík (1984) prove that the cation permeability of the inner membrane of maize root mitochondria is very low in the absence of energy. According to Jung and Brierley (1979) the low permeability of the mitochondrial membrane is either due to the absence of the system providing a cation-proton exchange or the system is "shuted down" blocking cation transport into mitochondria. The fast swelling of mitochondria supplied with energy proves the presence of the cation/proton exchange system in the mitochondrial membrane. Energy is necessary for its activation. Besides this way cations may enter mitochondria electrophoretically due to the pH-gradient between matrix and external space (Brierley, 1976). In maize root mitochondria however this pathway is rather slow. Even in the presence of ST we did not observe any stimulation of the mitochondria swelling. Our results show clearly that ST causes the swelling of mitochondria only in the presence of NADH and 100 mmol.l^{-1} KCl. ST added to mitochondria suspended in NaCl (with NADH) did not affect their size at all proving this the toxin action on the potassium uptake level. Since the swelling of mitochondria in the presence of ST was reversible for all concentration used (Fig. 6) let us assume that ST influences first of all the K^+/H^+ – antiporter. Because ST acts further as an uncoupler of the oxidative phosphorylation and as an ionophor increasing the membrane permeability to H^+ and OH^- it may very likely affect the pH-gradient between matrix and external space. As confirmed by Surico and DeVay (1982) it is the increased permeability

of the mitochondrial membrane to H^+ and OH^- which is responsible for the decline of the membrane potential after ST addition to the bathing solution. The use of the fungal toxin FC, a known stimulant of the plasmalemma H^+–ATPase, was without any effect on the transport processes in the mitochondrial membranes as shown by experiments with isolated mitochondria. It seems that FC exclusively influences the function of the H^+–ATPase localized in the plasma membrane.

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Literature

Bednarski, M. A., Izawa, S. and Cheffer, R. P. (1977): Reversible effects of toxin from *Helminthosporium maydis* race T on oxidative phosphorylation by mitochondria from maize. Plant Physiol. 59, 540–545.

Brierley, G. P. (1976): Monovalent cation transport by mitochondria. In: Packer, L., Gomez-Puyou, A. (eds): Mitochondria. Bioenergetics, Biogenesis and Membrane Structure. Acad. Press, New York, San Francisco, London, 3–20.

Day, D. A. and Hanson, J. B. (1977): On methods for the isolation of mitochondria from etiolated corn shoots. Plant Sci. Lett. 11, 99–104.

Douce, R., Mannella, C. A. and Bonner, V. D. (1973): The external NADH dehydrogenases of intact plant mitochondria. Biochim. Biophys. Acta 292, 105–116.

Estabrook, R. W. (1967): Mitochondrial respiratory control and polarographic measurement of ADP:0 ratios. In: Methods in Enzymology Vol. X, 41–56.

Gengenbach, B. G., Miller, R. J., Koeppe, D. E. and Arntzen, C. J. (1973): The effect of toxin from *Helmithosporium maydis* (race T) on isolated corn mitochondria: swelling. Can. J. Bot. 51, 2119–2125.

Gross, D. C., DeVay, J. E. and Stadtman, E. H. (1977a): Chemical properties of syringomycin and syringotoxin; toxigenic peptides produced by *Pseudomonas syringae*. J. Appl. Bacteriol. 43, 453–463.

Gross, D. C. and DeVay, J. E. (1977b): Population dynamics and pathogenesis of *Pseudomonas syringae* in maize and cowpea in relation to the in vitro production of syringomycin. Phytopathology 67, 475–483.

Hanchey, P. and Wheeler, H. (1979): The role of host cell membranes. In: Daly, J. M. and Uritoni, I. (eds): Recognition and specificity in plant-host parasite interaction. Japan Sci. Soc. Tokyo and University Press. Baltimore, 193–208.

Jung, D. W. and Brierley, G. P. (1979): Swelling and contracting of potato mitochondria. Plant Physiol. 64, 948–953.

Marre, E. (1979): Fusicoccin: a tool in plant physiology. Ann. Rev. Plant Physiol. 30, 31–43.

Mistrík, I. (1984): Niektoré štrukturálne; a funkčné aspekty mitochondrialnych membrán. PhD Thesis, Bratislava, 124 ff.

Pavlovkin, J., Novacky, A. and Ultrich-Eberius, C.-I. (1986): Membrane potential changes during bacteria-induced hypersensitive reaction. Physiol. Molec. Plant Pathol. 28, 125–135.

Sedmak, J. J. and Grossberg, S. E. (1977): A rapid sensitive and versatile assay for protein using coomise brilliant blue G-250. Anal. Biochem. 79, 544–552.

Spanswick, R. M. (1982): The electrogenic pump in the plasma membrane of Nitella. In: Slayman, C. L. (ed.): Current Topic in Membranes and Transport Vol. 16, Academic Press, New York 16, 35–47.

Spurr, A. R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res. 26, 31–43.

Surico, G. and DeVay, J. E. (1982): Effect of syringomycin and syringotoxin produced by *Pseudomonas syringae* pv. *syringae* on structure and function of mitochondria isolated from holcus spot resistant and susceptible maize lines. Physiol. Plant Pathol. 21, 39–53.

Tazawa, M. and Shimmen, T. (1982): Control of electrogenesis by ATP, Mg^{2+}, H^+ and light in perfused cells of Chara. In: Slayman, C. L. (ed.): Current Topics in Membranes and Transport Vol. 16, Academic Press. New York, 49–67.

Ziegler, W., Pavlovkin, J. and Pokorny, J. (1984): Effect of syringotoxin on the permeability of bilayer lipid membranes. Biológia (Bratislava) 39, 693–699.

Ziegler, W. and Pavlovkin, J. (1985): Syringotoxin, ein Phytotoxin von *Pseudomonas syringae* pv. *syringae*, erzeugt Ionenkanäle in bimolekularen Lipidmembranen. Acta Phytopathol. Acad. Sci. Hung. 20, 35–45.

Ziegler, W., Pavlovkin, J., Remis, D. and Pokorny, J. (1986): The anion/cation selectivity of the syringotoxin channel. Biológia (Bratislava) 41, 1091–1096.

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Fireblight of Pome Fruits: Genuinness and Spuriousness

NABIL S. FARAG

Plant Pathology Research Institute, Agric. Research Center, Giza, Egypt

Since the discovery of the bacterial nature of fireblight in pome fruits, an awesome amount of literature has been accumulated on the disease. The literature contains numerous unsubstantiated claims, contradictory reports and working hypotheses which with time and continuous subsequent citations became accepted as facts (41). Scanning literature for genuine characteristics of *Erwinia amylovora* (Burrill) Winslow et al. revealed numerous inconsistencies that account for contradictory description of the pathogen. Bacteriological differences among cultures collected from different locations were attributed to variability of the pathogen (2), though many of the variants showed salient characteristics of the genus *Pseudomonas*. Because of dissimilar characteristics, some cultures were classified as "English" (5) and "Egyptian" (16) isolates of the pathogen ... etc.

In the present paper the writer is endeavouring to point out the reported spurious characteristics of the fireblight bacterium that may help in discrimination of genuine ones.

I. The Bacterium

From the bacteriological point of view, it is a myth to consider variation in *E. amylovora* without considering the aspects of bacterial genetics. Bacterial culture in genetic sense is a heterogenous population, even it is started from a single-cell isolation. Heterogeneity may become apparent only under conditions that allow selective development of special variants in the population (47). Mutations are normally developed at low rate and are passed on unchanged to all generations (45). The latter together with recombination are the usual mechanisms for the development of new strains.

Despite the reported variability of *E. amylovora* (2), the English isolates of the pathogen showed considerable physiological homogeneity (5). Bacteriological characteristics of the latter isolates, however, were different to large extent from the original description (6). A critical consideration of certain important citations revealed a variety of pitfalls that may account for such inconsistent descriptions. Because of limited space, few examples will be presented.

1. Morphology and cell size

Cell size of *E. amylovora* was determined in Gram stained smears of single-celled cultures (2,22) and in parent cultures of the pathogen (5,22). The bacterium is described as Gram (–)ve rods (2) or Gram (–)ve to variable short rods (5).

Akadémiai Kiadó, Budapest

Because of differences in cell size, a correlation was made between morphovars and virulence, and the magnitude of length was in every case greater for weakly pathogenic cultures. Cell length of highly virulent isolates was recorded to range from 0.93–1.42 μ, while slightly virulent ones ranged from 1.61–1.71 μ (2,22). The non-virulent mucoid cultures were nearly twice the length (2.75 μ) of the highly virulent ones (22) and filamentous cells or long rods were observed in smears of pathogenic cultures (5). It is observed, however, that such correlations are inconclusive in light of methodologies followed.

Based on ten isolates collected from eight different hosts and six different localities, Ark (2) studied variability in cell size of the pathogen. Cell length determination was made after 3 passages into pear shoots, reisolation and incubation at 28 °C for 24 h. Although the repeated passage of isolates from different hosts into pear shoots and reisolation, it was concluded that there was no correlation between the host from which the isolate came and the length of the organism. Surprisingly, the cultures were different in their growth rates and some isolates showed fair growth at 15 °C and 19 °C; however, cell length determination was made for cultures grown at 28 °C. Other bacteriological characteristics of Ark's collection showed that 3 out of 10 isolates studied were consistently different in behavior. Obviously, the limited number of cultures tested and differences in physiological characteristics will increase the degree of skepticism about the taxospecies used.

Cultures from ten different hosts, assembled from USA, Canada, and New Zealand were studied by Hildebrand (22). Cultural purification involved the isolation of 5–20 single cells from each of 62 mother cultures, making a total of 720 cultures. Identical parent and single-celled cultures of the same source were reduced to a single representative for each cultural difference, thus the original selection was made for differences rather than identity of behavior. According to this method, the author concluded the relative stability of the fireblight bacteria and emphasized that the highly virulent cultures showed significantly short cell length.

Regardless of difficulties experienced in Gram staining of the English isolates (5), the determined cell length was reported to be 0.8–1.2 μ. Some cultures were either predominately coccoid or rod shaped with occasional filamentous cells or long rods. Billing et al. (5) reported, however, that 3 cultures showed predominately violet cells and small proportion of red cells. This may have resulted from the fact that Gram negative bacteria can be rendered positive by increasing alkalinity (43). Filament formation may be induced in rod shaped bacteria by various treatments that inhibit cell division but which do not inhibit growth, such as high incubation temperature or some nutritional dependency (20, 53). Filament formation in cultures of *E. amylovora* was reported, however, by

Goodman (19), Vörös and Goodman (52), Huang and Goodman (21). Moreover, it is known that some strains of *P. syringae* pv. *syringae* have cells longer than 4 μ (34) and filaments could be observed in cultures of *P. phaseolicola* (6). The first is pathogenic on pear (10) and the second has been reported to be associated with pear disorder in Egypt (18).

It seems probable that isolates assembled from different countries (22, 15), localities (2, 5), and host plants (2, 22, 5, 15) may involve some *Pseudomonas* strains producing symptoms simulating certain phases of fireblight (17), thus the bacteriological differences between the two genera have been considered as strain variation of *E. amylovora*.

2. Flagella

Arrangement and number of flagella are key characters in genus identification of phytopathogenic bacteria. Peritrichous flagellation of *E. amylovora* was reported by many investigators (7, 5, 15), and correlations between virulence and number and length of flagella were made. Hildebrand (22) indicated that the length of flagella seemed to bear some relation to virulence, because as virulence decreased the proportion of cells with short flagella increased. He reported that the highly virulent isolates showed the highest average number of flagella (3.35/cell) and the lowest (2.08/cell) was recorded for nonvirulent ones. Although these correlations, neither dominant number of flagella/cell nor position of flagella were unequivocally indicated.

The English isolates of *E. amylovora* stained by Gray's method showed 1–5 flagella (5). Although the carbol thionin method gave less clear background, there was better staining, hence, up to 8 flagella/cell were seen. The proportion of cells with a single flagellum, however, was not indicated.

In stained preparations, the small-sized lophotrichous rods are occasionally recognized as peritrichous; and polar flagella may be partly obliterated by the dense body of the organism thus apparently emerged laterally. Lateral flagella, however, may be associated with rod constriction, cross septa, or chain formation (35).

From the morphological point of view, it is known that cells of *Pseudomonas* spp. may have one or several polar flagella, though the point of insertion is not invariably polar for some species, being subpolar in some instances (34). Moreover, certain strains under some growth conditions produce lateral flagella which are easily shed, and in older cultures most cells retain only a single flagellum. The property of mixed flagellation is found not only in *Pseudomonas* but also in *Aeromonas* and *Chromobacterium* (44, 34). Early records on flagellation of *E. amylovora* indicated that the bacterium had 1–3 flagella at one pole

(24), a single polar flagellum (37), or peritrichous and polar flagella (7). More recently, Martinec and Kocur (31) reported 46 nonmotile cultures originally received from NCPPB, Harpenden, England. Such controversy on position and number of flagella may indicate dealing with other pathogens, possibly *Pseudomonas* spp. producing symptoms resembling fireblight (14, 17, 18, 28, 32, 38, 54).

3. Colony form

The usual "wild type" strains of bacterial pathogens, as isolated from infected plants, occurs in the mucoid or smooth phase with regular existence of rough forms (46). The rough form of *E. amylovora* was found to be stable in ordinary media and to revert to the smooth type when passed 4–6 times through 2% sucrose or 1% glucose nutrient broth (2). Colonies on sucrose nutrient agar (SNA) usually show characteristic features of diagnostic value for pathologists (2, 5, 29). Cream, domed, mucoid colonies develop on SNA after 3 days incubation at 25 °C, though some cultures may be nonmucoid or less mucoid (5). On the latter medium, the blossom blast pathogen, *P. syringae*, produces colonies similar to those of *E. amylovora* but with considerable differences in colour and elevation (29).

The production of levan type colonies by different varieties of *P. syringae* (18, 30), as well as by nonfluorescent *Pseudomonas* spp. (3) may be a primary source of confusion in fireblight diagnosis.

4. Oxygen requirements

Like other *Erwinia* spp., *E. amylovora* is described as facultative anaerobes (6, 17); however, the strong aerobic nature and weak growth of the pathogen under anaerobic conditions are also indicated (5).

Billing et al. (5) tested the oxygen requirements of the English isolates in sucrose nutrient agar and glucose yeastrel agar as shaken cultures. They reported profused growth at top of shaked tubes, with poor submerged growth that was detected with a hand lens, thus concluded the strong aerobic nature of the bacterium.

It is important to note that certain pathovars of *P. syringae* such as *P. phaseolicola* can grow under low oxygen tension, thus considered as microaerophilic (6). Moreover, the oxygen uptake by washed cells of *E. amylovora* was recorded to be similar to that of *P. phaseolicola*, and greater uptake was recorded for *P. syringae* (25). Oxygen requirements of *E. amylovora* must be seriously considered and compared with other pathogens associated with pome fruit disorders (6, 17, 18).

5. Mode of glucose utilization

Production of acid from glucose under anaerobic conditions is used for recognition of bacteria with fermentative physiology, and *E. amylovora* has been reported to utilize glucose fermentatively (5, 15).

The English isolates produced acid near the surface of unsealed Hugh and Leifson's medium (23) after 2 days, and the reaction in sealed one was similar but slower (5). Even after 6 days the reaction tended to be localized near the surface and was seldom as strong as in the unsealed medium. A complete acid reaction was eventually obtained under both circumstances after 2 weeks. Billing et al. (5) concluded that the intermediate behavior of *E. amylovora* in this test reflect its strongly aerobic nature as shown by the agar shake culture experiments used in studying aerobiosis.

The limited acid production and localization of growth near the surface of sealed medium after 6 days might not be indicative of fermentative utilization of glucose. It is known that certain *Pseudomonas* spp. such as *P. phaseolicola* can produce slight acid in sealed Thornley's peptone medium 1 (48). Hence, production of acid from peptone under anaerobic conditions must be examined for the English isolates.

6. Acid production from carbohydrates

Sugars incorporated in peptone-free basal medium or peptone broth are commonly used to test acid production (13, 12). Unfortunately, the nature of acid produced during growth is often not known, and some bacteria produce acids from substrates that are not used for growth, while others produce no acids from usable carbohydrates (34). Bacteria may produce acids from the L form of a sugar but not from the D form, or may utilize both forms (8). The Egyptian isolates of *E. amylovora*, however, were reported to produce acid from glucose but not from dextrose (16). Such specificity for different synonyms of one and the same substance is not known for *E. amylovora* or its variants (2, 6, 8).

It is known that acid production from lactose under anaerobic conditions is a character that differentiates the tribe *Erwinieae* from other tribes of *Enterobacteriaceae* such as proteeae and salmonelleae (6). Therefore, the inability of *E. amylovora* to produce acid from lactose under anaerobic (15) and/or aerobic conditions (5, 15, 8) must be reconsidered in light of earlier records on variable acid production from lactose (2, 6). It seems probable that cultures collected from different sources and different host plants may contain different taxospecies, thus variable results are encountered.

Plant pathogens in genus *Erwinia* normally do not require organic nitrogen for growth (6), and are characterized by production of acid from salicin in peptone-free basal medium (13). Organic nitrogen in the test medium may cause a neutralization reaction for the acid produced during the course of bacterial growth, thus negative results are recorded. Dye (15) reported that 21–79% of *E. amylovora* isolates produced acid from salicin in agar medium containing mineral salts and yeast extract, and from 0–20% produced acid in peptone water. This indicates that different media may lead to different conclusions.

7. Gelatin liquefaction

Liquefaction produced by different isolates of *E. amylovora* varied from no to very strong proteolysis (2, 22, 15). Greater liquefaction was correlated with weak pathogenicity (2, 22); however, high pathogenicity was correlated with earlier and greater liquifaction (5).

Colony form of the English isolates on SNA was correlated with rate of gelatin liquefaction (5). Isolates showing typical colonies produced liquefaction in 2–3 days, and that for a typical ones varied from 3–21 days. Such correlation in different from those reported by Ark (2) and Hildebrand (22).

It seems probable that the strong aerobic nature of the English isolates and localization of growth near the surface are contributing factors relating to such false liquefaction. Accumulation of capsular material near the surface of the medium will of course influence the degree of gelatin hardening in the vicinity of growth, thus false liquefaction may be recorded. This explanation is justified by their record on a noncapsulated variant, from originally capsulated culture, that failed to liquefy gelatin. Moreover, Billing et al. (5) concluded that those isolates which attacked gelatin slowly were also slow to produce ooze from fruits and were characterized by their low degree of capsulation.

Gelatin liquefaction produced by the so-called "Egyptian isolates" of *E. amylovora* was studied by El-Helaly et al. (16). They found that the bacterium could liquefy gelatin in 15 days after incubation. When 5% sucrose was added to the nutrient gelatin, liquefaction was almost complete within 4 days incubation at 30 °C. This indicates that presence of sucrose increases the rate of liquefaction, a condition which is contrary to what is known about protein-sparing action of carbohydrates. Such false gelatin liquefaction as a result of incorporating 5% sucrose in the test medium, may be attributed to luxurniat levan production that affects the degree of gelatin hardening in the vicinity of growth.

8. Litmus milk and casein hydrolysis

Milk indicates both saccharolytic and proteolytic properties of bacteria by detecting whether they ferment lactose or digest casein (12).

Gradual digestion of milk was reported for different isolates of *E. amylovora* (2, 5, 15); and the weakly pathogenic strains were reported to decolorize litmus indicator in a shorter time (2). Although 7 out of 10 of Ark's isolates were to ferment lactose (2), no indication was made on coagulation of milk protein indicating slight acid production. In this respect, Hildebrand (22) reported that all cultures fermented litmus milk, though some of them were unable to ferment lactose.

Billing et al. (5) reported that the English isolates produced slow alkaline reaction and reduced litmus indicator. Slight digestion of milk protein was observed extending to a maximum depth of 4 mm after 21 days. This reaction possibly relates to the intense growth of the organism near the surface, because of the strong aerobic nature of the English isolates. Meanwhile, they reported negative results for casein hydrolysis in milk agar, though results with the latter can be read in 24–48 h, which is shorter than the time necessary to see digestion in litmus milk (12). Such observation may justify doubts regarding the proteolytic ability of English isolates of *E. amylovora*.

The Egyptian isolates of the fireblight bacterium as reported by El-Helaly et al. (16) were able to curd litmus milk despite inability of the bacteria to ferment lactose. Because they made no indication to the changes in litmus colour, it might be difficult to assume it either acid or sweet, rennin, curdle. Meanwhile, peptonization was not indicated although emphasis was made on proteolytic ability of the tested bacteria.

Dye (15) reported an alkaline reaction in purple milk in 14–28 days, though milk agar plates confirmed no hydrolysis of casein; a case which is similar to that reported by Billing et al. (5). The inability to hydrolyze casein may raise some questions regarding the proteolytic ability of the tested bacteria in general and the cause of alkaline change in purple milk in particular.

9. Ammonia production

Ammonia was reported to be produced by different isolates of the pathogen (2, 22) and negative to weakly positive results were reported for the English isolates (5).

The English isolates were tested in 1% peptone water plus 0.5% yeast extract; and in 0.25% peptone water. Although the essential requirement of nicotinic acid, the isolates showed negative results in yeast extract-enriched

medium, and negative to weak positive results in media devoid of it. As far as it is known, yeast extract is a good source for nicotinic acid or its amides; and the latter acts as essential part of codehydrogenases I and II. It is not expected, however, that the carbohydrate fraction in 0.5% yeast extract incorporated in the medium, could affect ammonia production by the English isolates.

10. Nitrate reduction

The inability of *E. amylovora* to reduce nitrate to nitrite was reported by many investigators (22, 5, 15) and utilization of sodium, potassium, as well as ammonium nitrate(s) was indicated by Hildebrand (22). The isolates collected by Ark (2), however, produced ammonia in nitrate broth.

The detected ammonia in nitrate broth after 14 days as reported by Ark (2) may be caused by the action of bacteria on peptone in the test medium. It should be noted that 3 of Ark's isolates which failed to produce ammonia in nitrate broth, showed a very slight or negative ammonification in nutrient broth.

The reduction of nitrate to nitrite is known to be lost by many bacteria after repeated subculturing in ordinary media (34). Therefore, the inability of *E. amylovora* to reduce nitrate (22, 5, 15) and the ability to utilize nitrate salts (22) must be seriously considered in identification studies. It is known, however, that *P. syringae* as well as group Ia pathogenic pseudomonas are unable to reduce nitrate (30), thus consideration of this test in safe diagnosis of fireblight (33) is not recommended.

II. Diagnosis

Because of close similarity in symptoms of fireblight and blossom blast, particularly in the initial stages of pathogenesis, it is not possible to consider diagnosis of the first without considering the latter (29). Most diagnostic methods vary from the use of simple culture media to the use of immunofluorescent microscopy (33), and more recently by using dot ELISA (55) as well as fatty acids profiling (51). The use of anyone method alone is not recommended, but by a combination of methods accurate and rapid diagnosis is possible (33). Most methods, however, are practically important if comparison is made with authentic cultures as checks.

Presumptive diagnosis of fireblight is based largely on some cultural, pathological, and serological characters of the bacterium (29). Colony form on SNA, absence of fluorescent pigments, slide agglutination with specific antiserum, production of ooze from immature pear fruits are used to differentiate *E. amylovora* from *P. syringae*.

Confirmatory diagnosis may be needed by workers inexperienced in fireblight diagnosis. These tests include tube agglutination against specific antiserum, fermentative reaction in Hugh and Leifson's medium, reaction with one or more phage (29); production of acid from sucrose and failure to reduce nitrate (4). The latter two reactions were also reported for *P. syringae* as well as different pathogenic species (30) thus not recommended for confirmatory diagnosis.

Although fluorescent pigment is a diagnostic tool in identification of genus *Pseudomonas* (34), the poor agreement among different observers on recognition of fluorescence (30) makes it a hazardous tool in genus identification. The versatile pigment production by *P. syringae* pv. *phaseolicola* was reported by Farag et al. (18) and inability to observe the pigment produced by this pathogen was indicated by Király et al. (26). Therefore, the inability of observes to recognize fluorescence may be misleading in taxon determination.

Susceptibility of immature pear fruit to the fireblight pathogen is known to correlate with high asparagin content (2). The use of small-sized fruits, about 2 cm in diam., is essential for proper diagnosis (11, 42). However, inoculation of pear slices and fruits without considering the size became a common practice for ooze detection, thus misleading conclusions may be recorded.

Because of poor diagnostic methods and misinterpretation of results (16, 50, 51, 1) the pear decline in Egypt was attributed to fireblight, though symptoms were inexacting to the disease syndrome (18). Based on ooze production from pear slices, absence of fluorescence in King's medium B, growth on MS medium, and biochemistry of fatty acids, the bacteria were identified as *E. amylovora* (50). The same isolates, however, were identified as strains of *P. syringae* pv. *phaseolicola*, due to their ability to produce systemic infection in Kidney beans, inability to infect green lemon fruits or to produce ooze from small pear fruits, and their distinguished bacteriological characteristics (18). Production of ooze from pear fruits larger than 2 cm in diam., may be considered as the primary source of confusion in fireblight diagnosis. This indicates that routine diagnosis and biochemistry of fatty acids may be inconclusive under many circumstances.

Generally, it could be concluded that levan production, negative oxidase reaction, inability to rot potato slices, negative arginine dihydrolase, ability to induce HR in tobacco leaves, and inability to reduce nitrate to nitrite (29, 30) will not differentiate the nonfluorescent variants of *P. syringae* and/or pv. *phaseolicola* from *E. amylovora* (15, 29). Misinterpretation of results on aerobiosis, utilization of glucose under anaerobic condition and arrangement of flagella, as indicated previously, might be a serious sources of confusion in fireblight diagnosis.

In retrospect, endeavours to determine the genuine characteristics of *E. amylovora*, as a type species of other *Erwinias* have lead to provocative conclusions. Spuriousness might be due to poor methodologies, misinterpretation of results, and dependency on cultures collected from different sources without authentication. Consideration of various biovars of *P. syringae* together with proper pathological work may help in discriminating the genuine characteristics of *E. amylovora*. Diagnosis based on ooze production without consideration of fruit size will be misleading in fireblight diagnosis. Type culture collections of the pathogen must be screened up for genuine characteristics with consideration of possible involvement of nonfluorescent *Pseudomonas* spp.

Literature

1. Abo-El-Dahab, M. K., M. A. El-Goorani, H. M. El-Kasheir and Alia A. Shoeib (1983): Severe outbreaks of pear fireblight in Egypt. Phytopath. Medit. 22, 168–170.
2. Ark, P. A. (1937): Variability in the fire-blight organism, *Erwinia amylovora*. Phytopathology 27, 1–27.
3. Ballard, R. W., N. J. Palleroni, M. Doudoroff, R. Y. Stanier and M. Mandel (1970): Taxonomy of aerobic pseudomonas: *Pseudomonas cepacia*, *P. marginata*, *P. alliicola*, and *P. caryophylli*. J. gen. Microbiol. 60, 199–214.
4. Billing, Eve, J. E. Crosse and C. M. E. Garrett (1960): Laboratory diagnosis of fire blight and bacterial blossom blight of pear. Plant Path. 9, 19.
5. Billing, Eve, Lily A. E. Baker, J. E. Crosse and C. M E. Garrett (1961): Characteristics of English isolates of *Erwinia amylovora* (Burrill) Winslow et al. J. Appl. Bact. 24, 195–211.
6. Breed, R. S., E. G. E. Murray and N. R. Smith (1957): Bergey's Manual of Determinative Bacteriology, 7th ed., Williams and Wilkins, Baltimore.
7. Bryan, M. K. (1927): The flagella of *Bacillus amylovorus*. Phytopathology 17, 405–406.
8. Buchanan, R. E. and N. E. Gibbons (1974): Bergey's Manual of Determinative Bacteriology, 8th ed., Williams and Wilkins, Baltimore.
9. Burkholder, W. H. and M. P. Starr (1948): The generic and specific characters of phytopathogenic *Pseudomonas* and *Xanthomonas*. Phytopathology 38, 494–502.
10. Cameron, H. R. (1962): Diseases of deciduous fruit trees incited by *Pseudomonas syringae* van Hall. A review of the literature with additional data. Agricultural Experimental Station, Oregon State Univ., Corvallis.
11. Crosse, J. E., Margery Bennett and C. M. E. Garrett (1960): Investigation of fire-blight of pear in England. Ann. Appl. Biol. 48, 541–558.
12. Cruickshank, R., J. P. Duguid, B. P. Marion and R. H. A. Swain (1975): Medical Microbiology, 12th ed., vol. 2: The Practice of Medical Microbiology. Churchill Livingstone, Edinburgh London and New York.
13. Dowson, D. J. (1957): Plant Diseases Due to Bacteria. Cambridge at the University Press, London.
14. D'Yakova, G. A. (1955): Bacterial pear blight in the USSR and the biology of its causal organism. Leningrad Univ. Thesis (in van der Zwet and Keil, 1979).
15. Dye, D. W. (1968): A taxonomic study of the genus *Erwinia*. I. The "Amylovora" group. New Zeal. Jour. Sci. 11, 590–607.
16. El-Helaly, A. F., M. K. Abo-El-Dahab and M. A. El-Goorani (1964): The occurrence of fire blight disease of pear in Egypt. Phytopathologia Mediterranea Vol. III, 156–163.

17. Elliott, Charlotte (1951): Manual of Bacterial Plant Pathogens. Chronica Botanica Company, Waltham, Mass., USA.
18. Farag, N. S., Faiza G. Fawzi, F. Bishay, T. A. El-Sharkawy and K. Y. Mickail (1986): Highlights on pear disorder in Egypt. Acta Phytopathologica et Entomologica Hungarica 21, 99–108.
19. Goodman, R. N. (1965): In vitro and in vivo interactions between components of mixed bacterial cultures isolated from apple buds. Phytopathology 55, 217–221.
20. Grula, E. A. (1960): Cell division in a species of *Erwinia*. I. Initial observations relating to nutritional dependency. J. Bacteriol. 80, 369–374.
21. Huang, P. Y. and R. N. Goodman (1970): Morphology and ultrastructure of normal rod-shaped and filamentous forms of *E. amylovora*. J. Bacteriol. 102, 862–866.
22. Hildebrand, E. M. (1954): Relative stability of fire blight bacteria. Phytopathology 44, 192–197.
23. Hugh, R. and E. Leifson (1953): The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. Jour. Bact. 66, 24.
24. Jones, D. H. (1909): Bacterial blight of apple, pear and quince trees. Ontario Dept. Agr. Bul. 176, 63 pp.
25. Katznelson, H. (1955): The metabolism of phytopathogenic bacteria: I. Comparative studies on the metabolism of representative species. Jour. Bact. 70, 469–475.
26. Király, Z. (1974): Methods in plant pathology, with special reference to breeding for disease resistance. Elsevier Scientific Publishing Co. Amsterdam, London, New York.
27. Klement, Z. and R. N. Goodman (1966): Hypersensitive reaction induced in apple shoots by and avirulent form of *Erwinia amylovora*. Acta Phytopath. Acad. Sci. Hungaricae 1 (3–4), 177–184.
28. Lacey, Margaret S. and W. J. Dowson (1931): Studies in bacteriosis: XVIII. A bacterial canker of apple trees. Ann. Appl. Biol. 18, 30–36.
29. Lelliott, R. A. (1967): The diagnosis of fireblight *(Erwinia amylovora)* and some diseases caused by *Pseudomonas syringae*. Report of the International Conference on Fireblight, Canterbury, August 17–18, 1967. EPPO Publications, Series A. N°. 45–E.
30. Lelliott, R. A., Eve Billing and A. C. Hayward (1966): A determinative scheme for fluorescent plant pathogenic pseudomonas. Jour. Appl. Bact. 29, 470–489.
31. Martinec, T. and M. Kocur (1964): A taxonomic study of *Erwinia amylovora* (Burrill 1882) Winslow et al. 1920. International Bulletin of Bacteriological Nomenclature and Taxonomy. 14 (1), 5–14.
32. McKeen, W. E. (1955): Pear blast on Vancouver Island. Phytopathology 45, 629–632.
33. Miller, H. J. (1979): A review of methods used in laboratory diagnosis of fireblight. EPPO Bull. 9 (1), 7–11.
34. Palleroni, N. J. and M. Doudoroff (1972): Some properties and taxonomic subdivisions of the genus *Pseudomonas*. Ann. Rev. Phytopath. 10, 73–100.
35. Rhodes, Muriel E. (1958): The cytology of *Pseudomonas* spp. as revealed by a silver-plating staining method. Jour. Gen. Microb. 18, 639–648.
36. Rhodes, Muriel E. (1965): Flagellation for the classification of bacteria. Bacteriol. Rev. 29, 442–465.
37. Rosen, H. R. (1926): The number and arrangement of flagella of the fire blight pathogen, *Bacillus amylovorus*. Mycologia 18, 23–26.
38. Rosen, H. R. and W. L. Bleecker (1933): Comparative serological and pathological investigations of the fire blight organism and a pathogenic fluorescent group of bacteria. Jour. Agr. Res. 46, 95–119.
39. Salle, A. J. (1961): Fundamental Principles of Bacteriology. McGraw-Hill Company, Inc. New York, Toronto, London.
40. Schaad, N. W. (1980): Laboratory Guide for Identification of Plant Pathogenic Bacteria. Amer. Phytopath. Soc. St. Paul, Minnesota.
41. Schroth, M. N., S. V. Thomson, D. C. Hildebrand and W. J. Moller (1974): Epidemiology and control of fire blight. Ann. Rev. Plant Pathol. 12, 389–412.
42. Schroth, M. N. and D. C. Hildebrand (1980): *Erwinia amylovora* or true erwiniae group, pp. 26–30 (In: Schaad, 1980).

43. Stearn, E. W. and A. E. Stearn (1924): The chemical mechanism of bacterial behavior. I. Behavior toward dyes, factors controlling the Gram reaction. Jour. Bact. 4, 479.
44. Sneath, P. H. A. (1956): The change from polar to peritrichous flagellation in *Chromobacterium* species. J. Gen. Microbiol. 15, 99–105.
45. Sokatch, J. R. and J. J. Ferretti (1976): Basic Bacteriology and Genetics. Year Book Medical Publishers, Inc, Chicago.
46. Starr, M. P. (1959): Bacteria as plant pathogens. Ann. Rev. Microbiol., 13, 211–238.
47. Stolp, H., M. P. Starr and Nancy L. Baigent (1965): Problems in speciation of phytopathogenic pseudomonads and xanthomonads. Ann. Rev. Phytopathology 3, 231–264.
48. Thornley, Margaret J. (1960): The differentiation of pseudomonas from other Gram-negative bacteria on the basis of arginine metabolism. Jour. Appl. Bact. 23, 37–52.
49. van der Zwet, T. and H. L. Keil (1979): Fire Blight a Bacterial Disease of Rosaceous Plants. U. S. Dept. Agr., Handbook no. 510.
50. van der Zwet, T. (1983): Survey of the presence of fire blight in Egypt. U. S. Dept. Agric., Agric. Res. Service, Northeastern region. Travel Rept., May 24 –June 4, 1983.
51. van der Zwet, T. (1986): Identification, Symptomatology, and epidemiology of fire blight on Le Conte Pear in the Nile Delta of Egypt. Plant Disease 70, 230–234.
52. Vörös, J. and R. N. Goodman (1965): Filamentous forms of *Erwinia amylovora*. Phytopathology 55, 876–879.
53. Webb, M. (1953): Effects of magnesium on cellular division in bacteria. Science 118, 607.
54. Wilson, E. E. (1934): A bacterial canker of pear trees new to California. Phytopathology 24, 534–537.
55. Zutra, D., E. Shabi and G. Lazarovits (1985): Fire blight on pear: A new disease in Israel. Contribution No. 1530–E, 1985 series from the Agricultural Research Organization, Volcani Centre, Bet Dagan, Israel.

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Hosts and Non-Hosts in the Diagnostic Strategy of Plant Viruses[1]

J. HORVÁTH

Pannonian University of Agricultural Sciences, Institute for Plant Protection, Department of Plant Pathology, H-8361 Keszthely, P.O. Box 71, Hungary

Beside the increasingly sensitive and reliable methods available for the diagnosis and identification of the plant viruses, plants susceptible or resistant to one virus or virus group are not dispensable either. In the diagnostic strategy an essential viewpoint is to determine the common and differing characteristics of the individual viruses, in which important role is played by the knowledge of the natural and experimental host range and non-host range. In certain cases (e.g., discovery of resistance-breaking strains) a host range is more discriminating than other tests.

Besides emphasizing that the role of plants in diagnostics must not be overestimated, it can be established that in the case of 232 mechanically transmissible viruses belonging to 24 virus groups, 141 hosts rendering possible the positive diagnosis of the individual viruses could be selected. The 133 selected non-hosts, on the other hand, are suitable for the exclusion or negative diagnosis of the individual viruses.

Knowing the hosts and non-hosts of the various viruses we were able to select plants of which 79 enabled the positive diagnosis of the individual virus groups, while 59 plants were found to be suitable for the negative diagnosis of virus groups.

We should like to emphasize that the host plants and non-hosts alone cannot provide a reliable basis for the differential diagnosis of the viruses and virus groups. For this modern virological methods are required, though these methods cannot dispense with host plant information concerning the viruses and virus groups.

Biological research in recent years has contributed highly important results to the knowledge of natural sciences. Here belong various achievements in virus research, of which the detection and description of new viruses, the examination of the relationship between the viruses and the infected host cell, the knowledge acquired of the chemical structure of viruses, of their proteins and nucleic acids, and of their genetic information deserve special attention. As a consequence of all this, today one can speak of molecular virology. Now, when the virus as a biological model is in the centre of research at institutes and university departments for molecular virology, and the recent advances in virus detection and diagnosis are based on increased sensitivity of methods for the detection of protein and nucleic acid, whether the "oldest" questions of virology, the host-virus relations, the host plants and test plants of viruses still command interest? This is a very difficult question. Reassurance may be drawn from the reflections of Selye (1967): "... it seems to me that no matter how much we shall learn about the most intimate mechanisms of biological phenomena, we will always need the old-fashioned holistic approach; for an over-all view we will

[1]Dedicated to the late Dr. K. Schmelzer (1928–1976) on the occasion of his 65th birthday

Akadémiai Kiadó, Budapest

continue to depend upon the broad-scale correlation of simple observations in which the chips are handled as units, although we know that in fact, they are intricately-structured complexes."

This work is aimed at providing practical information on the host plants, non-hosts and test plants of viruses for those who while possessing minimum laboratory equipment have commited themselves to virology. The author, a member of this group of virologists, preserves as sacred the intellectual legacy that he received from Klaus Schmelzer (1928–1976) before his death as a dedication in one of Schmelzer's books (Schmelzer and Wolf, 1971): "Für denjenigen, dem die Pflanze in der Virologie das wichtigste ist, kann die Kentniss des Wirtspflanzenkreises eines Virus durch nichts ersetzt werden." The eternal value of these thoughts is proved by the fact that the last several years are witnesses of a true renaissance of the interest in virus hosts and non-hosts. It is not easy to say what gave the impulse to this. That much is sure, that in studying the plant viruses it is very important to know those systems from which they originate, in which they can be found, and with the help of which they can be detected and studied in the simplest way.

While on the origin of the viruses we only have hypotheses (Gibbs, 1969; Gibbs and Harrison, 1976; Matthews, 1981; Goodman et al., 1986) our knowledge of their occurrence and distribution is wide. Today we know that the viruses are widespread in prokaryotes (bacteria and blue-green algae) and in eukaryotic plants (algae, fungi, pteridophytes, gymnosperms, angiosperms) (Gibbs and Harrison, 1976; Horváth, 1977a, 1982a). Natural wild plants probably are the original, or at least the most recent source of many of our economically important viruses. In a discussion on the origin of new virus diseases Bennett (1952) points out that most virus diseases have occurred in the new agricultural regions. Considering that most of the viruses known at present are confined to angiosperms, where the agricultural, horticultural and the weed species that grow in cultivated areas belong the virologists who study the host-virus relations are interested in four main fields of research: (*1*) to detect and identify the viruses that cause economic losses in cultivated plants, (*2*) to find the reservoir plants that play a role in the survival of viruses, (*3*) to point out virus susceptible and resistant plants that serve as feed plants for the vectors, and (*4*) to seek out virus susceptible test and indicator plants, and virus resistant plants.

Better understanding of the relations between plants and viruses indispensably requires a systemic testing of the flora. The extent to which the expected results will contribute to the knowledge of virus-plant relations, which even in the molecular phase of virology may be surprisingly new for the science, depends on how wide the range of the flora within which so far unknown or insufficiently known host-virus relations are cleared up is.

Problems on Host-Virus Interactions

Host specificity and host range

The plant viruses are obligatory parasitic infectious agents which multiply in their hosts. For multiplication they totally depend on living host cells, though they contain their own genetic information. They actually are tiny packages of genetic material alien to their hosts, but capable of inciting the latter to provide the metabolic machinery for virus reproduction (Bos, 1983b).

An essential but very difficult question of understanding the host-virus relations is the knowledge of the factors that determine the host specificity of the viruses. In spite of the highly significant research results (Diener, 1963; Mundry, 1963) we still rely mainly upon suppositions and speculations (Atabekov, 1975; Zaitlin and Hull, 1987; Schoelz, 1990) when throwing up this problem. We do not know as yet what the exact role of the virus genome and host cell genome is in the infection or resistance to infection of a plant cell. It is, however, known that the host ranges of related viruses may be different and, on the other hand, those of non-related viruses may be similar. Therefore the attempts to point out correlations between the host range and other properties of a virus failed. In the course of studying a quasi-genetic model of the host plants of viruses Bald and Tinsley (1967a, 1967b, 1967c) found that the various taxonomic groups of plants significantly differed in virus susceptibility. The authors are of the opinion that "the most susceptible groups of plants were relatively advanced in phylogenetic, and, therefore probably in evolutionary sense" and that "the more primitive groups of families were generally less susceptible to the viruses examined". The statistical genetic model showed that the virus susceptibility of plants was determined by many kinds of property not uniformly found among the related groups of plants, and the properties responsible for the infectivity of the virus were not uniform either. The susceptibility of the host plant and the infectivity of the virus are less controlled than the processes submitted to the effect of the deoxyribose nucleic acid (DNA) of cell. Great advance has been made with the realization that viral gene products other than those to be found in the virus particle itself may play an important role in determining the host response to infection (Matthews, 1980). The factors determining the host range are probably complex and may well vary with viruses and hosts (Matthews, 1981; Culver et al., 1991). In one sense a great amount of progress has been made in identification of viral host determinants. It has been possible to map a determinant of host specificity to a single genome segment in many multicomponent plant virus groups. In several cases, determinants of host specificity have been mapped to single genes. However, it has been difficult to make any generalizations about

viral host determinants because virtually every viral gene in one study or another has been implicated as a host determinant (for review, see Schoelz, 1990).

The molecular biology undoubtedly has made a great progress in explaining the biology of the plant virus genomes and virus replication. Unfortunately, this remarkable progress has not been accompanied by an increase in the knowledge of the effect exercised by the virus infection on the physiology of the host plant (Diener, 1963; Goodman et al., 1986, 1991; Zaitlin and Hull, 1987). In consequence of these very complex mechanisms the plant is either susceptible to or immune of the virus. By susceptible plant such plants are meant in the cells of which the virus can reproduce irrespective of whether the reaction of the plant to the virus manifests itself in local or systemic symptoms. The reproduction of the virus in the inoculated cells of the plants is the only reliable criterion of susceptibility (Atabekov, 1975). By immune plant, on the other hand, such plants are meant into the cells of which the virus cannot penetrate, or even if it has entered the cells, cannot replicate in them (Hull, 1986). Dawson and Hilf (1992) distinguished various types of host-virus interactions: (*1*) *total susceptibility* (the virus can replicate and systemically invade most cells of the hosts), (*2*) *initial susceptibility* (the virus begins replicating but is subsequently limited to a few cells around the initial point of entry, usually by a hypersensitive response), (*3*) *limited susceptibility* (in which a reduced level of virus replication occurs in cells throughout the plant, or in which the virus can spread in inoculated leaves but cannot systemically infect the plant, or in which the virus replicated but is limited to the initially infected cells), (*4*) *resistance to infection* (where there is a tendency for a low percentage of plant to become infected), and (*5*) *true immunity* (where the virus is not able to replicate even in the cells where the virus introduced). The virus susceptibility and immunity of plants are important properties which can be used for the identification, characterization and differentiation of the viruses. Although Matthews (1981, 1991) points out that the host range can be a rather meaningless determination, since (*1*) in many of the reported host range studies only positive results were recorded, (*2*) the lack of symptoms following the inoculation of test plants was not always checked up through back-inoculation to an indicator species to test for masked infection, (*3*) the way of inoculation may affect the results, (*4*) when studying large numbers of species it is usually practicable to make tests under one set of conditions only, but it is known that a given species may widely vary in susceptibility to a virus depending on the conditions of growth, (*5*) even closely related strains of a virus may differ in the range of plants they infect, and (*6*) mesophyll protoplasts may be readily infected with a virus that causes little or no infection when applied to intact leaves. In spite of this, the host range (virus susceptible plants) and non-host range (plants immune of viruses) still play a role in the virus diagnosis, and from the point of

view of virus prognosis they may also be important (Horváth, 1993a). Matthews (1991) emphasizes that sometimes the host range is more discriminating than other tests. For example, some plants (cowpea, potato or raspberry) have been protected against cowpea chlorotic mottle *Bromovirus*, potato X *Potexvirus* or raspberry bushy dwarf *"Idaeovirus"* by the use of cultivars resistant to the common strains of the three viruses. Recently discovered resistance-breaking strains of the three viruses could not be distinguished from the common strains (Murant et al., 1968; Moreira et al., 1980; Jones, 1982; Adams et al., 1984; Jones, 1985; Pauguio et al., 1988; Jennings and Jones, 1989).

Natural host range

According to our present knowledge *Eupatorium chinense* var. simplicifolium was the first natural virus host. A poem written by the Empress Kōhen on the "yellow leaf" of *Eupatorium* in the year of 752 that appeared in "Manyoshu" would be the first record all over the world in the literature of the possible plant virus diseases (Inouye and Osaki, 1980). Later the "colour-breaking" of tulip (*Tulipa* spp.) petals infected with tulip mosaic in Holland early in the 17th century, the "degeneration disease" of potato *(Solanum tuberosum)* in the 18th century, the "leaf variegation" of the woody ornamental *Abutilon striatum* var. spurium known as var. thompsonii in the second half of the 19th century, then the "mosaic disease" of tobacco *(Nicotiana tabacum)* made the first natural virus hosts known (Gibbs and Harrison, 1976; Bos, 1983a; Walkey, 1985; Horváth and Gáborjányi, 1990).

The natural host range covers those plants which under natural conditions become infected by the virus spontaneously, without human intervention. With the growing intensity of virus research the natural host range of a given virus increases almost day by day, as shown by the fact that earlier unimaginable susceptible host-virus relations have lately come to light (Horváth, 1982a). Particularly important research results were obtained in studies on mechanically transmissible viruses occurring in ornamental and wild cruciferous plants. Shukla and Schmelzer (1975) reported that in the course of testing 191 cruciferous plant species or varieties belonging to 58 genera they isolated 10 viruses and distinguished 209 virus-host combinations, of which 185 were described for the first time. Thirty-nine species contained more than one virus at a time. The results show that the viruses of ornamental and wild cruciferous plants occur in plants of other families too. It can be established that the study of ornamental and wild cruciferous species have opened new vistas especially in natural host ranges and geography of viruses.

The *Potyvirus* group was established in 1959 (Brandes and Wetter, 1959). At that time the number of viruses recognized in the group was only 14. According to Hollings and Brunt (1981) between 1959 and 1979 the number of viruses belonging to the *Potyvirus* group grew from 16 to 73. By 1988 the number of potyviruses had increased to 175; this number is about 30 % of all known plant viruses (Shukla and Ward, 1989a, b; Edwardson and Christie, 1991). In the last 20 years (1971–1991) the virus groups have increased more than twofold in number and almost the same can be said of the recently described viruses and natural hosts (Harrison et al., 1971; Smith, 1972; Klinkowski, 1977; Matthews, 1981; Fenner and Gibbs, 1983; Richter and Spaar, 1983; Matthews, 1985; Brown, 1986; Francki et al., 1991; Matthews, 1991). Plant viruses are at present classified into about 36 groups (approximately equivalent to families). Within the last years the *Capillo-*, *Carmo-*, *Crypto-*, *Faba-*, *Furo-*, *Marafi-* and *Tenuivirus* groups have been definied, while at least three other groups (*Badna-*, *Umbra-* and *parsnip yellow fleck virus* group) are semi-officially recognized (Milne, 1990). In the course of detecting new viruses and virus strains in Hungary, and examining new natural host-virus relations 16 viruses of 11 virus groups have been isolated from 50 natural virus hosts in the last years (Horváth, 1976).

As regards the number of infected plants the natural host ranges of viruses greatly vary. There are viruses whose natural host range only covers a single plant. Such are for example the Andean potato mottle *Comovirus*, potato M *Carlavirus* and potato S *Carlavirus*, all three isolated exclusively from potato *(Solanum tuberosum)*, or the velvet tobacco mottle *Sobemovirus* and Nicotiana velutina mosaic ? *Furovirus*[2] isolated from tobacco *(Nicotiana velutina)*, or the alfalfa latent *Carlavirus*, and lucerne Australian latent *Nepovirus* isolated from lucerne *(Medicago sativa)*, or the hop latent *Carlavirus* isolated from hop *(Humulus lupulus)*, or the Melandrium yellow fleck *Bromovirus* isolated from the *Melandrium album* plant. Also, there is a virus (tobacco vein mottling *Potyvirus*) with two natural hosts known *(Lycopersicon esculentum, Nicotiana tabacum)*. On the other hand, the natural host range of some viruses covers numerous plants. Such are for example the cucumber mosaic *Cucumovirus* (476 species in 67 families), the alfalfa mosaic virus (46 species in 12 families), the lettuce mosaic *Potyvirus* (21 species in 9 families). While the watermelon mosaic virus 1 (papaya ringspot *Potyvirus*) has a natural host range confined to Cucurbitaceae the watermelon mosaic virus 2 *Potyvirus* infects species in other families as well (Horváth et al., 1975b; Purcifull et al., 1984a, b).

There are also considerable differences in the number of viruses that infect a plant. For example, with the natural hosts of the legume viruses taken into

[2]Question mark indicate uncertainty of taxonomic status of the virus

consideration it can be established that *Pisum sativum* is natural host for 27, *Vicia faba* for 26, *Phaseolus vulgaris* for 21, *Glycine max* for 19, *Trifolium subterraneum* for 8 legume viruses (Boswell and Gibbs, 1983).

We know viruses whose natural occurrence has been reported so far from a single country. For example, Maclura mosaic ? *Potyvirus* (Yugoslavia), pepper veinal mottle *Potyvirus* (Ghana), Peru tomato *Potyvirus* (Peru), Melandrium yellow fleck *Bromovirus* (Hungary), Scrophularia mottle *Tymovirus*, Erysimum latent *Tymovirus* (Germany), cowpea mottle ? *Carmovirus* (Nigeria), broad bean necrosis ? *Furovirus* (Japan). There are viruses found to occur in two countries (e.g., melon necrotic spot *Carmovirus* in Japan and Holland), while some viruses have been detected in many countries (e.g., beet necrotic yellow vein *Furovirus* in c. 22 countries) or even all over the world: carnation etched ring *Caulimovirus*, Dahlia mosaic *Caulimovirus*, bean common mosaic *Potyvirus*, bean yellow mosaic *Potyvirus*, cucumber mosaic *Cucumovirus*, lettuce mosaic *Potyvirus*.

Harrison (1981) distinguished CULPAD (*cul*tivated *p*lant-*ad*apted) and WILPAD (*wil*d *p*lant-*ad*apted) viruses according to their occurrence in different plant communities. Among the CULPAD viruses were placed (e.g., potexviruses, tobamoviruses, ilarviruses) which he mainly found to occur in field crops and other cultivated plant communities. In the WILPAD virus group viruses equally occurring in wild and cultivated plant communities (e.g., cucumoviruses, potyviruses, plant rhabdoviruses, tymoviruses). It can thus be established that some viruses are particulary apt to survive in cultivated plants, while others have become specialized for wild plant communities in the first place. A CULPAD virus e.g., the potato X *Potexvirus*, has recently been detected in artichoke (*Cynara* sp., Chabbouh et al., 1990), or a WILPAD virus, the Arkansas isolate of eggplant mosaic *Tymovirus*, naturally occurs in eggplant *(Solanum melongena)* and is pathogenic not only to cultivated plants (e.g., *Lycopersicon esculentum*) but to wild plants as well (De Souza et al., 1990).

Among the natural host plants of viruses highly important are the weed plants (Duffus, 1971; Thresh, 1981). Among the about 250 weed species of world-wide importance the *Plantago* species have special significance. The potential weight of the *Plantago* species as natural hosts (virus reservoirs) of economically important viruses is greater than that of many other weeds, since many *Plantago* species are perennial (Hammond, 1982).

The attention of virologists has recently turned to the waters and the aquatic and uliginous plants (Tomlinson et al., 1983; Koenig, 1986; Piazzolla et al., 1986). Some aquatic plants may actually have pathogens in common with terrestrial relatives (MacClement and Richards, 1956; Zettler and Freeman, 1972). Recently some macrophytes have been examined for virus infection. In *Myriophyllum verticillatum* and *Shinnersia rivularis* cucumber mosaic

Cucumovirus was identified by comparative analysis of double-stranded ribonucleic acid (dsRNA), enzyme-linked immunosorbent assay (ELISA), immunoelectron microscopy (IEM), and by infection of test plants through sap inoculation (Horváth, 1988a; Proeseler et al., 1990; Horváth, 1991e).

Experimental host range

The mechanical inoculation is the application of fluids containing virus to the surface of plant leaves so that the virus can enter the cells. If the plant is local susceptible (mostly chlorotic-necrotic local lesions) may appear on the inoculated leaves, and systemic symptoms may occur in other, non-inoculated parts of the plant, or the infection is latent and symptoms do not occur at all.

The experimental host range of a virus includes those plants which can be infected with it under controlled conditions through human intervention. The term host range is used to mean the different plant species that may be infected by a virus. The natural hosts of a virus can be infected with it experimentally too, so the experimental host range of a virus generally is wider than the natural one. For example, the natural hosts of the four viruses belonging to the *Bromovirus* group (brome mosaic, broad bean mottle, cowpea chlorotic mottle, and Melandrium yellow fleck) only number 27 plants from the families Caryophyllaceae, Fabaceae, Gramineae and Rosaceae, while their experimental host range covers much more plants: Melandrium yellow fleck *Bromovirus* 219 species, brome mosaic *Bromovirus* 164 species, cowpea chlorotic mottle *Bromovirus* 39 species, broad bean mottle *Bromovirus* 24 species (Horváth et al., 1988b).

Major studies on the experimental host ranges of viruses published between 1938 and 1978 were discussed in chronological order in detail in an earlier paper (Horváth, 1977a). In a paper written by us on the host range of cucumber mosaic *Cucumovirus* we gave a full list of the experimental hosts of the virus. According to the data of the comprehensive study the experimental hosts of cucumber mosaic *Cucumovirus* include some 536 species, 106 genera of 53 families (Horváth, 1979, 1980a). On testing the susceptibility of 456 plant species to 24 viruses 1312 new susceptible experimental host-virus combinations were discovered (Horváth, 1983b). Of the experimental hosts 248 were first tested in virology. When investigating the experimental host range of Melandrium yellow fleck *Bromovirus*, as a new member of the *Bromovirus* group, we found 219 out of 228 plants belonging to 20 families and 69 genera to be susceptible (Horváth et al., 1988b). Tomato spotted wilt *Tospovirus* has also an extensive host range that includes 192 dicotyledonous species in 33 families and 8 monocotyledonous species in 5 families (Best, 1968; Cho et al., 1987).

Invaluable is the importance of the series of CMI/AAB Descriptions of Plant Viruses that have been appearing since 1970, and similarly important are those recent publications supplying information related with the host plants of legume viruses, ornamental viruses, forage legume viruses and tropical plant viruses (Boswell and Gibbs, 1983; Hammond and Lawson, 1983; Edwardson and Christie, 1986; Brunt et al., 1990).

Some plant families specially are experimental hosts for viruses, such as Cruciferae (Brassicaceae), Solanaceae, Leguminosae (Papilionaceae), Gramineae (Poaceae), while others, Geraniaceae, Gesneriaceae, Boraginaceae, Ranunculaceae, are not. Some viruses do not infect Solanaceae (e.g., carnation necrotic fleck *Closterovirus*) and there are ones mostly restricted to Solanaceae (e.g., Andean potato mottle *Comovirus*) (Inouye, 1974; Fribourg et al., 1979). It might be remarkable that the belladonna mottle *Tymovirus* known so far to infect solanaceous plants, has recently become pathogenic to members of families other than Solanaceae, namely: Chenopodiaceae, Compositae, Malvaceae, Scrophulariaceae (Horváth et al., 1976).

The viruses belonging to the various virus groups differ in host range too. Narrow, incomplete and largely restricted is the host range e.g., for the geminiviruses and sobemoviruses. Of the latter the blueberry shoestring *Sobemovirus* and the ginger chlorotic fleck ? *Sobemovirus* have each but a single virus (Ramsdell, 1979; Harrison, 1985; Thomas, 1988). Host range studies for diagnostic purposes are most useful for those infecting a relatively narrow host range (Matthews, 1991). Moderately experimental host range is possessed by the furoviruses of which the beet necrotic yellow vein *Furovirus* is more or less restricted to inoculated leaves of hosts, and the broad bean wilt *Fabavirus* which causes systemic symptoms. Wide host range belongs in general to the ilarviruses, the tombusviruses (most hosts are not invaved systemically, only locally), the tobamoviruses (in many plants the viruses do not become systemic). There are virus groups in which some members have a narrow, others a wide host range. Among the bromoviruses the Cassia yellow blotch has a narrow, the Melandrium yellow fleck a wide host range. The bromoviruses generally posses monocotyledonous hosts, but the type member of the virus group, the brome mosaic *Bromovirus* infects a number of species in other families (Lane, 1981). In the *Nepovirus* and *Tymovirus* group too, a part of the viruses have a narrow host range (olive latent ringspot *Nepovirus*, cacao yellow mosaic *Tymovirus*), while others have a wide host range (e.g., arabis mosaic *Nepovirus*, cherry leaf roll *Nepovirus*, tobacco ringspot *Nepovirus*, belladonna mottle *Tymovirus*).

In respect of the host range very interesting is the *Potyvirus* group (Edwardson, 1974b; Hollings and Brunt, 1981). Host range and symptoms have played a significant role in the description of potyviruses and their strains in the

past and even today they remain the first important criteria for recognition of strains. Some members of the virus group only have hosts in the family Poaceae (Gramineae): Agropyron mosaic *Potyvirus*, oat mosaic *Potyvirus*, wheat streak mosaic *Potyvirus*. We even know a *Potyvirus* with a single experimental host (*Oryza sativa*, the host of rice necrosis mosaic *Potyvirus*). It is also remarkable that the henbane mosaic *Potyvirus* while experimentally infecting tobacco and potato plants has not been reported to cause natural infection to these species (Govier and Plumb, 1972; Horváth et al., 1988a). The potyviruses have been reported to infect 2026 species in 556 genera of 81 families (Edwardson and Christie, 1991).

Non-host range

The non-host range of a plant virus consists of a collection of plants which when inoculated with the virus under experimental conditions do not show symptoms, and the virus cannot be detected in them, nor back-inoculated from them to known indicator plants susceptible to the virus.

The viruses greatly differ in the taxonomic breadth of their non-host range. It is very difficult to establish whether a plant is non-host for a virus, therefore many virologists are not impressed of non-hosts (Fulton, 1991; Lister, 1991). In spite of this, the non-hosts, as sources of resistance, play a very important role in breeding for resistance. Host-virus research has recently been paying increasing attention to seeking out virus resistant plants and detecting the occurrence of virus resistance genes. The importance lies in the fact that the chemotherapic and physicotherapic procedures used in vivo against viruses cannot be applied in practice, the conventional methods of plant protection are not efficient enough, are undesirable from the point of view of environment protection, toxicologically dangerous, and expensive. These facts suggest that the resistance of plants to viruses is of invaluable theoretical and practical importance (Cooper and Jones, 1983; Horváth, 1983c; Atabekov and Dorokhov, 1984; Horváth, 1985b, 1986a, 1986b, 1986c, 1986d; Fraser, 1986; Ross, 1986; Horváth, 1988d; Fraser, 1990).

Since the behaviour of non-hosts varies with the host-virus relations, they can be used in the separation of viruses, and in combination with other methods (e.g., serology, electron microscopy) in the diagnosis of viruses and virus groups as well (Horváth, 1993a).

Virus Identification, Diagnostic Principles and Strategies

Historically, virus detection and identification was based on symptom development. Considering that the disease symptoms are greatly influenced by various factors (e.g., temperature, daylength, light intensity, age of plants, virus strains, plant cultivars and accessions), symptomatology in itself is not sufficient for the exact identification of viruses (Fulton, 1964; Holmes, 1964; Roberts, 1964; Ross, 1964; Bos, 1967; Horváth, 1972c; Noordam, 1973; Gibbs and Harrison, 1976; Matthews, 1980; Schmelzer, 1980; Matthews, 1991). Viruses (e.g., alfalfa mosaic virus, cassava common mosaic *Potexvirus*, Viola mottle *Potexvirus* and several carlaviruses) causing latent infections in most species cannot even studied symptomatologically (Costa and Kitajima, 1972; Schmelzer et al., 1973; Lisa et al., 1982). In the course of testing the susceptibility of 456 plant species to 24 viruses Horváth (1983b) discovered 1312 new susceptible host-virus combinations 13 % of which were latent infections. Not infrequent are those host-virus relations of which recovery is characteristic (Horváth 1976; Jaspars and Bos, 1980; Stace-Smith, 1985). The symptomatological identification of viruses is made very difficult by the fact that many times more than one virus occur together in a plant, and the symptoms become either milder or more severe. During the 10-year period of 1957–1967 62 % of the plant introductions indexed positive for one or more viruses (Kahn et al., 1967). The Iris mild mosaic *Potyvirus* often occurs together with the Iris severe mosaic *Potyvirus*, and then the *Iris* plants are more severely affected, while when alone the virus usually causes mild symptoms (Brunt, 1986). Carnation necrotic fleck *Closterovirus*, nerine X *Potexvirus*, plantain X *Potexvirus*, parsnip mosaic *Potyvirus*, parsnip yellow fleck and parsnip mottle viruses as well as potato M *Carlavirus* and potato S *Carlavirus* are among the numerous viruses whose symptomatological diagnosis is difficult, if possible at all. Certain symptoms characteristic of host-virus relations, for example, "flower-break" (*Chrysanthemum* spp. – cucumber mosaic *Cucumovirus*; *Matthiola incana*, *Petunia hybrida* – turnip mosaic *Potyvirus*; *Tulipa* spp. – tulip breaking *Potyvirus* syn.: tulip mosaic virus); "fern-leaf" (*Lycopersicon esculentum* – cucumber mosaic *Cucumovirus* and tomato mosaic *Tobamovirus*); "enation" (*Pisum sativum* – pea enation mosaic virus) or "veinal necrosis" (*Nicotiana tabacum* – veinal necrosis strain of potato Y *Potyvirus*) represent useful information in virus diagnostics (Bos, 1967, 1970a; Horváth, 1972c; Horváth et al., 1975a; Francki et al., 1979; Schmelzer, 1980; Jaspars and Bos, 1980; Matthews, 1981; Bos, 1983a; Matthews, 1991).

While plant viruses cannot be grown on cell-free artificial media, many viruses can now be isolated from their hosts, and then characterized and recognized by their biological and physico-chemical properties. The purified

virus can be back-inoculated into healthy specimens of its original host plant to produce disease. So Koch's postulates (Bos, 1981) can now be applied in a redefined form to virus diseases to demonstrate that the pathogen is a virus or a virus is pathogenic. Bos (1983a) redefined Koch's postulates in the following way: *(1)* the virus must be concomitant with the disease, *(2)* it must be isolated from the diseased plant (separated from concomitants, multiplied in a propagation host, purified physico-chemically, identified for its intrinsic properties), *(3)* when inoculated into a healthy host plant it must produce the disease, and *(4)* the same virus must be demonstrated to occur in and must be re-isolated from the experimental host.

In the identification of viruses Hamilton et al. (1981) distinguished a diagnostic phase and a descriptive phase. Experiences obtained in either phase are very important in the correct identification of viruses. When using crude virus preparations or virus in situ the questions to be answered are: *(1)* reproduction of the disease using the isolated virus, *(2)* host range, *(3)* symptom expression, *(4)* mode of transmission, *(5)* type(s) of virus particles, *(6)* cytopathology by light and electron microscopy, *(7)* reactions with likely antisera, *(8)* in vitro properties and cross protection tests. When using purified preparations the important points are: *(1)* fine structure of virus particles in the electron microscope, (2) serology, *(3)* sedimentation properties, *(4)* analysis of the nucleic acid, *(5)* analysis of the coat protein. However, joint application of the mentioned techniques is only possible in well-equipped virus laboratories.

No matter what methods are used in identifying the viruses, a basic point of view is to determine the common and the different properties. As it is known, a virus group consists of viruses characterized by common properties. Common properties may be: *(1)* virus particles with the same structure and with closely similar composition, *(2)* similar genome strategies, *(3)* similar ecological life cycles, *(4)* natural and experimental host range possessed in common, *(5)* similar symptoms caused in host species, and *(6)* the same vector species (Boswell and Gibbs, 1983, 1986). Considering that the virus groups differ in the above properties, therefore when identifying unknown viruses from a diagnostical aspect three points must be taken into consideration. First thing to do is to determine the natural and experimental host range and the non-host range of the unknown virus, as well as the symptoms caused in the host plants, and the vector species (if there are any). The second step is to examine the structure and composition of the virus particles, the composition of the genome and the ecological life cycle of the virus. Thirdly, on the basis of the results and conclusions of the first and second point specific diagnostical examinations, such as serological tests, nucleic acid hybridization tests, or comparison of nucleotide or amino acid sequences must be carried out. Realization of the above diagnostical

principles or diagnostical strategy is only possible in well-equipped laboratories. The natural and experimental host range of an unknown virus, and the symptoms and non-hosts supply very useful information as to whether or not the virus is an earlier described member of some virus group, therefore the host range is of great value for those who have no up-to-date laboratory facilities at hand and for technical reasons are not able to carry out the second and third points of diagnostic strategy. Although the diagnostic method based on host range studies is criticized by many virologists (Hamilton et al., 1981; Matthews, 1991), yet, it must be established that the most important "early" clues to an unknown virus are the natural host range, i.e. the plants in which it occurs, the experimental host range i.e. the plants suitable for comparison with test plants of already known viruses and indicative of potential hosts, as well as the symptoms induced in the test plants (Horváth, 1993a). When the host ranges of two or more unknown viruses are compared and found to be identical (uniform), further, the symptoms appearing in the hosts are the same, then it can be supposed that the unknown viruses are isolates of a certain virus. If both the host range and the symptoms are identical, but there is a difference in virulence between the viruses, then the virus concerned is supposed to be a strain of a certain virus. Here it must be emphasized, however, that the hosts range of a given virus, and the symptoms visible on susceptible plants are so-called "variable features" that may be changed by a single nucleotide mutation, or by the ecological conditions, or even by the genotypes of the test plants. It is known that the accessions or varieties of the same species may give different responses to the same virus (Kassanis and Selman, 1947; Horváth, 1968a, 1968b; De Bokx, 1970; Van der Want et al., 1975; Hoekstra and Seidewitz, 1987; Van Dijk et al., 1987). The identification of the virus is made difficult by the fact that the reactions of the test plants to the virus depend in a great measure on the plant in which the virus is propagated. Van der Meer et al. (1980) found that the *Chenopodium quinoa* never showed symptoms when inoculated with sap from *Lonicera* species infected by Lonicera latent *Carlavirus*, even though the inocula induced a large number of lesions in *Nicotiana megalosiphon*. However, when *Chenopodium quinoa* plants were inoculated with sap from infected *Nicotiana megalosiphon* or *N. clevelandii* a limited number of small lesions developed on the inoculated leaves. With further successive transmissions from *Chenopodium quinoa* to *C. quinoa* many more lesions were obtained and in these tests some isolates also caused systemic symptoms. Dijkstra (1983) stated that when the inoculum came from infected *Nicotiana rustica* in which the tobacco streak *Ilarvirus* had been propagated continuously, the symptoms on the test plants were quite different from those obtained when *N. clevelandii* was used for propagation of the virus, even suggesting the possibility of infection by another virus. Host passage effects are thought to be based on selection of mutants

(Yarwood, 1979). Considering the great intraspecific differences in sensitivity between test plants, only such plant species as generally known and reliable in virus identification should be used.

The role of plants in virus diagnostics must not thus be overestimated, but it would be a similar mistake to deny their importance. Besides the secondary criteria playing a role in identifying and unknown virus, such as host range, test plants, symptoms, no further diagnostic compromissum is possible. Such traditional tests as e.g., the thermal inactivation point, in vitro longevity or dilution end-point of viruses, which are connected with the infectivity and stability of the virus-containing sap, have limited value, or are unserviceable in virus diagnostics and unsuitable for characterizing the viruses (Francki, 1980; Bock, 1982). Boswell and Gibbs (1983) point out that e.g., the 55 legume viruses whose in vitro longevity ranges from 1 to 24 days may belong to 15 virus groups, or the 66 legume viruses with thermal inactivation points between 50 and 70 °C may belong to 17 virus groups. A further example to add to those listed: between the in vitro longevity of cowpea aphid-borne mosaic *Potyvirus* and watermelon mosaic virus 2 *Potyvirus* theoretically a 144-day difference is even possible. It must not be forgotten that beside other controlling methods these so-called "traditional" methods make it possible to separate mixtures of viruses, and indicate potentially useful clarification steps in purification procedures (Horváth, 1967; Purcifull et al., 1984b).

Cross protection tests, similarly to the methods based on in vitro properties, cannot be reliably used for diagnostic and taxonomic purposes, even if there are examples of good correlation between degrees of cross protection and serological relationships, there are also many cases of incomplete or not detectable cross protection between virus strains as well as examples of cross protection between obviously unrelated viruses (Matthews, 1949; Schmelzer et al., 1960; Köhler, 1962; Kassanis, 1963; Horváth, 1969d; Hamilton, 1980; Hamilton et al., 1981). According to Dodds (1982) different mechanisms are probably responsible for cross protection between different groups of viruses.

Recently Sarkar (1986) placed the methods of phytopathogenic virus diagnosis in four basic groups: *(1)* the method based on the biological activity of the virus, *(2)* that based on the physico-chemical properties of the virus, *(3)* chemical analysis of infected plant material, and *(4)* the method based on the immunological properties of the virus. Out of these methods the one based on the biological activity of the viruses (symptoms on diseased plants, indicator plants, changes at cellular level under microscope) has remained invariably important with the application of the up-to-date techniques, and helps in the diagnosis of viruses. It must be emphasized, that the biological techniques are the only ones which detect infectious entities.

Host or Test Plants in Diagnosis

The detection of viruses using test plants developed simultaneously with the plant virology. Mayer (1886) was the first to prove that the tobacco mosaic disease he examined was transmissible with the juice extract of the diseased plant to healthy tobacco plants. This discovery, with the famous test plant experiments of Johnson (1925), Holmes (1929) and Smith (1931), then with the host range studies of Price (1940) and Holmes (1946) made the way for attaining results of invaluable scientific and practical importance. In the past three-quarters of a century thousands of researchers examined the test plants of viruses and described standard test plants which have not even lost importance up to the present day. The ideal host gives an immediate and characteristic response to sap inoculation, preferably by showing local lesions on the inoculated leaves (Smith, 1968). True, though, the use of test plants for detecting and diagnosing viruses is both cumbrous and expensive, since growing the test plants requires greenhouse space, time and energy, yet, it is very important if only because in certain cases even the serological methods cannot be as reliable as the use of test plants. Serology has its limitations, for example, a precipitation test requires minimum 0.5 μg/ml of virus (Ball, 1974; Van Regenmortel, 1982). Some viruses, e.g., luteoviruses, rhabdoviruses, and most viruses of woody hosts are present at concentrations below the limit of detection by serological tests, which require visualization of the antigen-antibody complex (Martin, 1985). For several years more sensitive, reliable and quick detection procedures have undoubtedly been developed for the detection of plant viruses present in low concentrations in the host tissue (e.g. enzyme-linked immunosorbent assay, immune electron microscopy, and complementary DNA probes) but these methods have not been widely introduced and do not exclude the necessity of using test plants.

The test plants have, above all, three important functions: *(1)* testing for possible mixture by inoculation of a series of specific indicator plants, *(2)* separation of mixtures by using test plants or single local lesion passages, and *(3)* original host as test plant must be reinoculated, then observed for original symptoms of the disease.

Among the test plants there are diagnostic, propagation, assay, purification, filter and bridge hosts depending on what purposes they are most suitable for. With respect to viral host ranges, some plants appear to behave anomalously toward numerous viruses (anomalous hosts) (see Dawson and Hilf, 1992).

Another viewpoint not to be neglected either is the stage of development at which the plants are most suitable for inoculation. According to Pawley (1973) *Chenopodium amaranticolor*, C. *quinoa* and *Nicotiana clevelandii* are in that state with 10 leaves, *N. glutinosa* with 7 leaves, *N. tabacum* and *Gomphrena*

globosa with 4 leaves. With cucumber *(Cucumis sativus)*, squash *(Cucurbita pepo)* cotydedons, with bean *(Phaseolus vulgaris)* and cowpea *(Vigna sinensis)* primary leaves are usually inoculated, because older leaves of the plants are less or not susceptible to viruses. For example, in young cucumber inoculated with cucumber mosaic *Cucumovirus* the yield is likely to be high in the cotyledons and lower in the first-formed, systemically infected true leaves (Yarwood, 1971). Very young primary bean leaves and very young cucumber cotyledons are resistant to several viruses. These plants do not reach their optimum susceptibility until about 10 days from seeding, and become resistant after about 15 days from seeding. Tobacco leaves remain susceptible much longer (Yarwood, 1971). Further useful data are to be found in works by Noordam (1973) and Hill (1984) concerning the age (days from sowing at 20 °C) and proper stage for use of test plants.

The virus susceptibility of plants is influenced also by the leaf position (Horváth, 1969a, b) as well by the critical size of leaf at the time or infection above which mosaic disease does not develop (Matthews, 1980).

Also, the plants show great differences in the length of day required for their development. For example, *Chenopodium quinoa*, *Nicotiana clevelandii* and *Pisum sativum* are long-day, *C. album* and *Glycine max* are short-day plants, while *Cucumis sativus*, *Phaseolus vulgaris* and *Vicia faba* are indifferent to the length of day. *Chenopodium amaranticolor* demands little of sunshine, therefore in the winter months it requires supplementary lighting to prevent flowering. Supplementary lighting (400 W mercury lamps giving 3800 lux for *Nicotiana* species; and fluorescent tubular lamps, warm white plus daylight, giving c. 750 lux at pol level, for *Chenopodium* spp. and *Gomphrena globosa*) is necessary from the end of September to March (Pawley, 1973; Hill, 1984). The optimum temperatures for raising the plants and from the point of view of virus susceptibility are 18–21 °C in winter and 21–24 °C in summer. It must be noted, however, that test plants exposed to 36 °C 1–2 days before inoculation are generally more susceptible to infection, similar treatment after inoculation decreases the efficiency of infection (Kassanis, 1978).

Harrison and Jones (1971) studied the effects of light and temperature on the development of symptoms on tobacco leaves inoculated with potato mop-top *Furovirus*. They found necrotic lesions developing and the rate of virus accumulation increasing when inoculated plants were transferred from 22 °C in light (4320 lux) to 14 °C in dark, but no lesions appearing when the order of the treatment was reversed. Thus, the process of lesion formation is composed of an early phase requiring light and a subsequent phase in which low temperatures are required. We examined the role played by temperature and photoperiod in the formation of local lesions on leaf discs of *Nicotiana tabacum* cv. Xanthi-nc

infected with tobacco mosaic *Tobamovirus*. According to the result of the experiment temperature has a decisive effect on the development of local lesions. In the course of various photoperiod treatments it was found that at low temperatures the appearance of local lesions was promoted by light of longer duration, while at higher temperatures by light of shorter duration (Horváth and Pocsai, 1972).

Walker et al. (1985) studied the flowering, seed production and seed vigour of *Nicotiana* and *Chenopodium* species infected with spinach latent *Ilarvirus* and arabis mosaic *Nepovirus*. They found that infection frequently caused mother plants to produce flowers earlier than healthy plants, and arabis mosaic *Nepovirus* induced *Chenopodium amaranticolor*, which normally flowers only under short-day conditions, to flower is long-days. In some cases the viability of seeds from infected plants was slightly reduced.

From the point of view of virus transmission important role is played in some plants (e.g., *Capsicum*, *Chenopodium*, *Emex*, *Dahlia*, *Dianthus*, *Phytolacca*) by the inhibitors which make virus transmission from one plant to another difficult, or in many cases prevent it (Fulton, 1964; Horváth, 1972c; Gibbs and Harrison, 1976; Hansen, 1989). In a broad sense a virus inhibitor is an agent that interferes with the pathogenicity of a virus, as shown by a lack of response in the test plants (Cooper and Jones, 1983).

Between 1970 and 1988 some 339 plant viruses (or viroids) and virus groups were described (some of them in revised edition too) in the series CMI/AAB Descriptions of Plant Viruses. This series has been welcomed and widely used by plant virologists all over the world. In this series 446 plants belonging to 43 families and 163 genera are described as suggested test plants of 232 mechanically transmissible viruses from 24 virus groups, and 24 mechanically transmissible but unclassified viruses, described between 1970 and 1988. There is a considerable number of plants which are diagnostic, propagation or assay hosts for a single virus each (e.g., *Adansonia digitata*, *Apium graveolens* cv. Utah 10B, *Amaranthus retroflexus*). The most frequently used 15 species of them belong to 9 genera and 6 families (Table 1). The species *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana clevelandii*, *Phaseolus vulgaris* and *N. glutinosa* are of outstanding importance among them. It is remarkable that the frequency of *Chenopodium quinoa* and *C. amaranticolor* as diagnostic and assay species is almost the same, with the essential difference that *C. quinoa* as a propagation host is much more important than *C. amaranticolor*.

Table 1

Number of viruses known to infect some species of plants[1]

Plant species	Diagnostic species	Propagation species	Assay species	Total
Chenopodium quinoa	103	46	88	237
Chenopodium amaranticolor	103	6	82	191
Nicotiana clevelandii	31	57	8	96
Phaseolus vulgaris	36	16	22	74
Nicotiana glutinosa	36	17	11	64
Gomphrena globosa	24	8	17	49
Cucumis sativus	29	10	9	48
Datura stramonium	26	11	9	46
Pisum sativum	23	12	3	38
Nicotiana tabacum	17	12	7	36
Chenopodium murale	21	2	11	34
Tetragonia expansa	23	5	5	33
Nicotiana tabacum cv. White Burley	26	4	3	33
Nicotiana tabacum cv. Xanthi-nc	15	7	8	30
Vigna sinensis	15	3	9	27

[1]Information from CMI/AAB Descriptions of Plant Viruses (1970–1988)

Hosts and non-hosts in virus separation

Some plants are susceptible to some viruses and immune to others; this makes them suitable for separating viruses occurring in mixed infections and producing pure virus cultures with them (Horváth, 1967, 1977b). The virus separation is based on the fact that the plants are susceptible to certain viruses and immune to others, or locally susceptible to some viruses and systemically to others. Virus separation with susceptible and insusceptible plants has long since been known. Johnson (1925), then Köhler (1933) were the first to report the separability of potato X *Potexvirus* and potato Y *Potyvirus* in *Datura stramonium*, as this plant is susceptible to potato X and immune to potato Y. The local susceptibility of *Chenopodium murale* to potato X *Potexvirus* and its immunity to potato Y *Potyvirus* similarly make it possible to separate the two viruses (Horváth, 1969c).

Smith (1931) introduced the term "filter plant", which has since been generally used in virology. For some decades numerous studies have been published on the separation of viruses (Horváth, 1967, 1977b). In the course of studying the new experimental hosts and non-hosts of 24 plant viruses (12 of which are potato pathogenic viruses) from 9 virus groups we found 246 species of 58 genera from 21 families to be immune. Of the immune plants 111 were first tested in plant virology. For 246 plant species 664 incompatible relations with

viruses were pointed out. Among the viruses and plants 9958 combinations of virus separation were established (Horváth, 1983b). With only the data on potato viruses taken into consideration, 165 species of 38 genera from 15 families were found for the first time to be immune. Among immune species we established 337 new incompatible combinations. For the 12 potato viruses tested we found 3846 combinations that could be used to separate those viruses (Horváth, 1985c).

Hosts, viruses and their relationship

Numerous good indicators have been found in various plant families and genera. Data on less known host-virus relations, and on those recently detected but not generally known as yet may give a further impetus to research. We therefore give a survey of some plants and of their behaviour to viruses, which can be successfully used in virus diagnostics.

1. Amaranthus species

Many of the more than thousand annual, perennial, herbaceous, woody or shrub species from about 60 genera in the family Amaranthaceae occurring all over the world though mostly in tropical and subtropical regions play a highly important role in plant virology.

The importance of the *Amaranthus* species lies, on the one hand, in their role as test plants in detecting certain viruses. On the other hand, as natural hosts they have a part in the survival and circulation in nature of certain viruses. *Amaranthus hybridus*, *A. spinosus* and *A. viridis* are important weed hosts of tomato spotted wilt *Tospovirus* and western flower thrips *(Frankliniella occidentalis)* virus vector of vegetable-growing regions of Hawaii (Cho et al., 1987, 1989).

Particularly important are among them the perennial *Amaranthus bouchonii*, *A. deflexus*, *A. deflexus* var. rufescens and *A. retroflexus* (Schmelzer and Molnár, 1975; Lovisolo and Lisa, 1976; Horváth, 1983b, 1991a). The strong affinity between *Amaranthus* species and viruses is supported by the fact that 12 species are known to be natural virus hosts (Horváth, 1991b). The list is not complete, because in several known natural host-virus relations the species of the plant and/or the virus have not been identified (Phatak, 1965; Govindaswamy et al., 1967). According to our knowledge 63 *Amaranthus* species synonyms for 4 of them are: *angustifolius* = *graecizans*, *ascendens* = *blitum*, *caudatus* = *cruentus*, *angustifolus* = *tricolor*) have proved so far susceptible to 121 viruses (Horváth, 1991b). The number of host-virus relations exceeds 470. Eight of the 121 viruses (e.g., Amaranthus mottle, Amaranthus mosaic, Tropaeolom mosaic viruses) are not

exactly identified, 5 of them are known as strains of well-known viruses (e.g., Physalis shoestring mosaic virus is a strain of tobacco mosaic *Tobamovirus*), while cabbage black ring virus and red currant ringspot virus are synonyms for turnip mosaic *Potyvirus* and raspberry ringspot *Nepovirus*, respectively. The *Amaranthus* species differ in virus susceptibility. *Amaranthus caudatus* is susceptible to 83 viruses, while *A. aspera*, *A. aurora*, *A. blitoides* are susceptible to one virus each (beet yellows *Closterovirus,* Prunus necrotic ringspot *Ilarvirus* syn.: plum line pattern virus, and alfalfa mosaic virus). Species important for virus epidemiology and virus ecology are: *Amaranthus retroflexus* with susceptibility to 40 viruses, and *A. deflexus* and *A. deflexus* var. rufescens, as species susceptible to 7 viruses, among them such important ones as cucumber mosaic *Cucumovirus* and beet yellows *Closterovirus*. *Amaranthus bouchonii*, a species recently showing wide geographical distribution is susceptible to some 15 viruses (Horváth, 1991b). Viruses with the largest number of *Amaranthus* hosts known are: cucumber mosaic *Cucumovirus* (42), potato X *Potexvirus* (42), alfalfa mosaic virus (39), tobacco mosaic *Tobamovirus* (35), potato Y *Potyvirus* (34). Some viruses e.g., Arracacha A *Nepovirus*, carnation Italian ringspot *Tombusvirus (A. caudatus)*; papaya ringspot *Potyvirus (A. dubius)*; Wisteria vein mosaic virus *(A. hybridus)* have been known so far to have a single *Amaranthus* host each. In respect of the number of susceptible *Amaranthus* species the following order can be set up for the virus groups: *Potexvirus* (44), *Cucumovirus* and *Potyvirus* (42 each), *Tobamovirus* (40), alfalfa mosaic virus (38). The wide and unknown host-virus relations suggest that the virologically so far unknown 40 *Amaranthus* species also play an important role in the distribution and ecology of viruses (Horváth, 1991c). To detect the so far unknown host-virus relations is a research task to be solved in the future.

