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Contents

PROCEEDINGS

of the

5th Conference on Virus Diseases of Gramineae in Europe held in Budapest, Hungary 24—27 May, 1988

Editey by

I. Milinkó, R. Gáborjányi and P. D. Nagy


WELCOME TO THE
CONFERENCE ON
IN EUROPE

Acta Phytopathologica et Entomologica Hungarica, 26 (1—2), pp. 5—6 (1991)

5th CONFERENCE ON VIRUS DISEASES OF GRAMINEAE IN EUROPE

24–27 MAY, 1988
BUDAPEST, HUNGARY

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Acta Phytopathologica et Entomologica Hungarica 26, 1991
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Acta Phytopathologica et Entomologica Hungarica, 26 (1—2), pp. 7—7 (1991)

Preface

At the statutory meeting and 1st Conference of the European Working Group on Gramineae Viruses (EWGGV) in Belgrad (Jugoslavia) 1974 the founders decided to organize a European Gramineae Virus Conference every 3–4 years in a different member country. The subsequent conferences were held by the virus researchers of cereals in Montpellier (France) 1977, Rothamsted (England) 1980, Braunschweig (GFR) 1984. At this latter the leaders of the organization made the decision of inviting Hungary to organize the next virus conference. The organizing committee of Hungary considered it a great honour that after the previous excellently organized conferences the "5th *Conference on Virus Diseases of Gramineae in Europe*" could be held in Budapest on 24–27 May 1988.

Almost the full material of lectures delivered at the Budapest conference is contained in this volume published now for those interested in the subject.

Keszthely–Budapest, November 1988.

Editors

Acta Phytopathologica et Entomologica Hungarica 26, 1991
Akadémiai Kiadó, Budapest

Acta Phytopathologica et Entomologica Hungarica, 26 (1—2), pp. 9—13 (1991)

Nucleic Acid Hybridization Studies with Swedish BYDV Isolates

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RNA of a Swedish BYDV isolate of the MAV type was used as template for the synthesis of cDNA which was cloned in plasmic pUC19. Some clones hybridize specifically with MAV-type isolates, whereas others hybridize with both MAV- and PAV-like isolates. ^{32}P-labelled probes readily detect BYDV in plant sap and in extracts from single viruliferous aphids in filter hybridization tests. Biotinylated probes detect BYDV in sap samples of oats prepared by a simple procedure without background problems.

Barley yellow dwarf virus (BYDV), the type member of the luteovirus group (Matthews, 1982), is considered to be the economically most important virus infecting cereals (Burnett, 1983). Its serological and transmission characteristics have been studied in great detail and five different types of isolates have been described (Rochow, 1970; Rochow & Carmichael, 1979). More recently the technique of synthesising and cloning complementary DNA (cDNA) has been applied to isolates of BYDV (Waterhouse et al., 1986; Barbara et al., 1987). We have produced a cDNA library from the RNA of a Swedish isolate of the MAV type (Eweida & Oxelfelt, 1988). We describe here the use of radioactive and non-radioactive probes prepared from one of the clones for detecting BYDV in sap samples from infected oats and extracts of viruliferous aphids.

Materials and Methods

An isolate of the MAV type (specifically transmitted by Sitobion avenae) was used as the source of template RNA. Virus inoculation, harvest, and purification were as described previously (Eweida & Oxelfelt, 1985). Procedures for RNA extraction, gel electrophoresis, and synthesis of cDNA were as described by Maniatis et al. (1982). Second strand synthesis was according to Gubler & Hoffman (1983). The doublestranded cDNA was cloned into the EcoRI site of pUC19 which was then used to transform E. coli strain JM83 essentially as described by Maniatis et al. (1982). For further details see Eweida & Oxelfelt (1988). Radioactive labelling of DNA was carried out by nick-translation accord-

ing to Arrand (1985) and biotinylation according to Al-Hakim & Hull (1986) or Habili et al. (1987). Preparation of extracts and filter hybridization were as described previously (Eweida & Oxelfelt, 1988).

Results

The production of a cDNA library from the RNA of a Swedish BYDV isolate of the MAV serotype was described previously (Eweida & Oxelfelt, 1988). It was shown that some clones hybridized specifically with MAV-like isolates, whereas others hybridized with both MAV- and PAV-like isolates. One of the latter, named C10, was used in this study to produce ^{32}P-labelled and biotinylated probes for the detection of BYDV in clarified extracts of infected oat plants

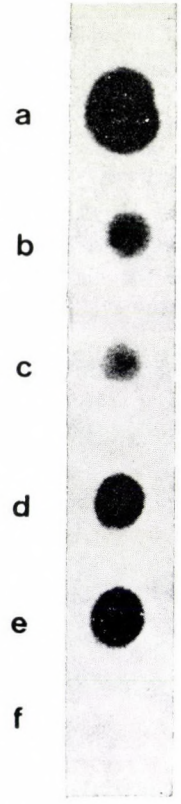

Fig. 1. Dot-blot of various samples using ^{32}p-labelled clone C10 as probe. The samples consisted of a) homologous pure virus, b) extract of aphids viruliferous with PAV-like isolate, c) extract of aphids viruliferous with MAV-like isolate, d) sap of oats infected with MAV-like isolate, e) sap of oats infected with PAV-like isolate, f) sap of healthy oats

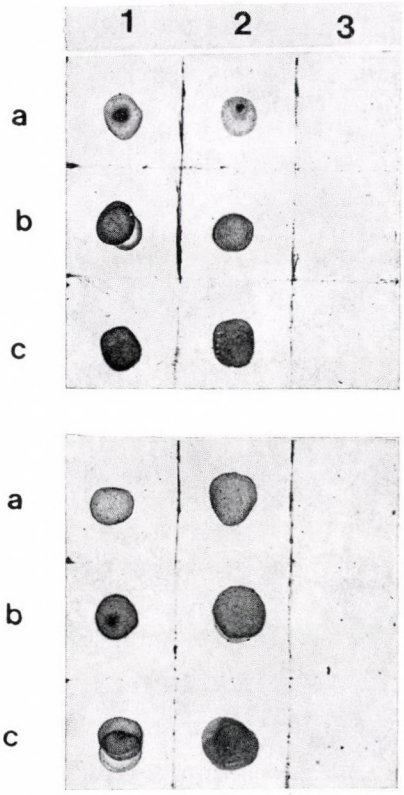

Fig. 2. Dot-blots of sap samples of infectec and healthy oats using biotinylated clone C10 as probe. Top photobiotin-labelled (Habili et al. 1987), bottom labelled according to Al-Hakim and Hull (1986). a) 4 μl, b) 8 μl, c) 12 μl per dot; 1 MAV, 2 PAV, 3 healthy

and viruliferous aphids. Fig. 1 shows a dot-blot of oat and aphid extracts using ^{32}P-labelled C10 as probe. Fig. 2 shows a dot-blot with oat leaf extracts probed with biotinylated C10. Aphid extracts were also tested in a dot-blot test with biotinylated probes but the reaction was equally strong with viruliferous and non-viruliferous aphids (not shown).

Discussion

As shown earlier (Waterhouse et al., 1986; Barbara et al. 1987; Eweida & Oxelfelt, 1988) both serotype-specific and more general cDNA clones can be obtained from BYDV. They can be used for the detection of BYDV in sap samples of infected plants with a sensitivity greater than that of ELISA (Barbara et al., 1987; Eweida & Oxelfelt, 1988). A very simple procedure for clarifying the sap

samples by freezing and a brief centrifugation was used with radioactive probes. As shown in the present study the same procuedure is sufficient to eliminate background problems also when using biotinylated probes in dot-blot tests on nitrocellulose filters. Nylon filters gave very high background and seem to require more efficient blocking procedures. The sensitivity of detection with biotinylated probes is comparable to that of radioactive probes.

The use of non-radioactive cDNA probes appears to be an attractive method for rapid diagnosis of BYDV infection in surveying the incidence of infection in perennial grasses and for screening in breeding work. Also, the fact that BYDV is detectable in single viruliferous aphids and the availability of isolate-specific probes offers the possibility to carry out epidemiological studies on aphids caught in suction traps. It was shown that ^{32}P-labelled probes could detect virus in aphid extracts (Fig. 1 and Eweida & Oxelfelt, 1988), however, when using biotinylated probes a more elaborate procedure for sample preparation is needed.

Acknowledgement

This work was supported by grants from the Swedish Council for Forestry and Agricultural Research. We thank Miss Anna-Karin Widmark for carrying out the tests with biotinylated probes.

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Acta Phytopathologica et Entomologica Hungarica, 26 (1–2), pp. 15–20 (1991)

Comparison between ELISA and Northern Dot Blot Hybridization Techniques for BYDV Detection in Triticinae Species

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Several plant progenies belonging to different genera of the subtribe Triticinae were tested for their response to BYDV infection using both "ELISA" and "Northern Dot Blot Hybridization" (NDBH) techniques. Seedlings, grown in the greenhouse, were infested with viruliferous *Rhopalosiphum padi* (L.) aphids carrying the M-PAV isolate of BYDV. The crude sap in extraction buffer of samples was used for both ELISA and NDBH. Both techniques are sensitive to BYDV in the screened genotypes. Sap extraction followed by chloroform clarification tend to improve the sensitivity of ELISA but not of NDBH, when the techniques are applied to *Avena byzantina* K. Koch. Except for *A. byzantina* the remaining infected progenies, tended not to show evident symptoms of BYD in the greenhouse, particularly those belonging to the genera Aegilops, Daspyrum and Secale.

The current most widely used diagnostic techniques for the rapid detection of Barley Yellow Dwarf Virus (BYDV) in plant extracts are the Enzyme Linked Immunosorbent Assay (ELISA) (Clark, 1981) and the Northern Dot Blot Hybridization technique (NDBH) (Gerlach et al., 1987). ELISA is a serological method that requires specific antisera for virus capsid protein sub-unit detection. NDBH is a nucleic acid hybridization method requiring a specific complementary DNA (cDNA) probe to detect the viral genome. Both techniques need appropriate preparation of plant sap extracts, careful reading of antisera- or cDNA-sap component reaction results, and an objective interpretation of results.

It is known that the nucleic acid probes are at least as sensitive as ELISA but the disadvantage at present is that the sap samples rquire a great amount of preparation for testing (Gerlach et al., 1987).

In the investigation reported in this paper a comparison between ELISA and NDBH techniques for detecting BYDV was made on sap from wheat and related species and *Avena byzantina* K. Koch.

Materials and Methods

Three different experiments were performed.

A) *ELISA and NDBH applied to 69 Triticinae species using extraction buffer* (PBS Tween pH 7.4, with 2% of PVP K-25). The genotypes studied con-

sisted of 45 tetraploid wheat (Triticum turgidum var. durum Desf.), 1 T. boeoticum Boiss, 1 T. urartu Thum, 1 T. monococcum L., 1 T. dicoccoides Korn, 1 T. timopheevi Zhuk, 1 T. polonicum L., 1 T. spelta L.; 1. T. aestivum L. cv. "Chinese Spring", 1 Aegylops squarrosa L., 1 A. speltoides Tausch, 6. Dasypyrum villosum L. and 3 Secale cereale L. progenies, 4 Chinese Spring-D. Villosum disomic addition lines (kindly provided by E. R. Sears), 1 Modoc × D. villosum hexaploid amphiploid.

The virus was a BYDV strain originally derivcd from maize (M-PAV) (Loi et al., 1985).

For each genotype two greenhouse grown potted plants were inoculated at the one leaf stage, using 3 viruliferous *Rhopalosiphum padi* (L.) per plant and one plant was maintained as negative control. The transmission method, described by Osler et al., 1985, was adopted. Twentyfour *A. byzantina* test plants, known to express typical symptoms of BYD in the greenhouse, were also inoculated; the same number of plants were not infested (negative controls). All the plants were checked for symptom expression. One month after inoculation, leaf samples were collected from all the inoculated and non-inoculated (controls) plants and stored at −25 °C. DAS ELISA (Double Antibodies Sandwich ELISA), was performed using an antiserum specific to the PAV strain of BYDV (sensu Rochow, 1979) provided by BIOREBA, Basel, Switzerland. Each sample was diluted 1 : 2 (w/v) in extraction buffer. Diagnosis by ELISA was based on the "3 × healthy background" criterium for a positive A_{405} value.

NDBH procedure (De Pace et al., 1987) was applied directly to the sap of the same test plants used for ELISA but without any chloroform clarification. The probe used was pBY82 (kindly provided by W. L. Gerlach, CSIRO, Australia) ^{32}P radiolabeled. The data record was made using a scintillation counting of the radiactive signal from NC filters. A sample was considered positive when it gave a value of at least 47% c.p.m. higher than the negative control (Casa et al., 1988).

B) *NDBH applied to 56 tetraploid wheat plants, using chloroform cleared sap.* In this experiment plants were infected using the same procedure described in A). The sap extraction and clarification with chloroform were carried out according to De Pace et al., 1987.

C) *ELISA and NDBH applied to A. byzantina using crude sap and chloroform cleared sap.* Six infected *A. byzantina* plants, with clear symptoms of BYD and six non-inoculated plants were each subjected to the two different diagnostic techniques. For each method either crude sap in extraction buffer or chloroform cleared sap was used.

Results

A) *ELISA and NDBH applied to 69 Triticinae species using extraction buffer.* Sixtyeight out of 69 inoculated plant genotypes assayed by ELISA gave a positive reaction (Table 1). Using the NDBH technique 48 out of 69 inoculated

Table 1

M-PAV detection in crude sap extracts of different Triticinae inoculated, using ELISA and NDBH

Detection method	No. genotypes		
	inoculated	positive	negative
ELISA	69	68	1*
NDBH	69	48	21

* Dasypyrum villosum, positive to NDBH.

plant genotypes were positive. The pairwise comparison between ELISA and NDBH applied to infected plants, showed that only 47 genotypes gave concordant results.

B) *NDBH applied to 56 tetraploid wheat plants, using chloroform cleared sap.* The autoradiographic signals showed significant differences in intensity

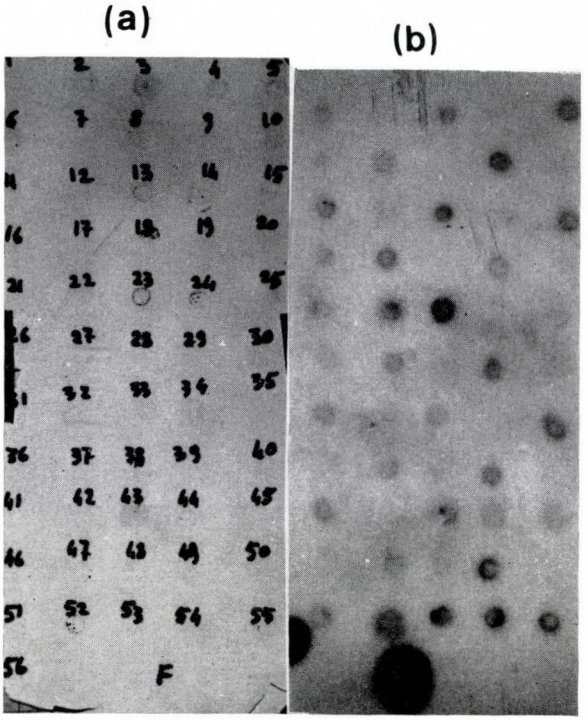

Fig. 1. Spotted filter (a) and related autoradiographic signals (b) after NDBH of cleared sample extracts from tetraploid wheat infected with M-PAV (Spots with even numbers up to 50 = negative controls)

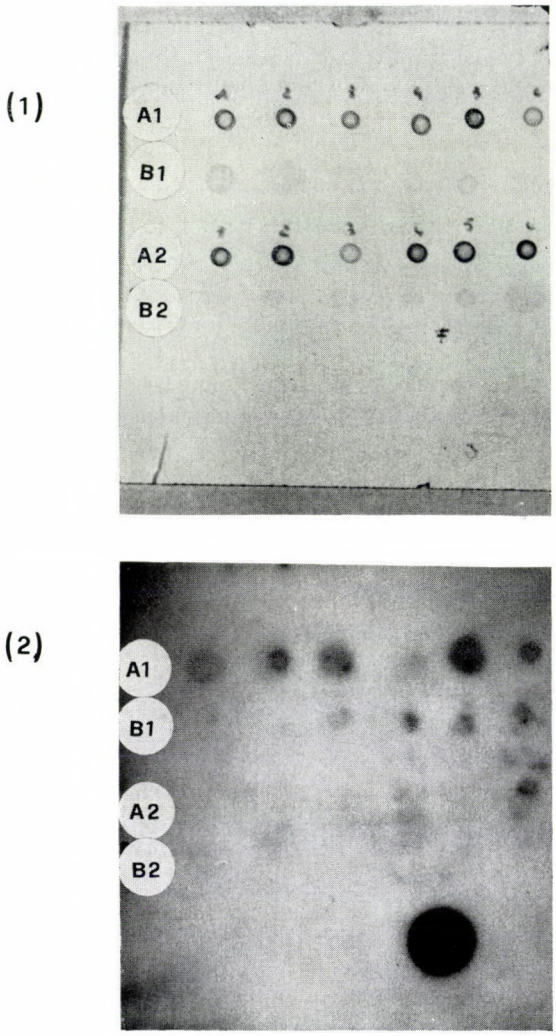

Fig. 2. Spotted filters (1) and related autoradiographic signals (2) after NDBH of crude (A1–A2) and cleared extracts (B1–B2) of 6 M-PAV infected (A1–B1) and 6 non infected (A2–B2) *A. byzantia* plants

between those from sap of inoculated and non inoculated plants. Only in one case (spot n. 23, Fig. 1) was the signal particularly high. Non infected plants gave scintillation readings about 25% higher than those from a non-spotted portion of the NC filter (background noise); this difference was slightly more evident by viewing the autoradiography after three days of exposure. The v.p.m.'s from Dot Blots of sap from inoculated plants were up to 144% higher than the controls.

C) *ELISA and NDBH applied to A. byzantina using crude sap and chloroform cleared sap*. Spots of crude sap extracts of infected plants (A1 dots, Fig. 2) always gave clear positive signals which were significantly different from crude of non infected plants (A2 dots, Fig. 2). However spots of cleared sap from infected plants gave positive autoradiographic signal only in 4 out of the six cases reported in Fig. 2 (B1 dots). Both crude and cleared sap extracts from non-infected plants (A2, B2 dots, Fig. 2) gave negative results with NDBH.

The ELISA reaction was definitely positive when applied to both crude and to clarified sap obtained from infected plants, while it was negative for allt he controls.

All the A. byzantina inoculated plants expressed evident symptoms of BYD in the greenhouse; the symptoms were much less clear on the remaining plant genotypes especially on those belonging to the genera Aegilops, Dasypyrum and Secale.

Discussion

In general it was observed in experiment A) (non clarified crude sap), that scoring the analyzed genotypes as belonging to the positive or negative class after NDBH gave in 32% of the cases (22 out of 69) discordant results when compared with the those obtained after ELISA. By using only extraction buffer the positive answers to NDBH were surprisingly much lower than expected if compared to the results obtained by ELISA. Using NDBH and chloroform cleared sap (B) gave generally good results both on scintillation readings and viewing of the autoradiography. The crude sap of non inoculated plants of the genotypes used in A) after NDBH, gave a c.p.m. readings not significantly different from those of sap obtained from the 21 inoculated plants found to be negative to the test. This fact may indicate that in the crude sap extract of leaves of wheat and related species there could be cellular components that interact with the cDNA probe causing non specific scintillation readings of the spots. When this occurs the signal of sap derived from non-inoculated plants increases and the differentiation among crude sap samples containing different amount of virus decreases. These cellular components are also suspected to be absent or drastically reduced in chloroform cleared sap extract of wheat genotypes (B). In fact when cleared sap extract are used, a good differentiation among saps from infected and non infected plants was achieved (Fig. 1). Moving to A. byzantina, the use of extraction buffer and NDBH (C) gave a clear differentiation among healthy and infected plants. It could be that in crude *A. byzantina* sap the interfering cellular components mentioned above, are absent or less represented than in wheat. The results obtained with ELISA of *A. byzantina* plants (C) were good both using sap in extraction buffer and chloroform cleared sap tending to be better with the latter.

The research is continuing to compare chloroform cleared sap of Triticinae in ELISA and in NDBH and the two techniques in different dilutions of *A. byzantina* and tetraploid wheat saps.

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Acta Phytopathologica et Entomologica Hungarica, 26 (1—2), pp. 21—26 (1991)

Barley Yellow Dwarf Virus Infection of the *Gramineae* in Tasmania

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Australia

Barley yellow dwarf (BYD) viruses have a world-wide distribution and infect plant species from several different subfamilies of the *Gramineae*. More than 70 species are known as experimental hosts of these viruses (Bruehl and Toko, 1957; Oswald and Houston, 1953) but relatively few of these have been found infected in the field.

There are many reports on the occurrence and effects of BYD viruses on the temperate cereal crops, oat *(Avena sativa)*, barley *(Hordeum vulgare)*, triticale (X *Triticosecale* spp.) and wheat *(Triticum aestivum)* (Burnett, 1984). There is also a growing understanding of these viruses in rice *(Oryza sativa)* (Osler, 1984) and maize *(Zea mays)* (Carroll, 1984).

It is likely that the BYD viruses and the vectors that occur here were introduced to Australia following European colonization some 200 years ago. Since introduction the viruses and their vectors have had many opportunities to interact in a new environment with important introduced crop plants, weeds and ornamentals in addition to members of the native flora. This paper examines the extent to which BYD viruses have spread to the native grass flora and whether the types of BYD viruses found in various species of the Gramineae reflect the botanical classification defined by Watson et al. (1985). The results are drawn from of a survey to identify the aphids infesting, and BYD viruses infecting, species of the *Gramineae* from a broad range of habitats and taxa.

Materials and Methods

Sampling

Annual and perennial grasses sampled from sites throughout Tasmania were, examined for infestation with aphids and tested for infection with BYD viruses. The sites included saline swamps, sand dunes, dry sclerophyll woodland, alpine areas, roadside verges, lawns, pastures, gardens and cereal crops. The number of species sampled from each subfamily reflected their occurrence in Tasmania and was therefore biased towards a higher proportion of Pooideae than occurs elsewhere in Australia (Hattersley, 1983) because many subtropical

genera are poorly represented in this State. A number of species was generally sampled at each site and included some known hosts of BYD viruses whenever possible.

Aphid Transmission Tests

Each sample was carefully examined for infestation with aphids. When found they were transferred to young oat indicator seedlings (cv. Algeribee) for 48 hr transmission feeds. They were then removed from the seedlings and preserved for identification.

Serological Tests

The double sandwich enzyme-linked immunosorbent assay (ELISA) system of Clark and Adams (1977) as modified by Guy et al. (1986) was used to test each sample serologically for infection with BYD viruses. Four antisera were used in the tests. Three were to the New York type isolates of MAV, PAV and RPV and the fourth was an antiserum to a Tasmanian PAV-like isolate (T-OA-6).

Results

Sampling

A total of *2977* plants was sampled and tested during the survey. They represented seven species of Arundinoideae (166 plants), two species of Bambusoideae (26 plants), four species of Chloridoideae (31 plants), ten species of Panicoideae (255 plants) and 33 species of Pooideae (1599 plants). See Guy et al. (1987) for a complete species list. These samples represented 20 native plant species and 36 introduced species. Only PAV, RPV and mixed infections of these two viruses infected the Gramineae in Tasmania.

Symptoms

Most plants infected with BYD viruses displayed no symptoms of infection. Only four of the 13 annual species infected with either PAV and/or RPV consistently displayed clear symptoms of leaf yellowing. These were *Hordeum vulgare*, *Triticum aestivum*, X *Triticosecale* spp. and *Vulpia bromoides*. Infected perennials: *Festuca arundinacea*, *L. perenne* and *Phalaris aquatica* plants occasionally showed mild symptoms.

Aphid Incidence

Five species of aphid (Table 1) were collected from Tasmanian Gramineae (1982–88). During the period 1982–85 *R. padi* was the most commonly collected

Table 1

Aphids collected from species of Gramineae in Tasmania and their effectiveness as vectors of barley yellow dwarf viruses

Aphid species	Natural plant hosts	Behaviour as vectors
Hyalopterus pruni	Phragmites australis	Non-vector of both PAV and RPV.
Metopolophium dirhodum	Arrhenatherum elatius Avena sativa Bromus spp. Dactylis glomerata Hordeum vulgare Phalaris spp. Poa pratensis X Triticosecale spp. Triticum aestivum	Vector of PAV and mixed PAV/RPB infections: field collections and glasshouse tests.
Rhopalosiphum maidis	Echinochloa crus-galli Hordeum vulgare Panicum capillare Zea mays	Non-vector of both PAV and RPV: field collections and glasshouse tests.
Rhopalosiphum padi	Arrhenatherum elatius Avena sativa Dactylis glomerata Deschampsia caespitosa Festuca arundinacea Hordeum vulgare Lolium perenne Phalaris aquatica Poa pratensis Trisetum spicatum X Triticosecale sp. Triticum aestivum	Vector of both PAV, RPV and mixed PAV/RPV infections: field collections and glasshouse tests
Sitobion fragariae	Avena sativa Bromus unioloides Deschampsia caespitosa Ehrharta erecta Hordeum vulgare Poa pratensis Trisetum spicatum X Triticosecale sp. Triticum aestivum	Some clones transmitted PAV and these occasionally also transmitted RPV with PAV as a mixed infection

species. *M. dirhodum* was first reported from Australia in 1984 (Waterhouse and Helms, 1985) and was first collected from Tasmanian *Gramineae* in 1985. Since then *M. dirhodum* has been observed infesting, particularly, barley and *Bromus* spp. and during the period 1986–88 was the most commonly collected species.

Virus Incidence and Relationship to Taxonomic Grouping

Both PAV and RPV were identified in plants from each of the five subfamilies represented in the survey. The incidence of infection within the subfamilies varied from 4–31%. There was no significant difference overall between virus incidence in the native and introduced species ($X^2 < 0.1$).

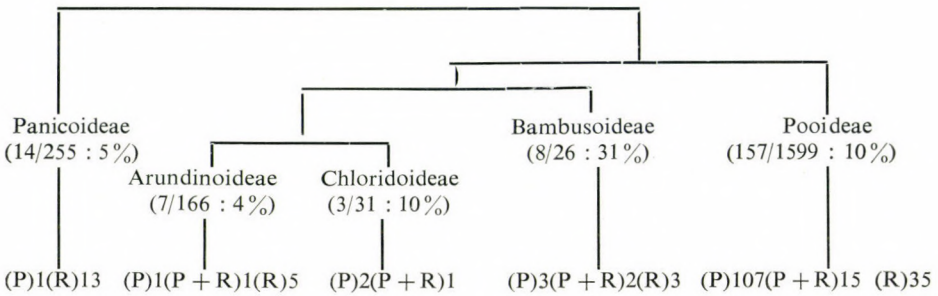

Diagram 1. Hierarchy of Poaceae (Gramineae) after Watson et al. (1985) showing the number and types of barley yellow dwarf viruses infecting each subfamily: (P) PAV, (R)RPV

Of the seven species of Arundinoideae sampled the native, *Danthonia tenuior*, was the only one found to be infected. It was predominantly infected with RPV viruses.

Virus incidence was highest in the Bambusoideae represented by two species of *Ehrharta*: infected with PAV, RPV and mixed infections.

Three of the four species of Panicoideae were infected exclusively with RPV viruses.

Two species of Pooideae, *Dactylis glomerata* and *Poa pratensis*, like the Panicoideae, were predominantly infected with RPV. These were clearly different from the other infected Pooideae which predominantly contained either PAV alone or else PAV/RPV mixed infections. The infected *D. glomerata* and *P. pratensis* plants were mostly sampled from sites where other Pooideae infected with PAV and PAV/RPV mixtures were common. The incidence of mixed infections in the Pooideae was far greater than would have occurred by chance had PAV and RPV infected the plants independently ($P < 0.001$). Similarly the proportion of mixed infections in the *Arundinoideae*, *Bambusoideae* and *Chloridoideae* was unusually high.

Discussion

Barley yellow dwarf virus was first recorded from Tasmania in 1959 when it was identified in barley, oats and wheat on the basis of host symptoms and its transmission to oat indicator seedlings with *R. padi*. Recently we identified

two BYD viruses occurring in two pasture grasses in Tasmania and defined them both biologically and serologically as similar to PAV and RPV (Guy et al., 1986). We have now extended these findings by establishing that natural hosts of BYD viruses in Tasmania include at least 32 species within the *Gramineae*. Eight of those hosts were Australian native species, none of which was previously known as a host of BYD viruses. Twenty four of the 38 introduced species collected during the survey were also shown to be infected and although some of them, particularly the cereals, are well known as hosts of BYD viruses in the field, many of the grasses which we identified as infected had also not been recorded as natural hosts previously.

Symptoms of BYD on cereals are well reported but there is little information regarding effects in other hosts. It was somewhat surprising to find that so few of the infected species displayed symptoms, especially among the Australian native hosts because they apparently evolved without exposure to BYD viruses. However it seems that few grasses are visibly affected by these viruses and that more critical studies of yield losses and changes in the infected plant's competitiveness in the sward are needed to assess the effects of infection.

The proportions of PAV and RPV in the samples of *Panicoideae* and *Pooideae* revealed dramatic differences in the host ranges of the two viruses. More than 90% of the infected Panicoideae contained RPV and 78% of the infected Pooideae contained PAV. A number of the sampling sites contained mixes of Panicoideae infected exclusively with RPV together with Pooideae infected almost exclusively with PAV. In other situations we found widespread infection of Pooideae with PAV alongside *Panicoideae* and other *Pooideae* that were healthy. The more limited information on infection in the *Arundinoideae*, *Bambusoideae* and *Chloridoideae* suggested they were intermediate between the *Panicoideae* and *Pooideae* with respect to the proportion of virus-infected plants. The differences in taxonomic host range provides a further line of evidence for the placement of PAV and RPV in two separate groups. This supports the evidence based on vector specificities, host reactions and cytopathology, enzyme immunoassays using polyclonal and monoclonal antisera, nucleic acid hybridization analyses and electrophoretic patterns of dsRNA extracts from infected plants (see Waterhouse et al., 1988).

Despite the general tendencies for the various subfamilies of *Gramineae* to be preferentially infected either with PAV or RPV, virus infection status was not a good character to use in isolation for classifying the species of Poaceae included in the survey. Notable exceptions to the broad generalizations were the tendency for two species of *Pooideae*, *Dactylis glomerata* and *Poa pratensis* to be almost exclusively infected with RPV viruses. This is in agreement with the findings of Fargette et al. (1982) and Paliwal (1982) that *Poa pratensis* in Indiana, Ontario and Quebec was predominantly infected with RPV whereas cereals in the same areas were predominantly infected with PAV.

All of the *Chloridoideae* and *Panicoideae* species collected for the survey had C_4 photosynthetic systems while those of the other three subfamilies were

all C_3 species. The data are therefore inconclusive in indicating the relative susceptibilities of C_3 and C_4 species to PAV and RPV. A study of the susceptibility of C_3, C_4 and C_3/C_4 intermediate species, is underway.

Acknowledgement

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Survey of Barley Yellow Dwarf Virus (BYDV) from Grasses in West of France

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Grasses are commonly grown in west of France. More than 40% of the growing area is occupied with fodder crops. Five species are commonly grown: *Bromus catharticus*, *Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne* and *Lolium italicum*.

Since 1984, samples of these grasses were collected in crops of west of France twice each year (in spring and in autumn). BYDV detection was achieved with ELISA using polyclonal antisera (B) and in 1986, using monoclonal antibodies (MAC 91, MAC 92 and MAFF 2).

BYDV was detected in four species out of five tested. Cocksfoot was the one found free of virus. The percentage of infected plants ranged from 4% in Italian ryegrass to 84% in fescue. In 1986, BYDV strains were assessed. The "PAV-like" one was found in the four infected species, the "MAV-like" one in brome and "RPV-like" one in ryegrass.

BYDV incidence could be related to aphids found on the crops during our study.

Barley yellow dwarf (BYD), an aphid transmitted disease attacks all of the small grain cereals (barley, wheat and oat). The virus causing this disease (BYDV) has a very large host range, and it infects more than a hundred grass species (Bruehl, 1961 and Oswald and Houston, 1953). The latter are host of several species of cereals aphids (Robinson and Hsu, 1963; Coon, 1959 and Kieckhefer and Lunden, 1983) and could play an important role in their annual life cycle (Wiktelius, 1987). Most of them are pluriannuals, and can therefore act as reservoirs of BYDV and aphids during a long period. BYDV has been reported from pastures specially from ryegrass in many countries (Doodson, 1967; Latch, 1977; Fargette et al., 1982; Grafton et al., 1982; and Guy et al., 1986).

In France, except Bayon studies (Bayon et al., 1983), there is no information on the occurrence of BYD in pastures. However, cultivated grasses take an important place in the agriculture of France and particularly in the west area.

We reported here the results of a study on the role of pastures as reservoirs of cereals aphids and BYDV in the area of "Ille et Vilaine" (west of France).

Materials and Methods

Five species of cultivated grasses were studied: brome (*Bromus catharticus* Vahl.), cocksfoot (*Dactylis glomerata* L.), fescue (*Festuca arundinacea* Schreb.), Italian ryegrass (*Lolium italicum* Lmk.) and perennial ryegrass (*Lolium perenne* L.). For these species, 19 to 43 pastures were sampled in the "Ille et Vilaine" area once in autumn (november-december) and once in spring (april-may) between 1984 and 1986. Number of aphids found on 100 to 500 tillers per fields were reported and five to ten samples of plant were collected in each field and then performed by ELISA (Enzyme linked immunosorbent assay).

For ELISA two kinds of antisera were used:

– in 1984, 1985 and spring 1986, antisera used came from BIOREBA*, and were prepared against a BYDV isolate which was transmitted non specifically by *Rhopalosiphum padi* L. and *Sitobion avenae* F. Thus this isolate equate to the PAV strain described by Rochow (1969). Because of the serological relationships between PAV and MAV strains, this antisera reacted weakly with the MAV strains (Rochow, 1979). ELISA procedure used was that described by Clarks and Adams (1977).

– in autumn 1986, all tests were done with three monoclonal antibodies supplied by Dr. L. Torrance (MAFF**). They were prepared against the english strains: G148 ("PAV-like"), R568 ("RPV-like") and F ("MAV-like") (Torrance et al., 1986b). ELISA procedure used was that described by Torrance et al. (1986a).

Results

Occurrence of aphids on pastures

Three cereals aphids were found on grasses: *R. padi*, *Sitobion avenae* F. and *Metopolophium dirhodum* Wlk.

In autumn, aphids were found in 59 of the 75 fields sampled. Percentage infestation varied from 1 to 79% for brome, 0 to 19% for cocksfoot, 0 to 11% for fescue, 0 to 7% for perennial ryegrass and 0 to 19% for Italian ryegrass. *R. padi* is then the most common aphid and his number represents 90.9%, 48.8% and 98.6% of the total number of aphids observed on grasses in autumn 1984, 1985 and 1986. In autumn 1985, *S. avenae* is numerous on brome (77% of total numbers of aphids). In spring, aphids are very scarse on grasses. They were only found on 7 of the 72 fields sampled.

* BIOREBA–BALE–SWITZERLAND

** MAFF — Ministry of Agriculture, Fisheries and Foods — Harpenden — ENGLAND.

Occurrence of BYDV in pastures

BYDV was detected in 4 of the 5 grass species: brome, fescue, Italian and perennial ryegrass. This virus was found in 98 of the 140 fields samples of these four species of grasses.

The percentage of infected samples for all fields together are very high:

- 30 to 90% for fescue
- 13 to 57% for brome
- 6 to 25% for Italian ryegrass
- 0 to 32% for perennial ryegrass.

We noticed that the percentage of infected samples per field varied with the grass species. In half of the fescue pastures it is very high (81 to 100% of infected samples). In the contrary, the percentage infection of ryegrass pastures are lower (0 to 40%). The percentage infection of brome are distributed in all infection levels (0 to 80% of infected samples).

Three fields of cocksfoot, fescue and brome were sampled from autumn 1984 to autumn 1986. We noticed that in fescue and brome's pastures, infected samples were collected at each sample date, even when no aphid were found on the crop. In cocksfoot pastures, we never found infected samples even when aphids were observed on the fields.

Identification of BYDV strains

In autumn 1986, "PAV-like", "MAV-like" and "RPV-like" strains were found in the collected samples. "PAV-like" was the predominant strain. The repartition of these different strains differ from one species of grasse to another:

- in brom, "PAV-like" and "MAV-like"
- in fescue, only "PAV-like"
- in ryegrass, "RPV-like", "PAV-like" and mixtures of these two strains.

Discussions

The results indicates that BYDV is a widespread disease of grasses in the area of "Ille et Vilaine". The incidence of infection with BYDV was within the range of that which has been reported from other countries. Catherall (1963) reported 22% and 77% infection levels and Doodson (1967) reported 93% in perennial ryegrass pastures of west Wales and Britain, Latch (1977) found 50% of infected samples in New-Zealand perennial ryegrass pasture and Guy et al. (1986) reported infection levels of 13% in Tasmanian pastures. High infection levels were also reported in others grass species like in fescue in the USA (Grafton et al., 1982 and Fargette et al., 1982).

The three strains "PAV-like", "MAV-like" and "RPV-like" were detected in the grasses of the "Ille et Vilaine" area in autumn 1986. These strains have

been reported from grasses: "PAV-like" and "MAV-like" by Holmes (1985) on ryegrass in England, "PAV-like" on fescue, and "PAV-like" and "RPV-like" on ryegrass, by Guy et al. (1986) in Tasmania and "RPV-like" on several grasses in USA by Fargette et al. (1982).

The distribution of BYDV strains differ from one grass species to another. The reason of that is unknown. It could be related to the species of aphids colonizing the crops:

– brome is infected by "MAV-like" and "PAV-like" strains, and also infested by *R. padi* and *S. avenae*,

– other grass species are mainly infested by *R. padi*, vector of "PAV-like" and "RPV-like" strains.

Cocksfoot was always found free of virus, in the fields sampled. This agree with Guy et al. (1986), who never detected BYDV in the 250 samples of cocksfoot tested. However, this species is known as susceptible to the RPV stain (Panayotou, 1985). Probably our ELISA method is not well adapted to detect BYDV in cocksfoot, because Eweida and Ryden (1984) had very good responses in ELISA with this plant.

Whether or not pastures are significant in the infection of cereals depend on whether viruliferous aphids move from these crops to cereals. If so, it would expected that the virus isolates from grasses and cereals would be of a similar type (Plumb, 1977). Results obtained in west of France suggest that the PAV and MAV isolates are the most abundant in cereals whereas the RPV one is the most common in pastures (mainly ryegrass) (Henry and Gillet, 1987). The virus reservoir in grasses in this area, therefore is not the major source of BYDV for spread directly to cereals. This suggestion agree with Paliwall (1982) in Canada.

Survey of both BYDV strains and aphids need to be conducted in grasses and cereals for several years before firm conclusions can be reached regarding the role of pastures in BYDV epidemiology.

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Barley Yellow Dwarf Virus in *Lolium* spp.

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Surveys of *Lolium perenne* crops in the west of Scotland between 1981 and 1987 showed that BYDV was widespread and infection in individual crops was often high. The RPV strain was found to predominate, although PAV was also widespread. The MAV strain was detected at relatively low levels, and the SGV and RMV strains were not found.

The preparation of leaves and the method of sap extraction had a major influence on the success of diagnosis of BYDV infection of *L. perenne* by ELISA. Fresh leaves ground first in liquid nitrogen, and then extraction buffer gave the highest rate of virus detection. The use of freeze dried leaves and rapid extraction methods sometimes resulted in the failure to detect the virus in samples known to be infected with BYDV.

The predominant agricultural crop in the west of Scotland, and indeed throughout the United Kingdom, is perennial ryegrass *(Lolium perenne)*. This has been found to be infected widely with barley yellow dwarf virus (Doodson, 1967; Holmes, 1985a). In Europe too it has frequently been identified in *L. perenne* and other agricultural grass species (Ruth, 1972; Lindsten & Gerhardsen, 1969). In most situations, the infection is symptomless, and it was not until the enzyme-linked immunosorbent assay technique was adapted for use with plant viruses (Clark & Adams, 1977), and found to be suitable for the detection of BYDV in grasses (Fargette et al., 1982; Holmes, 1985a), that progress was made in investigating infection of *L. perenne*.

BYDV-infected ryegrass constitutes a huge reservoir of virus for the infection of autumn-sown cereals (Plumb, 1977; Holmes, 1985b). Accurate information on the incidence of BYDV, and prevalence of the different strains of the virus has important implications for the prediction of the risk of serious infection of cereals in the autumn. In 1985 antisera to the five known strains of BYDV were made available by Professor W. F. Rochow, and these were used to reinforce and expand results of earlier surveys. These data together with information on the importance of sap extraction for BYDV detection in grass are presented in this paper.

Materials and Methods

Surveys

1981 and 1982. Samples were obtained from 1980-sown plots (5 m × 1.08 m) of a range of varieties of *L. perenne* and *L. multiflorum* located at Auchincruive, Ayr. In 1981 each sample consisted of one symptomless tiller. In 1982 a sample was composed of 10 symptomless leaves collected at random from each plot.

1983. Samples were collected from 13 crops of *L. perenne* located around Auchincruive. In each field an area of 3 ha was divided into 15 sub-areas and a bulk sample of 10 symptomless leaves collected from widely spaced points in each.

1985 and 1987. Farm crops located throughout the west of Scotland were sampled. A sample consisted of a tuft of grass approximately 7.5 cm in diameter. The tufts were potted in compost in 15 cm diameter clay pots and maintained in an unheated glasshouse. The grass was kept aphid-free by the regular application of pirimicarb.

Serological tests

Enzyme-linked immunosorbent assay (ELISA) was conducted according to the procedure described by Clark and Adams (1977). in 1981, 1982, 1983 and 1985 the antisera referred to as B and F were used. These kindly were supplied by Dr. M. F. Clark (East Malling Research Station). The B antiserum detected both the PAV and RPV strains described by Rochow (1969) and the F antiserum reacted principally with the MAV strain (Rochow, 1969), but gave a slight cross-reaction with the PAV strain. In 1985 and 1987 antisera to the PAV, RPV, MAV, RMV and SGV strains kindly were supplied by Professor W. F. Rochow (Cornell University, Ithaca, N. Y.).

Plants were considered to be infected when the absorbence reading (A405) exceeded that of healthy control wells plus three times their standard deviation.

Preparation of leaf material

Fresh leaves. These were tested immediately or stored at −18 °C until use.

Freeze dried. The leaves were chopped into 0.5 cm lengths and frozen at −18 °C for 24 h before freeze drying. Thus prepared, the samples were stored at −18 °C.

Partial purification. Ten grammes of fresh leaf material were ground in 40 ml of phosphate buffered saline (PBS), filtered through fine muslin and mixed with 20% v/v butanol + chloroform. The mixture was stirred for 10 min and then centrifuged for 30 min at 15,000 g. The supernatant was retained and centrifuged at 75,000 g for 5 h. The resulting pellet was resuspended in 1 ml PBS and kept at 4 °C until use.

Sap extraction

In 1981, 1982, 1983 and 1985 sap was extracted by grinding 1g of fresh leaf material in 2 to 5 ml of extraction buffer (PBS, pH 7.4, containing 2% polyvinyl pyrrolidone MW 44,000, 0.2% ovalbumin and 0.05% Tween 20). The sap was passed through fine muslin before placing in the microtitre plate.

In 1985, in addition to the above procedure, fresh (1g) or freeze dried (0.25 g) leaf samples were ground first in liquid nitrogen and then in PBS, or in liquid nitrogen, then 6 ml of chloroform and finally PBS. Samples thus prepared were centrifuged at 5000 rpm for 5 min before use.

In one series of tests a Polytron electric grinder was used. For this, 3 g of finely chopped leaf was placed in a 30 ml centrifuge tube together with 9 ml of PBS + Tween and ground for 6 sec. Chloroform then was added (9 ml), shaken and centrifuged as above.

From late 1985 on, the standard extraction procedure was to grind in a mortar 1 g of fresh leaf, first with liquid nitrogen and then 3 ml of extraction buffer.

Results

BYDV in Lolium perenne 1981–87

The incidence in *L. perenne* of strains of BYDV which reacted with the B antiserum remained consistent over the 7 year period (Table 1). In contrast, the detection of strains which reacted with the F antiserum fell after 1982 and remained low at around 8% of samples in subsequent surveys.

BYDV in Lolium perenne in 1985

BYDV was found in 28 of the 36 crops examined (78%). Eighty-one of the 181 samples tested (45%) were infected. In 53.6% of fields where infection was found 3 or more of the five samples collected from each field contained BYDV.

The majority of samples which gave a positive reaction did so with the B antiserum (Table 2). Strains reacting with the F antiserum were found in 17.3% of infected samples, and 2.5% contained strains of BYDV which reacted with both antisera.

ELISA tests

Tests in 1985 on 60 selected samples of fresh *L. perenne* leaves from the survey of farm crops using the B and F antisera showed that 37 contained PAV/RPV-type strains (B) and 10 MAV-type strains (F). No virus was detected in 13 samples (Table 3).

Table 1

Incidence of BYDV in *Lolium perenne* in 1981, 82, 83, 85 and 87

Year	Number of samples	% of samples tested	
		B (PAV/RPV)	F (MAV)
1981	47	27.7	27.7
1982	120	44.2	30.8
1983	195	33.0	8.2
1985	180	37.2	8.9
1987*	85	40.0	7.1

* PAV, RPV and MAV antisera used. For comparison, PAV and RPV found in one sample has been equated to 1 positive with the B antiserum.

Table 2

Incidence of BYDV strains in *Lolium perenne* — 1985

Reaction type	Number of samples giving reaction in ELISA test	% samples
PAV/RPV (B)	65/81	80.2
MAV (F)	14/81	17.3
MAV + PAV /RPV (B + F)	2/81	2.5

Table 3

Summary of ELISA tests on 60 fresh samples of *Lolium perenne*

Reaction type	Number of samples	Reaction type	Number of samples
B	7	PAV	15
		RPV	23
		MAV	0
F	10	RMV	0
		SGV	0
None	13	None	22
Total	60		60

Tests on leaves from the same plants with Rochow's antisera showed that the most prevalent strains was RPV (36%), with the PAV strain in 25% of samples. The MAV, RMV and SGV strains were not detected in any of the material tested.

Leaf preparation and the detection of BYDV by ELISA

The type of leaf material used in ELISA test was found to have an important influence on the subsequent detection of BYDV (Table 4). The highest detection rate was with fresh or partially purified virus preparations, although some PAV was lost with the latter procedure. Freeze drying of grass leaves resulted in loss of much of the RPV strain. The PAV strain was lost almost completely.

Table 4

Leaf preparation and detection of BYDV in *Lolium perenne* by ELISA

Leaf preparation*	Number of samples	% in which BYDV detected	% infected with	
			RPV	PAV
Fresh	60	63	38	23
Freeze dried	52	19	17	2
Partially purified	32	69	50	16

* Leaves for freeze drying and partial purification taken from same source of plants used for fresh material.

Table 5

Comparison of pestle and mortar extraction methods

Ryegrass sample	Liq. N_2 + Chloroform + Phosphate buffer		Liq. N_2 + Phosphate buffer		Phosphate buffer + PVP	
	RPV	PAV	RPV	PAV	RPV	PAV
6A	.420	.263	.216	.303	.189	.451
21D	.433	.069	.322	.101	.179	.096
8E	1.038	.058	.910	.057	.586	.045

Table 6

Comparison of Polytron and pestle and mortar extraction

Ryegrass sample	Mean A405 values			
	Polytron		Pestle and mortar	
	RPV	PAV	RPV	PAV
Healthy grass	.026	.003	.003	.013
9E	.062	.129	.156	.336
18B	.148	.131	.272	.292
4A	.022	.194	.036	.393

Sap extraction and the detection of BYDV by ELISA

Grinding leaf material with phosphate buffer + polyvinyl pyrrolidone gave absorbance readings which, generally, were about half those obtained when the leaves were ground first in liquid nitrogen (Table 5). The addition of a chloroform clarification stage further increased virus extraction by up to 50%. The use of more rapid grinding methods such as the Polytron often resulted in much reduced absorbance readings, even when compared with the basic pestle and mortar, phosphate buffer procedure (Table 6).

Discussion

BYDV has been found to occur widely in *L. perenne* crops in the west of Scotland. Individual crops may be extensively infected. The consistent level of detection of PAV/RPV strains over the 7 year period probably reflects the sub-lethal nature of BYDV in *L. perenne*. The reduced detection of MAV strains since 1983 may be due to the fact that in 1981 and 1982 the samples were collected from plots, but since then they have been taken from farm crops. The difference in incidence may reflect the influence of management on the populations of the aphid vectors. The plots were managed on a conservation regime, that is, cut every 6 weeks, with a lot of top growth developing between cuts. Farm crops are used for conservation only early in the season, when aphid populations are low, and then grazed for the rest of the year. The vector of MAV strains, *Sitobion avenae*, prefers to feed at the top of the plants, and thus would be favoured by the conservation regime in the plots. The principal vector of PAV and RPV strains is *R. padi*, and it prefers to feed at the base of the grass plants (George, 1978). Therefore, it is well adapted to survival in the farm situation.

The availability of antisera which can differentiate between the five known strains of BYDV enabled, for the first time, the identification of the strain spectrum in the west of Scotland. According to Plumb (1977), PAV and MAV-type strains seem to be most prevalent in Britain. This is not the situation in grass in the west of Scotland, for the RPV strain predominated and little MAV was found. This information may enable a more precise calculation of the risk of BYDV infection in the autumn in winter cereals than that used currently (Plumb et al., 1985), by allowing corrections to be made for vector species and for the prevalence of different strains in particular geographical areas. Neither the SGV or RMV strains were found in the samples reported on here, or in many other grass samples which were tested subsequently. The vector of SGV *(Schizaphis graminum)* does not occur in the west of Scotland, but that of RMV *(R. maidis)* does, although in relatively small numbers. The failure to find the RMV strain may be due to the grass species examined, or to the environmental conditions not favouring virus development. Weed grass species will be examined for RMV in the future.

BYDV often occurs in *L. perenne* at very low concentrations (Holmes,

1985a), and can give very marginal results in ELISA tests. Furthermore, the concentration of virus particles in grass plants varies greatly depending on the time of the year, and between leaves on the same plant (Holmes, unpublished data). This, combined with the absence of any visual symptoms on most infected plants, means that thorough extraction of virus is essential for accurate diagnosis. The use of liquid nitrogen in the standard pestle and mortar extraction has been found to provide maximum virus yield and, very importantly, a highly reproducable maceration of leaf tissue. Rapid extraction methods such as the Polytron (and the Pollahne leaf press) are not satisfactory for grass, although they have been used successfully for cereals. Freeze drying of leaves is unsuitable for grass, although it too has been effective with cereals (Rochow, personal communication).

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The Effects of MAV-, PAV- and RPV-like Isolates of BYDV on Spring and Winter Barley Cultivars

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Inoculation of the winter barley cultivars Igri and Vixen, and the spring barleys Atlas 68 and Atlas 57 with MAV-, PAV-, and RPV-like isolates of barley yellow dwarf virus from UK, showed that the Yd_2 gene was very effective in preventing damage caused by MAV- and PAV-like isolates but conferred little or no benefit when an RPV-like isolate caused infection. The breeding of resistant or tolerant barleys containing the Yd_2 and their deployment should take this into account.

There are, in current use, three methods of preventing or restricting crop loss due to BYDV i) avoidance, ii) chemical control of the aphid vectors and iii) breeding for resistance or tolerance. In Europe, avoidance of infection, unknowingly, used to be the principal method of control, but changes in cropping practice, especially the switch from spring to autumn sowing of cereals and the associated need to start autumn sowing earlier to complete the autumn programme, and to achieve full yield potential, has increased the frequency of infection with BYDV and the use of insecticides to control the aphid vectors. Resistant or tolerant cultivars have never played a part in control. Elsewhere, especially in California, the use of BYDV-tolerant spring barley is common: more than half the barley area is sown to cultivars containing the Yd_2 gene which confers tolerance. Tolerance is used to mean that plants are infected, that there is no apparent decrease in virus content but plants often show few symptoms and suffer much less yield loss than cultivars without the Yd_2 gene. In California near isogenic lines with Yd_2 outyielded those without by 35–45% (Schaller and Qualset, 1980).

However, most of the selection in the past has been done in field grown crops and the infecting virus isolates have rarely been defined, largely because no quick, reliable diagnostic methods were available. Indeed in 1959 when the Yd_2 gene in barley was reported (Rasmusson and Schaller, 1959), there was little realisation that barley yellow dwarf (BYD) was caused by more than one isolate, and even less that these isolates probably represent two distinct viruses (Plumb, 1989). Only recently, with the development of enzyme-linked immunosorbent assay (ELISA) for plant viruses, and the availability of supplies of polyclonal and, more recently, monoclonal antibodies to many, if not all, of the BYDV isolates, has regular and reliable typing of virus isolates been possible. Neverthe-

less, most screening is still done by natural infection and the selection of the best looking lines.

In United Kingdom there has been a small effort to incorporate the Yd_2 gene in spring barley cultivars but its expression was poor in the slow growing backgrounds of barley cultivars that give acceptable yields in our conditions (Jones and Catherall, 1970). However, a resistant spring barley, named Corris, then Coracle, was released in 1977 (Catherall, Hayes and Boulton, 1977). More recently the Yd_2 gene has been transferred into a winter barley background by a (Coracle × Igri × Igri) cross (Parry and Habgood, 1986). This cultivar, called Vixen, was provisionally recommended for use where BYDV is a particular problem, in 1987. The assessments reported by Parry & Habgood (1986) compared different sowing dates, different times of inoculation and different numbers of infecting aphids for cvs Igri and Vixen. The isolates of BYDV used were not defined serologically but were transmitted by *Rhopalosiphum padi* and defined as a mixture of 'mild' and 'severe' isolates. There were differences in response to inoculation time and numbers of aphids but the early October infection of early September sown plants had the most severe effect and Vixon consistently performed better than Igri, although less well than uninoculated plants, and more than 30% yield loss was reported (compared with more than 90% loss in Igri). While clearly a great improvement, such a result alone would not eliminate the need for insecticidal treatment, although the inoculation conditions were more extreme than would usually be experienced in field crops. Of equal importance to a cultivar's reaction when infected is its performance when uninfected, and there were no differences in the yield of uninoculated Vixen and Igri.

Experimentation

In 1985/6 we tested the response of Vixen and Igri to inoculation on four different dates with the Rothamsted isolate B of BYDV (Plumb, 1986). The results showed that Vixen yielded better than Igri irrespective of time of inoculation, but that losses suffered by Vixen were still unacceptably large. At the time that this experiment was done it was thought that B was a single isolate. Tests with monoclonal antibodies (Torrance et al., 1986) showed that it is a mixture of PAV and RPV isolates. Consequently, the experiment was repeated in 1986/7 using the three defined isolates of BYDV known to be present in UK, an MAV-like isolate (UK F), and RPV-like isolate (UK R568) and a PAV-like isolate (UK G). These were inoculated to cvs Igri or Vixen in the autumn or spring using five aphids per plant and *Sitobion avenae* to transmit MAV, and *R. padi* to transmit RPV and PAV. The results for cv Igri are given in Table 1 and for Vixen in Table 2.

Igri was severely damaged by all isolates but, as expected, most damage resulted from autumn inoculation. Whereas, the yield of Vixen was unaffected by inoculation with MAV in autumn or spring or by PAV inoculation in autumn. The most severe damage, equivalent to that in Igri, was caused by RPV infection.

Table 1

The effects of PAV-, RPV- and MAV-like isolates of BYDV on winter barley cv' Igri inoculated in the autumn or spring

Inoculation time and isolate	Yield (g/plant)	1000 grain wt. (g)	Height (cm)	Shoot number
Autumn				
PAV	0.00	0.00	0.00	0.00
MAV	5.87e[a]	31.08c	44.8c	17.8a
RPV	1.31f	24.61d	49.0c	7.1e
Spring				
PAV	7.66d	36.30b	72.2b	8.6d
MAV	8.37c	39.06b	67.6b	11.8c
RPV	10.95b	38.94b	88.6a	13.1bc
Uninoculated	14.41a	48.47a	93.0a	13.5b

[a] Values in each column followed by different letters are significantly different at $P = 0.005$ as determined by the Student-Newman test.

Table 2

The effects of PAV-, MAV- and RPV-like isolates of BYDV on winter barley cv' Vixen, containing the Yd_2 gene, inoculated in the autumn or spring

Inoculation time and isolate	Yield (g/plant)	1000 grain wt. (g)	Height (cm)	Shoot number
Autumn				
PAV	15.87[a]	41.75b	94.8b	15.4a
MAV	15.93a	42.63ab	94.7b	15.4a
RPV	1.25d	32.56b	52.1c	4.7c
Spring				
PAV	12.61b	34.80c	91.2b	15.0a
MAV	15.70a	45.12a	100.7a	14.6ab
RPV	10.18c	33.30c	94.5b	14.3ab
Uninoculated	15.62a	45.26a	104.0a	13.4b

[a] Values in each column followed by different letters are significantly different at $P = 0.005$ as determined by the Student-Newman test.

These experiments were done in large pots and may not accurately reflect the consequences of field infection. There is a suggestion that an increase in shoot number in inoculated plants compensated for decreases in 1000 grain weight. The effect of PAV inoculation in spring on Vixen appears anomalous and cannot be explained.

A similar experiment was done with the near isogenic spring barleys Atlas 68 ($+Yd_2$) and Atlas 57 ($-Yd_2$), although they are not cultivars well adapted

to UK conditions. The relative effects of the three isolates differed from their effects on winter barley but also showed that the presence of the Yd_2 gene had no effect on the damage done by isolate RPV but did decrease losses caused by PAV and MAV (Table 3).

Table 3

The effects of PAV-, MAV- and RPV-like isolates of BYDV on spring barley cvs Atlas 68 (+ Yd_2) and Atlas 57 (− Yd_2)

Cultivar Isolate	Yield (g/plant)	1000 grain wt. (g)	Height (cm)	Shoot number
Atlas 68				
PAV	19.13b[a]	44.19bc	108.9a	13.4b
MAV	36.93a	49.07ab	118.6a	18.9ab
RPV	16.37b	40.62c	106.a	12.2b
Uninoculated	39.37a	52.11a	115.9a	19.4a
Atlas				
PAV	0.00	0.00	0.0	0.0
MAV	11.21b	45.83b	106.4a	6.7b
RPV	13.96b	40.38c	105.9a	12.2b
Uninoculated	40.16a	49.47a	122.9a	22.4a

[a] Values in each column followed by different letters are significantly different at $P = 0.005$ as determined by the Student-Newman test.

Conclusion

Although these results require confirmation they strongly suggest that the Yd_2 gene, whether expressed in a spring or winter barley cultivar, is effective only against MAV- and PAV-like isolates. This would support other evidence that suggests that the isolates of BYDV should be grouped in two distinct viruses, separating MAV- and PAV-like isolates from RPV. Current evidence suggests, perhaps fortunately, that RPV-like isolates are the least common in Britain (Torrance et al., 1986). Nevertheless, this is not the case in all regions and the use of cultivars containing Yd_2 should be related, where possible, to the known prevalence of BYDV isolates. It is also unknown whether the widespread cultivation of varieties tolerant to MAV- and PAV-like isolates will change the frequency of isolates in that region.

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Different Levels of Tolerance to Barley Yellow Dwarf Virus (BYDV) in Some Hybrid Lines Barley

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Inoculation of Barley Yellow Dwarf Virus (BYDV) to offspring of two crosses of barley varieties with different levels of tolerance to the virus indicated the occurrence of daughter families with a level of tolerance to BYDV different from that of any of the parent varieties. It was found of interest to define these differences more exactly, and thus hopefully achieve a better understanding of their background.

The following is a preliminary report from this work.

Materials and Methods

The mother plants of both crosses were of the variety Lise. This is a 6-rowed, early maturing variety with virus strain dependent but generally low tolerance to BYDV infection. The tolerant variety was CI9654. This is a line of barley of Ethiopian origin known to be highly tolerant under different environmental conditions (Bruhel, 1961). Compared to other Ethiopian barleys CI9654 is an early maturing variety. As the sensitive parent the Black Hulless Barley CI666 was used. This is a fairly late maturing variety of Chinese origin.

The families used in these experiments are derived from single F_2-plants. They were selected on the basis of a visual evaluation of the symptom development in 8–12 inoculated plants (F_3) per family. The families that seemed most interesting in this preliminary screening, were multiplied once more to give a sufficient number of plants for further experiments.

The tolerance of parent varieties and selected progeny families was further evaluated by comparison of growth of uninoculated plants and plants inoculated with either of two virus isolates. The virus isolates used are both of the PAV type, but one (isolate 162) is mild and the other (isolate 163) is severe in the variety Lise, and they are earlier shown to interact differentially with varieties of barley and oats (Munthe, 1985).

The comparisons are performed in a greenhouse as block experiments with 10 plants per treatment. The experiments will be repeated four times with the blocks allocated to different times starting in October 1987. The plants are inoculated by 5–10 aphids *(Rhopalosiphum padi)* at the one leaf stage, and left in

a greenhouse at fluctuating temperatures for 5 weeks. At harvest symptoms are noted and the fresh weight of the green material (leaves and stems) are registered for each plant.

The main results from the experiments which are accomplished till now, are presented in tables 1 and 2 as the average fresh weight of inoculated plants in percent of uninoculated control plants.

Table 1

Effect of BYDV infection. Weight of inoculated plants in percent of uninoculated

Variety/family	
Lise	46
CI9654	91
665	25
175	51
419	76
882	79
426	93
1942	101

Table 2

Weight of plants of the varieties Lise and CI666 and some selected progeny families after inoculation with two isolates of BYDV given as percent of uninoculated control plants

Variety/family	Isolate	
	162	163
Lise	62	36
CI666	15	30
62	11	26
49	13	19
38	62	74
43	62	65
47	56	78
53	67	77

Results and Discussion

Lise × CI9654 families

Till now only two complete comparisons (blocks including both virus isolates) have been accomplished for Lise, CI9654 and the Lise × CI9654 families. To increase the number of replicates for this material, two earlier experiments including only virus isolate 163 have been added to give the results presented in Table 1.

The average weight of inoculated plants of the variety Lise was less than half that of uninoculated control plants of the same variety, whereas inoculated plants of the parent variety CI9654 were reduced by less than 10 percent.

Plants of the family 665 were even more damaged by infection than plants of the most sensitive of the parent varieties. This was the case in all of the six comparisons brought together in table 1 and is well in line with impressions from earlier visual evaluations. The magnitude and stability of this loss of tolerance compared to that of the variety Lise makes it more likely to be caused through the loss of a specific gene for tolerance than by a change in general genetic background.

In the earlier visual comparisons of the families, family 175 was rated as more tolerant than the variety Lise, as inoculated plants of '175' seemed less stunted than those of 'Lise'. This difference may then be caused by a difference in growth habit and not by any differences in specific genes for tolerance.

Characteristic for inoculated plants of the families 419 and 828 both in preliminary comparisons and in these experiments, was a mild discoloration of 1–2 leaves developing 2–3 weeks after inoculation without any noticeable growth reduction. This symptom differentiated these families clearly from any of the parent varieties. This symptom type seemed constant through F_3 and F_4 generations and under varying environmental conditions. It was also parallelled in these experiments by a significant reduction in weight of plants. Thus, at this stage of the experiments, it seems improbable that the families 419 and 828 should carry the same genes for tolerance as plants of the parent variety CI9654 and the sister families 1942 and 426.

The families 1942 and 426 which were selected as highly tolerant, gave even less yield reduction after inoculation than the parent variety CI9654 did, but the differences will hardly be significant. No plants of these families or the variety CI9654 developed visible symptoms during these experiments.

Lise × CI666 families

The inoculation experiments with the varieties Lise and CI666 and the Lise × CI666 families are replicated four times and the results are given in table 2 separately for each of the virus isolates 162 and 163.

For the parent varieties the results demonstrate a variety/virus isolate interaction similar to that described earlier (Munthe, 1985). Although disease development was generally too severe in these experiments to give a good seperation of low levels of tolerance, inoculated plants of the families 49 and 62 had clearly lower relative yields than those of any of the parent varieties for both virus isolates. This indicate that even the extremely sensitive variety CI666 has got some tolerance to BYDV.

Plants of the families 38, 43, 47 and 53 inoculated with virus isolate 162 yielded a weight of plants relative to uninoculated control plants which was very close to that of variety Lise. This is a level of tolerance which gives good protection under most practical growing conditions. In contrast to 'Lise', which is very sensitive to isolate 163, the families 38, 43, 47 and 53 were more tolerant to isolate 163 than to isolate 162. It therefore seems as if some trait, possibly a tolerance gene of little effect on its own, may have been added to the tolerance gene derived from variety Lise to give increased tolerance and make the tolerance less virus strain specific. Considering the obvious interaction between the two parent varieties and the virus isolates used in these experiments it is surprising that all of the Lise × CI666 families tested are most tolerant to the one of the two virus isolates.

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Plurilocal Assessment of the Risk of Primary Infection of Young Cereals by BYDV in Autumn in the West of France

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A two year study (1985–1986) allowed to show a certain geographical stability of the relationships observed during the autumn of the same year between weekly catches of *R. padi*, less males, at a suction trap sited in le Rheu and percentages of infestation by *R. padi* and infection by a PAV-like strain of BYDV, respectively, of barley pots located at different distances from the trap. These results increase the possibilities of practical use of forecasting methods based on suction trap catches of *R. padi* in so far as they seem at least available at the scale of a small region.

Five years of experiments in the area of Le Rheu (Brittany, western part of France) allowed to find in autumn a steady interannual relationship between weekly cumulated suction trap captures of the aphid *Rhopalosiphum padi* L. and levels of infestation by this aphid species of barley pots placed near the suction trap (Gillet et al., 1987; Henry et al., 1988), showing that barley infestation may be locally predicted by suction trap captures. In the opposite relationships between captures of *R. padi* and percentage of infection by BYDV of the barley seedlings were variable from one year to another as a symptom of large interannual variation of the quantity of virus carried and transmitted by a same amount of aphids. Nevertheless the vector-virus relationships seem to be stable during a whole cropping season in autumn (Dedryver et al., 1987), which can also be used for short term forecasts.

Such predictions may only be of practical interest if the observed relationships are regionally available. Therefore are reported in this paper experiments in which were compared infestation by *R. padi* and infection by BYDV of barley seedlings placed at different distances of the suction trap in le Rheu, to the *R. padi* captures.

Materials and Methods

The main species of aphids known to be potential vectors of BYDV, *Rhopalosiphum padi* L., *Sitobion avenae* F., and *Metopolophium dirhodum* Wlk., were counted in catches of a suction trap sited in Le Rheu (France, Ille-et-Vilaine).

This 12.2 m "Rothamsted-type" trap is a part of the french AGRAPHID network (Robert & Choppin De Janvry, 1977). Weekly catches of each species from mid-September to mid-December were considered. Only *R. padi* was taken into account in further analysis because it constituded more than 90% of the potential vectors catches. Males of *R. padi* were discarded because they may acquire virus from *Graminae* but migrate to their primary host *(Prunus padus* L.) and have an uncertain role in BYDV epidemiology (Plumb et al., 1986).

Infestation of winter barley by aphids is measured in three places located in a North-west transect (Figure 1): Le Rheu (100 m from the suction trap), Montauban de Bretagne (16 km from the suction trap) and Broons (32 km from the suction trap). In each location was placed on bare soil each week a batch of 50 (Montauban de Bretagne, Broons) to 100 (Le Rheu) pots with fine to six barley seedlings cv. Capri at the two leaf growth stage. At the end of the seven day exposure, aphids species and morphs were identified and counted and then killed by an aphicide spray (pyrimicarbe). The batch of pots were then removed and replaced by a new one. Each pot of seedlings was regarded as a sampling unit. In further analyses, only *R. padi* was taken into consideration.

BYDV infection of barley seedlings was estimated as follows: the barley test plants, after spraying with aphicide, were kept for a week at about 20 °C in the laboratory to allow multiplication of the eventually transmitted virus. The vegetal samples were then improved by the ELISA-method (Clark and Adams, 1977). The polyclonal rabbit antiserum anti-B (BIOREBA A. G., Bxsel, Switzerland) used detects a PAV-like isolate (Rochow, 1970) and weakly a MAV-like one. A sample was considered positive if O. D. was greater than the mean O.D values of control samples plus three time the standard deviation (Goumas et al., 1985).

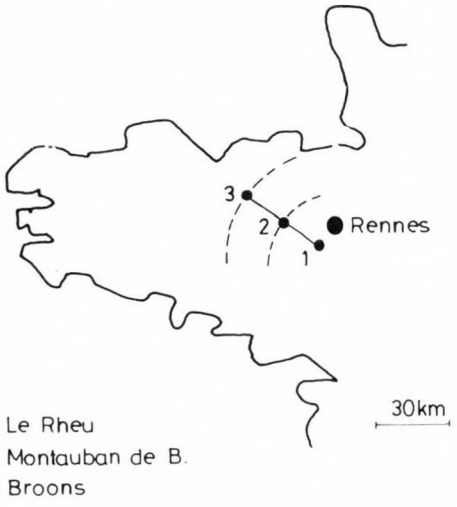

Fig. 1. Localisation of the three sites of experiment in Brittany

A statistical analysis was done with the scientific statistic package BIOM (Rohlf, 1984), on 4 variables (Table 1) belonging to the different series of observations.

Table 1

Description of variables and relevant transformations

RPST2	Weekly accumulated catches of *R. padi* by suction trap less males (Log (x + 1)
RPINF	Percentage of sampling units infested by *R. padi* (Arc sin $\sqrt{x}$)
RPAL	Number of alate *R. padi* foundcd on 100 sampling units (Log (x + 1)
VIRINF	Percentage of infected sampling units (Arc sin $\sqrt{x}$)

Results

Correlations between variables

Correlation coefficients between total number of *R. padi* trapped, less males, (RPST2) and percentage of infested samples by *R. padi* (PRINF) and percentage of infected samples by BYDV (VIRINF) respectively were generally very significant or highly significant (Table 2) in 1985 and 1986, whatever the

Table 2

Coefficients of principal correlation between variables for each location

1985	Le Rheu ddl = 8		Montauban de Bretagne ddl = 5		Broons ddl = 5	
RPST2/RPINF	0.910	HS	0.878	HS	0.936	HS
RPST2/RPAL	0.744	VS	0.897	HS	0.902	HS
RPST2/VIRINF	0.847	HS	0.741	S	0.758	VS
RPINF/VIRINF	0.841	HS	0.940	HS	0.887	HS
1986	**Le Rheu ddl = 10**		**Montauban de Bretagne ddl = 10**		**Broons ddl = 10**	
RPST2/RPINF	0.844	HS	0.764	HS	0.796	HS
RPST2/RPAL	0.515	NS	0.467	NS	0.567	NS
RPST2/VIRINF	0.726	HS	0.681	VS	0.758	HS
RPINF/VIRINF	0.860	HS	0.649	VS	0.914	HS

HS (highly significant, $\alpha = 0.01$), VS (very significant, $\alpha = 0.05$)
S (significant, $\alpha = 0.1$), NS (non significant).

location. It is the same for correlation coefficients between RPINF and VIRINF. At the opposite, the correlation coefficients between RPST2 and the number of alate *R. padi* infesting the samples (RPAL) are very or highly significant in 1985, but not significant in 1986. For 1985 it was hazardous to make comparisons between correlation coefficients because of the differences in degrees of freedom, but it could be done for the 1986 data. Table 2 shows that correlation coefficients between RPTS2 and PRINF, RPAL and VIRINF respectively are not different whatever the distance of the different locations to the suction trap.

Relationships between suction trap catches and barley plants infestation by R. padi

Relationships between suction trap catches of *R. padi*, less males (RPST2) and percentage of barley pots infested by *R. padi* (PRINF) are shown Figure 2. For 1985 it is possible to draw a regression line common to all the points, whatever the location (Table 3). It is not possible in 1986 because although the slopes of the different equations corresponding to each location are not significantly

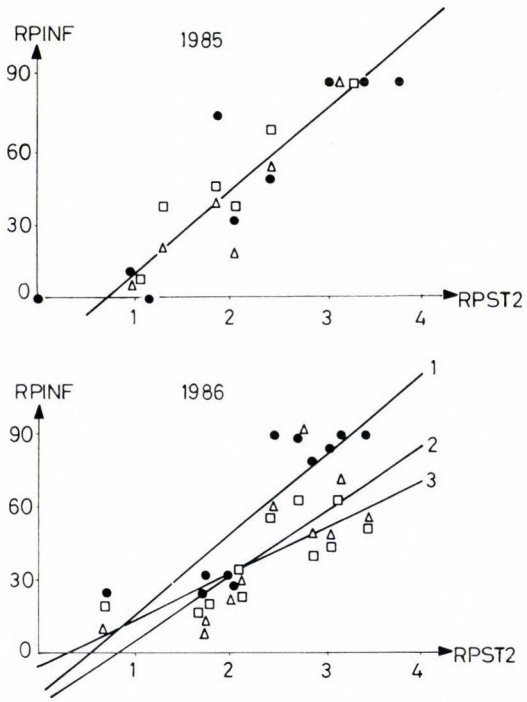

Fig. 2. The relationship of the weekly percentage of pots with infected barley test seedlings (RPINF) to the weekly accumulated catches of *R. padi* by suction trap less males (RPST2) at Le Rheu (●; 1), Montauban de Bretagne (△; 2) and Broons (□; 3)

different, the adjusted means of the samples are significantly different (Table 3). From one year to another, the difference between common slopes is small (33.9 in 1985; 26.16 in 1986).

Relationships between suction trap catches of R. padi and barley pots infection by BYDV

For 1985 it is possible to draw a regression line common to all the points whatever their location, between RPST2 and the percentage of barley pots with BYDV infected plants (VIRINF) (Figure 3, Table 3). It is not possible for 1986: slopes of the equations corresponding to the three location were not significantly different but adjusted means were. Indeed, for a given value of RPST2, VIRINF is in 1986 generally weaker at Montauban de Bretagne and Broons that at le Rheu.

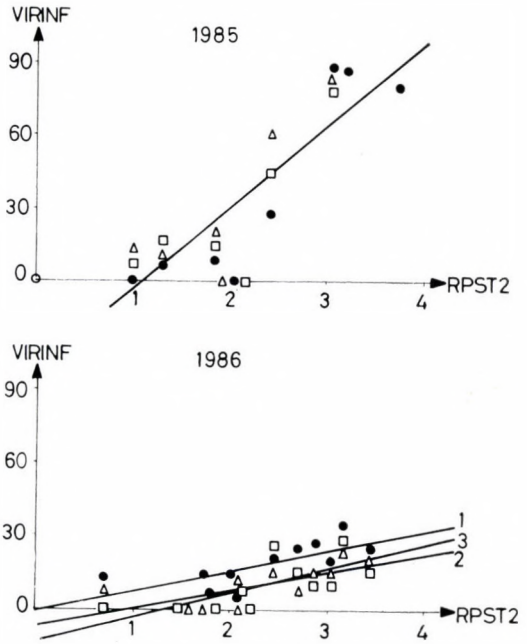

Fig. 3. The relationship of the weekly percentage of pots with infected barley tests seedlings (VIRINF) to the weekly accumulated catches of *R. padi* by suction trap less males (RPST2) at Le Rheu (●; 1), Montauban de Bretagne (△; 2) and Broons (□; 3)

Common slopes of the regressions are very different from one year to another (34.01 in 1985; 8.19 in 1986), which show that the *R. padi* were so numerous in 1986 than in 1985 but consistently less viruliferous.

Table 3

Characteristics of the regression between RPST2 and RPINF and VIRINF respectively

	Differences among slopes		Differences among adjusted means		Common slope		Common intercept	
	1985	1986	1985	1986	1985	1986	1985	1986
RPST2/RPINF	F = 0.24 (NS)	F = 1.4 (NS)	F = 0.64 (NS)	F = 5.6 (S)	33.9	26.16	−21.9	−14.5
RPST2/VIRINF	F = 0.13 (NS)	F = 0.23 (NS)	F = 0.13 (NS)	F = 6.5 (S)	34.01	8.18	−36.2	−6.2

S: significant (NS non significant) differences for = 0.05.

Discussion

Experiments which were designed at le Rheu during five consecutive years (1983–1987) allowed us to show a good interannual stability in the relationships between the weekly suction trap catches of *R. padi*, less males, and the percentage of barley pots infested by aphids *(R. padi)* during the same week. The above results show, in addition, a geographical stability of these relationships, at least for the same year, between locations distant from several tens of kilometers.

Weekly trap catches can thus be considered as representative of infestation of barley pots sited at 16 or 32 km from the suction trap, than of infestation of pots located at some tens of meters of the suction trap. This sort of result gives informations, on the geographical range of data given by suction trap in the case of *R. padi* in autumn and make possible the regional generalisation of forecasts of risks of primary contamination of barley by aphids.

The links between the percentage of infected plants by BYDV and suction trap catches of *R. padi*, less males, is very good in 1985 and good in 1986, but very different for both years. The fact that, at least in 1985, a common regression line could be drawn between all points whatever the locality they belong to, reveal a certain geographical stability, at least at the scale of our study, of the annual relationships between trap catches of *R. padi* and infestation of barley by BYDV. It shows that the relationships between the detected PAV-like isolate of BYDV and its main vector are more or less steady, for a given year, in a small region which is submitted to the same agroclimatic conditions and where the aphid life cycle is probably the same. The practical consequences of these findings result in possibility, for a given year, to extrapolate at a larger geographical scale the results of one only experimentation done near the suction trap (Dedryver et al., 1988).

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The Use and Validation of the Infectivity Index as a Method of Forecasting the Need to Control Barley Yellow Dwarf Virus in Autumn-sown Crops in the United Kingdom

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The infectivity index has been used for several years to guide decisions on the use of insecticides to control the aphid vectors of BYDV. The comparison of sequentially sown barley plots with the infectivity index in 1985/6 and 1986/7 showed that, at least for the area represented by Rothamsted, i.e. much of eastern England, the index gave a good forecast of virus infection and damage.

In Britain, the principal method for controlling barley yellow dwarf virus (BYDV) in autumn-sown cereals is the use of insecticides applied at, or near, the end of the autumn migration of its aphid vectors, usually at the end of October or the beginning of November. Although some regions of the country appear to be sufficiently prone to infection to justify routine treatment, in most areas the risk of infection differs from year to year. However, because the potential damage by BYDV is great and the cost of chemical treatment relatively small some farmers choose to apply insecticides prophylactically. To ensure that insecticides are applied only when necessary, which will decrease the risk of insecticide resistance developing in aphids and help conserve natural predators and parasites, we have been developing and implementing a forecasting system.

The Infectivity Index

Details of the infectivity index and how it is calculated have been given elsewhere (Plumb, 1983, Plumb, 1986, Plumb, Lennon and Gutteridge, 1986) but in outline it integrates potential vector populations, as sampled by the Rothamsted Insect Survey suction traps, with measurements of the infectivity of aphids caught alive and tested for BYDV by feeding on oat seedlings. The system is not perfect and recent results have suggested ways in which it might be improved by incorporating aspects of vector biology (Tatchell, Plumb and Carter, 1988). Nevertheless, for the area respresented by the Rothamsted rsults, which includes much of East Anglia, the principal and most productive cereal growing area in Britain, the forecast seems reliable. One reason for this is that a long run of data is available for Rothamsted and it has been possible to compare the infec-

tivity index of crops sown at different times over many years with their response to insecticide applied to control the aphid vectors. Consequently, a threshold value of 50 has been obtained for Rothamsted. For crops with an index above this an insecticide spray is advised. Crops with an index less than 50 are thought not to be at risk and no treatment is necessary. However, the index is a guideline that needs interpreting in the light of local experience so, ultimately, it is the farmer or his advisor who decides.

The index measures only primary infection, whereas most damage is caused by secondary spread within the crop. The insecticide treatment works efficiently because the extent of primary infection in our region is low. This is largely because the principal vector species, *Rhopalosiphum padi*, has a predominantly sexual population in the autumn and the number of aphids colonising cereals is generally small. There is usually little spread within crops until November. The index, as currently formulated, tends to over-estimate the risk of infection, as occasionally primary infection is not followed by spread in cold, wet weather.

We have tried to extend this system to other regions of Britain and at one time scientists at ten sites were trapping and testing aphids, calculating an index, and trying to relate this to crop infection. As part of this more extensive project an experiment was devised to try and validate the index for different sites.

Sequential Sowing Experiments

At each site where aphids were trapped and infectivity was measured, winter barley crops were sown at approximately 10 day intervals from early September. It was not possible to replicate the plots at all sites and only a Rothamsted was an insecticide treatment included in the experiment and so only the Rothamsted results are used to compare with the infectivity index.

The experiment, a factorial design of four replicates, has been done for three years, 1985/6, 1986/7 and 1987/8 but full results are available for only the first two years. The barley cultivar was Igri and the experiment was covered with a net before the crop ripened to avoid damage by birds. For each sowing date, the aphid population, incidence of BYDV and final yield were measured and compared with the infectivity index for each plot calculated in the usual way (Tables 1 and 2).

The sowing dates were almost identical in both years but the risk of BYDV, as measured by the infectivity index was very different. In the autumn of 1985 the maximum index was 95 and plots sown on the 13 September (87) and the 23 September (77) were above the threshold of 50. Aphid populations and numbers of BYDV-infected plants were greater on these plots than those sown later. The aphicide treatment (Cypermethrin at 25 g/ha) was applied approximately two weeks (14 November) later than the usual optimum date. Consequently, BYDV control was poorer than it should have been and only on the earliest sown crops was there a marked decrease in virus infection of sprayed plots. Aphid counts

were made after the crops had been sprayed, which showed that the insecticide treatment had been very effective. By contrast in autumn 1986 the infectivity index for all plots was well below the threshold and virus infection was negligible.

Table 1

Winter Barley — sowing date, infectivity index, BYDV and aphid incidence

1985/6					
Sowing date	Infectivity Index	Insecticide			
		−		+	
		Aphids/ Plant	Virus (%)	Aphids/ Plant	Virus (%)
13 Sept.	87	1.65	21.1	0	6.5
23 Sept.	77	0.59	4.5	0	6.1
2 Oct.	37	0.07	3.0	0	1.2
11 Oct.	30	0.05	0.5	0	0
23 Oct.	9	0	0	0	0

Table 2

Winter barley — sowing date, infectivity index, BYDV and aphid incidence

1986/7					
Sowing date	Infectivity Index	Insecticide			
		−		+	
		Aphids/ Plant	Virus (%)	Aphids/ Plant	Virus (%)
12 Sept.	12	0.33	<0.1	0	<0.1
22 Sept.	12	0 17	<0.1	0	<0.1
1 Oct.	3	0.18	0	0	0
10 Oct.	0	0.01	0	0	0
24 Oct.	0	0.01	0	0	0

Therefore, as expected from measurements of virus infection, only in 1985/6 were there increases in yield as a consequence of the insecticide treatment (Table 3). However, although insecticide treatment increased the yield of the 13 September-sown crop and had no significant effect on the 23 September crop, where BYDV had not been controlled, there was an increase in yield on the 2 October-sown crop where there was little virus infection on untreated plots. This result cannot therefore be explained in terms of control of BYDV.

In the 1986/7 crop, as expected, insecticide treatment did not increase the yield of any plots.

Table 3

Winter barley sowing dates and yield (t/ha)

Sowing date 1985/6	Yield (t/ha) Insecticide −	+	
13 Sept.	4.28	4.69	
23 Sept.	4.80	4.94	
2 Oct.	5.40	5.88	S.E.D. 0.201
11 Oct.	6.00	6.10	
23 Oct.	5.31	5.50	
1986/7			
12 Sept.	5.28	5.20	
22 Sept.	5.14	5.46	
1 Oct.	5.54	5.63	S.E.D. 0.225
10 Oct.	5.47	5.39	
24 Oct.	5.61	5.64	

Conclusion

The comparison of the infection by BYDV of sequentially sown barley plots supports the use of the infectivity index as currently the most reliable guide to likely infection by BYDV. Decisions based on the index will decrease the unnecessary use of insecticide and, at the same time, ensure that there is little yield lost to BYDV infection. Future studies seek to improve the index by incorporating results of biological studies of the aphid vector (Tatchell, Plumb and Carter, 1988), and by speeding up its availability through the increased use of serological diagnosis of BYDV, either in the test plants or in the aphids, in determining infectivity. We also feel that the methods developed for cereal aphids and BYDV may have wider application for other luteoviruses and possibly for the non-persistently aphid transmitted viruses.

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Comparison of the Most Common Aphid Species in Northern Italy as Vectors of a Maize Isolate of BYDV

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Two sets of parallel single and multiple transmissions of a maize isolate (M-PAV) of Barley Yellow Dwarf Virus (BYDV) were conducted under controlled environmental conditions. Nine of the most common aphid-species potential vectors of BYDV, present in Northern Italy, were used in the transmission trials. Their relative natural abundance was based on the pluriannual captures of the alatae, using two different suction traps. In the trials, in order not to lose the transmission capacity of the occasional or erratic vectors, besides one, three and nine aphids 15 individuals were also used/test plant. In the two sets of trials, sources of inoculum were *Avena byzantina* K. Koch plants, 28 and 12 days respectively after infection. The younger plants were the better sources.

Only *Rhopalosiohum padi* (L.), *Metopolophium dirhodum* (Walker), *Sitobion avenae* (F.) and *S. fragariae* (Walker) were vectors of M-PAV. The transmission-efficiency was in the above order. *Anoecia corni* (F.), *R. maidis* (Fitch), *Schizaphis graminum* (Rondani), *M. festucae* (Theobald) and *Myzus persicae* (Sulzer) on the other hand did not transmit the BYDV isolate. It is stated that there is a direct dependence between the length of the latent period of BYD in the plant and the efficiency with which the vector transmits its virus.

In Italy an isolate of BYDV (M-PAV) was originally transmitted from naturally infected maize plants, mainly using the aphid *Rhopalosiphum padi* (L.) as a vector, to *Avena byzantina* K. Koch and to 33–16 maize inbred line (Osler et al., 1985; Refatti et al., 1988). The virus isolate was identified as the PAV-type (sensu Rochow, 1969) on the basis of serological reactions (Loi et al., 1985; *L. Torrance*, personal communication) and after positive transmissions carried out by the aphids *R. padi*, *Sitobion avenae* (F.) and *Metopolophium dirhodum* (Walker) (Osler et al., 1988).

By using suction traps during a 6 year period, 13 different species of winged aphids potential vectors of BYDV were captured in Northern Italy (Peressini et al., 1988). Eight of them proved to be natural carriers of BYDV. In fact a variable proportion of individuals of such species infected potted *A. byzantina* plants after experimental transmissions under greenhouse conditions (Loi et al., 1988).

The present research was undertaken with the main purpose of ascertaining experimentally the relative ability of the most important aphid species present

and captured in Northern Italy – already known to be potential vectors of BYDV – to transmit the above mentioned M-PAV strain. A related aim was to compare the results obtained both using single and multiple transmission methods and sources of inoculum of two different ages.

Materials and Methods

The aphid species used were from clonal lines derived from a single agamic female captured directely on natural graminae plants or using suction traps. The virus-free colonies, obtained as described by Rochow (1959), were reared under appropriate conditions in a greenhouse (about 20 °C, 16 hrs/day of lighting, U. R. 60–80%).

The following nine species of aphids were artificially grown on suitable hosts and then tested for their M-PAV transmission capacity: *R. padi*, *S. avenae*, *Sitobion fragariae* (Walker), *Metopolophium festucae* (Theobald) and *M. dirhodum*, grown on oat; *Schizaphis graminum* (Rondani), on wheat; *Rhopalosiphum maidis* (Fitch), on barley; *Myzus persicae* (Sulzer), on beet; *Anoecia corni* (F.), on *Cornus sanguinea* L.

The M-PAV variant of BYDV was maintained in *A. byzantina* by using *R. padi* as a constant vector. Virus sources of inoculum were symptomatic *A. byzantina* plants used respectively 28 days (Set 1, Table 1) or 12 days (Set 2, Table 1) after they were infected. Potted *A. byzantina* at the one leaf stage was used as test plant. The two-day acquisition feeding period was on detached leaves of *A. byzantina* in Petri-dishes, placed in a growth-chamber at 20 °C and with constant light. The infection feeding was carried out in the greenhouse, for three days on single caged test plants. Only in the case of *A. corni*, were half of the test plants inoculated, in Petri dishes, through the roots.

The survival and the larviposition rate was estimated for all the aphid species used – except *A. corni* – only using the data gathered from the transmissions with 9 individuals/test plant.

At the end of the transmission, all the plants were treated with an insecticide and kept in the greenhouse for symptom expression.

The experiment consisted in two transmission sets, using in both cases the nine aphid species mentioned above. One, three, nine and 15 infective individuals per test plant were used for each aphid species. The number of test plants in each transmission trial ranged from 10 to 180 (according to the vector species and the number of test aphids/plant) in the first set (seee Table 1); in the second set, 24 test plants were inoculated, when one, three or nine aphids/test plant were used, and 12, with 15 aphids/test plant. An adequate number of check plants were infested with the corresponding number of virus-free aphids or kept uninfested.

Table 1

Results of transmission trials of the M-PAV strain of BYDV on A. byzantina carried out, using nine aphid species and different numbers of individuals per test plant

Aphids		I transmission set (XX) Test plants				II transmission set (XX) Test plants			
Species	No./plant	Inoculated	Symptoms + (%)	ELISA + (%)	M.I.P. (XXX)	Inoculated	Symptoms + (%)	ELISA + (%)	M.I.P. (XXX)
R. padi	1	64	44	44	16.6	23	83	78	12.2
	3	59	66	66	16.3	22	100	100	12.2
	9	38	97	97	12.3	24	100	100	9.8
	15	10	100	100	—	12	100	100	9.3
M. dirhodum	1	100	20	22	22.0	24	62	62	14.2
	3	20	25	40	18.8	22	77	77	12.6
	9	20	75	70	17.8	22	100	100	11.7
	15	10	90	100	—	12	100	100	10.9
S. avenae	1	100	5	4	20	23	22	22	13.4
	3	20	15	25	—	22	45	45	13.9
	9	20	50	65	18.7	24	100	100	13.3
	15	10	90	100	—	11	100	100	11.5
S. fragariae	1	180	0	0	—	24	0	0	—
	3	30	0	0	—	24	4	4	18.5
	9	30	10	10	20	23	43	48	17.5
	15	10	20	20	—	12	58	58	16.2
A. corni	3	70	0	0	—	—	—	—	—
M. festucae	1	70	0	0	—	24	0	0	—
	3	28	0	0	—	24	0	0	—
	9	24	0	0	—	24	0	0	—
	15	12	0	0	—	12	0	0	—
M. persicae	(X)	(X)	0	0	—	(X)	0	0	—
R. maidis	(X)	(X)	0	0	—	(X)	0	0	—
S. graminum	(X)	(X)	0	0	—	(X)	0	0	—

(X) Numbers of test aphifd and test plants, as M. festucae; (XX) Source of inoculum = A. byzantina plants 28 day after inoculated in set 1 and after 12 days in set 2; (XXX) Mean Incubation Period in days, in the plant

Daily observations were made on the test plants and the date of the incipient symptoms noted. Twenty-eight days after they had been inoculated all the plants were subjected to ELISA, using an antiserum corresponding to the "B" isolate of Plumb (1974), supplied by Bioreba, Switzerland.

Results

Table 1 shows the percentages of test plants infected by each of the nine aphid species used in both the two transmission sets. *R. padi* is the most efficient vector of the M-PAV strain of BYDV. *M. dirhodum* and *S. avenae* are moderately efficient. *S. fragariae* transmitted the M-PAV strain to an appreciable extent only when infections were carried out using nine or 15 viruliferous aphids/plant; in fact in the two sets only four plants out of the 54 inoculated became infected when 3 individuals/test plant were used and none of the 204 test plants reacted when each was inoculated with one aphid.

None of the remaining five aphid species demonstrated any vector capacity, either when a high number of test plants were used or when the number of aphids/test plant were increased. Not one of the plants used as control showed evident symptoms of BYD or reacted to ELISA.

In regard to the larviposition the average number of larvae for each adult female/day on *A. byzantina* was: 0.84 for *S. fragariae;* 0.79, *M. dirhodum;* 0.66, *R. padi;* 0.65, *S. avenae;* 0.60, *M. festucae;* 0.35, *S. graminum* (on wheat, 0.48); 0.24, *M. persicae* (on beet, 0.64); 0.10, *R. maidis* (on barly, 0.39).

The survival percentage of the test aphids after 2 and 3 days of inoculation, was as follows: *M. dirhodum*, 98–74; *S. avenae*, 97–94; *M. festucae*, 97–74; *S. fragariae*, 94–86; *M. persicae*, 91–41; *R. padi*, 84–84; *R. maidis*, 79–49; *S. graminum*, 77–48.

The average transmission efficiency of the four vector species was definitely higher in the second transmission set, where the plants used as a source of inoculum were younger than those of the first set. Almost 100% of the plants inoculated with 15 individuals of *R. padi*, *M. dirhodum* and *S. avenae* become infected in both the transmission sets.

The median incubation period of the disease in the test plants was progressively longer in plants infected respectively with *R. padi*, *M. dirhodum*, *S. avenae* and *S. fragariae*. Similarly, it became progressively longer in plants inoculated with 15, nine, three or one aphids.

The incubation period was longer also in the plants of the first set, than in the second one (Table 1).

Discussion

The data collected confirm that *R. padi* is the most efficient vector of the M-PAV strain of BYDV; *M. dirhodum* and *S. avenae* are also relatively active vectors. Among the remaining six species, only *M. fragariae* succeeded in transmitting the isolate.

In general, results based on symptom expression in the test plants were confirmed by the ELISA reaction. The adaptation of the test aphids to *A. byzantina*, based on the larviposition rate, was good for all the five aphid species nor-

mally grown on *A. byzantina (S. fragariae, M. dirhodum, R. padi, S. avenae, M. festucae);* the larviposition and survival rate at the end of the inoculation period were consistently lower for *M. persicae, S. graminum* and *R. maidis* as these aphids are not usually reared on oat.

On the grounds of the results obtained and particularly in regard to *S. fragariae*, it is evident that, when seeking to demonstrate the transmission capacity of a given unknown vector, it is not sufficient to simply increase the number of the test aphids using one individual per plant. On the contrary it is necessary to increase the number of aphids/test plant. This is especially true for transmissions using species with a low efficiency rate either due to the peculiar characteristics of the vector (i.e. *S. fragariae*) or to a poor source of inoculum (i.e. old plants, as in the first set, Table 1), or to any other cause.

When looking for relatively precise information on transmission efficiency of different vectors it is necessary to use a low number of test aphids/plant, preferably one per plant. Using groups of nine or 15 aphids and the multiple transfer method, the high percentage of the infected plants tends to mask possible differences among the transmission-rates of the vectors.

Considering the two sets of trials the highest transmission rates occurred when sources of inoculum were plants infected 12 instead of 28 days before.

The median incubation period of the disease in *A. byzantina* was shorter in the second transmission set, as it also was in plants infected by the most efficient vectors and when the number of the inoculative aphids per plant was increased.

These results, together with data already published (Osler et al., 1988), indicate that there is a direct relationship between the length of latent period of BYD in the plant and the efficiency with which the vector transmits the virus, "efficiency" being a consequence of any factor, like aphid species potency, quality of the source of inoculum, number of test aphids used per plant.

It is interesting to note that Sylvester et al. (1970) demonstrated a dosage effect on the incubation period of Sowthistle Yellow Vein virus in its aphid vector (= latent period). Osler et al. (1988) dealing with BYDV report that the median incubation period of the disease in the plant is correlated to the median latent period in the vector and to the transmission efficiency. It could mean that the latent period (in the aphid) and the incubation period (in the plant) as well as the transmission efficiency are all governed by a virus dosage.

From an epidemiological point of view it could be of interest that some aphid species such as *A. corni* and *R. maidis*, transmitted BYDV when captured in nature (Loi et al., 1988) but not the M-PAV strain in our artificial transmissions. This is an indication that in Northern Italy different BYDV-strains (besides M-PAV) and/or different aphid strains are present in nature.

It will be of interest in the future to compare the transmissibility – with at least the nine aphid species reported – of different strains of BYDV, including the RGV one (Rice "Giallume") isolated from rice (Belli et al., 1974).

Acknowledgement

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Monitoring the Barley Yellow Dwarf Virus Infectivity of Migrant Aphids Colonizing Oat Crops

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Investigations on the natural spread of barley yellow dwarf virus (BYDV) by aphids were carried out in 1982–1987 in a field close to the research station in Uppsala. Bait plants of oats were regularly placed next to a spring cereal crop from the beginning of June until the end of August. After one week's exposure the bait plants were examined for aphids, sprayed with an aphicide and then kept in a greenhouse to allow symptom of BYDV infection to develop.

Aphid frequency and spread of BYDV generally was highest during July. In two of the six years, 1982 and 1983, when most infected plants were found, the earliest BYDV-infection occurred in the second half of June. The number of aphids was highest in 1985 and 1986. Among the aphids found on the plants. *Rhopalosiphum padi* dominated in 1982, 1984, 1985 and 1987. *Sitobion avenae* in 1983 and *Metopolophium dirhodum* in 1986. The spread of virus was not correlated to the number of aphids found on the bait plants.

Barley yellow dwarf virus (BYDV) often occurs in spring cereal crops in Sweden. However, severe epidemics of BYDV are rare and only occur in one or two years of ten. Oat varieties cultivated show distinct symptoms and severe yield reduction when infected.

The dominant variant of BYDV in most years is PAV, non-specifically transmitted by *Rhopalosiphum padi*. However, the MAV-variant which is specifically transmitted by *Sitobion avenae* occasionally dominates (Rochow, 1979; Eweida et al., 1985a).

The biology of *R. padi* is well known and the size of populations can be forcasted by counting the eggs on *Prunus padus*. *S. avenae*, on the other hand, is less predictable as we do not exactly know, how it overwinters in Sweden.

To study the fluctuation of the infection pressure of BYDV within the growing season and between different years, investigations with oat bait plants in spring cereal crops were carried out in 1982–87.

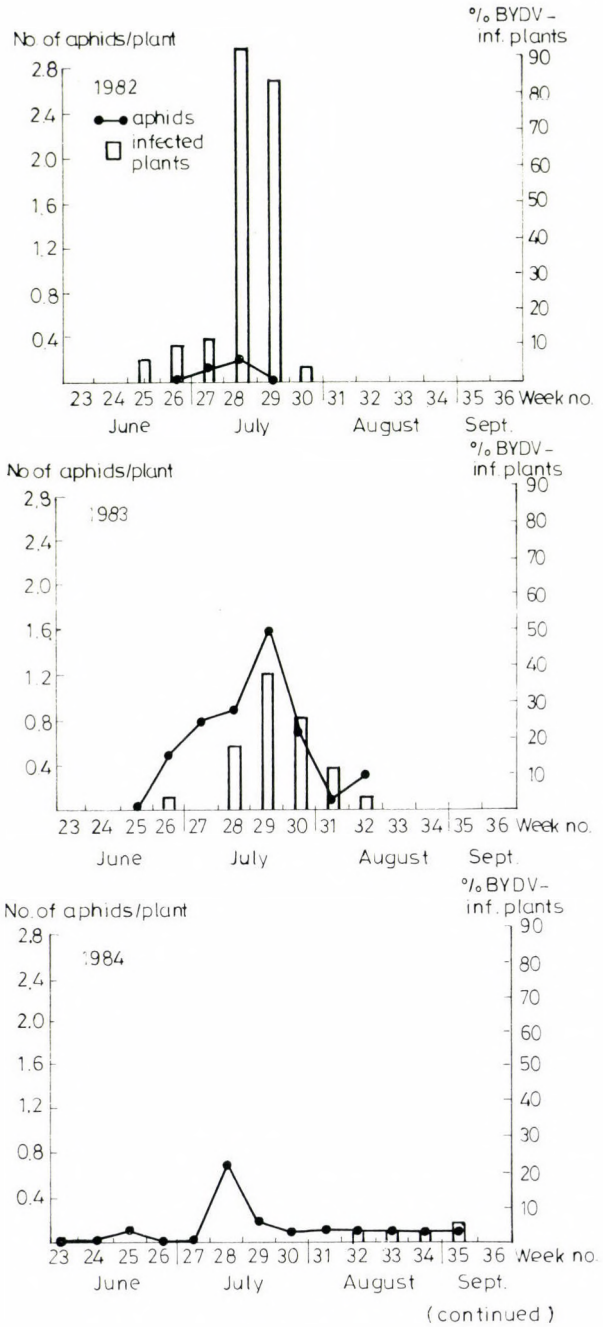
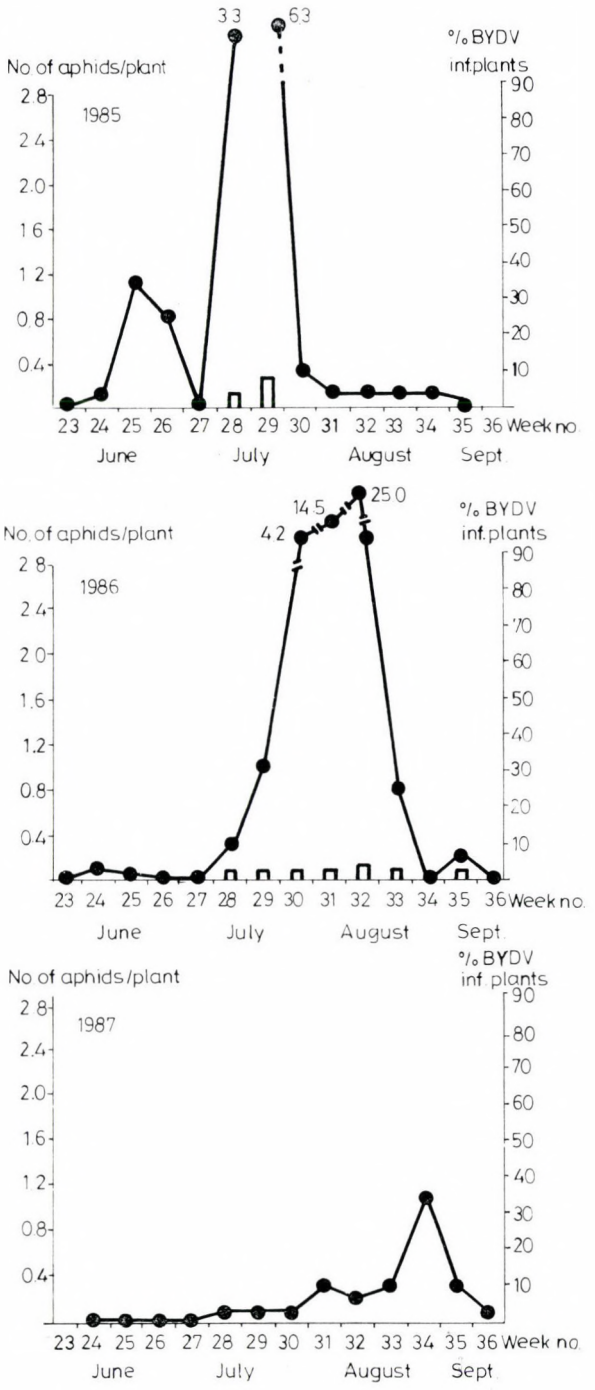


Fig. 1. Aphid activity and the spread of BYDV to bait plants of oats during the growing seasons of six years (1982–1987). Solid lines represent number of aphids per bait plant at the time of collection. Vertical bars represent percentage of plants showing symptoms of BYDV


No. of aphids/plant
% BYDV inf. plants
1985
33
63
1986
14.5
25.0
4.2
1987
23 24 25 26 27 28 29 30 31 32 33 34 35 36 Week no.
June
July
August
Sept.

Materials and Methods

Bait plants of oats were exposed at weekly intervals in an experimental field at the University of Agricultural Sciences in Uppsala during June, July and August each year. Three trays, with 50 oat plants in each, were placed at sites about 4 m apart and adjacent to spring oats or barley. The oat variety was Sol II, which is very susceptible to BYDV, and the plants were 11 days old when taken out into the field. To acoid damage by rooks a cage of chicken net was placed over each tray.

After one week's exposure plants in the trays were examined and the number of aphids recorded. The plants were sprayed with an aphicide and then placed in a greenhouse for four weeks to allow symptoms of BYDV infection to develop.

At the station there is a suction trap for aphids at a height of 12 m. By courtesy of Dr S. Wiktelius we could compare our counts with data from the suction trap.

To show which variant of BYDV dominated in different years, samples of oat plants with symptoms of BYDV were collected each year from different parts of southern Sweden. The samples were tested using aphid transmission to test plants and by ELISA, using antisera against a PAV-like and a MAV-like variant of BYDV produced by Eweida et al. (1985b).

Results

The results of each year are presented in Fig. 1.

1982 There were very few aphids on the bait plants, with the highest level of 0.2 aphids per plant in the second week of July. Nevertheless, 91% of the bait plants became infected the same week and 80% the following week. *R. padi*, the dominating aphid species, however, was abundant in the suction trap. PAV was the main variant of BYDV.

1983 S. avenae arrived early in the season and was relatively abundant in the suction trap. The highest level of aphids on the bait plants (1.6 per plant of mainly *S. avenae*), was found in the third week of July. The rate of infected plants the same week was 38%, which was the maximum value recorded. The dominating variant of BYDV was MAV.

1984 The number of aphids, mainly *R. padi*, was low on the bait plants as well as in the suction trap. The highest level of aphids, 0.7 per plant was found in the second week of July. BYDV infected plants did not occur until August, when about 5% of the plants became infected per week. PAV and MAV variants occurred to about the same extent.

1985 *R. padi* was the dominating aphid in this year as in 1982. The number of aphids on the bait plants was high with one peak in June (1.1 aphids per plant) and another peak in July (6.4 aphids per plant). However, very few

plants became infected, the highest level beeing 8% in the third week of July. PAV was the most frequent variant of BYDV.

1986 The number of aphids was very high with 4–25 aphids per plant in the end of July and the beginning of August. The most abundant aphid species was *Metopolophium dirhodum*, but many *R. padi* and *S. avenae* were also found on the bait plants. The BYDV infection was very low, with only 2–4% infected plants per week in July and August. The PAV variant of BYDV was dominating.

1987 Very few aphids were found this year and not a single bait plant became infected.

Discussion

The first bait plants were infected during June in 1982 and 1983, during July in 1985 and 1986 and during August in 1984. The two years 1982 and 1983 with the earliest infection of BYDV also had the highest infection rate in July. This is in agreement with previous experience that early migration of infective aphids and late sowing increase the risk of BYDV infection.

It seems to be no connection between the number of aphids on the bait plants and the spread of BYDV. For example, in 1982 most of the bait plants became infected in the middle of July, but the number of aphids was very low. As the aphids recorded on the plants after a week's exposure, is an estimate of aphid establishment rather than aphid visitations (Clement et al., 1986) many more aphids may have alighted on the plants and contributed to the spread of BYDV. Warm and sunny weather and high numbers of aphids found in the suction trap also indicate high activity of aphids during 1982.

On the other hand, in 1985 and 1986 there were a great number of aphids on the bait plants but infections were few. In 1983 only, there was a correspondence between number of aphids on the plants and virus infection. This last year, however, was also characterized by having MAV as the most frequent virus variant and *S. avenae* as the most important virus vector. It is possible that viruliferous *S. avenae* may have moved into Sweden from the south on wind currents in the spring of 1983, causing the outbreaks of MAV-isolates.

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Aphid Colonisation and Barley Yellow Dwarf Infections on Some Grass Species in North-East Italy

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Plots of *Festuca arundinacea* L., *Dactylis glomerata* L., *Bromus willdenovii* Kunt and *Lolium perenne* L. were cultivated in the experimental field and checked periodically over two years (1986 and 1987) in order to ecaluate the aphid colonisation and, if any, the infection due to barley yellow dwarf virus (BYDV).

The aphid colonisation was mainly seasonal in spring and in autumn. No colonies were observed in summer. *B. willdenovii* was the most colonised species, *D. glomerata* was more colonised than *F. arundinacea* and *L. perenne* the least. However a great difference in colonising aphid species was noted between the two years and between spring and autumn colonisation.

Sitobion (= Macrosiphum) avenae Fab. was the most common aphid species, followed by *Rhopalosiphum padi* L. and *Metopolophium dirhodum* W. *Rhopalosiphum maidis* Fitch, *Metopolophium festucae* T. and *Schizaphis graminum* R. were rerely found.

The BYDV presence in both *Avena byzantina* K. test plants and field plants was determined by ELISA.

The role of the grasses as a source both for aphid vectors and for virus inoculum in our agro-climatic situation was investigated.

Barley yellow dwarf virus (BYDV) can affect several Graminae and its epidemiology involves several aphid species (Oswald and Houston, 1953; Rochow, 1970; Plumb, 1977; Paliwal, 1982; Plumb, 1983). In our Region BYDV was identified for the first time in 1978 (Conti, 1978; Snidaro, 1978). The predominant BYDV strain has since been determined (Osler et al., 1984) and the most dangerous period for sowing autumn cereals has been ascertained (Snidaro, 1984; Peressini, 1987) also in respect of aphid migrations (Coceano, 1984; Peressini and Coceano, 1986). More recently studies have been concluded on the migration and on the infectivity of the main BYDV vectors (Caciagli et al., 1988; Loi et al., 1988; Peressini et al., 1988). The role of maize as a source both of BYDV and aphid vectors has been studied too (Osler et al., 1985; Coceano and Peressini, in preparation).

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Grasslands take up a little less than 1/5 of the area of the Friuli-Venezia Giulia Region and cover between 100,000 and 120,000 ha. A preliminary trial based on one hundred grass samples had collected without consideration of symptoms in different places of the Region had showed that about one third of samples was BYDV infected (Peressini, unpublished).

Taking into account such information and in order to improve our knowledge of BYDV epidemiology, the present work was undertaken to evaluate the role of grasses both as vector host and as a source of viral inoculum in our agri-climatic conditions.

Materials and Methods

Four species of grasses commonly grown in the Region, *Bromus willdenovii* K. (cv. Bellegarde); *Dactylis glomerata* L. (cv. Dora); *Festuca arundinacea* L. (cv. Dovey) and *Lolium perenne* L. (cv. Reveille), were sown in March 1986 in plots of 9×15 m, replicated three times for each species. The cultural methods used were the ones commonly followed in the Region.

To verify the aphid colonisation 30 potted plants of the same grass species were placed as bait-plants in every plot (total number 90 for each grass). Over two years eight checks before cutting the grasses were made, five in 1986 (in June, July, August, September and November) and three in 1987 (June, July and November). Meanwhile the migration of the aphid species colonising the grasses in the study area was recorded daily by a 12.20 m suction trap (Coceano, 1984). In each check all the bait-plants were observed individually. The check was made for colonisation and, if positive, the colonising aphids were classified and some of them (usually three wingless morphs) were transferred to a healthy potted *Avena byzantina* K. test plant at the growth stage of one and a half leaf. They were then taken into a glasshouse with 5 h of artificial light a day and a constant temperature of 19 °C. After an inoculation period of three days, the plants were treated with 0.05% Bromophos, a treatment repeated every 10 days. After 30 days during which the *A. byzantina* plants were periodically checked for BYDV symptoms, a portion of the leaves was taken from each test plant and tested in ELISA to diagnose the BYDV presence. After every check the colonised bait-plants were replaced in the field by healthy potted plants of the same age.

In autumn 1987 to verify the BYDV presence in our experimental plots 360 field grass plants of each species were sampled. For every bait-plant four plants were gathered from the positions North, South, East and West in respect to the bait-plants themselves.

