VOLUME 16 • NUMBER 1—2 • 1981

acta Phytopathologica

ACADEMIAE SCIENTIARUM HUNGARICAE

AKADÉMIAI KIADÓ
BUDAPEST

Acta Phytopathologica

Academiae Scientiarum Hungaricae

Szerkeszti:
KIRÁLY ZOLTÁN

A szerkesztő bizottság tagjai:

Jermy Tibor, Klement Zoltán, Matolcsy György, Milinkó István,
Nagy Barnabás, Szepessy István, Vörös József

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Megrendelhető a belföld számára az Akadémiai Kiadónál (1363 Budapest Pf. 24.), a külföld számára pedig a „Kultura" Külkereskedelmi Vállalatnál (1389 Bp. 62, P.O.B. 149).

Acta Phytopathologica Academiae Scientiarum Hungaricae. A quarterly review of the Hungarian Academy of Sciences.

Papers are published mainly in English.

Editorial Office: 1525 Budapest, P.O.B. 102.

Distributor: "Kultura" Foreign Company (1389 Budapest 62, P.O.B. 149) or its representatives abroad.

Acta Phytopathologica Academiae Scientiarum Hungaricae. Vierteljahresschrift der Ungarischen Akademie der Wissenschaften.

Aufsätze erscheinen in englischer bzw. in deutscher, französischer oder russischer Sprache.

Redaktion: 1525 Budapest, P.O.B. 102

Bestellbar bei »Kultura« Außenhandelsunternehmen (1389 Budapest 62, P.O.B. 149) oder seinen Auslandsvertretungen.

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Acta Phytopathologica

Academiae Scientiarum Hungaricae

Volume 16

Akadémiai Kiadó, Budapest

1981

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Pathogenicity of *Choanephora cucurbitarum* on Chilli *(Capsicum annuum)* and Possibility of Its Chemical Control

By

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Choanephora cucurbitarum was isolated from different diseased parts of chilli plants (*Capsicum annuum* L.), collected from blight affected crops. Reactions of *Balijhuri chilli*, a local cultivar of *Capsicum annuum*, to *C. cucurbitarum* were determined following inoculation of seeds, seedlings, mature plants and detached plant parts. The fungus did not affect seed germination, nor did it cause any pre- and post-emergence diseases. No evidence of seed transmission of the fungus was found. Inoculation of injured leaves, side shoots and fruits of nine week old plants resulted in infection. However, inoculation of injured stems did not cause infection. Die-back and fruit-rot symptoms observed following artificial inoculation in the glasshouse, were similar to the symptoms observed in the field.

Five fungicides (Copper oxychloride, Dithane M-45, Plantvax, Vitavax 200 and Brassicol) with four concentration levels (1000, 2000, 2500 and 5000 ppm) were assayed for their inhibitory effect on the growth of *C. cucurbitarum*. Copper oxychloride, Dithane M-45 and Plantvax proved ineffective. Five thousand ppm of Vitavax completely inhibited fungal growth and Brassicol arrested growth at 1000 ppm.

Chillies (*Capsicum* spp.) are reported to be attacked by *Choanephora cucurbitarum* at all stages of growth (TALUKDAR, 1968). The fungus is reported to cause blossom blight of peppers (WEBER, 1932), die-back (DASTUR, 1922), wet rot (SINHA, 1940) and a soft-rot of the stems or side shoots of chilli plants (TALUKDAR, 1968). Low temperature and high humidity greatly favour the asexual reproduction of the fungus (BARNETT and LILLY, 1955) and thereby an increase in disease severity. Such ideal weather conditions occasionally occur in Bangladesh during October through March when chilli is cultivated. The only report to date of the occurrence of *C. cucurbitarum* in Bangladesh is that of TALUKDAR (1974). In November, 1976 an epiphytotic was reported in the districts of Mymensingh and Bogra. The present work was designed to investigate: i) the disease on Balijhuri, a cultivar of chilli widely grown in Bangladesh, following inoculation with *C. cucurbitarum* and ii) evaluate in the laboratory, the efficacies of five fungicides in inhibiting the growth of the pathogen, *C. cucurbitarum*.

* This paper is based on the senior author's M. Sc. Ag. thesis, submitted to the Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Materials and Methods

Isolation of the pathogen was done by employing the tissue-plating method both on PDA (Potato Dextrose Agar) and in moist chambers. After single spore transfer to PDA, the fungus was identified as *Choanephora cucurbitarum* (Berk. and Rav.) Thaxt. (FITZPATRICK, 1930; BARNETT, 1960).

Inoculation of chilli cv. Balijhuri with C. cucurbitarum

Ripe chilli fruits were collected from growers in Marichar Char, Churkhai, and Boyra in the district of Mymensingh. Seed viability was tested using the standard blotter technique (ISTA, 1966) as well as by sowing seeds in pots containing sterile soil. Germination in the blotter and soil tests was 96% and 85%, respectively.

The capacity of the fungus to cause: 1. Seed-rot, pre- and post-emergence mortality of seedlings; 2. collar rot and 3. symptoms on stems, leaves, flowers and fruits was investigated:

1. Seed-rot, pre- and post-emergence mortality of seedlings was tested by both the blotter and pot-soil inoculation methods. Blotter technique: freshly extracted seeds of the test cultivar were plated on moist blotter previously dipped in a fungal suspension for one three different periods of time. Pot-soil method: a 5-day-old oat culture of the fungus was mixed with the top two inches of soil of individual pots at the rate of 0.5% by weight.

2. Collar rot: one week old seedlings were inoculated at the collar region with a 5-day-old oat culture of the fungus.

3. Three hundred and twenty four detached plant parts (72 pieces of cut stems, leaves, flowers and fruits each and 36 pieces of growing tips) from nine week old plants were placed into 108 moist chambers. Fifty per cent of each category was autoclaved (121 °C, 15 p.s.i. and 20 minutes) before inoculation. Inoculation was made with and without injury.

Ability of the fungus to cause infection of the aerial parts of whole potted plants was tested by inoculating different sites such as leaves, side shoots, flowers, fruits and stems at different plant ages (2, 4, and 9 weeks).

In all the above cases, inocula in the form of 5 mm ∅ mycelial blocks were prepared from 5-day-old PDA cultures of the fungus. Fungal suspensions were prepared in a Waring blender at 1500 rpm for 5 minutes. Following inoculation, both inoculated and non-inoculated plants were incubated under humid conditions for 24 hours by keeping them covered with polyethylene bags.

Laboratory assay of fungicides

Inhibition of mycelial growth of *C. cucurbitarum* was tested by five fungicides (Table 1). The concentrations used were 1000, 2000, 2500 and 5000 ppm. Both acidified (2 drops of 50% lactic acid per ml) and non-acidified PDA medium was used. Mycelial blocks (5 mm ∅) from 5-day-old PDA culture of the fungus,

Table 1

Specification of the fungicides

Commercial name	Chemical name	Group	Percentage of active ingredient
Copper oxychloride	Copper oxychloride	Copper fungicides	50
Dithane M-45	Manganous ethylene bis dithiocarbamate + Zinc ions	Dithiocarbamate	80
Plantvax	2,3-dihydro-S-carboxanilide-6-methyl-1,4-oxanthiin-4,4-dioxide	Oxanthiin	75 W. P.
Vitavax 200	5,6-dihydro-2-methyl-1,4-oxanthiin-3-carboxanilide	Oxanthiin	75 W. P.
Brassicol	75% Pentachloronitrobenzene	Benzene compounds	75

soaked completely with chemical were placed at the centre of the plates. Linear measurements of the growing colonies were taken at 24 hrs intervals. This assay was repeated twice.

Results

Reaction of chilli cv. Balijhuri to C. cucurbitarum

On moist blotter and in infested pot soil, there was no difference between treated and untreated regarding seed germination (Table 2) indicating that the fungus affected neither seed viability nor seed germination. Inoculation of one-week-old seedlings at the collar region did not result in any disease development.

The autoclaved plant parts failed to support fungal growth. Inoculation of the unautoclaved surface sterilized detached and injured plant parts resulted in infection, but uninjured plant parts were, with a single exception, not infected (Table 3). Fig. 1 shows infected fruits following inoculation after injury. The results of this experiment indicate that the ability of plant parts to support growth of *C. cucurbitarum* is lost in course of heating in the autoclave. Failure of infection in the uninjured plant parts indicates that infection mainly takes place through wounds.

Table 2

Effect of *Choanephora cucurbitarum* on germination of chilli seeds

Blotter test Intervals (hours)	Mean percentage of germination	
	Inoculated	Control
16	81.2	88.0
20	94.4	92.0
24	89.2	84.0
Inoculum mixed in soil		
Laboratory	71.2	73.4
Glasshouse	80.0	85.0

Table 3

Incidence of infection on unautoclaved and surface sterilized detached plant parts inoculated with *Choanephora cucurbitarum*

Place and mode of inoculation	Number of inoculations		Number of infection	
	A. Real	B. Control	A.	B.
Stem pieces				
Cut ends	15	3	15	0
Middle part injured	6	3	6	0
Middle part uninjured	6	3	1	0
Leaves				
Cut end of petiole	15	3	15	0
Lamina injured	6	3	6	0
Lamina uninjured	6	3	0	0
Growing Tips: Cut ends	15	3	15	0
Flowers				
Cut end of pedicel	15	3	15	0
Thallamus	15	3	15	0
Fruits				
Cut end of peduncle	15	3	12	0
Middle part injured	6	3	6	0
Middle part uninjured	6	3	0	0

Results of the inoculation of various parts of whole plants at different plant ages (Table 4) indicate that mature plants at the fruiting stage are much more susceptible than younger plants. In the case of nine-week-old plants, discolouration of inoculation sites on leaves, side shoots and fruits were observed. These then withered and later fell off. Symptoms developed faster on leaves and side shoots than on fruits. In the case of side shoots, infection started with an elongated

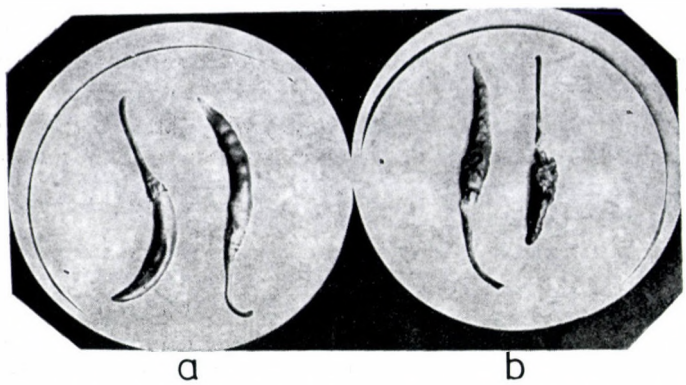

Fig. 1. Reaction of detached chilli fruits to *Choanephora cucurbitarum*. a. control, b. infected fruits showing lesion

brownish lesion around the point of inoculation. The lesion then extended in an upward and downward direction causing the shoots to blight. Stems did not become infected. Fungal growth, showing silvery white sporangiophores bearing sporangial heads was observed on the infected plant parts.

Re-isolation of the fungus from different infected plant parts confirmed the pathogenicity of *C. cucurbitarum*.

Table 4

Incidence of infection following artificial inoculation of chilli plants with *Choanephora cucurbitarum*

Age of plant (week)	Place and mode of inoculation	No. of inoculation	No. of infection	Method of inoculation
2	Growing tips uninjured	40	0	Mycelial block
	Control	20	0	Agar block
4	Growing tips injured	100	0	Mycelial block
	Leaves injured	100	27	Mycelial block
	Stem injured	33	0	Mycelial block
	Control	50	0	Agar block
	Whole plant uninjured	33	0	Suspension
	Control	11	0	Water
9	Leaves injured	60	60	Mycelial block
	Point of branching injured	30	30	Mycelial block
	Fruits injured	35	35	Mycelial block
	Shoots injured	20	20	Mycelial block
	Stem injured	6	0	Mycelial block
	Control	75	0	Agar block
	Whole plant injured	24	24	Suspension
	Control	6	0	Water

Discussion

It is the opinion of the chilli growers that crops planted during late October or early November are rarely attacked by the disease. The results of this study are in agreement with the findings of DASTUR (1922) who observed that the severity of die-back in chilli, caused by *C. cucurbitarum*, was less when the crop was transplanted late.

The weather data were collected for the day of the first report of the disease. They show that during the later part of the day, and early part of the night, the prevailing temperature was high. There was a sudden fall in the temperature late night accompanied by high humidity and dense fog. These conditions are ideal for abundant sporulation of *C. cucurbitarum* (BARNETT and LILLY, 1955); increasing the inoculum of the fungus tremendously. VESTAL's (1946) findings support this view. He reported from India on the abundant occurrence of *C. cucurbitarum* on chilli under low temperature and high humidity conditions.

Minute insect injury was observed at the base of young side shoots of infected plants in the field. The insects were not found on the infected plants during day time. It is presumed that such insect injury is necessary for subsequent fungal infection. In the present investigation, pin-prick injuries were necessary for infection. Results obtained by FERRY and CUTHBERT (1973), working on the incidence of pod rot of cowpea by *C. cucurbitarum*, are in close agreement with the observation of the author. According to them, insect damage provided points of entry for the fungus.

The result of the seed-inoculation experiments indicate that the fungus is not seed-borne. No information in the literature was found concerning seed-transmission of *C. cucurbitarum.*

The fungus is a facultative parasite and is able to survive on debris in the soil (SINGH, 1937). However, in the present study the fungus failed to grow on inoculated, autoclaved plant parts. The author can find no explanation of the failure of the fungus to grow on plant parts killed by heating in the autoclave.

Laboratory assay of fungicides

In preliminary tests, Copper oxychloride, Dithane M-45 and Plantvax proved ineffective in inhibiting fungal growth. Brassicol completely inhibited growth at all concentrations and Vitavax partly inhibited fungal growth.

The results of further tests with Vitavax 200 and Brassicol are given in Table 5. Vitavax 200 used at low concentration, was fungistatic, while at 5000 ppm it was fungicidal. Brassicol proved to be fungicidal even at a concentration of 1000 ppm. Vitavax and Brassicol are common seed-treatment fungicides against diseases such as loose smut and bunt of wheat (BABAYAN, A. *et al.*, 1977) and leaf stripe of barley (EFREMOVA, 1977) etc. While Vitavax as a foliar spray restricts the growth of *Alternaria alternata* on chilli (CRISAN, 1977), the usefulness of Vitavax and Brassicol in the control of Choanephora-blight of chilli under field conditions, has yet to be investigated.

Table 5

Effect of Vitavax 200 and Brassicol on the growth of *C. cucurbitarum* on PDA

Fungicide concentration (ppm)	Linear growth measured at 24 hrs intervals (cm)							
	Vitavax 200				Brassicol			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
0	2.2	4.0	6.1	7.2	2.2	4.0	6.1	7.2
1000	0.4	3.9	6.4	7.7	0.0	0.0	0.5	1.0
2000	0.2	3.0	5.5	7.0	0.0	0.0	0.0	0.0
2500	0.0	1.2	3.5	5.8	0.0	0.0	0.0	0.0
5000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Acknowledgements

The author extends his gratefulness to Professor dr. agro. J. E. HERMANSEN and Dr. JOHN HOCKENHULL, Department of Plant Pathology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark for their valuable suggestions, criticism and for meticulous correction of the present manuscript.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 9–14 (1981)

Control of *Fusarium* Wilt of Tomato with an Integrated Nitrate-Lime-Fungicide Regime

By

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Nitrate-nitrogen drenched on potted tomatoes grown on a high lime, benomyl regime, provided complete control of the symptoms of *Fusarium* wilt *(Fusarium oxysporum* f. sp. *lycopersici)* on the susceptible cultivar Primset by simultaneously using the following practices: (1) supplying nitrogen in the nitrate form at the rate of 420 ppm; (2) liming the soil to the range of pH 7.6 with $Ca(OH)_2$; and (3) drenching the roots with benomyl at a concentration of 300 mg/liter. The integrated application of nitrate, high lime, and fungicide appears to be the first report on the complete control of the symptoms of *Fusarium* wilt of tomato. Benomyl, lime and nitrate each contributes to partial disease control but only by using an integrated approach one can check the wilt symptoms almost perfectly.

Certain chemicals and nutritional factors reduce the development of *Fusarium* wilt in the host (ALBERT, 1946; GOULD and MILLER, 1970; JONES and OVERMAN, 1971; and WOLTZ and ENGELHARD, 1973). JONES and WOLTZ (1968) have demonstrated the control of *Fusarium* wilt (race 2) of tomato by liming the soil, WOLTZ and ENGELHARD (1973) had the same result with *Chrysanthemum.*

Nitrate nitrogen supply reduced the development of *Fusarium* wilt in tomato according to HUBER and WATSON (1974), and HUBER (1980), and in cotton according to ALBERT (1946). However, the severity of *Fusarium* wilt in other host plants, such as cabbage and watermelon, was increased by supplying nitrate nitrogen (HUBER, 1980). BIEHN and DIMOND (1970) and ATKINSON and ADAMSON (1977) reduced the symptoms of fusarial wilt of tomato with benomyl, and THANASSOULOPOULOS *et al.* (1970) had the same result with watermelon and tomato. According to the literature, no nutritional or fungicidal treatments, or combinations, have provided complete control of the symptoms of *Fusarium* wilt of tomato.

The aim of this research was to determine the ability of obtaining complete control of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. and Hans. race 1 of tomato in an integrated system in which the partially effective factors of a fungicidal chemical, lime and nitrate nitrogen were combined.

Materials and Methods

Greenhouse experiments

A wilt susceptible cultivar of tomato, Primset, was used. Seeds were sown into soil, two weeks later, seedlings were transplanted to a limed soil: sand mix. (3 : 1) in 10 cm pots. Plants, four-week-old, were inoculated by up-rooting them, dipping the roots in inoculum, and repotting the plants. The inoculum was a suspension of a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici.* The pathogen was grown on PDA at 28 °C. The treatments were replicated three times with four plants per replication. Dry $Ca(OH)_2$ at 2 g/kg of soil was thoroughly incorporated in the soil and allowed to equilibrate for one week prior to potting. Fungicide was evaluated in a high-lime, NO_3-nitrogen regime. The fungicide benomyl, in the form of Chinoin-Fundazol 50 WP, was used at the rate of 300 ppm. Pots were drenched with fungicide, one day before inoculation, at the rate of 60 ml/pot. Potted tomato plants were fertilized with Hogland's solution, according to our previous experiences (SARHAN and KIRÁLY, 1981), twice weekly with 40 ml/pot of nutrient solution at rate of 420 ppm nitrogen in nitrate form, plus micronutrients.

The severity of disease was assessed after four weeks from inoculation, using the leaf index and the vascular index. The stems were cut and examined for vascular browning after the fourth week. The leaf grade was determined by rating each leaf for severity of symptoms on a 0–100 scale according to EDGINGTON and WALKER (1958).

The pH of the soil was determined at planting time and at the end of the experiments. The soil samples for determination of pH were prepared with 50–50 (v/v) soil-deionized water which had equilibrated one hour. The initial pH of the soil before liming was 6.4 and after liming was 7.6.

Field test

A small area 3 × 10 m plowed to 20–25 cm deep, was used. The field was divided into three plots: control (untreated and uninfected), untreated but infected and infected and treated with and integrated nitrate-lime-fungicide regime. Tomato seedlings, 14-day-old, were transplanted into each plot. In the plot, treated with integrated systems soil was amended with dry $Ca(OH)_2$ at 0.5 kg/m^2, in the order to establish soil with high pH value. The dry $Ca(OH)_2$ was spread over the soil surface and immediately incorporated with a spade to a depth of 15 cm and allowed to equilibrate for one week prior planting. The soil moisture was then increased to approximately field capacity to facilitate the lime reaction and the concomitant pH change. Using the method described by GABAL (1980), nitrogen was added at level of 20 mgN/100 gm soil (or 20 gm N/m^2), in the nitrate form, $Ca(NO_3)_2$ and KNO_3, before the plot was set with tomato seedlings. The systemic

fungicide benomyl (Chinoin-Fundazol 50 WP) was applied to the soil as drenched 2 gm/m^2 of active ingradient, the fungicide was supplied one day before inoculation.

Each experimental plot consisted of four 3 meter rows with each row containing 10 plants which were set in the plots at May 8. In both the treated and untreated plots plants were inoculated 2 weeks after transplanting into plots. The roots of the plants were injured twice just prior to inoculation by using a small spade under soil level and about 10 cm deep on the opposite side of the plant. The conidial-mycelial suspension was poured into the slits, in the soil around each plant. The suspension was prepared from 10 days old shake culture grown at 28 °C according to WOLTZ and JONES (1968).

The incidence of *Fusarium* wilt was estimated two times through the experiment (16 August and 28 Sept.) by examining the plants for wilt symptoms. Internal vascular browning was detected by making a longitudinal section in the stems and measuring the discoloration. Also the tomato fruits were collected from each plant and weighted. Soil pH determinations were made as mentioned above.

Results

Greenhouse tests

The application of NO_3-nitrogen, hydrated lime, and benomyl decreased the rate of development and incidence of *Fusarium* wilt caused by race 1. The most significant result of this investigation was the demonstration that high NO_3-nitrogen supplied to potted plants grown under the high lime, benomyl-fungicide regime produced healthy plants which were completely free of leaf and stem symptoms of *Fusarium* wilt (Table 1 and Fig. 1) as compared to the untreated infected plants. Comparing the percentage of the vascular discoloration and the

Table 1

Effect of NO_3-nitrogen, benomyl fungicide, and lime on *Fusarium* wilt development in tomato

Treatments	Fusarium wilt[a] index	Vascular discoloration[b] in per cent
Control (H_2O)	77.5	90.5
Nitrate + benomyl	2.5	11.9
Nitrate + lime	12.5	23.3
Benomyl + lime	8.5	20.5
Nitrate + benomyl + lime	0	0
$LSD_{0.05}$	4.5	6.9

[a]Wilt index on 0—100 scale where 0 = no disease and 100 = dead plant.