2. *Capsicum species*

As far as we know at present, the genus *Capsicum* includes 21 wild species and 9 domesticated or cultivated species (Horváth, 1986a). The *Capsicum* species show strong affinity to plant viruses. At present 13 *Capsicum* species and 9 varieties are known for virus susceptibility. The *Capsicum*-virus relations include some 43 viruses (Horváth, 1986a). In spite of the wide range of the host-virus relations the *Capsicum* species are not frequently used as test plants; the reason for this is the strong inhibitor of the pepper which makes the virus transmission difficult (Fischer and Nienhaus, 1973; Hansen, 1989). In spite of this the *Capsicum annuum* is an indispensable test plant with some viruses (e.g., alfalfa mosaic virus, potato aucuba mosaic *Potexvirus*, tobacco etch *Potyvirus*; Horváth, 1964; Purcifull and Hiebert, 1982). On the basis of the latest research

results 144 susceptible host-virus relations have been established for some 21 *Capsicum* species (8 of them are new in the virological literature) and 14 viruses (Horváth, 1986g). The virological importance of the pepper lies in the first place in the fact that for some years and increasing number of new viruses pathogenic to pepper have been found to occur, and therefore breeding for resistance to pepper viruses has become imperative (Lana et al., 1975; Lee et al., 1975; Van Den Berkmortel, 1977; Boukema, 1980; Nagai et al., 1981; Rast, 1982; Csilléry et al., 1983; Wetter et al., 1984; Horváth, 1986a, 1986b; Fletcher et al., 1987; Wetter et al., 1987; Pares, 1988; Rast, 1988; Pirone, 1989; Tóbiás et al., 1989; Green and Kim, 1991).

Of the recent discoveries remarkable are a mechanically and typical whitefly-transmitted bipartite genome *Geminivirus* isolated from pepper (*Capsicum annuum*; Stenger et al., 1990), and a *Luteovirus*, probably the *Capsicum* strain of potato leafroll *Luteovirus* (Gunn and Pares, 1990).

3. Chenopodium species

The genus *Chenopodium* which is relatively rich in species is very important not only from an economic but also from a virological point of view (Waterworth and Povish, 1976). Some 30 *Chenopodium* species are known to be susceptible to viruses. There are, however, essential differences in virus susceptibility between the *Chenopodium* species. In the series CMI/AAB Descriptions of Plant Viruses nine *Chenopodium* species are described as diagnostic, propagation, assay and purification hosts (Table 2). The highly important role played by the *Chenopodium* species in plant virology is emphasized by Edwardson (1974a, 1974b) and Edwardson and Christie (1991) who gives account of 141 viruses in the *Potyvirus* group as pathogenic to plants belonging to the family Chenopodiaceae, 68 viruses of which are pathogenic to *Chenopodium amaranticolor* and 68 to *C. quinoa* plants. The brome mosaic *Bromovirus* is one of the few grass viruses that produce local lesions in *Chenopodium amaranticolor*, *C. hybridum* and *C. quinoa* (Lane, 1977), but there are viruses (e.g., alfalfa latent *Carlavirus*) which do not infect these *Chenopodium* species (Boswell and Gibbs, 1983). *Chenopodium album, C. ambrosioides, C. capitatum, C. foetidum, C. foliosum* and *C. hybridum* differ from the three *Chenopodium* species of outstanding importance (*amaranticolor*, *murale* and *quinoa*, see Table 2) mainly by their *(1)* lower susceptibility to viruses, *(2)* poor germinability, *(3)* irregular lesion formation and *(4)* small leaf area.

Table 2

The role of *Chenopodium* species as different hosts in virology[1]

Plant species	Diagnosis	Propagation	Assay	Purification	Frequency
Chenopodium album	7	0	4	0	11
C. amaranticolor	103	6	82	92	283
C. ambrosioides	1	0	0	0	1
C. capitatum	6	1	0	0	7
C. foetidum	1	0	2	0	3
C. foliosum	1	0	1	0	2
C. hybridum	2	0	3	0	5
C. murale	21	2	11	0	34
C. quinoa	103	46	88	34	271

[1]Information from CMI/AAB Descriptions of Plant Viruses (1970–1988)

The *Chenopodium* species play an important role not only in the isolation and diagnosis of viruses, they can be used for virus separation as well. In the course of our investigations we found about 25 possible separation combinations for 16 *Chenopodium* species and 7 viruses, in addition to the so far known 71 separation combinations (Horváth, 1976).

In recent years numerous publications have appeared on the virus susceptibility of *Chenopodium amaranticolor*. The importance of this species mainly lies in the fact that is more inclined than *C. quinoa* to forming local lesions. This fact perhaps explains why it is widely used as an assay host. In the case of certain viruses (e.g., wineberry latent *Potexvirus*) the advantage of *Chenopodium amaranticolor* over *C. quinoa* is that the virus yields from the former are generally greater than those from the latter (Jones et al., 1990). As a disadvantage, on the other hand, its leaves readily crumble, the plant often becomes contaminated with other viruses (e.g., sowbane mosaic *Sobemovirus*), and has a high inhibitor content (Hollings, 1956; Kado, 1966). *Chenopodium hybridum* also has recently been found to be naturally infected by sowbane mosaic *Sobemovirus* (Horváth et al., 1990, 1993). Together with *Chenopodium amaranticolor*, *C. album* and *C. quinoa* it is a natural host for alfalfa mosaic virus (Hull, 1969). In some publications there are data suggesting the virus resistance of *Chenopodium amaranticolor*. According to Mayhew et al. (1984) the plant is resistant to the corky ringspot strain of tomato mosaic *Tobamovirus*. Experimental results indicate that there are differences in the tobacco mosaic *Tobamovirus* reproduction inhibiting vigour of the inhibitor to be found in various leaf sequences (leaf position) of *Chenopodium amaranticolor* (Horváth, 1969b). The inhibitors contained in older leaves of the plants were found to inhibit virus reproduction more vigorously than those present in younger leaves.

The virological importance of *Chenopodium quinoa* also deserves attention (see Table 2). This plant is the only systemic, and local and systemic host for cactus X *Potexvirus* and Chenopodium necrosis virus, respectively (Bercks, 1971; Tomlinson et al., 1983), and the most sensitive indicator of viruses of woody plants (Schmelzer, 1971; Németh, 1986). It contains relatively less inhibitor, so its importance is very great (Schmelzer, 1961; Hollings, 1966). *Chenopodium quinoa* is mainly suitable for detecting the filamentous viruses, while with *C. amaranticolor* first of all the polyhedral viruses can be detected. It should be underlined that *Chenopodium quinoa* is suitable for detecting viruses present in low concentrations in the plants. *Chenopodium quinoa* and *C. amaranticolor* are useful for routine assay in the diagnostics of ornamental viruses. For example, carnation ringspot *Dianthovirus* causes necrotic local lesions in *Chenopodium quinoa* plants 2–4 days after the inoculation. It is also worth mentioning in this place that e.g., with the rhabdoviruses *Chenopodium amaranticolor* and *C. quinoa* yield local lesions only after 2–3 weeks. According to Hammond and Lawson (1983), of 25 bioassay hosts listed with 47 ornamental viruses *Chenopodium quinoa* is assay host for 19, *Chenopodium amaranticolor* for 12 viruses. For the sake of comparison it should be noted that plants as important in virology as *Cucumis sativus*, *Datura stramonium*, *Gomphrena globosa*, *Nicotiana tabacum* and *Tetragonia tetragonoides* are only known as assay plants for a single ornamental virus each. It is remarkable that *Chenopodium quinoa* is suitable for differentiating various strains of certain viruses. For example, *Chenopodium quinoa* is a suitable assay host of arabis mosaic *Nepovirus*. The rose isolate of the virus may cause no local lesions with systemic small chlorotic interveinal lesions that fade. Isolates from other hosts (e.g., lilies, tulip, narcissus) may produce necrotic local lesions and top necrosis (Thomas, 1983). *Chenopodium quinoa* plays a highly important role in studies of viruses in water and forest ecosystems too (Koenig, 1986; Büttner and Nienhaus, 1989a, b; Nienhaus et al., 1990).

Important role is played in the plant virology by *Chenopodium murale* too, which is known to be suitable for separating the often jointly occurring potato X *Potexvirus* and potato Y *Potyvirus* (Horváth, 1969c, 1983b). *Chenopodium murale* and *C. album* are important weed hosts of tomato spotted wilt *Tospovirus* and western flower thrips *(Frankliniella occidentalis)* vector (Cho et al., 1987, 1989).

The studies on the host-virus relations of *Chenopodium* species have largely contributed to our virological knowledge. It was through them, for example, that 42 compatible and 6 incompatible relations to viruses of 20 *Chenopodium* species became known (Horváth, 1983b). It is also a significant achievement that indexing the potato viruses with *Chenopodium* species has

become possible (Horváth, 1964; De Bokx, 1970; Horváth and De Bokx, 1972; Hiruki, 1975; Proll et al., 1978; Kenten and Jones, 1979; Horváth, 1985c). Susceptibility and immunity to 27 viruses of 8 virologically unknown or hardly known *Chenopodium* species have been revealed in the course of recent investigations (Horváth, 1986e). Between *Chenopodium* species and viruses 123 new host-virus relations (99 local and 24 local and systemic) have been detected.

4. Cucumis species

To the African and Asian origin *Cucumis* genus some 40 species belong. From an economic point of view *Cucumis anguria*, *C. melo* and *C. sativus* are the most important species. Out of them *Cucumis sativus* is the one best known for virus susceptibility. To our knowledge *Cucumis sativus* is susceptible to more than 60 viruses (Thornberry, 1966; Schmelzer and Wolf, 1977; Horváth, 1985b). The cucumber *(Cucumis sativus)* is very important in the isolation and identification of viruses, and as a so-called "bridge plant" plays an outstanding role in transmitting viruses that owing to the high inhibitor content of the donor plants can hardly, if at all, transmitted to other plants. *Cucumis sativus* is particularly susceptible to the members of certain virus groups. Nepoviruses generally cause local and systemic infection to the cucumber plants. The latter can be used also to separate certain viruses. The clover yellow mosaic *Potexvirus* infects the cucumber locally and systemically, while the white clover mosaic *Potexvirus* is apathogenic to it. Of the viruses belonging to the *Cucumovirus* group cucumber mosaic *Cucumovirus* causes systemic infection to cucumber, which, though, is not the best test plant of the virus, while the type strain of tomato aspermy *Cucumovirus* does not infect it. *Cucumis sativus* is therefore a separator or filter plant suitable for separating the two viruses (Hollings and Stone, 1971).

5. Cucurbita species

Among some 27 *Cucurbita* species (22 of which are not cultivated) *Cucurbita maxima*, *C. mixta*, *C. moschata* and *C. pepo* are of the greatest economic importance. Due to the research work of Provvidenti et al. (1978) fourteen wild *Cucurbita* species have been found to be susceptible or resistant, respectively, to viruses.

Studies on the virus susceptibility of 27 *Cucurbita* species and accessions (22 of which are test plants new in virology) revealed 60 new local and systemic relationships. Most of the findings are related with cucumber mosaic *Cucumovirus*, watermelon mosaic virus 2 *Potyvirus* and cucumber green mottle mosaic *Tobamovirus* (Horváth, 1985d).

6. Datura species

Datura species belonging to the family Solanaceae are popular test plants. Some 25 species are known in virology, but major importance is only attached to three of them *(Datura metel, D. meteloides, D. stramonium).* The best known of them is *Datura stramonium*, which is diagnostic host for 26, propagation host for 11 and assay host for 9 viruses (see Table 1). Also, it plays an important role in separating certain viruses (Horváth, 1967, 1983b, 1985c). *Datura metel*, whose leaves are less sensitive to mechanical inoculation, is important mainly in the identification of potato viruses (e.g., potato X *Potexvirus*, potato Y *Potyvirus*, potato M *Carlavirus*), but can be successfully used for the diagnosis of alfalfa mosaic virus and cucumber mosaic *Cucumovirus* too. *Datura meteloides* is not only an experimental host for several viruses, but, according to Nelson et al. (1982), it is an overwintering host of pepper mottle *Potyvirus*. The less known species include *Datura fastuosa*, which has lately been discovered as host of Datura distortion mosaic virus (Mali et al., 1985). Further species belonging to the less known ones are: *Datura sanguinea*, a host of tobacco mosaic *Tobamovirus* and belladonna mottle *Tymovirus* (Schmelzer and Wolf, 1971; Horváth, 1981), and several species described by Horváth (1981) and Paul et al. (1968), such as *Datura carolinianum, D. ceratocaula, D. chlorantha, D. discolor, D. leichardtii, D. quercifolia* and *D. rosei*, which are host for belladonna mottle *Tymovirus*.

According to our present knowledge there are some 120 susceptible host-virus relations in the 25 *Datura* species (Horváth, 1981, 1983b, 1985c). *Datura fastuosa* and *D. innoxia* have recently proved good experimental hosts for henbane mosaic *Potyvirus*, which naturally occurs in the species *Datura inermis* and *D. stramonium* (Horváth et al., 1988a).

7. Gomphrena species

Among the more than 100 species of the genus *Gomphrena* (family: Amaranthaceae) there was until recently a single species, *Gomphrena globosa*, for which data were published concerning its behaviour to viruses. According to the first experiment data (Wilkinson and Blodgett, 1948) more than forty years back, the *Gomphrena* plant is an excellent quantitative and qualitative indicator of the potato X *Potexvirus*, potato M *Carlavirus* and Melandrium yellow fleck *Bromovirus*. Recently it has been found unsuitable for detecting the so-called resistance-breaking strain (HB strain) of potato X *Potexvirus*, since it does not respond with local lesions to the inoculation (Moreira et al., 1980). To our present knowledge the species *Gomphrena globosa* is suitable for detecting some 100 viruses.

However, *Gomphrena* is not only an important test plant, it is also a natural host of some viruses (e.g., Gomphrena virus) (Kitajima and Costa, 1966). The importance of *Gomphrena* is increased by the fact that unlike many plants (e.g., *Chenopodium* spp.) it possesses few inhibitors, and is significant as a virus "source plant" and "bridge plant" as well. It is suitable for the detection of some 24 of the potyviruses (Edwardson, 1974a, 1974b). Beside its qualitative and quantitative properties it plays an important role in the separation of viruses. *Gomphrena globosa* has proved suitable for the separations of 22 viruses in 240 combinations (Horváth, 1976).

Among the new *Gomphrena* species *G. decumbens, G. diffusa* and *G. dispersa* deserve attention. *Gomphrena decumbens* and *G. diffusa* are susceptible to 22 viruses (Horváth, 1976, 1983b, 1985c). *Gomphrena dispersa* responded with local symptoms to 7 viruses, with local and systemic symptoms to 15 viruses, and was immune to 7 viruses (Table 3). *Gomphrena dispersa*, a plant with small leaves, tending to branch off and developing creeping shoots, while less suitable than *Gomphrena globosa* for virus diagnosis, deserves attention as a prognostic host.

Table 3

Gomphrena dispersa as a host and non-host plant of different viruses

Local host for	Local and systemic host for	Non-host for
Bean yellow mosaic *Potyvirus*	Alfalfa mosaic virus[1]	Bean common mosaic *Potyvirus*
Lettuce mosaic *Potyvirus*	Arabis mosaic *Nepovirus*	Cauliflower mosaic *Caulimovirus*
Potato M *Carlavirus*	Belladonna mottle *Tymovirus*	Celery mosaic *Potyvirus*
Potato S *Carlavirus*	Broad bean wilt *Fabavirus*	Cherry leaf roll *Nepovirus*
Potato X *Potexvirus*	Cucumber mosaic *Cucumovirus*	Cucumber green mottle mosaic *Tobamovirus*
Tobacco necrosis *Necrovirus*	Melandrium yellow fleck *Bromovirus*	Potato aucuba mosaic *Potexvirus*
Watermelon mosaic virus 2 *Potyvirus*	Potato Y *Potyvirus*	Turnip yellow mosaic *Tymovirus*
	Radish mosaic *Comovirus*	
	Tobacco mosaic *Tobamovirus*	
	Tobacco rattle *Tobravirus*	
	Tobacco ringspot *Nepovirus*	
	Tomato aspermy *Cucumovirus*	
	Tomato mosaic *Tobamovirus*	
	Tomato ringspot *Nepovirus*	
	Turnip mosaic *Potyvirus*	

[1]Monotypic group with no approved group name (see Hull et al., 1991).

8. Lycium species

Since the investigations by Dennis (1938) *Lycium barbarum* has been known to be locally susceptible to various strains of potato Y *Potyvirus*. Numerous studies have since been made of the virus susceptibility of various *Lycium* species. The intensity of research has increased with the realization that intact and detached leaves of different *Lycium* species as assay hosts can be successfully used for the quick identification of certain viruses (Chin, 1972).

The investigations were positively influenced by those examinations in the course of which some *Lycium* species turned out to be possible natural hosts of certain viruses (Schmelzer and Schmidt, 1968; Schmelzer, 1969; Plese and Miličić, 1974; Kröll, 1975; Horváth, 1983b).

A survey of the results attained so far in studies related with *Lycium* species shows that at present 15 *Lycium* species are known to be susceptible to viruses: *Lycium australe, L. barbarum* (syn.: *L. halimifolium*), *L. carolinianum, L. cestroides, L. chinense, L. europaeum, L. ferocissimum* (syn.: *L. campanulatum*), *L. flexicaule, L. horridum, L. mediterraneum, L. pallidum, L. rhombifolium, L. ruthenicum, L. turcomanicum* and *L. viscosa*. More than 150 host-virus relations of *Lycium* species to viruses have been described so far (Horváth, 1972b, 1991d). *Lycium* species as assay plants play an important role in the identification of viruses, and as perennial and woody species in the ecology of viruses. The widest known virus susceptible species are: *Lycium mediterraneum* and *L. pallidum* (22 viruses), *L. barbarum* (15 viruses), *L. chinense* and *L. europaeum* (12 viruses). The relations between *Lycium* species and viruses are mostly characterized by local symptoms manifested in chlorotic, sometimes chlorotic-necrotic lesions and leaf drop (hypersensitive reaction) (Ross, 1948; Horváth, 1991d). In some systemic host-virus relations (between *Lycium ruthenicum, L. turcomanicum* and alfalfa mosaic virus) the inoculated plants responded with systemic mosaic, leaf deformation and development of smaller and anisometrical leaves (Beczner and Horváth, 1972), while in other systemic host-virus relations (between *Lycium mediterraneum, L. pallidum* and lettuce mosaic *Potyvirus*) the infected plants showed no symptoms. The latent systemic susceptibility to lettuce mosaic *Potyvirus* and tobacco mosaic *Tobamovirus* is particularly important. The systemic susceptibility of these species to belladonna mottle *Tymovirus*, cucumber mosaic *Cucumovirus*, lettuce mosaic *Potyvirus*, tomato aspermy *Cucumovirus* and tobacco mosaic *Tobamovirus* is considered important.

With diagnostical and virus separation aspects taken into consideration it may also be important that both species appear to be suitable for separating the cucumber mosaic *Cucumovirus* and eliminating the celery mosaic *Potyvirus* in the case of a mixed infection by the two viruses. This is of practical significance,

because the two viruses in question frequently occur in mixed form in some horticultural crops (Wolf and Schmelzer, 1972).

9. Nicotiana species

We are indebted to Mayer (1886) for the possibility of transmitting tobacco mosaic disease to healthy tobacco *(Nicotiana tabacum)* plants in juice extracts taken from infected plants. Since his discovery tobacco as a virus host and test plant, respectively, together with other members of the family Solanaceae, have been in the forefront of research. Fernow (1925) and Price (1930) were the first to call attention to the fact that the tobacco mosaic *Tobamovirus* is able to infect plants *(Martina louisima, Phaseolus vulgaris)* belonging to two other families (Martinaceae and Leguminosae). These results did not however, divert attention off the very rich *Nicotiana* genus. The observation of Holmes (1929) that *Nicotiana glutinosa* produced countable necrotic lesions on leaves inoculated with tobacco mosaic *Tobamovirus* was of very great importance for plant virology. Since that time analogous local lesions have been described in various hosts (e.g., *Chenopodium, Cyamopsis, Phaseolus, Solanum*) inoculated with numerous different viruses, and local lesion counts provide the basis for most quantitative infectivity tests. Characteristically of the intensity of research related with the virus susceptibility of *Nicotiana* species, Holmes (1946) described 28 *Nicotiana* species as experimental hosts of tobacco mosaic *Tobamovirus* and tobacco etch *Potyvirus* as early as in 1946. There are among them such species as *Nicotiana clevelandii, N. glutinosa, N. rustica, N. sylvestris,* which have kept their importance in virology up to the present day. The *Nicotiana* species, especially *N. clevelandii, N. sylvestris, N. debneyi, N. glutinosa, N. tabacum, N. rustica, N. megalosiphon* and recently *N. benthamiana* and *N. quadrivalvis* have proved extremely valuable in virus work (Hollings, 1959, 1966; Thornberry, 1966; Schmelzer and Wolf, 1977; Christie and Crawford, 1978; Van Dijk et al., 1987).

Nicotiana clevelandii is a long since known test plant (Hollings, 1959). According to Schmelzer and Wolf (1977) it is susceptible to some 100 viruses. For several viruses it is an excellent assay and propagation host. It has special importance in the diagnostics and propagation of carlaviruses. *Nicotiana clevelandii* is the only non-leguminous species reported to be systemically infected by cassia yellow bloth *Bromovirus* (Dale, 1988). This plant is susceptible to 58 potyviruses (Edwardson and Christie, 1991). When using the plant one must known that dormancy in seeds must be broken by soaking the seed for two hours in gibberellic acid solution (1 g litre^{-1}) before sowing (Hill, 1984).

Nicotiana tabacum and its varieties are susceptible to some 90 viruses. There are among them good local lesions hosts (e.g., *Nicotiana tabacum* cv. Xanthi-nc) and systemic hosts (e.g., *Nicotiana tabacum* cv. Samsun). They have the advantages that they do not require special circumstances to grow, the seeds germinate much better, the plants develop faster and produce a larger mass of leaves than e.g., *Nicotiana clevelandii*.

According to the data of Christie (1969) the hybrid of *Nicotiana clevelandii* x *N. glutinosa*, which is equal in size to *N. glutinosa* and larger than *N. clevelandii*, is a very good test plant for several viruses.

Nicotiana glutinosa was found by Schmelzer and Wolf (1977) to be susceptible to some 80 viruses. It is a very good assay host for certain viruses (tobacco mosaic *Tobamovirus*) and plays important diagnostic role in differentiating the cucumber mosaic *Cucumovirus* strains (Richter, 1983).

Nicotiana rustica is a test plant well-known in plant virology, susceptible to more than 50 viruses (Schmelzer and Wolf, 1977). Its advantage over the other *Nicotiana* species is that it is earlier (by about 10 days) suitable for inoculation. The plant can be inoculated some 35 days after sowing.

Nicotiana megalosiphon belongs to those test plants which have mostly become known since the 1960s. Its susceptibility to alfalfa mosaic virus was first established as late as in 1963 (Schmelzer, 1963). It is a test plant particularly suitable for use in cross protection tests with various viruses causing ringspot symptoms. *Nicotiana megalosiphon* is a useful test plant in identifying fruit-tree viruses (Kegler et al., 1966). To our knowledge it is susceptible to about 40 viruses. Its importance is increased by the fact that it is resistant to the blue mold disease of tobacco *(Peronospora tabacina)* which is particularly dangerous for tobacco test plants in glasshouses. According to our investigations the plant is a good test plant for various strains and isolates of potato Y *Potyvirus* (Horváth, 1969e). It reacted with vein clearing and mosaic symptoms followed by leaf curl. As far as symptoms are concerned no difference could be established either for the isolates or for the strains of potato Y *Potyvirus* used.

Considering that the blue mold endangers the plant virology work in glasshouse, attention is worth being paid to the *Nicotiana species* (e.g., *Nicotiana debneyi, N. exigua, N. goodspeedii, N. megalosiphon, N. tabacum* cv. Resistant Hicks Fixed A2–426) resistant to blue mold but susceptible to viruses (Horváth, 1969e).

Nicotiana sylvestris is one of the less wide-spread test plants, though to our knowledge it is host for some 25 viruses (Schmelzer and Wolf, 1977). It has a significant role in differentiating tobamoviruses. The plant is locally susceptible to tobacco mosaic *Tobamovirus* ("Hungarian" and "Dahlemense" isolates) and ribgrass mosaic *Tobamovirus*, and locally and systemically susceptible to the

common strain of tobacco mosaic *Tobamovirus* (Mamula et al., 1974). *Nicotiana sylvestris*, together with other plants can be reliably used in separating several tobamoviruses (Table 4).

Table 4

Reaction of some differential test plants for tobamoviruses following inoculation with tobacco mosaic, tomato mosaic and ribgrass mosaic[1]

Test plants	Viruses[2, 3]			
	ToMV-H	ToMV-D	TMV	RMV
Nicotiana sylvestris	L	L	LS	L
Chenopodium amaranticolor	LS	LS	L	LS
Chenopodium quinoa	LS	LS	L	L
Lycopersicon esculentum	S	S	S	L
Plantago major	LS	LS	L	LS

[1]From Mamula et al. (1974). [2]ToMV, Hungarian isolate of tomato mosaic *Tobamovirus*; ToMV-D, Dahlemense isolate of tomato mosaic *Tobamovirus*; TMV, common strain of tobacco mosaic *Tobamovirus*; RMV, strain of Holmes' ribgrass mosaic *Tobamovirus*. [3]L, local symptoms; S, systemic symptoms; LS, local and systemic symptoms

Nicotiana debneyi plays an important role e.g., in the diagnosis of potato viruses. It is a reliable test plant for potato S *Carlavirus*, potato X *Potexvirus* and potato Y *Potyvirus* (Horváth, 1964; De Bokx, 1970; MacKinnon and Bagnall, 1972).

In recent years *Nicotiana benthamiana*, a popular test plant has become the centre of interest. Quacquarelly and Avgelis (1975) suggest that *Nicotiana benthamiana* may have a range of susceptibility equivalent to that of *Nicotiana clevelandii*. The real importance of *Nicotiana benthamiana* was first realized when it turned out to be a host for such viruses (e.g., blackeye cowpea mosaic *Potyvirus*, clover yellow mosaic *Potexvirus*, Desmodium mosaic virus, papaya mosaic *Potexvirus*, soybean mosaic *Potyvirus*, cucumber green mottle mosaic *Tobamovirus*) which earlier not had any host in the *Nicotiana* genus (Christie and Crawford, 1978; Horváth, 1993b). *Nicotiana benthamiana* is susceptible to more than 200 viruses belonging to 26 virus groups and an unclassified group (Horváth, 1993b). *Nicotiana benthamiana* is the test plant "number one" in virology (Horváth, 1993b). It is remarkable that the plant is hosts for some furoviruses (4), geminiviruses (9), ilarviruses (3), nepoviruses (11), plant rhabdoviruses (5) and sobemoviruses (2) too. Its importance is increased by the fact that it is not only a good test plant, but is also suitable for maintaining and/or purifying several viruses, and is a good source of protoplasts for the work of superinfection (Barker, 1989). In the last years a not exactly identified virus

containing bacilliform particles was isolated from drainage water in the Fraser delta region of British Columbia, which locally infected the *Nicotiana benthamiana* plant (Stace-Smith, 1989). *Nicotiana benthamiana*, as anomalous host, allows full and rapid systemic infection by numerous tobacco mosaic *Tobamovirus* mutants that fail to infect other plants effectively (reviewed by Dawson and Hilf, 1992). Mutant KK6 with delayed 30-kDa protein synthesis, odontoglossum ringspot *Tobamovirus*, and the tobamovirus hybrids with either the odontosglossum ringspot *Tobamovirus* 30-kDa gene or the odontosglossum ringspot *Tobamovirus* coat protein gene substituted into tobacco mosaic *Tobamovirus* can systemically infect only this plant efficiently.

In the course of studying the host-virus relations we established 97 new compatible and 47 new incompatible relations for 18 *Nicotiana* species, 12 cultivars and one hybrid (Horváth, 1983b). An excellent work published not long ago deals with the behaviour to 26 isolates of 7 viruses of 109 accessions of 67 *Nicotiana* species belonging to 3 subgenera (*Rustica*, *Tabacum* and *Petunioides*) and 12 sections of the *Nicotiana* genus (Van Dijk et al., 1987). The authors pointed out considerable differences in virus susceptibility possibly shown e.g., by the accessions. *Nicotiana benthamiana*-9, *N. miersii*-33, and *N. occidentalis*-37B seem to be suitable for routine inoculation tests, because of their larger virus ranges and sensitivity to different viruses.

10. Phaseolus species

Beans (*Phaseolus* species) have a high affinity to plant viruses. To our best knowledge the bean-virus relations cover 48 *Phaseolus* species and some 137 viruses (Horváth, 1986c, 1986f). The best known test species is *Phaseolus vulgaris*, which is susceptible to about 80 viruses. However, a large proportion of the relations between bean plants and viruses are sufficiently known, which renders it possible to identify the different viruses. *Phaseolus vulgaris* cv. Monroe, an effective local lesion host, is excellently suitable for identifying the bean common mosaic *Potyvirus* (Trujillo and Saettler, 1972; Castano et al., 1982), or clover yellows vein *Potyvirus* (Dwadash-Shreni and Stavely, 1984), and the variety Red Kidney can be used to detect potato M *Carlavirus*, potato S *Carlavirus* (Hiruki, 1970; Horváth, 1972a; Kowalska, 1977) and other viruses. In recent investigations *Phaseolus hysterinus* and *P. mungo* proved excellent assay plants for tobacco necrosis *Necrovirus* and Melandrium yellow fleck *Bromovirus*, and *Phaseolus mungo* for tobacco rattle *Tobravirus* too (Horváth, 1986d). When studying the responses of some 50 *Phaseolus vulgaris* varieties we found them suitable not only for the identification but also for the separation of many viruses (Horváth, 1972a, 1983b, 1985c). *Phaseolus vulgaris* cultivars as

differential hosts play a highly important role in identifying the strains of pathotypes of certain viruses too. Drijfhout (1978) tested about 450 bean cultivars and lines with strains of bean common mosaic *Potyvirus*. On the basis of their resistance or susceptibility to virus strains the cultivars could be placed in eleven groups. Cultivars in groups 1 to 6 never gave systemic necrosis response, but reacted with mosaic symptoms to one or more strains. The resistance group 7 responded neither with mosaic, nor with local and systemic necrosis. Cultivars of groups 8 to 10 reacted to some strains with systemic necrosis, but never with mosaic. Group 11 only gave local necrosis response. In various French bean cultivars Schmidt and Zobywalski (1984) could differentiate the pathotypes of 14 strains of bean yellow mosaic *Potyvirus*, on the basis of different reactions of the inoculated leaves (latent infection, chlorotic or necrotic spots partly changing in vein necrosis) and the deviating symptoms of the subsequent leaves, inclusively latent, systemic infections.

Some *Phaseolus vulgaris* cultivars are suitable for differentiating various strains of the same virus. The primary leaves of *Phaseolus vulgaris* cv. The Prince reacted with discrete local lesions to a strain of the group D of tobacco necrosis *Necrovirus*, and the plant showed systemic symptoms in response to the stipple streak strain of the virus (Kassanis, 1970). In the course of investigations into the host-virus relations *Phaseolus* species (e.g., *P. lunatus*) and cultivars inoculated with cucumber mosaic *Cucumovirus* showed considerable differences in symptoms (Provvidenti, 1976; Horváth, 1986f).

11. Pisum species

The genus *Pisum* which includes relatively few species is of importance not only in a classical genetic sense, but from a virological point of view too. According to Schmelzer and Wolf (1977) some 8 species are virologically known. The best known species is *Pisum sativum*, diagnostic host for 23, propagative host for 12 and assay host for 3 viruses (see Table 1). Hampton et al. (1978) studying the test plants of mechanically transmissible legume viruses and the responses of the hosts found that *Pisum sativum* was a particularly suitable test plant for detecting pea viruses. It must be emphasized, however, as pointed out by Bos (1970b) and Beczner et al. (1983) too, that the host reactions of *Pisum sativum* to the same viruses may greatly vary with the different authors. Such discrepancies, of course, greatly depend on the varieties tested. Concerning the virological importance of *Pisum sativum* and its role in the differentiation of viruses, as well as in connection with the virus resistant and susceptible germplasms detailed data are to be found in works by Bos (1970b), Beczner et al. (1983), Hampton (1980), Schmidt and Naumann (1981) and Hampton et al. (1981).

12. Physalis species

Various *Physalis* species have recently been playing an increasingly important role in virology as test plants, filter plants, production hosts and natural hosts, respectively. *Physalis floridana*, a well-known species susceptible, to our knowledge, to about 45 viruses and suitable for separating numerous viruses (Horváth, 1970, 1974), has lately been found to be a good production host for satsuma dwarf *Nepovirus* (Tanaka and Imada, 1974) and a natural host e.g., for groundnut eyespot virus (Dubern, 1981). *Physalis floridana* as donor host yielded slightly more tobacco mosaic *Tobamovirus* than tobacco, and about 2000 times more than bean (Yarwood, 1971). *Physalis floridana* and *P. angulata* plant are suitable for differentiating potato Y *Potyvirus* strains as well (Hein and Bartels, 1964; Horváth, 1967; Leiser and Richter, 1979). During investigations in the last several years *Physalis floridana* was playing an important role in identifying potato viruses occurring in the Andes (e.g., Andean potato mottle *Comovirus*, Andean potato calico strain of the tobacco ringspot *Nepovirus*, Andean potato latent *Tymovirus* and wild potato mosaic virus) (Fribourg, 1977; Fribourg et al., 1977a, 1977b; Jones and Fribourg, 1979). Further, the results obtained lately which show the species *Physalis floridana* and other *Physalis* species (e.g., *Physalis angulata, P. pubescens*) to be suitable for the postharvest indexing of certain potato viruses are also remarkable (Singh and Smith, 1981; Singh, 1982). *Physalis heterophylla* is more sensitive to potato leafroll *Luteovirus* than *P. pubescens*. This species *(Physalis heterophylla)* shows earlier (2-4 days) and more definied symptoms (stunting, interveinal chlorosis, and epinasty) of potato leafroll *Luteovirus* infection than *P. pubescens* (De Souza-Dias and Costa, 1991). The importance of the *Physalis* species is increased by the fact that many of them (e.g., *Physalis alkekengi, P. angulata, P. floridana, P. heterophylla, P. subglabrata*) are known as natural virus hosts (Lovisolo and Bartels, 1970; Feldman and Gracia, 1972; Moline and Fries, 1974; Peters and Derks, 1974; Joshi and Dubey 1976).

Some *Physalis* species are important in separating viruses. The less known *Physalis minima* is suitable for the separation of viruses that frequently occur in complex form (Table 5). The possibility of separating cucumber mosaic *Cucumovirus* from bean common mosaic *Potyvirus* and bean yellow mosaic *Potyvirus*, respectively, with the aid of *Physalis minima* deserves attention. It is a well-known fact that the appearance of cucumber mosaic *Cucumovirus* simultaneously with bean common mosaic *Potyvirus* and bean yellow mosaic *Potyvirus* in bean plants has recently become more and more frequent. In our opinion *Physalis minima* is of importance in differentiating cucumber mosaic *Cucumovirus* strains isolated from bean plants in several countries and described

as pathogenic to legumes (Horváth, 1983b), as well as bean common mosaic *Potyvirus* and bean yellow mosaic *Potyvirus*. The two strains of cucumber mosaic *Cucumovirus* isolated from bean plants in Hungary were found to be equally pathogenic to *Physalis minima*.

Table 5

Separation of different viruses with *Physalis minima*[1]

Separable viruses	Eliminated viruses
Alfalfa mosaic virus[2]	Bean common mosaic *Potyvirus*
Arabis mosaic *Nepovirus*	Bean yellow mosaic *Potyvirus*
Belladonna mottle *Tymovirus*	Carnation ringspot *Dianthovirus*
Broad bean wilt *Fabavirus*	Cauliflower mosaic *Caulimovirus*
Cucumber mosaic *Cucumovirus*	Celery mosaic *Potyvirus*
Melandrium yellow fleck *Bromovirus*	Cucumber green mottle mosaic *Tobamovirus*
Tobacco rattle *Tobravirus*	Radish mosaic *Comovirus*
Tobacco ringspot *Nepovirus*	Turnip yellow mosaic *Tymovirus*
Tomato aspermy *Cucumovirus*	Watermelon mosaic virus 2 *Potyvirus*
Tomato mosaic *Tobamovirus*	
Tomato ringspot *Nepovirus*	
Turnip mosaic *Potyvirus*	

[1]From Horváth (1983a). [2]Monotypic group with no approved group name (see Hull et al., 1991)

In recent years further *Physalis* species (e.g., *Physalis anisotrichus*, *P. curassavica, P. filiformis, P. glabripes, P. lanceifolia*) have become known as test plants. Characteristically of the intensity of studies on the *Physalis* host-virus relations, while in 1970 only 23 *Physalis* species were known for susceptibility to 67 viruses (Horváth, 1970), by 1988 the behaviour of 30 *Physalis* species to more than 100 viruses became known (Horváth, 1984, 1985a, e, 1988b, c).

13. Solanum species

In wild *Solanum* species many known and so far unknown viruses have recently been detected (Horváth, 1988d). Remarkable are the conclusions of those experiments in which the *Solanum* species proved to be not only natural virus hosts, but also susceptible to inoculation with many viruses occurring in the gene centres of potato; so these *Solanum* species are at the same time prognostic hosts too (Table 6). Recently the henbane mosaic *Potyvirus* has been found to be pathogenic to wild *Solanum* species (Horváth et al., 1988a, Horváth and Hoekstra, 1989).

Table 6

Virus susceptibility of some *Solanum* species

Solanum species	Experimentally transmitted viruses[1]	Literature
Solanum acaule	CMV, TMV, ToMV	Horváth (1968a), Schmelzer and Spaar (1975)
S. demissum	APLV, APMV, CMV, TMV, ToMV, PepMV, PVV	Horváth (1968a), Fribourg et al. (1977a,b), Schmelzer and Spaar (1975), Jones and Fribourg, (1978), Jones et al. (1980), Fribourg and Nakashima (1984)
S. phureja	CMV	Schmelzer and Spaar (1975)
S. spegazzini	PVM	Horváth (1982b)
S. stoloniferum	APLV, CMV, PepMV, PTV	Schmelzer and Spaar (1975), Jones and Fribourg (1978), Jones et al. (1980) Fribourg (1979)
S. tuberosum ssp. andigena	APLV, APMV, CMV, PTV, TRSV-APCS	Schmelzer and Spaar (1975), Fribourg (1977, 1979), Fribourg et al. (1977a,b) Fribourg and Nakashima (1984)
S. tuberosum ssp. tuberosum	APLV, PBRV, PTV, TRSV-APCS, PVV	Fribourg (1977, 1979), Fribourg et al. (1977b), Salazar and Harrison (1977, 1979), Fribourg and Nakashima (1984)
S. vernei	CMV, PVX-HB, TMV, ToMV, WPMV	Horváth (1968a), Schmelzer and Spaar (1975), Jones and Fribourg (1979), Moreira et al. (1980)

[1]APLV, Andean potato latent *Tymovirus*; APMV, Andean potato mottle *Comovirus*; CMV, Cucumber mosaic *Cucumovirus*; PBRV, Potato black ringspot *Nepovirus*; PepMV, Pepino mosaic *Potexvirus*; PTV, Peru tomato *Potyvirus*; PVX-HB, Resistance-breaking strain of potato X *Potexvirus*; PVM, Potato M *Carlavirus*; PVV, Potato V *Potyvirus*; TMV, Tobacco mosaic *Tobamovirus*; ToMV, Tomato mosaic *Tobamovirus*; TRSV-APCS, Andean potato calico strain of tobacco ringspot *Nepovirus*; WPMV, Wild potato mosaic virus

Solanum species reacting typically to certain viruses are used as test or indicator plants. For routine use the methods should not only be cheap and quick, but sensitive and reliable as well. Two plants deserve special attention: *Solanum demissum* A6 hybrid (*Solanum demissum* x *S. tuberosum* cv. Aquila) and *Solanum demissum* P.I. 230579 (Köhler, 1953; Webb and Wilson, 1978). The best known assay plant is *Solanum demissum* A6 hybrid, whose detached leaves render the quick detection of more than one virus possible (Köhler, 1953; De Bokx and Chrzanowska, 1972; Webb and Wilson, 1978). It is of special importance in the detection of potato Y *Potyvirus* and potato A *Potyvirus*, though it can be used for detecting such viruses as e.g., cucumber mosaic *Cucumovirus*

and henbane mosaic *Potyvirus* (Zschüttig and Horváth, 1968; Horváth and Pocsai, 1971; Horváth et al., 1988a). Attention has lately turned to a new *Solanum* accession: *Solanum demissum* P.I. 230579, a diagnostic plant for potato Y *Potyvirus* recently described by Webb and Wilson (1978), and Singh and Boiteau (1984) is more suitable for detecting the virus than the *Solanum demissum* A6 hybrid, the latter being smaller and slower growing. In recent experiments the *Solanum demissum* P.I. 230579 accession reacted with necrotic local lesions to alfalfa mosaic virus, cucumber mosaic *Cucumovirus*, henbane mosaic *Potyvirus*, potato M *Carlavirus*, tobacco mosaic *Tobamovirus*, tobacco rattle *Tobravirus*, tobacco ringspot *Nepovirus* and tomato mosaic *Tobamovirus*, and showed both local and systemic symptoms in response to infection by Andean potato mottle *Comovirus*, potato aucuba mosaic *Potexvirus* and tomato ringspot *Nepovirus* (Horváth 1990). The virus susceptibility of *Solanum demissum* P.I. 230579 enables a wider use of the plant in virus diagnostics.

14. Tetragonia species

Out of more than 2500 species of 130 genera in the family Aizoaceae, *Tetragonia tetragonoides* (syn.: *T. expansa, T. japonica, T. halimifolia, T. cornuta, T. quadricornis, Demidovia tetragonoides*), a member of the subfamily Tetragonoideae is unquestionably the best known in virology. To our knowledge, it is experimental host for more than 50 viruses (Horváth, 1983b), diagnostical host for some 23 viruses, and propagation and assay host, respectively, for 5 viruses each. According to Tamada (1973), of the strains of beet necrotic yellow vein *Furovirus* the YS strain infects the plant both locally and systemically, while other strains only cause local infection. In our experiments it was found to be a very good test plant for cucumber mosaic *Cucumovirus*. The symptoms appear not only on the inoculated leaves, but on axillary shoots too (Horváth, 1976).

Tetragonia echinata, another member of the subfamily Tetragonoideae, is also known to be susceptible to beet yellows *Closterovirus* (Brandes and Zimmer, 1955; Roland and Tahon, 1961; Horváth, 1973).

In the last years further two virus susceptible *Tetragonia* species became known. *Tetragonia eremaea* proved susceptible to two viruses (cucumber mosaic *Cucumovirus* and lettuce mosaic *Potyvirus*), while *T. crystallina* to 13 viruses (Horváth, 1980b, 1983b). *Tetragonia crystallina* and *T. echinata* are not only suitable for identifying various viruses, they also can be used as filter plants in separating a number of viruses.

15. Vigna species

In the genus *Vigna*, which includes some 150 species, the virologically known species are 16 in number. However, major importance in only attached to the following species: *Vigna cylindrica* (syn.: *V. sinensis* spp.), *V. radiata, V. sesquipedalis* (syn.: *V. sinensis* ssp.), *V. sinensis*, *V. unguiculata*. Among the species listed *Vigna sinensis* and *Vigna unguiculata* are the most important for virus diagnostics. *Vigna sinensis* is diagnostic host for 15, propagation host for 3 and assay host for 9 viruses (see Table 1). *Vigna sinensis* is susceptible to most polyhedral viruses and *ne*matode-transmitted viruses with *tu*bular particles (NETU viruses), usually giving local lesions. Among the *Vigna sinensis* cultivars "Blackeye" is the best known as test plant. *Vigna sinensis* is a filter plant suitable for differentiating two cucumoviruses (cucumber mosaic and Robinia mosaic). According to data by Schmelzer (1971) the cucumber mosaic *Cucumovirus* produces local, the Robinia mosaic *Cucumovirus* both local and systemic symptoms in *Vigna sinensis* plants.

Vigna unguiculata is diagnostic, propagation and assay host for numerous viruses. Among its cultivars the "Blackeye Early Ramsh" is the best known. According to Hampton et al. (1978) four clover viruses were virulent to *Vigna unguiculata*, while the five bean viruses lacked virulence to this host. The *Vigna unguiculata* test plant with its varied symptoms is important in the identification of cowpea viruses (Rossel and Thottappilly, 1985).

Vigna catjang, a species susceptible to some eight viruses, has become known as a new test plant in plant virology. Its immunity to tobacco mosaic *Tobamovirus* deserves attention (Horváth, 1983b).

Selected and Proposed Hosts and Non-Hosts of Viruses

This chapter sums up the selected and proposed hosts and non-hosts of those mechanically transmissible viruses, which were described in the series CMI/AAB Descriptions of Plant Viruses between 1970 and 1988, and can be found in the latest book of Matthews (1991) and in the paper of Hull et al. (1991). By listing the selected and proposed hosts and non-hosts we wish to sort out for the 232 mechanically transmissible viruses of 24 virus groups (Table 7) those plants which most frequently occur in virology and are the widest known and best test plants. The selection is based on the 421 hosts described in the series CMI/AAB Descriptions of Plant Viruses in the case of the 232 mechanically transmissible viruses. With viruses that practically have a single known host each, there

Table 7

Virus groups in which mechanically transmissible plant viruses are found[1]

Group	No. of member	No. of probable or possible member	Total
Alfalfa mosaic virus[2]	1	0	1
Bromovirus	5	0	5
Carlavirus	18	0	18
Carmovirus	9	5	14
Caulimovirus	5	0	5
Closterovirus	3	2	5
Comovirus	12	0	12
Cucumovirus	4	0	4
Dianthovirus	3	0	3
Furovirus	5	2	7
Geminivirus	4	0	4
Idaeovirus	1	0	1
Ilarvirus	12	0	12
Necrovirus	1	0	1
Nepovirus	24	3	27
Pea enation mosaic virus[2]	1	0	1
Potexvirus	17	1	18
Potyvirus	54	1	55
Sobemovirus	10	3	13
Tobamovirus	8	0	8
Tobravirus	2	0	2
Tombusvirus	3	0	3
Tospovirus	1	0	1
Tymovirus	12	1	13

[1]Collection from CMI/AAB Descriptions of Plant Viruses (1970–1988) and from Matthews (1991), and from Hull et al. (1991). [2]Monotypic groups with no approved group names (see Hull et al., 1991)

was no possibility for host selection (e.g., blueberry red ringspot *Caulimovirus*, ginger chlorotic fleck? *Sobemovirus*, Narcissus tip necrosis *Carmovirus*). In such cases we gave the single known host.

Considering that the series CMI/AAB Descriptions of Plant Viruses does not discuss the non-hosts of viruses, or makes references to non-hosts only in a few exceptional cases, therefore when listing the non-hosts of viruses we relied on the original sources, that for lack of space we cannot, unfortunately refer to, and on the helpful information given by virologist colleagues.

However, we should like to emphasize here, that there are essential differences in opinion among the virologists concerning the judgement and acceptability of the non-host of a given virus. For example, Fulton (1991) is of the opinion that it often depends on the adequacy of the technique whether or not

a plant is considered to be non-host for a virus. Fulton (1948) in an earlier work studied the host range of tobacco streak *Ilarvirus*, but did not give its non-hosts at that time. It was only thirty years later that he re-examined the same plants and found that those plants which earlier had been considered non-hosts, all proved hosts in the repeated experiments. In the opinion of Fulton (1991) this is due to the improved inoculating methods. He was unable to infect two species of fern and one species of Bryophyte, and next year someone with a better method could infect them. Lister (1991) holds the same view, namely, that it is difficult to establish whether a plant is non-host for a virus. It must also be emphasized that the variability of the strains and isolates of some viruses (e.g., cucumber mosaic *Cucumovirus*, tomato spotted wilt *Tospovirus*, or the ilarviruses) is very great, as shown in the host ranges too (Horváth, 1976; Francki and Hatta, 1981; Fulton, 1981; Kaper and Waterworth, 1981; Fulton, 1991). As for the differences between virus strains we may mention here that e.g. the common strain of beet necrotic yellow vein *Furovirus* causes local, while its YS strain both local and systemic infection to the *Tetragonia tetragonoides* plant (Tamada, 1975), or the Australian strains of lucerne transient streak *Sobemovirus* infect the *Chenopodium amaranticolor* systemically, but the New-Zealand strains are not able to do so (Forster and Jones, 1980). Of the qualitative differences between host plants and virus strains tulip breaking *Potyvirus* may be an example. According to Derks (1991) *Chenopodium amaranticolor*, *Nicotiana benthamiana*, and *N. clevelandii* are equally hosts for the lily isolates of tulip breaking *Potyvirus*, but are not hosts for the tulip isolates of the virus. *Lilium formosanum*, on the other hand, is host for both the lily and the tulip isolates (Table 8). Comparisons of some host ranges with non-hosts show that some species which have been reported to be hosts of a certain potyvirus are also described as being non-hosts of the same virus. Several factors may account for this situation such as *(1)* differences in virus strains, *(2)* differences in genotypes of samples of species inoculated with the same virus, *(3)* differences in inoculating technique, and *(4)* environmental influences on symptom expression (see Edwardson and Christie, 1991).

For the 232 mechanically transmissible viruses discussed in the series CMI/AAB Descriptions of Plant Viruses between 1970 and 1988 we have chosen 141 plants as proposed hosts. They are suitable for the safe isolation and positive diagnosis of the 232 viruses (see Table 8). For the 232 viruses we listed 133 non-hosts. The host plants and non-host plants listed are not, naturally, sufficient for the exact identification of the different viruses, yet they are suitable for detecting the presence of a given virus, or knowing the non-hosts of the given viruses establishing a probably correct diagnosis excluding the presence of other virus or viruses (negative diagnosis). For example, *Nicotiana benthamiana* is equally good host for the alfalfa mosaic virus and cucumber mosaic *Cucumovirus*, its

systemic symptoms are not, however, suitable for differentiating the two viruses. But the *Cucurbita pepo* as a cucumber mosaic *Cucumovirus* host and alfalfa mosaic virus non-host, as well as the *Beta vulgaris*, a non-host for cucumber mosaic *Cucumovirus* and host for alfalfa mosaic virus render the differentiation of the two viruses possible (Crill et al., 1970; Quacquarelli and Avgelis, 1975; Horváth, 1980a; see Table 8). To give one more example, *Chenopodium amaranticolor* and *C. quinoa* are proposed hosts for some 145 viruses, and at the same time non-hosts for 45 other viruses (see Table 8). Thus, when *Chenopodium amaranticolor* or *C. quinoa* is used alone as test plant, from a positive reaction the presence of some of at least 145 viruses can be reliably concluded on, while in the case of a negative reaction the presence of 45 viruses can be excluded, out of the 232 viruses. Certain viruses can be concluded on and others excluded from the diagnosis with a reliability much higher than that when other selected and proposed plants are also taken into consideration (see Table 8).

Selected and Proposed Hosts and Non-Hosts of Virus Groups

Division into virus groups has classically been based upon *(1)* type of nucleic acid, *(2)* particle morphology, *(3)* type of transmission, *(4)* cytopathology and finer divisions have largely been made according to host range (see Milne, 1990). From a survey of the host plants of viruses we can establish that there are species susceptible to 40–50 % or more of the viruses belonging to a given virus group (Table 9). And there are plants susceptible to all the viruses of a given virus group. For example, *Chenopodium quinoa* is susceptible to all the 27 members of the *Nepovirus* group (see Table 9).

Table 8

Suggested list of some selected and proposed host plants and non-host plants for use in studies on virus identification[1]

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
African cassava mosaic	GEMINI (II)	ACMV	DATST		SOL	AHLS	CHEGI		CHE
			NIOBE		SOL	DH, PH	CHEQU		CHE
			NIOCL		SOL	DH	GOMGL		AMA
							TEATE		AIZ
Agropyron mosaic	POTY (mite)	AgMV	AGRRE		GRA	DH, PH	CHEGI		CHE
			LOLMU		GRA	DH	CHEQU		CHE
			XXXXX		XXX	AH	DATST		SOL
							NIOTA		SOL
Alfalfa latent	CARLA	ALV	CASOC		LEG	ASL	CHEGI		CHE
			VICFX		LEG	DH	CHEQU		CHE
			PIBST		LEG	PH	CUMSA		CUC
							DATST		SOL
							GOMGL		AMA
Alfalfa mosaic	AMV group	AMV	CHEQU		CHE	DH	CUUPE		CUC
			NIOBE		SOL	PH	TEACR		AIZ
			PHSVX		LEG	AHL			
			VIGSI		LEG	AHL			
American hop latent	CARLA	AHLV	CHEQU		CHE	DH, PH	NIOCL		SOL
			DATST		SOL	AHL	NIOGT		SOL
							NIOTA	WHB	SOL
American plum line pattern	ILAR	APLPV	CUMSA		CUC	DH	GOMGL		AMA
			NIOSU		SOL	DH			
			NIOOC		SOL	PH			
			VIGSC		LEG	ASL			
Andean potato mottle	COMO	APMV	NICPH		SOL	DH	CHEGI		CHE
			NIOCL		SOL	AHS, PH	CHEQU		CHE

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Apple chlorotic leaf spot	? CLOSTERO	ACLSV	CHEGI		CHE	DH	CUMSA		CUC
			CHEQU		CHE	AHLS	GOMGL		AMA
			PHSVN	PIN	LEG	AHL	NIOTA		SOL
							VIGSI		LEG
Apple mosaic	ILAR	ApMV	CUMSA		CUC	DH, PH	CPSAN		SOL
			VIGSI		LEG	DH	NIOTA		SOL
			CMOTE		LEG	ASL	PEUHY		SOL
							VICFX		LEG
Arabis mosaic	NEPO	ArMV	CHEGI		CHE	AHLS	CPSAN		SOL
			CHEQU		CHE	DH	VICFX		LEG
			NIOCL		SOL	PH			
			PEUHY		SOL	PH			
Arracacha A	NEPO	AVA	CHEMU		CHE	DH	CPSAN		SOL
			CHEQU		CHE	AHL	PHSBX		LEG
			NIOCL		SOL	PH	VIGSI		LEG
			TEATE		AIZ	DH	ZIIEL		COM
Arracacha B	? NEPO	AVB	CHEGI		CHE	AHLS	CPSAN		SOL
			CHEMU		CHE	DH	DATME		SOL
			CHEQU		CHE	AHLS, PH	PHSVX		LEG
			TEATE		AIZ	PH			
Artichoke Italian latent	NEPO	AILV	CUMSA		CUC	DH	CPSAN		SOL
			CUUPE		CUC	PH	NIOGT		SOL
			GOMGL		AMA	DH	VICFX		LEG
			NIOTA	WHB	SOL	DH	ZIIEL		COM
			PHSVX	LAV	LEG	ASL			
Artichoke vein banding	? NEPO	AVBV	CHEGI		CHE	DH	NIOGT		SOL
			CHEQU		CHE	AHL, PH	NIOTA	XNC	SOL
			PHSVX		LEG	PH			

Artichoke yellow ringspot	NEPO	AYRSV	CHEGI		CHE	DH	BEAVA		CHE
			CHEMU		CHE	DH	BRSRA		CRU
			CHEQU		CHE	AHLS	CPSAM		SOL
			CUMSA		CUC	AHLS, DH, PH	CUUMA		CUC
			GOMGL		AMA	DH, PH	CUUPE		CUC
			NIOGT		SOL	AHL	SOLME		SOL
Asparagus virus 2	ILAR	AV2	CHEQU		CHE	DH	AMIMA		UMB
			GOMGL		AMA	DH	CUMME		CUC
			NIOTA	HFT	SOL	PH	CUMSA		CUC
			PHSVX		LEG	AHL	DATST		SOL
			VIGSI		LEG	AHL			
Barley yellow mosaic	POTY (fungus)	BaYMV	HORVX		GRA	AHS, DH, PH	CHEGI		CHE
							NIOTA		SOL
Bean common mosaic	POTY (aphid)	BCMV	CHEQU		CHE	AHL	CUMSA		CUC
			NIOCL		SOL	DH	DATST		SOL
			PHSVX		LEG	DH, PH			
Bean golden mosaic	GEMINI (II)	BGMV	PHSVV	TCR	LEG	AHS, DH, PH	GLXMA		LEG
							RHNMI		LEG
Bean mild mosaic	? CARMO	BMMV	CMOTE		LEG	DH	PHSLU		LEG
			DOLLA		LEG	DH	VICSS		LEG
			PHSVN	PIN	LEG	AHS, PH	VIGSI		LEG
Bean pod mottle	COMO	BPMV	CHEQU		CHE	PH	CUMSA		CUC
			GLXMA		LEG	DH	NIOGT		SOL
			PHSVX	BLV	LEG	PH			
			PHSVX	BTF	LEG	AHLS, DH			
			PHSVN	PIN	LEG	AHLS, DH			
Bean rugose mosaic	COMO	BRMV	CHEGI		CHE	DH	CUMSA		CUC
			PHSVX	PLT	LEG	PH	NIOTA	VHB	SOL
			PHSVV	TCR	LEG	AHL			
			VICFX		LEG	DH			
Bean yellow mosaic	POTY (aphid)	BYMV	CHEQU		CHE	AHL	CUMSA		CUC
			NIOCL		SOL	PH	DATST		SOL
			PHSVX		LEG	DH, PH	PIBST	PFC	LEG
			VICFX		LEG	PH			

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Bearded iros mosaic	POTY (aphid)	BIMV	BMCCH		IRI	AHLS, PH	CHEGI		CHE
			IRISS		IRI	DH	CUMSA		CUC
							NIOSU		SOL
							VIGSI		LEG
Beet mosaic	POTY (aphid)	BtMV	BEAVA		CHE	DH, PH	CUMSA		CUC
			CHEQU		CHE	AHL	DATST		SOL
			GOMGL		AMA	AHL	NIOGT		SOL
			SPQOL		CHE	DH			
Beet necrotic yellow vein	FURO	BNYVV	BEAMA		CHE	DH	CUMSA		CUC
			CHEQU		CHE	AHL	DATST		SOL
			NIOBE		SOL	PH	NIOGT		SOL
			TEATE		AIZ	PH	PHSVX		LEG
							VICFX		LEG
Beet yellows (= sugarbeet yellows)	CLOSTERO	BYV	BEAVA		CHE	DH, PH	DATST		SOL
			CHECA		CHE	DH	NIOGT		SOL
			CHEFO		CHE	AHS	PHSVX		LEG
			TEATE		AIZ	PH	VICFX		LEG
							VIGSI		LEG
Belladonna mottle	TYMO	BeMV	DATST		SOL	DH, PH	AMIMA		UMB
			NIOTA	SAM	SOL	PH	CHEGI		CHE
			XXXXX		XXX	AH	CUUPE		CUC
							PHSVN	PIN	LEG
Bidens mottle	POTY (aphid)	BiMoV	CHEQU		CHE	AHLS	LACSA	VAL	COM
			NIOHY	GTC	SOL	DH, PH	PIBST		LEG
			ZIIEL		COM	DH			
Black raspberry latent	ILAR	BRLV	CHEQU		CHE	AHLS	PIBST		LEG
			CUMSA		CUC	PH	VIGSQ		LEG

			GOMGL		AMA	PH		
			NIOTA	WHB	SOL	DH		
Blackeye cowpea mosaic	POTY (aphid)	BlCMV	CHEQU		CHE	AHL	CUMSA	CUC
			GLXMA		LEG	PH	NIOGT	SOL
			NIOBE		SOL	PH	NIOTA	SOL
			VIGSI		LEG	DH		
Blackgram mottle	? CARMO	BMoV	CMOTE		LEG	DH	CHESS	CHE
			PHSAU		LEG	DH	NIOCL	SOL
			PHSVX	BLV	LEG	AHS, PH		
			PHSVN	PIN	LEG	AHL		
Blueberry leaf mottle	NEPO	BLMV	CHEQU		CHE	AHLS	CUUMA	CUC
			NIOCL		SOL	AHLS, DH, PH	DATST	SOL
							PEUHY	SOL
							VIGSI	LEG
							ZIIEL	COM
Blueberry red ringspot	CAULIMO	BRRV	VACSS		ERI	DH	CHEQU	CHE
			XXXXX		XXX	AH	DATST	SOL
							NIOCL	SOL
							VIGSI	LEG
Broad bean mottle	BROMO	BBMV	CHEGI		CHE	AHL	NIOTA	SOL
			NIOCL		SOL	DH, PH		
			VICFX		LEG	PH		
			XXXXX		XXX	AH		
Broad bean necrosis	? FURO	BBNV	CHEQU		CHE	AHL	CUMSA	CUC
			NIOCL		SOL	DH	GOMGL	AMA
			PIBST		LEG	PH	NIOGT	SOL
			VICFX		LEG	DH	PHSAU	LEG
Broad bean stain	COMO	BBSV	PHSVV	TDG	LEG	AHL, DH	GOMGL	AMA
			PIBST		LEG	PH	NIOCL	SOL
			VICFX		LEG	AHS, PH		
Broad bean true mosaic	COMO	BBTMV	LTHOD		LEG	DH, PH	CHEQU	CHE
			PHSVX		LEG	DH	GOMGL	AMA
			VICFX		LEG	AHS	NIOCL	SOL
Brome mosaic	BROMO	BMV	CHEGI		CHE	DH	GOMGL	AMA
			CHEHY		CHE	AHL	NIOGT	SOL

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
			CHEQU		CHE	DH	NIOTA	SAM	SOL
			HORVX		GRA	PH	PEUHY		SOL
Cacao necrosis	NEPO	CNV	CHEQU		CHE	DH	DATST		SOL
			PHSVV	TPR	LEG	AHL, PH	PHYFL		SOL
							VICFX		LEG
Cacao yellow mosaic	TYMO	CYMV	CHEGI		CHE	AHLS	DATST		SOL
			CHEQU		CHE	AHLS, DH	GOMGL		AMA
			NICPH		SOL	PH	LYPES		SOL
			NIOCL		SOL	PH	NIOGT		SOL
							PEUHY		SOL
Cactus X	POTEX	CVX	AMACA		AMA	DH	NIOSS		SOL
			CHEQU		CHE	AHLS			
			GOMGL		AMA	AHL			
Carnation etched ring	CAULIMO	CERV	VAAPY	PIB	CAF	AHLS, PH	NIOGT		SOL
			SILAR		CAF	AHS, DH			
Carnation latent	CARLA	CLV	CHEGI		CHE	AHL	NIOGT		SOL
			CHEQU		CHE	AHL, DH	LYPES		SOL
			DINBA		CAF	PH			
Carnation mottle	CARMO	CarMV	CHEGI		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHL	NIOGT		SOL
			DINCA		CAF	PH	PIBST		LEG
			GOMGL		AMA	DH	VIGSI		LEG
			TEATE		AIZ	DH	VIGSI	BLE	LEG
Carnation necrotic fleck	CLOSTREO	CNFV	DINBA		CAF	AHL	CHEGI		CHE
			DINCA		CAF	DH, PH	CUMSA		CUC
							DATST		SOL
							NIOGT		SOL
							PHSVX		LEG

Carnation ringspot	DIANTHO	CRSV	CHEGI		CHE	AHL	GLXMA		LEG
			CHEQU		CHE	AHL	PIBST		LEG
			DINBA		CAF	DH, PH			
			GOMGL		AMA	DH			
			VIGSI		LEG	AHL			
Carnation vein mottle	POTY (aphid)	CVMV	CHEGI		CHE	AHL	GOMGL		AMA
			CHEQU		CHE	AHL	PHSVX		LEG
			DINBA		CAF	DH, PH			
Carrot thin leaf	POTY (aphid)	CTLV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEMU		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHL, PH	GOMGL		AMA
			DAUCS		UMB	DH	NIOGT		SOL
			NIOCL		SOL	DH, PH	PHSVX		LEG
							TEATE		AIZ
							VIGSI		LEG
Cassava common mosaic	POTEX	CsCMV	CHEGI		CHE	AHL	PHSVX		LEG
			CHESS		CHE	AHL	VIGSI		LEG
			CHEQU		CHE	AHL			
			MANES		EUP	PH			
			RIICO		EUP	PH			
Cassia yellow blotch	BROMO	CYBV	CASOC		LEG	PH	DATST		SOL
			CHEGI		CHE	AHL	PIBST		LEG
			NIOCL		SOL	DH, PH	VIGSI		LEG
Cauliflower mosaic	CAULIMO	CaMV	BRSRA		CRU	PH	CHEGI		CHE
			BRSCA	JTR	CRU	AHL	CHEQU		CHE
			CRMAB		CRU	PH	CUMSA		CUC
							DATST		SOL
							GOMGL		AMA
							NIOGT		SOL
							NIOTA	XNC	SOL
							PEUHY		SOL
Celery mosaic	POTY (aphid)	CeMV	CHEQU		CHE	AHL	DATST		SOL
			APUGD		UMB	AHS, DH, PH	GOMGL		AMA
			PAVSA		UMB	DH	NIOCL		SOL
							OCIBA		LAB

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Cherry leaf roll	NEPO	CLRV	CHEGI		CHE	AHLS, PH	CUUPE		CUC
			CHEQU		CHE	AHLS, PH	NIOGT		SOL
			CUMSA		CUC	DH	PEUHY		SOL
			NIOTA	WHB	SOL	PH	SOLCU		SOL
			PHSVV	TPR	LEG				
Cherry rasp leaf	? NEPO	CRLV	CHEGI		CHE	DH	SOLME		SOL
			CHEQU		CHE	DH, PH	SPQOL		CHE
			CMOTE		LEG	AHL	VESTH		SCP
			CUMSA		CUC	AHLS, PH			
Chicory yellow mottle	NEPO	ChYMV	CHEQU		CHE	AHL	PIBST		LEG
			CUUPE		CUC	DH, PH	VICFX		LEG
			PHSVX		LEG	DH			
Chrysanthemum B	CARLA	CVB	NIOCL		SOL	PH	CHEGI		CHE
			PEUHY		SOL	AHL, DH	CHEQU		CHE
			TEATE		AIZ	AHL, DH	CUMSA		CUC
							GOMGL		AMA
							VIGSI		LEG
Citrus leaf rugose	ILAR (II)	CiLRV	CHEQU		CHE	DH	CHEAL		CHE
			CUMSA		CUC	DH	DATST		SOL
			GOMGL		AMA	PH	PHSLU		LEG
			VIGSI		LEG	AHL			
Clitoria yellow vein	TYMO	CYVV	NIOCL		SOL	DH, PH	CHEGI		CHE
			PHSVX	LOT	LEG	AHLS	CHEQU		CHE
							DATST		SOL
							GOMGL		AMA
							NIOGT		SOL
Clower yellow mosaic	POTEX	ClYMV	CHEGI		CHE	AHLS	DATST		SOL
			GOMGL		AMA	AHLS, PH	NIOGT		SOL

			PIBST		LEG	PH	NIOTA	SOL
			VICFX		LEG	DH		
Clower yellow vein	POTY (aphid)	ClYVV	CHEQU		CHE	AHL	VIGSI	LEG
			NIOCL		SOL	PH		
			PHSVV	TPR	LEG	DH		
Cocksfoot mild mosaic	? SOBEMO	CMMV	DACGL		GRA	DH	FESPR	GRA
			LOLPS		GRA	AHLS	LOLPE	GRA
			SETIT		GRA	PH	NIOGT	SOL
Cocksfoot mottle	SOBEMO	CoMV	DACGL		GRA	DH, PH	CHEQU	CHE
			HORVX		GRA	DH	NIOCL	SOL
			XXXXX		XXX	AH	NIOGT	SOL
Cocksfoot streak	POTY (aphid)	CSV	DACGL		GRA	DH, PH	AGRIT	GRA
			PASDJ		GRA	AHS	DATST	SOL
							GOMGL	AMA
							NIOGT	SOL
Cowpea aphid-borne mosaic	POTY (aphid)	CABMV	CHEGI		CHE	AHL	DATST	SOL
(= azuki bean mosaic)			GLXMA		LEG	DH, PH	NIOGT	SOL
			PHSVX	BAT	LEG	PH	VICFX	LEG
			PEUHY		SOL	DH		
			VIGSI		LEG	DH, PH		
Cowpea chlorotic mottle	BROMO	CCMV	CHEHY		CHE	AHL	LYPES	SOL
			GLXMA		LEG	DH	NIOGT	SOL
			VIGSI		LEG	PH	PHSLU HEN	LEG
Cowpea mild mottle	CARLA	CPMMV	CHEQU		CHE	AHL	CUMSA	CUC
			NIOCL		SOL	DH, PH	DATST	SOL
			PHSVV	TPR	LEG	DH	NIOGT	SOL
Cowpea mosaic	COMO	CPMV	CHEGI		CHE	AHL	CUMSA	CUC
			VIGSI		LEG	DH, PH		
Cowpea mottle	? CARMO	CPMoV	CHEQU		CHE	AHL, DH	CUMSA	CUC
			PHSVX		LEG	PH	DATST	SOL
			VIGSI		LEG	PH	NIOCL	SOL
							VICFX	LEG
Cowpea severe mosaic	COMO	CPSMV	CHEGI		CHE	AHL	MEDSA	LEG
			VIGSI		LEG	DH, PH		

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Cucumber green mottle mosaic	TOBAMO	CGMMV	CHEGI		CHE	AHL	DATST		SOL
			CUMSA		CUC	DH, PH	NIOGT		SOL
			CUUPM		CUC	PH	PEUHY		SOL
							VIGSI		LEG
Cucumber leaf spot	? CARMO	CLSV	CHEQU		CHE	AHL, DH	DATST		SOL
			CUMSA		CUC	DH, PH	NIOGT		SOL
			NIOBE		SOL	PH	NIOTA	SAM	SOL
							PHSVX		LEG
							VICFX		LEG
Cucumber mosaic	CUCUMO	CMV	CHEQU		CHE	AHL	BEAVA		CHE
			NIOBE		SOL	PH			
			NIOCL		SOL	PH			
			NIOGT		SOL	DH			
			NIOSU		SOL	DH, PH			
			VIGSI		LEG	DH			
Cucumber necrosis	TOMBUS	CuNV	CHEGI		CHE	AHL	XXXXX	XXX	XXX
			CUMSA		CUC	DH, PH			
			GOMGL		AMA	AHL			
Cymbidium mosaic	POTEX	CyMV	CASOC		LEG	AH, DH	LYPES		SOL
			CHEGI		CHE	AHL, DH	NIOGT		SOL
			CMFSS		ORC	PH	PHSVX		LEG
			DATST		SOL	AHL, DH			
Cymbidium ringspot	TOMBUS	CyRSV	CHEQU		CHE	AHL	CUMME		CUC
			NIOCL		SOL	PH	CUMSA		CUC
			NIOGT		SOL	DH	PIBST		LEG
			PHSVX		LEG	AHL	SPQOL		CHE
			VIGSI	BLE	LEG	DH			

Dahlia mosaic	CAULIMO	DMV	AMACA		AMA	DH	CHEGI		CHE
			CHECA		CHE	DH	GOMGL		AMA
			VEEEN		COM	AHLS, DH, PH	NIOGT		SOL
			ZIIEL		COM	DH	PHSVX		LEG
							TEATE		AIZ
Daphne X	POTEX	DVX	CUMSA		CUC	AHL, DH	CPSFR		SOL
			GOMGL		AMA	AHL	VICFX		LEG
			NIOCL		SOL	DH, PH	ZIIEL		COM
Dasheen mosaic	POTY (aphid)	DsMV	PIOSE		ARA	AHS, PH	CPSAN		SOL
							CHEGI		CHE
							CUUPE		CUC
							DATST		SOL
							GOMGL		AMA
							NIOTA	SAM	SOL
							PHSVX	RKY	LEG
							VIGSI		LEG
Desmodium yellow mottle	TYMO	DYMV	DEDTO		LEG	AHL	CHEQU		CHE
			PHSVN	GRN	LEG	DH, PH	NIOGT		SOL
			VIGSI		LEG	DH			
Dioscorea latent	POTEX	DLV	DIUCO		DIO	DH	NIOGT		SOL
			NIOBE		SOL	DH	NIOTA		SOL
			NIOSU		SOL	AHLS, PH			
Eggplant mosaic	TYMO	EMV	CHEGI		CHE	AHLS	PHSVV	TPR	LEG
			CHEQU		CHE	AHLS			
			NIOCL		SOL	DH, PH			
Elderberry carla	CARLA	ECV	CHEGI		CHE	DH	DATME		SOL
(= Elderberry A)			CHEQU		CHE	DH	DATST		SOL
			GOMGL		AMA	AHL, DH, PH	NIOBE		SOL
							NIOCL		SOL
							NIOTA	XNC	SOL
Elderberry latent	? CARMO	ELV	CHEQU		CHE	AHLS, DH	PHSVV	TPR	LEG
			CUMSA		CUC	PH	PIBST		LEG
			DATST		SOL	PH			
			GOMGL		AMA	PH			
			NIOBE		SOL	PH			

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
			NIOCL		SOL	DH, PH			
			NIOGT		SOL	PH			
Elm mottle	ILAR (II)	EMoV	CHEMU		CHE	DH	BRSOX		CRU
			CHEQU		CHE	AHLS, PH	CITLA		CUC
			CUMSA		CUC	PH	TOPMA		TRP
			NIOCL		SOL	PH			
			NIOSU		SOL	DH			
			PHSVX		LEG	AH, DH			
			VIGSI		LEG	AH, DH			
Erysimum latent	TYMO	ErLV	BRSRA		CRU	AHLS	CHEGI		CHE
			BRSCH		CRU	DH, PH	CHEQU		CHE
			SINAL		CRU	DH, PH	NIOCL		SOL
							VIGSI		LEG
Foxtail mosaic	POTEX	FoMV	CHEGI		CHE	AHLS, DH	AGRSM		GRA
			CHEQU		CHE	AHLS	COMSS		CMM
			GOMGL		AMA	DH	CYPES		CYP
			NIOCL		SOL	DH	SORHA		GRA
			NIOSU		SOL	PH			
Frangipani mosaic	TOBAMO	FrMV	DATST		SOL	AHL, DH	CHEGI		CHE
			NIOGT		SOL	DH	CUMSA		CUC
			NIOTA	SAM	SOL	DH	PHSVX		LEG
Galingsoga mosaic	CARMO	GaMV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEQU		CHE	AHL, DH	DATST		SOL
			PHSVX		LEG	AHL, DH, PH	VICFX		LEG
			SPQOL		CHE	PH			
Ginger chlorotic fleck	? SOBEMO	GCFV	ZINOF[7]		ZIN	AH, DH, PH	CHEGI		CHE
							CHEQU		CHE
							NIOGT		SOL
							NIOTA		SOL

Grapevine Bulgarian latent	NEPO	GBLV	CHEQU		CHE	AHLS, PH	CUMSA		CUC
			GOMGL		AMA	DH	NIOTA	HFT	SOL
			NIOCL		SOL	DH	PHSVX		LEG
							VIGSI		LEG
Grapevine chrome mosaic	NEPO	GCMV	CHEQU		CHE	AHLS	CUUPE		CUC
			DATST		SOL	DH	NIOGL		SOL
			GOMGL		AMA	DH	NIOTA	SAM	SOL
			PHSVX		LEG	DH, PH	PEUHY		SOL
Grapevine fanleaf	NEPO	GFLV	CHEQU		CHE	DH, PH	CUUPE		CUC
			CUMSA		CUC	DH, PH	DATST		SOL
			GOMGL		AMA	DH, PH	NIOGT		SOL
			NIOBE		SOL	DH	PEUHY		SOL
			VITVI		VIT	AHS			
Guniea grass mosaic	POTY (aphid)	GGMV	SETIT		GRA	AHS, DH, PH	CHEQU		CHE
			ZEAMX		GRA	DH	DATST		SOL
			PANMA	KOE	GRA	PH	GOMGL		AMA
							NIOCL		SOL
							PHSVN	PIN	LEG
							VIGSI		LEG
Helenium S	CARLA	HVS	CHEAL		CHE	DH	CUMSA		CUC
			CHEGI		CHE	DH	NIOCL		SOL
			CHEQU		CHE	AHLS, DH, PH	PHSVX	RKY	LEG
							VIGSI		LEG
Henbane mosaic	POTY (aphid)	HMV	CHEGI		CHE	DH	CUMSA		CUC
			DATST		SOL	DH	GOMGL		AMA
			NIOSI		SOL	AHLS	PHSVX		LEG
			NIOTA	XNC	SOL	PH	VIGSI		LEG
			SOLHY	TUA	SOL	AHL			
Heracleum latent	? CLOSTERO	HLV	CHEMU		CHE	AHL	NIOCL		SOL
			CHEQU		CHE	DH, PH	NIOTA		SOL
Hibiscus chlorotic ringspot	CARMO	HCRSV	CHEQU		CHE	AHL	AMASS		AMA
			CMOTE		LEG	AHL	CPSSS		SOL
			HIBCA		MAV	PH	CUMSS		CUC
			HIBRS		MAV	DH, PH	DATSS		SOL
							NIOSS		SOL
							TEATE		AIZ

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Hibiscus latent ringspot	NEPO	HLRSV	CHEMU		CHE	AHL, DH	NIOSI		SOL
			CHEQU		CHE	DH	PHSVX		LEG
			GOMGL		AMA	DH	PHYFL		SOL
			HIBCA		MAV	PH	SOLHY	SDA	SOL
			NIOCL		SOL	DH, PH	VIGSI		LEG
Hippeastrum mosaic	POTY (aphid)	HiMV	CHEQU		CHE	AHL	DATST		SOL
			GOMGL		AMA	DH	NIOGT		SOL
			HPSHY		AMY	AHL, PH	TEATE		AIZ
			NIOCL		SOL	DH, PH	VICFX		LEG
Honeysuckle latent	CARLA	HnLV	CHEQU		CHE	AHLS	NIOTA	WHB	SOL
			NIOCL		SOL	DH, PH			
			NIOSU		SOL	PH			
Hop latent	CARLA	HpLV	CHEMU		CHE	AHL	CHEGI		CHE
			HUMLU		MOR	PH	CHEQU		CHE
			PHSVX		LEG	AHL	CUMSA		CUC
							DATST		SOL
							GOMGL		AMA
							NIOCL		SOL
							NIODE		SOL
							NIOGT		SOL
							NIORU		SOL
							PIBST		LEG
Hop mosaic	CARLA	HpMV	CHEAL		CHE	AHL	GOMGL		AMA
			CHEGI		CHE	AHL	NIOSI		SOL
			CHEMU		CHE	AHL	NIOTA	WHB	SOL
			CHEQU		CHE	AHL	PHSVX	KIW	LEG
			HUMLU		MOR	DH	TEATE		AIZ
			NIOCL		SOL	PH	VICFX		LEG
			NIODE		SOL	PH	VIGSI	BLE	LEG

Hydrangea ringspot	POTEX	HRSV	CHEQU		CHE	AHL	NIOGT		SOL
			GOMGL		AMA	AHL, DH	NIOTA	SAM	SOL
			PRIMA		PRI	PH			
Hypochoeris mosaic	? FURO	HyMV	CHEGI		CHE	AHLS	DATST		SOL
	(? TOBAMO)		CHEQU		CHE	AHLS	NIOBE		SOL
			CUMSA		CUC	DH	VIGSI		LEG
			GOMGL		AMA	DH			
			NIOCL		SOL	DH, PH			
Iris fulva mosaic	POTY (aphid)	IFMV	BMCCH		IRI	AHS, PH	CHEGI		CHE
			CHEQU		CHE	AHL, DH	GOMGL		AMA
			IRISS		IRI	PH	NIOCL		SOL
							PHSVX	BTF	LEG
							TEATE		AIZ
							VIGSI		LEG
Iris mild mosaic	POTY (aphid)	IMMV	CHEQU		CHE	AHL	DATST		SOL
			IRIHO	WED	IRI	DH, PH	GOMGL		AMA
			NIOCL		SOL	DH, PH	PEUHY		SOL
			TEATE		AIZ	AHL			
Iris severe mosaic	POTY (aphid)	ISMV	BMCCH		IRI	AHS	CHEGI		CHE
			CVOSS		IRI	DH	CHEQU		CHE
			IRIHO	WED	IRI	PH			
			IRISS		IRI	DH			
Kennedya yellow mosaic	TYMO	KYMV	DATST		SOL	DH	LYPES		SOL
			KENRU		LEG	PH	NIOCL		SOL
			PHSAU		LEG	AHLS			
			VIGSQ		LEG	PH			
Leck yellow stripe	POTY (aphid)	LYSV	ALLPO		LIL	DH, PH	ALLFI		LIL
			CHEGI		CHE	AHL	NIOCL		SOL
			CHEQU		CHE	AHL	NIOSU		SOL
							PEUHY		SOL
							PHSVX	BAT	LEG
							TEATE		AIZ
Lettuce mosaic	POTY (aphid)	LMV	CAUTI		COM	PH	DATST		SOL
			CHEQU		CHE	AHLS, PH	NIOGT		SOL
			GOMGL		AMA	AHL, DH	PEUHY		SOL
			LACSA		COM	DH	PHSVX		LEG

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Lilac chlorotic ringspot	CLOSTERO	LCLV	CHEQU		CHE	AHLS	BEAVA	MOB	CHE
			NIOCL		SOL	DH			
Lilac ring mottle	ILAR (VII)	LRMV	CHEQU		CHE	AHS	AMACA		AMA
			NIOGT		SOL	DH	CUMSA		CUC
			NIOTA	WHB	SOL	SH	LYPES		SOL
Lily symptomless	CARLA	LSV	LILLO		LIL	AHS, DH, PH	NIOCL		SOL
							NIOGT		SOL
							PHSVX		LEG
Lucerne Australian latent	NEPO	LALV	CHEGI		CHE	DH	CUUMA	BUT	CUC
			CHEQU		CHE	AHLS	VICFX		LEG
			GOMGL		AMA	DH, PH			
			PIBST		LEG	PH			
Lucerne transient streak	SOBEMO	LTSV	CHEGI		CHE	AHL(S)	CUMSA		CUC
			CHEQU		CHE	AHL(S)	GOMGL		AMA
			NIOCL		SOL	DH, PH	NIOGT		SOL
			PIBST		LEG	DH	PHSVX		LEG
							VIGSI		LEG
Maclura mosaic	? POTY (aphid)	MacMV	CHEGI		CHE	AHL	CUMSA		CUC
			NIOCL		SOL	DH, PH	GOMGL		AMA
			TEATE		AIZ	DH, PH	NIOSU		SOL
							PEUHY		SOL
							VIGSI		LEG
Maize chlorotic mottle	? SOBEMO	MCMV	ZEAMX		GRA	DH, PH	SORVU	AGB	GRA
			TRZAX	PAR	GRA	DH			
			XXXXX		XXX	AH			
Melandrium yellow fleck	BROMO	MYFV	CHEQU		CHE	AHL	BRSOX		CRU
			GOMGL		AMA	DH, PH	DINCA	WLS	CAF
			NIOCL		SOL	DH, PH	TRFIN		LEG
			TEATE		AIZ	DH			