ELISA test was performed in direct double antibody sandwich (DAS) ELISA (Clark and Adams, 1977) using an anti-BYDV PAV-type serum supplied by Bioreba AG (CH). Indirect ELISA, trapping the virus with rabbit polyclonal anti-BYDV antisera and using monoclonal antibodies for PAV, MAV and RPV types supplied by ADAS (UK) (Torrance et al., 1986), was used in 1987 only for testing the field samples.

All samples were stored at −20 °C until tested. One gram tissue samples were usually grinded in 1 ml of PBS containing 0.05% Tween 20, 2% polyvinyl-pyrrolidone (MW 25,000) and 0.02% sodium azide. ELISA values exceeding twice those for healthy control values were rated as positive.

The colonisation data was both considered as "effective" and also weighted with respect to the number of aphid catches in order to balance the difference in aphid migration which has been found between the two years. However, after the transformation the total number of aphid colonisation for each grass species remained of the same amount. After that, both "effective" and "weighted" data has been analysed using a log-linear analysis and using methods for testing association based on chi-square measure.

Results

Colonies on grasses were found only in spring and autumn periods, no definitive colonisation was recorded in the other checks.

Total catches, and also those related to the exposure periods of the bait-plants which were showed to have been colonised are listed in Tabl. 1. *Rhopalosiphum padi* L. and *Sitobion avenae* Fab. represented about 87% of total catches over the two years. *Metopolophium dirhodum* W. was consistently present only

Table 1

Total catches and catches recorded during bait-plants exposure periods of BYDV vector aphids colonising grasses. Suction trap 12.20 m. Pozzuolo del Friuli (UD) Italy (1986–1987)

Aphid species	Total catches (No.)	Catches of bait-plants exposure periods	
		spring	autumn
1986		21/4–12/6	15/9–19/11
Metopolophium dirhodum (Walk.)	10	10*	90
M. festucae (Theob.)	1	100	—
Rhopalosiphum maidis (Fitch.)	105	2	80
R. padi (L.)	1059	1	75
Schizaphis graminum (Rond.)	9	—	100
Sitobion avenae (Fab.)	489	73	9
1987		27/4–16/6	07/9–12/11
Metopolophium dirhodum (Walk.)	287	30	1
M. festucae (Theob.)	14	92	—
R. maidis (Fitch.)	98	17	22
R. padi (L.)	1793	2	28
S. graminum (Rond.)	18	5	39
S. avenae (Fab.)	466	47	4

* Expressed as percentage of total catches for each species.

in 1987 whereas amongst the other species examined only *Rhopalosiphum maidis* Fitch was regularly present, though in rather limited numbers. The migrations of the different species showed quite distinct periodic fluctuation: for *M. dirhodum* and *S. avenae* the spring migrations were heavier, while for *R. padi* the autumn ones were. A preliminary log-linear analysis based on the "effective" colonisation data as well as that based on "weighted" data (Table. 2) showed that a model with effects of first and second degreee seemed adquate to explain the experimental data (non reported). Nevertheless the analysis demonstrated it was better to consider together both the two spring periods and the two autumn periods. The autumn ones on the whole were greater than the spring ones. The analysis for testing association confirmed that the aphid activity, with regard to colonisation, was different (chi-square = 237.87, $P < 0.01$) and greater than all other species in *S. avenae*. In spring *S. avenae* was the most colonising species (55%) whereas in the autumn colonisation it was *R. padi* which colonised most (51%). The mixed colonies of *R. padi* and *S. avenae* represented about 14% of the autumn colonisation in 1986. On the other hand *M. dirhodum* took an a certain preminence in 1987 and made up 12% of the total spring colonisation. Moreover the four grass species showed a different behaviour to aphid colonisation (chi-square = = 95.77, $P < 0.01$). The relationship between the grasses under examination can be represented thus: *B. willdenovii* > *D. glomerata* > *F. arundinacea* > *L. perenne*.

No BYDV infection was found on the 515 aphid transmission on *A. byzantina* test plants with DAS ELISA using PAV-type serum. Total number of transmission greater than number of colonised bait-plants (Table 2) was due to mixed colonisations. Analysing the field plants (360 for each species of grass) only one of *F. arundinacea* resulted to be infected, both with DAS ELISA and indirect ELISA using anti-BYDV PAV-type sera. No infection was found using the monoclonal antisera MAV- and RPV-type in indirect ELISA.

Discussion

The colonisation data weighted with respect to the aphid catches was quite different from the "effective" colonisation data. However, the analysis confirmed that the aphid species most likely to colonise were *S. avenae* and *M. dirhodum*. On the contrary, *R. padi* seemed to be less inclined to colonise grasses.

The low percentage of BYDV infections found on the grasses during the two years can be explained, we believe, not only by the low annual average vectors infectivity but also by epidemiological implications in the spread of BYDV. The vector host-plant relationship in grasses in the study area was limited to the spring and autumn periods. *S. avenae* made up 55% of the total spring and about 34% of the autumn colonisation. This species has never been recorded to be infected in Friuli-Venezia Giulia (Loi et al., 1988). The same way be said of *M. dirhodum*, and therefore the contribution that these two species made to BYDV spread may be said to be practically of no value. *R. padi*, on the contrary,

Table 2

Spring and autumn colonisations by BYDV vector aphids on four grasses. Pozzuolo del Friuli (UD) Italy (1986–1987)

	Spring 1986 + 1987					Autumn 1986 + 1987					Total***
Grasses	A*	B	C	D	E	A	B	C	D	E	
B. willdenovii	60 (46)**	1 (1)	12 (16)	0	12	59 (76)	17 (0)	0	16	1	178/360
D. glomerata	21 (4)	7(23)	8 (7)	4	6	16 (1)	45 (20)	0	15	1	123/351
F. arundinacea	6 (0)	7 (13)	1 (0)	0	4	14 (38)	50 (86)	0	6	0	88/321
L. perenne	7 (1)	14 (18)	0	0	1	4 (6)	29 (27)	0	1	0	56/350
Total	94	29	21	4	23	93	141	0	38	2	445/1382
Percentage	55.0	17.0	12.3	2.3	13.4	33.9	51.5	—	13.9	0.7	

* Aphid species: A = *S. avenae;* B = *R. padi;* C = *M. dirhodum;* D = mixed colonies *R. padi* + *S. avenae;* E = others as *R. maidis*, *M. festucae*, *S. graminum* and mixed colonies different from *R. padi* + *S. avenae*.

** Numbers in brackets represent "weighted" colonisation data for the aphid species.

*** Number of colonised bait-plants / total number of bait-plants of each grass species. 9 of *D. glomerata*, 39 of *F. arundinacea* and 10 of *L. perenne* died before the checks.

with 17% of the total spring colonisation, 51% of the autumn colonisation and its high level of average infectivity noted until 1984 could be objectively considered the most important BYDV vector. In the last two years, however, its infectivity has decreased: 3% in 1985, 2% in 1986. For 1987 it was available only for October and November and reached 13%. This is confirmed by the low level of BYDV in the field plants noted at the end of the two year period. In a preliminary test made by one of us (Peressini, unpublished) both to verify the BYDV presence and to test that the ELISA could be applied, about one hundred samples of various grass species were collected from grasslands at least four years old. This analysis proved that 35% of *F. arundinacea* samples, 29% of *D. glomerata* and 70% of *B. willdenovii* were BYDV infected. It may therefore be considered quite probable that in our experimental field over the two years there was no virus accumulation required to reach such high BYDV infections.

This leads us to conclude that, as a source of vectors, grasses may take on a certain importance in some years, while their role as inoculum source is strictly connected to the infectivity of the colonising aphid population. However a definitive statement on this subject will required further studies.

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Virus Diseases of *Gramineae* in Hungary

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Virus diseases are playing a significant role in cereal pathology. This report gives a short summary on gramineaviruses in Hungary and a review of most important publications dealing with the problem of virus identification, transmission experiments, epidemiology and the resistance studies.

Barley yellow dwarf virus (BYDV) was described in Hungary by Szirmai in 1967. In this first report the symptoms, host range and vector transmission by *Macrosyphum graminearum*, *M. avenae* and *Rophalosyphum maidis* was described. Epidemiological problems, connected with the aphid transmission were described also by Szirmai in 1974a, b. Natural occurrence of BYDV in wheat, maize and rice was reported by Szunics and Szunicsné, 1980; Pocsai, 1983; Nagy and Milinkó, 1984; Pocsai et al., 1985, respectively. Yield loss caused by BYDV infection inw heat was calculated by Szunics and Szunicsné, 1980. The virus was considered as the most important gramineavirus.

Strains, BYDV have not yet been characterized. The epidemiology of the disease in Hungary is considerably different from those found in Northern or West-Europe (Nagy and and Milinkó, 1984). In our continental climate both the hard winter and hot summer can limit the distribution of the pathogen (Nagy and Milinkó, 1986). It spreads mainly by cereals and volunteer plants. Resistance study of different varieties based on aphid transmission test was made by Pocsay and Szirmai, 1985.

The virus was never purified, no antisera were produced. Serological detection by ELISA is based on antisera from different foreign sources.

Barley stripe mosaic virus (BSMV). The pathogen was found in the fields by Milinkó and Remete (1984). Recent evidences showed a presence of a new, Hungarian strain (BSMV-H). Infection of this strain causes very mild symptoms in barley and symptomless in wheats. In spite of the mild symptoms, the virus concentration is relatively high. The BSMV-H genom contains three genom and a small subgenomic RNAs (Nagy and Gáborjányi, 1988). The H-strain is virulent and infects those barley and wheat varieties, which were detected earlier (Pocsai, 1985) as resistant ones to the infection of BSMV-Russian strain (Pocsai et al., 1985).

Acta Phytopathologica et Entomologica Hungarica 26, 1991
Akadémiai Kiadó, Budapest

Brome mosaic virus (BMV). In 1986 Szirmai described this virus form *Dactyllis glomerata* in a complex infection with cocksfoot mottle virus, and later from wheat. According to the last years screening the BMV is the most commonly distributed virus of small grains (Gáborjányi and Nagy, 1988). Susceptibility of wheat and barley varieties was compared by Pocsai (1986). Seed transsmission was examined by gel-diffusion test (Pocsai, 1987). The abundance and rapid distribution of BMV in small grains seemed to be correlated to the gradation of *Oulema melanopus* and other *Oulema* species. Positive vector transmission was demonstrated from BMV infected wheats to wheat, but not from wheat to corn plants (Gáborjányi and Szabolcs, 1987, Szabolcs and Gáborjányi, 1988). The virus infection is easily detectable by SDS-polyacrylamide electrophoresis or by serological means.

Wheat streak mosaic virus (WSMV). The presence of this virus in Hungary was detected by serological diagnosis and electron microscopy (Gáborjányi and Nagy, 1988). The description of properties of our isolate is in progress.

Wheat dwarf virus (WDV). A newly recognized virus in Hungary. Adults and nymphs of the leafhopper *Psammotettix alienus* were able to transmit the pathogen from infected plants to young wheat seedlings with high frequency. Virus purification procedure was modified to get pure virion solution. Germinated particles were shown by electron microscopy. Presence of a single nucleic acid band resistant to RNAses and sensitive to DNAse was demonstrated. Hungarian isolate of WDV is serologically identical with the Czechoslovak standard strain (Bisztray et al., 1988).

Maize dwarf mosaic virus (MDMV). First occurrence of the pathogen was reported by Szirmai and Paizsné in 1963. Aphid transmission by *Doralis fabae*, and *Rophalosyphon maidis* was demonstrated. Population dynamics of vectors were studied by Milinkó et al. (1983). In Hungary the MDM seems to be the most widespread virus disease of maize. It infects maize, *Sorghum bicolor* and *S. halepense*. The later plays important role in the overwintering of the pathogen (Milinkó et al., 1979). Early works showed that all the varieties are susceptible to mechanical inoculation. Reactions of different corn varieties to MDMV are known (Szirmai, 1968, Milinkó et al., 1982). Resistance to MDMV infection may be due to at least for the most part of aphid resistance (Milinkó et al., 1983). In the past years the resistance of different varieties were compared in natural conditions, in which the infested *Sorghum halepense* served as natural source of inoculum (Milinkó et al., 1984). Yield loss caused by MDMV infection was studied by Peti (1983). AntiMDMV sera was produced by Sum et al. (1979) for ELISA.

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Appearance of Barley Yellow Dwarf Virus (BYDV) on Wheat in Hungary

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The barley yellow dwarf virus was first identified on wheat in the Martonvásár wheat breeding nursery in 1972. Since then it has been found almost every year. The virus causes a reduction in the height and yield ability of the infected plants, an increasc in the protein content and changes in the amino acid composition. There is a distinet difference in susceptibility between the genotypes.

Barley yellow dwarf virus was first found in Hungary on winter barley in 1966 (Szirmai, 1967), appearing on a large scale in 1982 (Pocsai et al., 1983). Substantial damage was first observed on wheat in 1972, since when it has been found in the Martonvásár nursery almost every year (Szunics and Szunics, 1980). Major infection was recorded in 1976, 1980, 1981, 1982 and 1986. In recent years small centres of infection have been observed in several wheatfields, but so far the spread of BYDV in Hungary is only a local problem.

Materials and Methods

The work was carried out at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, where the plants became spontaneously infected with barley yellow dwarf virus in the nursery. The degree of infection was scored on a 0 (resistant) to 9 (intensely susceptible) scale. For the various examinations plants characteristic of the variety and resistant to BYDV were taken as the control.

The quantity of protein in the grain yield was calculated by the Kjeldahl method ($N \times 5.7$) in terms of 86% dry matter content, and the amino acid composition was determined using a JEOL amino acid analyser.

Results

In recent years several thousand genotypes have been tested. Research on virus resistance has confirmed that there are distinct differences in susceptibility between the varieties. Under the same agroecological conditions some are infected

more intensely and others less severely. Among the better-known varieties the following can be regarded as resistant at Martonvásár so far: Bánkuti 1201, Bezostaya 1, Yubileinaya 50, Martonvásári 4, Martonvásári 7, Martonvásári 8 and Martonvásári 10. These can be used in breeding programmes as sources of resistance. The following varieties proved to be susceptible: NS Rana 1, NS Rana 2, Sava, Machvanka 2, Lonja, Super Zlatna, Ibis, Chul, Atlas 66, Arthur, Martonvásári 5, Martonvásári 9, Martonvásári 12, GK Csongor, GK Szeged, GK Boglár, GK Zombor, GK Basa, GK Minaret.

On the basis of data collected over a number of years for several varieties, the height of diseased plants was found to be 45.9–66.4%, the grain number 19.8–41.8%, the grain mass 10.8–39.5% and the thousand grain mass 56.9–94.3% that of their healthy counterparts.

The greater the extent of infection, the smaller and more shrivelled the wheat grain. This leads to an intense reduction in the endosperm and a consequent rise in the germ and husk percentage. There is an increase in the protein content of the grain (Table 1). The least change was recorded in GK Zombor (2.74%) and the greatest in Zagrepchanka (6.57%). In Table 2 the amino acid compositions of the varieties Martonvásári 13 and GK Boglár are presented on the basis of data from 1982 and 1986. As the result of BYDV infection the quantity of lysine, arginine and cistine decreased in both varieties. In other cases certain differences were found between the varieties and the years. For example, in the years examined the proline content of Martonvásári 13 rose as the result of infection, while that of GK Boglár decreased slightly in 1982 and rose substantially in 1986.

Of the 16 pairs of characters evaluated, 9 gave significant correlations, 2 of which were loose, 4 medium, 2 close and 1 very close (Table 3). An extremely close negative correlation was found between the degree of BYDV infection and plant height and a close negative correlation with grain number/plant and

Table 1

Effect of BYDV infection on protein content (%)
(Average of 1982, 1983 and 1986 data)

Variety	Healthy	Diseased	Deviation
	Plants		
Martonvásári 9	13.55	18.03	4.48
Martonvásári 12	13.27	17.90	4.63
Martonvásári 13	11.62	15.18	3.56
GK Csongor	12.25	18.68	6.43
GK Boglár	12.13	16.91	4.78
GK Zombor	12.19	14.93	2.74
Zagrepchanka	12.35	18.92	6.57
Lonja	12.17	17.88	5.71
Baranjka	12.50	15.25	2.75
Beauchamp	11.87	15.25	3.38

grain mass/plant. BYDV infection was in medium positive correlation with general tillering, protein and proline content and medium negative correlation with the thousand grain mass. The correlation coefficients for lysine and arginine quantities suggest a loose negative correlation with the degree of BYDV infection.

Table 2

Effect of BYDV infection on the amino acid compositions of Martonvásári 13 and GK Boglár (%)

Amino acid	Martonvásári 13				GA Boglár			
	1982		1986		1982		1982	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
1. Lysine	2.95	2.90	2.85	2.79	3.09	2.76	2.93	2.78
2. Histidine	2.38	2.42	2.38	2.33	2.57	2.37	2.44	2.31
3. Arginine	5.47	5.24	5.01	4.90	5.28	5.12	5.14	4.87
4. Cistine	2.11	2.08	2.04	2.00	2.21	2.04	2.10	1.99
5. Aspartic acid	4.64	5.27	5.18	4.77	5.63	5.22	5.57	5.86
6. Methionine	1.67	1.66	1.73	1.60	1.77	1.90	1.68	1.59
7. Threonine	2.11	2.84	2.76	2.48	3.18	2.62	2.87	2.82
8. Serine	4.04	4.99	4.44	4.48	5.19	5.05	4.14	5.03
9. Glutamic acid	33.97	33.37	32.84	35.02	35.51	32.78	33.72	31.95
10. Proline	9.47	10.56	9.44	10.64	11.38	10.83	9.18	11.44

Table 3

Correlation between the degree of BYDV infection and certain characters of wheat

Character	Correlation coefficient
1. General tillering	0.532^{+++}
2. Plant height	-0.918^{+++}
3. Grain number/plant	-0.820^{+++}
4. Grain mass/plant	-0.895^{+++}
5. Thousand grain mass/plant	-0.667^{+++}
6. Protein	0.670^{+++}
7. Lysine	-0.326^{+}
8. Histidine	0.061
9. Arginine	-0.357^{+}
10. Cistine	−0.195
11. Aspartic acid	−0.007
12. Methionine	−0.116
13. Threonine	−0.233
14. Serine	−0.044
15. Glutamic acid	−0.205
16. Proline	0.465^{++}

Discussion

BYDV is a potential danger to wheat in Hungary. In early wheatfields certain wheat varieties are strongly infected, particularly if the autumn is mild, providing favourable conditions for aphid gradations. As the result of infection there is a reduction in the height and productivity of the plants. The protein content of the shrivelled grains increases, but there is an unfavourable change in the amino acid composition, e.g. the quantity of lysine is lower. The cultivation of resistant varieties and their use as sources of resistance could be an important component of biological plant protection.

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Studies on UK Isolates of Barley Yellow Mosaic Virus

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Two strains of barley yellow mosaic virus, termed 'Streatley' and 'Wiltshire' occur throughout the winter barley growing regions of the UK. The 'Wiltshire' strain proved more sensitive to buffer pH in ISEM and could only reliably be detected in ELISA if full cream milk powder was substituted for ovalbumen in the extraction buffer. The 'Wiltshire' strain appeared to be the more frequent of the two, at least on feeding barleys, and has a temperature optimum below 15 °C.

Barley yellow mosaic virus (BaYMV) has been known in the UK since 1980 (Hill and Evans, 1980). It is now known to occur widely in southern and eastern England wherever winter barley is grown and causes significant yield losses. Two serologically distinct strain were reported in West Germany (Huth et al., 1984) and surveys in England have demonstrated a very similar situation (Adams, Swaby and Jones, 1987). The nomenclature of the strains and some other known properties are summarised in (Table 1).

This paper reports procedures for detecting the strains and some data on their distribution and properties.

Strain detection

Virus purification and antiserum production have been described previously (Adams, Jones and Swaby, 1987).

ISEM. In immunosorbent electron microscopy (ISEM) using homologous antisera fewer particles are generally trapped from the 'Wiltshire' than the 'Streatley' strain. Results from spray preparations suggest that this may be due to differences in virus concentration and not just in antiserum titres. When buffers of different pH were used for preparing leaf samples few particles of the 'Wiltshire' strain were trapped if the pH was below 7.0 but the 'Streatley' strain was trapped equally in all treatments (Table 2).

ELISA. For routine tests, the $F(ab')_2$ indirect ELISA system with an alkaline phosphatase assay has been used (Adams, Swaby and Jones, 1987). Good results were obtained with the 'Streatley' strain but using antisera raised to the 'Wiltshire'

Table 1

Strains of barley yellow mosaic virus

Strain names	I	II
UK	Streatley	Wiltshire
W. Germany	BaYMV-M	BaYMV-NM*; BgYMV-So^{+}
Related to		Japanese BaYMV and WYMV
Sap transmission	Yes	No / with difficulty

*, when occurring mixed with BaYMV-M; $^{+}$, when occurring on its own (see Huth, 1986).

Table 2

Effect of buffer pH on trapping of BaYMV in ISEM

Virus strain	pH				
	6.0	6.5	7.0	7.5	8.0
Streatley	>40	>40	>40	>40	>40
Wiltshire	0	1/5	9	7	7

0.06M phosphate buffer with 0.1M EDTA. Numbers are particles per field at 45000 magnification.

strain, non-specific reactions led to large absorbance values with healthy leaf sap and samples known to be positive (by ISEM) could not reliably be detected. The usual ELISA buffer containing 0.2% ovalbumen (Clark and Adams, 1977) was modified by substituting either skimmed milk powder (Five Pints, St. Ivel, Swindon, Wilts., England) or full cream milk powder (Nido, Nestlé, Croydon, England) at various concentrations. Non-specific healthy reactions were diminished by both skimmed and full cream milk powders. Best results were with full cream milk powder at 1.0% (or higher in other experiments not presented here), with which enhanced positive values were also obtained. Results with the 'Streatley' strain were also improved by this procedure (Table 3).

Strain differences

Occurrence in the UK. During winter and spring 1987 and 1988, samples of winter barley with symptoms of BaYMV were collected, or received from regional offices of the Agricultural Development and Advisory Service, Ministry of Agriculture. Strain diagnosis was usually on the basis of trapping in ISEM. In both years, the majority of samples received were of the 'Wiltshire' strain and a few samples had both strains present (Table 4).

Table 3

Effect of using ovalbumen, skimmed milk powder (SMP) or full cream milk powder (FCMP) in ELISA buffers for detecting two strains of BaYMV

		Streatley			Wiltshire		
		B	H	D	B	H	D
Ovalbumen	0.2%	0.172	0.365	2.830	0.228	0.979	1.197
	1.0%	0.159	0.361	2.869	0.326	0.907	1.021
SMP	0.2%	0.092	0.190	2.749	0.162	0.431	0.988
	1.0%	0.106	0.098	2.807	0.157	0.191	0.918
FCMP	0.2%	0.079	0.230	2.718	0.180	0.430	1.313
	1.0%	0.080	0.172	2.810	0.174	0.230	2.343

Values are A_{405} after overnight incubation; samples at 1/100. B, blank; H, healthy sap; D, diseased sap.

Table 4

Occurrence of strains of BaYMV in samples: 1987 and 1988

Strain	1987	1988
Wiltshire	38	28
Streatley	9	13
Both	8	6

In both years, most samples were received from the west of England (Wilts, Avon, Glos) and it is not known whether the relative proportions of the two strains is similar in eastern England where BaYMV is also prevalent.

Cultivar response. As reported by Adams, Swaby and Jones (1987), the samples of the feed barley cvs Igri and Panda received in 1987 were mostly of the 'Wiltshire' strain, whereas the 'Streatley' strain was the more common on the malting cvs Maris Otter, Halcyon and Pipkin. Because the 'Wiltshire' strain is not readily transmitted mechanically, vector zoospores containing this strain are needed before cultivars can be inoculated with both strains under controlled conditions.

Temperature optima. With the 'Streatley' strain, the most rapid symptom production occurred at 20–23 °C (Adams, Swaby and Macfarlane, 1986; Adams and Swaby, 1988). The 'Wiltshire' strain appears to have a lower temperature optimum (Adams, Swaby and Jones, 1987) and when field-grown plants have been transplanted to pots in the greenhouse, symptoms disappear rapidly when the temperature exceeds 15 °C.

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Studies on Strains of Barley Yellow Mosaic Virus (BaYMV)

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In the German Democratic Republic the BaYMV was detected for the first time in 1983 (Proeseler et al., 1984). After developing a suitable purification method we were able to produce antisera to BaYMV from field material, to the strain M and to one strain from Great Britain (Kühne et al., 1985). The latter strain was mechanically transmissible only with difficulties and was therefore designated as BaYMV-MD. It was serologically related to ByMV-Japan, BaYMV-So, wheat yellow mosaic and wheat spindle streak mosaic viruses, but not to BaYMV-M. By using immunoelectron microscopy we demonstrated the occurrence of two serologically unrelated strains of BaYMV in our country, too. (Stanarius et al., 1987).

Continuing our investigation on strains of BaYMV, we examined the behaviour of these strains in single winter barley plants (cv. 'Erfa') from an infested field at Aschersleben during the spring of 1987. After the snow had thawed by the end of March, 35 plants carrying MD particles were selected. Using immunosorbent electron microscopy with antibody decoration (ISEM-D), the occurrence of both strains in these plants was investigated in weekly intervals. If available, leaves with symptoms were used, or one young green leaf was sampled.

ISEM grids were prepared with mixed antisera to BaYMV-M and -MD, the strain-specific antisera were used to decorate the virus particles.

The plant material was homogenized with 0.05 M K_2HPO_4 buffer (pH 7.0) in the mortar, and ISEM grids were floated for two or three hours on drops of the homogenates. After washing with distilled water the particles were decorated with strain-specific antisera. The grids were washed with buffer and water and finally stained with uranyl acetate. They were examined in a JEOL 100 B electron microscope.

In Figure 1 the occurrence of the BaYMV strains at different dates of testing in relation to the dynamics of the mean temperature of the single week ago are shown. From April to July, short before the barley plants had finished their maturation, particles of both strains could be detected. Although, there were differences in the quantity of particles and in some cases one or both strains disappeared for a certain time, no plant was found carrying one of the both alone during the whole test period.

The MD particles seemed to be able to multiply even at temperatures near or below 0 °C, whereas the M particles occurred in very low concentration at that time. A rapid temperature rise in the week from 28th April to 5th May caused a drastic decrease in the concentration of MD particles. Slow temperature rise

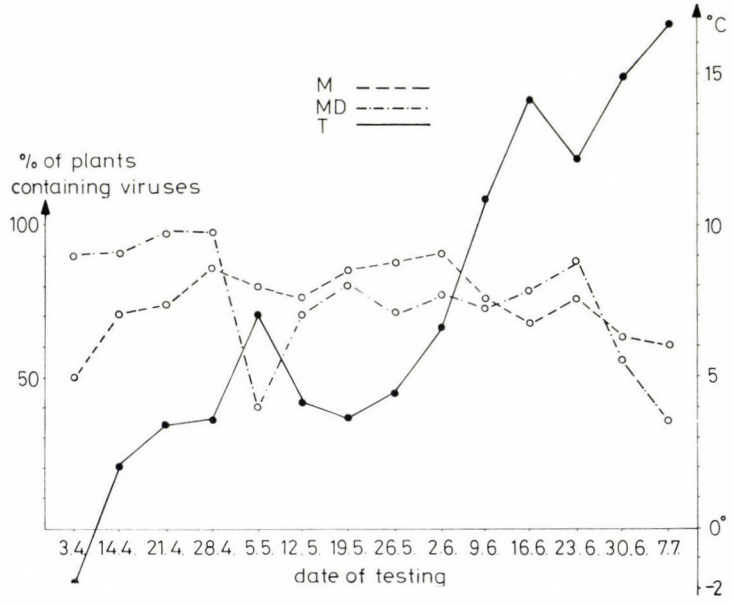

Fig. 1. Occurrence of BgYMV strains in winter barley plants cv. 'Erfa' of an infested field and the temperature dynamics in the spring of 1987

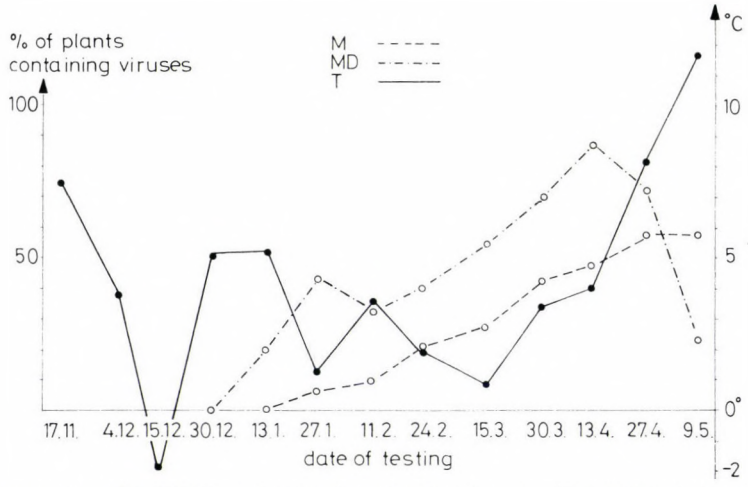

Fig. 2. Occurrence of BaYMV strains in winter barley plants cv. 'Erfa' of an infested field and the temperature dynamics from November 1987 till May 1988

did not have such a great influence on particle amount, but normally rising of temperature decreased the virus concentration, and symptoms disappeared.

For repeating our results from 1987 30 randomly selected winter barley plants (cv. 'Erfa') grown up in our infested field were examined by ISEM-D at the middle of November. At that time plants had developed some leaves and shoots. Because the winter time was mild in this year and snow had fallen only seldom it was possible to collect leaf material continuously in about two-weekly intervals. The results are shown in Figure 2. Although, there were great differences in weather conditions between 1987 and 1988 and the test periods were quite different, too, the results we obtained were rather equal. As in 1987, both strains were found together in the same plants and particle concentrations varied clearly depending on changes of temperature. But contrary to 1987, till now five plants were found containing the MD particles alone. In January and February the MD particles were preponderant, but the M particles could be detected in some plants alone, too.

Altogether, in 1988 the virus infection was lower than that in 1987. This is certainly attributed to the much higher temperature in early spring time of this year, which was double the size at Aschersleben.

Summarizing the two-year results (the results of 1987 are published in the meantime by Stanarius et al., 1988), the particles of both strains may occur in susceptible plants from winter time till maturation in summer. But the virus concentration depend on temperature dynamics. Rising in temperature decreased particles concentration and symptoms disappeared. The MD particles were much more sensitive than the M particles. Therefore, it would be possible to fail in virus diagnosis for a time, by that false conclusions can be make out.

In the past all attempts failed to obtain one own MD-like isolate. After mechanical inoculation of MD-containing material the M particles always appeared, too. Therefore, other plants in our infested field have been examined for symptoms since the end of November, because there are some indications from the literature for an earlier occurrence of MD particles opposite to the M particles.

Between 6th January and 4th February 18 plants with symptoms could be observed. 11 of them carried only the MD particles, 3 were mixed infected and in the other 4 plants only the M particles were found, indicating again that M particles may occur very early in the year, too. With the MD-containing and mixed-infected plants transmission tests were carried out. Altogether, 207 plants were inoculated. The infection rate of MD was 10 to 20%, but for M more than 50%. In one case, besides MD particles, the M particles could be found after inoculation of MD material in two plants out of 10, indicating that M particles must be present in the source at such a level not detectable by ISEM-D. But on the other hand in some cases after inoculation of mixed-infected material, there were plants carrying only the MD particles. After a repeated mechanical inoculation of sap from those plants only MD particles were present.

Some studies were initiated for comparing the properties of our new MD-like isolate with MD from Great Britain. Using ELISA and IEM no serological

differences were observed. On the other hand the biological behaviour seems to be different in some details. At present, attempts are being carried out to compare incubation time, infection rate, symptom development and intensity, and virus concentration by means of ELISA.

Summarizing our results, including those published in literature (e.g. Huth et al., 1984; Ehlers and Paul, 1986; Huth 1986; Adams et al., 1987), barley yellow mosaic syndrome in Europe seems to be caused by two serologically and biologically distinguished viruses inducing the same symptoms: the easily transmissible BaYMV-M and the BaYMV-MD which is difficult to transmit mechanically. The latter virus is represented by several isolates with very similar properties, e.g. BaYMV-GB, BaYMV-Japan, BaYMV-So, BaYMV-NM or BaYMV-As. Analogous conclusions were attained by Huth (pers. communication).

Finally, we approve of the proposal by Huth (1988, this report) to use the name barley mild mosaic virus instead of BaYMV-M in the future. All other BaYMV isolates (like MD) correspond to that BaYMV described in CMI/AAB Description of plant viruses No. 143 (1975).

Acknowledgements

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Possibilities of the BaYMV-Identification from the Soil by Means of Serological Methods

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The indentification of fungus-borne plant-pathogen viruses in soil takes place mainly by using indicator plants for the biological test ("Trap-plant method" – Hillmann, 1984; "biotest" – Kegler and Proeseler, 1987 (in press). The biological test consists of growing virus/vector host plants in infested soil, however, requiring several weeks and months, before virus identification by means of symptom verification and serological methods (DAS-ELISA – Clark and Adams, 1977) can take place. There are reports by Beemster and De Heij (1987) on direct ideintification of a plant-pathogen virus, beet necrotic yellow vein virus (BNYVV) in soil. They succeeded in detecting BNYVV by serological and hybridisation tests after incubation of eluates of infested soil at 40 °C.

We tried to apply the experiences gained by Beemster and De Heij (1987) in incubation to the identification of barley yellow mosaic virus (BaYMV).

Since September 1985 observations are being made on the occurrence of *Polymyxa graminis*, the vector of BaYMV, in the course of the autumn barley vegetation period. In the years 1985 to 1987, as arising from (Fig. 1) already 3 months after the emergence of winter barley, the first fungus attack by hand of resting spores in roots (Fig. 2) and virus infections by first symptoms and first positive values of ELISA (Proeseler et al., 1987) could be detected.

Comparing investigations carried out at the time of the enormous occurrence of yellow mosaic (in April) on the virus content in leaves, stalks and roots showed that BaYMV is detectable in high virus concentrations in the aerial plant organs, whereas, in very low amounts in roots (Fig. 3). The relatively low virus amount in roots was not to be realized mostly by help of the DAS-ELISA.

A DOT-Immunobinding assay (DIBA), described by Banttari and Goodwin (1985), modified for the BaYMV detection in roots and a immune electronmicroscopy technique with decoration (ISEM-D) gave better results. Both methods were parallely used at the same time. The well defined detection of BaYMV in roots of 8 winter barley varieties by DIBA and ISEM-D in contrast to DAS-ELISA is shown in Table 1.

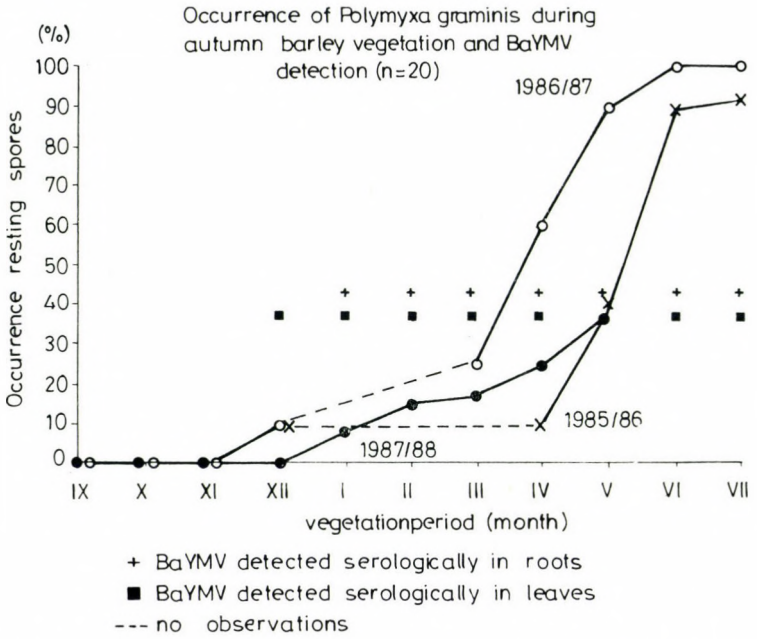

Fig. 1. Occurrence of *Polymyxa graminis* during autumn barley vegetation and BaYMV detection (n = 20)

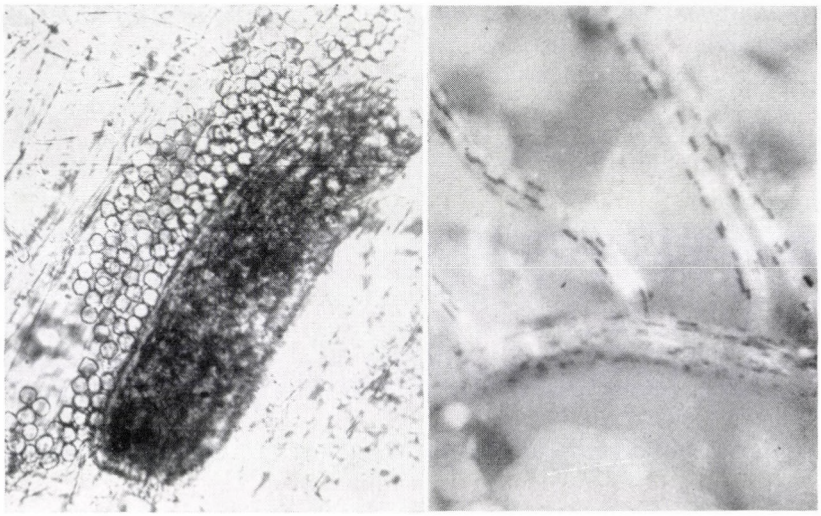

Fig. 2. Resting spores in root (126 x/16 x)

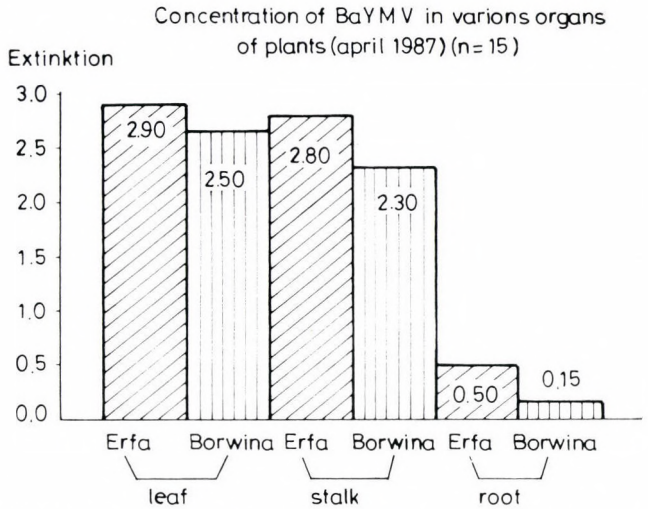

Fig. 3. Concentration of BaYMV in various organs of plants (April 1987) (n = 15)

Table 1

Comparing investigations on BaYMV-detection in roots of living plants of different winter barley varieties by means of DAS-ELISA, DIBA and ISEM-D (n = 10)

Varieties	Detection of BaYMV by means of					
	DAS-ELISA		DIBA		ISEM-D	
	leaf	root	leaf	root	leaf	root
'Borwina'	+	−	+	+	+	+
'Dilana'	+	−	+	+	+	+
'Erfa'	+	−	+	+	+	+
'Friberga'	++	−	+	+	++	+
'Leuta'	+	−	+	+	+	+
'Plana'	++	−	+	+	++	+
'Rubina'	++	−	+	+	++	+
'Vogelsänger Gold'	+	+	+	+	+	+

− No ByYMV-detection
+/++ BaYMV-detection of lower/mean intensity

We compared 2 methods for indirect detecting of BaYMV in soil samples:
– On the one hand, the root remnants were sieved from soil samples (1 kg). A third of washed roots was dried, another one was frozen and a further third was incubated at 28 °C for 48 hrs by adding root extracts. As the results show in Table 2, BaYMV being obviously degenerated completely was not detectable by means of the following serological and electron-

microscopic tests in dried roots. Whereas, the virus remained alive in low concentrations and in a low amount of particles, respectively, in the frozen samples. The incubation had a very favourable effect, as the germination of zoospores from resting spores existing in roots was stimulated. The virus could be released evidently from the vector. The detection of BaYMV by this method was especially possible from April to June.

– On the other hand, the checking of a possible detectability of the virus took place directly from soil eluates. Soil samples (100 g) containing many root remnants and taken from different locations were filled into a glass tube closed below by cork and soaked with root extract. Afterwards, the soil filled into this tube was incubated at 28 °C for 48 hrs. After adding tap water to the soil columns the eluate can be collected (Fig. 4) and examined (Tabl. 3). By the help of this method the virus could also be identified in the soil. The detection reliability in the eluates is still very low according to the investigations up to now reaching between 32% and 0%.
The reasons for that may be a low virus concentration in the samples, a low germination rate of the fungus, a small number of viruliferous zoospores as well as a partial degeneration of virus particles after release into the soil.

The shell test developed for detecting BNYVV in soil, after Beemster and De Heij (1987), failed in our trials. Only in those variants, in which the soil samples were preincubated before at 28 °C, an improved virus detection could be attained by the treatment at 40 °C.

The application of the methods described renders the direct detection of BaYMV in soil generally possible, independent of the winter barley vegetation period. They reduce the test time from some weeks up to 4 days, compared to the biotest. In consequence of the detection reliability still too low, these methods,

Table 2

Detection of BaYMV in sieved root relics from infested fields

Time of samplings 1987	Roots dried		Roots freezed		Roots incubated (Germination stimulated)	
	DIBA	ISEM-D	DIBA	ISEM-D	DIBA	ISEM-D
April–Juli	–	–	+	+	++	+
August–October	–	–	–	–	–	–
November	–	–	(+)	(+)	–	–

– No BaYMV-detection.
(+) Only single BaYMV-particles and low serological reactions.
+/++ BaYMV-detection of lower/mean intensity.

however, are not suitable yet for routine investigations in field crops. On the other hand, the advantage of the biotest is that growing plants in infested soil for weeks offers the possibility of an infection to the vector in any case.

Table 3

Detection of BaYMV in eluates of soil samples at various seasons by means of DIBA and ISEM-D

Time of sampling	Detection of BaYMV in soil eluates by means of DIBA abs.(a/b)	rel. (%)	ISEM-D abs.(a/b)	(rel.%)
June '87— November '87	28/105	27	26/105	25
December '87— Februar '88	0/54	0	2/54	4
March '88— April '88	27/83	32	25/83	30

a — Number of BaYMV-containing positive samples.
b — Total number of tested samples.

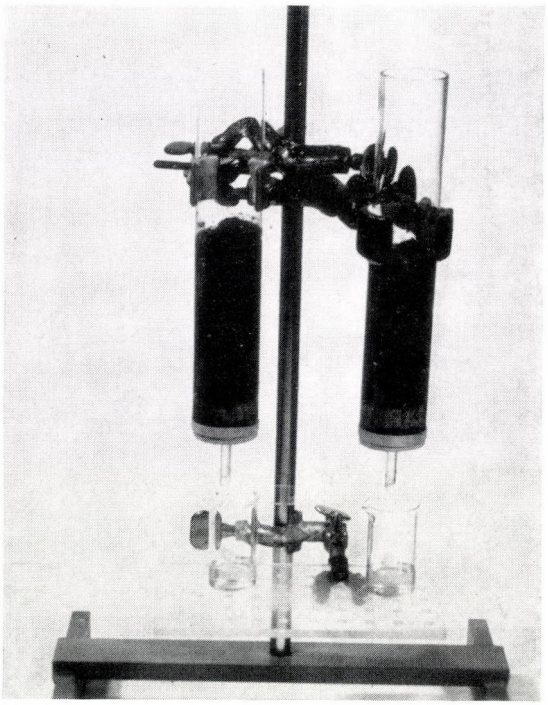

Fig. 4. Extraction of eluates

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Inheritance of Resistance to Barley Yellow Mosaic Virus

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Barley yellow mosaic virus is widely distributed in Central and Western Europe. Considerable yield losses caused by this virus can only be prevented by cultivation of resistant cultivars (Huth, 1984).

Among barley varieties differential reaction to this disease can be observed. Resistance genes are present in a number of German and European winter barley cultivars as well as in numerous stocks from different parts of the world (Takahashi et al., 1973; Friedt et al., 1985). Immunity to BaYMV of German cultivars is probably due to one identical recessive gene, because their crosses are resistant in F_1 and do not segregate in F_2, where all plants are resistant, too (Friedt and Foroughi-Wehr, 1986).

Besides the German resistant cultivars, an important source of resistance is the Chinese spring barley 'Mokusekko 3'. In earlier genetic analyses carried out in Japan, it was shown, that 'Mokusekko 3' has one dominant resistance gene, called *Ym1* (Takahashi et al., 1973). Hybrid plants (F_1) from crosses of German cultivars to Asian resistant parents, which carry the gene *Ym1*, like 'Mokusekkor 3', are all resistant and the respective F_2-populations do not segregate susceptible individuals (Friedt and Foroughi-Wehr, 1986). Therefore it can be concluded, that the respective resistance genes are either allelic or very tightly linked.

In order to identify the chromosomal location of the resistance gene of 'Mokusekko 3', Takahashi et al. (1973) studied genetic relationships between resistance gene *Ym1* and several marker genes on the individual barley chromosomes. The results of their cross-experiments show, that the gene *Ym1* is inherited independently from the genes *n* for naked kernels on chromosome 1, *V* for two-rowed spikes on chromosome 2, *B* for black kernels on chromosome 5, *o* for orange lemma base and nodes on chromosome 6 and *s* for short and hairy rachilla on chromosome 7. No marker gene for chromosome 3 was used. On the contrary, evidence for linkage of *Ym1* to *K* (hooded lemma) was found in the cross with 'Colsess IV', in which excessive numbers of parental character combinations were observed. The observed frequencies of the four phenotypes did not fit to the calculated numbers for independent segregation (9 : 3 : 3 : 1), and it was

concluded therefore, that the gene *Ym1* of 'Mokusekko 3' was located on chromosome 4.

So far, only a few stocks have been studied genetically (e.g. Friedt and Foroughi-Wehr, 1986). In order to clarify the genetic basis of resistance or immunity, different cross-experiments have been initiated. Marker- and trisomic-analyses were carried out to localize the gene for resistance of German resistant cultivars like 'Birgit', 'Franka', 'Ogra' and 'Sonate'.

Materials and Methods

For marker-analyses, multiple as well as simple genetic markers were used. Multiple genetic markers 'Nigrinudum' and 'Colsess orange lemma', kindly provided by the Institute of Agricultural and Biological Sciences, Okayama University, Japan were studied. 'Nigrinudum' carriers alleles *n* for naked kernel (chr. 1), *V* for two-rowed spikes (chr. 2) and *B* for black kernel (chr. 5). 'Colsess orange lemma' has *K* for hooded lemma (chr. 4) and *o* for orange lemma base and nodes (chr. 6). For testing linkage to the remaining chromosomes, the single genetic markers *yst* (yellow stipe, chr. 3) and *mt2* (mottled leaves, chr. 7) obtained from Dr. Tsuchiya, Department of Agronomy, Colorado State University, Fort Collins, USA, were used.

Trisomic-analysis was started with a complete trisomic set of the cultivated spring barley variety 'Shin Ebisu 16' kindly provided by Dr. Tsuchiya, too.

Tests for resistance (immunity) to BaYMV are carried out in the greenhouse by mechanical inoculation. By means of this artificial inoculation technique only BaYMV type M is transmitted. Details of inoculum preparation, plant inoculation and maintenance of inoculated plants have already been described by Friedt (1983; 1984). This technique has been improved by Umbach (1987) by application of an efficient and highly reliable air-brush (spray-gun) inoculation.

One month after inoculation all plants were examined serologically by ELISA-test (Casper and Meyer, 1981). The antiserum for this test was kindly provided by Dr. Huth, Biologische Bundesanstalt, Braunschweig, Germany F. R.

Results and Discussion

To identify the chromosomal location of the resistance gene of German varieties, cvs. 'Birgit' and 'Franka' were crossed to multiple genetic marker stocks like 'Colsess orange lemma' and 'Nigrinudum', as described above. The F_1-generation was susceptible to BaYMV, as expected. Segregation in F_2-generation (Table 1) indicates, that the German resistance gene is inherited independently of gene *n* (naked kernels) on chromosome 1, *V* (two-rowed spike) on chromosome 2, *B* (black lemma and pericarp) on chromosome 5, and *o* (orange lemma base and nodes) on chromosome 6.

Table 1

Linkage analysis of "German" BaYMV-M resistance by the use of genetic markers (Kaiser 1988)

Cross: Marker x	Marker[1] X	x	suscept. X	x	resist. X	x	Total	X_2	P
		Chrom.1[2]							
Franka x	N	n	299	121	118	44	582	6.07	.20—.10
Birgit x	N	n	305	95	94	40	534	2.02	.70—.50
		Chrom.2[3]							
Franka x	V	v	312	108	126	36	582	3.35	.50—.30
Birgit x	V	v	279	120	90	44	534	9.87	.025—.01*
		Chrom.4[4]							
Birgit x	K	k	318	110	88	46	562	6.45	.10—.05
Franka x	K	k	190	72	68	29	359	2.96	.50—.30
x Franka	K	k	263	98	91	34	486	1.35	.80—.70
		Chrom.5[5]							
Franka x	B	b	305	115	120	42	582	3.80	.30—.20
Birgit x	B	b	312	88	102	32	534	2.01	.70—.50
		Chrom.6[6]							
Birgit x	O	o	313	115	98	36	562	1.45	.70—.50
Franka x	O	o	190	72	67	30	359	3.58	.50—.30
x Franka	O	o	258	103	99	26	486	3.72	.30—.20

[1] 'Colsess-orange lemma' or 'Nigrinudum' [2] N = hulled, n = naked [3] V = two-rowed, v = six-rowed [4] K = Kapuze (hood), k = awned [5] B = black, b = yellow lemma and pericarp [6] O = yellow, o = orange lemma.

The marker gene *V* shows deviation from the expected independent segregation in the cross-combination with 'Birgit' (Table 1). This difference to the comparable cross with 'Franka' can be explained by difficulties in the classification of marker characters, because of the occurrence of various intermedium-types of spikes. In this cross-combination, *V* shows linkage to the genes *n* and *B*, too, which would not be expected, because the genes *n*, *V* and *B* are definitive located on different chromosomes. Segregation of *V* and *v* did not fit to the expected 3 : 1 ratio.

The marker-analyses in F_2-generation (Table 1) indicates also, that the German resistance gene is inherited independently of gene *K* for hooded lemma on chromosome 4. This result is in contradiction to earlier results, because the gene *Ym1* was said to be linked with the gene *K* on chromosome 4 (Takahashi et al., 1973) and the "German gene" has proved to be allelic to the gene *Ym1* (Friedt and Foroughi-Wehr, 1986). Therefore, it might be possible, that the gene *Ym1* is not located on chromosome 4. Our results could alternatively be explained by the presence of an additional, recessive gene in 'Mokusekko 3', which then should be allelic to the gene in the German cultivars (Friedt et al., 1987).

Results of linkage between the marker genes *yst* (yellow stripe, chromosome 3) and *mt2* (mottled leaves, chromosome 7) with the "German gene" for resistance are not available yet.

Marker-analysis has one main problem. If the marker gene and the gene in question are on the same chromosome, so that 50% recombination can occur, the data will show independent inheritance. Therefore, trisomic-analyses were carried out with a complete trisomic set of 'Shin Ebisu 16'. Trisomic plants of each barley chromosome were crossed as females to the German resistant cvs. 'Sonate' and 'Ogra'. In F_1-generation trisomic plants of each cross combination were identified by morphological and cytological examinations (Tsuchiya, 1963) and grown to maturity.

In F_1-generation the plants were morphological classified as disomics and trisomics two times (one day before and one month after infection); each of the F_2-populations has been heterogeneous because of the presence of disomic and trisomic plants. Heterogeneity was also evident between F_1-populations because of the different morphology of each trisomic itself.

In the 4- to 5-leaves stage the plants were infected mechanically with BaYMV-M. Transmission was not always complete, because of the heterogeneous populations and the weak growth habit of some trisomic types. Because of previous classification, it was possible to evaluate the segregation for reaction to BaYMV-M for trisomics and disomics separately. F_2-populations with less than 80% infection-rate in the control plants (cv. 'Gerbel') were excluded and populations with less than 100% infection were corrected arithmetically for the rate of escapes.

In the trisomic fractions, especially of the weakest trisomics 'Slender', 'Pale' and 'Semi-erect', unexpected segregations with an excess of resistant plants were found. This finding may be explained by deleterious effects of the severe inoculation in the weak trisomic plants. But for the disomic fractions, which were more uniform and vigorous, clear results were obtained. Among disomics of crosses with cv. 'Ogra' the theoretically expected segregations have been observed (Table 2). In all seven F_2-populations, except the one including 'Pale' as a parent, a good fit to the uncritical segregation (3 : 1) was found, whereas the F_2 derived from crosses to 'Pale' (trisomic for chromosome 3) a good fit to the critical segregation of 8 : 1 for the disomics was evident.

In the disomic fractions of F_2's of crosses with cv. 'Sonate', identical results were obtained (Table 3). Data of all F_2'-populations except the one of 'Pale' again indicate good fits to an expected uncritical segregation. Fit to the critical segregation (8 : 1) in disomic F_2's of 'Pale' was obtained, too.

Table 2

Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Ogra' (Kaiser, 1988)

Trisomic-type	Extra chrom.	Infect. rate (%)	Suscep. (n)	Resist. (n)	Total (n)	X_2 for 3 : 1 ratio	P
Bush	1	92	113	55	168	0.237	0.70—0.50
Slender	2	89	126	60	186	0.083	0.80—0.70
Pale	3	89	69	17	86	7.044	<0.01*
Robust	4	96	109	39	148	0.199	0.70—0.50
Pseudonormal	5	100	125	46	171	0.329	0.70—0.50
Purple	6	100	65	20	85	0.098	0.80—0.70
Semierect	7	96	113	39	152	0.414	0.70—0.50

n = number of plants examinated.
Test of 8 : 1 ratio for Pale (Chr. 3): $X^2 = 0.0649$; P = 0.90—0.80.

Table 3

Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Sonate' (Kaiser, 1988)

Trisomic-type	Extra chrom.	Infect. rate (%)	Suscep. (n)	Resist. (n)	Total (n)	X_2 for 3 : 1 ratio	P
Bush	1	98	55	27	82	1.739	0.20—0.10
Slender	2	93	91	42	133	0.111	0.80—0.70
Pale	3	90	110	20	130	17.359	small *
Robust	4	100	123	40	163	0.018	0.90—0.80
Pseudonormal	5	100	139	49	188	0.114	0.80—0.70
Purple	6	96	85	36	121	0.184	0.70—0.50
Semierect	7	100	78	19	97	1.516	0.30—0.20

n = number of plants examinated.
Test of 8 : 1 ratio for Pale (Chr.3): $X^2 = 1.7310$; P = 0.20—0.10.

Conclusions

As mentioned above, German resistant cultivars carry an identical, recessive resistance gene. From the data presented above, it can be concluded, that the gene for resistance to BaYMV-M of these cultivars is located on barley chromosome 3.

This conclusion is supported by recently published results of Konishi and Matsuura (1987). They found, that the Chinese landrace 'Mokusekko 3' and some

resistant cultivars derived from 'Mokusekko 3', always carry the same esterase isozyme pattern (of 'Mokusekko 3') after hybridizations to other cultivars. These results indicate, that the minor resistance gene in 'Mokusekko 3' would be linked to an esterase isozyme gene block at the terminal end of the long arm of chromosome 3.

Our own results, together with the results of Takahashi et al. (1973) and Konishi et al. (1987), can therefore be interpreted as follows: either the German gene for resistance is allelic to a minor gene of 'Mokusekko 3' on chromosome 3, or the "German gene" is allelic to the dominant gene *Ym1* of 'Mokusekko 3', which then could also be located on chromosome 3. Further analyses will be necessary to clarify this open question.

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Genetics of and Breeding for Resistance to BaYMV

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Yellow mosaic disease of barley is one of the most important cereal virus diseases in the Federal Republic of Germany and other European countries, like France, Belgium, England and the German Democratic Republic. The present area of distribution in Germany mainly covers the central and northern parts of the country, however, soil of some smaller areas in the south are infested, too (Huth, 1984).

This soil-borne disease is caused by barley yellow mosaic virus (BaYMV), which is transmitted by a fungus, *Polymyxa graminis*. The disease was first discovered in Japan in 1940 (Inouye and Saito, 1975) and became subsequently one of the major diseases of Japanese two-rowed malting barley (Usugi, 1987). Barley yellow mosaic virus frequently causes severe damage to susceptible winter barley crop with corresponding substantial reductions of grain yield (Table 1; see also Friedt and Götz 1987; Huth, 1984). Because of its soil-transmission, chemical measures against this virus disease are either inefficient or uneconomic. So far, yield losses can only be prevented by growing resistant cultivars.

Sources of resistance to BAYMV

After the virus had been discovered in the Federal Republic of Germany (Huth and Lesemann, 1978), many entries of various barley collections have been screened recently and numerous stocks from different parts of the world were found to be resistant or immune to BaYMV (Takahashi, 1983; Friedt et al., 1985), for example high-lysine barley 'Hiproly' from Ethiopia, 'Turkey Naked 2' (naked, black kernel) from Turkey, 'Anson Barley' (awnless) from the U.S.A. or the two-rowed barley cv. 'Palomino' from England (Table 2). But most of the identified, immune genetic stocks are descending from East Asia, like 'Mokusekko 3', 'Mihori Hadaka 3', 'Muju covered 2', and 'Asahi 9' (Table 2).

In Germany, several released, BaYMV-resistant cultivars are available now, i.e. 'Banjo', 'Brunhild', 'Birgit', 'Franka', 'Ogra' (six-rowed) and 'Diana',

'Sonate' (two-rowed). According to the nomenclature recommended by Cooper and Jones (1983), these cultivars may be considered as being immune to the virus.

Tests for resistance to BaYMV are carried out by mechanical inoculation in the greenhouse and laboratory respectively; details of inoculum preparation, plant inoculation and subsequent maintenance of inoculated plants have already been described earlier (Friedt, 1983; 1984). It is necessary to mention that only

Table 1

Grain yield of BaYMV-resistant as compared to susceptible winter barley cultivars in Northern Hesse, 1987

Cultivar	BaYMV-reaction	Grain yield*	
		dt/ha	relative
Six-row			
Franka	resistant or immune	48.2	100
Mammut	susceptible	19.8	41
Two-row			
Sonate	resistant or immune	46.1	100
Danilo	susceptible	24.9	54

* Gipper's Farm, Bellnhausen/Gilserberg: 10 m² plots, 3 replications; LSD 5% = = 7.8 dt/ha.

Table 2

Sources of resistance to BaYMV (FRIEDT & FOROUGHI-WEHR, 1987)

Variety	Origin	Reference (s)
Mokusekko 3	China	Takahashi et al., 1973
Resistant Ym No. 1	Japan	Muramatsu, 1976
Kanto nijo 19	Japan	Kitahara et al., 1982
Tochigi-strains	Japan	Kitahara et al., 1982
Mihori hadaka 3	Japan	Takahashi et al., 1973
Chikurin Ibar. 'Ea52'	Japan	Ukai, 1984
Hiproly (CI 3947)	Ethiopia	Yoshikawa & Kato, 1983
Kagoshima Kobai	Japan	Friedt et al., 1985
Muju covered 2	Japan	Friedt et al., 1985
Senbon hadaka	Japan	Kawada et al., 1982
Asahi 9	Japan	Sanada, pers. comm.
Nirasaki-strains	Japan	Sanada, pers. comm.
Iwate Mensury 2	Japan	Friedt et al., 1985
Miyako A	Japan	Friedt et al., 1985
Taisho-mugi	Japan	Kato, pers. comm.
Anson barley	USA	Murphy, 1983
Turkey Naked 2	Turkey	Friedt et al., 1985
Birgit, Franka, Ogra	FRG	Friedt et al., 1985
Diana, Gloria, Sonate	FRG	Friedt et al., 1985
Palomino	England	Friedt et al., 1985

the virus-type M (BaYMV-M) can be used for mechanical inoculation, while the other virus-types, which are known now, are not applicable in this test, because their infection-rate is too low.

Results of genetic analyses

Previous genetic studies have indicated, that immunity to BaYMV of German cultivars mentioned above is probably due to one recessive gene (Table 3). Resistance genes of the different adapted cultivars are obviously identical, because their crosses do not segregate in F_2, where all plants are resistant (Table 4). This "German gene" was probably derived from a commonly used cross parent, 'Ragusa', a Dalmatian land-race of spring-barley (Friedt, 1984).

In order to obtain a complete picture of genetic diversity of BaYMV-resistance in barley, the German resistant cultivars were crossed in various combinations to foreign carriers of BaYMV-resistance. By analyzing such crosses, different (non-allelic) resistance genes can be identified.

In earlier genetic analyses carried out in Japan, three different resistance genes were identified. A dominant gene was found in the Chinese spring barley 'Mokusekko 3' (Ym1), and another dominant gene was found in the Japanese naked spring barley 'Mihori Hadaka 3' (Ym2, Takahashi et al., 1973). The resistance gene of 'Mokusekko 3' was introduced into two-rowed malting barley like 'Resistant Ym No. 1', a BaYMV-immune strain with favourable agronomic and malting characteristics (Muramatsu, 1976). Recent investigations in Japan (Kawada et al., 1986) have shown, that other varieties also carry gene Ym1, e.g. 'Hakei I-41', although their resistance-gene was not derived from 'Mokusekko 3'.

Table 3

Genetics of BaYMV-Resistance: Mechanical Inoculation of crosses including "German resistance" as male parent (GÖTZ, 1989)

Female	F_2-Segregation resistant : susceptible	Interpretation
Franka	120 : 0	identical
Birgit	123 : 0	identical
Mokusekko 3	328 : 0	allelic
Hakei I-41	119 : 1	allelic
Iwate Mensury 2	119 : 0	allelic
Kagoshima Kobai 1	120 : 0	allelic
Kanto Nijo 19	116 : 0	allelic
Nirakei 31	119 : 0	allelic
S-1001	120 : 0	allelic
Turkey Naked 2	122 : 0	allelic
Anson barley	55 : 65	7 : 9

Table 4

Genetics of BaYMV-Resistance: Mechanical Inoculation of crosses including "*Ym1*" (GÖTZ, 1989)

Female	(Male: *Ym1*) resistant : susceptible	Interpretation
Diana	120 : 0	allelic
Franka	120 : 0	allelic
Hakei I-41	120 : 0	allelic
Iwate Mensury 2	120 : 0	allelic
Kagoshima Kobai 1	119 : 1	allelic
Kanto Nijo 19	118 : 0	allelic
Nirakei 31	116 : 0	allelic
S-1001	118 : 0	allelic
Turkey Naked 2	123 : 0	allelic
Anson barley	89 : 30	13 : 3 ?

Ukai (1984) reported that a recessive mutation causes BaYMV-immunity in the early mutant 'Ea52' of the Japanese six-rowed cv. 'Chikurin Ibaraki 1'; its gene was shown to be not allelic to the genes Ym1 and Ym2. Therefore, Ukai (1984) proposed the symbol Ym3 for this "new" gene. However, it does not provide resistance to BaYMV-strains prevailing in Germany (e.g. BaYMV-M), whereas the original variety 'Chikurin Ibaraki 1' proved to be resistant here (Friedt, 1985).

These varieties and numerous other resistant genetic stocks were used in the crossing programme mentioned above. Hybrid plants (F_1) from crosses of German cultivars like 'Ogra' to Asian resistant parents, which carry the gene Ym1, like 'Mokusekko 3', 'Resistant Ym No. 1' or 'Hakei I-41', were all resistant and the respective F_2-populations did not segregate susceptible individuals (Table 3). Since German varieties possess a recessive resistance gene, segregation into 3 susceptible and 13 resistant F_2-plants would be expected, provided that the genes were unlinked. The fact, that no one susceptible individual occurred among almost 4,000 F_2-plants indicates therefore, that the respective resistance genes are either allelic or very tightly linked.

Several other resistant stocks like 'Iwate Mensury 2', 'Nirakei 31' and 'Turkey Naked 2' were crossed to German cultivars. These crosses also did not segregate susceptible plants in F_2 and therefore, the respective resistance-genes must be allelic to the "German gene", too.

The cross between 'Diana' and 'Anson Barley' was the first one between a German cultivar and a foreign resistant variety which has segregated susceptible plants in the F_2-generation. The observed segregation (7 : 9) indicates that the resistance-genes are unlinked and that both act recessively.

Progeny of crosses of several resistant genetic stocks to either Ym1 or German cultivars lead to identical results, e.g. the F_2-generation did not segregate

in both cases (Table 4). This confirms the conclusion mentioned above, that the "German gene" and Yml are either allelic or very tightly linked. The observed F_2-segregation of the cross of Yml to 'Anson Barley' (Table 4, Table 5) confirms this conclusion. Finally, segregation in the F_2-generation of crosses of the "German gene" and Yml, respectively, to 'Anson Barley' clearly indicates that the resistance of the latter variety is inherited independently from the former genes.

Table 5

Anther-culture results of crosses including donors of dominant BaYMV-resistance derived from 'Mokusekko 3'

('*Mokusekko*' x '*Igri*') x	Anthers cultured	Callus-format. (%)		Green plants (%)		BaYMV-React. inocul. susc.	
							A_1
Strain 106/1430-2							
x Alraune	20,907	1,309	(6.3)	188	(0.9)	156	64
x Cosima	21,265	1,102	(5.2)	120	(0.6)	102	36
x Danilo	20,444	1,072	(5.2)	114	(0.6)	99	40
x Marinka	21,366	1,252	(5.9)	126	(0.6)	126	53
Stamm 106/1431-1							
x Harmonika	5,379	444	(8.3)	51	(0.9)	38	19
x Interbell	6,487	322	(5.0)	35	(0.5)	28	13
x Posaune	8,628	801	(9.3)	238	(2.8)	231	55
x Tamara	5,097	664	(13.2)	145	(2.8)	131	87
Total/Average	109,673	6,966	(6.4)	1,017	(0.9)	911	367

Breeding for BAYMV-resistance

Since various sources of monogenically inherited resistance or immunity are available (Table 2), rapid progress in breeding for BaYMV-resistance has already been made in recent years and can further be expected in the near future. Many of the resistant cultivars or stocks exhibit inferior agronomic performance, due to susceptibility to lodging or various diseases or, in total insufficient grain yield (Friedt and Götz, 1987). Therefore, extensive breeding activities are required for an improvement of agronomic value of these resistant materials.

In self-pollinating barley, new breeding lines and cultivars can either be developed by conventional selfing over several generations, i.e. pedigree-, bulk- or family-selection procedures or by using haploidy-techniques (e.g. via androgenesis, i.e. anther- or microspore-culture) for the production of dubled-haploid, i.e. homozygous lines. In the case of monogenic-recessive immunity, as many as 50% of homozygous resistant doubled haploids can be expected from an F_1-hybrid derived from an immune and a susceptible parent, whereas the selfed F_1-progeny yields only 25% homozygous recessive, i.e. BaYMV-immune plants in F_2. The results obtained from culturing anthers of various F_1-hybrids including

Ym1 is given in Table 5. Numerous doubled haploid lines have already been tested in field trials for agronomic performance. Many of them have already been handed over to breeding companies for further propagation and testing.

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Resistance to Barley Yellow Mosaic Virus in Triticale

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Several cultivars and breeding lines of Triticale were investigated by experimental inoculation and by growing in virus contaminated field soil with regard to their resistance to barley yellow mosaic virus. After mechanical inoculation the virus could be detected serologically in inoculated leaves of several plants howing no symptoms. According to this reaction it was concluded that Triticale is extremely resistant to BaYMV.

Triticale is an important food cereal in several countries and may be considered as a supplement to winter barley. Winter barley however, is threaten in some european countries by barley yellow mosaic virus (BaYMV). In order of its genetical descent from wheat and rye, Triticale does not seem being predestinated as a host plant for BaYMV. Because earlier investigations demonstrated BaYMV infections of single Triticale plants (Kegler et al., 1985) we included this cereal into our investigations on resistance to BaYMV.

Materials and Methods

The seeds of the investigated Triticale cultivars and breedings lines were placed at our disposal by the Institute of Plant Breeding Gülzow-Güstrow.The Triticale seedlings were inoculated two times mechanically with BaYMV 2 and were grown under controlled conditions (Proeseler and Kegler, 1987). Furthermore, the different genotypes were investigated by growing in field soils strongly contaminated by *Polymyxa graminis* and BaYMV. The Triticale plants were symptomatologically checked 3–6 weeks, after mechanical inoculation and in April/May after growing about 8 months in contaminated soil, respectively. All plants which have been mechanically inoculated and samples of such growing in the field were tested by direct double antibody sandwich ELISA for latent BaYMV infections.

Results and Discussion

Mechanically inoculated Triticale plants

Since 1984 we proved altogether 56 breeding lines and 2 cultivars of Triticale in regard to their resistance to BaYMV. Only 4 out of 90 plants from one breeding line developed mild symptoms similar to these appearing on winter barley. But our serological reindexing experiments more often indicated positive results few weeks after mechanical inoculation. Therefore, we investigated this phenomenon with the cv. 'Grado' and 2 breeding lines. It could be demonstrated that more or less plants of each genotype reacted positively in ELISA 3–6 weeks after inoculation so far inoculated leaves were tested (Table 1). Serological testing of noninoculated leaves and biological testing of inoculated and noninoculated leaves, respectively, by mechanical virus transmission experiments to the BaYMV-sensitive winter barley cv. 'Erfa', were negative in each case. Neither plants reacting positive in ELISA nor the other plants developed symptoms after inoculation. Nevertheless it can not be excluded that Triticale can be infected by ByYMV although it does not have any genetical relation to BaYMV-susceptible genera like Hordeum, Aegilops a.o. (Proeseler, 1988).

Table 1

Results of experimental inoculation of Triticale with barley yellow mosaic virus

Genotype	Inoculated plants	Number of plants reacting positive in ELISA after weeks			
	3	4	5	6	
Grado	30	2	1	1	1
Breeding line 1	80	—	22	—	—
	158	—	—	39	—
	158	—	—	—	1
	30	2	2	0	0
Breeding line 2	160	—	22	—	—
	13	—	—	4	—
	30	0	0	1	0

Residues of inoculum on the inoculated leaves of barley were serologically detected only 1 day p.i. by Huth (1985). Therefore, the serological detection of BaYMV some weeks p.i. in inoculated Triticale leaves may indicate a kind of subliminal infection of single cells but no or only a limited virus replication as well as virus transport. This reaction is typical for extreme resistance. Further investigations have to be carried out to clear up this phenomenon in detail.

Naturally inoculated Triticale plants

Two breeding lines and the triticale cultivar 'Grado' were grown in soil strongly infested by BaYMV and *Polymyxa graminis* from September 1987 to May 1988. No plant from the 108, 115 and 104 plants, respectively which growed in the vector and virus contaminated soil developed yellow mosaic symptoms.

From 25–37 plants of every genotype leaves and roots were tested by ELISA in regard to latent virus infection. Neither in leaves nor in the roots BaYMV was detected. Obviously, Triticale only may be infected by mechanical inoculation but not by the vector.

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Methods and Results of Resistance Screening of Barley to Barley Yellow Mosaic Virus

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Barley yellow mosaic virus (BaYMV) was first identified in the German Democratic Republic in 1983. Up to now about 50 infested fields have been detected, mainly at the western border to the Federal Republic of Germany. A high yield reduction was observed in two fields of two co-operative farms. The contamination of the soil decreased with the increase of depth, but was still detectable in a soil depth of 50 to 60 cm (Proeseler et al., 1988 a and b). Host plants of BaYMV are not only some Hordeum species but also some *Aegilops species*, *Eremopyrum hirsutum* (Bertol.) Nevski and *Lagurus ovatus* L. (Proeseler, 1988). These host plants recently discovered are not important for the spread of the virus.

Losses in yield can be avoided by growing resistant cultivars. Therefore, selection and breeding of resistant cultivars are very important and a main task of breeders for winter barley in our country.

Three methods of screening for resistance of barley to BaYMV have been applied at Aschersleben and at some other institutions:

- screening in a Dederon-gaze covered cage in spring and in autumn (pre-test),
- testing in a growth chamber (main-test),
- screening in field with infested soil (field-test).

In the pre- and main-test for each line, strain or cultivar 50 plants in the 3 leaves stage were infected by mechanical inoculation after a modified method described by Friedt (1984). The average infection rate in the pre- and main-test was about 50 and 80%, respectively, of the cultivar 'Erfa' as a sensitive standard. In field-tests the seeds of each line, strain and cultivar, respectively, were sown in plots of 1 m^2 in two or four replications (Proeseler and Kegler, 1987). Agreeing

with Friedt et al. (1985) at all times the results of laboratory and field tests corresponded. Barleys with an infection rate below 10% were selected as possibly resistant and their behaviour was tested in further experiments.

By using the three screening methods the resistance of investigated foreign cultivars could be confirmed.

Some resistant lines and strains with superior grain yield were included to breeding material of winter barley in the G.D.R. (Table 1). In 1988 the first resistant cultivar will be admitted.

Table 1

Susceptibility or resistance of barleys
(Results of screening at Aschersleben from 1984 to 1988)

	Breeding lines and strains of the G.D.R.	Collection of Gatersleben			
		winter barley		spring barley	
		cultivars	sources	cultivars	sources
Susceptible	583	116	121	51	64
Resistant*	881	29	51	14	20

* Infection rate below 10%

In the *Hordeum* and barley collection of the Central Institute of Genetics and Crop Research Gatersleben out of 466 accessions 114 were resistant to BaYMV. The resistance of the cultivars from the Federal Republic of Germany and of our first cultivar originates from the variety 'Ragusa' (Dalmatian landrace.). Ten sources and progenies of 'Ragusa' in the Gatersleben collection were found to be extremely or moderately resistant and susceptible, respectively (Table 2) (Proeseler and Lehmann, 1987).

Besides qualitative virus resistance, which is controlled by one gen and appears absolutely, as quantitative resistance to BaYMV, was also found in barley, i.e. the extension of incubation period is longer and the virus concentration, symptom intensity and infection rate are lower in 'Turkey 2' and other varieties than in the sensitive standard cultivar 'Erfa' (Table 3). The varieties with different intensity of symptoms were distinguishable by remission spectroscopy like other virus host combinations (Kegler et al., 1988). The importance of cultivars with quantitative resistance is low, because the infection potency of soil will not decrease by their cultivation (Proeseler et al., 1988).

Some barleys with resistance against BaYMV, furthermore, are resistant to Drechslera teres (Sacc.) Shoem. and *Puccinia hordei* Otth. (Proeseler et al., 1988).

Table 2

Susceptibility or resistance of sources and progenies of 'Ragusa'

1. *winter barley*
   Hordeum vulgare L. convar. hexastichon Alef. var. hybernum Vib.

collection number Gatersleben	cultivar	infection rate pre-test	infection rate main-test
HOR 3218	'Firlbecks Vierzeilige'		0.0%
HOR 308	'Ragusa b'	14.0%	13.6
HOR 2276	'Ragusa b'	0.0	0.0
HOR 305	'Ragusa (6–9)'	8.3	13.1
HOR 304	'Ragusa 34–40'	0.0	0.0
HOR 800	'Ragusa 415'	11.8	14.6
HOR 799	'Ragusa 416'	8.2	4.3
HOR 801	'Ragusa 420'	3.1	3.4

2. *spring barley*
   Hordeum vulgare L. convar. distichon Alef. var. nutans (Rode) Alef.

HOR 3878	'Peroga'	53.2
HOR 3790	'Stankas Frühgerste'	0.0
HOR 3789	'Sunna'	49.4

Table 3

Characteristics of quantitative resistance

Cultivar	Infection rate	Incubation visual	Symptom intensity visual	Symptom intensity remission spectroscopy a-value	ELISA A405 nm 5 weeks p.i.
Erfa (susceptible standard)	180 of 209 plants 86.1%	21.3 d	middle	15.50	1.36
Turkey 2	144 of 234 plants	28.6 d	slight	16.29	0.45

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Detection of Maize Dwarf Mosaic and Sugarcane Mosaic Viruses in the Federal Republic of Germany

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From potyvirus isolates obtained from maize in South-West Germany six were identified as sugarcane mosaic and one as maize dwarf mosaic virus, using ELISA and immunoelectron microscopical decoration techniques. Five species of perennial grasses were found to be hosts of the isolates in glasshouse tests.

Maize plants showing virus disease-like symptomes have been collected in South-West Germany since 1983. Electron microscopical examination of such plants revealed infections by potyviruses which in the following study were identified as maize dwarf mosaic (MDMV) and sugarcane mosaic (ScMV) viruses.

Methods

Virus isolated obtained from naturally infected field plants were propagated by mechanical inoculation of crude plant sap onto maize seedlings with two to three leaves. From infected plants viruses have been purified using the method developed to purify barley yellow mosaic virus (Huth et al., 1984).

Antisera were prepared by intramuscular injection of viruses emulsified in Freund's adjuvant. The first injection was done using the complete adjuvant, the following using the incomplate adjuvant. Bleedings were taken in two weeks intervals. IgG fractions were prepared according to Clark and Adams (1977).

The molecular weight of the coat protein was determined in 5% Polyacrylamid-gels (Laemmli and Favre, 1973). The splitting of the virus was done in 0.125 M Tris-borate buffer adjusted to pH 10 containing 8 M ureas, 1.1% sodium dodecyl sulfate and 0.15 M dithioerythrol.

The molecular weight of nucleic acids has been determined in 0.8% agarose gels under non-denaturing conditions according to Peacock and Dingmann (1968). The virus was splitted using the method of Hull and Lane (1973). Nucleic acids of TMV (2.1×10^6 d), potato virus Y (3.1×10^6 d) and brome mosaic virus (1.1×10^6 d, 1.0×10^6 d, 0.75×10^6 d, 0.3×10^6 d) have been used as markers. Virus particles were visualized in the electron microscope after adsorption to

carbon-coated Pioloform films from leaf squash extracts in 0.1 M phosphate buffer pH 7.0 and by negative staining with 2% uranyl acetate. Immunoelectron microscopical decoration tests (Milne and Luisoni, 1977) were used to evaluate serological relations among isolates.

Results

Symptoms of infected plants

Infected plants have been found for the first time near Ludwigshafen (upper Rhine valley) on fields where maize had been cultivated since several years. Plants in these fields showed two different types of symptomes. The first was characterized by a mosaic of small streaks along the veins especially of young leaves. On older leaves of such plants the whole surfaces appeared entirely chlorotic with only a few distinct, scattered, small darkgreen streaks as remnants of the normal leaf colour. The second type of symptoms comprized light green stripes up to 10 mm wide which continued from the tops to the bases of the leaves. Sometimes only one broad stripe was present on one leaf half. The stripes obviously enlarged during ageing of the leaves so that old leaves appeared very similar to those of plants showing the first symptom type on young leaves.

The two symptom types also developed on mechanically inoculated test plants grown in the glasshouse or in the field and were found to be specific for the virus isolates.

Serological reactions of different isolates

Antisera have been prepared against the German isolates 481 and 552 as representatives of the two symptomatologically differing virus types. Furthermore an antiserum has been prepared against isolate BG which had been collected from *Sorghum halepense* in Belgrad (Yugoslavia) in 1974. These antisera have been used to compare some other isolates collected from the same field where 481 and 552 came from. Results of this investigation by ELISA are given in Table 1 and show that most of the isolates reacted only with antiserum to 552, similarly as the well defined isolate MDMV-B from USA. The isolates 481 and BG on the other hand cross reacted only with each other.

In decoration tests, however, a distant serological relationship between isolates 552 and MDMV-B on one side and 481 on the other side could be detected. Antiserum to 552 had a homologous decoration titer of 1 : 5120 and a titer of 1 : 640 with isolate 481. Antiserum to 481 had a homologous titer of 1 : 2560 and a titer of 1 : 20 with isolate 552. Isolate MDMV-B had heterologous titers of 1 : 1280 and 1 : 20 with antisera to 552 and 481, respectively.

Antisera to isolates 552 and 481 were also used to study with ELISA and decoration tests the relationship to several other already defined isolates belonging

to the complex of potyviruses infecting maize, sorghum and sugarcane (Shukla et al., 1988) which were kindly provided to us by Dr. M. Tošić, Belgrade, Yugoslavia. From Table 2 it can be seen that antiserum to isolate 552 reacted with high ELISA values and strong decoration with the isolates related to MDMV-B, listed under the name of sugarcane mosaic virus (ScMV). In ELISA a weak reaction of antiserum to 552 with ScMV-H isolate of sorghum mottle virus (ScMV) indicated a distant relationship between ScMV and CrMV which is

Table 1

Serological reactions of several German isolates with antisera against the isolates 481, 552 and BG. Values represent optical densities in ELISA at 405 nm. Values representing positive reactions are underlined

Isolate	Antiserum against isolate Nr.		
	552	481	BG
420	*> 1.050*	0.000	0.005
480	*> 1.500*	0.014	0.005
481	0.071	*1.098*	*1.158*
505	*> 1.500*	0.005	0.012
552	*> 1.500*	0.015	0.003
576	*> 1.500*	0.014	0.001
667	*> 1.500*	0.007	0.024
BG	0.041	*0.765*	1.148
MDMV-B (USA)	*> 1.500*	0.028	—
Healthy maize	0.026	0.056	0.014

much more clearly demonstrated in the decoration test. Similarly the decoration test indicates a serological relation between ScMV and maize dwarf mosaic virus (MDMV), which is not evident from the ELISA results. The results presented in Table 2 for antiserum to 481 indicate a high specificity of this antiserum in ELISA and in decoration tests for isolates related to MDMV.

Some physical and chemical properties

The isolates 481, 552, BG and MDMV-B[5](USA) had buoyant densities of 1.329 g/cm^3 after isopycnic centrifugation in $CsCl_2$. The nucleic acids migrated as single bands upon electrophoresis in agarose gels and molecular weights of 3.7×10^6 d have been calculated for the isolates 552, 481, BG and MDMV-B (USA). In SDS-Page one major band was detected which corresponded for the isolates 481 and 552 to a coat protein molecular weight of 35.5×10^3 d, with isolate BG a value of 36.5×10^3 d, was obtained. Additionally to the major band weights were found.

Table 2

Serological comparison of the German isolates 481 and 552 with defined isolates ++++ of potyviruses infecting maize, sugarcane and sorghum using ELISA and immunoelectron microscopical decoration tests

Virus isolates	Antiserum against 552		481	
	ELISA[3]	Decoration[4]	ELISA	Decoration
JOHNSONGRASS MOSAIC[1]				
ScMV-JG (AU)[2]	0.055	−−	0.046	−−
MDMV-O (USA)	n.t.	−−	n.t.	−−
MAIZE DWARF MOSAIC				
MDMV-A (USA)	0.037	n.t.	0.930	n.t.
MDMV-A (YU)	0.044	++	2.000	+++
MDMV-A (IT, Bergamo)	n.t.	++	n.t.	+++
MDMV-A (IT, Rome)	n.t.	−−	n.t.	++
ScMV-JG (USA)	0.040	++	2.000	+++
SUGARCANE MOSAIC				
MDMV-B (USA)	0.758	++	0.024	−−
ScMV-A (USA)	n.t.	+++	n.t.	−−
ScMV-D (USA)	0.878	++	0.035	−−
ScMV-E (USA)	n.t.	+++	n.t.	−−
ScMV-BC (AU)	0.453	+++	0.033	−−
ScMV-SC (AU)	n.t.	+++	n.t.	−−
ScMV-Sabi (AU)	1.003	+++	0.016	−−
SORGHUM MOTTLE				
ScMV-H (AU)	0.128	++	0.026	−−
ScMV-J (AU)	0.038	+	0.014	−−
552	1.670	+++	0.037	−−
481	0.050	++	2.000	+++
Healthy maize	0.002	n.t.	0.003	n.t.

[1] Virus names as proposed by Shukla et al. (1988)
[2] AU = Australia; YU = Yugoslavia; IT = Italy
[3] Extinction values at 405 nm; n.t. = not tested
[4] Intensity of decoration: +++ strong; ++ medium; + weak; −− no decoration

Possible natural hosts of virus isolates in Germany

Numerous hosts for MDMV/ScMV have been published already (e.g. Tošić and Ford, 1972; Rosenkranz, 1987) but most of them are not common in central Europa where only perennial grasses can be of importance as overwintering hosts. In order to obtain informations on possible hosts in the source area of the German isolates these were mechanically inoculated onto 21 species of perennial grasses which were cultivated in the glasshouse. Five species became infected: *Bromus inermis*, *Br. secalinus* and *Br. sterilis* by isolate 552, *Dactylis glomerata* by isolate 481 and Echinochloa crus-galli by 481 and 552. The following

species were not infected: *Agropyron caninum*, *A. repens*, *Alopecurus geniculatus*, *Anthoxanthum odoratum*, *Bromus erectus*, *Calamagrostis epigeios*, *Cynosurus cristatus*, *C. echinatus*, *Festuca arundinacea*, *F. pratensis*, *Holcus lanatus*, *Phleum arundinacaea*, *P. phleodies*, *Poa palustris*, *P. pratensis*, *P. trivialis*. As a further probably not published host the annual *Bromus tectorum* was found after mechanical inoculations. None of the mentioned grasses have been found to be naturally infected up to now by the viruses discussed here.

Discussion

The occurrence of MDMV/ScMV types in Europe is known since the investigations of Dijkstra and Grancini (1960) on Sorghum red stripe virus. In the meantime different MDMV/ScMV strains became economically important pathogens in most South European countries where maize is one of the most important agricultural crops (see Conti, 1983; Signoret, 1983). In West-Germany similar viruses have relatively seldom been found on maize which is a relatively young crop there. Probably the small number of suitable perennial hosts may be a reason for the limited occurrence in this region. Also in the GDR the occurrence of MDMV/ScMV-related viruses has only recently been reported (Fuchs and Kozelska, 1984) and different virus types have been identified (Fuchs et al., 1989).

On the basis of serological properties Shukla et al. (1988) proposed that all known virus isolates which were up to now roughly classified as strains of MDMV or ScMV can now be subdivided in four distinct viruses, i.e. johnsongrass mosaic (JGMB), maize dwarf mosaic (MDMV), sugarcane mosaic (ScMV) and sorghum mottle (SrMV) viruses. According to this system MDMV-A and related viruses are combined to a newly named maize dwarf mosaic virus whereas MDMV-B and its related isolates are now called sugarcane mosaic virus. Following this taxonomy the German isolate 481 belongs to MDMV and isolate 552 to ScMV. Both are serologically only distantly related to each other and ScMV (552) is distantly related to SrMV. Both isolates did not show any serological relation to JGMV.

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Serological Comparison of some Hungarian and Cuban Potyviruses from *Sorghum halepense*

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Antisera produced to one Hungarian virus isolate from maize (MDMV-Hu) one Cuban MDMV isolate from Sorghum halepense (MDMV-Cu) and one SCMV isolate from sugarcane (SCMV-1) and in addition 3 antisera (MDMV-A, SCMV-A and SCMV-H) from the American Type Culture Collection were used to test serological activity in plant sap of S. halepense originating from Hungary and Cuba, respectively.

In three selected *S. halepense* plants from each country high serological activity was obtained not only to the MDMV-A group but also for antisera to the SMV-A group including SMV-1, and SCMV-H of a third serogroup. However, 3–4 weeks after mechanical inoculation of plant sap to maize plants ELISA only showed MDMV infection.

In contrast to the earlier reports, SCMV isolates belonging to the SCMV-A serogroup could easily be transmitted to S. halepense seedlings originating from Hungarian seeds. A possible explanation for these deviating findings may be the occurrence of hybrids between *S. halepense* and *S. bicolor*. The importance of this is somewhat discussed.

The severeness and spread of maize dwarf mosaic virus (MDMV) is much depending on the occurrence of Johnsongrass (*Sorghum halepense* (L.) Pers.) which is its main overwintering host plant in many countries including also Hungary (Milinkó et al., 1979).

MDMV belongs to the potyvirus group and is closely related to sugarcane mosaic virus (SCMV). The most common strain in maize, in Europe MDMV-A, seems to be identical with the Johnsongrass strain of SCMV (SCMV-Jg). A great number of strains within the SCMV complex has been reported to deviate concerning e.g. symptoms, host range and serological relationships (Persley et al., 1985).

Serological differences among strains seem to be common. However, four main serogroups can be distinguished (Jarjees et Uyemoto, 1984). Among common strains MDMV-A and SCMV-Jg are classified into one serogroup, MDMV KS 1 into a second, MDMV-B, SCMV-A, SCMV-B and SCMV-D into a third and SCMV-H, SCMV-I and SCMV-M in a fourth. Differences in symptoms and host range seem also to be in agreement with this classification.

Acta Phytopathologica et Entomologica Hungarica 26, 1991
Akadémiai Kiadó . Budapest

As far as the authors are a ware all earlier reports seem to be in agreement with Persley et al. (1985) that strains within the third group rarely infect S. halepense. However, on S. halepense originating from Hungarian seeds, deviating results were obtained in comparative tests in a glasshouse at Uppsala. Not only different isolates of SCMV from Cuba but also isolates from the American Type Culture Collection were used in these tests. This deviation may be due to origin of S. halepense, age of plants and growing conditions etc. Some preliminary results on possible infection of S. halepense also with other potyviruses are reported in here.

Materials and Methods

In connection with a research project supported by the Swedish research organization for developing countries (SAREC), Mrs. Ondina Leon, CENSA, Cuba, in 1984 brought some SCMV isolates and some virus infected S. halepense plants to Sweden. Transmission was made to sweet corn cv. Jubilee. From these materials the isolates SCMV-1, SCMV-2, SCMV-3 and MDMV-Cu, which was obtained from one of the S. halepense plants. MDMV-Hu originates from a maize plant brought to Sweden in 1985. At the same time also seeds of S. halepense were brought to Sweden from Hungary.

The isolates SCMV-A, SCMV-B, SCMV-D and MDMV-A as well as their antisera were bought from the American Type Culture Collection. Later also SCMV-H, SCMV-I and their corresponding antisera were bought from the same place.

During a joint visit in 1987 to some places in Hungary, we collected some S. halepense plants. Plant no. 25 (Martonvásár) and 39–40 (Baja). Plants 42, 62 and 69 originate from a group of about 50 plants randomously collected in March, 1988, at Madruga Breeding Station, Cuba. Plants no. 25, 40 and 69 have showed more or less clear mosaic symptoms but 39, 42 and 62 were symptomless.

As test plants, sweet corn cv. Jubilee and S. halepense from the Hungarian seeds have been used. Inoculation has taken place with the finger rub method using carborundum and 0.01 M phosphate buffer (pH 7), when the test plants were in the 2–3-leaf stage.

The isolates SCMV-1, MDMV-Cu and MDMV-Hu were all purified from infected sweet corn cv. Jubilee and antisera produced by injections into rabbits.

ELISA was carried out mainly according to Clark et Adams (1977). Plant sap was obtained by using a Pollähne press and 2 drops of plant sap diluted in 1 ml PBS-TPO buffer was used for each sample. Readings were taken in a Titertek Multiscan MC at 405 nm one hour after addition of substrate.

Results

Our own antisera reacted to the Cuban isolate from S. halepense (MDMV-Cu) and to the Hungarian maize isolate (MDMV-Hu) in both homologous and heterologous tests with ELISA as the MDMV-A antiserum from the American Type Culture Collection. The produced antiserum to the Cuban sugarcane isolate (SCMV-1) on the other hand, showed quite different ELISA reactions and was very similar to the American SCMV-A, SCMV-B and SCMV-D antisera.

Preliminary tests with ELISA carried out in 1987 at Keszthely (HUNGARY) showed that most of the 42 collected S. halepense plants with mosaic symptoms gave strong colour reactions for MDMV antiserum (MDMV-Hu) but none or only slight reactions to SCMV antiserum (SCMV-1). Only one S. halepense plant which in addition to strong reaction to MDMV antiserum also showed rather strong reaction to the SCMV antiserum.

After bringing the Hungarian plants to Uppsala, three of them no. 25 (from Martonvásár) and 39–40 (from Baja), have been studied more in detail and later also been compared with three Cuban S. halepense plants (Fig. 1).

As can be seen from Tables 1 and 2 the symptomless plant no. 39 has shown rather high ELISA readings not only for the MDMV-Hu antiserum but even higher for the SCMV antiserum. Also plant 25 has given obvious reaction to the SCMV antiserum.

Table 2 shows also that SCMV-1 transmitted to S. halepense seedlings, originated from Hungarian seeds, reacted strongly serologically with SCMV-1 antiserum.

In Fig. 1 a serological comparison of sap from the Hungarian S. halepense plants and three S. halepense plants with similar reactions from Cuba is shown. In this test the American MDMV-A antiserum was included instead of MDMV-Hu

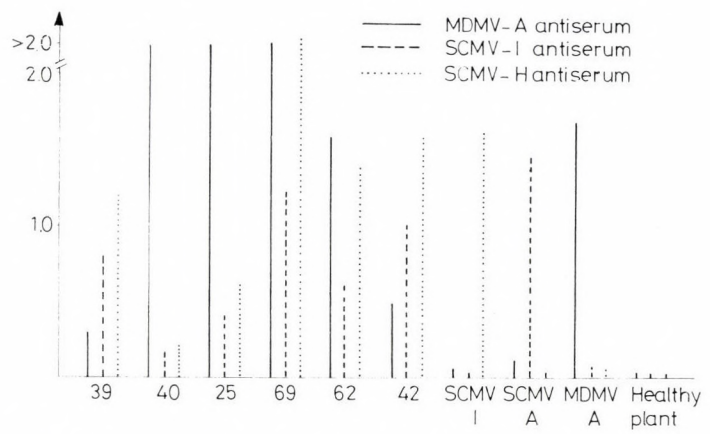

Fig. 1. ELISA of plant sap from Hungarian and Cuban *Sorghum halepense*. Isolates on maize from the American Type Culture Collection and healthy maize were also included

Table 1

ELISA results of the Hungarian S. halepense plants no. 25, 39 and 40 using one MDMV antiserum (MDMV-Hu) and one SCMV antiserum (SCMV-1)

Date of test	Antiserum	S. halepense plant no. 39	40	25	Antiserum
87. 07. 22.	MDMV-Hu	0.86	—	0.40	—
	SCMV-1	0.93	—	0.38	—
87 07. 24.	MDMV-Hu	0.40	0.23	0.25	0.03
	SCMV-1	0.42	0.20	0.28	—
87. 08. 27.	MDMV-Hu	0.34	0.20	0.51	0.03
	SCMV-1	0.65	0.06	0.53	0.03

Table 2

ELISA results of the Hungarian S. halepense plants using three different MDMV antisera (MDMV-Hu, MDMV-Cu and MDMV-A /and one SCMV antiserum/ SCMV-1). In addition SCMV-1 infected S. halepense from Hungarian seed was included

Date of test	Antiserum	S. halepense plant no. 39	40	25	SCMV-1	
87. 09. 25.	MDMV-Hu	0.68	0.78	1.03	0.01	0.0
	MDMV-Cu	0.33	0.47	0.70	0.03	0.0
	MDMV-A	0.94	1.27	1.94	0.03	0.0
	SCMV-1	1.15	0.46	0.87	1.04	0.0

as it was earlier found to give stronger ELISA readings (see Table 2). In addition, antiserum to the American type isolate SCMV-H was included. Again sap from the Hungarian plant no. 39 showed rather high readings for SCMV antiserum but the reaction to MDMV antiserum was for some unknown reason lower than usual. The highest readings after one hour at 405 nm were obtained for the SCMV-H antiserum which amounted to about 1.2.

Also for the included Cuban S. halepense plants, the ELISA readings for this latter antiserum became very high comparable to those of SCMV-I infected maize (Fig. 1).

Mechanical transmissions were carried out several times from the Hungarian plants to young maize plants (cv. Jubilee). In general symptoms were shown on most of the inoculated test plants of no. 25 and 40 but only at one occasion for plant no. 39. Successful transmission has also been made from the cuban plants no. 62 and 69 but so far not from 42. ELISA of mosaic showing test plants has, as a rule, given positive reactions for the MDMV antisera but so far, no indication of reactions for the other two serogroups.

Discussion

The preliminary results reported in here concerning serological reactions in *Sorghum halepense* seem to be some what contradictory and partly in disagreement with earlier reports dealing with viruses on this grass (cf. Persley et al., 1985). Before any definite conclusions can be made the serological tests must be repeated with additional plants and also complemented with e.g. ISEM. However a few attempts to use ISEM directly on plant sap from the Hungarian plants have been carried out. Even if the number of particles were few there were indications of decoration especially when SCMV-H antiserum was used on the grids to sap from the Hungarian plants no. 25 and 39.

Failure of mechanical transmission of any virus but the ordinary MDMV-A strain from all the tested S. halepense plants does not necessarily exclude that a mixed infection of virus strains may occur in them. In any case it was found in repeated experiments (unpublished) that several isolates of SCMV, including SCMV-A, SCMV-B and SCMV-D from the American Type Culture Collection can easily be mechanically transmitted to S. halepense originating from Hungarian seeds.

Possibly S. halepense is more heterogeneous than expected and strains with ability to act as hosts also for SCMV may occur. May be also hybrids of *S. halepense* and *S. bicolor*, which is a good host for most SCMV strains, can not be excluded also as perennial weeds. Interesting in this connection is that all hybrids between *S. halepense* and *S. bicolor* seem to produce rhizomes (L. Farády, 1988 pers. comm.) and therefore also may act as overwintering hosts for viruses.

Differences in aphid transmission seem to be common within the potyvirus group and have also been used when distinguishing strains within the MDMV-SCMV complex (Presley et al., 1985). If variants of S. halepense are possibly able to act as hosts also for SCMV strains this will potentially increase the dangerousness of this weed. However, crucial from a practical point of view is the transmissibility of virus to maize, sorghum and other arable crops. If the aphid transmission is non-effective then the practical importance will be low.

Further investigations seem to be justified to clarify the role of *S. halepense* as a possible host not only for MDMV but also for other members of the SCMV complex.

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Acta Phytopathologica et Entomologica Hungarica, 26 (1–2), pp. 137–146 (1991)

Distinction of Different Isolates of the Maize Dwarf Mosaic Virus (MDMV) in GDR

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Since 1985 we have observed two serologically different groups of MDMV-isolates in GDR. Serological, immune electron microscopical, and biological investigations raise the question, whether both serogroups represent strains or two closely related MDMV-similar viruses. The lack of serological relationships, the partial cross-protection as well as the lack of mixed infections in maize plants growing under open ground conditions speak for the latter assumption.

The maize dwarf mosaic virus (MDMV) has been appearing regularly in GDR since 1982. For this time we have checked the occurrence of MDMV in the southern and central regions of GDR. The portion of attacked maize crops differed in the singe years between 0% in 1984 and 75% in 1985. In case of an infection during the period from the end of May to early July it is to calculate a decrease in the plant height by 25%, the total plant weight by 38%, and in the corn-cob weight by 27%, respectively. According to our results published by Fuchs et al. in 1987 two serologically different serogroups of MDMV are present in GDR. This paper informs of comparative investigations using representatives of both serogroups.

Material and Methods

The partial purification of MDMV as well as the preparation of antisera followed Fuchs and Merker (1977). For ELISA we used a modified double-antibody-sandwich-variant described by Richter et al. (1977, 1979), in which conjugate and samples for testing are added simultaneously (Flegg and Clark, 1979). By means of preliminary trials we established the optimum Ig-concentration for "coating" and the optimum dilution of the Ig-enzyme-conjugate with alkaline phosphatase (produced by Institut for Phytopathology Aschersleben, GDR). Quantitative measurement of results was made with a spectrophotometer SPEKOL (Veb Carl Zeiß Jena, GDR) at 405 nm. The measuring values could differ between 0 and 2. The latex test was carried out according to Schade (1971). The immune electron microscopical investigations consisted of following steps: firstly – the

coating of grids with antiserum diluted 1 : 1000, secondly – the incubation of grids in each case with plant sap of homologous virus isolates for 30 min, thirdly – the decoration of grids with antiserum diluted 1 : 50.[1]

With regard to the biological differentiation of serogroups in each case 25 plants of altogether 28 species from the genera *Bromus*, *Digitaria*, *Echinochloa*, *Eleusine*, *Panicum*, *Phleum*, *Setaria*, and *Sorghum* were mechanically inoculated with the isolates Gerichshain 15 and Panitzsch 10 – representatives of serogroup 1 – as well as with the isolates Cunnersdorf 23 and Gerichshain 20 – representatives of serogroup 2. At the time of inoculation the plants had developed 3 leaves. The inoculated plants were visually evaluated 14 to 25 days after inoculation. The biological and serological testing was done 29 days p.i.

For cross-inoculation tests we used maize plants of the variety 'BEKE 245'. 14 days after primary infection all leaves showing typical symptoms were inoculated with an isolate belonging to the opposite serogroup. 20 days after the second inoculation we tested the plants separately by means of latex test using antisera of both serogroups. Furthermore we inoculated a number of plants simultaneously with opposite MDMV-isolates. These plants were tested 3 weeks p.i. In 1987 the influence of serogroups on growth and yield of maize plants was investigated in a field plot trial. The variety 'BEKE 245' was sown on April 27(th), 1987. The first mechanical inoculation was accomplished on June 3(rd), the second inoculation on July 13(th). We used the MDMV-isolates Bulg. (serogroup 1), and Seeh. (serogroup 2), respectively. The plants were harvested on September 30(th), 1987.

Results

The extinction values obtained from ELISA are listed in Table 1. In this connection only one isolate of serogroup 1-MDMV-Bulg., one isolate of serogroup 2-MDMV-Seeh., and the somewhat questionable isolate Rockend. as well as the adequate antisera may serve as example. Heterologous reactions between representatives of both groups can not be observed. The heterologous values are nearly identical with such of healthy plants. Obviously, the isolate Rockend. is a mixed isolate. Comparisons by means of latex test or agar gel double diffusion test using additional isolates, too, gave the same results.

The immune electron microscopical comparison was carried out with the 3 above-mentioned isolates. This test supplied results corresponding to those of ELISA. The application of homologous antisera resulted in a complete decoration (Fig. 1), but in the case of heterologous antisera generally no decoration took

[1] Special thanks are due to Dr. J. Hamacher, Rheinische Friedr. Wilhelms University Bonn, BRD, in whose laboratory the immunoelectronmicroscopial investigations were performed.

place (Fig. 2). In these experiments, too, the isolate Rockend. proved to be a mixed isolate. Only a part of particles was decorated with antisera of representatives of both serogroups. However, the decoration of the particles concerned was always complete (Fig. 3). A simultaneous treatment of this isolate with antisera of both groups resulted in a complete decoration of all particles (Fig. 4).

Table 1

E_{405mm}-values of homologous and heterologous reactions of both serogrups

Antisera	Virus isolates			Healthy plants
	MDMV-Bulg.	MDMV-Seeh.	MDMV-Rockend.	
489 serogroup 1 (MDMV-Bulg.)	1.90	0.04	−2.00	0.04
165 serogroup 2 (MDMV-Seeh.)	0.04	1.80	−2.00	0.04
115 serogroups 1 + 2 (MDMV-Rockend.)	1.85	1.75	−2.00	0.02

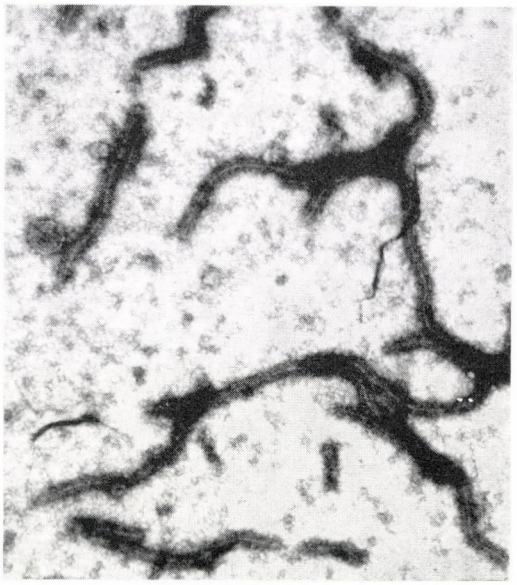

Fig. 1. Complete decoration of particles of the isolate MDMV-Seeh. using homologous antiserum

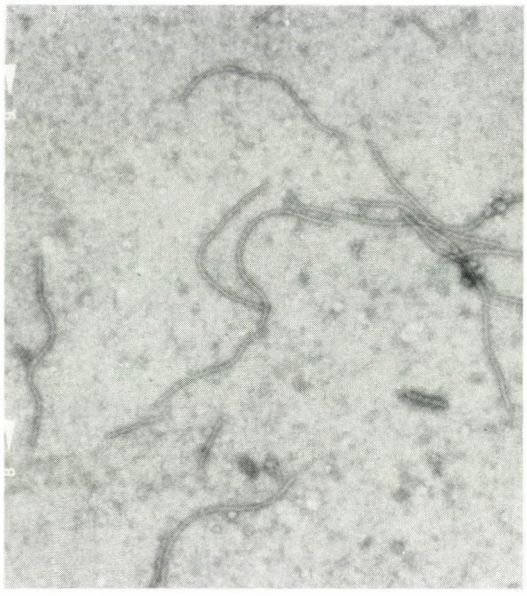

Fig. 2. No decoration of particles of the isolate MDMV-Seeh. using heterologous antiserum

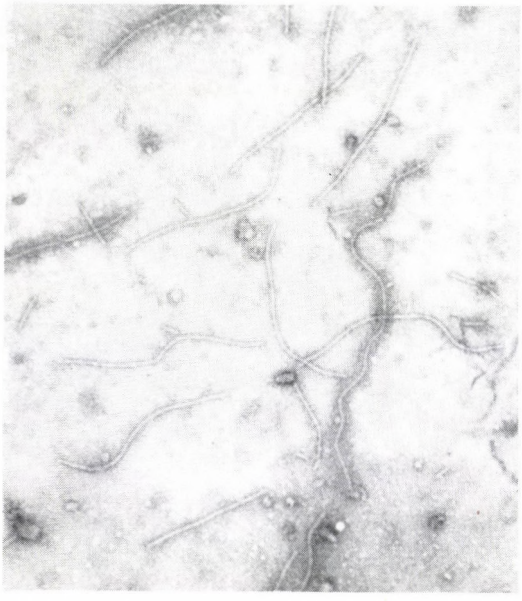

Fig. 3. Complete decoration of a number of particles of the mixt isolates MDMV-Rockend. using antiserum of isolate of serogroup 1

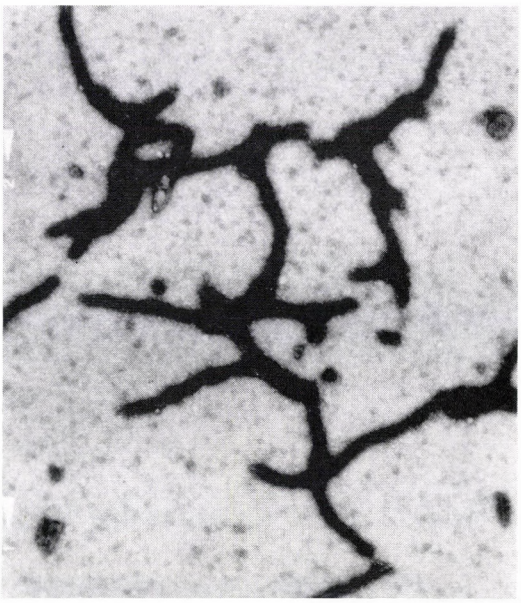

Fig. 4. Complete decoration of all particles of the mixt isolate MDMV-Rockend. using antisera of both serogroups

Already in 1987 Fuchs et al. reported on the simultaneous occurrence of representatives of both serogroups in maize crops. Even neighbouring plants can be infected by serologically opposite MDMV-isolates. The figures 5 and 6 show the percentage of serogroups 1 and 2 in attacked maize crops grown in the vicinity of Halle and Leipzig, respectively. Only in 3 crops we observed the serogroup 2 alone. The portion of serogroup 1 varied between 6% and 75%. In 1987 we found only representatives of serogroup 2. Mixed infections did not occur under open ground conditions. For this reason we carried out investigations to explain this premunity. The results are compiled in Table 2. If an isolate of serogroup 1 was used for the prior infection of maize plants and an isolate of serogroup 2 for the second infection, we could detect only serogroup 1 in 55% of individual plants and both serogroups in 45% of plants. The serogroup 2 alone could not be found. However, in the reverse case the serogroup 2 was prevalent with 96%. Only 4% of plants contained both serogroups. The results indicate a partial premunity, in which the representatives of serogroup 2 have obviously a stronger cross-protecting effect. The simultaneous inoculation resulted in a mixed infection in 74% of all plants. Serogroup1 alone was present in 19.5% of plants, serogroup 2 alone in 6.5%.