[b]The percentage of browning means the ratio of the length of stem with vascular discoloration to the whole stem length.

Fig. 1. Fusarial wilt symptoms of tomato plant as affected by NO_3-nitrogen, lime and benomyl regime. A: untreated infected plant shows the wilt symptoms. B: infected plant with integrated control shows no wilt symptoms

wilt index between the treatments (Table 1) demonstrated a good reduction in disease development by the use of high NO_3-nitrogen fertilization and benomyl as compared to the high NO_3-fertilization and high lime. Similar comparison between the plants grown under high lime and benomyl supply demonstrated the additional wilt control provided by the fungicide. The initial pH of the soil in the high lime treatments was 7.6 and at the end of the experiments was 7.9 whereas the unlimed treatments were 6.4 and 6.6 initially and at the end, respectively.

The additive effects of NO_3-nitrogen, benomyl, and the altered soil pH were evident because all plants in the four replications were free of symptoms of wilt only if they received a disease control with integrated systems.

Field test

The field experiment was carried out in order to apply integrated control measures under practical circumstances. With an integrated NO_3-nitrogen, lime, and benomyl regime, basically the same result was obtained in the field experiment (Table 2) as in the greenhouse. Inoculated plants in the plot with integrated systems

Table 2

Incidence of *Fusarium* wilt, vascular browning, and the yield of tomatoes as affected by integrated NO_3-nitrogen, lime, and benomyl regime in the field

Treatments	16 August		28 September	
	Mean vascular browning cm	Mean fruit weight kg/plant	Mean vascular browning cm	Mean fruit weight kg/plant
Control	0	0.240	0	0.356
Untreated-infected	11.60	0.163	25.2	0.240
Treated-infected (integrated systems)	0	0.398	0	0.581
$LSD_{0.05}$	1.45	0.040	3.13	0.080

were free of wilt symptoms as compared to the plants in the untreated plot checked twice during the season.

The average weights of the fruits of tomato increased significantly as compared to the untreated and to the control plots. The initial pH of the unlimed soil was 6.2 and the limed soil was 7.4. At the end of the experiment pH values were 6.5 and 7.6, respectively.

Discussion

Complete control of symptoms of *Fusarium* wilt of tomato was accomplished by simultaneously using factors or conditions which, separately, each provide a significant reduction in disease symptoms. Our previous results (SARHAN, BARNA and KIRÁLY, 1980; and SARHAN, 1980) demonstrated that *Fusarium* wilt of tomato, supplied with NO_3-nitrogen or grown in limed soil, can be significantly decreased. These results agree with those of ENGELHARD and WOLTZ (1973) on chrysanthemums. The effect of NO_3-nitrogen nutrition, lime, and benomyl in the present experiment was shown to be additive and resulted in controlling *Fusarium* wilt of tomato. Others (JONES and OVERMAN, 1971) have reported that *Fusarium* wilt of tomato was reduced by raising the soil pH plus fumigation. The nitrification increased as the soil pH was increased. In contrast, acidic soil, high nitrogen, warm, and moist weather were favourable for development of *Fusarium* wilt of safflower (CHAKRABARTI and BASUCHAUDHARY, 1978).

A clear reduction of wilt symptoms was evident in tomato plants drenched with benomyl before inoculation (BIEHN and DIMOND, 1970; and FUCHS et al., 1970). Benomyl acts fungistatically in the soil and in the vascular tissue of plant to prevent disease symptoms. Also, the juvenility and greening of the tomato leaves due to the high NO_3-nutrition may be associated with the high degree of resistance to *Alternaria solani* (KIRÁLY, 1976). High protein content may also have some role in the resistance of the plants (SARHAN and KIRÁLY, 1981).

The high nitrogen nutrition and the liming procedures and perhaps benomyl may alter the physiology and nutrition of both host and parasite in a complex manner.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 15—21 (1981)

The Effect of "N-serve" on Reducing Root-rot Disease of Wheat Seedlings Caused by *Fusarium graminearum*

By

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Greenhouse pot experiments were conducted to study the effect of nitrapyrin (N-serve) applied together with three levels of NH_4-N (70, 284 and 630 ppm where 284 ppm corresponds to the standard concentration) in Hoagland solution on the root-rot disease of a wheat cultivar caused by *Fusarium graminearum*. The results indicated that nitrapyrin reduced disease severity and therefore, increased the number of seedlings in the infected pots. Seedling vigour seemed to be less reduced in nitrapyrin-treated infected plants.

The nitrate reductase activity, free amino acids and protein content of the plants were determined. The enzyme activity as well as the contents in free amino acids and in protein appeared to be higher in the nitrapyrin-treated plants.

One may suppose that the effect of nitrapyrin on the protein metabolism of host plants may influence disease resistance to *Fusarium graminearum*, by maintaining the tissues of wheat plants in a juvenile state.

The relationship between the nutritional status of plant and disease severity has been pinted out by many workers investigating foot and root-rot diseases of wheat caused by *Fusarium* species (HUBER *et al.*, 1965, ONUORAH, 1969; SMILEY *et al.*, 1970; 1972; HUBER and WATSON, 1974). It was found that application of different chemical forms of nitrogen had different effects on severity of foot and root-rot diseases of wheat.

Several studies show that the NH_4-N form, when maintained by the use of a nitrification inhibitor (N-serve), may considerably reduce the severity of certain plant diseases (HUBER and WATSON, 1972). The ammoniacal nitrogen fertilizers, when applied under field conditions, are rapidly converted to NO_3-N fertilizers, but the addition of nitrapyrin (N-serve) markedly reduces the loss of NH_4-N applied in autumn (WARREN *et al.*, 1975). This may also be an effective measure for controlling fungal diseases (BUSSARD and RUSSELL, 1979).

Evidence exists which shows that the use of the nitrification inhibitor (N-serve) in combination with NH_4-fertilizer reduces the stalk root of corn caused by *Gibberella zeae* (WHITE, 1978), take-all of wheat caused by *Ophiobolus graminеaus* (LEMAIR and JOUN, 1976) and *Helminthosporium sativum* disease of winter barley (SAUR and SCHÖNBECK, 1972).

In the light of the results mentioned above, I decided to study the effect of nitrapyrin (N-serve) on disease incidence caused by *Fusarium graminearum* as characterized by some morphologic and metabolic parameters of the host plant.

Materials and Methods

Fifteen surface-sterilized seeds of winter wheat cultivar GKF_2 were sown in 125 gram of washed sand, normally 1.5 cm deep, in small pots of 7 cm in diameter. The experiment consisted of two parts: in one part plants were treated with NH_4-N only and in the other part plants were treated with NH_4-N and nitrapyrin (N-serve).

The nitrification inhibitor 2-chloro-6(trichloromethyl) pyridine produced by Dow Chemical Co. was diluted and applied to the sand (20 ppm) with a pipette. The sand of pots treated with nitrapyrin was then mixed thoroughly with 5 ml of the N-serve solution before sowing the seeds. Three levels of NH_4-N nutrient were used in the experiments, namely 70, 284, and 630 ppm in Hoagland solution, where 284 ppm corresponds to the standard concentration. The chemical composition of the solutions was formulated and modified according to MASHAAL *et al.* (1976).

The inoculum suspension of *Fusarium graminearum* was produced by a bubble-culture method as described by MESTERHÁZY (1974). The pathogenicity of the inoculum was tested before use, and the suspension contained about 6×10^5 conidia/ml.

There were 10 replicate pots for each level of NH_4-N nutrient treatment. Five pots were infected and five pots served as controls in which the infecting suspension was replaced by distilled water.

The surface of the sand was watered with 20 ml of the suspension of *Fusarium*, essentially as described by MESTERHÁZY (1978). The seeds were placed on the infected sand in each pot and covered with a 15 mm thick layer of sand which was then moistened with 20 ml of distilled water.

The pots were watered with NH_4-N nutrient at one of three different NH_4-N concentrations as applied by HOAGLAND and SNYDER (1933). All pots received 30 ml of their specific solution twice a week, and were watered regularly each day with 20 ml of tap-water to maintain a desirable moisture condition for seedling development.

Seedling growth observations were made by counting the number of seedlings that emerged and survived over a 20-day-period. The plants were harvested after 20 days and seedling disease was assessed visually by scoring coleoptile and root infection using a 0–9 scoring system as described by MESTERHÁZY and ROWAISHED (1977). Seedling vigour was determined by measuring shoot and root length and dry matter content of the healthy and infected pots.

The protein content of leaves from healthy plants was determined by the standard Kjeldhal method ($N \times 6.25$). The phosphorus and potassium content was also determined. Free amino acids were analyzed using the ninhydrin method of CHING *et al.* (1974). Nitrate reductase activity was determined in 20-day-old healthy plants treated with and without nitrapyrin according to the *in vivo* method of KLEPPER (1973).

Results and Discussion

The root-rot infection seemed to decrease with increasing the nitrogen concentration when plants were treated with the NH_4-N nutrient solution alone, but no significant differences were observed (Table 1). In the nitrapyrin-treated seedlings less infection was observed at the standard concentration of NH_4-N (284 ppm). The disease severity was more pronounced in plants grown in pots without nitrapyrin. The extent of the reduction in disease severity of root-rot as a result of nitrapyrin treatment, was 48% as compared to 78%, at the 284 ppm treated.

The number of germinated seeds after infection (Table 1) also indicates similar results. Thus, the number of germinating seeds increased with increasing nutrient concentration, and in the case of the nitrapyrin-treated plants, higher germination percentages occurred. The rate of germination was highest at the standard nutrient concentration (284 ppm).

Generally, shoot and root length and dry matter content of the healthy plants increased with increasing N-concentrations as shown in Table 2. Considering the infected plants, their shoot and root length and dry matter content was significantly reduced, while this reduction was less in the nitrapyrin treated plants. We observed again that the lowest damage caused by infection occurred at the standard NH_4-N concentration (284 ppm) in the presence of N-serve.

These morphological features clearly indicate that nitrapyrin has no direct effect on the plant vigour, but it can reduce the damage caused by *Fusarium* infection, especially at the optimal nutrient concentration.

The decrease of disease severity with increasing nitrogen concentrations agrees with the observations of some workers (BLOOM and WALKER, 1955; HUBER *et al.*, 1968) and may support the findings of KIRÁLY (1976) and GILLYNÉ (1978) that in diseases caused by facultative parasites, disease severity decreases with increasing nutrient concentration. The effect of nitrapyrin was not consistent with

Table 1

Number of germinated seeds in the infected pots and the disease severity in GKF_2 seedlings after treatment with nitrapyrin (NI) and different levels of NH_4-N nutrient (Data represent the average of 5 replicates)

Hoagland solution concentration ppm	Disease severity (0—9)				Germination of seeds (out of 15 seeds per pot)			
	NH_4-N	%	NH_4-N +(NI)	%	NH_4-N	%	NH_4-N +(NI)	%
70	8.62	86	6.09	61	3.85	25	8.80	58
284	7.75	78	4.78	48	4.60	31	10.20	68
630	6.85	69	5.68	57	5.80	39	8.40	56
$LSD_{5\%}$	0.96	—	0.68	—	0.92	—	0.52	—

this trend because greater reduction in disease incidence was obtained only at the standard nutrient concentration (284 ppm), Tables 1 and 2.

In general, nitrapyrin decreased the disease severity caused by root-rot fungi. WARREN *et al.* (1975) suggested that nitrapyrin decreases the ability of pathogens to infect a plant actively growing with an adequate supply of N (nitratpyrin-treated) as compared to the N-deficient (70 ppm), less vigorous plants. According to this explanation, keeping plants in the juvenile state as long as possible may help to reduce disease incidence.

In addition to the morphological parameters, we have studied some biochemical parameters of wheat plants in order to obtain a deeper insight into the mechanism of disease development as affected by the nitrapyrin treatment.

Table 3 presents the results of the biochemical analysis of healthy plants watered with three different concentrations of NH_4-N with and without nitrapyrin. The nitrate reductase activity increases with increasing nitrogen concentration; this is in agreement with the results of THAKUR *et al.* (1966). However, plants treated with nitrapyrin have a higher enzyme activity (Table 3). It is generally well known that nitrate reductase plays a key role in amino acid biosynthesis. Therefore, the increase in activity of this enzyme particularly in the case of nitrapyrin-treated plants, can probably contribute to the resistance of plants.

These findings led us to investigate the level of free amino acids in the host plant, as shown in Table 3. The results indicate that, there is a striking increase in free amino acids with increasing nutrient N-concentration. In the nitrapyrin-

Table 2

Shoot and root length and dry matter content of 20 day-old healthy and infected GKF_2 seedlings supplied with different concentration of NH_4-N and nitrapyrin (Data represent the average of 5 replicates)

Hoagland concentration ppm	Healthy seedlings			Infected seedlings		
	Shoot length cm	Root length cm	Dry matter g	Shoot length cm	Root length cm	Dry matter g
	Plants treated with NH_4-N					
70	16.47	8.07	0.47	3.32	2.27	0.11
284	22.61	14.41	0.70	3.34	2.07	0.16
630	21.58	12.26	0.66	6.71	3.79	0.25
$LSD_{5\%}$	0.48	1.81	0.03	0.94	0.78	0.03
	Plants treated with NH_4-N + nitrapyrin					
70	15.70	10.17	0.39	6.88	4.26	0.20
284	19.53	12.46	0.51	12.20	6.36	0.30
630	19.34	12.06	0.54	8.09	5.11	0.22
$LSD_{5\%}$	0.67	0.96	0.04	0.83	0.75	0.04

Table 3

Nitrate reductase activity, free amino acid, protein and P and K contents of healthy seedlings of 20-day-old GKF_2 plants treated with different levels of NH_4-N nutrient with and without nitrapyrin
(Data represent the average of 3 replicates)

Hoagland solution conc. ppm	NRA*-μM NO_2/gr fr.wt./h	Free amino acid μM/g	Protein %	P_2O_5 %	K_2O %
		Plants treated with NH_4—N			
70	0.051	177.48	24.91	1.88	7.63
284	0.084	198.53	29.40	1.76	8.16
630	0.118	215.00	29.40	2.89	7.56
		Plants treated with NH_4—N + nitrapyrin			
70	0.108	251.49	28.35	1.85	6.80
284	0.119	286.66	33.25	2.05	7.20
630	0.136	191.04	29.40	3.06	7.20

* NRA = nitrate reductase activity

treated plants higher amounts of free amino acids were obtained, but a sharp decrease occurred at high N-concentration (630 ppm). The NRA seemed to be well correlated with free amino acid content of the healthy plants, except in the NH_4-N and nitrapyrin variant at 630 ppm.

The total protein, phosphorus and potassium content was determined for the healthy plants as shown in Table 3. The results show that the protein content increased with N-concentration, but a greater increase was obtained with nitrapyrin. This results is in agreement with that of AYDENIZ *et al.* (1976), WALLS (1977) and HARRISON *et al.* (1977), who found that protein content increased by addition of nitrapyrin to the fertilizer. The highest protein content was obtained in nitrapyrin-treated plants at the standard nutrient concentration. This was found previously in the case of the morphological parameters. The phosphorus and potassium content were also slightly higher in the nitrapyrin-treated plants and increased with increasing N-concentrations, but changes in these ions were irregular in the NH_4-N treated plants.

The experimental evidence concerning the biochemical and morphological parameters related to the host resistance suggest that nitrapyrin may have an effect on the metabolism of the host plant which may influence disease resistance of the host.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 23—30 (1981)

Biochemical Bases of Resistance of *Venturia inaequalis* to Benomyl

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Biochemical factors of the resistance of *V. inaequalis* developed under natural conditions to benomyl have been studied. The comparison has been made between the uptake, metabolism of benomyl, its binding to cell nuclei in resistant and sensitive isolates. No differences in the extent of uptake and inactivation that would be responsible for benomyl resistance were found. On the bases of the experimental data it may be concluded that in the case of *V. inaequalis* the type of resistance to benomyl which has been formed is due to structural change within receptors. Comparison of the response of isolates of *V. inaequalis* to other antimitotic compounds also proved the validity of the receptor hypothesis.

The synergistic effect of serine on thiabendazole fungitoxicity was observed in the case of benomyl resistant *V. inaequalis* isolates, but not in the benomyl sensitive ones.

The common characteristic of earlier investigations of the biochemical causes of benzimidazole resistance is that the experiments were carried out on benomyl resistant strains formed under laboratory conditions. NACHMIAS and BARASH (1976) attributed the resistance of *Sporobolomyces roseus* strains to benomyl to the decrease of the uptake of active ingredient and so did GESSLER (1976) in the case of *Botrytis cinerea* strains. In the case mentioned above, however, the difference in uptake was ill-proportioned to the difference in sensitivity.

DAVIDSE and FLACH (1977) have shown that the benomyl resistance of *Aspergillus nidulans* is due to a decrease in the binding ability of the protein tubulin in the cell. From the experimental data, obtained by TRIPATHI and SCHLÖSSER (1980), it appears that resistance of *Botrytis cinerea* to carbendazim is going with a lesser uptake and reduced binding of the active ingredient to tubulin.

We have succeeded in elucidating the biochemical causes of the antifungal selectivity of some compounds on different fungal species (GASZTONYI and JOSEPOVITS, 1975, 1979; GASZTONYI, 1979). When studying benomyl, the investigations were also extended to the isolates of *V. inaequalis* which have become resistant under natural conditions.

Between these benomyl resistant and benomyl sensitive *V. inaequalis* isolates comparison in different respects has been made in this present work.

Materials and Methods

Materials

Benomyl, methyl-1-(buthylcarbamoyl)-2-benzimidazolecarbamate, pure (Chinoin Chemical and Pharmaceutical Works Ltd.); thiabendazole, 2-(4'-thiazolyl)-benzimidazole, pure (Chinoin); propham, isopropil-phenylcarbamate, pure (North-Hungarian Chemical Works); trifluralin, 2,6-dinitro-4-trifluoromethyl-N,N-dipropilaniline, pure (AGAN Chemical Manufactures Ltd.); serine (Reanal, Budapest).

Four benomyl resistant isolates of *V. inaequalis* were obtained from apple orchards where benzimidazole fungicides have been used for several years. Two benomyl sensitive *V. inaequalis* isolates originating from the orchard never treated with fungicides were also used.

Study on fungitoxic activity

The study of fungitoxic activity was accomplished using an agar-disc method (VÖDRÖS, 1973).

Incubation with fungal mycelia

Mycelial units pre-incubated for 5–6 weeks were added in an amount equivalent to about 1 g fresh weight to potato-dextrose medium (20 cm^3) containing benomyl (10 mg $litre^{-1}$) and incubated for 24 hrs at 25 °C. Simultaneously under identical conditions, benomyl solution was incubated without mycelium, and mycelium without benomyl. Each variation was replicated three times.

After termination of the incubation, the mycelium was separated from the incubation medium, ground with quartz sand and extracted with ethyl acetate in the presence of Na_2SO_4 and $NaHCO_3$. Subsequent partition of extract between the organic solvent and 1 M HCl solutions provided sufficient clean-up for assay by ultraviolet spectrophotometry. The incubation media after adjusting pH to 6.0 was also extracted with ethyl acetate and the latter with 1 M HCl. The recovery of MBC (methyl-benzimidazol-2-ylcarbamate) from mycelia was 80 per cent, and from the incubation media 92 per cent.

Incubation with nuclei fraction

Nuclei fraction of *V. inaequalis*, prepared as described for higher plants in Modern Methods for Plant Analysis (LISKENS and TRACEY, 1963) was dispersed in tris buffer (0.05 M, pH = 7.5) and incubated with benomyl (3 mg $litre^{-1}$) for 20 hrs. The processing has been made as it was described above for mycelia.

Determination of benomyl by u. v. spectrophotometry

The benomyl content of mycelium or incubation medium was determined by measuring the absorbance values of MBC in the 1 M HCl solutions at 250 nm, 282 nm and 300 nm.

Results and Discussion

The degree of sensitivity of V. inaequalis isolates to benomyl

As concerning the fungitoxic activity of benomyl against four benomyl resistant and benomyl sensitive isolates investigated with agar-disc method it has been observed that the growth of the benomyl sensitive *V. inaequalis* isolates was entirely inhibited by 0.1 mg litre^{-1} benomyl while growth of the resistant ones could be observed in the presence of even 100 mg litre^{-1} benomyl.

The benomyl resistant isolates of *V. inaequalis*, used in these experiments, have shown cross-resistance with both thiabendazole (TBZ) and thiophanate-methyl, i.e. in general they can be considered benzimidazole resistant.

Uptake of benomyl in sensitive and resistant isolates

Mycelia of benomyl sensitive and benomyl resistant isolates were incubated for 24 hrs with benomyl (10 mg litre^{-1}). The quantities of MBC, established in the incubation media, are shown in Fig. 1. As the hydrolysis of benomyl to MBC takes place in the course of incubation and processing, the values shown by the diagram, concern the MBC content in percentage of MBC corresponding to the

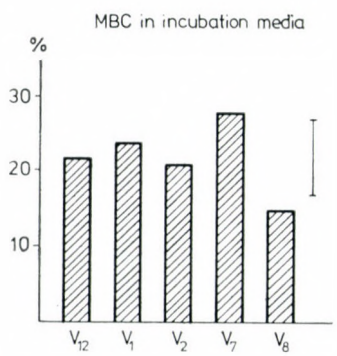

Fig. 1. MBC content in incubation media expressed as a percentage of MBC corresponding to the amount of benomyl added at the beginning of the incubation. (V_1, V_2, V_7, V_8) resistant isolates, (V_{12}) sensitive isolate

amount of benomyl added to the incubation medium. It can be seen that there is no significant difference between the various isolates of *V. inaequalis* in the degree of uptake of benomyl.