Melon necrotic spot	CARMO	MNSV	CUMAN	LGI	CUC	DH	LGNSI		CUC
			CUMME		CUC	AH, DH, PH	VIGSI	BLE	LEG
			CUMSA		CUC	AH, DH, PH			
			VIGSQ		LEG	AHL			
Mulberry ringspot	NEPO	MRSV	CHEQU		CHE	DH	CHEGI		CHE
			GLXMA		LEG	DH	NIOGT		SOL
			NIOCL		SOL	PH			
			VIGSI	BLE	LEG	AHLS			
Mungbean yellow mosaic	GEMINI (II)	MYMV	PHSVV	TCR	LEG	DH, PH	DOLLA		LEG
			PHSAU		LEG	AHS	PHSLU		LEG
							VIGSI		LEG
Myrobalan latent ringspot	NEPO	MLRSV	CHEQU		CHE	AHL	ZIIEL		COM
			NIOCL		SOL	PH			
			NIOTA	XNC	SOL	DH			
Narcissus latent	CARLA	NLV	NIOBE		SOL	DH	NIOTA	WHB	SOL
(= gladiolus ringspot)			NIOCL		SOL	DH, PH	PEUHY		SOL
			TEATE		AIZ	AHL	VICFX		LEG
Narcissus mosaic	POTEX	NMV	CHEGI		CHE	AHL	NIOGT		SOL
			GOMGL		AMA	DH, PH	NIOTA	WHB	SOL
			NIOCL		SOL	PH			
Narcissus tip necrosis	? CARMO	NTNV	NARSS		AMY	DH	CHEQU		CHE
			NARXX	BAB	AMY	DH	NIOTA		SOL
			XXXXX		XXX	AH			
Narcissus yellow stripe	POTY (aphid)	NYSV	NARPS		AMY	DH	XXXXX		XXX
			TEATE		AIZ	AHL, PH			
Nerine X	POTEX	NVX	CHEGI		CHE	DH	CUMSA		CUC
			CHEQU		CHE	AHL, PH	VICFX		LEG
			GOMGL		AMA	DH	ZIIEL		COM
			TEATE		AIZ	DH			
Nicotiana velutina mosaic	? FURO	NVMV	CHEQU		CHE	AHL	TEATE		AIZ
			GOMGL		AMA	DH			
			NIOGT		SOL	DH, PH			
Oat mosaic	POTY (fungus)	OMV	AVESA		GRA	AHS, DH, PH	HORVX		GRA
							TRZAX		GRA

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Oat necrotic mottle	POTY (? mite)	ONMV	AVESA		GRA	DH	HORVX		GRA
			BROMO		GRA	DH	SECCE		GRA
			LOLMU		GRA	DH	TRZAX		GRA
			XXXXX		XXX	AH			
Odontoglossum ringspot	TOBAMO	ORSV	CHEQU		CHE	AHL	DATST		SOL
			NIOCL		SOL	PH			
			NIOTA	XNC	SOL	AHL, DH			
Okra mosaic	TYMO	OkMV	CHEGI		CHE	DH	AMACA		AMA
			CUMSA		CUC	DH, PH	CPSAN		SOL
			NIOCL		SOL	DH	DATST		SOL
			VIGSI		LEG	DH	NIOGT		SOL
			XXXXX		XXX	AH	NIOTA	XNC	SOL
							PHSVV	TPR	LEG
							ZIIEL		COM
Olive latent ringspot	NEPO	OLRSV	CHEGI		CHE	DH	NIOGT		SOL
			CHEQU		CHE	DH, PH	NIOTA	SAM	SOL
			GOMGL		AMA	AHL	NIOTA	WHB	SOL
							PHSAU		LEG
Onion yellow dwarf	POTY (aphid)	OYDV	ALLCE		LIL	DH, PH	ALLPO		LIL
			CHEGI		CHE	AHL			
			CHEQU		CHE	AHL			
Panicum mosaic	? SOBEMO	PMV	PANMI		GRA	AHS	CHEGI		CHE
			SETIT		GRA	AHS	CHEQU		CHE
			ZEAMX		GRA	DH, PH	CUMME		CUC
							DATST		SOL
							GOMGL		AMA
							NIOGT		SOL
							TEATE		AIZ

Papaya mosaic	POTEX	PapMV	ATHMM		SCP	PH	DATST		SOL
			CIAPA		CAA	DH, PH	NIOGT		SOL
			CHEGI		CHE	DH	PHSVX		LEG
			GOMGL		AMA	AHL	TEATE		AIZ
Papaya ringspot (= watermelon mosaic virus 1)	POTY (aphid)	PRSV	CHEGI		CHE	AHL	NIOBE		SOL
			CHEQU		CHE	AHL			
			CUUPE		CUC	DH, PH			
			LUFAC		CUC	DH			
Parsnip mosaic	POTY (aphid)	ParMV	CHEGI		CHE	AHL	CUMME		CUC
			CHEQU		CHE	AHL, PH	CUMSA		CUC
			PAVSA		UMB	DH	DATST		SOL
							NIOCL		SOL
							NIOGT		SOL
							NIOTA	SAM	SOL
							PHSVX		LEG
							TEATE		AIZ
							VIGSI		LEG
Passionfruit woodiness	POTY (aphid)	PWV	CHEGI		CHE	AHL	DATST		SOL
			GLXMA		LEG	PH	NIOGT		SOL
			PAQED		PAS	DH, PH	VIGSI		LEG
			PHSVX	BTF	LEG	PH			
Pea early-browning	TOBRA	PEBV	CHEGI		CHE	AHL	LYPES		SOL
			NIOCL		SOL	DH, PH	MEDSA		LEG
			PHSVX		LEG	AHL			
			VICFX		LEG	DH			
Pea enation mosaic	PEMV group	PEMV	CHEGI		CHE	AH	CUMSA		CUC
			CHEQU		CHE	AH	DATST		SOL
			NIOCL		SOL	PH	NIOGT		SOL
			PIBST		LEG	DH, PH	TEATE		AIZ
			TRFIN		LEG	DH			
			VICFX		LEG	DH			
Pea seed-brone mosaic	POTY (aphid)	PSbMV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEQU		CHE	DH	DATST		SOL
			PIBST		LEG	DH, PH	NIOGT		SOL
			VICFX		LEG	DH, PH	NIOTA	XNC	SOL
							PHSVX		LEG

Table 8 (continued)

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Pea streak (= alfalfa latent)	CARLA	PeSV	CHEGI		CHE	AHL	CUMSA		CUC
			GOMGL		AMA	AHL	NIOGT		SOL
			PIBST		LEG	DH, PH	NIOTA		SOL
			VICFX		LEG	DH	PEUHY		SOL
Peach rosette mosaic	NEPO	PRMV	CHEGI		CHE	DH	VITLA	DEL	VIT
			CHEQU		CHE	AHLS, PH			
Peanut clump	FURO	PCV	CHEGI		CHE	AHLS	CUMME		CUC
			NIOBE		SOL	DH, PH	GOMGL		AMA
			NIOGT		SOL	DH	TEATE		AIZ
			THSVX		LEG	PH	VICFX		LEG
Peanut mottle	POTY (aphid)	PeMoV	CASOC		LEG	PH	CUMSA		CUC
			NIOCL		SOL	DH, PH	GOMGL		AMA
			PHSVV	TPR	LEG	AHLS			
Peanut stunt (= robinia mosaic)	CUCUMO	PSV	CHEQU		CHE	AHL	GLXMA		LEG
			GOMGL		AMA	PH	MEDSA		LEG
			NIOSU		SOL	PH			
			PHSVX		LEG	AHLS, DH			
			VIGSI		LEG	PH			
Pelargonium flower-break	CARMO	PFBV	CHEQU		CHE	AHLS	PELGR	NIL	GER
			GOMGL		AMA	DH			
			NIOCL		SOL	DH, PH			
Pepper mild mottle	TOBAMO	PMMV	CPSSS		SOL	AHS	LYPES		SOL
			CHEGI		CHE	DH	NIOGL		SOL
			CHEQU		CHE	DH			
			DATST		SOL	AHL, DH			
			NIOSI		SOL	AHL, DH			
			NIOTA	XNC	SOL	DH			
Pepper mottle	POTY (aphid)	PepMoV	CPSFR	TAB	SOL	AHL, DH	NIOTA	VTO	SOL
			CHEGI		CHE	AHL			

			NIOBE		SOL	PH			
			NIOGT		SOL	PH			
			NIOTA	XNC	SOL	PH			
Pepper veinal mottle	POTY (aphid)	PVMV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEQU		CHE	AHL	GOMGL		AMA
			NIOCL		SOL	DH, PH	PHSVX		LEG
			NIOSU		SOL	DH, PH	VICFX		LEG
Peru tomato	POTY (aphid)	PTV	CHEQU		CHE	AHL	SOLHY	TUA	SOL
			NIOOC		SOL	DH, PH			
			NIOTA	BUR	SOL	PH			
Plantain X	POTEX	PlVX	NIOCL		SOL	DH, PH	CHEQU		CHE
			NIOHY	EDW	SOL	AHLS, DH	GOMGL		AMA
							NIOGT		SOL
Plum pox	POTY (aphid)	PPV	CHEVU		CHE	AHL, DH	CUMSA	LGT	CUC
			NIOCL		SOL	PH	DATST		SOL
			NIOSU		SOL	PH	GOMGL		AMA
Poinsettia mosaic	? TYMO	PnMV	NIOBE		SOL	DH, PH	CHEGI		CHE
			XXXXX		XXX	AH	CHEQU		CHE
							NIOCL		SOL
							NIOGT		SOL
Pokeweed mosaic	POTY (aphid)	PkMV	CHEQU		CHE	AHL	CUMSA		CUC
			GOMGL		AMA	DH, PH	DATST		SOL
			PHTAM		PHY	DH	NIOCL		SOL
							NIOGT		SOL
							PHSVX		LEG
							VIGSI		LEG
Poplar mosaic	CARLA	PopMV	NIOGT		SOL	DH	CHEGI		CHE
			NIOSU		SOL	DH, PH	CHEQU		CHE
			PEUHY		SOL	DH, PH	PHSVX		LEG
			VIGSI		LEG	AHLS, PH			
Potato A	POTY (aphid)	PVA	LYPPI		SOL	DH, PH	DATME		SOL
			NICPH		SOL	PH	VIGSI		LEG
			NIOGT		SOL	PH			
			SOLHY	SDA	SOL	AHL			
			SOLHY	TUA	SOL	AHL			

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Potato aucuba mosaic	POTEX	PAMV	CPSAN		SOL	AHLS, DH, PH	CUMSA		CUC
			NIOGT		SOL	DH, PH	VIGSI		LEG
							ZIIEL		COM
Potato black ringspot	NEPO	PBRSV	CHEGI		CHE	AHL	SOLPJ	CPC	SOL
			CHEQU		CHE	AHL, DH	SOLAD	CPC	SOL
			CUMSA		CUC	PH			
			DATST		SOL	DH, PH			
			GOMGL		AMA	PH			
			NIOBE		SOL	PH			
			NIOCL		SOL	PH			
			NIOTA	XNC	SOL	PH			
			PHSVV	TPR	LEG	PH			
Potato M	CARLA	PVM	CHEQU		CHE	AHL	CUUPE		CUC
			DATME		SOL	AHL, DH	NIOTA	XNC	SOL
			GOMGL		AMA	AHL	PEUHY		SOL
			LYPES		SOL	PH	TINER		CMM
			NIODE		SOL	DH			
			PHSVX		LEG	AHL			
			SOLCU		SOL	DH			
Potato mop-top	FURO	PMTV	CHEGI		CHE	AHL	CHECA		CHE
			CHEQU		CHE	DH	CUMSA		CUC
			DATST		SOL	PH	GOMGL		AMA
			NIOBE		SOL	PH	PHSVX		LEG
			NIOCL		SOL	PH	VICFX		LEG
			NIODE		SOL	PH	VIGSI		LEG
			NIOGT		SOL	PH			
			NIOSU		SOL	PH			
			NIOSI		SOL	PH			
			NIOTA	XNC	SOL	DH, PH			

			TEATE		AIZ	DH			
Potato S (= pepino latent)	CARLA	PVS	CHEAL		CHE	AH	CUUPE		CUC
			CHEGI		CHE	AHL	NIOTA	XNC	SOL
			CHEQU		CHE	AHL	PEUHY		SOL
			NIOCL		SOL	PH			
			NIODE		SOL	DH			
Potato V	POTY (aphid)	PVV	NIOCL		SOL	AHS	CHEGI		CHE
			NIODE		SOL	AHLS, PH	CHEQU		CHE
			NIOGT		SOL	AHS	CUMSA		CUC
			NIOOC		SOL	DH, PH	DATST		SOL
			NIOTA	WHB	SOL	DH, PH	GOMGL		AMA
			SOLTU	MAP	SOL	DH	PHSVN	PIN	LEG
							VIGSI		LEG
Potato X	POTEX	PVX	DATST		SOL	DH, PH	CRMAB		CRU
			GOMGL		AMA	AHL	CUUPE		CUC
			NIOTA	XNC	SOL	PH	CUMSA		CUC
							GLXMA		LEG
Potato Y	POTY (aphid)	PVY	CHEGI		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHL			
			LYUSS		SOL	AHL			
			NIOTA	XNC	SOL	DH, PH			
			PHYFL		SOL	DH, PH			
			SOLHY	TUA	SOL	AHL			
Prune dwarf	ILAR (III)	PDV	CVTSP		LEG	AHL	CHEQU		CHE
			CUMSA		CUC	DH, PH	NIOTA		SOL
			CUUMA		CUC	DH			
Prunus necrotic ringspot	ILAR (III)	PNRSV	CUMSA		CUC	AHLS, DH, PH	NIOTA		SOL
(= some isolates of rose mosaic)				CMOTE		LEG	DH	SOLSY	
SOL									
Quail pea mosaic	COMO	QPMV	DOLLA		LEG	AHL	GOMGL		AMA
			GLXMA		LEG	AHL			
			PHSVX	BLV	LEG	PH			
			PHSVN	PIN	LEG	AHL			
Radish mosaic	COMO	RaMV	BRSRA		CRU	DH, PH	NIOTA	HFF	SOL
			CHEGI		CHE	AHL			
Raspberry bushy dwarf	"IDAEO"	RBDV	CHEGI		CHE	DH	CUMSA	SPM	CUC
			CHEMU		CHE	AHL	LYPES		SOL

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
			CHEQU		CHE	AHLS	NIODE		SOL
			PHSVV	TPR	LEG	AHL, DH	NIOSI		SOL
							NIOTA	SAM	SOL
							VIGSI	BLE	LEG
Raspberry ringspot	NEPO	RRSV	CHEGI		CHE	AHL	VICFX		LEG
			CHEQU		CHE	AHLS			
			NIOCL		SOL	PH			
			PEUHY		SOL	PH			
			PHSVV	TPR	LEG	DH			
Red clover mottle	CARMO	RCMV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEQU		CHE	AHL, DH	NIOGT		SOL
			GOMGL		AMA	DH			
			PHSVX		LEG	AHL			
			VICFX		LEG	DH, PH			
Red clover necrotic mosaic	DIANTHO	RCNMV	CHEQU		CHE	AHL	PEUHY		SOL
			GOMGL		AMA	DH			
			NIOCL		SOL	PH			
			PHSVX		LEG	PH			
Red clover vein mosaic	CARLA	RCVMV	CHEGI		CHE	AHL	CUMSA		CUC
			CHEQU		CHE	AH, DH	DATST		SOL
			GOMGL		AMA	AHL, DH	NIOGT		SOL
			PIBST		LEG	DH, PH	PHSAU		LEG
			VICFX		LEG	DH			
Ribgrass mosaic	TOBAMO	RMV	CHEGI		CHE	DH	PHSVX		LEG
			NIOGT		SOL	AHL			
			NIOTA	SAM	SOL	PH			
			NIOTA	TUR	SOL	AHL			
			NIOTA	XNC	SOL	AHL			

Rice necrosis mosaic	POTY (fungus)	RNMV	ORYSA		GRA	DH, PH	CHEGI		CHE
			XXXXX		XXX	AH	CHEQU		CHE
							NIOTA		SOL
Rice yellow mottle	SOBEMO	RYMV	ORYSA		GRA	DH, PH	AVESA		GRA
			XXXXX		XXX	AHL	NIOGT		SOL
							SECCE		GRA
							ZEAMX		GRA
Robinia mosaic	CUCUMO	RbMV	CHEMU		CHE	AHL, DH	AMARE		AMA
			CHEQU		CHE	AHLS	OCIBA		LAB
			NIOGT		SOL	PH			
			VIGSI		LEG	DH			
Ryegrass mosaic	POTY (mite)	RGMV	AGRRE		GRA	DH, PH	TRZAX		GRA
			LOLMU	STT	GRA	DH, PH			
			XXXXX		XXX	AH			
Saguaro cactus	CARMO	SCV	CHEGI		CHE	AHL	DATME		SOL
			CHECA		CHE	DH, PH	NIOGT		SOL
			CHEMU		CHE	PH	VIGSI	BLE	LEG
			CHEQU		CHE	AHL			
			GOMGL		AMA	DH			
Satsuma dwarf	? NEPO	SDV	CHEQU		CHE	AHL	CIDUN	MXL	RUB
			PHYFL		SOL	DH, PH	CIDUN	RGL	RUB
			VIGSI	BLE	LEG	DH			
Scrophularia mottle	TYMO	ScrMV	ATHMM		SCP	DH, PH	ZEAMX		GRA
			CHEQU		CHE	DH			
			DATST		SOL	DH, PH			
			VICFX		LEG	AHL			
Shallot latent	CARLA	SLV	ALLPO		LIL	PH	ALLNE		LIL
			CHEGI		CHE	AHL, DH			
			CHEQU		CHE	AHL, DH			
Soilborne wheat mosaic	FURO	SBWMV	CHEGI		CHE	AHL	NIOTA		SOL
			CHEQU		CHE	AHL	PHSVX		LEG
			TRZAX	MIA	GRA	DH, PH			
Solanum nodiflorum mottle	SOBEMO	SNMV	NIOCL		SOL	DH, PH	CHEGI		CHE
			NIODE		SOL	AHL	CHEQU		CHE
			NIOVE		SOL	DH	DATST		SOL

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
			SOLAM		SOL	DH, PH	NIOGT		SOL
							PHSVX		LEG
							VICFX		LEG
							VIGSI		LEG
Southern bean mosaic	SOBEMO	SBMV	GLXMA		LEG	DH	CASTO		LEG
			PHSVX		LEG	AHL, PH	CIEAR		LEG
			VIGSI		LEG	DH	LUPAL		LEG
							MEUAL		LEG
Sowbane mosaic	SOBEMO	SoMV	BEAVA		CHE	DH	CUMSA	DEL	CUC
			CHEGI		CHE	AHL	CUUMA		CUC
			CHEHY		CHE	PH	DATST		SOL
			CHEMU		CHE	DH, PH	NIOGT		SOL
							NIOSU		SOL
							NIOTA	XNC	SOL
							PHSVN	PIN	LEG
							VIGSI		LEG
Soybean chlorotic mottle	CAULIMO	SbCMV	DOLLA		LEG	DH	CHEGI		CHE
			GLXMA		LEG	AHS, PH	CHEQU		CHE
			XXXXX		XXX	AHL	CUMSA		CUC
			PHSVX	KIN	LEG	PH	DATST		SOL
							GOMGL		AMA
							NIOCL		SOL
							NIOTA		SOL
							TEATE		AIZ
Soybean mosaic	POTY (aphid)	SbMV	CHEQU		CHE	AHL, DH	CUMSA		CUC
			CMOTE		LEG	AHS	DATST		SOL
			DOLLA		LEG	AHS	GOMGL		AMA
			GLXMA		LEG	AHS, PH	NIOTA		SOL

			PHSVX		LEG	AHLS	VICFX		LEG
Spinach latent	ILAR	SpLV	BEAVA		CHE	DH	CUMSA		CUC
			CHEGI		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHLS, PH	LYPES		SOL
			NIOSS		SOL	PH	PEUHY		SOL
			PHSVX	BAT	LEG	AHL	PHSVV	TPR	LEG
Squash mosaic	COMO	SMV	CUMSA		CUC	DH	CITLA		CUC
			CUUPE		CUC	AHS, DH, PH			
			XXXXX		XXX	AHL			
Strawberry latent ringspot	? NEPO	SLRSV	CHEGI		CHE	AHLS	AMACA		AMA
			CHEMU		CHE	AHLS	CUUFI		CUC
			CHEQU		CHE	AHLS, DH			
			CUMME		CUC	PH			
			CUMSA		CUC	PH			
			DATST		SOL	AH			
			TEATE		AIZ	DH, PH			
Subterranean clover mottle	SOBEMO	SCMoV	PIBST		LEG	AHL, DH	CHEGI		CHE
			PRFSU		LEG	PH	CHEQU		CHE
							NIOCL		SOL
Sugarcane mosaic	POTY (aphid)	SCMV	SORVU	RIO	GRA	DH, PH	CHEGI		CHE
			ZEAMX		GRA	DH, PH	CHEQU		CHE
			XXXXX		XXX	AHL	DATST		SOL
							NIOGT		SOL
							VIGSI		LEG
Sunn-hemp mosaic	TOBAMO	SHMV	CMOTE		LEG	DH	CUMSA		CUC
			NIOGT		SOL	AHL			
			NIOTA	XNC	SOL	AHL			
			PHSVV	TPR	LEG	DH, PH			
Sweet clover necrotic mosaic	DIANTHO	SCNMV	CHEGI		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHL	PEUHY		SOL
			NIOCL		SOL	DH, PH	ZIIEL		COM
			NIOGT		SOL	DH			
			PHSVX	RKY	LEG	DH, PH			
Sweet potato mild mottle	POTY (whitefly)	SPMMV	CHEGI		CHE	AHL	CHECA		CHE
			CHEQU		CHE	AHL	CUMSA		CUC

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
			GOMGL		AMA	AHL	PHSVN	PIN	LEG
			IPONI	SOM	COM	DH	PHYFL		SOL
			NIOCL		SOL	PH	VICFX		LEG
			NIOGT		SOL	DH, PH	VIGSI	BLE	LEG
			NIOTA	WHB	SOL	DH, PH			
Tephrosia symptomless	? CARMO	TeSV	GLXMA		LEG	DH, PH	CHEQU		CHE
			PHSVX	LOT	LEG	DH	GOMGL		AMA
			PHSVV	TCR	LEG	DH			
			XXXXX		XXX	AHL			
Tobacco etch	POTY (aphid)	TEV	CHEGI		CHE	AHL	PIBST		LEG
			CHEQU		CHE	AHL	SPQOL		CHE
			DATST		SOL	DH, PH			
			NIOGT		SOL	PH			
			NIOSS		SOL	DH			
Tobacco mosaic	TOBAMO	TMV	CHEGI		CHE	AHL	PIBST		LEG
			CHEQU		CHE	AHL	TRFSS		LEG
			NIOGT		SOL	AHL, DH			
			NIOSI		SOL	DH			
			NIOTA	SAM	SOL	DH, PH			
			NIOTA	XNC	SOL	AHL, DH, PH			
			PHSVN	PIN	LEG	AHL			
Tobacco necrosis	NECRO	TNV	CHEGI		CHE	AHL	CUMSA		CUC
			NIOCL		SOL	PH	PIBST		LEG
			PHSAU		LEG	AHL			
			PHSVV	TPR	LEG	AHL, DH, PH			
Tobacco rattle	TOBRA	TRV	CHEGI		CHE	AHL	CHECA		CHE
			NIOCL		SOL	DH, PH	CUUPE		CUC
			PHSVX		LEG	AHL, DH			

Tobacco ringspot	NEPO	TRSV	CHEQU		CHE	AHL	LYPES		SOL
			CUMSA		CUC	PH			
			NIOCL		SOL	DH, PH			
			NIOSS		SOL	PH			
			PHSVX		LEG	DH			
			VIGSI		LEG	DH			
Tobacco streak	ILAR (I)	TSV	CHEQU		CHE	PH	NIOGT		SOL
			CMOTE		LEG	AHL	PEUHY		SOL
			DATST		SOL	PH	PHSMU		LEG
			GOMGL		AMA	AHL	SPQOL		CHE
			NIOTA		SOL	DH, PH	ZIIEL		COM
			PHSVX		LEG	AHL			
Tobacco vein mottling	POTY (aphid)	TVMV	NIOTA	BUR	SOL	AH, DH, PH	CPSAN		SOL
			NIOTA	KEN	SOL	AHS, PH	CPSFR		SOL
			XXXXX		XXX	AHL			
Tomato aspermy	CUCUMO	TAV	CHEGI		CHE	AHL	BRSOB		CRU
			CHEQU		CHE	AHL	CUUPE		CUC
			CUMSA		CUC	DH	PHSVN	PIN	LEG
			NIOCL		SOL	DH, PH	PIBST		LEG
			NIOGT		SOL	DH, PH	TOPMA		TRP
			VIGSI		LEG	AHL	VICFX		LEG
Tomato black ring	NEPO	TBRV	CHEGI		CHE	AHLS	MEDSA		LEG
			CHEQU		CHE	AHLS			
			NIOCL		SOL	PH			
			PEUHY		SOL	DH, PH			
			PHSVV	TPR	LEG	DH			
Tomato bushy stunt	TOMBUS	TBSV	CHEGI		CHE	AHLS	BRSPK		CRU
			CHEQU		CHE	AHLS	GLXMA		LEG
			DATST		SOL	PH	PIBST		LEG
			GOMGL		AMA	DH	TOPMA		TRP
			NIOCL		SOL	DH, PH	SOLNI		SOL
			NIOGT		SOL	AHLS, PH			
			PEUHY		SOL	PH			
Tomato golden mosaic	GEMINI (II)	TGMV	DATST		SOL	DH, PH	SOLNI		SOL
			NIOBE		SOL	AHLS, PH	SOLTU	MAB	SOL
			NIOGT		SOL	AHLS			

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Tomato mosaic	TOBAMO	ToMV	CHEGI		CHE	AHL	PHSVV	TPR	LEG
			CHEQU		CHE	AHL	VIGSI	BLE	LEG
			DATST		SOL	DH			
			NIOCL		SOL	PH			
			NIOGT		SOL	AHL			
			NIOTA	SAM	SOL	PH			
Tomato ringspot	NEPO	ToRSV	CHEQU		CHE	AHLS	TEATE		AIZ
			CUMSA		CUC	DH, PH			
			NIOCL		SOL	PH			
			NIOTA	XNC	SOL	DH			
			PEUHY		SOL	DH, PH			
			VIGSI		LEG	AHLS			
Tomato spotted wilt	TOSPO	TSWV	GOMGL		AMA	PH	TEATE		AIZ
			NIOCL		SOL	PH			
			NIOGT		SOL	DH			
			PEUHY		SOL	AHL			
			TOPMA		TRP	DH, PH			
Tulare apple mosaic	ILAR (II)	TAMV	NIOTA		SOL	DH, PH	CHEQU		CHE
			PHSVX	BTF	LEG	AHL			
Tulip breaking	POTY (aphid)	TBV	CHEGI[8]		CHE	PH	NIOTA	WHB	SOL
			LILFO[9]		LIL	DH	PHSVX		LEG
			NIOBE[9]		SOL	PH	VICFX		LEG
			NIOCL[8]		SOL	PH			
			XXXXX		XXX	AH			
Tulip X	POTEX	TVX	CHEGI		CHE	AHLS	NIOCL		SOL
			CHEQU		CHE	PH			
Turnip crinkle	CARMO	TCV	BRSPK		CRU	PH	BEAVA		CHE
			CHEGI		CHE	AHL	NICPH		SOL

			CHEMU		CHE	DH	NIOGT		SOL
			CHEQU		CHE	AHL	PIBST		LEG
			DATST		SOL	DH	TOPMA		TRP
			TEATE		AIZ	DH			
Turnip mosaic	POTY (aphid)	TuMV	BRSRA		CRU	PH	PHSVX		LEG
			BRSPK		CRU	PH	VICFX		LEG
			CHEGI		CHE	AHL			
			CHEQU		CHE	AHL			
			MTLIN		CRU	DH			
			NIOTA		SOL	AHL			
Turnip rosette	SOBEMO	TRoV	BRSPK		CRU	AHLS, PH	NIOTA		SOL
			NIOCL		SOL	DH			
Turnip yellow mosaic	TYMO	TYMV	BRSPK		CRU	DH	CHEGI		CHE
			BRSRA		CRU	DH	CUMSA		CUC
			CRMAB		CRU	PH	NIOGT		SOL
			XXXXX		XXX	AHL	NIOTA		SOL
							PHSVX		LEG
							PHYFL		SOL
Ullucus C	COMO	UVC	CHEGI		CHE	AHLS	CUMSA		CUC
			CHEMU		CHE	AHL, DH	DATST		SOL
			CHEQU		CHE	AHLS, PH	NIOGT		SOL
			TEATE		AIZ	AHL	NIOTA	XNC	SOL
Velvet tobacco mottle	SOBEMO	VTMoV	NIOCL		SOL	AHLS, PH	CHEGI		CHE
			NIOHY	GTC	SOL	DH	CHEQU		CHE
			NIOGT		SOL	DH	CUMSA		CUC
			NIOVE		SOL	DH	PHSVX		LEG
							VIGSI		LEG
							ZIIEL		COM
Viola mottle	POTEX	VMV	CHEGI		CHE	AHLS	CUMSA		CUC
			CHEQU		CHE	AHLS, PH	NIOGT		SOL
			GOMGL		AMA	AHLS	PEUHY		SOL
			NIOCL		SOL	DH	PHSVX		LEG
Voandzeia necrotic mosaic	TYMO	VNMV	CHEGI		CHE	AHL	CHEQU		CHE
			DOLLA		LEG	DH	CUMSA		CUC
			VIGSI		LEG	DH	GOMGL		AMA
			VOASU		LEG	PH	NIOGT		SOL

Table 8 (continued)

Virus name	Family/group or subgroup[2]	Acronym[3]	Code of hosts[4]				Code of non-hosts[4]		
			Code of botanical name	Cultivar or hybrid code	Code of family[5]	Code of host character[6]	Code of botanical name	Cultivar or hybrid code	Code of family[5]
Watermelon mosaic virus 2	POTY (aphid)	WMV2	CHEGI		CHE	AHL	DATST		SOL
			CHEQU		CHE	AHL	NIOGT		SOL
			CUUPE	SMS	CUC	DH, PH	PEUHY		SOL
			NIOBE		SOL	PH	RANSA		RAN
Wheat spindle streak mosaic	POTY (fungus)	WSSMV	TRZAX	KEN	GRA	DH, PH	AMARE		AMA
			TRZDU		GRA	DH, PH	CHEAL		CHE
			XXXXX		XXX	AHL			
Wheat streak mosaic	POTY (mite)	WSMV	AVESA		GRA	DH, PH	AGRRE		GRA
			HORVX		GRA	DH, PH	BROIN		GRA
			TRZAX		GRA	DH, PH	SORVU		GRA
			XXXXX		XXX	AHL			
White clover mosaic	POTEX	WClMV	CUMSA		CUC	DH	ATHMM		SCP
			PHSVX		LEG	AHLS, PH	CHEGI		CHE
			VICFX		LEG	DH	GOMGL		AMA
			VIGSI		LEG	AHLS			
Wild cucumber mosaic	TYMO	WCMV	CUMME		CUC	AHL	CHEGI		CHE
			CUMSA		CUC	DH	NIOTA	SAM	SOL
			CUUPE		CUC	DH, PH	VIGSI		LEG
Wineberry latent	POTEX	WLV	CHEGI		CHE	AHL, PH	NIOSS		SOL
			CHEQU		CHE	AHL, PH			
			GOMGL		AMA	DH			
Yam mosaic	POTY (aphid)	YMV	DIUCA		DIO	AHS, PH	DIUCO		DIO
			NIOBE		SOL	PH	DIUFL		DIO
			NIOSU		SOL	DH			

Zucchini yellow mosaic	POTY (aphid)	ZYMV	CHEGI	CHE	AHL	LVATR	MAV
			CHEQU	CHE	DHL		
			CUUPE	CUC	DH, PH		
			GOMGL	AMA	DH		
			LUFAC	CUC	DH, PH		

[1]Modified compilation from Horváth (1993a). [2]From Hull et al. (1991). Roman numerals (I, II, etc.) indicate recognized subgroups. The vector (e.g., mite, aphid, leafhopper, etc.) is indicated where of taxonomic importance. Question mark indicates uncertain taxonomic position, or other doubt (e.g., ? CLOSTERO, ? NEPO, etc.). [3]Proposed standard acronym of viruses (see Hull et al., 1991). [4,5]See the code of hosts, non-hosts and families by Anonymous (1992) and Horváth (1993c). [6]AH = Assay host, AHL = Local assay host, AHLS = Local and systemic assay host, AHL(S) = Local assay host, when some virus strains produce also systemic symptoms, AHS = Systemic assay host, DH = Diagnostic host, PH = Propagation host. [7]Only one known host. [8]Susceptible to many lili isolates, but no tulip isolates (Derks, 1991). [9]Susceptible to all lily and tulip isolates (Derks, 1991)

Table 9

Species reported susceptible to forty-fifty per cent or more of viruses in different groups

Group	No. of member	Species[1]
Alfalfa mosaic virus[2]	1	*Chenopodium amaranticolor*[3]
		Chenopodium quinoa
		Phaseolus vulgaris
		Vigna unguiculata
		Nicotiana benthamiana
		Nicotiana glutinosa
		Nicotiana tabacum
Bromovirus	5	*Chenopodium amaranticolor*
		Nicotiana clevelandii
		Pisum sativum
		Phaseolus vulgaris
		Chenopodium hybridum
		Chenopodium quinoa
Carlavirus	18	*Chenopodium quinoa*
		Chenopodium amaranticolor
		Nicotiana clevelandii
Carmovirus	14	*Chenopodium quinoa*
		Phaseolus vulgaris
		Chenopodium amaranticolor
Caulimovirus	5	0[4]
		Brassica campestris
		Saponaria vaccaria
		Zinnia elegans
		Phaseolus vulgaris
		Dahlia pinnata
Closterovirus	5	*Chenopodium quinoa*
		Chenopodium murale
		Chenopodium capitatum
		Chenopodium amaranticolor
		Beta vulgaris
Comovirus	12	*Phaseolus vulgaris*
		Chenopodium amaranticolor
Cucumovirus	4	*Chenopodium quinoa*[5]
		Lycopersicon esculentum[5]
		Vigna sinensis
		Chenopodium amaranticolor
		Phaseolus vulgaris
		Nicotiana glutinosa
		Nicotiana tabacum
		Cucumis sativus
		Nicotiana clevelandii
Dianthovirus	3	*Chenopodium quinoa*[5]
		Gomphrena globosa[5]
		Nicotiana clevelandii[5]
		Phaseolus vulgaris[5]
		Nicotiana tabacum

Group	No. of member	Species[1]
		Nicotiana glutinosa
		Cucumis sativus
		Vicia faba
		Vigna sinensis
		Chenopodium amaranticolor
Furovirus	7	*Chenopodium amaranticolor*
		Chenopodium quinoa
		Tetragonia expansa
Geminivirus	4	*Nicotiana benthamiana*
		Phaseolus vulgaris
		Datura stramonium
Idaeovirus	1	*Chenopodium amaranticolor*
		Chenopodium murale
		Chenopodium quinoa
		Phaseolus vulgaris cv. The Prince
Ilarvirus	13	*Nicotiana tabacum*
		Phaseolus vulgaris
		Chenopodium quinoa
		Cucumis sativus
		Vinca rosea
Necrovirus	1	*Nicotiana tabacum*[3]
		Chenopodium amaranticolor
		Nicotiana clevelandii
		Phaseolus vulgaris
Nepovirus	27	*Chenopodium quinoa*
		Chenopodium amaranticolor
		Phaseolus vulgaris
		Nicotiana tabacum
		Nicotiana clevelandii
Pea enation mosaic virus[2]	1	*Chenopodium amaranticolor*
		Chenopodium quinoa
		Nicotiana clevelandii
		Pisum sativum
		Trifolium incarnatum
Potexvirus	18	*Chenopodium amaranticolor*
		Gomphrena globosa
Potyvirus	55	*Nicotiana clevelandii*
		Chenopodium quinoa
		Chenopodium amaranticolor
Sobemovirus	13	0[4]
		Nicotiana clevelandii
Tobamovirus	8	*Nicotiana tabacum*
		Nicotiana glutinosa
		Chenopodium amaranticolor
		Phaseolus vulgaris
		Nicotiana clevelandii
		Nicotiana sylvestris
		Datura stramonium
		Chenopodium quinoa

Group	No. of member	Species[1]
Tobravirus	2	*Chenopodium amaranticolor*
		Phaseolus vulgaris
		Nicotiana clevelandii
		Cucumis sativus
		Nicotiana glutinosa
		Vicia faba
		Pisum sativum
Tombusvirus	3	*Chenopodium quinoa*
		Chenopodium amaranticolor
		Nicotiana clevelandii
		Phaseolus vulqaris
		Nicotiana glutinosa
		Nicotiana tabacum
		Gomphrena globosa
		Cucumis sativus
		Celosia argentea
Tospovirus	1	*Nicotiana clevelandii*
		Petunia hybrida
		Tropaeolum majus
Tymovirus	13	0[4]
		Nicotiana clevelandii
		Chenopodium amaranticolor

[1]Plants are listed in order of their susceptibility to the viruses. [2]Monotypic groups with no approved group names (see Hull et al., 1991). [3]For the monotypic groups (alfalfa mosaic AMV group, and pea enation mosaic PEMV group) as well as for those in which only one member in each was examined (see CMI/AAB Descriptions of Plant Viruses) only several major host species are listed. [4]In three virus groups *(Caulimovirus, Sobemovirus, Tymovirus)* the frequency of the test plants did not reach 20% *(Caulimovirus)* and 30% (*Sobemovirus* and *Tymovirus*), respectively. For these groups several test plants most characteristic of the members of the group were given. [5]Species susceptible to all members of the given virus group

However, some species are only suitable for detecting one or two viruses of those belonging to a given virus group. Among the 17 host plants (see relevant Description) of the five viruses of the *Caulimovirus* group (blueberry red ringspot, carnation etched ring, cauliflower mosaic, Dahlia mosaic and soybean chlorotic mottle) there is not a single species with which more than one *Caulimovirus* can be detected. With *Nicotiana clevelandii,* one of the 45 host plants of the thirteen sobemoviruses, only four sobemoviruses can be reliably detected, while two of the 41 tymovirus hosts, *Chenopodium amaranticolor* and *Nicotiana clevelandii*, render the reliable detection of four of the thirteen tymoviruses possible.

Thus, the virus group diagnosis with test plants encounters much difficulty and contains a great many factors of uncertainty, as pointed out by an excellent work published under the editorship of Kurstak (1981) too, which deals with the

diagnostic problems of some 25 virus groups. In this handbook Murant (1981) states for example, that the identification of the nepoviruses is usually not possible from symptoms alone, because different nepoviruses induce similar symptoms in many hosts. Stace-Smith (1981) also emphasize, that the comoviruses are particularly difficult to diagnose by host plant reaction, since for example, closely related strains of the same virus can induce markedly different symptoms in the same host. Fulton (1981, 1991) called attention to many problems making the group diagnosis difficult. He emphasizes that for example, the ilarviruses are definitely unstable in crude sap, and are essentially more variable than other viruses; their detection greatly depends on the method used in transmitting them. No less problem was pointed out by Martelli (1981) in the case of tombusviruses. He found differentiation among tombusviruses merely on the basis of host range responses to be difficult if not impossible.

Yet, beside the essential and difficult problems some statements of general validity can also be made. Murant (1981) e.g. emphasizes, that there are assay hosts *(Chenopodium amaranticolor, C. quinoa, C. murale, Phaseolus vulgaris, Vigna sinensis),* propagation plants *(Cucumis sativus, Nicotiana clevelandii)* and maintaining hosts *(Gomphrena globosa, Petunia hybrida)* that can be excellently used in the diagnosis of nepoviruses. In the diagnosis of tombusviruses Martelli (1981) considers the species *Ocimum basilicum* important, since certain viruses (e.g., tomato bushy stunt, Pelargonium leaf curl, artichoke mottle crinkle, carnation Italian ringspot, Petunia asteroid mosaic) can be differentiated with it from other members of the *Tombusvirus* group. In his opinion, among experimental hosts *Chenopodium amaranticolor* and *C. quinoa* with their reactions may assist in the preliminary identification of tombusviruses at the group level. All members of the group induce symptoms in the above-mentioned plants. From a diagnostic point of view it is also remarkable that nine species *(Chenopodium amaranticolor, C. quinoa, C. murale, Gomphrena globosa, Nicotiana clevelandii, N. tabacum, Petunia hybrida, Phaseolus vulgaris, Tetragonia expansa)* are the most useful hosts for the potyviruses (Hollings and Brunt, 1981). In the diagnosis of potexviruses some 40 plants are known to be susceptible to more than 4 potexviruses (Purcifull and Edwardson, 1981), among them *Chenopodium quinoa* is susceptible to 21, *Gomphrena globosa* to 18 and *Chenopodium amaranticolor* to 17 viruses. Out of the tobraviruses of major importance pea early browning *Tobravirus* and tobacco rattle *Tobravirus* can be differentiated with 6 species (Harrison and Robinson, 1981): *Chenopodium amaranticolor*, *Cucumis sativus*, *Nicotiana tabacum* cv. Samsun NN, *Phaseolus vulgaris* cv. The Prince, *Pisum sativum* cv. Onward and *Vicia faba* cv. The Sutton. According to Koenig and Lesemann (1981) most tymoviruses can be differentiated on the basis of host range and symptomatology; to this they suggest 12 species.

In giving selected and proposed hosts for the different virus groups we followed the guiding principle that the species listed should be suitable for detecting all members of the virus group concerned (Table 10). Consequently, the number of species taken into consideration veried with the virus groups, but this is far from meaning that in the case of a virus group consisting of numerous members the number of the species listed had to be proportionately larger. For example, in the *Nepovirus* group which includes 27 members, 9 hosts are sufficient for detecting all members, while for the detection of the members of the *Caulimovirus* group, which contains much fewer, same 5 members, at least 6 plants, and to detect the 3 members of the *Dianthovirus* group minimum 5 plants are required. In the case of the very rich and highly diversified *Potyvirus* group, which includes about 55 members, 41 is the number of species reliably detecting all members of the group. For the "positive" diagnosis of the 24 virus groups we consider 79 plants as selected and proposed hosts sufficient.

When compiling the list of non-hosts for the different virus groups we took the non-hosts of each member in a given group into consideration, and it was from them that we selected the few species found to be susceptible to none of the members (Table 10). We had to make a thorough selection work, as we found that plants immune to some members of a given virus group may have been susceptible to other members of the same group. For example, among the 50 non-hosts determined for the *Potyvirus* group we only found 5 species that were non-hosts for all members of the virus group. Remarkably enough, the 11 non-hosts established for the caulimoviruses are non-hosts for all members of the virus group. On the basis of the non-host lists of the virus groups 59 non-host plants can be reckoned with in the "negative" diagnosis of the 24 virus groups.

We should like to emphasize that the host plants and non-host plants in themselves do not provide a reliable basis for the differential diagnosis of virus groups. For this modern virological methods are required, though these methods cannot dispense with host plant information concerning the viruses and virus groups. In the diagnosis of viruses and virus groups, besides the host range data such important properties as the antigenic nature of plant viruses, the particle morphology and the cytopathic effects induced by some viruses are sufficiently characteristic to be used for diagnostic purposes at group level (Christie and Edwardson, 1977; Van Regenmortel, 1982; Gugerly, 1983; Clark and Bar-Joseph, 1984; Hiebert et al., 1984; Martelli and Russo, 1984; Milne, 1984; Francki et al., 1985; Halk and De Boer, 1985; Hampton et al., 1990).

Table 10

Suggested list of some selected and proposed host plants and non-host plants for use in studies on virus group identification[1]

Virus group[2]	Code of hosts[3]			Code of non-hosts[3]		
	Code of botanical name	Cultivar or hybrid code	Code of family	Code of botanical name	Cultivar or hybrid code	Code of family
Alfalfa mosaic	CHEGI		CHE	CUUPE		CUC
	CHEQU		CHE	PAZFA		SCP
	NIOBE		SOL			
	PHSVX		LEG			
	VIGSI		LEG			
Bromo	CHEGI		CHE	BRSOX		CRU
	CHEHY		CHE	DINCA	WLS	CAF
	NIOCL		SOL	NIOTA	SAM	SOL
	PIBST		LEG	PHSLU	HEN	LEG
	VIGSI		LEG			
Carla	CHEQU		CHE	CUMSA		CUC
	DATME		SOL	DATST		SOL
	HUMLU		MOR	NIOGT		SOL
	LILLO		LIL	NIOSI		SOL
	NIOCL		SOL	PHSVX	RKY	LEG
	PIBST		LEG	VIGSI		LEG
	NIOCL		SOL	PHSVX	RKY	LEG
Carmo	BRSPK		CRU	BEAVA		CHE
	CHEQU		CHE	DATME		SOL
	CUMSA		CUC	LGNSI		CUC
	GLXMA		LEG	NIOGT		SOL
	HIBRS		MAV	NIOTA		SOL
	NARXX	BAB	AMY	PELGR	NIL	GER
	NIOCL		SOL	PIBST		LEG
	PHSVX		LEG	TOPMA		TRP
	SPQOL		CHE	VICFX		LEG
	TEATE		AIZ			
Caulimo	BRSRA		CRU	CHEQU		CHE
	GLXMA		LEG	CUMSA		CUC
	PHSVX	KIN	LEG	DATST		SOL
	VAAPY	PIB	CAF	GOMGL		AMA
	VACSS		ERI	NIOGT		SOL
	ZIIEL		COM	VIGSI		LEG
Clostero	CHEGI		CHE	CUMSA		CUC
	CHECA		CHE	DATST		SOL
	CHEQU		CHE	NIOGT		SOL
	DINCA		CAF	VIGSI		LEG
	NIOCL		SOL			
Como	BRSRA		CRU	CITLA		CUC
	CHEGI		CHE	DATST		SOL
	CHEQU		CHE	GOMGL		AMA
	CUUPE		CUC	NIOGT		SOL

Virus group[2]	Code of hosts[3]			Code of non-hosts[3]		
	Code of botanical name	Cultivar or hybrid code	Code of family	Code of botanical name	Cultivar or hybrid code	Code of family
	NIOCL		SOL	NIOTA	HFF	SOL
	PHSVX		LEG	NIOTA	WHB	SOL
	VICFX		LEG	NIOTA	XNC	SOL
	VIGSI		LEG			
Cucumo	CHEQU		CHE	BEAVA		CHE
	CUMSA		CUC	CUMMY		CUC
	NIOBE		SOL	PIBST		LEG
	NIOGT		SOL	TOPMA		TRP
	VIGSI		LEG	VICFX		LEG
Diantho	CHEQU		CHE	DATST		SOL
	DINBA		CAF	GLXMA		LEG
	GOMGL		AMA	PEUHY		SOL
	NIOCL		SOL	PIBST		LEG
	TEATE		AIZ	ZIIEL		COM
Furo	CHEGI		CHE	CHECA		CHE
	CHEQU		CHE	CUMME		CUC
	NIOBE		SOL	DATST		SOL
	NIOCL		SOL	GOMGL		AMA
	NIOGT		SOL	VIGSI		LEG
	NIOTA	XNC	SOL			
	TEATE		AIZ			
Gemini	DATST		SOL	CHEGI		CHE
	NIOBE		SOL	CHEQU		CHE
	NIOCL		SOL	GOMGL		AMA
	PHSVX		LEG	TEATE		AIZ
	PHSAU		LEG	VIGSI		LEG
"Idaeo"	CHEGI		CHE	CUMSA	SPM	CUC
	CHEMU		CHE	LYPES		SOL
	CHEQU		CHE	NIODE		SOL
	PHSVV	TPR	LEG	NIOSI		SOL
	NIOTA	SAM	SOL			
	VIGSI	BLE	LEG			
Ilar	CHEQU		CHE	CUMME		CUC
	CUMSA		CUC	NIOSI		SOL
	NIOSU		SOL	NIOTA	SAM	SOL
	NIOOC		SOL	PHSMU		LEG
	NIOTA		SOL	VICFX		LEG
	PHSVX		LEG	VIGSI	BLE	LEG
Necro	CHEGI		CHE	CUMSA		CUC
	NIOTA		SOL	PHSAU		LEG
	PHSVX		LEG			
Nepo	CHEQU		CHE	CPSAN		SOL
	CUMSA		CUC	CUUMA		CUC
	GOMGL		AMA	DATME		SOL
	NIOCL		SOL	PHSAU		LEG
	NIOTA		SOL	SOLHY	SDA	SOL

Virus group[2]	Code of hosts[3]			Code of non-hosts[3]		
	Code of botanical name	Cultivar or hybrid code	Code of family	Code of botanical name	Cultivar or hybrid code	Code of family
	PHSVX		LEG	SOLAD	CPC	SOL
	PHYFL		SOL	VICFX		LEG
	TEATE		AIZ	ZIIEL		COM
Pea enation mosaic	CHEGI		CHE	CUMSA		CUC
	CHEQU		CHE	DATST		SOL
	NIOCL		SOL	NIOGT		SOL
	PIBST		LEG			
	TRFIN		LEG			
	VICFX		LEG			
Potex	CHEGI		CHE	BRSPK		CRU
	CUMSA		CUC	CRMAB		CRU
	DATST		SOL	CUUPE		CUC
	GOMGL		AMA	GLXMA		LEG
	NIOBE		SOL	PEUHY		SOL
	NIOCL		SOL	ZIIEL		COM
	NIOGT		SOL			
	NIOTA		SOL			
Poty	AGRRE		GRA	CUMSA		CUC
	ALLCE		LIL	MEDSA		LEG
	APUGD		UMB	PHSVX	RKY	LEG
	AVESA		GRA	PIBST	PFC	LEG
	BMCCH		IRI	VIGSI	BLE	LEG
	BEAVA		CHE			
	BRSRA		CRU			
	CPSFR	TAB	SOL			
	CHEGI		CHE			
	CHEVU		CHE			
	CHEQU		CHE			
	CUUPE		CUC			
	CUUPE	SMS	CUC			
	DACGL		GRA			
	DATST		SOL			
	DINBA		CAF			
	GLXMA		LEG			
	GOMGL		AMA			
	HORVX		GRA			
	LILFO		LIL			
	LOLMU	STT	GRA			
	NARPS		AMY			
	NIOBE		SOL			
	NIOCL		SOL			
	NIODE		SOL			
	NIOGT		SOL			
	NIOHY	GTC	SOL			
	NIOOC		SOL			
	NIOTA	HFF	SOL			

Virus group[2]	Code of hosts[3]			Code of non-hosts[3]		
	Code of botanical name	Cultivar or hybrid code	Code of family	Code of botanical name	Cultivar or hybrid code	Code of family
	NIOTA	KEN	SOL			
	NIOTA	SAM	SOL			
	ORYSA		GRA			
	PAVSA		UMB			
	PEUHY		SOL			
	PHSVX		LEG			
	PIOSE		ARA			
	PIBST		LEG			
	SETIT		GRA			
	SOLHY	TUA	SOL			
	SORVU	RIO	GRA			
	TEATE		AIZ			
	TRZAX	KEN	GRA			
	VICFX		LEG			
	VIGSI		LEG			
Sobemo	BRSPK		CRU	CUMME		CUC
	CHEGI		CHE	CUMSA	DEL	CUC
	DACGL		GRA	CUUMA		CUC
	GLXMA		LEG	DATST		SOL
	LOLPS		GRA	LOLPE		GRA
	NIOCL		SOL	NIOSU		SOL
	ORYSA		GRA	NIOTA	XNC	SOL
	PHSVX		LEG	PHSVN	PIN	LEG
	TRFSU		LEG	TEATE		AIZ
	ZEAMX		GRA	VICFX		LEG
	ZINOF		ZIN	ZIIEL		COM
Tobamo	CPSSS		SOL	PEUHY		SOL
	CHEGI		CHE	PIBST		LEG
	CUMSA		CUC	NIOGL		SOL
	NIOCL		SOL	TRFSS		LEG
	NIOGT		SOL	VIGSI		LEG
	NIOTA	SAM	SOL	VIGSI	BLE	LEG
	PHSVV	TPR	LEG			
Tobra	CHEGI		CHE	CUUPE	CAP	CUC
	NIOCL		SOL	LYPES		SOL
	PHSVX		LEG	MEDSA		LEG
Tombus	CHEQU		CHE	CUMME		CUC
	CUMSA		CUC	NIOTA	SAM	SOL
	GOMGL		AMA	PIBST		LEG
	NIOCL		SOL			
	OCIBA		LAB			
Tospo	NIOCL		SOL	TEATE		AIZ
	PEUHY		SOL			
	TOPMA		TRP			
Tymo	BRSPK		CRU	AMACA		AMA
	BRSCH		CRU	AMIMA		UMB

Virus group[2]	Code of hosts[3]			Code of non-hosts[3]		
	Code of botanical name	Cultivar or hybrid code	Code of family	Code of botanical name	Cultivar or hybrid code	Code of family
	CHEGI		CHE	CPSAN		SOL
	CUMSA		CUC	GOMGL		AMA
	CUUPE		CUC	NIOTA	XNC	SOL
	DATST		SOL	PHSVN	PIN	LEG
	NIOBE		SOL	PHSVV	TPR	LEG
	NIOCL		SOL	PHYFL		SOL
	NIOTA	SAM	SOL	ZIIEL		COM
	PHSAU		LEG			
	PHSVN	GRN	LEG			
	PHSVX	LOT	LEG			
	VIGSI		LEG			

[1]Modified compilation from Horváth (1993a). [2]Alfalfa mosaic virus group and pea enation mosaic virus group are monotypic groups with no approved group names (see Hull et al., 1991; Horváth, 1993b). [3]See the code of hosts, non-hosts and families by Anonymous (1991) and Horváth (1993b)

Conclusion

The fact that the host plants play an important role in the diagnosis of viruses was realized long ago. The "problem finders" have pointed out many biological, physiological and biochemical problems and have established many correlations. In spite of this, the "problem solvers" are still in debt with the explanation of numerous questions concerning the host-virus relations. Science is in debt with the answer, why some plants are susceptible to many viruses, while others only to a few, why some viruses possess a wide; while others a narrow host range, and what the reason for certain plant being susceptible to no known virus is. Further, science has to answer the question, why such special hosts as susceptible each to a single virus, or immune to a single virus have not been found so far. I think, such expectations are unreasonable and groundless. Science is based on the experience that to rational questions nature gives rational answers, consequently, if there is no answer, then the question must be wrong.

When we began to examine the host-virus relations, even the biological-statistical and mathematical methods failed to help us. We have to admit, that in the host-virus relations there are so many "unknown variables" that the question cannot even be approached and answered with mathematical methods.

I should have given, most gladly, information on such plants which would make the virus diagnosis simpler and more reliable, but alas, we still have to wait

for this. Until then so much can be said, that no one test or host plant species in itself gives reliable diagnostic reactions; more and more test plants, serological tests, electron microscopy and other modern tests are necessary for a reliable diagnosis.

Hosts, non-hosts, and symptomatology can play a very important role in the diagnostical strategy of plant viruses if the biochemical properties of the viruses are established first and then efforts are made to find differential hosts which can distinguish one virus or strain from the other similar virus(es) or strain(s).

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Literature

Adams, S. E., Jones, R. A. C. and Coutts, R. H. A. (1984): Occurrence of resistance-breaking strains of potato virus X in potato stocks in England and Wales. Plant Pathol. 33, 435–437.

Anonymous (1992): Important Crops of the World and their Weeds. Scientific and Common Names, Synonyms, and WSSA/WSSJ Approved Computer Codes. 2nd Ed., Business Group Crop. Prot., Bayer AG, Leverkusen, Germany 1992. 1682 pp.

Atabekov, J. G. (1975): Host specificity of plant viruses. Ann. Rev. Phytopathol. 13, 127–145.

Atabekov, J. G. and Dorokhov, Yu. L. (1984): Plant virus-specific transport function and resistanee of plants to viruses. Adv. Virus Res. 29, 313–364.

Bald, J. G. and Tinsley, T. W. (1967a): A quasi-genetic model for plant virus host ranges. I. Group reactions within taxonomic boundaries. Virology 31, 616–624.

Bald, J. G. and Tinsley, T. W. (1967b): A quasi-genetic model for plant virus host ranges. II. Differentiation between host ranges. Virology 32, 321–327.

Bald, J. G. and Tinsley, T. W. (1967c): A quasi-genetic model for plant virus host ranges. III. Congruence and relatedness. Virology 32, 328–336.

Ball, E. M. (1974): Serological test for the identification of plant viruses. Plant Virology Committee, Amer. Phytopathol. Soc., St. Paul, Minn., USA, 1974.

Barker, H. (1989): Specificity of the affect of sap-transmissible viruses in increasing the accumulation of luteoviruses in co-infected plants. Ann. Appl. Biol. 115, 71–78.

Beczner, L. and Horváth, J. (1972): *Lycium ruthenicum* Murr. and *Lycium turcomanicum* Turcz. as new experimental hosts of alfalfa mosaic virus. Acta Phytopath. Hung. 7, 465–468.

Beczner, L., Horváth, J. and Borbély, F. (1983): Susceptibility of *Pisum sativum* and *Lupinus* species and cultivars to some economically important viruses. Tag.-Ber., Akad. Landwirtsch.- Wiss. DDR, Berlin 216, 313–322.

Bennett, C.W. (1952): Origin and distribution of new or little known virus diseases. Plant Dis. Rep. 211, 43–46.

Bercks, R. (1971): Cactus virus X. CMI/AAB Descriptions of Plant Viruses. 58, 1–4.

Best, R.J. (1968): Tomato spotted wilt virus. Adv. Virus Res. 13, 65–145.

Bock, K.R. (1982): The identification and partial characterisation of plant viruses in the tropics. Tropical Pest Management 28, 399–411.

Bos, L. (1967): Methods of studying plants as virus hosts. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. I., Academic Press, New York and London, 1967. pp. 129–162.

Bos, L. (1970a): Symptoms of Virus Diseases in Plants. Centre for Agr. Publ. Doc., Wageningen, 1970.

Bos, L. (1970b): The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. Neth. J. Plant Pathol. 76, 8–46.

Bos, L. (1981): Hundred years of Koch's postulates and the history of etiology in plant virus research. Neth. J. Plant Pathol. 87, 91–110.

Bos, L. (1983a): Introduction to Plant Virology. Centre for Agr. Publ. Doc., Wageningen, 1983.

Bos, L. (1983b): Ecology and control of virus-induced diseases of plants: a critical synopsis. Adv. App. Biol. 7, 105–173.

Boswell, K. F. and Gibbs, A. J. (1983): Viruses of Legumes 1983. Descriptions and Keys from VIDE. Australian National Univ., Research School of Biol. Sci., Canberra, 1983.

Boswell, K. and Gibbs, A. (1986): The VIDE data bank for plant viruses. Developments in Applied Biology. l. Developments in Applications in Virus Testing. In: Jones, R.A.C. and Torrance, L. (eds), Assoc. Appl. Biologists, Wellesbourne U.K., 1986. pp. 283–287.

Boukema, I.W. (1980): Allelism of genes controlling resistance to TMV in *Capsicum* L. Euphytica 29, 433–439.

Brandes, J. and Zimmer, K. (1955): Elektronenmikroskopische Untersuchungen über die viröse Vergilbungskrankheit der Rübe (beet yellows). Phytopathol. Z. 211–215.

Brandes, J. and Wetter, C. (1959): Classification of elongated plant viruses on the basis of particle morphology. Virology 8, 99–115.

Brown, F. (1986): The classification and nomenclature of viruses: summary of results of meetings of the International Committee on Taxonomy of Viruses in Sendai. Intervirology 25, 141–143.

Brunt, A. A. (1986): Iris mild mosaic virus. CMI/AAB Descriptions of Plant Viruses. 324, 1–4.

Brunt, A. A., Crabtree, K. and Gibbs, A. (1990): Viruses of Tropical Plants. Descriptions and Lists from the VIDE Database. C. A. B. International, Wallingford, UK, 1990.

Büttner, C. and Nienhaus, F. (1989a): Virus contamination of soils in forest ecosystems of the Federal Republic of Germany. Eur. J. Forest. Pathol. 19, 47–53.

Büttner, C. and Nienhaus, F. (1989b): Virus contamination of waters in two forest districts of the Rhineland area (FRG). Eur. J. Forest Pathol. 19, 206–211.

Castano, M., Tamayo, P. J. and Morales, F. (1982): Monroe: a local lesion *Phaseolus vulgaris* assay for bean common mosaic and soybean mosaic virus. Turrialba 32, 329–332.

Chabbouh, N., Cherif, C. and Martelli, G.P. (1990): Natural infections of artichoke by potato virus X in Tunisia. J. Phytopathol. 129, 257–260.

Chin, W. (1972): *Lycium chinense*, a local lesion assay host for tobacco vein-banding mosaic virus. J. Agr. Assoc. China 79, 18–21.

Cho, J. J., Mau, R. F. L., Mitchell, W. C., Gonsalves, D. and Yudin, L. S. (1987): Host list of plants susceptible to tomato spotted wilt virus (TSWV). Univ. Hawaii Coll. Tropic. Agr. Hum., Resour. Ext. Series 078, 1–12.

Cho, J. J., Mau, R. F. L., German, T. L., Hartmann, R. W., Yudin, L. S., Gonsalves, D. and Provvidenti, R. (1989): A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Disease 73, 375–383.

Christie, R. G. and Edwardson, J. R. (1917): Light and electron microscopy of plant virus inclusions. Florida Agr. Exp. Stat., Monograph. Ser. 9, 1–150.

Christie, S. R. (1969): *Nicotiana* hybrid developed as a host for plant viruses. Plant Dis. 53, 939–941.

Christie, S. R. and Crawford, W. E. (1978): Plant virus range of *Nicotiana benthamiana*. Plant Dis. Rep. 62, 20–22.

Clark, M. F. and Bar-Joseph, M. (1984): Enzyme immunosorbent assays in plant virology. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. VII. Academic Press, Orlando, Florida, 1984. pp. 51–85.

CMI/AAB Descriptions of Plant Viruses (1970–1988): Commonwealth Mycological Institute, Association of Applied Biologists. Nos 1–339.

Cooper, J. I. and Jones, A.T. (1983): Responses of plants to viruses: proposals for the use of term. Phytopathology 73, 127–128.

Costa, A. S. and Kitajima, E. W. (1972): Cassava common mosaic virus. CMI/AAB Descriptions of Plant Viruses. 90, 1–4.

Crill, P., Hagedorn, D. J. and Hanson, E. W. (1970): Alfalfa mosaic, the disease and its virus incitant. Univ. Wisconsin, Res. Bull. 280, 1–40.

Csilléry, G., Tóbiás, I. and Ruskó, J. (1983): A new pepper strain of tomato mosaic virus. Acta Phytopath. Hung. 18, 195–200.

Culver, J. N., Lindbeck, A. G. C. and Dawson, W.O. (1991): Virus-host interactions: Induction of chlorotic and necrotic responses in plants by tobamoviruses. Ann. Rev. Phytopathol. 29, 193–217.

Dale, J. L. (1988): Cassia yellow blotch virus. CMI/AAB Descriptions of Plant Viruses. 334, 1–4.

Dawson, W. O. and Hilf, M. E. (1992): Host-range determinants of plant viruses. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43, 527–555.

De Bokx, J. A. (1970): Reactions of various plant species to inoculation with potato virus S. Neth. J. Plant Pathol. 76, 70–78.

De Bokx, J. A. and Chrzanowska, M. S. (1972): Symptom development on A6 following inoculation with various viruses. Potato Res. 15, 146–159.

Dennis, R. W. G. (1938): A new test plant for potato virus Y. Nature 142, 154 (Abstr.).

Derks, A. F. L. M. (1991): Personal communication.

De Souza–Dias, J. A. C. and Costa, A. S. (1991): A *Physalis* species more sensitive than *P. pubescens* as an indicator plant for potato leafroll virus. Plant Dis. 75, 101 (Abstr.).

De Souza, V. B. V., Gergerich, R. C., Kim, K. S. and Langham, M. A. C. (1990): Properties and cytopathology of a tymovirus isolated from eggplant. Phytopathology 80, 1092–1098.

Diener, T. O. (1963): Physiology of virus-infected plants. Ann. Rev. Phytopathol. 1, 197–218.
Dijkstra, J. (1983): Tobacco streak virus in sunflower *(Helianthus annuus)*. Neth. J. Plant Pathol. 89, 153–169.
Dodds, J. A. (1982): Cross-protection and interference between electrophoretically distinct strains of cucumber mosaic virus in tomato. Virology 118, 235–240.
Drijfhout, E. (1978): Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Centre for Agr. Publ. Doc., Wageningen, 1978.
Dubern, J. (1981): *Physalis floridana*, a natural host of groundnut eyespot virus. Phytopathol. Z., 100, 237–241.
Duffus, J. E. (1971): Role of weeds in the incidence of virus diseases. Ann. Rev. Phytopathol. 9, 319–340.
Dwadash-Shreni, V. C. and Stavely, J. R. (1984): Comparative resistance of *Phaseolus vulgaris* cultivars to clover yellow vein virus using various inoculation methods. Plant Dis. 68, 555–558.
Edwardson, J. R. (1974a): Some Properties of the Potato Virus Y-group. Monograph No. 4. University of Florida, Gainesville, USA, 1974.
Edwardson, J. R. (1974b): Host Ranges of Viruses in the PVY-Group. Monograph No. 5. University of Florida, Gainesville, USA, 1974.
Edwardson, J. R. and Christie, R. G. (1986): Viruses Infecting Forage Legumes. Vol. I–III., University of Florida, Gainesville, USA, 1986.
Edwardson, J. R. and Christie, R. G. (1991): The Potyvirus Group. Vol. 1–4, University of Florida, Gainesville, USA, 1991.
Feldman, J. M. and Gracia, O. (1972): Studies of weed plants as sources of viruses. III. Natural infections of some weeds with tobacco mosaic, cucumber mosaic and potato Y viruses. Phytopathol. Z. 73, 251–255.
Fenner, F. and Gibbs, A. (1983): Cryptograms – 1982. Intervirology 19, 121–128.
Fernow, K. H. (1925): Interspecific transmission of mosaic diseases of plants. Agr. Exp. Stat. Cornell, N. Y., Memoir, No. 96.
Fischer, H. and Nienhaus, F. (1973): Virushemmende Prinzipien in Paprikapflanzen *(Capsicum annuum* L.*)*. Phytopathol. Z. 78, 25–41.
Fletcher, J. T., Wallis, W. A. and Davenport, F. (1987): Pepper yellow vein, a new disease of sweet peppers. Plant Pathol. 36, 180–184.
Forster, R. L. S. and Jones, A. T. (1980): Lucerne transient streak virus. CMI/AAB Descriptions of Plant Viruses. 224, 1–4.
Francki, R. I. B. (1980): Limited value of the thermal inactivation point, longevity in vitro and dilution endpoint as criteria for the characterization, identification and classification of plant viruses. Intervirology 13, 91–98.
Francki, R. I. B. and Hatta, T. (1981): Tomato spotted wilt virus. In: Kurstak, E. (eds), Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam, 1981. pp. 492–512.
Francki, R. I. B., Mossop, D. W. and Hatta, T. (1979): Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses. 213, 1–4.
Francki, R. I. B., Milne, R. G. and Hatta, T. (1985): Atlas of Plant Viruses. Vols 1 and 2. CRC Press, Boca Raton, Florida, 1985.
Francki, R. I. B., Fauquet, C. M., Knudson, D.L. and Brown, F. (1991): Classification and Nomenclature of Viruses. 1st Rep. Internat. Comm. Taxonomy of Viruses. Arch. Virology, Suppl. 2., Springer–Verlag, Wien 1991. 450. pp.
Fraser, R. S. S. (1986): Genes for resistance to plant viruses. CRC Critical Rev. Plant Sci. 3, 257–294.
Fraser, R. S. S. (1990): The genetics of resistance to plant viruses. Ann. Rev. Phytopathol. 28, 179–200.
Fribourg, C. E. (1977): Andean potato calico strain of tobacco ringspot virus. Phytopathology 67, 174–178.
Fribourg, C. E. (1979): Host plant reactions, some properties and serology of Peru tomato virus. Phytopathology 69, 441–445.

Fribourg, C. E. and Nakashima, J. (1984): Characterization of a new potyvirus from potato. Phytopathology 74, 1363–1369.

Fribourg, C. E., Jones, R. A. C. and Koenig, R. (1977a): Andean potato mottle, a new member of the cowpea mosaic virus group. Phytopathology, 67, 969–974.

Fribourg, C. E., Jones, R. A. C. and Koenig, R. (1977b): Host plant reactions, physical properties and serology of three isolates of Andean potato latent virus from Peru. Ann. Appl. Biol. 86, 373–380.

Fribourg, C. E., Jones, R. A. C. and Koenig, R. (1979): Andean potato mottle virus. CMI/AAB Descriptions of Plant Viruses. 203, 1–4.

Fulton, R. W. (1948): Hosts of the tobacco streak virus. Phytopathology 38, 429–439.

Fulton, R. W. (1964): Transmission of plant viruses by grafting, dodder, seed, and mechanical inoculation. In: Corbett, M. K. and Sisler, H. D. (eds), Plant Virology. Univ. Florida Press, Gainesville, 1964. pp. 39–67.

Fulton, R. W. (1981): Ilarviruses. In: Kurstak, E. (eds), Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam 1981. pp. 377–413.

Fulton, R.W. (1991): Personal communication.

Gibbs, A. (1969): Plant virus classification. Adv. Virus Res. 14, 263–328.

Gibbs, A. and Harrison, B. (1976): Plant Virology, the Principles. Edward Arnold, London, 1976.

Goodman, R. N., Király, Z. and Wood, K. R. (1986): The Biochemistry and Physiology of Plant Disease. Univ. Missouri Press, Columbia, 1986. p. 433.

Goodman, R. N., Király, Z. and Wood, K. R. (1991): A beteg növény biokémiája és élettana (The Biochemistry and Physiology of Plant Disease). Akadémiai Kiadó, Budapest 1991. p. 829.

Govier, D. A. and Plumb, R. T. (1972): Henbane mosaic virus. CMI/AAB Descriptions of Plant Viruses. 95, 1–4.

Govindaswamy, C. V., Mariappan, V., Kandaswamy, T. K. and Padmanabhan, C. (1967): Mosaic disease of *Amaranthus* in Coimbatore (Madras State). Madras Agric. J. 54, 35–36.

Green, S. K. and Kim, J. S. (1991): Characteristics and control of viruses infecting peppers. A literature review. Asian Vegetables Res. and Development Center, A. V. R. D. C., Shanhua, Tainan, Technical Bull. 18, 1–60.

Gugerly, P. (1983): Use of enzyme immunoassay in phytopathology. In: Avrameas, S., Druet, P., Masseyeff, R. and Feldmann, G. (eds), Immunoenzymatic Techniques. Elsevier, Amsterdam, 1983. pp. 369–384.

Gunn, L. V. and Pares, R. D. (1990): *Capsicum* yellows – a disease induced by a luteovirus in glasshouse peppers *(Capsicum annuum)* in Australia. J. Phytopathol. 129, 210–216.

Halk, E. L. and De Boer, S. H. (1985): Monoclonal antibodies in plant-disease research. Ann. Rev. Phytopathol. 23, 321–350.

Hamilton, R. I. (1980): Defenses triggered by previous invaders: Viruses. In: Horsfall, J. G. and Cowling, E. B. (eds), Plant Disease. Vol. 5, How Plants Defend Themselves. Academic Press, New York, 1980. pp. 279–303.

Hamilton, R. L., Edwardson, J. R., Francki, R. I. B., Hsu, H. T., Hull, R., Koenig, R. and Milne, R. G. (1981): Guidelines for the identification and characterization of plant viruses. J. Gen. Virol. 54, 223–241.

Hammond, J. (1982): *Plantago* as a host of economically important viruses. Adv. Virus. Res. 27, 104–136.

Hammond, J. and Lawson, R. H. (1983): Diagnostic Methods for Viruses of Ornamental Plants. Florist and Nursery Crops Laboratory, USDA, BARC–West, Beltsville, USA, 1983.

Hampton, R., Beczner, L., Hagedorn, D., Bos, L., Inouye, T., Barnett, O., Musil, M. and Meiners, J. (1978): Host reactions of mechanically transmissible legume viruses of the northern temperate zone. Phytopathology 68, 989–997.

Hampton, R., Mink, G., Bos, L., Inouye, T., Musil, M. and Hagedorn, D. (1981): Host differentiation and serological homology of pea seed-borne mosaic virus isolates. Neth. J. Plant Pathol. 87, 1–10.

Hampton, R., Ball, E. and De Boer, S. (1990): Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens: A Laboratory Manual. APS Press, St. Paul, Minnesota, USA, 1990.

Hampton, R. O. (1980): Within-line heterogeneity for gene sbm in the U.S. *Pisum* Collection. The *Pisum* Newsletter 12, 27–28.

Hansen, A. J. (1989): Antiviral chemicals for plant disease control. CRC Critical Rev. Plant Sci. 8, 45–86.

Harrison, B. D. (1981): Plant virus ecology: ingrediens, interactions and environmental influences. Ann. App. Biol. 99, 195–209.

Harrison, B. D. (1985): Advances in geminivirus research. Ann. Rev. Phytopathol. 23, 55–82.

Harrison, B. D. and Jones, R. A. C. (1971): Effects of light and temperature on symptom development and virus content of tobacco leaves inoculated with potato mop-top virus. Ann. Appl. Biol. 67, 377–387.

Harrison, B. D. and Robinson, D. J. (1981): Tobraviruses. In: Kurstak, E. (eds.), Handbook of Plant Virus Infections. Comparative Diagnosis, Elsevier, North–Holland Biomedical Press. Amsterdam, 1981. pp. 515–540.

Harrison, B. D., Finch, J. T., Gibbs, A. J., Hollings, M., Shepherd, R. J., Valenta, V. and Wetter, C. (1971): Sixteen groups of plant viruses. Virology 45, 356–363.

Hein, A. and Bartels, R. (1964): Ein Tabakrippenbräune-Isolat aus Freilandtomaten. Phytopathol. Z. 49, 313–324.

Hiebert, E., Purcifull, D. E. and Christie, R. G. (1984): Purification and immunological analyses of plant viral inclusion bodies. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. VIII. Academic Press, Orlando, Florida, 1984. pp. 225–280.

Hill, S. A. (1984): Methods in Plant Virology. Blackwell Sci. Publ., Oxford, 1984.

Hiruki, C. (1970): Red Kidney bean a useful bioassay host for quantitative and qualitative work with potato virus M. Phytopathology 60, 739–740.

Hiruki, C. (1975): Factors affecting bioassay of potato virus S in *Chenopodium quinoa*. Phytopathology 65, 1288–1292.

Hoekstra, R. and Seidewitz, L. (1987): Evaluation data on tuber-bearing *Solanum* species. German–Dutch Curatorium Plant Genetic Resources, Braunschweig and Wageningen, 1987.

Hollings, M. (1956): *Chenopodium amaranticolor* as a test plant for plant viruses. Plant Pathol. 5, 57–60.

Hollings, M. (1959): *Nicotiana clevelandii* Gray. as a test for plant viruses. Plant Pathol. 8, 133–137.

Hollings, M. (1966): Local lesion and other test plant for the identification and culture of viruses. In: Beemster, A. B. R. and Dijkstra, J. (eds), Viruses of Plants. North-Holland Publ. Comp., Amsterdam, 1966. pp. 230–241.

Hollings, M. and Stone, O. M. (1971): Tomato aspermy virus. CMI/AAB Description of Plant viruses. 79, 1–4.

Hollings, M. and Brunt, A. A. (1981): Potyviruses. In: Kurstak, E. (eds): Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam 1981. pp. 731–807.

Holmes, F.O. (1929): Inoculating methods in tobacco mosaic studies. Bot Gaz. 87, 56–63.

Holmes, F.O. (1946): A comparison of the experimental host ranges of tobacco-etch and tobacco-mosaic viruses. Phytopathology 36, 643–659.

Holmes, F. O. (1964): Symptomatology of viral diseases in plants. In: Corbett, M. K. and Sisler, H. D. (eds), Plant Virology, Univ. Florida Press, Gainesville, 1964. pp. 17–38.

Horváth, J. (1964): Ergebnisse der Identifizierung von mechanisch übertragbaren Kartoffelviren an Testpflanzen, mit besonderer Rücksicht auf Vergleichsuntersuchungen. Acta Agr. Hung. 13, 103–134.

Horváth, J. (1967): Separation and determination of viruses pathogenic to potatoes with special regard to potato virus Y. Acta Phytopath. Hung. 2, 319–360.

Horváth, J. (1968a): Susceptibility and hypersensitivity to tobacco mosaic virus in wild species of potatoes. Acta Phytopath. Hung. 3, 35–43.

Horváth, J. (1968b): Susceptibility, hypersensitivity and immunity to potato virus Y in wild species of potatoes. Acta Phytopath. Hung. 3, 199–206.

Horváth, J. (1969a): Die Anfälligkeit und Symptomausprägung der Blätter von *Nicotiana tabacum* L. cv. Hicks Fixed A2–426 nach Infection mit dem tobacco mosaic virus in Abhängigkeit von ihrer Sequenz am Spross. Acta Phytopath. Hung. 4, 131–137.

Horváth, J. (1969b): Die Anfälligkeit von *Chenopodium amaranticolor* Coste et Reyn. gegenüber dem Kartoffel–Y–Virus im Hindblick auf die Blattsequenz. Acta Bot. Hung. 15, 71–77.

Horváth, J. (1969c): Trennung und Nachweiss des Kartoffel–X- und Kartoffel–Y–Virus mittels *Chenopodium murale* L. Z. PflKrankh. PflSchutz 76, 9–11.

Horváth, J. (1969d): Cross protection test with four strains of potato virus Y in *Nicotiana tabacum* L. cv. Samsun. Zbl. Bakt. Abt. II. 123, 249–252.

Horváth, J. (1969e): Symptoms evoked by different strains of potato virus Y on *Nicotiana* species resistant to *Peronospora tabacina* Adam. Acta Agr. Hung. 18, 55–66.

Horváth, J. (1970): Reaction of *Physalis* species to plant viruses. I. The cape gooseberry as a symptomless carrier of potato virus X and Y. Acta Phytopath. Hung. 5, 65–72.

Horváth, J. (1972a): Identification of potato virus M using various bean varieties (in Hungarian). Növénytermelés 21, 303–308.

Horváth, J. (1972b): *Lycium*-Arten als neue Testpflanzen für verschiedene Pflanzenviren. Acta Phytopath. Hung. 7, 343–351.

Horváth, J. (1972c): Plant Viruses, Vectors and Virus Transmission. Akadémiaii Kiadó, Budapest, 1972. p. 515.

Horváth, J. (1973): *Tetragonia echinata* Ait., als eine neue Testpflanze für das Gurkenmosaik-virus (cucumber mosaic virus). Z. PflKrankh. PflSchutz 80, 129–132.

Horváth, J. (1974): Reaction of *Physalis* species to plant viruses. II. Additional data on the virus susceptibility of *Physalis* spp. and new experimental results: *Physalis peruviana* L. var. macrocarpa and *Physalis pruinosa* L. Acta Phytopath. Hung. 9, 1–9.

Horváth, J. (1976): Host Ranges of Viruses and Virus Differentiation, D. Sc. Thesis. Budapest and Rostock, 1976. p. 607.

Horváth, J. (1977a): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. I. Historical review. Acta Phytopath. Hung. 12, 177–214.

Horváth, J. (1977b): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. II. General remarks and proposals on the experimental work. Acta Phytopath. Hung. 12, 215–246.

Horváth, J. (1979): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. X. Cucumovirus group: Cucumber mosaic virus. Acta Phytopath. Hung. 14, 285–299.

Horváth, J. (1980a): Viruses of lettuce. II. Host ranges of lettuce mosaic virus and cucumber mosaic virus. Acta Agr. Hung. 29, 333–352.

Horváth, J. (1980b): *Tetragonia eremaea* Ostenf. (Aizoaceae) as a new host plant of cucumber mosaic virus. 14th Conf. Polish Plant Virologists. Blazajevko, 1980. 291, 37 (Abstr.).

Horváth, J. (1981): New data on the virus susceptibility of plants. 1. Solanaceae (*Capsicum*, *Datura*, *Nicotiana*, *Petunia* and *Scopolia* species). Bot. Közlem. 68, 181–185.

Horváth, J. (1982a): Hosts and Non-Hosts of Plant Viruses. Today and Tomorrow's Printers et Publ., New Delhi, 1982. p. 77.

Horváth, J. (1982b): Unpublished data.

Horváth, J. (1983a): Reaction of *Physalis* species to plant viruses. VII. Additional data on the virus susceptibility of *Physalis minima* L. Acta Phytopath. Hung. 18, 209–216.

Horváth, J. (1983b): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. XVIII. Concluding remarks. Acta Phytopath. Hung. 18, 121–161.

Horváth, J. (1983c): The role of resistant plants in virology and plant breeding. Tag.-Ber. Akad. Landwirtsch.–Wiss. DDR, Berlin. 216, 201–216.

Horváth, J. (1984): Reaction of *Physalis* species to plant viruses. VIII. *Physalis glabripes* Pojark, as a new host of various plant viruses. Acta Phytopath. Hung. 19, 303–308.

Horváth, J. (1985a): Reaction of *Physalis* species to plant viruses. X. *Physalis lanceifolia* Nees, as a new host to some mechanically transmissible viruses. Acta Phytopath. Hung. 20, 103–107.

Horváth, J. (1985b): New artificial host-virus relations between cucurbitaceous plants and viruses. I. *Benincasa-*, *Bryonia-*, *Bryonopsis-* and *Citrullus* species. Acta Phytopath. Hung. 20, 225–251.

Horváth, J. (1985c): A check-list of new host plants for identification and separation of twelve potato viruses. Potato Res. 28, 71–89.

Horváth, J. (1985d): New artificial host-virus relations between cucurbitaceous plants and viruses. II. *Cucumis* and *Cucurbita* species. Acta Phytopath. Hung. 20, 253–266.

Horváth, J. (1985e): Reaction of *Physalis* species to plant viruses. IX. Additional data on the virus susceptibility of *Physalis aequata* Jacq. and *Physalis alkekengi* L. (syn.: *P. franchetti* Mast.). Acta Phytopath. Hung. 20, 97–101.

Horváth, J. (1986a): Compatible and incompatible relations between *Capsicum* species and viruses. I. Review. Acta Phytopath. et Entomol. Hung. 21, 35–49.