Table 3 shows the first results regarding the biological differentiation of both serogroups. We could demonstrate on the one side that only isolates of

Table 2

Investigation to premunity between both serogroups by means of mechanical inoculations

Succession of inoculations	Number of checked plants	Percentage of plants with the detection of		
		only serogroup 1	only serogroup 2	serogroups 1 + 2
At first serogroup 1, serogroup 2 two weeks later	73	54.8	0.0	45.2
At first serogroup 2, serogroup 1 two weeks later	138	0.0	95.6	4.4
Both serogroups at the same time	77	19.5	6.5	74.0

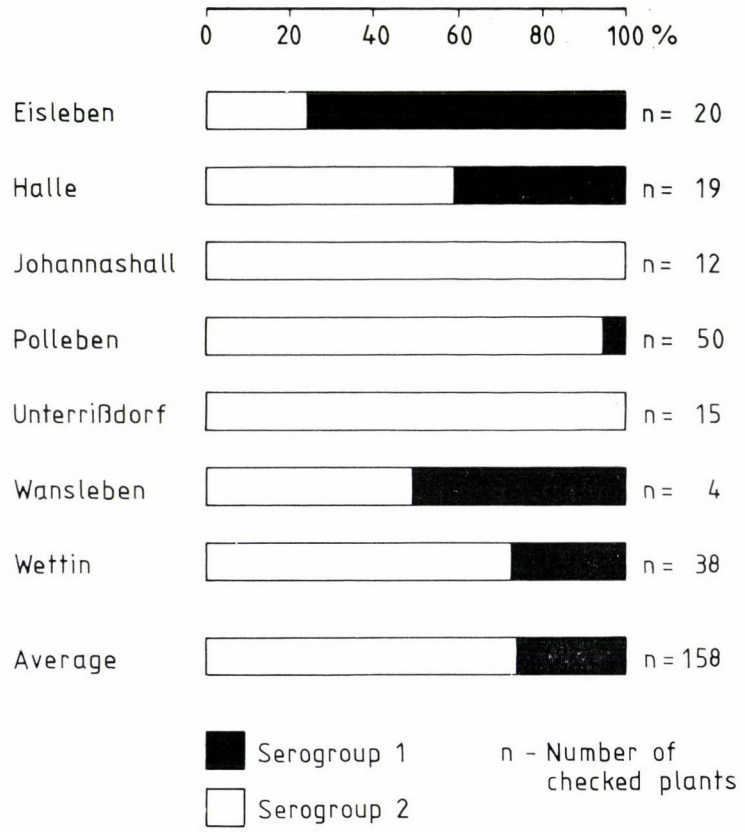

Fig. 5. Percentage of serogroup 1 and 2 of MDMV in maize crop grown in the vicinity of Halle/Saale in the summer of 1985

Table 3

Host plants for differentiation of both serogroups

Host plants	Isolates of	
	serogoup 1	serogroup 2
Sorghum almum Parodi	×	—
Sorghum dochna (Forsk.) Snowden	×	—
Sorghum halepense (L.) Pers.	×	—
Bromus hordeaceus L.	—	×
Bromus rubens L.	—	×
Setaria pumila (Poir) Roem. et Schult.	—	×

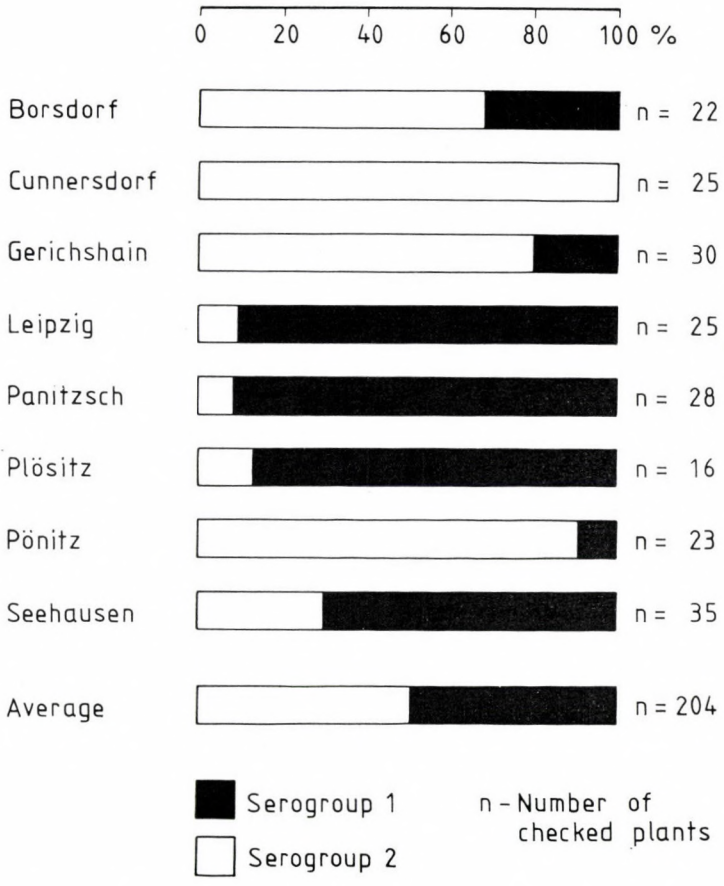

Fig. 6. Percentage of serogroup 1 and 2 of MDMV in maize crops grown in the vicinity of Leipzig in the summer of 1985

serogroup 1 are able to infect *Sorghum almum* Parodi, *S. dochna* (Forsk) Snowden, and *S. halepense* (L.) Pers. On the other side *Bromus hordeaceus* L., *B. rubens* L., and *Setaria pumila* (Poir) Roen. et Schult. are host plants only for isolates of serogroup 2. By means of a final trial we investigated the influence of MDMV-isolates Bulg. (serogroup 1) and Seeh. (serogroup 2) on plant height, plant weight, and corn-cob weight after mechanical inoculation. Between the isolates, but also between the both dates of inoculation, no significant differences could be observed (Figs 7 and 8). On an average of both infection dates the isolate

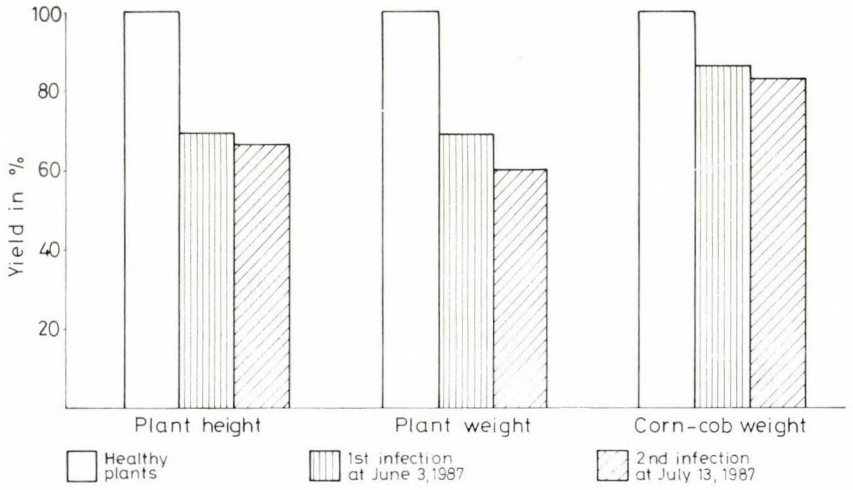

Fig. 7. Influence of MDMV-Bulg. (serogroup 1) on the yield of Zea mays L.

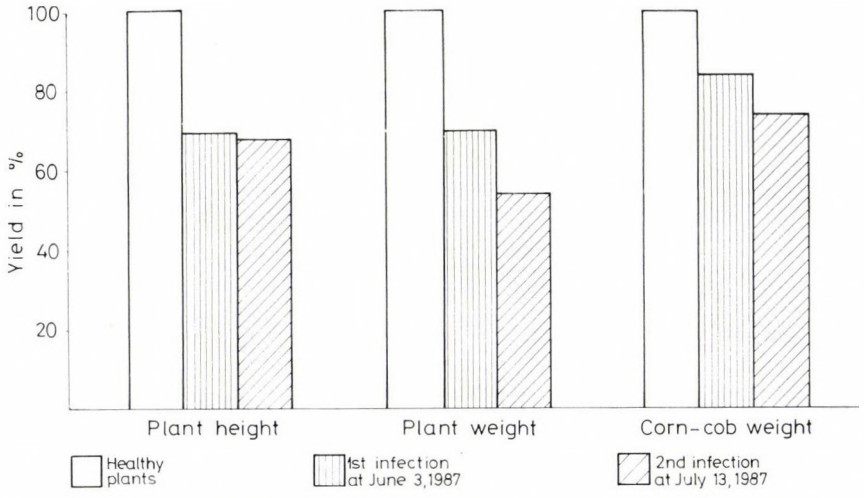

Fig. 8. Influence of MDMV-Seeh. (serogroup 2) on the yield of Zea mays L.

MDMV-Bulg. reduced the plant height by 32%, the total plant weight by 36%, and the corn-cob weight by 16%, respectively. The corresponding values of isolate MDMV-Seeh. ran to 32%, 39% and 21%, respectively.

Discussion

The occurrence of two serologically different groups of MDMV-isolates in GDR was the reason for starting investigations to characterize the groups exactly. We could detect the same both serogroups in other countries, too, f.e. in West Germany, Czechoslovakia and in Hungary, but the extent of these investigations was limited.

At present a well defined classification of the isolates proved by us to MDMV-strains as described in literature is not yet possible. The necessary material as a standard of comparison was not available to carry out a subdivision of isolates according to Gordon and Williams (1970) as well as Jarjes and Uyemoto (1984).

In our opinion the fact that only isolates of serogroup 1 are able to infect Sorghum halepense speaks for the occurrence of the MDMV-strains A and B (Tošić and Ford, 1972). We believe that both serogroups represent two different but closely related viruses. This assumption is based on the lack of serological relationships between both groups, on the observed partial cross-protection as well as on the lack of mixed infections in maize plants growing under open ground conditions. However, may I point out that the results concerning the premunity were obtained after mechanical inoculations. A direct comparison with the natural infection by aphides is not possible.

Future investigations will provide the answer to the question, whether different MDMV-similar viruses exist or not.

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Maize Dwarf Mosaic Virus, an Important Pathogen of *Sorghum* in Yugoslavia

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Maize dwarf mosaic virus causes mosaic, reddening and necrosis no leaves, as well as dwarfing, and intensive tillering of infected plants of sorghum, which sometimes die prematurely. Yield loss ranges up to, and over, 50%.

Sorghum is one of the most important cultivated plants. It is grown throughout the world on more than 70 million hectares (Józsa, 1976). In Europe, as well as in Yugoslavia, sorghum is planted on a relatively small scale. Each year in Yugoslavia sorghum is planted approximately on 10 to 25 000 hectares. Among the different kinds broom corn is a predominant one. In recent years other types of sorghum, as well as Sudan grass, are being grown on a larger scale, too.

The most important disease on sorghum in Yugoslavia is mosaic or sorghum red stripe, established for the first time in 1961 by Lovisolo and Aćimović (1961). This disease is caused by the virus which corresponds to the strain A of maize dwarf mosaic virus (MDMV-A) described in the USA and elsewhere (Tošić, 1965; 1974). Mosaic on sorghum in Yugoslavia can be epiphytotic, causing very important economic losses. Therefore, more and more attention is being drawn to this sorghum disease.

In this paper we shall present some results concerning incidences of MDMV on some sorghum cvs. in different localities, and its effect on sorghum yield.

Materials and Methods

Maize dwarf mosaic virus (MDMV) infection on sorghum was surveyed in three different localities: Bački Petrovac, Požarevac and Bogaraš. Within these localities the main difference was the presence of Johnson grass as a weed. Johnson grass was very abundant in the locality of Požarevac; it was not so abundant in the locality of Bački Petrovac, and it was not present in the vicinity of Bogaraš. As it is known (Šutić and Tošić, 1966) Johnson grass is mostly infected with MDMV and serves as a source for this virus under field conditions.

In each locality three surveys per year were done in order to record MDMV infection on sorghum plants. Each time 100 plants were observed for mosaic and other accompanying symptoms.

Etiology of observed symptoms was verified by virus isolation and its inoculation on maize, as well as on sorghum. Sorghum differential cvs. (Tošić and Ford, 1982) were used as test and indicator plants. Isolation of the virus was done by mechanical method under greenhouse conditions.

Intensity of infection, or in other words, frequency of infected plants was observed on some sorghum cvs. planted on experimental plots in each locality.

The effect of MDMV on yield of infected sorghum plants was tested in the locality of Bački Petrovac. The effect of MDMV on sorghum plants yield was evaluated by the weight of the brush with the grain. The difference in yield of mosaic and nonmosaic plants, as well as among different sorghum cvs. was checked by statistical analysis.

Results and Discussion

Symptoms on sorghum infected with MDMV and virus identification

The initial and the most frequent symptom observed on sorghum plants infected with MDMV, in the course of these investigations, was mosaic. On the most infected plants mosaic was accompanied with dwarfing, reddening of the leaves, intensive tillering and premature necrosis.

The first signs of mosaic could be observed at the base of the youngest leaf when plants are in the stage of 5–7 leaves, and later on, during vegetation. Mosaic begins as chlorotic dots and streaks, which spread along the leaf blade and appear on all leaves which develop after infection. Very often mosaic spreads on the whole leaf blade but many times it extends in stripes and bands. Soon after appearing, mosaic tissue becomes red and necrotic. Necrosis extends very often to the whole leaf blade and even sometimes to the whole infected plant, which dies prematurely.

Sorghum plants infected with MDMV are usually dwarfed; their height decreases by 20–50% or even more. Each sorghum plant infected with MDMV has 1.5 to 2.5 tillers, which is more than usual.

The symptoms we observed on sorghum plants infected with MDMV correspond to those described by Lovisolo and Aćimović (1961). Tošić (1965) described only mosaic on sorghum plants infected with MDMV under field conditions.

MDMV identification was done on the basis of virus transmission and reaction of test plants. The virus was easily isolated and transmitted by mechanical method. Symptoms caused by isolated virus on test plants under greenhouse conditions are presented in Table 1.

As it can be seen from the results presented in Table 1, broom corn cvs. Sava, Tisa, Neoplanta and Panonija reacted to the isolated virus by mosaic,

Table 1

Symptoms on maize and sorghum test plants caused by the virus isolated from sorghum plants showing mosaic, necrosis, dwarfing and intensive tillering

Test plant	Symptoms
Sweet corn	Mosaic
Broom corn cvs.:	
Sava, Neoplanta, Tisa and Panonija	Mosaic, reddening, dwarfing, necrosis
Sorghum differential cvs.:	
Martin, OKY 8, Q 7539, SC 0097	Mosaic
Atlas, Rio, SA 8735	Mosaic followed by weak necrosis
NM 31	Mosaic followed by pronounced necrosis

reddening, dwarfing and necrosis symptoms which were observed on these cvs. under field conditions. These results verify that observed symptoms on broom corn cvs. under field conditions are caused by isolated virus.

Reactions of sorghum differential cvs. Martin, OKY 8, Q 7539, SC 0097, Atlas, Rio, SA 8735 and NM 31 showed that isolated virus corresponds to the MDMV-A, which causes identical symptoms on these sorghum cvs. (Tošić and Ford, 1982).

Incidence of MDMV infection on some sorghum cvs. in different localities

An unusual outbreak of the MDMV infection on sorghum – broom corn occurred in 1984 in the locality of Požarevac. Therefore, in the following years infection on sorghum – broom corn was surveyed. Surveys were done in the localities of Bački Petrovac, Požarevac and Bogaraš. Obtained results are presented in Table 2.

As it can be seen from the results presented in Table 2, the incidence of MDMV infection on sorghum – broom corn varied across years, among localities, and to some extent, on sorghum cv. In the locality of Požarevac in 1984, infection varied from 78% to 92% depending on cv. Such a high incidence of MDMV on sorghum was probably due to favourable conditions for vectors' activity.

In 1986 the rate of infection was much lower and similar in the localities of Bački Petrovac and Požarevac, and it varied from 3.5% to 11.0%. The same year in the locality of Bogaraš the percentage of sorghum plants infected with MDMV was very low with an average of 0.2%. The low rate of infection in the locality of Bogaraš was, probably, due to the absence of Johnson grass as a source of the virus.

Table 2

Incidence of MDMV on some sorghum cvs. in different localities

Sorghum cv.	Locality, year, and % of infection			
	Bački Petrovac 1986	Požarevac 1984	Požarevac 1986	Bogaraš 1986
	% of infection			
Neoplanta	8.3	83	7.4	0.6
Panonija	7.8	78	4.1	0.0
Tisa	6.3	87	8.5	0.0
Sava	7.0	92	5.8	0.6
Jantar	6.5	—	9.0	0.0
NS-BP/CL 6	7.2	—	3.5	0.0
Szegedi Törpe	11.0	—	11.0	0.0
Szegedi 185	9.4	—	10.6	0.0
Jumak	6.6	—	8.5	0.0
Szegedi Szlovak	6.0	—	10.6	0.6

Effect of MDMV on productivity of infected sorghum plants

In 1984 when the rate of MDMV infection on sorghum was very high in the locality of Požarevac (78–92%) the percentage of first class of brush was very low (10–20%). This indicated that MDMV has a big effect on sorghum plants' yield. Because of that, some detailed research concerning the effect of MDMV on sorghum plants was done in 1986 in the locality of Bački Petrovac. Obtained results are presented in Table 3.

As it can be seen from Table 3, MDMV has an important influence on the productivity of sorghum plants. Decrease of yield due to infection varied from 31.6% to 58.6% concerning the weight of brush with grain. If we take into consideration the lower quality of brush from infected plant it comes out that damage caused by MDMV on sorghum is even higher than only the loss in weight. These results show that MDMV is one of the most important pathogens on sorghum in Yugoslavia.

Table 3

The effect of MDMV on yield of sorghum

Sorghum cv.	% of infection	healthy plant %	infected %*
Sava	7.8	57.2	23.7	41.4
Jumak	7.5	65.8	33.7	51.2
Szegedi Törpe	11.4	58.5	40.0	68.4
Tisa	6.7	61.7	25.6	41.5
Deer 418	12.1	55.4	37.8	68.2

* % = yield of infected plant comparing to the yield of healthy one

Conclusion

Maize dwarf mosaic virus is one of the most important pathogens of sorghum in Yugoslavia. This statement is based on the following data:

1. MDMV causes mosaic, reddening and necrosis on leaves, and dwarfing and intensive tillering of infected sorghum plants, which sometimes die prematurely.

2. Incidence of MDMV infection on sorghum cvs. differ, depending mainly on locality. Under conditions favourable for infection, the infection rate can be as high as 90%.

3. Yield of sorghum plants infected with MDMV is reduced by 31.6% to 58.6%, depending on the variety of sorghum.

4. Breeding for resistance is the most important way to control MDMV infection on sorghum.

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Albinism in some Krish Sorghum Genotypes and Resistance to Maize Dwarf Mosaic Virus

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Sublines derived from QL 2-6-1-2-2, which possess Krish type of resistance to MDMV, differ in how they inherit albinism as well as in resistance to MDMV. Under field conditions all derived sublines showed resistance to MDMV. But by mechanical inoculation and under greenhouse conditions sublines which inherit albinism at the ratio of 7 : 2 showed susceptibility to MDMV.

Albinism, as a consequence of lethal gene(s), frequently appears in spontaneous, as well as in cultivated plants. If albinism is controlled by one dominant factor, it is called "aurea" type, as it is case with albinism of *Antirrhinum* sp., *Urtica* sp. and other species (Dubovský and Maršálek, 1968). More frequently albinism is controlled by one or more recessive factors, and is usually inherited by inbreed lines. This type of albinism is known in corn and it is controlled by one recessive homozigous factor (Demerec, 1923, loc. cit. Tavčar, 1952; Borojević and Borojević, 1976), and in barley, controlled by two homozigous recessive factors (Gustafson, 1947, loc. cit. Tavčar, 1952). As a matter of fact, with albinism controlled by recessive factors, "xantha" plants appear at the ratio of 9 : 3 : 4 (no. of green: no. of "xantha": no of albino plants) (Borojević and Borojević, 1976).

In sorghum, however, albinism is very rare. The senior authors had few occasions to see sorghum albino plants during 25 years of research on sorghum. Albinism was very rarely noticed by two inbreed lines of broom corn (Exc × Tan 2.2, and Acme × Tan). More frequently, albinism appeared in some inbreed line, resistant to maize dwarf mosaic virus (MDMV), like QL 2–6–1–2–2, provided by the kindness of Prof. D. Weibel (Oklahoma Sta. Univ., Stillwater, Oklahoma, USA). The pedigree of that line is Krish × Tx7078[2] and that line has the gene for male sterility in "B" type of cytoplasm (Weibel, 1978). From the QL 2–6–1–2–2 few sublines were derived, some of which inherited albinism, but some did not.

In this paper we shall present some results concerning albinism in sorghum, its inheritance and correlation with resistance to MDMV.

Materials and Methods

These investigations have been carried out with sublines of QL 2–6–1–2–2 line. Under conditions of controlled self polination from QL 2–6–1–2–2 line, 43 sublines were derived during the period from 1981–1987. Obtained sublines differ only in having lethal gene(s) controlling albinism. Some investigations carried out in 1986 were done by sublines inheriting albinism. These trials were done under field conditions. For the sublines being tested the following criteria were applied: total number of plants, number of albino plants, and the ratio of normal (green) to albino plants. With 9 sublines having sufficient no. of plants, segregation was evaluated by Chi-square test.

Certain investigations were done concerning the inheritance of albinism and the inheritance of resistance to MDMV. These studies were done by crossing 3 sublines of QL 2–6–1–2–2, resistant to MDMV by 5 sorghum genotypes having different resistance to MDMV. By F_1, BCF_1, and in some combinations of BC_2F_1 and BC_3F_1, the ratio of green : albino plants was evaluated.

Some research concerning correlation of albinism to resistance to MDMV has also been done. For this purpose, some QL 2–6–1–2–2 sublines inheriting albinism and some crosses of these sublines (F_1–BC_3F_1) with some other sorghum genotypes have been tested with MDMV by mechanical inoculation under greenhouse conditions. This MDMV was a Yugoslav isolate which verified correspondence to MDMV-A (Tošić, 1974). Inoculation of seedlings was done at the three-leaves stage. Included in these investigations were 8 sublines without albinism, 4 ones inheriting albinism, 8 crosses (4 of F_1, 2 of BC_1F_1, one of BC_2F_1, and one of BC_3F_1).

Albino plants were recorded after emergence of seedlings by all investigated sublines and crosses. Inoculation with MDMV was done on both green and albino plants. Inoculated plants were observed for symptoms. Four weeks after inoculation, all seedlings without symptoms of MDMV were checked for the presence of MDMV by reinoculation on Atlas sorghum.

Results and Discussions

The Krish type sorghum line QL 2–6–1–2–2, which inherited resistance to MDMV (Weibel, 1978) was used as a source of resistance in breeding program of sorghum resistant to MDMV. This line appeared to inherit albinism, too. On the basis of the ratio of green: albino plants, Chi-square test and P, 14 sublines were selected for these investigations. Data concerning these 14 sublines of QL 2–6–1–2–2 are presented in Table 1.

All 14 sublines listed in Table 1, showed resistance to MDMV under field conditions.

According to the data presented in Table 1, albinism in sorghum is controlled by recessive homozigous gene (or genes). Green colour is dominant and is con-

Table 1

Frequency of albinism in sublines of QL 2—6—1—2—2

Subline	No. of observed plants	No. of green plants	No. of albino plants	The ratio of green : albino plants	Chisquare	P
161	43	38	5	7 : 1		
166	130	110	20	6 : 1		
188	59	49	10	5 : 1		
190	81	80	1	80 : 1		
193	117	92	25	7 : 2	0.05	0.9
194	111	86	25	7 : 2	0.01	0.9
200	125	95	30	3 : 1	0.07	0.9
202	226	180	46	7 : 2	0.46	0.5
203	130	100	30	7 : 2	0.06	0.9
204	126	96	30	3 : 1	0.0	0.9
205	197	156	41	7 : 2	0.23	0.7
207	158	122	36	7 : 2	0.00	0.9
208	147	110	37	3 : 1	0.00	0.9
209	93	88	15	6 : 1		

trolled by at least two factors. But, the expected ratio of 3 : 1 (green : albino plants) was found only in three sublines. This ratio of 3 : 1 is characteristic in cases of albinism in corn, where albinism is controlled by a recessive lethal factor (Tavčar, 1952; Borojević and Borojević, 1976).

With investigated sorghum sublines, the most frequent ratio of green : albino plants was around 4 : 1, or more precisely 7 : 2. Six out of 14 tested sublines showed this ratio. This ratio indicated that the presence of chlorophyll in sorghum plants is controlled by at least two factors and albinism is controlled by one out of two homozigous recessive factors. If factors controlling the presence of chlorophyll in sorghum are marked as $G_1G_1G_2G_2$, than albinism is controlled by $g_1g_1G_2G_2$, and by $G_1G_1g_2g_2$. Theoretically albinism in sorghum could be controlled by $g_1g_1g_2g_2$, $g_1g_1G_2g_2$, and $g_1G_1g_2g_2$ but these combinations cannot take place, since albino plants die at the 3–4 leaf-stage and have no progeny.

Under conditions of controlled polination, sublines with genom possessing one out of two recessive factors ($G_1g_1G_2G_2$, and $G_1G_1G_2g_2$) have progeny with a ratio of 3 : 1 of albinism, and sublines with two recessive factors ($G_1g_1G_2g_2$) follow a ratio of 7 : 2. Less frequently cases which have a ratio like 5 : 1, or 6 : 1, or 7 : 1, and 80 : 1 are to be explained in future research. All these 14 sublines, nevertheless of ratio of albinism, showed resistance to MDMV under field conditions, as it was pointed out before.

Three of the investigated sublines: 190, 193 and 204, having different ratios of albinism 80 : 1, 7 : 2 and 3 : 1, respectively, were crossed with different sorghum genotypes in order to find out the correlation of resistance to MDMV and the ratio of albinism in sorghum. Results obtained under field conditions are presented in Table 2.

Table 2

Inheritance of albinism and resistance to MDMV

Crossing		combination	F_1	Generation of crossing		
				BC_1F_1	BC_2F_1	BC_3F_1
Redlan* (−/S)	×	190 (80 : 1/R)	−/R	−/R	−/R	−/R
	×	193 (7 : 2/R)	−/R	10 : 1/R	7 : 2/R	7 : 2/R
	×	204 (3 : 1/R)	−/R	−/R		
OK 24 (−/S)	×	190	−/R	−/R		
	×	193	−/R	12 : 1/R		
	×	204	−/R	−/R		
AK 3003 (−/S)	×	190	−/R	−/R		
	×	193	−/R	−/R		
	×	204	−/R	−/R		
QL 19 (−/R)	×	190	−/R	−/R		
	×	193	−/R	−/R		
	×	204	−/R	−/R		
QL 21 (−/R)	×	190	−/R	−/R		
	×	193	−/R	−/R		
	×	204	−/R	−/R		

* − = no albinism (all seedlings green), 80 : 1, 10 : 1, 7 : 2, and 3 : 1 = ratios of albinism

S = susceptible to MDMV, R = resistant to MDMV

According to the results presented in Table 2, the resistance to MDMV is dominant in all generations of crosses tested (F_1, BC_1F_2, BC_2F_1 and BC_3F_1). But inheritance of albinism was not uniform. In F_1 albinism was absent, while in BC_1F_1 it appeared in two out of 15 possibilities. Inheritance of albinism was associated with crossing of Redlan and OK 24 by subline 193 and resulted in the ratios of 10 : 1 and 12 : 1, respectively. By BC_2F_1 and BC_3F_1 crossings of Redlan with subline 193, inheritance of albinism was at the ratio of 7 : 2. According to these data, inheritance of albinism was shown only in case of the subline which has two lethal recessive factors. These results indicate that further investigations of inheritance of albinism are necessary.

More detailed studies of the correlation of albinism and resistance to MDMV were done under greenhouse conditions. Included in these investigations were 12 sublines of QL 2–6–1–2–2, two crosses of Redlan by subline 193 and 200, two crosses of OK 24 by the same sublines, two BC_1F_1, one BC_2F_1 and one BC_3F_1. All emerged seedlings being green or albino were mechanically inoculated with MDMV. Obtained results are presented in Table 3.

As it can be seen from the results presented in Table 3, with 7 out of 20 tested sorghum genotypes, infection with MDMV was successful only on green plants which showed mosaic symptom; but it was not so in albino plants.

Table 3

Inheritance of albinism and resistance to MDMV in some sorghum sublines and hybrids

No.	Sublines or crosses	Albinism ratio/resistance to MDMV
1–8.	QL 2–6–1–2–2/162, 163, 165, 167, 168, 171, 180, 201	–/R*
9.	/200	3 : 1/R
10.	/207	3 : 1/R
11.	/202	7 : 2/S (mos)
12.	/203	7 : 2/S (mos)
13.	F_1 : Redlan × 200	–/R
14.	× 193	–/S (mos)
15.	OK 24 × 200	–/R
16.	× 193	–/S (mos)
17.	BC_1F_1 : Re× 200)× 200**	–/R
18.	(Re× 193)× 193	10 : 1/S (mos)
19.	BC_2F_1 : (BC_1F_1× 193)	7 : 2/S (mos)
20.	BC_3F_1 : (BC_2F_1× 193)	7 : 2/S (mos)

* – = no albinism (all seedlings green)
R = resistant to MDMV, S = susceptible to MDMV, (mos) = mosaic
** Re = Redlan cv.

Two of the tested sublines, 202 and 203, showed susceptibility to MDMV by mechanical inoculation under greenhouse conditions. Both of these sublines had albinism controlled by both factors, or in other words, these sublines had genetic constitution of two recessive factors like $G_1g_1G_2g_2$ and albinism with the ratio of 7 : 2.

As can be seen from the comparison of the results presented in Table 1 and Table 3, susceptibility to MDMV was shown in some lines (202, 203) and crosses (BC_1F_1: (Rex 193) × 193, BC_2F_1 × 193 and BC_3F_1: BC_2F_1 × 193), in which albinism was inherited, too.

Seedlings obtained by crossing and back crossing subline 193 by Redlan and OK 24 showed susceptibility to MDMV. With these seedlings albinism was not inherited by F_1. In the cases of BC_1F_1 albinism was inherited at the ratio of 10 : 1, but with BC_2F_1 and BC_3F_1 at the ratio of 7 : 2.

According to the previous research QL 2–6–1–2–2 possesses Krish factor of resistance to MDMV (Weibel, 1978), and it is considered to be dominant. On that basis, all sublines derived from that line should be resistant to MDMV. The results we obtained in these investigations are in dissagreement with the findings of some researchers (Conde et al., 1976; Toler, 1985). On the other hand, the results we obtained are in agreement with those obtained by Stakić (1984), who found that subline 111 is susceptible to MDMV under conditions of mechanical inoculation in greenhouse. These results indicate that the Krish type of

resistance to MDMV is not a clasical intraalel, a dominant-recessive one, in which the Krish gene should be dominant, but it is an interallelic (epistatic) type, which is inherited dominantly, but the presence of both recessive lethal factors for albinism block epistatic dominant effect.

Literature

In the sorghum genotype QL 2–6–1–2–2 having Krish type of resistance to MDMV, albinism appears frequently and is maintained by self polination. Under controlled conditions of self polination sublines were derived from QL 2–6–1–2–2, some of which inherit albinism.

With those sublines where it is inherited, albinism appears at the ratio of 3 : 1 and 7 : 2 (earlier approximation of 4 : 1); but other ratios are present, too. From these results it can be concluded that albinism in sorghum is controlled by two recessive lethal factors, and albinism will appear if one of these two factors is homozigous. Albinism is inherited with genotypes having $G_1g_1G_2G_2$ and $G_1G_1G_2g_2$ at the ratio of 3 : 1, and in genotype having $G_1g_1G_2g_2$ in the ratio of 7 : 2.

Out of 43 investigated sublines of QL 2–6–1–2–2 albinism was inherited by 14 of these.

All sublines showed to be resistant to MDMV under field conditions.

By mechanical inoculation under green house conditions, it was shown that some sublines with two heterozigous recessive factors inherit albinism at the ratio 7 : 2, and are susceptible to MDMV. Infection with MDMV was proved only in green plants.

Obtained results also indicate the possibility of a correlation between a certain kind of albinism and resistance to MDMV. This correlation should be more thoroughly studied by investigating factors which control inheritance of albinism, as well as resistance to MDMV

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Resistance Evaluation of Maize Hybrids and Inbred Lines to Maize Dwarf Mosaic Virus (MDMV) in Hungary

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The authors recommend to the growers modern maize hybrids (e.g. in FAO 200: Mona SC and Mirna SC, in FAO 300: Carla SC, in FAO 400: Pioneer 3747 SC and in FAO 500: Volga SC etc.) which were MDMV resistant under severe natural infection especially in 1986 and 1987. We are of the opinion, that MDMV is most dangerous for inbred lines, sweet corn and less for maize hybrids. On Tables 2 and 3 there are many inbred lines, which could be source of MDMV resistance. Finally in the paper there are some possibilities to improve the hybrid seed production.

In Hungary, maize dwarf mosaic virus (MDMV) was first published by Szirmai and Mrs. Paizs (1963), 25 years ago. Since that time it has distributed into most parts of the country and now it is the most important Gramineae pathogenic virus in Hungary. And this virus is especially, dangerous for the inbred lines (Milinkó et al., 1985 and 1986). Consequently, the highest yield losses can occur in the seed production.

Therefore our goals were:

- to evaluate the current maize hybrids to MDMV resistance,
- to find and breed new MDMV-resistant inbred lines,
- and to improve the hybrid seed production by controlling MDMV (Szirmai, 1968, Milinkó, 1977, 1978, Milinkó et al., 1979, 1981, 1982, 1983, 1984, 1985, 1986).

Materials and Methods

Our field experiments were carried out in Baja area where there is the highest natural MDMV infection pressure. Since 1981 in every year there was a small plot experiment with four repetition. Among and around the blocks there were 2-m-broad Sorgum halepense (L). Pers. stripes, which were allowed to develop to insure the natural infection. Field evaluation of maize hybrids and inbreds were made every year by a 0–5 scale. Some cases we checked the plants by ELISA-test too.

Results

On Table 1, MDMV resistance evaluations of current maize hybrids are shown. The most important result of these evaluations was that we could recommend the growers every year and in every maturity group (FAO 200–500) one or more modern MDMV resistant maize hybrids. On Table 1 it is also obvious

Table 1

MDMV resistance of maize hybrids
(Baja, 1985—1987)

FAO maturity group	Hybrid	Scale value 0—5			Reaction
		1985	1986	1987	
	Mona SC	*1*	*1*	*1*	*R*
	Sanora TC	1	3	—	
	3965 A MTC	2	—	—	S
200	*3950 MSC	2	4	4.5	S
	Mirna SC	*1*	*1*	*1*	*R*
	SC 3270	—	0	1	
	*3902 SC	—	—	1	
	Pannonia SC	0	1	1.5	
	Isela SC	0	0	1.5	
300	*Carla SC*	—	*1*	*1*	*R*
	*3901 SC	*1*	*1*	*1*	*R*
	*3732 SC	1	1	1	
	3747 SC	*0*	*0*	*1*	*R*
400	*3709 MSC	1	1	—	
	SzeDC 488	1	—	—	
	Sabrina SC	*1*	*1*	*1*	*R*
500	*Volga SC*	*0*	*1,5*	*1*	*R*
	Rosa MSC	—	—	1	

* = Pioneer hybrid
R denotes resistant
S denotes susceptible

that the slightest infection occurred in 1985 after a very cold winter and a rainy spring which were not favorable for virus vector aphids.

On Tables 2 and 3 there is a number of MDMV resistant inbreds. This is explained by the continuous removal of the susceptible inbreds from our collection during the previous years.

The damaging effects of MDMV are far more serious in single cross (SC) seed production, where the infection has the following main damaging effects:

Table 2

MDMV resistance of inbred lines
Baja, 1985—86

Scale value	Number of lines		Reaction*
	1985	1986	
0	*12*	*19*	*R*
1	15	9	R
2	4	1	MS
3	10	2	S
4	5	3	S
5	1	13	VS
Total number	47	47	

* R = resistant
MS = moderately susceptible
S = susceptible
VS = very susceptible

- increasing the heterogenity of maize stand,
- decreasing the co-flowering,
- cobs are smaller,
- seed setting is poor, causing more seed losses at harvest,
- increasing the ratio of large round seeds. Consequently the seed quality and yield will be poorer than those of healthy stands.

The possibilities in case of a SC seed production are shown in Table 4.

The most dangerous version when the female line is susceptible. That was situation e.g. in case of Pioneer 3732 SC in previous years. MDMV-infection of female line had reached almost 100% by mid-summer. The female line of this hybrid was sensitive to environmental factors, therefore the seed demand of Pioneer 3732 SC could not be supplied. For this reason the reciprocal seed production was introduced into technology. In this case the MDMV resistant parent line is the female. This modified version is in agreement with the results of our virus resistance evaluation.

The other dangerous situation is when the male line is to be planted much later than the female one, because of nick. Like in case e.g. of Mona and Dea SC hibryds. In this case the very susceptible male lines will grow under very heavy MDMV-infection pressure, where the pollen shedding and nick are not guaranteed.

Therefore for areas with heavy infection potentials we recommend to produce resistant SC or TC and DC hybrids, which are less susceptible to MDMV infection.

Table 3

MDMV resistance of inbred lines
Baja, 1985–86

Inbred lines	Scale value		Reaction*
	1985	1986	
A 375	0	0	R
A 634	0	0	R
CG 1	0	0	R
CM 105	0	0	R
Co 125	0	0	R
N 6 J	0	0	R
Ky 66-2500	1	0	R
Oh 509 A	0	1.5	R
Pa 405	0	1.5	R
Oh 514	1	1.5	R
Va 35	1	1.5	R
A 632	1	—	MS
A 654	1	—	MS
FC 1444	1	3	S
CM 7	—	4	S
F 2	2	5	S
Oh 41	2	5	S
Mo 17	3	5	S
W 401	3	5	S
W 64 A	3.5	—	S
W 153 R	4	—	S
N 20	4	5	VS
M 14	4	5	VS
W 117	5	—	VS

* R = resistant
MS = moderately susceptible
S = susceptible
VS = very susceptible

Table 4

Possibilities of MDMV resistance in a single cross(SC) seed production

♀		♂	
Susceptible	×	Susceptible	(partner of * 3965 A MTC)
Susceptible	×	Resistant	(* 3906 SC, Volga SC)
Resistant	×	Susceptible	(Mona SC, Dea Sc, Mirna SC, Isela SC, Carla SC, * 3901 SC, Pannónia SC, * 3732 SC Sabrina SC)
Resistant	×	Resistant	(aim of breeding)

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Observations and Studies of Maize Inbred Lines and Hybrids Naturally Infected by MDMV

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The virus infection of maize inbred lines and hybrids was studied in Martonvásár under field condition in 1987. In the diseased plants MDMV was indicated the most frequently by the ELISA test but BYDV occurred in many samples, too. In the inbred lines significant differences could be observed between the various relation groups. In the hybrids virus symptoms could be found only on plants planted the latest on June 23rd, not in earlier plantings. The degree of virus infection in the case of every hybrid increased as time passed. The results so far reveal that resistance is dominantly inherited.

The most important virus deasease of maize in Hungary is the MDMV disease, which was first reported by Szirmai and Paizs (1963). The spread, the epidemiology and the damage of the virus in Hungary were studied by Milinkó et al. (1983). The most effective means of protection against the disease is the development of resistant hybrids. Opinions differ regarding the character of the gene effects determining resistance. Certain authors (Loesch and Zuber, 1972, Zuber et al., 1973) presume a mainly additive gene effect, others demonstrated partial or complete dominance (Johnson, 1971, Milinkó et al., 1986).

In recent years in the maize breeding work being conducted at our institute the resistance study of breeding materials, inbred lines and hybrids has received special emphasis. In our 1987 investigations we had two objectives. On the one hand we wished to obtain information on the natural infection of breeding materials, the differences among them in susceptibility. On the other hand, in the case of hybrids the dynamics of virus infection within the growing season was studied as a function of the date of planting.

Materials and Methods

The virus infection of 410 inbred lines and breeding basic materials was studied in an experiment without replications, on 19 plants per plot. The observations were made late July and early August on the basis of visual symptoms. In order to study the MDMV infection of hybrids 15 of them were planted at three dates; May 6th, June 2nd and June 23rd in experiments with four replica-

tions. On three occasions during the growing season: July 27th, August 9th and September 19th the infection % of plants (I %) was determined on the basis of the symptoms seen on the leaves. At the last evaluation on September 19th the disease was determined, too, according to a simplified version (where 1 = no symptoms, 6 = most severe symptoms) of the rating system of Zeyen, Groth and Davis (1979). Diseased leaves were collected from the lines and hybrids under observation for indication of virus infection using the ELISA test on several occasions during the year.

Results and Discussion

1. Virus infection of inbred lines and breeding basic materials

The degree of MDMV infection of inbred lines according to three major relation groups is presented in Table 1. The infection data of closely related lines were summarized. The lines among which plants with very serious symptoms could be found were indicated separately.

Among the representatives of the ISSS (B) group two inbred lines were found which were consequently free from symptoms. These were the lines H Mv 1528 and H Mv 5403 developed at Martonvásár. This latter line, H Mv 5403 originates from the line B 37, the individuals of which often revealed serious symptoms at evaluation.

The data of the table show that lines in the relation group Lancaster Sure Crop (A) were the most strongly infected. Two lines were observed in this group, too, which were free of symptoms and had been developed at our institute. These were H Mv 5110 and H Mv 5404.

The X relation group is shown divided in four subgroups. In this group only the inbred lines developed from the two open pollinated hungarian varieties (MPS and MPF) were free of symptoms. Among them also in the source material (B 73 and F 2) of H Mv 73–118 and H Mv 2–118 seriously infected plants can often be found.

The majority of the lines in the X relation group were highly infected. Among them seriously infected plants were observed in eight inbred lines. These results, too, indicate, that due to the dominance of resistance resistant lines can be developed from susceptible sources.

2. Virus infection of hybrids

Parallel to the observation of lines the MDMV infection of 15 early maturing hybrids was studied in a planting date experiment. Noteworthy virus infection occurred only on plants planted at the third date on June 23rd. The infection of hybrids is summarized in Table 2 according to scorings on three occasions.

Table 1

The degree of MDMV infection of corn inbred lines Martonvásár, 1987

Inbred lines	%	Inbred lines	%
ISSS (B) Group		*X group*	
B 73	10.6*	H Mv 33	8.9
B 59	10.0	A 654	12.0
H Mv 1528	0.0	Co 120	13.3
B 37	11.9*	W 117	14.9
H Mv 5403	0.0	W 64 A	16.2*
FRA 634	12.0	W 153 R	18.9*
A 641	13.3	Syn 7	20.0
A 635	15.0	CM 7	22.1*
A 350 N	20.0	W 401	28.3*
A 665	24.7	Ep 1	33.3
B 14-CM 4	29.7*	MR 11	33.3*
		CK 22	40.0*
Lancaster Surecrop (A) group			
Mo 17	22.4*	F 7	15.6*
H Mv 5404	0.0	F 564	17.1
A 619	32.2	F 2	18.7*
H Mv 5110	0.0		
KI 57	34.7*	156 RF	0.0
C 123	37.2	H Mv 404	0.0
		0118a—124 A	0.0
		H Mv 73—118	0.0
		H Mv 2—118	0.0
		H Mv 118—65	0.0
		H Mv 118—78	0.0
		H Mv 5204	0.0

* Plants with very serious symptoms

The increase in infection was especially high in hybrids 1, 9, 10, 13, 14 and 15. The disease severity index (Di) expressing the severity of infection was the highest in these, too.

The virus infection of some closely related hybrids are presented graphically in Figure 1. The inbred line marked "R" proved to be relatively resistant already during line evaluation, whereas those marked "S" were more strongly infected (Table 1). The data of the figure seem to support the data of Johnson (1971), Milinkó et al. (1986) according to which resistance to virus infection is dominantly inherited (Graphs 1, 4, 6).

In order to confirm our data, however, further investigations are required on systematic genetic materials.

Table 2

The degree of MDMV infection of maize hybrids
Martonvásár, 1987

Hybrids	Infection % (I %)			Disease severity index (Di)
	27.07.	09.08.	19.09.	
Planting date: June 23rd, 1987. 1—6 severity scale				
1. Mv 210	5.8	13.5	24.0	1.56
2. Mv 211/A	11.4	14.0	17.5	1.40
3. Mv 224	2.3	6.6	7.5	1.22
4. Mv 225	2.7	6.2	10.6	1.27
5. Mv Exp. 60—87	5.1	8.1	9.1	1.29
6. Mv 243	1.8	4.6	4.6	1.11
7. Mv 245	3.6	14.3	16.1	1.51
8. Mv 246	0.1	5.2	8.7	1.22
9. Mv 255	6.2	14.2	22.1	1.60
10. Mv 250	11.3	23.5	25.2	1.95
11. No 410	10.5	14.9	17.5	1.53
12. Mv To 279	0.0	2.8	4.6	1.15
13. Bermarit	8.6	29.5	33.3	1.84
14. Bermasil	4.6	22.2	36.1	1.66
15. NK PX 14	10.4	34.0	40.2	2.01

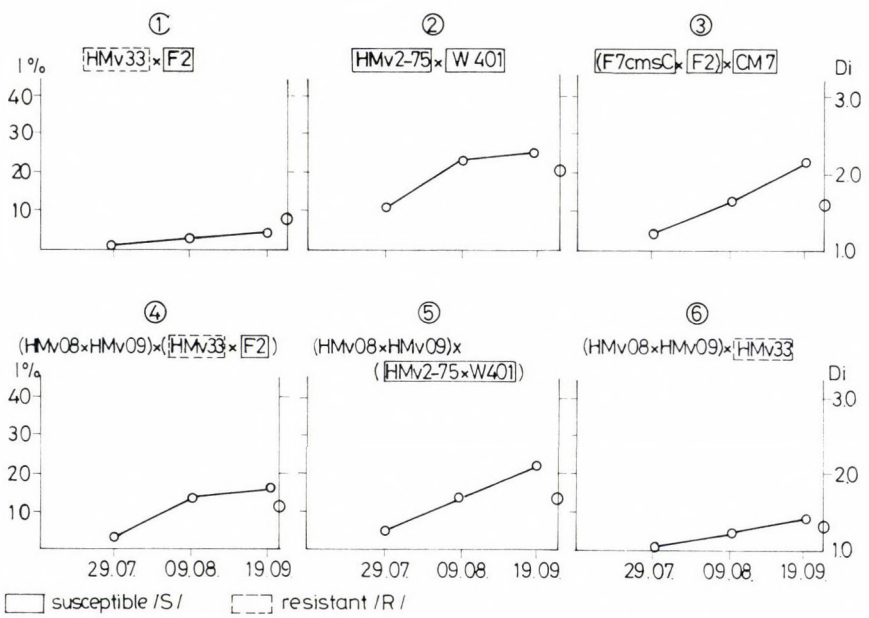

Fig. 1. The MDMV infection of corn hybrids Martonvásár, 1987

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Effects of Carbon Dioxide Anaesthesia on the Ability of the Planthopper *Laodelphax striatellus* to Vector Maize Rough Dwarf Virus

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It has been suggested that anaesthesia with carbon dioxide can influence the feeding behaviour of the planthopper *Laodelphax striatellus*, a vector of plant viruses within the Reoviridae, the Rhabdoviridae and the tenuivirus group. We have studied the influence of CO_2 anaesthesia on the acquisition and transmission in serial passages of maize rough dwarf virus, a phloem-limited member of the Reoviridae, which is transmitted propagatively by *L. striatellus*.

Anaesthetizing the insects before acquisition feeding significantly reduced ($p < 0.01$) the acquisition of the virus from infected barley and maize plants (from 47.37% to 25.00%).

In serial passage infectivity tests using 3-day inoculation feedings, the 36 infective hoppers not anaesthetized infected all the test plants of 5 passages. Four out of 52 insects failed to infect the test plants of the third passage when subjected to anaesthesia between the second and the third passage, although the four insects infected again the test plants during the fourth and fifth passages.

Since 80% of anaesthetized insects died when starved for 3 days and the survival of insects was not significantly affected by anaesthesia during serial transfer, it is concluded that CO_2-treatment can temporarily affect the ability of the *L. striatellus* to acquire and transmit MRDV, and that this is not due to fasting during the acquisition or inoculation access.

Maramorosch (1953) described the use of carbon dioxide as a harmless anaesthetic for handling insect vectors in virus transmission tests, and this technique has routinely been used for the planthopper *Laodelphax striatellus*, an important vector of plant viruses within the Reoviridae, the Rhabdoviridae and the tenuivirus group.

L. striatellus is a propagative vector of the phloem-limited maize rough dwarf virus (MRDV; Reoviridae) (Boccardo and Milne, 1984; Conti, 1984).

Caciagli and Casetta (1986) reported finding non-transmitter insects that were ELISA-positive for MRDV antigens, and they suggested that failure to transmit could have been caused by the effects of CO_2 anaesthesia on hopper feeding behaviour.

In this paper we describe the preliminary results of a study on the influence of CO_2 anaesthesia on the acquisition and serial transmission of MRDV.

Materials and Methods

A field isolate of MRDV was maintained in a mixed culture of barley and maize seedlings by successive transfers using *L. striatellus* reared in a glasshouse at 20–22 °C. MRDV-free hoppers were reared on MRDV-free barley plants (Milne et al., 1973).

For acquisition tests, male or female third instar nymphs, were anaesthetized with CO_2 (4 min in CO_2 atmosphere) before acquisition, and then allowed to feed for three days on infected maize and barley plants. Control insects were not anaesthetized before the acquisition feed. All hoppers were then transferred to healthy barley for two weeks, then were again transferred weekly to new healthy barley plants to prevent further access to the virus.

Tests to determine the proportion of hoppers that had acquired the virus were done 35 or more days after the start of the acquisition access period, by which time maximum infectivity has already been reached (Caciagli and Casetta, 1986). Insects to be tested were each transferred, without anaesthesia, in small cages to one barley seedling at the coleoptile stage and allowed to feed for three days.

For serial transfer transmission tests, the insects were allowed to acquire the virus without previous anaesthesia; they were handled as above and then transferred to new test plants every third day, until the death of the insect or for up to five passages. The insects were anaesthetized for 2 min or not for the transfer from the second to the third test plant.

At the end of the inoculation access period, the test plants were sprayed with insecticide and observed in the glasshouse for MRDV symptoms.

To test the survival of hoppers after a 3-day fast, two groups of 30 adult hoppers were anaesthetized or not as described, and then each group was kept for 3 days in a cage over a pot containing soil but no plants.

Results

Acquisition tests. The proportion of anaesthetized hoppers transmitting the virus was 25% (21 transmitters out of 84 insects tested) while 47.37% (54/114) of unanaesthetized hoppers transmitted the virus. The z test for the difference of the proportions (Fleiss, 1981) indicates that this difference is significant ($z = 3.0588$; $p < 0.01$).

Serial transfer transmission tests. Thirty-nine insects survived at least up to the fourth transfer (the second after the anaesthesia). Four insects did not transmit the virus in the transmission test immediately following the anaesthesia, although they transmitted again during subsequent tests. None of the 36 insects transferred without anaesthesia showed discontinuity in transmission.

Survival of hoppers. Only 6 of the 30 hoppers tested survived a 3-day fast after anaesthesia with CO_2, while 19 out of 30 survived in the unanaesthetized

group. The two proportions are significantly different in the z test (Fleiss 1981) ($z = 3.142$; $p < 0.01$).

The analysis of mortality of hoppers during the serial transmission tests did not reveal any significant differences between hoppers anaesthetized at the third transfer and unanaesthetized hoppers, when corresponding passages in the two treatments were compared.

Discussion

We have shown that anaesthesia with carbon dioxide influenced the ability of *L. striatellus* to acquire MRDV and transmit it to barley plants.

We know from previous work (Caciagli and Casetta, 1986) that infectivity test done 35 days after the start of acquisition and without anaesthesia before the transfer to test plants, is a suitable indicator of the efficiency of acquisition, since under these conditions all the hoppers that have acquired the virus (as judged by ELISA test) transmitted it to the test plants (Caciagli and Casetta, 1986). Whereas only 25% of anaesthetized insects acquired the virus from MRDV-infected barley and maize plants, more than 47% became infected when exposed to the virus without previous anaesthesia.

On the basis of acquisition tests, 8–9 hoppers would be expected to be non-transmitters after anaesthesia, but only 4 were. This could possibly be related to the longer exposure to CO_2 of hoppers anaesthetized before the acquisition access or to a stronger effect of CO_2 on third instar nymphs (the stage of insects at the moment of acquisition access) than on adults, which they had become at the time of the serial transfer.

The saltatory transmissions were not due to the fasting of hoppers as a consequence of anaesthesia, since the survival rate of anaesthetized insects to a 3-day fasting was low (20%), and there was no significant difference in survival in serial transmission tests, attributable to anaesthesia.

The CO_2 could possibly affect the precibarial chemosensilla, which guide the stylets to the vascular bundles (Backus, 1985), thus reducing the probability of acquisition and inoculation of MRDV.

The analysis of survival of hoppers during the serial transfers, although based on a small number of insects, confirms the conclusion of Maramorosch (1953) that CO_2 is a harmless tool for handling insects. However, in transmission tests with plant viruses, particular precautions should be taken, at least with MRDV, since the anaesthesia with CO_2 can cause saltatory transmission and reduction of percentage of acquisition.

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A Possible Barley Adapted Strain of Wheat Dwarf Virus (WDV)

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Two virus isolates, which may be a barley adapted strain of wheat dwarf virus (WDV), were found to easily infect barley but wheat *(Triticum aestivum)* and other Triticum spp. were not infected. On the other hand, two old wheat isolates of WDV had a high preference for wheat and rarely infected barley. *Poa annua, Lagurus ovatus* and *Avena sativa* seemed to be good hosts for all isolates tested. All WDV isolates were serologically closely related.

Wheat dwarf virus (WDV) is a leafhopper transmitted gemini-virus (Lindsten et al., 1980) which in the earlier part of this century caused very severe damage to wheat in Sweden (Lindsten et al., 1970). It was first reported as a virus from Czechoslovakia (Vacke, 1961) where it caused severe damage also recently, 1983–86.

WDV causes disease mainly on wheat but several gramineous plants including barley and oats are reported as hosts (Vacke, 1972). However, during the last years contradictory results were obtained concerning infection of barley. One Swedish isolate, kept in a glasshous since the end of the seventies, was found not to infect barley.

An exchange of isolates for comparison took place in 1985 and in 1987 also two WDV isolates from severely damaged winter barley in Bohemia, Czechoslovakia, were included.

Comparative studies of the isolates showed great differences in their ability to infect barley and wheat which are reported on here.

Materials and Methods

The Swedish WDM-isolate (WDV-S), originating from a wheat plant, which had been kept on wheat plants in a glasshouse since the end of the seventies was found to have more or less lost the ability to infect barley. In 1985 and exchange of this isolate with a Czech isolate from wheat (WDV-Ru) was made but also this was found to infect barley very poorly.

In 1987 we compared by using ELISA some Czech isolates from wheat and barley and also the Swedish isolate WDV-S. All the isolates tested were found to be serologically closely related. Two of the barley isolates, WDV-Er and WDV-Te, which were collected from severely damaged winter barley fields in Bohemia close to West Germany, were brought to Sweden in order to investigate if Swedish leafhoppers *(Psammotettix alienus)* possibly lacked ability to transmit to barley plants.

As test plants in Sweden were mainly seedlings of wheat (cv. Drabant) and barley (cv. Alva) used. The acquisition feeding period as well as the test feeding period were 2 days for each if not otherwise is mentioned. Both the hoppers and the test plants were kept isolated in insect-proof glasshouses or in a growing room with 16 hours of halogen lamp light and about 17 °C.

ELISA was carried out mainly according to Clark and Adams (1977). Plant sap was obtained by using a Pollähne press and 1 drop of plant sap diluted in 1 ml PBS-TPO buffer was used for each sample. Readings were taken in a Titertek Multiscan MC at 405 nm 30–60 minutes after addition of substrate. In general very clear ELISA readings were obtained for WDV-S with values around 0.01–0.05 for negative samples and >1–2 for positive samples.

Results

10 leafhoppers, 5 adults and 5 nymphs, were caged on each of the two Czech WDV-infected barley plants (WDV-Er and WDV-Te). In addition 10 hoppers were caged in a similar way on wheat plants infected with the Swedish isolate (WDV-S) and the Czech isolate WDV-Ru, respectively. After 2 days of acquisition feeding all the hoppers from each cage were transferred to pots with 3 test plants of wheat (cv. Drabant) and after 3 days of test feeding they were transferred for a second test feeding on barley plants (one plant of Alva, one of Borwina and one of Miraz). Further test feedings were then made to new test plants including also *Poa annua.*

As shown in Table 1 all three Drabant plants used for the isolates WDV-S and WDV-Ru, respectively, became infected (clear symptoms, ELISA readings >2) in the first test feeding. However, none of the wheat test plants for WDV-Er and WDV-Te showed any disease symptoms and the ELISA readings were very low (0.01–0.05). The results in the second test feeding when barley was used as test plant were quite the opposite. Similar results were obtained also in other test feedings of the same surviving hoppers as well as in new experiments with test feedings of individual hoppers. However, *P. annua*, was found to be a good host for all four virus isolates as shown by ELISA and in back-transmission experiments to wheat and barley, respectively, *P. annua* did not show any clear symptoms after infection and infected plants can therefore not be selected only by symptoms.

In Table 2 the results of back-transmission of the isolate WDV-Er from barley- and *P. annua* plants are shown.

In order to somewhat clarify the host range of barley isolates (cf. Vacke, 1972) an experiment with some possible hosts including 8 different Triticum spp.

Table 1

Results of test feeding with *Psammotettix alienus* after acquisition feeding on wheat dwarf virus (WDV) infected wheat and barley, respectively

	Acquisition feeding on wheat infected with isolate		Acquisition feeding on barley infected with isolate	
	WDV-S	WDV-Ru	WDV-Er	WDV-Te
1st test feeding on wheat (cv. Drabant)	1) 3/3	3/3	0/3	0/3
2nd test feeding on barley (cv. Alva, Borwina and Miraz)	0/3	0/3	3/3	3/3

[1] No of test plants infected/total no of test plants used

Table 2

ELISA results from testplants after back-transmission with *Psammotettix alienus* from WDV-Er infected plants

Acquisition from	Positive/total plants 1st testplant		Positive/total plants 2nd testplant	
Barley cv. Alva	barley Alva	4/10	wheat cv. Drabant	0/6
Barley cv. Alva	Drabant	0/10	Alva	5/8
Poa annua 1	Alva	6/10	Drabant	0/7
Poa annua 1	Drabant	0/10	Alva	6/8
Poa annua 2	Alva	5/10	Drabant	0/6
Poa annua 2	Drabant	0/10	Alva	8/9

was carried out (Table 3). In this experiment both the acquisition feeding and the test feeding period were increased each to 6 days. Especially *Avena sativa* (cv. Diadem) and *Lagurus ovatus* seemed to be good hosts for both isolates (WDV-Ru and WDV-Te) and infected plants showed typical symptoms. On the other hand, the results in Table 3 indicate that not only *T. aestivum* but also other Triticum spp. are non-hosts for barley isolates of WDV even when the infection rate for wheat isolates are very high.

Table 4 shows the transmission results to barley and wheat of WDV-isolates collected in some different localities of Czechslovakia. All isolates from the barley fields could easily be transmitted to test plants of barley but not to wheat plants. Isolates collected in wheat fields, on the other hand, were easily transmitted to wheat but not at all or only occasionally to barley.

Table 3

Results of inoculation of plant species with the isolates WDV-Ru and WDV-Te

Plant species	Plants infected / tested	
	Isolate WDV-Ru	Isolate WDV-Te
Avena fatua	2/10	5/10
A. sativa	9/10	9/10
A sterilis	1/10	1/10
A. strigosa	5/10	0/10
Lagurus ovatus	6/10	9/10
Lolium multiflorum	3/10	0/10
L. perenne	0/10	0/9
Secale cereale	4/10	0/10
Triticum dicoccoides	9/10	0/10
Tr. dicoccon	10/10	0/10
Tr. durum	10/10	0/10
Tr. durum	10/10	0/10
Tr. monococcum	10/10	0/10
Tr. polonicum	10/10	0/10
Tr. spelta	10/10	0/10
Tr. timopheevii	9/10	0/10
Tr. turgidum	10/10	0/10

Table 4

Transmission of different isolates of WDV to some test plants

Locality	Source of infection	Transmission to		
		winter barley cv. Borwina	spring barley cv. Koral	spring wheat cv. Jara
Erpuzice	winter barley	16/19	18/20	0/20
Kostelec	winter barley	20/20	17/20	0/20
Malovice	winter barley	18/20	14/20	0/19
Techlovice	winter barley	16/17	15/19	0/20
Tisova	winter barley	14/20	19/19	0/20
Erpuzice	winter wheat	1/20	0/20	17/18
Ksice	winter wheat	0/20	0/20	20/20

Discussion

Two different groups of WDV isolates concerning the ability to infect wheat and barley, respectively, were found. The possible barley adapted group seem to have completely lost the ability to infect *Triticum aestivum* and also other Triticum spp. seem to be non-hosts (Table 3). This is in contrast to what has been recently reported for a similar virus, maize streak mosaic virus (MSV), as all MSV isolates tested seem to be able to infect maize (Pinner et al., 1988).

The wheat isolates WDV-S and WDV-Ru, on the other side, showed a high preference to wheat and infected barley not at all or only occasionally. However, both these isolates have been kept in glasshouses for many years and may have underwent changes. Maybe also choice of barley cultivar will influence the infection rate. Thus experiments in progress indicate that the barley cv. Igri can be infected not only with WDV-Er but also with WDV-S according to the ELISA readings. However, no symptoms show up for the WDV-S isolate, in contrast to infection with WDV-Er which causes dwarfing and yellowing, and back-transmission with hoppers to test plants have, so far, been negative.

All WDV isolates tested with ELISA react strongly against an antiserum to WDV-S and all seem to be closely serological related. Also symptoms on host plants in common including *Avena sativa* and *Lagurus ovatus* are very similar and no differences in transmissibility have been noticed. This applies also to *P. annua* but *P. annua* is usually symptomless. Usually 2 days of acqusition feeding and 2 days of test feeding are enough for a high transmission rate but may sometimes be too short (cf. Tables 2 and 3).

The genomes of WDV-S and WDV-Ru have been sequenced and compared recently (Macdowell et al., 1985, Maatzeit, 1987 and Woolston et al., 1988). They have 2749 and 2750 nucleotides, respectively, and there are in all only 47 base changes between the isolates. WDV-Er, on the other hand, is more different as expected and there are about 200 base changes compared to WDV-Ru, the closest related isolate (B. Groonenborn, pers. comm.).

According to experiments in progress one and the same hopper specimen can pick up both virus variants (WDV-S and WDV-Er) and become infective to both barley and wheat in serial transmissions. In fact so far no evidence of cross-protection in the vector is found. Maybe mixed infections and/or recombinations of the virus can take place both in the vector and in certain host plants. In any case the findings reported on here may give help to explain the somewhat contradictory results obtained earlier concerning the host range of WDV.

The high specificity of the WDV isolates to barley and wheat, respectively, might be worth-wile considering also in breeding programmes and in the control of the disease. Maybe the disease in barley can easily be controlled by just substituting barley with wheat and vice versa.

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Wheat Dwarf Virus in Hungary

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Winter wheat plants showing symptoms of dwarfing, leaf mottling and yellowing were collected at Martonvásár (Central Hungary). Adults and nymphs of the leafhopper *Psammotettix alienus* Dahlb. were able to transmit the pathogen from diseased plants to young wheat seedlings with high frequency.

A combined method of extraction in liquid nitrogen and using ascorbic acid and 2-mercapto ethanol in the extraction buffer at pH 4.0 led to the best results. Electron microscopy of the purified preparation showed typical geminivirus particles, about 20× 30 nm in size.

Agarose gel electrophoresis of nucleic acid demonstrated the presence of a single stranded DNA (resistant to RNAse but sensitive to DNAses).

Results indicate that the causal agent of the disease is wheat dwarf virus.

A number of plant diseases suspected of being caused by viruses have been studied in Hungary. Nevertheless, the classification of the pathogens has proved difficult, especially because of the lack of mechanical transmissibility. One of these diseases shows characteristic severe dwarfing and yellowing of winter wheat plants commonly occurring in wheat growing areas of Hungary.

All attempts made to transmit the pathogen by mechanical inoculation have been unsuccessful. Last year the disease was successfully transmitted using the leafhopper *Psammotettix alienus*. Purification from plant material infected by insect transmission has led us to identify the pathogen as wheat dwarf virus.

Material and Methods

Intact winter wheat *(Triticum aestivum)* plants with symptoms of dwarfing, leaf mottling and yellowing, that were naturally infected early in the season were collected in the fields and transplanted to a greenhouse. Adults and nymphs from virus-free cultures of the leafhopper vector, *Psammotettix alienus* Dahlb. were used in transmission tests. After insect inoculation the experimental plan were kept in the greenhouse until the symptoms appeared. Wheat leaves from insect transmission experiments were stored frozen until used for virus purification.

A combined method of Lindsten et al. (1980) and Bock et al. (1974) was used to purify the pathogen. Before extraction 200 g of sample were frozen at −70 °C. Leaves were ground in liquid nitrogen and extracted with two parts (v/w) of 0.01 M sodium phosphate buffer (pH 4.0) containing 0.325 M ascorbic acid, 0.2% 2-mercapto-ethanol, 0.01 M EDTA and 0.1% thioglycollic acid. The homogenate was expressed through cheesecloth and after stirring with 7% v/v chloroform the crude juice was centrifuged at low speed (2.500 g, 20 min.). Virus particles were precipitated with 10% polyethylene glycoll (6000) and 0.2 M NaCl. After low speed centrifugation the pellet was suspended in extraction buffer overnight. After repeating the precipitation procedure the final pellet was resolved in 0.01 M phosphate buffer (pH 4.0) and diluted with extraction buffer up 50 ml. After clarification by low speed centrifugation (10.000 g; 15 min.) the supernatant was ultracentrifuged at 40.000 rpm for 5 hrs (Beckman Ti 50 rotor). Pellets were suspended in small amount of 0.01 M phosphate buffer (pH 4.0) and after low speed centrifugation (10.000 rpm, 10 min) the supernatant was layered onto a 10–40% sucrose gradient (solved in 0.01 M phosphate buffer, pH 4.0) and centrifuged for 16 hrs at 18.000 rpm in a SW 28 rotor in a Beckman LBM ultracentrifuge. A narrow opalescent virus layer was collected using an ISCO gradient collector. The purified virus was stored at −20 °C.

Electron microscopy was used for detection of virions in sucrose gradient fractions. All preparations were mounted on carbon-coated Formvar membranes, negatively stained in 2% uranyl acetate and examined with a Jeol JEM-100 C electron microscope.

Nucleic acids were extracted from the purified virus suspension by treatment with chloroform and phenol according to the protocol of Maniatis et al. (1982). Nucleic acids were run in a horizontal 1% agarose mini gel at 5 V/cm and stained with ethidium bromide (0.5 μg/ml). Gels were photographed using transmitted UV light (LKB transilluminator). Individual samples were treated with S_1 nuclease, DNAse and RNAse according to Maniatis et al. (1982).

Samples of purified virus were mixed with equal amount of sample buffer (Weber and Osborn, 1969) and denaturated in boiling water for 3 minutes. Ten μl samples were run in a vertical mini slab gel, containing 10% polyacrylamide according to Laemmli (1970). Gels were stained with Coomassie G-250. Coat proteins of purified brome mosaic virus, poa semilatent virus and barley stripe mosaic virus were used as molecular weight standards.

Results

Results from the study of leafhopper transmission of wheat dwarf agent suggests that the causal agent is the wheat dwarf virus. Both adults and nymphs of *Psammotettix alienus* readily transmitted the pathogen to spring wheat plants very easy. Proportion of infected test plants fluctuated between 56 and 100% in different transmission tests. Symptoms caused by the pathogen in wheat were

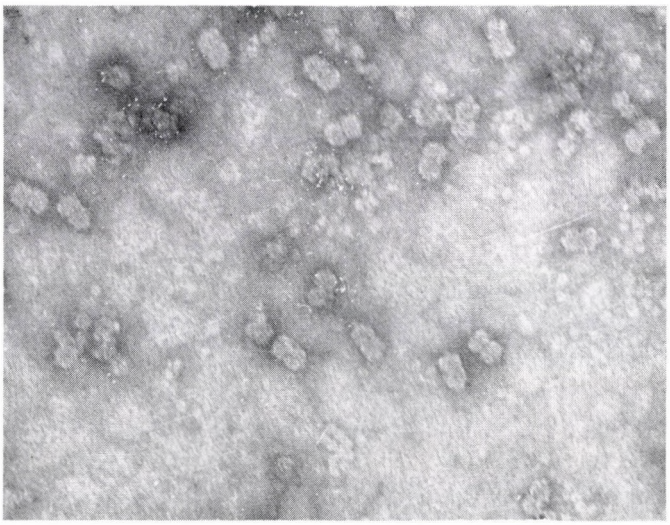

Fig. 1. Electron micrograph of purified WDV

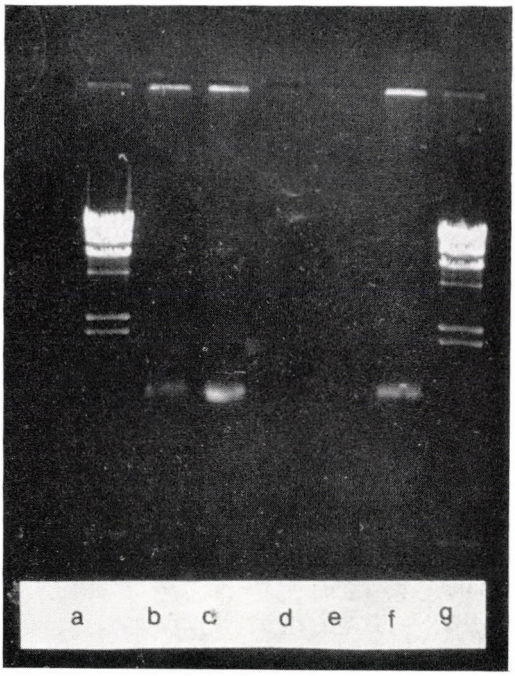

Fig. 2. Agarose gel electrophoresis of wheat dwarf virus nucleic acid (a, g phage, Hind III digested DNA marker; b, f. WDV DNA; c, WDV + RNAse; d, WDV + S_1 nuclease; e, WDV + DNAse)

similar to those ones induced by the type strain of wheat dwarf virus -Ru which was tested paralelly. Incubation period in plants lasted 10–14 days.

The purification protocol described in the Material and methods section resulted in a sharp band in the sucrose gradient and a higher virus concentration (about 2.5 mg/ml), without significant contamination.

Presence of quasi-isometric geminate particles of about 20×30 nms in the opalescent band of the sucrose gradient centrifugation supplied direct evidence that the pathogen should be classified as a geminivirus (Fig. 1).

Nucleic acids extracted from purified virus were run in agarose gels. Only one sharp band was observed in purified preparation (Fig. 2b, c, f) which was absent in the pattern of nucleic acids from control plants. This fast migrating band was a single stranded DNA, had a molecular weight approximately 7×10^5 Kd. and was resistant to RNAse (Fig. 2f) but sensitive to DNAse treatments.

SDS-polyacrylamide electrophoresis of viral protein showed only one band of approximately 28 Kd. which corresponds to the coat protein of wheat dwarf virus (Macdowell et al., 1985, Lazarowitz, 1987).

Discussion

The virus disease called "wheat dwarf" was first described in Czechoslovakia in 1961 (Vacke, 1961). Later on was also found in USSR (Pridanceva, 1965), Sweden (Lindsten et al., 1970) and in Bulgaria (Stephanov and Dimov, 1981) and now in Hungary. It is characterized by severe dwarfing of infected plants, mottling and yellowing of the leaves and suppressed heading.

The leafhopper-borne wheat dwarf virus is a possible member of geminiviruses (Lindsten et al., 1980). Based on insect transmission and host range this group can be divided into three subgroups (Stanley, 1985).

Among the leafhopper transmitted geminiviruses infecting monocotyledonous plants, wheat dwarf virus is distinct from other members such as chloris striate mosaic virus and maize streak virus because its only vector *Psammotettix alienus*.

Host range studies of Lindsten et al., 1970, Vacke, 1972 indicates the possibility of transmission of the pathogen to perennial weeds as well as overwintering of the pathogen in Hungary. The vector *Psammotettix alienus* is a very common in wheat and barley fields as well as in ruderal places. It has five developmental phases and two or three generations. First damage caused by these insects in wheat fields can be observed in April and May (Sáringer, 1988). Because of the abundance and permanent presence of the vector all posibilities are given to the spread of the wheat dwarf virus in our fields.

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Characterization of Barley Stripe Mosaic Virus Hungarian Strain

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Serologically the Hungarian isolate (BSMV-H) proved to be similar to the other BSMV strains and differed from PSLV.

Virions of BSMV-H contained three large genomic RNAs and an additional, probably subgenomic RNA. The large RNAs of BSMV-H was highly homologous to the RNAs of BSMV Argentina Mild strain for they hybridized to the cDNA probe prepared from RNAs of BSMV-Am. The small, encapsidated RNA (0.27×10^6 d) of BSMV-H hybridized to the full-length clone of RNA γ of BSMV-ND18 and it is thought to be subgenomic RNA. According to its properties BSMV-H is considered to be a new strain of BSMV.

Barley stripe mosaic virus (BSMV) is the type member of hordeivirus group. In many respects BSMV is a unique plant virus that has two to four large, electrophoretically different RNAs and rod-shaped virions. According to the recent results BSMV is thought to have tripartite genome (Jackson et al., 1983). There is a large number of BSMV strains distinguished on the basis of biological properties, like pathogenicity, symptomatology, seed transmissibility, etc. These strains are serologically very similar to one another and their nucleic acids seem to be highly homologous (Jackson and Lane, 1981). In the present study the Hungarian isolate of BSMV is described in details in respect of its physico-chemical properties.

Materials and Methods

Virus purification: Hungarian (H) isolate of BSMV was collected from spring barley in the field. Authentic strains were used for comparison. BSMV-H isolate and the standard strains were propagated on barley (*Hordeum vulgare* cv. GK Omega). All of the virus strains and the H isolate were purified from systemically infected leaves two or three weeks after inoculation following the method of Lane (1974). After the first high-speed centrifugation samples were layered on linear 10–40% sucrose gradients buffered with 1×TE (10 mM Tris, 1 mM EDTA pH 7.2). Gradients were centrifuged at 21 000 rpm for 105 min at 5 °C in a Beck-

man SW27 rotor. The virion zones were pelleted by high-speed centrifugation and resuspended in 1 × TE.

Electron microscopy: Samples of BSMV-H infected leaves or purified virions were attached to copper grids coated with formvar for 1 min and, after wash, stained with 2% uranyl acetate for 1 min. Preparations were examined in a JEOL 100XL electron microscope.

Serology: Antisera against Ru strain of BSMV and PSLV were produced in New Zealand White rabbits as described by Hunter et al. (1986). IgG fraction of antisera was prepared by the use of the method of Steinbuch and Audran (1969).

The serological relationship among H and Br isolates as well as other BSMV strains (Ru and AmIII) and PSLV was examined with two assays. The first assay was done with rocket immunoelectrophoresis (Gáborjányi and Tóbiás, 1984). Rocket immunoelectrophoresis was also used to measure virus concentration in infected plants. The second assay was the dot-immunobinding method. Indirect dot-immunobinding procedure (indirect DIA) of Powell (1987) was carried out.

Virion ssRNA isolation: RNA from purified virions was isolated by the use of the SDS-phenol-chloroform method (Schwinghamer and Symons, 1977). After ethanol precipitation, RNAs were resuspended in TE buffer and analysed in 1.5% agarose gel buffered with TBE (90 mM Tris, 90 mM boric acid, 10 mM EDTA, pH 8.3). Different RNA bands were visualized after ethidium bromide staining and photographed.

Hybridization of RNA: RNA extracts were denatured with formaldehyde and formamide, electrophoreses in 1.5% agarose gels in MAE buffer (0.04 M MOPS, pH 7.0 and 10 mM sodium acetate, 1 mM EDTA) containing 2.2 M formaldehyde (Maniatis et al., 1982) and blotted to nitrocellulose filters. Prehybridization and hybridization were done according to Wahl et al. (1979). Blots were washed four times, 10 min each, at 65 °C with 2 × SSC (0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) containing 0.1% SDS and autoradiographed. ^{32}P labelled cDNA to virion ssRNA of AmIII was prepared by random priming with sonicated salmon sperm DNA according to Taylor et al. (1976). Non-incorporated nucleotides were removed by ethanol precipitation.

RNA blots were also hybridized with biotin labelled full-length clone of RNA γ of BSMV-ND18 strain (kind gift of A..O. Jackson, Berkeley, California). Cloned DNA was biotinilated by nick translation following the procedure of the manufacturer (BRL-Gibco). Prehybridization, hybridization, filter washing and detection were done according to the procedure recommended by the manufacturer (BRL-Gibco).

Results

Hungarian isolate of BSMV was readily transmitted by mechanical inoculation. BSMV-H caused mild systemic mosaic symptoms and little yellow spots on barley and was symptomless on wheat plants.

Electron microscopy: Crude plant sap from plants infected with H isolate (Fig. 1) contained short rigid rods, similar in size to those measured by other authors

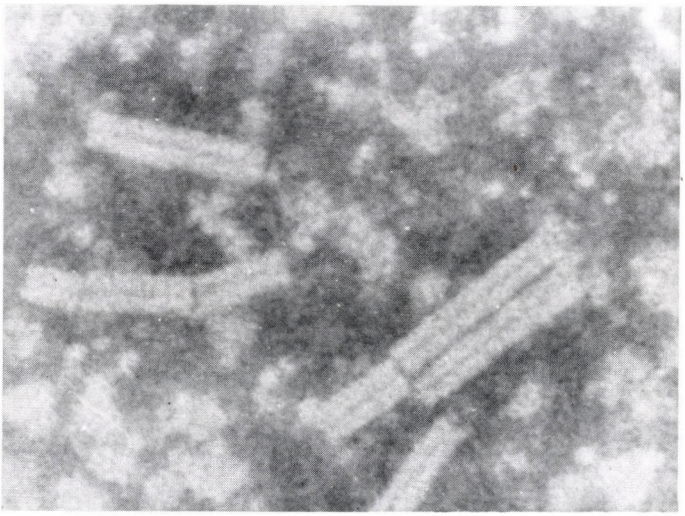

Fig. 1. Electron micrograph of purified virions of BSMV

(Brakke and Palomar, 1976; Hunter et al., 1986). Because of the end-to-end aggregation of virion particles, particle lengths could not be accurately measured.

Serology: Samples containing BSMV-H reacted positively (formed peak in the gel) only with antiBSMV IgG but gave aspecific reaction with antiPSLV IgG in rocket immunoelectrophoresis (data not shown). Rocket immunoelectrophoresis was found to be a valuable method to make a distinction between BSMV and PSLV.

The dot-immunobinding assay also proved that BSMV-H was serologically identical to other BSMV strains and differed from PSLV (data not shown). Our results with BSMV and PSLV are consistent with those obtained by Hunter et al. (1986).

Analysis of virion ssRNAs: Electrophoresis of extracted RNAs of H isolate was revealed four bands in agarose gel (data not shown). The three larges RNAs were consistent with the genomic RNAs of standard BSMV strains, and differed in electrophoretical mobility from the genomic RNAs of PSLV. The MW of

small RNA was about 0.27×10^6 d, similar to the 800 nucleotide-long subgenomic RNA reported by Jackson et al. (1983a) and Dolja et al. (1983). Hybridization studies with ^{32}P labelled cDNA copied from genomic RNAs of AmIII strain also showed the relationship of H isolate with other BSMV strains (data not shown). PSLV RNAs failed to hybridaze to the probe. The small RNA of BSMV-H is of viral origin.

The cloned RNA γ of BSMV-ND18 strain hybridized to the RNA γ and to the small RNA of BSMV-H (Fig. 2).

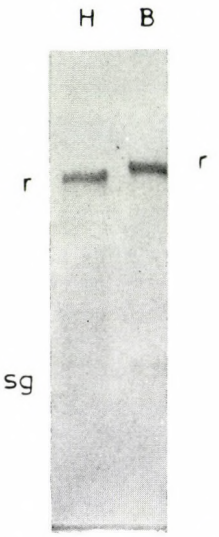

Fig. 2. Northern blot analysis of isolated virion RNAs of two strains of BSMV with biotin labelled RNA-i probe H = the Hungarian, B = the Braunschweig strains of BSMV

Discussion

Although BSMV can be well distinguished from the closely related PSLV and from other, not related plant viruses (Hunter et al., 1986), it is difficult to find great differences among BSMV strains on the basis of physico-chemical properties. One of the intriguing exception is the number of virion RNAs resolved with electrophoresis (Jackson and Brakke, 1973, Lane, 1974). Two, three and four component BSMV strains were described (Jackson and Lane, 1981). However, it is known that all BSMV strains are tripartite (reviewed by Atabekov and Dolja, 1986).

In connection with these properties, we were interested whether BSMV-H isolate could be distinguished from other BSMV strains.

The rod-shaped virions of H isolate are very similar to those of other BSMV strains or of PSLV.

In dot-immunobinding assay and rocket immunoelectrophoresis BSMV-H proved to differ from PSLV but not from BSMV strains.

On the basis of ssRNA/cDNA hybridization studies BSMV-H seems to belong to the orthotripartite BSMV strains (for the standard BSMV strains, see Jackson et al., 1983b). The hybridization results with cloned RNA γ also support this idea. Our results also proved the high sequence homology among BSMV strains (reviewed by Atabekov and Dolja, 1986). It is interesting to note that virions of BSMV-H contained a relatively large amount of a small RNA. As a similar subgenomic RNA was found studying other BSMV strains (Jackson et al., 1983a, Dolja et al., 1983) and as it was homologous to RNA γ of BSMV, this small RNA was thought to be sobgenomic one. Detection of this subgenomic-like RNA with ethidium bromide staining was not reliable, but frequent in the sample of the isolated virion RNAs of H isolate.

BSMV-H could be regarded as a mild isolate of BSMV. Surprisingly, the concentration of H isolate in infected barley and wheat plants was three to four times higher than that of other strains (data not shown).

According to our results BSMV-H isolate could be regarded as a new strain of BSMV. However, the characterization of this new strain also shows that in spite of high similarity in respect of nucleic acid sequences and of antigenicity of virions, yet there are great differences among BSMV strains in biological properties. Only a few bases in the genome are likely to have great importance in determining the biological properties (differencies) of BSMV strains.

Acknowledgement

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Evidence for Cross-Protection Between Strains of Barley Stripe Mosaic Virus

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Bilateral cross-protection studies between barley stripe mosaic virus (BSMV) B and H strains showed that the coat protein of the challenge strain could not be detected in protected plants. The severe symptoms of the B challenge strain did not appear in plants infected with BSMV-H. The cross-protection between BSMV-B and -H strains is reciprocal and complete.

Neither the symptoms, nor the coat protein of the W challenge strain were found in plants infected with the B strain. On contrary, a small amount of the coat protein of B challenge strain was detected in plants infected with the W strain 18 day after challenge inoculation. However, the symptoms of the challenge B strain never appeared. The W strain prevented the symptom development, but not the replication of the B challenge strain.

Cross-protection is the phenomenon whereby the activity of a virus in a plant prevents the expression of a subsequent releated virus (Hamilton, 1980, Sherwood, 1987). Although the specific mechanism of protection is unknown, cross-protection has been used successfully to control some viral diseases (Fulton, 1986).

Recently transgenic tobacco and tomato plants were generated that express the coat protein coding sequence of tobacco mosaic virus and alfalfa mosaic virus (Powell Abel et al., 1986; Loesch-Fries et al., 1987; Tumer et al., 1987). These transgenic plants were protected against an infection by the corresponding virus. The protection has some of the characteristics of cross-protection (Beachy et al., 1987). Thus, there is again an increasing interest for virologists to learn both the mechanism of cross-protection and the inducing molecule(s).

Barley stripe mosaic virus (BSMV) is the type member of hordeiviruses. BSMV has three positive strand RNA segments encapsidated into a rod-shaped virions (Atabekov and Dolja, 1986). It infects most of the monocots and some dicots, too. In nature, BSMV spreads mainly by seed transmission (Carroll, 1986).

In our recent paper, evidence of cross-protection between strains of barley stripe mosaic virus (BSMV) is presented.

Materials and Methods

Virus strains and inoculations

The Hungarian (H) strain of BSMV was collected from spring barley in the field and characterized (Nagy and Gáborjányi in press). BSMV-B was the kind gift from W. Huth (Braunschweig, F.R.G.). The Welsh (W) strain was kindly supplied by P. Catherall (Aberyswith, U. K.). Barley *(Hordeum vulgare* L. cv. GK. Omega) and wheat *(Triticum aestivum* L. cv. Martonvásári 8) plants were grown in a greenhouse. Plants in the one leaf stage were inoculated with the inducing virus strain. Ten days later, the second leaf was challenge inoculated. We used the following pairs of inducing and challenge strains in the experiments (the first letter shows the inducing strain and the second one is the challenge strain): 1. B–H; 2. H–B; 3. B–W; 4. W–B.

Control plants were inoculated only once with all of three strains, separately. The inoculations were carried out either on the day of the first or that of the challenge inoculation.

Plant saps of infected leaves diluted two fold with phophate buffer (0.1 M, pH 7.0) were used for inoculum.

Samples for virus purification were collected either 10 or 18 days post challenge inoculation (dpci).

Virus purification

BSMV was purified from 10–15 g of leaves following the method of Lane (1974). Virus pellets of the first high-speed centrifugation were resuspended in 1 ml of TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.2). After low-speed centrifugation of these virus suspensions, samples contained only a small amount of contaminants and were suitable for serological studies.

Serology

Purified virus preparations were treated with dissociation buffer at 100 °C for 2 min and loaded (0.5 μg/well) on a 12.5% SDS polyacrilamide gel (Laemmli, 1970). After electrophoresis, the transfer of proteins to the membrane was done according to Towbin et al. (1979). For immunological detection of viral coat protein, electro-blot immunoassay (EBIA) was employed (Burgermeister and Koenig, 1984) using the Bio-Rad GAR-HRP conjugate and color development reagent. Purified antiBSMV IgG was prepared as described earlier (Gáborjányi and Tóbiás, 1984).

Results

Three BSMV strains were chosen for our cross-protection studies. BSMV-B strain is different in some properties from the H and the W strain. BSMV-B causes much more severe symptoms on plants than the other two strains. The coat protein of BSMV-B is faster (23.5 kD) during SDS polyacrylamide gel electrophoresis than that of BSMV-H and W (25 kD) (Fig. 1). On the basis of these

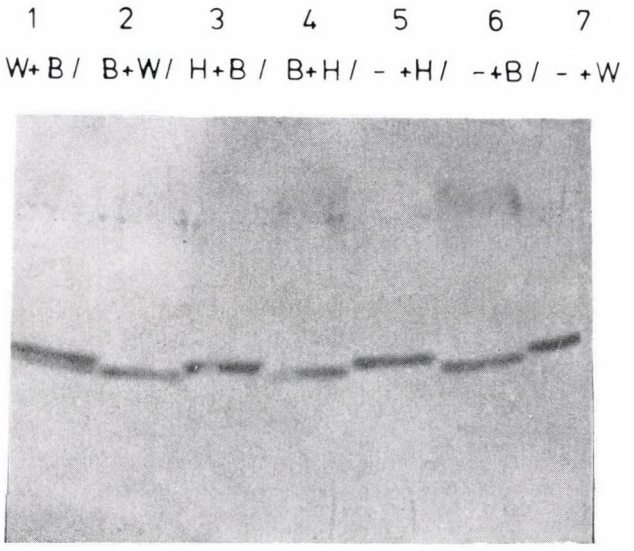

Fig. 1. Electro-blot immunoassay of virions of BSMV purified from doubly inoculated plants. Each well contains 0.5 μg of purified virions The first letter indicates the inducing strain, the second one (after + sign) shows the challenge strain. The last samples (5–7) are from the control experiments. Control plants were inoculated only with one strain at the time of the challenge inoculation

differences, it was possible to analyse the composition of the progeny virus in doubly inoculated plants.

Bilateral cross-protection studies between BSMV-B and -H showed that the coat protein of the challenge strain could not be detected in protected plants (Fig. 1). The severe symptoms of the B challenge strain did not appear in BSMV-H infected plants. These data demonstrate that there is reciprocal cross-protection between BSMV-B and -H strains.

Similarly, neither the symptoms, nor the coat protein of the challenge BSMV-W were found in plants infected with the B strain. On contrary, a small amount of the coat protein of B challenge strain was detected in plants infected with the W strain 18 dpci (Fig. 1). However, the symptoms of the challenge B

strain never appeared. The W strain prevented the symptom development, but not the replication of the B challenge strain.

The host plants (barley and wheat) did not influence the cross-protection phenomenon in our BSMV system.

Discussion

McKinney and Greeley (1965) found cross-protection between BSMV strains. However, they studied only the symptoms induced by the challenge strain. We reexamined their early results with the use of a sensitive serological method (EBIA) to learn whether there is inhibition of symptom development of the challenge strain with or without significant inhibition of replication of the challenge strain. The infection of plants with either BSMV-B or -H strains not only inhibited the symptom development of the challenge strain (BSMV-B, H and W, separately) but prevented its replication (coat protein production) as well. On contrary, the inhibition caused by the W strain on the coat protein production of BSMV-B challenge strain was not complete but considerable.

The cross-protection phenomena between BSMV-B and -H is similar to that found by Dodds et al. (1985) with cucumber mosaic virus strains. In that study, neither virions, nor dsRNA of the challenge strain were detected in protected plants. On the other hand, Cassels and Herrick (1977) as well as Burgyán and Gáborjányi (1984) demonstrated that the challenge strain of tobacco mosaic virus did accumulate in protected plants, as BSMV-B challenge strain accumulated in W strain infected plants in our study. However, they also found that the concentration of the challenge strain was higher (or at least equal) than that of the inducing strain. The symptoms of the challenge strain developed in young leaves. On the contrary, BSMV-B challenge strain accumulated only to a low extent and its symptoms never appeared in plants infected with the W strain.

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Ryegrass Mosaic and Ryegrass Cryptic Virus in Australia

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The *Gramineae* in Australia is comprised of some 1400 species representing 222 genera. The majority of these have never been surveyed for viruses. More than 50 viruses have been reported infecting the worlds cereals and grasses however less than 20 are reported infecting the *Gramineae* in Australia. Most of the economically important *Gramineae* in Australia are introductions from the northern hemisphere and it is probable that the introduction of other described *Gramineae* viruses into Australia, could have serious economic consequences.

Ryegrasses are important components of improved pasture throughout much of South-east Australia. On most farms Italian, short rotation and/or perennial ryegrasses, in combination with a legume species, are the predominant grasses sown to pasture. In November 1985 Tama ryegrass plants showing mosaic symptoms with necrotic flecking were collected from near Forth in northern Tasmania.

This paper reports on the identification of the two viruses associated with these symptoms as ryegrass mosaic and ryegrass cryptic viruses, and on a preliminary survey to establish virus incidence.

Materials and Methods

Immune serum electron microscopy (ISEM)

ISEM tests followed the method of Plumb and Lennon (1981) using coating antisera dilutions (1/200, 1/1000) and decorating dilution of 1/200 to the following viruses: Brome mosaic J, BMV-R, cocksfoot mild mottle, cynosurus mottle, maize dwarf mosaic-A, phleum mottle, rice necrosis mosaic, ryegrass mosaic and ryegrass cryptic, turnip yellow mosaic-RR, TYMV-LA, wheat streak mosaic. Double diffusion tests were done in 0.6% agar gels (150 m NaCl, 1.5 mM Na Citrate $\pm$ 5 mM Na_2EDTA) using sap and partially purified extracts.

Thin Sections

Leaf pieces were fixed in 5% (v/v) gluteraldehyde in 0.1 M phosphate buffer (pH 7) for 3 h at 20 °C (30 min in vacuo). The tissue was washed in three changes of buffer for 18 hr and then post fixed in 2% OsO_4 in 0.1 M phosphate buffer for 1 hr at 20 °C. The tissue was then dehydrated and embedded in Spurrs resin.

Ultrathin sections were stained for 15 min in saturated uranyl acetate in 40% (v/v) ethanol, followed by staining for 5 min in 0.05% (w/v) lead citrate in 0.02 M NaOH. Sections were examined in an Hitachi H-6005 electron microscope at 75 kV.

Mechanical Transmission Tests

Leaves from Tama ryegrass plants showing mosaic symptoms were ground in a mortar with two to five volumes of distilled water or acetate, borate, HEPES, phosphate, saline and Tris buffers ranging in molarity 20–100 mM and pH 6–8. PVP, cysteine, tween-20, triton X-100 or BSA were added to some of the buffers for some of the transmission tests.

Extracts of infected plants were mechanically inoculated to carborundum dusted plants of the following species: *Avena sativa* cvs; *A. strigosa* cv SAIA; *Dactylis glomerata* cv Porto; *Echinochloa frumentacea; Festuca arundinacea* cv Demeter; *Datura stramonium*, *Hordeum vulgare* cvs Proctos, Shannon, *Lolium multiflorum* cv S22, Tama, *L. multiflorum* × *perenne* cvs Ariki, New Zealand Manawa, *L. perenne* cv Victorian, *Nicotiana glutinosa*, *Oryza sativa*, *Orzopsis holciformis*, *Panicum* spp, *Setaria sphacelata*, *Sorghum bicolor* cvs Cargill CS150, Cargill Gunsynd, Pioneer pride, *Triticum aestivum* cv Bindawarra, X *Triticosecale* cv Coorong and *Zea mays* cvs Iochief, Supergold.

Aphid Transmission Tests

The aphid species *Metopolophium dirhodum*, *Myzus persicae Rhopalosiphum maidis*, *R. padi* and *Sitobion fragariae* were used in attempts to transmit the two viruses. Leaves from infected ryegrass plants were infested with each aphid species for 3 or 48 hr acquisition feeds. The aphids were then transferred (5–10 per plant) to the above species for 3 or 48 hr transmission feeds. The plants were then treated with insecticide and returned to an insect free glasshouse.

Survey of Tasmanian Ryegrass

Ryegrass tillers were collected at random from pastures and orchards in major agricultural regions of Tasmania. Each tiller was ground in a mortar and inoculated to three S22 ryegrass plants. Three weeks later the inoculated plants were checked for symptom development and a subsample was used to prepare leaf dips and examined in the electron microscope.

Seed transmission

Seed was collected from short rotation ryegrass infected with both viruses. This seed and seed from lines of *L. multiflorum*, *L. multiflorum* × *perenne* and *L. perenne* was sown in the glasshouse. Seedlings were removed from the pots at the 3–5 leaf stage and ground in 60 mM phosphate for use in ISEM tests.

Results

Grids coated with the serum (dilution: 1/1000) containing antibodies to ryegrass mosaic and ryegrass cryptic viruses trapped large numbers of rod-shaped and isometric virions from sap extracts of infected plants. The virions on these grids became heavily decorated with antibodies when further incubated with the serum (dilution: 1/200). Small numbers of virions were observed on uncoated grids and grids coated with the other antisera. No decoration was observed when these sera were incubated with virions captured by ryegrass mosaic and ryegrass cryptic virus antibodies. No precipitin bands formed in any of the double diffusion tests using sap and partially purified extracts. Therefore ISEM was used as the sole method for the serological detection of the two viruses.

Hereafter the rod shaped virus (730 nm) and the isometric virus (30 nm) are referred to as ryegrass mosaic (RyMV) and ryegrass cryptic viruses (RCV) respectively.

Pinwheel inclusions and bundles of virions were observed in the cytoplasm of Tama ryegrass mesophyll cells infected with RyMV. RyMV virions were not associated with particular organelles however RCV virions were associated with the nucleii of infected cells. RCV virions inside the nucleus were penetrated by stain and appeared empty whereas large aggregates of RCV virions in the cytoplasm adjacent to the nucleus were not penetrated by stain.

RyMV was mechanically transmitted to species from two subfamilies of the *Poaceae*. The virus was not transmitted by any of the aphid species. The use of buffers and additives did not improve the infectivity of leaf extracts. Symptoms appeared in the susceptible species 4–6 weeks after inoculation. Infected ryegrass plants *(L. multiflorum, L. multiflorum* × *perenne, L. perenne)* showed varying degrees of patchy chlorosis and sometimes necrosis and leaf distortion. Infected *A. sativa* cvs Blackbutt and Cooba showed strong mosaic symptoms and *Zea mays* cv Iochief developed occasional chlorotic streaks in systemically infected leaves.

RCV was not transmitted by any of the aphid species nor by mechanical inoculation of sap. The presence of the isometric virus in seedlings grown from, seed collected from short rotation ryegrass infected with both viruses, and from seedlines of *L. multiflorum* cvs S22, Tama *L. multiflorum* × *perenne* cvs Ariki, New Zealand Manawa and *L. perenne* cv Victorian was confirmed using ISEM. None of these plants showed any symptoms of virus infection.

RyMV was detected in ryegrass tillers from 7 of the 11 Tasmanian collection sites; 18% of the tillers (19/106) were infected. The virus has also been found infecting ryegrass at Leongatha in Victoria. RyMV was present in perennial, hybrid and Italian ryegrass. Field infected Tama plants only developed conspicuous symptoms during late spring (October-November). There were large variation in the effects of RyMV on ryegrass breeding lines. The majority of lines showed symptoms of similar severity to S22 and Tama ryegrasses. RyMV was latent in one line and caused very severe symptoms and was lethal to about 10% of seedlings from another line. RCV was present in all lines tested except for the one which supported a latent infection of RyMV.

Discussion

RyMV is widespread in Tasmania and probably occurs throughout temperate Australia. It may have been previously overlooked because of the short period of time that infected plants show symptoms. The main vector of RyMV, *Abacarus hystrix* has not been recorded in Australia (Schicha pers. comm.) therefore mechanical transmission is likely to be the only mode of spread.

It is impossible to predict the concequences of RyMV in Australian pastures. However the preliminary glasshouse studies are in line with studies in the UK which showed that RyMV can reduce dry matter yield by as much as 27% in sensitive lines of *L. multiflorum* and by 13–15% in more tolerant lines (Wilkins and Hides, 1976) as well as reducing water soluble carbohydrates (Holmes, 1977).

Interactions with other plant viruses have been recorded and Catherall (1967) found that even when mild isolates of RyMV were present with barley yellow dwarf viruses (BYDV) severe stunting and vastly reduced tiller numbers resulted. High levels of BYDV are present in Australian pastures. In a survey (Guy et al., 1986) no Tasmanian ryegrass pasture greater than six months old was free of infection and the levels in 2–4 year old pastures ranged from 2–70%. Similarly Eagling and Sward (pers. comm.) sampled 4 year old ryegrass trials in Western Victoria and found levels of 8–90% depending on cultivar and site.

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Brome Mosaic Virus Transmission by Cereal Leaf Beetle (*Oulema melanopus*, Coleoptera, Chrysomelidae)

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After one day feeding period adults of cereal leaf beetle were able to transmit the brome mosaic virus. Feeding on healthy plants the insects rapidly loosing their infectivity. The transmission experiments and the life cycle of the vector suggest the role of *Oulema melanopus* in the natural spread of the virus.

Brome mosaic virus (BMV) is a multicomponent, single stranded RNA virus with uniform isometric particles (Fig. 1) about 26 nm in diameter, a type member of bromovirus group (cf. Lane, 1977). The widely distributed pathogen infects more than 50 genera of Graminea. Easy to transmit by mechanical inoculation and succesfull attempts have been made with the nematoda *Xyphinema sp.*

The abundance and rapid distribution of BMV in small grains seems to be correlated to the gradation of cereal leaf beetle *(Oulema melanopus)*. Other members of bromoviruses, namely cowpea chlorotic mottle virus and broad bean mottle virus, are transmitted by Chrisomelid beetles: *Desmodium laevigatum*, and *Acalimma trivittata*, *Diabrotica indecimpunctata*, *Colapsis flavida*, respectively (Walters and Dodd, 1969; Walters and Surin, 1973). The similarity in properties of bromovirus group members suggested further studies on the role of cereal leaf beetles in the transmission of brome mosaic virus. Although Proesler in 1978 reported a positive transmission with *Oulema melanopus* in a preliminary report, the detailes of his experiments remained unknown.

Materials and Methods

Adults of *Oulema melanopus (Lema melanopus)* were collected from ruderal places near to Keszthely (Western Hungary). After one day starving period 20–20 insects were placed in plastic chambers containing winter wheat (*Triticum aestivum* cv. MV 8) seedlings systemically infected with a Hungarian isolate of brome mosaic virus (T 12). After one day acquisition the insects were transferred to healthy wheat plants and were removed after one day feeding, and the plants were kept in greenhouse until the systemic mosaic symptoms appeared. Two weeks later two grams of samples were harvested from the plants, homogenized

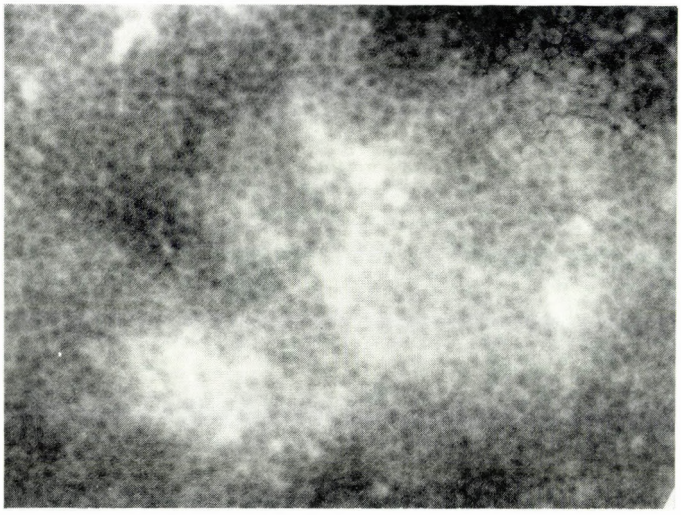

Fig. 1. Electron micrograph of purified BMV

Results

in equal volume of Sörensens 0.1 M phosphate buffer (pH 7.2). The presence of BMV was checked in 15 μl samples in 12% poliacrylamide gel by electrophoresis according to Laemmli, 1970. Accumulation of BMV coat protein in large amounts was characteristic for the infected plants. Purified BMV coat protein (20.3 kd Mwt) was used as standard.

Adults of *Oulema melanopus* were able to transmit the brome mosaic virus after one day acquisition period. The appearance of coat protein band of three replication is shown on the Fig. 2. If the viruliferous insects were fed on healthy plants for one day they lost their infectivity because they got rid the virions stuck on their mouth parts. However, consecutive feeding of insects on healthy plants did not result in identifiable virus transmittance (Fig. 2).

Occurrence of cereal leaf beetles in Hungary was first noted in the last century (Lovassy, 1983; Sajó, 1894). Depending on the gradation years this was an important pest of barley, oat and wheats. Serious yield losses have been detected (cf. Webster and Smith, 1979; Webster et al., 1980; 1982) when the flag leaves were damaged by larvae.

Adults appear early in the spring (April) and the population reaches a maximum in the middle of May. The peak of larval density is reported in the end of this month. First adults of the new generation appear in the middle of June and the population declines in the middle of July. Late summer (September) the adults are comming out from the soil for a short feeding period, but the diapause is starting also from this time.

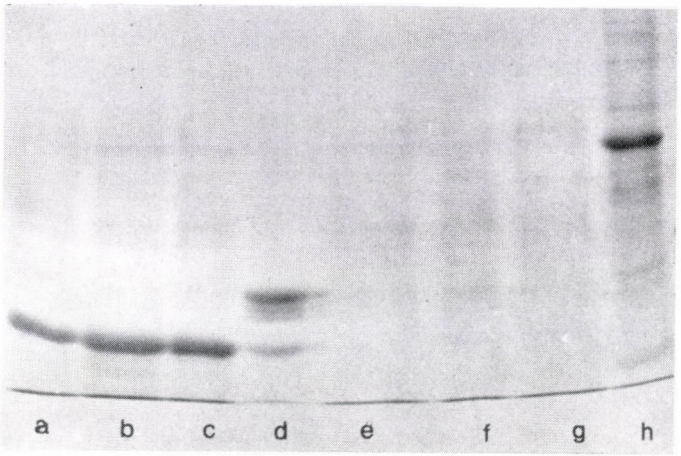

Fig. 2. Polyacrylamide gel electrophoresis of wheat extracts after insect transmission (a, b, c-plants tested after one day acquisition feeding, d, coat protein standards BSMV, PSLV, BMV, e, f, g-plants tested after overtransfering the viruliferous insects fed one day on healthy plants, h-healthy control)

Conclusions

The results of transmission experiments and the life cycle of cereal leaf beetles suggest that the good flying adults of *Oulema melanopus* can have a role in the natural spread of BMV in small grains. Between the harvesting period of cereals and the growth of new winter varieties the ruderal weeds and corn can be the reservoir of the virus. Other three species of the genus *(O. lichenis; O. rufocyanea; O. septentrionis)* are also occurring in wheat and corn fields (Szabolcs, 1974) were also tested. These species are relative small amount in the population but in the transmission experiments all of four species were able to transmit the BMV from wheat to wheat plants, but not to corn.

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Brome Mosaic Virus Infection in Different Cereal Breeding Materials

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Besides being a dangerous pathogen of grasses, brome mosaic virus may cause serious damages in cereal crops also. As far as the authors are aware and according to the available references, occurrence of the mentioned virus in barley and Triticale has not been yet investigated in Hungary. The present work, aiming to study the natural occurrence of brome mosaic virus in cereals in Hungary was carried out in the Research Institute of the University of Agricultural Sciences of Gödöllő in Kompolt and in the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár. Virus diagnosis was carried out both serological and biological tests.

Obtained results showed that natural infection of cereals by brome mosaic virus can be detected in Hungary.

The mentioned virus was detected in barley, wheat and Triticale. Virus infection of the examined cereal plants was found to fluctuate between 5–100%.

McKinney et al. (1942) were the firsts who described brome mosaic virus (BMV) as a viral disease of *Bromus inermis* in America. Since then, and because of the easiness of its mechanical transmission, BMV has been reported several times on different hosts in different countries. According to Lane (1977), the mentioned virus occurs in many States of USA, South Africa as well as in many countries in East Europe. In USA, BMV was reported on *Hordeum vulgare* (McKinney, 1953) and Zea mays (Stoner et al., 1967). In South Africa Von Wechmar and Van Regenmortel (1966) stated that *Agropyron distichum*, *Ammophila arenaria*, *Cynodon dactylon*, *Pennisetum clandestinum*, *Lolium multiflorum* and *Triticum aestivum* were found to be natural hosts of BMV. In East Germany the mentioned virus was reported on *Lolium multiflorum* (Klinkowski and Kreutzberg, 1958) and on *Hordeum murinum* (Ohmann-Kreutzberg, 1963). In the Soviet Union *Agropyron repens*, *Triticum aestivum* and *Rubus idaeus* were found to be naturally infected with BMV (Vlasov et al., 1965; Larina, 1968; and Vlasov and Teplouhova, 1971. respectively). In Yugoslavia Milicic et al. (1966) as well as Richter et al. (1966) had separately reported BMV on *Agropyron repens*. The virus was also reported there on wheat by Tošić (1971). In Hungary Szirmai (1986) was the first to report BMV on both wheat and *Dactylis glomerata*. The degree of infection of different rice varieties with BMV was studied by Pocsai and Mrs. Simon, Kiss I. (1986). Pocsai (1988a, 1986b, 1987a, 1987b) had determined

the degree of infection of other cereal plants with BMV under greenhouse conditions. According to his results, the degree of infection (artificial) of the examined barley and wheat varieties was found to be 100%.

Since 1985 cereal crops grown in the middle Transdanubian region have been serologically investigated for their natural infection with BMV. According to our results and up till now, we have been unable to detect any BMV natural infection of these crops.

In the year 1987 breeding materials of barley, wheat and Triticale were both serologically and biologically investigated for their natural infection with BMV. Results of these investigations are given in the present work which shows that the degree of natural infection of the mentioned breeding materials with BMV is remarkable.

Table 1)

Results of serological tests on winter barley samples showing different symptoms (Kompolt, 25. May 1987)

Sample label	Symptoms	Serological test BMV	BYDV
AT-81	yellow streak of leaf	+	−
Bulg. KT 20/16	necrotic brown stripe on leaf	−	−
CT-13 K 80/30	necrotic brown stripe on leaf	−	−
CT-14 K 72/3	yellow streak of leaf	−	−
CT-18 K 79/7	yellow streak of leaf	−	−
FIT 19 H 36	yellowing of leaf, stunting	−	+
Kompolt 4	yellowing of leaf, stunting	−	+
Kompolt early	yellowing of leaf, stunting	+	+
Kompolt early	yellow streak of leaf	−	−
K-4 SK	necrotic brown stripe on leaf	−	−
K 83/20	yellowing of leaf, stunting	−	+
K 83/28	yellowing of leaf, stunting	−	+

Materials and Methods

In the year 1987 our attention was attracted to remarcable symptoms on leaves of the breeding materials of barley, wheat and Triticale grown in both the experimental fields of the Research Institute of the University of Agricultural Sciences of Gödöllő Agriculture of the Hungarian Academy of Sciences in Martonvásár.

In Martonvásár the mentioned symptoms were equally observed on leaves of barley, wheat and Triticale. On the other hand in Kompolt such symptoms were only observed on leaves of winter and spring barley.

In Kompolt the first visual inspection was carried out on the 25th of Maay and the second one on the 25th of June, 1987. At the time of the first visual

inspection 12 breeding material samples of winter barley were collected for investigating them for their viral infection.

In Kompolt, on the 25th of June samples collected from breeding materials of winter and spring barley are in table 2.

In Martonvásár seven parcels of wheat, one of barley and one of Triticale were visually inspected on the 24th of June 1987 (Table 3).

Samples were collected from each of the inspected parcels for further examinations. Samples collected during the first visual inspection is Kompolt were serologically investigated for the presence of both BMV and barley yellow dwarf virus (BYDV). For BMV double diffusion test (Ouchterlony, 1958) was applied. For detection of BYDV ELISA test (Clark and Adams, 1977) was used. Samples collected in the course of the second visual inspection in Kompolt and those collected in Martonvásár were both serologically and biologically investigated for the presence of BMV only.

Table 2

Results of serological and biological tests on investigated breeding materials of winter and spring barley
(Kompolt, 25. June 1987.)

Sample label	Sample number	Varieties and crosses	Serological test	Biological test
Winter barley				
AT-38	K 85/12	(ALBM× Kompolt early× Sigra)	20/0	20/0
AT-41	K 85/38	(ALBM× FIT I-3) 2× Ogra× Sigra)	20/0	20/0
AT-46	K 83/12	(Odeszkij 2038× ST 2094)	20/0	20/0
AT-50		(KK× Igri)	20/0	20/0
AT-54	K 84/2	(K 80/30× Odeszkij 2087)	20/0	20/0
AT-59	K 84/10	(Kompolt early× K 78/4)	20/2	20/2
AT-69	K 84/32	(Kompolt 4× Cebeco)	20/0	20/0
AT-81		Pallideum 397/4	20/2	20/2
AT-93	K 83/26	(GK-22× K 71/26)× (GK-22× Rubin)	10/0	10/0
AT-104	K 84/30	(KT 1003× Igri)	5/2	5/2
AT-105	K 84/27	(K 71/16× Spartan× Igri× Matyo)	20/1	20/1
		Kompolt early	60/0	60/0
		Sigra	20/0	20/0
Spring barley				
TA-4		Matyo	10/2	10/2
TA-9	K 84/2	(K-1× Bonanza)	10/4	10/4
TA-15	FIT XVI-13	(Fatran× ST-6984)	10/9	10/9
TA-21	K 84/20	(Hevesi-1× Galt)	10/9	10/9
TA-41	K 84/16	(ST-23353× Matyo)	10/10	10/10
TA-79	K 86/29	(K-1× Fatran)× (HVS-827× Hevesi-1)	10/5	10/5
TA-81	K 86/51	(Malata× MV-82)× (K-80/23× Matyo)	10/8	10/8
TA-97	K 83/25	(K 71/16× Spontan× Igri)× Fatran	10/6	10/6

For the serological detection of BMV each plant samples was homogenized in a porcelain mortar together with 0.1 M Hepes buffer (pH: 5.0) in the ratio of 1 : 4 (W/v). The used BMV antiserum (titre 1024) was diluted to 1/32. Each serological test was repeated four times.

For the biological tests 1–3 leaf plants of the winter barley variety "Kompolt early" were used as test plants. The inoculum was prepared by homogenizing each examined sample in a porcelain mortar together with 0.02 M phosphate buffer (pH: 6.0) in the ratio 1 : 3 (W/v). As an abrasive 500 mesh celite was used on leaf surfaces of plants to be mechanically inoculated.

Table 3

Results of serological and biological tests on investigated breeding materials of different cereals
(Martonvásár, 24 June 1987)

Species	Sample label	Varieties, hybrids and crosses	Serological test	Biological test
Triticum aestivum	FD-5	Froucra	10/10	10/10
	H-51	(BU 886-5× Mv-14-83)× GK-34-82	10/0	10/0
	H-217	((Blue× Agent)× Mv-9)× Flamura 80	10/6	10/6
	H-555	(22-5-1× F-262)× (F-109-H-6-11× So 1586	10/0	10/0
	H-584	(Plaisman× Mv-8)× Mv-08-85	6/0	6/0
	H-5013	((Mv-10× GK-Tarjan)× Mv-10)× ((Kz-1× Zg-1477)× Kavkaz)	10/10	10/10
Triticum durum	H-664	(Mv-81× Parusz)× D-2	10/1	10/1
Triticale	654	Fundulea-1× Bolero	10/3	10/3
Hordeum vulgare	Mexico	Barley hybrids	10/7	10/7

Results and Discussion

According to the type of symptoms they showed samples of breeding material of winter barley collected in Kompolt on the 25th of May 1987, were differentiated into three groups. The first group consisting of plants showing yellow streaks on the leaves was the most frequent. The second group shows leaf yellowing and stunting. The third group consists of plants showing yellowing of leaves accompanied by necrotic striping spreading parallel to the veins. This type of symptoms was of the least frequency. Results obtained from the serological examinations of the above-mentioned groups are shown in Table 1.