The accumulation of MBC in nuclei

Nuclei fraction prepared from one sensitive and one resistant isolate each were incubated with benomyl (3 mg litre^{-1}) for 20 hrs and the uptake was determined. In lack of more measurement data the observation concerning this can be considered to be preliminary. According to those, there is no accumulation of benomyl in the nuclei either in the resistant or in the sensitive isolates. The uptake of benomyl by nuclei fraction and by whole mycelia has been found to be approximately equal.

Metabolism of MBC

From the point of view of resistance it was of primary importance to compare the degree of inactivation of MBC in the mycelia of different isolates, therefore the inactive metabolites produced in small amounts were not investigated. In the course of these investigations the significant decrease of MBC content due to degradation could not be detected within the incubation time. The MBC content in the mycelia of the examined *V. inaequalis* isolates have been found to be at equal rates (Fig. 2). Beside this inactivation another possible metabolic pathway of MBC has also been examined. In earlier experiments (GASZTONYI and JOSEPOVITS, 1970) the possibility of formation of β-alanine derivatives in plant and fungi as a result of reaction between thiabendazole (or MBC) and serine was investigated by the analogy with amitrole (3-amino-1,2,4-triazole) (MASSINI, 1963). It was also established that the fungicidal effect of TBZ on some fungal species was synergized by serine (GASZTONYI, 1974). One of the possible

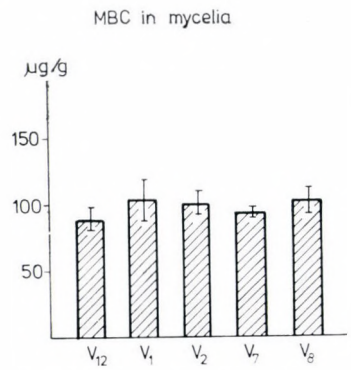

Fig. 2. MBC content (μg/g fresh weight) in mycelia of resistant (V_1, V_2, V_7, V_8) and sensitive (V_{12}) *V. inaequalis* isolates

explanations for this is the formation of a more active metabolite. In the present work the benzimidazole resistant and benzimidazole sensitive *V. inaequalis* isolates were compared from this point of view, too. In the case of resistant isolates the inhibition effect of TBZ was synergised by serine (Fig. 3), while this could not be observed in the case of sensitive isolates (Fig. 4). Moreover, it was found that the resistant isolates grow more intensively in the presence of serine than in control variation (Fig. 3). This was also not observed in the case of sensitive *V. inaequalis* isolates. It was assumed that there is enough endogenous serine in the sensitive *V. inaequalis* isolates (at least in relation to the low effective concentration of TBZ), so no synergistic effect between TBZ and serine added from outside can be noticed. The resistant isolates, on the other hand, requiring exogenous serine for growth can also use this in that mechanism which leads to the increase of the fungicidal effect of TBZ. This synergistic effect may reach even 1/2 – 1 order of magnitude but does not equalize the great difference between the antifungal effect on the sensitive and the resistant isolates. Consequently, the demand for serine is not the cause of the benomyl resistance of *V. inaequalis* but increases its level only.

Cross-resistance with antimitotic compounds

The binding of benomyl to tubulin in various *V. inaequalis* isolates has not been compared by direct chemical method. The attempt, however, has been made to obtain indirect information concerning this.

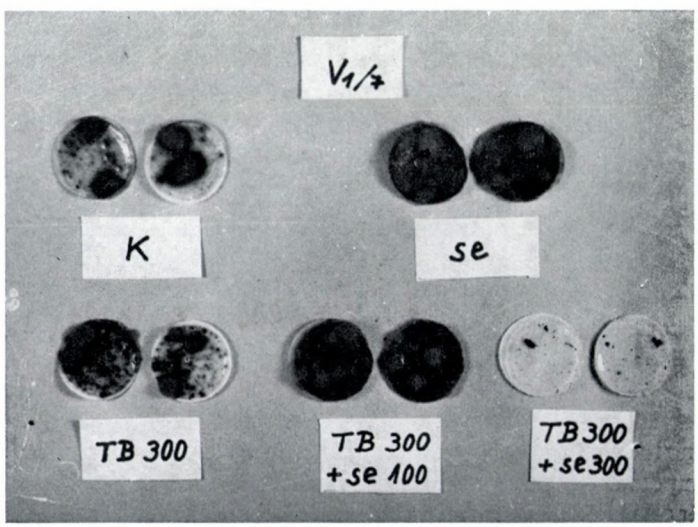

Fig 3. Effects of thiabendazole (TB 370 mg litre^{-1}), serine (Se 100 mg litre^{-1}) and their combinations (3 : 1 and 1 : 1) on the growth of resistant *V. inaequalis* isolate ($V_1/_7$), (K) control

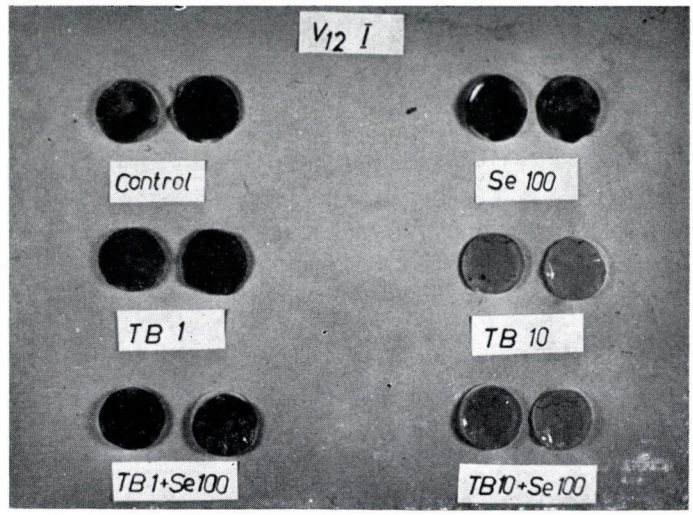

Fig. 4. Effects of thiabendazole 1 and 10 mg litre^{-1} (TB 1, TB 10); serine 100 mg litre^{-1} (Se 100) and their combinations (1 : 100 and 1 : 10) on the growth of sensitive *V. inaequalis* isolate (V_{12})

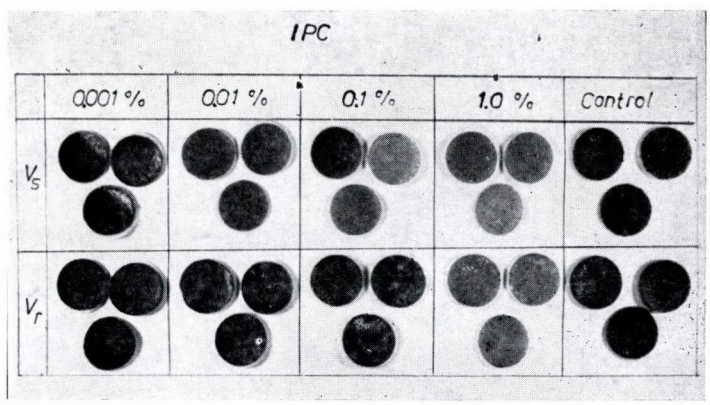

Fig. 5. Fungitoxic effect of propham (IPC, 0.001 %— 1.0 %) on sensitive (V_s) and resistant (V_r) *V. inaequalis* isolates

When studying the cross-resistance between benzimidazoles and other antimitotic compounds, LEROUX and GREDT (1980) observed negative cross-resistance between benomyl and propham in the case of some benomyl resistant strains of *Botrytis cinerea* and *Penicillium expansum*. Our experiments with *V. inaequalis* isolates carried out with agar-disc method showed definite positive cross-resistance between benomyl and propham (Fig. 5).

According to ECKERT and RAHM (1979) the formation of a hydrogen bond between the NH-group and the receptor is necessary for the exert of the activity both in the case of benzimidazole fungicides and phenylcarbamate herbicides. Therefore the cross-resistance observed here may be attributed to the changed ability of tubulin for forming hydrogen bonds.

Comparing experiments of this type were also carried out with trifluralin (another type of herbicide also having antimitotic activity), but it had no antifungal effect against all *V. inaequalis* isolates.

Conclusions

As to the results of our experiments the resistance that *V. inaequalis* acquired towards benomyl can be attributed neither to the decrease of uptake nor to an increased inactivation of active ingredient. The experiments support the change of binding to tubulin in the case of most *V. inaequalis* isolates and this manifests itself in the lower affinity to propham.

The resistant isolates of *V. inaequalis* need exogenous serine for their growth. The synergistic effect of serine on thiabendazole fungitoxicity has been more pronounced in the case of benomyl resistant *V. inaequalis* isolates than in the case of earlier investigated fungal species.

Acknowledgements

The authors are grateful to Dr. S. ROZSNYAI for supplying the *V. inaequalis* isolates used in this work and thank Mrs. M. VÖDRÖS for skillful technical assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 31—40 (1981)

Effects of Benomyl on *Venturia inaequalis* Cke. Isolates Resistant to Benomyl

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Of the isolates from orchards treated and untreated, respectively, with benzimidazole derivative fungicides the sensitive ones were inhibited by benomyl at concentrations as low as 0.01—0.1 ppm. Resistance to benzimidazole derivatives was observed up to a concentration 100 ppm and proved to be stable. Cross-resistance of benomyl resistant strains to carbendazim, thiabendazole and fuberidazole has been shown.

Benzimidazole derivatives at sublethal concentrations had some effect on the resistant isolates. On media containing 100 ppm benomyl the resistant strains differed from each other in their growth intensity. A few of them were inhibited by this concentration, whereas other not. When colonies kept for 30 days on a medium containing benomyl at 100 ppm were transferred onto media containing lower concentrations (0, 1, 5 ppm) of benomyl their growth recovered. Benomyl and other benzimidazole derivatives at 1—5 ppm concentrations stimulated mycelial growth in most of the resistant isolates. In some cases "cross-stimulation" by different benzimidazole derivatives were observed.

Conidia from benomyl resistant strains exhibited various level of resistance to benomyl. In benomyl resistant cultures we succeeded to find conidia being sensitive to and dependent on benomyl, respectively.

Some data on origin, pathogenicity, fungicide resistance, biological and physiological characteristics of our strains were published elsewhere (TÓTH *et al.* 1978; GASZTONYI *et al.*, 1979; GASZTONYI and JOSEPOVITS, 1979; OROS, 1979; GASZTONYI and JOSEPOVITS, 1980; OROS and ROZSNYAI, 1980).

In this study we present the effects of different benzimidazole derivatives on *Venturia inaequalis* Cke. strains isolated in Hungary.

Materials and Methods

The strains of *V. inaequalis* have been isolated from orchards treated and untreated with benomyl, respectively, by D. ROZSNYAI in 1976. Their resistance to benomyl were acquired by natural selection.

The sensitivity or resistance of strains to benomyl or to other benzimidazole derivatives have been evaluated by the method of YODER (1978).

A standard suspension of conidia have been prepared as described earlier (OROS and ROZSNYAI, 1980).

The ratio of benomyl "sensitive" conidia in the standard suspension have been determined as a difference between the number of conidia germinated on benomyl-free PDA and on PDA containing benomyl at 1 ppm, respectively.

The benomyl "dependent" monoconidial lines were isolated from the standard suspension of resistant strains onto PDA-CO containing benomyl at 1 or 5 ppm, respectively. Criteria of benomyl "dependence" were as follows: (i) the colony formed after germination of conidia is not able to continue to grow in the absence of benomyl or after two passages on benomyl-free media, respectively, and (ii) mycelial growth recovers in the presence of benomyl (1 or 5 ppm).

The ratio of benomyl "dependent" conidia have been determined in conidia production of the 11th laboratory passage on benomyl-free PDA-CO. 4×100 conidia of each *Venturia* strain have been investigated. The ratio of "dependent" conidia was calculated as a percentage of benomyl resistant conidia in the standard suspension.

The media used were potato dextrose agar (PDA) according to YODER (1978) and PDA completed with Czapek-Dox salts (PDA-CO) which latter consisted of the following components: sodium (α)glycerolphosphate $\cdot$ 6 H_2O – 0.66 g; $MgCl_2$ sicc. – 0.20 g; KCl – 0.19 g; $NaNO_3$ – 1.63 g; KNO_3 – 0.44 g; K_2SO_4 – 0.35 g; Sequestrene – 0.025 g; solution of microelements according to HOAGLAND – ARNON – 1.0 ml for 1 litre. The plates were incubated at 19 $\pm$ 1 °C in the dark.

Results and Discussion

In our earlier studies we have found that *V. inaequalis* strains can be divided into two physiological types. Type "A" is characterized by an intensive growth, grey colour and high sugar consumption. Type "B" with brown colour grows slower and consums less amount of sugar (OROS and ROZSNYAI, 1980).

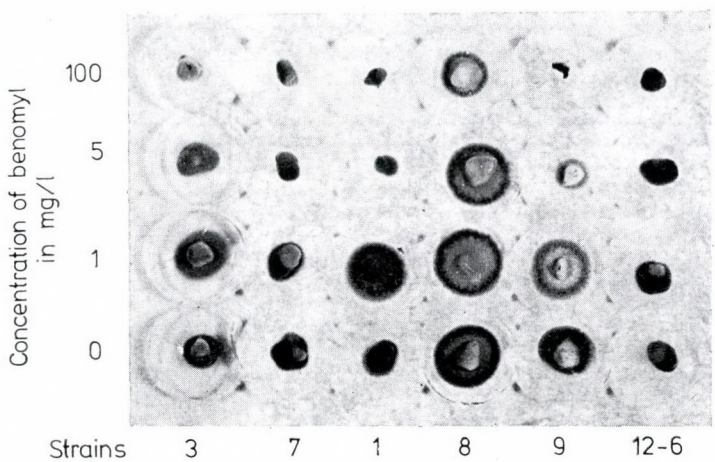

Fig. 1. The effect of different benomyl concentrations on the growth of *V. inaequalis* strains

Both types can acquire resistance to benomyl. The strains investigated by us resist not only benomyl but carbendazim, thiabendazole and fuberidazole, too. Naturally acquired resistance remained stabile even after ten laboratory passages on PDA-CO free of benomyl (Table 1).

Table 1

Sensitivity of different *venturia inaequalis* strains to benzimidazole derivatives

Strains	Origin	Physiol. type	Degree of resistance	Benomyl	Carbendazim	Thiabendazole	Fuberidazole
V-12-3	Central Hungary	A	1	+	+	+	+
V-12-6	Central Hungary	A	3	—	—	—	—
V-9-1	East H.	A	3	—	—	—	—
V-8-5	East H.	A	2	—	—	—	—
V-7-1	East H.	A	2	—	—	—	—
V-3-6	West H.	A	3	—	—	—	—
V-3-3	West H.	B	2	—	—	—	—
V-2-5	West H.	B	3	—	—	—	—
V-1-2	West H.	B	2	—	—	—	—
V-12-4	Central H.	B	1	+	+	+	+

Degree of resistance 1 — Benomyl has killing effect at 1 ppm, and morphopathological effect at subletal concentrations.
2 — Benomyl has no killing but fungistatic effect at 100 ppm, and has no morphopathological effect at subfungistatic concentrations.
3 — Benomyl has no effect at all, or retards fungal growth at 100 ppm.
+ : sensitive; — : resistant

Table 2

MIC values* of different fungicides for physiological types of benomyl sensitive *Venturia inaequalis* Cke. strains

Fungicides	Physiological types	
	A	B
Benomyl	0.01—0.1	0.01—0.1
Carbendazim	0.01—0.1	0.01—0.1
Thiabendazole	0.1—1	0.01—0.1
Fuberidazole	5—10	5—10
Tridemorph	0.1—1	0.1—1
Triforine	0.1—1	1—10
Dodine	>2	>2

* in ppm

The two physiological types showed a slight difference in sensitivity to benzimidazoles and other fungicides (BURCHILL and COOK, 1975; DRANDAREWSKY and SCHICKE, 1976; EVANS, 1971; FUCHS and DRANDAREWSKY, 1976; KERKENAAR *et al.*, 1979; GASZTONYI and JOSEPOVITS, 1975; MANTINGER, 1973; SISLER and RAGSDALE, 1977). Type "A" appeared to be less sensitive to thiabendazole, but more sensitive to triforine than "B" (Table 2). According to ARNESON (1974) and ROSS (1977) both types were resistant to dodine.

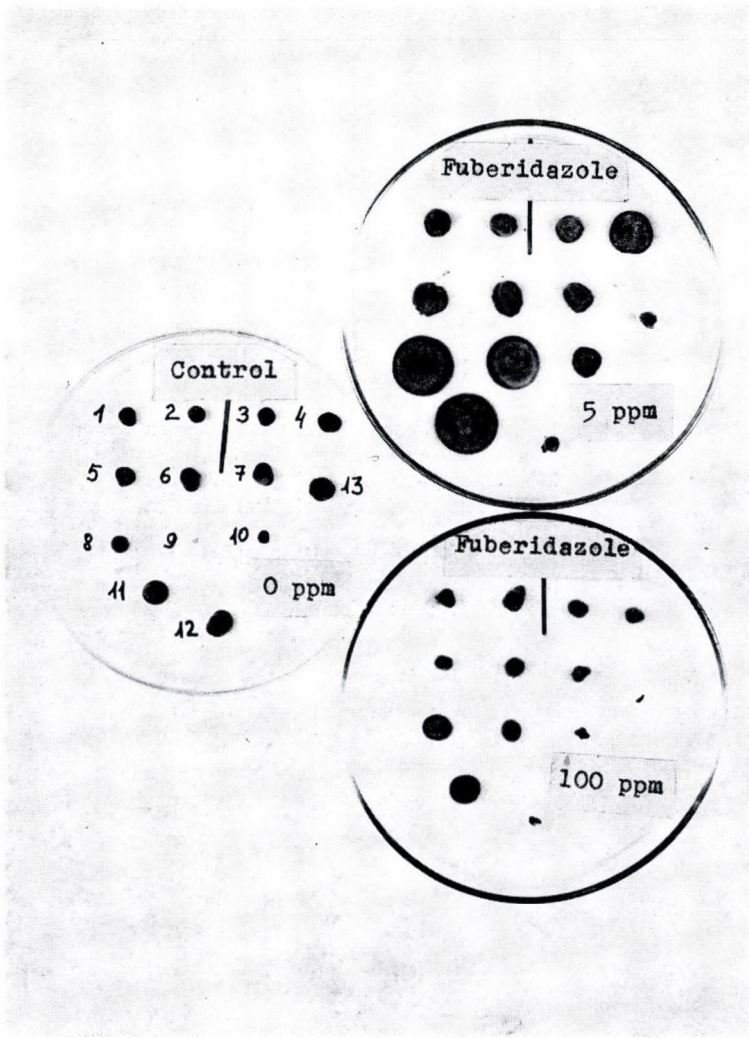

Fig. 2. Effect of fuberidazole on *V. inaequalis* strains: 1—7 — monospore lines of V-12-6; 8 — V-9-1; 9 — V-8-5; 10 — V-3-6; 11 — V-2-5; 12 — V-12-4; 13 — V-12-3

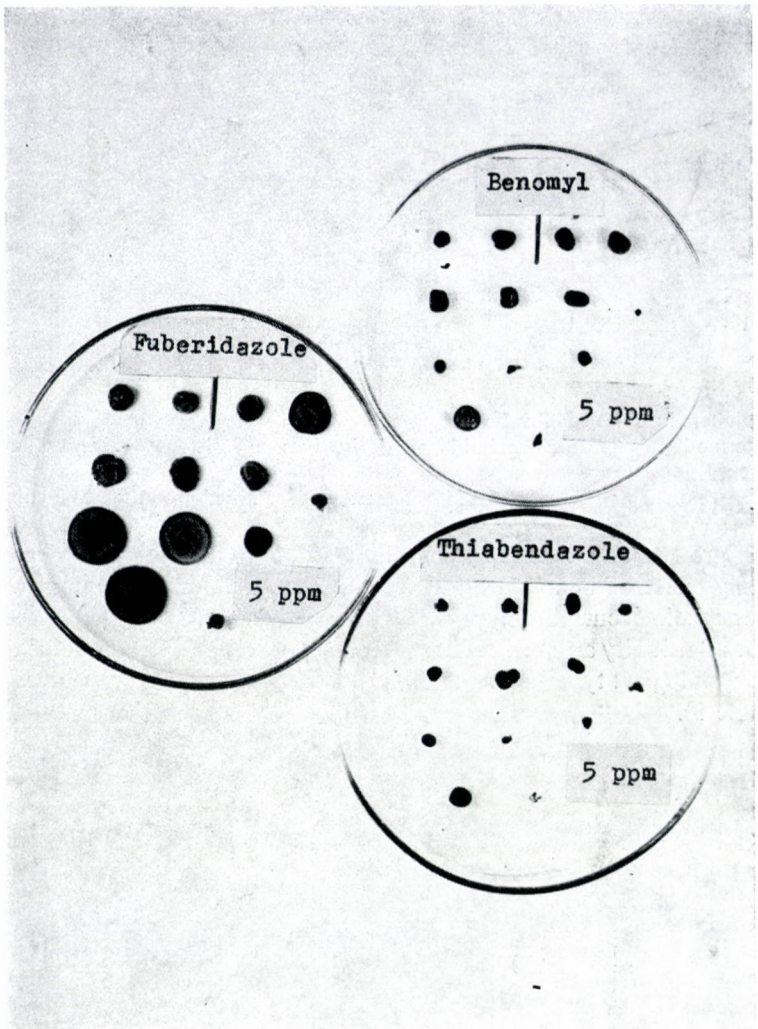

Fig. 3. Effect of benomyl, thiabendazole and fuberidazole on the growth of *V. inaequalis* strains. The location is the same as on the Figure 2

Within resistant strains of both types two groups should be distinguished on the basis of the mode of effect of benomyl. The first one is characterized by a fungistatic effect of the fungicide at 100 ppm, while the other by ineffectiveness or growth retarding effect, at the same concentration (Table 3 and Fig. 1). The thiabendazole proved to be fungistatic towards more *Venturia* strains than benomyl or carbendazim at this concentration, fuberidazole, however, slightly stimulated colony growth. Fuberidazole at lower concentrations (1 and 5 ppm) stimu-

Table 3

Effects of different fungicides on the growth of benomyl sensitive and benomyl resistant *Venturia inaequalis* Cke. strains

Strains Physiol. type	V-12-III A	V-9-1 A	V-8-5 A	V-3-3 B	V-2-5 B	V-12-IV B
Benomyl	0.01—0.1	NE	NE	FS	NE	0.01—0.1
Carbendazim	0.01—0.1	NE	NE	FS	NE	0.01—0.1
Thiabendazole	0.1—1	RG	FS	FS	FS	0.01—0.1
Fuberidazol	5—10	ST	ST	RG	ST	5—10
Tridemorph	0.1—1	0.1—1	<0.1	<0.1	0.1—1	0.1—1
Triforine	0.1—1	0.1—1	<0.1	0.1—1	1—10	1—10
Dodine	>2	>2	<0.1	0.1—2	>2	>2

Numbers indicate MIC values in ppm.