Horváth, J. (1986b): Compatible and incompatible relations between *Capsicum* species and viruses. III. New incompatible host-virus relations (resistant and immune plants). Acta Phytopath. et Entomol. Hung. 21, 59–62.

Horváth, J. (1986c): Compatible and incompatible relations between *Phaseolus* species and viruses. I. Review. Acta Phytopath. et Entomol. Hung. 21, 297–318.

Horváth, J. (1986d): Compatible and incompatible relations between *Phaseolus* species and viruses. III. New incompatible host-virus relations (resistant and immune plants). Acta Phytopath. et Entomol. Hung. 21, 329–336.

Horváth, J. (1986e): New data on the virus susceptibility of plants. 5. Chenopodiaceae (*Chenopodium* species) (in Hungarian). Bot. Közlem. 73, 229–242.

Horváth, J. (1986f): Compatible and incompatible relations between *Phaseolus* species and viruses. II. New compatible host-virus relations (susceptible plants). Acta Phytophath. et Entomol. Hung. 21, 319–327.

Horváth, J. (1986g): Compatible and incompatible relations between *Capsicum* species and viruses. II. New compatible host-virus relations (susceptible plants). Acta Phytopathol. et Entomol. Hung. 21, 51–58.

Horváth, J. (1988a): The role of waters, terrestrial, aquatic and uliginous plants in the ecology of plant viruses. 18th Congr. Hung. Biol. Soc., Budapest, 1988. p. 46. (Abstr.).

Horváth, J. (1988b): Reaction of *Physalis* species to plant viruses. XI. Additional data on the susceptibility of *Physalis ixocarpa*, *Physalis philadelphica* and *Physalis pruinosa*. Acta Phytopath. Hung. 23, 137–141.

Horváth, J. (1988c): Reaction of *Physalis* species to plant viruses. XII. *Physalis anisotrichus* and *Physalis filiformis* as new experimental plants in virology. Acta Phytopath. Hung. 23, 143–146.

Horváth, J. (1988d): Potato gene centres, wild *Solanum* species, viruses and aphid vectors. Acta Phytopath. et Entomol. Hung. 23, 423–448.

Horváth, J. (1990): *Solanum demissum* P.I. 230579 a true seed host for some potato viruses. 11th Triennial Conf., EAPR, Edinburgh (UK), 1990. pp. 267–268 (Abstr.).

Horváth, J. (1991a): *Amaranthus bouchonii* Thell. (Family: Amaranthaceae) a new adventive plant species in Hungary, and its reaction to some viruses. Acta Phytopath. et Entamol. Hung. 26, 371–378.

Horváth, J. (1991b): *Amaranthus* species (Family: Amaranthaceae) as hosts of plant viruses: A review. Acta Phytopath. et Entomol. Hung. 26, 385–422.

Horváth, J. (1991c): *Amaranthus mangostanus* L., *A. mitchelii* Benth, and *A. quitensis* H.B.K. (Family: Amaranthaceae) as new virus hosts. Acta Phytopath. et Entomol. Hung. 26, 379–384.

Horváth, J. (1991d): *Lycium* species (Family: Solanaceae) as new experimental hosts of plant viruses. Acta Phytopath. et Entomol. Hung. 26, 353–365.

Horváth, J. (1991e): Isolation of cucumber mosaic virus from a water plant living in the Lake Balaton. International Conf. on Virology in the Tropics. Lucknow (India), 1991.

Horváth, J. (1993a): Host plants in diagnosis. In: Matthews, R. E. F. (eds), Diagnosis of Plant Virus Diseases. CRC Press, Boca Raton, Florida 1992. pp. 15–48.

Horváth, J. (1993b): The role of *Nicotiana* species in plant virology with especial regards to *Nicotiana benthamiana* Domin: A review. Acta Phytopath. et Entomol. 28, 355–377.

Horváth, J. (1993c): A list of proposed letter codes for hosts and non-hosts of plant viruses. Acta Phytopath. et Entomol. 28, 21–58.

Horváth, J. and Pocsai, E. (1971): *Solanum demissum*-Hybride A6 als neue Testpflanze für den Nachweis des Gurkenmosaik-Virus (cucumber mosaic virus). Z. PflKrankh. Pflschutz. 78, 695–699.

Horváth, J. and De Bokx, J. A. (1972): Über die Anfälligkeit von *Chenopodium*-Arten und Varietäten gegen das M-virus (potato virus M). Phytopathol. Z. 73, 171–177.

Horváth, J. and Pocsai, E. (1972): Effect of temperature and photoperiod on local susceptibility of *Nicotiana tabacum* L. cv. Xanthi-nc tobacco leaf discs to tobacco mosaic virus (in Hungarian). A Növényvéd. Korsz. 5, 65–82.

Horváth, J. and Hoekstra, R. (1989): Reaction of some new Bolivian tuber-bearing *Solanum* species to different potato pathogenic viruses. Acta Phytopath. et Entomol. Hung. 24, 351–362.

Horváth, J. and Gáborjányi, R. (1990): Present situation, problems and development of the Hungarian plant virology. Növényvédelem 26, 193–206.

Horváth, J., Juretić, N., Besada, W. H. and Mamula, D. (1975a): Natural occurrence of turnip mosaic virus in Hungary. Acta Phytopath. Hung. 10, 77–88.

Horváth, J., Juretić, N., Besada, W. H. and Kuroli, G. (1975b): Two viruses isolated from patisson (*Cucurbita pepo* L. var. patissonina Greb. f. radiata Nois.), a new vegetable natural host in Hungary. I. Watermelon mosaic virus (general). Acta Phytopath. Hung. 10, 93–111.

Horváth, J., Mamula, D., Juretić, N. and Besada, W. H. (1976): Natural occurrence of belladonna mottle virus in Hungary. Phytopathol. Z. 86, 193–204.

Horváth, J., Salamon, P., Wolf, I. and Kölber, M. (1988a): Henbane mosaic potyvirus pathogenic to wild and cultivated potato. Potato Res. 31, 311–320.

Horváth, J., Hollings, M. and Barton, R. J. (1988b): Experimental host range of Melandrium yellow fleck virus. Acta Phytopath. et Entomol. Hung. 23, 449–463.

Horváth, J., Juretić, N., Wolf, I. and Pintér, Cs. (1990): Natural occurrence of sowbane mosaic virus in *Chenopodium hybridum* plant in Hungary. Növényvédelem 6, 260. (Abstr.).

Horváth, J., Juretić, N., Wolf, I. and Pintér, Cs. (1993): Natural occurrence of sowbane mosaic virus on *Chenopodium hybridum* L. in Hungary. Acta Phytopath. et Entomol. Hung. 28, 379–389.

Hull, R. (1969): Alfalfa mosaic virus. Adv. Virus Res, 15, 365–433.

Hull, R. (1986): The pathogenesis of cauliflower mosaic virus. In: Day, P.R. and Jellis, G.J. (eds), Genetics and Plant Pathogenesis. Blackwell, Oxford, 1986. pp. 25–32.

Hull, R., Milne, R. G. and Van Regenmortel, M. H. V. (1991): A list of proposed standard acronyms for plant viruses and viroids. Archs. Virol. 120, 151–164.

Inouye, T. (1974): Carnation necrotic fleck virus. CMI/AAB Descriptions of Plant Viruses. 136, 1–4.

Inouye, T. and Osaki, T. (1980): The first record in the literature of the possible plant virus disease that appeared in "Manyōshū", a Japanese classic anthology, as far back as the time of the 8th century. Ann. Phytopath. Soc. Japan 46, 49–50.

Jaspars, E. M. J. and Bos, L. (1980): Alfalfa mosaic virus. CMI/AAB Descriptions of Plant Viruses 229, 1–4.

Jennings, D. L. and Jones, A. T. (1989): Further studies on the occurrence and inheritance of resistance in red raspberry to a resistance-breaking strain of raspberry bushy dwarf virus. Ann. Appl. Biol. 114, 317–323.

Johnson, J. (1925): Transmission of viruses from apparently healthy potatoes. Wisc. Agr. Exp. Stat., Res. Bull. No. 63.

Jones, A. T., Mitchell, M. J., McGavin, W. J. and Roberts, I. M. (1990): Further properties of wineberry latent virus and evidence for its possible involvement in calico disease. Ann. Appl. Biol. 117, 571–581.

Jones, R. A. C. (1982): Breakdown of potato virus X resistance gene Nx: selection of a group four strain from strain group three. Plant Pathol. 31, 325–331.

Jones, R. A. C. (1985): Further studies on resistance-breaking strains of potato virus X. Plant Pathol. 34, 182–189.

Jones, R. A. C. and Fribourg, C. E. (1978): Symptoms induced by Andean potato latent virus in wild and cultivated potatoes. Potato Res. 21, 121–127.

Jones, R. A. C. and Fribourg, C. E. (1979): Host plant reaction, some properties, and serology of wild potato mosaic virus. Phytopathology 69, 446–449.

Jones, R. A. C., Koenig, R. and Lesemann, D. E. (1980): Pepino mosaic virus, a new potexvirus from pepino *(Solanum muricatum).* Ann. Appl. Biol. 94, 61–68.

Joshi, R. D. and Dubey, L. (1976): Some weed reservoirs of cucumber mosaic virus in Gorakhpur and adjacent areas. Indian Phytopathol. 28, 568. (Abstr.).

Kado, C. L. (1966): Purification and characterization of a semi-latent seed-borne virus in *Chenopodium amaranticolor.* Phytopathology 56, 884. (Abstr.).

Kahn, R. P., Monroe, R. L., Hewitt, W. B., Goheen, A. C., Wallace, J. M., Roistacher, C. N., Neuer, E. M., Ackerman, W. L., Winters, H. F., Seaton, G. A. and Pifer, W. A. (1967): Incidence of virus detection in vegetatively propagated plant introductions under quarantine in the United States, 1957–1976. Plant Dis. Rep. 51, 715–719.

Kaper, J. M. and Waterworth, H. E. (1981): Cucumoviruses. In: Kurstak, E. (eds), Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam, 1981. pp. 258–332.

Kassanis, B. (1963): Interactions of viruses in plants. Adv. Virus Res. 10, 219–255.

Kassanis, B. (1970): Tobacco necrosis virus. CMI/AAB Descriptions of Plant Viruses. 14, 1–4.

Kassanis, B. (1978): Forty years' research on plant viruses at Rothamsted Experimental Station. Rothamsted Rep., Part 2. Rothamsted, 1978, pp. 5–26.

Kassanis, B. and Selman, I. W. (1947): Variations in the reaction of White Burley tobacco to the tomato aucuba mosaic virus and some other strains of tobacco mosaic virus. J. Pomol. Hort. Sci. 23, 167–172.

Kegler, H., Richter, J. and Schmidt, H. B. (1966): Untersuchungen zur Identifizierung und Differenzierung des Blattrollvirus der Kirsche (cherry leaf roll virus). Phytopathol. Z. 56, 313–330.

Kenten, R. H. and Jones, R. A. C. (1979): Arracacha virus B, a second isometric virus infecting arracacha (*Arracacha xanthorrhiza*: Umbelliferae) in the Peruvian Andes. Ann. Appl. Biol. 93, 31–36.

Kitajima, E. W. and Costa, A. S. (1966): Morphology and developmental stages of Gomphrena virus. Virology 29, 523–539.

Klinkowski, M. (1977): Pflanzliche Virologie. Bände 2–4., Akademie Verlag, Berlin, 1977,

Koenig, R. (1986): Plant viruses in rivers and lakes. Adv. Virus Res. 31, 321–333.

Koenig, R. and Lesemann, D.-E. (1981): Tymoviruses. In: Kurstak, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press. Amsterdam, 1981. pp. 33–60.

Kowalska, A. (1977): Reaction of Red Kidney bean to potato virus S. Potato Res. 20, 85–88.

Köhler, E. (1933): Untersuchungen über die Viruskrankheiten der Kartoffel. 1. Versuche mit Viren aus der Mosaikgruppe. Phytopathol. Z. 5, 567–591.

Köhler, E. (1953): Der *Solanum demissum* Bastard 'A6' als Testpflanze verschiedener Mosaikviren. Züchter 23, 173–176.

Köhler, E. (1962): Über einige Probleme der Virusinterferenz bei Pflanzen. Zbl. Bakt. Abt. II. 117, 616–633.

Kröll, J. (1975): Studies on the host plant range of the plum pox virus (PPV). Zbl. Bakt. Abt. II. 130, 219–225.

Kurstak, E. (1981): Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam, 1981.

Lana, A. O., Gilmer, R. M., Wilson, G. F. and Shoyinka, S. A. (1975): Identification and characterization of a severe strain of pepper veinal mottle virus isolated from pepper (*Capsicum annuum* and *C. frutescens*) in Nigeria. Nigerian J. Plant. Prot. 1, 76–81.

Lane, L. C. (1977): Brome mosaic virus. CMI/AAB Descriptions of Plant Viruses. 180, 1–4.

Lane, L. C. (1981): Bromoviruses. In: Kado, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam, 1981. pp. 333–376.

Lee, R. F., Niblett, C. L. and Johnson, L. B. (1975): Properties of Kansas strain of belladonna mottle virus isolated from pepper. Proc. Amer. Phytopath. Soc. 2, 222. (Abstr.).

Leiser, R.-M. and Richter, J. (1979): Ein Beitrag zur Frage der Differenzierung von Stämmen des Kartoffel–Y–Virus. Arch. Phytopathol. Pflanzenschutz 15, 289–298.

Lisa, V., Boccardo, G. and Milner, R. G. (1982): Viola mottle virus. CMI/AAB Description of Plant Viruses. 247, 1–4.

Lister, R. M. (1991): Personal communication.

Lovisolo, O. and Bartels, R. (1970): On a new strain of henbane mosaic virus from *Physalis alkekengi*. Phytopathol. Z. 69, 189–201.

Lovisolo, O. and Lisa, V. (1976): Characterization of a virus isolated from *Amaranthus deflexus*., serologically related to bean yellow mosaic virus. Agr. Consp. Scient. 39, 553–559.

MacClement, W. D. and Richards, M. G. (1956): Virus in wild plants. Canad. J. Botany 34, 793–799.

MacKinnon, J. P. and Bagnall, R. H. (1972): Use of *Nicotiana debneyi* to detect viruses S, X and Y in potato seed stocks and relative susceptibility of six common varieties to potato virus S. Potato Res. 15, 81–85.

Mali, V. R., Nirmal, D. D., Patel, K. V. and Vyanjane, N. T. (1985): Studies on a virus causing distortion mosaic of *Datura fastuosa*. Indian Phytopath. 38, 413–417.

Mamula, D. Juretić, N., Horváth, J. and Librić, M. (1974): Identification and serological properties of tomato mosaic virus isolated in Hungary. Acta Phytopathol. Hung. 9, 261–276.

Martelli, G. P. (1981): Tombusvirus. In: Kurstak, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North–Holland Biomedical Press, Amsterdam, 1981. pp. 61–90.

Martelli, G. P. and Russo, M. (1984): Use of thin sectioning for visualization and identification of plant viruses. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. VIII. Academic Press, Orlando, Florida, 1984. pp. 143–224.

Martin, R. R. (1985): Recent advances in virus detection. HortScience 20, 837–845.

Matthews, R. E. F. (1949): Studies on potato virus X. II. Criteria of relationships between strains. Ann. Appl. Biol. 36, 460–474.

Matthews, R. E. F. (1980): Host plant responses to virus infection. In: Fraenkel-Conrat, H. and Wagner, R. R. (eds), Comprehensive Virology. Vol. 16. Virus-Host Interaction, Viral Invasion, Persistence, and Dia[illegible]nose. Plenum Press, New York and London, 1980. pp. 297–359.

Matthews, R. E. F. (1981): Plant Virology. 2nd ed., Academic Press, New York, 1981.

Matthews, R. E. F. (1985): Viral taxonomy. Microbiol. Sci. 2, 74–76.

Matthews, R. E. F. (1991): Plant Virology. 3rd ed., Academic Press, New York, 1991.

Mayer, A. (1886): Über die Mosaikkrankheit des Tabaks. Landwirtsch. Versuchsstat., 32, 451–467.

Mayhew, D. E., Hedin, P. and Thomas, D. L. (1984): Corky ringspot: a new strain of tomato mosaic virus in California. Plant Dis. 68, 623–625.

Milne, R. G. (1984): Electron microscopy for the identification of plant viruses in vitro preparations. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. VII. Academic Press, Orlando, Florida; 1984. pp. 87–120.

Milne, R. G. (1990): Plant virus taxonomy and nomenclature: What's new? Rev. Investig. Agr. 22, 206–224.

Moline, H. E. and Fries, R. E. (1974): A virus isolated from *Physalis heterophylla* in Iowa. Phytopathology 64, 44–48.

Moreira, A., Jones, R. A. C. and Fribourg, C. E. (1980): Properties of a resistance-breaking strain of potato virus X. Ann. Appl. Biol. 95, 93–103.

Mundry, K. W. (1963): Plant virus-host cell relations. Ann. Rev. Phytopathol. 1, 173–196.

Murant, A. F. (1981): Nepoviruses. In: Kurstak, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North–Holland Biomedical Press. Amsterdam, 1981. pp. 197–238.

Murant, A. F., Taylor, C. E. and Chambers, J. (1968): Properties, relationships and transmission of a strain of raspberry ringspot virus infecting raspberry cultivars immune to the common Scottish strain. Ann. Appl. Biol. 61, 175–186.

Nagai, Y., Takeuchi, T. and Tochihara, H. (1981): A new mosaic disease of sweet pepper caused by pepper strain of tobacco mosaic virus. Ann. Phytopath. Soc. Japan 47, 541–546.

Nelson, M. R., Wheeler, R. E. and Zitter, T. A. (1982): Pepper mottle virus. CMI/AAB Descriptions of Plant Viruses. 253, 1–4.

Németh, M. (1986): Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees. Akadémiaii Kiadó, Budapest, 1986. p. 841.

Nienhaus, F., Büttner, C. and Hamacher, J. (1990): Virus infection of forest trees by mechanical transmission. J. Phytopathol. 129, 141–150.

Noordam, D. (1973): Identification of Plant Viruses, Methods and Experiments. Centre for Agr. Publ. Doc., Wageningen, 1973.

Paguio, O. R., Kuhn, C. W. and Boerma, H. R. (1988): Resistance-breaking variants of cowpea chlorotic mottle virus in soybean. Plant Dis. 72, 768–770.

Pares, R. D. (1988): Serological comparison of an Australian isolate of Capsicum mosaic virus with Capsicum tobamovirus isolates from Europe and America. Ann. Appl. Biol. 112, 609–612.

Paul, H. L., Bode, O., Jankulowa, M. and Brandes, J. (1968): Untersuchungen über ein neues isometrisches Virus aus *Atropa belladonna* L. I. Symptomatologie, Reinigung, Morphologie, physikalische und chemische Eigenschaften. Phytopathol. Z. 61, 342–361.

Pawley, R. R. (1973): Year-round production of test plants for virology. Rep. Glasshouse Crops Res. Inst., Littlehampton, 1973. pp. 149–151.

Peters, D. and Derks, A. F. L. M. (1974): Host range and some properties of Physalis mosaic virus, a new virus of the turnip yellow mosaic virus group. Neth. J. Plant Pathol. 80, 124–132.

Phatak, H. C. (1965): Mosaic disease of *Amaranthus* – a new record for India. Current Sci. 22, 645–646.

Piazzolla, P., Castellano, M. A. and De Stradis, A. (1986): Presence of plant viruses in some rivers of Southern Italy. J. Phytopathol. 116, 244–246.

Pirone, T. P. (1989): Comparison of tobacco vein mottling and pepper veinal mottle viruses. Plant Dis. 73, 336–339.

Plese, N. and Miličić, D. (1974): Virusisolierungen aus gelbnetziger *Forsythia suspense* Vahl. und aus *Lycium halimifolium* Mill. Acta Bot. Croat. 33, 31–36.

Price, W. C. (1930): Local lesions on bean leaves inoculated with tobacco mosaic virus. Amer. J. Botany 17, 694–702.

Price, W. C. (1940): Comparative host ranges of six plant viruses. Amer. J. Botany 27, 530–541.

Proeseler, G., Stanarius, A., Kontzog, H.-G. and Barth, H. (1990): Virusbefall und Wasserpflanzen. Arch. Phytopathol. Pflanzenschutz 26, 19–24.

Proll, E., Ahrens, E. and Richter, J: (1978): Ein Beitrag zur Differenzierung von Isolaten des Kartoffel–Virus M. Arch. Phytopathol. Pflanzenschutz 14, 209–217.

Provvidenti, R. (1976): Reaction of *Phaseolus* and *Macroptilium* species to a strain of cucumber mosaic virus. Plant Dis. Rep. 60, 289–293.

Provvidenti, R., Robinson, R. W. and Munger, H. M. (1978): Resistance in feral species to six viruses infecting *Cucurbita*. Plant Dis. Rep. 62, 326–329.

Purcifull, D. E. and Edwardson, J. R. (1981): Potexviruses. In: Kurstak, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press. Amsterdam, 1981. pp. 627–693.

Purcifull, D. E. and Hiebert, E. (1982): Tobacco etch virus. CMI/AAB Descriptions of Plant Viruses. 258, 1–4.

Purcifull, D., Hiebert, E. and Edwardson, J. (1984a): Watermelon mosaic virus 2. CMI/AAB Descriptions of Plant Viruses. 293, 1–4.

Purcifull, D., Edwardson, J., Hiebert, E. and Gonsalves, D. (1984b): Papaya ringspot virus. CMI/AAB Descriptions of Plant Viruses. 292, 1–4.

Quacquarelli, A. and Avgelis, A. (1975): *Nicotiana benthamiana* Domin, as host for plant virus. Phytopath. Medit. 14, 36–39.

Ragetli, H. W. J. and Weintraub, M. (1973): The influence of inhibitors on the reaction of indicator plants. 2nd Internat. Congr. Plant Path., University of Minnesota, Minneapolis, Minnesota, USA, 1973. pp. 1–12.

Ramsdell, C. C. (1979): Blueberry shoestring virus. CMI/AAB Descriptions of Plant Viruses. 204, 1–4.

Rast, A. Th. B. (1982): Resistance of *Capsicum* species to tobacco, tomato and pepper strains of tobacco mosaic virus. Neth. J. Plant Pathol. 88, 163–169.

Rast, A. Th. B. (1988): Occurrence of pepper yellow vein in the Netherlands. Neth. J. Plant Pathol. 94, 311–313.

Richter, J. (1983): Differenzierung von Stämmen des Gurkenmosaik-Virus (cucumber mosaic virus). Arch. Phytopathol. Pflanzenschutz 19, 77–79.

Richter, J. and Spaar, D. (1983): Aktueller Stand der Virustaxonomie unter besonderer Berücksichtigung von Pflanzenviren. Biol. Rdsch. 21, 213–223.

Roberts, D. A. (1964): Local-lesion assay of plant viruses. In: Corbett, M. K. and Sisler, H. D. (eds), Plant Virology. Univ. Florida Press, Gainesville, 1964. pp. 194–210.

Roland, G. and Tahon, J. (1961): Quelques considerations sur les virus de la betterave. Rev. Agr. 14, 869–895.

Ross, A. F. (1948): Local lesions with potato virus Y. Phytopathology 38, 930–932.

Ross, A. F. (1964): Identification of plant viruses, In: Corbett, M. K. and Sisler, H. D. (eds), Plant Virology. Univ. Florida Press, Gainesville, 1964. pp. 68–92.

Ross, H. (1986): Potato Breeding, Problems and Perspectives. Verlag Paul Parey, Berlin and Hamburg, 1986.

Rossel, H. W. and Thottapilly, G. (1985): Virus Diseases of Important Food Crops in Tropical Africa. Internat. Inst. Tropical Agr., Ibadan (Nigeria), 1985. pp. 1–60.

Salazar, L. F. and Harrison, B. D. (1977): Two previously undescribed potato viruses from South America. Nature 265, 337–338.

Salazar, L. F. and Harrison, B. D. (1979): Potato black rinsgpot virus. CMI/AAB Descriptions of Plant Viruses. 206, 1–4.

Sarkar, S. (1986): Recent developments in the diagnosis of phytopathogenic viruses. In: Varma, A. and Varma, J. (eds.), Vistas in Plant Pathology. Malhotra Publ. House, New Delhi, 1986. pp. 503–511.

Schmelzer, K. (1961): Wirte und Wirtskreise pflanzlicher Viren. Tagungsber. 33, 43–56.

Schmelzer, K. (1963): Untersuchungen an Viren der Zier- und Wildgehölze. 4. Mitteilung: Versuche zur Differenzierung und Identifizierung der Ringfleckenviren. Phytopathol. Z. 46, 315–342.

Schmelzer, K. (1969): *Lycium halimifolium* Mill. – ein potentielles Reservoir des Gurkenmosaik-Virus. Zbl. Bakt. Abt. II. 123, 305–309.

Schmelzer, K. (1971): Ergebnisse elfjähriger Untersuchungen an Viren und Virosen der Zier-, Forst- und Wildgehölze. Tag.-Ber., Akad. Landwirtsch. Wiss. Berlin, DDR. 115, 35–49.

Schmelzer, K. (1980): Symptomatologie. a) Aussere Symptome. In: Klinkowski, M. (eds.), Pflanzliche Virologie. Band 1. Akademie Verlag, Berlin, 1980. pp. 21–83.

Schmelzer, K. and Schmidt, H. E. (1968): Untersuchungen an Viren der Zier- und Wildgehölze. 6. Mitteilung: Ergänzende Befunde an *Caryopteris* sowie Virosen an *Philadelphus*, *Aristolochia, Buddleja*, *Lycium* und *Aesculus*. Phytopathol. Z. 62, 105–126.

Schmelzer, K. and Wolf, P. (1971): Wirtspflanzen der Viren und Virosen Europas. Johann Ambrosius Barth. Leipzig, 1971.

Schmelzer, K. and Molnár, A. (1975): Der Rauchhaarige Fuchsschwantz (*Amaranthus retroflexus* L.), ein wichtiges Reservoir des Gurkenmosaik-Virus (cucumber mosaic virus) in der Ungarischen Volksrepublik. Acta Phytopath. Hung. 10, 231–233.

Schmelzer, K. and Spaar, D. (1975): Untersuchungen über die Reaktion von Kulturkartoffelsorten und Wildkartoffelarten gegenüber dem Gurkenmosaik-Virus. Potato Res. 18, 385–396.

Schmelzer, K. and Wolf, P. (1977): Wirtspflanzen und ihre Viren, Virosen und Mykoplasmosen. In: Klinkowski, M. (eds), Pflanzliche Virologie. Registerband: Verzeichnisse und Übersichten zu den Virosen Europa. Akademie Verlag, Berlin, 1977. pp. 53–189.

Schmelzer, K., Bartels, R. and Klinkowski, M. (1960): Interferenzen zwischen den Viren der Tabakätzmosaik-Gruppe. Phytopathol. Z. 40, 52–74.

Schmelzer, K., Schmidt, H. E. and Beczner, L. (1973): Spontane Wirtspflanzen des Luzernemosaik-Virus. Biol. Zbl. 92, 211–227.

Schmidt, H. E. and Naumann, K. (1981): Virus- und Bakterienkrankheiten der Erbse. Fortschr. Landwirtsch. Nahrungsgüterwirtsch. 19, 1–45.

Schmidt, H. E. and Zobywalski, S. (1984): Determination of pathotypes of bean yellow mosaic virus using *Phaseolus vulgaris* L. as a differential host. Arch. Phytopathol. Pflanzenschutz 20, 95–96.

Schoelz, J.E. C1990): Genetic basis of host specificity of plant viruses. In: Mandahar, C. L. and Sehgal, O. P. (eds.), Plant Viruses. Vol. II. Pathology. CRC Press, Inc., Boca Raton 1990. pp. 153–176.

Selye, J. (1967): In Vivo, the Case for Supramolecular Biology. Liveright Publ. Corp., New York, 1967.

Shukla, D. D. and Schmelzer, K. (1975): Studies on viruses and virus diseases of cruciferous plants. XIX. Analysis of the results obtained with ornamental and wild species. Acta Phytopath. Hung. 10, 217–229.

Shukla, D. D. and Ward, C. W. (1989a): Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Arch. Virol. 106, 171–200.

Shukla, D. D. and Ward, C. W. (1989b): Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. Adv. Virus Res. 36, 273–314.

Singh, R. P. (1982): *Physalis angulata* as a local lesion host for postharvest indexing of potato virus A. Plant Dis. 66, 1051–1052.

Singh, R. P. and Smith, E. M. (1981): *Physalis angulata* as a local lesion host for post harvest indexing of potato virus A. Amer. Potato J. 58, 518–519.

Singh, R. P. and Boiteau, G. (1984): Necrotic lesions host for potato Y useful in field epidemiological studies. Plant Dis. 68, 779–781.

Smith, K. M. (1931): On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators and selective methods of transmission. Proc. Roy. Soc. B109 No. 251.

Smith, K. M. (1968): Plant Viruses. 4th ed., Methuen and Co Ltd., London, 1968.

Smith, K.M. (1972): A Textbook of Plant Virus Diseases. Longman, London, 1972.

Stace-Smith, H. (1981): Comoviruses. In: Kurstak, E. (eds), Handbook of Plant Virus Infections. Comparative Diagnosis. Elsevier, North-Holland Biomedical Press, Amsterdam, 1981. pp. 171–195.

Stace-Smith, R. (1985): Tobacco ringspot virus. CMI/AAB Descriptions of Plant Viruses. 309, 1–4.

Stace-Smith, R. (1989): Isolation of a virus from drainage water in British Columbia. Phytopathology 79, 911–912.

Stenger, D. C., Duffus, J. E. and Villalon, B. (1990): Biological and genomic properties of a geminivirus isolated from pepper. Phytopathology 80, 704–709.

Tamada, T. (1975): Beet necrotic yellow vein virus. CMI/AAB Descriptions of Plant Viruses. 144, 1–4.

Tamada, T. and Baba, T. (1973): Beet necrotic yellow vein virus from rizomania affected sugar beet in Japan. Ann. Phytopath. Soc. Japan 39, 325–332.

Tanaka, H. and Imada, J. (1974): *Physalis floridana*, a good production host for satsuma dwarf virus. Plant Dis. Rep. 58, 603–605.

Thomas, B. J. (1983): Arabis mosaic virus. In: Hammond, J. and Lawson, R. H. (eds), Diagnostic Methods for Viruses of Ornamental Crops. Florist and Nursery Crops Laboratory, USDA, BARC–West, Beltsville, USA, 1983. pp. 3–4.

Thomas, J. E. (1988): Ginger chlorotic fleck virus. CMI/AAB Descriptions of Plant Viruses. 328, 1–4.

Thornberry, H. H. (1966): Index of Plant Virus Diseases. ARS, USDA, Agriculture Handbook, No. 307. Washington, 1966.

Thresh, J.M. (1981): Pests, Pathogens and Vegetation. Pitman, London, 1981.

Van den Berkmortel, L. G. (1977): Breeding pepper for resistance to a strain of TMV. Capsicum 77, 89–92.

Tóbiás, I., Fraser, R. S. S. and Gerwitz, A. (1989): The gene-for-gene relationship between *Capsicum annuum* L. and tobacco mosaic virus: effects on virus multiplication, ethylene synthesis and accumulation of pathogenesis-related proteins. Physiol. Molecular Plant Pathol. 35, 271–286.

Tomlinson, J. A., Faithfull, E. M., Webb, M. J. W. and Seeley, N. D. (1983): *Chenopodium* necrosis: a distinctive strain of tobacco necrosis virus isolated from river water. Ann. Appl. Biol. 102, 135–147.

Trujillo, G. E. and Saettler, A. W. (1972): Local lesion assay of bean common mosaic virus (BCMV) on Monroe bean. Plant. Dis. Rep. 56, 714–718.

Van der Meer, F. A., Maat, D. Z. and Vink, J. (1980): Lonicera latent virus, a new carlavirus serologically related to poplar mosaic virus: some properties and inactivation in vivo by heat treatment. Neth. J. Plant Pathol. 86, 69–78.

Van der Want, J. P. H., Boerjan, M. L. and Peters, D. (1975): Variability of some plant species from different origins and their suitability for virus work. Neth. J. Plant Pathol. 81, 205–216.

Van Dijk, P., Van der Meer, F. A. and Piron, P. G. M. (1987): Accessions of Australian *Nicotiana* species suitables as indicator hosts in the diagnosis of plant virus diseases. Neth. J. Plant Pathol. 93, 73–85.

Van Regenmortel, M. H. V. (1982): Serology and Immunochemistry of Plant Viruses. Academic Press, New York, 1982.

Walkey, D. G. A. (1985): Applied Plant Virology. Heinemann, London, 1985.

Walkey, D. G. A., Brocklehurst, P. A. and Parker J. E. (1985): Some physiological effects of two seed-transmitted viruses on flowering, seed production and seed vigour in *Nicotiana* and *Chenopodium* plants. New Phytol. 99, 117–128.

Waterworth, H. E. and Povish, W. R. (1976): *Chenopodium quinoa* as a host species for plant virus research. Indian Phytopathol. 29, 155–163.

Webb, R. E. and Wilson, D. R. (1978): *Solanum demissum* P.I. 230579 a true seed diagnostic host for potato virus Y. Amer. Potato J. 55, 15–24.

Wetter, C., Dore, I. and Bernard, M. (1987): Bell pepper mottle virus, a distinct tobamovirus infecting pepper. J. Phytopathol. 119, 333–344.

Wetter, C., Conti M., Altschuh, D., Tabillion, R. and Van Regenmortel, M. H. V. (1984): Pepper mild mottle virus, a tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74, 405–410.

Wilkinson, R. E. and Blodgett, F. M. (1948): *Gomphrena globosa*, a useful plant for qualitative and quantitative work with potato virus X. Phytopathology 38, 28 (Abstr.).

Wolf, P. and Schmelzer, K. (1972): Untersuchungen an Viruskrankheiten der Umbelliferen. Zbl. Bakt. Abt. II. 127, 665–672.

Yarwood, C. E. (1971): Procedures to increase virus yield from infected plants. In: Maramorosch, K. and Koprowski, H. (eds), Methods in Virology. Vol. V, Academic Press, New York 1971. pp. 451–479.

Yarwood, C. E. (1979): Host passage effects with plant viruses. Adv. Virus Res. 25, 169–187.

Zaitlin, M. and Hull, R. (1987): Plant virus-host interactions. Ann. Rev. Plant Physiol. 38, 291–315.

Zettler, F. W. and Freeman, T. E. (1972): Plant pathogens as biocontrols of aquatic weeds. Ann. Rev. Phytopathol. 10, 455–470.

Zschüttig, H.-G. and Horváth, J. (1968): Ein Beitrag zur Verwendung des *Solanum demissum* Bastards A6 als Testpflanze für den Nachweis des Kartoffel–Y–Virus. Acta Phytopath. Hung. 3, 311–320.

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The Role of *Nicotiana* Species in Plant Virology with Special Regard to *Nicotiana benthamiana* Domin: A Review[1]

J. HORVÁTH

Pannonian University of Agricultural Sciences, Institute for Plant Protection, Department of Plant Pathology, H-8361 Keszthely, P.O. Box 71, Hungary

Nicotiana species and cultivars have been used extensively in virus detection and propagation. The ten *Nicotiana* species and one hybrid (*Nicotiana clevelandii* × *N. glutinosa*) of major virological importance belong to six sections: Alatae *(N. sylvestris)*, Bigelovianae *(N. clevelandii)*, Genuinae *(N. tabacum)*, Rusticae (*N. rustica)*, Suaveolentes *(N. benthamiana, N. debneyi, N. exigua, N. goodspeedii, N. megalosiphon)* and Tomentosae *(N. glutinosa)*.

The study shortly deals with the role of the major *Nicotiana* species in virology, and gives a comprehensive survey of the virus susceptibility of *Nicotiana benthamiana*. Latter plant is susceptible to 203 viruses belonging to 26 virus groups and an unclassified group. As far as we know the *Nicotiana benthamiana* is susceptible to most viruses and thereby a first-class test plant in virology.

Nicotiana Species as Virus Hosts

Firstly, Mayer (1886) investigated a mosaic disease of tobacco *(Nicotiana tabacum)*, and found that the disease could be transmitted to healthy tobacco plants in juice extracts taken from infected plants. Since his discovery tobacco as a virus host and test plant, respectively, together with other members of the family Solanaceae, have been in the forefront of research. Fernow (1925) and Price (1930) were the first to call attention to the fact that the tobacco mosaic *Tobamovirus* is able to infect plants *(Martina louisima, Phaseolus vulgaris)* belonging to two other families (Martinaceae and Leguminosae). These results did not, however, divert attention of the very rich *Nicotiana* genus. The observation of Holmes (1929) that *Nicotiana glutinosa* produced countable necrotic lesions on leaves inoculated with tobacco mosaic *Tobamovirus* was of very great importance for plant virology. Since that time analogous local lesions have been described in various hosts (e.g., *Chenopodium, Cyamopsis, Phaseolus, Solanum*) inoculated with numerous different viruses, and local lesion counts provide the basis for most quantitative infectivity tests. Characteristically of the intensity of research related with the virus susceptibility of *Nicotiana* species, Holmes (1946)

[1]Dedicated to Prof. Dr. Gy. Sáringer, Keszthely (Hungary) on the occasion of his 65th birthday

Akadémiai Kiadó, Budapest

described 28 *Nicotiana* species as experimental hosts of tobacco mosaic *Tobamovirus* and tobacco etch *Potyvirus* as early as in 1946. There are among them such species as *Nicotiana clevelandii*, *N. glutinosa*, *N. rustica*, *N. sylvestris*, which have kept their importance in virology up to the present day. The *Nicotiana* species, especially *N. clevelandii*, *N. sylvestris*, *N. debneyi*, *N. glutinosa*, *N. tabacum*, *N. rustica*, *N. megalosiphon* and recently *N. benthamiana* and *N. quadrivalvis* have proved extremely valuable in virus work (Hollings, 1959; Hollings, 1966; Thornberry, 1966; Schmelzer and Wolf, 1977; Christie and Crawford, 1978; Van Dijk et al., 1987).

In the series of CMI/AAB Descriptions of Plant Viruses (1970–1988) some *Nicotiana* species are described but only five (*N. clevelandii, N. glutinosa, N. tabacum, N. tabacum* cv. White Burley, *N. tabacum* cv. Xanthi-nc) are the most frequent ones occurring as diagnostic, propagation or assay hosts (Table 1).

Table 1

Number of viruses known to infect some *Nicotiana* species of plants[1]

Plant species	Diagnostic species	Propagation species	Assay species	Total
Nicotiana clevelandii	31	57	8	96
N. glutinosa	36	17	11	64
N. tabacum	17	12	7	36
N. tabacum cv. White Burley	26	4	3	33
N. tabacum cv. Xanthi-nc	15	7	8	30

[1]Information from CMI/AAB Descriptions of Plant Viruses (1970–1988)

Nicotiana clevelandii is a long since known test plant (Hollings, 1959). According to Schmelzer and Wolf (1977) it is susceptible to some 100 viruses. For several viruses it is an excellent assay and propagation host. It has special importance in the diagnostics and propagation of carlaviruses. *Nicotiana clevelandii* is the only non-leguminous species reported to be systemically infected by cassia yellow bloth *Bromovirus* (Dale, 1988). When using the plant one must know that dormancy in seeds must be broken by soaking the seed for two hours in gibberellic acid solution (1 g litre^{-1}) before sowing (Hill, 1984).

Nicotiana glutinosa was found by Schmelzer and Wolf (1977) to be susceptible to some 80 viruses. It is a very good assay host for certain viruses (tobacco mosaic *Tobamovirus*) and plays important diagnostic role in differentiating the cucumber mosaic *Cucumovirus* strains (Richter, 1983).

Nicotiana tabacum and its varieties are susceptible to some 90 viruses. There are among them good local lesions hosts (e.g., *Nicotiana tabacum* cv. Xanthi-nc), and systemic hosts (e.g., *Nicotiana tabacum* cv. Samsun). They have the advantages that they do not require special circumstances to grow, the seeds germinate much better, the plants develop faster and produce a larger mass of leaves than e.g., *Nicotiana clevelandii.*

Nicotiana rustica is a test plant well known in plant virology, susceptible to more than 50 viruses (Schmelzer and Wolf, 1977). Its advantage over the other *Nicotiana* species is that it is earlier (by about 10 days) suitable for inoculation. The plant can be inoculated some 35 days after sowing.

Nicotiana megalosiphon belongs to those test plants which have mostly become known since the 1960s. Its susceptibility to alfalfa mosaic virus was first established as late as in 1963 (Schmelzer, 1963). It is a test plant particularly suitable for use in cross protection tests with various viruses causing ringspot symptoms. *Nicotiana megalosiphon* is a useful test plant in identifying fruit-tree viruses (Kegler et al., 1966). To our knowledge it is susceptible to about 40 viruses. Its importance is increased by the fact that it is resistant to the blue mold disease of tobacco *(Peronospora tabacina)* which is particularly dangerous for tobacco test plants in glasshouses. According to our investigations the plant is a good test plant for various strains and isolates of potato Y *Potyvirus* (Horváth, 1969). It reacted with vein clearing and mosaic symptoms followed by leaf curl. As far as symptoms are concerned no difference could be established either for the isolates or for the strains of potato Y *Potyvirus* used.

Nicotiana sylvestris is one of the less wide-spread test plants, though to our knowledge it is host for some 25 viruses (Schmelzer and Wolf, 1977). It has a significant role in differentiating tobamoviruses. The plant is locally susceptible to tobacco mosaic *Tobamovirus* ("Hungarian" and "Dahlemense" isolates) and ribgrass mosaic *Tobamovirus*, and locally and systemically susceptible to the common strain of tobacco mosaic *Tobamovirus* (Mamula et al., 1974). *Nicotiana sylvestris*, together with other plants can be reliably used in separating several tobamoviruses.

Nicotiana debneyi plays an important role e.g., in the diagnosis of potato viruses. It is a reliable test plant for potato S *Carlavirus*, potato X *Potexvirus* and potato Y *Potyvirus* (Horváth, 1964; De Bokx, 1970; McKinnon and Bagnall, 1972).

According to the data of Christie (1969) the hybrid of *Nicotiana clevelandii* x *N. glutinosa,* which is equal in size to *N. glutinosa* and larger than *N.* clevelandii, is a very good test plant for several viruses (e.g., cucum-

ber mosaic *Cucumovirus*, potato X *Potexvirus*, potato Y *Potyvirus*, tobacco mosaic *Tobamovirus*, turnip mosaic *Potyvirus*).

Considering that the blue mold endangers the plant virology work in glasshouse, attention is worth being paid to the *Nicotiana* species (e.g., *Nicotiana debneyi, N. exigua, N. goodspeedii, N. megalosiphon, N. tabacum* cv. Resistant Hicks Fixed A2–426) resistant to blue mold but susceptible to viruses (Horváth, 1969).

In the course of studying the host-virus relations we established 97 new compatible and 47 new incompatible relations for 18 *Nicotiana* species, 12 cultivars and one hybrid (Horváth, 1983). An excellent work published not long ago deals with the behaviour to 26 isolates of 7 viruses of 109 accessions of 67 *Nicotiana* species belonging to 3 subgenera (Rustica, Tabacum and Petunioides) and 12 sections of the *Nicotiana* genus (Van Dijk et al., 1987). The authors pointed out considerable differences in virus susceptibility possibly shown e.g., by the accessions.*Nicotiana benthamiana*–9, *N. miersii*–33 and N. occidentalis-37B seem to be suitable for routine inoculation tests, because of their larger virus ranges and sensitivity to different viruses.

Susceptibility of *Nicotiana benthamiana* to plant viruses

In recent years *Nicotiana benthamiana*, a popular test plant has become the centre of interest. Quacquarelly and Avgelis (1975) suggest that *Nicotiana benthamiana* may have a rate of susceptibility equivalent to that of *Nicotiana clevelandii*. The real importance of *Nicotiana benthamiana* was first realized when it turned out to be a host for viruses (e.g., blackeye cowpea mosaic *Potyvirus*, clover yellow mosaic *Potexvirus*, Desmodium mosaic virus, papaya mosaic *Potexvirus*, soybean mosaic *Potyvirus*, cucumber green mottle mosaic *Tobamovirus*) which earlier had not any host in the *Nicotiana* genus (Christie and Crawford, 1978; Horváth, 1993).*Nicotiana benthamiana* is susceptible to 203 viruses belonging to 26 virus groups and an unclassified group (Tables 2 and 3).*Nicotiana benthamiana* is the test plant "number one" in virology. It is remarkable that the plant is host for some furoviruses (4), geminiviruses (9), ilarviruses (3), nepoviruses (11), plant rhabdoviruses (5) and sobemoviruses (2) too (see Table 3).

Nicotiana benthamiana as a test plant plays a highly important role in studies on the viruses of forest and water ecosystems too (Nienhaus et al., 1990; Li et al., 1992; Büttner and Nienhaus, 1989a, b). The beet necrotic yellow vein *Furovirus*, of which mainly local hosts have so far been known,

causes systemic infection to *Nicotiana benthamiana*, and this fact makes the study of the virus easier in many respects (Horváth, 1989, Horváth et al., 1990). Its importance is increased by the fact that it is not only a good test plant, but is also suitable for maintaining and/or purifying several viruses, and is a good source of protoplasts for the work of superinfection (Barker, 1989). In the last years a not exactly identified virus containing bacilliform particles was isolated from drainage water in the Fraser delta region of British Columbia, which locally infected the *Nicotiana benthamiana* (Stace-Smith, 1989). In the identification of some new viruses (Ahlum waterborne *Carmovirus*, bean calico mosaic *Geminivirus*, cassava brown streak *Carlavirus*, cassava brown streak *Potyvirus*, grapevine Algerian latent *Tombusvirus*, onion mite-borne latent *Potyvirus*, Sikte waterborne *Tombusvirus*) *Nicotiana benthamiana* is a suitable test plant (Brown et al., 1990; Mali et al., 1991; Van Dijk et al., 1991; Li et al., 1992). In host-range experiment four viruses (cauliflower mosaic *Caulimovirus,* Erysimum latent *Tymovirus*, melon necrotic spot virus and turnip yellow mosaic *Tymovirus*) were apathogenic to *Nicotiana benthamiana* (Horváth, 1989). With respect to viral host ranges, some plants appear to behave anomalously toward numerous viruses. *Nicotina benthamiana*, for example, allows full and rapid systemic infection by numerous tobacco mosaic *Tobamovirus* mutants that fail to infect other plants effectively (see Dawson and Hilf, 1992). It is interesting to mention, that tomato golden mosaic *Geminivirus* is usually a phloem-associated virus, but in *Nicotiana benthamiana* it spreads to mesophyll cells (Rushing et al., 1987).

Table 2

Susceptibility of *Nicotiana benthamiana* Domin (Family: Solanaceae) to different plant viruses

Virus name	Acronym	Literature
African cassava mosaic *Geminivirus* III. (syn.: Cassava latent virus)	ACMV	Walter (1980), Bock and Woods (1983), Bock and Harrison (1985), Kounounguissa et al. (1989)
Ageratum yellow vein *Geminivirus*	AYVV	Tan and Wong (1993)
Ahlum waterborne *Carmovirus*	AWV	Li et al. (1992)
Alfalfa mosaic AMV group	AMV	Quacquarelli and Avgelis (1975), Savino and Gallitelli (1976), Mayoral et al. (1988)
Amaranthus mosaic *Potyvirus* (?)	AmaMV	Taiwo (1988)
Andean potato latent *Tymovirus*	APLV	Fribourg et al. (1977a)

Table 2 (continued)

Virus name	Acronym	Literature
Andean potato mottle *Comovirus* (Fig. 1A)	APMV	Fribourg et al. (1977b), Avila et al. (1984)
Artichoke Italian latent *Nepovirus*	AILV	Quacquarelli and Avgelis (1975)
Artichoke latent-	ALV	Quacquarelli and Avgelis (1975), Foddai et al. (1978), Rana et al. (1982)
Artichoke yellow ringspot *Nepovirus*	AYRSV	Rana et al. (1980), Avgelis and Vovlas (1989)
Artichoke mottle crinkle *Tombusvirus*	AMCV	Quacquarelli and Avgelis (1975)
Asparagus virus 3 *Potexvirus*	AV3	Fujisawa (1986)
Asystasia mottle-	AsMV	Thouvenel et al. (1988)
Bean calico mosaic *Geminivirus*	BCMoV	Brown et al. (1990)
Bean common mosaic *Potyvirus* (aphid)	BCMV	Christie and Crawford (1978), Lana et al. (1988)
Bean yellow mosaic *Potyvirus* (aphid)	BYMV	Christie and Crawford (1978), Makkouk et al. (1982), Hammond and Chastagner (1988), Hammond and Lawson (1988)
Beet curly top	BCTV	Stanley et al. (1992)
Beet mosaic *Potyvirus* (aphid)	BtMV	Porth et al. (1987)
Beet necrotic yellow vein *Furovirus* (Fig. 1B)	BNYVV	Horváth (1989), Horváth et al. (1990)
Belladonna mottle *Tymovirus* (Fig. 1C)	BeMV	Horváth (1989)
Bidens mottle *Potyvirus* (aphid)	BiMoV	Christie and Crawford (1978)
Blackeye cowpea mosaic *Potyvirus* (aphid)	BlCMV	Christie and Crawford (1978), Dijkstra et al. (1987), Lana et al. (1988), Zhao et al. (1991)
Blueberry scorch disease-	BSDV	MacDonald and Martin (1989)
Brinjal necrotic mosaic-	BNMV	Gupta (1989)
Broad bean mottle *Bromovirus*	BBMV	Makkouk et al. (1988)
Broad bean wilt *Fabavirus*	BBWV	Quacquarelli and Avgelis (1975)
Caper latent-	CapLV	Gallitelli and Di Franco (1987)
Caraway latent-	CarLV	Van Dijk and Bos (1989)
Carnation Italian ringspot *Tombusvirus*	CIRSV	Di Franco et al. (1984)
Carnation ringspot *Dianthovirus*	CRSV	Quacquarelli and Avgelis (1975), Koenig et al. (1988)
Carrot mottle-	CMoV	Van Dijk et al. (1987), Van Dijk and Bos (1989)
Carrot yellow leaf *Closterovirus*	CYLV	Van Dijk and Bos (1989)
Cassava African mosaic-	CAMV	Horvát and Verhoyen (1981)
Cassava American latent *Nepovirus*	CALV	Walter et al. (1989)
Cassava brown streak *Carlavirus*	CBSV	Mali et al. (1991)

Table 2 (continued)

Virus name	Acronym	Literature
Cassava brown streak *Potyvirus*	CBSV	Mali et al. (1991)
Cassava common mosaic *Potexvirus*	CsCMV	Zettler and Elliott (1986)
Cassava Ivorian bacilliform-	CaIBV	Fargette and Harrison (1990)
Cassava latent (syn.: African cassava mosaic *Geminivirus*, III)	CsLV	Bock et al. (1981), Adejare and Coutts (1982), Sequeira and Harrison (1982), Coutts and Buck (1987)
Cassava mosaic-	CsMV	Adejare and Coutts (1982)
Celery mosaic *Potyvirus* (aphid)	CeMV	Avgelis and Quacquarelli (1973b), Quacquarelli and Avgelis (1975)
Cherry leaf roll *Nepovirus*	CLRV	Savino et al. (1977)
Chickpea chlorotic dwarf *Geminivirus*	CCDV	Horn et al. (1993)
Chicory yellow mottle *Nepovirus*	ChYMV	Quacquarelli and Avgelis (1975), Vovlas and Quacquarelli (1975)
Chinese seed-borne-	CSBV	Barbara et al. (1985)
Chino del tomato-	CDTV	Brown and Nelson (1988)
Citrus ringspot-	CiRV	Timmer et al. (1978)
Clover yellow mosaic *Potexvirus*	ClYMV	Christie and Crawford (1978)
Clover yellow vein *Potyvirus* (aphid)	ClYVV	Provvidenti et al. (1984)
Cow parsnip mosaic-	CoPMV	Van Dijk and Bos (1989)
Cowpea aphid-borne mosaic *Potyvirus* (aphid)	CABMV	Lima et al. (1981)
Cowpea chlorotic mottle *Bromovirus*	CCMV	Christie and Crawford (1978)
Cowpea mosaic *Comovirus*	CPMV	Purcifull et al. (1981)
Cowpea severe mosaic *Comovirus*	CPSMV	Fribourg and Koenig (1985)
Cucumber fruit streak- (Fig. 1D)	CFSV	Di Franco and Martelli (1987), Horváth (1989)
Cucumber green mottle mosaic *Tobamovirus* (Fig. 2A)	CGMMV	Horváth (1989)
Cucumber leaf spot *Tombusvirus* (Fig. 2B)	CLSV	Di Franco and Martelli (1987), Horváth (1989), Vetten et al. (1989)
Cucumber mosaic *Cucumovirus*	CMV	Vovlas and Martelli (1973), Quacquarelli and Avgelis (1975), Purcifull et al. (1981), Valverde (1984), Xu (1984)
Cymbidium ringspot *Tombusvirus*	CyRSV	Martelli and Russo (1981)
Cymbidium mosaic *Potexvirus*	CyMV	Christie and Crawford (1978)
Cynara *Rhabdovirus*	CRV	Rana et al. (1988)
Dandelion *Carlavirus*	DCV	Dijkstra et al. (1985)
Dandelion yellow mosaic-	DaYMV	Bos et al. (1983)
Dasheen mosaic *Potyvirus* (aphid)	DsMV	Rana et al. (1983)
Desmodium mosaic-	DMV	Christie and Crawford (1978)
Dioscorea alata ring mottle-	DRMV	Porth and Nienhaus (1983)
Dioscorea green banding mosaic-	DGBMV	Reckhaus and Nienhaus (1981)
Dioscorea latent *Potexvirus*	DLV	Phillips et al. (1986), Phillips and Brunt (1988)
Dipladenia mosaic-	DiMV	Paludan (1988)
Eggplant green mosaic-	EGMV	Ladipo et al. (1988a)
Eggplant mosaic *Tymovirus*	EMV	De Souza et al. (1990)

Table 2 (continued)

Virus name	Acronym	Literature
Eggplant mottled dwarf *Rhabdovirus* (2)	EMDV	Quacquarelli and Avgelis (1975), Martelli and Cherif (1987), Al-Musa and Lockhart (1990)
Eggplant severe mottle-	ESMV	Ladipo et al. (1988b)
Elderberry carla *Carlavirus*	ECV	Van Lent and Dijkstra (1980)
Elderberry latent ? *Carlavirus*	ELV	Jones (1972)
Elm mottle *Ilarvirus*	EMoV	Jones and Mayo (1973)
Epirus cherry-	EpCV	Avgelis et al. (1989)
Euphorbia mosaic *Geminivirus* (III)	EuMV	Jaramillo and Lastra (1986)
Garlic common latent *Carlavirus*	GCLV	Van Dijk (1993)
Globe artichoke latent	GALV	Rana et al. (1989)
Grapevine A *Closterovirus* (-grapevine stem pitting associated *Closterovirus*)	GVA	Monette and James (1990)
Grapevine Algerian latent *Tombusvirus*	GALV	Li et al. (1992)
Grapevine fanleaf *Nepovirus*	GFLV	Quacquarelli and Avgelis (1975)
Grapevine little pattern-	GLPV	Lehoczki et al. (1987)
Groundnut rosette assistor *Luteovirus*	GRAV	Rajeshwari and Murant (1988)
Groundnut rosette-	GRV	Reddy et al. (1985), Murant et al. (1988)
Henbane mosaic *Potyvirus* (aphid)	HMV	Quacquarelli and Avgelis (1975)
Heracleum latent ? *Closterovirus*	HLV	Van Dijk and Bos (1989)
Hibiscus latent ringspot *Nepovirus*	HLRSV	Brunt et al. (1980)
Hydrangea mosaic *Ilarvirus*	HdMV	Thomas et al. (1983)
Hyppeastrum mosaic-	HyMV	Castellano and Rana (1982)
Impatiens necrotic spot *Tospovirus*	INS	Lawson et al. (1993)
Indian cassava mosaic-	ICMV	Roberts (1989)
Iris mild mosaic *Potyvirus* (aphid)	IMMV	Brunt (1975)
Ivy mosaic-	IMV	Castellano and Rana (1981)
Ivy vein clearing-	IVCV	Castellano and Rana (1981)
Kalanchoe isometric-	KIV	Izaguirre-Mayoral et al. (1990)
Lettuce big-vein-	LBVV	Huijberts et al. (1990)
Lettuce mosaic *Potyvirus* (aphid)	LMV	Quacquarelli and Avgelis (1975), Horváth (1980)
Lilac chlorotic leaf spot *Closterovirus*/? *Capillovirus*	LCLV	Brunt (1978)
Lychnis ringspot *Hordeivirus*	LRSV	Beczner et al. (1992)
Maracuya mosaic *Tobamovirus*	MaMV	Fribourg et al. (1987), Lawson et al. (1988)
Melandrium yellow fleck *Bromovirus* (Fig. 2C)	MYFV	Horváth et al. (1988)
Nandina mosaic *Potexvirus*	NaMV	Zettler et al. (1980)
Narcissus late season yellows	NLSYV	Mowat et al. (1988)
Narcissus latent *Carlavirus*	NLV	Brunt (1977)
Narcissus tip necrosis ? *Carmovirus*	NTNV	Mowat et al. (1977)
Nasturtion ringspot-	NRSV	Quacquarelli and Avgelis (1975)
Nigerian cassava mosaic-	NCMV	Adejare and Coutts (1982)
Nigerian yam-	NYV	Porth et al. (1987)
Odontoglossum ringspot *Tobamovirus*	ORSV	Christie and Crawford (1978)

Table 2 (continued)

Virus name	Acronym	Literature
Okra mosaic *Tymovirus*	OkMV	Edwardson and Christie (1986a)
Onion mite-borne latent *Potyvirus* (garlic strain)	OmbLV	Van Dijk et al. (1991)
Ourmia melon-	OuMV	Lisa et al. (1988)
Papaya mosaic *Potexvirus*	PapMV	Christie and Crawford (1978)
Parsley carrot-leaf-	PCLV	Quacquarelli and Avgelis (1975)
Parsnip mosaic *Potyvirus* (aphid)	ParMV	Van Dijk and Bos (1989)
Parsnip mottle-	PMV	Van Dijk and Bos (1989)
Parsnip yellow fleck-	PYFV	Van Dijk and Bos (1985), Van Dijk et al. (1987), Hemida and Murant (1989), Van Dijk and Bos (1989)
Passion-fruit ringspot-	PfRSV	De Wijds (1974)
Pea enation mosaic PEMV group	PEMV	Quacquarelli and Avgelis (1975)
Peanut clump *Furovirus*	PCV	Thouvenel and Fauquet (1981)
Peanut green mosaic-	PGMV	Boswell and Gibbs (1983)
Peanut mild mottle *Potyvirus* (aphid)	PMMV	Xu et al. (1983)
Peanut mottle *Potyvirus* (aphid)	PeMoV	Christie and Crawford (1978)
Peanut stripe-	PStV	Demski et al. (1984)
Peanut stunt *Cucumovirus*	PSV	Christie and Crawford (1978)
Pelargonium leaf curl *Tombusvirus*	PLCV	Vovlas and Grigoriu (1977)
Pelargonium leaf malformation-	PLMV	Vovlas (1974)
Pelargonium zonate spot ? *Tombusvirus*	PZSV	Vovlas and Di Franco (1987)
Pepper mottle *Potyvirus* (aphid)	PepMoV	Christie and Crawford (1978)
Pepper ringspot *Tobravirus*	PepRSV	Langenberg and Purcifull (1989)
Pepper veinal mottle *Potyvirus* (aphid)	PVMV	Atiri (1986), Pirone (1989)
Petunia asteroid *Tombusvirus*	PeAMV	Quacquarelli and Avgelis (1975)
Pittosporum vein clearing-	PVCV	Rana and Di Franco (1979), Di Franco et al. (1980)
Pittosporum vein yellowing *Rhabdovirus*	PVYV	Camele et al. (1992)
Plantain virus 5	PLV5	Hammond (1981)
Plantain virus 6	PLV6	Hammond (1981)
Plantain virus 7	PLV7	Hammond (1981)
Plantain virus X *Potexvirus*	PlVX	Hammond and Hull (1981)
Plum pox *Potyvirus* (aphid)	PPV	Gáborjányi (1993), Lecoq et al. (1993)
Poinsettia mosaic ? *Tymovirus*	PnMV	Lesemann et al. (1983), Koenig et al. (1986), Bellardi and Bertaccini (1989)
Poplar mosaic *Carlavirus*	PopMV	Rana et al. (1989)
Potato aucuba mosaic *Potexvirus* (Fig. 2D)	PAMV	Horváth (1989)
Potato black ringspot *Nepovirus*	PBRSV	Salazar and Harrison (1978)
Potato leafroll *Luteovirus*	PLRV	Barker (1989)
Potato mop-top *Furovirus*	PMTV	Harrison and Jones (1970)
Potato virus Y *Potyvirus* (aphid)	PVY	Christie and Crawford (1978), Savino and Di Franco (1980), Kim et al. (1987)
Potato virus X *Potexvirus*	PVX	Christie and Crawford (1978), Moreira et al. (1980)
Potato yellow dwarf *Rhabdovirus* (2)	PYDV	Lockhart (1989)
Potato yellow mosaic-	PYMV	Roberts et al. (1988)

Table 2 (continued)

Virus name	Acronym	Literature
Radish mosaic *Comovirus*	RaMV	Quacquarelli and Avgelis (1975)
Raspberry ringspot *Nepovirus*	RRSV	Barker (1975), Harrison et al. (1977), Barker and Harrison (1978)
Red clover necrotic mosaic *Dianthovirus*	RCNMV	Boswell and Gibbs (1983)
Roditis leaf discoloration-	RLDV	Rumbos and Avgelis (1989)
Rottboellia yellow mottle *Sobemovirus*	RYMV	Thottappilly et al. (1992)
Scrophularia mottle *Tymovirus*	ScrMV	Rana et al. (1988)
Shallot latent *Carlavirus* (Garlic strain)	SLV	Van Dijk (1993)
Sikte waterborne *Tombusvirus*	SWV	Li et al. (1992)
Sinapis mosaic-	SMV	Avgelis and Quacquarelli (1973a)
Soilborne wheat mosaic *Furovirus*	SBWMV	Rana and Lafortezza (1988)
Spinach yellow mottle-	SYMV	Kruppa et al. (1981)
Solanum apical leaf curling-	SALCV	Hooker (1983)
Sonchus yellow net *Rhabdovirus* (2)	SYNV	Christie and Crawford (1978)
Sorghum chlorotic spot-	SCSV	Langenberg et al. (1988)
Sowbane mosaic *Sobemovirus*	SoMV	Horváth et al. (1993)
Soybean mosaic *Potyvirus* (aphid)	SbMV	Christie and Crawford (1978), Rossel et al. (1983)
Sweet potato feathery mottle *Potyvirus* (aphid)	SPFMV	Anonymous (1992)
Sweet potato latent-	SPLV	Green et al. (1989)
Sweet potato mild mottle *Potyvirus* (whitefly)	SPMMV	Hollings et al. (1976)
Sweet potato sunken vein *Closterovirus*	SPSVV	Cohen et al. (1992)
Telfairia mosaic-	TeMV	Shoyinka et al. (1987)
Tobacco etch *Potyvirus* (aphid)	TEV	Christie and Crawford (1978)
Tobacco mosaic *Tobamovirus*	TMV	Quacquarelli and Avgelis (1975), Avgelis (1986), Paludan (1988)
Tobacco necrosis *Necrovirus*	TNV	Quacquarelli and Avgelis (1975), Horváth (1989), Nienhaus et al. (1990)
Tobacco rattle *Tobravirus*	TRV	Kurppa et al. (1981)
Tobacco ringspot *Nepovirus*	TRSV	Christie and Crawford (1978), Edwardson and Christie (1986b)
Tobacco streak *Ilarvirus*	TSV	Christie and Crawford (1978), Dijkstra (1983)
Tobacco stunt-	TStV	Hiruki (1975)
Tomato aspermy *Cucumovirus*	TAV	Christie and Crawford (1978)
Tomato big bud disease-	TBBV	Flores (1972)
Tomato bushy stunt *Tombusvirus*	TBSV	Christie and Crawford (1978), Koenig and Avgelis (1983), Koenig and Lesemann (1985)
Tomato golden mosaic *Geminivirus* (III)	TGMV	Hamilton et al. (1982), Buck and Coutts (1985), Petty et al. (1988)
Tomato leaf curl-	TLCV	Green and Sulyo (1987)
Tomato mosaic *Tobamovirus*	ToMV	Avgelis (1986)
Tomato mottle *Geminivirus*	TMotV	Polston et al. (1993)
Tomato ringspot *Nepovirus*	ToRSV	Bitterlin and Gonsalves (1988), Horváth (1989)

Table 2 (continued)

Virus name	Acronym	Literature
Tomato spotted wilt *Tospovirus*	TSWV	Cho et al. (1987), Latin and Ruhl (1990), Urban (1990)
Tomato vein-yellowing-	TVYV	Maataoui et al. (1985)
Tomato yellow leaf curl *Geminivirus*	TYLCV	Rochester et al. (1990)
Tulip breaking *Potyvirus* (aphid)	TBV	Dercks (1991)
Tulip habo necrosis-	THNV	Mowat (1971)
Turnip chlorotic blotch-	TCBV	Hammond and Chastagner (1988)
Turnip crinkle *Carmovirus*	TCV	Russo and Martelli (1982)
Turnip mosaic *Potyvirus* (aphid)	TuMV	Quacquarelli and Avgelis (1975), Hammond and Chastagner (1988)
UV	UV	Stace-Smith (1989)
Vanilla leaf distortion *Potyvirus* (aphid)	VIDV	Pearson et al. (1990)
Vanilla necrosis *Potyvirus*	VNV	Liefting et al. (1992)
Wa tulip-	WaTV	Hammond and Chastagner (1988)
Watermelon mosaic- 2 *Potyvirus* (aphid)	WMV2	Christie and Crawford (1978)
White clover mosaic *Potexvirus*	WClMV	Christie and Crawford (1978)
Wild cucumber mosaic *Tymovirus*	WCMV	Allen and Fernald (1971), Christie and Crawford (1978)
Wild potato mosaic-	WPMV	Jones and Fribourg (1979)
Yam mosaic *Potyvirus* (aphid)	YMV	Thouvenel and Fauquet (1979), Marchoux et al. (1979), Thouvenel and Fauquet (1986)
Zucchini yellow mosaic *Potyvirus* (aphid)	ZYMV	Hseu et al. (1985)

Table 3

Susceptibility of *Nicotiana benthamiana* to plant viruses in different virus groups

Group	Virus[1]
Alfalfa mosaic virus[2]	Alfalfa mosaic
Bromovirus	Broad bean mottle, Cowpea chlorotic mottle, Melandrium yellow fleck
Carlavirus	Cassava brown streak, Dandelion, Elderberry carla, Elderberry latent?, Garlic common latent, Narcissus latent, Poplar mosaic, Shallot latent
Carmovirus	Ahlum waterborne, Narcissus tip necrosis?, Turnip crinkle
Closterovirus	Carrot yellow leaf, Grapevine A, Heracleum latent?, Lilac chlorotic leafspot ?, Sweet potato sunken vein
Comovirus	Andean potato mottle, Cowpea mosaic, Cowpea severe mosaic, Radish mosaic
Cucumovirus	Cucumber mosaic, Peanut stunt, Tomato aspermy
Dianthovirus	Carnation ringspot, Red clover necrotic mosaic
Fabavirus	Broad bean wilt
Furovirus	Beet necrotic yellow vein, Peanut clump, Potato mop-top, Soilborne wheat mosaic

Table 3 (continued)

Group	Virus[1]
Geminivirus	African cassava mosaic (syn.: Cassava latent), Ageratum yellow vein, Bean calico mosaic, Beet curly top, Chickpea chlorotic dwarf, Euphorbia mosaic, Tomato golden mosaic, Tomato mottle, Tomato yellow leaf curel
Hordeivirus	Lychnis ringspot
Ilarvirus	Elm mottle, Hydrangea mosaic, Tobacco streak
Luteovirus	Groundnut rosette assistor, Potato leafroll
Necrovirus	Tobacco necrosis
Nepovirus	Artichoke Italian latent, Artichoke yellow ringspot, Cassava American latent, Cherry leaf roll, Chicory yellow mottle, Grapevine fanleaf, Hibiscus latent ringspot, Potato black ringspot, Raspberry ringspot, Tobacco ringspot, Tomato ringspot
Pea enation mosaic virus[2]	Pea enation mosaic
Potexvirus	Asparagus 3, Cassava common mosaic, Clover yellow mosaic, Cymbidium mosaic, Dioscorea latent, Nandina mosaic, Papaya mosaic, Plantain X, Potato aucuba mosaic?, Potato X, White clover mosaic
Potyvirus (aphid)	Amaranthus mosaic, Bean common mosaic, Bean yellow mosaic, Beet mosaic, Bidens mottle, Blackeye cowpea mosaic, Celery mosaic, Clover yellow vein, Cowpea aphid-borne mosaic, Dasheen mosaic, Henbane mosaic, Iris mild mosaic, Lettuce mosaic, Parsnip mosaic, Peanut mild mottle, Peanut mottle, Pepper mottle, Pepper veinal mottle, Plum pox, Potato Y, Soybean mosaic, Sweet potato feathery mottle, Tobacco etch, Tulip breaking, Turnip mosaic, Vanilia leaf distortion, Vanilia necrosis, Watermelon mosaic 2, Yam mosaic, Zucchini yellow mosaic
Potyvirus (mite)	Onion mite-borne latent
Potyvirus (whitefly)	Cassava brown streak, Sweet potato mild mottle
Rhabdovirus	Cynara, Eggplant mottled dwarf, Pittosporum vein yellowing, Potato yellow dwarf, Sonchus yellow net
Sobemovirus	Rottboellia yellow mottle, Sowbane mosaic
Tobamovirus	Cucumber green mottle mosaic, Maracuya mosaic, Odontoglossum ringspot, Tobacco mosaic, Tomato mosaic
Tobravirus	Pepper ringspot, Tobacco rattle
Tombusvirus	Artichoke mottle crinkle, Carnation Italian ringspot, Cucumber leaf spot, Cymbidium ringspot, Grapevine Algerian latent, Pelargonium leaf curl, Pelargonium zonate spot?, Petunia asteroid, Sikte waterborne, Tomato bushy stunt
Tospovirus	Impatiens necrotic spot, Tomato spotted wilt
Tymovirus	Andean potato latent, Belladonna mottle, Eggplant mosaic, Okra mosaic, Poinsettia mosaic ?, Scrophularia mottle, Wild cucumber mosaic
Unclassified viruses	Artichoke latent, Asystasia mottle, Blueberry scorch disease, Brinjal necrotic mosaic, Caper latent, Caraway latent, Carrot mottle, Cassava African mosaic, Cassava Ivorian bacilliform, Cassava mosaic, Chinese seed-borne, Chino del tomato, Citrus ringspot, Cow parsnip mosaic, Cucumber fruit streak, Dandelion yellow mosaic, Desmodium mosaic, Dioscorea alata ring mottle,

Table 3 (continued)

Group	Virus[1]
	Dioscorea green banding mosaic, Dipladenia mosaic, Eggplant green mosaic, Eggplant severe mottle, Epirus cherry, Globe artichoke latent, Grapevine little pattern, Groundnut rosette, Hippeastrum mosaic, Indian cassava mosaic, Ivy mosaic, Ivy vein clearing, Kalanchoe isometric, Lettuce big-vein, Narcissus late season yellows, Nasturtion ringspot, Nigerian cassava mosaic, Nigerian yam, Ourmia melon, Parsley carrot-leaf, Parsnip mottle, Parsnip yellow fleck, Passion-fruit ringspot, Peanut green mosaic, Peanut stripe, Pelargonium leaf malformation, Pittosporum vein clearing, Plantain 5, Plantain 6, Plantain 7, Potato yellow mosaic, Roditis leaf discoloration, Sinapis mosaic, Spinach yellow mottle, Solanum apical leaf curling, Sorghum chlorotic spot, Sweet potato latent, Telfairia mosaic, Tobacco stunt, Tomato big bud disease, Tomato leaf curl, Tomato vein-yellowing, Tulip habo necrosis, Turnip chlorotic blotch, Wa tulip, Wild potato mosaic.

[1]Viruses indicated by question mark are tentative allocations to a virus group (see Matthews, 1991). [2]Monotypic groups with no approved group names

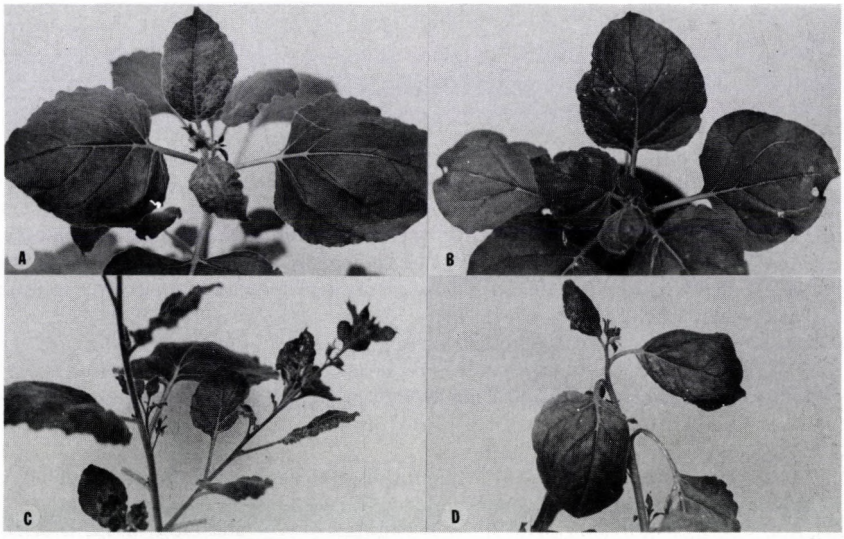

Fig. 1. Systemic symptoms on *Nicotiana benthamiana* Domin. inoculated by Californian isolate of Andean potato mottle *Comovirus* (A), beet necrotic yellow vein Furovirus (B), belladonna mottle *Tymovirus* (C) and cucumber fruit streak virus (D)

Fig. 2. Systemic symptoms on *Nicotiana benthamiana* Domin. inoculated by cucumber green mottle mosaic *Tobamovirus* (A), cucumber leaf spot ? *Tombusvirus* (B), Melandrium yellow fleck *Bromovirus* (C) and potato aucuba mosaic *Potexvirus* (D)

Literature

Adejare, G. O. and Coutts, R. H. A. (1982): Ultrastructural studies on *Nicotiana benthamiana* tissue following infection with a virus transmitted from mosaic-diseased Nigerian cassava. Phytopathol. Z. 103, 87–92.

Allen, T. C., Jr. and Fernald, E. K. (1971): Recovery and partial characterization of wild cucumber mosaic virus from *Marah oreganus*. Plant Dis. Rep. 55, 546–550.

Al-Musa, A. M. and Lockhart, B. (1990): Occurrence of eggplant mottled dwarf virus in Jordan. J. Phytopathol. 128, 283–287.

Anonymous (1992): International Potato Center. Ann. Rep. 1992. Lima, Peru 1992. p. 79.

Atiri, G. I. (1986): A disease of fluted pumpkin (*Telfairia occidentalis* Hook. f.) caused by a yellow veinclearing strain of pepper veinal mottle virus in Nigeria. J. Plant Prot. Tropics 3, 105–110.

Avgelis, A. D. (1986): A pepper strain of TMV who is new in Crete (Greece). Phytopath. Medit. 25, 33–38.

Avgelis, A. D. and Quacquarelly, A. (1973a): Virus diseases of vegetable crops in Apulia. XII. A mosaic of *Sinapis nigra* L. Phytopath. Medit. 12, 48–53.

Avgelis, A. D. and Quacquarelly, A. (1973b): Virus diseases of vegetable crops in Apulia. XIII. Two diseases of celery caused by strains of celery mosaic virus (CeMV). Phytopath. Medit. 12, 118–120.

Avgelis, A. D. and Vovlas, C. (1989): Artichoke yellow ringspot nepovirus naturally infecting cucumber in Crete. Neth. J. Plant Pathol. 95, 177–184.

Avgelis, A. D., Barba, M. and Rumbos, I. (1989): Epirus cherry virus, an unusual virus isolated from cherry with rasp-leaf symptoms in Greece. J. Phytopathol. 126, 51–58.

Avila, A. C., Salazar, L. F., Daniels, J. and Ortega, M. (1984): A new strain of Andean potato mottle virus from Brazil. Plant Dis. 68, 997–998.

Barbara, D. J., Ashby, S. C. and McNamara, D. G. (1985): Host range, purification and some properties of rubus Chinese seed-borne virus. Ann. Appl. Biol. 107, 45–55.

Barker, H. (1975): Effects of virus infection and polyacrylic acid on leaf protein. J. Gen. Virol. 28, 155–158.

Barker, H. (1989): Specificity of the affect of sap-transmissible viruses in increasing the accumulation of luteoviruses in co-infected plants. Ann. Appl. Biol. 115, 71–78.

Barker, H. and Harrison, B. D. (1978): Double infection, interference and superinfection in protoplasts exposed to two strains of raspberry ringspot virus. J. Gen. Virol. 40, 647–658.

Beczner, L., Hamilton, R. I. and Rochon, D. M. (1992): Properties of the mentha strain of Lychnis ringspot virus. Intervirology 33, 49–56.

Bellardi, M. G. and Bertaccini, A. (1989): Virus diseases of ornamental shrubs. III. Angular mosaic of *Euphorbia fulgens*. Phytopath. Medit. 28, 79–82.

Bitterlin, M. W. and Gonsalves, D. (1988): Serological grouping of tomato ringspot virus isolates: implications for diagnosis and cross-protection. Phytopathology 78, 278–285.

Bock, K. R. and Woods, R. D. (1983): Etiology of African cassava mosaic disease. Plant Dis. 67, 994–995.

Bock, K. R. and Harrison, B. D. (1985): African cassava mosaic virus. CMI/AAB Descriptions of Plant Virus Diseases. 297, 1–4.

Bock, K. R., Guthrie, E. J. and Figueiredo, G. (1981): A strain of cassava latent virus occurring in coastal districts of Kenya. Ann. Appl. Biol. 99, 151–159.

Bos, L., Huijberts, N., Huttinga, H. and Maat, D. Z. (1983): Further characterization of dandelion yellow mosaic virus from lettuce and dandelion. Neth. J. Plant Pathol. 89, 207–222.

Boswell, K. F. and Gibbs, A. J. (1983): Viruses of Legumes 1983. Descriptions and Keys from VIDE. The Australian National Univ., Research School of Biol. Sci., Canberra (Australia), 1983.

Brown, J. K. and Nelson, M. R. (1988): Transmission, host range, and virus-vector relationships of chino del tomato virus, a whitefly-transmitted geminivirus from Sinaloa, Mexico. Plant Dis. 72, 866–869.

Brown, J. K., Chapman, M. A. and Nelson, M. R. (1990): Bean calico mosaic, a new disease of common bean caused by a whitefly-transmitted geminivirus. Plant Dis. 74, 81 (Abstr.).

Brunt, A. A. (1975): Viruses and virus diseases of irises in Britain. Acta Hort. 47, 45–50.

Brunt, A. A. (1977): Some hosts and properties of narcissus latent virus a carlavirus commonly infecting narcissus and bolbous iris. Ann. Appl. Biol. 87, 355–364.

Brunt, A. A. (1978): The occurrence, hosts and properties of lilac chlorotic leafspot virus a newly recognised virus from *Syringa vulgaris*. Ann. Appl. Biol. 88, 383–391.

Brunt, A. A., Barton, R. J., Philips, S. and Lana, A. O. (1980): Hibiscus latent ringspot virus, a newly recognised virus from *Hibiscus rosa-sinensis* (Malvaceae) in Western Nigeria. Ann. Appl. Biol. 96, 37–43.

Buck, K. W. and Coutts, R. H. A. (1985): Tomato golden mosaic virus. CMI/AAB Descriptions of Plant Viruses. 303, 1–4.

Büttner, C. and Nienhaus, F. (1989a): Virus contamination of waters in two forest districts of the Rhineland area (FRG). Eur. J. For. Path. 19, 206–211.

Büttner, C. and Nienhaus, F. (1989b): Virus contamination of soils in forest ecosystem of the Federal Republic of Germany. Eur. J. For. Path. 19, 47–53.

Camele, I., Nuzzaci, M., Rana, G. L. and De Stradis, A. (1992): Identification of caper vein yellowing virus as an isolate of *Pittosporum* vein yellowing virus (PVYV). 5th Internat. Plant Virus Epidemiol. Symp., Valenzano, Bari, Italy 1992. pp. 13–14.

Castellano, M. A. and Rana, G. L. (1981): Transmission and ultrastructure of Ivy vein clearing virus infections. Phytopath. Medit. 20, 199–202.

Castellano, M. A. and Rana, G. L. (1982): Occurrence of Hippeastrum mosaic virus in Apulia. Informatore Fitopatol. 32, 37–39.

Cho, J. J., Mitchell, W. C., Mau, R. F. L. and Sakimura, K. (1987): Epidemiology of tomato spotted wilt virus disease on crisphead lettuce in Hawaii. Plant Dis. 71, 505–508.

Christie, S. R. (1969): *Nicotiana* hybrid developed as a host for plant viruses. Plant Dis. 53, 939–941.

Christie, S. R. and Crawford, W. E. (1978): Plant virus range of *Nicotiana benthamiana*. Plant Dis. Rep. 62, 20–22.

CMI/AAB Descriptions of Plant Viruses (1970–1988): Commonwealth Mycological Institute. Association of Applied Biologists. Nos 1–339.

Cohen, J., Franck, A., Vetten, H. J., Lesemann, D. E. and Loebenstein, G. (1992): Purification and properties of closterovirus-like particles associated with a whitefly-transmitted disease of sweet potato. Ann. Appl. Biol. 121, 257–268.

Coutts, R. H. A. and Buck, K. W. (1987): Cassava latent virus specific DNAs in mosaic diseased cassava of Nigerian origin. Neth. J. Plant Pathol. 93, 241–244.

Dawson, W. O. and Hilf, M. E. (1992): Host-range determinants of plant viruses. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43, 527–555.

Dahle, J. L. (1988): Cassia yellow blotch virus. CMI/AAB Descriptions of Plant Viruses. 334, 1–4.

De Bokx, J. A. (1970): Reactions of various plant species to inoculation with potato virus S. Neth. J. Plant Pathol. 76, 70–78.

Demski, J. W., Reddy, D. V. R., Sowell, G. and Bays, D. (1984): Peanut stripe virus – a new seed-borne potyvirus from China infecting groundnut *(Arachis hypogaea)*. Ann. Appl. Biol. 105, 495–501.

Derks, A. F. L. M. (1991): Personal communication.

De Souza, V. B. V., Gergerich, R. C., Kim, K. S. and Langham, M. A. C. (1990): Properties and cytopathology of a tymovirus isolated from eggplant. Phytopathology 80, 1092–1098.

De Wijs, J. J. (1974): A virus causing ringspot of *Passiflora edulis* in the Ivory Coast. Ann. Appl. Biol. 77, 33–40.

Di Franco, A. and Martelli, G. P. (1987): Comparative ultrastructural investigations on four soil-borne cucurbit viruses. J. Submicroscopic. Cytol. and Pathol. 19, 605–613.

Di Franco, A., Russo, M. and Martelli, G. P. (1980): Cell wall outgrowths associated with infection by a plant rhabdovirus. J. Gen. Virol. 49, 221–224.