These results show that the yellowing of leaves and plant stunting symptoms were caused by BYDV. Among the group of plants showing yellow streaks on

leaves two barley breeding materials proved to be infected with BMV. As far as the authors are aware and according to the available literature this is the first report about the natural infection of barley with BMV in Hungary.

Results approached by the serological and biological examinations of winter and spring barley samples collected in Kompolt on the 25th of June 1987 are shown in Table 2.

These data show that results of the serological tests were in complete correspondence with those of the biological ones.

Among viruses infecting cereals, BMV has the highest mechanical transmission. Pocsai (1987b) showed that the mechanical transmissibility of BMV was 100% in 14 barley and 32 wheat varieties which he had investigated for this respects.

Data of Table 2 show that among the 13 examined breeding materials of winter barley four proved to be infected with BMV. The degree of infection was found to range from 5–10%.

Data of this table show that all these breeding materials of spring barley proved to be infected with BMV. Their degree of infection was ranging from 20–100%.

Results obtained from the serological and biological tests carried out on samples of wheat, barley and Triticale, collected in Martonvásár on the 24th of June 1987, are shown in Table 3. Data of this table show that many examined samples were found to be infected with BMV.

In Hungary and because of its rare occurrence previously BMV was not considered to be among the dangerous cereal viruses. Up till now, its natural occurrence in Hungary was reported only on wheat and *Dactylis glomerata* (Szirmai, 1986).

The present work proves the natural occurrence of BMV in the breeding materials of wheat, barley and Triticale. Our results also show that the degree of infection in the mentioned materials is high enough. In the future and for the sake of proving the standard of cereal breeding works, we propose that the infection of breeding materials with BMV should be strictly considered.

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Distribution of the *Arrhenatherum* (Foddergrass) Blue Dwarf Disease Caused by Oat Sterile Dwarf Virus in the German Democratic Republic

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In surveys carried out during 1966/67 to 1987 all the districts of the German Democratic Republic (G. D. R.) were involved. Mainly plants of Arrhenatherum elatius (L.) M. et K. growing abundantly throughout the country at man made habitats like road side stripes and areas along railways — often within and in the rule nearby villages and city areas — were examined for the presence of infections by oat sterile dwarf virus (OSDV). Determination was by symptoms with histoid enations as diagnostic feature. Such material was taken for light microscopic investigation suitable as confirmation when directed to the presence of inclusion bodies characteristic for members of the genus Fijivirus of the Reoviridae to which OSDV belongs. OSDV infected *A. elatius* were found to be distributed all over the country, being obviously the most important reservoir of OSDV in the G. D. R. An abundant occurrence of severely diseased spontaneously growing *A. elatius* could be registered for some places within six districts of the country. Moreover, long lasting observations show for a single locality during the first observation period a zero level, then a moderate, and in some years during more recent times a serious occurrence of OSDV in *A. elatius*.

The occurrence of the disease of grasses called Arrhenatherum blue dwarf, foddergrass blue dwarf, virus dwarf of tall meadow oat-grass, or *Lolium enation* disease is well documented since 1941 from the geographical area of Central Europe (Schumann, 1962, 1971; Vacke, 1966; Vacke and Vostřák, 1974; Gardoš, 1966; Mühle and Kempiak, 1971; Huth, 1975). Serological investigations by Milne and Lesemann (1978) have shown for the first time that the causal virus should be included in the oat sterile dwarf virus (OSDV) which belongs to the genus Fijivirus of the Reoviridae. OSDV is known as a potential danger on the basis of severe symptoms in at least some members of both, cereals and grasses (Schumann and Spaar, 1972; Boccardo and Milne, 1980). Furthermore, the main vector planthopper *Javesella pellucida* (F.) is quite common and abundant in the G.D.R. (Kempiak, 1972). Some basis work on this dangerous virus, including diagnostic possibilities as well az epidemiological aspects, is necessary in the present and future time. In the following we would like to give a summary of surveys carried out during 1966/67 to 1987 on the territory of G.D.R. Results show a wide distribution of this virus in spontaneously growing populations of *A. elatius*.

Materials and Methods

In our surveys randomly chosen localities were involved. There was always the aim to visit areas not yet seen before in order to diminish 'unknown areas' on the map. In some instances Lolium species were examined. But in most cases mainly stands of *A. elatius* growing near the smaller railway stations, along roads, railways etc. were taken under proof. Such an examination of an Arrhenatherum population was mostly confined to 20 or 30 minutes. In this time diseased *A. elatius* must be found out by the symptoms of dwarfing and diminished shooting ability and/or by the presence of histoid enations taken as diagnostic feature. For confirmation such material was fixed in ethanol/acetic acid (conc.) (3 : 1) and stored in this solution or (mostly) in 70% ethanol. Later on it was examined by light microscopy for inclusion bodies, characteristic for OSDV infection and the other members of the genus Fijivirus, too (Lesemann and Huth, 1975; Milne and Lovisolo, 1977; Kempiak, 1977). Feulgen technique (e.g. Kempiak, 1977) was used only in a few cases. For staining plant nuclei borax carmine acc. to Grenacher was used (as a bulk staining). Thereafter cuttings were made by hand and counterstained for inclusion bodies with orange G (OG) or light green (LG) (Gerlach, 1969). In a number of cases material from ethanol was investigated without any staining and/or after staining only with OG or LG, respectively.

Results

A total of 111 stands of *Arrhenatherum elatius* were examined; 96.4% contains plants with enations (Fig. 1). From this material about 61% were examined by light microscopy for inclusion bodies. These proofs were successful in every case (100%), without any exception. Typical vacuolated inclusions could be found out in phloem tissue even without staining the material kept in ethanol. Staining with orange G or light green was giving good results with strong contrasts especially when previously a staining of the nuclei with borax carmine was performed. But the staining of nucleic could also be omitted.

With respect to nine villages or city areas representing six different districts seriously attacked stands of *A. elatius* were observed (Tab. 1). This means that at such localities in short time (10 minutes or less) a high number of diseased plants (20 or more) were found, including detection of the typical histoid enations on each of these plants. In this connection also dwarfing symptoms and delays in shooting ability of diseased specimens were always present.

Most stands indicated (Fig. 1) could be observed only once. Long lasting observations, which were every year or at least during several years repeated, could be carried out with respect to Leipzig-South East from 1966 to 1976, Leipzig-North from 1974 to 1987, Motterwitz (in the district of Leipzig) from 1966/67 to 1970 Magdeburg 1966/67 to 1987, and (see Tab. 1) Genthin 1981, 1982, Mühlhausen from 1985 to 1988.

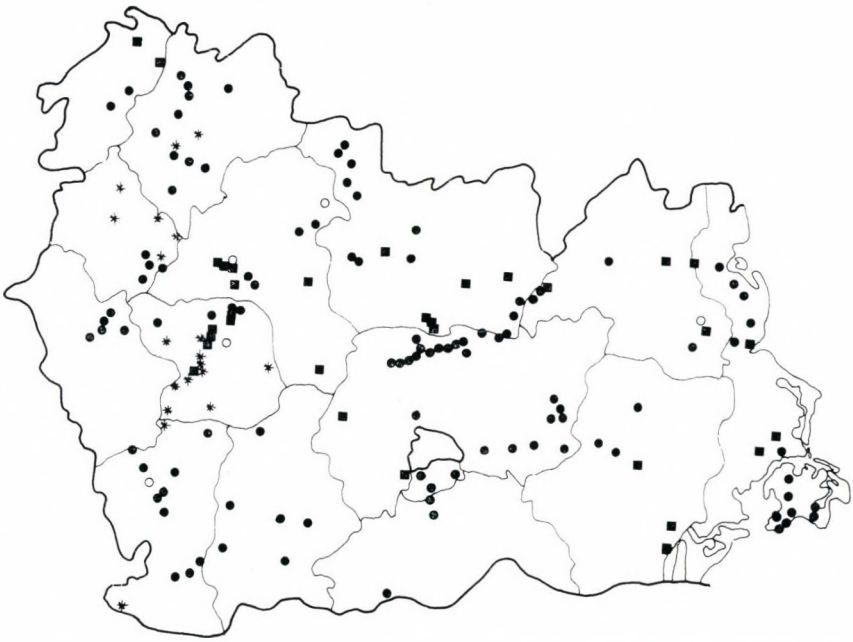

Fig. 1. Distribution map of oat sterile dwarf infections mainly on *Arrhenatherum elatius* (L.) M. et K. in the G. D. R. Findings from Gardoš (1966) are indicated as asterisks. The own data from Lolium spp. are given as open circles. Black circles (n = 111) represent records for *A. elatius* found diseased during 1980 to 1987. Black squares refer to data from *A. elatius* given already by Kempiak and Mühle (1981)

At Magdeburg the areas observed for more than 20 years (Tab. 1) where located at the northern boundary of the city along a main railway going in the direction to Stendal/Schwerin. One part of this remarkable area was a 3 to 5 m broad one hundred meter stripe along the railway indicated ('Magdeburg A', Tab. 1), the second part was the adjacent stripe, 200 to 300 m long and 2 to 3 m broad along the same railway ('Magdeburg B'), and the third part ('Magdeburg C', Table. 1) was going away rectangular from area A covering a stripe about 3 to 4 m deep between a street and the northern position of the backfront from garages build up during the observation time.

In A, B and C no diseased plants of *A. elatius* could be observed during 1966/67 until 1978 in the result of searching actions carried out every year. Firstly in 1979 some diseased plants were present, growing within the dense stand of the area A. In 1980 and 1981 the situation was somewhat the same; occasionaly, a few diseased plants could be found out within area B, with an increasing tendency during the following years until 1987. Only for the areas A and C – especially in 1982 and 1984 – the situation indicated in the Table was reached, showing some delay in 1983 and mainly in 1985 and 1986 in connection with

Table 1

Locations in different districts of the G.D.R. found out in surveys to harbour populations of *Arrhenatherum elatius* (L.) M. et K. severely affected by oat sterile dwarf virus. I.e. at the places indicated it was possible for one person within 10 minutes to found out easily 20 or more diseased plants, including the control of each specimen for the presence of histoid enations as diagnostic feature of the disease in this grass

District	Village or city area (areal)	Year(s)
Neubrandenburg	Usadel	1981
Schwerin	Güstrow	1980
Magdeburg	Magdeburg (A)[1]	1982, 1983[2], 1984
	Magdeburg (C)[1]	1982, 1983[2], 1984, 1987
	Genthin	1981[2], 1982
	Halberstadt	1981[2]
	Drübeck	1981[2]
Erfurt	Mühlhausen[3]	1985[2], 1986[2], 1987, 1988
Leipzig	Leipzig— Wahren	1980
Dresden	Arnsdorf	1981[2]

[1] For more details see text. [2] In this cases the amount of diseased plants were just reaching a level which was still high enough to be reported here. [3] The result for 1988 was obtained at 18th May.

a new increase in 1987 for area C. Thus, in the areas A and C in 1982 and 1984, for instance, nobody knowing the picture of severe OSDV infection in *A. elatius* could overlook the situation because seriously diseased specimens were in high numbers present and easily seen even without nearby inspection.

Findings regarding Lolium spp. in connection with symptoms of oat sterile dwarf could be obtained in only five cases (see Fig. 1). The number of controlled mostly meadow-like stands was not registered quantitatively owing to the very high number of negative results. In four of the five samples which were positive *Lolium perenne L.* was affected with conspicuous symptoms. This was i.a. true for the area of the Plant Breeding Institution at Gatersleben (district of Halle) in 1977. The fifth case was a very severe attack owing to numbers of diseased plants as well as symptoms seen in field grown *Lolium multiflorum Lamk.* in 1969 at Leipzig. A search for inclusion bodies was performed only in the case of *L. perenne* collected at Leipzig which proved to be positive.

Discussion

OSDV infected *Arrhenatherum elatius* was widely distributed in the G.D.R. Stands of this grass, which are – except on strong sandy soils – common throughout the country, seems to be the main reservoir for this dangerous virus under

our conditions. In about 96% of randomly chosen and examined populations of *A. elatius* mostly growing on areas not cultivated but typically man made (like road sides and so on) diseased plants could be found out.

In contrast to our findings with *A. elatius*, Lolium spp. with typical symptoms could be observed in only a few cases. But severe infections were included, too. A very serious attack in field grown *L. multiflorum* at Leipzig was also observed. Thus, in the whole we agree with comparable findings of other authors (Schumann, 1962, 1971; Gardoš, 1966; Huth, 1970, 1975). In our own infection trials (Kempiak, 1975), it was not so much easy to obtain OSDV infected Lolium spp. as it was the case with *A. elatius*. In detail, similar to results obtained by Lindsten (1961), only relatively few Lolium plants became infected with somewhat mild symptoms after prolonged incubation periods, suggesting at least some resistance to OSDV. Owing to this situation which contains conflicting aspects further experimental work should be done with respect to Lolium spp., because there are important fodder grasses and breeding parents, respectively. Moreover, such a need exist also in view on reports about infections in Lolium spp. caused by Macrosteles leafhoppers. Such infections are symptomatologically similar to oat sterile dwarf, but etiologically quite different (Vacke et al., 1978). With respect to *A. elatius* a similar situation does not exist up to now.

Since 1980 we have seen more and more cases for severely diseased spontaneous populations of *A. elatius* covering anthropogenic habitats. Instances from six districts could be found out, including the dramatical build up of a OSDV focus at the areas under observation for more than 20 years at Magdeburg, where the disease was not present at least from 1966/67 to 1978. Similar outbreaks were formerly only known from field grown *A. elatius* giving in some instances reason for destroying whole fields by ploughing (Gardoš, 1966; Schumann, 1971). Such an instance was also involved in the material presented by Kempiak and Mühle (1981) with the case from the Meiningen area (district of Suhl). But a report about seriously attacked *A. elatius* growing spontaneously was still not involved in this material nor in the results by Gardoš (1966). In fact, the data reported here seems to be the first ones in a somewhat new and astonishing direction toward an increasing importance of man made but not cultivated areas as reservoirs of the oat sterile dwarf virus in the G.D.R.

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Attempts to Reduce Damage Caused by Oat Sterile Dwarf Virus in Oats Using Virazole, 2,4-Dioxohexahydrotriazine, *Boerhaavia* Inhibitor, and Alkane-monosulfonate

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2,4-Dioxohexahydro-1,3,5-triazine (= DHT) applied as a granulated preparation was able to reduce significantly the stunting phenomenon caused by oat sterile dwarf virus in oats. Some activity in the same direction was registered after spraying DHT, or Boerhaavia Inhibitor, or Alkane-monosulfonate. Regarding the number of infected plants small therapeutic effects could be observed after application of Alkane-monosulfonate (= AMS), DHT, or DHT together with AMS. By monitoring the appearance of symptoms it could be shown that Boerhaavia Inhibitor decreased the percentage of diseased plants. But this was significant only at an early date in disease development.

The well-known damages caused by oat sterile dwarf virus (OSDV) consist mainly in pronounced stunging and sterility of diseased plants. Oats are the most severely affected cereal species (Lindsten, 1961; Catherall, 1970; Mühle and Kempiak, 1971; Milne and Lovisolo, 1977; Boccardo and Milne, 1980).

In the present investigation some tests were made to suppress the damages of the virus in oats by means of known antiphytoviral substances.

Ribavirin (= Virazole, 1-β-D-Ribofuranosyl-1,2,4-triazol-carboxamide), 2,4-Dioxohexahydro-1,3,5-triazine (= DHT) and Alkane-monosulfonate (= AMS, L333 or Emulgator E 30), alone and/or in combinations, are known to be active against some plant viruses (Schuster, 1986, 1987). The same is true for aqueous extracts from root powder of *Boerhavia diffusa* L. plants, growing as a weed in India (Verma and Awasthi, 1979, 1980; Verma et al., 1979). The Boerhaavia Inhibitor is known to contain a glycoprotein as the active principle. In some instances it was able to reduce disease symptoms as well as virus contents drastically. It is also known to induce systemic resistance against some plant viruses in dicots.

Material and Methods

The test plant species used was *Avena sativa* L. cv. Solidor. One plant was grown per pot (6 to 8 cm pot diameter). Infections were caused by larvae of *Javesella pellucida* (F.) caught during winter or early spring of the years 1979

to 1984 from hibernating places in a small plantation of *Arrhenatherum elatius* (L.) M. et K. at Leipzig suffering from Arrhenatherum blue dwarf disease. This is known to be caused by oat sterile dwarf virus on the basis of serological investigations (Milne and Lesemann, 1978).

There were one or two insect(s) caged for 4 to 6 days on each plant. This onset of infection feeding access time was always identical with the beginning of a trial. At this time plants were in every case in the early first-leaf stage. Only in one trial plants were fed on for five days by a total of five different specimens of *J. pellucida*, i.e. in this case each insect was allowed to stay on one plant for only one day, and on the following day it was in accordance with plant enumeration carried over to next plant, and so on.

After infection access time planthoppers were removed and plants kept under insect-proof conditions in a growth room at 18 C to 25 C. Light was by incandescent tubes (white) for daily 16 h.

Further details of the experiments including again some minor exceptions from the general scheme given above are shown in Tab. 1. For details regarding applied substances see Tab. 2. Light intensities were either about 4000 lux or nearly 9000 lux for trials 1, 2, 5, and 3, 6a, 6b, 7 (Tab. 1, 2), respectively. Experiment 4 was in a unshaded glasshouse.

Table 1

Trials carried out after infection feeding by specimens of *Javesella pellucida* (F.) taken from a population known to be carrying oat sterile dwarf virus. Time of substance application(s) in the course of each experiment are given. For data regarding substances see Table 2

Trial no.	No. of application of substances						Time span from beginning to the end of each experiment
	1[1]	2[2]	3	4	5	6	
1	1h	5d	8d	14d	—	—	42
2	2h	6d	10d	—	—	—	39
3	2h	6d	11d	16d	21d	—	39
4[3]	1h	5d	10d	15d	—	—	84
5	2h	5d	10d	—	—	—	56
6a	24h[4]	—[4]	—	—	—	—	55
6b	24h[4] and 2h[5]	—[4] 6d[5]	— 11d	— 16d	— 27d	— —	55
7	48h, 24h, and 20h	13d	27d	43d	—	—	58

[1] Application no. 1 was always some hours (h) earlier than the beginning of infection feeding time (BIFT). BIFT and onset of the trial were identical. [2] Applications no. 2 to 5 were in all cases after the end of infection feeding time (data are given in day (d) after BIFT). [3] As an exception this experiment was carried out with planthoppers which were already used for infection feeding (in identical variants) in trial no. 3. [4] Application of DHT as granular preparation to the soil. [5] Application of Alkane-monosulfonate as sprays.

Table 2

Percentages of oat sterile dwarf diseased oat plants (DP %) according to visual determinations at the end of each experiment as influenced or not by antiphytoviral substances. For further details see Table 1

Trial no. (plants per trial)		Substance(s) (concentrations of spray applications)	Results of variants (DP %)		Chi² values
			without substance(s)	with substance(s)	
1	(96)	Ribavirin (0.01%)[1]	36.2	40.8	
2	(97)	Ribavirin (0.03%)	38.3	38.0	
3	(99)	Alkane-monosulfonate (0.1%)	25.5	12.5	1.918⁻
4	(68)	Alkane-monosulfonate (0.1%)	27.8	9.4	2.616⁻
5	(94)	2,4–Dioxohexahydro-1,3,5 – triazine (DHT) (0.1%)	52.2	43.8	
6	(117)		38.5		
6a		DHT as granulated preparation (GP)[2]		17.5	3.34⁻
6b		DHT-GP[2] together with Alkane-monosulfonate (0.1%)		23.6	1.33⁻
7	(138)	Boerhaavia Inhibitor (1.25%)	27.9	20.0	

[1] In this trial sprays were mixed with an emulgator (0.2%).

[2] Granules with an active ingredient of 10% were given in an amount of 200 mg to the soil of each pot.

For taking results numbers of diseased plants were determined by visual proofs at least at the end of each experiment. Apart from this, measurements of plant height and mostly also fresh weight determinations were carried out. Plant height was estimated as the sum of leaf to leaf distances with measuring points always near the main axis of the plant. Thus, the distance between the basis of the oldest and the youngest leaf was considered as plant height.

Plants dying off prematurely and/or showing symptoms of European wheat striate mosaic (EWSM) disease (which is also vectored by *Javesella pellucida)* prior than exhibiting a clear picture of OSDV infection were in all cases excluded from calculation of results. Small or very small numbers of EWSM infected plants were present in all variants and in every experiment.

Results

As results of the different trials between about 9% and 52% diseased plants were obtained. Such plants could be detected easily by the main features of the oat sterile dwarf disease, which are stunting, incl. leaf shortening, dark green discoloration to some extent, and tiller production at a very early point in plant

development. Histoid enations, also a characteristic of OSD diseased oats were often but not in all cases observed.

With the exception of Ribavirin treatments in all experiments numbers of diseased plants were diminished more or less after administration of the anti-phytoviral substances being tested. But the differences between substance application versus lack of substance influence reached in no case the 5% significance level (Tab. 2). The most promising effect in this respect was seen after application of DHT as a granulated preparation.

By monitoring percentages of newly diseased plants on many days during the experiment after Boerhaavia Inhibitor application significantly fewer diseased plants were registered at day 26 after beginning of the infection feeding time, i.e. at a very early date in the course of the trial. Later on the amount of diseased plants increased also in the treated variant showing at the end of the experiment a still lower but not longer significant level (Fig. 1).

Apart from recording symptoms only visually a great number of measurings were carried out without taking notice from disease symptoms eventually present. Data obtained in this way were used for calculating cumulative percent slopes

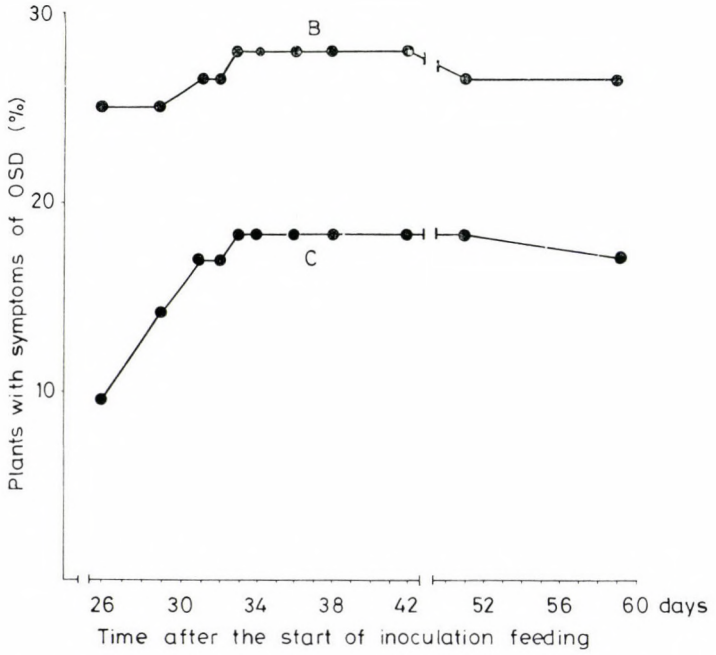
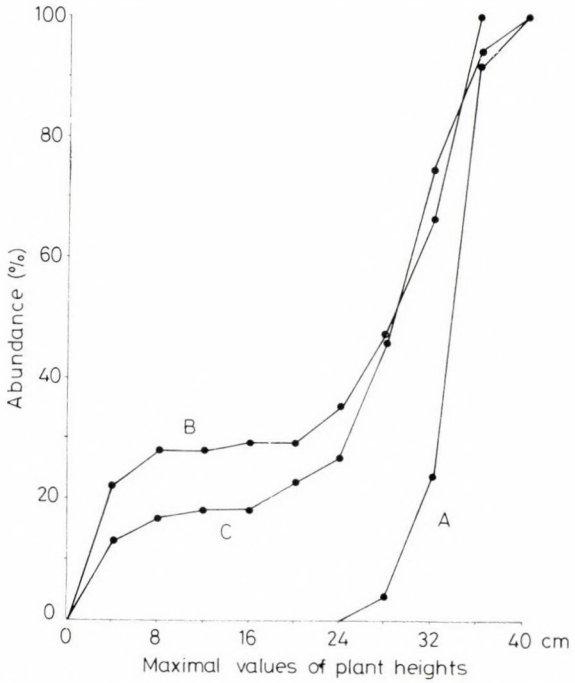


Fig. 1. Appearance of oat sterile dwarf symptoms at different times after the onset of inoculation feeding by *Javesella pallucida* (F.). The percentage of diseases plants was at all times reduced after Boerhaavia Inhibitor application (C), as seen by comparison with the phosphate buffer-treated (control-) variant (B). In every case a plant was considered diseases only when this observation was confirmed by two further examinations carried out at the following days

for each variant in the different experiments after dividing the material into 10 equal-sized classes of different maximal values, mostly for plant height (Figs. 2 to 4). Again with the exception of Ribavirin all antiviral treatments showed some increases in the height of plants, i.e. stunting effects induced by OSDV were



Figs 2 to 4. Differences in plant heights after different treatments of oats sucked (C, B) or not sucked (A) by specimens of *Javesella pellucida* (F.) taken from a population carrying the oat sterile dwarf virus. Plant groups (variants) received Boerhaavia Inhibitor (Fig. 2, C), DHT sprays (Fig. 3, C), or DHT granules (Fig. 4, C) via soil. Variants only treated with phosphate buffer (Fig. 2, B) or water (Fig. 3, B), respective without treatment (Fig. 4, B) were involved for comparisons. Variant A received no application. The cumulative percent slopes given for each of the variants indicated were constructed after dividing every material into 10 equal-sized classes of different maximal lengths

at least partly abolished. Similar to results obtained with Boerhaavia Inhibitor (Fig. 2), or Alkane-monosulfonate (= AMS), after application of 0.1% DHT sprays insignificant effects were present (Fig. 3). In this case some 21% of the untreated control plants, which were not infected, i.e. never fed on by plant-hoppers, reached a height of 14 cm or less. In contrast, the same was true for about 48% and nearly 67% of the DHT treated infected and not DHT treated infected specimens, respectively.

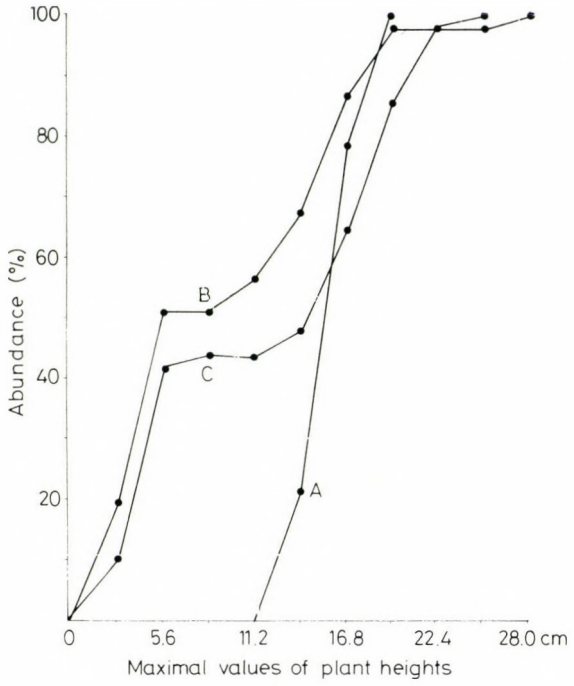


Fig. 3

Significance at the 5% level (Kolmogorov–Smirnov-test) with respect to plant height values could be observed after treatment with DHT granulates (Fig. 4). Without treatment some 43% of the oat plants visited by hoppers and therefore potentially infected reached a maximal height of only 20 cm. But after application of granulates the same occurred with as few as 18% of the plants. All other specimens in this variant were grown up to greater height. As in all the other trials OSDV infected plants diagnosed by visible symptoms were without exception included in those classes which contains the smallest measuring values. In the variant treated with both, DHT granules and AMS sprays, as indicated in Table 2, the positive and significant effect obtained for DHT granules was somewhat diminished.

A lowering of the positive effects of DHT granulate application when it is followed by AMS sprays was also obvious in the case of fresh weight determinations (Table 3). The fresh weight yield increase to 34.5% above the control obtained with DHT granules was going back to about 18% by additional AMS sprays. Giving AMS sprays or Boerhaavia Inhibitor sprays alone, respectively, only small fresh mass increases relative to the variants receiving no substance at all were measured.

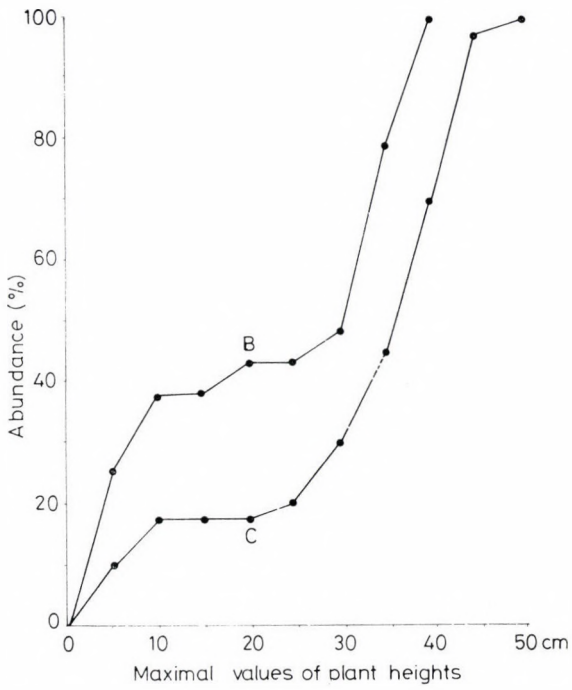


Fig. 4

Table 3

Fresh weight determinations from oat plants treated or not treated with antiphytoviral substances. All plants were previously fed upon by *Javesella pellucida* (F.) which were taken from a population known to be infected with oat sterile dwarf virus. For details, applied substances etc., see Table 1 and 2

Trial no.	Results of variants (fresh weight ± standard deviation)		Differences obtained after substance applications (without substance(s) = 100%)
	without substance(s)	with substance(s)	
6	1.97±1.0		
6a		2.65±0.88	134.5%
6b		2.32±1.03	117.8%
7	1.91±0.87	2.04±0.79	106.8%
4	1.17±0.40	1.19±0.25	101.7%

Discussion

Results presented here points especially to the usefulness of DHT granulates for preventing damages caused by viruses like OSDV. With respect to this substance, which lacks phytotoxic properties even at relatively high concentrations (Schuster, 1982, 1986), the same could be true for other viruses of cereals and foddergrasses, too. Further work is needed in every case, and in this connection data presented here may give some orientation. In every case it seems to be remarkable that with the exception of Ribavirin all the tested antiphytoviral substances exhibited at least small activities also against OSDV, respective the disease caused by it. According to results by Schuster (1976) Ribavirin should eventually be tested at higher concentrations than it was the case in the present work.

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Cereal Mosaic Virus

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A disease of oat, barley and wheat in the Soviet Far East is caused by the rhabdovirus ($420 \pm 50 \times 67 \pm 8$ nm) called as the cereal mosaic virus (CMV). The following symptoms are typical of this disease: striate mosaic, stunting, excess tillering, proliferation of oat panicles and in some cases the presence of large loop-like inclusions in epidermall cells of the infected plants. The infection is neither seed – and soil – borne, it is also not transmitted mechanically. The planthopper *Laodelphax striatellus* Fall. coming into complicated persistant relationships is a natural vector of CMV. Infection overwinters in nymphs. CMV is not transmitted transovarially. Infection of cereals of the late sowing tend to increase by late summer (frequently it is of epiphytotic type). This is connected with an increase in the number of vector and its virulence. Cereals of usual sowings are affected to a certain extent. It was shown experimentally that CMV affects a wide range of cereals, wild-growing grasses and weeds.

The virus disease of cereals, i.e. the oat pseudo-rosette is known long ago in Siberia and Far East (Bryzgalova, 1945; Pronicheva, 1929; Sukhov and Vovk, 1940). Resemblance in the external characters of plant disease and presence of one and the same vector – *Laodelphax striatellus* did not raise doubts in similarity, of this infection. However, studies carried out in Amur region (Krylov et ali., 1981) showed the other etiology of the disease with the patogen called as cereal mosaic virus (Borodina et al., 1982). The paper is devoted to biological characteristics of the virus.

Materials and Methods

Field investigations were carried out in Amur region, Primorye and Khabarovsk territories. Planthoppers were catched on crops of cereals and placed on the oat seedlings (cv. Selma and Orel) in the greenhouse (at 22–25 °C). Plants with insects were isolated with synthetic material or tubes of cellulose film. Infection in the vector and cereals under study was determined by symptoms appeared on indicator plants (oat). Electron microscopic tests of the ultrathin sections and of plant sap were conducted due to standard techniques. 0.1 M of phosphate buffer (pH 7.2) was used. To determine the latent period of CMV in vector and retention of infection and their dependence upon time of planthoppers infection on diseased plants (acquisition access period) the second and third instar nymphs

were used. After feeding of planthoppers on the diseased oat plants during one, three and seven days they were placed individually in a day (within the whole life) on new isolated healthy seedlings of oat. A day of placing an insect on discased plant was considered to the beginning of latent period. To determine the minimum acquisition access period and inoculation feeding period, the time of planthopper feeding from 5 min to 2 hours (on healthy plants in the first case and on diseased plants in the second) was estimated from the moment of insertion of the insect stylets into plant. To define the transovarial transmission of infection by a vector, the viruliferous planthoppers were placed on plants *Echinochloa crusgalli* (L.) Beauv. being immune against CMV wherein they lay their eggs. The hatched larvae were kept on the same plants and verified on indicator plants after alating. The percentage virulence of vector was estimated on 100–200 planthoppers collected on crops of oat, wheat and barley.

Results

The cereal mosaic, a disease of oat, barley and wheat in the Soviet Far East, is caused by rhabdovirus. Symptoms of this disease, namely: striate mosaic, stunting, abnormal tillering, proliferation of oat panicles, in some cases the presence of large looplike inclusions in epidermal cells of infected leaves, are similar to oat pseudo-rosette which was described in Siberia (Sukhov and Vovk, 1940).

Infection is neither soil- and seed-borne nor it is transmissible mechanically like for the oat pseudo-rosette. Natural vector of disease is *Laodelphax striatellus* Fall. *(Delphacidae)*.

Sizes of mature virus particles of cereal mosaic are measured $420 \pm 50 \times \times 67 \pm 8$ nm. At various stages of formation the particles are found in cytoplasm of epidermic, parenchymal, mesophyll and phloem cells and sometimes in xylem (Krylov et al., 1981).

Principally CMV affects the oat, whereas barley and wheat to a small extent. Usually infection of the cereal crops does not exceed some per cents and does not cause great damage. However in the late time of cereal sowing, i.e. the oat crops for green forage and windfallen grain, the outbreak of disease bearing the epiphytotic character is observed. Sometimes plant infection exceeds by 50%. This is connected with a sharp increase in the quantity of vector from middle summer.

It was shown experimentally that CMV affects a wide range of cereals, wild-growing grasses and weeds of the following genera: *Aegilops*, *Agropyron*, *Agrostis*, *Alopecurus*, *Anisantha*, *Avena*, *Brachypodium*, *Briza*, *Bromus*, *Chloris*, *Digitaria*, *Lolium*, *Panicum*, *Phalaris*, *Pleum*, *Secale*, *Setaria*, *Trisetum* and *Triticum*. Mosaic on the plants appear 5–10 day after infection. Some species of the genera: *Agrostis*, *Alopecurus*, *Calamagrostis*, *Eleisine*, *Lolium* and *Setaria* are infected latently and this is demonstrated by the retrograde transmission. In contrast to the oat pseudo-rosette, CMV does not affect rice and maize plants.

CMV circulation is directly connected with biology and the vector capacities of *L. striatellus* (Fig. 1).

A role of perennial grasses in reservation of virus infection is not yet studied sufficiently. However capacity of the virus to overwinter in nymphs of vector

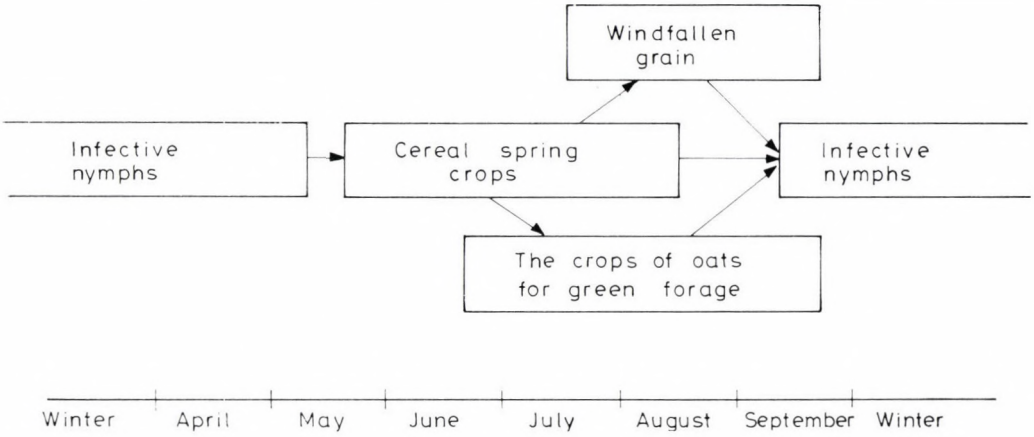

Fig. 1. CMV circulation

proved experimentally allows to conserve disease in agrocenoses and without involvement of wild-growing grasses. In Primorye and Khabarovsk territories where frequently crops of cereals are naturally isolated from each other and the number of vector is much lower than in Amur region, wild-growing grasses may by more essential for conservation of virus infection. CMV infection in those areas is negligible and outbreaks of the disease are not observed.

Studies on characteristics of disease spread by the vector showed that CMV and a planthopper had intricate persistant relationships. Probably the virus is reproduced in its own vector.

The planthopper in active state is able to retain infection and to affect plants during 68 days and often to the end of life. The virus is preserved during 9–10 months in the overwintering planthoppers. Both larvae and imagines can acquire virus from diseased plants. The minimum time for acquisition of infection by vector from the infected plant (acquisition access period) ranges from 2 to 6 h. The latent virus period in vector is about 15 days, and minimum 10 days. It was demonstrated experimentally that the acquisition access period does not influence upon the length of latent period and retention of infectivity by vector. After the end of latent period of the virus in a vector the planthoppers became viruliferous regardless of their instar. In some cases the feeding of viruliferous planthopper on plant for 5 minutes turned out to be sufficient for its infection. The effectiveness of virus transfer by vector is very great. We succeeded in infecting about 15 oat plants for 10 hours using one planthopper. Nevertheless as it was established

the infection transmission by vector was a discrete process. Occasionally the retardation up to 2–3 days is observed.

The degree of cereal infection is affected by the number of viruliferious planthoppers rather than an absolute quantity of the vector population. It was found that viruliferous population like the quantity of vector increased by the end of summer. Thus virulence of the overwintered planthoppers amounts to 4%, on the average, whereas virulence of summer generations (1st and 2nd) usually exceeds by 10% and in certain years was equal to 34%.

Discussion

The five rhabdoviruses transmitted by *L. striatellus* are known in addition to CMV: the oat pseudo-rosette virus of Siberia, sizes within $167 \pm 20 \times 57$ nm (Sukhov and Vovk, 1940; the wheat chlorotic streak mosaic virus of France, 335×55 nm (Signoret et al., 1977); the barley yellow striate mosaic virus of Italy, within $260–270 \times 55–60$ nm (Conti, 1969), $300–320 \times 40$ nm (Conti and Plumb, 1977); the wheat rosette stunt virus of China, within $320–400 \times 50–54$nm (Tian Bo et al., 1980); and northern cereal mosaic virus of Japan within $500–600 \times 40$ nm and $350\ (330–380) \times 68$ nm (Tian Bo et al., 1980).

NCMV and WRSV have the strongest resemblance to CMV in morphology, host range and relationships with a vector. All three viruses do not affect rice and maize plants. The transovarial transmission by planthopper was not observed for them.

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Contents

DISEASES

Contents

DISEASES.

INSECT PESTS

Acta Phytopathologica et Entomologica Hungarica, 26 (3-4), pp. 233-250 (1991)

Pathophysiological Aspects of Plant Disease Resistance

Z. Király, T. Érsek, B. Barna, A. Ádám and G. Gullner

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In the plant host/pathogen or plant/microbe relationships only a few *preformed* resistance substances have been demonstrated and only one case is known where substances of high specificity (e.g. lectins) could also be considered as preformed factors that might determine host/microbe interaction (legume-*Rhizobium* interaction).

In relation to the outcome of interaction, several data support the existence of both the "induced susceptibility" and the "induced resistance" hypotheses. However, research in relation to the induced susceptibility hypothesis has been neglected in the past. In the course of host/microbe interactions, a complex interplay of signals between the two organisms may participate in determining the compatible or incompatible reactions, i.e. susceptibility or resistance of the host. Several plant responses (phenolics, phytoalexins, lignin, callose, hydrolytic and oxidative enzymes, toxic free radicals etc.) that occur earlier or faster in resistant plants as compared to susceptible ones, were causally related to active resistance. However, these host reactions are non-specific stress responses. They are not causally but correlatively related to the phenomenon of resistance, therefore, these responses are likely to be consequences, rather than causes of specific mutual and early recognition between the host and the pathogen. It is suggested that a *two-phase mechanism* of *active resistance* exists: (1) A primary (determinative) phase occurs, which is a specific recognition phenomenon. As a result of recognition, pathogens can be either adversely affected (active or induced resistance) or perhaps promoted (induced susceptibility). (2) A secondary response of the host is activated by elicitors that are released from the pathogen, as a result of recognition. In this expressive phase the accumulating stress responses may contribute to the hypersensitive host cell necrosis (HR) and/or to the inhibition or killing of the pathogen. Of these stress responses, oxy free radicals are discussed in details. Free radicals can also function in the expression of the normosensitive (susceptible) necrotic symptoms of plants. Consequently, the free radical resistant crop plant varieties could be economically useful by resisting the normonsensitive necrotic symptoms, thereby avoiding or lessening damages caused by the pathogen. An oxy free radical resistant tobacco strain was characterized by an activated anti-oxidant system.

Acquired resistance develops after a primary infection and is effective against a *second* infection ("immunity"). A signal compound of acquired resistance is produced after the primary infection, which proved to be salicylic acid. The mechanism of acquired resistance itself is unknown, however cytokinins and lignification could be involved.

One can investigate and evaluate plant disease resistance from quite different points of view. Scientists working in the field of *plant pathology* are much interested in the action of disease resistance: whether it is effective (i) against infection and establishment of the pathogen in infected plants, or (ii) against colonization and sporulation, or (iii) in reduction of the adverse effects that cause yield loss. Accordingly, there are important differences between:

(1) Resistance to infection
(2) Resistance to colonization and sporulation
(3) Resistance to visible disease or yield loss.

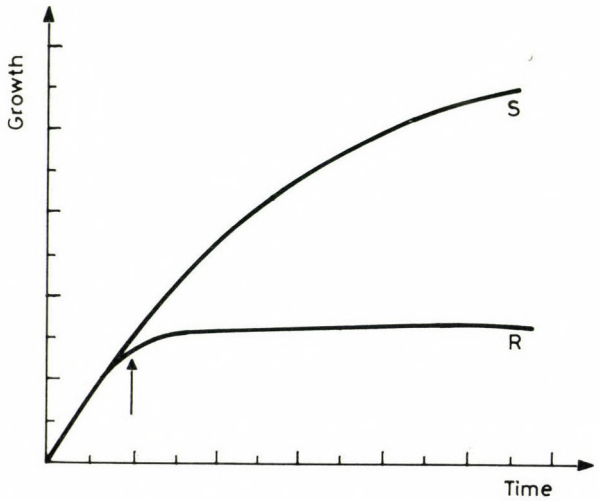

Fig. 1. Idealized curves for the pathogen's growth very early after infection according to the "active (induced) resistance" hypothesis. S=microbial growth in the susceptible plant. R=microbial growth in the resistant plant

Plant breeders and *geneticists are concerned with the* specificity of resistance to pathogenic races (strains) (Van der Plank, 1968). In existence are the so-called:

(1) Race-specific (vertical) resistance, and the
(2) Race-nonspecific (horizontal) resistance.

The former type of resistance is effective against only a few specific races of the pathogen, and it is usually very intensive. It is in most cases accompanied by an early and quick necrotic "hypersensitive" reaction (HR) that is a highly defined answer to infection. (The susceptible plant usually develops late and relatively slow responses as compared to the resistant one.) The second type of resistance is effective against several pathogenic races of the attacking microorganism. However, this resistance is not perfect and it is not asssociated with the hypersensitive reaponse (HR). As a rule, race-specific resistance is determined by a single or a few genes. On the other hand, race-nonspecific resistance is influenced by several genes.

The interest of *biologists* or *biochemists* in resistance is quite different. They speak about preformed (passive), active and acquired resistance.

(1) Preformed resistance

This is based on the existance of physical or chemical barriers. The latter consists of antimicrobial substances that are not induced as a result of infection. They exist in plants whether they are infected or not. At present we know a few preformed substances that are toxic to pathogens. Receptor molecules in the host (e.g. lectins, phytoagglutinins) could be regarded as preformed resistance factors if the connection of receptor with the carbohydrate ligand in the pathogen results in an incompatible interaction. Only one case is known when a receptor (lectin), that is specific to some carbohydrate sugars, might specifically determine the host/microbe relationship. In fact, legume host susceptibility with typical symptom expression (nodulation), rather than resistance, to infection by a *Rhizobium* strain is determined by such a lectin-carbohydrate interaction. Díaz et al. (1989) have shown that the introduction of the gene from pea roots, encoding a glucose/mannose-binding pea lectin, into white clover roots allows infection of clover by a strain, *Rhizobium leguminosarum* bv. *viciae*, which is specific for plants from the pea cross-inoculation group but not for white clover. Thus, the legume-*Rhizobium* symbiosis is determined by lectin-mediated interaction. We can only speculate that similar lectins with well defined sugar specificity are present in plants.

(2) Active resistance

Here we have to deal with a problem. It is not clear at present whether susceptibility or resistance (compatible or incompatible host/microbe interaction) is the characteristic phenomenon, i.e. the actively induced reaction in the plant/pathogen relationships.

Plant scientists were convinced in the past that plant resistance, rather than susceptibility, to pathogenic attack is specifically induced as a result of host/pathogen interaction. This was believed by implication, in spite of the fact that non-host as well as host resistance to microbial infections seemed to be the rule and susceptibility the exception. Scientists were convinced that the pathogen is actively inhibited in growth and multiplication or even killed by an active host reaction. Consequently, susceptibility is a rather passive phenomenon as compared to resistance. Quantitative measurements of bacterial multiplication in resistant as well as in susceptible hosts supported this contention (Klement et al., 1964) (Fig. 1). The number of bacteria in a resistant plant levels off relatively early after infection. It appears that something, a hypothetical reaction, actively inhibits the growth of the pathogen in the resistant plant. Precise methods for enumerating the population of bacteria in the host tissues permit this conclusion. However, in the case of fungus diseases, there are not satisfactory methods to follow the early growth or multiplication of pathogenic fungi in the resistant or susceptible host plants (Daly, 1972). Thus, it is difficult to show that the very early gowth of the fungus (particularly a biotrophic one) is really inhibited in a resistant plant or stimulated in a susceptible host. When a specific toxin induces disease it would seem unlikely that an active resistance mechanism would inhibit the incompatible pathogen and its toxic effects in the resistant plant. On the contrary, toxins probably can facilitate the growth of the pathogen in the suscep-

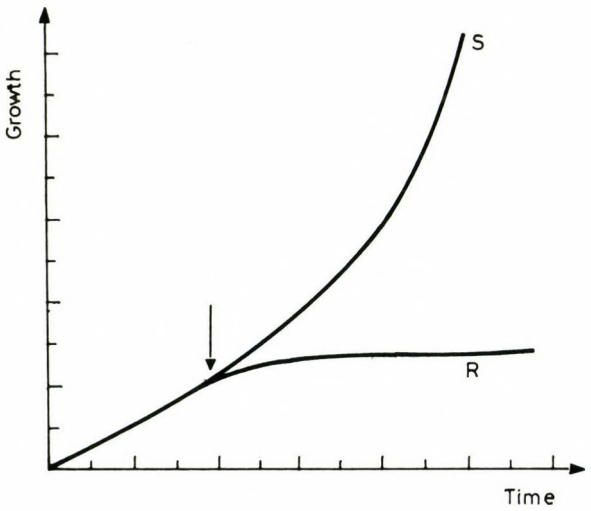

Fig. 2. Idealized curves for the pathogen's growth very early after infection according to the "induced (active) susceptibility" hypothesis. S=fungal growth in the susceptible host. R=fungal growth in the resistant plant

tible plant (Yoder, 1980). This would be a case of induced susceptibility. Fig. 2 represents this hypothetical situation.

In summary, one can not state with certainty whether infectious fungi in resistant plants are indeed inhibited as a result of an active mechanism (Fig. 1) or, conversely, the pathogen is actively stimulated in a susceptible host by several host responses (Fig. 2).

Interplay of signals between hosts and pathogens

The response of host plants as well as the reaction of pathogens are the result of a complex interplay of signals between the two organisms. In the past, some rapid responses of infected plants were in many cases causally related to disease resistance. Recently, it has been shown that early plant responses can affect biochemical activities of pathogens (cf. Dixon and Lamb, 1990).

(a) *Responses of pathogens.* Many infectious fungi secrete a cutinase enzyme to breach the intact plant cuticle and penetrate the host. Woloshuk and Kolattukudy (1986) demonstrated that cutin, and particularly cutin monomers, can induce the cutinase gene in pathogens and help to breach the cuticular barrier. The insertion of the cutinase gene of *Fusarium solani* f.sp. *pisi* into a *Mycosphaerella* sp. enabled the latter fungus, which is a wound pathogen, to penetrate the outer defensive barrier (cutin) of intact papaya fruits. The new gene in *Mycosphaerella* was inducible by cutin monomers of papaya cutin (Dickman et al., 1989). In most cases, the small amount of constitutive cutinase carried

by fungal spores is sufficient to release cutin monomers that trigger gene expression to levels required for succesful penetration.

Recently, it has been demonstrated that the stress response machinery of the host can produce factors that activate virulence genes in a bacterial pathogen, *Agrobacterium tumefaciens*, thereby facilitating the transfer of T-DNA into the plant cell. Phenolic compounds, such as acetosyringone produced as a result of wounding the host plant, signal the virulence gene activation in the pathogen (Stachel et al., 1985). A similar regulatory system signalled by isoflavonoids from legumes is involved in the activation of the *nod*-genes in *Rhizobium* spp. permitting thereby the first steps in specific initiation of nodulation (Peters et al., 1986, Györgypál et al., 1988). Common and host-specific *nod*-genes were shown recently to be involved in the production of a bacterial signal for specific root hair deformation, which is the first step in pathogenesis. This bacterial signal is a glucosamine oligosaccharide (probably a lectin ligand) (cf. Lerouge et al. 1990). Surprisingly, all of these initial signals are associated with susceptibility.

Specific signals may be required even at late stages of pathogenesis, not only for penetration but also for establishment of infection. An example is the accumulation of phytoalexins in infected plants that regulate the expression of a virulence gene of the pathogen. The phytoalexin-detoxifying enzyme in a *Fusarium* pathogen is induced by the phytoalexin pisatin, that accumulates in peas after infection. The above-mentioned enzyme contributes to the virulence of the fungal pathogen because it demethylates and thereby detoxifies pisatin in the host (Schäfer et al., 1989).

(b) *Responses of host plants.* As a result of successful infection, there are a series of metabolic changes in the host. Thus, we have to deal with multicomponent plant responses. These responses include accumulation of phenols, lignin, callose, phytoalexins, hydroxyproline-rich proteins, pathogenesis-related proteins, other stress proteins and stress compounds, deposition of silicium, activation of cutinase, chitinase, glucanases, cell wall-degrading enzymes, peroxidase, polyphenoloxidase, superoxid dismutase, catalase etc. (Fig. 3).

Some of the responses are demonstrable *early* in the course of pathogenesis, and those changes were frequently causally related to disease resistance. Some others appear only late after infection and these biochemical changes are associated, partly at least, with symptom expression. In other words, speed and timing of plant responses were causally related, by implication, with resistance or symptom expression. However, exact timing is extremely uncertain because of technical difficulties.

Several stress responses (phytoalexins, phenols, stress proteins and some enzymes) are regarded as specific reactions of the *resistant* plant. It is almost universally accepted that the visible symptoms of the resistant response is the hypersensitive reaction (cf. Király, 1980). Many plant pathologists believe, by implication, that if we know more about the mechanism of early plant response to pathogen challenge we will learn more about the mechanism of disease resistance.

However, two basic problems have emerged: First, the simultaneous appearance of some plant responses with the visible reaction of resistance is only a *correlative phenomenon.* It is difficult to provide good evidence for the role of an early plant response in disease resistance. It is hard to decide whether the simultaneous metabolic change in the infected resistant plant is a cause or a consequence of resistance. All of the metabolic

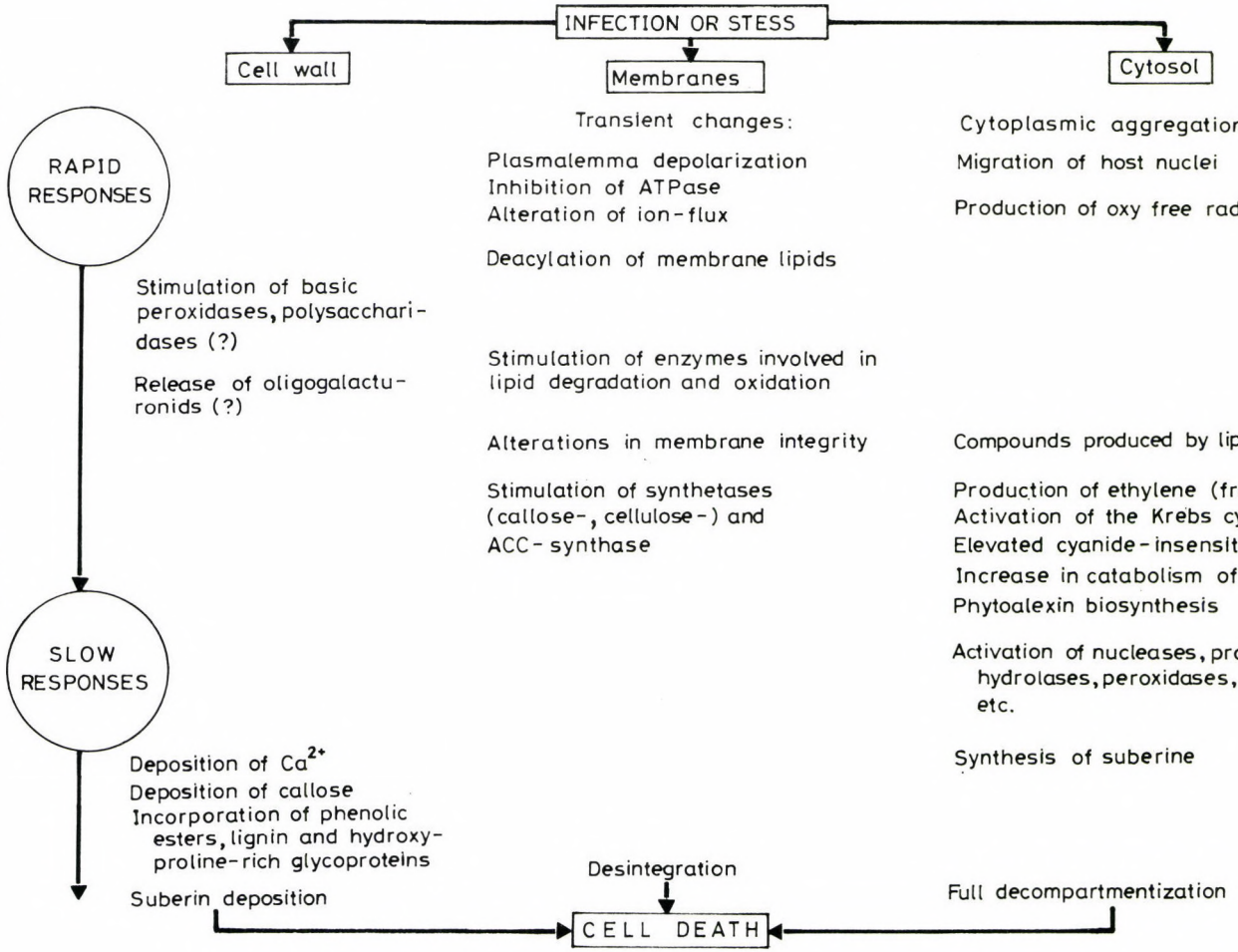

Fig. 3. Physiologic changes and metabolic responses in plant cell walls, membranes and in the cytosol as a result of infection or stress

changes associated with the hypersensitive reaction (activation of "defense" enzymes, synthesis and accumulation of "defense" compounds) or activation of "defense" genes could be the consequence of an early exchange of signals and an early damage to the pathogen, and, therefore, an early stress to plant. Consequently, the HR is not necessarily the cause of disease resistance (cf. Király et al., 1972, Daly, 1972). These situations seem to be true for phytoalexins, phenolics and enzymes involved in their synthesis, activation of chitinases, glucanases etc. In the past, in many case, it was not warranted to conclude that the resistant response necessarily referred to the mechanism of disease resistance.

In addition, practically all of the early and late plant responses after infection are *non-specific stress responses*. According to our experience, there is not a single reaction of the infected plant which is associated with resistance only and is independent from stress. The accumulation of stress compounds (phytoalexins etc.) in infected resistant as well as in susceptible host plants and the activation of stress-related enzymes represent aspecific responses of diseased as well as stressed plants (cf. Farkas, 1978, Goodman et al., 1986). There is a line of indirect evidence to believe that all of the metabolic changes (see Fig. 3) associated with resistance, or at least with the hypersensitive reaction, can be induced in infected susceptible plants if the pathogen is inhibited or damaged in the infected susceptible host. This phenomenon was shown first with potato infected by *Phytophthora infestans*. When the pathogen in susceptible potato tuber tissues was selectively inhibited by antibiotics, typical hypersensitive necrosis developed and the phytoalexin rishitin was accumulated in the infected susceptible host (Table 1). Similarly, susceptible wheat leaves infected with *Puccinia graminis* f.sp. *tritici*, susceptible bean leaves infected by *Uromyces phaseoli* developed hypersensitive type necroses when these fungi were inhibited or killed by heat treatment or by the application of selective chemicals (Király et al., 1972, Érsek et al., 1973, Barna et al., 1974). Interestingly, when the broad bean rust fungus (*Uromyces fabae*) was killed in the host by a rust eating bacterium,

Table 1

Activation of the hypersensitive reaction and phytoalexins in a compatible potato cultivar inoculated by a compatible race of *Phytophthora infestans* (Király et al., 1972)

Concentration of antibiotics (ppm)	Degree of necrosis "HR"	Rishitin content (μg/g fresh wt.)
0 (water control)	0	Trace
Chloramphenicol:		
50	+	41
100	+	62
200	++++	91
400	+++++	100
Streptomycin:		
3.125	+	12
6.250	+	45
12.500	++++	86
25.000	+++++	115

Erwinia uredovora, hypersensitive-like necroses developed in the susceptible host instead of the typical rust pustules (Hevesi and Mashaal, 1975). Several other researchers have reported that cell-free homogenate of the mycelium of the late blight fungus (*P. infestants*) can induce phytoalexin production and the hypersensitive reaction in potato tubers whether they are susceptible or resistant (cf. Goodman et al., 1986).

From the foregoing, we can conclude that the primary event in the naturally resistant host after infection is the adverse action of an unknown mechanism (recognition?) which alters or even inhibits the pathogen. The secondary event is a non-specific response of the resistant plant elicited by the damaged, inhibited or even the killed pathogen. This response could be associated with hypersensitive necrosis (HR), phytoalexin and aromatic accumulations etc. and the synthesis or activation of some enzymes as well as the genes encoding them. These genes are wrongly designated in some recent literature as "defense genes" because these genes are associated with aspecific stress responses, not necessarily with defense. This secondary and non-specific host response could be a consequence, not the primary cause, of plant resistance. It is worth to mention that inhibition of the hypersensitive plant's response in incompatible (resistant) tobacco leaves by infiltration of albumin into the intercellular space did not influence the multiplication of the inoculated incompatible bacteria in leaf tissues. In other words, in spite of the absence of the hypersensitive necrosis, multiplication of *Pseudomonas pisi* and *P. syringae* in the resistant tobacco still was inhibited (Király et al., 1977). This observation also supports the suggestion that the primary (basic) resistance reaction seems not to be related to the hypersensitive response and to other secondary biochemical responses.

It is logical to conclude that there exists a *two-phase mechanism* of resistance: (1) There is a primary (determinative) phase characterized by a *specific* recognition between the host and pathogen. In this phase the pathogen is inhibited or at least adversely affected by the resistant plant. The mechanism of this primary inhibition is unknown but likely to be the result of signal exchange. (2) The secondary, *non-specific* response is caused by elicitors from the damaged pathogen. In this expressive phase the characteristic symptoms of resistance (e.g. the HR) develop. The pathogen, however, could also be inhibited by the compounds accumulating as a result of non-specific, secondary responses (e.g. phenolics, phytoalexins etc.). However, the role of these non-specific plant responses in disease resistance remains a disputed question even today.

Recognition (primary determinative phase of resistance). As a result of the host/pathogen interaction the two organisms may recognize each other. For example, the host plant may recognize the "self" or the "not self" in the pathogen or vice versa, and the result is a compatible or incompatible host/pathogen relationship, respectively. For the first event, the best example could be the lectin/carbohydrate-mediated recognition of *Rhizobium* by legumes, as mentioned above. It was shown by Díaz et al. (1989) that susceptibility of a legume species to infection by a *Rhizobium* strain is determined by a host lectin/pathogen carbohydrate interaction (see also Lerouge et al., 1990). A host plant gene encoding a pea lectin allowed attachment, infection and colonization of white clover by a pathogenic strain originally specific for pea, but not for white clover, if that gene was transferred biotechnologically from peas to clover. In that case host susceptibility, rather than resistance, was determined by the lectin/carbohydrate interaction. In other words, recognition in this case determined compatibility!

Table 2

Inhibition of early attachment of *Pseudomonas syringae* pv. *glycinea* to isolated single leaf cells of two soybean cultivars in the case of incompatible host/pathogen interaction (Érsek et al., 1985)

Races of *Pseudomonas syringae* pv. *glycinea*	Cultivar Harosoy			Cultivar Flambeau		
	Plant response	Attachment	Inhibition by	Plant response	Attachment	Inhibition by
1	R (HR)	+	L-rhamnose	S	–	–
4	S	–	–	S	–	–
5	S	–	–	R (HR)	+	D-glucose
6	R (HR)	+	L-rhamnose	S	–	–

S = susceptible
R = resistant
(HR) = hypersensitive reaction

Only a few experiments with lectins or agglutinins have been performed where recognition determined incompatibility between the host and pathogen. Kojima et al. (1982) purified a spore-agglutinating factor from sweet potato root. This factor is an agglutinin-like pectic acid in host cell walls that inhibits the growth of incompatible strains of *Ceratocystis fimbriata*. The germinated spores of the compatible (pathogenic) strain of the fungus was insensitive to agglutination. Érsek et al. (1981) extracted an agglutination factor from soybean leaves that specifically agglutinated incompatible races of *Pseudomonas syringae* pv. *glycinea*, but not the compatible races. Another experiment has shown (Érsek et al., 1985) that the *P. syringae* pv. *glycinea* specifically attaches to isolated single leaf cells of resistant (incompatible) soybean cultivars as early as 30-180 min after inoculation. This early attachment can be inhibited by specific sugars in specific cultivars, which suggested that probably a lectin/carbohydrate recognition event determined incompatibility between soybean and *Pseudomonas*. For additional details see Table 2 and Fig. 4.

In conclusion, sugar specificity of the phenomenon suggests that lectins or lectin-like molecules can determine the specific attachment of the bacterium which is associated with incompatibility. However, the general role of phytoagglutinins is still unclear (Pueppke, 1984). Plant cell walls seem to be responsible for the early attachment phenomenon, because non-metabolizing or non-living plant cells are also able to bind the bacterial pathogen. Kojima et al. (1982) also believe that plant cell walls participate in the primary recognition process when hosts are infected with fungi. They suggest that agglutinins, as cell wall components, are widely distributed in the plant kingdom and may have role in recognition (resistance).

The non-specific, secondary response of host plants, as illustrated above, represents the expressive phase of disease resistance. In this phase the plant may respond with a rapid reaction associated with the hypersensitive reaction (HR) or with a late (slow) symptom development. Many investigators of disease resistance believe that the rapid

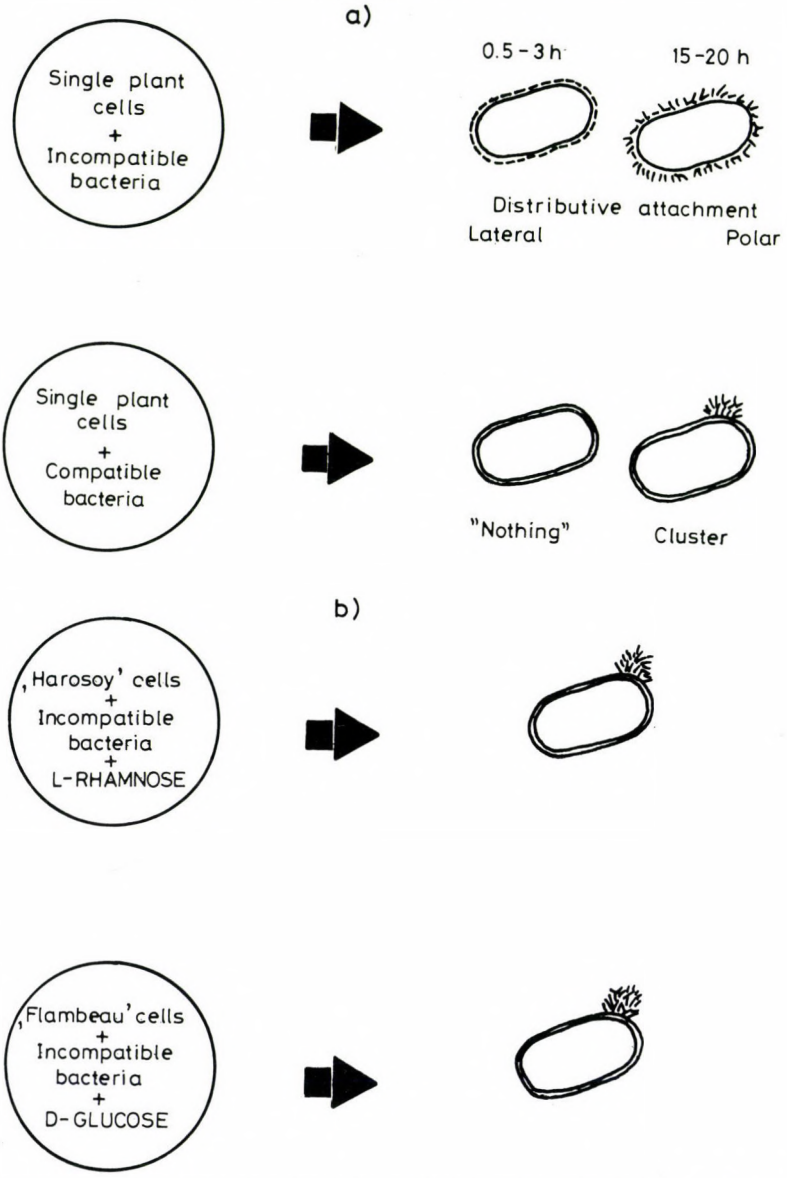

Fig. 4.a) Attachment of *Pseudomonas syringae* pv. *glycinea* to isolated single leaf cells from soybeans. Upper figure represents the combination of a resistant soybean cultivar and an incompatible strain of the bacterium. Under figure represents the combination of a susceptible host cultivar and a compatible bacterium. Note the early and distributive attachment of incompatible bacteria to the cell of a resistant cultivar. b): Inhibition of specific early attachment of incompatible bacterial strains by specific sugars in specific cultivars of soybean. Instead of the distributive attachment of bacteria to imcompatible (resistant) host cultivars bacterial clusters are formed on the resistant single soybean cells in the presence of L-rhamnose and D-glucose, respectively

hypersensitive response is causally related to resistance because of the simultaneous appearance of the two phenomena.

However, it must be realized that pathogens are damaged, prior to HR, (relatively early) in the resistant plants. Therefore, elicitors from the damaged pathogen may induce secondary non-specific plant responses relatively early in the course of pathogenesis. In other words, early induced plant response may be associated with early symptoms, not necessarily with the mechanism of resistance. Elicitation of rapid plant cell death (HR) is probably caused by the following sequence of events:

Reactive free radicals (e.g. $O_2^{\cdot-}$) → Lipid peroxidation → Membrane damage → Stress compounds (e.g. phytoalexins) → Cell death (HR)

The exact timing of the sequence of events is extremely difficult. Therefore, the cause- and effect relationships seem hard to be determined with certainty. Stress compounds, particularly phytoalexins, as early produced or accumulated anti-microbial agents are suspected to have a role in disease resistance. Schäfer et al. (1989) have shown recently that the gene encoding a phytoalexin demethylase enzyme in the pea pathogen, *Fusarium solani* may increase the ability of a fungal species (*Helminthosporium maydis*) non-pathogenic to peas to cause weak symptoms on pea if that gene is transformed into and is highly expressed in the originally non-pea pathogen, *H. maydis*. It was concluded from this experiment that phytoalexins can function in non-specific resistance of plants. This type of resistance is regarded as non-specific because a similarly transformed saprophytic fungus (*Aspergillus flavus*) also having the gene for phytoalexin demethylase was not able to affect pea. This demonstrated that this gene is not the specific gene for pathogenicity and that phytoalexins are involved in secondary, non-specific, rather than in primary, specific resistance of plants.

It has been shown recently that *active oxy free radicals* produced in the course of infection, can also be the elicitors of the non-specific, secondary response of host or non-host plants. Necrotic reactions associated with the hypersensitive rapid cell collapse (Doke, 1983, 1988, Keppler and Novacky, 1986, 1987, Aver'yanov et al., 1987, Ádám et al., 1989, 1990a, 1990b, Keppler and Baker, 1989, Croft et al., 1990, Király et al., 1990, Jordan és DeVay, 1990) as well as with normosensitive (compatible) necrotic disease symptom (Daub and Hangarten, 1983, Edlich et al., 1989, Popham et al., 1990) can be initiated by the production of reactive free radicals, such as $O_2^{\cdot-}$, $OH^{\cdot}$ etc., and/or by lipid peroxidation in infected plants.

In tobacco, before the appearance of the visible HR, an increased superoxide evolution was shown by the NBT reduction method. The hypersensitive tissue necrosis caused by the infection of *Pseudomonas syringae* pv. *syringae* was delayed by the application of oxy free radical *scavengers* or some other *anti-oxidants* (ascorbic acid, glutathione, albumin, Tiron, superoxide dismutase). A Tn5 transposon mutant of the bacterium could not induce $O_2^{\cdot-}$ production nor HR in inoculated leaves (Ádám et al., 1982), showing that oxygen radicals could be involved in the necrotic symptom expression and/or in the secondary resistance reaction of plants. Free radical production in potato tubers (Fig. 5) were also demonstrated with the ESR spectroscopic technique after treating tuber tissues with a crude elicitor from the pathogenic fungus, *Phytophthora infestans* (Király et al., 1990, Ádám et al., 1991).

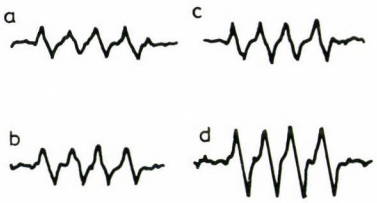

Fig. 5. ESR signals of enhanced free radical production in the microsomal fraction of potato tubers tissues (cultivar Magyar rózsa). a: Tiron only. b: Tiron + microsomal fraction of tuber tissue (3 h), c: Tiron + microsomal fraction of tuber tissue (24 h), d: Tiron + microsomal fraction + elicitor from *Phytophthora infestans* (24 h)

Thus, the hypothetical series of events that eventually lead to necrotic cell collapse could be initiated by free radicals. A similarly *non-specific* response, characterized by oxy free radical-induced deterioration of plant membranes, was also experienced as a result of senescence and wounding (Thompson et al., 1987). However, very few data are available as regards the mechanism of *in planta* production of oxy free radicals.

Whether the toxic free radicals cause only non-specific necrotic symptoms in plants or they also contribute to the resistance reaction of infected plants, remains to be seen. Our recent investigations (unpublished) support the hypothesis that oxy free radicals may inhibit or kill plant pathogens during the course of infection. In model *in vitro* experiments with *Pseudomonas syringae* pv. *syringae* and pv. *phaseolicola*, zoospores and cystospores of *Phytophthora infestans* and uredospores of *Puccinia graminis* f. sp. *tritici*, we demonstrated that the pathogens' spores or cells are adversely affected by the evolution of $O_2^{\cdot-}$ in a system of liquid or agar media that contained riboflavin and was illuminated. Thus, superoxide and other toxic free radicals may also serve as components of defense directly attacking the invading plant pathogens. It appears that they act both on host cells as well as on pathogenic cells, as they do in animals during the course of phagocytosis (Lachman, 1986, Prince and Gunson, 1987).

A few years ago Furusawa and co-workers (1984) selected a superoxide-resistant Samsun tobacco strain. In fact, tobacco callus tissue was selected *in vitro* against paraquat which is a known $O_2^{\cdot-}$-producing herbicide. In subsequent investigations we have shown (cf. Király et al., 1990) that in the superoxideresistant Samsun strain resistance to necrotic symptoms caused by viral, bacterial and fungal pathogens as well as by herbicides is also expressed. The superoxide resistant strain exhibited relative resistance even to the necrotic spots initiated by spraying the leaves with a 0.1% solution of $HgCl_2$. As compared to the sensitive leaves, necroses developed later and the spots were fewer and smaller whether they were caused by pathogens or $HgCl_2$.

The superoxide resistant strain exhibited several characters which were most probably associated with a more juvenile state, as compared to the sensitive one. Resistant plants were shorter, leaves were more green and, after detachment, senescence was delayed. Growth and maturation were also delayed, as compared to the sensitive strain.

The amount of phospholipids (and probably the stability of membranes) increased in the superoxide resistant strain (Ádám et al., 1990a).

The enzymatic as well as the non-enzymatic anti-oxidant system also seemed to be activated. The superoxide dismutase (SOD) activity was higher in the resistant than in the sensitive leaves. These results supported the original finding of Furusawa and co-workers (1984, 1988). On the other hand, glutathione reductase, glutathione-S-transferase and ascorbic acid peroxidase activities practically were the same in the uninfected or non-treated superoxide resistant plants as in the sensitive ones. However, the levels of non-enzymatic anti-oxidants (glutathione, ascorbic acid) were higher in the resistant strain (Gullner et al., 1991, Király, 1991). The high ascorbic acid level seemed particularly interesting. The proportion of the oxidized form (dehydroascorbic acid) in the resistant leaves was as high as 68% in contrast to the sensitive leaves where it was only 33%. Thus, oxidation of ascorbic acid and glutathione probably contributed to the high scavenging activity of the superoxide resistant leaves.

It has been shown that enzymatic defense (e.g. dismutation) against oxygen toxicity increases when endogenous levels of oxy free radicals are raised by paraquat treatment (Chia et al., 1981, Fridovich, 1978, Shaaltiel and Gressel, 1986). Treating tobacco leaves with paraquat and acifluorfen or infecting leaf tissues with a pathogen causing necrotic symptoms, the anti-oxidant systems were activated (Gullner et al., 1991, Király, 1991).

Particularly the glutathione oxidizing activity and the glutathione-S-transferase activity were increased after herbicide treatment. The latter enzyme is known to detoxify several toxic materials in plants and in animal tissues (Timmerman, 1989). The ascorbic acid content also increased after acifluorfen treatment or infection by tobacco necrosis virus (TNV).

We conclude that several stresses can induce endogenous anti-oxidant systems in tobacco. There are some differences between superoxide resistant and sensitive strains of tobacco after challenge by infections or herbicides. The higher *preformed* anti-oxidant power of resistant uninfected or non-treated plants probably also contributes essentially to the superoxide and disease resistance as well as to herbicide resistance of the Samsun tobacco strain, as compared to the sensitive strain which has a lower anti-oxidant capacity.

(3) Acquired resistance to secondary infections

This type of resistance develops after a primary infection and exerts its action on a second infection. Acquired resistance resembles immunity in animals, but the biochemical and physiological mechanism is quite different. The rather early experiments on *local* acquired resistance carried out by Müller and Börger (1941) called the attention to the role of the hypersensitive reaction and phytoalexin production of the host plant in acquired resistance to a subsequent infection. Incompatible potato tuber tissues were infected by an incompatible race of *Phytophthora infestans*. As a consequence of the incompatible reaction a protection developed in the plant tissue against a subsequent (secondary) infection by a compatible race of the same fungus and other pathogenic fungi. It was inferred by many investigators that the hypersensitive reaction or/and the production of phytoalexins inhibit the secondary challenge inoculum – the compatible pathogen – in addition to the initial (incompatible) pathogen.

Many non-host plants inoculated with non-pathogenic fungi and bacteria, as a result of acquired resistance, inhibit the diseases caused by the subsequently inoculated compatible pathogens. However, the mechanism of this type of resistance remained to be solved (cf. Ouchi et al., 1979, Matta, 1982).

The *systemic* acquired resistance is induced by a primary, mostly incompatible pathogen with the production of necrotic symptoms, and is effective agains secondary infections in distant plant organs. Thus, acquired resistance distributes to other plant organs, distant from the site of primary infection (Ross, 1966, Kuć, 1982).

Induction of systemic acquired resistance also elicits the incerased activities of peroxidase, β-1,3-glucanase and chitinase as well as the accumulation of pathogenesis-related proteins (Boller, 1987, Ye et al., 1989, 1990). The role of these alterations in acquired resistance seems controversial, although there are correlations between the appearance of acquired resistance and the activity of the above-mentioned enzymes and the accumulation of some stress proteins. However, Linthorst et al. (1989) for example have shown that expression of some genes of pathogenesis-related proteins in tobacco had no effect on susceptibility of the host to virus infection. Hammerschmidt and Kuć (1982) believe that some peroxidase-requiring biochemical changes, such as lignification, could be responsible for systemic acquired resistance.

Another finding is that cytokinin increases in the resistant leaves of tobacco, cucumber and barley, as a consequence of inoculation of the lower leaves by viruses, bacteria and fungi (Balázs et al., 1977, Sziráki et al., 1980, Doss et al., 1980, Doss, 1981, Sarhan et al., 1991). Whether the juvenility effect of the elevated hormon action or the oxygen radical scavenging properties of cytokinins (Leshem, 1984) have role in acquired resistance, remains to be demonstrated. Kuć (1985) supports the hypothesis that systemic acquired resistance is multicomponent. The multicomponent alterations in the sytemic protected leaves triggered by the primary (inducer) inoculation may explain the non-specific character of acquired resistance to several unrelated pathogens.

For a long time only little was known about the *signal transduction* pathways that are activated during systemic acquired resistance. Recently, a natural transduction signal was discovered by two research groups (Malamy et al., 1990 and Métraux et al., 1990). In tobacco infected by TMV and in cucumber infected by TNV and *Colletotrichum lagenarium* a fluorescent metabolite, salicylic acid was observed to increase transiently after the inducer primary inoculation. The peak of salicylic acid reached before the systemic acquired resistance was detected. It seems likely that salicylic acid, as a signal compound, activates the systemic acquired resistance mechanisms. In addition, correlative phenomena: the activities and genes of some pathogenesis-related proteins (e.g. chitinase, PR1 stress protein) are also stimulated. It is worth to mention that signal transduction and the mechanism of systemic acquired resistance are two distinct phenomena.

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Pathogenesis-related Proteins in *Nicotiana glutinosa* Leaves I. Effects of the Necrotic Process Induced by TMV or by Chemicals

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Changes in the soluble protein fraction from leaves of *Nicotiana glutinosa* inoculated with TMV or treated with $CuSO_4$ and HNO_3 were analysed by discontinuous-polyacrylamide gel electrophoresis. Only one b-protein fraction (Rf 0.71) increases in infected leaves parallel to the severity of the necrotic process and the maturation of local lesions. This b-protein is present also in leaves from healthy plants, though in lower amount. It is slightly increased by 3% $CuSO_4$, but not by 5% HNO_3. It is concluded that PR-proteins are of host origin and that their synthesis is stimulated, but not induced *de novo*, by the necrotic process due to the localization of the virus, whilst necrosis evoked by the chemical injury is far less or not effective.

The basic mechanisms underlying the process of virus localization in plants and formation of local necrotic lesions are far from well known (Kassanis, 1981; Sela, 1981; Goodman et al., 1986; Van Loon, 1987). Cell necrobiosis induces changes in the pattern of soluble leaf proteins (Van Loon, 1982; Redolfi, 1983). The involvement of host-specific, low molecular weight proteins – pathogenesis-related (PRs) or b-proteins – in the induction of both local and systemic resistance against a challenge inoculation is still debated (Sela, 1981; Fraser, 1982; Gianinazzi, 1982; Fraser and Clay, 1983; Van Loon, 1985 and 1987) and their role in the active defence mechanisms of the plant remains elusive.

It has been proposed that the induction of PRs might be the result of some type of biological or non-biological stress (see Gianinazzi, 1982; Van Loon, 1983 and 1985 for reviews). In fact, they can also be induced by various necrotizing and non-necrotizing chemicals. Moreover b-proteins have also been found in leaves of healthy plants during flowering (Fraser, 1981) and during natural leaf senescence (Conejero et al., 1979; Pennazio, 1981; Camacho Henriquez and Sänger, 1986).

This study was undertaken in the attempt to acquire more knowledge about the relationship between the course and severity of the necrotic process, its biological and non-biological induction, and induction of the pathogenesis-related proteins. We have investigated the changes of electrophoretic pattern of acid extractable proteins in *Nicotiana glutinosa* leaves following either inoculation with different concentrations of TMV under the effect of light and dark, or treatment with $CuSO_4$ and HNO_3.

Materials and Methods

Host plant and virus inoculation

Plants of *Nicotiana glutinosa* L. were grown in a green-house at 23-25°C and 60-80% r.h.. About 70 days after sowing, the growing tips and all the leaves but four intermediates were removed. The plants, selected for uniformity, were then transferred into a growth chamber at 22°C (±1), 60-80% r.h., 16 h photoperiod, and 6000 Lux light intensity at leaf level.

Five days later the plants were mechanically inoculated with a purified suspension of the common strain of tobacco mosaic virus (TMV-C), using carborundum 600 mesh as an abrasive. The virus was diluted with 0.01 M phosphate buffer *p*H 7.0; inocula of different concentrations (0.2 - 0.4 - 0.6 - 2.0 - 3.0 μg/ml) were used in the various experiments in order to induce increasing lesion numbers. Immediately after inoculation, the leaves were rinsed with tap water.

For time course experiment, one group of plants was kept under the light conditions described above; another group was transferred to a completely darkened section of the same growth chamber immediately after TMV inoculation (1 μg/ml). Thereafter, leaf samples were collected each 24 h, until the 8th day from illuminated plants whilst the sampling from plants kept in the dark lasted 96 h after inoculation due to the clear symptoms of withering shown by the plants in the following days.

Controls consisted of plants of the same age which received a mock inoculation with carborundum and buffer (rubbed controls). Other plants of the same group were kept untreated (untreated controls).

Copper sulphate and nitric acid treatments

Sixthy droplets (10 μl each) of a 3 % $CuSO_4$ solution or of a 5 % HNO_3 solution were applied to the upper leaf surface (3 leaves per plant) in order to induce necrotic lesions. Control leaves were similary treated with water droplets.

Extraction of soluble leaf proteins and discontinuous polyacrylamide gel electrophoresis (disc PAGE)

Extraction of soluble proteins was carried out by grinding leaf samples in a mortar at 4°C, using 1 ml/g of Mc Ilvaine buffer *p*H 2.8 containing 0.5 M sucrose and 0.3% (v/v) mercaptoethanol. The extracts were treated and analysed by nondenaturing disc PAGE in 7% gels (Davis, 1964). Aliquots corresponding to about 100 μg of proteins were applied to the gels and bromophenol blue was normally added as a marker.

Electrophoresis was carried out at 4°C in glass tubes (8.0×0.6 cm i.d.) using a Shandon kit at a constant current of 2 mA/tube for 20 minutes, thereafter increasing to 4 mA/tube until the bromophenol marker was a few millimeters from the end of the gel. The relative mobility of the protein bands (Rf values) was expressed taking as 1.0 the distance travelled by bromophenol blue. After electrophoresis, the gels were stained

overnight with 0.03 % Coomassie brilliant blue (CBB R 250) in methanol-acetic acid-water mixture (5:1:5) and destained in 10 % acetic acid. The discoloured gels were recorded by densitometry using a mod. DD2 Kipp and Zonen densitometer equipped with a mod. BD7 linear recorder.

Results

Changes related to the number of necrotic lesions induced by TMV

The counting of local lesions evoked by inocula of different concentrations was carried out 96 h after inoculation (Table 1). For the duration of the experiment, the plants were kept under the light conditions described above, and at the moment of the counting the lesions were completely mature and appeared as well defined, brownish small circular spots surrounded by a necrotic ring (Figs. 1.1 and 1.3).

At the same time, leaf samples were harvested for electrophoresis. The densitometer tracings of soluble proteins extracted from infected leaves showed quantitative changes in the b-proteins zone, as compared to mock-inoculated controls (Fig. 2). A peak with Rf 0.71 increased parallel to the intensity of the necrotic process, but when the leaf blade was almost completely necrotic (= uncountable lesions) no further increase was observed.

The electrophoretic pattern of soluble leaf proteins from mock-inoculated plants (Fig. 2d) and from untreated controls did not differ: in both samples the 0.71 band was present at a very low level.

Time course changes under the effect of light and dark

In the plants kept in the dark, lesions became visible later and were larger than in the illuminated plants. The lesions were grey and had a "water-soaked" appearance, without necrotic ring (Figs. 1.2 and 1.4). Lesions were counted 96 h after inoculation: their number/cm^2 was 3.6 and 3.2 in illuminated and darkened plants respectively.

Table 1

Necrotic lesions induced in *N. glutinosa* leaves by TMV inocula of increasing concentrations

TMV concentration (μg/ml)	Mean lesion number/cm^2
0.2	0.7
0.4	1.6
0.6	2.2
2.0	uncountable
3.0	//

Lesions were counted 96 h after inoculation
Each figure is the mean of 18 leaves taken from six plants

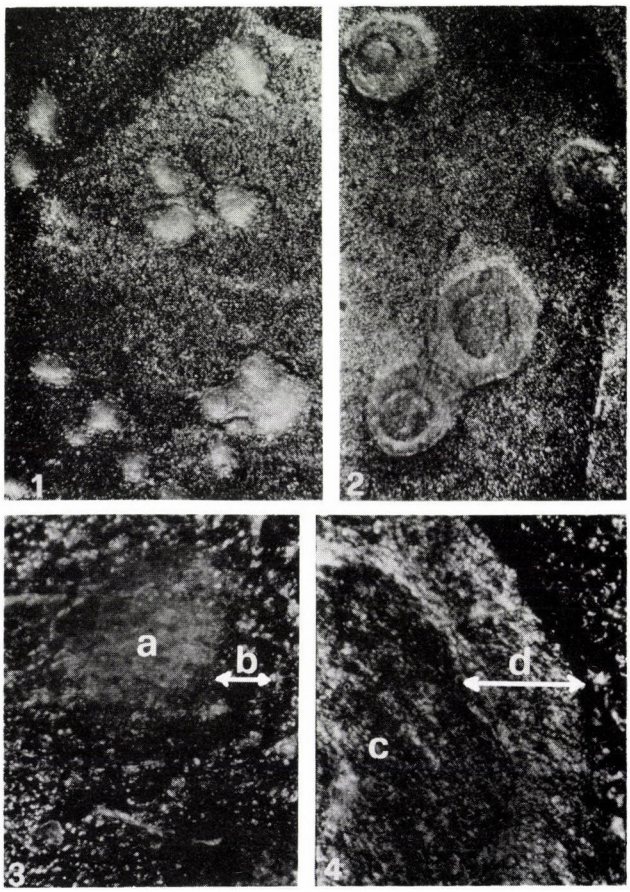

Fig. 1. TMV-induced local lesions in *Nicotiana glutinosa* leaves 96 h after inoculation. In illuminated plants (1.1 and 1.3) lesions are mature; the center is entirely collapsed (*a*) and surrounded by a well defined necrotic ring (*b*). In darkened plants (1.2 and 1.4) lesions are larger, have a "water-soaked" appearance (*c*) and the outer border is prominent but not necrotic (*d*). Photographs were taken under a Wild Makroskop M420 (1.1 and 1.2 = 23 x ; 1.3 and 1.4 = 82 ×)

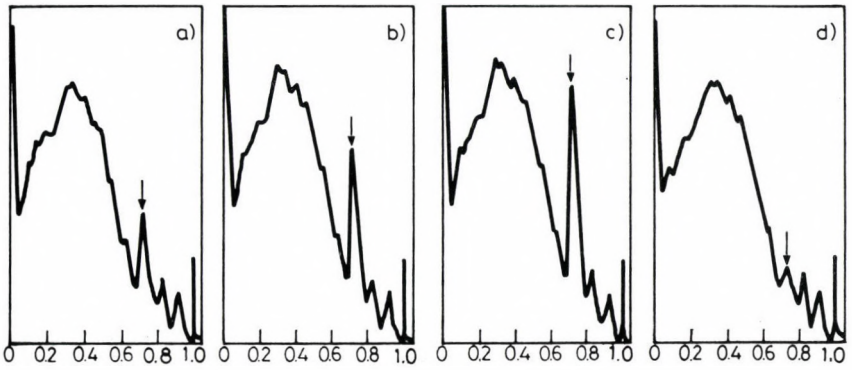

Fig. 2. Densitometer tracings of Disc-PAGE of soluble proteins (100 μg/gel; 7% acrylamide, stained with Coomassie blue) from *Nicotiana glutinosa* leaves 96 h after inoculation with increasing concentrations of TMV. *a*: 0.2 μg/ml; *b*: 0.6 μg/ml; *c*: 2.0 μg/ml; *d*: control (rubbed leaves). The arrow indicates the b-protein with Rf 0.71

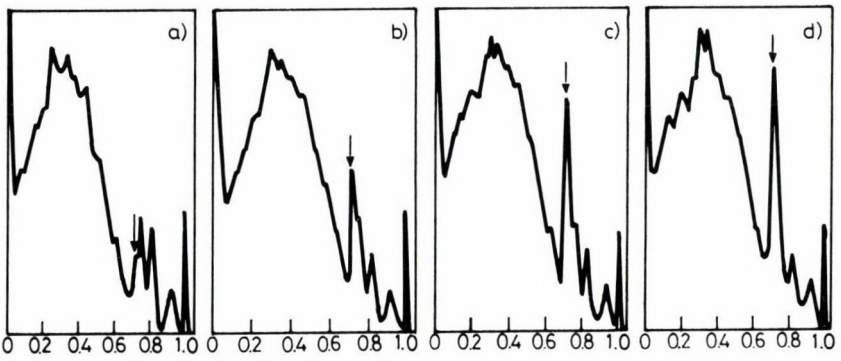

Fig. 3. Densitometer tracings of Disc-PAGE of soluble proteins (100 μg/gel; 7% acrylamide, stained with Coomassie blue) from illuminated leaves of *Nicotiana glutinosa* at different times after inoculation with TMV (1 μg/ml). *a*: 24 h; *b*: 72 h; *c*: 96 h; *d*: 192 h. The arrow indicates the b-protein with Rf 0.71. For rubbed control leaves (96 h) see Fig. 2*d*)

The electrophoretic pattern of soluble leaf proteins extracted from illuminated plants are reported in Fig. 3: 24 h after inoculation, when no symptoms were evident, the 0.71 band was very weak (Fig. 3a). In the following days the 0.71 band increased markedly, parallel to the maturation of the lesions (Figs. 3b and 3c). When the necrotic process was definitely established no further significative changes were evident (Fig. 3d).

Fig. 4 shows the protein pattern of plants kept in the dark: in the b-proteins zone no important changes were evident: the 0.71 band showed only a slight increase at the time of lesion appearance (Fig. 4b) and remained unchanged thereafter.

Effects of chemical treatments

The topical deposition of 3% $CuSO_4$ solution induced a localized necrotic reaction comparable (0.8 lesions/cm^2) to that evoked by TMV 0.2 μg/ml. Moreover, the lesions due to the chemical developed like those induced by TMV: they appeared 48 h after the deposition of the droplets and at the moment of the sampling (72 h after treatment) appeared already mature, as small circular spots surrounded by an evident necrotic ring.

At the same time samples were harvested from leaves treated with 5 % HNO_3 solution, that induced 1.4 lesions/cm^2. The necrotic reaction was in this case more severe, and the lesions appeared very soon after the localized deposition of the chemical; their aspect did not change in the following days.

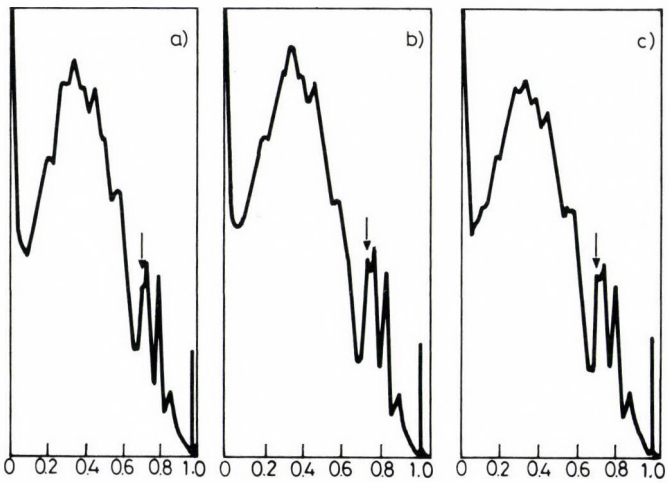

Fig. 4. Densitometer tracings of Disc-PAGE of soluble proteins (100 μg/gel; 7 % acrylamide, stained with Coomassie blue) from leaves of *Nicotiana glutinosa* kept in the dark at different times after inoculation with TMV (1 μg/ml). *a*: 24 h; *b*: 72 h; *c*: 96 h.
The arrow indicates the b-protein with Rf 0.71

The electrophoretic patterns of soluble proteins extracted from $CuSO_4$ or HNO_3 treated leaves are reported in Fig. 5, which shows that the two chemicals had a different effect on the b-proteins. $CuSO_4$ enhanced the 0.71 band (Fig. 5a) with an effect comparable to that of TMV 0.2 μg/ml (Fig. 2a). On the contrary, HNO_3, though inducing a higher number of necrotic lesions, had no noticeable effect on the same peak (Fig. 5b) and did not modify the intensity of the 0.71 band if compared with control (Fig. 5c).

Discussion

Our results clearly show that the hypersensitive reaction due to TMV infection in *N. glutinosa* leaves implies changes only in one component (Rf 0.71) in the b-proteins zone. This agrees with other statements showing that from this point of view *N. glutinosa* differs from *N. tabacum* Samsun NN and Xanthi *n.c.*, in which the appearance of 4 to 9 new b-proteins was shown (Van Loon and Van Kammen, 1970; Gianinazzi et al., 1970; Ahl and Gianinazzi, 1982; Ahl et al., 1982; Antoniw and White, 1987).

Contrasting results have been obtained in the attempt to elucidate if PR-proteins are normal constituents of the plant (Barker, 1975; Van Loon, 1976) or their synthesis is either stimulated or induced *de novo* by the hypersensitive reaction (Van Loon, 1985). Actually, small but significant amounts of PR-proteins, undetectable by Page, were found consistently in healthy leaves of *N. tabacum* Xanthi *n.c.* (Antoniw et al., 1985) and the presence of such proteins in healthy plants of different species during flowering (Fraser, 1981) or natural leaf senescence (Conejero et al., 1979; Pennazio, 1981; Camacho and Sänger, 1982; Nassuth and Sänger, 1986) has been shown.

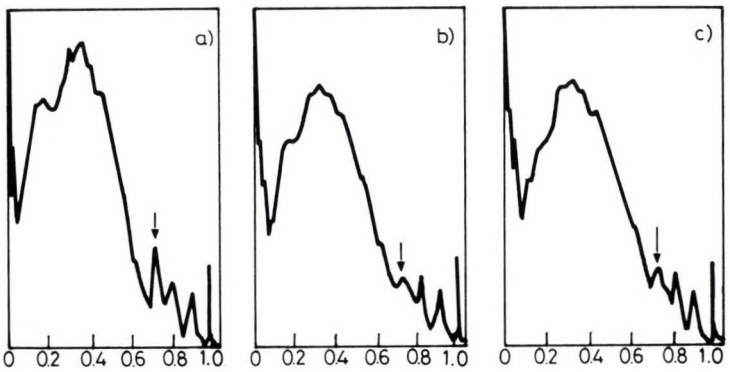

Fig. 5. Densitometer tracings of Disc-PAGE of soluble proteins (100 μg/gel; 7% acrylamide, stained with Coomassie blue) from *Nicotiana glutinosa* leaves 72 h after the treatment with 60 droplets (10 μl each) of 3% $CuSO_4$ (*a*) and 5% HNO_3 (*b*) per leaf. *c*: healthy control (60 droplets of tap water per unrubbed leaf)

Evidence presented here shows that a low amount of the b-protein with Rf 0.71 is also present both in untreated and in mock-inoculated leaves of healthy control plants. It seems therefore that the b-protein synthesis is stimulated but not induced *de novo* by TMV-evoked necrosis. Its increase is parallel to the severity of the necrotic process, *i.e.* to the number of local lesions and their maturation. The occurrence of the PR-protein we have found in healthy plants suggests that in our experimental conditions plant gene(s) encoding for this protein is normally operating. The m-RNA coding for PR-proteins (Carr et al., 1982) seems therefore to be activitated by the stimulus of viral infection.

Detectable amounts of PR-proteins in the intercellular fluids of leaves from hypersensitive *Nicotiana* species infected with TMV have been found by other authors (Parent and Asselin, 1984; Ohashi and Matsuoka, 1987; Grenier et al., 1988), whilst their presence in mock-inoculated healthy leaves is still debated (Parent and Asselin, 1984; Ohashi and Matsuoka, 1985).

In plants kept under dark conditions after inoculation necrosis is delayed, localization is hampered and the 0.71 b-protein remains almost unchanged. This result is in agreement with the data presented by Abad et al. (1986), who did not observe b-protein synthesis in Xanthi *n.c.* plants infected with TMV and kept in the dark, and enforces the conclusion by Asselin et al. (1985) that light is a major factor influencing the level of b-protein in the intercellular fluid of TMV-infected *Nicotiana* plants.

Pathogenesis related proteins can also be induced or stimulated by the application of a variety of chemicals, like polyacrylic acid (Gianinazzi and Kassanis, 1974; White et al., 1983), 2-chloroethylphosphonic, benzoic, salicylic and acetilsalicylic acids (Van Loon, 1977; White, 1979; Van Loon and Antoniw, 1982; Dumas and Gianinazzi, 1986), salicylates (Pennazio et al., 1987), mannitol (Pierpoint et al., 1981; Wagih and Coutts, 1981), mercuric chloride (AbuJawdah, 1982; De Tapia et al., 1986) and copper sulphate (Wagih and Coutts, 1982).

It seems therefore that induction and/or stimulation of b-proteins is the result of some type of stress (Pierpoint et al, 1981; Wagih and Coutts, 1982; Asselin et al., 1985) and that m-RNA coding for pathogenesis-related proteins is stimulated also by chemical treatments (Carr et al., 1982). However necrotic reaction evoked by infectious agents appears to be more efficient than necrosis induced by abiotic agents: the results presented here show that treatment with 3 % copper sulphate, though inducing a number of local necrotic lesions per leaf comparable to that produced by TMV 0.2 μg/ml, is far less effective than viral infection in stimulating the synthesis of the 0.71 b-protein. It would be interesting to investigate the effect of $CuSO_4$ in stimulating the production of ethylene, which is produced in plant tissues as a general reaction to stress and which is assumed to be the physiological inducer of PRs (Van Loon, 1985). However, if the necrotic process provokes the sudden death of treated cells – as is the case of 5 % HNO_3 – no stimulation of b-protein synthesis in the adjacent cells seems to be induced. This last result confirms that the production of PR-proteins is not a generalized stress response (White et al., 1986), but a specific reaction to certain forms of inducing stress.

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Use of Toxic Substances from *Phytophthora infestans* (Mont.) de Bary in Screening for Late Blight Resistant Potatoes

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Mycelial homogenate (MH) of *P. infestans* that elicits hypersensitive necrosis in potato leaves and tuber slices, as well as culture filtrate (CF) of the fungus that induces water soaking in leaves, caused a remarkable drop in the viability of potato root cap cells. The degree of response to both MH and CF of leaflets from different crosses inversely correlated with that of race-nonspecific resistance. Root cap cells from a cross of resistant parents were considerably less sensitive to the toxic materials than those from a cross of susceptible parents. Cells from a cross of resistant and susceptible parents, however, appeared to be the most sensitive. Race-nonspecific resistance to *P. infestans* thus seems be related to toxin damage to tissues. However, it remains to be determined whether susceptibility of root cap cells to *P. infestans* toxins can be used to asses late blight resistance.

The potato late blight fungus, *P. infestans*, has been reported to contain and to produce toxic substances that may contribute to the formation of symptoms caused by infection (Saveleva and Rubin, 1963; Sato et al., 1968; Varns and Kuc, 1971; Érsek, 1977; Bhenke and Lönnendonker, 1977; Bhenke, 1979; Bostock et al., 1981; Barna et al., 1987; Protsenko, 1987). Crude mycelium homogenate (MH) can elicit the hypersensitive response of tubers and leaves of potato, regardless of the race it derives from or the cultivar used (Varns et al., 1971). Tubers from cultivars with a high degree of race-nonspecific resistance[2] to *P. infestans* exhibit much weaker necrotic symptoms to MH than those with a low degree of resistance (Érsek et al., 1989). Culture filtrate of the fungus, on the other hand, is toxic to leaves, causing water soaking and wilting, symptoms characteristic of the fungal infection in susceptible cultivars. Interestingly, it does not induce any visible alterations in tuber tissues (Barna et al., 1987, Érsek and Barna, 1988). Preliminary tests have shown that leaves of cultivars possessing a high degree of race-nonspecific resistance are less sensitive to CF than those that are weakly resistant (Barna et al., 1987). Thus, the use of such toxic substances from *P. infestans* may help develop *in vitro* methods for selection of potato lines resistant to late blight.

Recent studies have explored the use of living single cells that separate naturally by sloughing off from the surface of root caps (Knudson, 1919; Hawes and Wheeler, 1982; Hawes, 1983). Viability of these root cap cells can be maintained for hours in water or for several weeks in a nutrient medium (Knudson, 1919). Moreover, they can be induced to divide and grow into callus and then develop into a full plant (Caporali, 1983; Hawes

1 To whom request for reprints should be addressed
2 Degree of race-nonspecific resistance is based on field test of spontaneous infection of foliage and on evaluation of tuber response to inoculation with a mixture of several races (Lönhárd, pers. comm.)

and Pueppke, 1986). In addition to the aforementioned features of root cap cells, they may also offer a suitable tool in selection for disease resistance. Susceptibility of corn and oat plants to *Helminthosporium* spp. was reported to be correlated with the sensitivity of root cap cells of these plants to toxins from the respective fungal species (Hawes, 1983, Hawes and Wheeler, 1982 and 1984).

The current work was conducted to test the toxicity of MH and CF on root cap cells, in comparison with leaflets of potato crosses from parents with various degrees of late blight resistance.

Materials and Methods

Fungal material

P. infestans, race 2.3.4 was maintained on pea-broth agar in 9-cm diam. Petri plates at 18°C in the dark. Two mycelial plugs of 8 mm diam. taken from the edge of 8-day-old cultures were transferred into 250 ml Erlenmeyer flasks containing 50 ml of synthetic, Henniger-type medium (Henniger, 1959).

At harvesting, cultures grown for 8 to 9 days at 18°C (in exponential growth phase) were filtered through Whatman paper then 0.45 μm pore size membrane filter (Paraplan, KIPSZER, Hung.). Combined filtrates designated as CF were stored at -20°C until use.

Mycelial mats were thoroughly washed with distilled water, squeezed then kept frozen. One g of frozen mycelium was homogenized in a mortar and pestle in 10 ml of ice-cold distilled water and sonicated at maximum intensity of an MSE sonifier for 10 min. The supernatant obtained after centrifugation at 6000 *g* for 20 min was autoclaved and designated MH (Érsek et al., 1989).

Plant material

Potato (*Solanum tuberosum* L.) crosses 79.16855 × Kondor (R×R), Ke.49 × 508 (R×S) and 78.6088 × 1188.N (S×S) were used. Parents of these crosses were race-non-specifically resistant (R) or susceptible (S) (Lönhárd, personal comm.). Seeds were surface-sterilized in 95% ethanol for 5 min then in 50% commercial bleach (approx. 6% sodium hypochlorite) for 5 min. This was followed by rinsing five times with sterile distilled water. After soaking for 1 h in distilled water, seeds were germinated at 22°C in Petri plates on 1% water agar overlaid with sterile filter paper. When roots were 10 to 20 mm long (5 to 6 days, depending on the growth rate), root cap cells were harvested. Five root tips were immersed in 200 μl water in each well of a microtiter plate. After 10 min, the water was agitated by taking it up and releasing it several times with a Pasteur pipette (Hawes and Pueppke, 1986). Cells were then collected by filtration of the suspension through a membrane filter (Paraplan) of 0.8 μm pore size and resuspended in distilled water so that a suspension of at least 2×10^3 root cap cells per ml was obtained.

After 3 days of incubation on water agar (before germination) seeds were planted in pots containing loam and grown in a glass-house. Terminal leaflets of second and third

leaves from 1-month-old plants (F_1 progeny) were excised and treated with toxic substances MH or CF from *P. infestans*.

Treatments

The bioassay with root cap cells was carried out in wells of a microtiter plate. Each well was filled with 90 μl of cell suspension to which 10 μl water or medium (as controls) or MH or CF was added. The mixtures were then incubated at 22°C. At various time intervals, 16 μl samples were taken and mixed with 4 μl of 2% (w/v) Evan's blue on a microscope slide then assessed for cell viability after 2 min. Viability was determined by the ability of cells to exclude the stain as observed microscopically and calculated as a percentage of the control.

Top leaflets of the excised leaves were immersed in vials containing CF and incubated at 20-22°C in glass-house. The volume of the CF was kept constant.

Leaflets, with abaxial surface up, were placed on moistened filter paper in Petri plates and 50 μl of MH was applied and spread over an area approximately one-third of the leaflet. Twenty-four h later, the drops were removed and the leaves were turned over.

Results and Discussion

Previous results and preliminary tests revealed that cultivars with a high degree of race-nonspecific resistance, as compared to those susceptible to races of *P. infestans*, were less sensitive to the *P. infestans*-derived toxic materials, MH and CF (Barna et al., 1987; Érsek et al., 1989). Furthermore, responses of root cap cells of certain plant species to toxins, bacterial binding and zoospores have been reported to be consistent with those of the whole plant (Hawes and Wheeler, 1982; Hawes and Pueppke, 1987; Goldberg et al., 1989). On this basis, we expected that root cap cells from resistant rather than from susceptible potatoes would be resistant to MH and CF. Root caps of potato crosses used yielded 4-12 cells per root (Fig. 1a and b). This yield, as compared to that of certain other plant species, is considered to be low (Hawes and Pueppke, 1986). The viability of cells obtained ranged from 65 to 72% and remained so for at least several hours. Thus the duration of assays (up to 2 h) did not affect viability.

The MH that elicits hypersensitive necrosis in tubers and leaves and the CF that causes water soaking and wilting in leaves of potato were found to decrease viability of potato root cap cells. The effect was diminished by dilutions of these materials (Table 1). Cells derived from a cross of susceptible (S) parents (78.6088 × 1188.N) were remarkably more sensitive to both MH and CF than those of a cross of late blight resistant (R) parents (79.16855 × Kondor) (Fig. 2a and b). Cells of a cross from resistant (R) and susceptible (S) parents (Ke.49 × 508), instead of reacting in an intermediate manner as anticipated, were even more sensitive to MH and CF than cells of the susceptible (S×S) cross. Cell viability decreased rapidly after treatments, and stabilized after approximately 20 min in each combination (Fig. 2a and b).

Table 1

Viability (%)[a] of root cap cells of a potato cross (78.6088 × 1188.N) susceptible to races of *P. infestans*, as affected by serial dilutions of culture filtrate (CF) and mycelial homogenate (MH) of the fungus

Dilutions[b]	CF	MH
10^{-1}	45.6±2.2[c]	44.2±2.4
10^{-2}	61.4±0.8	63.3±2.6
10^{-3}	88.0±1.0	85.2±2.6

[a]Cells excluding the dye Evan's blue were considered alive

[b]Dilutions indicated are the final ones in the suspensions. (Dilution series was made from non-diluted substances; each dilution became ten-fold diluted in the mixture.)