The mode of effect of benzimidazole derivatives on resistant strains was investigated at 100 ppm.

NE — no effect; FS — fungistatic effect; RG — retarded growth; ST — stimulation

lated the growth of all benomyl resistant strains but to various degrees. This stimulating effect of benomyl and carbendazim were less consistent, while that of thiabendazole occurred only in a few cases (Figs 1, 2, 3).

Both sensitivity and resistance to tridemorph, triforine and dodine depended on the physiological types and was not influenced by acquired resistance to benomyl (Table 3).

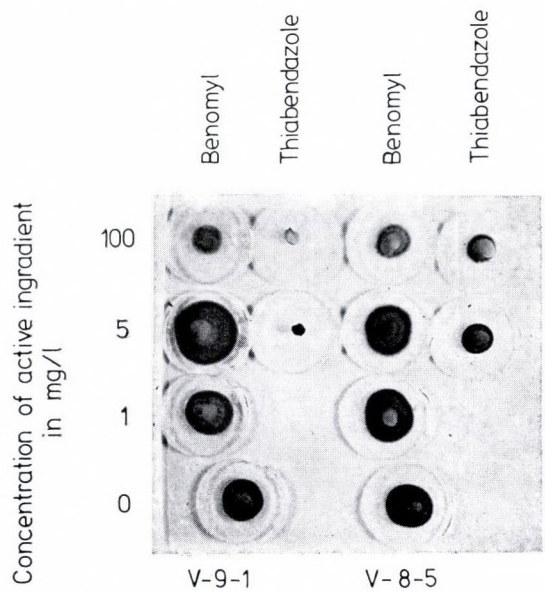

Fig. 4. Effect of different concentrations of benomyl and thiabendazole on benomyl resistant *V. inaequalis* strains in 11th passage

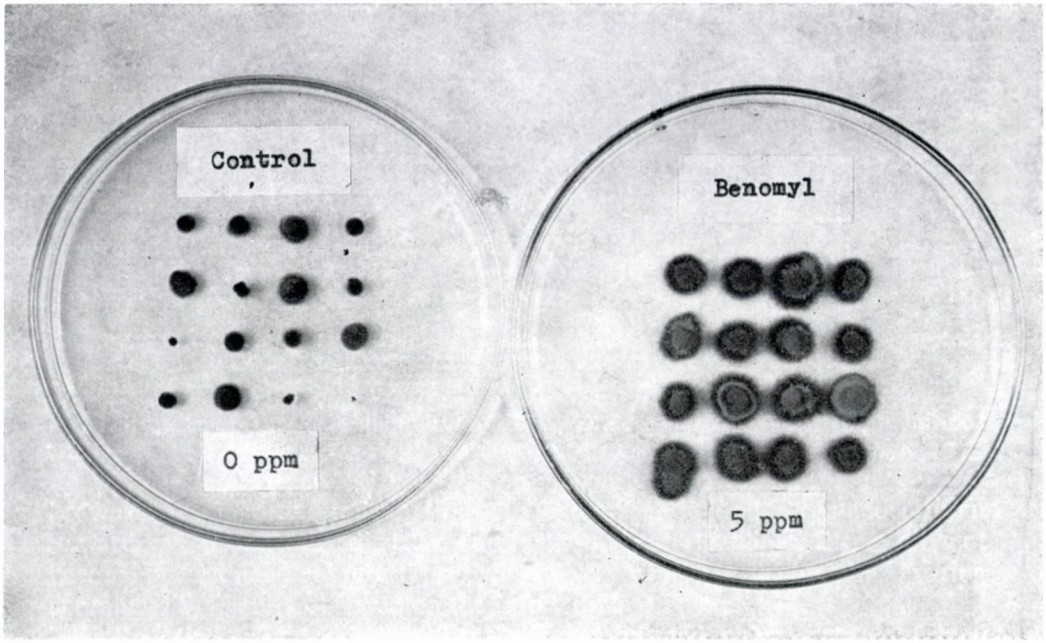

Fig. 5. Stimulation of the growth of monospore lines from V-12-6 strain by 5 ppm of benomyl

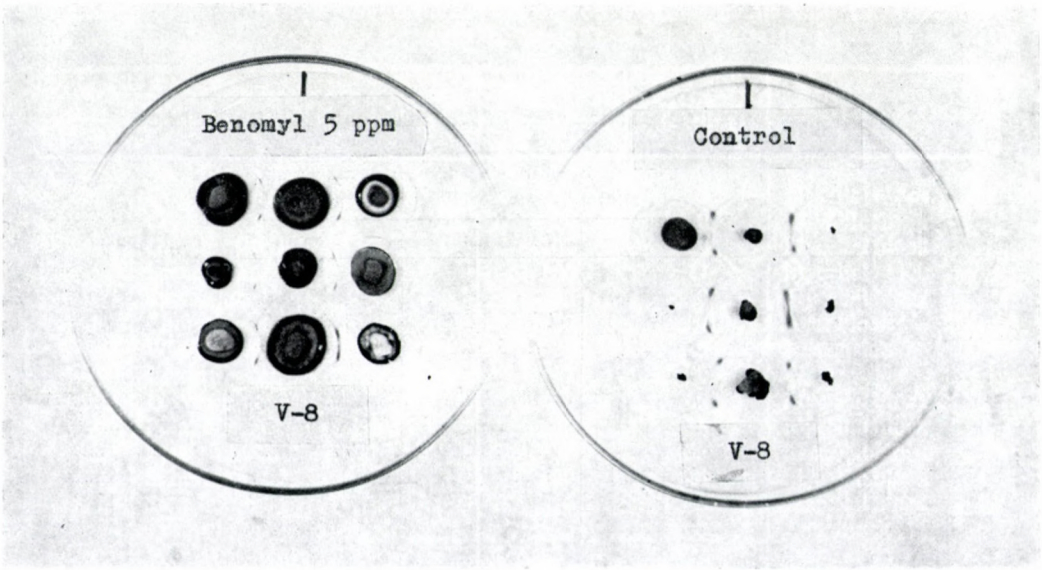

Fig. 6. Stimulation of the growth of monospore lines from V-8-5 strain by 5 ppm of benomyl

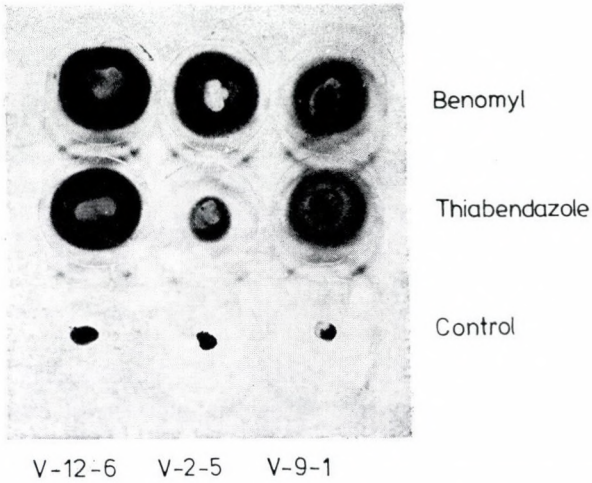

Fig. 7. Effects of benomyl and thiabendazole at 5 ppm on monospore lines from different strains

Conidia of benomyl resistant strains differed in resistance level to benomyl (Table 4). Both the benomyl sensitive and the dependent conidia could be found in all benomyl resistant strains (Figs 5 and 6, Table 5). Benomyl dependent lines produced by resistant strains were stimulated not only by benomyl but by other benzimidazoles too, at concentrations of 1 and 5 ppm (Figs 4 and 7). The phenomenon of cross-resistance was observed in all cases, but the "cross-stimulation" not (Figs 3 and 7).

Table 4

Effect of benomyl on germination of conidia of *Venturia inaequalis* Cke. strains

Strains	Physiol. type	Number of conidia germinated at different concentrations of benomyl			
		0 ppm	1 ppm	5 ppm	100 ppm
V-12-3	A	6852±2054	0	0	0
V-12-6	A	31869±4450	17852±2750	10500±1671	1566±655
V-9-1	A	356±27	315±22	12±10	0
V-8-5	A	83±2	18±3	3±2	0
V-7-1	A	2687±595	1470±623	1302±122	1361±117
V-3-6	A	0	1±3	0	0
V-3-3	B	2647±65	3036±550	1779±487	153±162
V-2-5	B	17814±2551	16334±5318	8316±1467	3401±1300
V-1-2	B	5063±1406	4492±827	3763±1398	58±12
V-12-4	B	8648±2232	0	0	0

n = 10

Table 5

Heterogeneity of conidial production

Strains	Benomyl sensitive conidia (%)	Benomyl dependent conidia (in % of resistant conidia produced)
V-12-3	100	0
V-9-1	10	— *
V-8-5	78	40
V-7-1	46	80
V-3-3	0	30
V-2-5	0	40
V-12-4	100	0

* Not evaluated

Conclusions

1. The benzimidazole derivative fungicides could stimulate the growth of benomyl resistant *Venturia inaequalis* Cke. strains in laboratory cultures.

2. The cross resistance was not related with stimulative effect of benzimidazole derivatives tested.

3. Among various benzimidazole derivatives tested the existance of "cross-dependence" was stated.

4. As regards benomyl resistance, the conidial production of benomyl resistant strains was not uniforme; sensitive as well as "dependent" conidia could be found.

5. The strongest stimulative and fungistatic effect was exhibited by fuberidazole and thiabendazole, respectively.

Acknowledgements

The author wish to thank G. Josepovits for his helpful and valuable advices, and S. Süle for helpful discussion of this paper.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 41–44 (1981)

Susceptibility of Sunflowers to Powdery Mildews Induced by *Plasmopara halstedii*

By

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During the recent years a considerable incidence of powdery mildew infection was observed on downy mildewed sunflower plants in some fields where open-pollinated cultivars, practically resistant to these pathogens were grown. The stunted plants were covered with mycelium of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*, and as a result, such plants could be noticed from afar by their white appearance. In case of leaf infection the symptoms were very remarkable, since the powdery mildew colonies mostly occurred on the chlorotic areas of the leaves, which had previously colonized by the downy mildew pathogen, *Plasmopara halstedii*.

This interaction became much more evident by making artificial inoculations. When young sunflower plants systemically infected by *P. halstedii* were inoculated with conidia of the powdery mildew fungi right after the appearance of leaf chlorosis, the spread of these fungi always followed the chlorotic areas, while other parts of the leaves remained free from their colonies.

Downy mildew is one of the most serious diseases of sunflowers, causing a considerable loss of yield from year to year. The diseased plants are stunted with chlorotic leaves and small, undeveloped heads, indicating the systemic infection by *Plasmopara halstedii*.

The powdery mildew, however, have not yet caused significant disease on sunflowers in Hungary. The traditional, open-pollinated cultivars, like Chakinskii 269, are practically resistant to powdery mildew fungi. The white cover of *Erysiphe cichoracearum* and rarely that of *Sphaerotheca fuliginea* can be observed only at the end of the vegetation period on the oldest leaves of sunflower plants (VIRÁNYI and SZ. NAGY, 1980).

In 1977 an interesting symptom was noticed on some downy mildewed sunflower plants. The leaves, petioles, and occasionally the stem of stunted plants were covered with mycelia and conidia of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*. Such plants could be noticed from afar by their white appearance. In case of leaf infection the symptoms were very remarkable, since the powdery mildew colonies mostly occurred on the chlorotic areas of the leaves, which had previously colonized by the downy mildew pathogen (Fig. 1).

This phenomenon has not been observed before, and has not been known in the literature as well. Therefore some field observations, microscopical examinations and artificial inoculations were carried out during the recent years.

Fig. 1. Simultaneous occurrence of symptoms on a sunflower plant caused by downy and powdery mildews

Material and Methods

Field observations in 1977–80 were made on various sunflower cultivars grown in different parts of Hungary. The sunflower plants showing typical symptoms were collected for microscopical observations.

Artificial inoculations were carried out in the greenhouse. The apical buds of sunflowers at two-leaf stage were inoculated with a spore suspension of *Plasmopara halstedii*, containing 10^5/ml sporangia. These sunflower seedlings were also inoculated with conidia of the powdery mildew fungi at the same time, as well as at different intervals, before and after the downy mildew infection.

Results

The field observations showed that this phenomenon occurred only on cultivars which are highly susceptible to downy mildew but resistant to powdery mildews. It was established, that the strong cover of powdery mildew developed only on the systemically infected, stunted plants; while the neighbouring plants which were free from downy mildew infection usually avoided the attack of powdery mildew fungi.

Microscopical observations revealed that powdery mildews covered not only the upper but also the lower surface of the leaves. Mycelia and conidia of powdery

Fig. 2. Sporangiophores and sporangia of *Plasmopara halstedii* as well as asci with ascospores and conidia of *Erysiphe cichoracearum* from the lower surface of a sunflower leaf

Fig. 3. Artificially inoculated sunflower leaf: the powdery mildew colonies follow the chlorotic areas caused by *Plasmopara halstedii*

mildews in large numbers could be found together with sporangiophores and sporangia of *Plasmopara halstedii* on the infected leaves. In addition the cleistothecia of both species *(E. cichoracearum* and *S. fuliginea)* also occurred on the upper and lower surfaces of such leaves (Fig. 2).

This interaction between downy and powdery mildews became much more evident by making artificial inoculations in the greenhouse. The infection with powdery mildew was successful, when it was made after the appearance of leaf chlorosis caused by *Plasmopara halstedii*. In this case the spread of powdery mildews always followed the chlorotic areas, while other parts of the leaves remained free from their colonies (Fig. 3).

Discussion

Interactions between different plant pathogens are well known in the literature. For instance, according to BLUMER (1967) certain obligate parasites, like viruses and rusts, can influence the susceptibility of the host plants to powdery mildews. Thus on cucumber plants, susceptible to powdery mildew, the virus infection inhibits the development of powdery mildew fungus *(Erysiphe polyphaga)* (BLUMER, STALDER and HARDER, 1955). In contrary, YARWOOD (1963 ap. BLUMER, 1967) reported, that the rust-infected bean plants which were resistant to powdery mildew became susceptible to *Sphaerotheca fuliginea*.

As far as we are aware, there is only one indication in the literature concerning a joined infection with powdery and downy mildew fungi. Recently COHEN, REUVENI and KENNETH (1975) reported, that tobacco plants systemically infected by *Peronospora tabacina*, became resistant to *Erysiphe cichoracearum*.

From all these data mentioned above completed with our observations it is clear, that any change in susceptibility or resistance to powdery mildews may vary according to the host-pathogens relationship.

Although the cause of this interaction between downy and powdery mildew of sunflower is still unknown, the induced susceptibility of sunflowers to the powdery mildew fungi is considered as a result of some changes in the metabolism of mildewed plant tissues.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 45—48 (1981)

Effect of Photosynthesis Inhibitors on Wheat Stem Rust Development

By

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The primary leaves of the wheat cultivars Little Club (susceptible) and Vernal (resistant) were inoculated with urediospores of *Puccinia graminis tritici* race 11. The inoculated leaves were excised on successive days after inoculation, then floated on water containing 30 ppm of benzimidazole.

Exposure of the detached leaf cultures to light conditions did not change the infection types. However, blocking the photosynthesis by dark or by addition of the photosynthesis inhibitors "DCMU" and "CMU" to the floating solutions changed the infection types of both cultivars to immune type reaction. When the detachment of the inoculated leaves was carried out on the 4th to 6th days after inoculation, neither the darkness treatment nor the presence of inhibitors had any effect on susceptibility or hypersensitivity.

When 3% sucrose (w/v) was added to the solutions containing inhibitors or to leaf-cultures held in dark, the original susceptibility of Little Club or the hypersensitive necrosis of Vernal was exhibited. The addition of sucrose to illuminated detached leaves resulted in an increase in the size of the urediopustules or that of the hypersensitive necrosis.

It seems that photosynthesis has an important role via the carbohydrate metabolism in the development of rust but does not influence compatibility or incompatibility.

It is a well-known phenomenon that in winter, when plants do not get enough light in the greenhouse, rusts develop slowly and week sporulation is experienced. HASSEBRAUK (1940) and SEMPIO (1950) investigated the effect of light on rust development in different parts of the incubation period and found that not only the incubation period is lengthened by suboptimal light, but rust reaction is also altered.

The increase in resistance with low light intensity is generally regarded as due to inadequate photosynthesis in the poorly lighted plants (STAKMAN and HARRAR, 1957; YARWOOD, 1976). This is also supported by the fact that rust develops on plants in the dark provided that they are fed sugar (SILVERMAN, 1960). Our aim was to investigate the effect of photosynthesis inhibitors on rust development in detached leaf culture.

Materials and Methods

Little Club *(Triticum compactum)* and Vernal *(Triticum dicoccum)* cultivars were grown under greenhouse conditions. The wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici* race 11 was used in the experiments. This race causes susceptible symptoms (3 – 4) on Little Club and resistant symptoms (0; – 1) on Vernal.

Eight days old seedlings were inoculated with race 11 by spraying the leaves uniformly with a spore suspension and held in moist chamber for 12 h. First leaves of seedlings were detached 1, 2, 3, and 4 days after inoculation and floated on 20 ml of benzimidazole solutions (30 ppm) in Petri dishes.

To examine the dark effect on rust development some of the Petri dishes were covered with aluminum foil to keep the leaves in dark. The light intensity was 5 – 6000 lux.

For the inhibition of photosynthesis, photosynthesis inhibitors (CMU in 20 ppm or DCMU in 15 ppm) were applied in addition to benzimidazole. CMU (3-)4- chlorophenyl(-1,1-dimethylurea) and DCMU (3-)3'-4'-dichlorophenyl(-1,1-

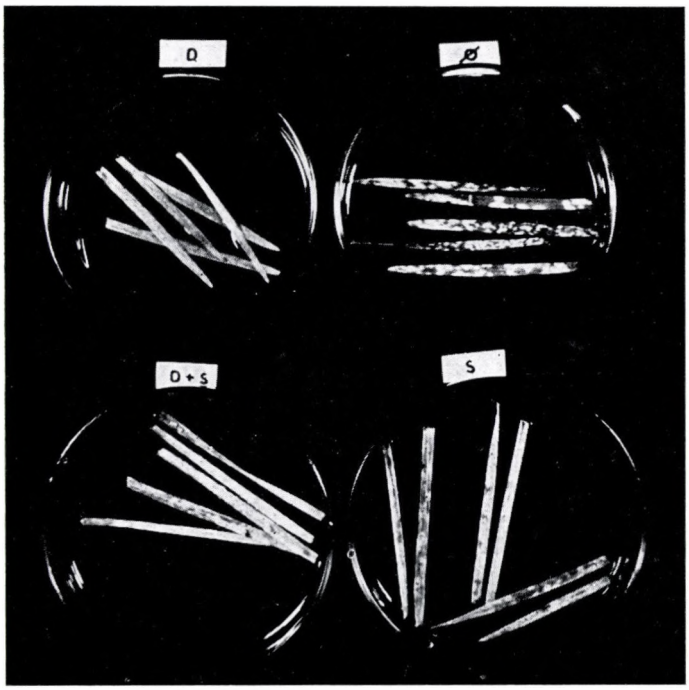

Fig. 1. Effect of dark and sucrose treatments on the reaction of Little Club wheat cultivar to stem rust race 11, ∅ : control, D: dark-treated, S: treated with sucrose, S+D: sucrose + dark treated leaves

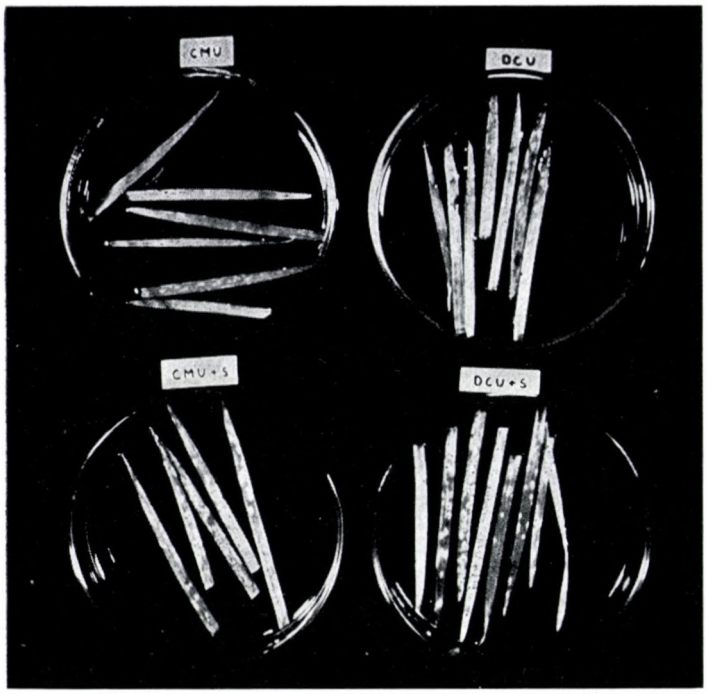

Fig. 2. Effect of photosynthesis inhibitors on the reaction of Little Club wheat cultivar to stem rust race 11. CMU: CMU-treated, DCU: DCMU-treated, CMU + S: CMU + sucrose-treated, DCU + S: DCMU + sucrose-treated leaves

dimethylurea) are extremly powerful inhibitors of the Hill reaction (GOOD and IZAWA, 1973).