Di Franco, A., Russo, M. and Martelli, G. P. (1984): Ultrastructure and origin of cytoplasmic multivesicular bodies induced by certain Italian ringspot virus. J. Gen. Virol. 65, 1233–1237.

Dijkstra, J. (1983): Tobacco streak virus in sunflower *(Helianthus annuus)*. Neth. J. Plant Pathol. 89, 153–169.

Dijkstra, J., Clement, Y. and Lohuis, H. (1985): Characterization of a carlavirus from dandelion *(Taraxacum officinale)*. Neth. J. Plant Pathol. 91, 77–92.

Dijkstra, J., Bos, L., Bouwmeester, H. J., Hadiastono, T. and Lohuis, H. (1987): Identification of blackeye cowpea mosaic virus from germplasm of yard-long bean and from soybean, and the relationships between blackeye cowpea mosaic virus and cowpea aphid-borne mosaic virus. Neth. J. Plant Pathol. 93, 115–133.

Edwardson, J. R. and Christie, R. G. (1986a): Viruses Infecting Forage Legumes. Vol. III, Monograph No. 14. University of Florida, Gainesville (USA), 1986.

Edwardson, J. R. and Christie, R. G. (1986b): Viruses Infecting Forage Legumes. Vol. II, Monograph No. 14. University of Florida, Gainesville (USA), 1986.

Fargette, D. and Harrison, B. D. (1990): Properties of cassava Ivorian bacilliform virus (CIBV). Scottish Crop. Res. Institute., Ann. Rep. 1989., Milne, Tannahill and Methven Ltd., Perth 1990.

Fernow, K. H. (1925): Interspecific transmission of mosaic diseases of plants. Agr. Exp. Stat. Cornell, N. Y., Memoir 96.

Flores, E. (1972): Observations and experiments on tomato big bud disease in Sao Paulo State Brazil. Agr. Tropical 22, 187–204.

Foddai, A., Marras, F. and Idini, G. (1978): Presence of a potyvirus on globe artichoke (*Cynara scolymus* L.) in Sardinia. Studi Sassaresi 25, 398–408.

Fribourg, C. E. and Koenig, R. (1985): Serotype III of cowpea severe mosaic virus in Peru. Phytopathol. Z. 113, 368–372.

Fribourg, C. E., Jones, R. A. C. and Koenig, R. (1977a): Host plant reactions, physical properties and serology of three isolates of Andean potato latent virus from Peru. Ann. Appl. Biol. 86, 373–380.

Fribourg, C. E., Jones, R. A. C. and Koenig, R. (1977b): Andean potato mottle, a new member of the cowpea mosaic virus group. Phytopathology 67, 969–974.

Fribourg, C. E., Koenig, R. and Lesemann, D.-E. (1987): A new tobamovirus from *Passiflora edulis* in Peru. Phytopathology 77, 486–491.

Fujisawa, I. (1986): Asparagus virus III: A new member of potexvirus from *Asparagus*. Ann. Phytopath. Soc. Japan, 52, 193–200.

Gallitelli, D. and Di Franco, A. (1987): Characterization of caper latent virus. J. Phytopathology 119, 97–105.

Gáborjányi, R. (1993): Personal communication.

Green, S. K. and Solyo, Y. (1987): Leaf curl virus on tomato in Taiwan Province. Outbreaks and New Rec. 35, 62.

Green, S. K., Luo, C. Y. and Lee, D. R. (1989): Elimination of sweet potato latent virus from *Nicotiana benthamiana* with ribavirin. Plant Prot. Bull. (Taiwan) 31, 310–315.

Gupta, V. P. (1989): Studies on a virus causing mosaic disease on brinjal (*Solanum melongena* L.). Ph. D. thesis. Aligarh Muslim Univ., Aligarh (India), 1989.

Hamilton, W. D. O., Bisardo, D. M. and Buck, K. W. (1982): Identification of novel DNA forms in tomato golden mosaic virus infected tissue, evidence for a two component viral genome. Nucleic Acid Res. 10, 4901–4912.

Hammond, J. (1981): Viruses occurring in *Plantago* species in England. Plant Pathol. 30, 237–243.

Hammond, J. and Hull, R. (1981): Plantain virus X: a new potexvirus from *Plantago lanceolata*. J. Gen. Virol. 54, 75–90.

Hammond, J. and Chastagner, G. A. (1988): Natural infection of tulips with turnip mosaic virus and another potyvirus isolate distinct from breaking virus in the U.S.A. Acta Hort. 234, 235–242.

Hammond, J. and Lawson, R. H. (1988): A strain of bean yellow mosaic virus is aphid-transmitted from orchid. Acta Hort. 234, 365–370.

Harrison, B. D. and Jones, R. A. C. (1970): Host range and some properties of potato mop-top virus. Ann. Appl. Biol. 65, 393–402.

Harrison, B. D., Hutcheson, A. M. and Barker, H. (1977): Association between the particles of raspberry ringspot and tobacco rattle viruses in doubly infected *Nicotiana benthamiana* cells and protoplasts. J. Gen. Virol. 36, 535–539.

Hemida, S. K. and Murant, A. F. (1989): Host ranges and serological properties of eight isolates of parsnip yellow fleck virus belonging to the two major serotypes. Ann. Appl. Biol. 114, 101–109.

Hill, S. A. (1984): Methods in Plant Virology. Blackwell Sci. Publ. Oxford, 1984.

Hiruki, C. (1975): Host range and properties of tobacco stunt virus. Canad. J. Botany 53, 2425–2434.

Hollings, M. (1959): *Nicotiana clevelandii* Gray. as a test for plant viruses. Plant Pathol. 8, 133–137.

Hollings, M. (1966): Local lesion and other test plant for the identification and culture of viruses. In: Beemster, A. B. R. and Dijkstra, J. (eds.), Viruses of Plants. North–Holland Publ. Comp., Amsterdam 1966. pp. 230–241.

Hollings, M., Stone, O. M. and Bock, K. R. (1976): Purification and properties of sweet potato mild mottle, a white-fly borne virus from sweet potato *(Ipomoea batatas)* in East Africa. Ann. Appl. Biol. 82, 511–528.

Holmes, F. B. (1929): Inoculating methods in tobacco mosaic studies. Bot Gaz. 87, 56–63.

Holmes, F. D. (1946): A comparison of the experimental host ranges of tobacco-etch and tobacco-mosaic viruses. Phytopathology 36, 643–659.

Hooker, W. J. and Salazar, L. F. (1983): A new plant virus from the high jungle of the Eastern Andes; Solanum apical leaf curling virus (SALCV). Ann. Appl. Biol. 103, 449–454.

Horn, N. M., Reddy, S. V., Roberts, I. M. and Reddy, D. V. R. (1993): Chickpea chlorotic dwarf virus, a new leafhopper-transmitted geminivirus of chickpea in India. Ann. Appl. Biol. 122, 467–479.

Horvát, F. and Verhoyen, M. (1981): Cytological modifications and presence of virus-like particles in cells of *Nicotiana benthamiana* (Domin) and *Manihot utilissima* (Pohl) infected with the geminivirus isolated from cassava infected with the cassava African mosaic disease. Parasitica 37, 119–130.

Horváth, J. (1964): Ergebnisse der Identifizierung von mechanisch übertragbaren Kartoffelviren an Testpflanzen, mit besonderer Rücksicht auf Vergleichsuntersuchungen. Acta Agr. Hung. 13, 103–134.

Horváth, J. (1969): Symptoms evoked by different strains of potato virus Y on *Nicotiana* species resistant to *Peronospora tabacina* Adam. Acta Agr. Hung. 18, 55–66.

Horváth, J. (1980): Viruses of lettuce. II. Host ranges of lettuce mosaic virus and cucumber mosaic virus. Acta Agr. Hung. 29, 333–352.

Horváth, J. (1983): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. XVIII. Concluding remarks. Acta Phytopath. Hung. 18, 121–161.

Horváth, J. (1989): Unpublished results.

Horváth, J. (1993) Host plants in diagnosis. In: Matthews, R. E. F. (eds.), Diagnosis of Plant Virus Diseases. CRC Press, Boca Raton, Florida 1992. pp. 15–48.

Horváth, J., Hollings, M. and Barton, R. J. (1988): Experimental host range of Melandrium yellow fleck virus. Acta Phytopath. et Entomol. 23, 449–463.

Horváth, J., Virág, J., Pocsai E. and Nyerges, K. (1990): New hosts and resistance sources of beet necrotic yellow vein virus. Növényvédelem 6, 259 (Abstr.).

Horváth, J., Juretić, N., Wolf, I. and Pintér, Cs. (1993): Natural occurrence of sowbane mosaic virus on *Chenopodium hybridum* L. in Hungary. Acta Phytopath. et Entomol. 28, in press.

Hseu, S. H., Wang, H. L. and Huang, C. H. (1985): Identification of a zucchini yellow mosaic virus from *Cucumis sativus*. J. Agr. Res. (China) 34, 87–95.

Huijberts, N., Blystad, D.-R. and Bos, L. (1990): Lettuce big-vein virus: mechanical transmission and relationships to tobacco stunt virus. Ann. Appl. Biol. 116, 463–475.

Izaguirre-Mayoral, M. L., Carballo, O. and Gil, F. (1990): Purification and partial characterization of isometric virus-like particles in Kalanchoe species. J. Phytopathol. 130, 303–311.

Jarmillo, S. and Lastra, R. (1986): Purification and properties of the geminivirus Euphorbia mosaic virus. J. Phytopathol. 115, 193–203.

Jones, A. T. (1972): Purification and properties of elderberry latent virus, one of four sap-transmissible viruses obtained from American elder (*Sambucus canadensis* L.). Ann. Appl. Biol. 70, 49–58.

Jones, A. T. and Mayo, M. A. (1973): Purification and properties of elm mottle virus. Ann. Appl. Biol. 75, 347–357.

Jones, R. A. C. and Fribourg, C.E: (1979): Host plant reactions, some properties, and serology of wild potato mosaic virus. Phytopathology 69, 446–449.

Kegler, H., Richter, J. and Schmidt, H. B. (1966): Untersuchungen zur Identifizierung und Differenzierung des Blattrollvirus der Kirsche (cherry leaf roll virus). Phytopathol. Z. 56, 313–330.

Kim, J. S., Kuo, Y. J. and Green, S. K. (1987): An unusual potyvirus from pepper in Taiwan. Korean J. Plant Pathol. 3, 261–269.

Koenig, R. and Avgelis, A. (1983): Identification of a virus similar to the BS3 strain of tomato bushy stunt virus in eggplant. Phytopathol. Z. 106, 349–353.

Koenig, R. and Leseman, D.-E. (1985): Plant viruses in German rivers and lakes. I. Tombusviruses, a potexvirus and carnation mottle virus. Phytopathol. Z. 112, 105–116.

Koenig, R., Lesemann, D.-E. and Fulton, R. W. (1986): Poinsettia mosaic virus. CMI/AAB Descriptions of Plant Viruses. 311, 1–4.

Koenig, R., Lesemann, D.-E. and Burgermeister, W. (1988): Isolation of carnation ringspot virus from a canal near a sewage plant: cDNA hybridization analysis, serology and cytopathology. J. Phytopathology 121, 346–356.

Kounounguissa, B. R., Givord, L., and Walter, B. (1989): African cassava mosaic virus (ACMV): stability of purified virus and improved conditions for its detection in cassava leaves by ELISA. J. Phytopathol. 127, 29–41.

Kurppa, A., Jones, A. T., Harrison, B. D. and Bailiss, K. W. (1981): Properties of spinach yellow mottle, a distinctive strain of tobacco rattle virus. Ann. Appl. Biol. 98, 243–254.

Ladipo, J. L., Lesemann, D.-E. and Koenig, R. (1988a): Further studies on a virus causing a green mosaic disease of eggplant in Nigeria. J. Phytopathol. 121, 159–165.

Ladipo, J. L., Lesemann, D.-E. and Koenig, R. (1988b): Host ranges, serology and cytopathology of eggplant and tomato strains of eggplant severe mottle virus, a new potyvirus from Nigeria. J. Phytopathol. 122, 359–371.

Lana, A. P., Lohuis, H., Bos, L. and Dijkstra, J. (1988): Relationships among strains of bean common mosaic virus and blackeye cowpea mosaic virus – members of the potyvirus group. Ann. Appl. Biol. 113, 493–505.

Langenberg, W. G. and Purcifull, D. E. (1989): Interactions between pepper ringspot virus and cylindrical inclusions of two potyviruses. J. Ultrastruct. Molec. Struct. Res. 102, 53–58.

Langenberg, W. G., Lommel, S. A. and Kendall, T. L. (1988): Structural proteins of Sorghum chlorotic spot virus attach to cylindrical inclusions of potato virus Y in doubly infected cells. Phytopathology 78, 1586 (Abstr.).

Latin, R. X. and Ruhl, G. E. (1990): Tomato spotted wilt virus on field-grown peppers in the Midwest. Plant Dis. 74, 183 (Abstr.).

Lawson, R. H. (1988): New viruses and virus strains. Acta Hort. 234, 23–30.

Lawson, R. H., Dienelt, M. M. and Hsu, H. T. (1993): Effects of passaging a defective isolate of impatiens necrotic spot virus at different temperatures. Phytopathology 83, 662–670

Lecoq H., Ravelonandro, M., Wipf-Scheibel, C., Monsion, M., Raccah, B. and Dunez, J. (1993): Aphid transmission of a non-aphid-transmissible strain of zucchini yellow mosaic potyvirus from transgenic plants expressing the capsid protein of plum pox potyvirus. Molec. Plant-Microbe Interact. 6, 403–406.

Lehoczky, J., Boscia, D., Martelli, G., Burgyán, J., Castellano, M. A., Beczner, L. and Farkas, G. (1987): Occurrence of the line pattern hitherto unknown virus disease of grapevine in Hungary. Kertgazdaság 19, 61–79.

Lesemann, D.-E., Koenig, R., Huth, W., Brunt, A. A., Phillips, S. and Barton, R.J. (1983): Poinsettia mosaic virus a tymovirus? Phytopathol. Z. 107, 250–262.

Liefting, L., Pearson, W. and Pone, S. P. (1992): The isolation and evaluation of two naturally occurring mild strains of vanilia necrosis *Potyvirus* for control by cross protection. J. Phytopathol. 136, 9–15.

Li, Yi, Lesemann, D.-E., Koenig, R., Rüdel, M. and Pfeilstetter, E. (1992): Isometric plant viruses in ditches and streams in agricultural areas: Recovery of previously found viruses and identification of hitherto unrecorded carmo- and tombusviruses including grapevine Algerian lantent virus. J. Phytopathology 134, 121–132.

Lima, J. A. A., Oliveira, F. M. E. W., Kitajima, E. W. and Lima, M. G. A. (1981): Biological, cytological and serological properties of a potyvirus isolated from cowpea in Ceará. Fitopatol. Brasil. 6, 205–216.

Lisa, V., Milne, R. G., Accotto, G. P., Boccardo, G. and Caciagli, P. (1988): Ourmia melon mosaic virus, a virus from Iran with novel properties. Ann. Appl. Biol. 112, 291–302.

Lockhart, B. E.-L. (1989): Recurrence of natively occurring potato yellow dwarf virus in Minnesota. Plant Dis. 73, 321–323.

Maataoui, M., Lockhart, B. E. L. and Lesemann, D. E. (1985): Biological, serological, and cytopathological properties of tomato vein-yellowing virus, a rhabdovirus occurring in tomato in Morocco. Phytopathology 75, 109–115.

MacDonald, S. G. and Martin, R. R. (1989): A spherical virus associated with scorch disease of highbush blueberry. Phytopathology 79, 910 (Abstr.).

Makkouk, K. M., Lesemann, D. E. and Haddad, N. A. (1982): Bean yellow mosaic virus from broad bean in Lebanon: incidence, host range, purification, and serological properties. Z. PflKrankh. PflSchutz 89, 59–66.

Makkouk, K. M., Bos, L., Rizkallah, A., Azzam, O. I. and Katul, L. (1988): Broad bean mottle virus: identification, host range, serology, and occurrence on faba bean *(Vicia faba)* in West Asia and North Africa. Neth. J. Plant Pathol. 94, 195–212.

Mali, V. R., Stanley, J. and Markhan, P. G. (1991): Some properties of brown streak viruses isolated from *Cassava* in India. Internat. Conf. Virology in Tropics. Lucknow 1991. p. 85 (Abstr.).

Mamula, D., Juretić, N., Horváth, J. and Librić, M. (1974): Identification and serological properties of tomato mosaic virus isolated in Hungary. Acta Phytopath. Hung. 9, 261–276.

Marchoux, G., Edwige, S. and Migliori, E.A. (1979): Some biological properties of mosaic of yam, *Dioscorea* sp. isolated in Guadeloupe. Phytopathology 11, 535–538.

Martelli, G. P. and Russo, M. (1981): Fine structure of Cymbidium ringspot virus in host tissues. I. Electron microscopy of systemic infections. J. Ultrastruct. Res. 77, 93–104.

Martelli, G. P. and Cherif, C. (1987): Eggplant mottled dwarf virus associated with vein yellowing of honeysuckle. J. Phytopathol. 119, 32–41.

Matthews, R. E. F. (1991): Plant Virology (3rd ed.). Academic Press, New York, 1991.

Mayer, A. (1886): Über die Mosaikkrankheit des Tabaks. Landwirtsch. Versuchsstat. 32, 451–467.

Mayoral, M. L., Uzcategui, R., Cropeza, T., Gil, F. and Garcia, V. (1988): Weeds and alfalfa cultivars as reservoirs of a temperature-sensitive strain of alfalfa mosaic virus in Venezuela. J. Plant Dis. Prot. 95, 635–641.

McKinnon, J. P. and Bagnall, R. H. (1972): Use of *Nicotiana debneyi* to detect viruses S, X and Y potato seed stocks and relative susceptibility of six common varieties to potato virus S. Potato Res. 15, 81–85.

Monette, P. L. and James, D. (1990): Detection of two strains of grapevine virus A. Plant Disease 74, 898–900.

Moreira, A., Jones, R. A. C. and Fribourg, C. E. (1980): Properties of a resistance-breaking strain of potato virus X. Ann. Appl. Biol. 95, 93–103.

Mowat, W. P. (1971): Stabilization, culture and some properties of tulip halo necrosis virus. Ann. Appl. Biol. 69, 147–155.

Mowat, W. P., Asjes, C. J. and Brunt, A. A. (1977): Occurrence, purification and properties of narcissus tip necrosis virus. Ann. Appl. Biol. 86, 189–198.

Mowat, W. P., Duncan, G. H. and Dawson, S. (1988): Narcissus late season yellows potyvirus: symptoms, properties and serological detection. Ann. Appl. Biol. 113, 531–544.

Murant, A. F., Rajeshwari, R., Robinson, D. J. and Raschké, J. H. (1988): A satellite RNA of groundnut rosette virus that is largely responsible for symptoms of groundnut rosette disease. J. Gen. Virol. 69, 1479–1486.

Nienhaus, F., Büttner, C. and Hamacher, J. (1990): Virus infection of forest trees by mechanical transmission. J. Phytopathology 129, 141–150.

Paludan, N. (1988): Viruses in *Diplandenia sanderi*. Acta Hort. 234, 53–59.

Paludan, N. and Begtrup, J. (1988): Strain of tomato bushy stunt virus, causing chlorotic mosaic and chlorosis in the leaves demonstrated in *Kalanchoe blossfeldiana*. Danish J. Plant Soil Sci. 92, 89–95.

Pearson, M. N., Brunt, A. A. and Pone, S. P. (1990): Some hosts and properties of a potyvirus infecting *Vanilla fragrans* (Orchidaceae) in the Kingdom of Tonga. J. Phytopathol. 128, 46–54.

Petty, I. I. T., Buck, K. W. and Coutts, R. H. A. (1988): Systemic infection of *Petunia* by mechanical inoculation with tomato golden mosaic virus. Neth. J. Plant Pathol. 94, 3–7.

Phillips, S. and Brunt, A. A. (1988): Dioscorea latent virus. CMI/AAB Descriptions of Plant Viruses 335, 1–2.

Phillips, S., Piggott, J. D'A. and Brunt, A.A. (1986): Further evidence that dioscorea latent virus is a potexvirus. Ann. Appl. Biol. 109, 137–145.

Pirone, T. P. (1989): Comparison of tobacco vein mottling and pepper veinal mottle viruses. Plant Dis. 73, 336–339.

Polston, J. E., Hiebert, E., McGovern, R. J., Stansly, P. A. and Schuster, D. J. (1993): Host range of tomato mottle virus, a new geminivirus infecting tomato in Florida. Plant Dis. 77, 1191–1184.

Porth, A. and Nienhaus, F. (1983): Dioscorea alata ring mottle virus, a new potyvirus of yam in Togo. Z. PflKrankh. PflSchutz 90, 352–362.

Porth, A., Lesemann, D.-E. and Vetten, H.J. (1987): Characterization of potyvirus isolates from West African yams (*Dioscorea* spp.). J. Phytopathol. 120, 166–183.

Price, W. C. (1930): Local lesions on bean leaves inoculated with tobacco mosaic virus. Amer. J. Botany. 17, 694–702.

Provvidenti, R., Gonsalves, D. and Humaydan, H.S. (1984): Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. Plant Dis. 68, 443–446.

Purcifull, D. E., Christie, S. R. and Lima, J. A. A. (1981): Detection of four isometric plant viruses in sodium dodecyl sulfate immunodiffusion tests. Phytopathology 71, 1221–1224.

Quacquarelli, A. and Avgelis, A. (1975): *Nicotiana benthamiana* Domin, as host for plant virus. Phytopath. Medit. 14, 36–39.

Rajeshwari, R. and Murant, A. F. (1988): Purification and particle properties of groundnut rosette assistor virus and production of a specific antiserum. Ann. Appl. Biol. 112, 403–414.

Rana, G. L. and Di Franco, A. (1979): Mechanical transmission of Pittosporum vein clearing virus. Phytopath. Medit. 18, 48–56.

Rana, G. L. and Lafortezza, R. (1988): *Nicotiana benthamiana* Domin, a new host of soilborne wheat mosaic virus (SBWMV). Informatore Fitopatol. 38, 46–48.

Rana, G. L., Vovlas, C. and Zettler, F. W. (1983): Manual transmission of dasheen mosaic virus from *Richardia* to nonaraceous. Plant Dis. 67, 1121–1122.

Rana, G. L., Di Franco, A. and Galasso, I. (1988): Further studies on cynara rhabdovirus. J. Phytopathol. 123, 147–155.

Rana, G. L., Castellano, M. A. and Koenig, R. (1988): Characterization of a tymovirus isolated from *Anagyris foetida* as a strain of Schrophularia mottle virus. J. Phytopathol. 121, 239–249.

Rana, G. L., Gallitelli, D., Kyriakopoulou, P. E., Russo, M. and Martelli, G.P. (1980): Host range and properties of artichoke yellow ringspot virus. Ann. Appl. Biol. 96, 177–185.

Rana, G. L., Russo, M., Gallitelli, D. and Martelli, G. P. (1982): Artichoke latent virus: characterization, ultrastructure and geographical distribution. Ann. Appl. Biol. 101, 279–289.

Rana, G. L., Piazzolla, P., Lafortezza, R. and Greco, N. (1989): Characterization of a latent elongated virus from globe artichoke (*Cynara scolymus* L.) in Italy. J. Phytopathol. 125, 289–298.

Reckhaus, P. and Nienhaus, F. (1981): Etiology of a virus disease of white yam (*Dioscorea rotundata*) in Togo. Z. PflKrankh. PflSchutz 88, 492–509.

Reddy, D. V. R., Murant, A. F. and Duncan, G. H. (1985): Viruses associated with chlorotic rosette and green rosette diseases of groundnut in Nigeria. Ann. Appl. Biol. 107, 57–64.

Richter, J. (1983): Differenzierung von Stämmen des Gurkenmosaik-Virus (cucumber mosaic virus). Arch. Phytopathol. Pflanzenschutz 19, 77–79.

Roberts, I. M. (1989): Indian cassava mosaic virus: ultrastructure of infected cells. J. Gen. Virol. 70, 2729–2739.

Roberts, E. J. F., Buck, K. W. and Coutts, R. H. A. (1988): Characterization of potato yellow mosaic virus as a geminivirus with bipartite genome. Intervirology 29, 162–169.

Rochester, D. E., Kositratana, W. and Beachy, R. N. (1990): Systemic movement and symptom production following agroinoculation with a single DNA of tomato yellow leaf curl Geminivirus (Thailand). Virology 178, 520–526.

Rossel, H. W., Thottappilly, G. and Huttinga, H. (1983): Soybeans. Ann. Rep., Internat. Inst. Tropical Agr., Ibadan (Nigeria). pp. 80–82.

Rumbos, I. C. and Avgelis, A. D. (1989): Roditis leaf discoloration – a new virus disease of grapevine: symptomatology and transmission to indicator plants. J. Phytopathol. 125, 274–278.

Rushing, A. E., Sunter, G., Gardiner, W. E., Dute, R. R. and Bisaro, D. M. (1987): Ultrastructural aspects of tomato golden mosaic virus infection in tobacco. Phytopathology 77, 1231–1236.

Russo, M. and Martelli, G. P. (1982): Ultrastructure of turnip crinkle and saguarco cactus virus-infected tissues. Virology 118, 109–116.

Salazar, L. F. and Harrison, B. D. (1978): Host range and properties of potato black ringspot virus. Ann. Appl. Biol. 90, 375–386.

Savino, V. and Gallitelli, D. (1976): *Cynanchum acutum* L., a new natural host of lucerne mosaic virus (LMV). Phytopath. Medit. 15, 63–64.

Savino, V. and Di Franco, A. (1980): Virus diseases of vegetable crops in Apulia. XXV. Malformations of tomato plants caused by potato virus. Informatore Fitopatol. 2, 7–10.

Savino, V., Quacquarelli, A., Gallitelli, D., Piazzolla, P. and Martelli, G. P. (1977): Cherry leaf roll virus in valnut. I. Identification and characterization. Phytopath. Medit. 16, 96–102.

Schmelzer, K. (1963): Untersuchungen an Viren der Zier- und Wildgehölze. 4. Mitteilung: Versuche zur Differenzierung und Identifizierung der Ringfleckenviren. Phytopathol. Z. 46, 315–342.

Schmelzer, K. and Wolf, P. (1977): Wirtspflanzen und ihre Viren, Virosen und Mykoplasmosen. In: Klinkowski, M. (ed.), Pflanzliche Virologie. Registerband, Verzeichnisse und Übersichten zu den Virosen Europa. Akademie Verlag, Berlin 1977. pp. 54–189.

Sequeira, J. C. and Harrison, B. D. (1982): Serological studies on cassava latent virus. Ann. Appl. Biol. 101, 33–42.

Shoyinka, S. A., Brunt, A. A., Phillips, S., Lesemann, D. E., Thottappilly, G. and Lastra, R. (1987): The occurrence, properties and affinities of Telfairia mosaic virus, a potyvirus prevalent in *Telfairia occidentalis* (Cucurbitaceae) in South Western Nigeria. J. Phytopathol. 119, 13–24.

Stace-Smith, R. (1989): Isolation of a virus from drainage water in British Columbia. Phytopathology 79, 911–912.

Stanley, J., Latham, J. R., Pinner, M. S., Bedford, I. and Markham, P. G. (1992): Mutational analysis of the monopartite geminivirus beet curly top virus. Virology 191, 396–405.

Taiwo, M. A. (1988): Studies on a virus disease of *Amaranthus hybridus* L. in Nigeria. Internat. J. Tropical Plant Dis. 6, 195–200.

Tan, H. N. P. and Wong, S. M. (1993): Some properties of Singapore Ageratum yellow vein virus (SAYVV). J. Phytopathology 139, 165–176.

Timmer, L. W., Garnsey, S. M. and McRitchie, J. J. (1978): Comparative symptomatology of Florida and Texas isolates of citrus ringspot virus on citrus and herbaceous hosts. Plant Dis. 62, 1054–1058.

Thomas, B. J., Barton, R. J. and Tuszynski, A. (1983): Hydrangea mosaic virus, a new ilarvirus from *Hydrangea macrophylla* (Saxifragaceae). Ann. Appl. Biol. 103, 261–270.

Thornberry, H. H. (1966): Index of Plant Virus Diseases. ARS, USDA. Agriculture Handbook No. 307. Washington 1966.

Thottappilly, G., Van Lent, J. W. M., Rossel, H. W. and Sehgal, O. P. (1992): Rottboellia yellow mottle virus, a new sobemovirus affecting *Rottboellia cochinchinensis* (Itch grass) in Nigeria. Ann. Appl. Biol. 120, 205–215.

Thouvenel, J.-C. and Fauquet, C. (1979): Yam mosaic, a new potyvirus infecting *Dioscorea cayenensis* in the Ivory Coast. Ann. Appl. Biol. 93, 279–283.

Thouvenel, J.-C.and Fauquet, C. (1981): Further properties of peanut clump virus and studies on its natural transmission. Ann. Appl. Biol. 97, 99–107.

Thouvenel, J.-C. and Fauquet, C. (1986): Yam mosaic virus. CMI/AAB Descriptions of Plant Viruses. 314, 1–4.

Thouvenel, J.-C., Fauquet, C. and Fargette, D. (1988): Identification and characterization of a newly described potyvirus in West Africa: Asystasia mottle virus. Ann. Appl. Biol. 112, 127–132.

Urban, L. A. (1990): Virion morphology differences between two tomato spotted wilt virus isolates. Phytopathology 80, 1022 (Abstr.).

Valverde, R. A. (1984): Unusual strain of cucumber mosaic virus causing flower-breaking symptoms in wild violets. Plant Dis. 68, 913–915.

Van Dijk, P. (1993): Carlavirus isolates from cultivated *Allium* species represent three viruses. Neth. J. Plant Pathol. 99, 233–257.

Van Dijk, P. and Bos, L. (1985): Viral dieback of carrot and other Umbelliferae caused by the Anthriscus strain of parsnip yellow fleck virus, and its distinction from carrot mottley dwarf. Neth. J. Plant Pathol. 91, 169–187.

Van Dijk, P. and Bos, L. (1989): Survey and biological differentiation of viruses of wild and cultivated Umbelliferae in the Netherlands. Neth. J. Plant Pathol. 95 (Suppl. 2), 1–34.

Van Dijk, P., Van der Meer, F. A. and Piron, P. G. M. (1987): Accessions of Australian *Nicotiana* species suitables as indicator hosts in the diagnosis of plant virus diseases. Neth. J. Plant Pathol. 93, 73–85.

Van Dijk, P., Verbeek, M. and Bos, L. (1991): Mite-borne virus isolates from cultivated *Allium* species, and their classification into two new rymoviruses in the family Potyviridae. Neth. J. Pl. Pathol. 97, 381–399.

Van Lent, J. W. M. and Dijkstra, J. (1980): Characterization of a carlavirus in elderberry (*Sambucus* spp.). Neth. J. Plant Pathol. 86, 117–134.

Vetten, H. J., Lesemann, D.-E. and Al-Musa, A. M. (1989): Comparative studies of cucumber leafspot virus isolates. 6th Conf. Recent Adv. Vegetable Virus Res., Asilomar, California (USA) 1989. pp. 33.

Vovlas, C. (1974): Leaf malformations, a new virus disease of *Pelargonium*. Phytopath. Medit. 13, 139–142.

Vovlas, C. and Martelli, G. P. (1973): Virus diseases of vegetable crops in Apulia. XI. The reddish ringspot of broad bean. Informatore Fitopatol. 5, 5–8.

Vovlas, C. and Quacquarelli, A. (1975): Virus diseases of vegetable crops in Apulia. XVII. A yellow mosaic of lettuce induced by chicory yellow mottle virus. Phytopath. Medit. 14, 144–146.

Vovlas, C. and Grigoriu, A. C. (1977): Occurrence of Pelargonium leaf curl virus (PLCV) in Greece. Phytopath. Medit. 16, 34–36.

Vovlas, C. and Di Franco, A. (1987): Nuovi dati sulla presenza di virus delle piante nei corsi d'acqua di Puglia e Bacilicita. Informatore Fitopatol. 3, 55–59.

Walter, B. (1980): Isolation and purification of a virus transmitted from mosaic-diseased cassava in the Ivory Coast. Plant Dis. 64, 1040–1042.

Walter, B., Ladeveze, I., Etienne, L. and Fuchs, M. (1989): Some properties of a previously undescribed virus from cassava: Cassava American latent virus. Ann. Appl. Biol. 115, 279–289.

Xu, Z. (1984): Identification of a cucumber mosaic virus strain from naturally infected peanuts in China. Plant Dis. 68, 386–389.

Xu, Z., Yu, Z., Lui, J. and Barnett, O. W. (1983): A virus causing peanut mild mottle in Hubei Province, China. Plant Dis. 67, 1029–1032.

Zettler, F. W. and Elliott, M. S. (1986): An antigenically distinct strain of cassava common mosaic virus infecting *Cnidoscolus aconitifolius*. Phytopathology 76, 632–638.

Zettler, F. W., Hiebert, E., Christie, R. G., Marciel-Zambolim, E. and Abo El-Nil, M. M. (1980): A potexvirus infecting *Nandina domestica* 'Harbor Dwarf'. Acta Hort. 110, 71–80.

Zhao, G. S., Baltensperger, D. D., Purcifull, D. E., Christie, R. G., Hiebert, E. and Edwardson, J. R. (1991): Host range, citology, and transmission of an alyceclover isolate of blackeye cowpea mosaic virus. Plant Dis. 75, 251–253.

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Natural Occurrence of Sowbane Mosaic Virus on *Chenopodium hybridum* L. in Hungary[1]

J. HORVÁTH[1], N. JURETIĆ[2], I. WOLF[3] and CS. PINTÉR[1]

Pannonian University of Agricultural Sciences, Institute for Plant Protection, Department of Plant Pathology, H-8361 Keszthely, P.O. Box 71, Hungary[1], University of Zagreb, Institute of Botany, CRO-41000 Zagreb, Marulicev trg. 20/II, Croatia[2] and Pannonian University of Agricultural Sciences, Department of Potato Research, H-8361 Keszthely, P.O. Box 71, Hungary[3]

From wild plant *Chenopodium hybridum* L., which had yellow flecking and severe mosaic, a isolate of sowbane mosaic virus (SoMV) was isolated. The virus isolate was identified on the basis of test plants, serology, electron microscopy and properties in vitro. *Nicotiana benthamiana* proved to be a new symptomless host of SoMV. The virus reacted with the antisera to the American (A) and Yugoslavian (J) isolates of SoMV and it could not be distinguishable from them. Isometric virus particles, c. 30 nm were found in partially purified preparations of SoMV-H. The thermal inactivation point was 88 °C, the dilution end-point 10^{-6} and the longevity in vitro 24 days. This is the first report of the natural occurrence of SoMV in *Chenopodium hybridum*.

Since its discovery (Silva et al., 1958) and description (Bennett and Costa, 1961) the sowbane mosaic virus (SoMV), one of the eleven viruses belonging to the *Sobemovirus* (*so*uthern *be*an *mo*saic *virus*) group, has been often in the centre of interest of plant virologists. This fact can be explained with the extremely high susceptibility of the *Chenopodium* species as very important diagnostical plants in virology to this virus. Moreover, SoMV is easily transmissible by seed, pollen and in mechanical way. Because of that it often causes contamination of chenopods in greenhouse. Further SoMV is high resistant to physical impacts (Kado, 1966, 1967, 1971; Engelbrecht and Van Regenmortel, 1968; Paul and Huth, 1970; Horváth, 1972; Schuster et al., 1973; Tremaine and Ronald, 1977; Sehgal, 1981; Francki and Miles, 1985; Francki et al., 1985; Lehoczky and Salamon, 1989).

The host range of SoMV includes 14 natural and 59 artificial hosts from 16 families (Table 1). Among the susceptible plants the *Chenopodium* species are the most important. According to literature 13 *Chenopodium* species can be infected artificially whereas only one species *(Chenopodium murale)* is known to be naturally affected by SoMV (see Table 1). From economically important cultivated plants sugar-beet, cherry, carnation, fig, apple, plum and grape-vine are known to be susceptible to SoMV (Kirkpatrick et al., 1965; Hollings and Stone, 1966; Bancroft and Tolin, 1967; Bercks and Querfurth,

[1]In memoriam Prof. Dr. J. Lehoczky (†1993)

Akadémiai Kiadó, Budapest

1969; Šarić, 1971; Quacquarelli, 1971; Pozděna et al., 1977; Buturović and Juretić, 1980; Šarić and Juretić, 1980). Concerning susceptibility of the individual plant species to SoMV the literature contains contradictions. According to recent data by Guy (1982) the susceptibility of certain plants to SoMV (e. g. *Beta vulgaris*, *Cucumis sativus*, *Nicotiana clevelandii*, *N. glutinosa*, *N. tabacum*, *Phaseolus vulgaris*) is not demonstrable (see Table 1).

Table 1

Survey of plant species susceptible to sowbane mosaic virus (SoMV)

Family and species	Type of host[1]	Literature
ACERACEAE		
Acer spp.	N	Erdiller (1986)
AIZOACEAE		
Tetragonia expansa (Pall.) O. Ktze	A, U	Juretić (1976), Buturović, and Juretić (1980)
AMARANTHACEAE		
Amaranthus caudatus L.	A	Schmidt (1977)
Gomphrena globosa L.	A	Teakle (1988), Schmidt (1977)
CARYOPHYLLACEAE		
Dianthus caryophyllus L.	N	Hollings and Stone (1966)
CHENOPODIACEAE		
Atriplex cordulata Jepson	A	Bennett and Costa (1961)
A. coulteri (Moq.) D.	A	Bennett and Costa (1961)
A. expansa S. Wats.	A	Bennett and Costa (1961)
A. hortensis L.	A	Bennett and Costa (1961), Zebzami et al. (1987)
A. inflata L. (syn.: A. halimoides Lindl.)	A	Guy (1982)
A. pacifica Nels.	A	Bennett and Costa (1961)
A. rosea L.	A	Bennett and Costa (1961)
A. semibaccata Guss.	A	Zebzami et al. (1987)
A. spongiosa F. Muell.	A	Guy (1982)
A. suberecta Verdoon	N	Guy (1982)
A. vesicaria Heward	A	Guy (1982)
Beta macrocarpa Guss.	A	Bennett and Costa (1961)
B. vulgaris L.	A	Bennett and Costa (1961), Kado (1971)
B. vulgaris L.	N	Buturović and Juretić (1980)
B. vulgaris L. cv. Blond Frisee	A	Zebzami et al. (1987)
B. vulgaris L. var. saccharifera Lange cv. Osjecka Poly l	A	Butrović and Juretić (1980)
Chenopodium spp.	N	Silva et al. (1958)
C. acutifolium Hook et Arm.	A	Kado (1967)
C. album L.	A	Bennett and Costa (1961), Juretić (1976), Buturović and Juretić (1980), Zebzami et al. (1987)

Table 1 (continued)

Family and species	Type of host[1]	Literature
C. amaranticolor Coste et Reyn	A	Bancroft and Tolin (1967), Diaz and Waterworth (1967), Quacquarelly (1971), Schuster et al. (1973), Juretić (1976), Zebzami et al. (1987)
C. ambrosioides L.	A	Juretić (1976)
C. anthelminticum L.	A	Kado (1967)
C. bonus-henricus L.	A	Kado (1967)
C. botrys L.	A	Schmidt (1977)
C. capitatum Aschers.	A	Bennett and Costa (1961)
C. foetidum Schrad.	A	Schuster et al. (1973), Juretić (1976), Buturović and Juretić (1980)
C. foliosum Aschers.	A	Juretić (1976)
C. hybridum L.	A	Kado (1967)
C. murale L.	A	Schuster et al. (1973), Juretić (1976), Šarić and Juretić (1980), Buturović and Juretić (1980)
C. murale L.	N	Bennett and Costa (1961), Schuster et al. (1973), Juretić (1976)
C. opulifolium Schrad.	A	Schmidt (1977)
C. polyspermum L.	A	Kado (1967)
C. quinoa Willd.	A	Bennett and Costa (1961), Bancroft and Tolin (1967), Dias and Waterworth (1967), Quacquarelli (1971), Jankulowa (1972), Schuster et al. (1973), Juretić (1976), Francki and Miles (1985), Zebzami et al. (1987), Lehoczky and Salamon (1989)
C. schraderianum Roem et Schult.	A	Kado (1967)
C. trigonon Roem et Schult.	A	Teakle (1968)
C. urbicum L.	A	Bennett and Costa (1961)
C. vulvaria L.	A	Schmidt (1977)
Kochia scoparia (L.) Schrad.	A	Bennett and Costa (1961)
Monolepis nuttalliana (Schultes) Greene	A	Bennett and Costa (1961), Schmidt (1977)
Spinacia oleracea L.	A, N	Schuster et al. (1973), Juretić (1976), Francki and Miles (1985), Zebzami et al. (1987)
COMPOSITAE		
Chrysanthemum indicum L.	N	Schmidt (1977)
Lactuca sativa L.	A	Bercks and Querfurth (1969)
CRUCIFERAE		
Brassica chinensis L.	A	Bercks and Querfurth (1969)
CUCURBITACEAE		
Cucumis sativus L.	A, SlH	Teakle (1968)
Cucurbita maxima L.	A	Schmidt (1977)

Table 1 (continued)

Family and species	Type of host[1]	Literature
LEGUMINOSEAE		
Cyamopsis tetragonoloba (L.) Taub	A	Schmidt (1977)
Dolichos biflorus L.	A, SlH	Teakle (1968)
Phaseolus vulgaris L. cv. Bountiful	A, SlH	Teakle (1968)
P. lathyroides L.	A, SlH	Teakle (1968)
Vigna sinenses (L.) Savi ex Hassk. cv. Blackeye	A, SlH	Teakle (1968)
MORACEAE		
Ficus altissima Blume	N	Quacquarelli (1971)
PRIMULACEAE		
Primula malacoides Franch.	A	Bercks and Querfurth (1969)
PORTULACEAE		
Talinum paniculatum (Jacq.) Willd.	A	Bercks and Querfurth (1969)
ROSACEAE		
Cerasus acida (Dum.) Dostál.	N	Šarić (1971), Németh (1986)
Malus pumila Mill.	N	Kirkpatrick et al. (1965), Bancroft and Tolin (1967), Engelbrecht and Van Regenmortel (1968), Németh (1986)
Prunus cerasus L. cv. Marasca	N	Šarić (1971), Šutić and Juretić (1976), Šarić and Juretić (1980)
P. domestica L.	N	Šutić and Juretić (1976), Šarić and Juretić (1980), Németh (1986)
P. spinosa L.	N	Németh (1986)
SCHROPHULARIACEAE		
Antirrhinum majus L.	A	Bercks and Querfurth (1969)
SOLANACEAE		
Capsicum annuum L.	A	Bercks and Querfurth (1969)
Nicotiana benthamiana Domin.	A, SlH	Horváth et al. (1990)
N. glutinosa L.	A, SlH	Teakle (1968)
N. clevelandii Grey.	A, SlH	Teakle (1968)
N. tabacum L. cv. Turkish	A, SlH	Teakle (1968)
Petunia hybrida Vilm.	A, SlH	Teakle (1968)
Physalis floridana Rydb.	A	Bercks and Querfurth (1969)
Solanum lycopersicon L.	A	Bercks and Querfurth (1969)
VITACEAE		
Vitis vinifera L.	N	Bercks and Querfurth (1969), Pozděna et al. (1977), Šarić and Juretić (1980)

[1]Abbreviations: A - artificial host, N - natural host, U - uncertain host, SlH - symptomless host

As far as we know there are no data on the natural susceptibility of *Chenopodium hybridum* to SoMV, with the exception of an earlier our preliminary report (Horváth et al., 1989). Considering that in the past several years we observed SoMV infection on *Chenopodium hybridum* occurring in Hungarian potato-weed communities, we thought it reasonable to carry on etiological studies on the disease of this important weed plant in potato fields and establish its role in virus ecology.

Materials and Methods

The virus infected exemplars of *Chenopodium hybridum* were found in potato fields in the southern part of Hungary. The investigated virus isolate was marked SoMV-H.

Diseased *Chenopodium hybridum* leaves (Fig. 1) were ground in 0.02 M phosphate buffer, pH 7.2, and the sap was transmitted to carborundum-dusted leaves of *Chenopodium amaranticolor*, *C. hybridum*, *C. quinoa* and other test plants (e.g. *Nicotiana benthamiana*, *Spinacia oleracea*) by mechanical inoculation. Inoculated and non-inoculated, apical leaves were assayed on virus-free *Chenopodium quinoa* 3 to 4 weeks after inoculation.

For the serological comparison two other isolates of SoMV were included in the experiments. One of them was the American isolate (SoMV-A) supplied by Dr. H. E. Waterworth and the other one was SoMV-J found in sowbane *(Chenopodium murale)* in Yugoslavia (Juretić, 1976). In serological tests two immune sera to SoMV were used: one of them was sent us by Dr. H. E. Waterworth (titre 1/1024) and the other was prepared against SoMV-J earlier by the second author (titre 1/1024). Serological experiments were performed by means of double radial immunodiffusion technique (Juretić and Mamula, 1978, 1980). Immunoelectrophoretical analysis was carried out by LKB electrophoresis apparatus type 6800-Al. Veronal buffer in agar and in electrode vessels had pH 8.8 and ionic strength 0.1. The experiments were done at room temperature without cooling, under a potential of 8 V/cm 5 hours. In all serological experiments crude infective sap was used.

Partial purification of isolate SoMV-H was done after the chloroform-butanol method of Steere (1959). The virus was further purified by centrifugation on sucrose density gradients of 7–25 per cent at 25 000 rpm for 3.5 hours in Spinco SW 25.1 rotor. The gradients were prepared in 0.03 M phosphate buffer at pH 7.0. Purified virus was analysed using electron microscope and spectrophotometer.

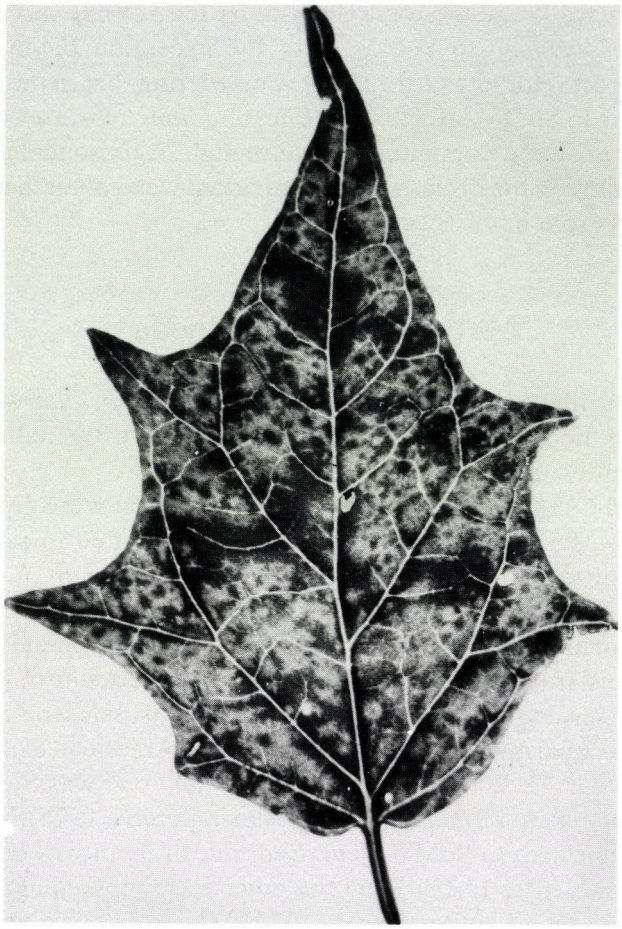

Fig. 1. Symptoms on the leaf of *Chenopodium hybridum* L. naturally infected with sowbane mosaic virus (SoMV)

The properties *in vitro* (thermal inactivation point, dilution end-point, longevity *in vitro*) of the isolate SoMV-H were established in extracted *Chenopodium quinoa* leaf sap, and *C. quinoa* was used as indicator plant.

Results and Discussion

Reaction of test plants

Sap from *Chenopodium hybridum* and/or other two *Chenopodium* species *(C. amaranticolor, C. quinoa)* was rubbed on 17 indicator plants belonging in 5 different families. Symptoms were developed only on several *Chenopodium* species, *Gomphrena globosa*, and *Spinacia oleracea* cv. Matador (Table 2). All test plants, of the family Chenopodiaceae were susceptible to the isolate SoMV-H and among the *Chenopodium* species examined *Chenopodium quinoa* was the most susceptible. This statement agrees with earlier observations by Zebzami et al. (1987). These authors also pointed out that – unlike *C. quinoa* – *Atriplex hortensis*, *A. semibaccata*, *Chenopodium album* and *C. amaranticolor* were found to be suitable for the symptomatological differentiation of some SoMV isolates. Ten plant species in four plant families showed no symptoms after inoculation with isolate SoMV-H (Table 2). With the exception of the apical leaves of *Nicotiana benthamiana* no virus could be back-transmitted to *Chenopodium quinoa*. Consequently, *Nicotiana benthamiana* is a new latent systemic host of SoMV-H.

In the course of investigations related with five symptomless SoMV susceptible plants (*Cucumis sativus*, *Nicotiana clevelandii*, *N. glutinosa*, *N. tabacum* and *Vigna sinensis* cv. Blackeye) detected by Dias and Waterworth (1967) and Teakle (1968), and the susceptible *Cucurbita maxima* reported by Schmidt (1977), we have come to the conclusion that the mentioned plants are non-hosts for the SoMV-H isolate (see Table 2).

Serological relationship

The SoMV-H was compared serologically with two isolates of SoMV (SoMV-A and SoMV-J). In serological tests performed by agar-gel double diffusion method SoMV-H did not differ from the SoMV-A (Fig. 2A) and SoMV-J either. The same results were obtained in tests carried out by single radial immunodiffusion technique (Fig. 2B). Also, SoMV-H could not be distinguished electrophoretically from SoMV-A (Fig. 2C) and SoMV-J. These results are in accordance with the results of other authors, namely, that there is no serological difference between the SoMV isolates (Bercks and Querfurth, 1969; Engelbrecht and Van Regenmortel, 1968; Kado, 1967; Buturović and Juretić, 1980). It should be noted, however, that in the case of a North-American and two Moroccan SoMV isolates Zebzami et al. (1987) found serotype differences. Seven unique antigenic determinants were detected in their studies.

Table 2

Reactions of various plant species to mechanical inoculation with the Hungarian isolate of sowbane mosaic virus (SoMV-H)

Family and species	Reaction of[1]	
	Inoculated leaves	Non-inoculated, apical leaves
AMARANTHACEAE		
Gomphrena globosa L.	RL, Ri	M
CHENOPODIACEAE		
Chenopodium album L.	CL	B, M
C. *amaranticolor* Coste et Reyn.	CL	B, LeD
C. *hybridum* L.	CL	B, LeD
C. murale L.	CL	M, LeD
C. quinoa Willd.	CL	M, B, LeD
Spinacea oleracea L. cv. Matador	CL	Vc, B, M, LeD
CUCURBITACEAE		
Cucumis sativus L. cv. Delicatess	0	0
Cucurbita maxima L.	0	0
LEGUMINOSEAE		
Phaseolus vulgaris L. cv. Pinto	0	0
Pisum sativum L.	0	0
Vigna sinensis (L.) Savi ex Hassk. cv. Blackeye	0	0
SOLANACEAE		
Datura stramonium L.	0	0
Nicotiana benthamiana Domin.	0	SlH
N. glutinosa L.	0	0
N. megalosiphon Heurck. et Muell.	0	0
N. *tabacum* L. cv. Xanthi-nc	0	0

[1]Abbreviations: B - blotching, CL - chlorotic lesions, LeD - leaf deformations, M - mosaic, 0 - no infection detected (back inoculation to *Chenopodium quinoa* is unsuccessful), SlH - symptomless host, Ri - rings, RL - red lesions, Vc - vein clearing

Purification, UV adsorption and electron microscopy

Source of the virus for purification was leaf tissue of *Chenopodium quinoa* plants systemically infected with SoMV-H. After partial purification according to Steere (1959), the virus suspension was further purified by sucrose density gradient centrifugation. During this proceeding the virus was not separated into more opalescent zones but only into one showing that it is one component virus. Purified virus showed a typical absorption curve with maximum in ultraviolet at 260 nm. Ratio A260/280 of the preparations was

between 1.45 to 1.48. This number indicated that the ratio of SoMV-H was very close to that of the typical SoMV (see Kado, 1971).

Electron microscope analysis of partially purified SoMV-H which was negative stained with 4 per cent phosphotungstate revealed isometric virus particles of about 30 nm in diameter (Fig. 2D). The particles could correspond to virus particles of typical SoMV.

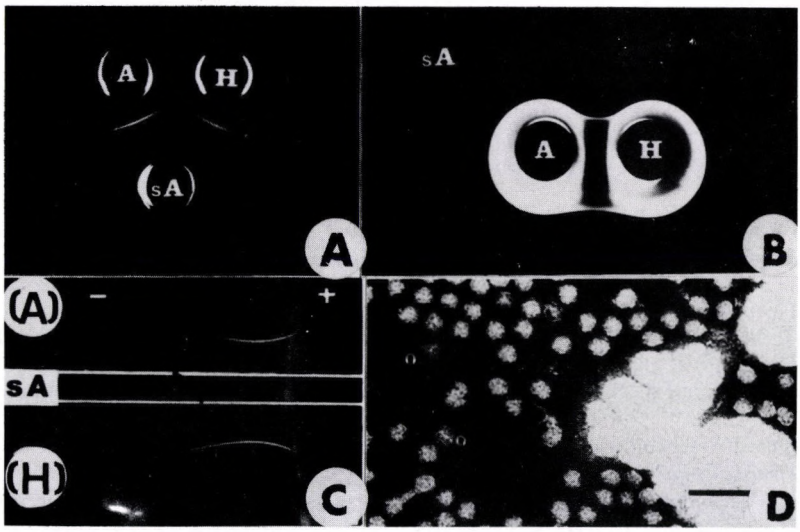

Fig. 2. Serological and immunoelectrophoretic comparison of the SoMV-H (H) with SoMV-A (A) using antiserum to SoMV-A (sA) and virus particles of SoMV-H. *A*, reactions obtained in double radial immunodiffusion test; *B*, single radial immunodiffusion test; *C*, immunoelectrophoretic test; *D*, isometric particles of SoMV-H in partially purified preparation. Bar represents 100 nm

Properties in vitro

The experiments showed that SoMV-H had thermal inactivation point 88 °C, dilution end-point 10^{-6} and longevity *in vitro* 24 days. The above data are in concordance with the results reported for SoMV by some authors (Teakle, 1968; Bercks and Querfurth, 1969; Kado, 1967, 1971, Jankulowa, 1972; Buturović and Juretić, 1980).

Conclusion

SoMV infection of *Chenopodium hybridum* plants appears in masses in potato-weed communities in Hungary. The effect of these infections on

potato is not known. Therefore, it would be important to known, whether the aforenamed virus is artificially transmissible and naturally infectious to potato. If it proved true, essential changes ought to be made in the seed potato certification system, too.

Acknowledgements

We express our thanks to Dr. M. H. V. Van Regenmortel, Institut de Biologie Moléculaire et Cellulaire du CNRS, 67084 Strasbourg Cédex, France for the virus isolate A (SoMV-A), to Dr. H. E. Waterworth, USDA, Glen Dale, Maryland, USA for sending the SoMV antiserum. We are indebted to Miss K. Molnár for her technical assistance.

Literature

Bancroft, J. B. and Tolin, S. A. (1967): Apple latent virus 2 is sowbane mosaic virus. Phytopathology 57, 639–640.

Bennett, C. W. and Costa, A. S. (1961): Sowbane mosaic caused by a seed-transmitted virus. Phytopathology 51, 546–550.

Bercks, R. and Querfurth, G. (1969): Über den Nachweis des sowbane mosaic virus in Reben. Phytopath. Z. 66, 365–373.

Buturović, D. and Juretić, N. (1980): Finding of sowbane mosaic virus in sugar beet. Acta Bot. Croat. 39, 9–13.

Dias, H. F. and Waterworth, H. E. (1967): The identity of a seed-borne mosaic virus of *Chenopodium amaranticolor* and *C. quinoa*. Canad. J. Botany 45, 1285–1295.

Engelbrecht, D. J. and Van Regenmortel, M. H. V. (1968): The problem of sowbane mosaic virus contamination. Phytopath. Z. 63, 10–15.

Erdiller, G. (1986): *Acer* virus diseases in Turkey. J. Turkish Phytopath. 15, 46–59.

Francki, R. I. B. and Miles, R. (1985): Mechanical transmission of sowbane mosaic virus carried on pollen from infected plants. Pl. Path. 34, 11–19.

Francki, R. I. B., Milne, R. G. and Hatta, T. (1985): Sobemovirus group. Atlas of Plant viruses. Vol. 1. CRC Press Inc., Boca Raton, Florida, 1985. pp. 153–169.

Guy, P. (1982): A disease of *Atriplex* caused by sowbane mosaic virus. Austr. Pl. Path. 11, 51–52.

Hollings, M. and Stone, O. M. (1966): Carnation viruses. Rep. Glasshouse Crops Res. Inst., Littlehampton, Sussex, 1966. p. 92.

Horváth, J. (1972): Vírusok, vektorok, vírusátvitel (Viruses, vectors and virus transmission). Akadémiai Kiadó, Budapest, 1972. p. 515.

Horváth, J., Juretić, N., Wolf, I. and Pintér, Cs. (1990): A *Chenopodium* mozaik vírus (sowbane mosaic virus) természetes előfordulása Magyarországon *Chenopodium hybridum* növényben (Natural occurrence of sowbane mosaic virus on *Chenopodium hybridum* in Hungary). Növényvéd. Tud. Napok, Budapest 1990. p. 72.

Jankulowa, M. (1972): Nachweiss und Identifizierung von *Chenopodium* mosaic virus (sowbane mosaic virus) in Bulgarien. Phytopath. Z. 74, 314–317.

Juretić, N. (1976): Some data on sowbane moasic virus isolated from *Chenopodium murale* in Yugoslavia. Acta Bot. Croat. 35. 33–39.

Juretić, N. and Mamula, D. (1978): Studies in the application of single radial immunodiffusion in the quantitation of several isometric plant viruses. Phytopath. Z. 93, 26–34.

Juretić, N. and Mamula, D. (1980): Application of single radial immunodiffusion for qualitative comparison of plant virus particles. Intervirology 13, 209–213.

Kado, C. I. (1966): Purification and characterization of a semi-latent seed borne virus in *Chenopodium amaranticolor*. Phytopathology 56, 884 (Abstr.).

Kado, C. I. (1967): Biological and biochemical characterization of sowbane mosaic virus. Virology 31, 217–229.

Kado, C. I. (1971): Sowbane mosaic virus. CMI/AAB Descriptions of plant viruses 64, 1–4.

Kirkpatrick, H. C., Mink, G. I. and Lindner, R. C. (1965): Purification of a second mechanically transmissible virus latent in apple. Phytopathology 55, 286–290.

Lehoczky, J. and Salamon, P. (1989): A *Chenopodium* mozaik vírus terjedésének potenciális forrása a fertőzött növény pollene (Pollen of diseased *Chenopodium quinoa* is a potential source and mode of natural spread of sowbane mosaic virus). Kertgazdaság 21, 29–40.

Németh, M. (1986): Virus, mycoplasma and rickettsia disease of fruit trees. Akadémiai Kiadó, Budapest. p. 841.

Paul, H. L. and Huth, W. (1970): Untersuchungen über das cocksfoot mild mosaic virus. II. Vergleich des cocksfoot mild mosaic virus mit dem brome mosaic virus, dem cocksfoot mottle virus, dem sowbane mosaic virus. Phytopath. Z. 69, 1–9.

Pozděna, J., Vanek, G. and Filigarová, M. (1977): Sowbane mosaic virus auf der Weinrebe in der CSSR. Sbor. UVTIZ–Ochr. 1977. Rostl. 23, 197–200.

Quacquarelli, A. (1971): Il mosaico del fico e il virus latente del *Chenopodium* (Fig mosaic and a latent virus of *Chenopodium*). Phytopath. medit. 10, 283–286.

Šarić, A. (1971): La présence de sowbane mosaic virus chez le cerisier acide (Some properties of a new virus isolate in naturally infected plum trees). VIII[e] Symp. européen sur les maladies á virus des arbres fruiters. Ann. Phytopathol. hors. sér. 3, 156–158.

Šarić, A. and Juretić, N. (1980): Natural hosts of sowbane mosaic virus in Yugoslavia. Plant Virology, Proc. 8th Conf. Czechoslovak Plant Virologists. Bratislava, 1976. VEDA Publ. House of the Slovak Acad. Sci. Bratislava, 1980. pp. 411–415.

Schmidt, H. E. (1977): Krautige Wildpflanzen. In: Klinkowski, M. (ed.), Pflanzliche Virologie., Band 4. Die Virosen and Zierpflanzen, Gehölzen und Wildpflanzen in Europa. Akademische Verlag, Berlin 1977. pp. 406–528.

Schuster, V., György, K. and Achmet, S. (1973): A *Chenopodium* mozaik vírus megjelenése és leküzdésének lehetősége Magyarországon (Appearance of the *Chenopodium* mosaic virus and possibility of its control in Hungary A Növényvéd. Korsz. 7, 145–149.

Sehgal, O. P. (1981): Southern bean mosaic virus group. In: Kurstak, E. (ed.), Handbook of plant virus infections and comparative diagnosis. Elsevier/North-Holland, Biomedical Press, Amsterdam, 1981. pp. 91–121.

Silva, D. M., Meneghini, M. and De Souza-Santos, H. (1958): Purificacao e propriedades do virus mosaico de quenopodio (Purification and properties of *Chenopodium* mosaic virus). Bragantia 17, 167–176.

Steere, L. R. (1959): The purification of plant viruses. Adv. Virus Res. 6, 1–73.

Šutić, D. and Juretić, N. (1976): Occurring of sowbane mosaic virus in plum tree. Mitt. biol. BundAnst. Land- u. Forstw., Berlin-Dahlem, Heft 170, 43–46.

Teakle, D. S. (1968): Sowbane mosaic virus infecting *Chenopodium trigonon* in Queensland. Austr. J. Biol. Sci. 21, 649–653.

Tremaine, J. H. and Ronald, W. P. (1977): Assembly of particles from Southern bean mosaic virus and sowbane mosaic virus components. Canad. J. Botany 55, 2274–2277.

Zebzami, M., Lockhart, B. E. L. and Hill, J. H. (1987): Identification of three serotypes of sowbane mosaic virus. Phytopathology 77, 571–574.

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Incidence of Cucumber Mosaic Virus in *Commelina communis* L. in Croatia*

N. JURETIĆ[1] and J. HORVÁTH[2]

University of Zagreb, Institute of Botany, CRO-41000 Zagreb, Marulicev trg 20/II, Croatia[1] and Pannonian University of Agricultural Sciences, Institute for Plant Protection, Department of Plant Pathology, H-8361 Keszthely, P.O. Box 71, Hungary[2]

A virus isolated from *Commelina communis* showing mild mosaic and leaf deformation was identified as a isolate of cucumber mosaic virus. Virus identification was based on reactions of test plants, electron microscopy, serology, aphid transmission and cross protection.

This is the first report of natural infection of *Commelina communis* by cucumber mosaic virus.

The number of plant species belonging to the world-known monocot family of *Commelinaceae* is about 650. There are among them commercially important annual and perennial herbaceous weed, ornamental and medicinal plants. From a virological point of view the *Commelinaceae* family has recently become a major subject of interest (Beczner and Schmelzer, 1972; Lockhart and Betzold, 1980; Hunst and Tolin, 1982; Horváth, 1983; Valverde, 1983; Baker and Zettler, 1988; Kitajima, 1988). According to our knowledge data on virus susceptibility have so far been available for 37 plant species belonging to 12 genera. Of the 40 genera belonging to the family *Commelinaceae* the *Commelina* genus is virologically the best known. In this genus 16 plant species are known for virus susceptibility, while in eleven other genera only 18 plant species are recorded as susceptible to viruses (Tables 1 and 2).

From a virological point of view the *Commelina* species are of special importance. The species of the genus *Commelina* are recognized as sources of various viruses. Specially, notable number of viruses have been isolated from *Commelina communis* and *C. diffusa* (Migliori and Lastra, 1978; Morales and Zettler, 1977; Migliori et al., 1978; Waterworth et al., 1978; Stone, 1980; Adlerz, 1981; Lockhart et al., 1981; Valverde, 1985; Baker and Zettler, 1986, 1988).

The *Commelina* species (e.g., *C. benghalensis, C. clandestina, C. communis, C. diffusa, C. elegans, C. erecta, C. graminifolia, C. nudiflora, C. tuberosa*) are particularly susceptible to cucumber mosaic virus (see Table 2,

*In memoriam Prof. Dr. D. Miličić (†1993)

cf. Wellman, 1972). It is remarkable that *Commelina diffusa* plants doubly infected with *Commelina* mosaic and cucumber mosaic viruses are better sources of cucumber mosaic virus inoculum for aphid vectors than are singly infected plants (Morales and Zettler, 1977).

Table 1

Susceptible commelinaceous plants (except *Commelina* species) to different viruses

Plant species	Viruses[1] and/or virus groups	Literature
Aneilema aequinoctiale	AV (potyvirus)	Baker and Zettler (1988)
	Potyvirus	Lockhart et al. (1981)
A. clarkei	AV (potyvirus)	Baker and Zettler (1988)
A. hockii	AV (potyvirus)	Baker and Zettler (1988)
A. sebitense	AV (potyvirus)	Baker and Zettler (1988)
A. succulentum	AV (potyvirus)	Baker and Zettler (1988)
A. zebrinum	AV (potyvirus)	Baker and Zettler (1988)
Cyanostis villosa	AV (potyvirus)	Baker and Zettler (1988)
Gibasis geniculata	BYMV (potyvirus)	Hunst and Tolin (1982)
	CYVV (potyvirus)	Baker and Zettler (1988)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
G. pellucida	CYVV (potyvirus)	Baker and Zettler (1988)
Hadrodemas warszewiczianum	AV (potyvirus)	Baker and Zettler (1988)
Murdannia nudiflora	CYVV (potyvirus)	Baker and Zettler (1988)
	CMV (cucumovirus)	Baker and Zettler (1988)
	Potyvirus	Baker and Zettler (1988)
Phaeosphaerion rufipes	AV (potyvirus)	Baker and Zettler (1988)
Rhoeo spathaceae (syn.: *R. discolor*)	AMV (monotypic group)	Beczner and Schmelzer (1972)
	CMV (cucumovirus)	Baker and Zettler (1988)
	CoMV (potyvirus)	Baker and Zettler (1988)
	CYVV (potyvirus)	Baker and Zettler (1988)
	Potyvirus	Lockhart et al. (1981)
	TRV (tobravirus)	Schmelzer (1957)
	TMV (tobamovirus)	Gordon and Smith (1961), Bawden (1961), Schmelzer and Wolf (1977), Lockhart and Pfleger (1977), Thompson and Corbett (1985), Baker and Zettler (1988)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
Rhopalephora scaberrima	AV (potyvirus)	Baker and Zettler (1988)
R. vitiensis	AV (potyvirus)	Baker and Zettler (1988)
Tinantia erecta	AMV (monotypic group)	Beczner and Schmelzer (1972), Horváth (1975, 1983)
	AV (potyvirus)	Baker and Zettler (1988)

Table 1 (continued)

Plant species	Viruses[1] and/or virus groups	Literature
	CYVV (potyvirus)	Baker and Zettler (1988)
	DGMV (potyvirus)	Reckhaus and Nienhaus (1981)
	BYMV (potyvirus)	Horváth (1983)
	CLRV (nepovirus)	Horváth (1983)
	PVY (potyvirus)	Horváth (1983)
	TMV (tobamovirus)	Horváth (1975, 1983)
	TNV (nepovirus)	Horváth (1983)
	ToMV (tobamovirus)	Horváth (1983)
	TRSV (nepovirus)	Horváth (1975, 1983)
	TRV (tobravirus)	Horváth (1983)
	TuMV (potyvirus)	Horváth (1983)
Tradescantia albiflora	BYMV (potyvirus)	Hunst and Tolin (1982)
	CYVV (potyvirus)	Baker and Zettler (1988)
	Potyvirus	Lockhart and Betzold (1980), Lockhart et al. (1981), Atyukova et al. (1988)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
T. blossfeldiana	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
T. fluminensis	BYMV (potyvirus)	Hunst and Tolin (1982)
	Potyvirus	Lockhart et al. (1981)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
T. ohiensis	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
Tradescantia spp.	CMV (cucumovirus)	Kitajima (1988)
	Potyvirus	Lockhart and Betzold (1980)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
Zebrina pendula	Potyvirus	Lockhart and Betzold (1980), Lockhart et al. (1981)
	TMV (tobamovirus)	Baker and Zettler (1988)
	TV (potyvirus)	Baker and Zettler (1988)
	ZV (potyvirus)	Baker and Zettler (1988)
Zebrina spp.	Potyvirus	Lockhart and Betzold (1980)

[1]AMV, alfalfa mosaic virus; AV, *Aneilema* virus; BYMV, bean yellow mosaic virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; CoMV, *Commelina* mosaic virus; CYVV, clover yellow vein virus; DGMV, *Dioscorea* greenbanding mosaic virus; PVY, potato virus Y; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; TRSV, tobacco ringspot virus; TRV, tobacco rattle virus; ToMV, tomato mosaic virus; TuMV, turnip mosaic virus; TV, *Tradescantia* virus; ZV, *Zebrina* virus

Table 2

Commelina species as hosts of plant viruses

Plant species	Viruses[1] and/or virus groups	Literature
Commelina benghalensis	AV (potyvirus) *,**	Baker and Zettler (1988)
	CMV (cucumovirus) *,**	Baker and Zettler (1988)
C. bracteose	AV (potyvirus)*	Baker and Zettler (1988)
C. clandestina	CLRV (nepovirus)**	Horváth (1983)
	CMV (cucumovirus)**	Horváth (1983)
	TMV (tobamovirus)**	Horváth (1983)
	TNV (nepovirus)**	Horváth (1983)
	ToMV (tobamovirus)**	Horváth (1983)
	TRSV (nepovirus)**	Horváth (1983)
C. coelestis	CLRV (nepovirus)*,**	Schmelzer (1966), Horváth (1983)
	MYFV (bromovirus)**	Horváth et al. (1988)
C. communis	AV (potyvirus)**	Baker and Zettler (1986)
	BMV (bromovirus)*,**	Valverde (1983)
	CMV (cucumovirus)**	Thornberry (1966)
	CeMV (potyvirus)*	Thornberry (1966)
	TMV (tobamovirus)*,**	Baker and Zettler (1988), Horváth (1983)
	CLRV (nepovirus)**	Horváth et al. (1974), Horváth
	TNV (nepovirus)**	Horváth (1983)
	ToMV (tobamovirus)**	Horváth (1983)
	TRSV (nepovirus)**	Horváth (1983)
C. diffusa	AV (potyvirus)**	Baker and Zettler (1986, 1988)
	BMV (bromovirus)*	Valverde (1983)
	CX (potexvirus)*,***	Stone (1980)
	CoMV (potyvirus)*,**	Morales and Zettler (1977), Migliori et al. (1978), Migliori and Lastra (1978), Lockhart et al. (1981), Baker and Zettler (1986)
	CDV (potyvirus)*,****	Migliori and Lastra (1978, 1980)
	CoYMV (badnavirus)*	Lockhart and Khaiess (1988), Lockhart et al. (1989), Lockhart (1990)
	CMV (cucumovirus)*,**	Thornberry (1966), Adlers (1981), Baker and Zettler (1988), Shanmugasundarum et al. (1969), Migliori et al. (1978), Waterworth et al. (1978)
	Potyvirus*	Barton et al. (1980), Stone (1980), Baker and Zettler (1988)
	SBLV (bromovirus)*	Valverde (1985)
	TVT (potyvirus)*,**	Baker and Zettler (1988)
	ZV (potyvirus)*,**	Baker and Zettler (1988)
C. elegans	CMV (cucumovirus)*	Thornberry (1966)
C. eckloniana	AV (potyvirus)*	Baker and Zettler (1988)

Table 2 (continued)

Plant species	Viruses[1] and/or virus groups	Literature
C. erecta	AV (potyvirus)**	Baker and Zettler (1988)
	CMV (cucumovirus)*	Thornberry (1966), Fernández and Palaez (1982)
	Potyvirus*	Baker and Zettler (1988)
C. gigas	CMV (cucumovirus)*	Thornberry (1966)
C. graminifolia	CLRV (nepovirus)**	Horváth et al. (1974) Horváth (1983)
	CMV (cucumovirus)**	Horváth (1983)
	TMV (tobamovirus)**	Horváth (1983)
	TNV (nepovirus)**	Horváth (1983)
C. jacobii	CoMV (potyvirus)	Padmanabhan et al. (1972)
C. nudiflora	CeMV (potyvirus)*	Thornberry (1966)
	CMV (cucumovirus)*	Doolittle and Webb (1960), Schmelzer and Wolf (1971)
C. palludosa	AV (potyvirus)*	Baker and Zettler (1988)
C. thwaitesii	AV (potyvirus)*	Baker and Zettler (1988)
C. tuberosa	CLRV (nepovirus)**	Horváth et al. (1974), Horváth (1983)
	CMV (cucumovirus)**	Horváth (1983)
	TMV (tobamovirus)**	Horváth (1983)
	TNV (nepovirus)**	Horváth (1983)
	ToMV (tobamovirus)**	Horváth (1983)
	TRSV (nepovirus)**	Horváth (1983)
Commelina spp.	CMV (cucumovirus)*	Wellman (1972)

[1]AV, *Aneilema* virus; BMV, brome mosaic virus; CeMV, celery mosaic virus; CDV, *Commelina* diffusa virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; CoMV, *Commelina* mosaic virus; CoYMV, *Commelina* yellow mottle virus; CX, *Commelina* X-virus; MYFV, Melandrium yellow fleck virus; SBLV, Spring beauty latent virus; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; TRSV, tobacco ringspot virus; TVT, *Tradescantia* T-virus; ToMV, tomato mosaic virus; ZV, *Zebrina* virus. Viruses marked with one asterisk were isolated from spontaneously infected plants, while those marked with two asterisks could be transmitted to the given plants only under experimental conditions. The potexvirus marked with three asterisks reacted with sera to papaya mosaic virus and *Ullucus* virus X. It is probably not a European virus. *Commelina diffusa* is a symptomless host of the mentioned potexvirus. The *Commelina diffusa* virus (CDV) marked with four asterisks is morphologically similar to cacao swollen shoot virus (CaSSV)

The various *Commelina* species are, however, not only natural host plants, but as test plants play an important role in plant virology too (Horváth et al., 1974; Horváth, 1975, 1983; Baker and Zettler, 1988). For virus differentiation important are the incompatible host-virus relations detected between some nine *Commelina* species *(C. benghalensis, C. clandestina, C. coelestis, C. communis, C. diffusa, C. erecta, C. graminis, C. sikkimensis, C. tuberosa)* and fifteen viruses (alfalfa mosaic, bean common mosaic, bean

yellow mosaic, Commelina mosaic, clover yellow vein, cucumber mosaic, potato aucuba mosaic, potato M, potato S, potato Y, radish mosaic, tobacco mosaic, tobacco rattle, turnip mosaic, turnip yellow mosaic viruses; Beczner and Schmelzer, 1972; Horváth, 1983; Baker and Zettler, 1988).

On field specimens of *Commelina communis* mild mosaic and leaf deformation symptoms characteristic of virus have been observed for years. Considering that the etiology of the disease is unknown, we carried out investigations to determine the pathogen. Present paper gives account of the identification and some characteristics of the virus causing the disease.

Materials and Methods

The virus isolate denoted *Co* was found in infected *Commelina communis* plants spontaneously growing in a garden in Zagreb (Croatia). This virus was obtained for the further investigations from single lesion of inoculated *Chenopodium quinoa*. The *Co* isolate was maintained either in *Nicotiana tabacum* cv. Samsun or in *Nicotiana megalosiphon*. For plant reaction trials the virus isolate was transmitted mechanically in the standard way. Infectiveness was controlled by back inoculations from test plants to *Cheponodium quinoa*. Aphid transmission tests were performed by *Myzus persicae* aphids. They were starved for 4 h before acquisition access period and inoculation feeding period which lasted 10 and 20 min, respectively. In these tests tobacco plants served as the source of the virus and also as plants for aphid inoculation. For aphid inoculation 5 individuals of *Myzus persicae* per plant were used.

In cross protection tests a total of ten young tobacco (*Nicotiana tabacum* cv. Xanthi-nc) plants were inoculated by *Co* isolate. Ten days later plants out of them were challenge-inoculated with the white *(W)* strain of cucumber mosaic virus. Healthy tobacco plants inoculated by *W* strain alone served as control.

The virus was partially purified after the method of Habili and Francki (1974) including modifications described by Juretić and Horváth (1984), and was examined by means of eletcron microscope.

The serological experiments were carried out by means of the agar-gel double diffusion test using infective plant sap and partially purified virus suspensions. Antiserum to cucumber mosaic virus of titer 1/64 was kindly supplied by Dr. E. Luisoni.

The physical properties (thermal inactivation point, longevity in vitro) of the *Co* isolate was determined in the sap of infected leaf material of tobacco plant as source. *Chenopodium quinoa* were used as indicator plant.

Results and Discussion

Symptom on test plants

The isolate *Co* was mechanically transmitted to some diagnostic test plants. Symptoms of these plants are shown in Table 3. These were similar to those induced by cucumber mosaic virus (Gibbs and Harrison, 1970; Horváth, 1976; Francki et al., 1979).

Aphid transmission

Our *Co* isolate was easily transmissible by *Myzus persicae*. From 20 tobacco plants included in the test 16 plants, were infected by aphids.

Cross protection

The *Co* isolate from *Commelina communis* protected tobacco plants against the infection of *W* strain of cucumber mosaic virus.

Purification, ultraviolet absorption and electron microscopy

The purification procedure after the modified method of Habili and Francki (1974; see Juretić and Horváth, 1984) was rather successful because yield of about 70 mg per kilogram of tobacco tissue was obtained. Ratio A_{260}/A_{280} of the purified virus suspension was 1.60.

Electron microscopy of purified preparations revealed isometric particles about 28 nm in diameter (Fig. 2A).

Serological properties

Sap of infected plants as well as purified preparations reacted with antiserum to cucumber mosaic virus (Fig. 2B). In the tests performed by infective plant sap nonspecific precipitin patterns produced by host proteins could be observed.

Table 3

Reactions of test plants inoculated with *Co* isolate of cucumber mosaic virus

Family and species	Symptoms[1]
AIZOACEAE	
Tetragonia tetragonoides	LoChlSp (Fig. 1A)
CHENOPODIACEAE	
Chenopodium amaranticolor	LoChlNL
C. quinoa	LoChlNL (Fig. 1B)
COMMELINACEAE	
C. clandestina	SMo, ChlP
C. communis	SMo, ChlP
C. diffusa	SMo, ChlP (Fig. 1C)
C. graminifolia	SMo, ChlP
CUCURBITACEAE	
Cucumis sativus	SMo (Fig. 1D)
Cucurbita pepo convar. patissonina f. radiata	SMo
FABACEAE	
Vigna sinensis	LoBrNL
V. unguiculata	LoBrNL
SOLANACEAE	
Datura stramonium	SMo, Def
Lycopersicon esculentum	SMo, FLe
Nicotiana clevelandii	SMo, Def
N. glutinosa	SMo
N. .negalosiphon	SMo
N. tabacum cv. Samsum, Xanthi-nc	SMo
Petunia hybrida	SMo

[1]Br, brown; Chl, chlorotic; Def, deformation; Fle, fern leaf; Lo, local, Mo, mosaic; NL, necrotic lesions; P, pattern; S, systemic; Sp, spots

Stability in plant sap

Co isolate had the thermal inactivation point between 60 and 64 °C and its infectiveness was retained at least 3 days at room temperature.

Based on all the above quoted data it may be concluded that *Co* isolate represents a variant of cucumber mosaic virus. Consequently, this seems to be the first finding of cucumber mosaic virus in *Commelina communis*. It should be pointed out that occurrence of cucumber mosaic virus in *Commelina commumis* was established only in one of the five gardens inspected in Zagreb.

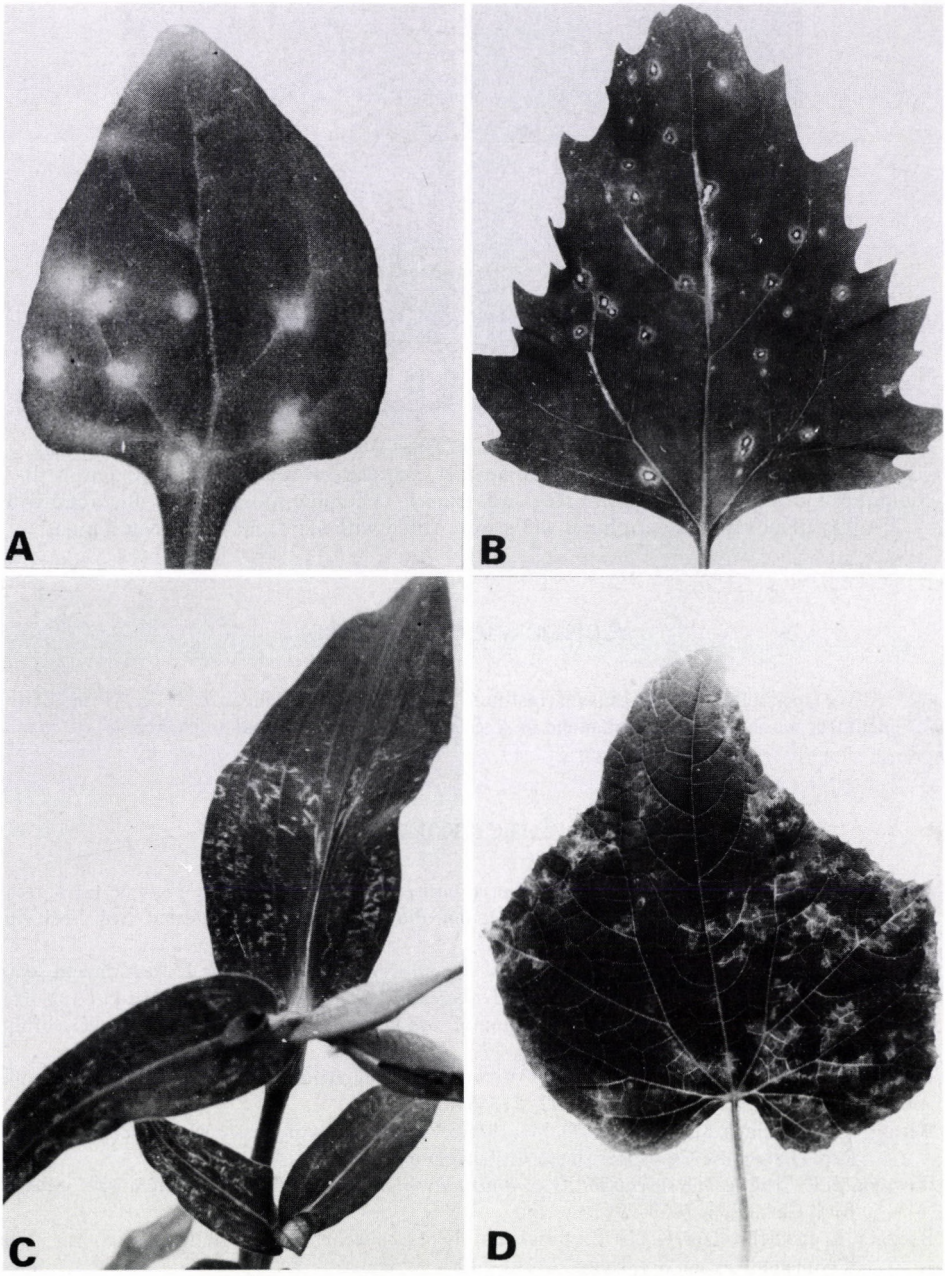

Fig. 1. Local (A, B) and systemic symptoms (C, D) in different test plants inoculated with cucumber mosaic virus. A, *Tetragonia tetragonoides*; B, *Chenopodium quinoa*; C, *Commelina diffusa*; D, *Cucumis sativus*

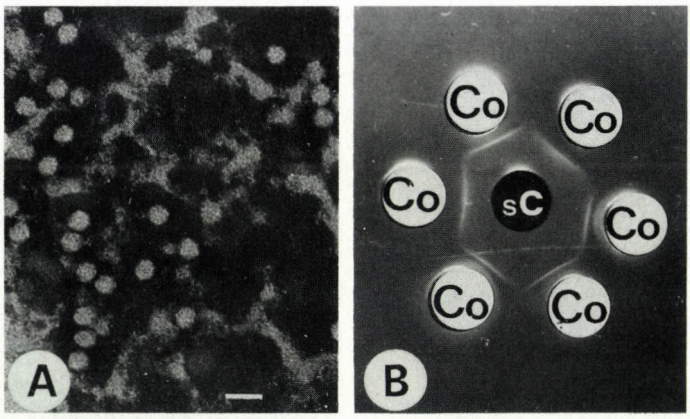

Fig. 2. A - particles of the virus isolate *Co* from *Commelina communis* in a partially purified preparation (bar represents 50 nm); B - serological reaction of *Co* isolate with cucumber mosaic virus antiserum: sC - antiserum to cucumber mosaic virus absorbed with healthy plant sap, the peripheral wells were filled with sap from *Co*-infected plants

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Literature

Adlerz, W. C. (1981): Weed hosts of aphid-borne viruses of vegetable crops in Florida. In: Thresh, J. M. (ed.), Pests, pathogens and vegetation. Pitman Publ. Inc., Marshfield, MA 1981. pp. 467–478.