[c]Data are means (±SE) of 3 separate experiments and expressed as percentages (±SE) of corresponding controls (100%) after a 40 min treatment

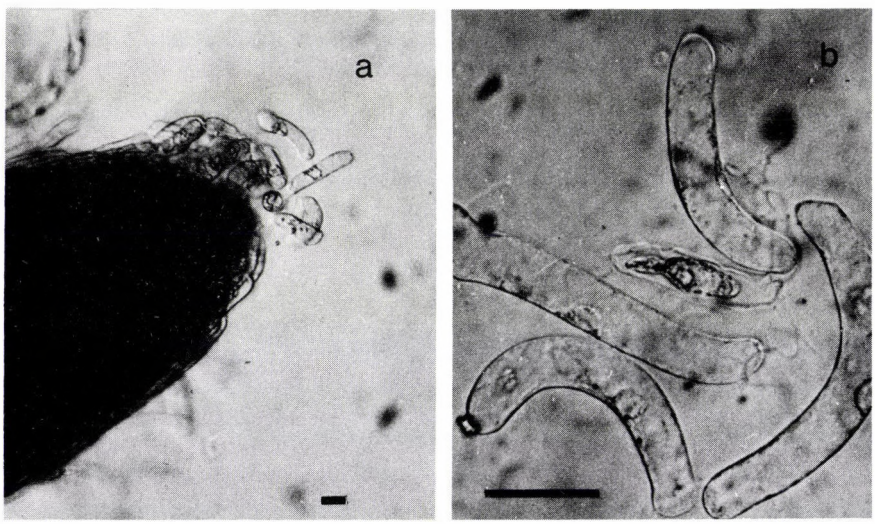

Fig. 1. Root tip of a potato seedling 2 min after the addition of water (a) and sloughed peripheral root cap cells (b). Scale 50 μm

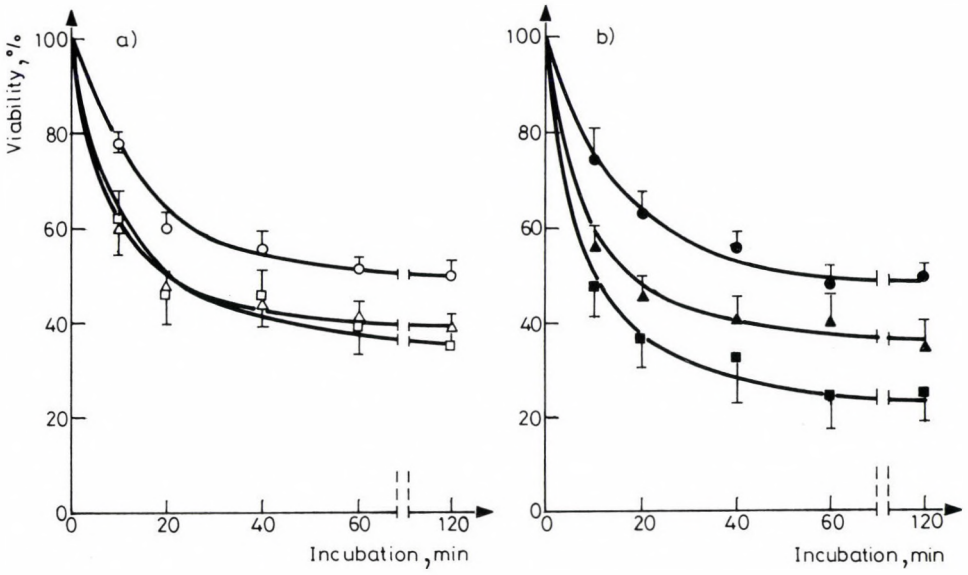

Fig. 2. Effect of mycelial homogenate (a) and culture filtrate (b) from *P. infestans* on the viability of potato root cap cells obtained from crosses of parents race-nonspecifically resistant (R) and/or susceptible (S) to the fungus ○, ● ; □,■ and △, ▲ refer to crosses R×R, R×S and S×S, respectively. Symbols (±SE) represent counts of at least 300 cells in 6 separate experiments, and expressed as percentages of controls

Furthermore, the bioassay was extended to determine whether/or not the phenotype of root cap cells accurately reflects that of the plant of the respective cross. The degree of response of leaflets to both MH and CF inversely correlated with resistance to the fungus (Fig. 3a and b). The leaflets, contrary to root cap cells, of the cross Ke. 49 × 508 (R×S) exhibited responses intermediate between those of fully susceptible (S×S) and fully resistant (R×R) crosses.

Although the use of root cap cells seems to provide a means to easily and economically test toxic substances and select pathogen-resistant plants, it remains to be further studied whether this tool is applicable to *P. infestans*-produced toxic substances. The fact, however, that resistance of leaflets to MH and CF and that of tubers to MH, at least in extreme cases, correlates with race-nonspecific resistance of intact potato plants gives rise to the hope that it is a general phenomenon. If so, this would alleviate the routine screening for late blight resistant potatoes.

Several types of toxic materials of *P. infestans* that cause destructive changes in various organs of potato have been reported (cf. Protsenko, 1987). The toxic activity of

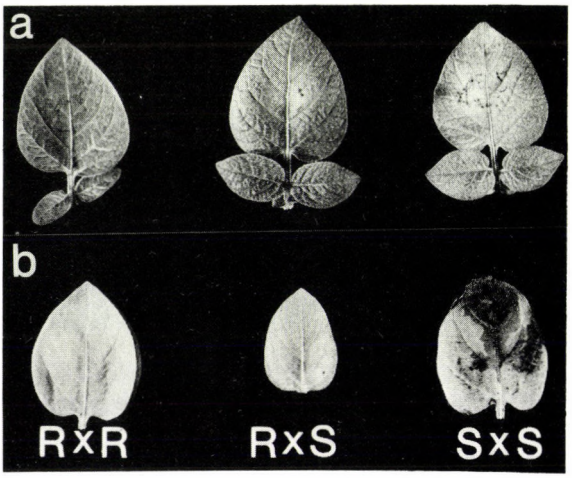

Fig. 3. Effect of mycelial homogenate (a) and culture filtrate (b) from *P. infestans* on leaflets of potato crosses of parents race-nonspecifically resistant (R) and/or susceptible (S) to the fungus. Note pin-point necroses (a) and water soaking (b), best seen in cross S×S. Photographs were taken 3 days after treatments

MH seems to be due to arachidonic and eicosapentaenoic acids, the activities of which are enhanced by glucans (Preisig and Kuc, 1985).

The substance causing water soaking of leaves upon CF-treatment is as yet unknown for *P. infestans*. Preliminary results indicate that the water soaking-active material is a glycoprotein of high molecular mass (Barna et al., unpublished). This would be in agreement with findings concerning other *Phytophthora* spp. (Csinos and Hendrix, 1977; Plich and Rudnicki, 1979; Breiman and Galun, 1981; Strange et al., 1982; Shohet and Strange, 1989).

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Vertical Resistance or Tolerance, a Methodical Challenge?

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The tolerance was studied for stem rust, powdery mildew in seedling and adult stages as well as for *Fusarium* head blight in wheat. Genotypes can be identified with tolerance, e.g. the infection severity is not significantly different while the yield reaction differences are highly significant.

The tolerance could be demonstrated over several years tests only in the case of the head blight tests, in the other cases its presence is possible. According to our present knowledge the selection strategy have to remain based on the durable resistance determined by major genes, gene combinations and/or supported with slow rusting genes, which can be identified relatively easily.

Until now the tolerance can be identified only after several year testing. It is useful to cross parents where beside major genes also tolerance exists. It seems that tolerance is more or less independently inherited from resistance and it does not only correlate for the extensive, low yielding genotypes, but also highly productive varieties may have it. These are two arguments more to continue the research work on it.

In the past two decades the non specific resistance became an important part of the research program not only in the Cereal Research Institute, but a world-wide interest can be recognized. It was not an accident, since as the resistances determined by major genes often become ineffective due to new mutant races of the pathogens. The problem is most severe in powdery mildew, less in brown rust and the most stable host-pathogen relationship was recorded for wheat-stem rust aegricorpus according to Loegering (1984), Loegering and Sears (1981). The non-specific forms of the self defence seemed to give a solution for the problems, since they do not inhibit a low incidence and do not allow a high selection pressure.

Because the inheritance of the tolerance is more complex, the selection pressure is much lower than that experienced in the case of major genes. Consequently the pathogenic population can be kept more stable and the low incidence does not generally cause a significant yield reduction. Another consquence is from breeding point of view that the selection for tolerance needs a more skilled work than that for the vertical resistance.

A special form of this non-specific resistance is the tolerance which according to Caldwell (1968), Simons (1966, 1969), Robinson (1969) can be characterized by the same level of the disease and even differing yield reactions. We should refer to the warning of Mac Key (1986) who emphasized that tolerance should be considered as a primitive form of resistance which occurs first of all in extensive varieties where vast biological reserves can be identified.

The Center Pivot method (Barabás et al., 1974) enabled us to study the build-up of a stem rust epidemic. Generally the yield and 1000 grain withts decreased most seriously at the spreader where the epidemic started and correlated well with the infection severity. The genotypes with vertical resistance were practically free of stem rust infection. Among the susceptible genotypes both tolerant and non tolerant genotypes,

could be differentiated by comparison of the yield reduction. Surprisingly, sometimes the symptomless, resistant cultivars also showed a yield reduction, but only in the sector or subplot in the vicinity of the spreader (Matuz et al., 1979). The changig climatic conditions affected significantly the results from year to year, so we do not have enough data to demosntrate the multi year tolerance by the Center pivot method. Having been collected some basic information, even the method was suitable to test the necessary parameters, the high costs of the test pressed us to turn back to the serial work based on the twin plot method.

During the test methodical problems arise to find proper way of tests by that the tolerance can be identified with sufficient certainty to make selection in this direction.

Summarizing the experimental hypothesis, we hoped that the extensive usage of non-specific resistance forms will solve some of the problems originated from the use of vertical resistance.

Material and Methods

The tests were performed with three pathogens of wheat, *Fusarium* head blight, powdery mildew and stem rust. In the '70-s the tolerance to stem rust was tested by the divided plot and the twin plot methods of Simons (1969), modified by Brönnimann and Fossati (1974). They have several disadvantages, as the presence of other diseases and pesticides used may disturb the yield responses. The tolerance and field resistance can not be distinguished from each other and the developmental diffrences among genotypes cannot be followed.

A fundamentally new method was described to test race-non-specific resistance by Barabás et al. (1974). In the middle of a 1 ha field a spreader of 10 m in diameter will be sown and 10 genotypes can be tested in four replicates in plots 40 m long, divided at harvest into five subplots (8 m).

In the 80's we used also the twin plot method, in spite of the fact that the information obtained was less precise than in the case of the Center Pivot method, but several methodical changes helped to improve the reliability of method. Here we had 1,5 m^2 plots in four replicates in protected (Bayleton, 0,4 kg/ha, 4-5 times in a season) and not protected variants.

The root reduction test with stem rust is based on the observation that some genotypes have a good root development even when the leaves are heavily infected, and others not (Mashaal and Király, pers. comm., 1982). We performed several seedling tests to investigate whether this phenomenon is connected with tolerance or not. Similar test with powdery mildew was made to check the root reduction in wheat seedlings, too. The usual inoculation technique has been applied. The plants were kept in nutrient solution in conditioned (Henssler) green-house environment.

Concerning head blight tests, the results of 6 years' experiments in which 25 genotypes were treated yearly with four different isolates of *Fusarium* species in three replicates were summarized to find a possible way to distinguish tolerant and non-tolerant genotypes.

Table 1

Effect of stem rust epidemics on the yield of winter wheat varieties (1986)

Variety	ACI index 3rd evaluation	Yield difference between protected and infected treatments %
Kincső	0	-14.87
Mini Manó	0	+3.35
Mv 13	0	-4.62
Szemes	0.20	-7.41
Ságvári	0.88	-17.64
Zombor	2.44	-20.74
Réka	7.8	-12.58
Bu 20	9.76	-13.98
Boglár	11.20	-10.79
Csongor	13.60	-24.19
Lili	14.40	-18.38
Koppány	15.00	-23.65
Mv 8	23.20	-25.12
Laborc	24.00	-27.60
Korány	33.20	-22.41
GK 9	40.00	-22.06
Napfény	43.20	-23.24
Sarolt	43.20	-31.24
István	53.50	-38.06
Bokros	68.00	-41.97
LSD 5 %		10.34

ACI = mean infection coefficient

Results

The *field test* for *stem rust* in 1986 and 1987 are shown in Tables 1 and 2. The following conclusions were reached: Under heavy infection pressure, the varieties having effective vertical genes had no visible symptoms. In spite of this fact a significant yield reduction may occur, which shows that the resistance costs energy. Generally, there is a correlation between infection severity and yield reduction. But genotypes can be found having the same infection level (ACI index), but differing in yield response. In 1986 Boglár and Csongor cvs did not show a difference in the level of infection, but differed in yield reaction. Similar data pairs are genotype numbers 17 and 18 or in 1987 Réka and Boglár. Réka was tolerant in both years, but no other genotype had the same reaction.

Table 2

Effect of stem rust epidemics on the yield of 20 winter wheat varieties (1986)

Variety	ACI index 3rd evaluation	Yield difference between protected and infected treatments %
Kincső	0	-14.87
Mini Manó	0	+3.35
Mv 13	0	-4.62
Szemes	0.20	-7.41
Ságvári	0.88	-17.64
Zombor	2.44	-20.74
Réka	7.8	-12.58
Bu 20	9.76	-13.98
Boglár	11.20	-10.79
Csongor	13.60	-24.19
Lili	14.40	-18.38
Koppány	15.00	-23.65
Mv 8	23.20	-25.12
Laborc	24.00	-27.60
Korány	33.20	-22.41
GK 9	40.00	-22.06
Napfény	43.20	-23.24
Sarolt	43.20	-31.24
István	53.50	-38.06
Bokros	68.00	-41.97
LSD 5 %		10.34

ACI = mean infection coefficient

It should be emphasized that Réka is an intensive variety with a high yielding ability, so it seems that the concept of Mac Key (1986) can be reconsidered again.

Under field conditions the experimental data can not be precisely reproduced from year to year; the severity of infection and consequently the level of tolerance also differs.

The *powdery mildew field tests* (Table 3) provide in some respect similar observation. Genotypes were found which had a significant yield loss in spite of the low infection severity. Zombor and Réka did not show yield reduction, but Öthalom and Csürös cvs did it with similar infection severities. Kaláka proved to be more tolerant than cvs Kalangya, in spite of the fact that they are sister lines. We should mention Arthur (USA) which is susceptible, but no yield reduction was measured. Here the theory of Mac Key (1986) seems to work, the extensive (lower yielding) genotype has enough reserve to

Table 3

Tolerance to powdery mildew in wheat

Genotype	Mean infection severity		Yield reaction		1000 Kernel weight	
	Prot.	Inf.	Prot.	Inf.	Prot.	Inf.
Mini Manó	2.41	4.38*	3.48	3.03*	32.35	31.43
Bu-20	4.43	9.73*	4.25	3.90*	47.03	46.33
Boglár	2.88	8.00*	3.55	3.30	40.40	39.10
Kincső	2.99	7.84*	3.55	3.43	34.95	34.83
SGV-Bty	2.81	7.15*	3.30	3.18	42.38	41.50
Pu	4.53	12.14*	3.90	3.63	41.38	41.05
Öthalom	5.58	13.65*	3.90	3.63	46.88	45.50*
Zombor	9.83	19.78*	3.45	3.53	42.43	38.45*
Csürös	7.62	18.05*	3.73	3.48	41.43	41.28
Réka	4.29	13.05*	3.58	3.63	42.63	43.70
Bence	6.21	13.71*	3.65	3.48	40,28	39.93
Szeged	7.39	22.23*	3.25	2.90*	49.23	45.15*
Mv 15	11.69	22.07*	3.83	3.53*	44.43	41.95*
Mv 8	12.58	30.63*	3.25	3.00	46.09	44.65
Jbj-50	13.69	28.71*	3.55	3.33	52.33	49.28*
Barna	13.73	28.68*	3.65	3.45	43.38	42.55
Kalangya	11.54	30.49*	3.93	3.55*	47.25	44.33*
Arthur	13.08	27.15*	2.60	2.60	43.85	43.58
Kaláka	16.15	30.19*	3.75	3.55	45.50	43.18
Őrzse	10.68	24.68*	3.80	3.55	46.68	46.05
István	24.90	40.34*	3.53	3.08*	41.45	40.13
Csanád	28.25	49.25*	3,40	3.03*	46.83	43.35*
Ságvári	30.38	45.28*	3.33	2.75*	46.65	38.70*
Mean	10.77	22.25*	3.57	3.32	43.73	42.00

*The difference between protected and infected is significant at P = 5 %

withstand the yield loss. But Zombor, which is a high yielding, intensive variety, has an excellent adaptability and also a satisfactory tolerance.

In the *root reduction* test with *stem rust* (Table 4) the root and leaf weight of the highly resistant group (no visible symptoms at all, reaction type RR) significantly increased, compared to the control. In the root weight of the resistant group (R) no decrease, but rather an increase was observed. In the moderate susceptible (MS) group both high losses (34%) and an increase (7.7%) were recorded, the latter form is the tolerant version.

Table 4

Tolerance to stem rust is seedling stage measured by root volume (1988)

Genotype	Reaction type Stakman scale	Root volume as a % of the control
Csongor	RR	107.9
SC 821520	RR	118.3
Arthur	RR	124.8
TR 880152	R	99.4
Kalangya	R	102.9
SC 821524	R	111.5
FR 84.3	R	111.6
SMH 1749	MS	66.3
Rana 2	MS	77.5
HD 1	MS	96.9
TX 76402	MS	107.6
SMH 1739	S	72.7
JKM-Rna2*Grn-D1	S	85.5
Jbj-50	S	104.7
LSD 5 %		14.3

The No. of pustuls/leaf did not show significant differences within group

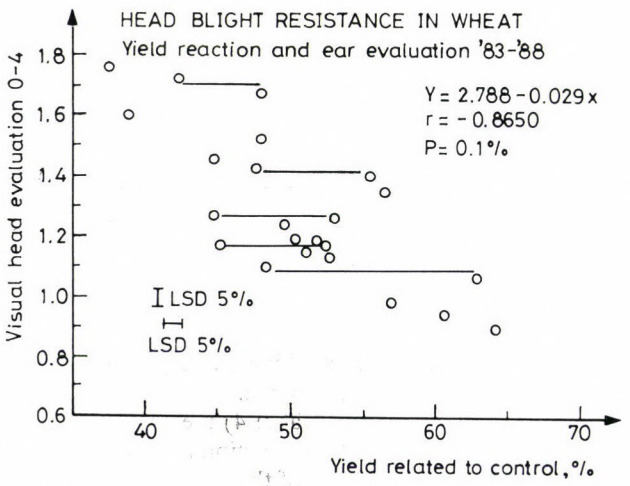

Fig. 1. Six years' averages of yield and visual head ratings.
Inoculation was made every year by four *Fusarium* isolates in three replications

Table 5

Tolerance to powdery mildew in wheat in seedling stage measured by root volume (1988)

Genotype	Infection type 0–4	Root volume as a % of the check
Tx 76 402	0	91.67
SMH 1739	0	76.00
SMH 1739	0	75.00
SC 821850	0.52	76.47
GP 7523	1.35	78.95
SC 821854	1.70	68.75
TR 42072	2.90	81.25
Arthur	3.20	76.92
FR 84.3	3.40	72.22
Kalangya	3.60	92.31
HD Kő/Gt-Pdj*Uhr	4.00	83.33
Jbj-50	4.00	68.75
Csongor	4.00	62.50
TR 880152	4.00	53.33
HD 1	4.00	52.94
Rana 2	4.00	52.63
Jkm-Rna2*Grn-D1	4.00	40.00
LSD 5 %		20.70

Similarly the S group had genotypes with high reduction 27.3% and an increase 4.7% was found for Yu- 50, which is a known tolerant genotype for several diseases. The results are encouraging, but several years of experience will be needed to select for this character and clearly demonstrate a genetically determined tolerance.

Similar results were achieved with *powdery mildew*, too. In Table 5 each infection severity group contains genotypes with significantly different yield responses. Further the task is to establish how stable should be the results at climatically different conditions to allow genetical conclusions.

The *Fusarium head blight* results (Fig. 1) demonstrated significant differences in resistance, and highly significant deviations in yield response were recorded, which is an indication of significant tolerance differences. This is the first case that tolerance and resistance could be demonstrated for scab in wheat. 6 year' averages prove that here genetically determined tolerance differences exist.

Conclusions

The former experiences clearly show that tolerance to any of the diseases can not be proven in a single test today. With the presently known methods several years and tests are necessary to prove unanimously the presence of tolerance. The results change from year to year or experiment to experiment in field conditions and a precise laboratory test is also lacking.

Therefore the early identification of the tolerance is hardly done with the field methods used until now. So according to our present knowledge it is a very hard task to make effective field selection for tolerance. Under controlled environment, we think, a better differentiation is possible, but this work is yet before us. We need a specific way of selection for this character, but we do not have the reasonable methods to score it until now. The results of the seedling tests are interesting, but here the identification of tolerance in seedling and adult stages is a future task. If a good correlation would exist, the seedling test could have a practical value. To add to these questions, the seedling test has a considerable value in the seedling resistance for vertical genes. But currently turned out that its practical value seems to be less than thought before (Knott, 1989).

But at the same time, the vertical resistance research achieved excellent results, too. It has been proven that genes with durable effect exist and there are genes and gene combinations which give stable resistance for decades. Such is among others the well known, today classic 1B/1R rye translocation Sr 31 gene responsible for resistance to stem rust, or the gene combination in Selkirk (Van der Plank 1968).

Resistance mechanisms were also detected like slow rusting (Wilcoxson 1981), partial resistance (Parlevliet, 1985), lengthened latent period (Roelfs, 1985) which might be connected to vertical or horizontal genes. The work with identified resistance genes has an old tradition. As the recent development shows, the challenge of new races could be answered positively. The individual seletion gave good results, the reactions are more or less reducible. Therefore we think that the race specific resistance will have its significance in the future. In our opinion tolerance can exist not only in extensive, but also in intensive cultivars. Therefore tolerance will have importance in spite of the fact that Knott (1989) as well as Simmonds and Rajaram (1988) do not mention tolerance as a valuable trait with future prospects.

We can not select directly for tolerance at single plant level, but a mass selection can be useful for this trait. Where tolerance exists, it ensures the prolonged cultivation time of a variety. For practical purposes the old method of strong selection for non shrivelling will be applied. According to our experience, in wide spaced stand the shrivelling is much more intensive than that observed at high plant densities, so the selection is more effective.

Especially interesting is the case of the head blight because we do not have vertical resistance at all. All of the data discussed refer to a non-specific type of resistance. In this case the tolerance can open a new reasonable way of research. The tolerance might be connected with both vertical and horizontal resistance, and many other important factors.

Our original question was, whether the tolerance is a methodical challenge or not? Actually the answer is: yes. Moreover, it remains a challenge, a problem to solve for the

future, to have develop more precise techniques. Certainly, the question is not whether to choose tolerance or resistance. We need both. One should not forget, that both resistant and susceptible genotypes with and without tolerance were found. The resistance programs should be concentrated on the resistance genes, but the breeders need more. They, and the growers need resistant forms which are able to tolerate the epidemic events without or only minimal yield reductions. One should not forget to use parents in which tolerance was once discovered. One of the main goals of the near future is to develop resistant varieties together with tolerance in complementary forms.

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Susceptibility of Certain German Carnation Cultivars to Five Isolates of *Fusarium oxysporum* f. sp. *dianthi*

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The susceptibility of 4-week old carnation cuttings of four German cultivars to five isolates of *F. o.* f. sp. *dianthi* was assessed at 22-28°C for 30 days. The four cultivars varied in their susceptibility, e.g. cv. Desio was the most susceptible, cv. Candy was less susceptible or moderately susceptible, cv. Chinera and cv. Francesco were susceptible. The five isolates differed also in their virulence, e.g. the French, German, Italian and Netherlands isolates were virulent as they caused severe wilt and death to all cultivars after 23 days of inoculation. On the other hand, the Egyptian isolate was mild in infection as it showed slight to moderate wilt symptoms on plants of the four cultivars up to 30 days after inoculation and did not cause death to plants. Wilt symptoms began from 12 days after inoculation, became more conspicuous from 16 to 23 days after which plants suffered severe wilt and died in case of the virulent isolates. Virulent isolates caused marked reduction in height of infected plants, whereas the mild isolate had little effect in this concern. Symptom severity was associated with the extent of stem colonization. Colonization with virulent isolates reached up to 100% after inoculation, meantime, plants expressed severe wilt symptoms. Stem colonization and wilt symptoms with Egyptian isolate, on the other hand, did not differ much throughout the whole experimental period. Vessel browning in longitudinal cutting of cv. Candy plants 23 days after inoculation reached up to more than 80% of plant height in case of the virulent isolates, but did not extend more than 8.3% with the Egyptian isolate.

The occurrence of carnation wilt incited by *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans. was previously reported from different parts of the world. During the last two decades, the disease became the most dangerous in production of carnation. This was reported from U.K. (Fletcher and Martin, 1972), France (Tramier et al., 1979), Italy (Pergola et al., 1979), and Egypt (Sheir et al., 1982). Breeding programmes for producing *Fusarium* wilt-resistant carnations were established in Netherlands (Sparnaaij and Demmink, 1977), USA (Carrier, 1977) and U.K. (Arthur and Mathews, 1980). The presence of several isolates of the pathogen and rapid development of new ones complicated the search of resistant cultivars (Garibaldi, 1983).

The present work aims to test certain German carnation cultivars to different geographical isolates of *F. o.* f. sp. *dianthi*.

Materials and Methods

Four carnation (*Dianthus caryophyllus* L.) cultivars, namely Candy, Chinera, Desio and Francesco were used throughout this work. Four-week old rooted cuttings of these

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cultivars were supplied by a private commercial grower in Germany during mid September 1990. Five isolates of *Fusarium oxysporum* Schlecht. f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans. each from Egypt, France, (1587) Germany, (1569), Italy (1577) and Netherlands (1584) were used. The Egyptian isolate was obtained from vascular wilted mature carnation plant (cv. White Scania) from Sabahiya Horticulture Experiment Station, Alexandria, during July 1990. Roots of cuttings were dipped in a spore suspension (10^6 ml) of a given isolate of *F. o.* f. sp. *dianthi* for 15 minutes prior to planting in pots (20 cm diameter) nearly filled with TKS (peat cultivation substrate). Cuttings were planted at the rate of three cuttings/pot in case of cv. Chinera and cv. Francesco, and five cuttings/pot in case of cv. Candy and cv. Desio. Roots of cuttings of the cultivars used dipped in water served as check. Five replicates (5 pots) were used for each treatment. Pots were put in greenhouse (22-28°C).

For preparing spore suspension, 1 litre conical flasks, each containing 500 ml Czapek Dox liquid medium were autoclaved, then singly inoculated with the five isolates of *F. o.* f. sp. *dianthi*. Inocula (4-mm diam) were taken from the growing margin of each isolate on PDA. Flasks were agitated on a shaker (Cartomat) at 200 rpm for six days at room temperature (21-25°C). Cultivars were filtered through sterile lens cleaning tissue and the suspension was adjusted to 10^6 spores/ml using a haemocytometer (Thomas).

Plants were observed for wilt symptoms at 1 to 3 day-intervals for 30 days after inoculation. Each plant was assigned to one of the following disease classes: healthy, slight wilting, moderate wilting, severe wilting and dead. For each cultivar/treatment, a disease index was calculated. The number of plants in the first class was multiplied by 0, those in the second class by 1, in the third class by 2, in the fourth class by 3, in the fifth class by 4. The sum of the products multiplied by 100 was divided by 4× the total number of plants to secure the index. Thus the index secured ranges from 0 indicating all plants healthy to 100 indicating all dead (Walker and Foster, 1946).

In order to assess the extent of colonization by the different isolates of *F. o.* f. sp. *dianthi*, re-isolations were achieved from one to two inoculated plants and one from the check plants after 5, 9, 16, 23 and 30 days of inoculation. Isolations were made from the stem after removing the root and leaves. The stem was surface-sterilized in 1% sodium hypochlorite (chlorox) for 3 minutes, rinsed in sterile water, blotted dry on sterile filter papers, cut into 2 cm segments, plated on PDA and incubated at 22°C under 12 hours alternating cycles of NUV light and darkness. The height to which the fungus had reached up in the stem was expressed as a percentage of the total height of the stem, and a mean value was obtained for the two plants.

Results

The four cultivars, namely, Candy, Chinera, Desio and Francesco showed varied degrees of susceptibility to the five isolates of *F. o.* f. sp. *dianthi* used. Desio cultivar was the most susceptible to these isolates, whereas cv. Candy was relatively less susceptible or moderately susceptible, the other two cultivars were susceptible.

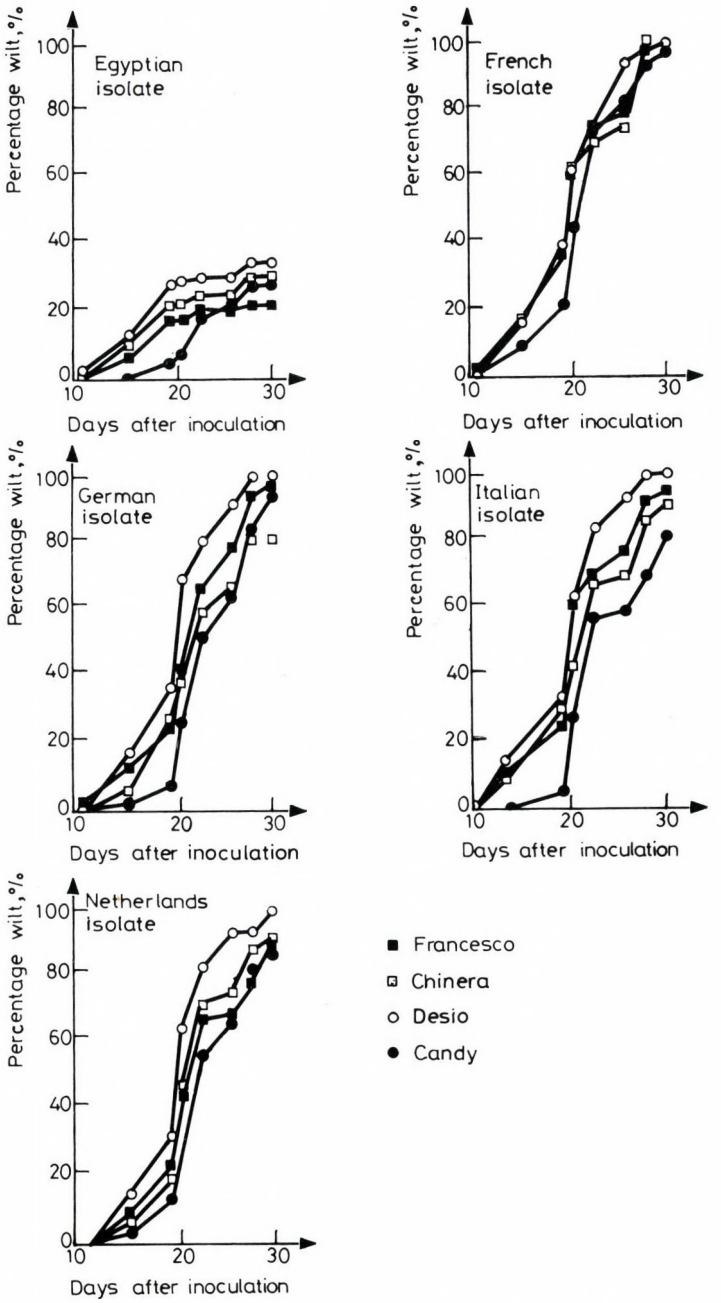

Fig. 1. Symptom development in carnation cultivars inoculated with different isolates of *F. o.* f. sp. *dianthi* grown at 22-28°C.

The isolates used differed in virulence. The French, German, Italian and Netherlands isolates were virulent, whereas, the Egyptian isolate was mild (Fig. 1). Wilt symptoms began to appear on plants of all cultivars used from 12 to 16 days after inoculation. From the 16 to 23 days after inoculation, very prominent wilt symptoms developed on carnation plants of all cultivars inoculated with the virulent isolates. From 23rd to 30th day, plants inoculated with the virulent isolates suffered sever wilt and died. Plants inoculated with the Egyptian isolate, on the other hand, showed slight to moderate wilt symptoms all throughout the whole experimental period and did not die. In cv. Candy, which is moderately susceptible, wilt symptoms began to appear late after 20 days of inoculation with the Italian and the Egyptian isolates (Fig. 1). It is noteworthy to mention that the virulent isolates had a marked effect on reducing the hight of plants 23 days after inoculation, however, the mild isolate had little effect (Fig. 2).

Stem colonization of carnation plants of the four cultivars by *F. o.* f. sp. *dianthi* at different heights at different periods from inoculation was assessed (Fig. 3). The fungus

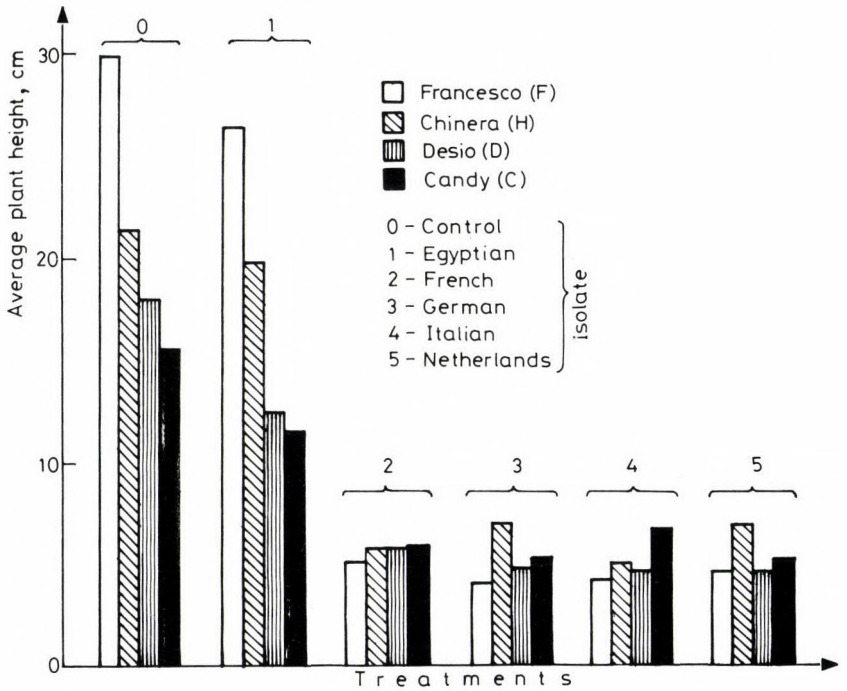

Fig. 2. Effect of inoculation with different isolants of *F. o.* f. sp. *dianthi* on height after 23 days grown at 22-28°C.

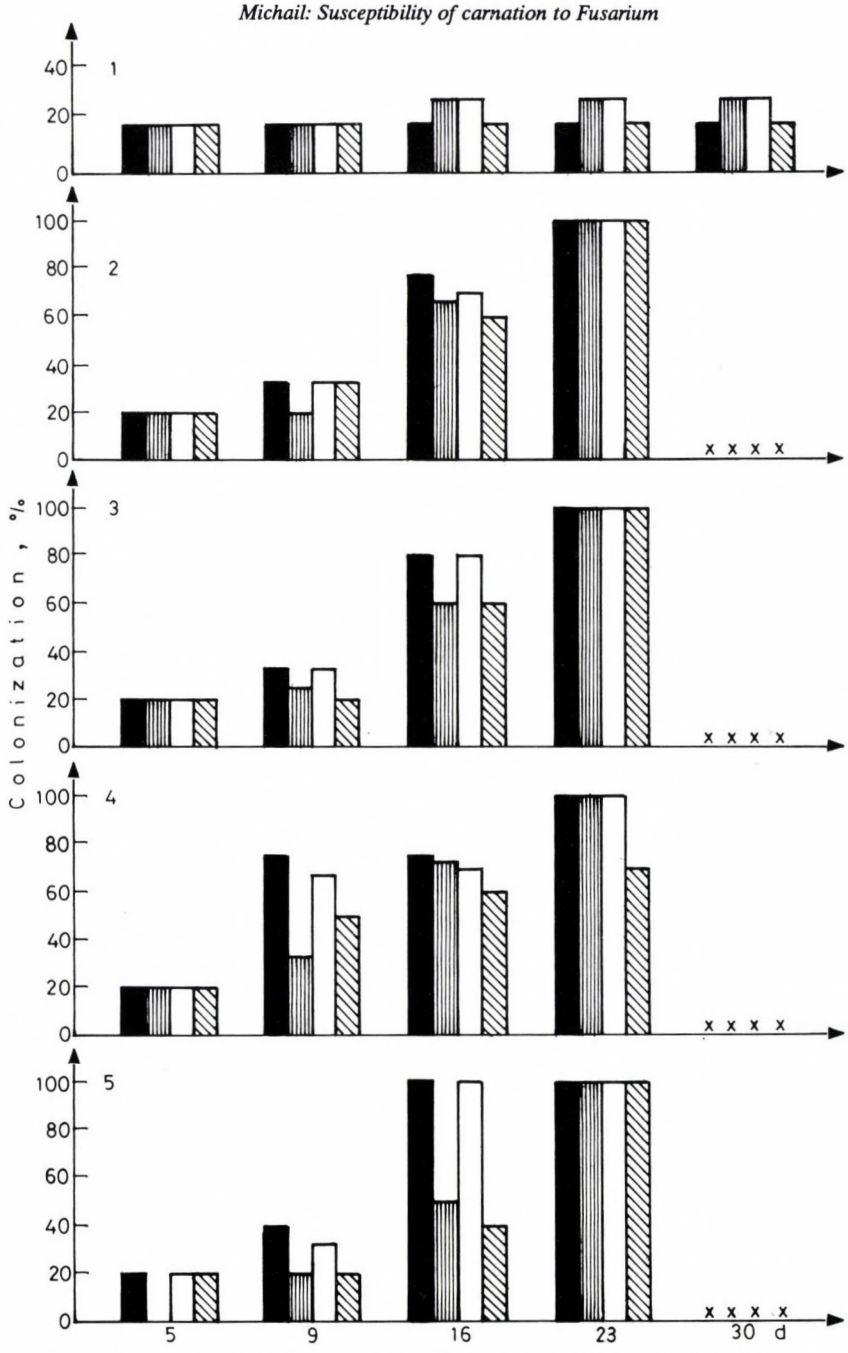

Fig. 3. Stem colonization of carnation plants at different heights after different periods form inoculation with five isolates of *F. o.* f. sp. *dianthi*

was found colonizing the basal part of stem after five days of inoculation with the isolates used. The fungus reached up different heights on the 9th day after inoculation with the virulent isolates. It reached up to 75% in Desio cultivar with the German isolate. By the 16th day from inoculation 100% stem colonization was attained in cv. Desio and cv. Francesco with the Netherlands isolate. On 23rd day, plants of all cultivars used showed 100% stem colonization with the virulent isolates. On the other hand, the percentage of stem colonization did not differ much and not exceed 35% from the 5th up to 30th day after inoculation with Egyptian isolate.

Detection of vessel browning in longitudinal cutting of cv. Candy plants 23 days after inoculation reached up to 81.2, 81.2, 88.0, and 84.2% of plant height with the French, German, Italian and Netherlands isolates, respectively. Brown discolouration with the Egyptian isolate extended only 8.3% of plant height.

Discussion

The present work showed differences in susceptibility of certain German carnation cultivars to *F. o.* f. sp. *dianthi*. Desio cultivar was the most susceptible, cv. Candy was less or moderately susceptible, Chinera and Francesco cultivars were susceptible. Such differences in susceptibility to carnation *Fusarium* wilt isolates support the work of Pawliczuk and Orlirowsk (1987) who found only two clones tolerant to *F. o.* f. sp. *dianthi* out of 249 clones examined, and the work of Garibaldi and Rossi (1987) and Garibaldi and Gullino (1988) who screened many carnation cultivars against common Italian isolates and found few cultivars resistant to all such isolates.

Wilt symptoms manifested three stages of development. Symptoms were slight in the first stage, became very prominent in the second stage, very severe and plants died in the third stage. Symptom severity was associated with the extent of stem colonization. The fungus was colonizing the basal stem part after five days of inoculation, and reached up different heights on the 9th day with the virulent isolates. By the 16th day, stem colonization attained 100% in cv. Desio which expressed conspicuous wilt symptoms. By the 23rd day, plants of all cultivars inoculated with the virulent isolates showed 100% stem colonization, where wilt symptoms were very severe and plants died. Stem colonization and wilt symptoms in plants inoculated with the Egyptian isolate did not differ much throughout all the experimental period and did not cause death of plants. Such association of wilt symptoms with the extent of stem colonization substantiates the findings of Harling et al. (1988). The considerable variation in virulence among the isolates used in this work agrees with the findings of Arthur et al. (1977).

The short lasting of plants (23 days) after inoculation might be attributed to the method of inoculation used and temperature under which plants were grown. The root-dip inoculation method is more quicker in showing up symptoms as compared to soil infestation method (Hood and Stewart, 1957). The temperatures under which plants were grown ranged from 22-28°C which favored wilt expression. This is in line with the findings of Harling et al. (1988) who found that at 22 and 26°C carnation cultivars exhibited clear

symptoms, and at 22°C plants could be differentiated into resistant, partially resistant and susceptible to the pathogen.

Although a limited number of carnation cultivars was tested in this work for *Fusarium* wilt resistance, yet a large number of German carnation cultivars should be screened along with a long-term breeding programme.

Acknowledgement

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Effect of Inoculation of Potato Tuber Discs by Non-Pathogenic Fusaria on *Fusarium* Tuber Rot

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The interaction between non-pathogenic fusaria of potato tuber discs on disease incidence causes by *Fusarium oxysporum* was studied. Blasticidin-S resistant mutant of the pathogen, *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium roseum* were choosen. Discs individually inoculated by such non pathogenic fungi were inoculated by the pathogen at different intervals (i.e. 0, 3, 6 and 9 h) from inoculation by the non-pathogens. Phytoalexin rishitin was determined as evidence for enhancement of defence reaction. Data obtained indicated that Blasticidin-S resistant mutant and *F. lycopersici* (closely related to the pathogen) completely reduced disease incidence especially when inoculation by the pathogen was carried out 3 h after inoculation by the non patoghens.

Fusarium roseum slightly reduced disease incidence even when the pathogen was inoculated 9 ha after inoculation by the non-pathogen. The obtained data led to postulate that the compounds accumulate in potato tuber in respons to infection by non-pathogens play a secondary role in limitation the challenge. The prime factor determins the state of interaction between pathogenic fusaria and potato tuber may be the recognition site between host and pathogen.

The biochemical nature of potato varietal resistance against *Fusarium* tuber rot are subject of research in many laboratories. According to Allen and Kuc (1968) steroid alkaloids play a role in resistance of potato tubers against *Fusarium* infection. Defago and Schwarz (1983) using a mutant resistant to glycoalkaloids of potato demonstrated that such compounds have effect in the resistance of potato tubers against *Fusarium* infection.

The role of phenolic compounds in resistance was also studied. It was found that free phenols and phenylalanine ammonia lyase (PAL) activity were higher in state of resistance than in susceptibility (Corsini and Pavec, 1980; Ashour and Mostafa, 1983). Recently, Hammerschmidt (1984) found in the study of lignin deposition that non-pathogenic fungi of potato tuber, *Fusarium roseum* and *Cladosporium cucumerinum* caused a yellow discolouration in the host cell walls. Such detection was firstly found 8-10 h after inoculation, he also found that the yellow discolouration in the cell walls stained positively for lignin. The pre-treatment of the tissue with aminooxyacetic acid a competitive inhibitor of PAL or abscisic acid, induced a state of susceptibility in the tissue to *C. cucumerinum*, and this was associated with a lack of lignin deposition. The role of lignin in restriction the challenge is not only found in local resistance but also in systemically protected leaves (Hammerschmidt and Kuc, 1982).

Therefore, it was proposed that if the elicitation of defence reaction plays the first role in disease resistance of potato against *Fusarium* tuber rot, then, these fungi which stimulate defence reaction might be approximately equally effective in limitation the challenge.

The fungal pathogen isolated from rotted potato tuber was identified as *Fusarium oxysporum*. Three fungi, one is closely related to the isolated fungus-blasticidin-S resistant

mutant of wild isolate and one which is *Fusarium oxysporum* f. sp. *lycopersici*, and a third one is *Fusarium roseum* were used to test such hypothesis.

The previously mentioned fungi were inoculated individually with the causal organism in order to study the effect of presence of such fungi on disease incidence. Phytoalexin rishitin was determined as an evidence for the enhancement of defence reaction of potato tuber tissue during infection (Corsini and Pavek, 1980).

Materials and Methods

Potato tubers: Potato tubers King Edward variety were obtained from Institute of Vegetable Research, Ministry of Agriculture, Egypt. Tubers were stored at 4°C up till used. Discs (2 cm in diameter and 1 cm in width) were prepared and used.

Fungi: *Fusarium oxysporum* was isolated from rotted potato tubers, the fungus was cultured on potato dextrose agar medium (PDA). *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium roseum* were obtained from Agric. Botany and Plant Pathology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

Blasticidin-S resistant mutant was obtained by culturing a very high density of *F. oxysporum* spores on PDA containing 20 ppm (a.i.) of Blasticidin-S (4 ppm inhibits fungal growth completely). After 20 days small colonies have appeared. The obtained mutant was cultured on PDA containing 20 ppm of Blasticidin-S. The mutant losted its pathogenicity 4 months after preservation, therefore, it used in this study.

Fungal spore suspension: The previously mentioned fungi were cultured on PDA for 10 days at 25°C in the light. Spore suspension was prepared in sterilized distilled water. Number of spores were counted, then adjusted to 5×10^5 spore/0.5 ml for each fungi.

Discs inoculations: The prepared discs were washed in sterilized distilled water then placed in Petri dishes (15 cm in diameter) contained two wetted filter papers. In every dish 10 discs were placed. Three replicates for every particular treatment were used. After one hour, discs were inoculated by 0.5 ml/disc of Blasticidin-S mutant, *F. lycopersici* or *F. roseum*. The inoculated discs were divided into 4 groups. The first was inoculated after 15 min. by 0.5 ml/disc of *F. oxysporum*. The 2^{nd}, 3^{rd} and the 4^{th} group were inoculated 3 h, 6 h, and 9 h, respectively by *F. oxysporum*. Three dishes only inoculated by *F. oxysporum* as a control.

Disease incidence and determination of rishitin: After 48 h of inoculation with Blasticidin-S mutant, *F. lycopersici* or *F. roseum* every disc was divided into two equal portions. The first was taken to determine rishitin content and the other part was left to accomplish 48 h from inoculation with *F. oxysporum*. For determination of rishitin, the upper 2 mm of the half disc was removed then extracted in chloroform. Extraction of 5 halves was collected together. Chloroform extract was concentrated to minimum volum. Rishitin was separeted on silica gel G plates (0.25 mm) using chloroform:acetone (85:15). The corresponding band of rishitin was scrushed then rishitin was extracted in chloroform. Chloroform evaporated till dryness and using sulfuric acid conc., the density of violet colour produced was measured by spectrophotometer (type UV-01). The amount

Table 1

Effect of inoculation of potato tuber discs by non-pathogenic *Fusaria* on disease incidence causes by *Fusarium oxysporum*. Number of spores/discs ($x10^5$) 48 h after inoculation by the pathogen at 25±1°C in the dark

Time after inoculation (h)	Blasticidin-S mutant		*F. lycopersici*		F. roseum	
	EXP I	EXP II	EXP I	EXP II	EXP I	EXP II
Control*	14,33±2,01	10,65±1,33				
0	7,80±1,23	4,19±1,10	13,04±2,01	7,71±1,59	6,61±2,32	6,09±2,88
3	1,03±0,55	0,16±0,10	0,05±0,02	0,02±0,02	10,12±3,55	3,89±2,99
6	0,18±0,10	0,09±0,06	0,02±0,15	0,0	3,35±2,05	2,84±1,89
9	0,03±0,01	0,14±0,09	0,0	0,0	4,42±3,14	3,04±2,84

* Discs inoculated by the pathogen only

of rishitin was calculated on standard curve of pure rishitin. Rishitin was expressed as mg/disc (Horikawa et al., 1976).

The second halves were taken after 48 h after inoculation by *F. oxysporum*. The upper surface was separated (2 mm) then grinded in 5 ml distilled water. Using Hemiacitometer, on light microscope, number of spores was counted and the results were expressed as number of spores/disc. Experiments were repeated twice.

Results

Inoculations of potato tuber discs with spore suspension of *Fusarium oxysporum* caused severe rot, this was associated with appearance of fungal mycelial mats on colo-

Table 2

Effect of double inoculation of potato tuber discs by pathogenic and non-pathogenic *Fusaria* on rishitin production after 48 h at 25±1°C in the dark

Time after inoculation by non pathogen (h)	Blasticidin-S mutant		*F. lycopersici*		F. roseum	
	EXP I	EXP II	EXP I	EXP II	EXP I	EXP II
Control*	71,44	127,84				
0	123,18	139,70	85,23	105,02	130,08	102,16
3	89,09	96,24	90,09	99,27	134,92	97,43
6	121,51	96,24	99,56	72,24	147,69	162,87
9	78,74	107,24	135,65	116,46	96,66	100,84

* Discs inoculated by the pathogen only

nized tissue. In such rotted tissue, rishitin was accumulated in high amount (99.6 mg/disc). On the other hand, discs inoculated with Blasticidin-S resistant mutant, *Fusarium oxysporum* f. sp. *lycopersici* or *Fusarium roseum* showed hypersensitive like reaction with different degrees of discolouration. Such colouration was more pronounced in *F. roseum* inoculated discs. Rishitin was also accumulated and its amount reached 98, 85 and 120 mg/disc for Blasticidin-S resistant mutant, *F. lycopersici* and *F. roseum*, respectively. Double inoculation at different intervals showed very high amount of rishitin. The phytoalexin was detected in all cases (colonization or hypersensitive like reaction) and no any correlation was found between state of interaction and phytoalexin accumulation (Table 2).

Concerning the effect of double inoculation on disease incidene, determined as number of spores/disc, non pathogenic fungi showed different effects. The closely related fungi are more effective in limitation the causal organism (*F. oxysporum*). Blasticidin-S resistant mutant greatly reduced disease incidences when inoculation with the pathogen was carried out at the same time. When inoculation of discs previously inoculated with *F. lycopersici* was carried out at the same time the reduction in disease incidence was less than that found in the case of Blasticidin-S mutant. Inoculation by the pathogen after 3, 6 and 9 h of inoculation by Blasticidin-S mutant or *F. lycopersici* strongly reduced disease incidence. *F. lycopersici* was more effective in this respect.

Fusarium roseum reduced disease incidence when inoculations were done at the same time and the same result was received at 3 h interval. After 6 and 9 h intervals disease was slightly reduced. The reduction induced by *F. roseum* was smaller than that obtained by inoculation with other non-pathogenic fungi.

It could be stressed that no any synergistic effect was found between the pathogen and non-pathogenic fungi (Table 1).

Discussion

As a result of resistance of potato tubers to *Fusarium* dry rot, several compounds accumulate in the tissue. The role of these compounds in resistance remained a disputed question. Király et al. (1972) raised the question about the role of defence compounds in the resistance of potato to *Phytophthora infestans*. The present investigations attempted to answer the question: whether the defence compounds can inhibit the challenge infection or their accumulation is only a consequence of the phenomenon of resistance?

Three non-pathogenic *Fusarium* fungi were applied to tuber tissues prior to the inoculation with the pathogen. According to Friend et al. (1973) and Hammerschmidt (1974) lignin-like materials deposit 8-12 h after inoculation by incompatible races of *Phytophthora infestans* or non-pathogenic fungi. This was the reason that in our study the inoculation by the pathogen was carried out 0-9 h after the inoculation with the non-pathogenic fusaria. The Blasticidine-S resistant mutant of the pathogen and *F. lycopersici* strongly reduced the disease caused by the pathogenic *Fusarium*. This was convincing especially when infection by the pathogen occurred 3 h later than the inoculations with the non-pathogens. Supposedly, during that period the defence reaction of the host did

not started yet. According to Green et al. (1975) PAL and TAL activities in wheat leaves increased significantly only 4 h after inoculation with an incompatible strain of *Erysiphe graminis* f. sp. *tritici*. Ashour and Mostafa (1983) also have shown that in potato tuber tissues the activity of PAL increased 4 h after inoculation by a non-pathogenic *Fusarium*.

The third non-pathogenic fungus, *F. roseum* behaved differently. This fungus was able to reduce disease incidence caused by *F. oxysporum* when the latter inoculation was carried out 0-3 h after the treatments with the non-pathogens. However, if the pathogen was applied 6 and 9 h after the treatment with the non-pathogens, the tubers tissue slightly rotted as a consequence of the inoculation with the pathogenic *F. oxysporum*. If the accumulation of the hypothetical defence compounds after the treatment with the non-pathogens would be responsible for the restriction of the challenge pathogenic fungus, then *F. roseum* could inhibit the pathogen, especially in the case when the inoculation with the pathogen was carried out 9 h after the inoculations with the non-pathogens. The hypothetical defence compounds, which accumulate in tissues after the treatment with the non-pathogens, may play only a secondary role in the limitation or inhibition of the pathogen. This conclusion agrees with the finding of Érsek et al. (1973) who found that the hypersensitive tissue necrosis of potato tuber tissue is a consequence, rather than the cause, of resistance to *Phytophthora infestans*.

As regards the ability of the non-pathogens to inhibit the infection of the challenge pathogenic fungus, one can speculate on the role of a competition between the pathogen and the non-pathogens for recognition sites in the host. Such recognition sites were studied in several earlier experiments (Sequeira, 1978; Keen and Legrand, 1980; Ouchi, 1983).

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Differences in Susceptibility of Some Potato Varieties to *Phoma exigua* var. *foveata* Foist in Laboratory Test

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In laboratory test 31 varieties and new potato lines were inoculated with *Phoma exigua* var. *foveata* to test their susceptibility to gangrene. Unlike varieties, the new potato lines contain genes from four wild potato species (*Solanum demissum, S. andigenum, S. acaule* and *S. stoloniferum*) consequently possess a relatively high level of resistance to viruses. Some of the varieties and lines tested showed a higher degree of susceptibility (e.g. Gülbaba, Agria, Somogy gyöngye and No. 84.1544), while other showed a lower degree. There was no variety or new potato line showing full resistance, while some of the new lines proved to have a lower susceptibility than that of the varieties. There was no correlation found between skin and flesh colour and susceptibility to gangrene, or level of resistance to viruses and level of susceptibility to gangrene. The data showed, that there was a difference in susceptibility between the stem-end and rose-end of the tubers.

Phoma spp. cause considerable losses of potatoes during storage in many European countries, therefore effective treatments are to be taken to reduce the losses, and testing the susceptibility of the varieties is very important (Volovik, 1973; Pietkiewicz and Jellis, 1975, Nielsen, 1977; Kubiczki and Kuzniewicz, 1978; Kapsa, 1979; Seppänen, 1980; Scheitza and Hoffmann, 1986; Kapsa, 1986; Fischl, 1989; Carnegie et al., 1989).

According to Carnegie et al. (1989) *Phoma* rot is the major fungal storage disease in the United Kingdom, therefore testing varieties for their reaction to gangrene is an important part of both breeding and statutory test programmes.

In Hungarian potato production mainly virus diseases and in some cases, *Phytophthora infestans* causes considerable losses in the field. During storage, rotting on average do not cause considerable losses (Horváth, 1985). Nevertheless, in some "wet" years, and because of incorrect treatments, infection by late blight and *Fusarium* spp. can cause severe losses in commercial storehouses. Infection by *Erwinia* spp. is not common, but occurs mainly in imported seeds or in the first field production. Tuber rot caused by *Phoma* spp. was unknown in Hungary, its first occurrence in an imported seed lot was observed by Pintér and Horváth (1981). After 1981, *Phoma* was observed only sporadically in many areas in Hungary.

The occurrence of *Phoma* initiated this study to test varieties and new potato lines to their reaction against the fungus. Besides investigations on varietal susceptibility, another aim was to get data on tuber susceptibility differences between varieties with *Solanum tuberosum*-backgrounds and new virus resistant potato lines having genes from four wild potato species, too. Tuber tissue differences within the tested tubers were also investigated.

Materials and Methods

Midsized tubers from 31 potato varieties and potato lines were inoculated in the tests. Tubers were grown in a sandy soil, harvested by hand in August, 1989. The varieties, except Ciklamen, as far as the author knows are bred from *S. tuberosum*-varieties, while the new potato lines (marked with numbers in Table 1) of the Department of Potato Research of the Pannonian University of Agricultural Sciences of Keszthely are having genes from the wild *Solanum* species (*S. demissum, S. andigenum, S. acaule* and *S. stoloniferum*).

They originated from 7^{th}-8^{th} back-cross generations with *S. tuberosum*. The variety Ciklamen has the same genetical background as the new lines.

Five midsized tubers from each genotype were inoculated with *Phoma exigua* var. *foveata*. The inoculum was prepared by cutting mycelial discs from three week old agar cultures. After washing and superficial disinfection of the tubers a 10 mm long and 6 mm in diameter tissue cylinder was cut out with a disinfected cork-borer from each tuber both at stem- and rose-end. PDA discs down with their mycelial surface were taken in the hole and closed again with the tissue cylinder. Than tubers were placed into aluminium-trays (26×16×5 cm) and closed with polyethylene sheets to keep high humidity and prevent drying out. Tubers then were incubated for 10 days at 10°C. After incubation the tubers were cut through the inoculation point.

Rotting surface was covered with transparent sheet, and rotting contours outlined. This was copied onto paper by xerox and contours were cut out from the paper. The surface of these paper discs was measured with a leaf-area-meter (LI-COR, Model LI 3000/A) and values given as cm^2. This resulted in 10 data from each variety and potato lines, from which 5 data represented tissue susceptibility of the stem-end, and 5 data that of the rose-end of the tubers.

Biometrical analysis was made by the Computer Centre of University by using different programs, among them a graphical program, as well.

Results and Discussion

The means of rotting surface in cm^2 of inoculated cut tubers of the tested potato genotypes are shown in Table 1 and Fig. 1. There are considerable differences in the mean values between the genotypes tested, ranging from 1.32 cm^2 to 3.24 cm^2. In this investigation the most susceptible were the varieties Gülbaba, Agria, Somogy gyöngye, Parade and among the new potato line No. 84.1544, while the lowest values were observed with some lines: No. 84.1319, No. 82.1947, No. 84.1689, No. 84.1976 and No. 83.238. Variation coefficients (CV %) are shown in Fig. 2. Considering both Fig. 1 and Fig. 2 it is evident that there are genotypes having relatively low rotting values with a CV value less than 10% (i.e. No. 84.1319, No. 84.2227).

They are presumably better for a breeding program than genotypes with higher values. Higher tuber tissue susceptibility to *Phoma* was observed with the variety Agria, with relatively low CV %. Differences in rotting values of cm^2 are significant at $P_{5\%}$ level ($P=0.4063$).

Table 1

Rotting values in cm^2 and variation coefficiens in CV% of some potato varieties and new-bred lines infected with *Phoma exigua* var. *foveata* on the rose-end and stem-end of their tubers (Means of each 5 tubers)

Serial	Varieties or lines	Rotting values in cm^2 and variation coefficients (CV %)			
		Rose-end of the tubers		Stem-end of the tubers	
		cm^2	CV %	cm^2	CV %
1.	Somogy gyöngye	2.34	28.38	2.87	24.46
2.	Somogyi kifli	1.49	27.22	1.89	35.97
3.	Gülbaba	2.80	14.11	3.24	10.74
4.	Ciklámen	1.98	24.71	2.20	29.48
5.	Rusett Burbank	1.82	16.95	2.31	21.16
6.	Fanfare	1.57	8.00	2.06	29.19
7.	Asterix	1.80	11.10	1.82	18.94
8.	Maradonna	1.97	40.45	2.08	53.68
9.	Parade	2.12	17.23	2.97	34.10
10.	Desíree	1.63	20.06	1.81	13.56
11.	Flamenco	1.83	23.13	2.06	15.91
12.	Obeliszk	2.16	16.81	2.52	24.86
13.	Agria	2.93	11.38	2.90	7.88
14.	No. 82.1947	1.41	37.99	1.52	17.43
15.	No. 82.1959	1.32	23.07	2.06	12.38
16.	No. 83.238	1.36	16.49	1.94	21.91
17.	No. 84.779	1.46	13.97	1.87	20.86
18.	No. 84.1319	1.36	4.26	1.51	11.23
19.	No. 84.1335	1.68	22.24	2.11	22.23
20.	No. 84.1527	1.75	15.91	2.07	13.80
21.	No. 84.1544	2.17	26.90	2.87	11.30
22.	No. 84.1609	2.10	21.73	2.21	34.67
23.	No. 84.1689	1.55	14.7	1.69	19.55
24.	No. 84.1884	1.86	23.59	2.22	20.90
25.	No. 84.1976	1.61	21.21	1.69	9.48
26.	No. 84.1977	1.97	14.63	2.18	28.59
27.	No. 84.2164	2.26	20.14	2.32	26.30
28.	No. 84.2227	1.64	6.61	1.69	10.24
29.	No. 84.2367	1.75	17.92	2.04	23.32
30.	No. 84.2520	1.84	19.68	2.01	11.13
31.	No. 84.2527	1.71	13.27	2.21	21.74
Main means		1.85	19.16	2.16	21.19

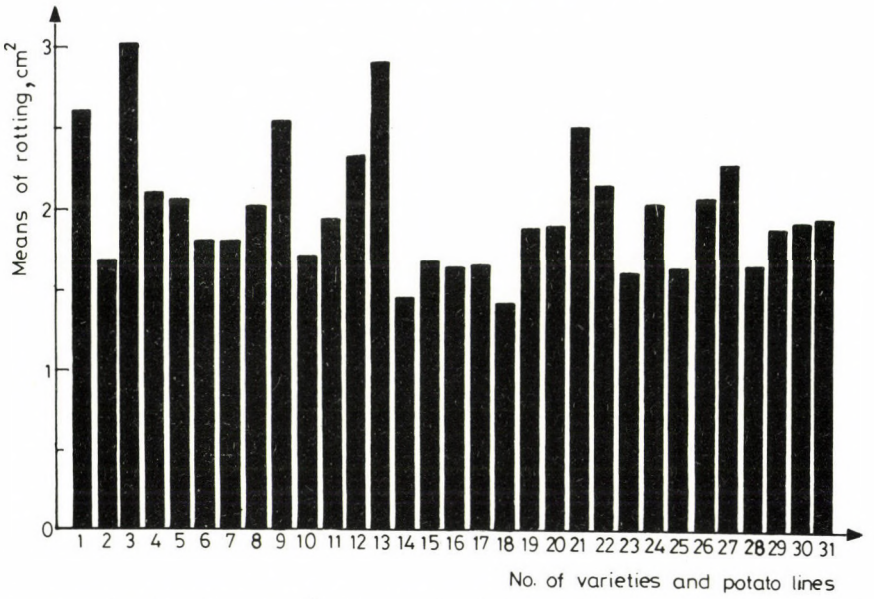


Fig. 1. Means of rotting values in cm^2 of the tested varieties and lines inoculated with *Phoma exigua* var. *foveata* (Numbers to serial No. of the varieties and lines given in Table 1)

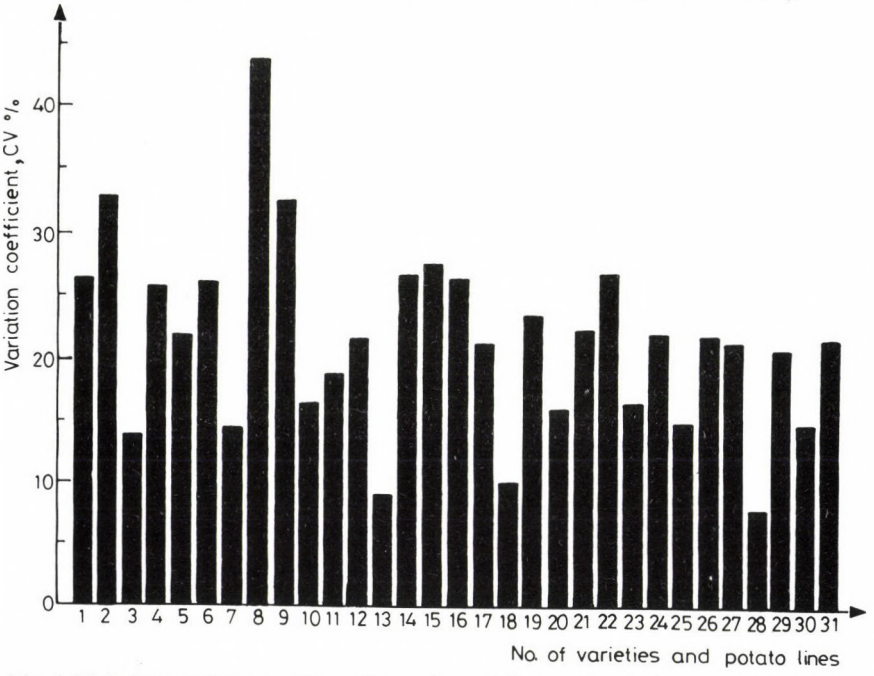


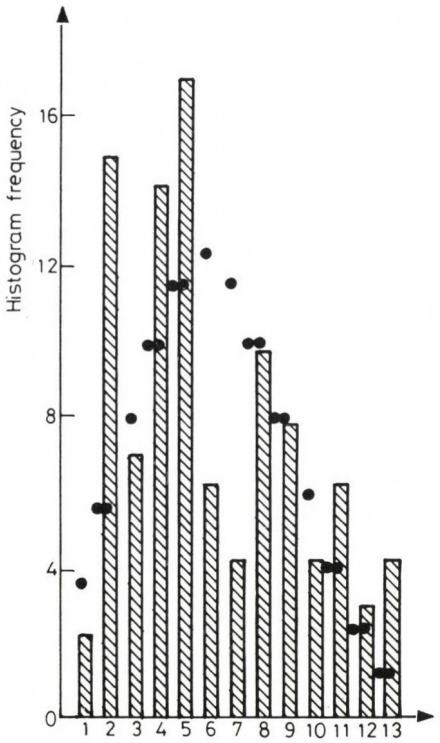
Fig. 2. Variation coefficients of the rotting values of the tested varieties and potato lines (No. of the varieties and lines given in Table 1)

The aim of measuring both stem-end and rose-end of the tubers was to study differences in rotting susceptibility of younger and older tuber tissues. The mean values of 5 tubers of each genotype are shown in Table 1. The mean values for stem-end for all the varieties tested is 2.16 cm^2, while for rose-end 1.85 cm^2 only, CV-values at the stem-end are also higher, i.e. variations between tubers were higher when inoculated at the stem-end. Highest CV-values are observed in the case of variety Maradonna and Somogyi kifli. The variety Somogyi kifli develops very long-shaped tubers, the ratio between length (width is greater than 5/1).

The colour tuber skin and tuber flesh can be divided as follows:

white skin/yellow flesh - 10 varieties
white skin/yellow flesh - 4 varieties
red skin/yellow flesh - 7 varieties
red skin/white flesh - 10 varieties.

Fig. 3 shows frequency-distribution of white-skinned/yellow fleshed genotypes by using a histogram. A greater part of the values belongs to the lower ranges (46 cases),



	Count	Value
1	<1.2	2
2	1.2 - 1.4	15
3	1.4 - 1.6	7
4	1.6 - 1.8	14
5	1.8 - 2.0	17
6	2.0 - 2.2	6
7	2.2 - 2.4	4
8	2.4 - 2.6	10
9	2.6 - 2.8	8
10	2.8 - 3.0	4
11	3.0 - 3.2	6
12	3.2 - 3.4	3
13	> 3.4	4

Fig. 3. Frequency distribution of white-skinned/yellow-fleshed potato varieties and lines according to their rotting values

which also be seen on the histogram. Though the distribution in the other skin/flesh groups is very similar. The biometrical analysis did not show any correlation between susceptibility of tuber to gangrene and different colour of skin or tuber flesh.

The wild potato genes containing new potato lines, together with the newly released Hungarian variety Ciklamen, showed on average 0.30 cm^2 lower values than that of the varieties considered to have only *S. tuberosum*-genes. This small difference does not allow us to state that resistance to gangrene could surely be increased by using genes from the mentioned wild potato species, but it is evident they do not decrease level of resistance.

The wild gene possessing lines tested here belong to 7 crosses and, as a consequence, there were few lines examined per crossing. Between values of sister-lines (for example under point 14 and 15, or 18 and 19, or 28 and 29 in Table 1) there was as large difference as between values of non-sister varieties. This is a consequence of the heterozygosity of the potato and of the fact that neither parents nor progeny have been tested for susceptibility before and selection has been made. The differences in susceptibility between genotypes refer to the possibility of decreasing varietal susceptibility by breeding work. However, choosing the correct method and testing throughout years is very important because there is a strong interaction between cultivars and years as Carnegie et al. (1989) reported. The season has an effect on the physiological status of the tubers, which can affect test values and the range of the genotypes tested. This is supported also by the fact, that even within a tuber, the stem-end is more sensitive than the physiologically different rose-end.

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Resistance of Wild *Cucumis* Species to Gummy Stem Blight (*Didymella bryoniae*)

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Nineteen accessions of eight *Cucumis* species were tested for resistance to *Didymella bryoniae*, the causative agent of gummy stem blight of cucumbers. Plants were inoculated both in the glasshouse and under growth chamber conditions. Resistance was found in *C. ficifolius* (PI 273648), *C. myriocarpus* (PIVT 0182) and *C. melo* var. *Agrestis* (PIVT 1165).

Gummy stem blight caused by *Didymella bryoniae* (Auersw.) Rehm (syn. *Mycosphaerella melonis* (Pass.) Chin & Walker) is highly destructive in many European countries (Van Steekelenburg, 1982). In Czecho-Slovakia the first occurrence of severe infestation of greenhouse cucumbers was reported in the early 80's (Lebeda, 1985). The sources of resistance in *Cucumis sativus* are rather limited (Boos and Belova, 1974; Prochorova, 1975; Van der Meer et al., 1978; Wyszogrodska et al., 1986). Resistances were described in related species, viz. *C. melo* (Pi 140471) (Prasad and Norton, 1968), *C. melo* cv. Tainan 2 (Sowel and Corley, 1974), *C. africanus* (Kiessling et al., 1985), and further in a related genus *Citrullus vulgaris* (PI 271778) (Sowel, 1975).

The objective of this investigation was to search for resistance in a yet untested set of *Cucumis* species and accessions which were not studied till now.

Materials and Methods

Nineteen accessions belonging to eight *Cucumis* species were tested (Table 1). For inoculation we used the isolates M-75-2 of *Didymella bryoniae* which was kindly provided by Dr N. A. M. Van Steekelenburg, Research Institute for Plant Protection (IPO), Wageningen, The Netherlands. The fungus was grown on potato-dextrose agar. Inoculum was prepared using 10-day-old culture.

Inoculation of plants in the glasshouse. In the glasshouse resistance was tested at the stage of adult plants which were raised in pots filled with garden soil. Leaves of seven week old plants were mechanically treated by abrasive paper and than inoculated with an agar block with sporulating mycelium. Air humidity was approximately 90%.

Inoculation of plants in the growth chamber. Pregerminated seeds were raised in plastic pots. For inoculation served plants at the stage of 2-4 leaves. For the inoculation the modified method as Svedelius and Unestam (1978) was used. Leaves were mechanically injured by pressing the surface with a 15 mm cork borer. Injured leaf area was inoculated with a droplet of spore suspension, the concentration being 2×10^6 spores per ml. Control plants of *C. sativus* were injured but not inoculated. Afterwards the inoculated plants were incubated at 24°C and 95% air humidity, under the 12-hour-photoperiod.

Table 1

Resistance of *Cucumis* species to gummy stem blight (*Didymella bryoniae*) in the glasshouse and growth chamber test

Cucumis species (genebank number)	Disease intensity	
	Glasshouse	Growth chamber
C. africanus		
PI 203974	nt	2
PI 274036	nt	1
PI 299569	1	2
C. dipsaceus		
PI 390450	2	nt
PIVT 1728	2	2
C. ficifolius		
PI 273648	0	0
C. figarei		
PIVT 1705	0	nt
C. melo var. *agrestis*		
PIVT 1165	0	nt
C. melo spp. *flexuosus* "Adzur"		
CUM 234/1973	2	2
C. metuliferus		
PIVT 0164	nt	2
PIVT 1747	nt	1
C. myriocarpus		
PI 203975	1	nt
PI 282449	1	1
PIVT 0182	nt	0
PIVT 0202	1	nt
C. zeyheri		
PI 299570	1	1
PIVT 0181	nt	2
PIVT 1053	nt	2
PIVT 1750	1	1
C. sativus (Trix F1, Fortuna F1, Leda F1, Sandra F1)	3	2

nt = not tested
Seeds were obtained from:

PI = Germplasm Resources Laboratory, USDA, ARS, Beltsville, USA;

PIVT = Centre for Plant Breeding Research (formerly Institute for Horticultural Plant Breeding), Wageningen, The Netherlands;

CUM = Central Institute for Genetics and Research of Crop Plants, Gatersleben, Germany

There were five plants per test (genotype) with a total of two leaves being inoculated in each plant by 40 μl inoculum droplets. Both tests were replicated 2 times. The plants in a glasshouse were evaluated on the basis of a 0-3 score scale (Van der Giessen and Den Nijs, 1981). Growth chamber tests were evaluated on the basis of a 0-2 score scale:

0 = no visible symptoms;
1 = slight yellowing;
2 = intensive yellowing or dry lesions.

Results and Discussion

The results from the glasshouse and growth chamber tests are summarized in Table 1. The occurrence of first disease symptoms in the glasshouse was recorded on control cultivars five days after inoculation. The disease progress was being assessed for about 6 weeks. Wild *Cucumis* species appeared to be more resistant compared to all cultivars of *C. sativus*. The accessions of *C. melo* var. *agrestis* (PIVT 1165) and *C. ficifolius* (PI 273648) were free of infection. Most tested accessions are characterized by slight infection (Table 1). Majority of the accessions showed slight or intermediate reactions. Heavy infection was not found. A resistant reaction was detected in *C. ficifolius* (PI 273648) and *C. myriocarpus* (PIVT 0182).

Heavy infection was only found in *C. sativus*. Slight and moderate infections are characteristic of several related species. In our study absence of infection was found in three accessions of three *Cucumis* species. A comparison of our results with other published data seems rather difficult. Leppik (1966) mentioned one sources of resistance without a precious description. A high degree of tolerance to *D. bryoniae* in *C. africanus* was recorded by Kiessling et al. (1985). Recent results support our previous assumption (Láska and Lebeda, 1989; Lebeda, 1984) that the widest resistance spectrum can be found in *C. ficifolius* and *C. africanus*. It is wishful to study the heritability of the resistances discovered here. This will prove the resistance and will elucidate its value for breeding. Further exploitation of wild *Cucumis* species as sources of resistance is related to overcoming the hybridization problems (Den Nijs and Custers, 1990).

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Virulenzdynamik des Gerstenmehltaus (*Erysiphe graminis* DC. f. sp. *hordei* March.) in Sortenmischungen der Sommergerste und deren Komponenten im Reinanbau

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Vier polyresistente Sortenmischungen der Sommergerste und deren 11 Komponenten im Reinanbau wurden anhand der Ergebnisse von Virulenzanalysen bei Mehltau verglichen. Der befallsreduzierende Effekt der Sortenmischungen gegenüber den Komponenten im Reinbestand ging einher mit einer relativen Verminderung der Häufigkeiten bestimmter Virulenzen. Besonnders bedeutsam ist die relative Häuftigkeitsverminderung der "seltenen" Virulenzen, da hierin die Schutzwirkung polyresistenter Sortenmischungen für Sorten mit noch nicht überwundener Resistenz zum Ausdruck kommt.

Polyresistente Sortenmischungen des Getreides wurden in erster Linie entwickelt, um unter Berücksichtigung epidemiologischer Aspekte zu dauerhafterer Resistenz gegen Blattkrankheiten zu gelangen (Wolfe und Barrett, 1981). Als besonders wirksam haben sich polyresistente Sortenmischungen der Sommergerste im Vergleich zu den Reinbeständen zur Senkung des Mehltaubefalls erwiesen (Wolfe, 1978; Zimmermann, 1982; Ibenthal u.a., 1985; Hengstmann, 1986).

Der befallsreduzierende Effekt polyresistenter Sortenmischungen wird in siner quantitativen Ausprägung von zahlreichen Faktoren beeinflusst. Unter diesen ist die resistenzgenetische Grundlage der Mischungspartner von vorrangiger Bedeutung. Die genaue Kenntnis der daraus resultirenden virulenzgenetischen Vorgänge in der Mehltaupopulation kann wichtige Hinweise für die Züchtung und zum Anbau geben und bei der Auswahl geeigneter Mischungspartner als Entscheidungshilfe dienen.

Material und Methoden

In einem randomisierten Parzellenversuch (Grösse pro Parzelle 8×2 m, vier Wiederholungen pro Variante) wurden vier Sortenmischungen und deren 11 Komponenten im Reinbestand angebaut (Tab. 1). Zur Isolierung der Parzellen dienten Hafer-Trennstreifen von 1 m Breite. Ab Epidemiebeginn wurden in 14-tägigen Abständen aus den Parzellen Mehltauproben entnommen und zur Virulenzanalyse verwendet. Dazu dienten Töpfe mit Fangpflanzen der universell-anfälligen Sorte 'Hana', die - in Bestandeshöhe inseriert - jeweils 6 Stunden in den Parzellen belassen wurden. Die Fangpflanzen wurden anschliessend acht Tage unter kontrollierten Bedingungen (18±2°C, 8/16-Stunden Licht/Dunkelrhythmus) inkubiert. Von den entstandenen Kolonien ausgehend wurde pro Variante eine wässrige Konidiensuspension definierter Konzentration (250-300 Konidien/cm^2) hergestellt, die nach einer standardisierten Inokulationsmethode über liegenden Primärblattsegmenten der Test- und Standardsorten versprüht wurde (10 Wie-

Tabelle 1

Sortenmischungen und ihre Komponenten (Feldversuch 1986)

Sortenmischung	Komponenten	Resistenzgrundlage
Ami	1. Salome	mlo+Ml a7
	2. Femina	Ml a13
	3. Ilka	Ml a H.spiti+Ml a7
	4. Dorina	Ml a3+Ml a7
	5. Nebi*	Ml a12
Bemi	1. Salome	mlo+Ml a7
	2. Defra	Ml a13+Ml a7
	3. Dera*	Ml a7+Ml a12
	4. Nebi*	Ml a12
Cemi	1. Salome	mlo+Ml a7
	2. Defra	Ml a13+Ml a7
	3. Ilka	Ml a H.spiti+Ml a7
	4. Nebi*	Ml a12
M 6	1. Elgina*	Ml a4+Ml a7
	2. Grit*	Ml a7+Ml a12
	3. Nebi*	Ml a12
	4. Trumpf*	Ml a7+Ml Ab
	5. Hana*	ohne bekannte vertikale Resistenzgene

* = vollanfällige Komponenten

derholungen) (Tab. 2). Die Inkubation der Testsortimente erfolgte unter den o.g. kontrollierten Bedingungen, die Auswertung am 9. Tag nach der Inokulation. Dazu wurde die durchschnittliche Pustelanzahl pro Blatt und Sorte ermittelt. Danach wurde die Pustelanzahl pro Testsorte prozentual zur durchschnittlichen Pustelanzahl auf den drei Standardsorten (≙ 100%) ausgedrückt.

Die so erhaltenen Werte repräsentieren die relativen Häufigkeiten der jeweils korrespondierenden Virulenzen in der Mehltaupopulation (Wolfe und Schwarzbach, 1975). Dreijährige Untersuchungen führten zu gleichsinnigen Aussagen. Für den vorliegenden Beitrag wurden die statistisch ausgewerteten Ergebnisse des Versuchsjahres 1986 verwendet.

Ergebnisse

Vergleich der Sortenmischungen mit den Reinbesänden ihrer Komponenten auf der Stufe der korrespondierenden Virulenzen.

Zur Auswertung diente ein parameterfreier Vergleich zweier Scharen von Verlaufskurven. In der Sortenmischung 'Ami' traten vier von fünf korrespondierenden Virulen-

Tabelle 2

Testsortiment zur Virulenzanalyse

Sorte	Resistenzgrundlage	Häufigkeit der Virulenzen
1. Goldfoil	Ml g	
2. Amsel	Ml g+Ml a7+1 dom.Gen	
3. Emir	Ml a12	
4. Europa	Ml g+Ml a12	häufig
5. HOR 1063	Ml a4	
6. Voldagsen 8141	Ml a6	
7. Maris Canon	Ml g+Ml a6	
8. Gerlinde	Ml a7+Ml a12	
9. Elgina	Ml a4+Ml a7	
10. Tamina	Ml a7+Ml a12+Ml a13	
11. Karat	Ml a13	
12. Defra	Ml a13+Ml a7	
13. HVS 19905/82	Ml a13+Ml a12	selten
14. Salome	mlo+Ml a7	
15. Ilka	Ml a H.spiti+Ml a7	
16. Dorina	Ml a3	
17. Monte Cristo	Ml a9+Ml a4	
18. Haisa	ohne bekannte vertikale Resitenzgene	
19. Hana	ohne bekannte vertikale Resitenzgene Standard	
20. Proctor	ohne bekannte vertikale Resitenzgene	

zen weniger häufig auf (gesichert bei $\alpha = 1\%$) als in den Reinbeständen der entsprechenden Komponenten. Die fünfte Virulenz zeigte die gleiche Tendenz. Während die relative Häufigkeitsverminderung der weitverbreiteten Virulenz V al2 mit Fortschreiten der Epidemie zurückging, nahm die der vier selteneren Virulenzen zu (Abb. 1). In der Sortenmischung 'Bemi' traten drei von vier korrespondierenden Virulenzen weniger häufig auf, eine häufiger (gesichert bei $\alpha = 1\%$) als in den entsprechenden Reinbeständen (Abb. 2). In der Sortenmischung 'Cemi' traten zwei von vier korrespondierenden Virulenzen weniger häufig auf (gesichert bei $\alpha = 1\%$) als in den Reinbeständen der entsprechenden Komponenten, die anderen zwei zeigten die gleiche Tendenz (Abb. 3). In der Sortenmischung 'M 6', die als einzige nur aus vollanfälligen Komponenten bestand, trat nur eine von vier korrespondierenden Virulenzen (V al2) weniger häufig auf (gesichert bei $\alpha = 1\%$) als im entsprechenden Reinbestand (Abb. 4). Nach der durchschnittlichen Häufigkeit der korrespondierenden Virulenzen hatten die Sortenmischungen die Rangordnung 'Ami' (34% des Standards), 'Cemi' (56% des Standards), 'Bemi' (60% des Standards) und 'M 6' (112% des Standards).

Vergleich der Sortenmischungen, die die Komponente 'Salome' enthalten, mit dem Reinbestand dieser Komponente auf der Stufe der korrespondierenden Virulenz (V lo+a7).

Die Auswertung erfolgte als parameterfreier Vergleich zweier Scharen von Verlaufskurven. In keiner der drei Sortenmischungen trat die Virulenz V lo+a7 so häufig auf wie im Reinbestand der Komponente 'Salome' mit der korrespondierenden Resistenz (gesichert bei $\alpha = 1\%$). Über vier Fünftel des Beobachtungszeitraumes war die relative Häufigkeitsverminderung in der Sortenmischung 'Bemi' am stärksten ausgeprägt, gefolgt von 'Cemi', 'M 6' und 'Ami'.

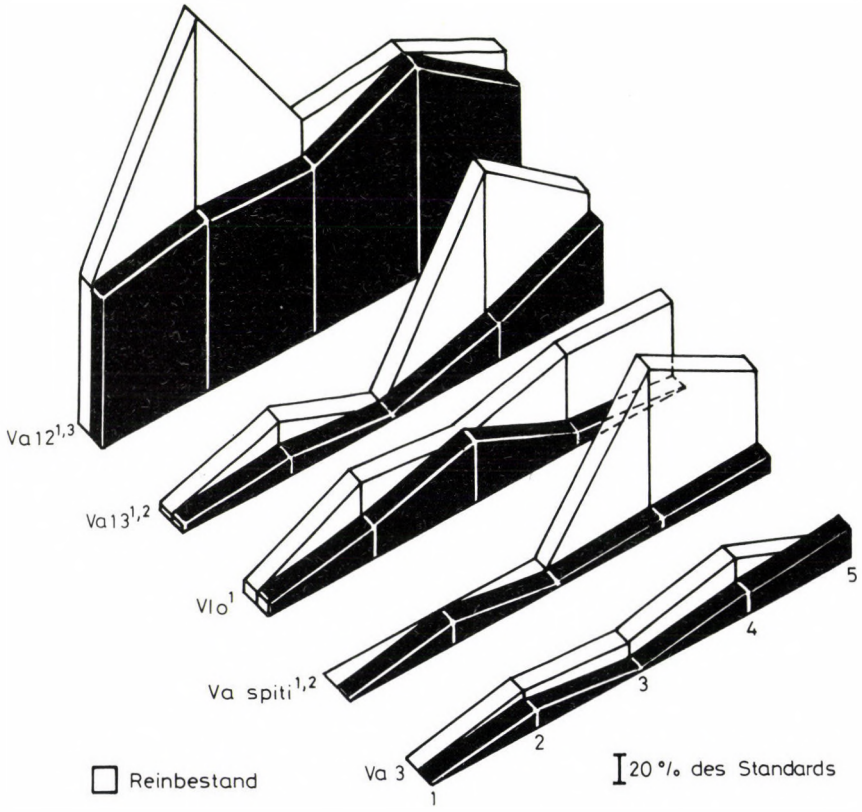

Abb. 1. Vergleich der Sortenmischung 'Ami' mit den Reinbeständen ihrer Komponenten 'Nebi', 'Femina', 'Salome', 'Ilka' und 'Dorina' auf der Stufe der jeweils korrespondierenden Virulenzen (V a12, V a13, V1 o, V a spiti und V a3). 1-5: Probenahme in Abständen von 14 Tagen signifikanter Unterschied ($\alpha = 1\%$) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

Zwischen den Häufigkeitsveränderungen der Virulenz V lo+a7 im Reinbestand der Komponente 'Salome' und in der Sortenmischung 'Ami' bestanden in frühen Phasen der Epidemie auffallende Ähnlichkeiten, jedoch vollzogen sich die Veränderungen in 'Ami' zeitlich verzögert und auf niedrigerem Niveau (Abb. 5).

Vergleich der Sortenmischungen, die die Komponente 'Nebi' enthalten, mit dem Reinbestand dieser Komponente auf der Stufe der korrespondierenden Virulenz (V a12).

Die Auswertung erfolgte als parameterfreier Vergleich zweier Scharen von Verlaufskurven (über die Termine) bzw. als einfaktorielle Varianzanalyse (pro Termin). Die Virulenz V a12 trat im Reinbestand der Komponente 'Nebi' durchschnittlich häufiger auf als in der Sortenmischungen 'Ami', 'Bemi' und 'M 6' (gesichert bei $\alpha = 1\%$), in der Sortenmischung 'Cemi' nur zu Beginn der Epidemie (gesichert bei $\alpha = 5\%$). In frühen

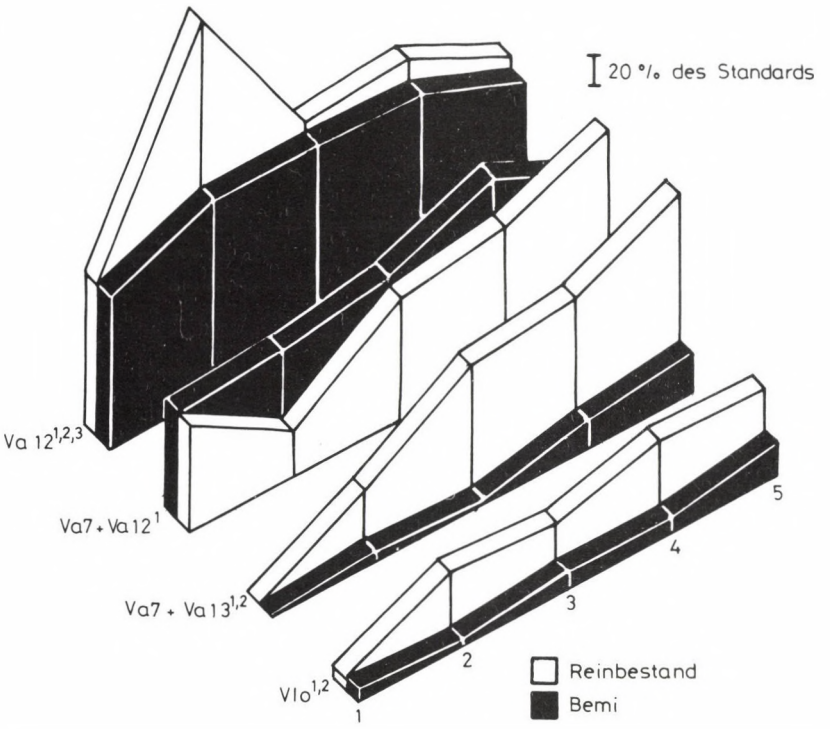

Abb. 2. Vergleich der Sortenmischung 'Bemi' mit den Reinbeständen ihrer Komponenten 'Nebi', 'Dera', 'Defra' und 'Salome' auf der Stufe der jeweils korrespondierenden Virulenzen (V a12, V a7 + a12, V a7 + a13 und V1 o). 1-5: Probenahmen in Abständen von 14 Tagen signifikanter Unterschied ($\alpha = 1\%$) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

Phasen der Epidemie war die relative Häufigketisverminderung der Virulenz V a12 gegenüber dem Reinbestand am ausgeprägtesten in der Sortenmischung 'Ami', gefolgt von 'M 6', 'Bemi' und 'Cemi'. Während des gesamten Beobachtungszeitraumes zeigte die Virulenz V a12 in der Sortenmischung 'Bemi' die geringste, in 'Cemi' dagegen die stärkste Bewegung. Der Unterschied der Sortenmischung 'Cemi' zum Reinbestand der Komponente 'Nebi' bestand vorallem in der zeitlich verzögerten Häufigkeitszunahme der Virulenz V a12 (Abb. 6).

Vergleich der Sortenmischungen mit dem Mittel ihrer vollanfälligen und dem ihrer weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand auf der Stufe von neun 'häufigen' Virulenzen.

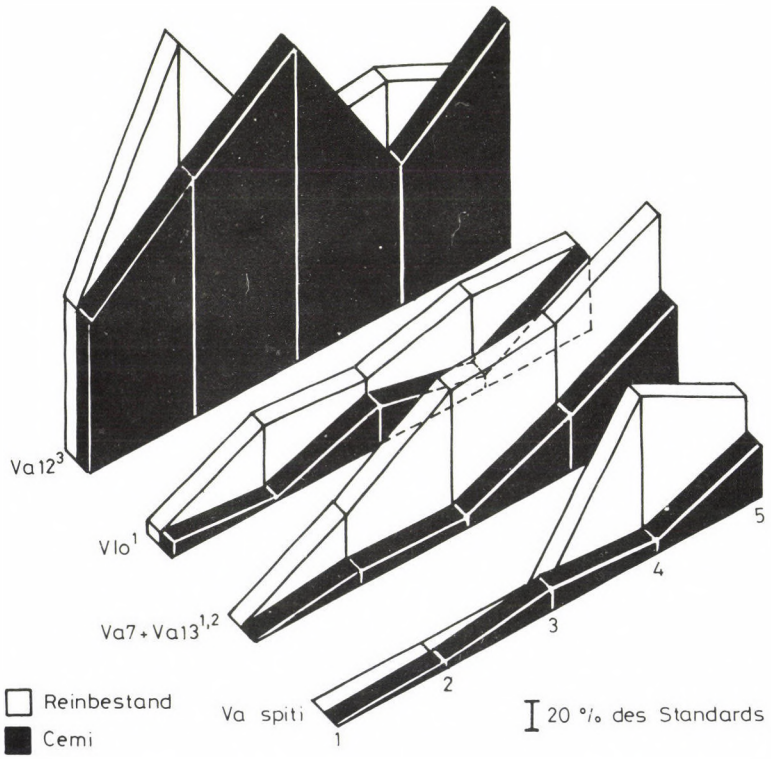

Abb. 3. Vergleich der Sortenmischung 'Cemi' mit den Reinbeständen ihrer Komponenten 'Nebi', 'Salome', 'Defra' und 'Ilka' auf der Stufe der jeweils korrespondierenden Virulenzen (V a12, Vl o, V a7 + a13 und V a spiti). 1-5: Probenahmen in Abständen von 14 Tagen signifikanter Unterschied (α = 1%) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

Die Auswertung erfolgte als einfaktorielle Blockanlage. Die o.g. Gruppe von Virulenzen trat in der Sortenmischung 'Ami' zu Beginn der Epidemie weniger häufig auf (gesichert bei $\alpha = 5\%$) als in der vollanfälligen Komponente 'Nebi' im Reinbestand. Gegenüber dem Mittel ihrer vier weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand war kein gesicherter Unterschied vorhanden (Abb. 7). Gegenüber dem Mittel ihrer beiden vollanfälligen Komponenten war die o.g. Gruppe von Virulenzen in der Sortenmischung 'Bemi' bei Epidemiebeginn weniger häufig (gesichert bei $\alpha = 5\%$) vertreten.

Gegenüber dem Mittel ihrer beiden weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand lag kein gesicherter Unterschied vor (Abb. 7). In der Sortenmischung 'Cemi' trat die o.g. Gruppe von Virulenzen zu Beginn der Epidemie weniger häufig auf (gesichert bei $\alpha = 5\%$) als in ihrer vollanfälligen Komponente 'Nebi' im Reinbestand. Gegenüber dem Mittel ihrer drei weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand war kein gesicherter Unterschied vorhanden (Abb. 7). In der Sortenmischung 'M 6', die nur aus vollanfälligen Komponenten bestand,

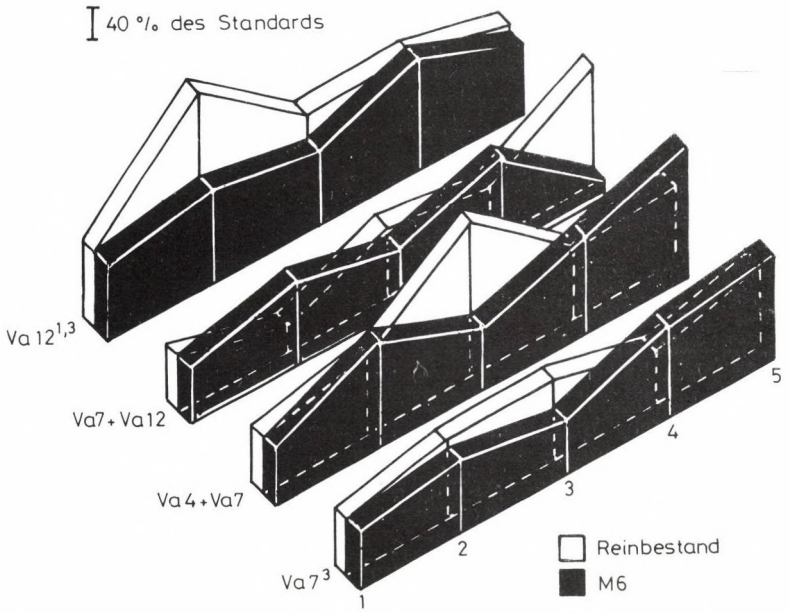

Abb. 4. Vergleich der Sortenmischung 'M 6' mit den Reinbeständen ihrer Komponenten 'Nebi', 'Grit', 'Elgina' und 'Trumpf' auf der Stufe der jeweils korrespondierenden Virulenzen (V a12, V a7 + a12, V a4 + a7 und V a7). 1-5: Probenahmen in Abständen von 14 Tagen signifikanter Unterschied ($\alpha = 1\%$) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

blieb die Häufigkeit dieser Gruppe von Virulenzen überwiegend unter dem durchschnittlichen Niveau der Reinbestände (gesichert bei $\alpha = 5\%$ für den 3. Termin) (Abb. 7).

Vergleich der Sortenmischungen mit dem Mittel ihrer vollanfälligen und dem ihrer weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand auf der Stufe von acht 'seltenen' Virulenzen.

Die Auswertung erfolgte als parameterfreier Vergleich zweier Scharen von Verlaufskurven. Die o.g. Gruppe von Virulenzen trat gegenüber dem Mittel der weitgehend resistenten bis schwachanfälligen Komponenten im Reinbestand in allen Sortenmischungen (ausser 'M 6', die nur vollanfällige Komponenten enthielt) mit geringerer Häufigkeit auf (gesichert bei $\alpha = 1\%$). Gegenüber dem Mittel der vollanfälligen Komponenten im Reinbestand war diese Gruppe von Virulenzen in den Sortenmischungen 'Bemi', 'Cemi'

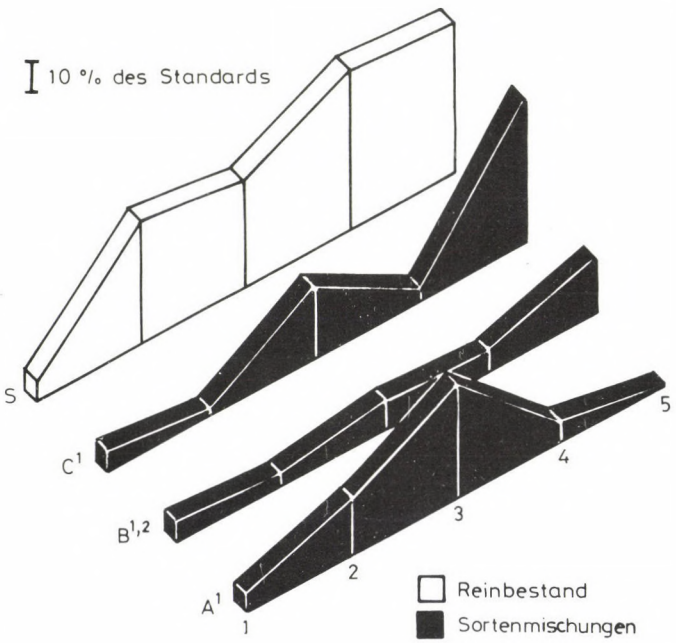

Abb. 5. Vergleich der Sortenmischungen 'Ami' (A), 'Bemi' (B) und 'Cemi' (C), die die Komponente 'Salome' enthalten, mit dem Reinbestand dieser Komponente (S) auf der Stufe der korrespondierenden Virulenz (V1 o). 1-5: Probenahmen in Abständen von 14 Tagen signifikanter Unterschied ($\alpha = 1\%$) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

und 'M 6' häufiger vorhanden (gesichert bei α = 1%), kein gesicherter Unterschied bestand diesbezüglich bei der Sortenmischung 'Ami' (Abb. 8).

Diskussion

Die Mehltaupopulation in Mitteleuropa stellt eine epidemiologische Einheit dar, weil die Konidien mit dem Wind über weite Strecken transportiert werden können, ohne ihre Vitalität zu verlieren und weil in der europäischen Gerstenzüchtung weitverbreitet die gleichen wenigen Resistenzgene in grossflächig angebaute Sorten eingelagert wurden. Durch langjährige Anpassung der Erregerpopulation an die Wirtspopulation dominieren

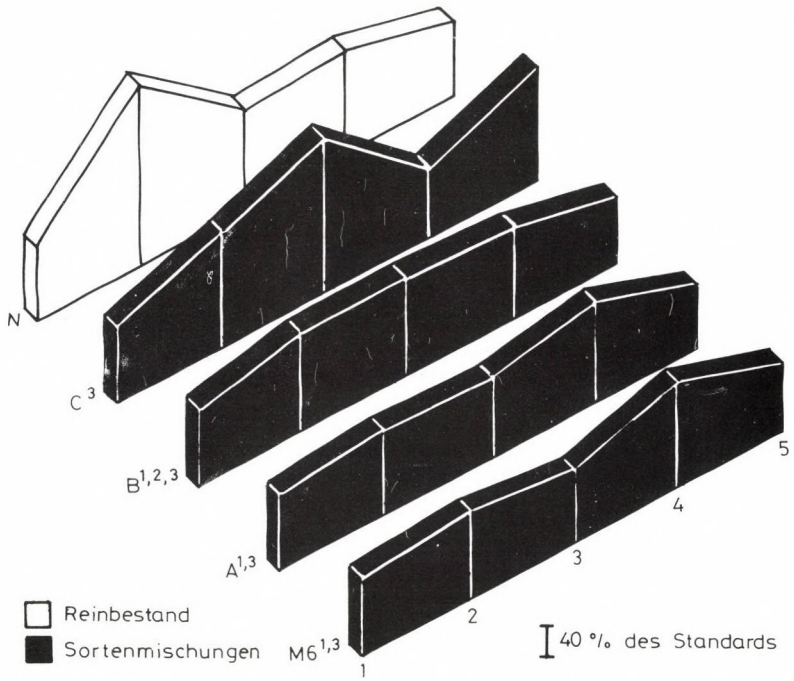

Abb. 6. Vergleich der Sortenmischungen 'Ami' (A), 'Bemi' (B), 'Cemi' (C) und 'M 6', die die Komponente 'Nebi' enthalten, mit dem Reinbestand dieser Komponente (N) auf der Stufe der korrespondierenden Virulenz (V a12). 1-5: Probenahmen in Abständen von 14 Tagen signifikanter Unterschied (α = 1%) zwischen den Mittelwerten ([1]), den Flächen unter der Kurve ([2]) bzw. den Kurvenanstiegen ([3])

in ihr die korrespondierenden Virulenzen der vorherrschend genutzten Resistenzen (Limpert und Schwarzbach, 1979, 1981a und 1981b; Welz und Kranz, 1983; Zwatz, 1982). Trotz der über Jahre relativ stabilen Grundstruktur der Mehltaupopulation vollziehen sich während der Epidemie z.T. bedeutende Verschiebungen der Häufigkeiten einzelner Virulenzen. Grad, Richtung und Beständigkeit dieser Veränderungen werden von zahlreichen Faktoren beeinflusst, unter denen die Resistenz und die Anbauform der Wirtspopulation eine besondere Rolle spielen (Barrett, 1980; Munk, 1983; Eckardt, 1984; Chin und Wolfe, 1984; Heun, 1987). Die dargelegten Ergebnisse haben gezeigt, dass analog zur relativen Befallsreduktion in den Sortenmischungen die Häufigkeiten bestimmter

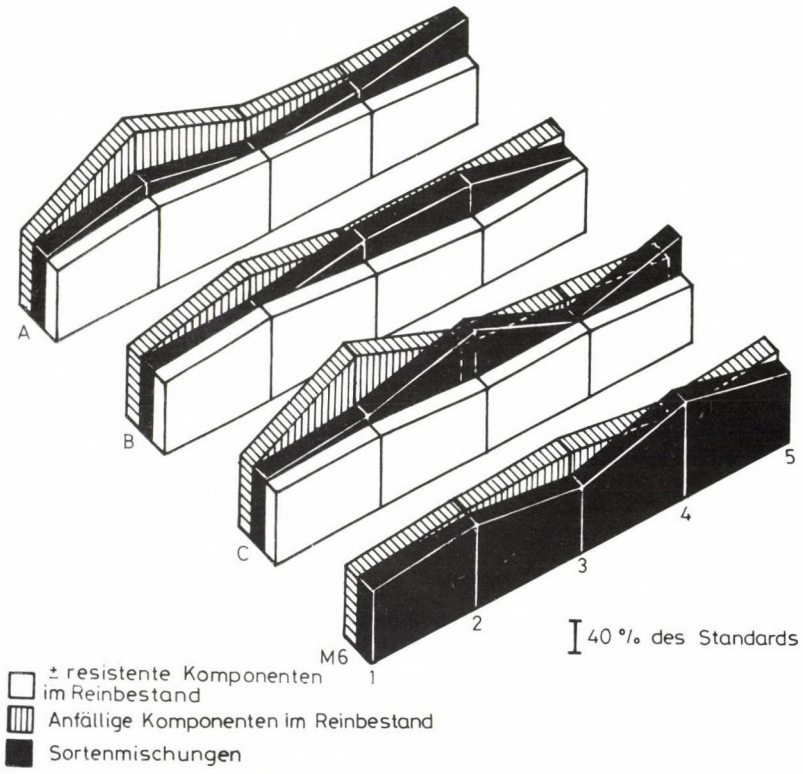

Abb. 7. Vergleich der Sortenmischungen 'Ami' (A), 'Bemi' (B), 'Cemi' (C) und 'M 6' mit dem Mittel ihrer vollanfälligen und dem ihrer weitgehend resistenten Komponenten im Reinbestand auf der Stufe von 9 'häufigen' Virulenzen. 1-5: Probenahmen in Abständen von 14 Tagen

Virulenzen gegenüber den Reinbeständen der Komponenten vermindert waren (Abb 1 bis 4). Dafür gibt es unterschiedliche Ursachen. Zu Beginn der Epidemie wirkt sich in jedem Falle die verminderte Dichte kompatibler Wirtspflanzen im heterogenen Bestand aus. Je geringer der Anteil einer anfälligen Komponente in der Mischung ist, desto stärker kommt dieser Effekt zur Geltung. Nach Wolfe und Minchin (1977) besteht ein direkter Zusammenhang zwischen der Verminderung des Befalls einer anfälligen Komponente und der Abnahme ihres Anteils in einem Gemisch mit einer resistenten Komponente. Dieser Zusammenhang wird durch die dargelegten Ergebnisse eindeutig bestätigt. Die Sortenmischung 'Ami' enthielt den geringsten Anteil vollanfälliger Komponenten (eine von fünf), gefolgt vom 'Cemi' (eine von vier), 'Bemi' (zwei von vier) und 'M 6' (fünf von fünf). Dem entsprach die Rangordnung der Sortenmischungen sowohl nach der Stärke der Befallsreduktion als auch nach dem Grad der durchschnitt-

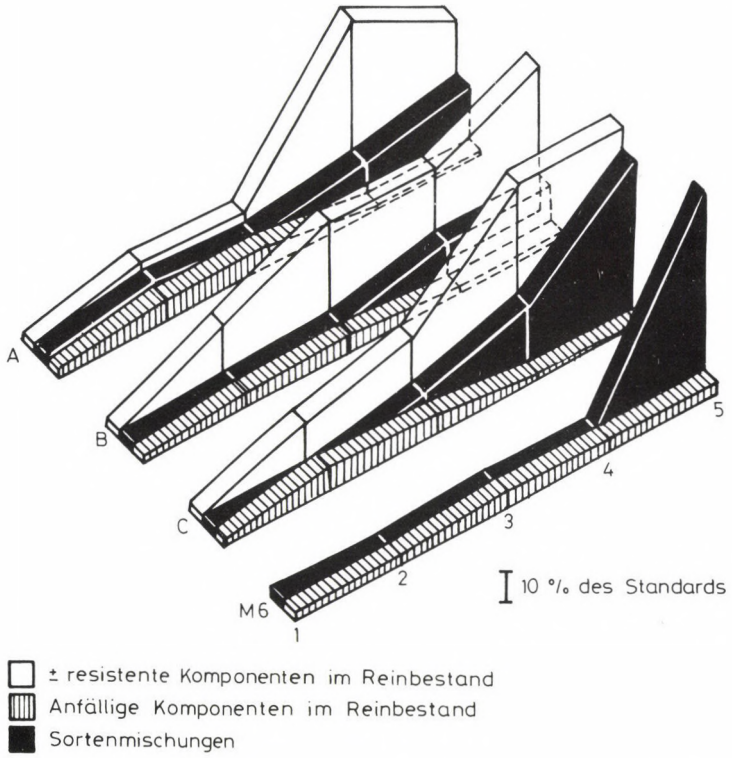

Abb. 8. Vergleich der Sortenmischungen 'Ami' (A), 'Bemi' (B), 'Cemi' (C) und 'M 6' mit dem Mittel ihrer vollanfälligen und dem ihrer weitgehend resistenten Komponenten im Reinbestand auf der Stufe von 8 'seltenen' Virulenzen

lichen Häufigkeitsverminderung der korrespondierenden Virulenzen. Bedingt durch die hohe Reproduktionsrate gut angepasster Rassen auf ihren kompatiblen Wirten nimmt die Wirkung der verminderten Dichte kompatibler, vollanfälliger Pflanzen in der Sortenmischung mit dem Fortschreiten der Epidemie mehr oder weniger rasch ab.

Hinzu kommen vielfältige Wechselwirkungen, durch die die Häufigkeitsveränderungen der Virulenzen beeinflusst werden. Die dargelegten Ergebnisse unterstreichen, dass unter diesen der Resistenzinduktion durch avirulente Mehltaurassen besondere Bedeutung zukommt (Chin u.a., 1984; Wolffgang und Pelcz, 1986; Pelcz und Wolffgang, 1986; Pelcz, im Druck). So war zum Beispiel die Häufigkeit der Virulenz V lo+a7 in den drei Sortenmischungen gegenüber dem Reinbestand der Komponente 'Salome' mit der korrespondierenden Resistenz in unterschiedlichem Masse verringert. Am stärksten war dies in 'Bemi' der Fall, gefolgt von 'Cemi' und 'Ami' (Abb. 5). Diese Reihenfolge entsprach nicht dem Anteil der Komponente 'Salome' in den drei Sortenmischungen, der in 'Ani' ein Fünftel, in 'Bemi' und 'Cemi' jeweils ein Viertel betrug, sondern dem gleichzeitig vorhandenen unterschiedlichen Anteil vollanfälliger Komponenten. Aus dem unterschiedlichen Anteil der vollanfälliger Komponenten resultierte eine entsprechend unterschiedlich grosse Menge avirulenten Inokulums, dem u.a. die Komponente 'Salome' ausgesetz war. Demgemäss hatte die Resistenzinduktion auf der Komponente 'Salome' in der Sortenmischung 'Bemi' die relativ stärkste Wirkung, gefolgt von 'Cemi' und 'Ami'. Die Richtigkeit dieser Deutung wird zusätzlich durch die Tatsache gestützt, dass auch die Virulenz V a13+a7 gegenüber dem Reinbestand der Komponente 'Defra' in der Sortenmischung relativ stärker vermindert war als in 'Cemi' (Abb. 2 und 3).

Durch Einteilung der Komponenten in vollanfällige und weitgehend resistente bis schwachanfällige bzw. der Virulenzen in 'häufige' und 'seltene' und den getrennten Vergleich der Gruppen konnten die vielschichtigen Zusammenhänge zwischen Wirts- und Erregerpopulation differenzierter verdeutlicht werden. Es zeigte sich, dass die 'häuftigen' Virulenzen, die Bestandteile weitverbreiteter, gut angepasster Rassen darstellen, in frühen Phasen der Epidemie in den Reinbeständen der vollanfälligen Komponenten stärker vertreten waren als in den Sortenmischungen (Abb. 7). Dieser Effekt beruht auf der bereits erwähnten Verminderung der Dichte kompatibler Wirtspflanzen im heterogenen Bestand und verliert mit der fortschreitenden Reproduktion des Mehltaus auf den anfälligen Komponenten an Bedeutung.

Im Unterschied zu den 'häufigen' sind die 'seltenen' Virulenzen an Rassen gebunden, die in der Erregerpopulation noch einen weit geringeren Anteil einnehmen und der Wirtspopulation weniger gut angepasst sind. Deshalb nimmt die Häufigkeit der 'seltenen' Virulenzen viel langsamer zu als die der 'häufigen'. Besonders verzögert wird dieser Vorgang in Sortenmischungen, da hier neben der verminderten Dichte kompatibler Wirtspflanzen der Einfluss avirulenten Inokulums unvergleichlich stärker wirkt als in den Reinbeständen durch Konidienzuflug von aussen. Deshalb blieb die Häufigkeit dieser Virulenzen in den Sortenmischungen permanent unter dem Niveau der Reinbestände der weitgehend resistenten bis schwachanfälligen Komponenten. Durch den Nachweis dieses Zusammenhanges wird die Richtigkeit der Entscheidung, in der DDR neuzugelassene Sorten mit schwacher Anfälligkeit oder noch nicht überwundener Resistenz nur als Mischungspartner anzubauen, auch aus virulenzgenetischer Sicht unterstrichen. Die festgestellte geringe Häufigkeit der 'seltenen' Virulenzen in den Reinbeständen der voll-

anfälligen Komponenten beruht darauf, dass sie hier überflüssig und der Konkorrenz durch die vorherrschenden Virulenzen nicht gewachsen waren. Gegenüber der Resistenzinduktion durch avirulente Mehltaurssen (Hwang und Heitefuss, 1982) spielen andere Wechselwirkungen wie Anfälligkeitsinduktion durch virulente Mehltaurassen, physikalische Interferenzen zwischen verschiedenen virulenten Rassen (Chin u.a., 1984) und Antagonismen zwischen bestimmten Virulenzen (Eckardt u.a., 1983; Limpert und Schwarzbach, 1981; Vanderplank, 1982; Smedegaard-Petersen und Telstrup, 1985) und der in späten Phasen der Epidemie wirksam werdende Barriereneffekt (Wolfe und Minchin, 1977) sicher eine untergeordnete Rolle. Zur gezielten Nutzung der Resistenzinduktion als Schutzfaktor weitgehend resistenter Komponenten ist offensichtlich ein gewisser Anteil anfälliger Komponenten in der Sortenmischung von Vorteil.