To prevent the effect of the dark or photosynthesis inhibitors on rust development 3% sucrose was added to the solutions.

Results

All treatments, namely covering the dishes by alufoil and the photosynthesis inhibitors, induced resistance to rust. When the leaves were detached only 3 days after inoculation chlorotic flecks appeared, but rust development stopped at this stage. When detachment occurred 1 or 2 days after inoculation rust development was not visible on the leaves (Fig. 1).

The inhibition of rust was experienced in all of the susceptible and resistant combinations. In the case of covering the Petri dishes, depending on the length of dark treatment, chlorosis appeared on the leaves. This was never experienced when photosynthesis inhibitors were applied.

In the case of simultaneous application of one of the above treatments plus sucrose, the inhibitory effect was always overcome. Sucrose alone accelerated sporulation and prevented the inhibition of rust development caused by the dark treatment or by photosynthesis inhibitors (Fig. 2). We got similar results in resistant combinations. However, the dark treatment often caused yellowing in the infected leaves.

Discussion

The result strengthened the earlier idea on the importance of carbohydrates in rust development. Considering that the rate of photosynthesis (the major source of carbohydrates) is decreasing in the sporulation phase, rust development is very sensitive to any further decrease of light at this stage.

By the inhibition of the Hill reaction we could simulate the dark effect and inhibition of rust development. This inhibition can be reversed in every case by adding sucrose. These facts suggest that any disturbances in carbohydrate production by the host plant may led to starving of the pathogen and led to an apparent resistance to it.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 49—57 (1981)

The Role of Protein Metabolism of Wheat in Amino Acid Induced Resistance to Rust

By

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Amino acids in excess can induce resistance in detached wheat leaves to stem rust *(Puccinia graminis tritici)*, but the mechanism is still unknown. Earlier experiments indicated, that protein metabolism of the plant is involved in the mode of action.

Although the effect of the amino acid treatments on the total protein content of wheat leaves was very slight, the polyacrylamide gel electrophoresis showed qualitative changes in protein composition. It is difficult to relate alteration of a special protein band to changes in resistance. Still, these data present evidences that amino acid treatments cause disturbances in protein metabolism. Disturbances are not expressed in amino acid composition of the total protein extract, but in the inhibition of incorporation of ^{14}C-leucine into the protein fraction.

The data suggest, that high concentrations of amino acids, which are almost toxic to the plant, cause changes in protein composition and a general reduction in protein synthesis and/or an induction of protein degradation. It is unfavourable for the development of the rust. This effect can be reduced by inhibiting the induction of proteases and nucleases with protein synthesis inhibitors.

A relatively great amount of studies have been done on the role of different amino acids in plant diseases. An excess of some amino acids may induce rust resistance in different wheat cultivars in detached leaf culture too (SAMBORSKI and FORSYTH, 1960; VAN ANDEL, 1966).

Our previous results show, that protein synthesis inhibitors can decrease the resistance induced by amino acids and suggest that protein metabolism of the host plant may be responsible for changes of resistance (BARNA *et al.*, 1977). However, it is not clear how the general protein metabolism is affected by the application of amino acids in excess. The purpose of this study was to obtain information on the possible changes in the protein composition, in the incorporation of labelled amino acid and in the amino acid composition of proteins of uninfected wheat leaves treated by amino acids and to examine the relationship between this possible changes of protein metabolism and changes in resistance.

Materials and Methods

Little Club (C. I. 4066) wheat cultivar was grown in greenhouse. For the experiments primary leaves of 8 days old plants were used.

Infection

In order to control the resistance of plants in each experiment additional plants were inoculated with uredospore suspension of *Puccinia graminis* f. sp. *tritici* race 11 and held in a moisture chamber for 20 hrs. The reaction types were determined 12 days after inoculation according to STAKMAN *et al.* (1962).

Treatments of leaves in detached culture

Five centimeter long first leaves of 8-days-old seedlings were detached one day after inoculation and floated on 20 ml of different solutions in Petri dishes. All solutions contained 40 ppm benzimidazole, in addition to the amino acids and/or the protein synthesis inhibitors. The dishes were kept in greenhouse. Amino acids and protein synthesis inhibitors applied were as follows: L-histidine and L-methionine in 1000 ppm, DL-isoleucine in 2000 ppm and DL-serine in 2000 ppm, Actinomycin-D, cycloheximide and chloramphenicol. The Petri dishes were sterilized before use, the chemicals were solved in deionized, sterilized water, and we applied every day fresh solutions to avoid contaminations.

Protein extraction and determination

Protein contents for determination were extracted by the method of SCHMIDT and TANNHÄUSER (1945) modified by FLETCHER and OSBORN (1965).

Uninoculated leaves were macerated and extracted with 80 per cent ethanol, then with 10 per cent TCA at 4 °C and finally with the mixture of absolute ethanol: ether (3 : 1 v/v). The residue remaining from the final extraction was subjected to N NaOH and incubated overnight at 37 °C. An aliquot of the solution was assayed by the method of LOWRY *et al.* (1951).

Polyacrylamide gel electrophoresis

Two-g samples of uninoculated leaf tissue after the different treatments were homogenized with 6 ml of 0.15 *M* phosphate buffer (pH 7.2) and quartz sand at 4 °C. The sap was expressed through four layers of cheesecloth and then centrifuged at 6000 *g* for 30 min at 0 °C, and the supernatant ultracentrifuged (50 000 *g*) for 1 h. Protein content of the supernatant was determined as described in the previous part. Aliquots of supernatants were used for electrophoresis. The polyacrylamide gel electrophoretic method worked out by DAVIS and ORNSTEIN was followed as described earlier (BARNA *et al.*, 1975). Evaluation was made by scanning the gels in a Joyce Chromoscan densitometer.

Protein hydrolysis

Proteins from treated and untreated leaves were extracted as described above. The residue remaining after extraction with ethanol: ether 3 : 1 were

dissolved in 100–200 fold excess of 6N HCl in a tube, evacuated and the tube was sealed. Tubes were kept at 105 °C for 24–72 hours. After hydrolisation acid was removed in a Petri dishes in vacuum desiccator over solid P_2O_5.

GLC method

To detect the different amino acids in protein hydrolysate we used the GLC method developed and studied by GEHRKE and his coworkers (1969, 1970).

We put about 0.1–0.1 mg amino acid standards or equivalent protein hydrolysate into screw-cap septum vial, added 25 µl acetonitrile and 25 µl BSTFA (N, O-bis-trimethylsilyl-trifluoroacetamide). The vials were heated for one and a half hours at 135 °C. The conditions for the gas chromatographical detection were as follows:

Detector type: flame ionization detector.

Column: 180 cm long, glass with 4 mm internal diameter, packed with 5% OV-17 on silylated Chromosorb W 60–80 mesh.

Injector temperature: 215 °C. Detecto rtemperature: 215 °C. Sensitivity: 1×10^{-9}. Column temperature: 80–210 °C, 2°/min. Carrier gas: N_2, min.

^{4}C-leucine incorporation studies

Uninoculated leaves were floated on 20 ml of the appropriate solutions containing 1.8 ml ^{14}C-leucine of 10 µC/ml activity (specific activity 10.46 mC/mM) for 1, 4 or 20 hours. The preincubation with the test solutions before addition of the radioactive isotop lasted for 2, 24 or 48 hours. The extraction method was the same described in chapter protein determination. After incubation, the leaves were rinsed three times with distilled water and blotted dry. Since most of the radioactivity was present in the ethanol soluble fraction and in the NaOH soluble fraction only these fractions were collected. Activities were determined using a GM-counter. In every case protein determination was performed from the protein containing fraction. Activity was counted as cpm/g fresh weight or as cpm/mg protein, and was expressed as per cent of the control.

Results

Protein changes in detached leaf cultures

Total protein contents did not change significantly after amino acid treatments. Even cycloheximide caused only 10–15 per cent decrease in protein content after a 4-day-treatment (Table 1). On the other hand, slight qualitative changes were found in polyacrylamide gel after electrophoresis (Fig. 1). However, this

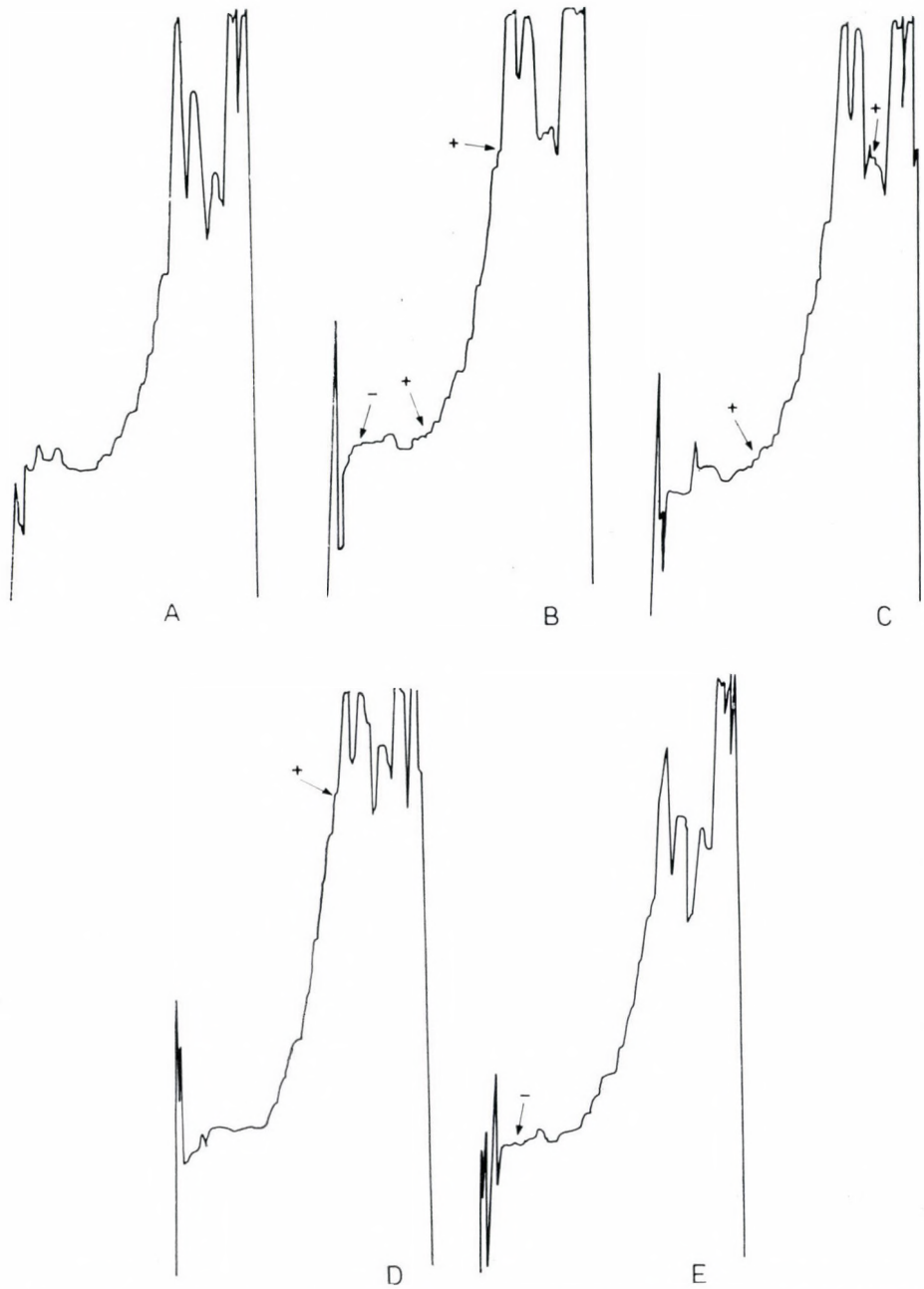

Fig. 1. Protein bands after polyacrilamid gel electrophoresis of wheat leaf extracts. Leaves were floating for 5 days on the experiment solutions. One sample on each tube contained 200–300 μg protein. Gels were stained with 1% amidoblack and evaluated by Joyce Chromoscan densitometer. A = control, B = histidine, C = methionine, D = serine, E = isoleucine treatment, + = new band appeared, − = one band disappeared

Table 1

The total protein content in Little Club wheat leaves treated by different amino acids and/or protein synthesis inhibitors. Leaves were floating for 4 days on the experiment solutions

Treatment	Protein content %
Control (0)	100
+ actinomycin D (1 ppm)	94.8
+ cycloheximide (0.5 ppm)	88.1
+ chloramphenicol (40 ppm)	101.9
L-Histidine	101.3
+ actinomycin D	105
+ cycloheximide	93.6
+ chloramphenicol	99.3
L-Methionine	94.5
+ actinomycin D	88.4
+ cycloheximide	92.1
+ chloramphenicol	95.6
DL-Serine	101.7
+ actinomycin D	103.2
+ cycloheximide	91.1
+ chloramphenicol	105.3
DL-Isoleucine	95.5
+ actinomycin D	97.1
+ cycloheximide	89.1
+ chloramphenicol	88.7

changes were different in each amino acid treatment. Some bands strengthened or appeared some decreased or disappeared as compared to the control, only benzimidazole treated sample. Naturally, the mechanism of induced rust resistance is not necessarily the same, but it still seems difficult to relate changes of one or two protein bands to resistance.

Uptake of ^{14}C-leucine as influenced by amino acid treatments

The total activities were strongly decreased as an effect of each amino acid treatment (Fig. 2), which refers to the inhibition of amino acid uptake. Activities, expressed in per cent of the control were similar after 14 or 20 hours incubation both in the alcohol and in NaOH soluble fractions. As a result of treatments with different amino acids, the aggregate sum of activities of the fractions suggests that uptake of ^{14}C-leucine into the leaves was inhibited approximately by the same extent by each amino acid. This lends to support the hypothesis that L-amino acids have the same carrier system. Treatments with protein synthesis inhibitors did not affect the uptake of the labeled amino acid.

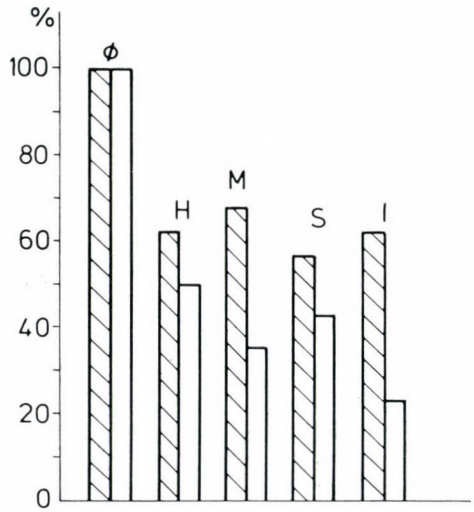

Fig. 2. Incorporation of ^{14}C DL-leucine into the different fractions of wheat leaves treated in detached leaf culture. The pretreatment with amino acids lasted for 48 hours, the incubation with the radioactive material and the amino acid lasted for 20 hours. ■ activity of the sum of the fractions in per cent of the control, □ activity of the protein containing fraction in per cent of the control the absolut activity of the control: □ 2879 cpm/g fresh weight, ■ 4827 cpm/g fresh weight, ø = control, H = histidine, M = methionine, S = serine, I = isoleucine treatment

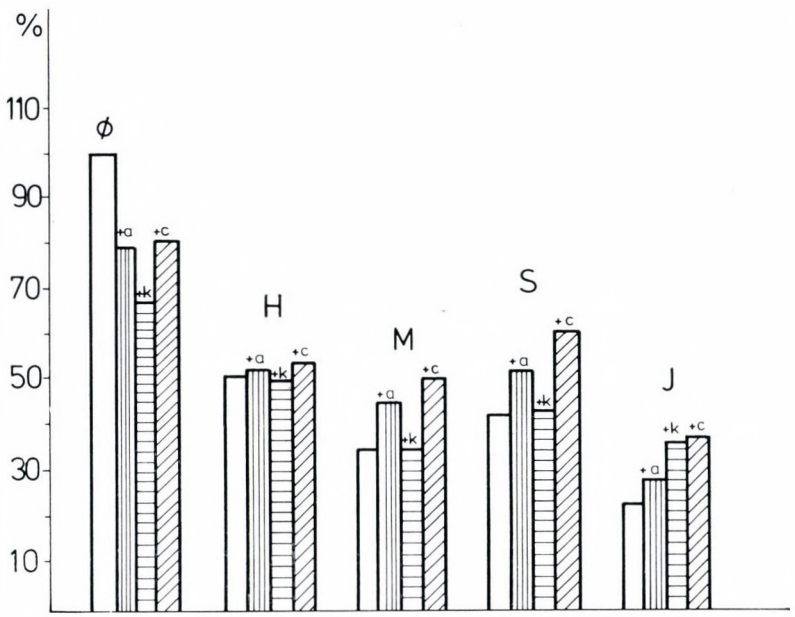

Fig. 3. Incorporation of ^{14}C-leucine into the protein containing fraction as influenced by amino acid and/or protein synthesis inhibitor treatments in per cent of the control. ø = control, H = histidine, M = methionine, S = serine, I = isoleucine, a = actinomycin D (1 ppm), k = chloramphenicol (40 ppm), c = cycloheximide (0.5 ppm). Activity of the control = 24.4 cpm/mg protein

Incorporation of ^{14}C-leucine into the protein fraction as influenced by amino acid and/or protein inhibitor treatments in detached leaf cultures

Incorporations of ^{14}C-leucine into the protein containing fraction of wheat leaves are seen on Fig. 2 and Fig. 3. Activities are decreased in consequence of amino acid treatments. However, this decrease is partly the consequence of the inhibition of ^{14}C-leucine uptake. On the other hand, all of the amino acids, but especially L-methionine and DL-isoleucine in addition to the decreased uptake, still inhibited the incorporation of ^{14}C-leucine into the protein fraction (Fig. 2). Protein synthesis inhibitors alone decreased the incorporation by 25–35 percent. However, amino acid-protein inhibitor combinations caused inhibitions of incorporation only about the same extent as amino acids alone, or relatively increased the incorporation (Fig. 3). Especially cycloheximide was effective in raising the amino acid-decreased incorporation activity and at the same time cycloheximide was the most active in decreasing the amino acid induced resistance to rust (BARNA *et al.*, 1977). None of the inhibitors changed remarkably the incorporation of ^{14}C-leucine in the presence of histidine. The length of preincubation before addition of the radioactive materials did not effect significantly the incorporation. In conclusion, data with the radioactive material show, that all the amino acids inhibited the incorporation into the protein containing fraction, which refers to the inhibition of protein synthesis.

Amino acid analysis by GLC

The retention times of the amino acids examined are presented in Table 2. In order to control the method we added different quantity of serine, isoleucine and methionine to the mixture of standard amino acids. In every case we were

Table 2

The retention times of different amino acids in GLC analysis

Amino acid	Time (minutes)
alanine	4.5
glycine	5.5
valine	7.0
leucine	8.5
iso-leucine	9.5
aspartic acide	11.0
serine	12.0
threonine	15.0
hydroxyproline	16.0
cisteine	17.0
glutamic acide	19.0
methionine	20.0
phenylalanine	22.0

able to detect the adequate increase in the quantity of the corresponding amino acid.

In spite of this, we did not find differences in the amino acid quantities of protein hydrolisates of wheat leaves treated and nontreated by amino acid.

We controlled the GLC method by amino acid analyser. The analysis gave the same result as the GLC, that treatment of leaves by amino acid solutions does not change the amino acid composition of proteins.

Discussion

In spite of the several observations, when amino acid treatments increased resistance, the mode of action is generally not clear. In some cases the amino acids have a direct chemotherapeutic effect, as in the case of apple leaves infected with *Venturia inaequalis*, or when cucumber seedlings are infected with *Cladosporium* etc., but we have indications that amino acids have no direct inhibitory effect on the development of *Puccinia graminis tritici* and that the protein metabolism of the plant is involved in the mode of action (BARNA *et al.*, 1977).

Although the effect of the amino acid treatments on the total protein content of wheat leaves was very slight the gel electrophoresis showed qualitative changes in protein composition.