Artyukova, E. V., Moiseenko, L. I., Tolkach, V. F. and Krylov, A. V. (1988): Extraction and some properties of potyvirus isolated from *Tradescantia albiflora* Kunth. Biol. Nauki 8, 30–34.

Baker, C. and Zettler, F. W. (1986): Distinction of five viruses naturally infecting members of the *Commelinaceae*. Phytopathology 76, 372.

Baker, C. A. and Zettler, F. W. (1988): Viruses infecting wild and cultivated species of the *Commelinaceae*. Plant Disease 72, 513–518.

Barton, R. J., Hollings, M. and Stone, O. M. (1980): Virus diseases of glasshouse flower crops. Ann. Rep. Glasshouse Crops Res. Inst., Littlehampton 1980. pp. 143–144.

Bawden, F. C. (1961): The susceptibility of *Rhoeo discolor* to infection by tobacco mosaic virus. J. Biol. Chem 236, 2760–2761.

Beczner, L. and Schmelzer, K. (1972): Susceptibility of *Commelinaceae* to alfalfa mosaic virus. Acta Phytopathol. Acad. Sci. Hung. 7, 213–219.

Doolittle, S. P. and Webb, R. W. (1960): A strain of cucumber virus 1 infections to blight resistant spinach. Phytopathology 50, 7–9.

Fernández, F. and Palaez, A. I. (1982): *Commelina erecta*, a natural reservoirs of cucumber mosaic virus. Agrotecnia de Cuba 14, 79–91.

Francki, R. I. B., Mossop, D. W. and Hatta, T. (1979): Cucumber mosaic virus. C. M. I./A. A. B. Descriptions of plant viruses. 213 (No. 1 revised), 1–6.

Gibbs, A. J. and Harrison, B. D. (1970): Cucumber mosaic virus. C. M. I./A. A. B. Descriptions of plant viruses 4, 1–4.

Gordon, M. P. and Smith, C. (1961): The infection of *Rhoeo discolor* by tobacco mosaic virus. J. Biol. Chem. 236, 2762–2763.

Habili, N. and Francki, R. I. B. (1974): Comparative studies on tomato aspermy and cucumber mosaic viruses. I. Physical and chemical properties. Virology 57, 392–401.

Horváth, J. (1975): New host plants of three isometric plant viruses. Acta Agr. Acad. Sci. Hung. 24, 387–392.

Horváth, J. (1976): Vírus-gazdanövénykörök és vírusdifferenciálás (Host range of viruses and virus differentiation). Akad. Dokt. Ért. (D. Sc. Thesis). Budapest-Keszthely 1976. p. 607.

Horváth, J. (1983): New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses. XVIII. Concluding remarks. Acta Phytopath. Acad. Sci. Hung. 18, 121–161.

Horváth, J., Schmelzer, K. und Juretić, N. (1974): Isolierung des Kirschenblattroll-Virus (Cherry leaf roll virus) aus *Sambucus nigra* L. in Ungarn. Acta Phytopath. Acad. Sci. Hung. 9, 209–218.

Horváth, J., Hollings, M. and Barton, R. J. (1988): Experimental host range of *Melandrium* yellow fleck virus. Acta Phytopath. et Entomol. 23, 449–463.

Hunst, P. L. and Tolin, S. A. (1982): Bean yellow mosaic virus isolated from *Gibasis geniculata*. Plant Dis. 66, 955–958.

Juretić, N. and Horváth, J. (1984): Isolation of cucumber mosaic virus from pomegranate (*Punica granatum* L.) in Yugoslavia. Acta Phytopath. Acad. Sci. Hung. 19, 309–313.

Kitajima, E. W. (1988): Present status of the studies on ornamental plant diseases in Brasil. Acta Hort. 234, 451–456.

Lockhart, B. E. L. (1990): Evidence for a double-stranded circular DNA genome in a second group of plant viruses. Phytopathology 80, 127–131.

Lockhart, B. E. and Pfleger, F. L. (1977): Properties of a strain of tobacco mosaic virus occurring in *Rhoeo discolor* in commercial greenhouses. Proc. Am. Phytopathol. Soc. 4, 203.

Lockhart, B. E. L. and Betzold, J. A. (1980): Some properties of a potyvirus occurring in *Tradescantia* and *Zebrina* spp. Acta Hort. 110, 55–57.

Lockhart, B. E. L. and Khaless, N. (1988): *Commelina* yellow mottle virus a non-enveloped bacilliform virus containing double-stranded DNA. Phytopathology 78, 1548 (Abstr.).

Lockhart, B. E., Betzold, J. A. and Pfleger, F. L. (1981): Characterization of a potyvirus causing a leaf distortion disease of *Tradescantia* and *Zebrina* species. Phytopathology 71, 602–604.

Migliori, A. and Lastra, R. (1978): Etude de virus présents chez *Commelina diffusa* Burm. en Guadeloupe. Ann. Phytopathol. 10, 467–477.

Migliori, A. and Lastra, R. (1980): Characterization of a virus of *Commelina diffusa* (CDV). Ann. Phytopath. 12, 145–151.

Migliori, A., Marchoux, G. and Quiot, J. B. (1978): Dynamique des populations du virus de la mosaique du concombre en Guadeloupe. Ann. Phytopathol. 10, 455–466.

Morales, F. J. and Zettler, F. W. (1977): Characterization and electron microscopy of a potyvirus infecting *Commelina diffusa*. Phytopathology 67, 839–843.

Olszewski, N. E., Medberry, S. L. and Lockhart, B. E. L. (1989): Molecular characterization of non-enveloped bacilliform viruses. Phytopathology 79, 1158 (Abstr.).

Padmanabhan, C., Jaganathan, T. and Kandaswamy, T. K. (1972): A note on a mosaic disease of *Commelina jacobii*. Madras Agr. J. 59, 361–362.

Reckhaus, P. and Nienhaus, F. (1981): Etiology of a virus disease of white yam (*Dioscorea rotundata*) in Togo. J. Plant Dis. Prot. 88, 492–509.

Schmelzer, K. (1957): Untersuchungen über den Wirtspflanzenkreis des Tabakmauche-Virus. Phytopath. Z. 30, 281–314.

Schmelzer, K. (1966): Untersuchungen an Viren der Zier- und Wildgehölze. 5. Mitt.: Virosen an *Populus* und *Sambucus*. Pytopath. Z. 55, 317–351.

Schmelzer, K. and Wolf, P. (1971): Host plants of the viruses and virus diseases of Europe. Nova Acta Leopoldina, Suppl. 2., Johann Ambrosius Barth, Leipzig 1972. p. 262.

Shanmugasundarum, S., Ishii, M., Gilbert, J. C. and Nagai, H. (1969): Cucurbit virus studies in Hawai. Plant Dis. Rep. 53, 70–74.

Stone, O. M. (1980): Two new potexviruses from nonocotyledons. Acta Hort. 110, 59–63.

Thompson, S. M. and Corbett, M. K. (1985): Mosaic disease of *Rhoeo discolor* caused by a strain of tobacco mosaic virus. Plant Dis. 69, 356–359.

Thornberry, H. H. (1966): Index of plant virus diseases. Agric. Res. Serv. U. S. Dept. Agric., Agricultural Handb. 307, 1–466.

Valverde, R. A. (1983): Brome mosaic virus isolates naturally infecting *Commelina diffusa* and *C. communis*. Plant Dis. 67, 1194–1196.

Valverde, R. A. (1985): Spring beauty latent virus: A new member of the bromovirus group. Phytopathology 75, 395–398.

Waterworth, H. E., Tousigmant, M. E. and Kaper, J. M. (1978): A lethal disease of tomato experimentally induced by RNA-5 associated with cucumber mosaic virus isolated from *Commelina* from El Salvador. Phytopathology 68, 561–566.

Wellman, F. L. (1972): Tropical American plant disease. Scareclow Press, Metuchen, New Jersey 1972. p. 989.

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Reactions of Sixty-Seven Accessions of Twelve *Cucumis* Species to Seven Viruses

J. HORVÁTH

Pannonian University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P.O. Box 71, Hungary

Four varieties, eight cultivars and 67 accessions of twelve *Cucumis* species originating from 45 countries were examined for reaction to seven viruses (cucumber green mottle mosaic *Tobamovirus*, CGMMV; cucumber leaf spot *Carmovirus*, CLSV; cucumber mosaic *Cucumovirus*, CMV; melon necrotic spot *Carmovirus*, MNSV; Melandrium yellow fleck *Bromovirus*, MYFV; watermelon mosaic-2 *Potyvirus*, WMV-2; zucchini yellow mosaic *Potyvirus*, ZYMV). Out of the 410 host-virus relationships 219 were compatible and 191 incompatible.

From the point of view of resistance to the viruses examined *Cucumis africanus* G1. 2302 proved the best, being immune to five viruses (CGMMV, CLSV, CMV, WMV-2, ZYMV). Good resistance qualities were shown by *Cucumis melo* P. I. 217974, which was immune to four viruses (CLSV, CMV, WMV-2, ZYMV) and hypersensitively resistant to two viruses (MNSV, MYFV). *Cucumis melo* P. I. 161375, an accession known thought by the literature, but so far unknown for its behaviour to certain viruses was immune to four viruses (CGMMV, MNSV, WMV-2, ZYMV), and showed hypersensitive resistance to MYFV.

The highest susceptibility to viruses was found in *Cucumis dipsaceus* G1. 0255, which proved susceptible to all viruses included in the study. The second in order of susceptibility was *Cucumis melo* P. I. 288988, which was susceptible to 5 out of 6 viruses (CGMMV, CLSV, CMV; WMV-2, ZYMV), and showed hypersensitive resistance to one virus. Next from the point of view of susceptibility was *Cucumis metuliferus* G1. 1775, which was susceptible to 5 out 6 viruses (CGMMV, CLSV, CMV, WMV-2, ZYMV), and resistant to one virus, *Cucumis melo* P. I. 116479 was susceptible to 6 out of the 7 viruses (CGMMV, CLSV, CMV, MNSV, WMV-2, ZYMV) and immune to one virus.

Cucumis species originated in tropical and subtropical Africa, which is the primary center of diversity. The secondary centres of diversity are China, Iran and Community of Independent States (Kallo, 1988). From an economic point of view *Cucumis anguria*, *C. melo* and *C. sativus* are the most important species. From a virological point of view *Cucumis sativus* is the best known species, susceptible – to our knowledge – to more than 60 viruses (reviewed by Horváth, 1985a). In the course of recent investigations, which cleared up the behaviour of several *Cucumis* species (*C. anguria*, *C. dipsaceus*, *C. melo* ssp. conomon, *C. melo* ssp. dudaim, *C. melo* ssp. melo, *C. metuliferus*) to 12 viruses, 16 new host-virus relationships were described (Horváth, 1985b). Remarkable are the investigations concerned with the virus resistance of various *Cucumis* species. For example, in earlier experiments we found *Cucumis myriocarpus* to be resistant to cucumber mosaic *Cucumovirus* (CMV) (Horváth, 1975, 1983). Webb (1979) detected watermelon mosaic-1

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Potyvirus (WMV-1) resistance transmitted by one gene in a line P. I. 180280 of *Cucumis melo*, while Bohl et al. (1980) found the WMF 29 strain of *Cucumis melo* to be resistant to WMV-1 and tolerant to WMV-2. Resistance to zucchini yellow mosaic *Potyvirus* (ZYMV) has been found in the P. I. 414723 accession of muskmelon *(Cucumis melo)*. These plants exhibit no symptoms and there is no systemic spread of the virus (Pitrat and Lecoq, 1984). Resistance to ZYMV (strain E15) in this accession from India is governed by one dominant gene *(Zym)* according to segregations observed in F_1, F_2 and BC_1 progenies. It is also resistant to WMV-1 as well as to melon aphid, *Aphis gossypii* (Kishaba et al., 1971; Pitrat and Lecoq, 1984). The resistance of *Cucumis metuliferus*, *C. melo*, *C. sativus* to WMV-1 and WMV-2 is very significant (Provvidenti and Gonsalves, 1982), since it is known that very little genetic material resistant to WMV-2 and the never cucurbit viruses (e.g. ZYMV, squash leaf curl *Geminivirus*, SLCV) is available. Weber and Kegler (1982), Weber (1983) inoculated 35 cucumber varieties with CMV and found all of them to be susceptible, though to different degrees. "Resistance" was found to be characterized by reduced virus multiplication in inoculated cotyledons (Weber et al., 1985a). According to data by Moyer et al. (1985) the breeding line 91213 of *Cucumis melo* inoculated with WMV-2 showed a lower titer than other varieties of *Cucumis melo*.

Due to resistance research, today a number of *Cucumis* cultivars possess resistance to CMV, WMV-1, WMV-2 and ZYMV (Cohen et al., 1971; Provvidenti et al., 1983; Wang et al., 1984). It now is possible for cucumber breeders to combine genes for resistance to four viruses. No doubt, however, that investigations on resistance to some important viruses (e.g. cucumber green mottle mosaic *Tobamovirus*, CGMMV; cucumber leaf spot *Carmovirus*, CLSV; melon necrotic spot *Carmovirus*, MNSV) are still very deficient.

Considering that the virus susceptibility and virus resistance of various accessions of the individual *Cucumis* species to a given virus or to viruses are different or are not known at all, we have examined several species for reaction to some viruses.

Materials and Methods

Sixty-seven accessions of twelve *Cucumis* species (four varieties and eight cultivars) from 45 countries were inoculated with seven viruses (Tables 1 and 2). All seed of *Cucumis melo* and other *Cucumis* spp. was treated with

0.5% sodium hypochlorite for 5 minutes followed by a 30 min. rinse in running tap water (Sowell, 1987). The seed samples originating from Germplasm Resources Laboratory, U. S. D. A., A. R. S., Beltsville, Maryland (USA) were marked with the letters P. I. (*P*lant *I*ntroduction), those from the Institute for Horticultural Plant Breeding, Wageningen (The Netherlands) were marked G1. Data concerning the methods of inoculation, detection of susceptibility and resistance, and back-inoculation are to be found in details in an earlier publication (Horváth, 1993). As propagative host for Melandrium yellow fleck *Bromovirus* (MYFV) (see Hollings and Horváth, 1981; Horváth et al., 1988) we used *Gomphrena globosa*, while as its assay host *Chenopodium quinoa* was employed.

Table 1

Viruses used in the experiments

Viruses and (acronyms)	Strains or isolates[1]	Literature[2]
Cucumber green mottle mosaic *Tobamovirus* (CGMMV)	IP-A	*Schmelzer* (1967), Hollings et al. (1975)
Cucumber leaf spot *Carmovirus* (CLSV)	Common	*Weber* et al. (1982, 1985b, 1986), Weber and Stanarius (1984), Weber (1986)
Cucumber mosaic *Cucumovirus* (CMV)	U/246	Schmidt and Horváth (1982)
Melon necrotic spot *Carmovirus* (MNSV)	Cu18	Gonzales-Garza et al. (1979), *Bos* et al. (1984), Teakle and Thomas (1985), Hibi and Furuki (1985), Tomlinson and Thomas (1986), Avgelis (1989)
Melandrium yellow fleck *Bromovirus* (MYFV)	LB	Hollings and Horváth (1981), Horváth et al. (1988)
Watermelon mosaic-2 *Potyvirus* (WMV-2)	PW	Horváth et al. (1975)
Zucchini yellow mosaic *Potyvirus* (ZYMV)	E15	*Lecoq* et al. (1981, 1983), Lisa et al. (1981), Lisa and Lecoq (1984)

[1]IP-A obtained from Institute of Phytopathology, Aschersleben, Germany. [2]Names printed in italics are the sender of the virus

Results and Discussion

Between 67 accessions of twelve *Cucumis* species, four varieties, eight cultivars and seven viruses 410 host-virus relationships were established, of which 219 were compatible (local and/or systemic susceptibility) and 191 incompatible (resistant or immune) (see Table 2). For technical reasons 60 host-virus relationships could not be studied.

Immunity to CGMMV was detected in seven accessions: *Cucumis africanus* (G1. 2302), *C. ficifolius* (G1. 1803), *C. meeusli* (G1. 1800) and *C. melo* (P. I. 108902, P. I. 161375, P. I. 207009, P. I. 212211). Hypersensitive reaction was obtained in the P. I. 190554 and P. I. 222187 accession of *Cucumis melo*. Attention is to be paid to *Cucumis africanus*, as some of its accessions are resistant to cucumber powdery mildew *(Erysiphe cichoracearum)* (Lebeda, 1984).

To CLSV some six accessions of four *Cucumis* species (*C. africanus*, *C. anguria* var. anguria, *C. melo*, *C. myriocarpus*) showed immunity, while the P. I. 190554 and P. I. 224769 accession of *Cucumis melo* exhibited hypersensitive resistance to it. As for resistance to cucumber powdery mildew, mention should be made of some accessions of *Cucumis africanus*, *C. anguria* var. anguria, *C. myriocarpus* (Lebeda, 1984).

To the bean strain of CMV 15 accessions of seven *Cucumis* species were found to be immune (see Table 2). Of the immunity of *Cucumis myriocarpus* to CMV we gave an account in an earlier paper too (Horváth, 1975). Hypersensitive resistance could not be detected in any relationship between plant and virus.

To MNSV certain accessions of three *Cucumis* species (*C. ficifolius*, *C. figarei*, *C. melo*) were immune. *Cucumis melo* P. I. 161375, one of the breeding lines best known in literature is – according to Risser et al. (1977) – most resistant but not immune or hypersensitive resistant to CMV. This accession, while according to Tóbiás and Velich (1983) tolerant, proved susceptible to CMV in our experiments. It is remarkable that 50 out of the total number of some 67 accessions examined showed hypersensitive resistance to MNSV. Out of 45 accessions of *Cucumis melo* 35 exhibited hypersensitive resistance.

In the course of experiments with MYFV 12 accessions of *Cucumis melo* showed immunity, while 41 accessions hypersensitive resistance (see Table 2).

To WMV-2 immunity was detected in 3 varieties and 2 cultivars of 8 species *(Cucumis africanus*, *C. anguria*, *C. ficifolius*, *C. meeusli*, *C. melo*, *C. myriocarpus*, *C. sativus*, *C. zeyheri)* and in some 29 *Cucumis melo* accessions.

Table 2
Reaction of different accessions of *Cucurbita* species to viruses

Cucumis species	Plant introduction (P. I. or Gl.) number and origin[1]	Reaction[2] to viruses[3]						
		CGMMV	CLSV	CMV	MNSV	MYFV	WMV-2	ZYMV
C. africanus	Gl. 2302 The Netherlands	I	I	I	L+S	Ni	I	I
C. anguria var. *anguria*	Gl. 1970 The Netherlands	S	I	I	L+S	Ni	I	I
C. anguria var. longipes	Gl. 1790 The Netherlands	S	L+S	I	L+S	Ni	I	S
C. dipsaceus	Gl. 0255 The Netherlands	S	L+S	S	L+S	Ni	S	L+S
C. ficifolius	Gl. 1803 The Netherlands	I	Ni	Ni	I	Ni	I	I
C. figarei	Gl. 2071 The Netherlands	S	Ni	Ni	I	Ni	Ni	S
C. meeusli	Gl. 1800 The Netherlands	I	L+S	Ni	L	Ni	I	L+S
C. melo	P. I. 93438 China	L+S	L+S	S	L	L	I	S
	P. I. 102077 Morocco	L+S	L+S	S	L	L	S	S
	P. I. 108902 CIS	I	L+S	L+S	I	L	I	I
	P. I. 109479 Turkey	L+S	L+S	I	L	I	Ni	L+S
	P. I. 116479 India	L+S	L+S	S	L+S	I	S	S
	P. I. 116917 Afghanistan	L+S	Ni	Ni	Ni	I	L+S	L+S
	P. I. 128901 France	L+S	L+S	S	L	L	I	L
	P. I. 136171 Canada	S	I	S	L	I	I	L+S
	P. I. 140762 Iran	L+S	L+S	S	L	I	I	L+S
	P. I. 161375 Korea	I	L+S	S	I	L	I	I
	P. I. 162668 Argentina	L+S	L+S	S	L	L	S	L+S
	P. I. 165450 Mexico	S	S	S	L	L	S	S
	P. I. 181748 Syria	L+S	Ni	Ni	L	I	S	I
	P. I. 183221 Egypt	S	L+S	S	L	I	I	Ni
	P. I. 183256 Saudi Arabia	S	L+S	S	L	L	L+S	S
	P. I. 190554 Iraq	L	L	S	I	L	I	I
	P. I. 193495 Ethiopia	L+S	L+S	S	L	L	I	L+S
	P. I. 197077 Spain	Ni	Ni	I	Ni	L	Ni	S
	P. I. 200819 Burma	S	Ni	I	Ni	L	I	I
	P. I. 201581 Portugal	L+S	Ni	S	L	L	I	L+S

	P. I. 207009 Peru	I	Ni	S	I	I	I	L+S
	P. I. 207662 Japan	L+S	S	S	L	L	S	L+S
	P. I. 208741 Cuba	S	S	I	L	L	I	L
	P. I. 211115 Israel	S	Ni	I	L	L	S	L
	P. I. 212211 Greece	I	Ni	Ni	Ni	I	Ni	L
	P. I. 217523 Pakistan	S	L+S	S	L	L	S	S
	P. I. 217974 Dominican Republic	S	I	I	L	L	I	I
	P. I. 222187 Algeria	L	L+S	L+S	L	L	S	S
	P. I. 224769 USA	S	L	S	L	I	I	S
	P. I. 236355 Great Britain	S	L+S	S	L	L	I	L+S
	P. I. 241690 Ecuador	L+S	L+S	S	L	L	I	L
	P. I. 247957 Finland	S	L+S	I	L	L	I	L+S
	P. I. 249560 Thailand	S	S	S	L	L	I	L+S
	P. I. 255946 Germany	Ni	Ni	Ni	L	L	Ni	Ni
	P. I. 261644 The Netherlands	L+S	L+S	I	L	L	L+S	L+S
	P. I. 261760 Belgium	S	L+S	I	I	L	S	L+S
	P. I. 273438 Switzerland	L+S	L+S	S	L	L	I	L
	P. I. 288988 Hungary	S	L+S	L+S	Ni	L	S	L+S
	P. I. 304745 El Salvador	L+S	L+S	S	L	L	I	L+S
	P. I. 320993 Canada	L+S	Ni	S	L	Ni	Ni	Ni
	P. I. 323427 Austria	L+S	L+S	S	L	L	I	S
	P. I. 357755 Yugoslavia	L+S	L+S	L+S	L	L	I	L+S
	P. I. 360713 Brasil	L+S	I	Ni	L	I	I	I
	P. I. 385965 Kenya	L+S	L+S	S	L	L	S	S
	P. I. 403994 Colombia	S	S	S	L	L	S	I
C. melo var. agrestis	Gl. 1816 The Netherlands	L+S	L+S	I	L	Ni	S	L+S
C. melo var. flexcuosus	Gl. 1767 The Netherlands	S	Ni	Ni	L	Ni	I	L+S
C. metuliferus	Gl. 1775 The Netherlands	L+S	L+S	S	L	Ni	L+S	S
C. myriocarpus	Gl. 1979 The Netherlands	S	I	I	L	Ni	I	L+S
C. pubescens	Gl. 1756 The Netherlands	S	L+S	S	Ni	Ni	S	L+S
C. sativus	P. I. 234517 USA-SC	S	S	S	L	L	S	S
C. sativus cv. Alko Bush Cucumber	P. I. 267747 USA-OK	L+S	L+S	S	L	L	S	S
C. sativus cv. Apple Shape	P. I. 135122 New Zealand	S	L+S	S	L	L	I	S
C. sativus cv. Hermaphrodite Cucumber	P. I. 292012 Israel	S	L+S	S	L	L	S	S

C. sativus sikkinensis cv. Khira Balam	P. I. 165509 India	S	L+S	I	L	L	S	S
C. sativus cv. Manchuko Wonder	P. I. 114339 Japan	L+S	Ni	Ni	L	L	I	S
C. sativus cv. Pepino Verde Comprido	P. I. 118279 Brazil	S	L+S	L+S	L	L	S	S
C. sativus cv. Shogoin Aonaga Fushinari	P. I. 390265 Japan	Ni	S	L+S	L	L	S	L+S
C. sativus cv. Supercrop	P. I. 233932 Canada	S	L+S	L+S	L	L	S	S
C. zeyheri	Gl. 0181 The Netherlands	L+S	Ni	Ni	Ni	Ni	I	L+S

[1]CIS, Community of Independent States (earlier USSR). [2]I, immune; L, local; S, systemic; Ni, not investigated. [3]CGMMV, cucumber green mottle mosaic *Tobamovirus*; CLSV, cucumber leaf spot *Carmovirus*; CMV, cucumber mosaic *Cucumovirus*; MNSV, melon necrotic spot *Carmovirus*; MYFV, Melandrium yellow fleck *Bromovirus*; WMV-2, watermelon mosaic-2 *Potyvirus*; ZYMV, zucchini yellow mosaic *Potyvirus*

Particularly remarkable were the P. I. 108902, P. I. 161375, P. I. 207009, P. I. 217974, P. I. 360713 accessions of *Cucumis melo*, which besides their immunity to WMV-2 were immune to further three viruses (see Table 2). Hypersensitive resistance was shown of the accessions.

Immunity to ZYMV was detected in *Cucumis africanus* Gl. 2302, *C. anguria* var. anguria Gl. 1970, *C. ficifolia* Gl. 1803, and in 8 accessions of *Cucumis melo* out of which the breeding line P. I. 161375 deserves special attention. As regards complex resistance the following proved best: *Cucumis africanus* Gl. 2302, which besides its immunity to ZYMV showed complex immunity to further 4 viruses (CGMMV, CLSV, CMV, WMV-2), *C. anguria* var. anguria Gl. 1970 and *C. melo* P. I. 217974 with their complex immunity to three viruses (CLSV, CMV, WMV-2), or *C. ficifolius* Gl. 1803, *C. melo* P. I. 161375 which also exhibited complex immunity to 3 viruses (CGMMV, MNSV, WMV-2). Hypersensitive resistance could be detected in 6 accessions of *Cucumis melo* (see Table 2).

On examining the reactions (immunity and hypersensitive resistance) of various accessions of the *Cucumis* species, varieties and cultivars to viruses, an order of importance can be set up for the plants. This order is based on the number of viruses to which a given plant is immune or resistant. In Table 3 the 15 plants most important from the point of view of resistance

Table 3

Order of importance between *Cucumis* species according to the immunity and hypersensitive reaction

Cucumis species and accessions	Plant reaction against viruses		Total
	Immune	Hypersensitive	
Cucumis africanus, Gl. 2302	5	0	5
C. melo, P. I. 217974	4	2	6
C. melo, P. I. 161375	4	1	5
C. melo, P. I. 108902	4	1	5
C. melo, P. I. 360713	4	1	5
C. melo, P. I. 207009	4	0	4
C. anguria var. anguria Gl. 1970	4	0	4
C. ficifolius, Gl. 1803	4	0	4
C. melo, P. I. 190554	3	3	6
C. melo, P. I. 136171	3	1	4
C. melo, P. I. 200819	3	1	4
C. myriocarpus, Gl. 1979	3	1	4
C. melo, P. I. 208741	2	3	5
C. melo, P. I. 224769	2	2	4
C. melo, P. I. 247957	2	2	4

are listed. *Cucumis africanus* Gl. 2302 was placed first by its immunity to five viruses (CGMMV, CLSV, CMV, WMV-2, ZYMV). Its importance is increased by the fact that some of its accessions are resistant to *Sphaerotheca fuliginea* (see Lebeda, 1984). The second place is taken by *Cucumis melo* P. I. 217974, which proved immune to four viruses (CLSV, CMV, WMV-2, ZYMV) and showed hypersensitive resistance to two viruses (MNSV, MYFV). The third place is taken by *Cucumis melo* P. I. 161375, a host well-known, though, in plant virology but so far not examined for reaction to certain viruses; it exhibited immunity to four viruses (CGMMV, MNSV, WMV-2, ZYMV) and hypersensitive resistance to one virus (MYFV). The order of importance of the plants was decided by the number of viruses to which a given plant showed immunity and hypersensitive resistance, respectively, rather than by the total number of viruses to which it was resistant.

In the course of studying various accessions of *Cucumis* species, varieties and cultivars we established 219 compatible host-virus relationships of which 115 were systemic and 104 local and systemic (see Table 2). It is remarkable that all accessions of *Cucumis sativus* were susceptible to CLSV. For some viruses recovery was pointed out. *Cucumis sativus* P. I. 116479 plants inoculated with MNSV responded with characteristic necrotic local lesions followed by the appearance of severe systemic chlorotic and necrotic lesions. The systemic symptoms were followed by recovery; from the symptomless leaves the virus could be back-inoculated. Recovery was found again in the *Cucumis melo* P. I. 304745 accession inoculated with CMV. Nevertheless, recovery could not be generally observed, it only appeared in 1-2 plants. The *Cucumis* species generally responded with very severe symptoms to CMV, CLSV, CGMMV and ZYMV, but WMV-2 did not cause serious symptoms except one cultivar (*Cucumis sativus* cv. Supercrop).

On the basis of compatible host-virus relations the plants can be placed in an order of virus susceptibility. This order is based on the number of viruses to which a given plant was susceptible the lager the number of viruses to which it is susceptible and the fewer the viruses to which it is resistant are. According to our investigations *Cucumis dipsaceus* Gl. 0255 was the most susceptible of the plants tested; it was susceptible to all viruses included in the experiment, and was not resistant to any of them (Table 4). The second place is taken by *Cucumis melo* P. I. 288988; it was susceptible to 5 out of 6 viruses and resistant (hypersensitive resistance) to a single virus. *Cucumis metuliferus* Gl. 1775 stands third in the order of susceptibility; it was susceptible to 5 out of 6 viruses and resistant only to one virus. The fourth place is taken by *Cucumis melo* P. I. 116479, which exhibited susceptibility to 6 out of 7 viruses while immunity only to one virus. For the plants from 5 to 15 order of

susceptibility is difficult to determine (unless the severity of the viruses is taken into consideration, which, however, may vary from plant to plant), since all of them were susceptible to 5 out of 7 viruses and resistant each to two viruses (see Table 4).

Table 4

Order of susceptibility between *Cucumis* species according to systemic susceptibility and resistance to six or seven viruses

Cucumis species and accessions	Viruses inoculated	Susceptible against viruses	Resistance against viruses[1]
Cucumis dipsaceus, Gl. 0255	6	6	0/0
C. melo, P. I. 288988	6	5	0/1
C. metuliferus, Gl. 1775	6	5	0/1
C. melo, P. I. 116479	7	6	1/0
C. melo, P. I. 102077	7	5	0/2
C. melo, P. I. 162668	7	5	0/2
C. melo, P. I. 165450	7	5	0/2
C. melo, P. I. 183256	7	5	0/2
C. melo, P. I. 207662	7	5	0/2
C. melo, P. I. 385965	7	5	0/2
C. sativus, P. I. 234517	7	5	0/2
C. sativus cv. Alko Bush Cucumber, P. I. 267747	7	5	0/2
C. sativus cv. Hermaphrodite Cucumber, P. I. 292012	7	5	0/2
C. sativus cv. Pepino Verde Comprido, P. I. 118279	7	5	0/2
C. sativus cv. Supercrop, P. I. 233932	7	5	0/2

[1]Immunity/hypersensitivity against virus(es)

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Literature

Avgelis, A. D. (1989): Watermelon necrosis caused by a strain of melon necrotic spot virus. Plant Pathol. 38, 618–622.

Bohn, G. W., Kishaba, A. N. and McCreight, J. D. (1980): WMR 29 muskmelon breeding line. HortScience 15, 539–540.

Bos, L., Van Dorst, H. J. M., Huttinga, H. and Maat, D. Z. (1984): Further characterization of melon necrotic spot virus causing severe disease in glasshouse cucumbers in the Netherlands and its control. Neth. J. Pl. Path. 90, 55–69.

Cohen, S., German, E. and Kedar, N. (1971): Inheritance of resistance to melon virus in cucumbers. Phytopathology 61, 253–255.

Gonzales-Garza, R., Gumpf, D. J., Kishaba, A. N. and Bohn, G. W. (1979): Identification, seed transmission and host range pathogenicity of a California isolate of melon necrotic spot virus. Phytopathology 69, 340–345.

Hibi, T. and Furuki, I. (1985): Melon necrotic spot virus. AAB Description of Plant Viruses 302, 1–4.

Hollings, M. and Horváth, J. (1981): Melandrium yellow fleck virus. *C.* M. I./A. A. B. Descriptions of Plant Viruses 236, 1–4.

Hollings, M., Komuro, Y. and Tochihara, H. (1975): Cucumber green mottle mosaic virus. CMI/AAB Description of Plant Viruses 154, 1–4.

Horváth, J. (1975): *Cucumis myriocarpus* Naud., *Gomphrena decumbens* Jacq. and *Solanum rostratum* Dun. as new virus-susceptible and -immune plants. Ann. Inst. Prot. Plant. Hung. 13, 189–197.

Horváth, J. (1983): New artificial host and non-hosts of plant viruses and their role in the identification and separation of viruses. XVIII. Concluding remarks. Acta Phytopath. Acad. Sci. Hung. 18, 121–161.

Horváth, J. (1985a): New artificial host-virus relations between cucurbitaceous plants and viruses. I. *Benincasa*, *Bryonia*, *Bryonopsis* and *Citrullus* species. Acta Phytopath. Acad. Sci. Hung. 20, 225–251.

Horváth, J. (1985b): New artificial host-virus relations between cucurbitaceous plants and viruses. II. *Cucumis* and *Cucurbita* species. Acta Phytopath. Acad. Sci. Hung. 20, 253-266.

Horváth, J. (1993): Reaction of thirty-nine accessions of four *Cucurbita* species from different origin to seven viruses. Acta Phytopath. et Entomol. 28, 415–425.

Horváth, J., Juretić, N., Besada, W. H. and Kuroli, G. (1975): Two viruses isolated from patisson (*Cucurbita pepo* L. var. patissonina Greb. f. radiata Nois.), a new vegetable natural host in Hungary. I. Watermelon mosaic virus (general). Acta Phytopath. Acad. Sci. Hung. 10, 93-111.

Horváth, J., Hollings, M. and Barton, R. J. (1988): Experimental host range of Melandrium yellow fleck virus. Acta Phytopath. et Entomol. 23, 449–463.

Kalloo (1988): Vegetable Breeding. Vol. I-III. CRC Press, Inc. Boca Raton, Florida 1988.

Kishaba, A. N., Bohn, G. W. and Toba, H. H. (1971): Resistance to *Aphis gossypii* in muskmelon. J. Econ. Entomol. 64, 935–937.

Lebada, A. (1984): Screening of wild *Cucumis* species for resistance to cucumber powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). Scientia Horticulturae 24, 241–249.

Lecoq, H., Pitrat, M. and Clément, M. (1981): Identification et caractérisation d'un *Potyvirus* provoquant la maladie du rabougrissement jaune du melon. Agronomie 1, 827–834.

Lecoq, H., Lisa, V. and Dellavalle, G. (1983): Serological identity of muskmelon yellow stunt and zucchini yellow mosaic viruses. Plant Dis. 67, 824–825.

Lisa, V. and Lecoq, H. (1984): Zucchini yellow mosaic virus. CMI/AAB Descriptions of Plant Viruses 282, 1–4.

Lisa, V., Boccardo, G., Agostino, G., Dellavalle, G. and d'Aquilo, M. (1981): Characterization of a *Potyvirus* that causes zucchini yellow mosaic. Phytopathology 71, 667–672.

Moyer, J. W., Kennedy, G. G., and Romanow, L. R. (1985): Resistance to watermelon mosaic virus II multiplication in *Cucumis melo*. Phytopathology 75, 201–205.

Pitrat, M. and Lecoq, H. (1980): Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. Phytopathology 70, 958–961.

Pitrat, M. and Lecoq, H. (1984): Inheritance of zucchini yellow mosaic virus resistance in *Cucumis melo* L. Euphytica 33, 57–61.

Provvidenti, R. and Gonsalves, D. (1982): Resistance to papaya ringspot virus in *Cucumis metuliferus* and its relationship to resistance to watermelon mosaic virus 1. J. Heredity 73, 239–240.

Provvidenti, R., Gonsalves, D. and Humaydan, H. S. (1983): Occurrence of zucchini yellow mosaic virus in the United States. Cucurbit. Genet. Coop. 6, 99.

Risser, G., Pitrat, M., Rode, J. C. (1977): Etude de la résistance du Melon (*Cucumis melo* L.) au virus de la Mosaique du concombre. Ann. Amélior. Plantes 27, 509–522.

Schmelzer, K. (1967): Some more recent knowledge on cucurbit viruses. Proc. 6th Conf. Czechoslovak Plant Virologists. Olomouc 1967. pp. 206–210.

Schmidt, H. E. and Horváth, J. (1982): Natural occurrence of cucumber mosaic virus on bean (*Phaseolus vulgaris* L.) in Hungary (in Hungarian). Növényvéd. Tud. Napok. Budapest 1982. p. 3.

Sowell, G., Jr. (1987): Personal communication.

Teakle, D. S. and Thomas, B. J. (1985): Effect of heat on zoospore motility and multiplication of *Olpidium radicale* and *O. brassicae*. Ann. Appl. Biol. 107, 11–15.

Tóbiás, I. and Velich, I. (1983): Identification of the CMV on muskmelon and testing of resistance sources. Bull. Vegetable Crops Res. Inst., Kecskemét 1983. 16, 13–16.

Tomlinson, J. A. and Thomas, B. J. (1986): Studies on melon necrotic spot virus disease of cucumber and on the control of the fungus vector *(Olpidium radicale)*. Ann. Appl. Biol. 108, 71-80.

Wang, Y.-J., Provvidenti, R. and Robinson, R. W. (1984): Inheritance of resistance to watermelon mosaic virus 1 in cucumber. HortScience 19, 587–588.

Webb, R. E. (1979): Inheritance of resistance to watermelon mosaic virus 1 in *Cucumis melo* L. HortScience 14, 265–266.

Weber, I. (1983): Resistenzprüfung von Freilandgurken gegenüber dem Gurkenmosaik-Virus (cucumber mosaic virus). Tag.-Ber., Akad. Landwirtsch.-Wiss. 216, 307–312.

Weber, I. (1986): Cucumber leaf spot virus. AAB Descriptions of Plant Viruses 319, 1–4.

Weber, I. und Kegler, H. (1982): Resistenzprüfung von Freilandgurken gegenüber dem Gurkenmosaik-Virus (cucumber mosaic virus). I. Charakterisierung von Virusstämmen und Zuchtmaterial. Arch. Gartenbau (Berlin) 30, 377–385.

Weber, I. and Stanarius, A. (1984): Comparison of cucumber leaf spot virus with four newly isolated viruses from cucumber by immunoelectron microscopy. Arch. Phytopathol. Pflanzenschutz 20, 447–448.

Weber, I., Proll, E., Ostermann, W. D., Leiser, R. M., Stanarius, A. and Kegler, H. (1982): Charakterisierung des Gurkenblattflecken-Virus (cucumber leaf spot virus), eines bisher nicht bekannten Virus an Gewächshausgurken (*Cucumis sativus* L.) Arch. Phytopathol. Pflanzenschutz 18, 137–154.

Weber, I., Döring, U., Meyer, U. und Richter, J. (1985a): Ein Beitrag zur Bewertung der Resistenz von Gurken-Genotypen gegenüber dem Gurkenmosaik-Virus (cucumber mosaic virus) anhand der Virusvermehrung. Arch. Pytopathol. Pflanzenschutz 21, 251–257.

Weber, I., Stanarius, A., Kegler, H. and Kleinhempel, H. (1985b): Wechselwirkungen des Gurkenblattflecken-Virus (cucumber leaf spot virus) und des Gurkengrünscheckungsmosaik-Virus (cucumber green mottle mosaic virus) bei der Infektion und Ausbreitung auf Testplanzen. Arch. Phytopathol. Pflanzenschutz 24, 347–351.

Weber, I., Stanarius, A. and Kalinina, I. (1986): Weitere Untersuchungen zur serologischen Verwandtschaft zwischen dem Gurkenblattflecken-Virus (cucumber leaf spot virus) und dem Gurkenfruchtstreifen-Virus (cucumber fruit streak virus). Arch. Phytopathol. Pflanzenschutz 22, 169–170.

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Reactions of Thirty-Nine Accessions of Four *Cucurbita* Species from Different Origin to Seven Viruses

J. HORVÁTH

Pannonian University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P.O. Box 71, Hungary

In the course of examining the relation of 30 accessions of 3 *Cucurbita* species *(C. moschata, C. ecuadorensis, C. texana)* and 9 cultivars of *Cucurbita pepo* to 7 viruses we detected 249 host-virus relations of which 103 were resistant or immune and 146 susceptible.

From the point of view of resistance remarkable was the *Cucurbita moschata* of which 24 accessions were immune to the cucumber green mottle mosaic *Tobamovirus* (CGMMV), 16 accessions to the cucumber mosaic *Cucumovirus* (CMV), 9 accessions to the cucumber fruit streak virus (CFSV), 3 accessions to the cucumber leaf spot *Carmovirus* (CLSV) and 2 accessions to the melon necrotic spot *Carmovirus* (MNSV) and 2 to the zucchini yellow mosaic *Potyvirus* (ZYMV). The susceptible accessions were: 23 to watermelon mosaic-2 *Potyvirus* (WMV-2), 20 to CLSV, 18 to MNSV, 13 to CFSV, 8 to CMV and 1 to CGMMV. Four accessions of *Cucurbita ecuadorensis* were immune to CGMMV, 2 accessions to ZYMV, while 1 accession of it to CLSV and again 1 to MNSV. The 285213 accession of *Cucurbita texana* was immune to CFSV, CGMMV and MNSV, and susceptible to CLSV, CMV, WMV-2 and ZYMV. Immunity to CFSV was shown by some *Cucurbita pepo* cultivars (Yellow Oval, Green Marrow, Salad Melon, Calabasa). All *Cucurbita pepo* cultivars were immune to CGMMV. Some cultivars (Little Gem, ovifera, Salad Melon, Super Squash, Calabasa) were found to be immune to CMV, and further cultivars (Yellow Oval, Green Marrow, Little Gem, Calabasa, Omaha Pumpkin) showed immunity to MNSV.

All *Cucurbita pepo* cultivars were susceptible to CLSV, WMV-2 and ZYMV. To 6 of the 7 viruses the following species and accessions were susceptible: *Cucurbita moschata* (172344, 200822, 267754, 286421, 357918), *C. ecuadorensis* (432444, 432445). To 5 viruses susceptibility was shown by *Cucurbita moschata* (163288, 165033, 192942, 199014, 306125, 369346),*C. ecuadorensis* 432442, *C. pepo* cv. ovifera 196309 and *C. pepo* cv. Omaha Pumpkin 302418. Further 18 accessions proved susceptible to four, 3 accessions to three and 2 accessions to two viruses.

The occurrence of pathogens (viroids, viruses, mycoplasmas, bacteria, fungi) infecting cucurbitaceous plants has recently become more and more frequent, and they have caused considerable quantitative and qualitative damages to crops (see Horváth, 1985a). As it is known the number of viruses infecting cucurbitaceous plants exceeds 60. Among the viruses of various characteristic there are about 8 potyviruses (Lockhart and Fischer, 1979; Lovisolo, 1980; Purcifull et al., 1984). It deserves special attention that in cucurbitaceous plants four so-called soil-borne viruses (cucumber fruit streak virus, CFSV; cucumber leaf spot *Carmovirus*, CLSV; cucumber soil-borne *Carmovirus*, CSBV; melon necrotic spot *Carmovirus*, MNSV) were detected in the short period of the past several years (Gonzales-Garza et al., 1979; Koenig et al., 1982; Weber et al., 1982; Gallitelli et al., 1983).

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The high virus susceptibility of the *Cucurbita* species made it necessary to examine the species for resistance. The investigations were promoted by those works which pointed out resistance to various viruses in some species. For example, resistance was detected in *Cucurbita maxima*, *C. moschata* and *C. pepo* to squash mosaic *Comovirus* (SMV), in *C. ecuadorensis*, *C. foetidissima* and *C. martinezi* to WMV-1, WMV-2 and CMV (Salama et al., 1968; Sitterly, 1972; Munger, 1976; Provvidenti et al., 1978; Pink and Walkey, 1984; Maluf et al., 1986; Paris et al., 1988; Horváth and Nagy, 1990; Horváth, 1991a). It is remarkable that *Cucurbita moschata* showed resistance to powdery mildew *(Sphaerotheca fuliginea)*, and *C. andreana, C. ficifolia, C. lundelliana, C. maxima, C. pepo* to bacterial wilt *(Erwinia tracheiphila)* (Watterson et al., 1971; Sowell and Corley, 1973). In earlier studies covering some 27 *Cucurbita* species resistance could not be detected (Horváth, 1985b).

Considering that the *Cucurbita* species, varieties, accessions and places of origin may show great differences in virus susceptibility and resistance even to different strains of the same viruses (e.g., Demski and Sowell, 1970; Schmidt et al., 1980; Weber, 1983; Pink and Walkey, 1984; Walkey and Pink, 1984; Maluf et al., 1986), we examined the behaviour of some *Cucurbita* species originating from different places towards various viruses.

Materials and Methods

A total of 30 accessions belonging to three *Cucurbita* species *(C. moschata, C. ecuadorensis, C. texana)* and 9 cultivars belonging to *C. pepo* have been tested for reaction to seven viruses (Table 1). The different *Cucurbita* species originated from 27 countries (Table 3). The seed samples originating from Germplasm Resources Laboratory, U. S. D. A., A. R. S., Beltsville, Maryland (USA) were marked with the letters P. I. (*P*lant *I*ntroduction).

Cotyledons of young seedlings of *Cucurbita* plants were inoculated with seven viruses maintained in different propagation hosts (see Table 1). Six young plants per viruses were inoculated in two replications with the carborundum (500 mesh) -spatula technique so that the virus-containing tissue sap was diluted with distilled water at a ratio of 1 : 1. Following the inoculation the leaves of the inoculated plants were sprayed with tap water. The susceptibility, resistance or immunity of the plants were determined symptomatologically and by back-inoculation tests. In back-inoculation tests tissue saps were prepared both from the inoculated leaves of the infected plants and from non-inoculated top leaves, and rubbed onto assay plants

Table 1

Viruses used in the experiments

Viruses[1] and (acronyms)	Strains or isolates	Propagation host	Literature[3]
Cucumber fruit streak ? (CFSV)	Common	*Nicotiana megalosiphon*	*Gallitelli* et al. (1983)
Cucumber green mottle mosaic *Tobamovirus* (CGMMV)	IP-A[2]	*Cucumis sativus*	*Schmelzer* (1967); Hollings et al. (1975)
Cucumber leaf spot *Carmovirus* (CLSV)	Common	*Cucumis sativus*	*Weber* et al. (1982, 1985, 1986); Weber and Stanarius (1984); Weber (1986)
Cucumber mosaic *Cucumovirus* (CMV)	U/246	*Nicotiana tabacum* cv. Xanthi-nc	Schmidt and *Horváth* (1982)
Melon necrotic spot *Carmovirus* (MNSV)	Cu18	*Cucumis melo* (cotyledons)	Gonzales-Garza et al. (1979); *Bos* et al. (1984); Teakle and Thomas (1985); Hibi and Furuki (1985); Tomlinson and Thomas (1986); Avgelis (1989)
Watermelon mosaic-2 *Potyvirus* (WMV-2)	PW	*Ammi majus*	*Horváth* el al. (1975)
Zucchini yellow mosaic *Potyvirus* (ZYMV)	E15	*Cucurbita pepo*	*Lecoq* et al. (1981, 1983); Lisa et al. (1981); Lisa and Lecoq (1984)

[1]Question mark indicate uncertainty of the taxonomic status of the virus. Cucumber fruit streak virus and cucumber leaf spot *Carmovirus* were found serologically related but not identical by immunoelectron microscopical and agar gel double diffusion tests (see Weber et al., 1986). [2]Obtained from Institute of Phytopathology, Aschersleben, Germany. [3]Names printed in italics are the sender of the virus

which provided the quickest and most reliable way of pointing out the virus (see Table 2). Previously the surfaces of the leaves were disinfected in a 2% solution of NaOH then washed with a jet of water.

Table 2

Assay hosts used in back-inoculation tests

Viruses[1] and (acronyms)	Assay hosts
Cucumber fruit streak ? (CFSV)	*Gomphrena globosa*
Cucumber green mottle mosaic *Tobamovirus* (CGMMV)	*Chenopodium amaranticolor*
Cucumber leaf spot *Carmovirus* (CLSV)	*Chenopodium quinoa*
Cucumber mosaic *Cucumovirus* (CMV)	*Chenopodium quinoa*
Melon necrotic spot *Carmovirus* (MNSV)	*Cucumis sativus*
Watermelon mosaic - 2 *Potyvirus* (WMV-2)	*Chenopodium amaranticolor*
Zucchini yellow mosaic *Potyvirus* (ZYMV)	*Chenopodium amaranticolor*

[1]Question mark indicates uncertainty of the taxonomic status of the virus. See footnote in Table 1 (Weber et al., 1986)

In the course of a studying the plant-virus relationships we determined the virus susceptibility, resistance or immunity for both the inoculated and the non-inoculated leaves. We obtained data in the first case on the localization of the virus in the inoculated, rubbed leaves, in the second case on the generalization of the virus in the fully developed plant. Those relationships in which only the inoculated leaves of the plants showed susceptibility (necrotic lesions, leaf drop; coalesce and cotyledons desiccated by MNSV and CLSV), while those developed after the inoculation remained free from virus were regarded as local host or resistant plant. Those plants which when inoculated remained free from viruses both locally and systemically (back-inoculation tests were also negative) were regarded as immune plants. Virus susceptible plants showed characteristic symptoms (vein clearing, mosaic) or in the case of latent infection the viruses could be back-inoculated to assay hosts.

Results and Discussion

Table 3 shows the reactions of *Cucurbita* species to seven viruses. In 30 accessions of three *Cucurbita* species *(C. moschata, C.ecuadorensis, C.texana)* and 9 cultivars of *Cucurbita pepo* 249 host-virus relations to seven viruses were revealed. For technical reasons 24 host-virus relationships were not established. According to the results of the experiment, between the

Cucurbita species and viruses tested susceptible host-virus relationship could be detected in the highest percentage, in some 146 cases. Out of them 61 were so-called "total susceptibility" (the inoculated plants were both locally and systemically susceptible to the given virus) and 85 systemic susceptibility. In 92 cases of the 103 incompatible relationships the result of test was complete absence of symptoms and negative back-inoculation (immunity), while in 11 cases resistant relationship (only local susceptibility or hypersensitive reaction).

To CFSV 14 plants (9 *Cucurbita moschata* accessions, 4 *C. pepo* cultivars and 1 accession of *C.texana*) were immune and 3 were resistant (2 *Cucurbita moschata* accessions and 1 *C. pepo* cv. Summer Squash).

As regards reaction to CGMMV *Cucurbita moschata* deserves attention. Out of its 25 accessions only the P. I. 286421 was susceptible, the other 24 accessions were immune (see Table 3).

To CLSV three *Cucurbita moschata* accessions (193499, 244707, 286421) were immune, while two accessions (135375, 163288) resistant. In the course of host range studies we found the species *Atriplex littoralis* to be excellent assay host, and *Nicotiana benthamiana* a systemic host for CLSV (Horváth, 1991b,1993).

To the bean strain of CMV 16 accessions of *Cucurbita moschata* and 5 cultivars of *C. pepo* were immune. Two *Cucurbita moschata* accessions,one *C. ecuadorensis* and one *C. texana* accession, and 6 cultivars of *Cucurbita pepo* proved immune to MNSV. According to Thomas and Tomlinson (1985) *Cucurbita pepo* appears to be immune to all isolates of MNSV. However, 5 accessions of *Cucurbita moschata* and *C. pepo* cv. Summer Squash P. I. 136448 showed resistance (see Table 3). As opposed to the data of Tomlinson and Thomas (1986) *Nicotiana benthamiana* is a very good systemic host (vein clearing, mosaic, leaf deformation) for MNSV (Avgelis, 1989; Horváth, 1993).

To WMV-2 only one *Cucurbita moschata* accession (P. I. 209116) was immune, the other plants examined were equally susceptible (see Table 3).

Immunity to ZYMV could be detected only in certain accessions of *Cucurbita moschata* (P.I. 200736, 349352) and *C. ecuadorensis* (P. I. 432243, 432445) all the other plants examined were susceptible.

From the point of view of general and complex virus resistance the P. I. 349352 accession of *Cucurbita moschata* proved the best; it was immune to five viruses (CFSV, CGMMV, CMV, MNSV, ZYMV) and susceptible only to two viruses (CLSV, WMV-2). Out of other accessions of the latter species the P. I. 193499 was immune to 4 viruses (CFSV, CGMMV, CLSV, CMV), the 135375 and 244707 accessions to 3 viruses, respectively (CFSV,

Table 3

Reaction of *Cucurbita* species to viruses

Cucurbita species	Plant introduction (P. I.) number and origin	Reaction[1] to viruses[2]						
		CFSV	CGMMV	CLSV	CMV	MNSV	WMV-2	ZYMV
C. moschata	135375 Afghanistan	I	I	L	I	L+S	L	S
	141646 Iran	S	I	S	I	L	S	S
	162889 Paraguay	I	I	L+S	I	L+S	L+S	S
	163228 India	L+S	I	L	S	L+S	S	S
	165033 Turkey	L+S	I	L	S	L+S	S	S
	172344 Mexico	L+S	I	L+S	S	L+S	S	S
	183258 Saudi Arabia	L+S	I	L+S	I	L	S	S
	192942 China	L+S	I	L+S	I	L+S	S	S
	193499 Ethiopia	I	I	I	I	L	S	S
	194570 Guatemala	I	I	L+S	S	I	S	S
	199014 Africa	L+S	I	L+S	I	L+S	S	S
	200736 El Salvador	L+S	I	L+S	I	L+S	S	I
	200822 Burma	S	I	L+S	S	L+S	L+S	S
	209116 Puerto Rico	I	I	L+S	I	L+S	I	S
	234251 Japan	I	I	L+S	S	L	S	S
	244707 Brazil	I	I	I	S	L	S	S
	267752 USA	L	I	L+S	I	L+S	S	S
	267754 Italy	L+S	I	L+S	S	L+S	S	S
	286421 Nepal	Ni	S	I	Ni	S	Ni	S
	288239 Egypt	L	I	L+S	I	L+S	S	S
	298036 Australia	I	I	L+S	I	L+S	S	S
	306125 Bulgaria	S	I	L+S	I	L+S	S	S
	349352 Ecuador	I	I	L+S	I	I	S	I
	357918 Yugoslavia	L+S	I	L+S	S	L+S	S	S
	369346 Costa Rica	L+S	I	L+S	I	L+S	S	S
C. ecuadorensis	432442 Ecuador	Ni	I	I	Ni	Ni	Ni	Ni
	432443 Ecuador	Ni	I	Ni	Ni	I	Ni	I
	432444 Ecuador	Ni	I	Ni	Ni	Ni	Ni	Ni

	432445	Ecuador	Ni	I	Ni	Ni	Ni	Ni	I
C. pepo cv. Yellow Oval	267663	USA-CT	I	I	L+S	S	I	S	S
C. pepo cv. Green Marrow	257287	Spain	I	I	L+S	L+S	I	S	S
C. pepo cv. Little Gem	234614	S. Africa	L+S	I	L+S	I	I	S	S
C. pepo cv. ovifera	196309	Mexico	S	I	L+S	I	L+S	S	S
C. pepo cv. Salad Melon Special	192941	China	I	I	L+S	I	Ni	S	S
C. pepo cv. Summer Squash	136448	Canada	L	I	L+S	I	L	S	S
C. pepo cv. Calabasa	318830	Mexico	S	I	L+S	I	I	S	S
C. pepo cv. Calabasa	311102	Guatemala	I	I	L+S	S	I	S	S
C. pepo cv. Omaha Pumpkin	302418	USA-ND	L+S	I	L+S	S	I	S	S
C. texana	285213	USA-TX	I	I	S	S	I	S	S

[1]Reaction of *Cucurbita* plants to viruses: I, immune; L, local; S, systemic; Ni, not investigated. [2]Name of viruses: CFSV, cucumber fruit streak virus; CGMMV, cucumber green mottle mosaic *Tobamovirus*; CLSV, cucumber leaf spot *Carmovirus*; CMV, cucumber mosaic *Cucumovirus*; MNSV, melon necrotic spot *Carmovirus*; WMV-2, watermelon mosaic-2 *Potyvirus*; ZYMV, zucchini yellow mosaic *Potyvirus*

CGMMV, CMV or CFSV, CGMMV, CLSV). In the course of testing 68 accessions of *Cucurbita moschata* Paris et al. (1988) found four accessions to be resistant to ZYMV. The mode of inheritance for resistance to ZYMV in *Cucurbita moschata* was determined from F_1, F_2 and backcross progenies of the cross between the susceptible cultivar Waltham Butternut and a resistant inbred line of the Menina cultivar. Resistance to ZYMV in Menina was conferred by a single dominant gene designated *Zym* (see Paris et al., 1988). From a genetic point of view it is remarkable that the *Cucurbita moschata* is readily crossed with *C. ecuadorensis*, *C. pepo, C. mixta, C. maxima* and *C. foetidissima*. Greber and Herrington (1980) examined the reaction of interspecific hybrids between *Cucurbita moschata*, *C. ecuadorensis* and *C. maxima*. The hybrid plants of *C. ecuadorensis* x *C. moschata* produced many female flowers, while male flowers were extremely rare and were not able to achieve fertilization to produce F_2 seed from this cross. However, some fertility was maintained in seed resulting from the backcross to *Cucurbita moschata* and the outcross to *C. pepo*. Greber and Herrington (1980) hope these progeny will provide a pathway for incorporation of WMV resistance into these species. The P. I. 432443 of *Cucurbita ecuadorensis* was immune to CGMMV, MNSV and ZYMV. The immunity of *Cucurbita pepo* cv. Yellow Oval 267663 and *C. pepo* cv. Green Marrow 257287 to CFSV, CGMMV and MNSV, of *C. pepo* cv. Little Gem 234614 and *C. pepo* cv. Calabasa 318830 to three viruses (CGMMV, CMV, MNSV) as well as the immunity of *C. pepo* cv. Calabasa 311102 and *C. texana* 285213 to three viruses (CFSV, CGMMV, MNSV) also deserve attention (see Table 3).

Of the plants tested the following were the most susceptible: *Cucurbita moschata* (P. I. 172344, 200822, 267754, 286421, 357918) and *C. ecuadorensis* (432444 and 432445) to six viruses, *Cucurbita moschata* (163288, 165033, 192942, 199114, 306124, 369346), *C. ecuadorensis* (P. I. 432442), *C. pepo* cv. ovifera (P. I. 196309), *C. pepo* cv. Omacha Pumpkin (P. I. 302418) to five viruses. Further, 18 accessions (10 of *C. moschata*, 1 of *C. ecuadorensis*, 6 of *C. pepo* cultivars and 1 of *C. texana*) were susceptible to four, three accessions (2 of *C. moschata*, 1 of *C. pepo* cv. Summer Squash) to three and two acces-sions (of *C. moschata*) to two viruses.

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Literature

Avgelis, A.D. (1989): Watermelon necrosis caused by a strain of melon necrotic spot virus. Plant Pathol. 38, 618–622.

Bos, L., Van Dorst, H.J.M., Huttinga, H. and Maat, D.Z. (1984): Further characterization of melon necrotic spot virus causing severe disease in glasshouse cucumbers in the Netherlands and its control. Neth. J. Plant Path. 90, 55–69.

Demski, J.W. and Sowell, G. (1970): Susceptibility of *Cucurbita pepo* and *Citrullus lanatus* introductions to watermelon mosaic virus-2. Plant Dis. Rep. 54, 880–881.

Gallitelli, D., Vovlas, C. and Avgelis, A. (1983): Some properties of cucumber fruit streak virus. Phytopath. Z. 106, 149–162.

Gonzales-Garza, R., Gumpf, D.J., Kishaba, A.N. and Bohn, G.W. (1979): Identification, seed transmission and host range pathogenicity of a California isolate of melon necrotic spot virus. Phytopathology 69, 340–345.

Greber, R.S. and Herrington, M.E. (1980): Reaction of interspecific hybrids between *Cucurbita ecuadorensis*, *C. maxima* and *C. moschata* to inoculation with cucumber mosaic virus and watermelon mosaic virus 1 and 2. Austral. Plant Pathol. 9, 1–2

Hibi, T. and Furuki, I. (1985): Melon necrotic spot virus. AAB Descriptions of Plant Viruses 302, 1–4.

Hollings, M., Komuro Y. and Tochihara, H. (1975): Cucumber green mottle mosaic virus. CMI/AAB Descriptions of Plant Viruses 154, 1–4.

Horváth, J. (1985a): New artificial host-virus relations between cucurbitaceous plants and viruses. I. *Benincasa*, *Bryonia*, *Bryonopsis* and *Citrullus* species. Acta Phytopath. Acad. Sci. Hung. 20, 225–251.

Horváth, J. (1985b): New artificial host-virus relations between cucurbitaceous plants and viruses. II. *Cucumis* and *Cucurbita* species. Acta Phytopath. Acad. Sci. Hung. 20, 253–266.

Horváth, J. (1991a): A *Cucurbita foetidissima* HBK és a *Cucurbita sororia* Bailey vad fajok vírusfogékonysága és vírusrezisztenciája. Növényvéd. Tud. Napok, Budapest 1991. p. 86.

Horváth, J. (1991b): Host-virus relations between leafy vegetables and viruses. Acta Phytopath. et Entomol. 26, 339–346.

Horváth, J. (1993): The role of *Nicotiana* species in plant virology with special regard to *Nicotiana benthamiana* Domin: A Review. Acta Phytopath. et Entomol. 28, 355–377.

Horváth, J. and Nagy, Gy. (1990): Resistance of *Cucurbita* maxima Duch. var. oblonga „Óvári Hengeres" to cucumber mosaic virus. Növényvédelem 6, 261 (Abstr.).

Horváth, J., Juretić, N., Besada, W. H. and Kuroli, G. (1975): Two viruses isolated from patisson (*Cucurbita pepo* L. var. patissonina Greb. f. radiata Nois.), a new vegetable natural host in Hungary. I. Watermelon mosaic virus (general). Acta Phytopath. Acad. Sci. Hung. 10, 93–111.

Koenig, R., Lesemann, D.E., Huth, W. and Makkouk, K.M. (1982): A new cucumber soil-borne virus compared with tombus-, diantho-, and other similar viruses. Phytopathology 72, 964 (Abstr.).

Lecoq, H., Pitrat, M. and Clément, M. (1981): Identification et caractérisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. Agronomie 1, 827–834.

Lecoq, H., Lisa, V. and Dellavalle, G. (1983): Serological identity of muskmelon yellow stunt and zucchini yellow mosaic viruses. Plant Dis. 67. 824–825.

Lisa, V. and Lecoq, H. (1984): Zucchini yellow mosaic virus. CMI/AAB Descriptions of Plant Viruses 282, 1–4.

Lisa, V., Boccardo, G., Agostino, G., Dellavalle, G. and d'Aquilo, M. (1981): Characterization of a potyvirus that causes zucchini yellow mosaic. Phytopathology 71, 667–672.

Lockhart, B.E.L. and Fischer, H.U. (1979): Host range and some properties of Bryonia mottle virus, a new member of the potyvirus group. Phytopath. Z. 96, 244–250.

Lovisolo, O. (1980): Virus and viroid disease of cucurbits. Acta Horticulturae 88, 33–82.

Maluf, W.R., de Melo Moura, W., da Silva, I.S. and Castelo-Branco, M. (1986): Screening of *Cucurbita* spp. accessions for resistance to vatermelon mosaic virus-1. Rev. Brasil. Genet. 1, 161–167.

Munger, H.M. (1976): *Cucurbita martinezii* as a source of disease resistance. Veg. Impr. Newsl. 18, 4.

Paris, H.S., Cohen, S., Burger, Y. and Yoseph, R. (1988): Single-gene resistance to zucchini yellow mosaic virus in *Cucurbita moschata*. Euphytica 37, 27–29.

Pink, D.A.C. and Walkey, D.G.A. (1984): Resistance in marrow (*Cucurbita pepo* L.) to different strains of cucumber mosaic virus. J. Agr. Sci. Camb. 103, 519–521.

Provvidenti, R., Robinson, R.W. and Munger, H.M. (1978): Resistance in feral species to six viruses infecting *Cucurbita*. Plant Dis. Rep. 62, 326–329.

Purcifull, D.E., Adlerz, W.C., Simone, G.W., Hiebert, E. and Christie, S.R. (1984): Serological relationships and partial characterization of zucchini yellow mosaic virus isolated from squash in Florida. Plant Dis. 68, 230–233.

Salama, E.A. and Sill, W.H., Jr. (1968): Resistance to Kansas squash mosaic virus strains among *Cucurbita* species. Trans. Kans. Acad. Sci. 71, 62–72.

Schmelzer, K. (1967): Some more recent knowledge on cucurbit viruses. Proc. 6th Conf. Czechoslovak Plant Virologists. Olomuc 1967. pp. 206–210.

Schmidt, H.E. and Horváth, J. (1982): Natural occurrence of cucumber mosaic virus on bean (*Phaseolus vulgaris* L.) in Hungary (in Hungarian). Növényvéd. Tud. Napok. Budapest 1982. p. 3.

Schmidt H.E., Schubert, L., Weber, I., Fabig, F. and Arndt, H. (1980): Bedeutung und Ergebnisse der Virus-Resistenzzüchtung bei Gemüsekulturen. Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin 184, 177–195.

Sitterly, W.R (1972): Breeding for disease resistance in cucurbits. Ann. Rev. Phytopathology 10, 471–487.

Sowell, G., Jr. and Corley, W.L. (1973): Resistance of *Cucurbita* plant introductions to powdery mildew. HortScience 8, 492.

Teakle, D.S. and Thomas, B.J. (1985): Effect of heat on zoospore motility and multiplication of *Olpidium radicale* and *O. brassicae*. Ann. Appl. Biol. 107, 11–15.

Thomas B.J. and Tomlinson J.A. (1985): Studies on melon necrotic spot virus and its vector. Occurrence and transmission by the fungus *Olpidium radicale*. Glasshouse Crops Res. Inst., Littlehampton., Ann. Rep. 1984., Littlehampton 1985. pp. 108–114.

Tomlinson J.A. and Thomas B.J. (1986): Studies on melon necrotic spot virus disease of cucumber and on the control of the fungus vector *(Olpidium radicale)*. Ann. Appl. Biol. 108, 71–80.

Walkey, D.G.A. and Pink, D.A.C. (1984): Resistance in vegetable marrow and other *Cucurbita* spp. to two British of cucumber mosaic virus. J. Agr. Sci. Cambridge 102, 197–205.

Watterson, J.C., Williams, P.H. and Durbin, R.D. (1971): Response of *Cucurbita* to *Erwinia tracheiphila*. Plant Dis. Rep. 55, 816 – 819.

Weber, I. (1983): Rezistenzprüfung von Freilandgurken gegenüber dem Gurkenmosaik-Virus (cucumber mosaic virus). Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin 216, 307–312.

Weber, I. (1986): Cucumber leaf spot virus. AAB Descriptions of Plant Viruses 319, 1–4.

Weber, I. and Stanarius, A. (1984): Comparison of cucumber leaf spot virus with four newly isolated viruses from cucumber by immunoelectron microscopy. Arch. Phytopathol. Pflanzenschutz 20, 447–448.

Weber, I., Proll, E., Ostermann, W.D., Leiser, R.M., Stanarius, A. and Kegler, H. (1982): Charakterisierung des Gurkenblattflecken-Virus (cucumber leaf spot virus), eines bisher nicht bekannten Virus an Gewächshausgurken (*Cucumus sativus* L,). Arch. Phytopathol. Pflanzenschutz 18, 137–154.

Weber, I., Stanarius A., Kegler, H. and Kleinhempel, H. (1985): Wechselwirkungen des Gurkenblattflecken-Virus (cucumber leaf spot virus) und des Gurkengrünscheckungsmosaik-Virus (cucumber green mottle mosaic virus) bei der Infektion und Ausbreitung auf Testpflanzen. Arch. Phytopathol. Pflanzenschutz 24, 347–351.

Weber, I., Stanarius, A. and Kalinina, I. (1986): Weitere Untersuchungen zur serologischen Verwandtschaft zwischen dem Gurkenblattenflecken-Virus (cucumber leaf spot virus) und dem Gurkenfruchtstreifen-Virus (cucumber fruit streak virus). Arch. Phytopathol. Pflanzenschutz 22, 169–170.

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Detection of Organophosphorus Resistance in the Spider Mites *Tetranychus urticae* Koch in Pear Orchards in Egypt

MAHMOUD HASSAN TAG EL-DIN and MOHAMED FADEL SHADY

Plant Protection Department, Faculty of Agriculture, Tanta University, Kafr El-Sheikh, Egypt

It became essential to develop resistance management to delay the evolution of resistance. Resistance management requires simple and quick detection methods by which resistance pests can be detected in field and in the early stages of resistance development. Because of the sensitivity to acetylcholinesterase is the most common cause of organophosphorus resistance in spider mites *T. urticae*, the activity of acetylcholinesterase and its inhibition with paraoxon were measured in spider mites susceptible strain S, resistante strain R, and in two field strains collected from pear trees in Rashid area, Beheira province BR and El-Mamouraa area, Alexandria province AR in Egypt. The resistant strain R was 102-fold resistant to parathion, while the two field strains BR and AR were 59-fold and 11-fold resistant to parathion, respectively. The activity of acetylcholinesterase in R strain and in the two field strains BR and AR are significantly lower than in the S strain, where the percent of activity to that of S strain was 60%, 70% and 81% for R, BR and AR strains, respectively. The reduction in rate of acetylcholinesterase inhibition was 30-times, 29-times and 17-times in R, BR and AR strains for paraoxon, respectively. The broken curves for the two field strains showed heterogeneity in spider mites population, where percent of resistant portion was 92% and 55% for BR and AR strains, respectively. This rapid and simple technique can be directly performed for the field strains and provides us with reproducible data about the development of resistance to organophosphorus insecticides in the field.

Development of resistance to organophosphorus insecticides "OP_s" in spider mites *T. urticae* is a well known phenomenon that started in the fifties (Matsumura and Voss, 1964; Herne and Brown, 1969; Croft, 1982 and Helle, 1984). Resistance of insects against insecticides, documented in at least 447 species and mites by 1984 (Rousch and McKenzie, 1987), is increasingly acknowledged as a major challenge for agriculture. Mites acquire increasing economic importance due to their developing resistance to the pesticides used intensively on some crops and fruit trees. The available genetic and biochemical evidence strongly supports the view that the most common cause of OPS resistance in*T. urticae* was found to be an altered acetylcholinesterase „AChE" wich proved to be 150-times and 600-times less vulnerable to inhibition by diazoxon and paraoxon, respectively, in comparison to normal enzyme (Smissaert, 1964 and Smissaert et al., 1970). Detection of resistant pests is prerequisite to delay the evolution of resistance, therefore detection methods need to be simple, to provide reproducible results and to simulate field

treatment conditions as possible (Walker et al. , 1973). Acetylcholinesterase with decreased sensitivity to inhibition by OP_s and Carbamate insecticides has been implicited in resistance of many arthropods to these chemicals (Devonshire, 1980). The slide-dip method "SDM" recommended by FAO has gained acceptance as the proved method for detection of resistant spider mites (Busvine, 1980). In recent years, population of spider mites *T. urticae* has detectable level of OP_s resistance in cotton fields and fruit orchards in Egypt. Despite the presence of resistant individuals, OP_s compounds are still used to maintain spider mites and other harmful pests below economic thresholds in cotton fields and fruit orchards in Egypt. In this case, the main purpose of resistance management program is to indicate which fields or orchards have developed relatively homogeneous population of resistant spider mites and are at greatest risk for the intensification of resistance and also, to avoid the economic loss in the yield. It is generally considered important in pest control, when insecticides are indispensable in complex pest situations to have such a rapid biochemical and bioassay method for detection of resistance in the field strains of spider mites. The present work reports on the successful application of a quick technique, based on the measurement of insensitive AChE to detect OPS resistance in field strains of *T. urticae* in pear orchards in Egypt.

Materials and Methods

Chemicals

Paraoxon of more than 99% purity was obtained from Bayer Company and the other insecticide of more than 97% purity was obtained commercially.

Strains

A suspectible strain S of *T. urticae* was collected in Beheira province on soybean plants and the resistant strain R was collected in Kafr El-Sheikh province on cotton plants. Mites of both strains were bred on bean plants at 25 °C and 80% RH with 16-hours light / day. The resistant strain R was treated with parathion in the laboratory for a number of generations and its resistance was measured with slide-dip method and was found to be 102-fold resistant to parathion (LD_{50} of S and R strains where 5.8 ppm and 590 ppm parathion, respectively). The field strains collected from Rashid area BR, Beheria province and from El-Mamouraa area AR, Alexandria province by random

way (three orchards in each province) in summer of 1989. A 50-leaf sample was taken from each orchard before any pesticide application in these orchards, and this 150-leaf sample for each province divided randomly to two parts (75-leaf/each). The first part used for obtaining a number of 700 surving adult females of spider mites for bioassay tests, where five concentrations of parathion were used (10, 50, 100, 500 and 1500 ppm parathion). Each concentration was tested with two replicates (50 adult females per replicate), and the treated mites were kept beside the control mites at 25 °C and 80% RH with 16-hours light per day. Mortality percents were recorded after 24 hours. The second part of leaf sample used for collecting the adult females by air suction device and stored at –15 °C measurement of AChE activity and its inhibition. Mites were homogenized in a 1-ml potter tube with a teflon peste in buffer at 0 °C. The crude homogenats of the four strains were centrifuged for 30 min in a fuge at 9000 g at 4 °C either kept at 4 °C for use on the same day or at –20 °C for several days.

Measurement of acetylcholinesterase activity and its inhibition

AChE activity was measured according to Ellman et al. (1961) and the inhibition constants "Ki" were measured according to Aldridge (1950). Five hundred adult females of S, R, KR and BR strains were collected (500 adult females per strain), and mites of each strain were macerated in 500 µl 0.1 M potassium phosphate buffer, pH 7.5. One hundred microliter supernatant after centrifugation was used. Paraoxon dissolved in 10 µl 20% acetone was added to 100 µl supernatant for measurement of the rate of AChE inhibition. The mixture was incubated for 5 min at 25 °C in shaking water bath. After incubation the mixture was diluted by adding 375 µl buffer and 25 µl of 1:1 mixture of 20 mM ASCH in water and 10 mM DTNB in acetone, so that the final ASCH concentration was 10^{-4}***M***. This dilution and addition of substrate stopped further inhibition. The mixture was introduced into the spectrophotometer and equilibrated for 1–5 min after which activity of AChE was constant and measured for further 10-min period. Uninhibited activity was measured after the same incubation period with 10 µl 20% acetone. Blanks without mites or substrate were used to correct for nonenzymatic activity. Other experiment was carried out with a 1:1 mixture of supernatants of S and R strains to exclude other differences between the two strains such as indetoxication or binding, as the cause of a difference in inhibition of the AChE by paraoxon. The broken curve for S and R strains confirm that difference in the rate of inhibition of the two strains is completely due to a difference in their AChE's.

Results

In Table 1 the AChE activity (after 5 min incubation with 20% acetone at 25 °C) is shown for adult females of S strain, R strain and two field strains BR and AR. The activity of AChE in R strain and in the two field strains BR and AR are significantly lower than in the S strain, where the percents of activity were 60%, 70% and 81% for R, BR and AR strains, respectively. The results of the inhibition of AChE with paraoxon for S and R strains are shown in Fig. 1, where the rate of AChE inhibition of the R strain is 34-fold lower than that of S strain. The values of I_{50}, were 10^{-6}, 30×10^{-6}, 29×10^{-6} and 17×10^{-6} ***M*** paraoxon for S, R, BR and AR strains, respectively, as indicated in Table 2. The obtained results emphasized that the insensitive AChE is the most common cause of organophosphorus resistance in *T. urticae*. Also, the obtained broken curves as in Figs 1 and 2 confirm that the difference in the

Table 1

Acetylcholinesterase activity (nmol/mite/h) in adult females of four strains of spider mites *T. urticae*

Strains	AChE activity "mean ± SE"
S	0.67 ± 0.06
R	0.40 ± 0.03
BR	0.47 ± 0.05
AR	0.54 ± 0.04

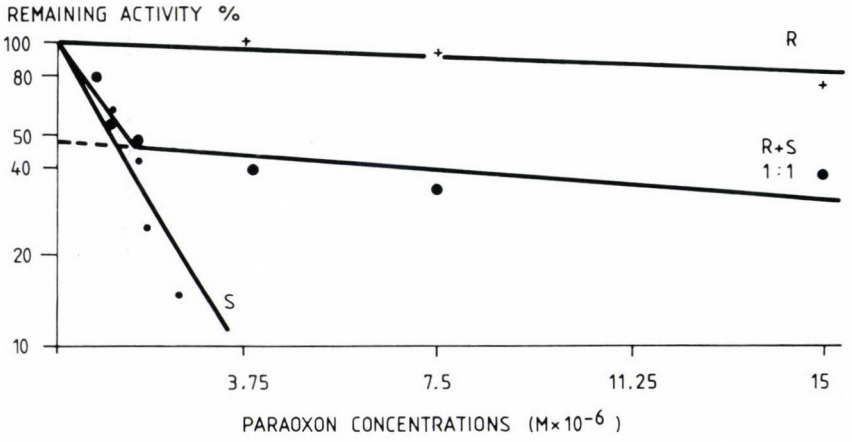

Fig. 1. Inhibition of AChE by paraoxon in supernatants of S and R strains of *T. urticae* and in mixture of their supernatants

Table 2

I_{50} values (M paraoxon) for adult females of four strains of spider mites *T. urticae*

Strains	I^{50} value "M paraoxon"
S	01.00×10^{-6}
R	29.75×10^{-6}
BR	29.00×10^{-6}
AR	17.00×10^{-6}

AChE sensitivity is the main cause of the difference of the rate of AChE inhibition of these strains. The broken curve in Fig. 2 indicated that the field strains BR and AR were not pure and showed heterogeneity, and at the same time provided us with data about the field resistant individuals of spider mites, where the percent of resistant individuals in the field strains was 92% and 55% for BR and AR strains, respectively. The Ki value of S enzyme for paraoxon was 13.1×10^4 M^{-1} min^{-1}, and that of R enzyme was 0.38×10^4 M^{-1} min^{-1}, this indicated previously that the R enzyme is 34-fold slower than that of the S enzyme. Beside this biochemical evaluation, the bioassay slide-dip method was used for susceptibility measurements. The susceptibility results for spider mite adult females revealed that LD_{50}, s were 5.8, 590, 342 and 62.8 ppm parathion for S, R, BR and AR strains, respectively, these findings showed that R, BR and AR strains were 102-fold, 59-fold an 11-fold more resistant to parathion than S strain.

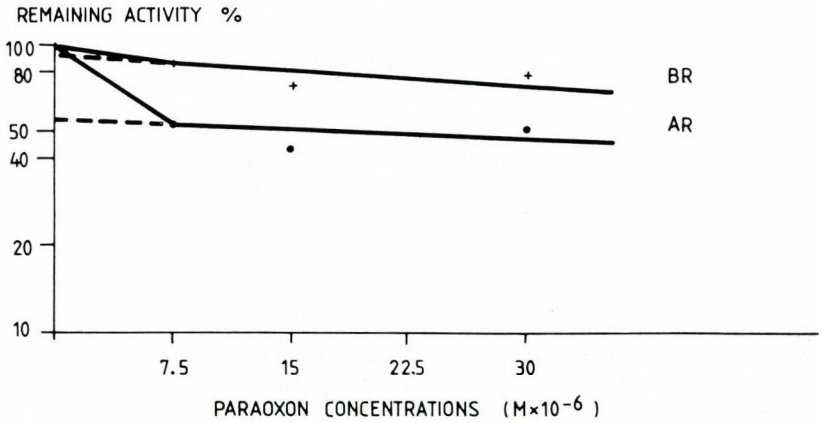

Fig. 2. Inhibition of AChE by paraoxon in supernatants of the BR and AR field strains of spider mites *T. urticae*

Discussion

The broken curves that resulted prove beyond doubt that the difference in the rates of AChE inhibition in susceptible and resistant strains, and in the two field strains is completely due to a difference in their AChE's. The relation between their insensitivity to AChE and the degree of resistance is known to be a complex relation, but the finding of 34-fold slower in rate of AChE inhibition by paraoxon in R strain of *T. urticae* in a monogenic parathion-resistant strain, and also, the reduction of AChE inhibition by 30-, 29- and 17-times in R, BR and AR strains than that of S strain leave no doubt that this is the main cause of spider mites resistance to organophosphorus compounds. These data are in complete agreement with many studies on the resistance to OP_s in many pest species based on reduced sensitivity of AChE by Ayad and Georghiou (1975), Devonshire (1975 and 1980), Hama (1977) and Voss (1980). Also, the susceptibility data for *T. urticae* confirm that the two field strains are more resistant to parathion with 59-times and 11-times than that of susceptible strain, and these findings are in harmony with those of Mansour (1988) and Grafton-Cardwell et al. (1989). According to all these studies and the present study, this technique applied is very simple, rapid, requires a simple apparatus and minimum materials, and can prove very useful in case of field strains to choose the effective acaricide treatment should quickly be made to avoid any economic losses caused by the resistant spider mite individuals in fruit orchards.