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Survival of *Alternaria helianthi*, the Leaf-Spot and Blight Pathogen of Sunflower in Kota, India

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Alternaria helianthi, the leaf-spot and blight pathogen of sunflower, is both externally and internally seed-borne. It is the first report showing that internally seed-borne inoculum is also important. Studies show that the pathogen can oversummer in the form of plant debris at Kota. It has been noted for the first time that air-borne inoculum has no role in its recurrence. At Kota, no collateral host of the pathogen was found excepting the garden sunflower.

Alternaria helianthi (Hansf.) Tubaki and Nishihara causes a severe leaf-spot and blight disease of sunflower (*Helianthus annuus* L.) in many countries. Sackston (1981) expressed reservation about the crop's future due to this disease. This pathogen is said to be soil-borne in Australia (Allen et al., 1983), soil-borne (Jeffrey et al., 1984) as well as seed-borne (Herr and Lipps, 1982) in the U.S.A., and soil-borne in Yugoslavia (Islam and Maric, 1980). From India, Bhaskaran and Kandaswamy (1977) indicated that in Karnataka, it perpetuates either through plant debris in soil or through seed, generally found externally seed-borne but rarely internally seed-borne also.

The role of collateral host(s), if any, is equally useful in providing information on the survival of a pathogen. From India, *Helianthus agrophyllus* and *H. debelis* (Anilkumar et al., 1976), and a *Tridex* sp. (Reddy, 1976) have been reported as additional hosts of *A. helianthi*. Allen et al. (1983) were able to artificially inoculate *Carthamus tinctorius* (safflower), *Xanthium pungens* and *X. spinosum* with the pathogen which was readily re-isolated.

Investigations were carried out to find the mode of survival of *Alternaria helianthi* in Kota, India.

Materials and Methods

Sunflower cv. 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.

Role of seed-borne inoculum

Fifty unsterilized and 50 surface-sterilized (with 0.1% $HgCl_2$) seeds from sunflower plants severely infected with *A. helianthi* were sown in sterilized soil in separate 23-cm

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pots (10 seeds/pot). The pots were covered with a cloth-chamber for protection from air-borne inoculum, if any. They were timely watered with sterile water. One month after sowing, the plants were examined. Controls consisted of seeds (from apparently disease-free plants) sown in sterilized soil.

Unsterilized and surface-sterilized seeds from severely diseased plants and unsterilized seeds from apparently disease-free plants (controls) were also plated in Petri plates on moist sterilized filter papers. Fungal colonies, which developed on these seeds, were examined and *A. helianthi*-type colonies were cultured on oatmeal agar and pathogenicity tested of the spores obtained from them.

Role of plant debris

Sunflower crop was grown in June 1978 (kharif) in a 5×3 m^2 plot (designated plot A) and was inoculated artificially with a spore suspension of *A. helianthi* in the third week of July which resulted in an epidemic out-break of the disease. The crop was harvested in the last week of September and the plant debris, including the blighted stem-pieces, was left in plot A. It was again sown with sunflower in the first week of November 1978 (rabi). No artificial inoculation was done this time. Disease incidence was noted on 15 randomly selected plants in the second week of December 1978 after the onset of winter rains and the development of a very high humidity. Half of the diseased stubbles were left in soil in plot A and the other half were collected and immediately spread in another plot B (5×3 m^2). Sunflower crop was sown during the kharif 1979 only after hot summer (in the second week of July) in both the plots A and B. The incidence of the disease on 15 randomly selected plants in each of the plots A and B were recorded in August 1979. Disease ratings (Percent Disease Indices, PDIs) were estimated as reported earlier (Ghemawat et al., unpublished).

Immediately after collection, the left-over plant debris, including the blighted stem pieces, of rabi 1978-79 crop from both the plots A and B (collected in the third week of June 1979 after the severe summer but before the next kharif planting) was examined microscopically.

Role of air-borne inoculum

Fields adjoining plots A and B were examined periodically for finding the role of air-borne inoculum.

Role of collateral hosts

Weeds of the family Asteraceae (Compositae) growing in and around Kota were examined periodically for *A. helianthi* infection. Weeds {*Ageratum conyzoides*, *Bidens pilosa*, *Blumea laciniata*, *Echinops echinatus*, *Eclipta alba*, *Gnaphalium pulvinatum*, *Launaea resedifolia*, *Sonchus oleraceus*, *Tridex procumbens*, *Vernonia cinerascens* and *Xanthium strumarium*} and ornamental plants {*Calendula arvensis*, *Carthamus tinctorius*, *Chrysanthemum indicum*, *Cosmos bipinnatus*, *Dahlia rosea*, *H. annuus* (the garden sun-

flower), *Tagetes erecta* and *Zinnia elegans*}, belonging to the family Asteraceae and growing at the Agricultural Research Stations, Borkheda and Karkhanabagh, Kota and the Government College, Kota were inoculated in situ with *A. helianthi* spores. The inoculated plants were maintained at >95% relative humidity for two days by covering them with thick cloth-chambers which were continuously kept moist. The inoculated plants were examined upto 10 days, and the incidence of the disease, if any, was noted. This experiment was repeated three times.

Results

Role of seed-borne inoculum

Infection developed on 17.02% plants when unsterilized seeds from severely diseased plants were sown in sterilized soil while only 8.33% plants developed infection when the surface-sterilized seeds from the same source were used (Table 1). None of the control plants showed any infection.

Unsterilized and surface-sterilized seeds from severely diseased plants, when plated on moist sterilized filter papers in Petri plates, showed 22% and 6%, respectively, developing *A. helianthi* while the controls (seeds from apparently disease-free plants) developing no *A. helianthi* (Table 2).

Role of plant debris

PDI on 15 randomly selected plants ranged from 25.2% (rabi 1978-79) to 13.3% (kharif 1979) in plot A and 10.4% (kharif 1979) in plot B. Seedling mortality of a few plants was also noted.

The left-over plant debris from both the plots A and B (after the severe summer) showed desiccated *A. helianthi*-type spores which could not be germinated. Isolations from the blighted stem pieces after surface sterilization, however, yielded *A. helianthi* whose pathogenicity was confirmed on sunflower. The pathogen could also be isolated in July 1979 from the diseased leaves and stems (from kharif 1978 from the plot A) preserved dry in the laboratory since October 1978.

Role of air-borne inoculum

The suflower crop in the fields adjoining the plots A and B, which was without any *A. helianthi*-infected plant debris, developed only traces of infection (Disease Rating =1; PDI = <1%) only on a few plants and that too only in late September/early October.

Role of collateral hosts

Natural infection of *A. helianthi* was not observed on any weed or ornamental plant belonging to the family Asteraceae at Kota. After artificial inoculations, none of 11 local

Table 1

Development of *Alternaria helianthi* infection on sunflower plants (cv. EC 68414) from seeds obtained from severely diseased plants

Seeds	No. seeds sown	No. seedlings emerged	No. *A. helianthi*-infected plants	% *A. helianthi*-infected plants
Unsterilized seeds from severely diseased plants	50	47	8	17.02
Surface-sterilized seeds from severely diseased plants	50	48	4	8.33
Control (unsterilized seeds from apparently disease-free plants)	50	48	0	0

weeds and eight ornamental plants of family Asteraceae, showed any infection, except the garden sunflower. The leaf-spot symptoms produced on the garden sunflower varied from those produced on the cultivated sunflower in being different in shape and bigger in size (generally round and 0.6-0.95 cm in diameter as compared to those on cultivated sunflower: more or less rectangular and 0.29-0.3×0.23-0.27 cm). However, both had halos around the leaf-spots. No symptom developed on stems of the garden sunflower while minute spots were observed on those of the cultivated sunflower.

Discussion

Role of seed-borne inoculum

Tables 1 and 2 show that perennation, recurrence and spread of the pathogen is possible through the host seed, confirming Bhaskaran and Kandaswamy's (1977) and Herr and Lipps' (1982) results but refuting those of Islam and Maric (1980). The pot

Table 2

Development of *Alternaria helianthi* colonies on seeds obtained from severely diseased sunflower plants (cv. EC 68414)

Seeds	No. seeds planted	No. seeds with *A. helianthi* colonies	% seeds with *A. helianthi* colonies
Unsterilized seeds from severely diseased plants	50	11	22
Surface-sterilized seeds from severely diseased plants	50	3	6
Control (unsterilized seeds from apparently disease-free plants)	50	0	0

experiment showed 17.02% infected plants from the unsterilized seeds obtained from severely diseased plants (Table 1) which should be from both the externally and the internally seed-borne inocula. Bhaskaran and Kandaswamy's (1977) results are not comparable as they used surface-sterilized seeds which were then inoculated with *A. helianthi*.

From the surface-sterilized seeds from the severely diseased pants 8.33% getting infected (Table 1), indicating it to be from the internally seed-borne inoculum. The difference between the two percentages (17.02-8.33=8.69%) can possibly be attributed to the externally seed-borne inoculum.

In Petri plates, unsterilized seeds from severely diseased plants developed *A. helianthi* on 22% seeds (Table 2) which should be due to both the externally and the internally seed-bone inocula. From the surface-sterilized seeds from severely diseased plants, 6% developing *A. helianthi* (Table 2) should be due to the internally seed-borne inoculum. The difference between these two percentages (22-6=16%) can possibly be attributed to the externally seed-borne inoculum. Bhaskaran and Kandaswamy's (1977) lower figures (12% and 1%, respectively) may be due to different experimental conditions, cv. and/or fungal isolate. Further, they do not mention the number of seeds tested and that number (if small) may be responsible for such a low percentage (1%) from the internally seed-borne inoculum.

Role of plant debris

Diseased plant debris left in plots A and B at Kota during summer (avg. weekly max. temp. 45°C) could cause infection in the kharif plants, indicating the pathogen's oversummering under the local conditions. However, the spores in the plant debris were found to be desiccated after summer and they could not be germinated. But such plant debris after surface sterilization produced *A. helianthi* with pathogenicity to sunflower, indicating oversummering of the pathogen possibly in the form of mycelium. Our studies confirm the results of Islam and Maric (1980), Allen et al. (1983) and Jeffrey et al. (1984) that the fungus perpetuates through plant debris in soil. Bhaskaran and Kandaswamy (1977) do not mention the period of year and hence their conclusion that the pathogen survives for at least 20 weeks in soil is of no practical importance.

Role of air-borne inoculum

The trace infection in the adjoining fields, without any diseased plant debris, appearing naturally but quite late in the season, is most probably due to secondary air-borne inoculum from the severely diseased crop of the nearby infested fields including the plots A and B.

Role of collateral hosts

Local common weeds and ornamental plants of family Asteraceae were neither naturally infected with *A. helianthi* nor could be artificially infected by it. The only

exception was the garden sunflower. The slight differences in symptoms on garden sunflower may be due to different anatomy, but this aspect was not investigated further.

Our results indicate that at Kota, *A. helianthi* is probably restricted only to *H. annuus*.

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Rhizosphere Competence of Two Selected *Trichoderma* Strains

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Rhizosphere competence of *Trichoderma* spp. was measured for roots of pepper when soil and seeds were treated with conidia of fungus in autoclaved and nonautoclaved soil.

In the autoclaved soil the activity of minor pathogens was reduced soil was more expressed than in nonautoclaved soil.

In seed treatments we had higher seed germination than in soil treatments. This result is correlated with R ratio, which compares the rhizosphere (r) population with the nonrhizosphere soil (s) population.

The use of *Trichoderma* spp. as biological control agents are clearly established, however there are some experiments without success (Vajna, 1987). Therefore the researchers pay more attention to the study of mechanisms of action of *Trichoderma*, mainly to the rhizosphere competence, which is related to plant growth (Ahmad and Baker, 1985, 1987; Chao, Nelson, Harman and Hoch, 1986; Papavizas, 1981).

Microorganisms in the rhizosphere, especially antagonistic species, often have a strong growth and development stimulating effect on plants. This is because many of them are active producers of vitamins, amino acids, auxins, and enzymes, etc.. At the same time some of these species can reduce the growth and reproduction of phytopathogenic organisms.

The purpose of our study was to establish the rhizosphere competence of two selected antagonistic strains of *Trichoderma*: T-14 (*Trichoderma harzianum*) and Tv-5 (*Trichoderma viride*); and their influence on the germination of pepper seeds.

Materials and Methods

Soil. Sandy peat soil was collected from Hódmezővásárhely, and passed through a 4-mm mesh screen. One half of soil was autoclaved for 48 hr before use.

The soil had the following characteristics: *p*H: 7.25, humus 5.06%, NO_3-N 89%, P_2O_5 456 ppm, K_2O 495 ppm, Zn 9.1 ppm, Fe 334 ppm.

Trichoderma spp. *Trichoderma harzianum* (T-14) was isolated from carnation soil in Szentes and *Trichoderma viride* (Tv-5) was isolated and identified by Vajna, L. Both strains had good effect in controlling some of the soil-borne phytopathogens and also increased seed germination and enhanced plant growth (Anonym, 1986, 1987).

Seed treatment. Strains of *Trichoderma* spp. were grown on potato dextrose agar (PDA), plates were incubated for 7 days at 25°C, then flooded with sterile distilled water and conidia were gently freed from culture surface with brush. Density of conidia was adjusted to 10^8 per milliliter with the aid of Bürker chamber.

Seeds of pepper (*Capsicum annuum* L.) were surface disinfected for 10 minutes in 1.1% sodium hypochlorite solution and air-dried. Seeds were treated with conidia suspensions of *Trichoderma* spp. in water containing 3% FH sorbent (Bácska Mgtsz, Vaskút, Hungary, consisting of carboxyl-methyl-cellulose) as a spreader or sticker. Conidia density was adjusted to 105 per seed. The control seeds were treated only with FH sorbent.

The conidial density per seed was determined by suspending the inoculated seeds in sterile distilled water. The conidia counted in the washed water by using Bürker chamber and serial dilution-planting method on *Trichoderma*-selective medium (Davet, 1979).

Soil treatment. The conidial suspensions of *Trichoderma* spp. were obtained from potato-dextrose agar (PDA), were grown on cooked barley seeds for highly mass production (Anonym, 1985).

Production of conidia of the T-14 strain was 5×10^9 and of Tv-5 strain 2×10^9 conidia per gram on barley. The powder of air-dried barley was used in soil treatments.

Rhizosphere competence assay. Plastic culture boxes (30×90×12 cm) were divided into two halves for two replicates. One treatment had four replicates. All of boxes were filled with autoclaved and nonautoclaved soil. Half of the boxes were filled with treated soil inoculated by *Trichoderma* strains. *Trichoderma* powder was added (109 conidia per kg of soil) to soil and mixed thoroughly just before the boxes were filled. 20 untreated seeds were placed on these boxes. The other half of the boxes were filled with untreated soil, and 20 treated seeds (105 conidia per seed) were placed on them.

Seedlings were sampled at 10 days after gemination, 10 seedlings were carefully removed from soil, then as much of the soil adhering to the roots as possible was removed carefully by washing the roots with running tap water. This preliminary root washing was followed by serial washing the roots with sterile water (Harley and Waid in: Parkinson and Pearson, 1963).

After washing, excess water was removed from the roots with sterile filter paper. The roots were then cut into 1-mm segments with a sterile scapel. The root segments with their adhering rhizosphere soil were air-dried in the room temperature. Each unit was weighed and transferred to 200 ml Erlenmayer flasks containing 30 ml of sterile distilled water. The flasks were shaked with 200 f/h speed for 30 minutes. The colony-forming units (CFU) of *Trichoderma* contained in the rhizosphere soil at each root samples of one treatment were determined by plating dilutions from the flasks on *Trichoderma*-selective medium.

Plates were incubated at 25 °C for 5 days, counts of *Trichoderma* colony-forming units per milligram of rhizosphere soil were made with four replicates per treatment.

Nonrhizosphere soil colonization tests. Nonrhizosphere soil was sampled at 10 days after germination, too. 10 gram of soil was taken from each replicate of the treatments. The samples of soil of one treatment were mixed up and 10 gram was weighed from mixed soil and transferred to 250 ml Erlenmeyer flasks containing 100 ml sterile distilled water. The flasks were shaked for 30 minutes.

The colony-forming units of *Trichoderma* contained in the nonrhizosphere soil at each treatment were determined by plating dilutions from the flasks on *Trichoderma*-selective medium.

Table 1

The effect of *Trichoderma* treatments on the germination of pepper seeds

Treatment	Method of treatment	Autoclaved soil						Nonautoclaved soil					
		replicates				aver-age	%	replicates				aver-age	%
		1	2	3	4			1	2	3	4		
1. Untreated control	-	15	18	18	16	16.75	83.75	16	17	15	14	15.5	77.5
2. *Trichoderma viride* (Tv-5)	seed treatment	20	18	19	19	19.0	95.0	17	18	18	15	17.0	85.0
3. *Trichoderma harzianum* (T-14)	seed treatment	18	16	20	20	18.5	92.5	19	16	15	17	16.75	83.75
4. *Trichoderma viride* (Tv-5)	soil treatment	13	15	18	12	14.5	72.5	12	14	13	13	13.0	65.0
5. *Trichoderma harzianum* (T-14)	soil treatment	19	15	18	16	17.0	85.0	14	16	17	15	15.5	77.5
		P 5% = 2,209						P 5 % = 2,416					

After that the R ratio was found, which compared the rhizosphere (r) population with the nonrhizosphere soil (s) population (Curl, 1982).

Results

Seed germination stimulating effect. The two *Trichoderma* showed to have stimulating effect to seed germination as seed treatment. In sterile soil, when pepper seeds were treated with conidia of Tv-5, the germination was 95% and the treatments with conidia of T-14, the germination was 92.5%. The germination of untreatments had only 83.75% (Table 1).

In nonsterile soil, when conidias of Tv-5 were applied as seed coats of pepper, it produced 109.6% and T-14 resulted 108.1% of germination when compared with germination of the untreated seeds.

In soil treatments both of two *Trichoderma* did not make better results than the control. Moreover the Tv-5 reduced the germination of seed in both of sterile and nonsterile soils.

Rhizosphere competence effect. In all of the seed and soil treatments, the population densities of *Trichoderma* with rhizosphere competence were higher in sterile soil than in nonsterile soil.

In the nonrhizosphere soil the populations of *Trichoderma* were formed in another way, population densities were higher in nonsterile soil than in sterile soil. We suppose that it could occurred because the soil microorganisms had a high entire effect on the rhizosphere competence and on the formation of *Trichoderma* population.

Table 2

Population of *Trichoderma* spp. in autoclaved soil (CFU ømg soil×10^2)

Treatment	Method of treatment	Population of *Trichoderma* spp. in rhizosphere soil					Population of *Trichoderma* spp.in nonrhizosphere soil					Total	R
		replicates				average	replicates				average		
		1	2	3	4		1	2	3	4			
1. Untreated control	-	0.19	0.19	0.19	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.14	.
2. *Trichoderma viride* (Tv-5)	seed treatment	0.62	0.74	0.12	0.14	0.40	0.00	0.26	0.26	0.26	0.19	0.62	2.2
3. *Trichoderma harzianum* (T-14)	seed treatment	0.23	1.16	1.16	0.46	0.75	0.80	0.00	0.26	0.00	0.26	1.01	2.0
4. *Trichoderma viride* (Tv-5)	soil treatment	3.33	5.18	3.33	2.96	3.70	12.26	17.60	12.08	12.80	13.86	17.5	0.2
5. *Trichoderma harzianum* (T-14)	soil treatment	1.78	1.78	2.85	1.43	1.96	4.53	5.60	5.33	5.86	5.33	7.29	0.4
		P5% =0.643					P5% =1.365						

Table 3

Population of *Trichoderma* spp. in nonautoclaved soil (CFU/mg soil×10^2)

Treatment	Method of treatment	Population of *Trichoderma* spp. in rhizosphere soil					Population of *Trichoderma* spp.in nonrhizosphere soil					Total	R
		replicates				average	replicates				average		
		1	2	3	4		1	2	3	4			
1. Untreated control	-	0.00	0.00	0.00	0.58	0.14	6.66	5.6	6.4	5.86	6.13	6.27	0.02
2. *Trichoderma viride* (Tv-5)	seed treatment	0.52	0.00	0.00	0.00	0.13	2.13	1.6	0.26	2.4	1.59	1.72	0.08
3. *Trichoderma harzianum* (T-14)	seed treatment	0.52	0.00	0.52	0.00	0.26	3.46	3.73	3.73	5.06	3.99	4.25	0.06
4. *Trichoderma viride* (Tv-5)	soil treatment	3.33	0.00	3.33	5.00	2.91	11.73	12.26	12.00	12.53	13.13	15.0	0.23
5. *Trichoderma harzianum* (T-14)	soil treatment	0.00	0.00	1.53	0.76	0.57	23.46	19.20	20.00	16.53	19.79	20.3	0.09
		P5% =1.21					P5% =1.99						

When the germination of peppers (Table 1) was compared to colonies of *Trichoderma* (CFU) (Table 2 and 3) it showed that the R ratio closely related to seed germinations and the total number of colonies of *Trichoderma* (r+s) or only the colony number in the rhizosphere *Trichoderma* somehow had only indirect effect on it.

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Numerical Analysis of *Pseudomonas fluorescens-putida* Rhizoplane and Tuber Surface Populations of the Potato Cultivar Hungarian Rosa (Contributions to the Bacteriology of Potato I.)

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Typical members of the *Pseudomonas putida* biovar-A predominate against *Erwinia carotovora* subsp. *carotovora* active fractions of the rhizoplane and tuber surface epiphytic pseudomonad populations of the studied potato cultivar Hungarian Rosa. Chiefly the forms of biovar-A may be responsible for the iron dependent protection of this potato cultivar against pathogens. The numerical analysis, however, revealed, that besides *P. putida*, the members of *P. fluorescens* furthermore different interspecific intermediate forms also participate in the composition of these antagonistic pseudomonad communities. Our findings clearly demonstrate the wide range of variability of these organisms and the existence of continuous sequences of species-interconnecting varieties within their individual natural populations. The *Pseudomonas fluorescens-putida* group can be considered as a hardly separable large assemblage of very closely related variable organisms.

Although the resistance of potato against pathogenic organisms (Elesawy and Szabó, 1979) and the latter behaviour in its root-zones and tuber surface regions have been thoroughly studied by many workers (Kloepper et al., 1980; Kloepper and Schroth, 1981a, 1981b; Schroth and Hancock, 1982; Burr et al., 1978; Misaghi et al., 1981), tili now little is known on the complex species or biotype composition and particular community metabolism of its indigenous rhizoplane and tuber's periderm bacterial populations. E.g. in the future such investigations could perhaps clarify the reasons for the at present still incomprehensible fact that in the very same and heavily infected soil the tubers of the same potato cultivar can be attacked by *Streptomyces scabies* very differently: strongly damaged tubers can be closely associated with well developed completely healthy ones. Presumable the differences in the composition of epiphytic bacterial communities on the individual tubers are responsible for this heterogeneity in the sensibility against the pathogen as it was found by Tóth A. L. (personal communication) during his latest studies.

We intend to carry out a series of detailed numerical analyses on potato bacterial communities and to study the interrelationships among the members of the latter and the invader pathogens. Below, we present the first results of our numerical analyses carried out on the against *Erwinia carotovora* active fractions of the rhizoplane and tuber surface pseudomonad populations of the potato cultivar Hungarian Rosa.

Materials and Methods

The studied potato-cultivar. The Hungarian Rosa. *Isolations of bacteria from the rhizoplane.* The root washing method was employed to liberate the collected mass of fine potato roots from soil particles. The roots washed seven times by sterile tapwater were

mechanically homogenized, serially diluted, plated onto King-B agar plates and incubated at 28°C for 2-3 days. Colonies were isolated on a random manner.

Isolations of bacteria from tuber surfaces. Tubers, after removing soil particles from their surfaces, were shaked in sterile water on a shaker and the obtained suspensions were diluted and plated onto King-B agar plates (proteose pepton N° 3. 20.0g; glycerol 10.0g; $K_2HPO_4 \times 3H_2O$ 2.0g; $MgSO_4 \times 7H_2O$ 1.5g; agar 20.0g; dist. water 1000ml). Isolations were carried out after 2-3 days incubation at 28°C. Maintenance of strains: on with 1% glycerol enriched nutrient agar slants.

Cultural-morphological studies of selected strains. Colour, elevation and viscosity of colonies on different diagnostic media were observed and recorded. The shape, size of the cells and their cilia were studied partly by light microscopical studies partly by electronmicroscopic observations. Pyocianin pigment production characteristic on *Pseudomonas aeruginosa* was studied on King-A medium and the diffusible pyoverdin pigment excretion as well as fluorescein production on King-B medium (King et al., 1954).

Methods used to study the physiological-biochemical activities of the selected strains. Pathogenicity was detected partly by inoculation of tuber pieces in Petri dishes with the suspension of the individual strains, partly with inoculated tuber pieces in nutrient broth. In both cases the decay of the tuber tissues were studied; antibiosis-test, sensibility of *Erwinia carotovora* ssp. *carotovora* was studied on King-B agar plates in Petri-dishes against the individual strains using the cross-streak method. The radius of the inhibition zones was measured. For detailed further studies were selected only the non pathogenic but active antagonistic strains; sensibility of our strains against 25 different antibiotics was detected on nutrient agar plates using the Resistest disks (Human, Budapest); catalase activity, ten per cent H_2O_2 was added onto 24-48 hr. old cultures, and intensity of gas production was observed; oxidase activity was detected by cytochrome oxidase paper strips (Pathotec Co.); Voges-Proskauer reaction and methyl-red test were carried out in glucose-phosphate broth; acetoin was detected by Barrit's modification; nitrate reduction was tested in Bacto Nitrate Broth, detection according to Cowan and Steel (1965); denitrification activity was detected according to Stanier et al. (1966); liquefaction of gelatin was studied with the aid of Charcoal discs (Oxoid); oxidative and fermentative degradation of glucose was tested by the method of Hugh and Leifson (1953); urease activity was detected by Christensen's method (1946), with Bacto Urea Base; the method of arginine hydrolysis offered by Thornley (1960) was employed after 3-7 days incubation; lipolytic activity was observed with Tween 40, 60 and 80 on the Sierra-medium (Sierra, 1957); starch hydrolysis was tested with soluble starch containing agar plates by Lugol's iodine solution; DN-ase and RN-ase tests were carried out according to Jeffries et al. (1957); growth on peptone-yeast extract agar (PY medium) at temperatures 4°C, 41°C and 46°C; NaCl tolerance (0%, 4% and 6.5% NaCl) and growth at different *p*H values (3-5-8 *p*H) were detected in nutrient broth; for the phosphatase production were used nutrient agar plates containing sodium phenolphtalein-phosphate and for sulphatase production was used nutrient medium containing potassium phenolphtalein-disulphate as described by Cowan and Steel (1965); ammonia production was observed in pepton-water cultures, with Nessler's reagent; indol production was detected with Kovacs's reagent; growth on Bacto MacConkey agar was observed after 6 days; utilization of carbohydrates as sole carbon sources was studied in a medium recommended by Palleroni

and Doudoroff (1972): for the reduction of methylene blue was employed nutrient broth containing 0.0066% methylene blue; aesculin hydrolysis was showed according to Cowan and Steel; sensitivity to lysozyme was detected in 0.001% lisozyme containing nutrient broth; casein hydrolysis was observed on Bacto Skim Milk agar plates; tyrosine and xantin decompositions were studied with 4 weeks old cultures on L-tyrosine and xantin containing agar plates; hydrogen sulphide production in nutrient broth was tested with lead acetate paper strips; chitinolytic activity was detected in nutrient agar plates containing purified and partially hydrolised chitin; lecitinase activity was detected on glucose phosphate agar plates containing 5% Oxoid Egg Yolk Emulsion (Klinge and Gräf, 1959).

Numerical analysis of the obtained data. Our selected and thoroughly studied 96 representative potato bacterial strains were compared, on the basis of results of simultaneous laboratory studies, with 20 authentic reference strains obtained from the Czecho-Slovakian National Culture Collection using computer aided numerical methods. The comparisons of strains were based on 141 coded diagnostic features and the levels of similarities were expressed in per cent calculated according to Sokal and Michener (1958) using an IBM PC AT-type computer. The cluster analysis and the construction of a dendrogram were based on more than 16356 data. The computer program was written by G. Lőrinc.

Results and Discussion

All of the selected representative strains of typical oxidative metabolism stained Gram negatively, moved actively with polar flagella at one end of the rod shaped cells of 1-3 μm length, showed positive catalase and oxydase reactions and did not ferment glucose. Except for two strains all of them produced pyoverdin, but none of them pyocyanin. Considering their key-characters they were identified as the members of the *Pseudomonas fluorescens-putida* group (Krieg and Holt, 1984). The results of the cluster analysis on the compared representative strains are presented in Fig. 1. As can be seen the computer created at relatively high similarity levels (>90 per cent) one large (group 4) and three small (groups 1, 2 and 3) assemblages of similar strains. Besides 12 authentic *Pseudomonas*- and one *Agrobacterium* strains furthermore our *Pseudomonas* sp. strain PB 85.b remained unclustered and were placed by the computer one after the other successively at the left side of the dendrogram. Similarly strain *P. fluorescens* CCEB 762 and two of our strains (Nos PB 74.a and PB 9.) were placed in intergroup positions. The overwhelming majority of our representative strains (82 ones: >80% of the total) belonged to the A-biovar of *P. putida* and were placed together into the group 4. Members of this large group did not show lecitinase reaction, all of them hydrolysed arginine, did not reduce nitrates except one and apart from five strains did not show gelatinase activity. Authentic strains (BKMB 1301 and CCEB 520) of *P. putida* biovar-A, which were also incorporated by the computer in group 4 can automatically identify the taxonomic position of all members of this group. Eight strains were identified as *P. fluorescens*, namely strains PB 69.b, PB 24.b and PB 24.a as the members of *P. fluorescens* biovar II. (group 1), strain PB 74.a (in intergroup position) as that of biovar V., furthermore strains PB

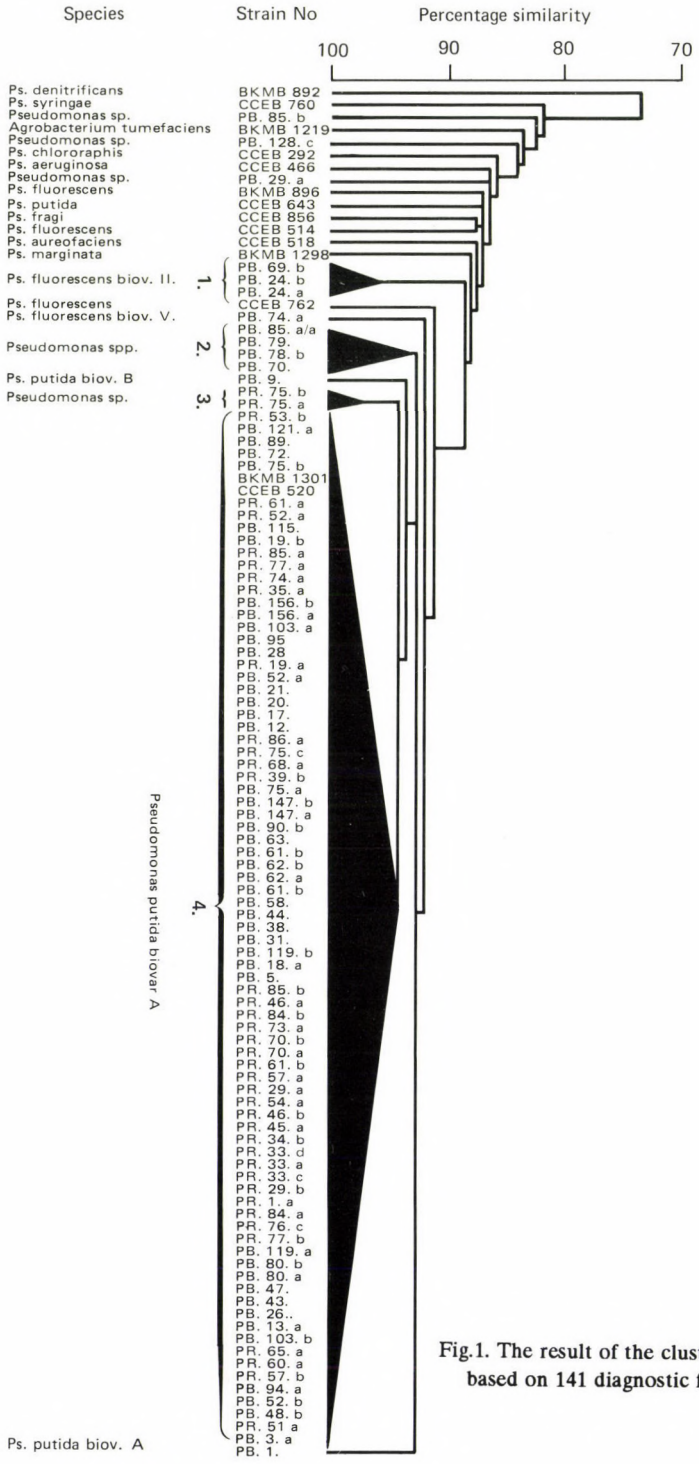

Fig.1. The result of the cluster analysis based on 141 diagnostic features

85.a/a, PB 79, PB 78.b and PB 70 forming a well separated similarity group *(Pseudomonas* spp. - group 2) characterized by transitional diagnostic features between *P. fluorescens* biovars III. and V. Finally one representative strain (PB 9.) proved to be the member of the B-biovar of *P. putida* and at the same time it can be considered as an intermediate form between the species *P. putida* and *P. fluorescens*.

The presented data clearly show that in the rhizoplane and on the tuber surfaces of cultivar Hungarian Rosa the members of *P. putida* biovar-A predominate and these organisms may chiefly be responsible for the iron dependent protection of this cultivar against pathogens. But both from taxonomic and ecological point of view it seems important to emphasize that in the very same natural root pseudomonad population simultaneously occur not only typical members of different biovars of species *P. putida* and *P. fluorescens* but also interspecific intermediate forms.

This finding could be trace back to the fact that the *P. fluorescens-putida* group can be considered as a hardly separable large assemblage of very closely related variable organisms.

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Host-Virus Relations Between Leafy Vegetables and Viruses[1]

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In the course of experiments on the virus susceptibility and resistance of eight leafy vegetable plants we detected 38 susceptible host-virus relations for the plants in question. The number of systemically, and of locally and systemically susceptible relations was 16, while the local relations were 22 in number. In 38 cases resistant relations were pointed out. In the experiment 31.5 per cent of the susceptible host-virus relations were symptomless (latent).

The susceptible and resistant host-virus relations play an important role in virus identification and plant breeding.

Vegetable production has recently increased to a considerable extent in Hungary. This can be explained partly by the fact that with the refinement of the eating habits the vegetable plants grown so far in gardens of seasonal character have been introduced in field- and greenhouse production, partly by a substantial improvement in the preservation of vegetables. The leafy vegetables – 21 in number – found in Hungary belong to 10 plant families (Somos, 1983; Horváth, 1987). However, only 5 of them (*Lactuca sativa, Spinacia oleracea, Rumex rugosus, Brassica pekinensis, B. campestris* var. *chinensis*) are of major importance. For 20 years viruses have caused serious problems in the production of vegetable plants including leafy vegetables in Hungary (Horváth and Beczner, 1983). The most important viruses are broad bean wilt virus, cauliflower mosaic virus, celery mosaic virus, cucumber mosaic virus, lettuce mosaic virus, radish mosaic virus, turnip mosaic virus and turnip yellow mosaic virus.

The appearance of vegetables' virus pathogens already known and recently become known, respectively (e.g. cucumber fruit streak virus, cucumber soil-borne virus, cucumber leaf spot virus, *Erysimum* latent virus, melon rugose mosaic virus, melon necrotic spot virus, Ourmia melon virus; see Shukla and Schmelzer, 1972; Gonzales-Garza et al., 1979; Weber et al., 1982; Galliteli et al., 1983; Koenig et al., 1983; Weber and Stanarius, 1984; Hibi and Furuki, 1985; Jones et al., 1986; Tomlinson and Thomas, 1986; Weber, 1986; Weber et al., 1986; Lisa et al., 1988) urged us to show up new scientific results concerning the virus susceptibility and virus resistance of some less known leafy vegetables.

1 Presented in abbreviated form as a poster at the XII. EUCARPIA Congress, Göttingen, Germany (February 27-March 4, 1989).

Materials and Methods

Seeds of the various leafy vegetables were obtained from the Seed Production and -Marketing Enterprise, Budapest, Hungary. The plants were grown in a sterilized soil mixture (loam, peat and sand; 3:2:1, v/v/v) in clay or plastic pots in the greenhouse at

Table 1

Viruses used in the experiments

Viruses	Strains or[1] isolates	Literature[2]
Alfalfa mosaic virus (AMV)	K2	*Beczner* (1972)
Arabis mosaic virus (AraMV)	Phil.2	*Schmelzer* (1968)
Bean common mosaic virus (BCMV)	F23	Horváth (1976)
Bean yellow mosaic virus (BYMV)	S	Horváth (1976)
Beet mosaic virus (BeMV)	IP-A	*Schmelzer* (1959)
Beet necrotic yellow vein virus (BNYVV)	H	Horváth et al. (1989)
Belladonna mottle virus (BMV)	H	Horváth et al. (1976a)
Broad bean wilt virus (BBWV)	Tm	Horváth and Szirmai (1975)
Carnation ringspot virus (CaRSV)	IP-A	*Fritzsche* and Schmelzer (1967)
Cauliflower mosaic virus (CaMV)	DH	Horváth et al. (1980a)
Celery mosaic virus (CeMV)	P	Horváth et al. (1976b)
Cucumber fruit streak virus (CFSV)	Common	*Gallitelli* et al. (1983)
Cucumber green mottle mosaic virus (CGMMV)	IP-A	*Schmelzer* (1967)
Cucumber leaf spot virus (CLSV)	Common	*Weber* and Stanarius (1984)
Cucumber mosaic virus (CMV)	U/246	Schmidt and Horváth (1982)
Erysimum latent virus (ELV)	IP-A	Schukla and Schmelzer (1972)
Melon rugose mosaic virus (MRMV)	Common	*Jones* et al. (1986)
Melandrium yellow fleck virus (MYFV)	LB	Hollings et al. (1978), Horváth et al. (1988)
Lettuce mosaic virus (LMV)	Mk-4	Horváth et al. (1981)
Potato virus X (PVX)	G	Horváth (1976)
Potato virus Y (PVY)	CNRAn	Horváth (1976)
Radish mosaic virus (RaMV)	HS7	Horváth et al. (1973)
Tobacco mosaic virus (TMV)	U1	*Siegel* and Wildman (1954)
Tobacco necrosis virus (TNV)	f	*Szirmai* (1964)
Tobacco rattle virus (TRV)	H	Horváth (1976)
Tobacco ringspot virus (TRSV)	D	Horváth (1976)
Tomato aspermy virus (TomAV)	Tm12	Horváth et al. (1980b)
Tomato mosaic virus (ToMV)	H	Mamula et al. (1974)
Tomato ringspot virus (TomRSV)	IP-B	*Nienhaus* (1979)
Turnip mosaic virus (TuMV)	All	Horváth et al. (1975a)
Turnip yellow mosaic virus (TYMV)	HB	Horváth et al. (1973), Juretić et al. (1973)
Watermelon mosaic virus (WMV)	PW	Horváth et al. (1975b)
Zucchini yellow mosaic virus (ZYMV)	E15	Lecoq et al. (1981, 1983)

[1] Meaning of abbreviations: IP-A, Biologische Zentralanstalt Berlin, Institut für Phytopathologie, Aschersleben, Deutschland; IP-B, Institut für Pflanzenkrankheiten, Bonn, Deutschland

[2] Names printed in italics are the sender of the viruses

Table 2

Leafy vegetables and their reaction to viruses[1]

Viruses	*Atriplex littoralis*	*Basella alba*	*Brassica campestris*	*Cichorium endivia*	*Emex spinosa*	*Spinacia oleracea*	*Tetragonia tetragonoides*	*Valerianella locusta*
AMV					L			
AraMV					R			
BCMV					R		R	
BNYVV						S	L	
BYMV					L			
BeMV					*L+S*			
BMV					R			
BBWV					R			
CaRSV					R			
CaMV			*L+S*		R			
CeMV					R			
CFSV	R	R		R			R	R
CGMMV	L (Fig. 2B)	R		L	*L+S*		R	*L+S*
CLSV	L (Fig. 2A)	R		R			*L+S*	*S*
CMV	L (Fig. 2C)	R	R	R	*L*		L (Fig.1D)	R
ELV			*L+S*					
MRMV	R	R		R				*L*
MYFV	L (Fig. 2E)			R	*L+S*			R
LMV					R			R
PVX					*L*			
PVY					R			
RaMV			L+S		*S*		L	
TMV					L			
TNV					L			
TRV					L			
TRSV					L			
TomAV					R			
TomMV					L		L	
TomRSV					*L+S*			
TuMV			L+S			L		
TYMV			L+S			*L+S*		
WMV-2	L (Fig.2D)	R		R		R	R	S
ZYMV	L (Fig.2F)	R		R			R	R

[1]Meaning of abbreviations. *Viruses:* AMV, alfalfa mosaic virus; AraMV, *Arabis* mosaic virus; BCMV, bean common mosaic virus; BNYVV, beet necrotic yellow vein virus; BYMV, bean yellow mosaic virus; BeMV, beet mosaic virus; BMV, belladonna mottle virus; BBWV, broad bean wilt virus; CaRSV, carnation ringspot virus; CaMV, cauliflower mosaic virus; CeMV, celery mosaic virus; CFSV, cucumber fruit streak virus; CGMMV, cucumber green mottle mosaic virus; CLSV, cucumber leaf spot virus; CMV, cucumber mosaic virus; ELV, *Erysimum* latent virus; MRMV melon rugose mosaic virus; MYFV, *Melandrium* yellow fleck virus; LMV, lettuce mosaic virus, PVX, Potato virus X; PVY, potato virus Y; RaMV, radish mosaic virus; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus, TRV, tobacco rattle virus; TRSV, tobacco ringspot virus; TomAV, tomato aspermy virus; TomMV, tomato mosaic virus; TomRSV, tomato ringspot virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV-2, watermelon mosaic virus; ZYMV, Zucchini yellow mosaic virus. *Plant reactions:* L, local; S, systemic; L+S, local and systemic susceptibility. Letters printed in italics indicate latent infection. R, resistant (no visible symptoms, results of back-inoculations and/or serological investigations are negative)

average temperatures of 22-25 °C. Eight leafy vegetables plants (*Atriplex littoralis, Basella alba, Brassica campestris* var. *chinensis* cv. *Japro RS 2701, Cichorium endivia, Emex spinosa, Spinacia oleracea, Tetragonia tetragonoides, Valerianella locusta*) were inoculated with one or another of a total of 33 viruses (Table 1). The inocula were prepared by grinding leaves of virus source plants with Sörensen phosphate buffer (150 mM, *p*H 7.5). The inoculations were made by the Carborundum (400 mesh) gauze-pad method. To dectect the infections of leafy vegetable plants symptom expression was checked, and in some cases serological agar gel-diffusion as well as back-inoculation tests were performed. To counteract virus inhibitors in *Spinacia* and *Basella* leaf saps (see Baillis and Okonkwo, 1979; Ushasri et al., 1982) inoculations were made with sap obtained by grinding leaves (1:5, v/v) in 0.067 M phosphate buffer (*p*H 7.5) containing bentonite and celite.

Results and Conclusions

Between the 8 leafy vegetable plants and the 33 viruses 38 susceptible host-virus relations were established of which 22 were local, 4 systemic and 12 local and systemic susceptibility (Table 2). In 38 cases resistant relation was pointed out. Out of the systemic host-virus relations attention should be paid to the susceptibility of *Brassica campestris* (to cauliflower mosaic, *Erysimum* latent, radish mosaic, turnip mosaic, turnip yellow mosaic viruses; Fig. 1 A,B,C), *Emex spinosa* (to beet mosaic, cucumber green mottle mosaic, *Melandrium* yellow fleck, radish mosaic, tomato ringspot, turnip yellow mosaic viruses) and *Valerianella locusta* to three viruses (cucumber green mottle mosaic, cucumber leaf spot, watermelon mosaic) as well as to the beet necrotic yellow vein virus susceptibility of *Spinacia oleracea*. From the point of view of both virology and breeding it is important that 31.5 per cent of the susceptible host-virus relations examined are latent. The latent virus susceptibility (to beet mosaic, cucumber green mottle mosaic, cucumber mosaic, *Melandrium* yellow fleck, potato X, radish mosaic, tomato ringspot, turnip yellow mosaic viruses) of *Emex spinosa* is of ecological importance. *Emex spinosa* is the first plant in the family of Polygonaceae found to be virus susceptible (e.g. to cucumber mosaic virus; cf. Horváth, 1986). With the susceptibility of *Cichorium endivia, Emex spinosa* and *Valerianella locusta* to cucumber green mottle mosaic virus ascertained the host-range of the virus has been extended to 3 new families (Asteraceae, Polygonaceae and Valerianaceae).

Remarkable is the resistance of *Basella alba* and *Cichorium endivia* to 7 viruses (see Table 2).

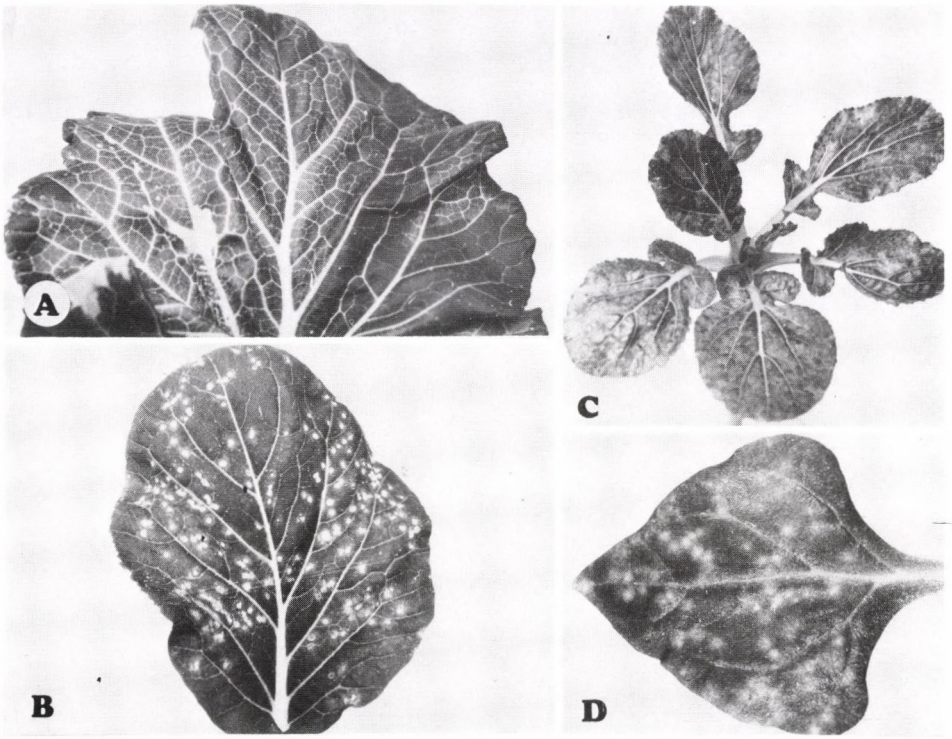

Fig. 1. Symptoms on *Brassica campestris* var. *chinensis* cv. *Japro RS 2701* (A,B,C) and on *Tetragonia tetragonoides* (D). A: natural inoculation of turnip yellow mosaic virus (TYMV). B: Local lesions of *Erysimum* latent virus (ELV). C: Systemic, yellow mosaic symptoms of *Erysimum* latent virus (ELV). D: Local, chlorotic lesions of cucumber mosaic virus (CMV)

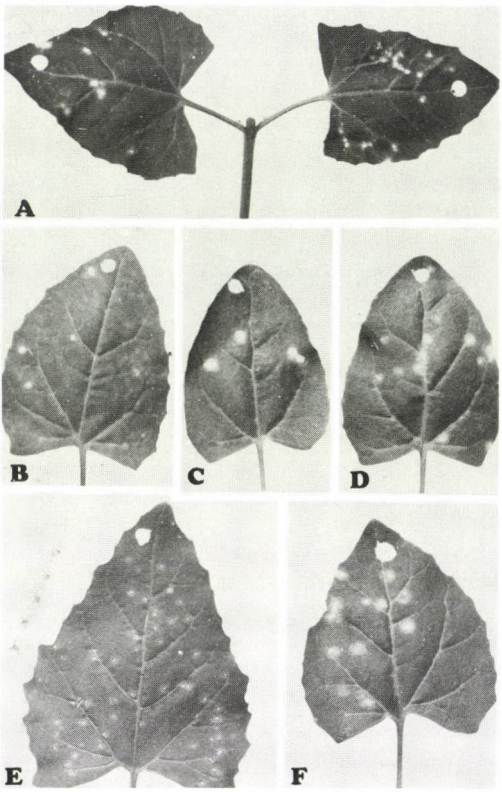

Fig. 2. Local symptoms on *Atriplex littoralis* plants with different viruses. A: Cucumber leaf spot virus (CLSV), B: Cucumber green mottle mosaic virus (CGMMV), C: Cucumber mosaic virus (CMV), D: Watermelon mosaic virus, (WMV), E: *Melandrium* yellow fleck virus (MYFV), F: Zucchini yellow mosaic virus (ZYMV)

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Unknown Compositae (Asteraceae) Hosts of Lettuce Mosaic Potyvirus

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In the course of experiments related with the virus susceptibility of six Compositae plants so far unknown or hardly known in plant virology we found the *Emilia flammea* Cass., *Iva xanthiifolia* Nutt. and *Layia elegans* Torr. et Gray to be latent local and systemic hosts for the lettuce mosaic potyvirus. The species *Madia elegans* Lindl. and *Linheimera texana* A. Gray showed systemic susceptibility to the virus. *Tridax trilobata* Hemsl. responded with severe vein clearing and mosaic. On the basis of the intensity of the symptoms induced in the latter plant and the high virus concentration of the inoculated and non-inoculated leaves *Tridax trilobata* is regarded to be not only a good diagnostical but also an important propagative host for the lettuce mosaic potyvirus.

According to a review of earlier studies on the host-range of lettuce mosaic potyvirus, a pathogen known wherever *Lactuca sativa* L. is grown in the world, the natural hosts of the virus extend to 21 species from 19 genera of 9 families, and its artificial hosts cover 121 species from 60 genera of 16 families (see Horváth, 1979, 1980). Most of the host plants – 9 natural hosts [e.g. *Lactuca sativa, Senecio vulgaris* L., *Sonchus asper* (L.) Hill etc.] and 82 artifical hosts – belong to the family Compositae (Asteraceae). As it is known, the Compositae plants – compared to those belonging to the families Solanaceae and Chenopodiaceae – are less suitable for pointing out viruses, it seems, thus, that a special affinity exists between the relatively large number of virus susceptible plants in the family and the lettuce mosaic potyvirus. Among the recently established members of the wide host-range of the polyphagous lettuce mosaic potyvirus *Gomphrena decumbens* Jacq. (family: Amaranthaceae), *Chenopodium multifidium* L., *C. pumilio* R.BR and *C. rigidum* Lingelsch. (family: Chenopodiaceae) are of outstanding importance (Horváth, 1983a,b, 1986). *Gomphrena decumbens* – a plant free from inhibitors similarly to *G. globosa* L. – is exceedingly suitable for the propagation of the virus (propagative host) and is important as a bridge plant too (cf. Horváth, 1977).

The so-called *Chenopodium* test earlier used to detect infection by lettuce mosaic potyvirus (see Marrou and Messiaen, 1967; Rohloff, 1962, 1966, 1968; Hall and Benett, 1970; Kimble et al., 1975) has not lost in importance in spite of the fact that recently more sensitive and economical methods (e.g. enzyme-linked immusorbent assay, ELISA; enzyme-linked fluorescent assay, ELFA; radioimmunosorbent assay, RISA), filter paper sero-assay (FiPSA) have become known for pointing out virus infection in lettuce seeds (Jafarpour et al., 1979; Ghabrial and Sheperd, 1980; Falk and Purcifull, 1983; Van Vuurde and Maat, 1983; Vetten, 1984; Dolores-Talens and Hill, 1985; Dolores-Talens et al., 1989, Haber and Knapen, 1989). However, not every virological laboratory has been able to prepare itself for the use of these methods of high instrumentation, so with the new *Chenopodium* assay plants the so-called *Chenopodium* test may gain a wider application. In order to get further information about the affinity between species belonging to the family Compositae and the lettuce mosaic potyvirus we carried out experiments with so far unknown or little known plants.

Materials and Methods

The lettuce mosaic potyvirus (isolate Mk-4; Horváth et al., 1981) was isolated from *Lactuca sativa* cv. *Május királya* in the fields of Georgikon Garden of the University at Keszthely showing severe mosaic symptoms. The virus was maintained by periodic sap inoculation under greenhouse conditions on *Chenopodium quinoa* Willd. and *Lactuca serriola* Torn., as well as by dehydrating the infected tissues over anhydrous calcium chloride ($CaCl_2$; cf. Horváth and Besada, 1980). Symptomatic leaves of *Chenopodium quinoa* were triturated in 0.05 M phosphate buffer, *p*H 7.2, containing 0.01 M Na_2SO_3. Mechanical inoculations were made by gently rubbing the extract on leaves of Compositae plants, *Emilia flammea* Cass. *Iva xanthiifolia* Nutt., *Layia elegans* Torr. et Gray, *Madia elegans* Lindl., *Lindheimera texana* A. Gray, *Tridax trilobata* Hemsl. in the greenhouse. Ten to 15 plants of each test species were inoculated with the virus for studies. All inoculated plants were weekly checked symptomatologically. Four weeks after inoculation return inoculations were made from inoculated and uninoculated leaves of the investigated plants to appropriate indicator plant (*Chenopodium quinoa*) to test for infection.

Results and Conclusion

Out of the Compositae plants so far unknown or little known in plant virology the *Emilia flammea, Iva xanthiifolia* and *Layia elegans* did not show symptoms of disease after inoculation. Virus back-inoculation separately from inoculated and non-inoculated leaves to *Chenopodium quinoa* plants was successful. This test results indicates that the above three plants are both locally and systemically latent hosts for the lettuce mosaic potyvirus. Among the above plants *Iva xanthiifolia* [syn.: *Cyclachaena xanthiifolia* (Nutt.) Fresen], a new adventive weed plant in Hungary is particularly important (Schermann, 1951; Baráth, 1953; Priszter, 1962; Terpó-Pomogyi, 1971). It is significant from a virus geographic point of view as well, since this native plant of North-America occurs in many European countries (e.g. Poland, Switzerland, Holland, Soviet Union, Austria, Germany, Czechoslovakia, Romania) apart from Hungary, and is included in the list of quarantine plants. The latent relation between *Iva xanthiifolia* and the lettuce mosaic potyvirus may be the reason that the natural host-virus relation has not been disclosed so far. Among the hosts of the latent lettuce mosaic potyvirus the *Emilia flammea* is also important. Owing to its latent virus susceptibility this species has no virus diagnostical value, yet, the fact that unlike many other plants belonging to the family Compositae (cf. Hollings and Stone, 1963) it contains little if any inhibitor makes it worth attention in plant virology. According to our knowledge *Emilia flammea* is susceptible to tobacco mosaic virus too (Holmes, 1946). Further species in the genus *Emilia* on the virus susceptibility of which data are available are *E. sagittata* DC., *E. sonchifolia* Hort. and *E. coccinea* (Sims) Sweet. *Emilia sagittata* is susceptible to five viruses (*Chrysanthenum* stunt, cucumber mosaic, tobacco mosaic, tomato aspermy and tomato spotted wilt viruses; Linford, 1932; Grant, 1934; Wellman, 1934; 1935; Brierley, 1953; Brierley et al., 1955). According to the data of Hollings and Stone (1963) outside the above viruses *E. sagittata* is susceptible to further

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21 viruses. The host-virus vector relation between *Emilia sonchifolia* and the tomato spotted wilt virus was pointed out by Sakimura (1940, 1956, 1961). *Emilia coccinea* has quite recently become known as a new natural host for alfalfa mosaic virus (Mayorai et al., 1988). In earlier investigations we found that *Emilia coccinea* was a local host for tobacco necrosis virus, responding with large necrotic lesions, and a local as well as systemic latent host for tobacco mosaic virus (Horváth, unpublished data). As far as we known, the virus susceptibility of *Layia elegans* has been reported so far only by Gardner et al. (1935) who found this plant to be susceptible to tomato spotted wilt virus.

Further two plant species – *Madia elegans* and *Lindheimera texana* – responded with systemic mosaic to lettuce mosaic potyvirus. In the course of back-inoculation the *Chenopodium quinoa* assay plants gave positive reactions only in case the virus was re-isolated from uninoculated leaves. These results are indicative of a systemic host-virus relation between the plants in question and the lettuce mosaic potyvirus.

In the case of *Tridax trilobata* we succeeded in detecting an excellent lettuce mosaic potyvirus susceptible plant. Latter plant responded with severe systemic vein clearing and mosaic to the inoculation. These symptoms were remarkably intense on the axillary shoots of the plant too. In the course of back-inoculating the virus we found that the inoculated leaves (which otherwise were free from symptoms of disease) also had latent infection. On this basis it can be established that *Tridax trilobata* is a new locally and systemically susceptible artificial host for lettuce mosaic potyvirus, and at the same time not only a highly important plant for virus diagnosis, but – owing to its high virus concentration – a significant propagation plant too.

Considering that the lettuce mosaic potyvirus is a naturally occurring pathogen e.g. of spinach (*Spinacia oleracea* L.) and pea (*Pisum sativum* L.) too (see Provvidenti and Schroeder, 1972; Provvidenti, 1973; Kuida et al., 1977; Schmidt and Naumann, 1981), it would be important to examine the relations between the more than 30 viruses belonging to some 14 virus groups and the assay plants of the family Compositae in question. These investigations may lead to disclosing artificial host-virus relations new from the point of view of diagnosis, on the one hand, and may call attention to possible natural relations, on the other hand; and last but not least they would increase the possibilities of virus separation. One of the facts on which the latter supposition is based is that e.g. among the *Emilia species E. sagittata* is resistant to bean yellow mosaic and beet mosaic viruses, which are pathogenic for *Pisum sativum*, and susceptible to turnip mosaic, tobacco necrosis, alfalfa mosaic, tomato black ring, tomato spotted wilt viruses (see Hollings and Stone, 1963; Schmidt and Naumann, 1981).

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Lycium Species (Family: Solanaceae) as New Experimental Hosts of Plant Viruses

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In the course of studying the responses of four *Lycium* species (*L. australe, L. cestroides, L. mediterraneum, L. pallidum*) to 24 viruses we established 56 new host-virus relations (25 local, 15 local and systemic, 16 resistant). From the point of view of virus ecology particularly remarkable is the systemic susceptibility showed by the four species examined to cucumber mosaic virus, by *Lycium cestroides* to belladonna mottle virus, and by *L. mediterraneum* and *L. pallidum* to belladonna mottle virus, lettuce mosaic virus and tobacco mosaic virus.

On the basis of the earlier and present research results it can be stated that *L. australe* is susceptible to 11, and *L. mediterraneum* and *L. pallidum* each to 15 viruses. *Lycium cestroides*, a species so far unknown in plant virology proved susceptible to six plant viruses (belladonna mottle, carnation ringspot, cucumber mosaic, *Melandrium* yellow fleck, tobacco rattle and tomato mosaic viruses). On the basis of their systemic susceptibility to cucumber mosaic virus and resistance to celery mosaic virus *Lycium mediterraneum* and *L. pallidum* seem to be suitable for separating the cucumber mosaic virus and eliminating the celery mosaic virus in the case of mixed infections.

Since the investigations of Dennis (1938), for some 50 years *Lycium barbarum* has been known to be locally susceptible to various strains of potato virus Y. Since that time numerous studies have been made on the virus susceptibility of various *Lycium* species. The intensity of research was increased by the realization that intact and detached leaves of the different *Lycium* species as assay plants are suitable for the quick identification of certain viruses (Bode, 1954; 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 (Kovachevsky, 1965; Schmelzer and Schmidt, 1968; Schmelzer, 1969; Plese and Miličić, 1974; Kröll, 1975; Akhatova et al., 1979; Horváth, 1983a).

With a survey of the results attained so far in investigations related with *Lycium* species it can be established that about 14 *Lycium* species are known at present to be susceptible to viruses (Table 1). Between various *Lycium* species and viruses 98 host-virus relations have been described so far (see Table 1); 64 of them were detected in our earlier investigations (Horváth, 1972; Beczner and Horváth, 1972; reviewed by Horváth, 1983b, 1985). In earlier experiments we also examined *L. australe, L. horridum, L. mediterraneum, L. flexicaule* and *L. pallidum.*

Considering that the *Lycium* species play a very important role as assay plants in the identification of plant viruses, and as perennial and woody plants in the ecology of viruses, the aim of our recent investigations was partly to study further *Lycium* species, partly to point out new host-virus relations for the known species.

Table 1

Susceptibility of *Lycium* species to plant viruses

Lycium species	Viruses[1]	Literature
Lycium asutrale F. Muell	PVX	Horváth (1978a)
	PVY	Horváth (1979a)
	TNV	Horváth (1982b)
	TMV	Horváth (1978b)
	TRSV	Horváth (1979b)
L. barbarum L. (syn.: *L. halimifolium* Mill.)	AMV	Romo (1961)
	AV-2	Weissenfells et al. (1978)
	BMV	Horváth (1979c)
	CMV	Bode (1954), Pleše and Miličić (1974), Horváth (1979d)
	PPV	Kröll (1975)
	PVA	MacLachlan et al. (1953)
	PVX	Ladeburg et al. (1950)
	PVY	Dennis (1938), Ross (1948)
	TomBRV	Bode (1954)
	TMV	Bode (1954)
	TNV	Bode (1954)
	TRV	Bode (1954)
	TuMV	Walker et al. (1945)
	TRSV	Horváth (1979b)
	TSV	Fulton (1948)
L. carolinianum Walt.	AMV	Horváth (1981)
	BMV	Horváth (1979c)
	CMV	Horváth (1979d)
	PVX	Horváth (1978a)
	PVY	Horváth (1979a)
	TRSV	Horváth (1979b)
L. chinense Mill.	ALMV	Lovisolo and Lisa (1976, 1979)
	AMV	Romo (1961)
	BBWV	Schumann (1963)
	BMV	Horváth (1979c)
	CMV	Horváth (1979d)
	PVX	Horváth (1978a)
	PVY	Ross (1948), Akhatova et al. (1979)
	TEV	Ross (1948)
	TMV	Ross (1948)
	TRSV	Horváth (1979b)
	TRV	Horváth (1978c)

Table 1 (continued)

Lycium species	Viruses[1]	Literature
	TVBMV	Chin (1972)
L. europaeum L.	AMV	Cervantes and Larson (1961), Romo (1961)
	BBWV	Horváth (1982a)
	BMV	Horváth (1979c)
	CMV	Horváth (1972)
	PAMV	Horváth (1978a)
	PVX	Horváth (1972, 1978a)
	PVY	Horváth (1972, 1979a)
	TMV	Horváth (1972, 1978b)
	TNV	Horváth (1982b)
	TRV	Horváth (1978c)
	TRSV	Horváth (1979b)
	TomMV	Horváth (1978b)
L. ferocissimum Miers. (syn.: *L. campanulatum* Miers.)	AMV	Cervantes and Larson (1961), Romo (1961)
	TMV	Schmidt (1957)
	TomSWV	Klinkowski and Uschdraweit (1952)
L. flexicaule Pojark	AMV	Horváth (1981)
	BMV	Horváth (1979c)
	CMV	Horváth (1979d)
	PVX	Horváth (1978a)
	PVY	Horváth (1979a), Akhatova et al. (1979)
	TMV	Horváth (1978b)
	TRSV	Horváth (1979b)
L. horridum Thbg.	AMV	Horváth (1981)
	BBWV	Horváth (1982a)
	BMV	Horváth (1979c)
	CMV	Horváth (1972, 1979d)
	PAMV	Horváth (1972, 1978a)
	PVX	Horváth (1972, 1978a)
	PVY	Horváth (1972, 1979a)
	TMV	Horváth (1972, 1978b)
	TRSV	Horváth (1979b)
L. mediterraneum Dun.	BBWV	Horváth (1982a, 1983b)
L. pallidum Miers.	BBWV	Horváth (1982a, 1983b)
L. rhombifolium (Moench.) Dippel	CMV	Bode (1954)
	PVA	MacLachlan et al. (1953)
	PVY	Darby et al. (1951), Hutton and Peak (1952)

Table 1 (continued)

Lycium species	Viruses[1]	Literature
	TomBRV	Bode (1954)
	TNV	Bode (1954)
	TMV	Bode (1954)
	TRV	Bode (1954)
L. ruthenicum Murr.	AMV	Beczner and Horváth (1972), Horváth (1981)
	BBWV	Horváth (1982a)
	BMV	Horváth (1979c)
	CMV	Horváth (1972, 1979d)
	PAMV	Kollmer and Larson (1960), Horváth (1972, 1978a)
	PVX	Horváth (1972, 1978a)
	PVY	Horváth (1972, 1979a)
	TMV	Horváth (1972, 1978b)
	TomMV	Horváth (1978b)
	TRSV	Horváth (1979b)
L. turcomanicum Turcz.	AMV	Horváth (1981)
	BMV	Horváth (1979c)
	BBWV	Schmelzer (1970)
	CMV	Horváth (1972, 1979d)
	PAMV	Horváth (1972, 1978a)
	PVX	Horváth (1972, 1978a)
	PVY	Horváth (1972, 1979a), Akhatova et al. (1979)
	TMV	Horváth (1972, 1978b)
	TRSV	Horváth (1979b)
L. viscosa Link	PVA	MacLeod (1962)

[1] Meaning of abbreviations: ALMV – *Amaranthus* leaf mottle virus, AMV – alfalfa mosaic virus, AV-2 – *Asparagus* virus-2, BMV – belladona mottle virus, BBWV – broad bean wilt virus, CMV – cucumber mosaic virus, PAMV – potato aucuba mosaic virus, PPV – plum pox virus, PVA – potato virus A, PVX – potato virus X, PVY – potato virus Y, TEV – tobacco etch virus, TMV – tobacco mosaic virus, TNV – tobacco necrosis virus, TRV – tobacco rattle virus, TRSV – tobacco ringspot virus, TSV – tobacco streak virus, TVBMV – tobacco vein-banding mosaic virus, TomBRV – tomato black ring virus, TomMV – tomato mosaic virus, TomSWV – tomato spotted wilt virus, TuMV – turnip mosaic virus

Materials and Methods

In our experiments aimed at studying new host-virus relations in *Lycium* species four species were included (*L. australe, L. cestroides, L. mediterraneum, L. pallidum*) of which *Lycium cestroides* is an experimental plant so fark unknown in virology.

Young *Lycium* seedlings were inoculated with different viruses at the 6-8-leaf stage. The viruses were obtained from the gene bank of our virus laboratory (Table 2). The 400

Table 2

Viruses used in experiments

Viruses	Strains or isolates[1]	Literature
Alfalfa mosaic virus (AMV)	K2	Beczner (1972)
Bean common mosaic virus (BCMV)	F23	Horváth (1977)
Bean yellow mosaic virus (BYMV)	S	Horváth (1976)
Belladonna mottle virus (BMV)	H	Horváth et al. (1976a)
Broad bean wilt virus (BBWV)	Tm	Horváth and Szirmai (1975)
Carnation ringspot virus (CaRSV)	IPA	Fritzsche and Schmelzer (1967)
Celery mosaic virus (CeMV)	P	Horváth et al. (1976b)
Cucumber green mottle mosaic virus (CGMMV)	IPA	Schmelzer (1967)
Cucumber mosaic virus (CMV)	U/246	Schmidt and Horváth (1982)
Lettuce mosaic virus (LMV)	MK	Horváth et al. (1981)
Melandrium yellow fleck virus (MYFV)	LB	Hollings et al. (1978), Hollings and Horváth (1981), Horváth et al. (1988)
Potato virus X (PVX)	G	Horváth (1976)
Potato virus Y (PVY)	C,N,R,An	Horváth (1976, 1977)
Radish mosaic virus (RMV)	HS7	Mamula et al. (1972), Horváth et al. (1973)
Tobacco mosaic virus (TMV)	U1	Siegel and Wildman (1954)
Tobacco necrosis virus (TNV)	f	Szirmai (1964)
Tobacco rattle virus (TRV)	H	Horváth (1976, 1977)
Tobacco ringspot virus (TRSV)	D	Horváth (1976,1977)
Tomato aspermy virus (TomAV)	Tm12	Horváth et al. (1980)
Tomato mosaic virus (TomMV)	H	Horváth and Beczner (1973), Mamula et al. (1974), Juretić et al. (1977)
Tomato ringspot virus (TomRSV)	VgB-B	Nienhaus (1979), Virus-gene bank, Bonn (Germany)
Turnip mosaic virus (TuMV)	All	Horváth et al. (1975a), Juretić et al. (1976)
Turnip yellow mosaic virus (TYMV)	HB	Juretić et al. (1973), Horváth et al. (1973)
Watermelon mosaic virus (WMV)–2	PW	Horváth et al. (1975b)

[1] IPA, Institute of Phytopathology, Aschersleben (Germany); VgB-B, Virus-gene bank of the Institute for Plant Disease, Department of Virology, Bonn (Germany)

mesh Carborundum used in the course of the inoculation was applied onto the leaves with a special duster before the infection. Inoculation was carried out with virus-containing tissue sap diluted with distilled water at a ratio of 1:1. After the inoculation the infected *Lycium* plants were sprayed with tap water. The virus resistance or virus suscep-

Table 3

Reactions of *Lycium* species to plant viruses

Viruses	*Lycium* species and their reactions[1]			
	australe F. Muell	*cestroides* Schlecht	*mediterraneum* Dun.	*pallidum* Miers.
Alfalfa mosaic virus			Lcl; Smo	Lcl; Smo
Bean common mosaic virus			R	R
Bean yellow mosaic virus			R	R
Belladonna mottle virus		Lcl; Smo	Lcl; Smo	Lcl; Smo
Broad bean wilt virus	Lcl			
Carnation ringspot virus		Lnl	R	R
Celery mosaic virus			R	R
Cucumber green mottle mosaic virus			R	R
Cucumber mosaic virus	LcL, Ledr; Smo	Lcl; Smo	Lcl; Smo	Lcl; Smo
Lettuce mosaic virus			L(1); S(1)	L (1); S(1)
Melandrium yellow fleck virus	Lnl	Lc-nl	Lc-nl	Lc-nl, Ledr
Potato virus X			Lc-nl	Lc-nl
Potato virus Y			Lc-nl, Ledr	Lc-nl, Ledr
Radish mosaic virus			R	R
Tobacco mosaic virus			Lcl, Ledr; S (1)	Lcl, Ledr; S (1)
Tobacco necrosis virus			Lnl	Lnl
Tobacco rattle virus	Lcl	Lcl	Lcl	Lcl
Tobacco ringspot virus			Lc-nl	Lc-nl
Tomato aspermy virus			Lcl; Smo	Lcl; Smo (Fig. 1)
Tomato mosaic virus	Lnl, Ledr	Lc-nl		
Tomato ringspot virus			Lc-nl, Ledr	Lc-nl, Ledr
Turnip mosaic virus	Lcl, Ledr		Lcl, Ledr	Lcl, Ledr
Turnip yellow mosaic virus			R	R
Watermelon mosaic virus–2			R	R

[1] Meaning of abbreviations: Lcl – local chlorotic lesions, Lnl – local necrotic lesions, Lc–nl – local chlorotic–necrotic lesions, Ledr – leaf drop, L (1), locally latent infection, R – resistant, Smo– systemic mosaic, S (1) – systemically latent infection.

tibility of symptomless plants was decided by inoculating virus specific assay plants with water diluted tissue sap (1:1, v/v) from leaves of the inoculated plants surface disinfected with 2% NaOH (Horváth, 1977). The experiments were carried out in vector-free glasshouse.

Results and Discussion

Between the four *Lycium* species and 24 viruses 56 new host-virus relations were established (Table 3), of which 25 were local and 15 local and systemic. In the other 16 cases the host-virus relations was incompatible or resistant.

The local host-virus relations were characterized by chlorotic, sometimes chlorotic-necrotic, necrotic lesions developing on the inoculated leaves, and by leaf drop, while in the systemic host-virus relations mosaic was the characteristic symptom. In some systemic host-virus relations (e.g. *Lycium mediterraneum, L. pallidum*–lettuce mosaic virus, tobacco mosaic virus) the plants showed latent infection. For these plants the systemic disease was pointed out by back inoculation.

In the case of *Lycium australe* six new host-virus relations were revealed. It is of particular importance that this plant — similarly to the other 3 *Lycium* species included in our experiments — is susceptible to cucumber mosaic virus systemically too (see Table 3). These examination results suggest that in the ecology of the cucumber mosaic virus

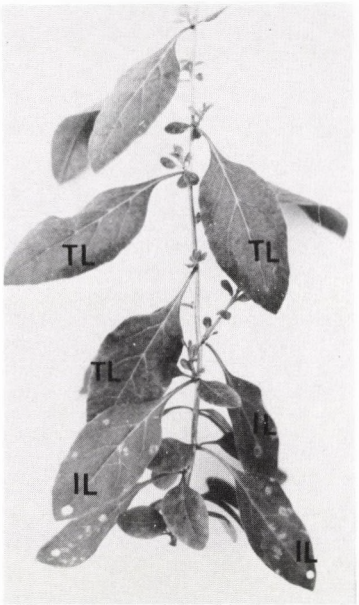

Fig. 1. Local chlorotic lesions on the inoculated leaves (IL) and systemic mosaic symptoms on the non-inoculated tip leaves (TL) on *Lycium pallidum* Miers. infected with tomato aspermy virus

not only the *Lycium halimifolium* (see Schmelzer, 1969) but the *L. australe, L. cestroides, L. mediterraneum* and *L. pallidum* too may play an important role. According to the present and earlier results of investigations the *Lycium australe* is susceptible to 11 viruses (see Table 1 and 3).

Lycium cestroides — a plant so far unknown in plant virology — poved susceptible to six viruses (Table 3). Particularly remarkable is its systemic susceptibility to belladonna mottle virus and cucumber mosaic virus.

In the case of *Lycium mediterraneum* and *L. pallidum* 22 new host-virus relations for each were pointed out (Table 3). The systemic susceptibility of these species to belladonna mottle, cucumber mosaic, lettuce mosaic, tomato aspermy (Fig. 1), and tobacco mosaic viruses is regarded as of importance. The latent systemic susceptibility to lettuce mosaic virus and tobacco mosaic virus is particularly improtant. Both plants proved resistant to bean common mosaic, bean yellow mosaic, carnation ringspot, celery mosaic, cucumber green mottle mosaic, radish mosaic, turnip yellow mosaic and watermelon mosaic viruses. Considering that — according to our present knowledge — only the broad bean wilt virus susceptibility of the two *Lycium* species in question is known (cf. Horváth, 1982a, 1983b), the recent data might be important from a general virological point of view as well. With diagnostical and virus differentiation aspects taken into consideration it may also be important that both species seem to be suitable for separating the cucumber mosaic virus and eliminating the celery mosaic virus in the case of a complex infection by the two viruses. Moreover, this is of practical significance, since the mentioned two viruses often occur in mixed form in some horticultural crops (see Wolf and Schmelzer, 1972).

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Isolation of Cucumber Mosaic Virus from *Lagenaria siceraria* convar. *clavatina* in Yugoslavia

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Mild mosaic, chlorotic spotting, stunting and mild distortion symptoms were observed on leaves of *Lagenaria siceraria* (syn.: *L. vulgaris*) convar. *clavatina* in the vicinity of Zagreb, Yugoslavia. From the infected plants a virus was isolated and identified as cucumber mosaic virus (CMV) based on test plant reactions, cross protection, serology, virus stability in sap, aphid and seed transmissibility, and particle morphology. The virus isolate is designated CMV-LY. This is the first report on natural occurring of CMV in genus *Lagenaria* in Yugoslavia and also the first datum on natural infection of *Lagenaria siceraria* convar. *clavatina*, in general.

The *Lagenaria* species belonging to the family Cucurbitaceae are becoming increasingly important as vegetable crops. On the basis of literary reviews made earlier as well as on the evidence of our own experiments it can be established that the *Lagenaria siceraria* (bottle gourd) and some of its varieties and convars (*clavatina, longissima*) are susceptible to more than 20 viruses (Horváth, 1983, 1985a,b). Considering that *Lagenaria siceraria* is not known for natural virus susceptibility in Yugoslavia and Hungary, we carried out investigations concerning possible virus infections in the above plant.

In 1988 virus symptoms on c. 30% of *Lagenaria* plants in the vicinity of Zagreb were observed. Preliminary investigations (e.g. host range) have shown that the causal virus can be cucumber mosaic virus (CMV). CMV is one of the most widespread plant virus. As far as we known and according to the available literature 476 species in 67 taxonomic plant families are natural hosts, and 536 species in 53 plant families are artificial hosts of CMV (Horváth, 1979, 1980). This virus was detected in *Lagenaria vulgaris* as well (Lovisolo, 1977). However, there is no data on natural infection of *Lagenaria siceraria* convar. *clavatina* with CMV. On the other hand it has been established experimentally that this plant is susceptible to CMV and to some other viruses (Horváth, 1985b). *Lagenaria siceraria* is a more and more popular vegetable plant in Yugoslavia. Usually, it is cultivated near cucumber and pumpkin fields. These two cultivated plants as well as perennial wild species can serve as reservoir hosts for aphid transmission of CMV and some other viruses (Lovisolo, 1980).

Our present study describes the identification and some properties of the CMV isolated from naturally infected *Lagenaria siceraria* convar. *clavatina* in Yugoslavia.

Materials and Methods

The subject of this paper is a virus isolate found in *Lagenaria siceraria* convar. *clavatina* specimens grown in a field in the vicinity of Zagreb, Yugoslavia (Fig. 1). The

isolate marked CMV-LV was passed through two single local-lesions transfers on *Chenopodium amaranticolor* and then maintained on *Nicotiana megaloshiphon*.

Inocula for host range experiments were prepared by grinding infected leaves of *Nicotiana megalosiphon* in 0.01 M sodium phosphate buffer, *p*H 7.0 which contained 0.02 per cent thioglicolic acid. The inocula were mechanically transferred to test plants by Carborundum.

In cross protection tests *Nicotiana tabacum* cv. *Xanthi-nc* plants in the 4 to 6-leaf stage were first mechanically inoculated with undiluted sap from *Xanthi-nc* tobacco infected with CMV-LY. After two weeks the tobacco plants were superinoculated with undiluted tobacco sap infected with an indicator white strain of CMV (CMV-W). In parallel experiments tobacco plants were first inoculated with CMV-W and the same plants were superinoculated after two weeks with CMV-LY.

Serological experiments were carried out by agar gel double-diffusion method using antiserum to CMV (titer 1/64) prepared by Dr. E. Luisoni.

The virus stability was established in extracted *Nicotiana megalosiphon* sap and *Chenopodium quinoa* was used as indicator plant.

Aphid transmission tests were performed by green peach aphids (*Myzus persicae*). The starvation period lasted 3 hr. Then the aphids were placed on CMV-LY infected cucumber (*Cucumis sativus*) plants and allowed a 5 min acquisition access period. Subsequently, the aphids were transferred to 20 healthy cucumber plants for a 15 min inoculation access period.

The *Lagenaria* plants to be grown in the field for the seed transmission experiments were obtained from a seed company which retails seeds in small pouchs. The small pouch bought contained about 50 *Lagenaria* seeds. Approximately, half of them were sowed in the field and the other half was retained in the small pouch. About three months later

Fig. 1. Naturally infected leaf of *Lagenaria siceraria* convar. *clavatina* with chlorotic spotting and mild distortion caused by the CMV-LY

when CMV-LY was identified in *Lagenaria* plants growing in the field, 25 of the remaining seeds were tested for possible virus infection. The investigated seeds were sown in pots in greenhouse and the generated plants were tested by infective experiments on test plants and serologically.

For electron microscope investigations of the virus morphology and size the CMV-LY isolate was purified using the procedure described by Habili and Francki (1974) with modification done by Juretic and Horváth (1984). The virus pellet was resuspended after the first and second high-speed centrifugation in 0.03 M phosphate buffer pH 7.2 which contained 1% of formaldehyde. A finer purification by density gradient centrifugation was omitted. The CMV-LY was investigated with a Siemens Elmiskop I electron microscope.

Results and Discussion

1. Host range and symptomatology

The CMV-LY was easily sap-transmitted to 22 species in five plant families (Table 1). On the basis of host range and symptoms presented in the Table 1, CMV-LY isolate could belong to CMV (cf. Francki et al., 1979). It is important to stress that our isolate CMV-LY did not cause systemic symptoms on cowpea (*Vigna sinensis*) but only local ones. Therefore, this isolate probably is not an isolate of peanut stunt virus (PeSV; Mink, 1972; Francki et al., 1979; Ahmad and Scott, 1985).

2. Cross protection

In the experiments tobacco plants inoculated first with CMV-LY and superinoculated with CMV-W were found to be protected against the infection of the challenging virus. Between the CMV-W and CMV-LY there was no cross protection if the tobacco plants were first inoculated with CMV-W. The partial cross protection between CMV-LY and CMV-W suggests that our isolate from *Lagenaria* plant is closely related to CMV.

3. Serology

The virus reacted positively with antiserum to CMV (Fig. 2). The used antiserum contained some antibodies to plant protein and it was, therefore, previously absorbed by sap of healthy plants.

4. Stability in sap

CMV-LY had the thermal inactivation point (TIP) between 60 and 65 °C. Longevity in vitro (LIV) at room temperature was 5 days. These data are very well in concordance with those of CMV (cf. Gibbs and Harrison, 1970).

Table 1

Reactions of test plants inoculated with isolate LY of cucumber mosaic virus (CMV-LY) from *Lagenaria siceraria* convar. *clavatina*

Test plants	Symptoms[1]	
	inoculated leaves	uninoculated tip leaves
AIZOACEAE		
Tetragonia expansa	ChlLL	SMo
CHENOPODIACEAE		
Chenopodium amaranticolor	Chl-NLL	NID
C. quinoa	Chl-NLL	NID
CUCURBITACEAE		
Cucumis sativus	Chl-LSp	SMo
Cucurbita maxima var. *oblonga* (cv. *Óvári hengeres*)	Chl-NLL	NID
C. pepo convar. *pepo* provar. *giromontiina* (zucchini)	Chl-NLL	Smo
C. pepo convar. *pepo* provar. *giromontiina* (kokocella)	Chl-NLL	SMo
C. pepo convar. *patissonina* provar. *torticollis* (crook-neck)	Chl-NLL	SMo
C. pepo convar. *patissonina* provar. *torticollis* (straight-neck)	Chl-NLL	SMo
C. pepo convar. *patissonia* f. *radiata* (patisson)	Chl-LSp	SMo
Lagenaria siceraria convar. *clavatina*	NID	SMo, Sp
FABACEAE (LEGUMINOSAE)		
Phaseolus vulgaris cv. *Red Kidney*	SlVc, Vn, NLL	NID
Vigna sinensis	NLL	NID
SOLANACEAE		
Datura stramonium	NID	SMo
Lycopersicon esculentum	NID	SMo
Nicotiana benthamiana	NID	SMo
N. clevelandii	ChlLSp	SMo
N. debneyi	NID	SMo
N. glutinosa	NID	SMo
N. megalosiphon	NID	SMo
N. tabacum cv. *White Burley*	NID	SMo
N. tabacum cv. *Xanthi-nc*	NID	SMo

[1]Meaning of abbreviations: ChlLL, chlorotic local lesions; Chl-NLL, chlorotic-necrotic local lesions; ChlLSp, chlorotic local spots; NID, no infection detected; NLL, necrotic local lesions; SlVc, star-like vein clearing; SMo, systemic mosaic; Sp, spot(s); Vn, veinal necrosis

5. *Aphid transmission*

The CMV-LY was easily transmitted in a non-persistent manner by *Myzus persicae.*

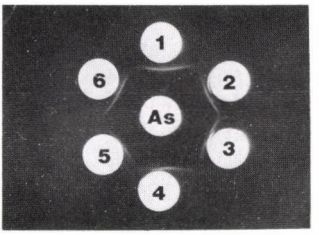

Fig. 2. Agar gel double-diffusion test with infective sap of the CMV-LY: As = antiserum to cucumber mosaic virus, 1,3,5 = infective sap from *Lagenaria siceraria* convar. *clavatina*, and 2,4,6 = infective sap from *Nicotiana tabacum* cv. *White Burley*

6. Seed transmission

All plants grown in greenhouse were virus free. On the basis of these data it was concluded that the plants in the field (see Materials and Methods) had not been infected via seeds; they were infected later in some other way, most likely by aphids.

7. Electron microscopy

Electron microscope analysis of purified CMV-LY (the ratio of UV absorbance at 260/280 nm was 1.68) revealed isometric virus particles about 28 nm in diameter (Fig. 3), close to the reported value of CMV (Francki et al., 1979).

CMV isolated from *Lagenaria siceraria* convar. *clavatina* in Yugoslavia proved to be different from the virus isoldated in 1986 from *Lagenaria siceraria* convar. *turbinata* in Hungary (Horváth et al., unpublished result). One of the most important differences is that while some plant species are susceptible to the virus isolated from *Lagenaria* in

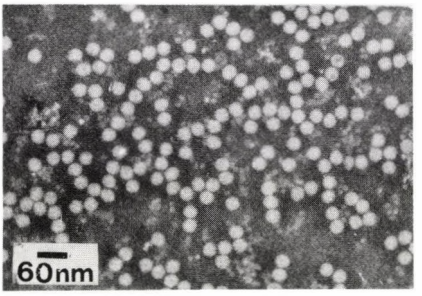

Fig. 3. Particles of the CMV-LY in partial purified preparation. Scale bar = 60 nm

Yugoslavia, there are not susceptible to the *Lagenaria* virus of Hungary. Identification of the virus isolated from *Lagenaria* plant in Hungary is now in process.

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Amaranthus bouchonii Thell. (Family: Amaranthaceae) a New Adventive Plant Species in Hungary, and its Reaction to Some Viruses

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In the course of investigations into the virus susceptibility of *Amaranthus bouchonii*, a new adventive plant species first appearing in Hungary in 1979 we found that it was locally and systemically susceptible to nine viruses (belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, henbane mosaic virus, potato virus X, potato virus Y, tobacco rattle virus, tobacco ringspot virus, tomato mosaic virus), and locally susceptible to three viruses (beet necrotic yellow vein virus, tomato aspermy virus, zucchini yellow mosaic virus). The affinity of the plant in question to various viruses is very strong. Considering that *Amaranthus bouchonii* mostly occurs on ruderal areas where the various European autochtohonous and archaeophythonous virus susceptible plants are also encountered, its virus ecological importance is considerable.

Of the about 100 species of the *Amaranthus* genus some 60 have been reported so far to be virus susceptible (Thornberry, 1966, Horváth 1975, 1976a,b, 1983a,b, 1985; Schmelzer and Wolf, 1977; Edwardson, 1974a,b; Edwardson and Christie, 1986a,b,c). Particularly important are those research results according to which certain *Amaranthus* species are not only experimental hosts for some viruses, they are natural virus hosts and/or virus reservoirs too (Table 1). Considering that the affinity of the various *Amaranthus* species to viruses is very strong, we started experiments to study the little known virus susceptibility of *Amaranthus bouchonii*, the new adventive *Amaranthus* species appearing in Hungary in 1979 (cf. Solymosi and Priszter, 1984). During the first experiments carried out with the plant we found it to be locally susceptible to cucumber mosaic virus, potato virus M, potato virus X, potato virus Y and tobacco mosaic virus, and systemically susceptible to alfalfa mosaic virus (Horváth, 1975, 1976a,b). We then repeated several earlier experiments and established further host-virus relations. To begin with, we should like to note that there are alternative hosts (e.g. *Amaranthus hybridus, A. spinosus*) which when infected with lettuce speckles virus or tobacco bushy-top virus by the *Myzus persicae* aphid became diseased, while in the case of mechanical infection they did not show symptoms and did not become infected (Falk et al., 1979; Chapola, 1980).

Materials and Methods

With eleven viruses obtained from our virus gene bank (Table 2) and with zucchini yellow mosaic virus placed at our disposal by Dr. H. Lecoq (INRA, Centre de Recherches Agr. d'Avignon, Montfavet, France) young *Amaranthus bouchonii* plants were inoculated by the Carborundum gauze-pad method known in plant virology. The virus-containing

Table 1

Amaranthus species as natural hosts of plant viruses

Amaranthus species	Viruses[1]	Literature
Amaranthus albus	AMV	Kaiser and Hannan (1983)
A. blitum	UV	Phatak (1965)
A. caudatus	UV	Govindaswamy et al. (1967)
	TuMV	Schmelzer and Wolf (1977)
A. deflexus	ALMV	Lovisolo and Lisa (1976, 1979)
	UV	Gracia and Feldman (1972)
	CMV	Horváth (1983c)
A. gangeticus	UV	Govindaswamy et al. (1967)
A. gracilis	AmaMV	Quereshi and Mahmood (1980)
A. hybridus	AmaMoV	Taiwo (1988)
	TRSV	Sammons and Barnett (1987)
	TSWV	Cho et al. (1986)
A. lividus	UV	Rubio-Huertos and Vela-Cornejo (1966)
	CMV	Rist and Lorbeer (1989)
	BBWV	Rist and Lorbeer (1989)
A. palmeri	TRSV	McLean (1962b)
A. paniculatus	TRSV	Wingard (1928)
A. powelii	TRV	Locatelli et al. (1978)
A. quitensis	UV	Gracia and Feldman (1972)
A. retroflexus	TRSV	McLean (1962a)
	BeYV	Gorjusin (1964)
	CMV	Dashkeeva et al. (1966), Häni (1971), Schmelzer and Molnár (1975)
	BeMV	Schmelzer and Wolf (1977)
	PVX	Locatelli et al. (1978)
	TRV	Locatelli et al. (1978)
	BeWYV	Boswell and Gibbs (1983), Timmerman et al. (1985)
A. spinosus	TSWV	Cho et al. (1986)
A. viridis	PiMV	Singh et al. (1972)
	TSWV	Cho et al. (1986)

[1]Meaning of abbreviations: AMV – alfalfa mosaic virus, ALMV – *Amaranthus* leaf mottle virus, AmaMoV – *Amaranthus* mosaic virus, AmaMV – *Amaranthus* mottle virus, BeMV – beet mosaic virus, BeWYV – beet western yellows virus, BeYV – beet yellows virus, BBWV – broad bean wilt virus, CMV – cucumber mosaic virus, PiMV – pingweed mosaic virus, PVX – potato virus X, TRV – tobacco rattle virus, TRSV – tobacco ringspot virus, TSWV – tomato spotted wilt virus, TuMV – turnip mosaic virus, UV – unidentified virus

tissue sap used for the inoculation was diluted with distilled water at a ratio of 1:5. The local and/or systemic infection of the plants was determined symptomatologically, or in the absence of symptoms by virus back-inoculation to assay plants (e.g. *Chenopodium quinoa,* see Horváth, 1977).

Table 2

Viruses used in the experiments

Viruses	Strains or isolates	Literature
Belladonna mottle virus	H	Horváth et al. (1976)
Beet necrotic yellow vein virus	HSoP	Horváth et al. (1989)
Broad bean wilt virus	Tm	Horváth and Szirmai (1975)
Cucumber mosaic virus	U/246	Schmidt and Horváth (1982)
Henbane mosaic virus	W/H	Horváth et al. (1988)
Potato virus X	*"Erstling"*	Kegler et al. (1987)
Potato virus Y	*"Mona Lisa"*	Beczner et al. (1984)
Tobacco rattle virus	H	Horváth (1976c)
Tobacco ringspot virus	D	Horváth (1976c)
Tomato aspermy virus	Tm12	Horváth et al. (1980)
Tomato mosaic virus	H	Mamula et al. (1984)
Zucchini yellow mosaic virus	E15	Lecoq et al. (1981, 1983)

Results and Discussion

When compared to the virus susceptibility of about 38 *Amaranthus* species included in our earlier experiments (see Horváth, 1975, 1976a,b) the affinity of *Amaranthus bouchonii* to the viruses examined can be said to be very strong. Nine of the 12 viruses (belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, henbane mosaic virus, potato virus X, potato virus Y, tobacco rattle virus, tobacco ringspot virus, tomato mosaic virus) induced severe local and systemic disease symptoms in the inoculated plants. The symptoms were: necrotic ring-shaped local lesions and systemic mosaic, followed by necrotic lesions. The local necrotic lesions were brown, while the systemic necrotic ones were mostly red (henbane mosaic virus, tobacco rattle virus). The plants inoculated with tobaco ringspot virus showed a considerable growth reduction in addition to the above symptoms compared to the healthy control plants. The present research results concerning potato virus X and potato virus Y partly differ from what was earlier published for the two viruses (cf. Horváth, 1976a). In our earlier experiments with the G-strain of potato virus X an C-, N-, R- and An-strains of potato virus Y *Amaranthus bouchonii* only gave local responses. From symptomless non-inoculated leaves neither potato virus X nor potato virus Y could be reisolated. In our present experiment the potato virus Y strain (veinal necrosis strain, cf. Beczner et al., 1984) causing the so-called tuber necrotic ringspot disease of *Solanum tuberosum* L. cv. *Mona Lisa,* and the so-called B212 strain of potato virus X (Virus-gene bank, Aschersleben, Germany) were used for the inoculation. As mentioned before, both viruses – unlike in the earlier experiments – induced systemic symptoms too in the *Amaranthus bouchonii* plants. It appears that the various potato virus X and potato virus Y strains differ in pathogenicity in the case of *Amaranthus bouchonii*. It is not out of the question either that the *Amaranthus bouchonii* seeds obtained from various sources (in 1972: Botanical Garden, Budapest, Hungary;

and in 1985: Central Institute for Genetic and Crops Research, Gatersleben, Germany) differ in virus susceptibility. It is also possible, that in the 1972-1973 experiments potato virus X and potato virus Y induced systemic latent infection in the *Amaranthus bouchonii* plants, but the reisolation of the viruses was unsuccesful owing to virus inhibitors occurring in the *Amaranthus bouchonii* plants. This hypothesis is supported by reports on virus inhibition by tissue saps from *Amaranthus caudatus* (Hollings, 1966; Klas, 1977), *A. albus, A. aureus, A. hypochandriacus, A. retroflexus* (Smookler, 1971) and from *A. mangostanus* (Choi and Jung, 1984).

The *Amaranthus bouchonii* plants inoculated with beet necrotic yellow vein virus, tomato aspermy virus and zucchini yellow mosaic virus reacted with necrotic local lesions. Systemic symptoms did not develop following the inoculation. The above three viruses were not transmissible from the top leaves of *Amaranthus bouchonii* by back-inoculation to assay plants (*Chenopodium quinoa*). These experiments suggest that there is a local host-virus relation between *Amaranthus bouchonii* on the one hand, and beet necrotic yellow vein virus, tomato aspermy virus and zucchini yellow mosaic virus on the other. According to our knowledge, in the case of tomato aspermy virus there are no experimental data on the virus susceptibility of the *Amaranthus* species. Zucchini yellow mosaic virus is known to induce systemic infection only in some species of the family *Cucurbitaceae*, while *Gomphrena globosa*, the only species of the family *Amaranthaceae* known so far to be susceptible to zucchini yellow mosaic virus is only locally infected by it (cf. Lisa et al., 1981).

The earlier and present results of experiments in Hungary concerning the virus susceptibility of *Amaranthus bouchonii*, the new adventive species are important, since the species occurring on ruderal areas among the European autochthonous or archaeophythonous weed plants may easily be infected under spontaneous conditions by the virus susceptible plants growing there. From a virological point of view *Amaranthus bouchonii* may be an important host plant not only in Hungary but also in France, Germany and Switzerland where it has been encountered since the 1920s, 1930s and 1950s, respectively. It is possible that the plant was introduced in Europe from the tropical and subtropical regions of America, though more probably it is an European mutation of *Amaranthus chlorostachys*, a species from the tropiccs of America becoming by now a cosmopolite (Solymosi and Priszter, 1984). Considering that *Amaranthus chlorostachys* is already known for susceptibility to several viruses (Horváth, 1985, 1976a,b), it would be interesting to extend the virological investigations to cover the above two species as well as the *Amaranthus bouchonii* hybrids (*A. bouchonii* x *A. chlorostachys*, *A. bouchonii* x *A. retroflexus*) discribed by Allen (1961).

According to the results of investigations made so far there are differences in inhibitory effect between the virus inhibitors of the *Amaranthus* species. Further, it would be important to examine the tissue saps of the *Amaranthus* species and hybrids concerned for inhibitory effect on virus infection, as it is known that there may be essential differences in virus inhibition between the tissue saps of plant species belonging to the same genus (e.g. the tissue saps of *Chenopodium quinoa* contains much less inhibitor than the tissue sap of *C. amaranticolor*; cf. Hollings, 1966). To settle this question would be necessary partly because the most virophobous species whose tissue sap has the lowest inhibitory effect on virus infection could thereby be picked out, partly because this

species would be the most suitable for carrying out standard virological examinations with. The tissue sap of *Amaranthus mangostanus* proved to have a 90 per cent while that of *A. viridis* a 72 per cent effect of virus inhibition in the *Nicotiana glutinosa* – tobacco mosaic virus relation (Hollings, 1966; Choi and Jung, 1984). The above may be the explanation for the growing importance of *Amaranthus viridis* as an experimental plant in virology.

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Amaranthus mangostanus L., *A. mitchelii* Benth. and *A. quitensis* H. B. K. (Family: Amaranthaceae) as New Virus Hosts

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Between *Amaranthus mitchelii*, a plant unkown for plant virology, and the hardly known *A. mangostanus* and *A. quitensis* on the one hand, and 12 viruses on the other hand, 36 compatible host-virus relations were detected, of which 5 were local, 22 local and systemic. In nine cases incompatible realations were pointed out.

Local and sytemic relation was established between all the three *Amaranthus* species and belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco ringspot virus and tomato mosaic virus, as well as between *Amaranthus quitensis* and henbane mosaic virus. Local host-virus relation was found between *Amaranthus mangostanus* and beet necrotic yellow vein virus and tobacco rattle virus, *A. mitchelii* and beet necrotic yellow vein virus and *A. quitensis* and beet necrotic yellow vein virus as well as tomato aspermy virus.

Incompatible relation was pointed out between *Amaranthus mangostanus* and henbane mosaic virus, tomato aspermy virus, zucchini yellow mosaic virus; *A. mitchelii* and henbane mosaic virus, tobacco rattle virus, tomato aspermy virus, zucchini yellow mosaic virus; and between *A. quitensis* and tobacco rattle virus as well as zucchini yellow mosaic virus.

In earlier publications we called attention to a strong affinity between various *Amaranthus* species and viruses (Horváth, 1975, 1976a,b, 1983a,b, 1985). When studying the host-virus relations of the new adventive *Amaranthus bouchonii* we found the species to be highly susceptible to the economicaly important viruses, and among the *Amaranthus* species known in plant virology it is one of the best test plants (Horváth, 1991a). In the course of further investigations into the relations of *Amaranthus* species and viruses we examined new species unknown or hardly known so far to plant virology.

Materials and Methods

In our experiments three *Amaranthus* species unknown or hardly known so far to plant virology (*Amaranthus mangostanus*[1], *A. quitensis*[2], *A. mitchelii*) were inoculated with 12 viruses: beet necrotic yellow vein virus, belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, henbane mosaic virus, potato virus X, potato virus Y, tobacco rattle virus, tobacco ringspot virus, tomato aspermy virus, tomato mosaic virus,

1 Two data are known in the literature on the virus susceptibility of *Amaranthus mangostanus*. Phatak (1965) succeeded in infecting *Amaranthus mangostanus* plants with a not closely identified virus isolated from *Amaranthus blitum* and *A. viridis*. The virus described by Singh et al. (1972) under the name pingweed mosaic virus – and is supposed to be identical with the virus mentioned by Phatak (loc. cit.) – proved pathogenic for *Amaranthus mangostanus*.

2 *Amaranthus quitensis* is a natural host for a virus not closely identified so far (see Gracia and Feldman, 1972).

zucchini yellow mosaic virus. To the origin of the viruses reference was made in our previous publication (cf. Horváth, 1991a). The virus-containing tissue sap uses for the inoculation at a 1:5 rate of dilution with distilled water was obtained from various propagative virus hosts kept under controlled conditions. The young *Amaranthus* plants were sprinkled with Carborundum (500 mesh) by means of a special sprinkling apparatus, then inoculated with the gauze-pad method and sprayed with water. The infection of the inoculated plants was established symptomatologically, or in the absence of symptoms by back-inoculation to assay plants (Horváth, 1977).

Results and Discussion

1. Amaranthus mangostanus

Amaranthus mangostanus showed local and systemic susceptibility to seven viruses (belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco ringspot virus, tomato mosaic virus), and responded with local symptoms to beet necrotic yellow vein virus and tobacco rattle virus. The plants inoculated with henbane mosaic virus, tomato aspermy virus and zucchini yellow mosaic virus did not display disease symptoms. Back-inoculation of the latter viruses from leaves of the inoculated plants to *Datura stramonium* and *Chenopodium quinoa* assay plants was unsuccessful. The mentioned responses of *Amaranthus mangostanus* mostly agree with those described in our earlier publication for *A. bouchonii*, except the resistance to henbane mosaic virus, tomato aspermy virus and zucchini yellow mosaic virus, and the local susceptibility to tobacco rattle virus (cf. Horváth, 1991a).

2. Amaranthus mitchelii

In the course of studying the host-virus relations we found that belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco ringspot virus and tomato mosaic virus induced local and systemic symptoms in the inoculated plants. These symptoms agreed with those observed for *Amaranthus mangostanus* and *A. bouchonii* (Horváth, 1991a). The plant proved resistant to henbane mosaic virus, tobacco rattle virus, tomato aspermy virus and zucchini yellow mosaic virus and gave local responses to beet necrotic yellow vein virus. The host-virus relations of *Amaranthus mitchelii* differed from those pointed out for *A. bouchonii* in the case of four viruses (henbane mosaic virus, tobacco rattle virus, tomato aspermy virus and zucchini yellow mosaic virus; cf. Horváth, 1991a). *Amaranthus mitchelii* proved resistant to henbane mosaic virus, tomato aspermy virus and tobacco rattle virus and zucchini yellow mosaic virus, *A. bouchonii*, on the other hand, gave local and systemic responses to henbane mosaic virus and tobacco rattle virus, and local response to tomato aspermy virus.

3. Amaranthus quitensis

Amaranthus quitensis gave local and systemic responses to eight viruses (belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, henbane mosaic virus, potato virus X, potato virus Y, tobacco ringspot virus, tomato mosaic virus). Beet necrotic yellow vein virus and tomato aspermy virus induced local symptoms in the plant in question, while tobacco rattle virus and zucchini yellow mosaic virus proved apathogenic for it. The response of the plant in question differed in the case of two viruses (tobacco rattle virus, zucchini yellow mosaic virus) from that observed with *Amaranthus bouchonii*. Tobacco rattle virus proved apathogenic for *Amaranthus mitchelii*, while *A. bouchonii* was locally and systemically susceptible to it. *Amaranthus mitchelii* was resistant to inoculation with zucchini yellow mosaic virus, while *A. bouchonii* responded with local symptoms to it.

As for local and systemic susceptibility, the host-virus relations of *Amaranthus bouchonii,* the species discussed in an earlier paper (see Horváth, 1991a) and of *A. mangostanus, A. mitchelii* and *A. quitensis* fully agreed in the case of the following viruses: belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco ringspot virus, tomato mosaic virus. The four *Amaranthus* species examined gave different responses to henbane mosaic virus, tobacco rattle virus, tomato aspermy virus and zucchini yellow mosaic virus. Henbane mosaic virus proved apathogenic for *Amaranthus mangostanus* and *A. mitchelii*, while *A. bouchonii* and *A. quitensis* were locally and systemically susceptible to it. *Amaranthus mitchelii* and *A. quitensis* were resistant to the tobacco rattle virus, while *A. mangostanus* responded locally, and *A. bouchonii* locally and systemically to it. *Amaranthus mangostanus* and *A. mitchelii* were resistant to tomato aspermy virus, while *A. quitensis* and *A. bouchonii* were locally susceptible to it. Zucchini yellow mosaic virus proved apathogenic for *Amaranthus mangostanus*, *A. mitchelii* and *A. quitensis,* and *A. bouchonii* showed local susceptibility to it.

The remarkable differences in the host-virus relations call attention to the different behaviour of the *Amaranthus* species to the same virus. Of the new artificial host-virus relations those concerning broad bean wilt virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco rattle virus and tobacco ringspot virus deserve special attention. Namely, between the viruses mentioned and the various *Amaranthus* species many natural relations have been described (reviewed by Horváth, 1991a,b). Natural virus infection has been detected in *Amaranthus lividus* by broad bean wilt virus, in *A. deflexus*, *A. lividus, A. retroflexus* by cucumber mosaic virus; in *A. retroflexus* by potato virus X; in *A. powelli, A. retroflexus* by tobacco rattle virus; in *A. hybridus, A. paniculatus* and *A. retroflexus* by tobacco ringspot virus (Wingard, 1928; McLean, 1962a,b; Dashkeeva et al., 1966; Häni, 1971; Locatelli et al., 1978; Horváth 1983c; Sammons and Barnett, 1987; Rist and Lorbeer, 1989). Thus, the recently examined *Amaranthus* species can be regarded as potential natural virus hosts in the case of the above viruses. The data on the systemic susceptibility to belladonna mottle virus, henbane mosaic virus, potato virus Y, tomato mosaic virus of the new *Amaranthus* species call attention to the possibility for a natural host-virus relation to develop, or even to its occasional presence so far unknown to us.

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Amaranthus Species (Family: Amaranthaceae) as Hosts of Plant Viruses: A Review

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The *Amaranthus* species as test plants, perennial virus hosts and inhibitor-containing plants are equally important in virus research. Of the about 1000 species of the genus *Amaranthus* 63 species (including four synonymous species) have so far been found to be susceptible to viruses according to our knowledge. The *Amaranthus* species are known to be susceptible to 121 viruses, and some 470 host-virus relations have been detected among them. There are remarkable differences in virus susceptibility between the *Amaranthus* species; some species, e.g. *Amaranthus caudatus* (syn.: *A. cruentus*) are susceptible to some 84 viruses while others (e.g. *Amaranthus aurora, A. blitoides* etc.) only to one each. Out of the viruses cucumber mosaic virus has the larges number of *Amaranthus* hosts (42 species), while e.g. *Arracacha* virus A and carnation Italian ringspot virus each has but a single *Amaranthus* host.

Out of the 63 *Amaranthus* species susceptible to 121 viruses belonging to 24 virus groups (and to one "unidentified" and one "complex or mixed" virus group) the largest number of susceptible *Amaranthus* plants are known in the potyvirus group.

Economic and virological importance of the *Amaranthus* species

Among the more than 1000 annual, perennial, herbaceous, woody or shrub species of some 60 genera in the family Amaranthaceae occurring all over the world through mostly in subtropical and tropical regions many play a highly important role in plant virology.

From the *Amaranthus* genus which includes some 100 species only *Amaranthus graecizans* (syn.: *A. angustifolius*) var. *sylvestris* and *A. lividus* ssp. (var.) *ascendens* are indigenous in Europe (Solymosi and Priszter, 1984). Aellen (1959, 1961) the monographist of name listed about 50 species in Europe – including a large number of hybrids (cf. Priszter, 1985) – introduced from America in the last two centuries. Particularly noteworthy are the ornamentals known as the oldest cultivated plants on the Earth (*Amaranthus caudatus, A. cruentus*) – produced as grain crops for the protein, starch and oil content of their seeds and used for human nutrition too –, and the leaf vegetable *Amaranthus tricolor* grown in South-China and India. None the less is wide-spread *Amaranthus viridis* important; it is not a mere weed plant but also a green vegetable locally known as "Cholai" in several parts of Delhi and adjacent areas in India. These plants together with other species play a very important role in plant virology (Hollings, 1965; Edwardson, 1974a,b; Horváth, 1975, 1976a,b, 1983a,b, 1985, 1991a,b; Edwardson and Christie, 1986a,b,c).

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 host plants they have a part in the survival and circulation in nature of certain viruses (reviewed by Horváth, 1991a). Among them the perennial *Amaranthus deflexus* and *A. deflexus* var.

rufescens are particularly important (see Lovisolo and Lisa, 1976, 1979; Horváth, 1983b). According to Schmelzer and Molnár (1975) in Hungary *Amaranthus retroflexus* is a highly important reservoir for cucumber mosaic virus. The strong affinity between *Amaranthus* species and viruses – as pointed out earlier (reviewed by Horváth, 1983a,b, 1985, 1991a,b) – is ocnfirmed by the fact that 12 *Amaranthus* species are known as natural virus hosts: *A. albus, A. caudatus, A. deflexus, A. gracilis, A. hybridus, A. lividus, A. palmeri, A. paniculatus, A. quitensis, A. retroflexus, A. spinosus* and *A. viridis* (Horváth, 1991a). The list is not complete, since in several known natural host-virus relations the species of the plant and/or the virus have not been identified (see Phatak, 1965; Rubio-Huertos and Vela-Cornejo, 1966; Govindaswamy et al., 1967; Gracia and Feldman, 1972 etc.).

In addition to the above the *Amaranthus* species are important model plants in the virus inhibitor research (Hollings, 1966; Smookler, 1971; Ragetli and Weintraub, 1974; Noronha et al., 1980; Choi and Jung, 1984). Considerable inhibitory effect is exercised by the tissue sap of *Amaranthus caudatus* on infection by tobacco mosaic virus, tobacco necrosis virus and alfalfa mosaic virus, and by the tissue sap of *A. deflexus* and *A. mangostanus* on tobacco mosaic virus infection. This fact often has a deceptive effect on the results of the virus transmission and back-inoculation methods used in classical plant virology. This might be the reason why the literary data are not free from contradictions (cf. Horváth, 1991a).

Virus susceptibility of *Amaranthus* species

Considering that as far as we know comprehensive work has not been published on relations between *Amaranthus* species and viruses, we try to fill in the gaps with the present paper (Table 1).

In the table 63 *Amaranthus* plants are listed. Four of them (*angustifolius* = *graecizans, ascendens* = *blitum*, *caudatus* = *cruentus*, *gangeticus* = *tricolor*) can be regarded as synonyms. *Amaranthus sylvestris* has the synonym *A. graecizans* var. *sylvestris*, but under this name no virus susceptible plant can be found in the literature. Eight of the 121 viruses (e.g. *Amaranthus* mottle virus, *Amaranthus* mosaic virus, *Tropaeolum* mosaic virus etc.) are not exactly identified, five of them are known as strains of authentic viruses contained in Table 1 (e.g. *Physalis* shoestring mosaic virus is a strain of tobacco mosaic virus, cabbage black ring virus is a strain of turnip mosaic virus), while one virus (red current ringspot virus) is a synonym of raspberry ringspot virus in Table 1. Between the *Amaranthus* species and viruses listed some 470 host-virus relations are known (see Table 1). The *Amaranthus* species differ in virus susceptibility. *Amaranthus cadatus* (syn.: *A. cruentus*) is susceptible to 84 viruses while *A. aspera*, *A. aurora*, *A. blitoides* etc. are – to our best knowledge – susceptible to a single virus each (see Table 2). Species important from the point of view of virus epidemiology and virus ecology are: *Amaranthus retroflexus* with its susceptibility to 40 viruses, and *A. deflexus* and *A. deflexus* var. *rufescens* susceptible to 7 viruses, among them such important ones as cucumber mosaic virus and beet yellow virus. *Amaranthus bouchonii*, a species recently showing wide geographical distribution is susceptible to some 15 viruses (see Tables 1 and 2).