It is difficult to relate alteration of a special protein band to changes in resistance. Still, these data present evidences that amino acid treatments cause disturbances in protein metabolism of wheat leaves. These disturbances are not expressed in amino acid composition of the total protein extract (GLC analysis), but in experiments with labeled amino acid. The inhibition of incorporation of ^{14}C-leucine into the protein containing fraction refers to the inhibition of protein synthesis, which can lead to resistance to rust. Similarly, the inhibition of the growth of *Marchantia polymorpha* gemmalings by lysine and threonine was correlated with an inhibition of protein synthesis (DUNHAM and BRYAN, 1971). The paradoxical results with protein synthesis inhibitors can be explained by the fact, that protoplasts from cycloheximide pretreated leaves are more active in incorporating labeled L-leucine, uridine and thymidine into TCA insoluble materials (KAUR-SAWHNEY *et al.*, 1976). This effect is due to the inhibition of post excision formation of proteases and nucleases in the floated leaves.

In conclusion, the high concentrations of amino acids, which are almost toxic to the plant, cause changes in protein composition and a general reduction in protein synthesis and/or an induction of protein degradation. This is unfavourable for the development of the rust. The effect can be reduced by inhibiting the induction of proteases and nucleases with protein synthesis inhibitors. These results suggest, that changes in protein metabolism of wheat leaves caused by amino acids in excess, are in correlation with changes in resistance. Similar conclusion was drawn by YAMAMOTO *et al.* (1976) and TANI *et al.* (1978) with oat rust resistance.

Acknowledgements

The authors thank Mrs. HANNA HENGER and Mrs. ERZSÉBET VÖDRÖS for technical assistance in the isotope work, Mrs. ERZSÉBET NESZMÉLYI for the automatic amino acid analysis and for Miss MARIAN VINCZE for her technical assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 59–65 (1981)

The Role of Polyphenols, Oxidative and Macerating Enzymes in Onion Bulb Cultivars Infected with *Botrytis allii*

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Three cultivars of onion differed in their susceptibility or resistance to the neck rot disease incited by *B. allii*. Behairi cultivar was susceptible, Giza 6 was moderately susceptible and Taliani Red was resistant. The differential response to the disease is attributed to higher content of polyphenols and the higher activity of the oxidases in the resistant cultivar which inhibit the macerating enzymes excreted by *B. allii* that induce neck rot.

Onion (*Allium cepa* L.) is one of the main export crop in Egypt. It is rated the third exported crop after cotton and rice (ANON., 1972). More than 100 thousand tons of fresh onion and 3 thousand tons of dried onions are exported every year (ANON., 1972). Neck rot of onion incited by *Botrytis allii* is a common post-harvest disease which was reported from the growing areas of onion all over the world. The literature on the occurrence of the disease is extensive (KAMEL, 1952, and EL-HELALY *et al.*, 1962), however, the work on the varietal resistance and the enzymatic activities of the fungus is rather limited (HANCOCK *et al.*, 1964, and HANCOCK and MILLAR, 1965 a & b).

The present work was undertaken (i) to investigate the response of certain cultivars of onion to the disease and (ii) to determine the polyphenols, the oxidases, and the macerating enzymes which might be involved in disease response.

Materials and Methods

Medium size onion bulbs of certain cultivars, namely, Behairi, Giza 6, and Taliani Red were used. Onion bulbs were surface sterilized by dipping in 0.1% mercuric chloride solution for 3 minutes then rinsed in sterile water. Such bulbs were inoculated by inserting a 6 mm disc from the advancing edge of *B. allii* previously isolated from Giza 6 diseased bulbs-grown on PDA, 3 mm deep near the neck of the bulb. Bulbs were kept in perforated polyethylene bags at room temperature (24–31 °C) until symptom development. Bulbs served as checks were similarly treated except using fungus-free PDA discs. Four bulbs were used in each replicate (bag) and four replicates consisted a treatment.

Phenolic compounds were determined in the *B. allii* artificially-infected and healthy onion bulbs of three cultivars by adopting the method of KHALIFA *et al.* (1968).

Determination of enzymes

Pectolytic [polymethyl-galacturonase (PMG), and pectinmethyl-esterase (PME)], cellulase, polyphenoloxidase, peroxidase and catalase enzymes were determined in tissues of healthy and *B. allii* artificially-infected onion bulbs of the three cultivars tested. The assay of the pectolytic enzymes and the cellulase were determined according to the method used previously (WASFY *et al.*, 1977).

Polyphenoloxidase assay

For determination of phenoloxidase, the method described by Braesch (1954) was used. Phenoloxidase activity was determined in inoculated and non-inoculated onion bulbs after 16 days. Enzyme activity was measured by grinding 10 g of inoculated and non-inoculated tissues in a mortar with 14 ml borate buffer (pH 9). Extracts were centrifuged for 15 minutes at 4,000 rpm. The supernatant was diluted by adding 8 ml distilled water to 2 ml supernatant. Aliquots of diluted supernatants were assayed for phenoloxidase activity using spekol colorimeter at 575.5 nm. The reaction mixture consisted of 2 ml borate buffer (pH 9), 1 ml of 1 % P-aminobenzoic acid, 2 ml of 1 % catechol and 0.2 ml extract. After mixing for 45 minutes, a red colour was formed. The activity of the enzyme was measured as relative deepness in red colour, by optical density. Control treatment was done similarly except that extracts were previously boiled. Four replicates were used in each treatment.

Peroxidase assay

For determination of peroxidase, the method described by FEHRMANN and DIMOND (1967) was used. Peroxidase activity was determined in inoculated and non-inoculated onion bulbs after 16 days. Enzyme activity was measured by grinding 10 g of inoculated and non-inoculated tissues in a mortar with 14 ml phosphate buffer (pH 6). Extracts were centrifuged for 15 minutes at 4,000 rpm. The supernatant was diluted by adding 8 ml distilled water to 2 ml supernatant. Aliquots of diluted supernatant were assayed for peroxidase activity using spekol colorimeter at 470 nm. The reaction mixture consisted of 1.5 ml of 0.04 M catechol solution, 1.5 ml H_2O_2 (20 volume), 1.5 ml phosphate buffer (pH 6) and 0.2 ml of extract. The control treatment was done similarly except the extract was previously boiled. Four replicates were used in each treatment. The difference in optical density between the reaction mixture and that of the control was taken as a measure of the activity of the reaction. Enzyme activities were expressed as the increase in optical density from 60 – 120 seconds after the substrate was added.

Catalase assay

Catalase activity (COLOWICK and KAPLAN, 1955) of inoculated and non-inoculated onion bulbs were determined. Enzyme activity was measured by grinding 10 g of tissues in a mortar with 14 ml phosphate buffer (pH 7). Extracts were centrifuged for 15 minutes at 4,000 rpm. Aliquots of the supernatants were assayed for catalase activity, by the addition of 4 ml of the extract to 60 ml of 0.01 N H_2O_2 solution. The mixture was incubated at 25 °C for 55 min. The decomposition of H_2O_2 was measured by titrating the remaining substrate with 0.0052 N potassium permanganate solution after stopping the enzymatic reaction with 5 ml 2% (v/v) sulfuric acid. A sample of 5 ml was taken from each assay mixture at 2-min. at first and 5-min. intervals, and the remaining H_2O_2 was titrated. Four replicates were used in each treatment.

Results

Pathogenicity tests

Disease symptoms of *B. allii* appeared on the susceptible cultivars after 16 days of inoculation, with no symptoms on the check. Symptoms of artificially-inoculated bulbs were similar to those obtained naturally. Onion cultivars tested differed in their response to the tested isolate of *B. allii*. Behairi cultivar was highly susceptible, Giza 6 was moderately susceptible, and Taliani Red was resistant as it showed no disease symptoms after the same period of incubation (Fig. 1).

Fig. 1. Onion bulbs of three cultivars artificially-inoculated with *B. allii* and cut lengthwise after 16 days, showing neck rot on Behairi (left) and Giza 6 (middle), with no symptoms on Taliani Red (right)

Phenolic compounds

Determination of phenolic compounds in the *B. allii* artificially-infected and healthy onion bulbs of the three cultivars showed higher polyphenolic content in the Taliani Red than in Behairi and Giza 6. This result is true in both artificially-infected and healthy bulbs (Table 1).

Table 1

Phenolic compounds in *B. allii* artificially – infected and healthy onion bulb varieties after 16 days at room temperature (24–31 °C)

Cultivar	mg/100 g dry weight	
	Treatment	
	Check	Infected
Behairi	12.7	19.3
Giza 6	13.2	19.5
Taliani Red	20.4	21.1

Enzyme activities

Determination of the activities of the macerating and oxidative enzymes in healthy and *B. allii* artificially – inoculated onion bulbs showed that the activities of such enzymes were higher in the inoculated than in healthy bulbs. The macerating enzymes (PMG, PME and cellulase) were much higher in the Behairi and Giza 6 than in Taliani Red cultivar (Table 2). On the other hand, the activity of the oxidative enzymes was more marked in the Taliani Red than in either Behairi or Giza 6 cultivars (Tables 3 and 4).

Table 2

PMG, PME and cellulase activities in onion bulbs of three cultivars artificially-inoculated with *B. allii* after 16 days at room temperature (24–31 °C)

Cultivar	Treatment	PMG[1]	PME[2]	Cellulase[1]
Behairi	Non-inoculated (check)	2.91	3.21	2.43
	Inoculated	63.98	16.66	98.48
Giza 6	Non-inoculated (check)	3.13	3.51	2.91
	Inoculated	49.67	10.00	85.17
Taliani Red	Non-inoculated (check)	1.93	2.44	1.60
	Inoculated	2.11	3.16	2.78

[1] Reduction in viscosity %
[2] μ equivalent/min/ml.

Table 3

Polyphenoloxidase and peroxidase activities in onion bulbs of three cultivars artificially-inoculated with *B. allii* after 16 days at room temperature (24—31 °C)

Cultivar	Treatment	Optical density	
		Polyphenol-oxidase	Peroxidase
Behairi	Non-inoculated (check)	0.150	0.020
	Inoculated	0.280	0.120
Giza 6	Non-inoculated (check)	0.110	0.050
	Inoculated	0.310	0.145
Taliani Red	Non-inoculated (check)	0.290	0.150
	Inoculated	0.320	0.155

Table 4

Activity of catalase in *B. allii* artificially infected and healthy onion bulb varieties after 16 days at room temperature (24—31 °C)

Cultivar	Treatment	Mg H_2O_2 Decomposed after (min)					
		2	5	10	15	20	25
Behairi	Non-inoculated (check)	0.406	0.666	0.908	1.133	1.271	1.470
	Inoculated	0.611	1.617	1.945	2.144	2.170	2.256
Giza 6	Non-inoculated (check)	0.510	0.951	1.340	1.522	1.686	1.893
	Inoculated	0.626	0.991	1.491	1.600	1.824	1.954
Taliani-Red	Non-inoculated (check)	0.562	1.046	1.513	1.755	1.962	2.075
	Inoculated	0.593	1.177	1.611	1.821	1.989	2.089

Cultivar	Treatment	Mg H_2O_2 Decomposed after (min)					
		30	35	40	45	50	55
Behairi	Non-inoculated (check)	1.703	1.859	1.962	2.118	2.222	2.300
	Inoculated	2.274	2.291	2.308	2.326	2.343	2.360
Giza 6	Non-inoculated (check)	1.962	2.118	2.170	2.248	2.291	2.334
	Inoculated	2.118	2.205	2.239	2.274	2.308	2.342
Taliani-Red	Non-inoculated (check)	2.118	2.205	2.239	2.300	2.306	2.331
	Inoculated	2.145	2.224	2.251	2.300	2.326	2.351

Discussion

The differential response of the three onion cultivars to the neck rot disease incited by *B. allii* could be explained in the light of the polyphenol content, oxidative and macerating enzyme activities. Pathogenicity tests showed that Behairi

cultivar was susceptible, Giza 6 moderately susceptible and Taliani Red was resistant. The macerating enzymes (PMG, PME and cellulase) activity was higher in the *B. allii*-inoculated bulbs of Behairi and Giza 6 than in those of Taliani Red. However, the polyphenols and the oxidases (polyphenoloxidase, peroxidase, and catalase) were higher in the *B. allii*-inoculated bulbs of Taliani Red than in Behairi or Giza 6. Greater accumulation of polyphenols in the resistant than in the susceptible cultivars was supported by HAMPTON (1962), KIRÁLY and FARKAS (1962), PATIL *et al.* (1962), and ABD-ELRASIK *et al.* (1972). Meantime, such polyphenols were most likely inhibitory to the macerating enzymes excreted by the fungus as such enzymes decreased in the resistant as compared with the susceptible cultivars. These results agree with those obtained by BYRDE (1957; 1963) and BYRDE *et al.* (1960), DEESE and STAHMANN (1963), and WILLIAMS (1965) who stated that the oxidized phenols are strongly inhibitors of extracellular pectolytic enzymes of fungi causing wilt and soft rots.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 67–72 (1981)

A Simplified Method for Isolating and Detecting the Frequency of Occurrence of Free Living *Streptomyces scabies* in Infected Soils

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Based on the cultural-morphological and physiological properties of the neotype (ATCC 33282) and type (ATCC 33281) strains of *Streptomyces scabies* and *Str. scabies* var. *achromogenes*, respectively, the authors suggest a usable scheme of isolations, simultaneous comparative investigations and clustering of soil streptomycetes for detecting the presence and frequency of occurrence of this species in samples of infected soils. To demonstrate the usefulness of this scheme, *Streptomyces scabies* strains were selected from the complex streptomycete population (composed of 23 identified species and the members of an unidentified heterogeneous group of streptomycetes) of a scabies-infected potato-free soil of the experimental farm of the Potato Research Department of the Agricultural University, Keszthely, Hungary.

Potato scab is a wide-spread disease causing a considerable economic loss in agriculture. Among the microorganisms responsible for scab production *Streptomyces scabies*, this extensively studied species of the genus *Streptomyces*, is without doubt the most important. Unfortunately, only little is known on the ecology of this species and on its frequency of occurrence in different soil types and after the application of various agricultural techniques. This lack of available information is due chiefly to the uncertainty on its systematic position (HÜTTER, 1967; BERGEY's Manual, cf. BUCHANAN and GIBBONS, 1975), and to the fact that in the scabs and on the surface of infected tubers, apart from the true pathogenic agent, many different species of *Streptomyces* may occur simultaneously (and generally in law number) which were isolated, studied and regarded by different authors as true representatives of "*Str. scabies*". This is the reason why although there are many data in the literature on the behaviour in the soils of this "species", the majority of these data is hardly evaluable.

In 1979, studying the scabies-populations of scab lesions of infected tubers of different potato cultivars, and taking the various species descriptions of many workers dealing with the scabies problem into consideration, we have selected, from among 452 "scab-strains", a strain available for designating as the neotype strain of *Str. scabies*, which was then deposited in the American Type Culture Collection as ATCC 33282. At the same time, the type strain (ATCC 33281) of an achromogenic variety *(Str. scabies* var. *achromogenes)* of this species was also designated and described. Detailed simultaneous comparative investigations

of fresh streptomycete isolates with these neotype and type strains may give us now more possibilities to detect the occurrence in various habitats of these organisms, capable also of saprophitically living in the soils which we can consider the most important source of the infection of potato.

The purpose of this paper is to present a simplified scheme of a particular strain selection process, that we can use with good results on trying to answer the question whether *Streptomyces scabies* type organisms are or are not present in a given soil sample, and if yes, how large is their frequency of occurrence in it?

Materials and Methods

To detect the presence of *Str. scabies* in the soil (A-horizon of a brown forest soil) of the experimental farm of the Potato Research Department (Keszthely, Hungary) soil samples were collected at three sampling sites from about 5–10 cm depth. The individual samples were then pooled to give a composite sample from which soil dilution plates were prepared using casein-starch agar and glycerol-arginine agar for isolation of streptomycetes. A representative random sample of isolates (190 purified *Streptomyces* strains) was then submitted to a selection procedure described under Results of this paper.

For the comparative investigations of strains, methods (morphological sections of SHIRLING and GOTTLIEB; colour wheals developed by TRESNER and BACKUS; determination of carbon utilization pattern on PRIDHAM and GOTTLIEB's basal salts medium; indicator pigments detected by observing the effect of 0.05 N NaOH and 0.05 N HCl on the colour of the reverse side of colony, etc.) and media (yeast extract-malt extract agar; oatmeal agar; inorganic salts-starch agar; glycerol-asparagine agar; tyrosine agar; peptone-yeast-iron agar) employed by the collaborators of the International Streptomyces Project (ISP) were used (SZABÓ and MARTON, 1976). Nitrite produced from nitrates in nitrate broth was demonstrated by the Griess–Ilosvay method. Hydrogen sulfide test was studied by the method recommended by Küster and Williams. The hydrolysis of Tweens was tested by the methods described by SIERRA. Hydrolysis of starch and cellulose, decomposition of gelatin, urease production, utilization of nitrate as sole source of nitrogen, growth on paraffin and in the presence of 3–10% NaCl were tested by methods used in our laboratory (SZABÓ, 1974). The taxonomic identifications were carried out on the basis of ISP criteria using the ISP-redescriptions of the type and neotype strains of Streptomyces spp.

Results

The scheme offered here for a rational selection of *Str. scabies* strains from soils and followed while studying the scabies-population of the infected soil of the experimental farm of Potato Research Department is presented in Table 1. The obtained representative collection of about 190 streptomycete strains, isolated

Table 1

A simplified scheme of the rapid selection of Streptomyces scabies strains from among the members of a randomly isolated representative collection of Streptomyces strains

1. *Initial Step* Isolation on a random basis of a representative collection of soil strains from media used in practice for plate counting of soil actinomycetes	→ All of the isolates (true bacteria and actinomycetes) which do not belong to the genus Streptomyces can be excluded from further work
2. *Second Step* Separation for further investigations only the strains which produce spore mass colour in the Gray-series according to the Tresner-Backus colour-wheels	→ All of the strains belonging to Yellow-, White-, Green-, Red-, Purple-, and Blue-series can be excluded from further investigations
3. *Third Step* Selection for strains producing spirale sporophores	→ All of the strains producing rectus-flexibilis spore chains can be excluded from further comparisons
4. *Fourth Step* Selection for strains having a complete ISP carbon utilizing spectrum	→ All of the strains having incomplete ISP carbon utilization spectra can be excluded from further observations
5. *Fifth Step* Selection for strains producing only smooth spores observed in electron microscope	→ All of the strains having spiny or hairy spore surface ornamentation can be excluded from further work
6. *Sixth Step* Selection for strains that cannot decompose cellulose, paraffin, do not grow at temperatures over 42 °C and at about 8—10% NaCl concentration	→ All of the strains which decompose cellulose and/or paraffin, capable of growing over 42 °C and at 8—10% NaCl can be excluded from further work
7. *Seventh Step (ultimate Step)* Special-purpose investigations (e.g. inoculation of plants, etc.) with the selected Str. scabies strains	→ Selection of chromogenic and achromogenic, apathogenic, etc. variants

on a random basis, was at first divided into the following two large clusters: a group of typically gray sporulated streptomycetes on the one hand, and a very heterogeneous group of streptomycetes possessing mature aerial mycelium characteristic for the Yellow, Red, Blue and White series of the TRESNER – BACKUS colour wheels on the other. Moreover, we separated also a third group of streptomycetes (altogether 10 strains) the sporulation of the members of which was very poor and unsuitable for typifying. Certain properties of these strains, however, revealed that taxonomically they were distinct from *Str. scabies*. Consequently, just as the strains that belonged to the Yellow, Red-, Blue- and White colour series, they were excluded from the further comparisons. But in order to obtain an insight into the specific structure of this local streptomycete community, we identified them. They proved to be the representatives of species *Str. bluensis*, *Str. roseolus*, *Str. parvus*, *Str. viridochromogenes*, *Str. levoris*, *Str. pilosus*, *Str. violaceus*, *Str. lavendulae*, *Str. rimosus*, *Str. lipmanii*, *Str. phaeochromogenes* and *Str. collinus*.

From among the total of 112 strains (59%) which possessed gray spore mass colour, 100 produced spirale sporophores and 12 rectus-flexibilis type straight spore chains. As the members of species *Str. phaeopurpureus*, *Str. avellaneus* and *Str. zaomyceticus*, the latter were similarly excluded from further comparison. As *Str. scabies* has a complete ISP carbon utilization spectrum (D-glucose, L-arabinose, D-xylose, i-inositol, D-mannitol, D-fructose, rhamnose, sucrose and raffinose are all utilized for growth), and this spectrum proved to be extraordinarily stable at its strains, we have also excluded from the further selection process all gray sporulated streptomycetes which produced spirale sporophores and possessed more or less deficient carbon utilization spectra. Among our isolates, such combinations of diagnostic features showed, the representatives of the following streptomyces species: *Str. griseoflavus*, *Str. flaveolus*, *Str. violaceochromogenes*, *Str. argenteolus* and *Str. endus*. Thereafter, from among these strains of a complete ISP C-utilization spectrum we separated and excluded again on the basis of electron microscopic observations a representative of the spiny ornamented spore producing species *Str. ganmycicus*. Finally, from among the remained gray sporulated, smooth spores and spirale sporophores producing strains capable of utilizing all ISP diagnostic carbon compounds we have separated three strains (No 127.62 and 23) which were cellulose and paraffin negative and could not grow at temperatures over 42 °C and in higher NaCl concentrations such as 7 per cent. These are the true representatives of species *Str. scabies*. The very closely related strains which can grow within a temperature range of between 42 – 50 °C and at the same time, decompose cellulose and/or paraffin and tolerate 8 – 10% NaCl concentrations are belonging to the variable group a *Str. diastatochromogenes*. This extraordinarily common species characterized by a world-wide distribution shows a frequent occurrence also in the investigated soil of the Potato Research Department.