Acknowledgement

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Literature

Aldridge, W. N. (1950): Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl-nitrophenylthiophosphate and analogues. J. Biochem. 46, 451–460.

Ayad, H. and Georghiou, G. P. (1975): Resistance to organophosphates and carbamate in *Anopheles albimanus* based on reduced sensitivity of acetylcholinesterase. J. Econom. Entomol. 68, 295–297.

Busvine, J. R. (1980): Recommended methods measurement of pest resistance to pesticides. FAO Plant Prod. Prot. Pap. 21.

Croft, B. A. (1982): Arthropod resistance to insecticide. A key to pest control failures and successes in North American apple orchards. Entomol. Exp. Appl. 31, 88–110.

Devonshire, A. L. (1975): Studies of the acetylcholinesterase from housefly *Musca domestica* L. resistant and susceptible to organophosphorus insecticides. Biochem. J. 149, 463–469.

Devonshire, A. L. (1980): Insecticide resistance caused by decreased sensitivity of acetylcholinesterase to inhibition. Insect Neurobiology and pesticide action, "Neurotox. 70" Meeting York Proc. Soc. Chem. Ind. Symp., 473–480.

Ellman, G. L., Courtney, K. D., Anders, V. and Featherstone, R. M. (1961): A new rapid colorimeteric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Grafton-Cardwell, E. E., Granett, J., Leigh, F. T. and Normington, M. S. (1989): Development and evaluation of a rapid bioassay for monitoring propargite resistance in *Tetranychus species* "Acari: Tetranychidae" on cotton. J. Econom. Entomol. 82, no. 3, 706–715.

Hama, H. (1977): Cholinesterase activity and its sensitivity to inhibition in resistant and susceptible strains of green rice leafhoper, *Nephotettix cincticeps* Uhler. Botyu Kagaku. 42, 82–89.

Helle, D. H. (1984): Aspects of pesticide resistance in mites. In: Griffiths, D. A. and Brownman, C. E. (eds): Acarology VI. Vol. 1, 122–131, Ellis Horwood, Chichester.

Herne, D. H and Brown, W. A. (1969): Inheritance and biochemistry of OP-resistance in New York strain of the two-spotted spider mites. J. Econom. Entomol. 62, 205–209.

Mansour, F. (1988): Field and laboratory experiments on the response and development of resistance to pesticides and population density of *Tetranychus cinnabarinus* in Israel. Phytoparasitica 16 no. 3, 239–245.

Matsumura, F. and Voss, G. (1964): Mechanism of malathion and parathion resistance in the two-spotted spider mites., *T. urticae*. J. Econom. Entomol. 57, 911–916.

Rousch, R. T. and McKenzie, J. A. (1987): Ecological genetics of insecticide and acaricide resistance. Ann. Rev. Entomol. 32, 361–380.

Smissaert, H. R. (1964): Cholinesterase inhibition in spider mites susceptible and resistant to organophosphate. Science 143, 129–131.

Smissaert, H. R., Voerman, S., Oostenbruge, L. and Renooy, N. (1970): Acetylcholinesterase of Organophosphate-Susceptible and resistant spider mites. J. Agric. Food Chem. 18, 66–75.

Voss, G. (1980): Cholinesterase autoanalysis: A rapid method for biochemical studies on susceptible and resistant insects. J. Econom. Entomol. 73, 189–192.

Walker, W. F., Boswell, A. L. and Smith, F. F. (1973): Resistance of spider mites to acaricides. Comparison of slide-dip and lead-dip methods. J. Econom. Entomol. 66, 549–550.

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Monitoring the Currant Borer, *Synanthedon tipuliformis* Cl. (Lepidoptera: Sesiidae) by Pheromone Traps in Bulgaria

MITKO SUBCHEV[1], ELENA TZOLOVA[2], GÁBOR SZŐCS[3] and MIKLÓS TÓTH[3]

[1]Institute of Zoology, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria [2]Experimental Station of Small Fruits, 2230 Kostinbrod, Bulgaria [3]Plant Protection Institute, Hungarian Academy of Sciences, Budapest, P.O. Box 102, H-1525, Hungary

Pheromone traps baited with a 100:3 mixture of (*E,Z*)-2,13-octadecadien-1-ol acetate and (*E,Z*)-3,13-octadecadien-1-ol acetate were used for monitoring the flight of the currant borer, *Synanthedon tipuliformis* Cl. (Lepidoptera: Sesiidae) in two black currant fields in Bulgaria in 1989 and 1990. It was shown that these traps are appropriate for season-long monitoring of the pest. In the years investigated the flight of the currant borer started at the beginning of May and lasted 78–91 days.

In Bulgaria the currant borer, *Synanthedon tipuliformis* Cl. (Lepidoptera: Seseiidae) causes damages mainly on black currant (*Ribes nigrum* L.). In some years damage levels can be as high as 100%. Since the larval stage and pupae live inside shoots and cannot easily be detected, the adult stage is most suitable for monitoring the occurrence of the pest. As with many other moths, males of adult currant borer are attracted to specific sex pheromone emitted by females, and traps baited with a synthetic pheromone are ideally suited for monitoring.

(*E,Z*)-2,13-octadecadien-1-ol acetate (E2Z13-18Ac) has been described as an attractant for male currant borer (Voerman et al., 1984). This compound was also detected in the female emitted sex pheromone of the species, together with the monounsaturated analogue, (*Z*)-13-octadecen-1-ol acetate (Z13-18Ac) (Szőcs et al., 1985). The addition of a low amounts (3 to 10 %) of positional isomer of the pheromone's main component, (*E,Z*)-3,13-octadecadien-1-ol acetate (E3Z13-18Ac) resulted in significant increase of catches (Priesner et al.,1986, Szőcs et al., 1990a). This synergistic effect of E3Z13-18Ac has been verified in field tests in Europe, in Belgium, Bulgaria, Denmark, Germany, Hungary, Italy, Norway, Switzerland, Lithuania and Central Russia, and the United Kingdom (Szőcs et al., 1991).

In the present paper we describe results of season long monitoring of currant borer in Bulgaria with traps baited with the above synergistic mixture of E2Z13- and E3Z13-18Ac.

Akadémiai Kiadó, Budapest

Materials and Methods

Dispensers loaded with 30 μg of E2Z13-18Ac and 0.9 μg of E3Z13-18Ac were prepared at the Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary. Ten μl of hexane containing the above amounts of the compounds was applied to the surface of 1 × 1 cm pieces of rubber tubing (Taurus, Budapest, Hungary; No. MSZ 9691/6; rubber dispensers where extracted 3 times in methylene chloride overnight before use). Dispensers were individually wrapped in pieces of aluminium foil and sent air mail to Bulgaria. Dispensers were stored at –20 °C until use. Compounds were gifts from Meyer Schwarz (Insect Chemical Ecology Laboratory, USDA, Beltsville, U.S.A.).

Observations were conducted in two black currant fields: 1. Experimental Station of Small Fruits (ESSF), Kostinbrod, 3 ha; 2. Institute of Mountain Agriculture and Stock-breading (IMASB), Trojan, 3 ha. Three pheromone traps were placed at each site.

Traps were visited each day before the beginning of the flight, then, as first captures were recorded, 3 times weekly. When captures exceeded ten males in a trap, the sticky insert was replaced a new one. Old traps were discarded after 3 weeks, and new ones were set up. All checks and other manipulation with the traps were made in the morning, since daily flight activity of males towards pheromone source takes place in the afternoon (Szőcs et al., 1990a).

In analysing trap captures, mean catch per day per trap was calculated from individual capture records.

Meteorological data were received from the nearest meteorological stations to the experimental plots.

Results and Discussion

In 1989 the beginning date of male flight, May 4, was recorded only at site 1, since at site 2 currant borers were already flying by the time the first traps were set up (Fig. 1). In 1990, the flight started one week later, than in the previous year at site 1, and on May 4 at site 2 (Fig. 2). Last catches were observed in 1989 on August 2, and July 21, and in 1990 on August 8, and July 20, at sites 1 and 2, respectively. Consequently the flight period of currant borer in the two years and at these sites lasted 78 to 91 days, which is shomewhat longer than the 70 days described earlier from Bulgaria (Dirimanov and Sengalevich, 1975). In the eastern part of Germany, the flight recorded by

pheromone traps was reported to last from June to September in 1987 (Szőcs et al., 1991). In Hungary, the flight lasted from May 10 to July 23, in 1989, as determinated by pheromone traps (Szőcs et al., 1990b). Apart from *S. tipuliformis*, traps baited with synthetic sex attractants were shown to be a suitable tool for monitoring the seasonal flight of a number of sesiids (Snow et al., 1989; Meyer et al., 1988). In a comparison of the feasibility of trapping with synthetic sex pheromone and counting of pupal skins found on peach trees for monitoring the seasonal distribution of lesser peachtree borer, *Synanthedon pictipes* Grote & Robinson, both methods were found to be suitable only at high population density (Yonce et al., 1977).

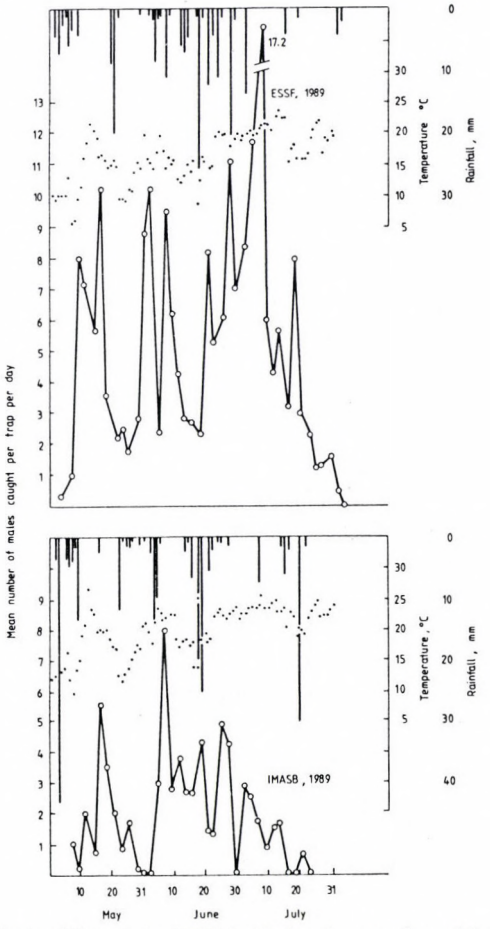

Fig. 1. Flight of *S. tipuliformis* in 1989 in Bulgaria, monitored by pheromone traps. ESSF – Experimental Station of Small Fruits, Kostinbrod; IMASB – Institute of Mountain Agriculture and Stock-breading, Trojan. Dots signify temperature, bars show rainfall

Our results show that the beginning of the flight of *S. tipuliformis* in the two years correlated with the temperature conditions. At site 1 the mean temperatures for March and April (when the caterpillars more probably began to develop after winter diapause) were higher in 1989 than in 1990: 7.9 vs. 13.1 °C, and 8.2 vs. 9.8 °C respectively, and the first catch was registrated earlier in 1989 as compared with 1990. On the other hand the same temperatures in 1990 were higher in site 2 as compared with site 1: 8.7 and 10.7 °C for March, and 8.2 and 9.8 °C for April, respectively, and the flight in the former locality began earlier than in the latter one. Our results clearly support those of Scott and Harrison (1979), who stated that the flight of *S. tipuliformis* started earlier in years when the mean temperatures were higher during two

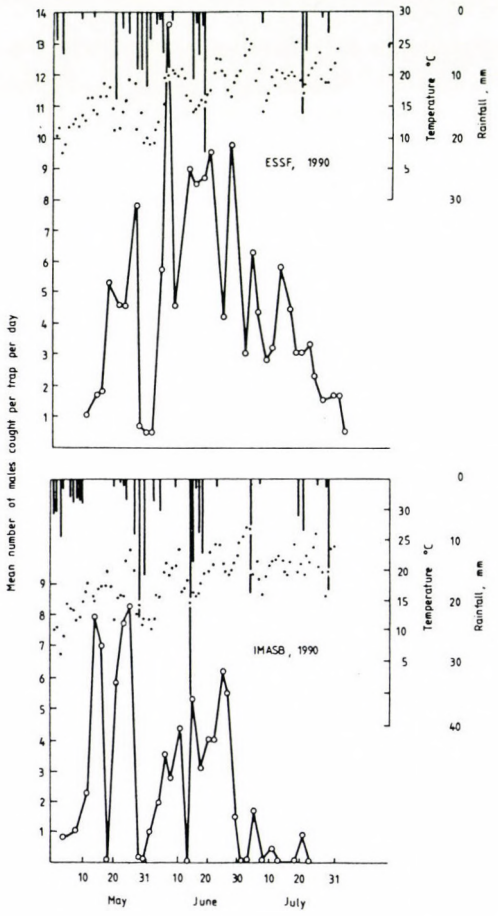

Fig. 2. Flight of *S. tipuliformis* in 1990 in Bulgaria, monitored by pheromone traps. For further explanation: see Fig. 1

months before the emergence of adults. Higher mean monthly temperature at a southern location as compared to a northern location in Colorado (U.S.A.) during the month preceding the emergence of adults of another sesiid, *Podosesia syringae* Harr., was found to correlate with two week's earlier record in captures of pheromone traps (Meyer et al., 1988). However, no such correlation was found in the same experiment for further sesiid, *Synanthedon exitiosa* Say.

In our experiments, the fluctuation in trap captures of *S. tipuliformis* in successive days correlated with the change of meteorological parameters. As compared to preceding or subsequent days, low captures were recorded on those days when the temperature dropped. Similarly, low captures were recorded on days when heavy rainfall and/or strong wind occurred. Although these tendencies could be observed in most of the cases, the correlations were not always clearcut. More data would be needed to analyse in details the combined effect of these meteorological parameters on trap captures.

Conclusion

1. Pheromone traps baited with a 100 : 3 mixture of E2Z13-18Ac and E3Z13-18Ac are appropriate for season-long monitoring of the flight of the currant borer.

2. In the fields observed in Bulgaria in 1989 and 1990 the flight of the currant borer started at the beginning of May and lasted for 78 –91 days.

Literature

Dirimanov, M. and Sengalevich, G. (1975): Dangerous enemies on *Ribes niger* and their control. Plant Protection (Bulgaria), 1, 34–37 (in Bulgarian).

Meyer, W. L., Cranshaw, W. S. and Eichlin, T.D. (1988): Flight patterns of clearwing borers in Colorado based on pheromone trap captures. Southw. Entomol., 13, 39–46.

Priesner, E., Dobler, G. and Voerman, S. (1986): Synergism of positional isomers in sex attractant systems of clearwing moths (Sesiidae). Entomol. Exp. Appl., 41, 311–313.

Scott, R. and Harrison, R. (1979): The biology and life history of currant clearwing, *Synanthedon tipuliformis*, (Lepidoptera: Sesiidae), in Canterbury. N. Z. J. Zool., 6, 145–163.

Snow, J. W., Schwarz, M. and Eichlin, T. D. (1989): Captures of clearwing moths (Lepidoptera: Sesiidae) with various octadecadienyl acetates and alcohols in Central Georgia during 1983–1985. Environ. Entomol., 18, 216–222.

Szőcs, G., Schwarz, M., Sziráki, Gy., Tóth, M., Klun, J. A. and Leonhardt, B. A. (1985): Sex pheromone of the female currant borer, *Synanthedon tipuliformis*: identification and field evaluation. Ent. Exp. Appl., 39, 131–133.

Szőcs, G., Miller, L. A., Thomas, W., Vickers, R. A., Rothschild, G. H. L., Schwarz, M. and Tóth, M. (1990a): Compounds modifying male responsiveness to main female sex pheromone component of the currant borer, *Synanthedon tipuliformis* Clerk (Lepidoptera: Sesiidae) under field conditions. J. Chem. Ecol., 16, 1289–1305.

Szőcs, G., Tóth, M. and Szarukán, I. (1990b): Monitoring the currant borer by pheromone traps. Tessedik S. Mezőgazd. Tud. Napok, Debrecen, pp. 255–256 (in Hungarian).

Szőcs, G., Buda, V., Charmillot, P., Esbjerg, P., Freier, B., Gottwald, R., Kovalev, B., Maini, S., Solomon, D. G., Sorum, O., Subchev, M., Tóth, M. and Van de Veire, M. (1991): Field tests of E,Z-3,13-octadecadien-1-ol acetate: a sex attractant synergist for male currant borer, *Synanthedon tipuliformis*. Ent. Exp. Appl., 60, 283–288.

Voerman, S., Persoons, C. and Piesner, E. (1984): Sex attractant of currant clearwing moth, *Synanthedon tipuliformis* (Lepidoptera: Sesiidae). J. Chem. Ecol., 10, 1371–1376.

Yonce, C. E., Gently, C. R., Tumlinson, H. J., Doolittle, R. E., Mitchell, E. R. and McLaughlin, J. R. (1977): Seasonal distribution of the lesser peachtree borer in Central Georgia as monitored by pupal skin counts and pheromone trapping techniques. Environ. Entomol. 6, 203–206.

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Study of Migratory *Lepidoptera* on the Northern Slopes of the Caucasian Mountains*

Z. MÉSZÁROS[1], B. HERCZIG[2], K. SZEŐKE[3], T. S. KOROLJ[4], D. I. USAKOV[4]

[1]Department of Entomology of the University of Horticulture and Food Industry, Budapest, [2]Plant Health and Soil Conservation Station of County Komárom–Esztergom, Tata, [3]Plant Health and Soil Conservation Station of County Fejér, Velence, Hungary
[4]Checheno-Ingush State University, Groznij, Russia

The authors studied the routes of migration of migratory Lepidoptera under different weather conditions in the territory north of the Caucasus Mountains. The work of the expedition represented actually a continuation of the surveys commenced earlier in Hungary and on the Balcan Peninsula.

In the lepidopteran fauna of Middle and North Europe numerous species are known that do not overwinter in these geographical regions. These migratory species that arrive mostly in the first part of the year from South Europe or from the direction of Asia Minor. During the vegetation period they produce one or two generations and the adults of the last generation leave again towards the souths. Under European climatic conditions these species are unable to overwinter under natural conditions only under special ones like e.g. in glasshouses. The regular annual changes in the ecological conditions (e.g. in daylength) do not trigger off a diapause in these species as shown by the uninterrupted development of their progeny that had managed to find its way into glasshouses. Other species migrate only within their own area and their "unexpected" damage caused by the larvae is easily explained by the migratory habit of their parents.

The result of present survey have already been published in an abbreviated form in Hungarian (Herczig et al., 1989).

Literature Review

In an earlier paper (Mészáros, 1986) the scope of the subject studied has already been treated, so in present paper only some relevant definitions will be discussed.

*Results of the Checheno-Ingush and Hungarian zoological expeditions, No. 2. The studies reported in present paper were supported by the OTKA research foundation (OTKA I/3, 1749)

In the matter of lepidopteran migration the autors generally accept the classification of Vojnits (1966) and Gyulai (1978).

The categories of Vojnits (1966) are the following:

1. True migrants that are not, or only under exceptional condition able to overwinter in any developmental instar in the northern regions of Middle Europe, they are, nevertheless, regularly present there during the vegetation period.

2. Guests. These are occasional immigrants that – by taking advantage of the favourable weather conditions – stride over the limits of their habitual areal and appear thus from time to time also in Middle Europe.

3. Internal migrants that displace themselves within the limits of their area, but their population may be occasionally supplemented by numerous individuals that had immigrated from the south, southeast and east.

The classification of Gyulai (1978) is more detailed. The author (who works in North Hungary) grouped the migratory lepidoptera into following types:

A. Mediterranean immigrants, the individuals of which leave their areal in some years and manage to reach longer distances, but are unable establish there continous populations because their overwintering fails in any developmental instar (*Peridroma saucia* Hbn., *Heliothis armigera* Hbn.) This group is practically identical with the "guests" of Vojnits (1966).

B. Species with fluctuating areals. One part of the species belonging here (B_1) arrive regularly each year in a given territory and their progenies establish populations the individuals of which either perish in the autumn or leave (*Agrotis ipsilon* Hufn., *Autographa gamma* L.). This group corresponds the most to the "true migrants" of Vojnits (1966).

The other part of these species (B_2) constitute very restricted, local populations in their "temporary areal" but then disappear from the given region, mostly resulting from a climatic factor (e.g. unusually severe winter). The species is then absent for longer period from the given area (*Polia cappa* Hbn., *Epilecta linogrisea* Den. et Schiff.).

C. Expensive species. These species appear and settle down every year in further new areas and their progenies constitute new points of departure for subsequent migrations (*Cucullia fraudatrix* Ev., *Hyssia cavernosa* Ev.).

D. Species migrating inside their areal. Their migrations take place within the limits of their habitual area and result in an interbreeding between their populations (*Agrotis segetum* Den. et Schiff., *Xestia c-nigrum* L.). This group corresponds to the "internal migrants" of Vojnits (1966).

E. Mountain-valley migrants. Into this group univoltine alpine or mountain species belong that migrate twice a year in opposing vertical

directions. The first migration takes place (in North Hungary) by the end of June–July. The adults fly to higher elevations, some of the species to the high mountains (*Rhyacia lucipeta* Den. et Schiff., *Rhyacia simulans* Hufn.).

The adults then fall – mostly in groups – into a resting stage in sheltered places. According to Gyulai (1978) this is an imaginal reproductional diapause (aestivatio). The second vertical, downward migration takes place in North Hungary from the end of August until September–October. All adults participating in the latter migration are females. The repeated two migrations are regular phenomena in these species and occur also under adverse weather conditions. The species of this type belong to systematically quite narrow group of noctuid moths: *Noctuinae* and *Amphypyrinae* subfamilies.

More recently Gatter (1981) brought into correlation the different migration types with the diapause conditions of the migrating species. It was a merit of Gatter that he extended his studies beyond *Lepidoptera* also to other migrating insect groups. His migrational grouping is the following:

1. Anemomigration – drifting. The flying insects are swept away by stronger winds, without conscious orientation.

2. Dismigration – spreading. It cannot be regarded as an active migration but more as a passive dispersion. Two types where distinguished: expansive and seasonal dismigration.

3. Migration, i.e. direction-oriented displacement. This type of migratory urge is endogenously programmed into the individuals with a definite direction and sense of orientation towards it. Here also two types were distinguished: expansive and seasonal directional migration.

Aims of present studies

In course of our studies we tried to find answers to the following questions:

– will it be possible to observe migrating populations (individuals) in the passes or elevations of the Caucasian Mountains?

– will it be possible to differentiate the populations of migrating populations from the non-migrating species by nightly collections?

– can connections be found between the weather situations and the migration of lepidoptera?

– what are the similarities (or differences) between the migrating populations of South Europe and the Caucasus?

Areas studied, survey methods

Similar studies were already made in the Balcan Mountains in Bulgaria (Mészáros, 1986) but it was our first possibility to carry out faunistical and ecological surveys in the Caucasus on the territory of the Chechen-Ingush Republic. It represented at the same time the first step in the realization of a co-ordinated research agreement between the Tolstoy State University of the Chechen-Ingush Republic and the Station of Plant Hygiene and Soil Protection, Tata.

The areas surveyed lie in three groups, all of them north of the main mountain range of the Caucasus Mountains.

I. Territory

Southwestern border of the Chechen-Ingush Republic (further Ch.-I.R.), near to the borders of the Grusian Republic and Northern Osset Republic. In this area the following sites were designed for collection:

2. Furtoug (1100 m). Open valley near to the Terek river, rocky terrain (3.8.1988).

3. Torgim (1180 m). In the valley of the Terek river that flows to north, in a region used once for agriculture, barren rocky terrain with occasional small stands of pine *(Pinus sosnovstii)* (4–7.8.1988).

4. Skolnij pereval (Skolnij Pass) (2100 m). On the divide of Terek and Assa rivers, open terrain with occasional patches of *Rododendron flavum* (5.8.1988).

6. Meeting point of Assa and Nelh rivers (1300 m). Closed mixed forest of deciduous and needle species (*Pinus sosnovstii, Tilia cordata, Alnus glutinosa, Ulmus montana, Fraxinus excelsior, Acer platanoides* etc.) (7.8.1988).

II. Territory

Valley of the Argun river in the middle of Ch.-I.R.

7. Kerigo (1000 m). Open valley of Argun river, zone of deciduous forests (9–10.8.1988).

8. Itum-Kale (850 m). Open valley of Argun river, northern slope covered with deciduous forest (11.8.1988).

III. Territory

Southeastern border of Ch-I.R., near to the frontier of Dagestan Republic.

9. Ozero (Lake) Kezeloj-am (1800 m). Some hundred meters from the lake, open rocky terrain (13.8.1988).

10. Harachoj (1100 m). Above the village in the valley of the stream Hulhulau, deciduous forest zone (14.8.1988).

(The sites designed by Nos 1, 5 and 11 were outside the Caucasian region, their data are planned to be published in a separate paper.)

In all these collecting sites the collections were made by daylight and in the night. In the nightly collections Tungsram mercury vapour lamp (250 W) was used, fed by Honda generator. During collections we tried to observe each individual, to determine exemplars of common species on the spot, to collect and store all other lepidoptera. We tried to collect also other insects attracted by the light, especially those belonging to Trichoptera and Neuroptera. From among the climatic factors we measured temperature and wind velocity, other factors were esteemed; also the weather front situations were registered.

In the nightly collections 4–5 persons were continuously engaged; in spite of that it was sometimes nearly impossible to perform all the planned objectives due to the enormous quantities of insects attracted onto the collecting sheet.

Results

We could observe migratory lepidoptera in course of all 14 days and 11 nights spent in the field surveys. On one occasion (the night of 5 August, Skolnij Pass) a mass-migration of lepidoptera was observed, the details of which will be separately discussed at the end of this paper.

First the enumeration of migratory species will be given in the order of collecting sites.

2. Furtoug (1100 m). In the afternoon of 3rd August a warm weather front arrived with clouds and rain. On the hillside exemplars of *Vanessa cardui* L., *Autographa gamma* L. and *Loxostege sticticalis* L. were active mostly near to the soil level, without noticeable directional pattern.

The nightly collection had begun at 8.30 p.m. in a drizzling rain (15°C). The rainfall continued throughout the night with changing intensity, accompanied by occasional gusts of wind (2–4 m/sec). The collection was finished at 2.30 a.m. in a pouring rain (12 °C). The flight intensity of moths

– although diminished – did not stop and the light attracted many lepidoptera. Individuals of the following migratory species were collected: *Celerio lineata livornica* Hbn. (many), *Herse convolvuli* L. (1 specimen), *Agrotis ipsilon* Hufn. (some), *Noctua pronuba* (some), *Parexarnis sollers* Chr, *Xestia c-nigrum* L. (many), *Mythimna vitellina* Hbn., *Heliothis peltigera* Den. et Schiff. (1–1 specimen), *Autographa gamma* (some), *Trichoplusia ni* L. (some), *Hypena proboscidalis* L. (1 specimen).

In the forenoon of 4th August the flight of insects was sporadic on the hillside (cloudy weather, drizzling rain). From among the migratory species some *Vanessa cardui* L., *Aglais urticae* L. and *Autographa gamma* L. were flying.

3. Torgim (1180 m). During the day on 4th August the weather corresponded to meteorological situation characteristic behind a cold front, with pelting showers from labile clouds.

The nightly collection was started at 8.45 p.m.(13 °C) in a calm weather and finished after 11 p.m. (9 °C). The light attraction of moths was quite strong, especially in the first one and a half hours. The following migratory species were collected: *Agrotis ipsilon* Hufn. (some), *Ochropleura flammatra* Den. et Schiff (1 specimen), *Noctua pronuba* L. (some), *Xestia c-nigrum* L. (many), *Parexarnis sollers* Chr., *Autographa gamma* L. (1 specimen), *Hypaena proboscidalis* L. (1 specimen), *Nomophila noctuella* (some).

On the 6th August a typical anticyclonal weather situation prevailed during the day. The nightly collection was started at 8.45 p.m. and finished after 1 a.m with gentle wind without constant direction (12 °C). The swarming was quite strong, especially in the first hours. Comparatively few migratory species were observed and collected: *Celerio lineata livornica* Hbn. (some), *Herse convolvuli* L. (1specimen), *Agrotis ipsilon* Hufn. (some), *Noctua pronuba* L. (some), *Xestia c-nigrum* L. (some), *Autographa gamma* L. (some), *Trichoplusiani* L. (1specimen).

On 4th, 5th and 6th August in the surrounding area active exemplars of the following migratory species were observed and collected during the daylight hours: *Colias croceus* Fourcr. (many), *Vanessa cardui* L. (sporadic), *Celerio alecto* (1specimen), *Macroglossum stellatarum* L. (some), *Heliothis peltigera* Den.et Schiff. (1specimen).

4. Skolnij pereval (Skolnij Pass) (2100 m). In the night of 5th to 6th August the flight of the moths towards the light source was extremely strong. Migratory species appeared also in high numbers on the collecting sheet and their behaviour differed markedly from that of the non-migratory ones (the differences will be mentioned again at the end of the paper).

5. Meeting point of Assa and Nelh rivers (1300 m). On 7th August strong northern wind prevailed during the day, that ceased in the closed valley by the evening. The nightly collection was started at 9 p.m. (17 °C) and finished at 1 a.m. (12 °C). The flight of moths was quite strong, although migratory species were nearly absent. The following migratory species were collected: *Celerio lineata livornica* Hbn. (some), *Agrotis ipsilon* Hufn. (1 specimen), *Xestia c-nigrum* L. (1 specimen).

6. Kerigo (1000 m). On 9th August a warm front arrived with temporary rains that stopped by the evening. The evening collection was started at 8 p.m. (17 °C) and finished at 1 a.m. (16 °C). During the night a continuous, gentle western wind prevailed (from the mountainside) with occasional gusts (3–5 m/sec). The flight was quite strong, although with only a few migratory species. The following were collected: *Celerio lineata livornica* Hbn. (some), *Noctua pronuba* L. (some), *Protoschinia scutosa* Den et Schiff. (some), *Celerio vespertilio* (1 specimen), *Agrotis ipsilon* Hufn. (some), *Macdounnoughia confusa* Steph. (some), *Trichoplusia ni* Hbn (some), *Prodotis stolida* F. (some), *Loxostege sticticalis* L.(some), *Nomophila noctuella* (some).

On 10th August a strong wind prevailed (arrival of a cold front?), by the evening the wind stopped. The nightly collection was started at 9 p.m. (20 °C) and finished (due to mechanical failure) at 11.30 p.m. (16 °C). During the evening a gentle breeze blew without constant direction. The attraction to light was again strong, without many migrating species. The following were collected: *Agrotis ipsilon* Hufn (some), *Noctua pronuba* L. (some), *Xestia c-nigrum* L. (some), *Macdounnoughia confusa* Steph. (some), *Trichoplusia ni* Hbn. (some), *Prodotis stolida* F. (many).

On 9th and 10th during the day in the region the following migratory butterflies were observed: *Colias croceus* Fourcr. (many), *Vanessa cardui* L. (some) *Macroglossum stellatarum* L. (many).

7. Itum-Kale (850 m). On 11th August strong wind blew and in the afternoon a strong thunderstorm activity appeared; this kept until the night but on the collecting site only a very sporadic rain was noticed. The nightly collection was started at 9 p.m. in gentle wind (23 °C) and finished at 3 a.m. (17 °C). During the night a calm prevailed but the tendency to thunderstorms showed itself in strong gusts of wind. The flight to the light was strong and steady, with many migratory species appearing. The following species were collected: *Celerio lineata livornica* Hbn. (some), *Agrotis ipsilon* Hufn. (some), *Noctua pronuba* L. (some), *Xestia c-nigrum* L. (some, after 11 p.m. many), *Helicoverpa armigera* Hbn. (some), *Macdounnoughia confusa* Steph. (some), *Autographa gamma* L. (some) *Trichoplusiani* Hbn. (many), *Loxostege sticticalis* L. (many) *Nomophila noctuella* (some).

On 11th August in the afternoon between Karigo and Itum-kale in Argun valley high numbers of *Colias croceus* Fourcr., *Macroglossum stellatarum* L. and sporadically, *Vanessa cardui* L. individuals were observed.

8. Ozero Kezenoj-am (1800 m). On 13th August during the day cyclonal weather prevailed with changing, mostly dense clouds. The nightly collection was started at 9 p.m. calm weather (13 °C). During the night a strong dew formation was noticed. The collection was finished at 3.30 a.m. (4 °C). The flight of moths to the collecting sheet was comparatively low but many migratory species appeared. The following were collected: *Celerio lineata livornica* Hbn. (many), *Herse convolvuli* L. (many, about 20 specimens), *Agrotis ipsilon* Hufn. (some) *Noctua pronuba* L. (some), *Mythimna vitellina* Hbn. (some), *Helicoverpa armigera* Hbn. (many, dominant from among the migratory species), *Heliothis peltigera* Den. et Schiff. (some), *Protoschinia scutosa* Den. et Schiff. (many), *Autographa gamma* L. (some), *Trichoplusiani* Hbn. (many), *Loxostege sticticalis* L. (many), *Nomophila noctuella* (many).

9. Harachoj (1100 m). On 14th August in the afternoon sunny weather prevailed with gradually increasing cloud cover in the afternoon and completely overcast sky in the evening. The night collection was started at 8.30 p.m. in calm weather (18°C) and finished at 3 a.m. (16 °C). During the night gentle wind blew from the mountainside. The flight was quite strong with many migratory species, the following were collected: *Celerio lineata livornica* Hbn. (some), *Herse convolvuli* L. (1 specimen), *Noctua pronuba* L. (some), *Xestia c-nigrum* L. (many), *Mythimna conigera* Den. et Schiff. (some), *Phlogophora meticulosa* L. (some), *Helicoverpa armigera* Hbn. (many), *Protoschinia scutosa* Den. et Schiff. (many), *Prodotis stolida* F. (some), *Loxostege sticticalis* L. (many), *Nomophila noctuella* (many).

On 14th August during the day many exemplars of the following migratory species were observed in active flight: *Colias croceus* Fourcr. (some), *Vanessa cardui* L. (some), *Vanessa atalanta* L. (1 specimen), *Macroglossum stellatarum* L.(some), *Autographa gamma* L. (some).

Observations on migratory lepidoptera on the Skolnij Pass

On the night of 5th to 6th August the collection was made on the Skolnij Pass (2100 m) under the Cejlam Peak. The nightly collection started at 8.45 p.m. (12 °C) in a 2–4 m/sec wind that blew from the hillside (west). The swarming started around 9 p.m. from the lower altitudes (valley) and the individual number of months was enormous. On the collecting sheet thousands of moths were moving or sitting. The observation, counting and partial collection of the individuals, measuring of temperature, wind velocity besides

writing of notes gave enough continuous work to four persons. The very strong flight decreased somewhat between 1 and 2 a.m. at 5–7 °C. The observations were finished at 3.15 a.m., when the activity of lepidoptera abatted.

A considerable fraction of the many thousand moths swarming on the collecting sheet represented individuals of the local fauna (dominant was *Lithosia quadra* L. that appeared always in thousands). The migratory species constituted the other part of the mass; hundreds appeared at the same time on the illuminated sheet. Their movement was directed well noticeably from the valley upward, although the wind direction and velocity had changed many times during the night.

Members of the local fauna and the migrants, respectively, were well discernible by their behaviour. The local one subsided within few minutes after their arrival and rested on the sheet or on the soil surface in surrounding light circle.

Even the migratory ones could be ranged into two groups after their behaviour. The individuals of one group flew in high numbers onto the sheet, then after a while (15 minutes to 1–2 hours) their majority left individually, barely noticeably and resumed the flight. This behavioural pattern was shown by the dominant *Noctua pronuba* L. and the nearly identically numerous *Trichoplusiani* Hbn. The exemplars of the latter group arrived also in large numbers from the direction of the valley, then after some hours hid themselves (also in masses) in the shadowed places around the light source (grass, collecting gear, bags). This latter behaviour was typical to mountain-valley migrants like *Agrotis clavis* Hufn. or species of the genera *Euxoa*, *Parexarnis*.

Discussion

1. The movement of the mass of lepidoptera showed a direction at the time of our survey from the inner (higher) regions of the mountain towards the lower territories. Although the "stream" of moths arrived from the direction of the valley (from southeast) seemingly in an upward flight, if however, the whole territory is considered, these animals arrived from the direction of the mountain crest and their route was directed towards the passes and lower regions. This was in accordance with the earlier data according to which the migration is directed in the second half of the summer from higher altitudes towards the lower regions.

2. In the enormous multitude of lepidoptera the forms belonging to different behavioural types could be well distinguished, even within migratory ones. Under the influence of labile weather situations (on previous day a cold front, on the next day many other fronts passed over the region) the behaviour of the "true migrants" (Vojnits, 1966), "species with fluctuating areals" (Gyulai, 1978) or the "direction oriented migrants" (Gatter, 1981) was the most conspicuous; although they alighted on the illuminated surface, they were very restless, vibrated their wings and practically all of them left before midnight to continue their flight. This arrival and leaving took place continually. The disengagement of the light and recommencement of the migration was especially conspicuous in case of the large *Hyles (Celerio) lineata livornica* Hbn. specimens.

3. The "mountain to valley migrants" hid themselves near to the light source in shadowed places and showed noticeably no intention to resume their flight.

4. Recording the conditions of the migration the experiences gained in the Caucasus were in accordance with the Bulgarian observations (Mészáros, 1986).

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Literature

Gatter, W. (1981): Insektenwanderungen. Kilda Verlag, Greven, pp.94.

Gyulai, P. (1978): The migration of Noctuids in North Hungary (in Hungarian). Növényvédelem 14 (8), 337–341.

Herczig, B., Mészáros, Z., Szeőke, K., Korolj, T. S., Usakov, D. I. (1989): Study of migrating Lepidoptera in northern Caucasus (in Hungarian). Növényvédelem 29 (6), 246–250.

Mészáros, Z. (1986): Study of migratory Lepidoptera on the Balcan-Peninsula. Acta Phytopath. Acad. Sci. Hung. 21 (3–4), 347–357.

Szeőke, K., Dulinafka, Gy. (1987): Occurrence of *Helicoverpa armigera* Hübner 1808 in Hungary and its damage in sweet corn (in Hungarian). Növényvédelem 23 (10), 433–438.

Vojnits, A. (1966): The "true" migrant butterflies. Fol. Ent. Hung., 19 (8), 167–175.

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Insecticidal and Haemolytic Characterization of the Fractions of *Bacillus thuringiensis* subsp. *israelensis* Toxin

A. Bozsik[1,4], P. Kiss[2], F. Fábián[3], L. Szalay-Marzsó[4] and M. Sajgó[3]

[1]Institute for Plant Sciences Agricultural Biotechnology Center, Gödöllő [2]Institute for Biochemistry and Protein Research Agricultural Biotechnology Center, Gödöllő [3]Department of Biochemistry University of Agricultural Sciences, Gödöllő [4]Department of Plant Protection University of Agricultural Sciences, Gödöllő, Hungary

Solubilized crystal protein of *Bacillus thuringiensis* subsp. *israelensis* was fractionated by Sephadex G-200 column chromatography and assayed against mosquito larvae and human erythrocytes. The fraction containing the major polypeptides 130 kDa, 68 kDa and the polypeptide 35 kDa was found to be nonhaemolytic but to retain nearly full toxicity. The fractions that contained also the 27 kDa protein showed haemolytic activity and their mosquitocidal toxicity was approximately in proportion to the quantity of the 68 kDa protein. The results suggested that the polypeptides 130 kDa and 68 kDa were responsible for the dipteran toxicity and the 27 kDa polypeptide had the haemolytic activity. However, it is possible that the 27 kDa protein may contribute, as a synergistic factor to the larvicidal activity.

A Gram-positive, sporulating bacterium strain (ONR-60A), which was specifically toxic to mosquito larvae, was isolated by Goldberg and Margalit (1977) from samples at a mosquito breeding site in the Negev desert and subsequently described as *Bacillus thuringiensis* subsp. *israelensis* (Bti), serotype H-14 (de Barjac, 1978). During sporulation the bacterium forms proteinaceous parasporal body similarly to the other *Bacillus thuringiensis* strains (Tyrell et al., 1979; Luthy, 1980; Charles and de Barjac, 1981; Thomas and Ellar, 1983). The crystalline inclusion is active against the larvae of Diptera especially Nematocera but not to lepidopteran larvae (Goldberg and Margalit, 1977; de Barjac, 1978). Bti is of considerable importance mainly in the tropics because of its potential as biological insecticide against *Anopheles* spp., *Aedes* spp., *Culex* spp. and *Simulium damnosum* which are the vectors of such devastating diseases as malaria, yellow fever, encephalitis, filariasis and river blindness (Davis and Shannon, 1930; LaMotte, 1960; Grassi et al., 1898 in Mihályi and Gulyás, 1963; Undeen and Nagel, 1978). In our country it can be regarded as natural alternative of the mosquito control because it is harmless for the non-target organisms of the water fauna (Szalay-Marzsó and Gharib, 1983).

A prerequisite of insecticidal effect of Bt isolates is the ingestion of parasporal body by the target insects. After ingestion the alkaline gut juice

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and the gut proteinases dissolve and activate the protein crystal (Charles and de Barjac, 1983). In the nematocerous larvae the midgut and caeca epithelium is the primary site of action for the toxic polypeptides. Pathological changes start with cell hypertrophy and the damage of microvilli in the caeca and in all regions of the midgut and continue with cell lysis and complete desintegration of the epithelial monolayer leading to ion imbalance, paralysis and decay (de Barjac, 1978; Charles and de Barjac, 1981, 1983; Lahkim-Tsror et al., 1982). As the mechanism of action of Bti the hypothesis of Knowles and Ellar (1987) may be accepted in which, after binding a specific plasma membrane receptor, the "attack" of toxic proteins may generate small pores in the plasma membrane which will lead to the mentioned colloidosmotic lysis.

The parasporal body is globular in shape, consist of different inlcusions and contains several polypeptides. The most importants of those are the polypeptides of 27 kDa, 68 kDa, 130 kDa and 135 kDa (Tyrell et al., 1981; Thomas and Ellar, 1983). The protein crystal possesses not only entomocidal activity but also haemolytic and cytolytic activities. Several reports have suggested that the 27 kDa protein is responsible for the general cytolytic activity, as well as for the mosquitocidal toxicity. However, more recent studies found evidences that the greather-molecular-mass proteins (68 kDa, 130 kDa) are associated with the nematocerous toxicity (Table 1).

In this paper we examine the toxicity and the haemolytic ability of different fractions of Bti crystal protein in order to identify which subunits are responsible for its entomocidal and haemolytic activity.

Materials and Methods

Microorganism

Biomass of Bti was obtained from the Microbiological Laboratory of the Chemical Works of Gedeon Richter LTD. Budapest.

Dissolution of the toxin protein

Before dissolution cell debris and putative surface-bound protease were removed by washing with 1 M NaCl (4 ×) and distilled water (4 ×) at 0 °C. The resultant precipitate was separated by centrifugation in a Janetzky

type centrifuge with 5000 g. Toxin proteins were dissolved at 1/50 (w/v) ratio with Tris buffer, pH 12.0, for 5 h, at 0 °C. Insoluble material was removed by centrifugation at 4000 g for 20 min at 4 °C and the supernatant containing soluble parasporal inclusions was removed for subsequent examinations.

Table 1

Effect of toxic subunits of *B. thuringiensis* subsp. *israelensis*

Polypeptides (kDa)					References
27	68	130	27+68	27+130	
M					Thomas and Ellar, 1983
MHC					Yamamoto et al., 1983
MHC					Davidson and Yamamoto, 1984
	M				Kim et al., 1984
		nM			Schnell et al., 1984
M C					Ward et al., 1984
MHC					Amstrong et al., 1985
MHC					Ellar et al., 1985
H	M				Hurley et al., 1985
M					Insell and Fitz-James, 1985
	M				Lee et al., 1985
H		M			Visser et al., 1985 in Waalwijk et al., 1985
nM	M?	M?	M	M	Wu and Chang, 1985
nMH		M			Bourgouin et al., 1986
MHC	M				Chilcott and Ellar, 1986
MHC					Ellar et al., 1986
nMH					Held et al., 1986
MH		M?			Ibarra and Federici, 1986
H	M?	M?			Pfannenstiel et al., 1986
H					Walther et al., 1986
H		M			Visser et al., 1986
H	M				Hurley et al., 1987
HC	M	M			Lereclus et al., 1989

M = mosquitocidal; H = haemolytic activity; C = cytolytic; nM = no mosquitocidal activity; M? = possible mosquitocidal activity.

Column chromatography

The dissolved toxin proteins were separated by gel filtration. 20 mg sample in 4 ml volume was loaded onto a Sephadex G-200 column (2 × 150 cm), equilibrated and eluted with 0.1 M ammonium-hydrogen-carbonate buffer, pH 9.0. The flow rate was 18 ml/h and 9 ml fractions were collected. Fractions were analyzed for protein concentration (optical density at 280 nm).

Polyacrylamide gel electrophoresis (PAGE)

SDS polyacrylamide gel electrophoresis of the solubilized crystal proteins and various column fractions were carried out using 1 mm thick gels containing 12.5% (w/v) polyacrylamide in the presence of 0.1% sodium-dodecyl-sulfate according to O'Farrel (1975).

The gels were stained in Coomassie brillant Blue R-250. Molecular weight standards were human serum albumin (68 kDa), γ-globulin (50 and 27 kDa), cytochrome C (14 kDa).

Protein estimation

Protein concentration was determined by the method of Lowry et al. (1951), and it was calibrated with human serum albumin.

Test animals

Larvae of the yellow fever mosquito, *A. aegypti*, were reared on pulverized dog chow at 24 ± 2 °C with a 16L : 8D photoperiod. Adult mosquitoes from the same colony were maintained at 24 ± 2 °C and fed 10 with % sucrose solution.

Mosquito bioassay

Ten early fourth-instar of *A. aegypti* larvae were placed in a test tube (8 × 12 mm) with 1 ml distilled water, to which the toxin or toxin fraction preparations were added. Each test tube were made up to a final volume of 2.5 ml. Six concentrations were tested for each sample. All treatments, including the control, were replicated four times, and two replicates were conducted on a different day. Larvae were incubated at 24 ± 2 °C, without diet and mortality was scored at 24, 48 and 72 h postinoculation. The LC 50 and 95% FL were calculated by probit analysis (Finney, 1971). LC 50's were considered significantly different if their 95% FL did not overlap. Control larvae incubated in the absence of toxin showed no mortality in the observed period. Similarly, the buffer control did not cause larval mortality in the absence of toxin protein.

Haemolysis test

Haemolytic activity of the samples was estimated with use of human erythrocyte concentrate obtained from the Blood Bank of Gödöllő. The test was performed as described by Jones and Elliot (1969), modified by Sztanó et al. (1988). Erythrocytes were washed with physiological salt solution, then the concentration of the blood cell suspension was set for A 1 cm 415 nm = 41.0. The test was carried out using such serial dilution that the concentration of erythrocyte suspension was increased and that of the protein samples was decreased. After incubation (37 °C, 1h) samples were centrifugated supernatants diluted 41-fold and the amount of haemoglobin released was estimated by measuring the absorbance of the supernatants at 415 nm. The haemolytic activity was characterized with the protein quantity of the samples causing 50% haemolysis. The EC 50 was calculated by probit transformation.

Results

The fraction S1 contained the polipeptides of 130 kDa, 68 kDa, 35 kDa and 27 kDa. The last one was present only in extremely little quantity but the 130 kDa and 68 kDa subunits dominated. In the fraction S2 the proteins of 68 kDa and 27 kDa were enriched, but the 68 kDa protein was predominant. Considerable amount of the polypeptides of 35 kDa and 27 kDa with very small amount of 68 kDa were found in the fraction S3. The fraction S4 contained no polypeptides (Figs 1 and 2).

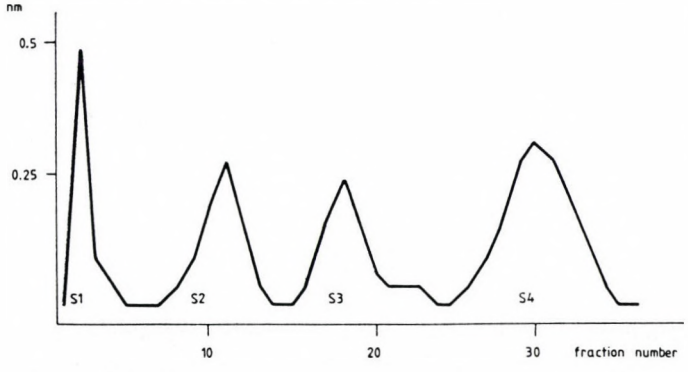

Fig. 1. Sephadex G-200 gel filtration of the supernatant of solubilized Bti crystal proteins. The column (2×150 cm) was equilibrated with 0.1 M ammonium-hydrogen-carbonate buffer, pH 9.0, and eluted by the same buffer. Nine-ml fractions were collected at a flow rate of 18 ml/h

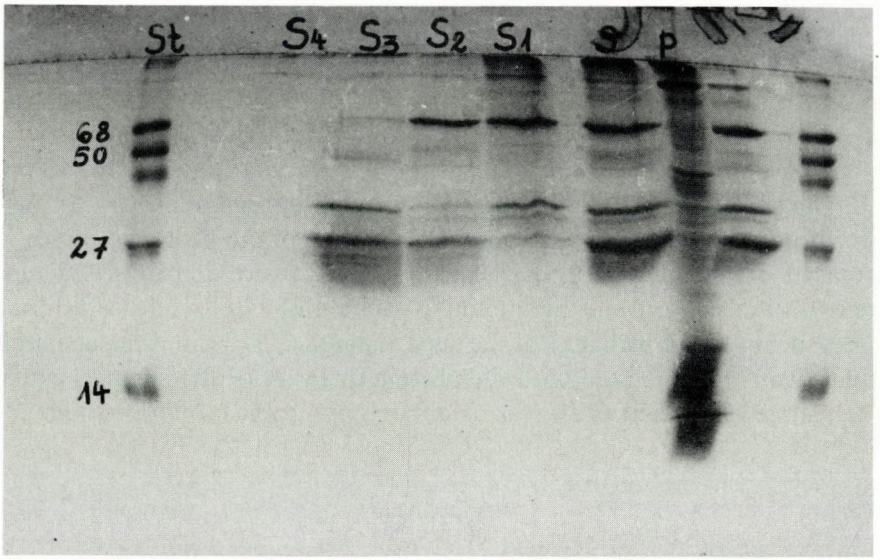

Fig. 2. SDS-PAGE analysis of the supernatant (S), precipitate (P) of solubilized Bti crystal proteins, and fractions (S1, S2, DS3, S4) of S. Electrophoresis was carried out in a 12.5% polyacrylamide gel containing SDS as described in the text. Protein standards (St): human serum albumin (68 kDa), γ-globulin (50 and 27 kDa), cytochrome C (14 kDa)

The fraction S1 did not show any haemolytic activity. The fractions S2 and S3 lysed erythrocytes and the 27 and 35 kDa enriched fraction (S3) was more than 2-fold as haemolytic as the other (Table 2). In contrast, the fraction S1 was quite effective against the larvae of *A. aegypti* and also the lower-molecular-weight enriched fractions possessed larvicidal activity. The LC 50's of each fractions differed significantly (Table 2).

As expected, the fraction S4 did not show either haemolytic nor mosquitocidal activity.

Discussion

We could not separate perfectly the main polypeptides by gel filtration. It can be explained by the high affinity of the toxin proteins for one another while the fractions were found to consist of protein mixtures. The fractions contained the main polypeptides but their proportion to each other differed remarkably, so according to these proportions we could conclude the particular

effects of each subunit. Fig. 2 shows that fraction S1 contained a slight amount of 27 kDa protein which was present in considerable quantity in fraction S2, and in the greatest quantity in fraction S3. The greater-molecular-weight proteins (130 and 68 kDa) being almost absent from fraction S3 cannot have any part in the haemolytic activity. Thus, it is most possible that the 27 kDa protein is responsible for the haemolytic activity of the parasporal body of Bti. This result is agreement with several earlier studies (Thomas and Ellar, 1983; Davidson and Yamamoto, 1984; Pfannenstiel et al., 1986 and others see Table 1).

Table 2

Mosquitocidal and haemolytic activity of the fractions of *B. thuriniengsis* subsp. *israelensis* crystal proteins

Fraction	Polypeptides (kDa)				Haemolytic activity (EC 50 µg/ml)	Larvicidal[a] (LC 50 µg/ml)
S1	130	68	35	27	n	17.06 (11.609 – 25.078)
S2	–	68	35	27	17.86	29.95 (25.525 – 35.154)
S3	–	68	35	27	8.40	78.81 (54.377 – 113.075)
S4	–	–	–	–	n	n
S	130	68	35	27	nd	16.88 (10.736 – 26.538)
P	130	120	48	–	nd	1.99 (1.337 – 2.965)

S = supernatant of solubilized crystal proteins, P = precipitate of solubilized crystal proteins, S1, S2, S3, S4 = fractions of S, [a] = mortalities were read after 48 h; nd = not determined; n = no activity; _ = dominant polypeptide; – = polypeptide not found

According to our toxicity results it is most likely that the larvicidal activity is associated with the 130 kDa and 68 kDa proteins and it is due mainly to the 130 kDa protein. Also the relatively high activity of the precipitate which contained most of the 130 kDa protein leads to this conclusion. Although almost every fraction possessed larvicidal activity the toxicity level of the fractions differed significantly from each other and the higher toxicities belonged to the fractions containing greater-molecular-weight proteins. The comparison of toxicity level of the supernatant and the fraction S1 (their protein composition differed only in the quantity of the 27 kDa protein) confirms the role of the 130 kDa and 68 kDa proteins (Table 2).

All these data are confirmed by studies of Bourgouin et al. (1986); Visser et al. (1986); Hurley et al. (1987).

The fraction S3 containing although only 35 kDa and 27 kDa proteins in greater amount showed "quite high" toxicity in comparison with the toxicity of S1 and S2 fractions. It may be due to the synergistic effect between the 27 kDa protein and the 86 kDa protein being present in slight amount (Wu and Chang, 1985).

The 35 kDa protein was observed in considerable quantity in the fraction S1. Cheung and Hammock (1985) thought it to be the cause of larval toxicity. In contrast, Insell and Fitz-James (1985) reported that it had no larvicidal activity. Pfannenstiel et al. (1986) did not analyse its toxicity though they observed it among the other protein subunits. According to our results the 35 kDa protein may not have haemolytic activity but it is not impossible that it contributes to the larval toxicity.

The lower larval toxicity of the samples examined found its explanation in the fact that we used solubilized crystal proteins for determination of the biotoxicity and solubilized Bti crystals are less toxic to mosquito larvae because of their feeding behavior (Schnell et al. 1984).

Our study demonstrates that the mosquitocidal activity is due mainly to the 130 kDa and 68 kDa proteins but also the 27 kDa protein may have a part in the toxicity as synergistic factor. Furthermore, we concluded that the 27 kDa protein is responsible for the haemolytic activity of the crystal. We could not find any evidence on the role of 35 kDa protein.

Literature

Armstrong, J. L., Rohrmann, G. F. and Beaudreau, G. S. (1985): Delta endotoxin of *Bacillus thuringiensis* subsp. israelensis. J. Bacteriol. 161, 39–46.

de Barjac, H. (1978): Un nouveau candidat à la lutte biologique contre les moustiques: *Bacillus thuringiensis* subsp. *israelensis*. Entomophaga, 23, 309–319.

Bourgouin, C., Klier, A. and Rappaport, G. (1986): Characterisation of the genes encoding the haemolytic toxin and the mosquitocidal delta-endotoxin. Mol. Gen. Genet. 205, 390–397.

Charles, J. F. and de Barjac, H. (1981): Histopathologie de l'action de la δ-endotoxine de *Bacillus thuringiensis* var. *israelensis* sur les larves d'*Aedes aegypti* (Dip.: Culicidae). Entomophaga, 26, 203–212.

Charles, J. F. and de Barjac, H. (1983): Action des cristaux de *Bacillus thuringiensis* var. *israelensis* sur l'intestin moyen des larves de *Aedes aegypti* L., en microscopie èlectronique. Ann. Microbiol. (Inst. Pasteur) 134 A, 197–218.

Cheung, P. I. K. and Hammock, B. D. (1985): Separation of three biologically distinct activities from the parasporal crystal of *Bacillus thuringiensis* var. *israelensis*. Curr. Microbiol. 12, 121–126.

Chilcott, C. N. and Ellar, D. J. (1986): Toxicity of the polypeptides isolated from *Bacillus thuringiensis* var. *israelensis* crystals. Proc. of the 4th Int. Coll. of Invert. Pathol. Veldhoven 18–22 August, 1986. P. 33.

Davidson, E. W. and Yamamoto, T. (1984): Isolation and assay of the toxic component from the crystals of *Bacillus thuringiensis* var. *israelensis*. Current Microbiology 11, 171–174.

Davis, N. C. and Shannon, R. C. (1930): The location of yellow fever virus in infected mosquitoes and the possibility of hereditary transmission. Am. J. Hyg. 11, 335–344.

Ellar, D. J., Thomas, W. E., Knowles, B. H., Ward, S., Todd, J., Drobniewski, F., Lewis, J., Sawyer, T., Last, D. and Nichols, C. (1985): Biochemistry, genetics, and mode of action of *Bacillus thuringiensis* δ-endotoxins. In: Hoch, J. A., Setlow, P. (eds): Molecular Biology of Microbial Differentiation, American Society for Microbiology, Washington, D.C. pp. 230–240.

Ellar, D. J., Ward, E. S. and Todd, J. A. (1986): Structural and functional analysis of the gene coding for the 27 kDa dipteran toxin from *Bacillus thuringiensis* var. *israelensis*. Cloning and expression in sporogenic and asporogenic strains of *Bacillus subtilis*. Proc. of the 4th Int. Coll. of Invert. Pathol. Veldhoven 18–22 August, 1986. p. 33.

Finney, D. J. (1971): Probit analysis. Cambridge University Press, London–New York, p.1–66.

Goldberg, L. H. and Margalit, J. (1977): A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergenti*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti* and *Culex pipiens*. Mosq. News 37, 355–358.

Held, G. A., Huang, Y. and Kawanishi, C. Y. (1986): Effect of removal of the cytolytic factor of *Bacillus thuringiensis* subsp. *israelensis* on mosquito toxicity. Biochem. Biophys. Res. Commun. 141, 937–941.

Hurley, J. M., Lee, S. G., Andrews, R. E., Klowden, M. J. and Bulla, Jr. L. A. (1985): Separation of the cytolytic and mosquitocidal proteins of *Bacillus thuringiensis* subsp. *israelensis*. Biochem. Biophys. Res. Commun. 126, 961–965.

Hurley, J. M., Bulla, Jr. L. A. and Andrews, Jr. R. E. (1987): Purification of the mosquitocidal and cytolytic proteins of *Bacillus thuringiensis* subsp. *israelensis*. Appl. Environ. Microbiol. 53, 1316–1321.

Ibarra, J. E. and Federici, B. A. (1986): Isolation of a relatively non-toxic 65-kilodalton protein inclusion from the parasporal body of *Bacillus thuringiensis* subsp. *israelensis*. J. Bacteriol. 165, 527–533.

Insell, J. P. and Fitz-James, P. C. (1985): Composition and toxicity of the inclusion of *Bacillus thuringiensis* subsp. *israelensis*. Appl. Environ. Microbiol. 50, 56–62.

Jones, M. and Elliot, F. C. (1969): Two rapid assays for saponin in individual alfalfa plants. Crop. Sci. 9, 688–691.

Kim, K.-H., Obha, M. and Aizawa, K. (1984): Purification of the toxic protein from *Bacillus thuringiensis* serotype 10 isolate demonstrating a preferential larvicidal activity to the mosquito. J. Invertebr. Pathol. 44, 214–219.

Knowles, B. H. and Ellar, D. J. (1987): Colloid-osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* δ-endotoxins with different insect specificity. Biochem. Biophys. Acta 924, 509–518.

Lakhim-Tsror, L., Pascar-Gluzman, C., Margalit, J. and Barak, Z. (1983): Larvicidal activity of *Bacillus thuringiensis* subsp. *israelensis*, serovar H14 in *Aedes aegypti*: histopathological studies. J. Invertebr. Pathol. 41, 104–116.

LaMotte, L. C. (1960): Japanese B encephalitis virus in the organs of infected mosquitoes. Am. J. Hyg. 72, 73–87.

Lee, S. G., Eckblad, W. and Bulla, Jr. L. A. (1985): Diversity of protein inclusion bodies and identification of mosquitocidal protein in *Bacillus thuringiensis* subsp. *israelensis*. Biochem. Biophys. Res. Commun. 126, 953–960.

Lereclus, D., Bourgouin, C., Lecadet, M. M., Klier, A. and Rapaport, G. (1989): Role, structure and molecular organisation of the genes coding for the parasporal δ-endotoxins of *Bacillus*

thuringiensis. pp. 255–276. In: Smith, R. A. Slepecky and P. Setlow (eds): Regulation of Procaryotic Development. American Society for Microbiology, Washington, DC 20006

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951): Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luthy, P. (1980): Insecticidal toxins of *Bacillus thuringiensis* FEMS Microbiol. Lett. 8, 1–7.

Mihályi, F. and Gulyás, M. (1963): Magyarország csípő szúnyogjai. Akadémiai Kiadó, Budapest, p. 229.

O'Farrel, P. H. (1975): Two dimensional gel electrophoresis. J. Biol. Chem. 250, 4007–4021.

Pfannenstiel, M. A., Couche, G. A., Ross, E. J. and Nickerson, K. W. (1986): Immunological relationship among proteins making up the *Bacillus thuringiensis* subsp. *israelensis* crystalline toxin. Appl. Environ. Microbiol. 52, 644–649.

Schnell, D. J., Pfannenstiel, M. A. and Nickerson, K. W. (1984): Bio-assay of solubilized *Bacillus thuringiensis* var. *israelensis* crystals by attachment to latex beads. Science 223, 1191–1193.

Szalay-Marzsó, L. and Gharib, A. H. (1983): Effectiveness of *Bacillus* thuringiensis var. *israelensis* (H–14 Serotype) against mosquito larvae and non-target organisms, with special regard to chironomidae. P. Int. Conf. Integr. Plant Prot., 4, 17–23. Budapest, 4th–9th July.

Sztanó, P., Fábián, F. and Sajgó, M. (1988): A lucerna szaponintartalmának meghatározása hemolitikus módszerrel. Agricultural Scientific Days, Abstracts p. 21. Gödöllő, 6th–7th April.

Thomas, W. E. and Ellar, D. J. (1983): Mechanism of action of *Bacillus thuringiensis* var. *israelensis* insecticidal δ-endotoxin. FEBS Lett. 154, 362–368.

Tyrell, D. J., Davidson, L. I., Bulla, Jr. L. A. and Ramoska, W. A. (1979): Toxicity of parasporal crystals of *Bacillus thuringiensis* subsp. *israelensis* to mosquitoes. Appl. Environ. Microbiol. 38, 656–658.

Tyrell, D. J., Bulla, Jr. L. A., Andrews, R. E., Kramer, K. J., Davidson, L. I. and Nordin, P. (1981): Comparative biochemistry of entomocidal parasporal crystals of selected *Bacillus thuringiensis* strains. J. Bact. 145, 1052–1062.

Undeen, A. H. and Nagel, W. L. (1978): The effect of *Bacillus thuringiensis* ONR 60 strain (Goldberg) on Simulium larvae in the laboratory. Mosq. News. 38, 524–527.

Visser, B., van Workum, M. E. S., Dullemans, A. M. and Waalwijk, C. (1986): The mosquitocidal activity of *Bacillus thuringiensis* var. *israelensis* is associated with Mr 230 000 and 130 000 crystal proteins. FEMS Microbiol. Lett. 30, 211–214.

Waalwijk, C., Dullemans, A. M., van Workum, M. E. S. and Visser, B. (1985): Molecular cloning and the nucleotide sequence of the Mr 28 000 crystal protein gene of *Bacillus thuringiensis* subsp. *israelensis*. Nucleic Acids. Res. 13, 8207–8217.

Walther, C. J., Couche, G. A., Pfannenstiel, M. A., Egan, S. E., Bivin, L. A. and Nickerson, K. W. (1986): Analysis of mosquito larvicidal potential exhibited by vegetative cells of *Bacillus thuringiensis* subsp. *israelensis*. Appl. Environ. Microbiol. 52, 650–653.

Ward, E. S., Ellar, D. J. and Todd, J. A. (1984): Cloning and expression in *Escherichia coli* the insecticidal δ-endotoxin gene of *Bacillus thuringiensis* var. *israelensis*. FEBS Lett. 175, 377–382.

Wu, D. and Chang, F. N. (1985): Synergism in mosquitocidal activity of 26 and 65 kDa proteins from *Bacillus thuringiensis* subsp. *israelensis* crystal. FEBS Lett. 190, 232–236.

Yamamoto, T., Iizuka, T. and Aronson, J. N. (1983): Mosquitocidal protein of *Bacillus thuringiensis* subsp. *israelensis*: identification and partial isolation of the protein. Current Microbiology 9, 279–284.

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Effect of Mineral Nutrition and Herbicide Mixtures on the Absorption and Translocation of Bensulfuron Methyl in Rice*

J. C. CHUN, K. W. HAN and J. K. YEO

Department of Agricultural Chemistry Jeonbug National University Jeonju 560-756, Korea

Absorption and translocation of bensulfuron methyl {methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]methyl] benzoate} in rice (*Oryza sativa* L.) as affected by mineral nutrition and herbicide mixtures were determined using ^{14}C-labeled herbicide in culture solution. Absorption of ^{14}C-bensulfuron methyl by roots decreased with increasing concentration of bensulfuron methyl. However, increase in the application concentration did not affect movement of the ^{14}C-label to shoots. There was no difference in total amount of ^{14}C-bensulfuron methyl taken up between absorption periods of 12 and 48 hours, whereas ^{14}C-bensulfuron methyl translocated to shoots increased with increasing the absorption period. When mixtures of bensulfuron methyl were applied, butachlor [N-(buthoxymethyl)-2-chloro-N-(2,6-diethylphenyl)acet-amide] did not affect absorption and translocation of ^{14}C-bensulfuron methyl. However, quinclorac (3,7-dichloro-8-quinoline carboxylic acid) mixed at a high concentration resulted in decrease in absorption and translocation of ^{14}C-bensulfuron methyl. Nutritional disorder such as deficient or excess supply of mineral nutrients caused to depress absorption of ^{14}C = bensulfuron methyl. The greatest decrease and delay of ^{14}C-bensulfuron methyl absorption and/or translocation occurred in N-deficiency and S-excess supply conditions.

Bensulfuron methyl, a sulfonylurea herbicide, is widely used in transplanted rice to selectively control most annual and perennial broadleaf weeds and sedges (Takeda et al., 1985). To increase the spectrum of weed control, however, bensulfuron methyl is applied in combination with a 'grass' effective herbicide such as butachlor or quinclorac. When the mixtures are used as pre-emergence treatment, an adverse effect may occur in rice plants aside from an improvement in weeding effect.

Interactions of herbicides with other herbicides or fertilizers have long been recognized and determined by several workers (Hance, 1981; Hatzios and Penner, 1985). Hatzios and Penner (1985) indicated that interactions between agrochemicals may result in adverse effects and these may be due either to alternations of the absorption, and biotransformation of one component from another, or to combinations of their actions or effects on target or nontarget species. Enhanced uptake and/or transport of picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) is evident in the presence of

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Akadémiai Kiadó, Budapest

phenoxyalkanoic acid herbicides (Davis et al., 1968; Agbakoba and Goodin, 1970). Addition of ammonium nitrate or calcium chloride also results in increases in herbicide penetration and translocation (Brady, 1970; Stanley, 1975).

The objective of this research was to investigate the absorption and translocation patterns of bensulfuron methyl in rice as affected by mineral nutrition and herbicide mixtures. Experiments were carried out to: a) determine the effect of application time and contrentration of bensulfuron methyl, b) evaluate the effect of butachlor and/or quinclorac mixtures, and c) observe the effect of deficiency or excess supply of major mineral nutrients.

Materials and Methods

Growth of rice plants. Rice (cv. Choochung) seeds were selected, sterilized, and soaked in distilled water prior to pre-germination. When the coleoptile reached 5 mm long, ten uniform seedlings were selected and transfered to a plastic pot (15 by 20 cm) containing washed sands and culture solution (Yoshida et al., 1972), and raised by 2-leaf stage in a greenhouse where the temperature varied between 21 and 30 °C and natural daylight provided approximately 270 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation during the experimental period. The seedlings were subjected to grow under different conditions of the culture solutions: full-strength, nutrient deficienty, and excess supply of nutrients. Nutrient deficient condition was prepared by deleting respective major nutrients from fullstrength solution, whereas for condition of excess supply of nutrients the respective major nutrients were added twofold as muchs as required by fullstrength solution. Nutrient solution decreased was added daily. At 2-leaf stage of the seedlings all the culture solutions were changed with the corresponding fresh solutions and the seedlings were grown for another three days prior to application of herbicides. There were four replications.

^{14}C-treatment solution. ^{14}C-bensulfuron methyl, labeled at the phenyl ring and having a specific activity of 14.9 μCi/mg, was used. After ^{14}C-bensulfuron methyl was dissolved in acetonitrile, this was re-diluted in deionized water. Five ml of the labeled herbicide and the required volume of unlabeled bensulfuron methyl prepared from 93% technical grade were added to the nutrient solution to have 0.05 μCi per pot. The inclusion of unlabeled bensulfuron methyl was to adjust herbicide concentrations. The final herbicide concentrations in the treatment solution were adjusted to 0.1, 1, 10, and 100 μM.

Experimental treatments. Three experimental conditions were employed: 1) The rice seedlings were treated with concentrations of bensulfuron methyl ranging from 0.1 to 100 μM. The plants were allowed to take up the nutrient solution over a period of 24 h and then harvested. To determine the effect of application time, bensulfuron methyl at 10 μM was applied to the plants for 12, 24, and 48 h uptake period. 2) Herbicide mixtures of bensulfuron methyl used were butachlor and quinclorac. The respective herbicide at 0.1, 1, and 10 μM was combined with 10 μM bensulfuron methyl. The plants were harvasted after completion of the 24 h uptake period. 3) Nutritional stresses (deficiency and/or excess supply) subjected to the plants were for N, P, K, Ca, Mg, and S. Bensulfuron methyl at 10 μM was treated to the plants for 12 and 48 h.

Plant harvest and ^{14}C assay. After harvest, the plants were rinsed twice with deionized water to remove unabsorbed herbicide and divided into roots and shoots. The plant parts were dried at 80 °C for 3 days and weighed. Two hundreds mg of the dried sample were combusted in a biological sample oxidizer. The liberated $^{14}CO_2$ was collected in 7 ml of a scintillant cocktail containing a CO_2 absorbent and radioassayed using a liquid scintillation spectrometry (Beckman LS 5100).

Results and Discussion

Absorption and translocation. Uptake of bensulfuron methyl was affected by concentration of the herbicide (Fig. 1). Total amount of ^{14}C absorbed by the root decreased with increasing the application concentration. After absorption, about two-thirds of ^{14}C was found in the roots and the rest in the shoots. The ^{14}C translocated to the shoot was not greatly influenced by the application concentration, whereas the ^{14}C remained in the root was dependent upon the total ^{14}C absorbed. This result agrees with the findings of Yuyama et al. (1987), indicating a limited translocation of absorbed ^{14}C-bensulfuron methyl from rice roots to shoots.

Root absorption of bensulfuron methyl occurred within a relatively short period of time (Table 1). There was no significant difference in ^{14}C absorbed between 12 h and 48 h absorption periods. However, the amount of ^{14}C-bensulfuron methyl translocated to the shoots increased as the exposure time increased, resulting in decrease in ^{14}C of the roots. Yuyama *et al.* (1987) reported that the uptake of bensulfuron methyl in paddy rice rapidly increased by 6 h and thereafter showed a gradual increase by 24 h.

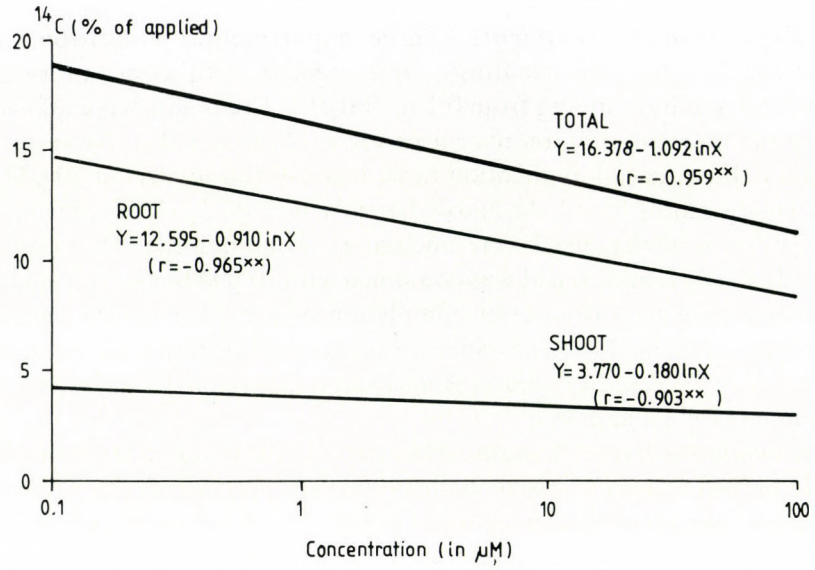

Fig.1. Distribution of ^{14}C-bensulfuron methyl as affected by time of application

Table 1

Absorption and translocation of ^{14}C-bensulfuron methyl as affected by time of application

Time after application (h)	Distribution of radioactivity (% of applied)		
	Absorption	Root	Shoot
12	10.8	9.2	1.6
24	11.7	8.8	2.9
48	11.7	8.2	3.5
LSD (0.05)	1.0	0.6	0.3

The regression analysis of the data showed a negative linear relationship between absorption of bensulfuron methyl and bensulfuron methyl concentration in the ambient solution ($r = -0.959$, $p < 0.01$). This negative linearity indicates that the mechanism of absorption may not be based on a simple diffusion process against a concentration gradient. Devine et al. (1979) determined chlorsulfuron {2-chloro-N-[[(4-methoxy-6-methyl-1,3,5,-triazin-2-yl)amino] carbonyl] benzenesulfonamide} uptake into excised pea root tissue over a concentration range from 0.001 to 1 mM was linear and suggested that the absorption mechanism was a nonfacilitated process. The difference may be due either to different absorption process itself between the herbicides or to difference in plant materials used. In this experiment intact rice plants were used.

Effect of herbicide mixtures Absorption and translocation of ^{14}C-bensulfuron methyl varied with the kind of herbicide mixtures combined (Table 2). Butachlor at the application concentrations ranging from 0.1 to 10 μM did not greatly affect the total amount of ^{14}C-label absorption, whereas a significant decrease in absorption of ^{14}C-bensulfuron methyl was found when 10 μM quinclorac was mixed.

Table 2

Effect of herbicide mixtures on absorption and translocation of ^{14}C-bensulfuron methyl in rice

Herbicide mixture	Concentration (μM)	Distribution of radioactivity (% of applied)[1]		Root/Shoot Ratio
		Root	Shoot	
Control	–	10.5 ab	3.7 ab	2.8
Butachlor	0.1	11.3 a	3.8 a	3.0
Butachlor	1	10.5 ab	3.3 b	3.2
Butachlor	10	10.2 bc	3.6 ab	2.8
Quinclorac	0.1	10.4 b	3.7 ab	2.8
Quinclorac	1	10.2 bc	2.8 c	3.6
Quinclorac	10	9.0 c	2.4 c	3.8

[1]Means followed by a common letter are not significantly different at 5% level by Duncan's Multiple Range Test.

The pattern of translocation of ^{14}C-bensulfuron methyl was related with ^{14}C-label absorbed. Butachlor mixture did not alter the root/shoot ratio as compared with the control. However, increase in the ratio occurred when greater than 1 μM of quinclorac was applied, resulting in decrease in translocation of ^{14}C-bensulfuron methyl absorbed. This indicated that quinclorac caused to decrease both absorption and translocation of bensulfuron methyl.

Based on the results obtained, it is assumed that both the herbicide mixtures employed do not affect membrane integrity of the root cells. If the membranes disrupted mechanically by the herbicide mixtures, amounts of ^{14}C-label absorbed and translocated would have rather been increased as compared with the control. Instead the decreased absorption and translocation of ^{14}C-bensulfuron methyl by means of quinclorac may be due either to competition for site of uptake or to interfering result on absorption process of bensulfuron methyl.

Effect of mineral nutrition. Absorption and translocation of ^{14}C-bensulfuron methyl was affected by mineral nutrition of the rice plants (Figs 2, 3). When the rice plant was grown under deficient conditions of mineral nutrients, the absorption of ^{14}C-label decreased and delayed. The greatest

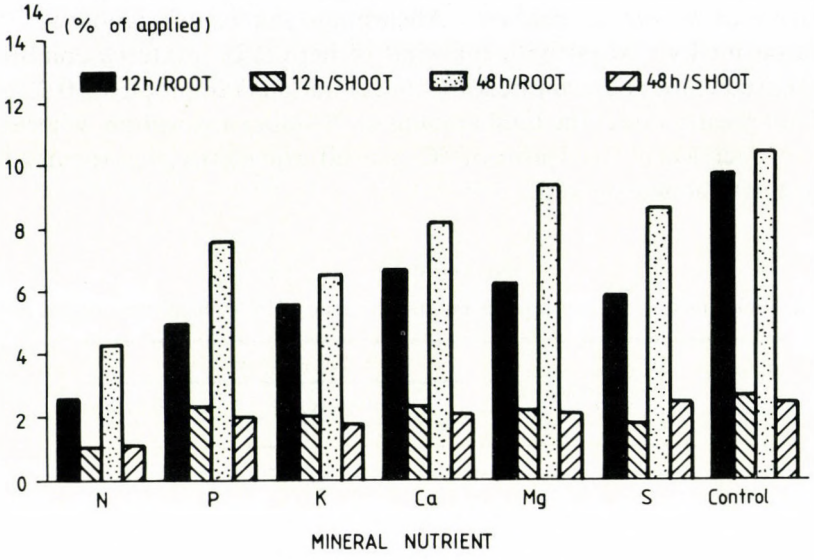

Fig. 2. Effect of deficiency of mineral nutrients on distribution of ^{14}C-bensulfuron methyl after 12-h and 48-h absorption periods

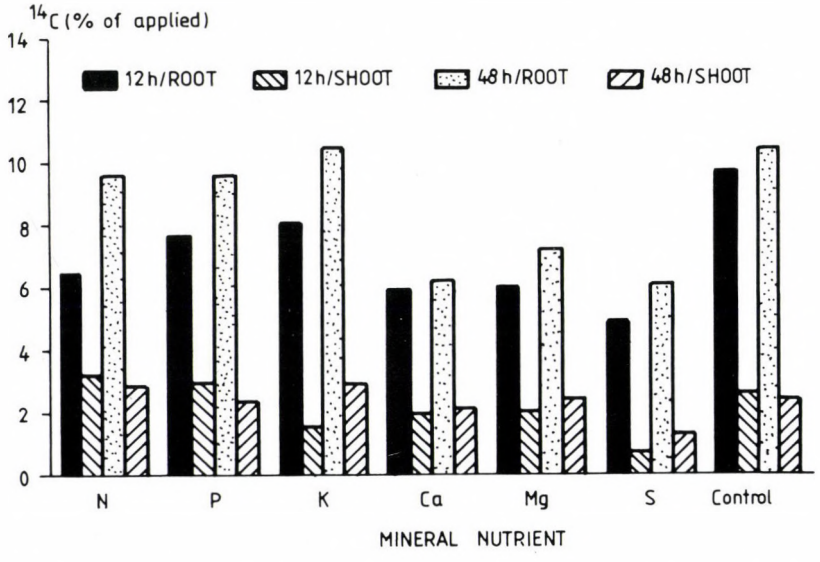

Fig. 3. Effect of excess supply of mineral nutrients on distribution of ^{14}C-bensulfuron methyl after 12 h and 48 h absorption periods

decrease in the absorption occurred in N-deficiency, but the least in Mg-deficiency (Fig. 2). In addition, the absorption process tended to be delayed. Unlike a rapid absorption of ^{14}C-bensulfuron methyl in the control, deficient mineral nutrition resulted in continuous absorption of ^{14}C-label by 48 h absorption period. However, the translocation of the ^{14}C-label absorbed was not much affected by the mineral nutrition, except for N-deficiency. The ^{14}C translocated under N-deficient condition was reduced as compared with control.

Excess supply of N, P, and K caused a delayed absorption of ^{14}C-bensulfuron methyl, but did not affect the total amount of the herbicide absorbed (Fig. 3). In contrast, excess supply of Ca, Mg, S resulted in reduction of the absorption of ^{14}C-bensulfuron methyl. Translocation of the absorbed ^{14}C was not affected by excess supply of the mineral nutrients, except for S. Decrease in the ^{14}C-label translocated was found in S excess supply.

These results obtained indicate that absorption and translocation of bensulfuron methyl is influenced by the application concentration, kind of herbicide mixtures, and mineral nutrition of rice. Whatever the factors employed are involved, amount of ^{14}C-bensulfuron methyl absorption and translocation tends to be decreased. This suggested that phytotoxic mode of action of bensulfuron methyl in rice is not associated with the total amount of bensulfuron methyl absorbed and translocated. Pyon and Kwon (1989) reported that differential selectivity to the herbicide between rice and *Cyperus serotinus* was due not to the absorption and translocation, but to the metabolism.

Acknowledgement

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Literature

Agakoba, C. S. O. and Goodwin, J. F. (1970): Picloram enhances 2,4-D movement in field bindweed. Weed Sci. 18, 19–21.

Brady, H. A. (1970): Ammonium nitrate and phosphoric acid increase 2,4,5-T absorption by tree leaves. Weed Sci. 18, 204–206.

Davis, R. S., Bovey, R. W. and Merkle, M. G. (1968): Effect of paraquat and 2,4,5-T on the uptake and transport of picloram in woody plants. Weed Sci. 26, 336–339.

Devine, M. D., Bestman, H. D. and Vanden Born, W. H. (1987): Uptake and accumulation of the

herbicide chlorsulfuron and clopyralid in excised pea root tissue. Plant Physiol. 85, 82–86.
Hance, R. J. (1981): Effect of pesticides on plant nutrition. Residue Rev. 78, 13–41.
Hatzios, K. K. and Penner, D. (1985): Interactions of herbicides with other agrochemicals in higher plants. Rev. Weed Sci. 1, 63.
Pyon, J. Y. and Kwon, C. S. (1989): Selective mode of action of bensulfuron methyl in rice and two paddy weeds. Proc. II 12th Asian-Pac. Weed Sci. Soc. Conf., Seoul, 471–478.
Stanley, R. A. (1975): Interaction of calcium and 2,4-D on Eurasian watermilfoil. Weed Sci. 23, 182–184.
Takeda, S., Yuyama, T., Ackerson, R. C., Weigel, R. C., Sauers, R. F., Neal, W., Gibian, D. G. and Tseng, P. K. (1985): Herbicidal activities and selectivity of a new rice herbicide DPX-F 5384. Weed Res. (Japan) 30, 284–289.
Yoshida, S., Forno, D. A., Cock, J. H. and Gomez, K. A. (1972): Laboratory manual for physiological studies of rice. Int. Rice Res. Inst., Los Banos, Philipp. 70 p.
Yuyama, T., Takeda, S. and Ackerson, R. C. (1987): Uptake and distribution of bensulfuron methyl (DPX-F 5384) in paddy rice. Proc. I 11th Asian-Pac. Weed Sci. Soc. Conf., Taipei, 145–151.