Table 1

Susceptible *Amaranthus* species to plant viruses

Amaranthus species	Viruses*	Literature
Amaranthus ssp.	*Amaranthus* mottle virus[1]	Qureshi and Mahmood (1980)
	Ivy vein clearing virus	Castellano and Rana (1981)
	Physalis shoestring mosaic virus[6]	Verma and Chowdhury (1984)
	Tobacco streak virus	Costa and Carvalho (1961)
	Tomato spotted wilt virus	Anonymous (1988)
A. albus	Alfalfa mosaic virus	Kaiser and Hannan (1983)
	Beet mosaic virus	Schmelzer and Wolf (1977)
	Beet yellows virus	Thornberry (1966)
	Broad bean wilt virus	Schmelzer and Wolf (1977)
	Strawberry latent ringspot virus	Schmelzer and Wolf (1977)
A. albus var. *roseus*	Beet mosaic virus	Schmelzer and Wolf (1977)
A. angustifolius (syn.: *A. graecizans*)	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. ascendens (syn.: *A. blitum*)	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
	Turnip mosaic virus	Kovachevsky (1975)
A. aspera	Beet yellows virus	Schmelzer and Wolf (1977)
A. atropurpureus	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. aureus	Alfalfa mosaic virus	Horváth (1975, 1981)
	Beet mosaic virus	Schmelzer and Wolf (1977)
	Beet yellows virus	Thornberry (1966)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. aurora	Plum line pattern virus	Schmelzer and Wolf (1977)
A. blitoides	Alfalfa mosaic virus	Horváth (1975)
A. blitum (syn.: *A. ascendens*)	*Amaranthus* mosaic virus[1]	Phatak (1965)
	Cucumber mosaic virus	Horváth (1975)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus X	Nagaich and Upreti (1965)
	Tobacco mosaic virus	Horváth (1975)
A. bouchonii	Alfalfa mosaic virus	Horváth (1981)

Table 1 (continued)

Amaranthus species	Viruses	Literature
	Beet necrotic yellow vein virus	Horváth (1991a)
	Belladonna mottle virus	Horváth (1991a)
	Broad bean wilt virus	Horváth (1991a)
	Cucumber mosaic virus	Horváth (1975, 1991a)
	Henbane mosaic virus	Horváth (1991a)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a, 1991a)
	Potato virus Y	Horváth (1976a, 1991a)
	Tobacco mosaic virus	Horváth (1975)
	Tobacco rattle virus	Horváth (1991a)
	Tobacco ringspot virus	Horváth (1991a)
	Tomato aspermy virus	Horváth (1991a)
	Tomato mosaic virus	Horváth (1991a)
	Zucchini yellow mosaic virus	Horváth (1991a)
A. caracu	Alfalfa mosaic virus	Horváth (1975)
	Beet yellows virus	Thornberry (1966), Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
	Tomato black ring virus	Thornberry (1966), Schmelzer and Wolf, (1977), Edwardson and Christie (1986b)
A. carneus	Beet yellows virus	Thornberry (1966), Schmelzer and Wolf (1977)
A. caudatus (*A. cruentus*)	Alfalfa mosaic virus	Price (1940)
	Amaranthus leaf mottle virus	Casetta et al. (1986)
	Amaranthus mosaic virus[1]	Gowindaswamy et al. (1967)
	Andean potato latent virus	Fribourg et al. (1977)
	Anemone brown ring virus	Hollings (1958)
	Anemone mosaic virus	Hollings (1957)
	Arabis mosaic virus	Schmelzer and Wolf (1977)
	Arracacha virus A	Jones and Kenten (1978)
	Arracacha virus B	Kenten and Jones (1979)
	Artichoke Italian latent virus	Savino et al. (1977)
	Bean yellow mosaic virus	Hollings (1959)
	Beet curly top	Thornberry (1966)
	Beet mosaic virus	Russel (1971)
	Beet yellows virus	Schmelzer and Wolf (1977)
	Black locust true virus	Boswell and Gibbs (1983)
	Broad bean wilt virus	Schmelzer (1960)
	Cactus virus X	Bercks (1971), Attathom et al. (1978)
	Cabbage black ring virus[2]	Brčak and Procházková (1976)
	Carnation Italian ringspot virus	Hollings et al. (1970)
	Carnation mottle virus	Hollings and Stone (1965)
	Carnation ringspot virus	Hollings and Stone (1965)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Carnation vein mottle virus	Hollings (1959)
	Celery latent virus	Bos et al. (1978)
	Celery yellow vein virus[3]	Hollings (1965)
	Chrysanthemum latent virus	Schmelzer and Wolf (1977)
	Cowpea aphid-borne mosaic virus	Quantz (1968)
	Clower yellow mosaic virus	Boswell and Gibbs (1983)
	Clover yellow vein virus	Lisa and Dellavalle (1983)
	Cucumber mosaic virus	Price (1940), Horváth and Szirmai (1973), Sharma and Chowfla (1987)
	Cymbidium ringspot virus	Hollings and Stone (1965)
	Dahlia mosaic virus	Brunt (1971)
	Dogwood mosaic virus	Barnett et al. (1989)
	Elderberry carlavirus	Van Lent et al. (1980)
	Elm mottle virus	Schmelzer and Wolf (1977)
	Grapevine fanleaf virus	Schmelzer and Wolf (1977)
	Heracleum latent virus	Bem and Murant (1979)
	Humulus japonicus virus	Adams et al. (1989)
	Hydrangea ringspot virus	Schmelzer and Wolf (1977)
	Iris fulva mosaic virus	Barnett and Alper (1977), Barnett (1986)
	Iris mild mosaic virus	Schmelzer and Wolf (1977)
	Iris mosaic virus	Brunt (1968)
	Lamium mild mosaic virus[4]	Lisa et al. (1982b)
	Lettuce mosaic virus	Hollings (1959)
	Narcissus mosaic virus	Schmelzer and Wolf (1977)
	Narcissus tip necrosis virus	Mowat et al. (1977)
	Okra mosaic virus	Givord (1979)
	Pea common mosaic virus	Hollings (1959)
	Pea early browning virus	Schmelzer and Wolf (1977)
	Pea mosaic virus	Hollings (1959)
	Peanut stunt virus	Waterworth et al. (1973), Boswell and Gibbs (1983)
	Pelargonium leaf curl virus	Hollings and Stone (1965)
	Pepper veinal mottle virus	Brunt and Kenten (1970), Atiri (1986)
	Pingweed mosaic virus	Singh et al. (1972)
	Plantain virus X	Hammond and Hull (1981)
	Potato black ringspot virus	Salazar and Harrison (1978)
	Potato leafroll virus	Schmelzer and Wolf (1977), Harrison (1984)
	Potato virus N[7]	Agur (1975)
	Potato virus X	Horváth (1976a), Moreira et al. (1980)
	Potato virus Y	Horváth (1976a)
	Raspberry bushy dwarf virus	Schmelzer and Wolf (1977)
	Red clover necrotic mosaic virus	Boswell and Gibbs (1983)
	Red clover vein mosaic virus	Schmelzer and Wolf (1971)
	Robinia mosaic virus	Schmelzer and Wolf (1977)
	Sowbane mosaic virus	Schmelzer and Wolf (1977)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Strawberry latent ringspot virus	Schmelzer and Wolf (1977)
	Tobacco etch virus	Hollings (1959)
	Tobacco mosaic virus	Schmelzer and Wolf (1977)
	Tobacco necrosis virus	Price (1940)
	Tobacco rattle virus	Schmelzer (1957)
	Tobacco ringspot virus	Price (1940)
	Tomato aspermy virus	Schmelzer and Wolf (1977)
	Tomato black ring virus	Schmelzer and Wolf (1977)
	Tomato bushy stunt virus	Hollings and Stone (1965)
	Tomato spotted wilt virus	Best (1968)
	Tropaeolum mosaic virus[1]	Delhey and Monasterios (1977)
	Turnip crinkle virus	Hollings and Stone (1965)
	Turnip mosaic virus	Thornberry (1966), Lisa and Lovisolo (1976)
	UV-potexvirus[1]	Fudl-Allah et al. (1983)
	Viola mottle virus	Lisa and Dellavalle (1977), Lisa et al. (1982a)
	Watermelon mosaic virus (strain 2)	Molnár and Schmelzer (1964)
	White clover virus	Hollings and Stone (1965)
A. caudatus cv. *Atropurpureus*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. chlorostachys	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. chlorostachys f. *strictus*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Tobacco mosaic virus	Horváth (1975)
A. chlorostachys var. *powellii*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. cruentus (syn.: *A. caudatus*)	Alfalfa mosaic virus	Horváth (1975, 1981)
	Amaranthus mosaic virus[1]	Phatak (1965)
	Beet yellows virus	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975, 1981)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. deflexus	*Amaranthus* leaf mottle virus	Lovisolo and Lisa (1976, 1979)
	Beet curly top virus	Thornberry (1966)
	Beet yellows virus	Schmelzer and Wolf (1977)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Cucumber mosaic virus	Horváth (1979, 1983b)
	Potato virus X	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
	UV[1]	Gracia and Feldman (1972)
A. deflexus var. *rufescens*	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. dubius	Alfalfa mosaic virus	Horváth (1975, 1981)
	Cucumber mosaic virus[10]	Horváth (1975, 1981)
	Papaya ringspot virus	Sanchez de Luque and Martinez-López (1977)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. edulis	Andean potato latent virus	Fribourg et al. (1977)
	Arracacha virus B	Kenten and Jones (1979)
	Maracuja (*Passiflora edulis*) mosaic virus	Fribourg et al. (1987)
	Potato virus X	Nagaich and Upreti (1965)
A. emarginatus	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. flavus	Broad bean wilt virus	Schmelzer and Stahl (1977), Edwardson and Christie (1986a)
A. gangeticus (syn.: *A. tricolor*)	*Amaranthus* mosaic virus[1]	Govindaswamy et al. (1967)
	Beet curly top virus	Thornberry (1966)
	Beet yellows virus	Thornberry (1966)
	Cucumber mosaic virus	Horváth (1975)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato aucuba mosaic virus	De Bokx (1975), Schmelzer and Wolf (1977)
	Potato virus X	Thornberry (1966)
	Tobacco ringspot virus	Sastry and Nayudu (1976)
A. gangeticus var. *multicolor* hort.	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. gracilis	*Amaranthus* mottle virus[1]	Qureshi and Mahmood (1980)
A. graecizans (syn.: *A. angustifolius*)	Alfalfa mosaic virus	Horváth (1975)
	Beet curly top virus	Thornberry (1966)
	Beet yellows virus	Thornberry (1966)
	Cucumber mosaic virus	Horváth (1975)
	Plum line pattern virus	Schmelzer and Wolf (1977)
	Potato leafroll virus	Schmelzer and Wolf (1977)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Holmes (1938)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Tomato black ring virus	Edwardson and Christie (1986b)
	Tomato spotted wilt virus	Best (1968), Edwardson and Christie (1986c)
A. hybridus	Alfalfa mosaic virus	Kaiser and Robertson (1976)
	Amaranthus leaf mottle virus	Casetta et al. (1986)
	Amaranthus mosaic virus[1]	Taiwo (1988)
	Artichoke Italian latent virus	Savino et al. (1977)
	Broad bean wilt virus	Boccardo and Conti (1973)
	Cactus virus X	Schmelzer and Wolf (1977)
	Carnation mottle virus	Schmelzer and Wolf (1977)
	Carnation ringspot virus	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Schmelzer and Wolf (1977)
	Lettuce speckles virus[5]	Falk et al. (1979)
	Potato virus X	MacLeod (1962)
	Tobacco mosaic virus	Schmelzer and Wolf (1977)
	Tobacco ringspot virus	Sammons and Barnett (1987)
	Tomato spotted wilt virus	Cho et al. (1986)
	Viola mottle virus	Lisa and Dellavalle (1977)
	Wisteria vein mosaic virus	Conti and Lovisolo (1969), Schmelzer and Wolf (1977)
A. hybridus spp. *incurvatus*	Alfalfa mosaic virus	Kaiser and Robertson (1976)
A. hybridus var. *hypochondriacus*	Broad bean wilt virus	Schmelzer and Wolf (1977)
	Celery latent virus	Brandes and Luisoni (1966), Bos et al. (1978)
	Raspberry ringspot virus	Rana et al. (1985)
A. hypochondriacus	Alfalfa mosaic virus	Horváth (1975, 1981)
	Cucumber mosaic virus	Horváth (1975, 1981)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975, 1981)
A. hypochondriacus cv. *Monstrosus*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. leucocarpus	Alfalfa mosaic virus	Nagaich and Giri (1968), Horváth (1975)
	Amaranthus mosaic virus[1]	Phatak (1965)
	Cucumber mosaic virus	Nagaich and Giri (1968), Horváth (1975)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus M	Horváth (1976b)
	Potato virus X	Nagaich and Upreti (1965), Horváth (1976a)
	Potato virus Y	Horváth (1976a)
A. lividus	Alfalfa mosaic virus	Horváth (1975), Schmelzer (1970)
	Amaranthus lividus virus[1]	Rubio-Huertos and Vela-Cornejo (1966)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Beet yellows virus	Horváth (1975), Schmelzer and Wolf (1977)
	Broad bean wilt virus	Rist and Lorbeer (1989)
	Cactus virus X	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975), Rist and Lorbeer (1989)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Strawberry latent ringspot virus	Schmelzer and Wolf (1977)
	Tobacco mosaic virus	Schmelzer and Wolf (1977)
	Watermelon mosaic virus (strain 2)	Yohana et al. (1977)
A. lividus var. *ascendens*	*Amaranthus lividus* mottle virus[1]	Schmelzer and Wolf (1977)
A. mangostanus	*Amaranthus* mosaic virus[1]	Phatak (1965)
	Beet necrotic yellow vein virus	Horváth (1990b)
	Belladonna mottle virus	Horváth (1991b)
	Broad bean wilt virus	Horváth (1991b)
	Cucumber mosaic virus	Horváth (1991b)
	Henbane mosaic virus	Horváth (1991b)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus X	Horváth (1991b)
	Potato virus Y	Horváth (1991b)
	Tobacco rattle virus	Horváth (1991b)
	Tobacco ringspot virus	Horváth (1991b)
	Tomato aspermy virus	Horváth (1991b)
	Tomato mosaic virus	Horváth (1991b)
	Zucchini yellow mosaic virus	Horváth (1991b)
A. mantegazzianus	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
A. mitchelii	Belladonna mottle virus	Horváth (1991b)
	Broad bean wilt virus	Horváth (1991b)
	Cucumber mosaic virus	Horváth (1991b)
	Henbane mosaic virus	Horváth (1991b)
	Potato virus X	Horváth (1991b)
	Potato virus Y	Horváth (1991b)
	Tobacco rattle virus	Horváth (1991b)
	Tobacco ringspot virus	Horváth (1991b)
	Tomato aspermy virus	Horváth (1991b)
	Tomato mosaic virus	Horváth (1991b)
	Zucchini yellow mosaic virus	Horváth (1991b)
A. monstrosus	Beet mosaic virus	Schmelzer and Wolf (1977)
A. oleraceus	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Potato virus Y	Horváth (1976a)
A. palmeri	Beet yellows virus	Schmelzer and wolf (1977)
	Tobacco ringspot virus	MacLean (1962b)
A. paniculatus	Alfalfa mosaic virus	Horváth (1975)
	Beet mosaic virus	Schmelzer and Wolf (1977)
	Beet yellows virus	Schmelzer and Wolf (1977)
	Carnation ringspot virus	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco broad ringspot virus[9]	Thornberry (1966)
	Tobacco mosaic virus	Horváth (1975)
	Tobacco necrosis virus	Kegler et al. (1969), Edwardson and Christie (1986c)
	Tobacco ringspot virus	Wingard (1928)
	Tobacco stunt virus	Hiruki (1975)
	Tomato black ring virus	Schmelzer and Wolf (1977)
	Turnip mosaic virus	Kovachevsky (1975)
A. paniculatus var. *cruentus*	*Cactus* virus X	Schmelzer and Wolf (1977)
A. paniculatus var. *flavescens*	*Cactus* virus X	Schmelzer and Wolf (1977)
A. paniculatus var. *flavus*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. paniculatus cv. *Roter Dom*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. paniculatus cv. *Roter Paris*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. paniculatus cv. *Sanguineus nanus*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. patulus	Alfalfa mosaic virus	Horváth (1975)
	Beet yellows virus	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975)
	Grapevine fanleaf virus	Schmelzer and Wolf (1977)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)

Table 1 (continued)

Amaranthus species	Viruses	Literature
A. powellii	Tobacco rattle virus	Locatelli et al. (1978)
A. quitensis	Belladonna mottle virus	Horváth (1991b)
	Broad bean wilt virus	Horváth (1991b)
	Cucumber mosaic virus	Horváth (1991b)
	Henbane mosaic virus	Horváth (1991b)
	Potato virus X	Horváth (1991b)
	Potato virus Y	Horváth (1991b)
	Tobacco rattle virus	Horváth (1991b)
	Tobacco ringspot virus	Horváth (1991b)
	Tomato aspermy virus	Horváth (1991b)
	Tomato mosaic virus	Horváth (1991b)
	UV[1,5]	Gracia and Feldman (1972)
	Zucchini yellow mosaic virus	Horváth (1991b)
A. retroflexus	Alfalfa mosaic virus	Schmelzer (1963), Horváth (1975)
	Apple stem groowing virus	Németh (1986)
	Arabis mosaic virus	Klinkowski and Uschdraweit (1968)
	Artichoke Italian latent virus	Savino et al. (1977)
	Asparagus virus 2	Weissenfels et al. (1978)
	Barley stripe mosaic virus	Schmelzer and Wolf (1977)
	Beet curly top virus	Thornberry (1966)
	Beet mosaic virus	Russel (1971)
	Beet western yellows virus	Boswell and Gibbs (1983), Timmerman et al. (1985)
	Beet yellows virus	Gorjushin (1964)
	Broad bean wilt virus	Schmelzer (1960), Weidemann et al. (1975), Jankulowa and Kaitazowa (1979)
	Cactus virus X	Schmelzer and Wolf (1977)
	Carnation mottle virus	Schmelzer and Wolf (1977)
	Carnation ringspot virus	Schmelzer and Wolf (1977)
	Celery latent virus	Brandes and Luisoni (1966), Bos et al. (1978)
	Cucumber mosaic virus	Doolittle and Walker (1923), Hein (1957), Dashkeeva et al. (1966), Häni (1971), Schmelzer and Molnár (1975)
	Elm mottle virus	Schmelzer and Wolf (1977)
	Grapevine fanleaf virus	Schmelzer and Wolf (1977)
	Hydrangea ringspot virus	Schmelzer and Wolf (1977)
	Iris ringspot virus	Thornberry (1966)
	Large nettle yellow mottle virus	Schmelzer and Wolf (1977)
	Lilac chlorotic leafspot virus	Brunt (1978)
	Okra mosaic virus	Givord (1979)
	Pepper veinal mottle virus	Atiri (1986)
	Plantago severe mottle virus	Rowhani and Peterson (1980)
	Potato aucuba mosaic virus	MacLeod (1962)
	Potato leafroll virus	Lebedeva (1975), Horváth (1976a), Locatelly et al. (1978)
	Potato virus M	Horváth (1976b)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Potato virus X	MacLeod (1962), Horváth (1975), Horváth (1976a)
	Prunus necrotic ringspot virus	Smith and Skotland (1986)
	Spinach latent virus	Štefanac and Wrischer (1983)
	Strawberry latent rinspot virus	Hein (1957), Dashkeeva et al. (1966), Häni (1971), Schmelzer and Molnár (1975)
	Tobacco broad ringspot virus[9]	Thornberry (1966)
	Tobacco mosaic virus	Horváth (1975)
	Tobacco rattle virus	Locatelli et al. (1978)
	Tobacco ringspot virus	McLean (1962a)
	Tomato black ring virus	Schmelzer and Wolf (1977)
	Tomato ringspot virus	Schmelzer and Wolf (1977)
	Tomato spotted wilt virus	Milbrath (1939)
	Turnip mosaic virus	Kovachevsky (1975)
A. silvester	*Cactus* virus X	Schmelzer and Wolf (1977)
A. speciosus	Alfalfa mosaic virus	Horváth (1975)
	Beet yellows virus	Schmelzer and Wolf (1977)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. spinosus	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Pingweed mosaic virus	Singh et al. (1972)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco bushy-top virus[11]	Chapola (1980)
	Tobacco mosaic virus	Horváth (1975), Schmelzer and Wolf (1977)
	Tomato spotted wilt virus	Cho et al. (1986)
A. sylvetris	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
A. tricolor (syn.: *A. gangeticus*)	Alfalfa mosaic virus	Horváth (1975)
	Amaranthus leaf mottle virus	Casetta et al. (1986)
	Apple stem groowing virus	Sawamura and Osada (1980)
	Artichoke latent virus	Rana et al. (1982)
	Artichoke yellow ringspot virus	Rana et al. (1980)
	Cucumber mosaic virus	Price (1940), Horváth (1969), Schmelzer and Wolf (1977)
	Potato virus M	Horváth (1976b)
	Potato virus X	MacLeod (1962), Horváth (1976a)
	Potato virus Y	MacLeod (1962), Horváth (1976a)

Table 1 (continued)

Amaranthus species	Viruses*	Literature
	Red currant ringspot virus[8]	Thornberry (1966)
	Spinach latent virus	Van der Meer (1968)
	Tobacco mosaic virus	Holmes (1946)
	Tobacco ringspot virus	Schmelzer and Wolf (1977)
	Tomato ringspot virus	Thornberry (1966)
	Turnip mosaic virus	Price (1940), Horváth (1969), Schmelzer and Wolf (1977)
A. tricolor cv. *Malten Fire*	Alfalfa mosaic virus	Horváth (1975)
	Cucumber mosaic virus	Horváth (1975)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth) (1975)
A. tricolor var. *splendens*	Tobacco ringspot virus	Tuite (1960)
A. viridis	Alfalfa mosaic virus	Horváth (1975)
	Amaranthus mosaic virus[1]	Phatak (1965)
	Artichoke Italian latent virus	Savino et al.(1977)
	Cucumber mosaic virus	Horváth (1979)
	Eggplant mottled crinkle virus	Raj et al. (1989)
	Pingweed mosaic virus	Singh et al. (1972), Shamsher and Verma (1976)
	Potato virus M	Horváth (1976b)
	Potato virus X	Horváth (1976a)
	Potato virus Y	Horváth (1976a)
	Tobacco mosaic virus	Horváth (1975)
	Tomato spotted wilt virus	Cho et al. (1986)

*Key to numbers by viruses: 1 – exactly unidentified virus(es), 2 – strain of turnip mosaic virus, 3 – strain of tomato black ring virus, 4 – related to broad bean wilt virus, 5 – pathogenic after aphid transmission but not mechanically, 6 – strain of tobacco mosaic virus, 7 – strain of cucumber mosaic virus, 8 – syn.: raspberry ringspot virus, 9 – strain of cucumber mosaic virus, or more likely one of the nematode-transmitted viruses, 10 – after Migliori et al. (1978) is not pathogenic to *Amaranthus dubius*, 11 – constituents: tobacco vein distorting and tobacco mottle viruses

Viruses and their *Amaranthus* hosts

For the sake of a better survey we have compiled a table for the *Amaranthus* host of the individual viruses (Table 3). As seen from the table the following viruses have the larges number of *Amaranthus* hosts known: cucumber mosaic virus (42), potato virus X (42), alfalfa mosaic virus (39), tobacco mosaic virus (35), potato virus Y (34). There are viruses (e.g. *Arracacha* virus A, carnation Italian ringspot virus, papaya ringspot virus, *Wisteria* vein mosaic virus etc.) known so far to have a single *Amaranthus* host each.

Table 2

Number of viruses infected *Amaranthus* species

Amaranthus species	Number of viruses	*Amaranthus* species	Number of viruses
Amaranthus ssp.	5	*A. hybridus* var. *hypochondriacus*	3
A. albus	5	*A. hypochondriacus*	6
A. albus var. *roseus*	1	*A. hypochondriacus* cv. *Monstrosus*	6
A. angustifolius (syn.: *A. graecizans*)	5	*A. leucocarpus*	7
A. ascendens (syn.: *A. blitum*)	7	*A. lividus*	11
A. aspera	1	*A. lividus* var. *ascendens*	1
A. atropurpureus	6	*A. mangostanus*	14
A. aureus	7	*A. mantegazzianus*	5
A. aurora	1	*A. mitchelii*	11
A. blitoides	1	*A. monstrosus*	1
A. blitum (syn.: *A. ascendens*)	5	*A. oleraceus*	5
A. bouchonii	15	*A. palmeri*	2
A. caracu	8	*A. paniculatus*	16
A. carneus	1	*A. paniculatus* var. *cruentus*	1
A. caudatus (*A. cruentus*)	84	*A. paniculatus* var. *flavescens*	1
A. caudatus cv. *Atropurpureus*	6	*A. paniculatus* var. *flavus*	6
A. chlorostachys	4	*A. paniculatus* cv. *Roter Dom*	6
A. chlorostachys f. *strictus*	4	*A. paniculatus* cv. *Roter Paris*	5
A. chlorostachys var. *powellii*	4	*A. paniculatus* cv. *Sanguineus nanus*	5
A. cruentus (syn.: *A. caudatus*)	9	*A. patulus*	7
A. deflexus	7	*A. powellii*	1
A. deflexus var. *rufescens*	2	*A. quitensis*	12
A. dubius	8	*A. retroflexus*	40
A. edulis	4	*A. silvester*	1
A. emerginatus	4	*A. speciosus*	7
A. flavus	1	*A. spinosus*	9
A. gangeticus (syn.: *A. tricolor*)	8	*A. sylvestris*	6
A. gangeticus var. *multicolor* hort.	6	*A. tricolor* (syn.: *A. gangeticus*)	15
A. gracilis	1	*A. tricolor* cv. *Malten Fire*	5
A. graecizans (syn.: *A. angustifolius*)	11	*A. tricolor* var. *splendens*	1
A. hybridus	16	*A. viridis*	11
A. hybridus ssp. *incurvatus*	1		

Table 3

Viruses and their *Amaranthus* hosts

Viruses*	*Amaranthus* hosts
Alfalfa mosaic virus	*Amaranthus albus*
	A. angustifolius (syn.: *A. graecizans*)
	A. ascendens (syn.: *A. blitum*)
	A. atropurpureus
	A. aureus
	A. blitoides
	A. bouchonii
	A. caracu
	A. caudatus (*A. cruentus*)
	A. caudatus cv. *Atropurpureus*
	A. chlorostachys
	A. chlorostachys f. *strictus*
	A. chlorostachys var. *powellii*
	A. cruentus (syn.: *A. caudatus*)
	A. dubius
	A. emarginatus
	A. gangeticus var. *multicolor* hort.
	A. graecizans (syn.: *A. angustifolius*)
	A. hybridus
	A. hybridus ssp. *incurvatus*
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. leucocarpus
	A. lividus
	A. mantegazzianus
	A. oleraceus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. paniculatus cv. *Roter Paris*
	A. paniculatus cv. *Sanguineus nanus*
	A. patulus
	A. retroflexus
	A. speciosus
	A. spinosus
	A. sylvestris
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
	A. viridis
Amaranthus leaf mottle virus	*A. caudatus* (*A. cruentus*)
	A. deflexus
	A. hybridus
	A. tricolor (syn.: *A. gangeticus*)
Amaranthus lividus virus[1]	*A. lividus*
Amaranthus lividus mottle virus	*A. lividus* var. *ascendens*
Amaranthus mosaic virus[1]	*A. blitum* (syn.: *A. ascendens*)
	A. caudatus (*A. cruentus*)
	A. cruentus (syn.: *A. caudatus*)
	A. gangeticus (syn.: *A. tricolor*)

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. hybridus
	A. leucocarpus
	A. mangostanus
	A. viridis
Amaranthus mottle virus[1]	*A.* ssp.
	A. gracilis
Andean potato latent virus	*A. caudatus* (*A. cruentus*)
	A. edulis
Anemone brown ring virus	*A. caudatus* (*A. cruentus*)
Anemone mosaic virus	*A. caudatus* (*A. cruentus*)
Apple stem grooving virus	*A. retroflexus*
	A. tricolor (syn.: *A. gangeticus*)
Arabis mosaic virus	*A. caudatus* (*A. cruentus*)
	A. retroflexus
Arracacha virus A	*A. caudatus* (*A. cruentus*)
Arracacha virus B	*A. caudatus* (*A. cruentus*)
	A. edulis
Artichoke Italian latent virus	*A. hybridus*
	A. retroflexus
	A. tricolor (syn.: *A. gangeticus*)
	A. viridis
Artichoke latent virus	*A. tricolor* (syn.: *A. gangeticus*)
Artichoke yellow ringspot virus	*A. tricolor* (syn.: *A. gangeticus*)
Asparagus virus 2	*A. retroflexus*
Barley stripe mosaic virus	*A. retroflexus*
Bean yellow mosaic virus	*A. caudatus* (*A. cruentus*)
Beet curly top virus	*A. caudatus* (*A. cruentus*)
	A. deflexus
	A. gangeticus (syn.: *A. tricolor*)
	A. graecizans (syn.: *A. angustifolius*)
	A. retroflexus
Beet mosaic virus	*A. albus*
	A. albus var. *roseus*
	A. atropurpureus
	A. caudatus (*A. cruentus*)
	A. monstrosus
	A. paniculatus
	A. retroflexus
Beet necrotic yellow vein virus	*A. bouchonii*
	A. mangostanus
Beet western yellows virus	*A. retroflexus*
Beet yellows virus	*A. albus*
	A. aspera
	A. aureus
	A. caracu
	A. carneus
	A. caudatus (*A. cruentus*)
	A. cruentus (syn.: *A. caudatus*)
	A. deflexus
	A. gangeticus (syn.: *A. tricolor*)

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. graecizans (syn.: *A. angustifolius*)
	A. lividus
	A. palmeri
	A. paniculatus
	A. patulus
	A. retroflexus
	A. speciosus
Belladonna mottle virus	*A. bouchonii*
	A. mangostanus
	A. mitchelii
	A. quitensis
Black locust true virus	*A. caudatus* (*A. cruentus*)
Broad bean wilt virus	*A. albus*
	A. bouchonii
	A. caudatus (*A. cruentus*)
	A. flavus
	A. hybridus
	A. hybridus var. *hypochondriacus*
	A. lividus
	A. mangostanus
	A. mithcelii
	A. quitensis
	A. retroflexus
Cabbage black ring virus[2]	*A. caudatus* (*A. cruentus*)
Cactus virus X	*A. caudatus* (*A. cruentus*)
	A. hybridus
	A. lividus
	A. paniculatus var. *cruentus*
	A. paniculatus var. *flavescens*
	A. retroflexus
	A. silvester
Carnation Italian ringspot virus	*A. caudatus* (*A. cruentus*)
Carnation mottle virus	*A. caudatus* (*A. cruentus*)
	A. hybridus
	A. paniculatus
	A. retroflexus
Carnation ringspot virus	*A. caudatus* (*A. cruentus*)
	A. hybridus
	A. paniculatus
	A. retroflexus
Carnation vein mottle virus	*A. caudatus* (*A. cruentus*)
Celery latent virus	*A. caudatus* (*A. cruentus*)
	A. hybridus var. *hypochondriacus*
	A. retroflexus
Celery yellow vein virus[3]	*A. caudatus* (*A. cruentus*)
Chrysanthenum latent virus	*A. caudatus* (*A. cruentus*)
Clover yellow mosaic virus	*A. caudatus* (*A. cruentus*)
Clover yellow vein virus	*A. caudatus* (*A. cruentus*)
Cowpea aphid-borne mosaic virus	*A. caudatus* (*A. cruentus*)
Cowpea mosaic virus	*A. caudatus* (*A. cruentus*)

Table 3 (continued)

Viruses*	*Amaranthus* hosts
Cucumber mosaic virus	*A. angustifolius* (syn.: *A. graecizans*)
	A. ascendens (syn.: *A. blitum*)
	A. atropurpureus
	A. aureus
	A. blitum (syn.: *A. ascendens*)
	A. bouchonii
	A. caracu
	A. caudatus (*A. cruentus*)
	A. caudatus cv. *Atropurpureus*
	A. chlorostachys
	A. chlorostachys f. *strictus*
	A. chlorostachys var. *powellii*
	A. cruentus (syn.: *A. caudatus*)
	A. deflexus
	A. dubius
	A. emarginatus
	A. gangeticus (syn.: *A. tricolor*)
	A. gangeticus var. *multicolor* hort.
	A. graecizans (syn.: *A. angustifolius*)
	A. hybridus
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. leucocarpus
	A. lividus
	A. mangostanus
	A. mantegazzianus
	A. mitchelii
	A. oleraceus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. paniculatus cv. *Roter Paris*
	A. paniculatus cv. *Sanquineus nanus*
	A. patulus
	A. quitensis
	A. retroflexus
	A. speciosus
	A. spinosus
	A. sylvestris
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
	A. viridis
Cymbidium ringspot virus	*A. caudatus* (*A. cruentus*)
Dahlia mosaic virus	*A. caudatus* (*A. cruentus*)
Dogwood mosaic virus	*A. caudatus*
Eggplant mottled crinkle virus	*A. viridis*
Elderberry carlavirus	*A. caudatus* (*A. cruentus*)
Elm mottle virus	*A. caudatus* (*A. cruentus*)
	A. retroflexus

Table 3 (continued)

Viruses*	*Amaranthus* hosts
Grapevine fanleaf virus	*A. caudatus* (*A. cruentus*)
	A. retroflexus
	A. patulus
Henbane mosaic virus	*A. bouchonii*
	A. mangostanus
	A. mitchelii
	A. quitensis
Heracleum latent virus	*A. caudatus* (*A. cruentus*)
Humulus japonicus virus	*A. caudatus* (*A. cruentus*)
Hydrangea ringspot virus	*A. caudatus* (*A. cruentus*)
	A. retroflexus
Iris fulva mosaic virus	*A. caudatus* (*A. cruentus*)
Iris mild mosaic virus	*A. caudatus* (*A. cruentus*)
Iris ringspot virus	*A. retroflexus*
Ivy vein clearing virus	*A.* spp.
Lamium mild mosaic virus[4]	*A. caudatus* (*A. cruentus*)
Large nettle yellow mottle virus	*A. retroflexus*
Lettuce mosaic virus	*A. caudatus* (*A. cruentus*)
Lettuce speckles virus[5]	*A. hybridus*
Lilac chlorotic leafspot virus	*A. retroflexus*
Maracuja (*Passiflora edulis*) mosaic virus	*A. edulis*
Narcissus mosaic virus	*A. caudatus* (*A. cruentus*)
Narcissus tip necrosis virus	*A. caudatus* (*A. cruentus*)
Okra mosaic virus	*A. caudatus* (*A. cruentus*)
	A. retroflexus
Papaya ringspot virus	*A. dubius*
Pea common mosaic virus	*A. caudatus* (*A. cruentus*)
Pea early browning virus	*A. caudatus* (*A. cruentus*)
Pea mosaic virus	*A. caudatus* (*A. cruentus*)
Peanut stunt virus	*A. caudatus* (*A. cruentus*)
Pelargonium leaf curl virus	*A. caudatus* (*A. cruentus*)
Pepper veinal mottle virus	*A. caudatus*
	A. retroflexus
Physalis shoestring mosaic virus[6]	*A.* spp.
Pingweed mosaic virus	*A. blitum* (syn.: *A. ascendens*)
	A. caudatus (*A. cruentus*)
	A. cruentus (syn.: *A. caudatus*)
	A. dubius
	A. gangeticus (syn.: *A. tricolor*)
	A. leucocarpus
	A. mangostanus
	A. spinosus
	A. viridis
Plantain virus X	*A. caudatus* (*A. cruentus*)
Plantago severe mottle virus	*A. retroflexus*
Plum line pattern virus	*A. aurora*
	A. graecizans (syn.: *A. angustifolius*)
Potato aucuba mosaic virus	*A. gangeticus* (syn.: *A. tricolor*)
	A. retroflexus
Potato black ringspot virus	*A. caudatus* (*A. cruentus*)

Table 3 (continued)

Viruses*	*Amaranthus* hosts
Potato leafroll virus	*A. caudatus* (*A. cruentus*)
	A. graecizans (syn.: *A. angustifolius*)
	A. retroflexus
Potato virus M	*A. ascendens* (syn.: *A. blitum*)
	A. atropurpureus
	A. bouchonii
	A. caracu
	A. caudatus cv. *Atropurpureus*
	A. chlorostachys f. *strictus*
	A. cruentus (syn.: *A. caudatus*)
	A. dubius
	A. gangeticus var. *multicolor* hort.
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. leucocarpus
	A. mantegazzianus
	A. oleraceus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. retroflexus
	A. speciosus
	A. spinosus
	A. sylvestris
	A. tricolor (syn.: *A. gangeticus*)
	A. viridis
Potato virus N[7]	*A. caudatus* (*A. cruentus*)
Potato virus X	*A. angustifolius* (syn.: *A. graecizans*)
	A. ascendens (syn.: *A. blitum*)
	A. atropurpureus
	A. aureus
	A. blitum (syn.: *A. ascendens*)
	A. bouchonii
	A. caracu
	A. caudatus (*A. cruentus*)
	A. caudatus cv. *Atropurpureus*
	A. chlorostachys
	A. chlorostachys var. powellii
	A. cruentus (syn.: *A. caudatus*)
	A. deflexus
	A. dubius
	A. edulis
	A. emarginatus
	A. gangeticus (syn.: *A. tricolor*)
	A. gangeticus var. *multicolor* hort.
	A. graecizans (syn.: *A. angustifolius*)
	A. hybridus
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. leucocarpus

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. lividus
	A. mangostanus
	A. mantegazzianus
	A. mitchelii
	A. oleraceus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. paniculatus cv. *Roter Paris*
	A. paniculatus cv. *Sanquineus nanus*
	A. patulus
	A. quitensis
	A. retroflexus
	A. speciosus
	A. spinosus
	A. sylvestris
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
	A. viridis
Potato virus Y	*A. angustifolus* (syn.: *A. graecizans*)
	A. ascendens (syn.: *A. blitum*)
	A. atropurpureus
	A. aureus
	A. bouchonii
	A. caracu
	A. caudatus (*A. cruentus*)
	A. caudatus cv. *Atropurpureus*
	A. cruentus (syn.: *A. caudatus*)
	A. deflexus var. *rufescens*
	A. dubius
	A. gangeticus var. *multicolor* hort.
	A. graecizans (syn.: *A. angustifolius*)
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. leucocarpus
	A. lividus
	A. mangostanus
	A. mantegazzianus
	A. mitchelii
	A. oleraceus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. paniculatus cv. *Roter Paris*
	A. paniculatus cv. *Sanguineus nanus*
	A. patulus
	A. quitensis
	A. speciosus
	A. spinosus
	A. sylvestris

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
	A. viridis
Prunus necrotic ringspot virus	*A. retroflexus*
Raspberry bushy dwarf virus	*A. caudatus* (*A. cruentus*)
Raspberry ringspot virus	*A. hybridus* var. *hypochondriacus*
Red clover necrotic mosaic virus	*A. caudatus* (*A. cruentus*)
Red clover vein mosaic virus	*A. caudatus* (*A. cruentus*)
Red currant ringspot virus[8]	*A. tricolor*
Robinia mosaic virus	*A. caudatus* (*A. cruentus*)
Spinach latent virus	*A. retroflexus*
	A. tricolor (syn.: *A. gangeticus*)
Sowbane mosaic virus	*A. caudatus* (*A. cruentus*)
Strawberry latent ringspot virus	*A. albus*
	A. caudatus (*A. cruentus*)
	A. lividus
	A. retroflexus
Tobacco broad ringspot virus[9]	*A. paniculatus*
	A. retroflexus
Tobacco bushy-top virus[11]	*A. spinosus*
Tobacco etch virus	*A. caudatus* (*A. cruentus*)
Tobacco mosaic virus	*A. angustifolius* (syn.: *A. graecizans*)
	A. ascendens (syn.: *A. blitum*)
	A. atropurpureus
	A. aureus
	A. blitum (syn.: *A. ascendens*)
	A. bouchonii
	A. caracu
	A. caudatus (*A. cruentus*)
	A. caudatus cv. *Atropurpureus*
	A. chlorostachys
	A. chlorostachys f. *strictus*
	A. chlorostachis var. *powelii*
	A. cruentus (syn.: *A. caudatus*)
	A. deflexus
	A. deflexus var. *rufescens*
	A. dubius
	A. emarginatus
	A. gangeticus var. *multicolor* hort.
	A. graecizans
	A. hybridus
	A. hypochondriacus
	A. hypochondriacus cv. *Monstrosus*
	A. lividus
	A. paniculatus
	A. paniculatus var. *flavus*
	A. paniculatus cv. *Roter Dom*
	A. paniculatus cv. *Roter Paris*
	A. paniculatus cv. *Sanquineus nanus*
	A. retroflexus

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. speciosus
	A. spinosus
	A. sylvestris
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
	A. viridis
Tobacco necrosis virus	*A. caudatus* (*A. cruentus*)
	A. paniculatus
Tobacco rattle virus	*A. bouchonii*
	A. caudatus (*A. cruentus*)
	A. mangostanus
	A. mitchelii
	A. powellii
	A. quitensis
	A. retroflexus
Tobacco ringspot virus	*A. bouchonii*
	A. caudatus (*A. cruentus*)
	A. gangeticus (syn.: *A. tricolor*)
	A. hybridus
	A. mangostanus
	A. mitchelii
	A. palmeri
	A. paniculatus
	A. quitensis
	A. retroflexus
	A. tricolor (syn.: *A. gangeticus*)
	A. tricolor cv. *Malten Fire*
Tobacco streak virus	*A.* spp.
Tobacco stunt virus	*A. paniculatus*
Tomato aspermy virus	*A. bouchonii*
	A. caudatus (*A. cruentus*)
	A. mangostanus
	A. mitchelii
	A. quitensis
Tomato black ring virus	*A. caracu*
	A. caudatus (*A. cruentus*)
	A. graecizans
	A. paniculatus
	A. retroflexus
Tomato bushy stunt virus	*A. caudatus* (*A. cruentus*)
Tomato mosaic virus	*A. bouchonii*
	A. mangostanus
	A. mitchelii
	A. quitensis
Tomato ringspot virus	*A. retroflexus*
	A. tricolor (syn.: *A. gangeticus*)
Tomato spotted wilt virus	*Amaranthus* sp.
	A. caudatus (*A. cruentus*)
	A. graecizans
	A. hybridus

Table 3 (continued)

Viruses*	*Amaranthus* hosts
	A. retroflexus
	A. viridis
Tropaeolum mosaic virus[1]	*A. caudatus* (*A. cruentus*)
Turnip crinkle virus	*A. caudatus* (*A. cruentus*)
Turnip mosaic virus	*A. ascendens* (syn.: *A. blitum*)
	A. caudatus (*A. cruentus*)
	A. paniculatus
	A. retroflexus
	A. tricolor (syn.: *A. gangeticus*)
UV[1]	*A. deflexus*
UV-potexvirus[1]	*A. caudatus* (*A. cruentus*)
UV[1,5]	*A. quitensis*
Viola mottle virus	*A. caudatus* (*A. cruentus*)
	A. hybridus
Watermelon mosaic virus (strain 2)	*A. caudatus* (*A. cruentus*)
	A. lividus
White clover virus	*A. caudatus* (*A. cruentus*)
Wisteria vein mosaic virus	*A. hybridus*
Zucchini yellow mosaic virus	*A. bouchonii*
	A. mangostanus
	A. mitchelii
	A. quitensis

*See the explanation at the end of the Table 1.

Table 4

Susceptible *Amaranthus* species in different virus groups

Virus group	Number of viruses (Members of group)*	*Amaranthus* species
Alfalfa mosaic virus (group)	1 (1)	*Amaranthus albus*
		A. angustifolius (syn.: *A. graecizans*)
		A. ascendens (syn.: *A. blitum*)
		A. atropurpureus
		A. aureus
		A. blitoides
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. chlorostachys
		A. chlorostachys f. *strictus*
		A. chlorostachys var. *powellii*
		A. cruentus (syn.: *A. caudatus*)
		A. dubius
		A. emarginatus
		A. gangeticus var. *multicolor* hort.
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. hybridus ssp. incurvatus
		A. hypochondriacus
		A. hypochondriatus cv. *Monstrosus*
		A. leucocarpus
		A. lividus
		A. mantegazzianus
		A. oleraceus
		A. paniculatus
		A. paniculatus var. *flavus*
		A. paniculatus cv. *Roter Dom*
		A. paniculatus cv. *Roter Paris*
		A. paniculatus cv. *Sanquineus nanus*
		A. patulus
		A. retroflexus
		A. speciosus
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
		A. viridis
Apple stem groowing virus (group)	1 (1)	*A. retroflexus*
		A. tricolor (syn.: *A. gangeticus*)
Carlavirus	4 (14a, 44, 80, 88)	*A. ascendens* (syn.: *A. blitum*)
		A. atropurpureus
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. chlorostachys f. *strictus*

Table 4 (continued)

Virus group	Number of viruses (Members of group)*	*Amaranthus* species
		A. cruentus (syn.: *A. caudatus*)
		A. dubius
		A. gangeticus var. *multicolor* hort.
		A. hypochondriacus
		A. hypochondriacus cv. *Monstrosus*
		A. leucocarpus
		A. mantegazziaunus
		A. oleraceus
		A. paniculatus
		A. paniculatus var. *flavus*
		A. paniculatus cv. *Roter Dom*
		A. retroflexus
		A. speciosus
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. viridis
Caulimovirus	1 (42)	*A. caudatus* (*A. cruentus*)
Closterovirus	3 (23, 48, 60)	*A. albus*
		A. aspera
		A. aureus
		A. caracu
		A. carneus
		A. caudatus (*A. cruentus*)
		A. cruentus (syn.: *A. caudatus*)
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus
		A. gangeticus (syn.: *A. tricolor*)
		A. graecizans (syn.: *A. angustifolius*)
		A. lividus
		A. palmeri
		A. paniculatus
		A. patulus
		A. retroflexus
		A. speciosus
Comovirus	1 (39)	*A. caudatus* (*A. cruentus*)
Cucumovirus	5 (40, 69, 81, 90, 103)	*A. angustifolius* (*A. graecizans*)
		A. ascendens (syn.: *A. blitum*)
		A. atropurpureus
		A. aureus
		A. blitum (syn.: *A. ascendens*)
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. chlorostachys
		A. chlorostachys f. *strictus*
		A. chlorostachys var. *powellii*
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus

Table 4 (continued)

Virus group	Number of viruses (Members of group)*	*Amaranthus* species
		A. dubius
		A. emarginatus
		A. gangeticus (syn.: *A. tricolor*)
		A. gangeticus var. *multicolor* hort.
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. hypochondriacus
		A. hypochondriacus cv. *Monstrosus*
		A. leucocarpus
		A. lividus
		A. mangostanus
		A. mantegazzianus
		A. mitchelii
		A. oleraceus
		A. paniculatus
		A. paniculatus var. *flavus*
		A. paniculatus cv. *Roter Dom*
		A. paniculatus cv. *Roter Paris*
		A. paniculatus cv. *Sanquineus nanus*
		A. patulus
		A. quitensis
		A. retroflexus
		A. speciosus
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
		A. viridis
Dianthovirus	2 (31, 87)	*A. caudatus* (*A. cruentus*)
		A. hybridus
		A. paniculatus
		A. retroflexus
Furovirus	1 (21)	*A. bouchonii*
		A. mangostanus
Geminivirus	1 (19)	*A. caudatus* (*A. cruentus*)
		A. deflexus
		A. gangeticus (syn.: *A. tricolor*)
		A. graecizans (syn.: *A. angustifolius*)
		A. retroflexus
Hordeivirus	1 (17)	*A. retroflexus*
Ilarvirus	7 (16,45, 49, 76, 84, 91, 101)	*A.* spp.
		A. aurora
		A. caudatus (*A. cruentus*)
		A. graecizans (syn.: *A. angustifolius*)
		A. retroflexus
		A. tricolor (syn.: *A. gangeticus*)
Luteovirus	2 (22, 79)	*A. caudatus* (*A. cruentus*)
		A. graecizans (syn.: *A. angustifolius*)
		A. retroflexus
Necrovirus	1 (98)	*A. caudatus* (*A. cruentus*)

Table 4 (continued)

Virus group	Number of viruses (Members of group)	*Amaranthus* species
		A. paniculatus
Nepovirus	15 (11, 12, 13, 14, 15, 34, 42a, 46, 78, 86, 89, 93, 100, 104, 106a)	*A. albus*
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. edulis
		A. gangeticus (syn.: *A. tricolor*)
		A. hybridus
		A. hybridus var. *hypochondriacus*
		A. lividus
		A. mangostanus
		A. mitchelii
		A. palmeri
		A. paniculatus
		A. patulus
		A. quitensis
		A. retroflexus
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
Potexvirus	11 (28, 36, 50, 62, 74, 75, 77, 82, 112, 114, 116)	*A. angustifolius* (syn.: *A. graecizans*)
		A. ascendens (syn.: *A. blitum*)
		A. atropurpureus
		A. aureus
		A. blitum (syn.: *A. ascendens*)
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. chlorostachys
		A. chlorostachys var. *powellii*
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus
		A. dubius
		A. edulis
		A. emarginatus
		A. gangeticus (syn.: *A. tricolor*)
		A. gangeticus var. *multicolor* hort.
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. hypochondriacus
		A. hypochondriacus cv. *Monstrosus*
		A. leucocarpus
		A. lividus
		A. mangostanus
		A. mantegazzianus
		A. mitchelii
		A. oleraceus
		A. paniculatus
		A. paniculatus var. *cruentus*
		A. paniculatus var. *flavescens*

Table 4 (continued)

Virus group	Number of viruses (Members of group)	*Amaranthus* species
		A. paniculatus cv. *Roter Dom*
		A. paniculatus cv. *Roter Paris*
		A. paniculatus cv. *Saquineus nanus*
		A. patulus
		A. quitensis
		A. retroflexus
		A. silvester
		A. speciosus
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
		A. viridis
Potyvirus	24 (2, 5, 18, 20, 27, 32, 37, 38, 47, 51, 52, 53, 58, 65, 66, 68, 71, 73, 83, 96, 110, 115, 117, 118)	*A. albus*
		A. albus var *roseus*
		A. angustifolius (syn.: *A. graecizans*)
		A. ascendens (syn.: *A. blitum*)
		A. atropurpureus
		A. aureus
		A. blitum (syn.: *A. ascendens*)
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus
		A. deflexus var. *rufescens*
		A. dubius
		A. gangeticus (syn.: *A. tricolor*)
		A. gangeticus var. *multicolor*
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. hypochondriacus
		A. hypochondriacus cv. *Monstrosus*
		A. leucocarpus
		A. lividus
		A. mangostanus
		A. mantegazzianus
		A. mitchelii
		A. monstrosus
		A. oleraceus
		A. paniculatus
		A. paniculatus var. *flavus*
		A. paniculatus cv. *Roter Dom*
		A. paniculatus cv. *Roter Paris*
		A. paniculatus cv. *Sanquineus nanus*
		A. patulus
		A. quitensis
		A. retroflexus
		A. speciosus

Table 4 (continued)

Virus group	Number of viruses (Members of group)*	*Amaranthus* species
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
		A. viridis
Rhabdovirus	1 (55)	*A.* spp.
Sobemovirus	1 (92)	*A. caudatus* (*A. cruentus*)
Tobamovirus	4 (61, 72, 97, 106)	*A.* spp.
		A. angustifolius (syn.: *A. graecizans*)
		A. ascendens (syn.: *A. blitum*)
		A. atropurpureus
		A. aureus
		A. blitum (syn.: *A. ascendens*)
		A. bouchonii
		A. caracu
		A. caudatus (*A. cruentus*)
		A. caudatus cv. *Atropurpureus*
		A. chlorostachys
		A. chlorostachys var. *powellii*
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus
		A. deflexus var. *rufescens*
		A. dubius
		A. edulis
		A. emarginatus
		A. gangeticus var. *multicolor* hort.
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. hypochondriacus
		A. hypochondriacus cv. *Monstrosus*
		A. lividus
		A. mangostanus
		A. mitchelii
		A. paniculatus
		A. paniculatus var. *flavus*
		A. paniculatus cv. *Roter Dom*
		A. paniculatus cv. *Roter Paris*
		A. paniculatus cv. *Sanquineus nanus*
		A. quitensis
		A. retroflexus
		A. speciosus
		A. spinosus
		A. sylvestris
		A. tricolor (syn.: *A. gangeticus*)
		A. tricolor cv. *Malten Fire*
		A. viridis
Tobravirus	2 (67, 99)	*A. bouchonii*
		A. caudatus (*A. cruentus*)
		A. mangostanus
		A. mitchelii

Table 4 (continued)

Virus group	Number of viruses (Members of group)*	*Amaranthus* species
		A. powelii
		A. quitensis
		A. retroflexus
Tospovirus	1 (107)	*A. caudatus* (*A. cruentus*)
		A. graecizans (syn.: *A. angustifolius*)
		A. hybridus
		A. retroflexus
		A. viridis
Tombusvirus	8 (29, 30, 41, 43, 63, 70, 105, 109)	*A. caudatus* (*A. cruentus*)
		A. hybridus
		A. paniculatus
		A. retroflexus
		A. viridis
Tymovirus	3 (7, 24, 64)	*A. bouchonii*
		A. caudatus (*A. cruentus*)
		A. edulis
		A. mangostanus
		A. mitchelii
		A. quitensis
		A. retroflexus
Ungrouped or unidentified (unclassified) viruses	18 (3, 4, 5, 6, 9, 25, 26, 33, 35, 54, 56, 57, 59, 85, 94, 102, 108, 111)	A. spp.
		A. albus
		A. blitum (syn.: *A. ascendens*)
		A. bouchonii
		A. caudatus (*A. cruentus*)
		A. cruentus (syn.: *A. caudatus*)
		A. deflexus
		A. flavus
		A. gangeticus (syn.: *A. tricolor*)
		A. gracilis
		A. hybridus
		A. hybridus var. *hypochondriacus*
		A. leucocarpus
		A. lividus
		A. lividus var. *ascendens*
		A. mangostanus
		A. mitchelii
		A. paniculatus
		A. quitensis
		A. retroflexus
		A. viridis
Komplex or mixed virus	1 (95)	*A. spinosus*

Table 4 (continued)

Key: 1 – Alfalfa mosaic, 2 – *Amaranthus* leaf mottle, 3 – *Amaranthus lividus*, 4 – *Amaranthus lividus* mottle, 5 – *Amaranthus* mosaic, 6 – *Amaranthus* mottle, 7 – Andean potato latent, 8 – *Anemone* brown ring, 9 – *Anemone* mosaic, 10 – Apple stem groowing, 11 – *Arabis* mosaic, 12 – *Arracacha* A, 13 – *Arracacha* B, 14 – Artichoke Italian latent, 14a – Artichoke latent, 15 – Artichoke yellow ringspot, 16 – *Asparagus* 2, 17 – Barley stripe mosaic, 18 – Bean yellow mosaic, 19 – Beet curly top, 20 – Beet mosaic, 21 – Beet necrotic yellow vein, 22 – Beet western yellows, 23 – Beet yellows, 24 – Belladonna mottle, 25 – Black locust true, 26 – Broad bean wilt, 27 – Cabbage black ring, 28 – *Cactus* X, 29 – Carnation Italian, ringspot, 30 – Carnation mottle, 31 – Carnation ringspot, 32 – Carnation vein mottle, 33 – Celery latent, 34 – Celery yellow vein, 35 – *Chrysanthenum* latent, 36 – Clover yellow mosaic, 37 – Clover yellow vein, 38 – Cowpea aphid-borne mosaic, 39 – Cowpea mosaic, 40 – Cucumber mosaic, 41 – *Cymbidium* ringspot, 42 – *Dahlia* mosaic, 42a – Dogwood mosaic virus, 43 – Eggplant mottled crinkle, 44 – Elderberry carla, 45 – Elm mottle, 46 – Grapevine fanleaf, 47 – Henbane mosaic, 48 – *Heracleum* latent, 49 – *Humulus japonicus*, 50 – *Hydrangea* ringspot, 51 – *Iris fulva* mosaic, 52 – *Iris* mild mosaic, 53 – *Iris* mosaic, 54 – *Iris* rings pot, 55 – Ivy vein clearing, 56 – *Lamium* mild mosaic, 57 – Large nettle yellow mottle, 58 – Lettuce mosaic, 59 – Lettuce speckles, 60 – Lilac chlorotic leafspot, 61 – Maracuja (*Passiflora edulis*) mosaic, 62 – *Narcissus* mosaic, 63 – *Narcissus* tip necrosis, 64 – Okra mosaic, 65 – Papaya ringspot, 66 – Pea common mosaic, 67 – Pea early browning, 68 – Pea mosaic, 69 – Peanut stunt, 70 – *Pelargonium* leaf curl, 71 – Pepper veinal mottle, 72 – *Physalis* shoestring mosaic, 73 – Pingweed mosaic, 74 – Plantain X, 75 – *Plantago* severe mottle, 76 – Plum line pattern, 77 – Potato aucuba mosaic, 78 – Potato balck ringspot, 79 – Potato leafroll, 80 – Potato M, 81 – Potato N, 82 – Potato X, 83 – Potato Y, 84 – *Prunus* necrotic ringspot, 85 – Raspberry bushy dwarf, 86 – Raspberry ringspot, 87 – Red clover necrotic mosaic, 88 – Red clover vein mosaic, 89 – Red currant ringspot, 90 – *Robinia* mosaic, 91 – Spinach latent, 92 – Sowbane mosaic, 93 – Strawberry latent ringspot, 94 – Tobacco broad ringspot, 95 – Tobacco bushy-top, 96 – Tobacco etch, 97 – Tobacco mosaic, 98 – Tobacco necrosis, 99 – Tobacco rattle, 100 – Tobacco ringspot, 101 – Tobacco streak, 102 – Tobacco stunt, 103 – Tomato aspermy, 104 – Tomato black ring, 105 – Tomato bushy stunt, 106 – Tomato mosaic, 106a – Tomato ringspot, 107 – Tomato spotted wilt, 108 – *Tropaeolum* mosaic, 109 – Turnip crinkle, 110 – Turnip mosaic, 111 – unidentified virus-1, 112 – unidentified potex, 113 – unidentified virus-5, 114 – *Viola* mottle, 115 – Watermelon mosaic (strain 2), 116 – White clover, 117 – *Wisteria* vein mosaic, 118 – Zucchini yellow mosaic viruses.
More information see in Key of Table 1.

Amaranthus hosts in different virus groups

The 121 viruses infecting *Amaranthus* species belong to 24 virus groups, one "unidentified" and one "complex or mixed" virus group. With the number of susceptible *Amaranthus* species taken into consideration the following order of succession can be established for the virus groups: potexvirus group (44), cucumovirus and potyvirus groups (42 each), tobamovirus group (40), alfalfa mosaic virus group (38). Considering that the virus groups significantly differ in the number of viruses included in them (1-23 in the above virus groups), the relative virus-host index – obtained by dividing the number of susceptible *Amaranthus* species listed for a given virus group by the number of viruses included in it – will be the following: alfalfa mosaic virus group 38, tobamovirus group 10, cucumovirus group 8.4, potexvirus group 4 and potyvirus group 1.8. Major importance would naturally be attached to such a numerical expression of host-virus or virus-host relations only if identical number of viruses were contained in the different virus groups.

In Table 4 only two virus groups (carlavirus group and tobamovirus group) can be found for which *Amaranthus* hosts of equaly 4 viruses are listed. If these viruses and the number of their *Amaranthus* hosts are compared, it can be established that the virus-host index is 10 for the tobamoviruses and 6 for the carlaviruses. A further question may be whether in the study of hosts for tobamoviruses and carlaviruses the *Amaranthus* species were uniformly subjects of examinations.

Conclusions

The affinity of *Amaranthus* species to plant viruses is highly remarkable. The cosmopolitan *Amaranthus* species which show an ever wider distribution, together with the various perennial species and the recently appearing *Amaranthus bouchonii* – supposedly introduced from the USA – play an increasing role in the spreading an survival of viruses. These wide and known host-virus relations suggest that the virologically so far unknown *Amaranthus* species – some 40 in number – also have an important part in the distribution and ecology of viruses. A future task of scientific research is to detect these so far unknown host-virus relations.

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Some Data on Adsorption of Two Plant Viruses to Soil

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The degree of adsorption of tobacco mosaic virus (TMV) particles and turnip yellow mosaic virus (TYMV) particles to two types of soil (humic and sandy soil) was established by laboratory tests. Both types of soil adsorbe in high percentage TMV (c. 95 %) as well as TYMV (nearly 100 %). Infectivity of the humic soil which contained the adsorbed TMV particles was completely reduced.

It is well known that the bentonite, a clay material, adsorbs proteins as well as virus particles and because of that it can be used in virus purification (Gibbs and Harrison, 1976). When virus particles get to the soil from a plant organism they can be adsorbed to soil components. Blanco-Sanches et al. (1986) have established that different types of soils adsorb tobacco mosaic virus (TMV) particles differently depending on the colloidal complex of each type of soil.

In the last several years it was demostrated repeatedly that certain plant viruses could be isolated from the drainage water (Teakle, 1986) or from waters of lakes and rivers nearly over the world (Koenig, 1986; Koenig et al., 1988; Kontzog et al., 1988). In order to detect the plant viruses in water environments the water samples were concentrated either by ultracentrifugation (Tošić and Tošić, 1984; Koenig and Lesemann, 1985; Juretić et al., 1986; Horváth et al., 1986) or by virus concentrators (Tomlinson et al., 1983). However, Piazzola et al., (1986) have changed the procedure of isolation of plant viruses from water environments. They did not concentrate the virus from water by ultracentrifugation but by means of a low speed centrifugation (5000 g). In this manner they obtained the sediments which were infective. Besides TMV, the collected sediments contained cucumber mosaic virus (CMV) and also tow other unidentified viruses. The authors supposed that these viruses were at least partially adsorbed to soil sediments occurring in water.

Above data inspired us to find out the degree of adsorption of two viruses to two specific types of soil.

Materials and Methods

Two viruses were used: tobacco mosaic virus (TMV, type strain) and turnip yellow mosaic virus (TYMV, strain 1). TMV was maintained in tobacco (*Nicotiana tabacum* cv. *Samsun*) and TYMV in turnip (*Brassica rapa* cv. *rapa*). The experiments were carried out with infective plant sap previously filtered through gauze and diluted with tap water (pH 7.2) at a ratio of 1:1000 for TMV and 1:100 for TYMV.

Experiments of adsorption of the viruses to soil were performed in to manners. In one case diluted infective sap was filtered through a moist soil-bed made in a column (25×1.5 cm). One type of sterilized soil consisted of 2 parts of common field soil, 1 part of compost and 10% sand (humic soil, pH 7.7) and the other type of used soil consisted of about 95 % sand and 5 % minute soil dust (sandy soil, pH 8.2). Infective samples (10 ml) were applied on the top of the column and eluted by 20 ml of water flow. The first 10 ml eluted liquid were tested on plants. In the other case diluted infective sap was homogenized with sterilized soil and steered 8 hr. After low speed centrifugation, the infectivity of the supernatant was tested on plants.

Following the treatments with soil, the infectivity of TMV preparations was assayed on half-leaves of *Chenopodium murale, Datura stramonium* and *Nicotiana glutinosa* as locally reacting hosts (Latin square design): four inocula were compared by randomizing them among halves of two leaves on four plants (*Datura stramonium* and *Nicotiana glutinosa*) or four leaves on four plants (*Chenopodium murale*) so that each inoculum was applied to one or two half-leaves of each plant. The infectivity of TYMV preparations was assayed on young exemplares of *Brassica rapa* cv. *rapa*. In these experiments whole plants were used as infectable units (systemically reacting host); each inoculum was applied to 50 plants allocated at random to each group.

Results

1. Adsorption of TMV to soil

Three specimens of TMV infective sap were tested after their treatment with soil. Together with the control specimen four samples in total were assayed. They were: sample A – untreated infective plant sap, sample B – infective sap homogenized with the humic soil, sample C – infective sap homogenized with the sandy soil, and sample D – infective sap filtered through humid soil. The results obtained are presented in the Figure 1. The curve in the Figure 1 is a resultant of the number of lesions appearing on all half-leaves of three test plants. In relation to the control (speciment A), the infectivity of all treated sap specimens (B, C, D) was esentially reduced. The average number of lesions in the three repeated experiments was: A = 1684, B = 340, C = 89, D = 92. The number of lesions varied depending on test plant species. However, the relative relations in the number of lesions produced by particular specimens were similar at all three test plant species (*Nicotiana glutinosa, Datura stramonium, Chenopodium murale*). The most susceptible test plant was *Datura stramonium* and the other two plants reacted similarly.

2. Adsorption of TYMV to soil

The adsorption of TYMV to the humic and the sandy soil was similar to that of TMV. However, as it can be seen from the Figure 1 the adsorption of virus particles of the speciments C and D was complete. Namely, from 50 inoculated plants the specimen A infected 50 plants, the specimen B 1 plant, the speciment C no plant and the specimen

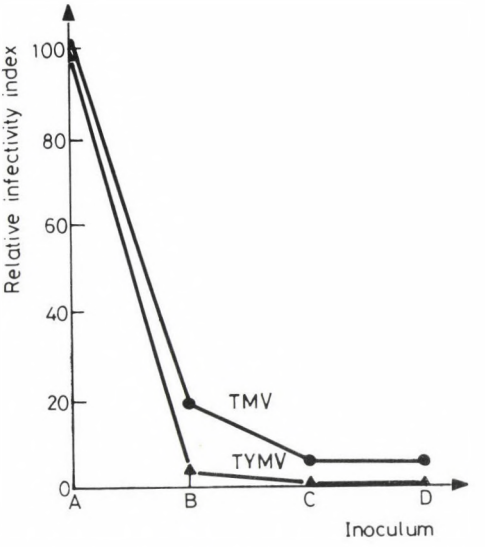

Fig. 1. Adsorption of TMV and TYMV to two types of soil: A – untreated infective plant sap, B – infective sap homogenized with the humid soil, C – infective sap homogenized with the sandy soil, D – infective sap filtered through humic soil. TMV samples were diluted with water 1 : 1000 and TYMV samples 1 : 100

D again no plant. These experiments with TYMV were done in the same manner as the experiments with TMV but in this case the infectivity tests were performed by *Brassica rapa* cv. *rapa* as a test plant systemically reacting to TYMV.

3. Infectivity of the soil adsorbing virus particles

To reveal the degree of the infectivity of the soil adsorbing virus particles, the following assayes were done. The humid soil with adsorbed TMV was dried by standing at room temperature for 48 hr. After that, soil was homogenized with a smoll quantity of water and the mixture was stirred for 4 hr. After low speed centrifugation the supernatant was inoculated to test plants (*Datura stramonium, Nicotiana glutinosa*). However, in three repeated tests no infectivity was detected. The results showed that our humic soil had a great capacity of inactivating the virus (cf. Blanco-Sanches et al., 1986).

Discussion

Obviously, the soils used are highly charged materials with a large surface area. Because of that they are good adsorbens for virus particles. It means that the soils contained much colloid matrices. Blanco-Sanches et al. (1986) established that their

carbonate soil adsorbed much more TMV particles then sandy soil did. Paradoxical datum obtained in our experiments that the sandy soil used adsorbed virus particles similarly as the humic soil did, could be explanated by the fact that the sandy soil had relatively enough minute pulverized soil which probably contained colloidal components responsible for the adsorption of virus particles.

It can be concluded that a large part of the plant viruses occurring in surface waters reach the soil and accumulate in its upper layers where they are adsorbed to colloid matrices. When a virus reaches underground water which passes through the soil, it can be adsorbed to soil colloids. Therefore, the virus inflow to rivers or lakes in this way is limited. The viruses found in rivers probably origin from surface waters. Our results agree with the finding of Piazzola et al. (1986) that plant viruses can be adsorbed in river waters to their sediments which have their origin in the soil components.

Acknowledgements

We are most grateful to Drs. I. Šugar and V. Hršak for valuable discussions.

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Distribution and Frequency of *Streptomyces scabies* on the Tubers of Two Scab-susceptible Potato Cultivars

(Contributions to the Bacteriology of Potato II)

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The presence and population densities of *Streptomyces scabies* the causal agent of common potato scab and other streptomycetes were detected in scabby tissues and healthy periderm on tubers of two scab-susceptible cultivars of potato ("Romano" and "Desiree") cultivated in sandy (rust-brown forest-) soils in SW-Hungary. The isolated *Str. scabies* strains were identified according to the determining key offered by Elesawy and Szabó in 1981. The population densities of *Str. scabies* proved to be the largest in scabby tissues, but the common occurrence of it was frequently detected also in the healthy periderm of the partly or completely undamaged tubers too. In some cases healthy tubers were colonized only by bacteria while streptomycetes lacked completely in such epiphytic microbiotas. Presumedly the bacterial members of the latters may be responsible for the local protection of the tuber tissues of the scab-susceptible cultivars against the attack of *Str. scabies*.

Among *Streptomyces* spp. which can induce potato scab, *Str. scabies* is the most common pathogen. The members of this species produce spirale sporophores, smooth spores and able to utilize many carbon compounds as sole sources of C in the medium (Elesawy and Szabó, 1979, 1981; Loria et al., 1986). Due to the difficulties in the rapid taxonomic identification of fresh streptomycete isolates there is little information in the literature on the population dynamics and ecology of *Str. scabies* in the rhizoplane and on the tuber's surface of potato. The presence of *Str. scabies* on the roots of potatoes in infested soil has been detected by Vruggink (1976) while Adams and Lapwood (1978) reported on actinomycetes which colonize the lenticels of developing potato tubers. Differences in certain physiological abilities of the members of *Str. scabies* populations associated with different scabby tissues were demonstrated by Elesawy and Szabó (1979). The frequency of occurrence of actinomycetes, among them *Str. scabies*, on the surfaces of tubers of the scab-susceptible cultivar Chippewa and the resistant cultivar Superior, furthermore the correlation of scab severity with the population densities of total actinomycetes were studied by Keinath and Loria (1989).

The aim of this study presented below was to clarify the distribution and frequency of occurrence of *Str. scabies* and other actinomycetes on the surfaces of attacked and completely healthy tubers of scab-susceptible potato cultivars. This would be the first approach to the problem what microbiological factors are responsible for the protection of individual scab-susceptible tubers or at least local surface microregions on these in soils heavily infected with *Str. scabies.*

Materials and Methods

Healthy and damaged tubers of the scab-susceptible potato cultivars Romano and Desiree were collected from cultivated and with *Str. scabies* heavily contaminated sandy soils in Somogy (SW Hungary) in 1987. From the aseptically removed scabby tissues or healthy tuber-surface regions, after homogenization, dilution series were prepared and from these 0.2 ml samples plated on glycerol-glycine-, starch-casein- and nutrient-agar media in Petri-dishes. After incubation lasted for 5-14 days both actinomycete and bacterial colonies were isolated randomly in large numbers. Bacterial isolates were maintained mostly on nutrient slants while the actinomycete ones on oatmeal-agar slants. On the results of our studies on tuber-surface bacteria we will report later. The actinomycete isolates were tentatively grouped into similarity groups on the basis of a limited number of easily accessible cultural-morphological properties. From every group of similar isolates representative strains were selected for further detailed studies. Taxonomic identification of *Streptomyces* strains was carried out by using the diagnostic criteria and standard methods recommended by the *International Streptomyces Project* (ISP) furthermore the determining key offered by Szabó et al. (1975). Selected isolates of *Streptomyces scabies* and members of other isolated *Streptomyces* spp. were tested in the greeenhouse for pathogenicity against potato. To obtain inoculum, *Streptomyces* cultures were grown on oatmeal-agar slants at 28°C for 10 days. Spore masses were removed from the agar surface and suspended in sterile tap water. The susceptibility of the scab-susceptible potato cultivar Desiree was tested for pathogenicity. From potato tissue-cultures devoid of virus were the test-plants bearing young tubers cultivated in pots containing heat sterilized soil samples wetted periodically with sterile tap-water. Inoculation was carried out with 2 ml of a spore suspension with $>10^4$ cfu/ml. Tubers were studied for scab severity 8-10 weeks after inoculation.

Results and Discussion

From the scabby tissues and the healthy tuber surfaces of the two potato cultivars altogether 753 *Streptomyces* isolates were obtained and from among the latters total 100 and among these 75 representative *Str. scabies* strains were separated and studied. Table 1 shows their taxonomic position, origin, frequency of occurrence and distribution. As can be seen the scabby tissues of both cultivars contained large numbers (common occurrence: Table 1) of *Str. scabies* the studied strains of which showed practically identical diagnostic characteristics with those of the suggested neotype strain of *Str. scabies* (Table 2). *Str. scabies* strains were frequently isolated from the seemingly healthy tuber surfaces around the severe scabs (Table 1: IV, V and VI) and also from surfaces of completely healthy tubers of the cultivar Romano (Table 1: X and XI). But they lacked of the healthy tuber surfaces of the cultivar Desiree (Table 1: XII and XIII). In two cases (Table 1: IX and XIII) only bacteria were isolated from the healthy surfaces of Romano and Desiree tubers. Other species of *Streptomyces* occurred in relatively low numbers both on healthy tuber surfaces and in scabby tissues. These proved to be common soil streptomycetes which may be considered, at least in the by *Str. scabies* attacked tissues,

Table 1

The distribution of selected 100 representative strains of *Streptomyces* spp. isolated from healthy surface regions or severe scab lesions on tubers of two potato cultivars, according to their taxonomic position*, number**, origin*** and frequency of occurrence****

Source of strains *Streptomyces* species	I	II	III	IV	V	VI	IX	X	XI	XII	XIII	XIV
Str. aburaviensis	1 st. Trac.	–	–	–	–	–	–	1 st. Trac.	–	–	–	–
Str. actuosus	–	–	2 st. Trac.	–	–	–	–	–	–	–	–	–
Str. avellaneus	–	–	–	–	–	–	–	1 st. Trac.	2 st. Trac.	–	–	
Str. exfoliatus	–	1 st. Trac.	–	–	–	–	–	–	–	–	–	–
Str. flaveolus	–	–	–	–	2 st. Trac.	–	–	–	–	–	–	–
Str. griseoflavus	–	–	–	–	1 st. Trac.	–	–	–	–	–	–	–
Str. griseus	–	–	–	–	–	2 st. Fre.	–	–	2 st. Fre.	3 st. Fre.	–	–
Str. omiyaensis	–	–	–	–	–	3 st. Trac.	–	–	1 st. Trac.	–	–	–
Str. scabies	9 st. Com.	16 st. Com.	11 st. Com.	4 st. Com.	11 st. Com.	5 st. Com.	–	3 st. Com.	6 st. Com.	–	–	10 st. Com.
Str. violaceus-ruber	–	–	–	–	–	–	–	–	–	–	–	1 st. Trac.
Streptomyces sp.	–	–	1 st. Trac.	–	–	–	–	–	–	1 st. Trac.	–	–

* Strains were identified according to the species redescriptions of the *International Streptomyces Project*, using the determining key of Szabó et al. (1975).

** The number of the thoroughly studied representative strains (st.) are given.

*** I-XI: tubers of the cultivar "Romano"; XII-XIV tubers of cultivar "Desiree". I-III: strains were isolated from individual scab lesions; IV-VI: from seemingly healthy tuber surfaces around of scab lesions; IX-XI: from the complete surface region of a healthy tuber; XII-XIII: from complete surfaces of more than one healthy tubers; XIV: from many different, distinct scab lesions of the very same potato tuber.

**** Trac.= occurrence in traces; Fre. = frequent occurrence; Com. = common occurrence (the frequency was estimated according to the species-distribution among all of the isolates)

mostly as random contaminants. The true *Streptomyces* partners of the metabolising tuber surface microbiotas are until now only less known. Interestingly among the strains of non "scabies-type" streptomycetes a few ones proved also to be potentially pathogen and caused in sterile soil severe scabs on the tubers of the tested potato. Such *Streptomyces* species were however never detected in large population densities in scabby tissues which clearly shows that they can not attack the tubers in the presence of the complex natural microbiota. In the future we will try to obtain more data on the pathogenity of non

Table 2

A comparison of selected diagnostic properties of 75 representative *Streptomyces scabies* strains isolated from potato tubers collected from sandy (cultivated roust-brown forest) soils in SW-Hungary with those of the suggested neotype strain (ATCC 33282) of *Str. scabies* (In parentheses the numbers of positive strains are given in per cent)

Diagnostic properties	Neotype strain ATCC 33282 of *Str. scabies*	Data on 75 *Str. scabies* strains isolated by us
Colour of aerial mycelium	Gray-series	Gray-series (100)
Colour of substrate mycelium	Yellow-brown series	Yellow-brown series(100)
Soluble pigment	–	–
Morphology of sporophores	Spiral	Spiral(100)
Spore surface (electr.opt.)	Smooth	Smooth(100)
Melanoid pigments on Iron-pepton agar	+	variable(42)
C-source utilization		
Arabinose	+	+(100)
Fructose	+	+(100)
Galactose	+	+(100)
Glucose	+	+(100)
Inositol	+	+(100)
Mannitol	+	+(100)
Raffinose	+	+(100)
Rhamnose	+	+(100)
Sucrose	+	+(100)
Xylose	+	+(100)

"scabies-type" streptomycetes. Among *Str. scabies* isolates studied by Keinath and Loria (1989) only about 5 per cent proved to be pathogenic against potato in separated pathogenicity tests. Among our *Str. scabies* strains tested against potato the majority caused scabs on the developing tubers.

On the basis of our findings we suppose, that the protective activities of certain tuber surface bacterial communities of particular species compositions can be responsible for the observed resistance of many local tuber surface microregions or complete tubers of scab-susceptible potato cultivars in with *Str. scabies* heavily infected soils. In the second step of our studies we will analyse qualitatively the composition of bacterial communities of completely healthy tuber surfaces and try to prepare inocula from their members to study the existence and mechanisms of the tuber-epiphytic bacterial anti-scabies activities.

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The Network on Insect-Plant Interactions of the European Science Foundation

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European Science Foundation Network Operations were authorized by mandate of the General Assembly to the Executive Council in November 1984.

Network concept

Networks are primarily orientated towards coordinating activities in order to stimulate and consolidate the scientific community in specific fields. A main idea of a Network is to foster mutual awareness and to promote mobility, which are matters that pertain to individual scientists and to their organisations in building scientific communities on a European scale. This includes the organization of workshops in specific fields as well as other kinds of international meetings. In addition to these types of activities, small collaborative research projects may be undertaken in order to strengthen the construction of scientific communities. In certain instances, Networks may also lead to the establishment and implementation of larger collaborative research enterprises.

The Network on Insect-Plant Interactions was approved for launching by the Executive Council in March 1990.

General description

The direct interaction between the plant world and the animal kingdom is greatly influenced by the primary position of insects. They form by far the most important group of animals consuming living plant tissues and, by virtue of this, have been a major force throughout evolution in shaping and maintaining the appearance and chemical constitution of the present-day plant world. Insect-plant studies therefore have a key position in the biological subdiscipline of fundamental ecology. Knowledge of the principles governing insect-plant interactions exceeds the realm of pure scientific interest, because insect herbivory poses an everlasting problem in agricultural production; the study of insect-plant relationships is therefore considered to represent "the very heart of agricultural entomology". The need to curtail the use of insecticides and to find more environmentally acceptable control measures is universally recognized and new ideas should be based on sound knowledge of insect-plant relationships.

Insect-plant interactions have received relatively little attention, probably because they require a concerted approach by botanists and zoologists. Until recently botany and

zoology have traditionally been separated fields. There is increased awareness of the fact that the combined input from both disciplines may lead to the discovery of important new biological concepts as well as principles applicable to crop protection. Much may be gained for biology in general by promoting cooperation between these two groups of scientists.

A basic element in all insect-plant studies is the notion that insects are with only few exceptions highly selective in their food choice. Since this selectivity is manifest in any insect-plant system it requires special attention when elucidating the principles underlying these systems. Insect food selectivity is influenced by two sources of variability: it involves (i) variation in insect behaviour, and (ii) qualitative and quantitative variation in plant constituents, rendering a plant more or less acceptable to insects. Behavioural variation and plant chemical variability are considered to be the most basic aspects of insect-plant relationships and have therefore been selected for concerted study.

Scope of the network

The activities of the Network will focus on problems concerning insect host plant detection and utilization on the one hand, and plant chemistry and phyisiological responses to insect damage on the other. Within these general areas special attention will be devoted to the following topics, which are considered particularly crucial to furthering our insight into insect-plant relationships. Emphasis has been put on physiological aspects at the level of individuals, rather than on ecological relationships.

1. Chemoreception in herbivorous insects.

Feeding insects recognize their food plants primarily on the basis of olfactory and gustatory stimulation. It is therefore essential to know which plant substances stimulate their chemoreceptors. In particular, the sense of smell and the principles underlying the perception of mixtures of chemicals (which reflect the natural situation) urgently await elucidation. In many cases host plant selection is primarily accomplished by the gravid female, thus decreasing the risk that her offspring, due to limited mobility, die of starvation. Despite this fact ovipositon behaviour has received relatively little attention, because of practical reasons. Research on plant factors governing oviposition behaviour, characteristics of the sense organs involved in host plant recognition and the mechanisms underlying variability in oviposition behaviour warrant increased consideration.

2. Variability in feeding behaviour.

Variability in insect behaviour reflects a basic biological phenomenon, which affects the results of all insect-plant studies. Genetically based variability has been detected both within populations and between geographically isolated populations. In addition, behavioural variability may arise from differences in previous experience, thus reflecting

learning processes. Changes in chemoreceptor sensitivity, due to previous stimulation, age and nutrient history can contribute significantly to behavioural variability.

3. Variability of plant chemistry.

The chemical constitution of plants may be altered due to insect attack, and/or other stress factors. Both qualitative and quantitative changes must be determined under standardized conditions to evaluate their importance in terms of energetic investment by the plant, and in terms of negative (or positive) responses by the infesting insects. Plant susceptibility to insect damage is influenced by many factors, including age and the presence of allelochemics, substances which are deterrent or toxic to insects, often at low concentrations. In order to construct a realistic model of the physiological relationship between an herbivorous insect species and its host plant, it is necessary to determine the chemical nature of the allelochemics and the energetic costs incurred in their production.

Objectives

The objectives of the Network include (1) concentrating the research efforts of different groups on some major problems in the analysis of insect-plant interactions; (2) promoting integrated research in insect-plant systems by entomologists and plant physiologists; (3) increasing the scientific value of results obtained by individual scientists, by agreement on standardization of physiological and behavioural methods and procedures; and (4) establishing a network of interacting scientists and of shared training facilities, with the expectation that such co-operation will greatly enhance the scientific productivity of all participants.

Implementation

To reach the goals set for the Network, several activities have been planned in order to promote interactions between scientists. They include (1) small workshops aimed at developing collaborative strategies to tackle some major problems in the field of insect-plant relations; (2) short exchange visits on an individual basis, e.g. to exchange experiences on methodological problems or to harmonize parallel projects; (3) production of an inventory of European scientists active in the field of insect-plant interactions. A newsletter will be circulated at regular intervals among scientists engaged in this field. This type of communication is important since this Network involves researchers from different disciplines who to a large extent use different communication channels; and (4) an open meeting at the end of Phase 1 to present the results of the collaborative programme on insect-plant interactions to the scientific community.

Coordination committee

Professor L. M. Schoonhoven (Agricultural University Wageningen, The Netherlands) (Chairman)
Dr. W. M. Blaney (Birkbeck College, London, United Kingdom) (Secretary)
Professor G. Bergström (University of Göteborg, Sweden)
Professor F. Camps (Centro de Investigacion y Desarollo, Barcelona, Spain)
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The Effects of Two Fungicides, Fenarimol and Nuarimol, on Larval Stages of *Neobellieria bullata* (Dipt., Sarcophagidae)

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When larva of *Neobellieria bullata* were fed a 0.2% fenarimol treated diet from hatching (0 hr) they did not moult. Sixty percent of the larvae had visible but unsclerotized anterior spiracles. This suggested that an unknown factor controls the apolyzis and the tracheal epithelium differentiation of the prothoracic spiracles. This control factor is independent of the cytochrome P-450-dependent monooxygenase system. Cuticle secretion and pre-ecdysial tanning of the prothoracical spiracles is controlled by 20-OH ecdysone.

Fenarimol inhibits the cytochrome P-450-dependent monooxygenase system, and 20-OH ecdysone synthesis. This effect in wandering stage larvae can be reversed by treatment with 20-OH ecdysone. First instar larvae were more sensitive than older larvae to fenarimol and nuarimol. Fenarimol showed a stronger physiological activity than nuarimol.

In the future of pesticide chemistry the synthesis of agents that affect reproduction, development and growth, other insecticides that do not act on cholinesterase, and compounds that are activated *in vivo*, will become more important.

20-OH ecdysone (Koolman, 1982) is known to be important in insect development, reproduction and embryogenesis. Ecdysteroids are common in invertebrates and plants (Karlson, 1983). The well known function of 20-OH ecdysone along with juvenile hormone is in the control of moulting in insects (Riddiford, 1981). They must moult in order to grow. During moulting ecdysteroids are responsible for apolysis, cell division, differentiation of tissues, and deposition of the new cuticule (Riddiford and Truman, 1978). The newly deposited cuticle is tanned (post-ecdysial tanning) shortly after the moult and the process is mediated by a polypeptide hormone, bursicon (Fraenkel and Hsiao, 1965).

Insects are incapable of *de novo* synthesis of the steroid ring. The precursor of 20-OH ecdysone may be one of several steroids in the food of insects. The possible biosynthetic pathway from dietary steroids to 20-OH ecdysone appears to occur *de novo* through cholesterol (Wilkinson, 1985). Thus biosynthetic pathway to 20-OH ecdysone constitutes a potential mechanism for hormone regulation. It is likely that many of the hydroxylation reactions involved are catalyzed by mitochondrial and microsomal cytochrome P-450-mediated monooxygenases (Bollenbacher et al., 1977; Smith et al., 1977; Mayer et al., 1978).

Cytochrome P-450 is a somewhat atypical "b" type cytochrome with a protoporphyrin IX prosthetic group, first described in insects by Ray (1965, 1967). The cytochrome P-450-dependent monooxygenase system plays an important physiological role in the biosynthesis of insect hormones (juvenile hormones, ecdysteroids), and pheromones, in feeding, in activation and detoxification of xenobiotics, in resistance and tolerance to toxicants (Hodgson, 1983).

Inhibition of steroidogenesis in insects (Lafont et al., 1986) through the action of cytochrome P-450 might be an interesting way to disrupt their development and reproduction.

Svoboda and Robbins (1967) found that two vertebrate hypocholesterolemic agents, triparanol and 22,25-diazacholesterol, inhibit growth and maturation in insects. This inhibitory effect is due to the reduction of desmosterol to cholesterol, as in mammalian steroidogenesis.

Because the final step in fungal and insect steroidogenesis is thought to be analogous Matolcsy et al. (1975) examined the effect of triarimol, an inhibitor of ergosterol biosynthesis in *Ustilago maydis* (Ragsdale and Sisler, 1973), on pupariation in *N. bullata*. Fenarimol has a similar activity (Tóth et al., 1977). The anti-ecdysterone activity of the triarimol derivatives may be related to their lipophilic character (Lukovits et al., 1978). In addition, triarimol and fenarimol inhibit the moulting in 1st instar larvae of *N. bullata* (Kulcsár et al., 1983; Fónagy et al., 1986). However, triarimol does not inhibit the *in vitro* activity of fucosterol-24(28)-epoxide cleavage enzyme in sitosterol dealkylation in *Spodoptera littoralis* larvae (Clarke et al., 1985).

Pirimidines are known to interact with the heme moiety of cytochrome P-450. This result from direct reversible ligand interaction between the sp2 or sp3 electrons of nitrogen atoms with the fifth or sixth ligand of the heme iron of cytochrome P-450, the same ligand responsible for oxygen binding (Wilkinson and Murray, 1984).

The aim of this study was to determine the effects of the fungicides fenarimol and nuarimol on different larval stages of *N. bullata*, and whether these fungicides interfere with the *in vivo* biosynthesis of 20-OH ecdysone.

Materials and Methods

1. Insects

Stock cultures of the ovoviviparous fleshfly (*N. bullata*) were reared in a constant temperature room maintained at 25±0.5°C and 50% r.h., with a daily photophase of 16 hr. Groups of about 200 adults were fed on a mixture of saccharose and milk powder (1:1, w/w). On the 14th day of adult life females were made to lay eggs by gently squeezing their abdomens. The larvae were reared under the same conditions on homogenized beef liver and 1% agar-agar (1:1, v/v). For our experiments we used newly hatched 1st, 24-hour-old (after hatching) 2nd, and 48-hour-old 3rd instar larvae.

2. Application

2.1. First, second and third instar larvae

In all cases the active ingredients of fenarimol or nuarimol (Elanco Co., USA) were dissolved in acetone and added to the diet to give a concentration of 0.2% (w/w). After the evaporation of the acetone, larvae were fed on the diet from a particular instar until they reached the wandering stage. Bran was placed under the diet in order to protect the larvae from drowning. All treatments were replicated 5 times (50-50 larvae per replicate). In 4 of the replicates the survival of the larvae was cheched daily and the

weights of 10 larvae per treatment were measured. In the fifth replicate a random sample of 5 larvae/day was taken and their cuticule mounted on a slide in lactic acid. Slides were examined under a light microscope to determine the state of moult. The remaining insects were reared to the gravid stage when 10 ovarioles containing live larvae and white (dormant) eggs were dissected. The data were analyzed using a one way ANOVA.

2.2. Wandering stage

Before treatment larvae were immobilized by placing them in ice-cold water for 2-5 minutes. The chemicals were injected in 3 μl of modified Locust-Ringer and 0.1% Tween 80, a non-ionic detergent. In each treatment (25 larvae, average weight of individuals: 120 mg) the chemicals were injected into the centre of their abdominal-cavity by means of a micro-injector and 26G1/2 record needle (Medicor, Hungary). After treatment the larvae were placed in Petri-dishes and kept at room temperature (20-24°C) in total darkness. Activity was assessed as described elsewhere (Darvas et al., 1987).

In the reversal study 20-OH ecdysone (Sigma Chem. Co., FRG) was simultaneously injected with the chemicals into wandering stage larvae, the dose chosen was the physiological level in the white prepupal stage (Wenthworth et al., 1981).

3. Compounds

The following inhibitors of cytochrome P-450 monooxygenases (Fig. 1) were used:
a) Fenarimol = alfa-(2-chlorophenyl)-alfa- (4-chlorophenyl) -5-pyrimidinemethanol,
b) Nuarimol = alfa-(2chlorophenyl)-alfa- (4-fluorophenyl)- 5-pyrimidinementhanol.

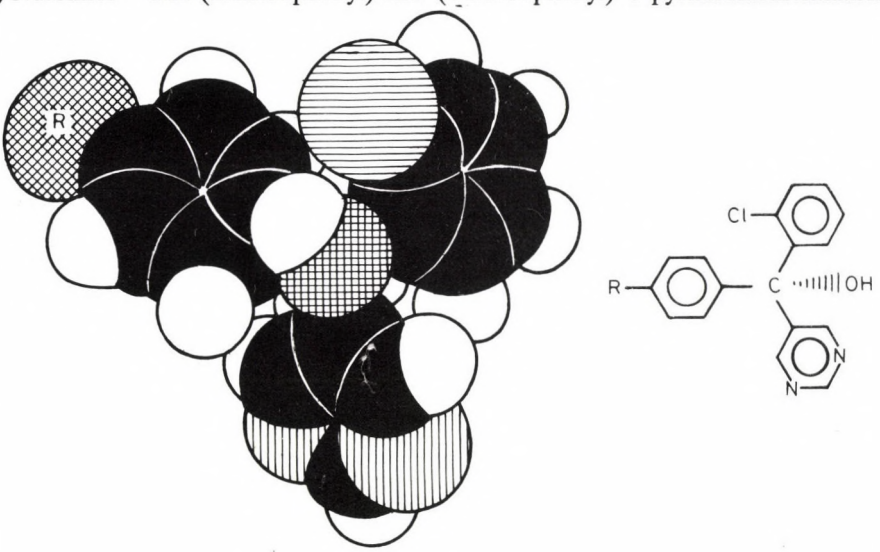

Fig. 1. Fenarimol (R = Cl) and nuarimol (R = F)

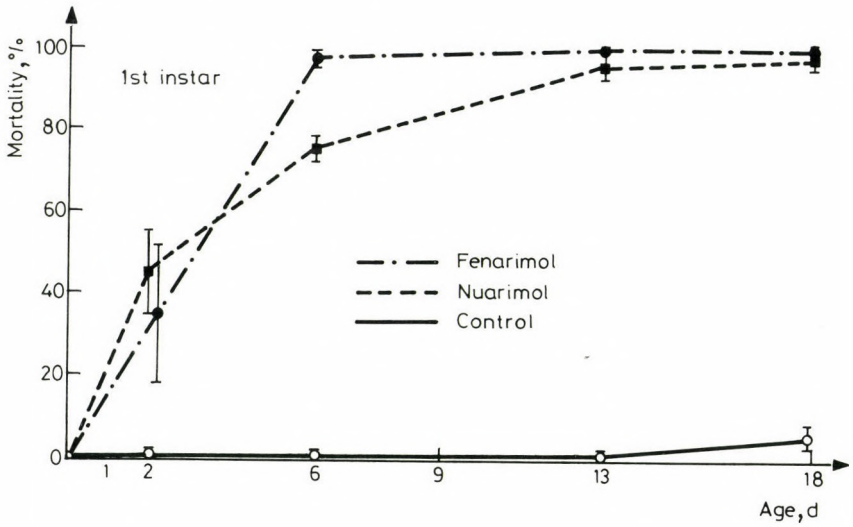

Fig. 2. Mortality of *N. bullata* reared from 1st instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements.
Solid line control; broken line nuarimol; semidotted line fenarimol.
SD 1% = 2nd day: 26.51. SD 0.1% = 6th day: 8.94; 13th day: 9.29; 18th day: 7.12

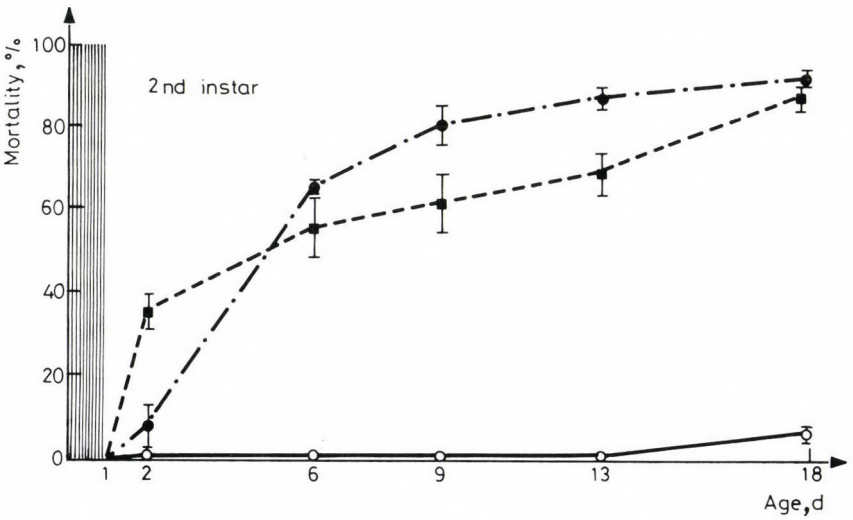

Fig. 3. Mortality of *N. bullata* reared from 2nd instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements.
Solid line control; broken line nuarimol; semidotted line fenarimol.
SD 0.1% = 2nd day: 23.71; 6th day: 28.03; 9th day: 33.69; 13th day: 24.27; 18th day: 13.66

Table 1

Length of the larval development in *N. bullata* reared on a diet containing 0.2% fenarimol or nuarimol

Treatment	Time of treatment (from)	Length of larval development	
		Average day ±S.E.[i]	% of control
Fenarimol	L1	10.58±0.76 b	–
	L2	9.87±1.67 b	163
	L3	9.43±1.37 b	152
Nuarimol	L1	9.43±1.37 b	145
	L2	8.72±0.44 ab	134
	L3	8.47±0.08 ab	130
Control	–	6.51±0.07 a	100
S.D. 0.1%		2.64	

[i] Values in a column followed by the same letter are not significantly different at the 0.1% level (one way ANOVA)

Results

First instar larvae

By the third day fenarimol and nuarimol had caused significant mortality (Fig. 2). In the nuarimol treatment the larvae that survived moulted on the third day, but none of the larvae treated with fenarimol moulted. In the latter case the larvae did not develop beyond the 1st instar although some survived for as long as 8 days (permanent 1st instar larvae). They developed prothoracic spiracles and rudimentary spiracular chambers after 2 days, but did not secrete a new cuticle.

On the 8th day the weight of fenarimol treated larva was hardly greater than their weight at hatching (Fig. 5).

The survival of nuarimol treated larvae was very low. The 1st moult ensued two days later than normal. The nuarimol treated larvae reached the white prepupal stage 3 days later than the controls (Table 1). The weight of the surviving insects at the white prepupal stage and the fecundity of the nuarimol treated insects were half that of the control (Fig. 5; Table 2).

Second instar larvae

Fenarimol and nuarimol killed fewer 2nd than 1st instar larvae (Fig. 3). The larvae that survived moulted only 2 (nuarimol) and 3 (fenarimol) days later than the controls. Some of the fenarimol treated larvae failed to moult their anterior spiracles.

The larvae treated with fenarimol or nuarimol reached the white prepupal stage 4 and 2 days later than the controls (Table 1), respectively. The weight of the treated larvae was half that of the controls (Fig. 6) and their fecundity was lower (Table 2). Some

Table 2

Survival and number of progeny produced by *N. bullata* reared as larvae on a diet containing 0.2% fenarimol or nuarimol

Treatments	Time of treatment (from)	Emerge rate (%)	Progeny (mean/female ±S.E.[i]) Larvae	White eggs
Fenarimol	L1	–	–	–
	L2	64	0.00[ii]	55.00[ii]
	L3	77	75.30±19.57 b	10.50±16.25
Nuarimol	L1	40	–	–
	L2	44	41.00±25.38 c[iii]	21.30±24.75
	L3	88	89.10±8.12 ab	3.10±3.67
Control	–	94	114.10±10.12 a	5.40±6.00
S.D. 0.1%			27.68	–
F values t=5%				2.86
exp.				2.83

(i) Values in a column followed by same letter are not significantly different at the 0.2% level (one way ANOVA)
(ii) Only 2 females
(iii) Examined one week later than the others

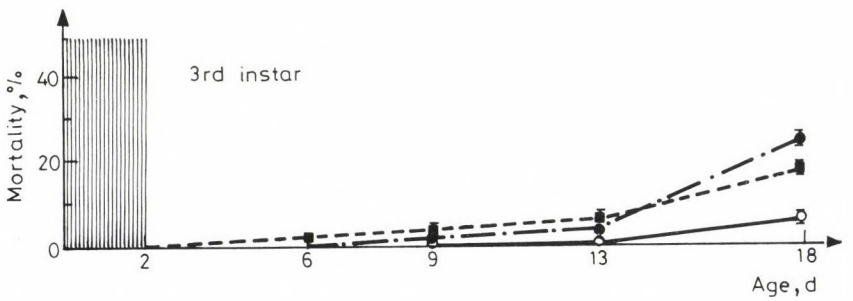

Fig. 4. Mortality of *N. bullata* reared from 3rd instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements. Solid line control; broken line nuarimol; semidotted line fenarimol. The F for this experiment was not significant ($P \leq 1$)

of the ovarioles of the treated females contained a lot of milk-white, normal sized eggs, but their development had stopped (Table 2).

Third instar larvae

Fenarimol and nuarimol did not cause significant mortality in this instar (Fig. 4). The fenarimol and nuarimol treated larvae reached the white prepupal stage 3 and 2 days later than the controls (Table 1). The weight of the treated larvae was lower (20-30%) than that of the controls (Fig. 7). The production of progeny by females treated with fenarimol in the 3rd instar was less than that of females treated with nuarimol and the controls (Table 2).

Wandering stage larvae

A 0.4 nmole dose of fenarimol slightly delayed the onset of pupariation in *N. bullata*. This effect was reversed by a 0.338 pmol dose of 20-OH ecdysone. A 0.4 nmole dose of nuarimol did not delay pupariation, but inhibited the pupariation stimulating effect of 20-OH ecdysone (Table 3).

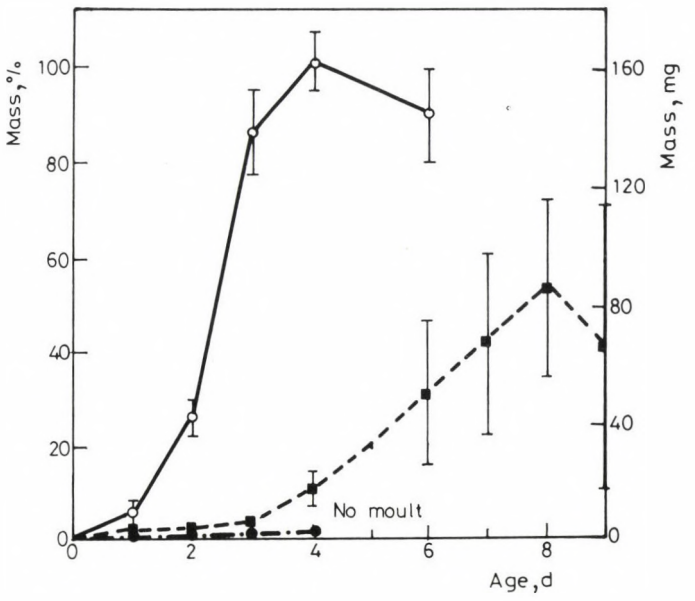

Fig. 5. Mass (mg) of *N. bullata* reared from 1st instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements. Solid line control; broken line nuarimol; semidotted line fenarimol. SD 0.1% = 2nd day: 6.34; 3rd day: 14.73; 4th day: 11.22

Table 3

The action of 20-OH ecdysone in reversing the effect of fenarimol or nuarimol in *N. bullata*

Treatment[i]	Mortality %[ii]		Retardation
	L3-Pupa	L3-Adult	ratio
Fenarimol 0.4 nmole	20	76	1.28
Nuarimol 0.4 nmole	36	48	1.08
Fenarimol 0.4 nmole +20-OH ecdysone 0.338 pmole	16	52	0.87
Nuarimol 0.4 nmole +20-OH ecdysone 0.338 pmole	28	64	0.98
20-OH ecdysone 0.338 pmole	20	48	0.87
Locust-Ringer+Tween 80	8	12	1.00
Untreated control	8	8	0.97

[i] Treatment with 3 μl modified Locust-Ringer + 0.1% Tween 80
[ii] After treat 25 wandering stage larvae

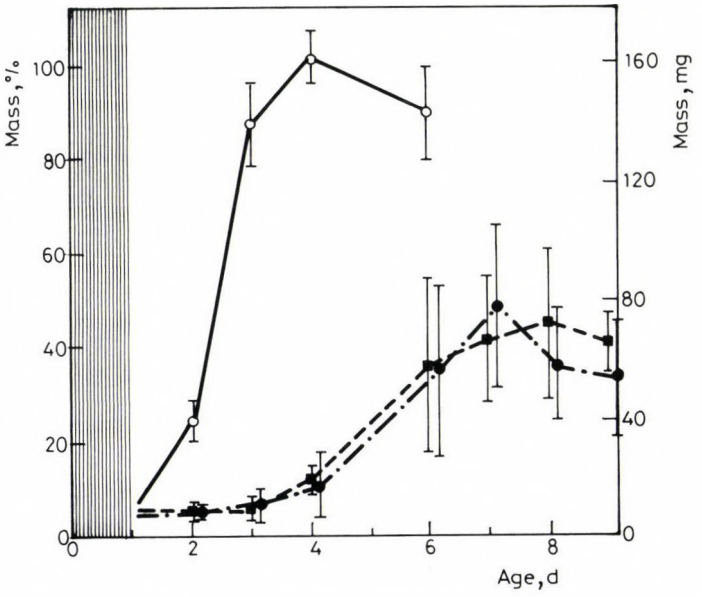

Fig. 6. Mass (mg) of *N. bullata* reared from 2nd instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements. Solid line control; broken line nuarimol; semidotted line fenarimol. SD 0.1% = 2nd day: 10.71; 3rd day: 14.95; 4th day: 15.58; 6th day: 43.36

Discussion

Distinct peaks in ecdysteroid titres occur during the larval development of *N. bullata*, one in the middle of the 1st and another in the 2nd larval stage (Roberts et al., 1980) with smaller peaks occurring a few hours prior to each ecdysis (Wenthworth et al., 1981). The first major ecdysteroid peak occurs within 14 hr of birth (Roberts et al., 1982). For up to 16 hr after birth, the first tracheal metamere remains undifferentiated. After 16 hr apolyzis occurs, and by 18 hr the tracheal epithelium is fully differentiated into the spiracle, spiracular chamber and trachea of the 2nd instar. Cuticule secretion begins after 20 hr. The first minor ecdysteroid release occurs before 22 hr, and sclerotization is noted between 24 hr and 28 hr at the time of the first ecdysis (Roberts, 1981). Pre-ecdysial tanning of the cuticule has been noted in many flies. For exemple, the larval mandibles (Smith, 1933; Roberts, 1976), and posterior spiracles (Roberts, 1981), are fully sclerotized by the 20th hour, some time before the first ecdysis occurs.

When larvae were fed the fenarimol treated diet from birth, 40% died by the 2nd day and before they had developed prothoracic spiracles. After the 2nd day the surviving larvae (Fig. 2) had unsclerotized anterior spiracles, but lacked new posterior spiracles and mandibles. Nevertheless, the pre-ecdysial tanning of the anterior spiracles did not occur in either case. In addition, some of the fenarimol treated 2nd instar larvae did not moult their anterior spiracles, although they had normal posterior spiracles and mandibles.

Ryerse and Locke (1978) have demonstrated that in *Calpodes ethlius* the apolysis of the tracheae, both *in vivo* and *in vitro*, is a result of ecdysteroid action. Our experiments provide two possible explanations:

(I) – Very low levels of 20-OH ecdysone induce the apolysis and the differentiation of the tracheal epithelium of the anterior spiracles, which is itself insufficient (De Loof, 1986) for secretion of the cuticule of the anterior spiracles, and also for the apolysis and epithelium differentiation of posterior spiracles and mandible formation.

(II) – An unknown control factor is responsible for the apolysis and differentiation of the tracheal epithelium of the anterior spiracles. This control factor is independent of cytochrome P-450-dependent monooxygenases. Thus fenarimol cannot inhibit the factor which organizes the apolysis and differentiation of the tracheal epithelium, but can inhibit the 20-OH ecdysone-dependent cuticule secretion (which requires substantial synthesis of cuticular protein, and of chitin and lipid components by epithelial cells) and pre-ecdysial sclerotization.

Our hypothesis is partly supported by the results of Matolcsy et al. (1986), who noted that alkylene-bis-isothiocyanates caused irreversible damage to the epidermis of the spiracles so that the spiracles were malformed or missing at the following moult in *Manduca sexta*. Their histological work revealed the lack of a junction between the trachea and body wall in the treated insect, suggesting inhibition of differentiation of the treacheal epithelium. Also crochets were missing from one or several prolegs of many of the treated larvae. The factor that organizes the apolyzis and differentiation of the tracheal epithelium of the new spiracles in *M. sexta* may thus be inhibited by alkylene-bis-isothiocyanates.

Roberts et al. (1982) suggested that the pre-ecdysial tanning of hooks and spiracles is mediated by an agent other than bursicon. In the *Brachycera* an ecdysteroid mediated pre-ecdysial tanning is associated with larva to larva moults similar to the puparial tanning that occurs prior to the larva to pupa transformation. Our results agree with these conclusions in that the pre-ecdysial tanning in *N. bullata* is inhibited by the cytochrome P-450 inhibitor, fenarimol.

Fenarimol showed a stronger physiological activity than nuarimol. None of the larvae that were fed the 0.2% fenarimol treated diet from birth moulted. Thus we suppose fenarimol inhibited the cytochrome P-450-mediated monooxygenase system, that controls the cholesterol – 20-OH ecdysone biosynthetic pathway (probably: one or more of the C-2, C-14, C-25, C-22, C-20, C-26 monooxygenases). Presumably fenarimol inhibits the ecdysone 20-monooxygenase, which is a well known microsomal and mitochondrial cytochrome P-450-dependent enzyme that possesses properties similar to vertebrate steroid hydroxylases (Smith and Mitchell, 1986), although the inhibitory effect of fenarimol on cytochrome P-450 forms may well be broader.

The 1st moult was a critical period in the life of the test insects. The 2nd instar (2nd moult) was less sensitive than the 1st, while the 3rd instar was hardly affected by the fenarimol and nuarimol treatments (Figs 2, 3 and 4). There are stage-dependent

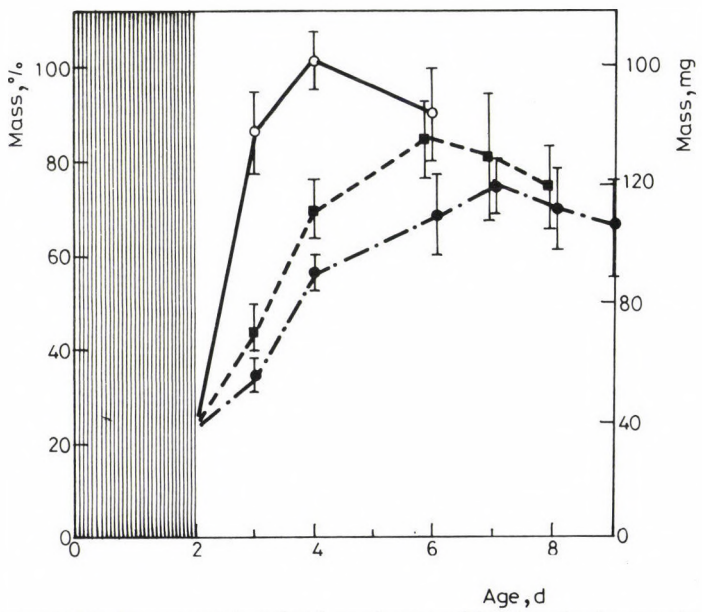

Fig. 7. Mass (mg) of *N. bullata* reared from 3rd instar larva on 0.2% fenarimol or nuarimol treated diet. Each point represents the mean ±S.E. of measurements. Solid line control; broken line nuarimol; semidotted line fenarimol. SD 0.1% = 3rd day: 17.37; 4th day: 15.67; 6th day: 29.97

differences in the cytochrome P-450 sets (Wilkinson and Brattsten, 1972) or the systems belonging to them. By inhibiting ecdysteroidogenesis in insects the next important cytochrome P-450-dependent physiological processes in larval development may be disturbed:

(I) – The final epoxidation step (methylfarnesoate epoxidase) in juvenile hormone biosynthesis (Pratt and Tobe, 1974; Hammock, 1975; Yu and Terriere, 1978). The juvenile hormone plays an important regulatory role only in larva to larva moults, and juvenile hormone synthetic activity is lower in the last larval stage than it is in the younger stages.

(II) – Adaptation to diet (including secondary compounds and xenobiotics). In the 1st instr the monooxygenase activity appears to below (Gould, unpubl. data, cyt. Hodgson, 1983). The cytochrome P-450-dependent monooxygenase system is induced by compounds in an insect's diet (Yu et al., 1979), which results in an increase in its xenobiotic metabolizing activity.