The selected *Str. scabies* strains showed completely identical diagnostic features with those of the neotype strain ATCC 33282 of *Str. scabies:* Spore chain morphology: Section spirales. Mature spore chains generally long, often with

more than 50 spores per chain. This morphology is seen on oatmeal agar, salts-starch agar and glycerol-asparagine agar. Spore surface: smooth. Aerial mass colour in the gray colour series on oatmeal agar, salts-starch agar and glycerol asparagine agar. Reverse side of colony: no distinctive pigment (Yellow-brown colour series); substrate pigment is no pH indicator. Melanoid pigments formed in peptone-yeast-iron agar and tyrosine agar. Pigments other than melanoids not formed in yeast-malt agar, oatmeal agar, salts-starch agar or glycerol asparagine agar. Carbon utilization: D-glucose, L-arabinose, D-xilose, D-fructose, rhamnose, sucrose, i-inositol, D-mannitol, raffinose, lactose, maltose, starch, inulin and dextrin are all utilized for growth. Dulcitol is not utilized. Growth on melibiose is variable. Tween 40 and 60 are hydrolised. Urease positive. Nitrate reduction variable. Cellulose and paraffin not decomposed. Nitrate as sole source of nitrogen is utilized. H_2S produced from peptone and L-cysteine. Growth at 40 °C but not at 42 °C. Gelatin hydrolysed.

Discussion

Above we have demonstrated that with an adequate scheme of strain-selection (Table 1) it is possible to isolate Str. scabies strains from potato tuber free, but infected soil samples. The complete lack of such organisms among the members of an isolated representative collection of Streptomyces isolates (for which case we have also examples in our laboratory) makes very probable that this infective agent is either completely lacking from the investigated soil sample, or it occurs only in such a low number, that we can hardly detect it with these methods. Naturally, by increasing the number of isolated and investigated strains the probability also increases that we will catch it after all. Another problem is that at present we cannot solve the task of the quick separation of pathogenic and apathogenic variants of this species on the basis of cultural-physiological investigations, and for this reason plant reinoculation tests are necessary. The production of melanoid pigments in tyrosine- and/or in peptone-iron agar media as a diagnostic criterium is not an available tool either for species differentiation or for detection of pathogenic abilities. Further investigation is also necessary to clarify the taxonomic relationships between *Str. scabies* and the variants (and many synonyms) of *Str. diastatochromogenes.*

The proposed scheme may also be suitable for estimating the relative frequency of occurrence of *Str. scabies* in soils. In the case of our investigated soil, this organism amounts to a few percent of the soil streptomycete flora. But calculating with 10^6 streptomycete germ count per 1 g dry soil, Str. scabies may be present in the soil of the experimental farm of the Potato Research Department at about $1-3\times10^4$/g dry soil, which we can consider already a very high inoculum-potential, supposed that this number represents chiefly the pathogenic variant. In the future, we intend to estimate the Str. scabies content of different soil types, and to find quick methods for separating the pathogenic and apathogenic variants of this species for soil population density estimations.

Acknowledgements

The authors gratefully acknowledge Professor I. SÁRVÁRI and Dr. M. LÖNHARD (Potato Research Department, Agricultural University, Keszthely, Hungary) for their suggestions in the course in this work.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 73–76 (1981)

b-Protein Variation in Virus-Infected Intraspecific Tobacco Hybrids

By

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TMV infection of tobacco hybrids resulting from crosses between *N. tabacum* cv. Burley 49 (hypersensitive) and cv. Judy's Pride Burley (sensitive) results in the appearance of an additional b-protein (b_1) as compared to the hypersensitive parent. Evidence that TMV-sensitive tobaccos possess the information necessary to code for b-proteins is discussed in relation to the expression of virus resistance and the presence of gene N.

The localisation of tobacco mosaic virus (TMV) by the hypersensitivity reaction in *Nicotiana tabacum* cv. Xanthi-nc is accompanied by the appearance of four new soluble proteins, b_1, b_2, b_3 and b_4 (GIANINAZZI, MARTIN and VALLÉE, 1970; GIANINAZZI and MARTIN, 1976; GIANINAZZI *et al.*, 1977). At least three of these new proteins ($b_1 - b_3$) can also be induced together with virus resistance by polyacrylic acid, showing that they are coded by the host plant (GIANINAZZI and KASSANIS, 1974). The four new proteins have similarly been found in *N. tabacum* cv. Samsun NN after TMV infection (VAN LOON and VAN KAMMEN, 1970), or following treatment with 2-chloroethylphosphonic acid (VAN LOON, 1977). This paper confirms the presence, after TMV infection, of the b-proteins in another hypersensitive cultivar of *N. tabacum*, Burley 49, and reports for the first time the appearance of an additional new b-protein in TMV-infected hybrids originating from crosses between Burley 49 and a TMV-sensitive "nn" cultivar of *N. tabacum*, Judy's Pride Burley.

8- to 10-week-old *N. tabacum* cv. Burley 49, cv. Judy's Pride Burley and the two hybrids resulting from reciprocal crosses were inoculated with TMV. All the inoculated leaves produced local necrotic lesions (hypersensitive necroses) except those of Judy's Pride Burley, which showed only slight chlorosis at the site of virus infection. Experiments were carried out in a constant environment room (16 hrs photoperiod, 12000 lux, day 23 °C/70 % humidity, night 17 °C/90 % humidity). Soluble leaf proteins from healthy and infected tobaccos were extracted after 1, 2, 4 and 7 days in phosphate-citrate buffer, pH 2.8, and analyzed by disc-electrophoresis in 10 % (w/v) polyacrylamide gel at 4 °C as previously described (GIANINAZZI *et al.*, 1977).

The soluble leaf proteins of all the hypersensitive cultivars (Burley 49 and both hybrids) were qualitatively modified by TMV infection. As shown in Fig. 1,

3 new soluble leaf proteins appeared in TMV-infected Burley 49 whilst 4 were detected in the hybrids, independent of the direction of the cross. Co-electrophoresis with extracts of TMV-infected *N. tabacum* cv. Xanthi-nc or cv. Samsun NN showed that b_1, b_2 and b_3 of Fig. 1 correspond to the bands b_1, b_2 and b_3 previously reported by GIANINAZZI *et al.* (1970, 1977), whilst the band $b_{1'}$, appears to be specific to the hybrids. No b-proteins were found 1 day after virus inoculation. b_1 and $b_{1'}$ were detected after 2 days, 7 to 9 hours after necroses appearance, whilst b_2 and b_3 were visible on gels only 3 days after infection. In TMV-sensitive cv. Judy's Pride Burley no such changes in soluble leaf proteins occurred, even 7 days after TMV inoculation (Figs 1 and 2).

The hypersensitive reaction to TMV in Xanthi-nc, Samsun NN and Burley 49 is thought to be controlled by the same dominant gene N originating from *N. glutinosa*. However, the proteins that are induced in the latter after TMV infection are completely different (VAN LOON and VAN KAMMEN, 1970) from those found in the 3 former varieties, showing that b-proteins are not directly coded by the gene N. The present work demonstrates that TMV infection of hybrids between hypersensitive and sensitive hosts can result in the production of new proteins, $b_{1'}$ for Burley $\vec{\times}$ Judy's Pride Burley, that are not present in either parent

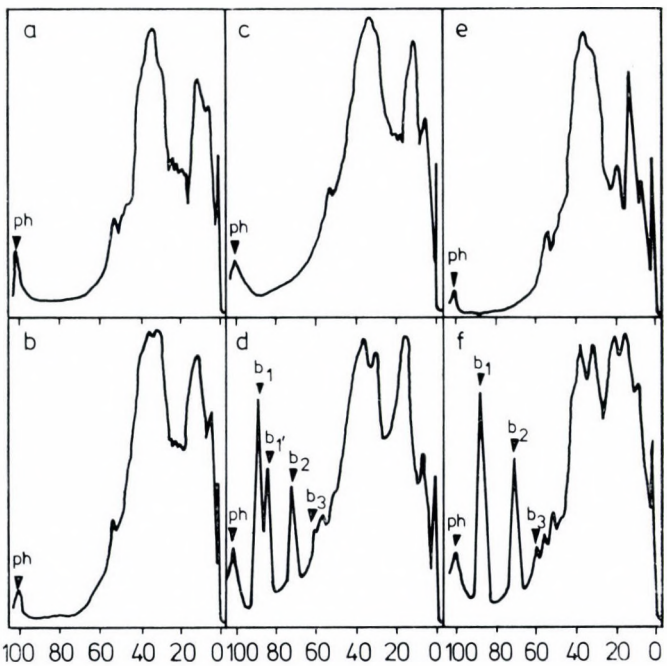

Fig. 1. Densitometer tracings of electrophoretic patterns of soluble leaf proteins 4 days after water inoculation (a, c and e) and TMV infection (b, d and f) of *M. tabacum* cv. Judy's Pride Burley (a and b), Judy's Pride Burley ♀× Burley 49 ♂ hybrid (c and d) and cv. Burley 49 (e and f). b_1, $b_{1'}$, b_2 and b_3, new protein components; ph, bromophenol blue

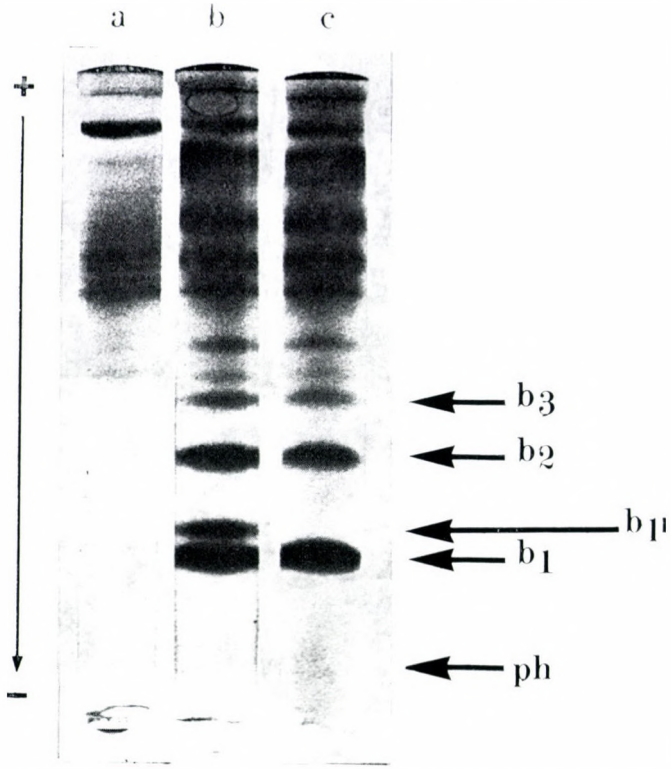

Fig. 2. Electrophoresis of soluble leaf protein extracts of TMV-infected *N. tabacum* cv. Judy's Pride Burley (a), Judy's Pride Burley ♀× Burley 49 ♂ hybrid (b) and cv. Burley 49 (c). b_1, $b_{1'}$, b_2 and b_3, new protein components; ph, bromophenol blue

when infected by the same virus. The fact that both hybrids produced the same new b-proteins suggests that the DNA of the TMV sensitive Judy's Pride Burley must also possess the information necessary to code for b-proteins. In fact leaf necroses induced by fungal infection of this same cultivar are accompanied by the synthesis of the b-proteins b_1, $b_{1'}$, b_2 and b_3 (S. GIANINAZZI, A. CASSINI and A. CORNU, unpublished results). Furthermore, GIANINAZZI (1978) has reported the induction of b-proteins, together with necroses, in TMV sensitive Samsun "nn" by temperature transfer from 25 °C to 11 °C. It is therefore clear that the information necessary to code for the b-proteins is independent of the presence of the gene N.

In spite of the fact that considerable amounts of b-proteins can be detected in the living tissues surrounding local lesions, their relevance to virus resistance is still unknown. It would appear from the present results that genetical studies could provide a useful approach to this problem.

The authors are grateful to Mr. J. DELBUT for the photography.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 77–84 (1981)

Der Einfluß von Virusaggregationen auf die Ergebnisse der Rocket-Immunelektrophorese

Von

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In connection with two plant viruses (barley stripe mosaic virus — BSMV, carnation ringspot virus — CRSV) a clear influence of the degree of aggregation of the virus particles on the results of the rocket immunoelectrophoresis (R-IEP) has been demonstrated. This should be taken into account in experiments for differentiation of virus strains or for the quantitative determination of viruses by this method. The degree of aggregation of both viruses can be influenced by rising the temperature or by addition of Igepon T-73 to the gel respectively of saccharose or potassiumchlorid to the virus sample. With BSMV a natural aggregation or an appearing one during virus purification seems to play a role. Four different strains of BSMV could not be differentiated by R-IEP. However, there are indications for the possibility of using this method for the identification of a reversible aggregation of virus particles.

In neuerer Zeit hat die Rocket-Immunelektrophorese (R-IEP) nach LAURELL (1966) auch in die Untersuchungen pflanzenpathogener Viren Eingang gefunden. HAVRANEK (1978, a, b, c) verwendet sie zum Nachweis partikulärer und löslicher Antigene bei Gurkenmosaik-Virus-Suspensionen bzw. zum quantitativen Nachweis des Gurkenmosaik- und des Tabakmosaik-Virus. Bei vergleichenden Untersuchungen zur Bestimmung der Konzentration des Tabakmosaik-Virus in *Capsicum annuum* L. erhalten TÓBIÁS u. a. (1979) mit der R-IEP Resultate, die denen von Lokalläsionstests auf Xanthi-nc-Tabak bzw. Datura stramonium vergleichbar sind. Von REICHENBÄCHER u. a. (1979), GNUTOVA u. a. (1980) und RICHTER u. a. (unveröffentlicht) wird die R-IEP erstmals auch für die Differenzierung von Tobamoviren bzw. Vertretern der Cucumovirus-Gruppe eingesetzt. Bei letzterer Anwendungsmöglichkeit wandern hochgereinigte und auf eine gleiche Konzentration eingestellte Viruspräparate entsprechend ihrer verwandtschaftlichen Stellung gegenüber dem zur Antiserumherstellung verwendeten Isolat unterschiedlich weit unter Ausbildung von Präzipitaten mit verschiedener Intensität in das serumhaltige Trägergel ein. Heterologe Antigene ergeben dabei aufgrund der Tatsache, daß sie vergleichsweise zu den homologen Antigenen erst später den Äquivalenzbereich mit den Antikörpern erreichen, höhere und weniger intensive Präzipitate als die Präparate, die zur Antiserumherstellung verwendet wurden bzw. mit diesen nahe verwandt sind.

Im Hinblick auf eine weitere Anwendung dieser Methode überprüften wir ihre Eignung bei Stämmen des barley stripe mosaic virus (BSMV) und am Beispiel des carnation ringspot virus (CRSV), wobei insbesondere der Einfluß einer Aggregation auf die Präzipitatausbildung von Interesse war.

Material und Methoden

Beim BSMV wurden die vier Stämme »Norwich«, »Type«, »Russian« und »Rothamsted« (siehe bei McKINNEY u. GREELEY, 1965; ATABEKOV u. NOVIKOV, 1966; KASSANIS u. SLYKHUIS, 1959 und LANE, 1974) geprüft, die serologisch bislang nicht differenziert werden konnten (BOJKOV u. a., 1977; STANARIUS u. a., im Druck) und stark zur Aggregation neigen (BRAKKE, 1959; LANE, 1974).*

Die Reinigung der Virusstämme erfolgte aus Hordeum vulgare L., Sorte 'Xenia', in Anlehnung an LANE (1974) unter Verwendung eines Tris-HCL-Puffers (pH 7.2). Antiseren wurden in Kaninchen mittels einer intravenösen und einer intramuskulären Injektion (dazwischen 4 Wochen Pause) mit Freund'schem inkompletten Adjuvant hergestellt.

Im Fall des CRSV wurde ein von Nelke isolierter Stamm der ATCC** auf Phaseolus vulgaris L., Sorte »Pinto«, vermehrt und nach einer von PROLL (unveröffentlicht) ausgearbeiteten Methode aufgearbeitet. Hochgereinigt lag das Virus in Phosphatpuffer pH 5 vor. Da das CRSV unter den für die R-IEP erforderlichen Pufferbedingungen von pH 8 nicht stabil ist, wurde das in pH 5 vorliegende Virus zunächst mit 2% Formaldehyd fixiert (siehe z. B. bei RICHTER u. a., 1972 a, b) und nachfolgend gegen einen 0.05 M Phosphatpuffer pH 8 dialysiert. Um den Einfluß eines unterschiedlichen Aggregationsgrades untersuchen zu können, wurde die Viruslösung geteilt und die Teilproben wurden mit so viel Saccharose bzw. KCL versetzt, daß sie 10%ig an Saccharose bzw. 1 molar an KCL waren. Diese Proben wurden mit einer unbehandelten Probe sowohl bei +22 °C als auch bei +35 °C verglichen.

In einer Reihe von Versuchen war für das CRSV (KÜHNE, in Vorbereitung) die von TREMAINE u. a. (1976) beschriebene Fähigkeit zur reversiblen endothermen Aggregation bestätigt und ein differenziertes Aggregationsverhalten in Abhängigkeit vom Medium festgestellt worden. Danach wird die Zusammenlagerung der Partikeln in Lösungen hoher Ionenstärke begünstigt und durch Saccharosezugabe stark gehemmt. Die Reinheit aller Virusproben wurde UV-spektrophotometrisch überprüft. Die Einstellung auf gleiche Viruskonzentration erfolgte unter Verwendung der Extinktionskoeffizienten von $E^{0,1\%}_{1\,cm} = 2{,}6$ bei 260 nm für das BSMV (ATABEKOV u. NOVIKOV, 1971) bzw. von $E^{0,1\%}_{1\,cm} = 6{,}45$ bei 260 nm für

* Die Stämme des BSMV wurden uns von Prof. J. G. ATABEKOV und Dr. M. K. BRAKKE überlassen.

** Für die Überlassung des Isolates danken wir Dr. C. OERTEL.

das CRSV (KALMAKOFF u. TREMAINE, 1967). Die so verbereiteten Virusproben* wurden mit verschiedenen Antiserumkonzentrationen** wie bei REICHENBÄCHER u. a. (1979) beschrieben mit der R-IEP überprüft.

Ergebnisse und Diskussion

Versuche mit Stämmen des barley stripe mosaic virus

Bei den Versuchen mit diesen Virusstämmen zeigten sich zunächst deutliche Stammunterschiede, wenn nur ein Antiserum verwendet wurde. Diese Unterschiede traten jedoch unabhängig von den verwendeten drei Antiseren auf, so daß eine Differenzierung der Stämme nach unseren Erfahrungen (siehe Einleitung) so nicht möglich ist (Abb. 1a und 1b). Die relative Höhe der Präzipitate untereinander war im übrigen bei allen Seren annähernd gleich (»Russian« 43%, »Type« 100%, »Norwich« 32%),*** was auf nur geringfügige oder nicht vorhandene Stammunterschiede hindeutet. Die unterschiedliche Höhe und Intensität der Präzipitate mußte daher andere Ursachen haben als antigene Differenzen. Die Vermutung lag nahe, daß aggregierte Partikeln im Vergleich zu weniger oder nicht aggregierte Viruspartikeln früher den Äquivalenzbereich erreichen und ihre Präzipitate daher auch dichter bzw. intensiver erscheinen, dabei ihnen nur eine geringere Anzahl von antigenen Determinanten für die Antikörper verfügbar ist. Um diese Vermutung zu überprüfen, versuchten wir, Aggregationen der Viren entweder nahezu auszuschließen bzw. wieder aufzulösen oder aber sie hervorzurufen. Zu diesem Zwecke wurden die Versuche mit den Monomeren der Virusstämme wiederholt, wie sie nach zusätzlicher Zentrifugation in einem Saccharose-Dichtegradienten abgetrennt werden können (BRAKKE, 1959).

Abbildung 1c zeigt eine deutliche Angleichung der ehemals beobachteten Peak-Unterschiede zwischen den drei Virusstämmen. Die eindeutigsten Ergebnisse wurden jedoch mit gereinigten Präparaten nach Elektrophorese bei +22 °C bzw. +40 °C und einem Zusatz von Igepon T-73 zum Agarosegel erhalten. Das Detergens Igepon T-73 kann in geeigneten Konzentrationen bereits vorhandene Aggregate wieder auflösen und/oder eine Aggregation verhindern (BRAKKE, 1959), hingegen verursacht eine Temperaturbehandlung zwischen +40 und +50 °C eine deutliche Aggregation der Partikeln (KASSANIS und SLYKHUIS, 1959). Für unsere Versuche erwies sich nach Vorversuchen eine 0.05%ige Igeponkonzentration im Agarosegel als geeignet. Bei dieser Konzentration zeigten sich gleich hohe und auch gleich intensive Präzipitations-Peaks der drei Stämme, wie sie bei serologisch identischen Stämmen zu erwarten sind (Abb. 1d). Ähnliche Ergebnisse wurden bei einem späteren Versuch mit vier BSMV-Stämmen und Kombinationen von

* BSMV — 0,9 mg/ml; CRSV — 0,75 mg/ml.