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Maximizing the Performance of Antagonistic Mixtures*

JERRY M. GREEN and KOFI S. AMUTI

Stine-Haskell Resarch Center, Agricultural Products Department, E.I. du Pont de Nemours & Company, Newark, Delaware 19714, U.S.A.

Herbicide antagonism is beneficial when it reduces crop injury and detrimental when it reduces weed control. Commercial successes where antagonism safens crops are far outnumbered by failures where it reduces weed control. Because mixtures are becoming more complex and more common, antagonism is more important to understand than ever before.

Factors that affect antagonism usually can be identified and managed to maximize performance. These factors include herbicide selection, differential species sensitivity, differential species joint action, rate adjustment, formulation, adjuvants, mode of action, timing, placement, stage of growth, and environmental factors. The degree of antagonism, not whether it occurs, usually determines whether the mixture is agronomically useful.

In the major herbicide markets, the potential number of herbicide combinations is so large that comprehensive empirical testing is impractical. Field tests with new herbicide candidates only determine their performance alone and allow us to make single response equations, but such tests do not adequately evaluate combinations or quantify joint action.

These response equations, in conjunction with similar equations for established herbicides, help potential mixtures. Appropriate tests then can be designed to quantify joint action. This joint action with the response equations is used in models to predict mixture performance and select mixtures that meet performance criteria. These criteria include weed control, crop injury, and economic and environmental limits. These models focus further field efforts on those mixtures most likely to succeed.

Herbicide mixtures are commonly used in all major agronomic crops to broaden weed spectrum, improve efficacy, and reduce costs. In soybeans *(Glycine max)*, U.S. growers use 150 different mixtures each season (Delvo, 1987). New active ingredients now are being introduced to markets in mixture only (Gerwick and Kleschick, 1990, Green et al., 1990). In addition to more mixtures, the number of components is increasing also. Such trends dramatically increase the chance of unexpected and unwanted mixture interaction.

Caution needs to be exercised with herbicide mixtures because antagonism occurs commonly. Antagonism is beneficial when it reduces crop injury without reducing weed control but detrimental when it reduces weed control. In cases where antagonism reduces weed control, factors usually can be identified and managed to decrease its impact on weed control. The degree of antagonism, not whether it occurs, usually determines whether a mixture is useful.

*Based on a paper presented at the International Conference on Herbicide Safeners, 12th–15th August, 1990. Budapest, Hungary

Akadémiai Kiadó, Budapest

Definition of Terms

Herbicides interact with other herbicides and with nonherbicides. They interact outside and inside the plant, before, during and after application.

The 3 types of herbicide-herbicide mixture responses are antagonism, additivity, and synergism (Fig. 1). Additivity occurs when herbicides can be substituted for each other at equivalent biological rates and still obtain the same biological response (Akobundu et al., 1975). If the response curves are parallel, linear contour lines define additivity. Antagonism occurs when activity is less than expected and the contours bend outward. Synergism is the reverse.

Herbicides also interact with chemicals that have no herbicide activity, nonherbicides (Fig. 2). A nonherbicide does not affect the herbicide is inert. A herbicide antidote, or safener, reduces herbicide activity; a synergist increases activity. Reducing crop injury is desirable, but not weed control.

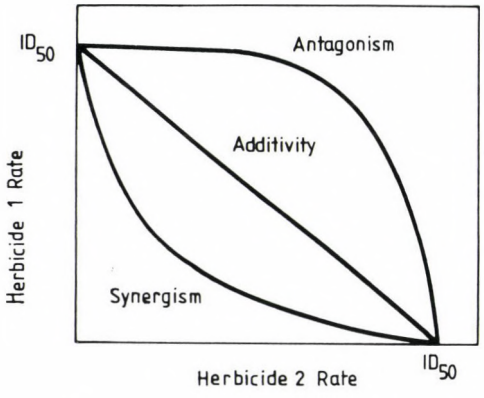

Fig. 1. Herbicide-herbicide mixture responses expressed graphically

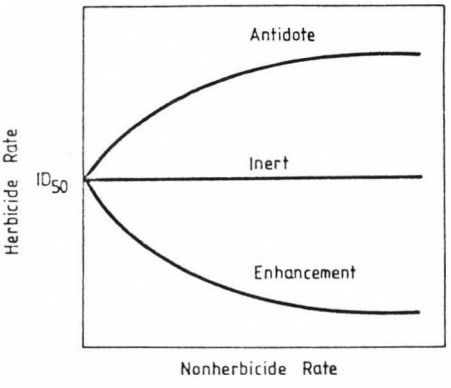

Fig. 2. Herbicide and nonherbicide mixture responses expressed graphically

Managing and Avoiding Antagonism

Herbicide-Herbicide Antagonism. Factors that affect herbicide antagonism usually can be managed so the herbicide(s) retains most of its (their) efficacy. These factors include: using herbicides with different spectrums and modes of action; using species differences with respect to sensitivity and joint

action; adjusting rates, formulations, adjuvants, timing, stage of growth and environmental factors.

The most common way to overcome antagonism is to increase the rate of the antagonized herbicide. For example, most postemergence broadleaf herbicides antagonize most grass herbicides in cereals and soybeans. Increasing the grass herbicide rate 30 to 100% generally overcomes this antagonism. Doubling the rate of the ethyl ester of quizalofop {(±)-2-[4-[(6-chloro-2-quinoxalinyl)-oxy]phenoxy]propanoic acid} overcomes bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] antagonism on barnyardgrass *(Echinochloa crus-galli)* (Green, 1989).

A better way is to select a herbicide that does not antagonize the other herbicide(s). Herbicide commonly antagonize each other in proportion to their biological activity. For example sulfonylurea broadleaf herbicides antagonize diclofop {(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid} on wild oat *(Avena sativa)* in proportion to their activity on wild oat. At equal rates the methyl ester of metsulfuron {2[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]amino]-sulfonyl]benzoic acid} is not active on wild oat and antagonizes diclofop most, clorsulfuron {2-chloro-*N*[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl-benzene-sulfonamide} is intermediate and methyl ester of thifensulfuron {3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-amino]sulfonyl]-2-thiophenecarboxylic acid} is least active and least antagonistic (Green, 1989).

A similar case occurs in soybeans with quizalofop. At 4 g/ha thifensulfuron has no activity on grass weeds and thus does not antagonize quizalofop. However, at the same rate, the ethyl ester of chlorimuron {2-[[[[(4-chloro-6-methoxy-2 pyrimidinyl) amino] carbonyl] amino] sulfonyl] benzoic acid} inhibits some grasses and strongly antagonizes quizalofop on these grasses.

Differences in the speed and type of activity make it very difficult to mix many herbicides. Slow-acting, translocated herbicides like the methyl ester of sulfometuron {2-[[[[4,6-dimethyl-2-pyrimidinyl) amino] carbonyl] amino] sulfonyl]benzoic acid} are almost always antagonized by fast-acting, burndown herbicides like paraquat (1,1'-dimethyl-4,4'-bipyridium ion). Such mixtures often are very effective, giving quick kill and residual control, but their joint action is technically antagonistic.

Creative uses of antagonism can result in long-term synergism. For example paraquat is used in South America with low rates of diuron [*N*'-(3,4-dichlorophenyl-*N*,*N*-dimethylurea] where uptake has been optimized through formulation and fine granulation. The diuron inhibits photosynthesis for a

few days while the weed takes up and translocates paraquat. When the level of diuron decreases, paraquat acts throughout the plant and kills it.

Commonly, one herbicide antagonizes the other but not the reverse. Such joint action is termed asymmetric. There are numerous examples in cereals where wild oat herbicides are antagonized by broadleaf herbicide, but the broadleaf herbicide is not antagonized by the wild oat herbicide. Similar examples occur in soybeans. For example, chlorimuron antagonizes quizalofop on barnyardgrass, but quizalofop does not antagonize chlorimuron on ivyleaf morninglory *(Ipomoea hederacea).*

Application method can affect how herbicides interact. For example, metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] and chlorimuron are additive preemergence, but they are antagonistic postemergence.

Antagonism between herbicides often can be avoided if the herbicides are applied sequentially. For example, chlorimuron antagonizes quizalofop on grass weeds when tank-mixed, but not when the herbicides are applied three days apart.

Selective antagonism of only one species is rare, but one successful commercial example occurs between the sulfonylurea, the methyl ester of bensulfuron {2-[[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] amino] carbonyl] amino]-sulfonyl]methyl]benzoic acid}, and thiocarbamate herbicides like thiobencarb {*S*-[(4-chlorophenyl)methyl]diethylcarbamothioate} and dimepiperate [1-piperidineacarbothioic acid *S*-(1-methyl-1-phenylethyl) ester]. Bensulfuron can injure Japonica rice in paddies, but thiobencarb or dimepiperate safen rice to an acceptable level without antagonism of weed control (Yuyama, Shirakura and Ishizuka, 1990).

Herbicide-Nonherbicide Antagonism. Herbicides commonly are applied with surfactants herbicide antidotes, fertilizers, formulants, and other pesticides. As the number of chemicals in the spray tank increases, the likelihood of an unexpected antagonism increases. Therefore, each new herbicide should be extensively field tested with possible mixture partners before it is marketed.

Dramatically different herbicide interactions occur with a commonly used insecticide, phorate [*O*,*O*-diethyl-*S*-(ethylthio-methyl) phosphorodithoate] insecticide. Phorate strongly synergizes metribuzin on corn (*Zea mays* 'Cargill 937'), but phorate strongly antagonizes clomazome {2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone} (Fig. 3).

Finding antidotes for herbicides that do not have sufficient crop selectivity involves: 1) random chemical screening, 2) analoging inactive herbicides, and 3) evaluating previously known antidotes. The ability to

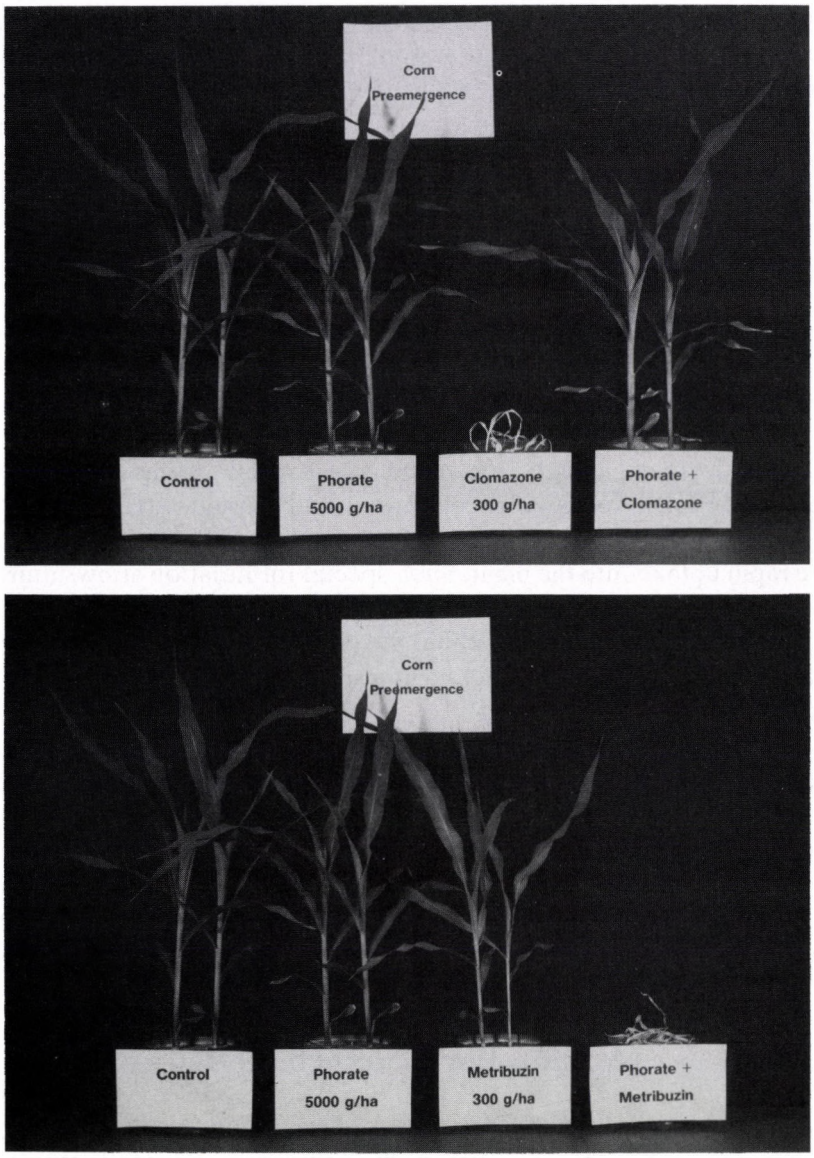

Fig. 3. The effect of the insecticide phorate on corn with clomazone and metribuzin. Preemergence phorate at 5000 g/ha was applied with 300 g/ha of clomazone and metribuzin

antidote different herbicide classes varies greatly. For example triazines are difficult, but thiocarbamates and sulfonylureas are easy (O. L. Hoffmann, personal communication).

Random chemical screening can identify antidotes for chlorsulfuron on sorghum *(Sorghum bicolor).* These can be inactive chlorsulfuron analogues like DPX-D5293 [*N*-(aminocarbonyl)-2-chlorobenzene-sulfonamide], previously known antidotes like NA (1,8-naphthalic anhydride), or entirely unrelated chemical classes.

Inactive racemic isomers are potential herbicide antidotes. When only one isomer is active, the inactive isomer can complete for the active site or turn on some other mechanism(s) like metabolism to reduce crop injury (Muir and Hansch, 1955, Rummens et al., 1975).

Formulation is an effective way to control herbicide uptake and thus increase or decrease antagonistic responses. An interesting example is the recently developed formulation of diuron and paraquat (Hayward et al., 1988). Diuron is finely granulated and formulated with special adjuvants to ensure rapid uptake into the plant. Such special formulation allows diuron to inhibit photosynthesis and before paraquat acts and damages the leaf tissue. Paraquat then translocates throughout the plant. Diuron is used at a low rate so that after two or three days, photosynthesis resumes and paraquat even kills unsprayed portions of the weeds.

Adjuvants currently are used to reduce the antagonism between the sodium salt of bentazon and sethoxydim {2[1-ethoximino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one}. One theory suggest that the adjuvant reduces the formation of the poorly absorbed, sodium salt of sethoxydim (Penner, 1989). Another theory suggests that this adjuvant improves sethoxydim uptake more than other adjuvants, masking but not changing the antagonism (Zoner et al. 1989).

Mixture Performance Model

Single Herbicide Response. Small tests with only one or two herbicide rates do not test a wide enough rate range to separate effects due to the sigmoidal shape of the response curve with joint action. Such small tests demonstrate mixture performance and should not be interpreted with a simple formula to determine synergism or antagonism.

For example DPX-E9636 {N-(((4,6-dimethoxypyrimidin-2-yl)-amino-carbonyl)-3-ethylsulfonyl)-2-pyridinesulfonamide) controls giant foxtail *(Setaria faberi)* at 2 g/ha, but it takes 50 to 100 g/ha to injure corn. DPX-

D5293 [*N*-(aminocarbonyl)-2-chlorobenzene-sulfonamide] safens SPX-E9636 on both species in a similar manner.

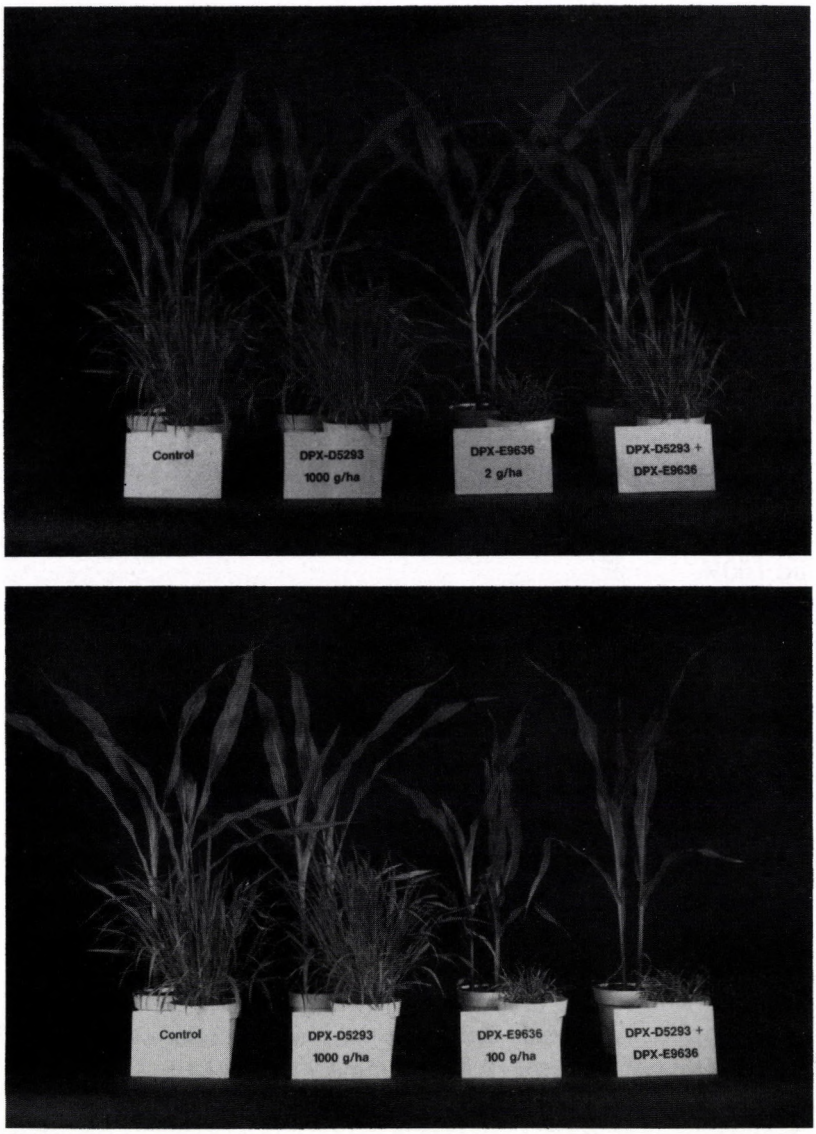

Fig. 4. The effect of the herbicide antidote DPX-D5293 on corn and giant foxtail with a high and a low rate of DPX-E9636. Postemergence DPX-E9636 at 2 and 100 g/ha was applied with and without 1000 g/ha DPX-D5293. All treatments had 0.25% v/v surfactant

If a test conducted at 2 g/ha DPX-E9636, safening is observed only on giant foxtail while at 100 g/ha safening is only corn (Fig. 4). The joint action is the same, the visual effect depends whether the study is on a level or steeply sloping part of the response curve.

Numerous models relate the rate of a single herbicide to its response. These models have the form Y=F(X) where Y is usually a transformation of the response, y; F is a function of the rate, x, or a transmission of rate, X. We commonly use a log-logit model where

$$Y = \log(y/100-y) = a + b\log(x).$$

Herbicide-Nonherbicide Mixtures. To achieve better herbicide performance, nonherbicides commonly are mixed with herbicides to enhance weed control or reduce crop injury. Models for such mixtures are more complex than for a single herbicide, but less complex than two herbicides. These models should start with the herbicide response equation and modify it with respect to the rate and activity of the nonherbicide.

In essence for a herbicide antidote, the herbicide response curves shift in a parallel fashion, depending on the rate and activity of the antidote (Streibig, 1981) (Fig. 5). A rate factor within the response equation makes the mixture sensitive to the slope and shape of the response curve and asymptotic to 0 and 100%.

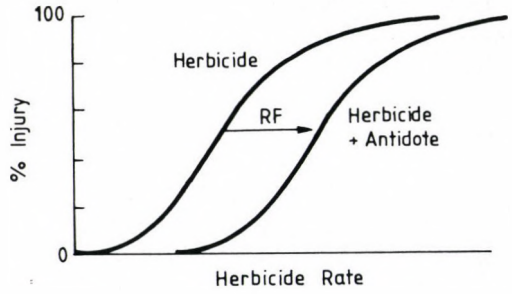

Fig. 5. RF defined graphically with parallel response curves

Two models with a rate factor are the proportionate rate factor (PRF) and the asymptotic rate factor (ARF) models (Green and Bailey, 1988). The PRF model is

$$Y=a_1 + b_1\log(x_1(1+\delta x_2)^{\gamma})$$

and the ARF model is

$$Y = a_1 + b_1 \log(x_1 ((1+\delta x_2)/(1+x_2))^{\gamma})$$

where Y is the mixture response expressed as logit, x_2 is the nonherbicide rate, and δ is the activity parameter for the nonherbicide. The exponential parameter, γ, can be set at 1 for synergists, –1 for herbicide antidotes, or allowed to vary.

The PRF model is appropriate when the herbicide antidote increases its activity as its rate increases. In the ARF model, the activity of the antidote plateaus with increasing rate. Whole plant tests usually cannot make these distinctions.

Figure 6 plots such a model with the herbicide butachlor [*N*-(buthoxymethyl)-2-chloro-*N*(2,6-diethylphenyl)acetamide] and the herbicide antidote, phenyl-2-chloro-4-trifluoromethyl-5-thiazole-carboxylate. The data is from a factorial experiment on rice *(Oryza sativa)* published in patent (Howe and Lee, 1980). The antidote does not injure rice, and thus no contours intersect the horizontal axis. The response curve for butachlor is on the vertical axis. The contours radiating out from this axis show an upward bend and thus indicate the antidote reduced the rice phytotoxicity.

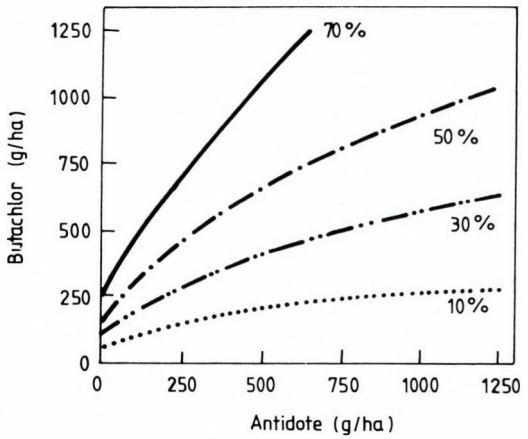

Fig. 6. Mixture response of a herbicide antidote, phenyl-2-chloro-4-trifluoromethyl-5-thiazolecarboxylate, and butachlor, on rice

Herbicide-Herbicide Mixtures. A more complex mathematical model is needed to quantify the joint action of two herbicides. The model should start with the individual herbicide response equations and be based on the*additivity* of doses or rates, not the *additivity of effects* (Green and Streibig, in press). Several additive dose models (ADM) do this, but they usually require parallel response curves.

We commonly use the equvivalents model (EQM) because it does not require parallel response curves and can quantify joint action (Green and Bailey, 1988).

EQM uses each herbicide response equation so that with a log-logit equation the mixture model is

$$Y = p(a_1 + b_1\log(x_1 + \Theta x_{1(2)})) + q(x_2 + b_2\log(x_2 + \Theta x_{2(1)}))$$

where Y is the mixture response expressed as logit, x_1 and x_2 are the herbicide rates, a_1 and a_2 are the intercepts and b_1 and b_2 are the slopes of the log-logit response equations, $x_{1(2)}$ and $x_{2(1)}$ are equivalent rates of each herbicide in terms of the other (Fig. 7), and Θ is the joint action parameter. The p and q terms always add to one and weight the individual response equations in proportion to their biological activity.

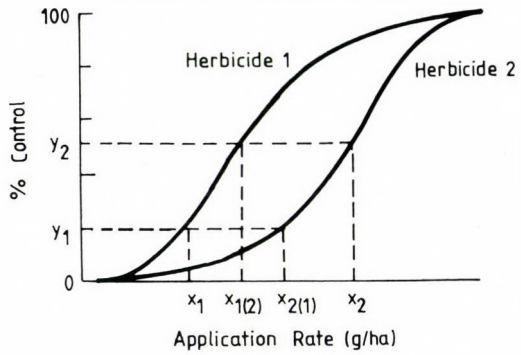

Fig. 7. EQM terms defined graphically

In the expected or additive case, Θ equals 1. If Θ is greater than 1, it is synergistic; if Θ is less than 1, it is antagonistic. If the response curves are parallel, this definition corresponds exactly to Figure 1.

Some mixtures are very antagonistic and require the inclusion of a rate factor like PRF in EQM. When Θ reaches 0, EQM is simplified to

$$Y = p(a_1 + b_1\log(x_1)) + q(a_2 + b_2\log(x_2)).$$

Incorporating a rate factor term EQM evolves to

$$Y = p(a_1 + b_1\log(x_1/(1-\Theta x_{1(2)})) + q(a_2 + b_2\log(x_2/(1-\Theta x_{2(1)}))$$

and can model strongly antagonistic mixtures[1].

Conclusions

Many factors that affect herbicide joint and reduce weed control can be identified and managed. A basic physiological understanding of new herbicides is needed so these factors can be rapidly identified and solutions sought early in their product development.

With more herbicides and the use of more complex mixtures, our historical empirical approach to optimize mixture performance will not work because there are too many possibilities to test. A more efficient approach is needed that incorporates empirical testing, physiological understanding, and matemathical modeling.

Such an approach will start with our knowledge of how the herbicide(s) perform alone. This can be determined from one of the large computer databases now available that contain data for herbicide performance from thousands of field tests. From this data, rate response equations can be determined for each herbicide under appropriate conditions. The strengths and weaknesses of the herbicides can be matched systematically and logical mixture partners selected.

Experiments then can quantify the joint action of these mixtures. Models using this joint action and the rate response equations can accurately project biological mixture performance. These projections, in conjunction with economic analyses and comparison with existing products, can focus further product develoment effort on the mixture rates and rations most likely to succeed.

Literature

Akobundu, I. O., Sweet, R. D. and Duke, W.B. (1975): A method of evaluating herbicide combinations and determining herbicide synergism. Weed Sci. 23,20.

Delvo, H. W. (1987): Herbicide use in wheat and soybean production, Proc. North Cent. Weed Control Conf., 42,28

[1]Program is SAS[R], registered trademark of SAS Institute, Inc., Cary, North Carolina, U.S.A., is available from authors.

Gerwick, B. C. and Kleschick, W. A. (1990): DE-498: a new broadspectrum herbicide for soybeans and other crops, Proc. North Central Weed Sci. Soc.

Green, J. M. (1989): Herbicide antagonism at the whole plant level, Weed Technology, 3, 217.

Green, J. M. and Bailey, S. P. (1988): Herbicide interaction with herbicides and other agricultural chemicals. In Methods of Applying Herbicides, McWhorter, C. G. and Gebhardt, M. R. (eds): Weed Science Society of America, Champaign.

Green, J. M., Leek, G. L., Strachan, S. D., Palm. H. L. and Rowe, S. W. (1990): DPX-79406 - a new postemergence herbicide product for corn, Abstr. Weed Sci. Soc. Am., 30, 4.

Green, J. M. and Streibig, J. C.: Herbicide Mixtures, in Herbicide Bioassays, Streibig, J. C. and Kudsk, P. (eds), CRC Press, Boca Raton, Florida, in press.

Hayward. D. M., Colby, S. R. and Parham, M. R. (1988): Enhancement of paraquat activity with photosyntethic inhibitors, Abstr. Weed Sci. Soc. Am., 28, 5.

Howe, R. K. and Lee, L. F. (1980): 2-4-Disubstituted-5-thiazole-carboxylic acids and derivatives. U. S. Patent 4, 199, 506.

Muir, R. M. and Hansch, C. (1955): Chemical constitution as related to growth regulator action. Ann. Rec. Plant Physiol., 6, 157.

Penner, D. (1989): The impact of Adjuvants on Herbicide Antagonism, Weed Technology, 3, 227.

Rummens, F. H. A., Rummens-Ditters, D. C. M. and Smith, A. E. (1975): The effects of diallate and its isomers on the growth of wild oats, Weed Sci., 23, 11.

Streibig, J. C. (1981): A method for determining the biological effect of herbicide mixtures, Weed Sci., 29, 469.

Yuyama, T., Shirakura, S. and Ishizuka, K. (1990): Mode of safener action of dimepiperate, a thiocarbamate herbicide, on bensulfuron methyl activity. In International Symposium on Herbicide Safeners: Chemistry, Mode of Action, and Applications, Hatzios, K. K. and Kőmíves, T. (eds). Budapest, Hungary.

Zoner, P. S., Evans, J. R., Gourd, D. R. and Carlson, D. R. (1989): A basis for eliminating bentazon-induced antagonism of sethoxydim with spray additives, Second International Symposium on Adjuvants for Agrichemicals, Foy, C. L. (ed.) Blacksburg, Virginia.

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Safening of Corn Against Clomazone Injury with Naphthalic Anhydride: Examination of Possible Hybrid Effects*

DAVID W. KEIFER

FMC Corporation, Agricultural Chemicals Group, Box 8, Princeton, New Jersey 08543, USA

Clomazone, (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) causes injury to corn that in the early stages is characterized by the presence of white or discolored tissue due to the inhibition of pigment synthesis. Corn hybrids differ in the sensitivity to clomazone injury as assessed by this early injury (Keifer, 1989). This difference in sensitivity is a property of the hybrid that can be observed in either greenhouse or field trials, and is a trait that is inherited. Seed treatment with naphthalic anhydride (0.5% w/w) provides added protection to corn (*Zea mays* L.) against this clomazone injury, in both greenhouse (3 to 4X safening) and field trials (2 to 4X safening).

In order to answer concerns about the spectrum of corn hybrids on which this treatment would provide protection, the degree of this protection, and whether there was any interaction with the genetics, an extensive number of corn hybrids were tested in the greenhouse with and without naphthalic anhydride as a 0.5% seed treatment. The test (fundamentally as described in the paper cited above) had five rates of clomazone and five replicates. This test was designed to evaluate the sensitivity of the hybrids by examining the rate response of the early discoloration injury. The treatments with and without naphthalic anhydride were in separate flats but the flats were paired to get the best possible determination of the treatment effect. We tested 255 hybrids one time each, and tested three hybrids multiple (15–22) times. All hybrids were safened, on average, by a factor of 3.4-fold (meaning that in the presence of the naphthalic anhydride a 3.4 fold higher rate of herbicide can be tolerated by the corn for the same level of injury).

When the data, on the sensitivity with the naphthalic anhydride treatment, were examined in terms of the inherent sensitivity of the hybrids, it was found that more sensitive hybrids were safened slightly more than the more tolerant hybrids (Fig. 1). This result suggests that the mechanism of tolerance may

*Based on a paper presented at the International conference on Herbicide safeners, 12th–15th August, 1990. Budapest, Hungary

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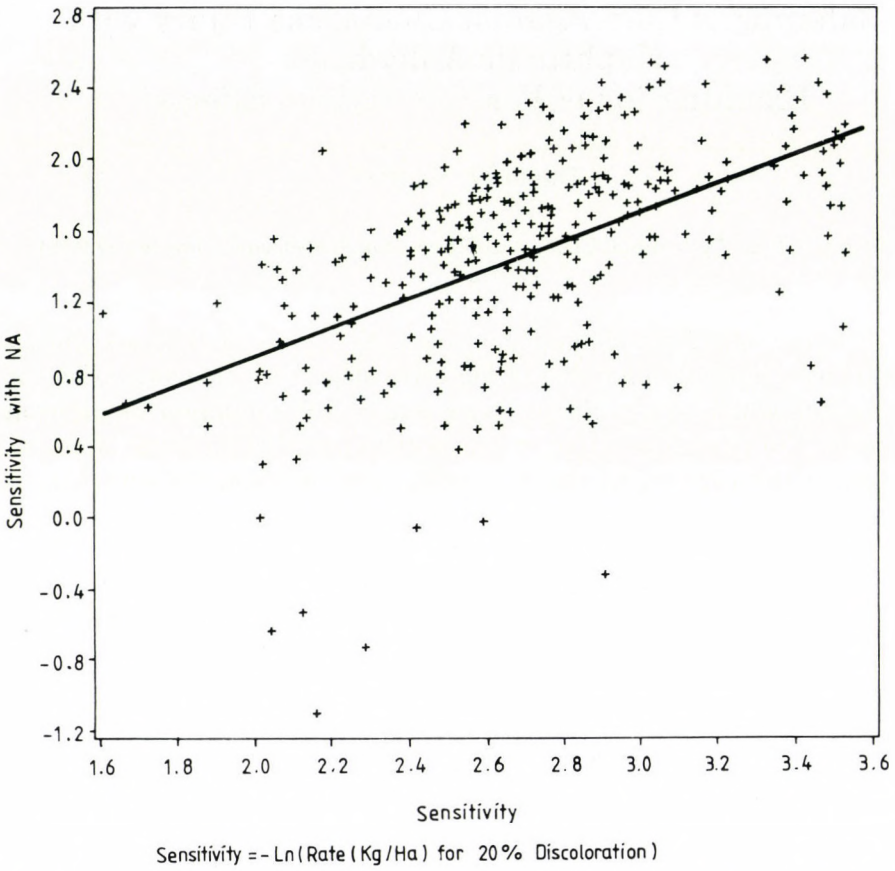

Fig. 1. Corn hybrid sensitivity with and without naphthalic anhydride, for 258 different hybrids. The relationship is highly significant (PR > F of 0.0001). The linear regression has an R^2 of 27%, an intercept of –0.71, and a slope of 0.80

have more potential for enhancement in the more sensitive hybrids. A tolerant hybrid with the safener typically remained more tolerant of clomazone than a sensitive hybrid with safener.

The scatter in the data is mainly a result of an inability to more precisely quantify the sensitivity of the hybrid. Figure 2 shows the results of multiple measurements made on three hybrids and illustrates the range of repeat measurements. This scatter limits our ability to discriminate small differences, so we conclude that there seems to be a single distribution about the mean, with no indication that there could be more than one mechanism of safening response for the hybrids of different clomazone sensitivity.

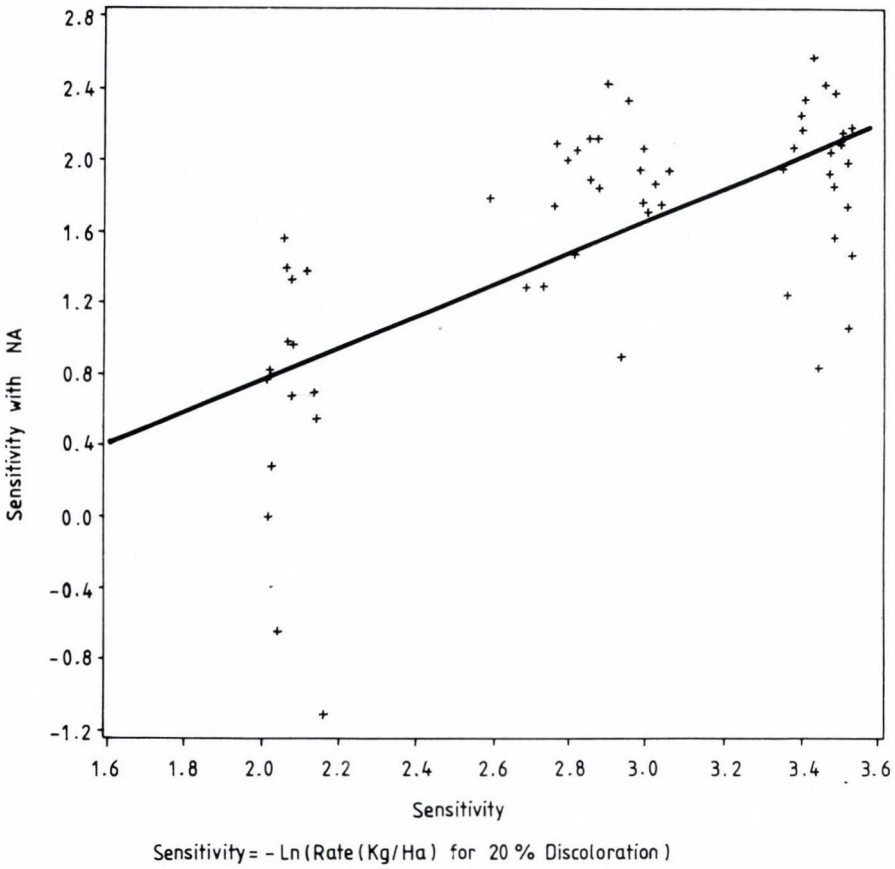

Fig. 2. Corn hybrid sensitivity with and without naphthalic anhydride, for 258 different hybrids. The relationship is highly significant (PR > F of 0.0001).
The linear regression has an R of 27%, an intercept of -0.71, and a slope of 0.80

While naphthalic anhydride treatment decreased the sensitivity of all the hybrids, the extent of safening (3.4-fold) was less than the range of inherent sensitivity (7.5-fold) of the hybrids in these tests. This means that a tolerant hybrid without safener, could still be more tolerant than a sensitive hybrid with the safener.

Acknowledgements

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Literature

Keifer, D. W. (1989): Tolerance of corn lines to clomazone. Weed Sci., 37, 622.

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Effect of Herbicide Safener on Rice (*Oryza sativa* L.) Sprouted Seedlings for Machine Transplanting in Korea*

KIL-UNG KIM, SOON-TAE KWON, DONG-HYUN SHIN and NOH-WON JUNG

Department of Agronomy, College of Agriculture, Kyungpook National University, Taegu, 702-701, Republic of Korea

Out of 12 paddy herbicides tested, pyrazolate [4-(2,4-dichloro- benzoyl)-1,3-dimethyl-5-pyrazolyl-p-toluenesulfonate], pyrazoxyfen[1,3- dimethyl-4-(2,4-dichlorobenzoyl)-5-phenacycloxy-pyrazole], the mixture of pyrazoxyfen at 1.8kg a.i./ha with quinclorac(3,7-dichloro-8-quinoline carboxylic acid) at 210g a.i./ha, the mixture of bentazon[3-isopropyl-1H-2, 1, 3-benzothiadiazin-4(3H)-one-2,2-dioxidel] at 3.0kg a.i./ha with quinclorac at 300s a.i./ha, and the mixture of bensulfuron-methyl [methyl-2-((((((4, 6-dimethoxy-2-pyrimidinyl) amino) carbonyl) amino) sulfonyl) methyl) benzoate] at 51g a.i./ha with mefenacet (2-benzothiazol-2-yloxy-N-methylacetanilide) at 750s a.i./ha and with dimepiperate(MY 93) at 2.1 kg a.i./ha, and pretilachlor at 300s a.i./ha with a safener, fenclorim at 100g a.i./ha, appeared to be promising herbicides for the sprouted seedlings (known as the young rice seedlings) culture although they caused a slight injury on them. Particularly, an application of dymron [1-(α, α-dimethylbenzyl)-3-(p-tolyl)urea] at 300–450g a.i./ha almost completely reduced bensulfuron-methyl/mefenacet mixture injury to the young rice seedlings, exhibiting acceptable safening effect on rice plant. A new growth regulator, homobrassinolide (22s, 23s-homobrassinolide) at 0.01–1.0 ppb appeared to be effective for safening the young rice seedlings against the mixture of bensulfuron-methyl with mefenacet and pyrazoxyfen with quinclorac. Homobrassinolide pretreatment of 1.0 ppb markedly reduced herbicidal injury of bensulfuron-methyl/mefenacet at 30kg prod.(0.17+2.5% a.i.)/ha and promoted plant height of the young rice seedlings treated with pyrazoxyfen/quinclorac at 30kg prod.(6.0+0.7% a.i.)/ha.

Management of rice nurserybed for either hand transplanting or machine transplanting required a lot of labor to rear rice seedlings under Korean conditions.

Recently rice sprouted seedling culture [known as the young rice seedlings (8–10 days old) having 1.5–2.0 leaf stage at time of transplanting has received a great attention due to economization of nurserybed management. It is known that the machine transplanting of the young rice seedlings can markedly reduce the cost of nurserybed management as much as 54%, as compared to the ordinary nurserybed management (3.5 leaf stage) for the machine transplanting in Korea (5).

*Based on a paper presented at the International conference on Herbicide safeners, 12th–15th August, 1990. Budapest, Hungary

It was suggested that the young rice seedling culture has the following advantages such as firstly easy rearing of seedlings, needed only 8–10 days after emergence, secondly desirable plant height of seedlings for machine transplanter, thirdly high adaptability to unfavorable environmental conditions, especially low and high temperature, and disease etc., fourthly rapid early growth immediately after transplanting due to nutritional supply from 30–50% remained endosperm etc. (5). Due to these advantages, it is expected that the area transplanted with the young rice seedlings will be expanded to about 25% of the total area of paddy field in Korea, reaching to 300,000 ha in 1991.

However one of the most difficult problems encountered in the young seedling culture, although it has several advantages, is weed control, As of 1990, 42 herbicides were recommended to be used in rice field in Korea. Over 90% of them is the preemergence soil applied herbicides which are applied at 5 to 7 days after transplanting. No herbicide among the preemergence type herbicides available today in market can be recommended to these transplanted young rice seedlings under field conditions in the rice field, considering weed control efficacy and rice injury. Thus successful introduction of the young seedling culture in Korea largely depends upon recommendation of appropriate herbicides which are very safe and at the same time exert satisfactory weed control.

In this regard, an alternative way for increasing rice safety may be the use of safener/herbicide combinations. At present, the most widely used herbicides are the chloroacetanilide type, covering over 70% of the paddy herbicides(1) either used in single formulation or in combination with sulfonylurea type herbicides which are effective to perennial weeds. Recently, the sulfonylurea group herbicides like bensulfuron-methyl, and pyrazosulfuron which require very low dose per unit area and possess extremely high herbicidal activity and very low mammalian toxicity, have been introduced in paddy rice field. The mixtures of these herbicides with other annual herbicides are receiving a great attention because they can be regarded as one-shot herbicide which can control both annual and perennial weeds at one time application. Thus it seems to be important to evaluate the safening effects of the young rice seedlings against these groups of herbicides.

There are some reports available about safening effects of some chemicals and growth regulators against rice injury caused by these group of herbicides treatments. Quandranti and Ebner (6) reported the first commercially developed safener, fenclorim which protects direct seeded rice against injury from the chloroacetanilide herbicide pretilachlor. Yuyama et al. (9) reported that thiocarbamate herbicides such as thiobencarb, dimepiperate (MY 93), isopropylate (CH-83) and SC-2957 significantly reduced bensulfuron-methyl

injury to the paddy rice plants. Brassinolide, a new plant growth regulator, has been also reported to exert safening of rice against butachlor, pretilachlor and simetryn when applied at seed or root (8). There was a comprehensive review article about rice safening against herbicides in Japan made by Matsunaka and Wakabayashi (4).

This study has attempted to evaluate visual injury of the young rice seedlings to the most commonly used soil applied type herbicides in Korea and the influence of different rates of dymron on safening of bensulfuron-methyl/mefenacet and homobrassinolide on safening of bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac herbicides against the young rice seedlings.

Materials and Methods

Rearing of the young rice seedlings. Two rice varieties such as O-dae (Japonica type) and Samgang (JaponicaxIndica) which were harvested in fall, 1989, were provided by the Youngnam Crop Experiment Station, Rural Development Administration. Rice seeds were sterilized in NaOCl for 15 min and soaked for 3 days at 25 °C for pregermination and pregerminated seeds with uniform appearance were seeded in nursery boxes with size of 30 cm to 60 cm which were filled with nurserybed soil. 200–220 g of rice seeds per box were seeded requiring 15 boxes per 1/10 ha. The rice plant were grown in nursery boxes under a growth chamber with 16 h light at 25 °C and 8 h dark at 18 °C. Difference of rearing of the young rice seedling as compared to the ordinary seedling culture for the machine transplanting is presented in Fig. 1. Hardening stage is missed in the young rice seedling culture as one of

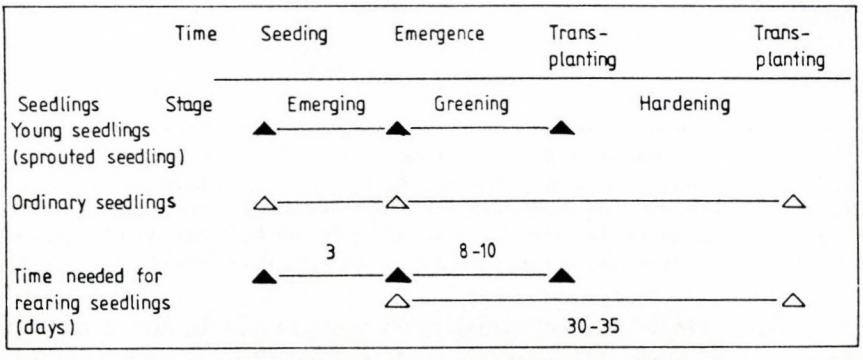

Fig. 1. Rearing of the young rice seedlings in comparison with ordinary nursery

great advantages. Eight days old rice seedlings with 1.5–2.0 leaves were transplanted in a 1/5,000a (1a = 1/100 ha) Wasner pot in May 18, 1990. The pots were placed in the green house where temperature was ranged from approximately 18 to 30 °C during the experimental period. Water was maintained at a depth of 2 cm after herbicide applications. The soil used was clay-loam with organic matter content of 2.5% and with pH of 5.6. Nitrogen fertilizer was applied at 0.2 g per 1/5,000a as basal applications.

Herbicides. Twelve herbicides such as pyrazolate, pyrazoxyfen, and pyrazoxyfen plus quinclorac, bensulfuron-methyl plus butachlor and plus pretilachlor and plus quinclorac, and plus mefenacet, and plus dimepiperate (MY 93), and plus mefenacet, mefenacet single application, bentazon plus quinclorac, and pretilachlor with fenclorim etc. were used to evaluate their effects on visual injury against the transplanted young rice seedlings. Herbicidal dose and application times are presented in Table 1. A Japonica type rice variety, 0-dae only was used for visual evaluation of the young rice seedling against 12 herbicides.

Table 1

Visual injury of the young rice seedlings planted in pot affected by various herbicides in green house[1]

Herbicides	a.i. (%)	Visual injury rating (0–9)[2] 0 — 1 — 2
Bensulfuron-methyl/butachlor	0.17+2.5	XXX
Bensulfuron-methyl/dimepiperate[3]	0.13+7.0	XXX
Bensulfuron-methyl/mefenacet	0.17+2.5	XXX
Bensulfuron-methyl/pretilachlor	0.17+1.0	XXX
Bensulfuron-methyl/quinclorac	0.17+1.0	XXX
Bentazon/quinclorac	10.0+1.0	XXX
Mefenacet	4.0	XXX
Pretilachlor/fenclorim[3]	2.0+0.7	XXX
Pyrazolate	6.0	XXX
Pyrazoxyfen[3]	10.0	XXX
Pyrazoxyfen/quinclorac[3]	6.0+0.7	XXX

[1]All herbicides applied at the rate of 30kg, prod./ha at 5 DAT except for bensulfuron-methyl/quinclorac and pyrazoxyfen/quinclorac applied at 10 DAT, bentazon quinclorac applied at 15 DAT. [2]Visual injury : 0 represents no injury, 9 represents plant death, determined at 15 days after herbicide application. [3]Dimepiperate, pretilachlor/fenclorim, pyrazoxyfen/quinclorac and pyrazoxyfen single formulation are not yet registered in Korea (as of 1990)

Safener. On the basis of visual injury screening of the above-mentioned herbicides, two herbicide mixtures such as bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac were selected as the promising herbicides to be

used in the young rice seedlings for further study to determine safening effects of dymron and brassinolide on injury of these specific mixture herbicides.

Various rates of dymron at 0, 300, 450, 600, and 700g a.i./ha applied at the same time of bensulfuron-methyl/mefenacet mixture application at 30 kg prod. (0.17/2.5% a.i.)/ha.

Effects of homobrassinolide as a synthesized brassinolide, on safening of the young rice seedlings against bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac were examined. The young rice seedlings were pulled out and their roots were soaked in homobrassinolide solution at the concentration of 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} ppm for 24 h just before transplanting. At 5 days after transplanting (DAT), two herbicide mixtures were applied for evaluating their effects on dry weight and plant height of two rice varieties.

Data taken and analysis of results. In the green house, all experiments were completely randomized designs with treatments replicated at three times, however five experiments were installed for visual injury evaluation. Visual injury evaluations were made at 20 DAT assigning a value from 0 to 9 (0 represents no injury to crop, and 9 represents plant death). Dry weight was obtained at 25 DAT for each variety by harvesting 1/5,000a Wagner pot. Plant height was determined at 5 days intervals till 25 DAT to check the influence of homobrassinolide pretreatment at 10^{-3} ppm on the growth of rice plants treated with the above two selected herbicides applied at 5 DAT. An analysis of variance was performed on all data and means were compared by either Duncan's multiple range test or LSD value calculated 0.05 level of significance.

Results and Discussion

Visual injury. In pot experiment under greenhouse, all the herbicides applied resulted in slight visual injury to the young rice seedlings (Table 1). Out of all the herbicides tested, pyrazolate and pyrazoxyfen appeared to be the most safe herbicides showing less than 1 degree of injury, followed by bentazon/quinchlorac, and pyrazoxyfen/ quinclorac and bensulfuron-methyl/ dimepiperate showing injury near to 1. The last two of them were not yet registered in Korea (Table 1). The rest showed 1 to 2 degrees of injury although it was known that they exerted less than 1 degree of injury when used in the ordinary seedlings (3 to 4 leaf stage) planted by machine transplanter in the paddy field. However, it was somewhat interesting to know that most of herbicides used in this pot experiment exerted no injury on rice under the

field conditions in rice growing season in 1990 (data not shown). It seems to be rather difficult to assess precisely herbicidal injury or phytotoxicity because it can be greatly affected by environmental factors.

Based on visual observation, bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac mixtures were selected for further study to evaluate safening effect of dymron and brassinolide on the young rice seedlings.

Safener effectiveness. Dymron reduced injury of the young rice seedlings caused by preemergent application of bensulfuron-methyl/mefenacet, regardless of varieties (Table 2). Thus dymron can be regarded as a safener against this mixture herbicide. A Japonica type rice variety, 0-dae in terms of dry weight showed approximately 20% reduction by the bensulfuron-methyl /mefenacet treatment. Out of dymron rates used the best safening effect was observed at 450g a.i./ha showing complete reduction of injury by bensulfuron-methyl/mefenacet treatment, followed by 300g a.i./ha (Table 2). However, no safening effects were observed at more than 600g a.i./ha of dymron. The similar trend of injury was observed in plant height in both Japonica type rice variety, 0-dae and Samgang, an Indica type.

Table 2

Safening effect of dymron on rice injury caused by bensulfuron-methyl/mefenacet herbicide mixture at 30kg prod. (0.17+2.5% a.i.)/ha[1]

Rice varieties Rates of dymron (g a.i./ha)	0-dae		Samgang	
	Plant height[2]	Dry weight[2]	Plant height	Dry weight
		% of control		
0	78.5c	83.7bc	81.3bc	82.6b
300	90.4ab	86.4b	87.4b	97.7a
450	93.0a	96.7a	95.6a	99.2a
600	81.7e	78.8c	75.6c	60.0c
750	84.2bc	81.6bc	78.4c	68.9c

[1]Means within columns followed by same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test. [2]Determined at 25 days after transplanting.

It can be concluded that mixtures of dymron at 300–450g a.i./ha with bensulfuron-methyl at 51g a.i./ha /mefenacet at 750g a.i./ha would be safe for use on the young rice seedling culture. Yuyama et al. (9) reported the safening effect of several thiocarbamate herbicides against bensulfuron-methyl. Increased metabolism of bensulfuron-methyl upon treatment of thiobencarb herbicide can be an important safening mechanism of rice against bensulfuron-methyl(7). Mechanism of dymron as a safener of rice against injury from the

herbicide bensulfuron-methyl/mefenacet mixture is not known. Further study is needed to clarify its safening mechanism.

It was known that brassinolide, a new growth regulator, has little activity on crop plants which are growing normally, but does affect them when they grow under various stress, such as low temperature, herbicide treatment, disease, cutting, or salt accumulation (2). Homobrassinolide at 10^{-2} to 10^{-4} ppm significantly reduced the injury of the transplanted young rice seedling of 0-dae (Japonica type) by bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac treatments (Table 3). Response of an Indica type, Samgang, was much less than that of 0-dae, showing no significant role of brassinolide in reducing injury on Samgang variety. The highest brassinolide concentration, 10^{-1} ppm reduced dry weight more than that of the untreated one in both herbicide treatments.

Table 3

Safening effect of homobrassinolide (HBR) pretreated rice seedlings on herbicides, bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac herbicides applied at 30kg prod./ha[1]

Herbicides	Bensulfuron-methyl/ mefenacet		Pyrazoxyfen/ quinclorac	
Conc. of HBR (ppm)	Varieties 0-dae	Samgang	0-dae	Samgang
		g / 6 plants		
0	4.06d	3.37b	4.59c	3.97ab
10^{-4}	5.42a	4.77a	5.35a	4.19a
10^{-3}	4.98ab	4.75a	5.23ab	4.17a
10^{-2}	5.28a	3.53b	4.97abc	4.37a
10^{-1}	4.16cd	3.44b	3.63d	3.47b
Untreated con.	4.67bc	4.32a	4.67bc	4.32a

[1]Means within columns followed by same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

The safening effect of brassinolide on rice injury by simetryn was reported to be caused by inhibited absorption of simetryn (8). There were reports available regarding effectiveness of the growth regulator, brassinolide for safening rice against butachlor and pretilachlor when treated to seed or root (8). It is not clear yet about a promoting or safening effect of homobrassinolide against these two mixture herbicides. The data obtained in this experiment proved an important role of homobrassinolide in safening the young rice seedlings against bensulfuron-methyl/mefenacet and pyrazoxyfen/ quinchlorac herbicides.

Figures 2 and 3 showed that homobrassinolide pretreated seedlings at 10^{-3} ppm greatly increased plant height of the transplanted young rice seedlings as the time went by, as compared to the untreated control. Bensulfuron-methyl/mefenacet treatment in the transplanted young rice seedlings inhibited the plant height of both varieties, showing greater inhibition in a Japonica type, 0-dae. The similar trend of homobrassinolide effect on plant height was also observed in pyrazoxyfen /quinclorac treatment, but there was no difference in plant height between pyrazoxyfen/quinclorac treated and the untreated one, meaning that pyrazoxyfen/quinclorac mixture may be more safe to the young rice seedlings as compared to bensulfuron-methyl/quinclorac treatment as already mentioned in visual injury evaluation.

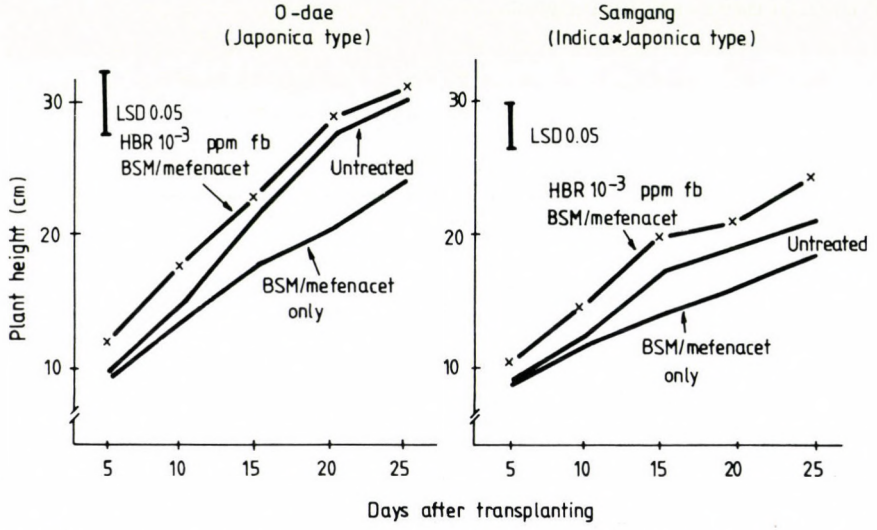

Fig. 2. Changes in plant height of the transplanted young rice seedlings as affected by homobrassinolide (HBR) pretreatment at 10^{-3} ppm followed by bensulfuron-methyl (BSM)/mefenacet applied at 30 kg prod. (0.17 + 2.5 %) a.i.)/ha

Based on the above observations, it can be summarized that dymron exerted the safening of rice against bensulfuron-methyl/mefenacet herbicide mixture when used in the transplanted young rice seedlings (8-day-old seedlings), and homobrassinolide pretreatment for 24 h before transplanting of the young rice seedling markedly increased dry weight and plant height of both varieties when treated with bensulfuron-methyl/mefenacet and pyrazoxyfen/quinclorac herbicide mixture. No study has been made to elucidate the mechanism of action of dymron and a growth regulator, homobrassinolide regarding their safening effect of these young rice plants.

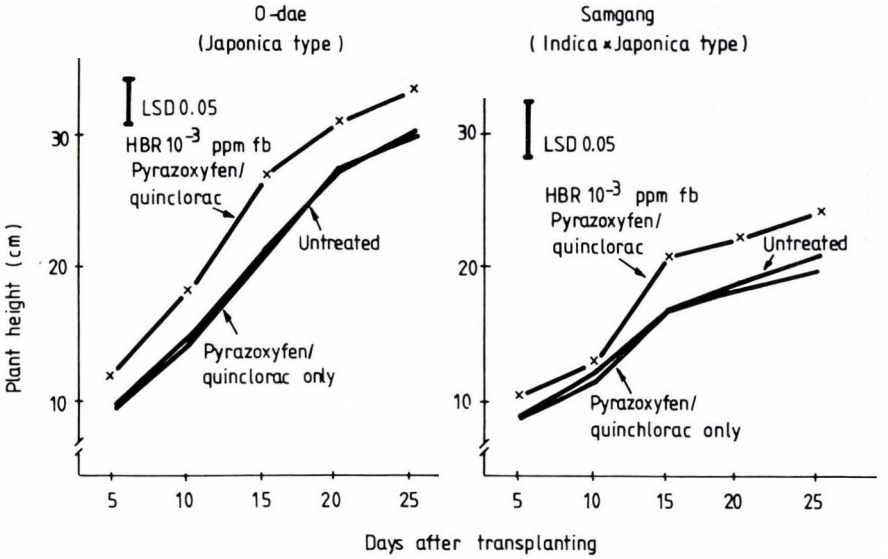

Fig. 3. Changes in plant height of the transplanted young rice seedlings as affected by homobrassinolide (HBR) pretreatment at 10^{-3} ppm followed by pyrazoxyfen/quinclorac at 30 kg prod. (6.0+0.7 %) a.i.)/ha

As described by Hatzios (3), safeners are believed to act either as "bio-regulators" influencing the amount of a given herbicide that reaches its target site in an active form or as "antagonists" of herbicidal effects at a common site of action.

Further studies on the main mechanisms of safening by dymron and homobrassinolide may shed more light on the establishment of weed control method based on herbicide for the young rice seedling culture in Korea.

Literature

1. Agrochemical Year Book. (1990): Korean Agricultural Chemicals Industrial Association. Munsan Publishing Co. 16 (Korea).
2. Fujita, F. (1985): Kagaku to Seibutsu (in Japanese). 23, 717.
3. Hatzios, K. K. (1989): Crop Safeners for Herbicides, ed. by K. K. Hatzios and R. E. Hoagland. Academic Press, Inc. 88.
4. Matsunaka, S. and Wakabayashi, K. (1989): Crop Safeners for Herbicides (eds): K. K. Hatzios and R. E. Hoagland. Academic Press, Inc. 47
5. Park, R. K. (1990): New Technology of Rice Machine Transplanting Culture. Crop Experiment Station, Rural Development Administration. Munyoung Publishing Co. 15 (Korea)
6. Quadranti, M. and Ebner, L. (1984): Proc. Asian-Pac. Weed Sci. Soc. Conf. 9th. 1983, 405.
7. Takeda S., Erbes. D. L. Sweetser, P. B., Hay, J. V. and Yuyama, T. (1986) Weed Res. (Tokyo) 31. 157.
8. Uesono, T., Nishi S., Fujiwara S. and Hamada K. (1985): 10th Annu. Meet. Pestic. Sci. Soc. Japan, Abstr. No. A108(in Japanese).
9. Yuyama T., Sweetser P. B., Ackerson, R. C. and Takeda, S. (1986): Weed Res. (Tokyo) 31. 164.

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Safening of Fluorochloridone by DKA-24 in Corn (*Zea mays*, L.)[1]

IMRE LÁNSZKY[a] and TAMÁS KŐMÍVES[b]

[a]Ministry of Agriculture, H-1053 Budapest, P.O.B. 73, Hungary; [b]Plant Protection Institute, Hungarian Academy of Science, H-1525 Budapest, P.O.B. 102, Hungary

Phytotoxicity of fluorochloridone with and without added DKA-24 safener to ten hybrids of corn was studied in the field. For comparison the effects of thiolcarbamate-based herbicides (EPTC, EPTC + AD-67 safener, and EPTC + DKA-24 safener) were investigated. Fluorochloridone, when applied at the recommended dose for efficient control of weeds causes phytotoxic symptoms on leaves of corn. These symptoms (appearance of chlorotic areas on the leaves exposed to strongest sunlight) were primarily manifested on corn hybrids with narrower leaves. The application of DKA-24 in a 4:1 herbicide-safener ratio significantly reduced the phytotoxic symptoms of fluorochloridone on leaves of corn, and led to higher yields as well.

Fluorochloridone (1-[3-trifluoromethylphenyl]-3-chloro-4-chloromethyl-2-pyrrolidone) is a new herbicide active ingredient developed for controlling broad-leaved weeds in a number of crops, such as cotton, carrots, potatoes, etc., and is recently applied in Hungary in rapeseed and sunflowers. Unlike the traditional chlorotriazine herbicides, it is a low-dose compound without weed resistance and environmental problems. Therefore, its introduction to control of weeds in corn would be unquestionably beneficial. To achieve this, application of safeners (such as AD-67 [N-dichloroacetyl-1-oxa-4-aza-spiro-4,5-decane], DKA-24 [N,N'diallyl-N'-dichloroacetyl-glycinamide] or MG-191 [2-dichloromethyl- 2-methyl-1,3-dioxolane) already in widespread use in Hungary to counteract chloroacetanilide and thiolcarbamate herbicide damage in this crop [Kőmíves and Iatzios, 1991]) offers a good possibility. This paper reports results of a field study to evaluate the above concept for potential use of fluorochloridone with and without safener in corn against weeds not adequately controlled by thiolcarbamate and/or chloroacetanilide herbicides.

Experimental

Phytotoxicity tests in the field were carried out in a randomized complete block design with four replications as described earlier (Lánszky,

[1]Based on a paper presented at the International Conference on Herbicide Safeners, Budapest, August 13-15, 1990.

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1979). Herbicide damage to the corn plants was characterized according to the EWRC scale as well as by the reduction in the crop yield.

Evaluation of herbicidal efficacy of the treatments was based on phytotoxicity observed on five weed species (*Amaranthus chlorostachys* Willd., *Chenopodium album* L., *Echinochloa crusgalli* L., *Gallinsoga parviflora* C.A.B., *Hibiscus trionum* L.) and ten corn hybrids were (Pioneer 0047 SC, Pioneer 3747 SC, Pioneer 3747 SC CmB/H/1, Pioneer 3707 SC, Pioneer 3732 SC, Pioneer 3925 SC, Pioneer 3839 SC, Pioneer 3901 SC, Pioneer 3906 SC, Ykon [sweetcorn]).

The following commercial emulsifiable concentrates (obtained from North-Hungarian Chemical Works, Sajóbábony, Hungary) were used for preplant incorporated and preemergence treatments of soil throughout the study :

SH-372 90 EC: 70% EPTC + 8% DKA-24 + 12% BB extender
SH-376 80 EC: 72% EPTC + 8% DKA-24
Alirox 80 EC: 72% EPTC + 8% AD-67
Witox 72 EC: 72% EPTC
Racer 25 EC: 25% fluorochloridone
DKA-24 50 EC: 50% DKA-24.

Results and Discussion

Herbicide safeners offer a unique possibility to expand the use spectrum of existing herbicides (Kőmíves and Hatzios, 1991). In accord with previous findings (Stephenson and Yaacoby, 1991), our data clearly indicate that the dichloroacetamide safeners AD-67 and DKA-24 dramatically reduce the phytotoxicity of the thiolcarbamate herbicide EPTC (N,N-dipropyl-S-ethyl-thiolcarbamate) to all the corn hybrids and varieties studied (Table 1). More importantly, phytotoxicity of fluorochloridone to corn hybrids was significantly reduced by DKA-24 (Table 1).

Research findings using a variety of plant-herbicide-safener systems showed that dichloroacetamide safeners exert their protective action by increasing the crop plant's capacity to detoxify the herbicide (Kőmíves and Dutka, 1989a; Kőmíves and Hatzios, 1991). The most important routes of (bio)chemical transformations of herbicides in plants leading to inactive products or derivatives of significantly lower phytotoxicity are oxidative and conjugation processes. Importance of the conjugation reaction (Equation 1) with glutathione in the detoxication of a number of herbicides (e.g., chlorotri-

$$GSH + X-R \xrightarrow{-HX} GSR \qquad \text{Equation 1}$$

GSH = glutathione
X-R = herbicide
(X = leaving group)

Table 1

Weed control and crophytotoxicity of the herbicides studied

	Herbicide	Method of application[a]	Dose l/ha	Weed control[b], (EWRC scale)	Phytotoxicity to corn (EWRC scale)	Corn yield[c] kg/plant
I.	a. SH-372 90 EC	ppi	8.0	1.0	1.0	0.222
	b. SH-372 90 EC	ppi	16.0	1.0	1.0	0.234
II.	a. SH-376 80 EC	ppi	8.0	1.0	1.0	0.246
	b. SH-376 80 EC	ppi	16.0	1.0	1.0	0.195
III.	a. Alirox 80 EC	ppi	8.0	2.1	1.0	0.230
	b. Alirox 80 EC	ppi	16.0	1.0	2.1	0.208
IV.	a. Witox 72 EC	ppi	8.0	1.0	5.6	0.195
	b. Witox 72 EC	ppi	16.0	1.0	7.4	0.169
V.	a. Racer 25 EC	ppi	2.5	2.3	2.5	0.201
	b. Racer 25 EC	ppi	5.0	1.6	4.0	0.188
VI.	DICA-24 50 EC	ppi	0.65	9.0	0.1	0.153
VII.	Va+VI	ppi	2.5+0.65	1.9	1.3	0.247
VIII.	a. Racer 25 EC	pre	2.5	2.2	3.2	0.195
	b. Racer 25 EC	pre	5.0	1.9	4.3	0.181
IX.	VIIIa+VI	pre	2.5+0.65	1.8	1.6	0.267
X.	Untreated control					0.231

[a]ppi=preplant incorporated, pre=preemergence; [b]Average for five weed species; [c]Average for ten hybrids

azines, thiolcarbamates and chloroacetanilides) has been demonstrated (Kőmíves and Dutka, 1989b).

Increased levels of the enzyme glutathione transferase (EC 2.3.4.5) and its cofactor glutathione have been detected in safener treated corn plants (Kőmíves and Dutka, 1989b). Involvement of cytochrome P-450 containing monooxygenases catalyzing oxidative transformations of herbicides in plants has also been implicated in safener mode of action (Kőmíves et al., 1985). We believe that in the safening of fluorochloridone by DKA-24 in corn the conjugation process with glutathione is of importance. Support to this explanation comes from an investigation of the chemical structure of this herbicide: it contains a labile chlorine atom, prone to conjugation with nucleophilic reagents.

Our data suggest that safened fluorochloridone has promising characteristics for possible use in corn. Further research is needed to clarify herbicidal properties of fluorochloridone with other safeners, as well as its combinations containing mixtures with safened thiolcarbamates and/or chloroacetanilides.

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Literature

Dutka, F. and Kőmíves, T. (1987): MG-191: a new selective herbicide antidote. In "Pesticide Science and Biotechnology", Ed. R. Greenhalgh and T. R. Roberts, Pergamon Press, Oxford, pp. 201–204.

Kőmíves, T. and Dutka, F. (1989a): Mode action of the antidote fenclorim in rice. Biochem. Physiol. Pflanzen, 184, 475–477.

Kőmíves, T. and Dutka, F. (1989b): Effects of herbicide safeners on levels and activities of cytochrome P-450 and other enzymes of corn. In "Herbicide Safeners: Development, Uses and Modes of Action" (eds): Hatzios, K. K. and Hoagland, R, E. Academic Press, New York, pp. 129–145.

Kőmíves, T. and Hatzios, K. K. (1991): Chemistry and structure-activity relationships of herbicide safeners, Z. Naturforsch. 46c, 798-804.

Kőmíves, T., Kőmíves, A. V., Balázs, M. and Dutka, F. (1985): Role of glutathione-related enzymes in the mode of action of herbicide antidotes. In "Proc. 1985 British Crop Protection Conference - Weeds, Vol. 3, pp. 1155–1162.

Lánszky, I. (1986): Field studies with herbicides containing the safener DKA-24 using different hybrids of corn. PhD Thesis, University of Debrecen, Hungary.

Stephenson, G. R. and Yaacoby, T. (1991): Milestones in the development of herbicide safeners. Z. Naturforsch. 46c, 867–871.

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Comparative Rates of safener and Acetanilide Movement in two Soil Types under Laboratory Conditions

W. T. MOLIN*, G. M. DILL and E. F. SANDERS

Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, USA

The relative mobility of two thiazolecarboxylic acid safeners and alachlor was examined in columns of two loam soils under laboratory conditions to determine the potential of the thiazolecarboxylates as tank mix safeners. Test compounds were leached through 6.4 cm × 6.0 mm columns of either Muscatine loam or Dupo silt loam soil with 20 ml of water. Column effluent was collected using a fraction collector and quantitation performed via HPLC. Flurazole, a seed applied safener, did not elute from either of the two soil types tested. The parent acid (2-chloro-4-(trifluoro-methyl)-5-thiazolecarboxylic acid) of flurazole eluted with the void volume well in advance of alachlor. Data suggest that understanding the physical properties of safeners relative to their soil mobility is essential in predicting their efficacy as tank mixture or seed treatment safeners.

One of the properties of a safener that distinguishes it as either a soil applied (preemergence) product or as a seed treatment is its mobility in the soil (Stephenson and Ezra, 1983). In practice, safeners protect sensitive species from the toxic effects of a herbicide rather than providing an antidotal or remedial effect. Hence, to be effective as a soil-applied treatment, the safener must move through the soil profile in advance of, or possibly at the same rate as, the herbicide. In contrast, seed applied safeners must remain in the proximity of the seed for sufficient time until the seedling has acquired the capacity to overcome the toxic effects of the herbicide (Pallos and Casida, 1978). The safening activity of flurazole and its free acid against alachlor are comparable, yet only flurazole has utility as a seed treatment safener. This report compares the mobility of flurazole, its acid, and alachlor in two soils and relates these findings to their utility for use as either soil or seed applied safeners.

Materials and Methods

Alachlor (2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide), flurazole (2-chloro-4-trifluoromethyl)-5-triazole-carboxylic acid (phenyl ester), and the free acid of flurazole (2-chloro-4-trifluoromethyl)-5-

*Based on a paper presented at the International Conference on Herbicide Safeners, 12th–15th August (1990) Budapest, Hungary

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triazole-carboxylic acid) were technical grade (>95% purity). A Muscatine loam and a Dupo silt loam soil were used in this study. Ten grams of sifted soil were loaded into each of 6.4 cm × 6.0 mm columns. A 0.2 ml aliquot from 100 µg/ml stock solution of the compound in question was loaded onto a column and the solvent was allowed to evaporate. Each column was eluted with 20 ml of distilled water and 0.5 ml fractions were collected. Each fraction was concentrated and purified by affinity chromatography on Sep Pak C18 columns. Samples were analyzed by HPLC with a Waters system equipped with a 3.9 mm × 25 cm µBondapak C_{18} column, a WISP autoinjector, two M6000 pumps, a Lambda Max 480 detector and a Data Module. Elution was performed under isocratic conditions with a 70:30 mixture of acetonitrile:water at a flow rate of 1 ml/min. Detection was accomplished via absorbance at 340 nm and quantitation via peak areas compared to standard curves generated for each compound. Experiments were repeated three times.

Results and Discussion

The elution profile of alachlor, flurazole, the acid of flurazole, and a hypothetical soil applied safener are shown in Figs 1 and 2. Alachlor was pre-

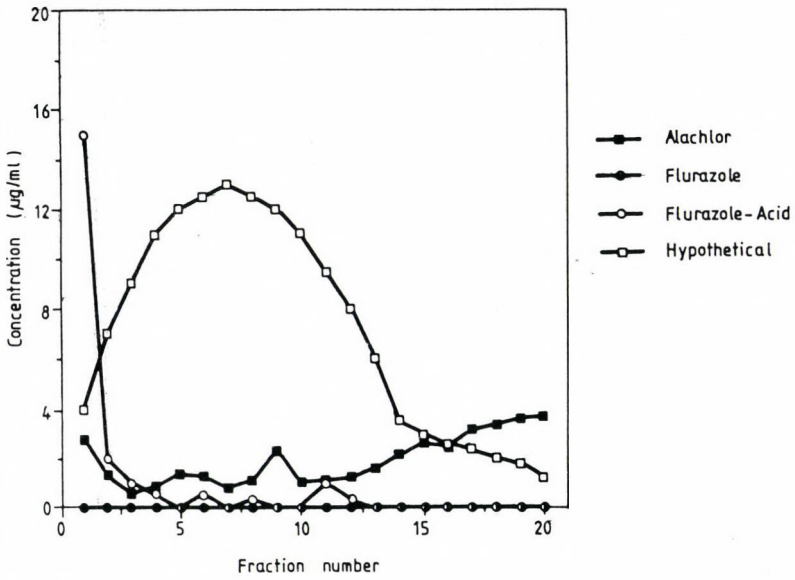

Fig. 1. Plot of alachlor, flurazole, and flurazole-acid concentration after elution through a Dupo silt loam column

sent in all fractions eluted from the column of Dupo silt loam soil (Fig. 1) with concentration increasing gradually with increasing fraction number. On the column of Muscatine loam soil (Fig. 2), alachlor eluted as a broad peak beginning with fraction 6. Flurazole bound tenaciously to both soil types and did not elute from either column. If used as a soil applied treatment, flurazole would not arrive at the seed in advance of the herbicide. Thus, little benefit would be gained from a preemergence application of flurazole. The acid of flurazole eluted with the solvent front in both soils (Figs 1 and 2) and was present in only the first fraction. Hence, although a good safener, the free acid would wash past the seed in the solvent front affording little opportunity to elicit its safening activity. An elution pattern for a hypothetical soil applied safener is drawn in both figures and represents a discovery target. The desired hypothetical safener would be more mobile than alachlor thereby arriving at the seed in advance of the herbicide.

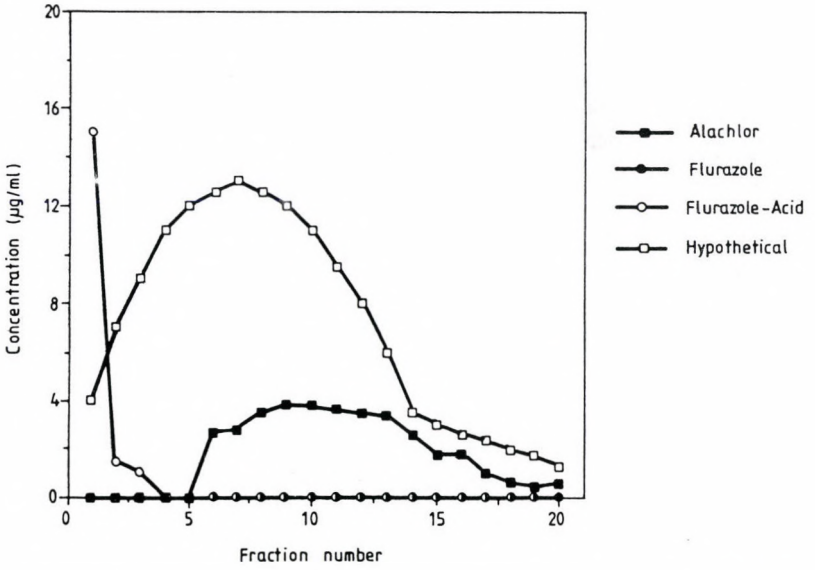

Fig. 2. Plot of alachlor, flurazole, and flurazole-acid concentration after elution through a Muscatine loam soil column

Although flurazole and its acid both have safening properties, the high soil mobility of the acid and the low soil mobility of flurazole negate their utility as soil applied safeners. Flurazole would not move in advance of the herbicide and would not be available to seedlings during ecesis. However, the

low soil mobility of flurazole demonstrates the desired characteristics for a seed treatment safener (i.e. retention of safener in the zone of seed germination), and indeed, its commercial potential as such has been realized.

Literature

Pallos, F. M. and Casida, J. E. (eds): Chemistry and Action of Herbicide Antidotes. Academic Press, New York (1978).

G. R. Stephenson and Ezra, G. (1983): Herbicide antidotes – A New Era in Selective Chemical Weed Control. *In* Plant Growth Regulating Chemicals. Vol. II, Chapt. 11, pp. 193–212.

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Use of Herbicide Safeners in Seed Corn Production*

E. SZÉLL

Cereal Research Institute, Szeged, H-6701, Hungary

Weed control efficacy and crop selectivity of herbicides and herbicide-safener mixtures in corn seed production have been comparatively investigated in the field. Experiments with 12 corn inbred lines and 12 hybrids in two soils indicate that highest corn yields with dependable control of weeds and negligible crop injury can be achieved by using herbicides containing safened thiolcarbamate and chloroacetanilide active ingredients.

Control of weeds during production of seed corn is hindered by the relatively high susceptibility of some corn parental lines to herbicide damage as compared to their hybrids (Keifer, 1989). Therefore, application of strictly selective herbicides for the above process is of vital importance. Traditionally, photosynthesis inhibiting herbicides and compounds with hormone action are used for weed control in hybrid corn seed production (Keifer, 1989). In recent years, however, weed resistance and environmental pollution problems hinder the use of chlorotriazines, while several parental lines of corn are only marginally tolerant to other "standard" maize herbicides (Keifer, 1989). Our project was aimed at the comparative evaluation of herbicides and safeners-herbicide mixtures for potential use in corn seed production.

Experimental

Phytotoxicity tests in the field were carried out in a randomized complete block design with four replications as described earlier (Széll et al., 1985). Herbicide damage to the corn plants was characterized by the number of damaged plants as well as by the reduction in the crop yield. All data were evaluated statistically (Széll et al., 1986).

*Based on a paper presented at the International Conference on Herbicide Safeners, 12th–15th August (1990) Budapest, Hungary

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Results and Discussion

Application of safeners to increase herbicide selectivity is a highly successful concept in weed control (Dutka and Kőmíves, 1987; Kőmíves, 1989; Kőmíves and Dutka, 1988, 1989; Széll et al., 1985, Széll et al., 1986). Herbicide safeners offer a unique possibility to control unwanted vegetation in corn (Kőmives and Hatzios, 1991; Széll et al., 1985, Széll et al., 1986), including volunteer corn plants from previous plantation (Stephenson and Yaacoby, 1991). This is especially critical in case of seed corn production, where genetic homogeneity is of particular importance.

Our experiments clearly indicate that genetic lines of corn are significantly more susceptible to herbicide damage than their hybrids (Table 1). Selectivity of herbicides recommended for use in corn varied from fair to excellent (Table 1).

Table 1

Average corn yields of ten inbred lines and twelve hybrids following weed control with different herbicides in 1987–89 (Újszeged; loamy soil, humus content = 2.4 %)

Herbicide group*	Dose**	Relative yield***	
		Inbred lines	Hybrids
A. Thiolcarbamates and chloroacetanilides + safeners	R	97	98
	RR	90	95
B. Postemergence hormonal compounds	R	85	103
	RR	74	94
C. Postemergence contact compounds	R	96	99
	RR	92	92
D. Photosynthesis inhibitors	R	88	93
	RR	72	85
Mean	R	92	98
	RR	82	92

*A: EPTC, butylate, acetochlor, alachlor; B: 2,4-D, dicamba; C: bromoxynil, pyridate; D: chlorbromuron, linuron, terbutrin. **R = recommended dose, RR = twofold of the recommended dose; ***Yield of hand-weeded control = 100%.

Highest corn yields with dependable weed control efficiency and negligible crop injury were achieved by using thiolcarbamates and chloroacetanilides together with safeners. Protective efficiencies of the two safeners currently produced in Hungary were determined in calcareous sandy soil very poor in humus, which provides rigorous conditions for evaluating herbicide phyto-

toxicity. As indicated by the data in Table 2, AD-67 exhibits good safening activity but the ability of MG-191 to protect twelve corn parental lines against EPTC damage is undoubtedly superior to that of AD-67.

Table 2

Susceptibility of corn parental lines to EPTC safened with AD-67 and MG-191 (Zsombó, 1988; calcareous sandy soil, 0.6% humus content)

Corn parental line	Percentage of damaged plants*;			
	EPTC + AD-67		EPTC + MG-191	
	6 l/ha	12 l/ha	6 l/ha	12 l/ha
1	4	4	1	6
2	9	7	2	0
3	0	6	0	0
4	6	9	5	4
5	3	24	5	5
6	11	26	0	8
7	12	24	4	3
8	7	15	2	5
9	2	2	0	0
10	1	2	3	3
11	1	5	5	5
12	1	7	6	6
Mean	5	11	3	4

*Observations made at the 4 to 5 leaf stage of the plant.

Literature

Dutka, F. and Kőmíves, T. (1987): MG-191: a new selective herbicide antidote. In "Pesticide Science and Biotechnology" (eds): R. Greenhalgh and T. R. Roberts, Pergamon Press, Oxford, pp. 201–204.

Keifer, D. W. (1989): Tolerance of corn *(Zea mays)* lines to clomazone. Weed Science, 37, 622–628.

Kőmíves, T. (1989): Mode action of the antidote fenclorim in rice. Biochem. Physiol. Pflanzen, 184, 475–477.

Kőmíves, T. and Dutka, F. (1988): Mode of action of the safener MG-191. Proc. EWRS Symp. "Factors Affecting Herbicidal Activity and Selectivity", Wageningen, pp. 381–385.

Kőmíves, T. and Dutka, F. (1989): Effects of herbicide safeners on levels and activities of cytochrome P-450 and on the enzymes of corn. In "Herbicide Safeners: Development, Uses and Modes of Action" (eds): K. K.Hatzios and R. E. Hoagland, Academic Press, New York, pp. 129–145.

Kőmíves, T. and Hatzios, K.K. (1991): Chemistry and structure-activity relationships of herbicide safeners. Z. Naturforsch. 46c, 798–804.

Széll, E., Csala, I., Fodor, F., Kőmives, T. and Dutka, F. (1985): Comparative study of a new class of herbicide antidotes. Cereal Res.Commun., 13, 55–58.

Széll, E., Csala, I., Fodor, F., Hulesch, A., Kőmíves, T. and Dutka, F. (1986): Effectiveness of new herbicide antidotes against alachlor phytotoxicity to corn (*Zea mays* L.) Cereal Res. Commun., 14, 397–402.

Stephenson, G. R. and Yaacoby, T. (1991): Milestones in the development of herbicide safeners. Z. Naturforsch. 46c, 794–797.

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