In vivo inhibition of steroidogenesis in phytophagous insects, Clarke et al. (1985) found that feeding last instar larvae of *S. littoralis* with a diet containing triadimefon (cytochrome P-450 inhibitor) caused larval mortality, delayed pupation, and reduced growth. Our results were similar for the 3rd instar larva, but the differences were greater in the 1st and 2nd instar (Figs 5, 6 and 7; Table 1).

Last but not least, fenarimol delayed pupariation when the wandering stage larvae were treated. The retardation ratio (Table 3) of the fenarimol in Tween 80 was lower that given by Tóth et al. (1977) for this compound in DMSO. Darvas (1989) showed that the DMSO may improve the penetrability of fenarimol *in vivo*. The effect of fenarimol was reversed by 20-OH ecdysone (Table 3), while nuarimol only inhibited the stimulatory effect on pupariation of 20-OH ecdysone. These results suggest that some of the effects may have been caused by changes in ecdysterone biosynthesis and metabolism.

Further studies in this area will be reported subsequently.

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Use of Copper Sulphate and Calcium Oxide as Repellents on Adults of *Leptinotarsa decemlineata* Say (Coleoptera, Chrysomelidae) and their Effects on *Solanum tuberosum* L. Production

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This paper reports results of an experiment conducted in 1986 in a potato-growing zone of central Italy. Trials were carried out to evaluate the repelling action of copper sulphate and calcium oxide, compared to deltamethrin in controlling adults of *L. decemlineata*.

Statistical analysis showed that copper sulphate gave an adequate repellent action against adults of the Colorado beetle. The first treatment was done before the appearance of the adults, when plants were 8-10 cm high. Two other treatments, 15 days apart, were sufficient to protect the crop. From the production point of view, moreover, copper sulphate and deltamethrin were equivalent, but, while the yield of the former was more heterogeneous, that of the latter was greater in the range 70 to 250 grams, which is more important commercially.

Results relative to calcium oxide, on the other hand, were disappointing. Further research is required to verify the results on a larger scale and to encourage farmers to produce healthy tubers without residues, suitable to be sold as a "biological" product.

Chemical treatment has been the only valid method of controlling the Colorado potato-beetle until now. However, indiscriminant use of insecticides has given rise to the phenomenon of phytophagous resistance (Simonsberger, 1969; Atak and Atak, 1977; Martel and Daneau, 1979; Harris and Svec, 1981). Furthermore, the ecological, toxicological and economical situation has been aggravated.

Up to the present time, satisfactory results have not been obtained using biological control methods (parasites, predators, nematodes and entomopathogenic fungi). For these reasons, a research program was initiated for the purpose of developing methods to control Chrysomelidae using chemicals with active ingredients having low residual effect and limited environmental interference. Therefore, copper sulphate and calcium oxide were tried to test their repelling capacity on populations of *L. decemlineata*.

The effectiveness of these compounds was compared to deltamethrin, a well-known chemical used to control Chrysomelidae (Malinowski and Kroczynski, 1983; Volovik and Glez, 1984; Kurilov, 1984).

Materials and Methods

Studies were carried out in 1986 at Sugano near Orvieto, Province of Terni (Umbria, central Italy), on a parcel of land cropped to *Solanum tuberosum* cv Manna. The 2 ha field was divided into 4 homogeneous plots (for exposure, soil conditions and moisture capacity) of 0.5 ha, each; three were used for treatments (deltamethrin 1.4 gr.

a. i. per hl of water; $CuSO_4$, 1.5% and a water solution of CaO, 0.8%) and the fourth was used as control.

Copper sulphate and calcium oxide were applied three times during the crop cycle. The first treatment was applied at the time the aduslts first appeared when the plants were, on the average, about 8–10 cm high (May 13). The successive two treatments were applied 15 days apart (May 28, June 13). Regarding deltamethrin two treatments were applied in order to obtain a completely insect-free crop. Treatments were carried out on May 26 and June 16 when average adult populations were 1.3 and 0.8 adults per plant, respectively.

In each plot, two parts, A and B, sufficiently far from one another, were chosen and 60 plants were sampled in each. Every three days, on sampled plants in part A, the number of adults and the number of egg masses were counted and removed to measure the repelling effect of the tested products. Sampled plants in part B were used to measure the number and weight of tubers at harvest time.

Analysis of data was carried out using statistical methods described in the next sections.

Results

Repellence

During this period, weather conditions were particularly favorable for both the crop and the insect due to high rainfall during the summer months (18 mm in May; 121 mm in June; 62 mm in July) as well as high light intensity.

Figures 1 and 2 report the trends of the numbers of adults and egg masses taken from sample plants in parts A of the plots treated with CaO, $CuSO_4$ and the control. With respect to the control, CaO and even more so, $CuSO_4$ seemed to provide a repulsive effect on the insect and on the deposition of eggs. This effect was also observed on samplings after June 18, where, because of the second generation of the insect, infestation reached decisively high levels. Nevertheless, the first generation causes the greatest damage to production and is, therefore, in the focus of attention. In the plot treated with deltamethrin the numbers of adults and egg masses in the sampled plants were never greater than two.

Yield

Yield analysis was carried out in order to better evaluate treatments. Data included the number and weight of tubers from the 60 sample plants in part B of each plot. Treatments are denoted as follows: C = control CO = calcium oxide, CS = copper sulphate and DE = deltamethrin.

Descriptive Analysis. Table 1 reports the mean and, between brackets, the standard deviation of the number (N) and the weight (P) of tubers produced by each plant, and the yield (PT) per plant for eacht treatment. It can be seen that calcium oxide did not

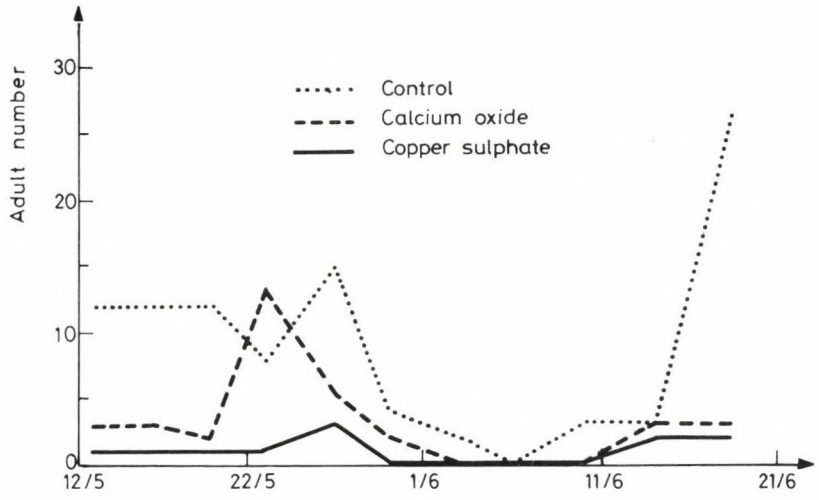

Fig. 1. Trend of the number of adults

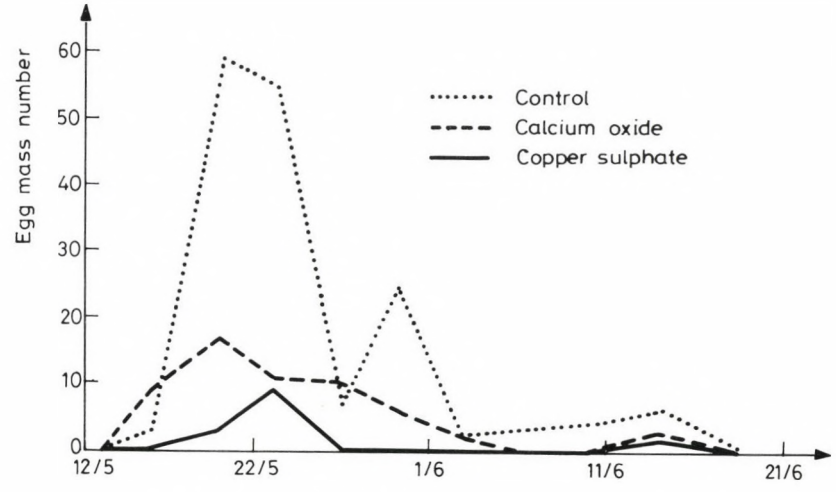

Fig. 2. Trend of the number of egg masses

Table 1

Mean and standard deviation of tubers per plant (N), of tuber weight (P) and yield per plant (PT)

Treatment	N		P		PT	
C	4.59	(1.72)	89.56	(65.23)	462.0	(206.6)
CO	5.78	(1.98)	99.79	(76.92)	577.0	(212.8)
CS	6.37	(1.46)	159.50	(108.60)	1015.0	(299.6)
DE	6.65	(1.59)	142.40	(90.10)	946.0	(299.6)

give good results. On the other hand, copper sulphate and deltamethrin were more effective with the former being slightly better. In fact, plants treated with CS produced, on the average, 6.37 tubers with an average weight of 159.5 gr for a total of 1015 gr per plant. The corresponding values for plants treated with DE were 6.65, 142.4 and 946.1

However, this first analysis can not be considered sufficient since the quality of the yield is judged on the basis of other factors, such as univormity, weight class and number of tubers. For this reason, it is appropriate to introduce the yield function $N(p)$ – nonnegative and monotone nonincreasing – which associates to every value of weight p, the number of tubers with weight greater than p produced by a single plant. Given $N(p)$, we define the yield density function $n(p)$ to be

$$n(p) = \lim_{h \to 0} \frac{N(p) - N(p+h)}{h} = \frac{dN(p)}{dp}, \qquad p > 0. \tag{1}$$

This function can be interpreted as the number of tubers produced within an interval of one unit. Function $n(p)$ is nonnegative and, given an interval (a,b), the integrals

$$\int_a^b n(p)\, dp$$

and

$$\int_a^b pn(p)\, pd, \tag{2}$$

give, respectively, the number and the total weight of tubers having a weight included in the interval (a,b) produced by a single plant. The function $n(p)$ is, therefore, adequate to describe the yield of the plants submitted to the same treatment since it permits the evaluation of the range of weight within which the tubers are concentrated.

On the basis of available data, it is possible to construct a graphic representation of the empirical density function using the histogram technique. For this purpose, 44 weight classes were established and the tubers produced by the 60 plants, submitted to the same treatment, were classified according to the weight class to which they belonged. To smooth the histograms, yield frequency was equalized using a three-term moving average. In the graph, the histograms were substituted with a line connecting the points

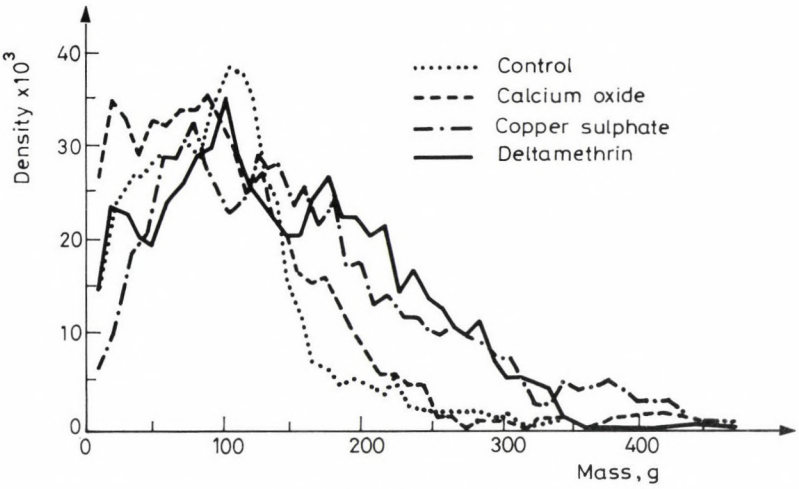

Fig. 3. Empirical density curves

whose coordinates are the central values of the weight classes and the yield density values in the classes. This was done in order to see the trend of the density function better and to draw the four curves relative to the different treatments in one graph.

In Figure 3, the density curves of C and CO appear to be the same. The curves of CS and DE are better than the others since they are higher when p is greater than 150. In particular, the curve relative to CS is lower than that of DE in the range (180, 250) and higher for p values greater than 350, signifying lesser homogeneity of the tubers.

A Theoretical Model. Data allow a theoretical yield curve to be modelled which is only slightly sensitive to random effects. Empirical values of the function $N(p)$ were calculated for each plant at the low extrems, pi, of the specified weight classes. For each p_i value, the mean $\bar{N}(p_i)$ and the variance $V[N(p_i)]$ were then calculated for the 60 values available for each treatment.

Using the methodology of the generalized linear models (McCullagh and Nelder, 1983, Chap. 2), the functional link between the variance and the mean of $N(p)$ was studied. The best description of data was given by the following relationship

$$V[N(p)] = \sigma^2 \log[1 + \bar{N}(p)], \tag{3}$$

were σ^2 is a scale factor. Adopting the variance function (3), the method of maximum quasi-likelihood (McCullagh and Nelder, 1983, Chap. 8) was used to fit the following theoretical yield function

$$N(p) = \exp[\beta_0 + \beta_1 p + \beta_2 p^2 + \beta_3 p^3]. \tag{4}$$

Table 2

Estimated parameters of yield functions

Treatment	β_0	$\beta_1 \times 10^2$	$\beta_2 \times 10^4$	$\beta_3 \times 10^6$
C	1.5760	–0.1094	–0.9316	0.1286
CO	1.7508	–0.4672	–0.4911	0.0704
CS	1.8782	–0.2429	–0.2329	0.0200
DE	1.8905	–0.2584	–0.1624	–0.0266

Table 2 reports the estimated values of the β_0, β_1, β_2 and β_3, parameters for each treatment. By substituting these values in expression (4), the estimated theoretical yield function can be obtained.

The estimated yield density function can be obtained by the derivative with respect to the independent variable, p , of the estimated yield function, that is:

$$n(p) = -\frac{dN(p)}{dp} = -(\beta_1 + 2\beta_2 p + 3\beta_3 p^2) N(p) \tag{5}$$

Figure 4 reports the theoretical density curves for the four treatments, while Figure 5 reports the empirical and theoretical density curves for CS and DE in order to show the goodness of fit. The theoretical density curves in Fig. 4 confirm the suppositions given by the empirical curves. In particular, plants treated with DE, with respect to those treated with CS, gave higher yields in the range (100, 250) and lower yields for p values greater than 300.

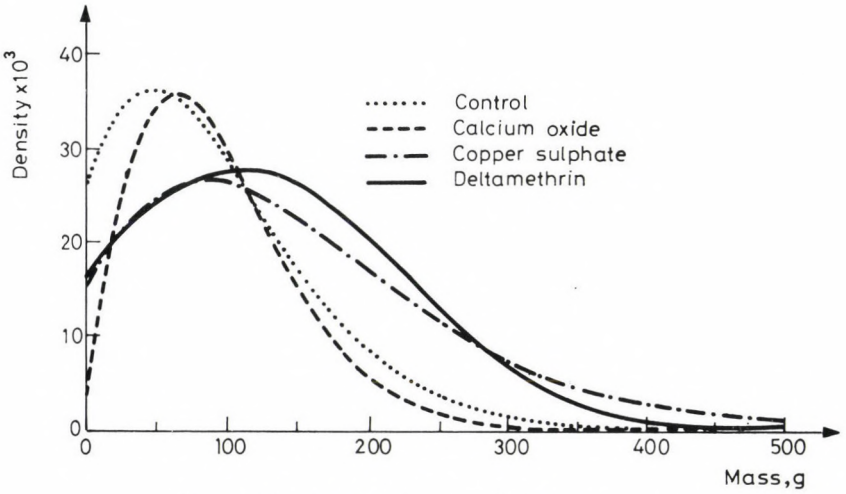

Fig. 4. Estimated theoretical density functions

Table 3

Predicated (T) and observed (E) yield in some ranges

	0- 70		70- 250		>250	
Treatment	T	E	T	E	T	E
C	77	69	358	368	23	25
CO	86	80	407	421	70	76
CS	59	62	568	560	377	393
DE	58	62	638	643	249	244

The reader can apply the procedure to any range

Commercially, yield in the range (70, 250) is the most profitable. By means of the theoretical density function (5), substituting the values from Table 2 for the parameters, it is easy to calculate with integral (2), evaluated with numerical methods, the total weight of the tubers produced in a given range by a plant subjected to different treatments. Theoretical (T) and empirical (E) values of the total yield in the ranges (0, 70), (70, 250) and (>250)[1] are reported in Table 3. In particular, referring to the theoretical values, it can be observed that DE gave a 12% greater yield with respect to that of CS in the range (70, 250), but 34% less in the range (>250).

Finally, the statistical significance of the difference between the 4 estimated theoretical density curves should be tested. The problem is somewhat complex because of the dependent structure of data. Here our approach will consist of statistical tests on yield

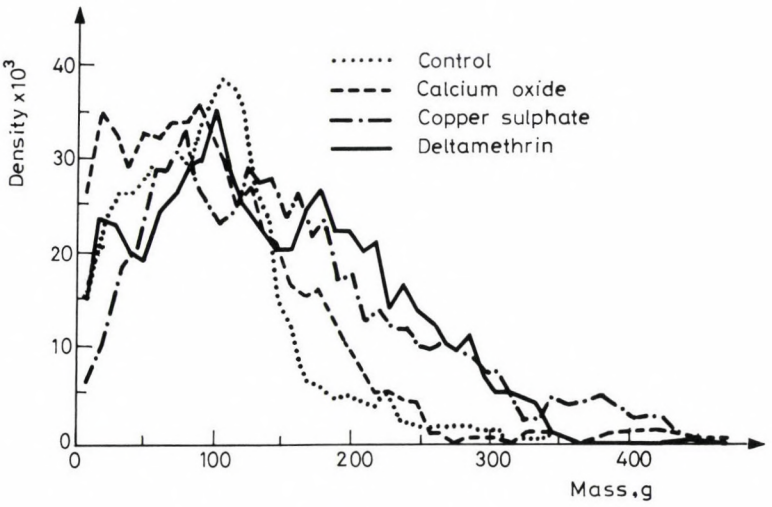

Fig. 5. Empirical and theoretical density curves

Table 4

Analysis of variance for yield and significant contrasts

Range	F-Test	Significance	Contrast (α=5%)
0- 40	2.79	5%	CO–CS
40- 80	1.26	n.s.	–
80- 120	2.45	n.s.	–
120- 160	1.00	n.s.	–
160- 200	9.35	1%	C–CS, C–DE
200- 250	16.75	1%	C–CS, C–DE, CO–CS, CO-DE
250- 300	12.23	1%	C–CS, C–DE, CO–CS, CO–DE
300- 350	6.33	1%	C–CS, C–DE, CO–CS
>350	11.07	1%	C–CS, CO–CS, CS–DE

differences in predetermined classes. This means to control the level of significance of the statistical test in single classes but not in all the classes simultaneously.

Table 4 reports the values of an asymptotic Fisher's F test (Scheffé, 1959) from the one-way analysis of variance (treatments), assuming as response variable the number of tubers produced by each plant in the predetermined classes. When the F test resulted significant, the treatments that had contributed to such significance were determined using the Scheffé test (1953). The last column of the table reports the significant contrasts (at the 5% level) between treatments compared two by two.

The following conclusions can be drawn: Calcium oxide did not differ significantly from the control in any class. On the contrary, yields relative to CS and DE were significantly different from C for p values greater than 160. Regarding the comparison between CS and DE, there was a significant yield difference for p values greater than 350. The same F tests were then calculated assuming as response variable the plant yield in the predetermined classes, but identical results were obtained.

In summary, calcium oxide did not differ significantly from the control. The opposite was true for copper sulphate and deltamethrin which, from a production point of view, were similar. However, the yield using copper sulphate was more heterogeneous, while deltamethrin gave a higher yield in the weight range more important commercially.

On the basis of these encouraging results, it is projected to conduct an experiment on a larger scale and in different locations to verify if copper sulphate could effectively be used as an alternative to common insecticides. Even if the plants treated with copper sulphate gave a product of lesser quality, this could be compensated for by selling them as "biological".

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Toxic and Morphogenetic Activity of 24 Novel 2,2-Dimethylchromene Derivatives on Larvae of *Pieris brassicae* and *Oncopeltus fasciatus*

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In *P. brassicae* no morphogenetic action but high toxicity was observed with 6-methoxy-7-prop-2-ynyloxy- and 6,7-diprop-2-ynyloxy-2,2-dimethylchromene, both showing significantly lower ED_{50} values than Precocene 3, while seven more new derivatives proved to be more toxic than Precocene 2. Considerable morphogenetic activity was obtained in *O. fasciatus* with six different 2,2,5-trimethylchromenes bearing various alkoxy groups at C6 and C7 positions.

Ageratochromenes – also termed as 2,2-dimethylchromenes – are naturally occurring compounds, isolated from *Ageratinae* (Eupatorinae) species (Alertsen, 1961), were first (re)discovered by Bowers and coworkers (1976). They have been found to possess allatotoxix activity in certain Hemipteran and Orthopteran species and due to their ability to induce precocious metamorphosis (Bowers, 1976; Bowers et al., 1976; Bowers and Martinez Pardo, 1977), resulting in sterile adultiforms, the term "precocenes" (P) has been adopted.

In "sensitive" Exopterygota species, studies focused on structure-activity relationship and metabolism of P, have indicated that these compounds are most likely biotransformed, within the *corpora allata* (CA), into highly reactive 3,4 epoxides by microsomal cytochrome P-450-dependent monooxygenases (Pratt et al., 1980; Pratt, 1983). The reactive derivatives (epoxides) alkylate nucleophilic substrates causing rapid irreversible degradation of parenchimal cells of the juvenile hormone producing CA (Unnithan et al., 1977). A number of structure optimizing studies underline that 3,4 double bond and the 2,2-dimethyl groups are indispensable, while C6 and C7 substituents may also bear crucial importance in causing the allatocidal effect (Bowers 1976; Chenevert et al., 1980; Brooks et al., 1985 Camps, 1985). On the contrary, some other quantitative structure-activity relationship (QSAR) studies do not support the epoxide formation theory (Dinya et al., 1986). Alternative models, such as possible conversion into reactive quinone-methids (Bowers et al., 1982), or formation of conjugates (Bergot et al., 1980) have also been suggested. It is generally accepted, that P (as suicide substrates) become active in CA, therefore the term "pro-allatocidines" seems to be a more appropriate expression for them (Pratt, 1983).

Besides morphogenetic (precocious) effects, acute toxicity of P is also known in Exopterygotes (Friedman-Cohen et al., 1984; Brooks et al., 1985; Darvas et al., 1985) and

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in "insensitive" Endopterygotes (Cupp et al., 1977; Unnithan, 1983; Wisdom et al., 1983). The possible explanation of the mechanism of acute, nonspecific toxicity, is that similar monooxygenase system is present in fat body, gut tissue cell microsomes and the CA cells, the formation of reactive epoxides of different P is the most likely, although short-lived, and are the most probable contributors to nonspecific high-dose mortality (Staal 1986). In this respect, the difference between the CA and other tissues may be in the presence or absence of epoxide hydrases (which are held to be responsible for detoxifying intermediate reactive epoxides into excretible polar nontoxic metabolites) (Pratt, 1983). According to Pratt (1983), not the presence of monooxygenases, but the absence of hydrases explain specific allatocidal effects, i.e. P "sensitive". Consequently, the observed allatocidal effects may be sublethal manifestations of general high dose toxic effects (Brooks et al., 1985). It is likely, that in Endopterygotes, the threshold level for precocious metamorphosis induction is very close to toxic concentrations (Kikuchi, 1982; Bowers 1985).

In the framework of an extended study (Kiss et al., 1988; Darvas et al., 1989; Fónagy et al., 1991) the aim of our present work was to evaluate the effects of various substitutents and their combinations on 24 chromene derivatives with respect to toxicity and precocene activity as measured on *Pieris brassicae* (Lep., Pieridae) and *Oncopeltus fasciatus* (Hem., Lygaeidae) larvae. Besides confirming some previous observations, the modifying role of C5, C6 and C8 substituents on the biological activity of the C7 substituted chromenes was further evaluated.

Materials and Methods

The original procedure for the synthesis of the new alkoxychromenes was based on the reduction of the appropriate alkoxy-4-chromanone to the corresponding alcohol, the dehydration of which was expected to yield the alkoxychromenes.

For the preparation of 7-O-substituted derivatives we used the previously reported method (Timár et al., 1988 a) and for the 7-O-substituted 5-methyl derivatives we refer to the article of Timár and coworkers (1988 b). The synthesis of 6,7-di- and 5,6,7-trisubstituted analogues (Timár and Jászberényi, 1988) 6,7,8-trisubstituted, 5,7,8 trisubstituted and halogenated chromenes (Sebők et al., 1988) was based on our recent works.

A general formula of 2,2-dimethylchromenes is given in Fig. 1, while different substitutents are listed on Table 1 and the analogues are cited by their serial numbers between square brackets in the text.

Caterpillars of *P. brassicae* were reared on cabbage (savoy) plants at 25°C (LD 18:6). Freshly moulted 4th instar larvae were topically treated with the acetone solution of substances. Duplicated groups of 20 larvae were treated with dosages ranging from 0.02 to 200 μg/sp). Toxicity was expressed as the percentage of the treated larvae which died during the first five days. LD_{50} values were calculated by linear regression of log dose-probit mortality data, and expressed in μg/insect.

Larvae of *O. fasciatus* were reared on sunflower seeds at 25°C (LD 18:6). Duplicated groups of 20 newly moulted second instar larvae were assessed for morphogenetic

Table 1

Number	Substituens				QSAR	Toxic and morphogenetic activity					
	R	R1	R2	R3		*P. brassicae*		*O. fasciatus*			
						LD50 (μg/spec.)	Larval mort.	Normal ad.		Prec. ad.(%)[4]	
1[1]	H	MeO	MeO	H	–	110.9	42.5	57.5	0.0		
2[2](FI-121)	H	H	prop-2-ynylO	H	–	5.9	85.0	15.0	0.0		
3	H	H	CH2CF3O	H	+	129.8	47.5	52.5	0.0		
4	H	H	cycloheptylO	H	+	88.0	45.0	55.0	0.0		
5 (P2)	H	MeO	MeO	H	–	39.5	45.0	0.0	22.5	27.5	5.0
6 (P3)	H	MeO	EtO	H	–	24.5	20.0	0.0	60.0	5.0	15.0
7	H	MeO	prop-2-ynylO	H	+	6.1	60.0	40.0	0.0		
8	H	prop-2-ynylO	prop-2-ynylO	H	+	6.9	55.0	45.0	0.0		
9	H	tBu	cyclopentylO	H	–	> 200.0	60.0	40.0	0.0		
10	H	H	cyclopentylO	MeO	+	29.1	90.0	10.0	0.0		
11	Me	H	cycloheptylO	H	+	14.3	37.5	62.5	0.0		
12	Me	MeO	MeO	H	+	96.5	30.0	0.0	25.0	45.0	0.2
13	Me	MeO	EtO	H	+	88.3	5.0	0.0	0.0	95.0	0.0
14	Me	MeO	PrO	H	+	32.7	15.0	5.0	0.0	80.0	0.0
15	Me	MeO	prop-2-ynylO	H	+	29.9	30.0	50.0	0.0	0.0	20.0
16	Me	MeO	nsBuO	H	+	20.0	47.5	15.0	0.0	0.0	37.5
17	Me	MeO	cyclopentylO	H	+	22.4	52.5	47.5	0.0		
18	Me	EtO	MeO	H	+	71.0	52.5	10.0	0.0	37.5	
19	H	tBu	MeO	MeO	+	29.1	47.5	52.5	0.0		
20	H	tBu	prop-2-ynylO	prop-2-ynylO	+	49.2	60.0	40.0	0.0		
21	H	tBu	cyclopentylO	cyclopentylO	+	> 200.0	40.0	60.0	0.0		
22	H	MeO	MeO	Br	+	> 200.0	47.5	52.5	0.0		
23	H	Br	MeO	MeO	+	59.5	40.0	60.0	0.0		
24	H	Cl	prop-2-ynylO	prop-2-ynylO	+	48.2	72.5	27.5	0.0		
25	MeO	Br	MeO	Br	+	55.7	27.5	72.5	0.0		
26	Me	H	MeO	MeO	+	52.5	35.0	65.0	0.0		
27[3]	Me	H	MeO	MeO	–	> 200.0	100.0	0.0	0.0		
Control							15.0	85.0	0.0		

[1] At position 1 the O atom is replaced by S atom
[2] At position 3 and 4 the H is replaced by Cl atoms
[3] At position 4 the H is replaced by Cl atom
[4] The occurrence of precocious metamorphosis is indicated for different larval stages (i.e. third, fourth and fifth instars)

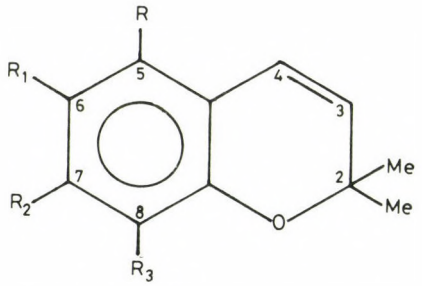

Fig. 1. 2,2-Dimethylchromenes

activity by contact treatment (3 μg/cm^2 in Petri dishes). The frequency of adultoid appearance (in third, fourth and fifth instars, respectively) and mortality was recorded daily till imaginal moult and were expresses as a percentage of treated larvae. The toxicity results obtained in *O. fasciatus* are only indicative and should be regarded as such.

QSAR study was made to relate the chemical descriptors of new derivatives to biological activity (LD_{50}). The following chemical parameters were considered for each (R-R_3) of the substituents:

π, (Hansch-Fujita's substituent constant characterizing hydrophobicity);

MR, (molar refractivity of the substituent);

F & R, (Swan-Lupton's electronic parameters, characterizing the field effect and resonance effects, respectively);

Es, (Taft's constant characterizing the steric effects of the substituent);

B1 & B4, (STERIMOL steric parameters representing the smallest and largest width of the substituent perpendicular to the bond connecting its α-atom to the rest of the compound);

I, (indicator variable 1 for proton acceptor capacity of the substituent).

Compounds involved in the analysis are indicated with + in the Table 1.

Results and Discussion

Our results are summarized in the Table 1.

Toxic effects

1. Monosubstitution at C7: Among the C7 substituted 2,2 dimethylchromenes – even though larger groups were introduced ([3], [4]) – low or medium toxicity was recorded in *P. brassicae*. When O was replaced by S in the ring [1] no increased action was observed, although C7 was substituted by MeO in this case, but it did not, however,

reach the effectiveness of P1 (Darvas, 1989), which is to be considered as its parent compound. Mortality in *O. fasciatus* was around 45%.

Among the C7 monosubstituted compounds the 3,4-dichlorochromene derivate [2] may be regarded as a reference toxic P derivative (also known as FI-121 see Darvas et al., 1989). It is noteworthy that an intruduction of a single 2-propynylosy (prop-2-ynylO) at C7 significantly increases toxicity, which by itself may also be responsible of masking any possible activity modifying effects of other groups as it was previously suggested (Darvas et al., 1989). The prop-2-ynylO functional group of several compounds is a known inhibitor of cytochrome P-450, exerting its action by alkylating the heme-containing active center (Ortiz de Montellano and Correia, 1983; Wilkinson and Murray, 1984). On the contrary, the introduction of Cl, however, may also influence the electric and steric properties of the 3,4 bond which effect should not be excluded either. Very high toxicity was equally obtained in *O. fasciatus* larvae, where 82,5% of them died within 48 hours. These results are in agreement with previous observations, stating that compounds bearing prop-2-ynylO group at C7 exibit consistently high toxicity on this species (Brooks, 1985).

2. Disubstitution at C5 and C7, C6 and C7 or at C7 and C8: Compound [11] may easily be compared to its monosubstituted pair [4]. The introduction of a Me group at C5 significantly increased toxicity. These data are in good accordance with previous obserations (although cycloalkoxy substituens were studied in our case), that the size of a single 7-alkoxy substituent itself does not play a significant role in the determination of direct toxicity in *P. brassicae*, but C5 Me strikingly enhances toxicity (Darvas et al., 1989). The case was different with *O. fasciatus*, where the toxicity decreased slightly, suggesting that the introduction of Me at C5 may be responsible for reducing toxicity (see point 3).

In the group of C6 and C7 dialkoxy substituted compounds P2 and P3 were regarded as general references. In this group on the other hand, toxicity of compounds increased by the introduction of larger groups at C7 position. Among all the C6 MeO substituted compounds [7] proved to be most toxic one. Even by replacing the C6 MeO group with prop-2-ynylO [8] the toxicity practically did not change strengthening the hypothesis that the length and the size of C7 alkoxy substituent is quite determining and besides, the presence of a short alkoxy group at C6 is quite favourable (Darvas et al., 1989). The presence of the prop-2-ynylO group at C7 [7], [8]) should be well considered too, since it is likely to be responsible for toxicity of the molecules (Ortiz de Montellano and Correia, 1983; Wilkinson and Murray, 1984).

In case of compound [9], where a non alkoxy group (tBu) was introduced to C6 position, leaving the molecule a solitary cyclopentylO group, resulted in the total loss of toxicity in *P. brassicae*. Comparing compounds [9] and [11] it should be noted that both have a O-linked group at C7, the latter bearing the larger (which although cyclic may also be important), but also having a Me at C5 which significantly enhances toxicity. These observations are in agreement with Darvas and co-workers (1989) who also recorded the importance of C5 Me of 2,2-dimethylchromenes bearing alkoxy groups at C7. Compounds [7], [8] (underlining previous suggestions) and [9] caused high toxicity in *O. faciatus*, as well.

The situation changed to its extremity in *P. brassicae* larvae with compound [10] when compared to [9]. In both cases the C7 cyclopentylO remained but an alkoxy group

(MeO) group was introduced to C8, which resulted in a high toxicity. This observation may be an additional information to previous statements, that an increase of toxicity may be achieved if a MeO substituent is introduced to C8 (Darvas et al., 1989) as a second substituent. The same significant increase was recorded in *O. fasciatus*, where already 82,5% of larvae died in L2. Moreover, the presence of a tBu at C6 [9] may not be neglected as a modifying factor, although it does not seem to be determining by itself (see point 4). The presence of the small C8 alkoxy group is rather favourable for the manifestation of the cyclopentylO.

3. Trisubstitution at C5, C6 and C7: Among these trisubstituted chromenes toxicity increased via introduction of sterically larger O linked groups (Me, Et, Pr, prop-2-ynyl, nsBu, cyclopentyl). Results obtained in this group strengthens previous observations that the presence of C5 Me may maintain (and/or increase) toxicity in *P. brassicae*, if it is combined with the introduction of larger dialkoxy groups at C6 and C7 [14], [16], [17]. These observations should be corraleted to values obtained with compounds [4] and [11] too, where a proportionally large cyclic group was present but in the latter case with Me at C5. On the other hand, it should be noted that when P2, P3 and compound [7] was methylated at C5 toxicity decreased. Our data are not sufficient to estimate correctly the role of C5 Me and further investigations are requered in this respect.

In *O. fasciatus* toxicity is in good accordance with morphogenetic effect (see below), pointing out that the most optimal compounds have shown medium or low toxicity. As Fónagy and co-workers (1991) have already found, the C5 Me is more likely a moderator for toxic effect in this species and as such, seems to have been able to counteract (sterically influence) the manifestation of the prop-2-ynylO (and cyclic) group, too.

4. Trisubstitution at C6, C7 and C8: Among these molecules [19], [20] and [21] form a separate subgroup which is also closely related to compound [9]. Compounds [9] and [21] showed no activity, indicating that the introduction of cyclopentylO itself could not improve activity and also that the presence of tBu may cause steric hindrance.

When larger rings are replaced by smaller alkoxy groups, the molecules became highly active. It's noteworthy that when cyclopentylO was at C7 position, but surrounded by smaller groups ([10], [17]) the cyclopentylO, MeO substitutions could readily exert high toxicity. The presence of MeO at C8 [19] may also bear importance ([9] versus [10]), since it may enhance toxicity. Results in *O. fasciatus* do not seem to correlate with results on *P. brassicae* giving further evidence to previous assumptions, how differently P are sequestrated and detoxified by different insects, prossessing or not, potent xenobyotic-metabolizing enzymes (Pratt, 1983; Haunerland and Bowers, 1985), which recognize specific substituent configurations.

In compounds [22] and [23] Br and MeO group positions were changed providing a very striking difference in activity. Since in the former case P2 may be regarded as the closest reference, the sole Br at C8 negatively influences the toxic effect of the molecule. When a MeO was introduced at C5 to the trisubstituted molecule ([25]; the only molecule bearing 4 substituents in this study) the effects can readily be compared to [23]. Slightly higher toxicity was obtained with compound [24] where prop-2-ynylO groups were at C7 and C8, but also bearing a C1 group at C6. In *O. fasciatus* moderate or low toxicity was

recorded with compounds [22], [23] and [25] respectively, but high with [24] due to the prop-2-ynylO (Brooks et al., 1985) and also to the Cl group.

5. Trisubstitution at C5, C7 and C8: Two compounds [26], [27] were studied in this group where the chlorinated compound lost all its activity compared to its non-chlorinated pair in case of *P. brassicae*, while it exerted high toxicity in *O. fasciatus*. This unequivocally points to what Cl itself may cause (especially those being close to 3,4 double bond) if it cannot be handled by detoxifying enzymes.

In QSAR studies the following highly significant linear regression equation was calculated for LD_{50} values for *P. brassicae* by stepwise regression analysis:

$$LD_{50}=145.36(\pm16.49)\pi_{R3}-25.69(\pm5.18)B4_{R3}+173.39(\pm70.87)F_{R2}-54.74(\pm9.15)B4_{R2}-10.67(\pm4.95)B4_{R1}-144.84(\pm26.83)I_R+254.79(\pm45.86)$$

$n=21$; $r=0.940$; $s=22.62$; $F=17.72$ ($p < 0.01$)

We may conclude that the requirements for high toxic activity of for the studied compounds were the following: Hydrogen bonding ability of the R substituent; R1 substituent with large B4 value; electron withdrawing R2 substituent with large B4 value; hydrophilic R3 with large B4 value.

Morphogenetic effect

Among the total of 24 new derivatives only six different C5, C6 and C7 trisubstituted new chromenes showed morphogenetic activity in *O. fasciatus* larvae in the applied concentration, but no morphogenetic activity was observed on *P. brassicae*. We already reported our findings earlier concerning these active compound (Fónagy et al., 1991). Equally high activity was decected with P2 and P3 as reference compounds, though the Me group is lacking at C5 position. Our comparative results in *O. fasciatus* further support previous observations pointing out to the importance of C6, C7 alkoxy groups necessary for morphogenetic action, as it was found in *Locusta migratoria* (Kiss et al., 1988), but does not underline the suggestion that the C7 chain must be larger than C6. Further evidence was given to the conclusions that precocious metamorphosis occurs if C6 alkoxy group is not larger than EtO, although it is not as significant as in case of MeO substitution ([12] versus [18]). These data may be attributed to the fact that our active compounds always possessed a Me at C5 which itself may explain manifestation of morphogenetic effects (Kiss et al., 1988), when accompanied by C7 substitution important for precocious metamorphosis induction (Bowers, 1976; Chenevert et al., 1980; Brooks et al., 1985) and by a small alkoxy group at C6 which is known for its anti-juvenile hormone activity (Bowers, 1976). On *L. migratoria* no significant activity was detected with compound bearing more than two substituents (Kiss et al., 1988), but it is not the case with *O. fasciatus* since it is likely, that the combination of favourable conditions – providing considerably low toxicity with high precocious activity – yielded morphogenetically active compounds.

No clearly applicable quantitative structure-activity relationships were found for the morphogenetic activity of the compounds against *O. fasciatus*. On the other hand, the data presented in this paper give further evidence regarding the importance of necessary groups at C5, C6 and C7 positions. Results of a larger group of studied compounds revealed that the following qualitative features seem to be essential for morphogenetic

activity: R=Me; R1=MeO or EtO group; R2=alkoxy group with 1-3 carbon atoms; R3=H.

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Biology and Control of *Ceutorhynchus maculaalba* Herbst (Coleoptera: Curculionidae)

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In Hungary *C. maculaalba* is the most dangerous pest of *Papaver somniferum* L. Its damage was first recorded in 1884. Poppies grown in lowlands are attacked by the pest in the first place. In some years the extent of damage may even reach 50 per cent. *C. maculaalba* has a single generation a year. It is only able to place its eggs in 1 to 2-day old capsules. The excitation mechanism of egg laying is: 1. in response to the fragrance of flowers as an olfactostimulus the female adults gather in the poppy flowers; 2. the specific stimulatns of the capsule, as contact chemostimuli induce mastication of the adults, then with the third stimulus – the roundness of the capsule as a form stimulus – added to the former two the female adult is moved to pierce the capsule with its proboscis and place its eggs through the hole thus made in the capsule. The antennae also play a decisive role in egg laying. Even such adults as possessing mere scapes are able to lay eggs, while those without scape do not lay eggs. The temperature threshold for eggs and larvae is 13°C. The effective heat sum calculated for the period of embryonal and larval development is only correct when based on temperature values obtained inside the capsule. In Hungary the species *Bracon longicaudis* (Hym., Braconidae) was raised from *C. maculaalba* larvae. Twenty-one per cent of the larvae were parazitized. The paper also discusses the gradological factors and prognostic methods, and finally describes the methods of control used with success in small- and large farms.

Poppy is one of the oldest cultivated plants, even mentioned in works by Greek and Roman authors alike (de Candolle, 1894). On the beginning of its cultivation in Hungary there are no exact data (Mándy, 1972). According to Mórász (1979) in 1974 poppies were grown on 3584 ha in Hungary. *C. maculaalba* as the most dangerous pest of poppy was already mentioned by Horváth (1884).

According to Jourdheuil (in: Balachowsky, 1963) the pest occurs in Central- and Southern Europe, in the Caucasus, in Turkestan, Asia Minor and North Africa. In Hungary it causes damages mainly in the lowlands, in the mountains the individual number decreases with height (Kuthy, 1900; Szelényi, 1951). From France Balachowsky (1947), from Germany Eichler (1949) reported first its occurrence. In Romania it is a frequent pest in Moldavia, Transylvania and the Banat (Manolache et al., 1961). Dieckmann (1972) gives information on its distribution over the Earth. Its presence in Italy is mentioned by Rizza et al. (1980, 1982), in Austria by Berger and Komarek (1989).

Feed plants

According to the opinion of Szelényi (1951) *C. maculaalba* is a typical example of a species changing from wild plants over to cultivated ones, since the insect concerned is at present only able to live on *Papaver somniferum* L. Originally – as the author says – it lived on *Papaver rhoeas* L. On the latter *Ceutorhynchus albovittatus* Germ. lives today.

Since *C. maculaalba* develops on *P. somniferum* in Hungary, its individual density depends on the size of the areas sown to poppy.

Rizza et al. (1980) in Italy carried out detailed laboratory examinations to determine the range of feed plants.

Jourdheuil (in: Balachowsky, 1963) mentions the mass occurrence of *C. maculaalba* early in spring on the following plants: *Sisymbrium officinale* Scop., *Carduus acanthoides* L. and on various Boraginaceae: *Cynoglossum* sp., *Echium* sp. and *Symphytum* sp. He does not, however, speak of the damage done by the pest to these species.

Symptoms of damage

The main pest is the larva; its damage becomes visible when the capsule is opened. In the course of their development the larvae chew the seeds and the septa of the capsule. The latter shows tissue proliferation as a consequence of chewing.

At the beginning the adult chews the part of stalk below the bud, the veins of the thicker leaves and the leaf blade. Its presence is indicated by longitudinal traces of skinning. In case of a large number of insects doing damage the plant sheds the bud (Reichart, 1961). Immediately after the flowers have opened the adult visits the young capsule and begins to peel it; then later pierces through the wall of the capsule. From the injured capsule milk-white sap containing opium oozes that the insect spreads about with its proboscis. The sap having come into contanct with air soon turns brown, then black. These black spots are sure signs of the pest's presence.

The tiny round holes visible on the mature capsule are made by the larvae crawling out from the capsule.

C. maculaalba causes considerable losses first of all in poppies grown in small plots (home gardens), though it may be dangerous for poppy fields of large-scale farms too. In Hungary the losses may be 15-20, or here and there even 30-50 per cent (Huzián, 1965). The industrial value of the capsule is also affected (Rónay, 1941; Győrffy, 1941).

According to Sáringer (in: Balás and Sáringer, 1984) the difference in seed yield is much greater between the healthy capsule and the one infested by a single larva than between a capsule infested by one larva and another in which eight larvae are developing. If in a capsule more than eight larvae are developing the seeds produced in it will be essentially fewer.

The damage caused by *C. maculaalba* is considerably increased by the fact that it offers possibility for egg laying by *Dasyneura papaveris* Winnertz. Latter causes in many cases greater losses than *C. maculaalba*.

Annual cycle of development

In Hungary the pest has a single generation a year. Overwintering takes place in adult form, in cocoons prepared in the soil of the poppy field (Szelényi, 1935, 1939). Manloche et al. (1961) found the average depth of overwintering to be 8-10 cm, though

they found overwintering insects even at a depth of 35 cm. Information on their appearance in spring is given by the data of Fig. 1. Accordingly, 1-2 adults (usually males) emerge 10-20 days before the beginning of mass flying, then in the second half of May the adults appear in large numbers. Mass flying ends in the first decade of June, later only sporadical flying can be observed. While at the time of the flying peak males and females appear in nearly identical numbers, towards the end of flying, like at the beginning, the males outnumber again the females. The length of the flying period varies from year to year; it was shortest – 47 days altogether – in 1966, and longest – 81 days – in 1963. On the average of years it has been 57 days. Out of the population overwintering in imaginal diapause 13.1 per cent remained in diapause over two years, in 1965-67 (Sáringer, 1970b). The adults overwinter in the soil of the former poppy field, and seek out the newly sown poppies by flying. According to Manolache et al. (1961) they fly in masses at 15°C. Szelényi (1935) observed their slow gathering in poppy approaching the stage of flowering. Two to three days after the first flowers have opened their number suddenly rises which proves that the fragrance of the poppy flower has an alluring effect on the insects. Szelényi (1935) found that the adults preferred the white-flowered French poppy to varieties with red flower and open capsule.

According to Sáringer (1976) egg laying by *C. maculaalba* takes place under the control of the following mechanism of excitation: 1. in response to the fragrance of the poppy flower as olfactostimulus the female adults found the poppy flowers and gather in them; 2. the specific chemical stimulants of the capsule, as contact chemostimuli induce the peeling activity of the adults, then the contact chemostimulus together with the third stimulus, the roundness of the capsule as formal stimulus moves the adult to pierce through the capsule with its proboscis and place its eggs in it through the hole. The antennae also play a decisive role in egg laying. Adults with mere scapes are already capable of laying eggs, while those without scapes do not lay eggs (Sáringer, 1970c). The adults couple in freshly opened flowers, then egg laying begins. The female is only able to place its eggs in 1-2 day old capsules. Having pierced the wall of the capsule through the female turns round beside the opening thus produced, sinks its pigidium into it and places an egg inside the capsule. The proboscis is of no importance in introducing the egg into the capsule. According to investigations by Sáringer (in: Balás and Sáringer, 1984) in the upper half of the capsule an average of 11.1±3.4 per cent more eggs are placed than in the lower half. Through an opening maximum 3 eggs are placed in the capsule. More than one female can lay eggs at a time or one after the other in the same capsule. Egg laying is continued throughout the whole flowering period. In capsules older than 2 days eggs are naturally no longer laid. A poppy field may even flower for 40-45 days.

Sáringer (1970b) found that hte diapausing adults without hibernation (winter cold) did not lay eggs after they had come out. In the laboratory experiments of Sáringer (in: Balás and Sáringer, 1984) the number of eggs laid by one female in 1962 was 94 at 18°C, 89 at 22°C and 98 at 28°C. At the beginning of egg laying the number of eggs rose by leaps at all the three temperatures. Maximum was reached in the middle of the egg laying period, then a sudden decrease set in followed by a repeated increase lasting up to the end. According to observations in the field the egg laying period may even be

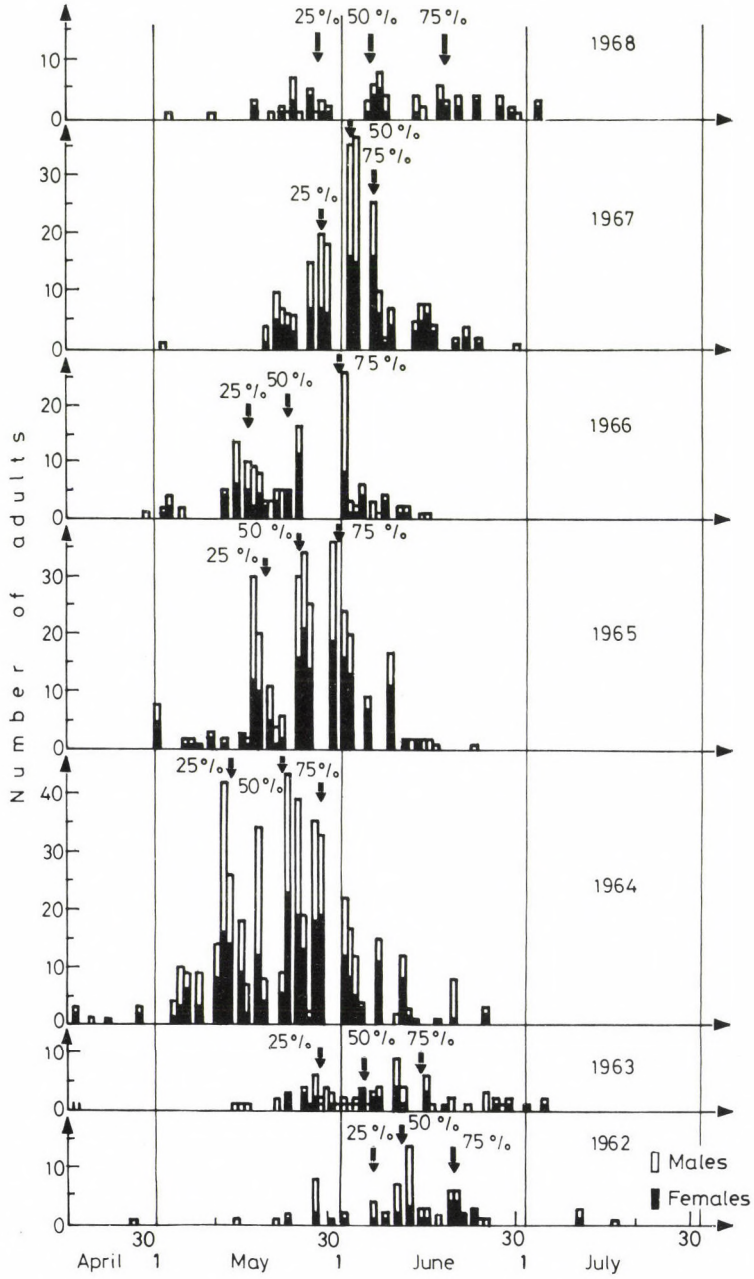

Fig. 1. Spring appearance of *C. maculaalba* in 1962-1968. Thick arrows indicating percentage of emerged adults (After Sáringer, 1970b)

Table 1

Development data for *C. maculaalba* Herbst at various constant temperatures (Original)

Develop-ment state	18°C Development time in days			22°C Development time in days			28°C Development time in days		
	shortest	longest	average	shortest	longest	average	shortest	longest	average
Ovum	7	8	7.1	5	6	5.6	3	4	3.9
L_1	2	4	2.8	2	3	2.6	1	2	1.5
L_2	2	3	2.6	2	3	2.7	1	2	1.5
L_3	5	6	5.3	3	4	3.4	3	3	3.0
Praepupa	13	18	15.5	12	16	14.1	9	14	11.5
Pupa	17	21	19.0	12	17	14.5	9	14	11.5
Total	46	60	52.3	36	49	42.9	26	39	32.9

longer than 5-6 weeks. The data of ontogeny in three thermostats with constant temperatures are contained in Table 1.

The threshold of development temperature is 12.1°C for the eggs and 13.6°C for the larvae of *C. maculaalba*. The temperature threshold of development for eggs and larvae can be roughly taken for 13°C.

The effective heat sum calculated for the time of embryonal development from daily means of temperatures measured by thermistor in the southern half of the capsule was 107.0°C. When calculated from values obtained in the northern half of capsule it was 98.4°C. The difference is 5.5 per cent. When calculated from values obtained outside the capsule in the poppy field, the effective heat sum was 89.9°C, while the daily mean temperatures measured in the meteorological cabin gave 65.6°C for effective heat sum. According to the data only the effective heat sum calculated from temperature values obtained in the capsule is a reliable value.

The fully developed larva gnaws its way through the wall of the capsule and lets itself drop to the ground where it at once makes its way in and soon transforms into an adult. The adult does not come to the surface, but stays in diapause until next spring.

On the cycle of development of *C. maculaalba* data were published by Jablonowski (1903), Győrffy (1936, 1938), Zsoár (1950, 1960), Reichart (1961), and Szücs (1962) – besides the above mentioned authors – from Hungary, and from the neighbouring countries by Lisitzuina (1935), Blattny (1938), Pape (1944), Kotte (1948), Nolte (1952a,b), Schrödter and Nolte (1952), Miller (1956) and Grümmer (1966). With its cycle of development in Italy Rizza et al. (1982) dealt in detail.

Factors influencing the population dynamics

Szelényi (in: Scsegolev, 1951) wrote: "... the more intenzive the cultivation of poppy and the more its production area increases from year to year, the larger the overwintering population of *C. maculaalba* will be and the easier the number of insects

Table 2

Emergence data of *C. maculaalba* Herbst and meteorological data in 1962-1968 at Keszthely (After Sáringer, 1970a)

Year	50% emergence of the population	Effective heat amount in °C at 10 cm soil depth starting with April until emergence of 50% of the population	Precipitation in mm between 1 April – 15 May	Opening of first poppy blossoms
1962	11 June	111.2	46.4	20 June
1963	5 June	135.6	46.5	17 June
1964	23 May	40.7	76.6	24 June
1965	25 May	32.8	136.5	18 June
1966	23 May	57.3	129.1	16 June
1967	2 June	103.4	35.8	17 June
1968	7 June	152.0	1.1	7 June

Precipitation mean for 50 years at Keszthely between 1 April and 15 May: 92 mm

rises extremely high under the influence of other favourable environmental factors (weather etc.)."

According to Zsoár (in: Manninger, 1960) during the development of *C. maculaalba* there are two critical points of time which are of decisive importance in respect of reproduction. "One of them is the time of egg laying, the other is the time when larvae mature for pupation move into the soil. In the first case an average temperature of 20-25°C and a warm, sunny weather, while in the second case humid, warm, rainy weather is favourable, because then the mature larva easily makes its way into the soil, taking advantage of the tiny cracks and cavities."

In the course of investigations between 1962 and 1968 Sáringer (1970a) found a relationship between the time of the adult population's 50% emergence and the meteorological factors (Table 2.). From the data he established the following relationship: the emergence of 50 per cent of the adult population occurs when the between 1 April and 15 May measured in the amount of precipitation of the many-years average exceed in number or it is about and from 1 April at 10 centimetre soil depth effective heat sum calculated from values of temperature reaches 35-60°C.

If the precipitation is less than half of the many-year average, that is there is a drought in spring, the appearance of 50 per cent of the adults occurs at a time when the effective heat sum is between 100 and 150°C. Accordingly, the time of the adults' appearance cannot be determined by mere temperature values, because the current conditions of precipitation also play a decisive role in the reactivization of the overwintering adults.

Natural enemies

In Hungary Sáringer (in: Balás and Sáringer, 1984) raised the species *Bracon longicaudis* Ratz. (Hym.: Braconidae, det.: Dr. Jenő Papp) from *C. maculaalba* larvae collected at Egregy. Twenty-five per cent of the larvae were parazitized.

The idea of the biological control of *C. maculaalba* was considered by Rizza et al. (1980) in Italy.

Prognostic studies

The optimum time of control can be determined with the following methods elaborated by Sáringer (1970a):

– tent isolator observation of overwintering adults,
– effective heat sum calculation,
– signalization.

Tent isolator method. In the first half of July 50-100 poppy capsules containing fully developed larvae are collected. The capsules are placed on the surface of an earthen vessel filled with soil from the poppy field. The larvae move into the soil and transform there into adults. The adults overwinter in the earth cocoon.

In spring of the next year (in mid-May at the latest) a tent-shaped trap made of black linen cloth is mounted on to the pot sunk into the ground. The adults caught in the catch-bottle of the tent-trap are removed every second day.

Control is reasonably started in the poppy field 10-14 days after the beginning of flying, that is when the adults appear in masses.

Prognosis by effective heat sum calculation. Near the tent-isolator the temperature of soil at a depth of 10 cm is taken every day form 1 April, at 7 a.m. and 2 and 9 p.m. Further, the amount of precipitaion is continuously recorded. The biological zero point serving for the basis of the effective heat sum calculation is 13°C. Sáringer (1970a) found that the amount of precipitation influenced the effective heat sum required for the adults to swarm. If the amount of precipitation between 1 April and 15 May is more than 90 mm, the appearance of 50 per cent of the adults can be expected when the effective heat sum is 35-60°C. If, on the other hand, the amount of precipitation is low (does not even exceed 45 mm), the adult's flying peak can only be expected when the effective heat sum reaches 100-150°C. Control is best started with the flying peak.

Signalization. It is important to survey the poppy stand regularly so as to complete the prognostic studies. The observations start at the beginning of May, when the adults appear, and are continued up to the end of May, or until mid-June in case swarming is protracted. The observations had better be made at about noon, when the sunlight is the most intensive. Adults occurring on a 1 m^2 area of each of several places from the margin to the centre of the field are counted. If their number is an average 2/m^2 the control must be started without delay.

Control methods

In large-scale fields the control operations are carried out using chemical insecticides, dusts and sprays containing methyl-parathion, dioxacarb and toxaphen as active agent. Only the adults can be controlled. The first treatment should be carried out at the

"crook" stage of the plant, the second treatment at the beginning of the flowering period. If necessary, a third treatment is carried out in full blossom. For the second and third treatment only bee-sparing insecticides and techology can be used. Since the surface of the plant is covered with wax, dusting is more efficient than spraying (Reichart, 1961; Zsoár, 1961, 1966; Sáringer and Zsoár, 1963; Sándor, 1978).

In case of a mild infestation the adults are in larger numbers on that side of the field which is nearer to the previous year's poppy filed. This fact makes it possible to protect the whole poppy field by dusting the critical edge in time. A severe infestation almost uniformly affects the whole field. Révy (1931) and Baja (1943) found in every case a greater extent of infestation at the edge of the field. In large-scale poppy fields uncultivated strips are usually left for the dusting machine in order to avoid damages caused by treading. In large, 50-100 ha fields the insecticide is distributed by helicopter.

The decoy strip sown 1.5 - 2 weeks earlier lures the swarming adult populations with its flowers opening earlier. By dusting this strip a large number of adults can be destroyed.

In home gardens protection against the pest is ensured by the removal of the poppy petals. The insect probably keeps out of capsules deprived of petals because such capsules do not offer a hiding place. The removal of petals can be disadvantageous because the stamina may be injured whereby the rate of fertilization will decrease (namely the poppy is not only a self-fertile plant!). By removing the petals Bogyai (1933) successfully controlled the pest.

The agrotechnical factors (careful soil preparation, fertilization etc.) even when optimum do not give protection, as the insects visit first of all the best developed capsules with the purpose of egg laying.

According to Sáringer (1988) if early in spring, as soon as the ground is suitable for walking, the poppy is sown, flowering will take place at a time when the adults do not swarm in masses yet. In home gardens of some regions when the winter is mild the way of protection from the damage of the pest is to sow the poppy already at the end of February, occasionally over the thin snow cover.

The autumn poppy grown in Hungary only on a small area mostly flowers in spring before the adults begin to swarm, so it practically remains free from the infestation (Sáringer, 1964).

The repelling effect on *C. maculaalba* adults of naphthalene spread between the rows of poppy was reported by Dworák (1934) and Szelényi (1935).

Control methods used in Hungary were described by Anonym (1918), Szelényi (1943) and Legány (1945) too.

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Changes in Food-related Behavioural Patterns of Some Phytophagous Insect Species Following Exposures to an Antifeedant

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Laboratory experiments were conducted to determine the effects of a feeding deterrent, copper sulphate (applied as Bordeaux-mixture), on the food-related behaviour of last larval instars of two oligophagous species, *Pieris brassicae* and *P. napi*, and two polyphagous ones, *Mamestra brassicae* and *M. oleracea*, as well as third larval instar of the oligophagous *Leptinotarsa decemlineata*. The larval feeding behaviour of all species followed recurring cycles of palpation, biting, feeding, locomotory activity and rest.

The oligophagous species invariably reacted to the presence of the deterrent by shortening the feeding periods, increasing locomotory activity and showing irregular behavioural sequences. To the contrary, the polyphagous species showed high individual variability in their responses. Disturbances in food-related behavioural sequences, changes in duration of behavioural patterns, as well as normal feeding behaviour all occurred within the scale.

The concentrations eliciting feeding inhibition were higher for the polyphagous species than for the oligophagous ones. With all species, the first meal taken after moulting was always longer and larger than the subsequent ones.

Phytophagous insects show various levels of specializations in their feeding habits. Those termed oligophagous species usually feed on a restricted range of taxonomically related plant species. Others, such as the polyphagous ones, consume a wide array of unrelated plant species. Within a given class of phytophages, there are variability in the degree of food specialization between individuals, sometimes differring in orders of magnitude with various species. Polyphagous species often show greater individual variability in feeding behaviour than oligophagous ones (Simmonds and Blaney, 1984; Blaney and Simmonds, 1987).

An increase in host-plant specialization is connected with an increased sensitivity of the chemoreceptors to feeding inhibitors. Therefore, polyphagous insects are less easily deterred by specific allelochemics in plants than the more specialized ones (Jermy, 1966). The same was found by Bernays and Chapman (1978) on graminivorous Acridoidea and by All and Benjamin (1976) and Ohigashi et al. (1981) on larvae of sawflies.

As for behavioural changes in the presence of antifeedants, bioassays with deterrent chemicals include examples where a pronounced antifeedant effect declined with time (e.g. Yamiza et al., 1969; Schoonhoven, 1969, 1977; Munakata, 1977; Jermy et al., 1982; Szentesi and Bernays, 1984), as well as cases where more reduction in the deterrent activity of a given insect species was observed (Hosozawa et al., 1974a,b; Schoonhoven and Jermy, 1977).

After contacting a feeding deterrent, an insect may not only interrupt food intake, but may also show increased locomotory activity (Jermy, 1971). Jermy also reported that Colorado potato beetle larvae and adults, having tasted a cooper solution, moved around

for some time. The same was found by Schoonhoven (1982). Barton Browne (1975) suggested that feeding and locomotion were in certain insects at least, antagonistic behaviours, and this may explain why movement increases in the presence of an antifeedant.

Blaney et al. (1973) and Simpson (1982) demonstrated in *Locusta migratoria* that feeding on normal substrate occurred in discrete bouts, separated by periods of "roosting" when the nymphs climbed the cage walls. Simpson (1982) also demonstrated a daily feeding rhythm, with reduced feeding activity at night. Reynolds et al. (1985) showed that feeding of *Manduca sexta* larvae on tobacco was restricted to distinct bouts, and the larvae spent over 80% of their time feeding. The same was found by Ma (1972) with the caterpillars of *Pieris brassicae*.

One of the intriguing questions is to know the extent and nature of changes taking place in the highly stereotyped food-related behavioural patterns and in sequences of such patterns with insect groups when either the host-plant species are made unpalatable or plant species normally found outside the sphere of acceptable hosts are offered. Modelling the first case, laboratory experiments were conducted to determine the effects of a feeding deterrent, cooper sulphate, on the food-related behaviour of two polyphagous and three oligophagous insect species.

Material and Methods

Insects

The insects used were final larval instars of the polyphagous *Mamestra brassicae* L. and *M. oleracea* (Lep., Noctuidae), and the oligophagous *Pieris brassicae* L. and *P. napi* L. (Lep., Pieridae), and the third larval instar of *Leptinotarsa decemlineata* (Say) (Col., Chrysomelidae). All were reared in the laboratory: *M. brassicae* and *M. oleracea* on a semisynthetic diet (Nagy, 1970), *P. brassicae* and *P. napi* on cabbage (*Brassica oleracea* cv. capitata) and *L. decemlineata* on green house-grown potato (*Solanum tuberosum* L. cv. Desirée). Observations were conducted with newly moulted instars.

Plants

Individual full-grown but not senescent leaves, picked from one or different plants (cabbage or potato) having similar age and shape were treated by painting the leaf on both surfaces with Bordeaux-mixture ($CaSO_4$: $Cu(OH)_2$: $3Ca(OH)_2$ complex) spread with a glass rod. The leaves were dried in a cool airstream. Leaves treated only with distilled water were used as a control (0.3% Tween was applied as a surfactant for control and treated cabbage leaves).

Experimental arrangement

One larva was placed into a plastic dish (height: 30 mm × diam.: 100 mm) containing a piece of wet filter paper attached to the wall, and a single (cabbage or potato,

control or treated) leaflet with the petiole immersed into a water-filled vial and fixed with a cotton plug, so that the leaflet remained fresh throughout the observation period. The dishes were covered with glass plates of 110 × 110 mm, and kept at 23-25°C, and under illumination of approx. 1000 lux provided by fluorescent tubes.

Comparison of behaviours of different species using a given concentration of the feeding deterrent (Bordeaux-mixture containing 0.1M $CuSO_4$. $5H_2O$)

Daily observation usually began at 10.00 a.m. For behavioural observation 4 replicates (dishes) of 2 variables (control and antifeedant-treated leaves) were arranged on a table, placed close to each other so that the observer could scan all of them quickly, repeatedly and without disturbing the insects.

The following behavioural patterns were differentiated and their duration and sequence during the observation period recorded with each individual: palpation (P), biting (B), feeding (F), locomotory activity (L) and "resting" (R).

They are defined as follows. Palpation is described as repetitive contacts with the substrate by the maxillary palpi. Palpation is usually followed by biting. Through biting the larvae take a mere mouthful or make several small bites, immersing the mandibles into the leaf tissue and removing a bit or only crashing the tissues, possibly letting the plant sap ooze out. Feeding is characterized by repetitive bites. Feeding bouts are terminated normally in one of the followings: walking (locomotory activity) and rest. Locomotion comprise here all the locomotory activities performed on the leaflet or elsewhere within the box, while rest excludes any other behavioural patterns related to feeding and locomotion.

For recording the time elapsed during any one behavioural pattern a stop watch was switched on at the beginning of a given behavioural pattern and switched off at its termination, and switched on again at the beginning of another one, and so on. The observations were continued for 4 days, and the data collected during 6 hours/day were used to give an analysis of the feeding behaviour for each species. Altogether 23 individuals of *L. decemlineata*, and 21 individuals of *M. brassicae, M. oleracea* and *P. brassicae* were observed for each variable (control and antifeedant-treated plant).

On the basis of the raw observational data the food-related behaviour was characterized by the followings:

(1) the frequency of meal size and interfeed length;
(2) the percentage time spent with each activity;
(3) the frequency of transitions, i.e. changing of one behavioural pattern to another.

The assessment of concentration for feeding inhibition

In contrast to the preceding experiment where 0.1M Bordeaux-mixture was uniformly used, in this test concentrations inhibiting feeding by 70-80% as compared to the control were determined for each species (in no-choice test and using final instar larvae; Jermy et al., 1982), and behavioural observations were also carried out at these concentrations. For *L. decemlineata* and *P. napi* 0.05M, for *P. brassicae* 0.1M, for *M. brassicae*

Table 1

Feeding activity (min/h) of larvae of some phytophagous insect species during 4 days (6 hours/day) on control- and treated-leaves (mean ±S.D.)

Treatment	Days	Insect species			
		L. decemlineata	*P. brassicae*	*M. oleracea*	*M. brassicae*
Control	1st	24.1±4.2b	22.9±4.2b	17.4±1.6b	9.7±1.7b
	2nd	30.4±6.7a	25.9±3.9a	18.4±1.9a	10.4±1.9b
	3rd	31.4±9.3a	27.7±3.3a	18.7±1.9a	13.2±2.1a
	4th	28.9±6.4a	28.9±2.5a	18.9±1.9a	13.5±2.0a
Treated	1st	1.5±1.4c	4.1±2.8c	6.2±2.8c	5.5±4.8c
	2nd	1.0±0.6d	3.4±3.4c	3.3±1.2d	3.7±4.9b
	3rd	0.6±0.3e	2.4±1.8d	4.3±1.6d	4.7±5.8b
	4th	0.6±0.7e	2.5±3.5d	5.2±1.6c	17.0±8.7a

Means signed with the same letter(s) within columns do not differ significantly at 1% level of probability (DNMR-test).

and *M. oleracea* 0.2M $CuSO_4.5H_2O$ suspensions were used. Number of insects: 23 larvae of 3rd-instar *L. decemlineata*, 21 larvae of 5th-instar *P. brassicae* and 6th-instar *M. brassicae* and 15 larvae of 6th-instar *M. oleracea*.

Results

With all insect species sequences of feeding behaviour on normal plant followed recurring cycles consisting of palpation, biting, feeding, locomotory activity and rest. Degree of acceptance of the antifeedant-treated plant varied among insect species according to inhibitory levels.

Feeding

L. decemlineata: the feeding behaviour of larvae on control-plants generally began with a short palpation period. Palpation was followed by repeated biting at a single site then feeding began. Feeding always lasted for a relatively long period, but the quantity actually consumed (measured as the decrease of surface area) was small. Larvae on control leaves ate more or less uniformly throughout the observation periods in 4 days. The mean period spent feeding/hour on control plant seemed to be constant with a tendency of increase in time from the first to the fourth day, whereas feeding on treated-plant decreased severely (Table 1). The feeding behaviour on control leaf was always followed by very short periods of locomotion then rest in the same place, while insects

Table 2

Locomotory activity (min/h) of larvae of some phytophagous insect species during 4 days (6 hours/day) on control- and treated-leaves (mean ±S.D.)

Treatment	Days	Insect species			
		L. decemlineata	*P. brassicae*	*M. oleracea*	*M. brassicae*
Control	1st	1.5±1.9b	6.4±0.9b	3.4±1.4b	2.8±1.2c
	2nd	0.4±0.2c	3.0±0.5c	3.2±1.1b	2.9±1.3c
	3rd	0.4±0.3c	2.3±0.2d	3.0±1.0b	3.0±0.9c
	4th	0.3±0.1d	1.2±0.2e	4.0±1.2b	4.0±0.9b
0.1M Copper sulphate	1st	14.2±6.3a	8.1±8.8b	3.5±1.1b	8.3±5.8a
	2nd	13.2±12a	13.3±9.6a	3.6±2.6b	10.8±6.8a
	3rd	16.2±15a	15.2±9.6a	6.9±4.3a	3.8±1.8b
	4th	1.5±3.3b	14.5±12a	8.4±5.1a	3.6±1.6b

Means signed with the same letter(s) within columns do not differ significantly at 1% level of probability (DNMR-test)

consuming bits of treated-plants showed an increase in the length of locomotory (Table 2), and resting periods (Fig. 1).

Feeding on control leaves by lepidopterous larvae was discontinuous. There were bouts of active feeding separated by periods of inactivity.

P. brassicae: the lengths of feeding periods of larvae fed control plant were relatively long, increasing gradually from the first to the fourth day, and also large amounts were eaten. Meals were followed by short periods of locomotion and rest at an adjacent place. Feeding on treated-plant evoked an opposite tendency, i.e. a decrease in the length of feeding periods (Table 1). A more drastic change was found in locomotory activity which increased gradually from the first to the fourth day (Table 2). *P. napi* feeding behaviour was distributed invariably during any observation periods. At the beginning, feeding was relatively long, followed by a short period of locomotion then rest.

M. brassicae: feeding period increased on normal plant fom 9.7±1.7 min on the 1st day to 13.5±2.0 min on the 4th day (Table 1). On treated-plant duration of feeding periods decreased on the second day, then once again they began to increase, gradually becoming similar to that of the control on the fourth day: 17±8.7 min. Locomotory activity showed significant differences between control- and treated-plant-feeding insects (Table 2), while rest did not change (Fig. 1).

M. oleracea: in spite of being a polyphagous feeder, it showed a behaviour similar to oligophagous species both on control- and treated plants. The feeding behaviour consisted of a sequence of repeated activities: palpation for a short period of time, continued in feeding, then in locomotion. Feeding periods on control plants seemed to be constant during the four-day observation period. Insects on treated-plants decreased the feeding periods from the first to the second day, similarly to *M. brassicae*. However,

they did not show a similarly high consumption level on the last day (Table 1). Locomotory activity increased with time following feeding on treated-plant (Table 2), while time spent resting showed an opposite trend, i.e. it increased on the first and second day then decreased again on the third and fourth (Fig 1).

Meal frequency

The mean number of meals taken by control insects was always greater than those of insects feeding on treated-plants (mean number of meals/insect/observation period) (Fig. 2a). Generally, the first meal taken after moulting was the longest and the largest. The number of meals per observation period, as percentage of the total, differed between insects fed control- and treated-plants.

Meal-length

L. decemlineata larvae, when feeding on control plants, distributed meals from 3-6 to 15-18 min/meal. However, an examination of the number of meals/day showed a higher proportion of sheort meals (1-3 min) on treated-plants. *P. brassicae* showed a similar behaviour to those of the CPB larvae both on control and treated-leaves (Fig. 2a). Polyphagous species showed another kind of distribution of meal length, namely the differences between individuals were very pronounced both for control- and treated-plant-feeding insects. They showed a trend for decreasing the meal-length, but dissimilarly to the oligophagous ones, 50% of the individual meals-lengths of *M. brassicae* were 1-5 min (Fig. 2a).

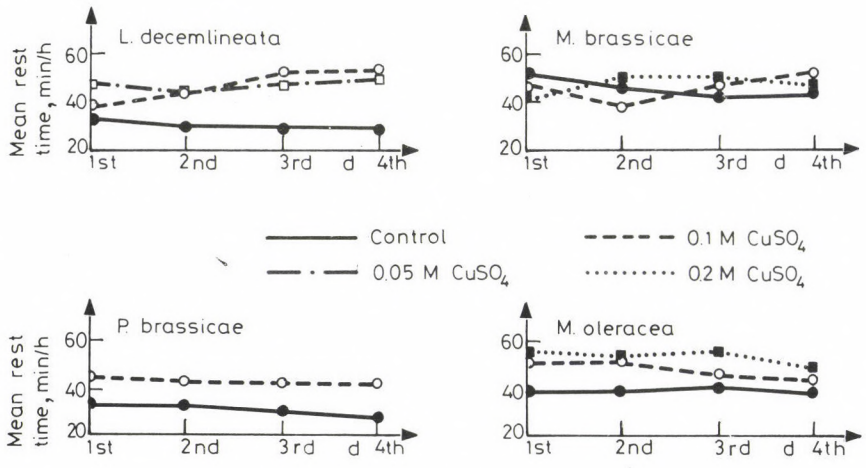

Fig. 1. Rest periods of four phytophagous species on control- and treated plant leaves during 4 days (6 hours/day observation periods)

Interfeeds

The frequency of interfeeds of different lengths also changed with feeding on treated-plants, especially for oligophagous species (Fig. 2b). Insects on treated-plants showed very short (1-15 min) or very long (>40 min for *L. decemlineata* and >50 min for *P. brassicae*) interfeed periods. While interfeeds in the range of 15-45 min were more frequent in control-plant-feeding insects, *M. oleracea* showed another kind of distribution of interfeed periods, i.e. the frequency of short periods was significantly (p=1%) higher

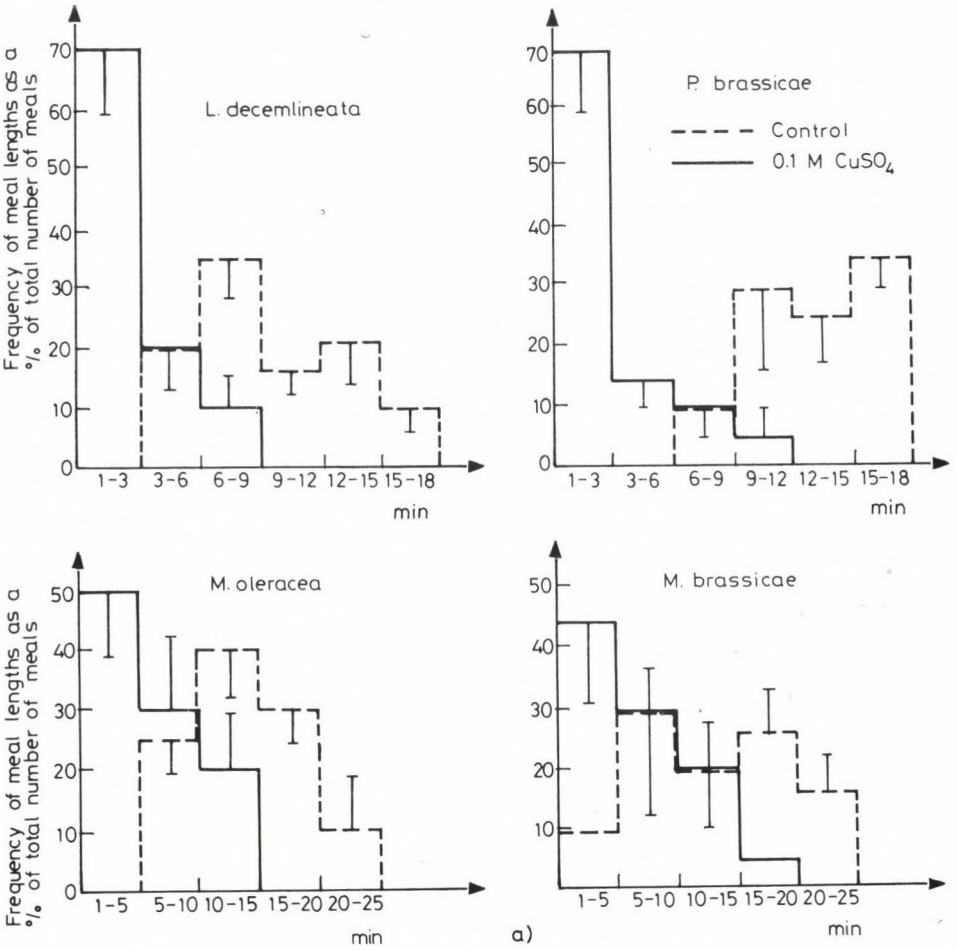

Fig. 2a. Meal size distribution of insects on control- and treated-plants (means of 4 days observations). Bars are S.D.-s

Table 3

Distribution of transitions among behavioural patterns of some phytophagous insect species on control and antifeedant-treated leaves

Species	Percentage of individuals showing a transition between two behavioural patterns			
	1–2	1–3	2–3	2–1
L. decemlineata				
control	10	90	100	0
0.05M $CuSO_4$	25	75	50	50
0.1M $CuSO_4$	30	70	40	60
P. brassicae				
control	20	80	85	15
0.1M $CuSO_4$	40	60	70	30
M. oleracea				
control	10	90	100	0
0.1M $CuSO_4$	30	70	90	10
0.2M $CuSO_4$	40	60	80	20
M. brassicae				
control	5	95	100	0
0.1M $CuSO_4$	10	90	95	5
0.2M $CuSO_4$	30	70	70	30

1=feeding, 2= locomotory activity, 3= resting

in insects having fed on treated-plants, while those on untreated ones were tending to take longer periods of interfeeds. These differences disappeared with *M. brassicae* between the control- and treated-plant-feeding insects (Fig. 2b).

Transitions

The percentage of time spent in each activity, and also the number of individuals that displayed the various transitions among behavioural patterns are shown in Table 3. The time spent feeding by *L. decemlineata* seemed to be 50% of the observation periods/day, and it decreased to 2% after feeding on treated-plant, however, resting increased and so did locomotion (Fig. 3).

The feeding inhibitory level of copper sulphate

The concentrations eliciting 70-80% feeding inhibition were higher for the polyphagous species than for the oligophagous ones (see Table 4).

Behavioural responses observed at concentrations giving 70-80% inhibition

L. decemlineata: Larvae sampled the leaves at different sites, palpated and bit frequently, then fed for short periods of time, finally either moved on or took a rest (Table 4). The percent feeding time decreased with exposure to antifeedant-treated plant (Fig. 3).

The exposure to the feeding deterrent with the inhibitory concentrations resulted in changes regarding transitions between different behavioural patterns (Table 3). *P. brassicae*: the locomotory activity decreased with time, and its proportion to other activities was 5.4% (Fig. 3). Relatively small amounts were eaten during the first day observation period, what even decreased with repeated exposures to the treated-plant. Locomotory activities increased, and larvae sampled treated-leaves more frequently. Interfeed periods were relatively longer. The concentration to elicite the same responses was less than with *P. brassicae*. *M. oleracea*: on treated-plant, the duration of the first meal was

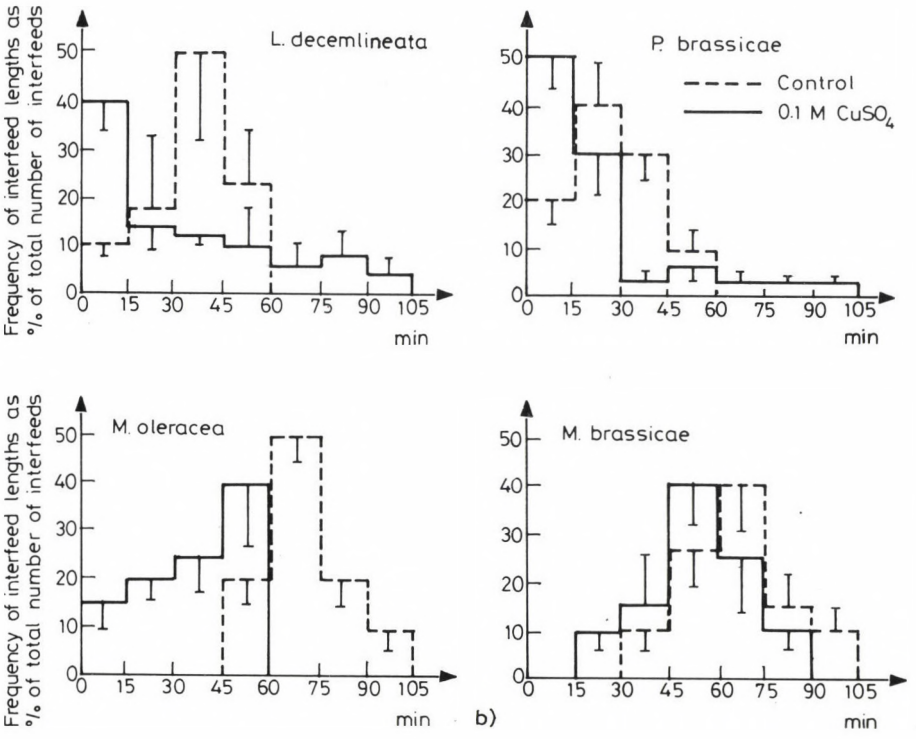

Fig. 2b. Interfeed length distribution of insects on control and treated plants (means of 4 days observation). Bars are S.D.-s

Table 4

Duration of feeding and locomotory activities (min/h) of some phytophagous insect species on treated plants with the concentrations eliciting 70-80% feeding inhibition (mean ±S.D.)

Species		Days	Locomotory activity	Feeding activity
L. decemlineata				
	N=23	1st	14.3±6.2a	1.3±0.6a
	0.05M	2nd	13.5±12.5a	1.0±0.8b
	$CuSO_4$	3rd	5.8±4.8b	0.9±0.7b
		4th	7.5±8.4b	0.7±0.8c
P. brassicae				
	N=21	1st	8.1±8.8b	4.1±2.8a
	0.1M	2nd	13.3±17.6a	3.4±3.4a
	$CuSO_4$	3rd	15.2±9.6a	2.4±1.8b
		4th	14.5±15.1a	2.8±2.9b
M. oleracea				
	N=15	1st	2.0±1.0b	3.9±1.8a
	0.2M	2nd	2,8±1.6b	2.7±1.6b
	$CuSO_4$	3rd	3.2±3.4a	3.4±1.4a
		4th	4.0±2.1a	3.6±1.5a
M. brassicae				
	N=15	1st	11.0±5.1a	6.3±4.4b
	0.2M	2nd	6.8±4.1b	5.0±2.3b
	$CuSO_4$	3rd	8.8±11.5b	7.6±2.4b
		4th	4.9±1.3c	10.6±9.5a

Means signed with the same letter(s) within columns (for each species) do not differ significantly at 1 % level of probability (DNMR-test)

reduced by about 30% as compared with the control. Relatively small amounts were eaten during the subsequent feeding periods. The lenghts of feeding periods decreased in the second day, then increased again in the third and fourth day, resp. Locomotory activity showed the same trend with control-leaf feeding insects. The period of rest increased then decreased again with time, but it was higher than with the control insects (Fig. 3). *M. brassicae*: this species showed another kind of behaviour, namely, on treated-plant the larvae ate more and faster in comparison with other species. Locomotory activity increased in the first day, and so did the length of rest (Fig. 3).

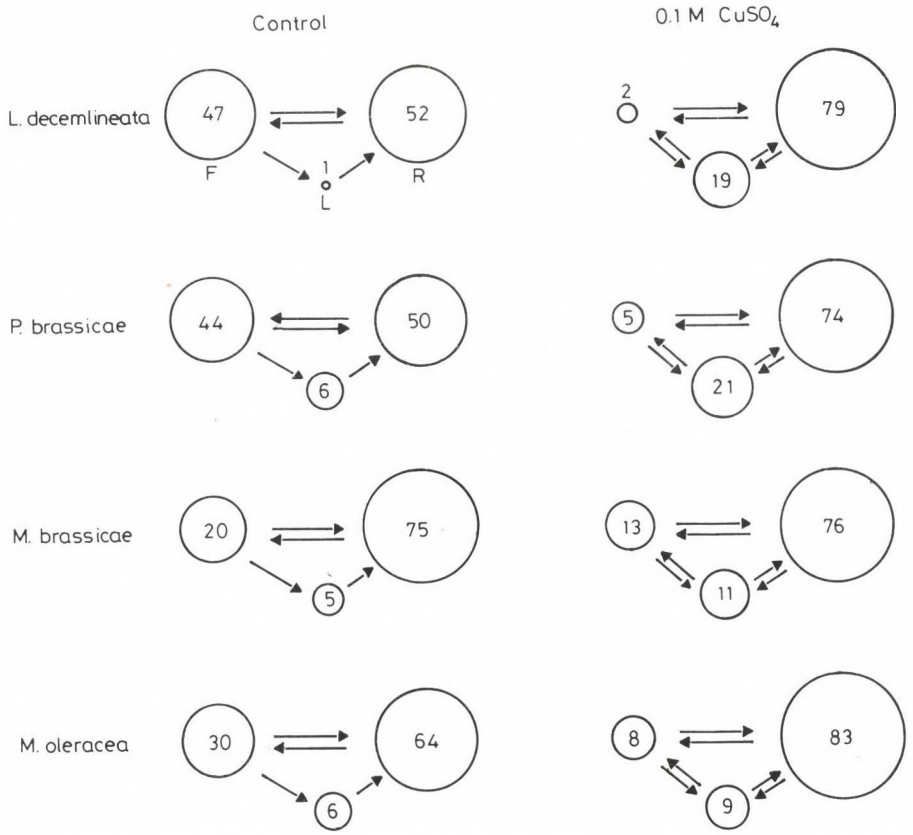

Fig. 3. Relationship between 3 behavioural patterns of two oligo- and two polyphagous insect species on control and antifeedant-treated host plants. (Any numerical value corresponds to the proportion of an observed behaviour as related to all activity occurred). F=feeding, L=locomotory activity, R=rest

Discussion

From the direct observation of behavioural responses it could be concluded that different species often reacted differently to the same feeding deterrent, and also the concentration giving a feeding inhibitory level of 70-80% differed from one species to the other.

The larval feeding behaviour of all species on control plants followed a recurring cycle of behaviour involving, palpation, biting, feeding, locomotory activity and rest. All these patterns may cycle for relatively long periods of time. However, meal-length were

not similar with all species. The occurrence of discrete feeding bouts was also demonstrated by Ma (1972), Blaney et al. (1973) and Reynolds et al. (1985). Meal length depends on the preceding interfeed period, i.e. a long feeding is followed by a long interfeed period and vice versa. This is in agreement with the finding of Bernays and Simpson (1982). They also found that volumetric feedback could account for the dependence of meal length on the previous interfeeding periods.

Polyphagous species usually tolerate higher concentrations of the same deterrent than oligophagous ones. This supports the view that different species have their specific deterrent receptors, what is in accordance with the findings of Schoonhoven and Jermy (1977). They found that the deterrent effect of copper ions varied with insect species. Whereas *L. decemlineata* larvae were deterred by 0.002M $CuSO_4$, larvae of *P. brassicae* readily engorged copper sulphate solutions at concentrations of 0.1M or higher. This was in accordance with our results. Feeding of *L. decemlineata* was inhibited by 0.05M, that of *P. brassicae* deterred by 0.1M, whereas *M. brassicae* and *M. oleracea* were deterred only by 0.2M $CuSO_4$, and also the responses differed.

Dethier (1980) concluded that choice by monophagous and oligophagous herbivores was based strictly on specific stimulants and that polyphagous species ate any plant lacking deterrents or repellents. This explains the responses of the different species to copper sulphate. While oligophagous species were sensitive to low concentrations, polyphagous ones were only inhibited by higher concentrations. With oligophagous species locomotory activity increased gradually especially in *P. brassicae*. After long exposure to the deterrent the length of resting increased, meal-length decreased, then larvae stopped feeding. Also an increase in the frequency of palpation could be observed. Jermy (1971) reported that CPB larvae and adults having tasted a copper sulphate-treated plant immediately stopped feeding and started feeding again only after a comparatively long time, although the optimal feeding stimuli were present. However, locomotory activity increased. Jermy (1971) also suggested that the deterrent evoked a central inhibitory state, which caused a prolonged inhibition of the feeding centre in the central nervous system which may concomitantly release locomotory behaviour normally shown only during interfeed periods. On the other hand, polyphagous species showed another kind of behaviour, i.e. they ate the treated-plant on the first day (although small quantities and during short periods of time, and with a high individual variability), nevertheless, long exposure to the deterrent resulted in an increased consumption. This was in accordance with the results found by Szentesi and Bernays (1984), i.e. that behavioural habituation of *Schistocerca gregaria* occurred when the insects were forced to feed on treated food for longer periods during several days.

The change in the mean number of meals taken by insects (on control- and treated-plants) may be attributable to the variability of different species in their responses to 0.1M $CuSO_4$, nevertheless, oligophagous species were more sensitive than polyphagous ones. This was also demonstrated by Schoonhoven and Jermy (1977). They reported that azadirachtin (0.002M) solution offered to larvae of *Pieris brassicae*, deterred the insects from drinking. Larvae of *Hypsipyla grandella* (Lepidoptera) and *Leptinotarsa decemlineata* also appeared to perceive the chemical and did not drink the solution. The same is stated by Blaney et al. (1987). They revealed a greater susceptibility in the feeding behaviour of the oligophagous *Spodoptera exempta* compared with the polyphagous

species. With locusts, the same was found with the oligophagous *Locusta migratoria* and the polyphagous *Schistocera gregaria* (Blaney, 1981). Blom (1978) also added that *Mamestra* is much less sensitive to feeding deterrents than *Pieris*, what probably underlies to a certain extent, the differences in behavioural reactions to different food plants. Schoonhoven (1982) demonstrated that different insects greatly differred in their responses to feeding deterrents. The length of the intermeal period was found to depend on the previous meal. A similar behaviour has been recorded with *Manduca sexta* (Bowden, 1987; Reynolds et al., 1986).

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Field Trapping Investigations with the European Goat Moth, *Cossus cossus*

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(*Z*)-5-Dodecenyl acetate (*1a*), (*Z*)-5-dodecenol (*2a*), (*Z*)-3-decenyl acetate (*1b*), and (*Z*)-3-decenol (*2b*) were synthesized to obtain an effective lure, proper for mass-trapping of European goat moth (*Cossus cossus* L.). It is concluded, that the mass-trapping as a control method may be effective only at very low population density of pest. The applied lure was 1 mg of compound *1a*, 0.25 mg of *1b*, and 0.25 mg of *2a*. The possible effect of trap design and the question of non-target moth species flown into the traps are discussed.

The European goat moth, *Cossus cossus* L., lives in Europe (except its northernmost territories), Western Siberia, Caucasus and Northern Africa. It causes serious damage on deciduous fruit trees, first of all in southern parts of Europe. This large moth has a number of host plants, such as willow, poplar, elm, alder, birch, oak, ash, beech, maple, mulberry tree, larch (trees of forests and parks), as well as apple, apricot, quince, pear, almond and sweet cherry (fruit trees) (Gozmány and Szőcs, 1965; Zagulyaev, 1978). According to our preliminary survey, *C. cossus* may be relatively frequent within Hungary in the Danube valley and between the rivers Danube and Tisza, which is demonstrated by some data in Table 1.

The usual chemical control (sprayings with insecticides) are ineffective, because of the long swarming period of adults, and the hidden manner of life of larvae. The local treatment with high doses of insecticides might be effective (Audemard, 1974), but in large-scale orchards there is no manpower enough to perform it.

The first attempts for mass trapping of *C. cossus* by sex attractants was accomplished in Italy (Pasqualini et al., 1982a, 1982b). One year later Capizzi and his co-workers isolated and identified the sex pheromone components of the European goat moth (Capizzi et al., 1983). The main constituent, which proved to be (*Z*)-5-dodecenyl acetate (*1a*), showed only weak attractive effect in pure form. A minor substance of the natural blend is (*Z*)-5-dodecenol (*2a*). (*Z*)-3-Decenyl acetate (*1b*), not detected in the female tip washings, significantly improved the attractiveness of the principal active component *1a*.

Here we report the synthesis of these compounds, along with the related (*Z*)-3-decenol (*2b*).

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Table 1

Percentage of *Cossus*-infected trees in some Hungarian orchards

Culture	Locality	Year	Percentage of infection (%)
Apple	Halásztelek*	1988	18
	Kecskemét-Szarkás**	1987	14
	Érd-Levira-Major*	1980	3
Pear	Nagykovácsi*	1987	0
	Budakeszi*	1984	0.5
Apricot	Kecskemét-Szarkás**	1988	2
	Érd-Elvira-major*	1980	3
	Dunaharaszti*	1987	4

* = Bács-Kiskun-county
** = Pest-county

Lures baited with their different mixtures were investigated to find a synergistic composition proper for mass-trapping under our conditions. The possible effect of two trap design was studied, and mass-trapping experiments were carried out.

Materials and Methods

Synthesis

The crude products were purified by flash chromatography (Still et al., 1978). IR spectra were recorded on a Zeiss Specord-75 IR apparatus. ^{1}H- and ^{13}C-NMR spectra were obtained with a Varian XL-100 spectrometer ($CDCl_3$/TMS). Capillary GC-analyses were carried out on a Perkin Elmer F22 gas chromatograph (60 m × 0.25 mm ID FSOT column coated with SPB-2 [Supelco], carrier gas: nitrogen, FID). Compounds shown in Fig. 1 displayed the expected spectral and physical characteristics in accord with literature data.

Literature survey revealed numerous Witting (Maurer and Grieder, 1977; Schaub et al., 1985; Kovalev et al., 1987) and acetylenic (Warthen and Jacobson, 1968; Steck et al., 1978, 1980; Arsura et al., 1982; Capizzi, 1983) synthetic approaches to pheromone components *1a* and *2a*, the latter ones being preferred for our purposes to exclude the formation of the corresponding (*E*) isomer in noticeable amount. Thus, the components *1* and *2* (see Fig. 1) admixed for pheromone traps were prepared by routine acetylenic routes (Henrick, 1977) though with some differences compared to the known syntheses of *1a*, *2a* (cited above), *1b* and *2b* (Wilson and Prodan, 1976; Arsura et al., 1982; Capizzi et al., 1983; Yadagiri and Yadov, 1983). 1-Octyne (*3*), the common precursor for substances *1* and *2*, gave the acetylenic tetrahydropyranyl ethers *6* on successive lithiation and

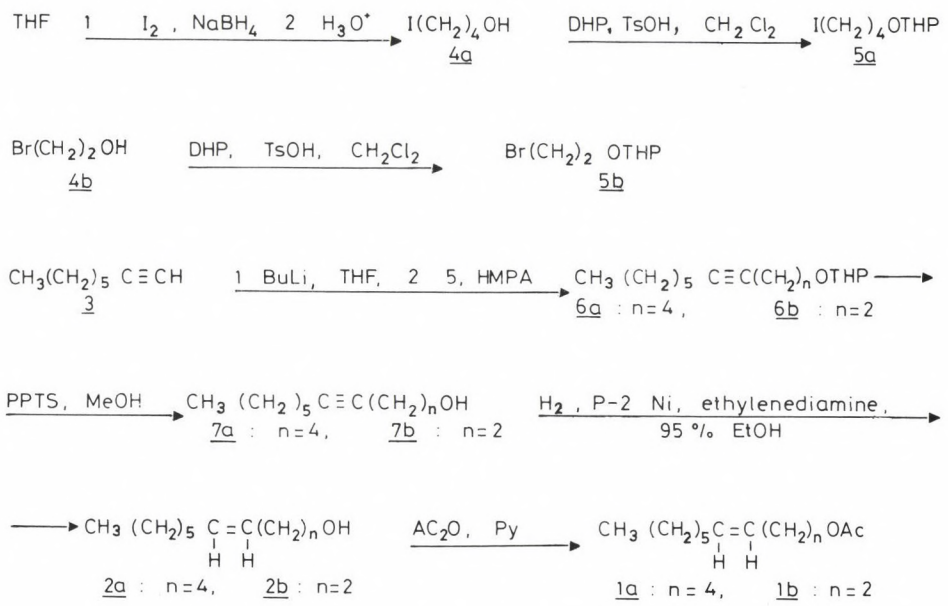

Fig. 1. Syntheses of the pheromone components *1* and *2*
Abbreviations used: Ac: CH_3CO, Buli: butyllithium, DHP: 3,4-dihydro-2*H*-pyran, EtOH: ethanol, HMPA: hexamethyl-phosphoramide, MeOH: methanol, PPTS: pyridinium *p*-toluene-sulfonate, Py: pyridine, THF: tetrahydrofuran, THP: tetrahydropyranyl group, TsOH: p-toluenesulfonic acid

alkylation in HMPA/THF mixture (Sakai et al., 1986) in good yield. The protecting group was removed following the established protocol (Sakai et al., 1986; Camps et al., 1988). The resulted alchols *7* were partially hydrogenated over P-2 nickel with high stereoselectivity (Brown and Ahuja, 1973; Jonston and Dehlschlager, 1982), yielding essentially homogeneous products (2) which were finally converted into the corresponding acetates *1*.

The bromo ether *5b* was accessed by a routine procedure (Newkome et al., 1985; Anantanarayan et al., 1986). The iodo ether *5a* was obtained similarly from 4-iodo-1-butanol (*4a*), which, in turn, was prepared with an optimized literature method (Long and Freeguard, 1965; Odham and Samuelsen, 1970). The ring opening of tetrahydrofuran in this way was convenient, and led to the almost pure form of the crude alcohol *4a*. Compound *5a*, a useful synthetic intermediate is applied widely in pheromone research (Oshima et al., 1973; Hayashi and Midorikawa, 1975; Sakai et al., 1986).

2(4-Iodobutoxy)tetrahydro-2H-pyran (5a). Glassware and sodium borohydride used in this experiment were dried at 110°C for 2 hours. THF was freshly distilled from lithium aluminium hybride. A solution of iodine (5.08 g, 20 mmol) in THF (20 mL) was added dropwise to the stirred and cooled suspension of sodium borohydride (0.57 g, 15 mmol) in THF (5 mL) at +10°C under nitrogen. After ceasing of the violent hydrogen evolution

Table 2

Efficiency of different lures on capturing of males of *Cossus cossus*

Sign of the blend	Compounds and doses	Captured males/trap
A	*1a*, 200 μ + *1b*, 50 μ	2.0
B	*1a*, 200 μ + *1b*, 50 μ + *2a*, 50 μ	2.6
C	*1a*, 200 μ + *1b*, 50 μ + *2b*, 50 μ	2.2

Number of replications: 5

Table 3

Catches of microlepidopterous species in traps baited with components of synthetic sex pheromone of *Cossus cossus* (20 June – 11 July 1986, Halásztelek)

Lure	Number of males in the traps			
	Caryocolum alsinellum	*Scrobipalpa atriplicella*	*Depressaria depressana*	*Coleophora versulella*
1a 200 μg	24	3	0	0
1b 50 μg	0	0	128	16

Number of replications: 3

Table 4

Efficiency of mass-trapping at Kecskemét-Szarkás (9 June – 2 September 1988), and at Halásztelek (10 June – 1 September 1988)

Locality	Culture	Territory (ha)	Total number of males in the traps	Number of males/trap	Number of male pupae skins
Kecskemét-Szarkás	Apricot	4	20	5.0	12
Halásztelek	Apple	36	184	5.1	360

the reaction mixture was heated to reflux temperature, stirred under reflux until the brown color of iodine faded (1-2 hours), and then cautiously poured into a stirred mixture of 10% hydrochloric acid, crushed ice, and ether. The organic phase was separated, the acidic aqueous layer was extracted with ether. The combined organic solutions were washed with saturated $NaHCO_3$ solutions, 10% $Na_2S_2O_3$ solution, brine, dried over $MgSO_4$ and evaporated under reduced pressure. The oily residue, crude 4-iodo-butanol (*4a*), was dissolved in dry dichloromethane (60 mL), DHP (3.3 mL, 36 mmol) and TsOH (0.2 g) was then added with cooling. The solution was stirred under nitrogen for 3 hours, washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and finally concentrated

under diminished pressure. The resulted crude product was purified by flash chromatography with benzene as eluent to give oily *5a* (6.1 g, 72%). IR (film) ν_{max}: 1140, 1090, 1040 cm^{-1}; ^{1}H-NMR : 1.3-2.2 (10H, m, CH_2), 3.1-4.1 (6H, m, CH_2I, CH_2O), 4.6 (1H, m, CHO_2).

Field tests

Trials with different attractant blends were carried out between 20 June and 11 July, 1986 at Halásztelek (Pest-county, near Budapest) in an old, rather infected large-scale apple orchard with different apple varieties. The tested blends are shown in Table 2. The pheromone dispensers here and in our other trials were rubber septa. The number of replications was 5. The traps were arranged in one line, at a distance of 53 m from each other. The trap design was "Reanal" type fully-roofed trap (Sziráki, 1987). The sticky bottom of the traps was weekly changed.

In our trial in 1987 we investigated which compounds attract the captured non-target species flown regularly into the traps. Because the traps baited with blend "A" captured the same non-target species as the traps baited with the other two blends, in this trial we investigated only the attractant effect of the compounds *1a* and *1b*. In this case the number of replications was 3.

In the trap design trial we compared the half-roofed traps with the fully-roofed ones. The shape of the tested traps was such as written earlier (Sziráki, 1987), but the surface of the sticky bottom of the half-roofed traps for the goat moth was the same as that of the fully-roofed ones (480 cm^2), usually only 220 cm^2. The number of replications was 6, the working period 9 June – 17 August, 1987; culture: apple, locality: Halásztelek. The compounds of the lures were the same as in blend "B", but their amount was five-times more, i.e.: 1 mg of *1a*, 0.25 mg of *1b*, and 0.25 mg of *2a*.

The mass-trapping trials were carried out in 1988 at Halásztelek, in an isolated apple orchard of 36 ha, and at Kecskemét-Szarkás (Bács-Kiskun-county) in an isolated apricot orchard of 4 ha territory.

The trap density was 1 pheromone trap/ha in both orchards. These traps worked from the end of May to the end of August. The traps were baited with dispensers containing 1 mg of compound *1a*, 0.25 mg of *1b*, and 0.25 mg of *2a*. The dispensers were changed in every 3 weeks. At Halásztelek the sticky bottom of the traps was renewed weekly, while at Kecskemét-Szarkás in every 3 weeks. On the research field at Kecskemét-Szarkás we examined all of the trees, and counted the male pupae skins to assess the population density of the goat moth. At Halásztelek we investigated only 10% of the trees and from these figures we calculated the total number of the male pupae skins.

Results and Discussion

The captures by the three examined compositions are shown in the Table 2.

The blend "B" proved to be the optimal one. Addition of *2a* alcohol to lure "A", which was almost the same as the lure used by Pasqualini and his co-workers in their

mass-trapping trials (Pasqualini et al., 1982a, 1982b, 1985; Bratti et al., 1988), increased the captures. The difference between blends "A" and "B" is significant ("t"-test, p = 1%). Addition of *2b* alcohol to the lure "A" does not increased the male captures significantly.

In the sex pheromone traps for *C. cossus* we regularly found four non-target moth species (Table 3). *Caryocolum alsinellum* and *Scrobipalpa atriplicella* (both *Gelechiidae*) were lured by *1a*, while *Depressaria depressana* (*Oecophoridae*) and *Coleophora versulella* (*Coleophoridae*) by compound *1b*. These microlepidopterus species are not of economic importance, but significant part of the sticky surface may be occupied by them in the goat moth traps.

The coumpound *1a* as attractant of *S. atriplicella* (Roelofs and Comeau, 1970), and *1b* as attractant of *C. versulella* (Capizzi et al., 1985) were described before. Sex attractant for *D. depressana* and *C. alsinellum* was not reported before.

The results of the trap design trial showed, that the catches by the tested type of traps do not differ significantly from each other. In average 3.4 males flew into the half-roofed-, and 3.1 males into the fully-roofed traps.

The results of mass-trapping of European goat moth with pheromone traps (Table 4) show that this method might be effective only at very low population density.

In Italy, where all of the trees in the *Cossus*-infected apple orchards are usually damaged by the caterpillars of this moth, only 35% decrease of population density was achieved in this manner by about 15-20 traps/ha (Pasqualini et al., 1985) and 50% by 30 traps/ha (Bratti et al., 1988).

At Kecskemét-Szarkás, where 2% of trees was infected by goat moth, the four traps caught more males (20 exemplares) than the number of pupae skins (12 exemplares) found in the apricot orchard of 4 ha territory. (It is possible that some males flew into our traps from other territories – in spite of the isolated situation of the orchard.)

Pasqualini and his co-workers concluded that the goat moth pheromone traps have a very limited range of attraction; not much more than the width of a tree canopy (Pasqualini et al., 1982a). The results of our trial at Kecskemét-Szarkás show that at low population density this range might be probably more than 100 m.

At Halásztelek, where 18% of the trees was infected by *C. cossus*, the number of caught males was only about half of the number of males emerged on the research field (Table 4). Probably in the case of a higher trap density the percentage of caught goat moths would be higher also here, but it could not be economical in practice in Hungarian large-scale orchards.

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Comparative Population Studies on the Eriophyid Mite *Eriophyes vitis* (Pgst.) Infesting Vineyards in Egypt

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Eriophyes vitis (Pgst.) is host-specific mite and considered to be a serious pest infesting vineyards.

Three physiological races of this mite are known; they are morphologically identical and can be separated from one another only by the type of damage that they cause.

The grape vine varieties comparatively differed in their susceptibility. Banaty was highly infested with grape bud mite followed by Romey while Balady was slightly infested. This race reached its peak of infestation in March.

Regarding the grape erineum mite results showed that Balady was heavily infested followed by Romey, while Banaty was the least infested variety.

Concerning the grape curl mite, data showed that Romey was the most infested variety followed by Balady while Banaty was moderately infested.

In recent years the eriophyid mite *Eriophyes vitis* (Pgst.) become more numerous in vineyards. This probably due mainly to the increased use of none acaricide and fungicides, although other factors such as the more luxuriant tree growth in modern orchards and the use of pesticides that are toxic to predators have also contributed (Newkirk and Keifer, 1971; Carmona, 1971 and Osman, 1975a).

Three physiological races of this mite are known, they are morphologically identical and can be separated from one another only by the type of damage that they cause. One race damage the buds another produces galls on leaves causing erineum patches on the leaves and the third causes leaf curl.

The gall of this study is to throw the light on the population abundance of these races and the susceptibility of different grape vine varieties for eriophyid mites.

Materials and Methods

This work carried out in Menoufia Governorate. Three grape vine varieties which widely cultivated and about 5 years old were chosen for this investigation, these are Balady, Banaty and Romey.

Samples of twenty buds and forty leaves were randomized collected from each of the three grape vine varieties at regular intervals of two weeks allover the year. The collected buds were examined as well as the both surfaces of the leaves for the three physiological races of *Eriophyes vitis* (Pgst.) by the aid of stereomicroscope.

Results and Discussion

The results obtained on the population densities are shown in Tables 1, 2 and 3.

The incidence of the race known grape bud mite has become serious in several viticultural regions, feeding by this mite causes a distortion of the primordial buds and disrupts subsequent growth, shoots are distorted and tend to become flattened. Also, mites damaged to buds give rise to stunted bunches with a poor berry set (Osman, 1985b and Krantz, 1978). Data in Table 1 indicated that grape vine varieties comparatively differed in their susceptibility for the bud mite. Banaty was heavily infested, Romey was moderately while Balady was slightly infested. This race showed that the peak of infestation occurred in March.

The feeding activities of the grape erineum mite on the undersurface of the leaf gives rise to the characteristic galls on the upper surface of the leaf. Wherever the mite feeds, identations appear which develop into galls and damaged leaf cells multiply and produce fine white hairs that form a wolly covering. The results obtained in Table 2 showed that Balady was highly infested with this race of mite followed by Romey while Banaty was slightly infested. The peak of this race occurred in July.

The grape curl race merely causes the leaves to curl and does not produce the appearance of abnormal hair growth or the formation galls on the leaves. Romey was highly infested with this race followed by Balady while Banaty was moderately infested (Table 3). The peak infestation occurred in August (Chakrabarti and Mondal, 1983).

Table 1

Average number of grape bud mite (*Eriophyes vitis*) per one bud during the year

Month	Average number of mites per bud		
	Grapevine Varieties		
	Balady	Banaty	Romey
January	1.4	5.6	2.5
February	1.4	8.7	3.9
March	4.2	15.8	6.7
April	2.5	14.5	4.2
May	2.2	10.2	3.7
June	1.9	7	1.6
July	1.1	5.5	1.4
August	0.8	4.4	1.3
September	0.5	3.2	1.1
October	0.0	2.4	0.8
November	0.0	1.3	0.0
December	0.0	0.0	0.0
Total	16.1	78.6	27.2

Table 2

Average number of grape erineum mite (*Eriophyes vitis*) per one leaf during the year

Month	Average number of mites per leaf		
	Grapevine Varieties		
	Balady	Banaty	Romey
January	3.2	0.0	0.0
February	50.8	10.8	15.9
March	100.6	15.9	20.5
April	120.5	18.4	40.5
May	150.7	20.3	50.6
June	185.8	22.4	100.5
July	200.5	25.3	150.7
August	180.9	25.2	80.8
September	100.7	10.5	20.6
October	50.4	–	–
November	–	–	–
December	–	–	–
Total	1144.1	148.8	480.1

Table 3

Average number of grape curl mite (*Eriophyes vitis*) per one leaf during the year

Month	Average number of mites per leaf		
	Grapevine Varieties		
	Balady	Banaty	Romey
January	1.9	0.0	3.9
February	15.2	9.4	30.4
March	30.2	14.3	50.6
April	50.6	25.4	70.4
May	80.4	40.6	100.9
June	95.4	80.9	150.4
July	160.7	90.4	200.9
August	200.5	112.4	250.4
September	150.4	80.6	200.9
October	40.2	20.2	100.8
November	–	–	–
December	–	–	–
Total	825.5	474.2	1159.6

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