** Ein Antiserum gegen CRSV wurde uns freundlicherweise von Dr. H. Kegler zur Verfügung gestellt.

*** Mittel aus 6 getrennten Versuchen; Höhe des »Type«-Präzipitates = 100%.

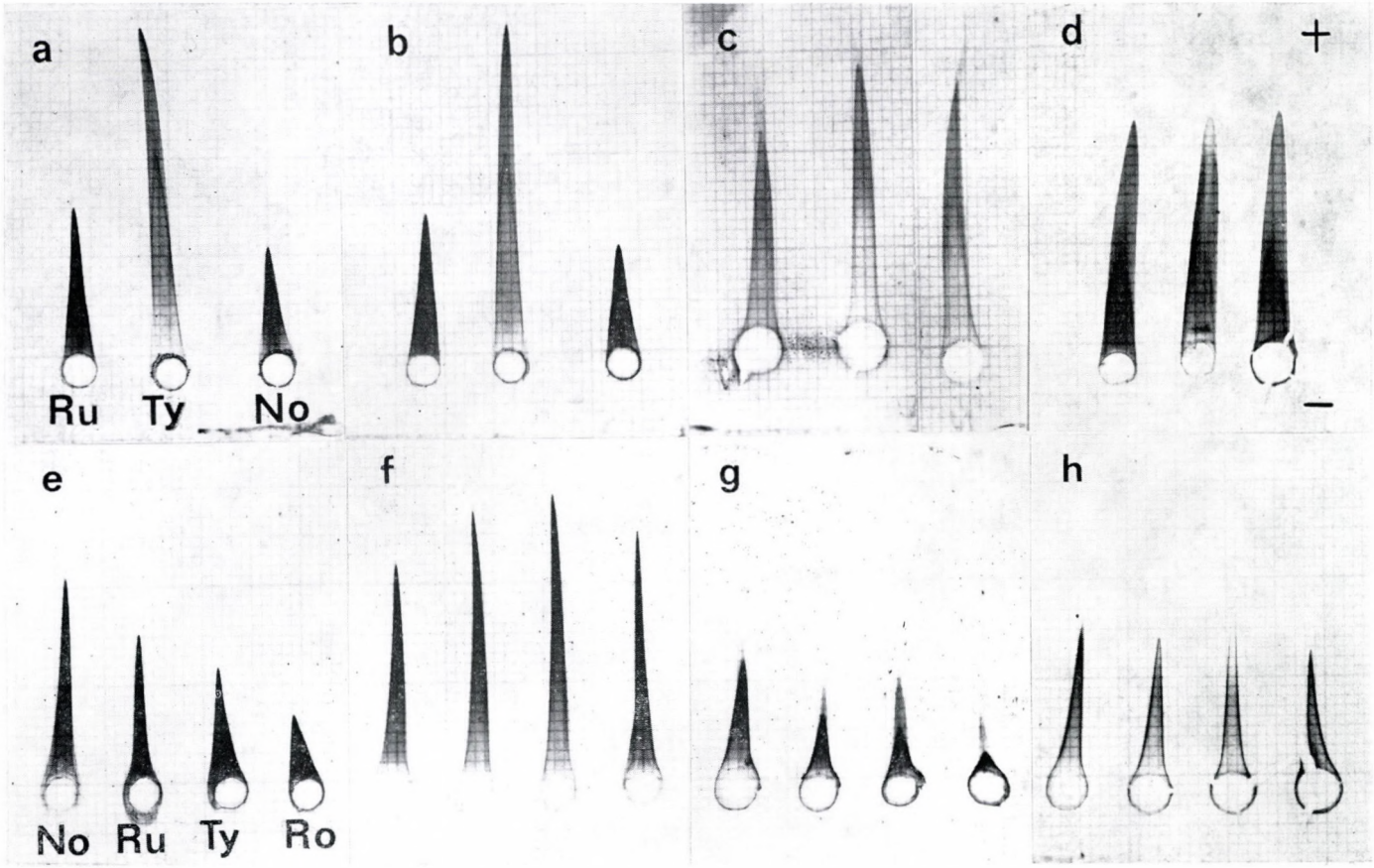

Abb. 1. Vergleich von unterschiedlich aggregierten Viruspartikeln bei Stämmen des BSMV No = »Norwich«; Ru = »Russian«; Ty = »Type«; Ro = »Rothamsted«; b = 5% Antiserum gegen BSMV (»Type«) im Agarosegel, sonst bei a–h 3% Antiserum gegen BSMV (»Norwich«) c = Monomere der Stämme (nach Abtrennung über einen Saccharose-Dichtegradient) d, f, h = 0.05% Igepon T-73 im Agarosegel; e, f = Elektrophorese bei +22 °C (wie bei a–d); g, H = Elektrophorese bei +40 °C

Igepon und den oben erwähnten Temperaturvarianten (Elektrophorese bei +22 oder +40 °C) erhalten (Abb. 1e bis 1h). Der Einfluß einer unterschiedlichen Aggregation, hervorgerufen durch eine Temperaturbehandlung oder Igeponzugabe bzw. eine Kombination beider, wird hier augenfällig an den unterschiedlichen Präzipitaten sichtbar. Offenbar konnte bei diesen Viruspräparaten ein unterschiedlicher Aggregationsgrad der Stämme des BSMV, der auch von demjenigen der zuerst untersuchten Präparate abweicht (vgl. Abb. 1a und 1e), durch die Igeponzugabe nicht in allen Fällen gleich gestaltet werden, so daß nach Detergensbehandlung wenn auch generell höhere, so doch unterschiedliche Präzipitate erhalten werden können (siehe Abb. 1f). Dieser Befund weist darauf hin, daß auch nach einer Detergensbehandlung das Problem der Ausschaltung von Aggregationen nicht ohne weiteres zu lösen ist.

Insgesamt kann jedoch aus den vorliegenden Ergebnissen geschlußfolgert werden, daß die geprüften BSMV-Stämme somit auch nach dem Einsatz der R-IEP serologisch nicht differenzierbar sind. Eine Beeinflussung der elektrophoretischen Beweglichkeit der BSMV-Stämme konnte mit der Immunelektrophorese nach SCHEIDEGGER (1955) bei der gewählten Igeponkonzentration und bei +40 °C nicht festgestellt werden.

Versuche mit dem carnation ringspot virus

Die Untersuchung des isometrischen CRSV erbrachte Ergebnisse, die denen analog sind, die bei den Versuchen mit dem stäbchenförmigen BSMV erhalten wurden. Auch hier ergaben aggregierte Viruspartikeln (hervorgerufen durch einen

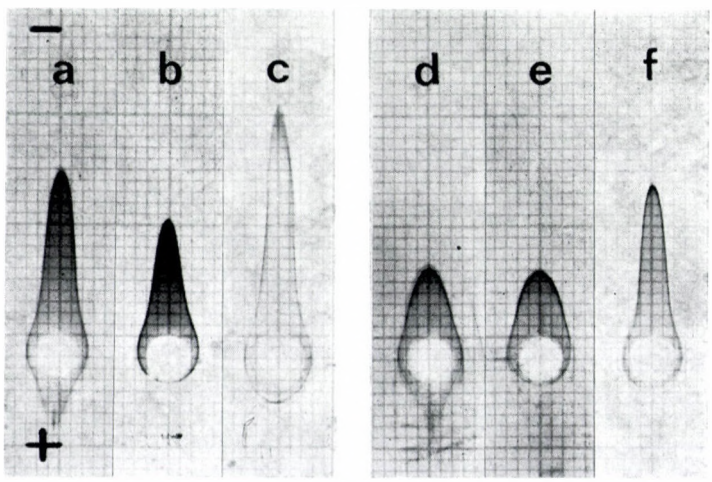

Abb. 2. Vergleich von unterschiedlich aggregierten Viruspartikeln des CRSV. a, b, c = Elektrophorese bei +22 °C; d, e, f = Elektrophorese bei +35 °C; a, d = unbehandelte Probe; b, e = Probe mit 1 m/l KCl; c, f = Probe mit 10% Saccharose und Agarosegel mit 10% Saccharose (bei a bis f 0.25% Antiserum gegen CRSV im Agarosegel)

Zusatz von Kaliumchlorid oder eine Temperaturerhöhung) kürzere und intensivere Präzipitate als solche, die durch Saccharosezugabe stabilisiert worden waren (Abb. 2).

Die entgegengesetzte Wirkung einer Behandlung mit Saccharose bzw. Kaliumchlorid konnte für die gegebene Viruskonzentration durch die zwei Temperaturvarianten (+22 °C, Abb. 2a bis 2c und +35 °C, Abb. 2d bis 2f) noch deutlicher sichtbar gemacht werden. Wie beim BSMV bereits erwähnt, beeinflußten die gewählten Behandlungsvarianten (Saccharose, Kaliumchlorid und Temperatur) die elektrophoretische Beweglichkeit der Partikeln nicht.*

Die außerdem beim CRSV durchgeführte Überprüfung der Partikelmorphologie mit Hilfe des Elektronenmikroskopes sowie sein Sedimentationsverhalten in der analytischen Ultrazentrifuge erbrachten ebenfalls keine Hinweise auf veränderte oder gar denaturierte Viruspartikeln (KÜHNE, unveröffentlicht). Aus diesem Grunde müssen die hier mit der R-IEP erhaltenen Ergebnisse ihre kausale Ursache in einem unterschiedlichen Aggregationszustand der Partikeln haben.

Nach den vorliegenden Untersuchungen an einem stäbchenförmigen und einem isometrischen Pflanzenvirus ist bei der Interpretation von Ergebnissen der R-IEP zur Differenzierung oder quantitativen Bestimmung von Viren, die stark zur Aggregation neigen, eine gewisse Vorsicht geboten (HEUCK u. a., 1979). Neben antigenen Differenzen oder Konzentrationsunterschieden können die Ergebnisse, die mit der R-IEP erhalten werden, durch den Aggregationsgrad der Viruspartikeln stark beeinflußt werden. Dies unterstreichen auch die Untersuchungen von TÓBIÁS u. a. (1979) am Tabakmosaik-Virus. Nach einer zusätzlichen Reinigung an Hydroxylapatit erhaltene nichtaggregierte Partikeln ergaben bei gleicher Konzentration höhere Präzipitate als aggregierte Vergleichsproben. Um bei Versuchen zur Stammdifferenzierung von Viren mit der R-IEP Fehlinterpretationen zu vermeiden, ist die Verwendung von mehreren Antiseren daher auch aus diesem Grunde notwendig. Andererseits deuten sich Möglichkeiten an, mit der R-IEP gewisse Aussagen zum Aggregationsgrad von Viren zu treffen, wenn andere Ursachen (z. B. Denaturierungen) ausgeschlossen werden können.

Zusammenfassung

Am Beispiel von zwei Pflanzenviren (barley stripe mosaic virus – BSMV, carnation ringspot virus – CRSV) wurde ein deutlicher Einfluß des Aggregationsgrades der Viruspartikeln auf die Ergebnisse der Rocket-Immunelektrophorese (R-IEP) festgestellt. Dies ist bei Versuchen zur Differenzierung von Virusstämmen oder der quantitativen Bestimmung von Viren mit dieser Methode zu berücksichtigen.

* Geringe Anteile der Partikeln wandern aufgrund des Isoelektrischen Punktes des CRSV (er liegt im Neutral-Bereich, ist genau jedoch nicht bekannt) und der gewählten Pufferbedingungen auch zur Anode.

Der Aggregationsgrad beider Viren kann durch Temperaturerhöhung sowie den Zusatz von Igepon T-73 zum Gel bzw. von Saccharose oder Kaliumchlorid zur Virusprobe beeinflußt werden. Beim BSMV spielt offenbar eine natürliche bzw. während der Virusreinigung auftretende Aggregation eine Rolle. Vier unterschiedliche Stämme des BSMV konnten mit Hilfe der R-IEP nicht differenziert werden. Es deuten sich jedoch Möglichkeiten an, die Rocket-Immunelektrophorese zum Nachweis einer reversiblen Aggregation bei Viruspartikeln einzusetzen.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 85—96 (1981)

Characterization of the Hungarian *Datura innoxia* Mosaic Virus

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A potyvirus isolated from *Datura innoxia* from an experimental plot of the Research Institute for Medicinal Plants in Budapest, is tentatively named Hungarian *Datura innoxia* mosaic virus (HDMV). Seedlings of *D. innoxia* mechanically inoculated with HDMV expressed systemic mosaic symptoms with strong foliar distortion. This virus infected *Datura stramonium, Lycopersicon esculentum, Nicotiana debneyi, N. glutinosa, N. megalosiphon, N. rustica, N. sylvestris, N. tabacum, Petunia hybrida, Physalis floridana* (mosaic and/or systemic necrosis). *Capsicum annuum, C. frutescens, N. glauca, Solanum dulcamara, S. melongena, S. tuberosum, Chenopodium* species, *Cucumis sativus* and *Gomphrena globosa* were not susceptible. In negatively stained leaf extracts typical potyvirus particles were measured. In ultrathin section of infected *N. tabacum* leaves, pinwheels, scrolls and short laminated aggregates were observed. Cytoplasmic granular inclusions resembling those of other potyviruses were seen by light and electron microscopic techniques. On the basis of the pathogenicity and host range studies HDMV was different from those viruses that were isolated and described from Datura species and also from potato virus Y (PVY) and tobacco etch virus (TEV). There was no any serological relationship between HDMV and PVY or TEV. The HDMV had a thermal inactivation point of 58—60 °C, an longevity *in vitro* of 72 hours at 20—24 °C, a dilution end-point of 10^{-4}. There was no protection among HDMV, PVY and TEV in cross protection tests.

Datura innoxia Mill is among the most important *Datura* species cultivated on a large scale for their alkaloids. Several viruses have been reported on these plants (Table 1). The Research Institute for Medicinal Plants (Budapest) sent several plant material showing mosaic and very strong malformation of the leaves to our institute for virus identification. Sap inoculation from a number of *D. innoxia* to herbaceous plants revealed that these plants contained a virus which had a narrow host range restricted only to Solanaceae species. The host range tests and the electron-microscopical investigation proved that this virus was a potyvirus and differed from the all potyviruses reported from Datura species so far, and also from potato virus Y and tobacco etch virus.

This paper reports the identification of a virus tentatively named Hungarian *Datura innoxia* mosaic virus (HDMV).

Table 1

List of the viruses reported from Datura species and Solanum jasminoides*

Virus	Original host	Properties of isolated virus		Literature
		morphology of particula	most important hosts	
Datura virus 3.	D. alba D. innoxia	unknown	*D. alba*, *D. innoxia*, *Nicotiana tabacum*, *Lycopersicon esculentum*	CAPOOR and VARMA, 1948, 1951.
Datura virus 3A	D. innoxia	unknown	*D. innoxia*, *Petunia hybrida* (no more host)	GARGA, 1958.
Datura enation mosaic virus	D. metel	unknown	*D. metel* var. *fastuosa*, *N. glutinosa*, *N. tabacum*, *N. plumbaginifolia*, *P. hybrida*, *S. nigrum*	VERMA and VERMA, 1963.
Columbian Datura virus (CDV)	D. candida D. sanguinea	flexous (721 nm)	*D. metel*, *S. stramonium*, *Gomphrena globosa*, *N. tabacum*, A6 hybrid	KHAN and BARTELS, 1968.
Datura shoestring virus (DSV)	Salanum jasminoides	flexous (770 nm)	*D. metel*, *N. glutinosa*, *N. rustica*, *N. tabacum*	GIRI and AGRAWAL 1971.
Datura mosaic virus (DMV)	D. metel	flexous (712 nm)	*D. innoxia*, *D. stramonium*, Nicotiana species	QURESHI and MAHMOOD 1978.
Mosaic virus of D. metel (tobacco mosaic virus)	(D. metel	rod (300 nm)	*N. glutinosa*, *N. sylvestris*	HOTIN and PROCENKO 1973.
Mosaic virus of D. innoxia (potato Y-virus, PVY)	D. innoxia	flexous	*D. innoxia*, *Physalis floridana*, *N. sylvestris*, *N. tabacum*	BLASZCZAK, 1976.

* Mechanically not transmissable and leafhopper transmissable viruses are not mentioned.

Materials and Methods

Isolations and host-range experiments. The virus (HDMV) was isolated from *Datura innoxia* in 1964 and 1965 and maintained in *D. innoxia.* It was also stored in a cold room in infected leaf material desiccated over calcium chloride to prevent any change or contamination. In a host range study, mechanical inoculations were made by dusting plant species with 400-mesh carborundum and rubbing the leaves with sterile glass spatulas. Inocula were prepared by grinding infected leaves of *D. innoxia*, 10–20 days after infection together with 0.067 M Na-phosphate buffer, pH 7.0 in sterile mortars. Inoculated plants were rinsed with tap water immediately after rubbing.

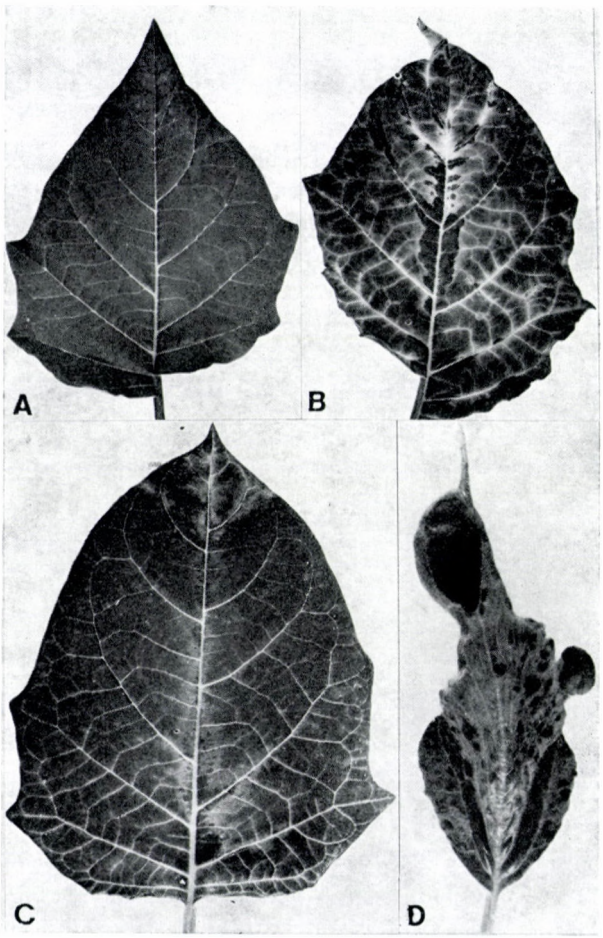

Fig. 1. A = Healthy leaf of *Datura innoxia.* B = Symptoms on the naturally infected *D. innoxia.* C, D = Systemic symptoms on *D. innoxia* experimentally infected with HDMV

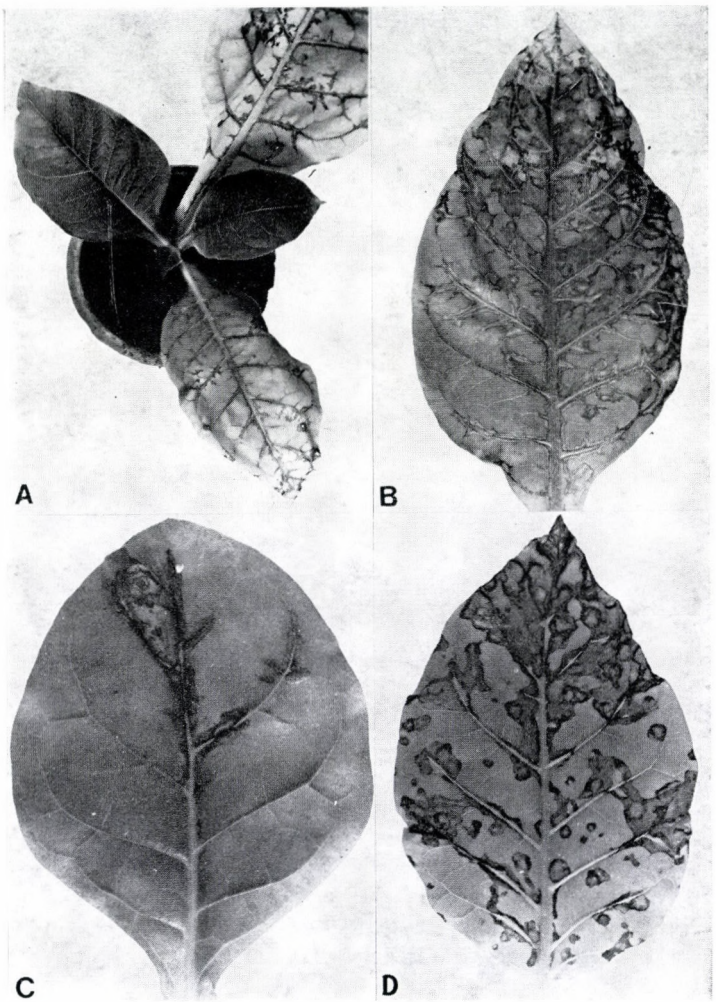

Fig. 2. A, B = Systemic, C = local symptoms on *Nicotiana tabacum* cv. Bel 61-10, D = systemic symptoms on *N. tabacum* cv. Xanthi 21 days after inoculation of HDMV

A minimum of 6 individuals of each test plants were inoculated. The symptoms in these plants were recorded and afterward, the rubbed and the top leaves of each test plants were checked for virus infection. Assay plant was *D. innoxia* and *Nicotiana megalosiphon*. The physical properties of the virus were studied as outlined by Bos *et al.* (1960) using *N. megalosiphon* and *D. innoxia* plants for assaying infectivity.

Cross-protection. In a cross-protection experiment with *Nicotiana tabacum* cv. Xanthi or/and Bel. 61–10 (breeding line), 6–6 plants, first inoculated with

HDMV, or potato virus Y (PVY), or tobacco etch virus (TEV), were super-inoculated 7 days later with PVY, TEV or HDMV separately.

Purification. The method of BECZNER *et al.* (1976) was followed. The harvested leaves of *N. megalosiphon* were stored overnight at 4 °C and homogenized in McIlvaine's phosphate-citric acid buffer (0.18 M, pH 7.0). For homogenization and for resuspending the first high-speed sediment, 0.1 % thioglycolic acid and 0.02 M sodium diethyldithio-carbamate were added. Every 100 g plant material was homogenized together with 300 ml of buffer, after that 50 ml of chloroform

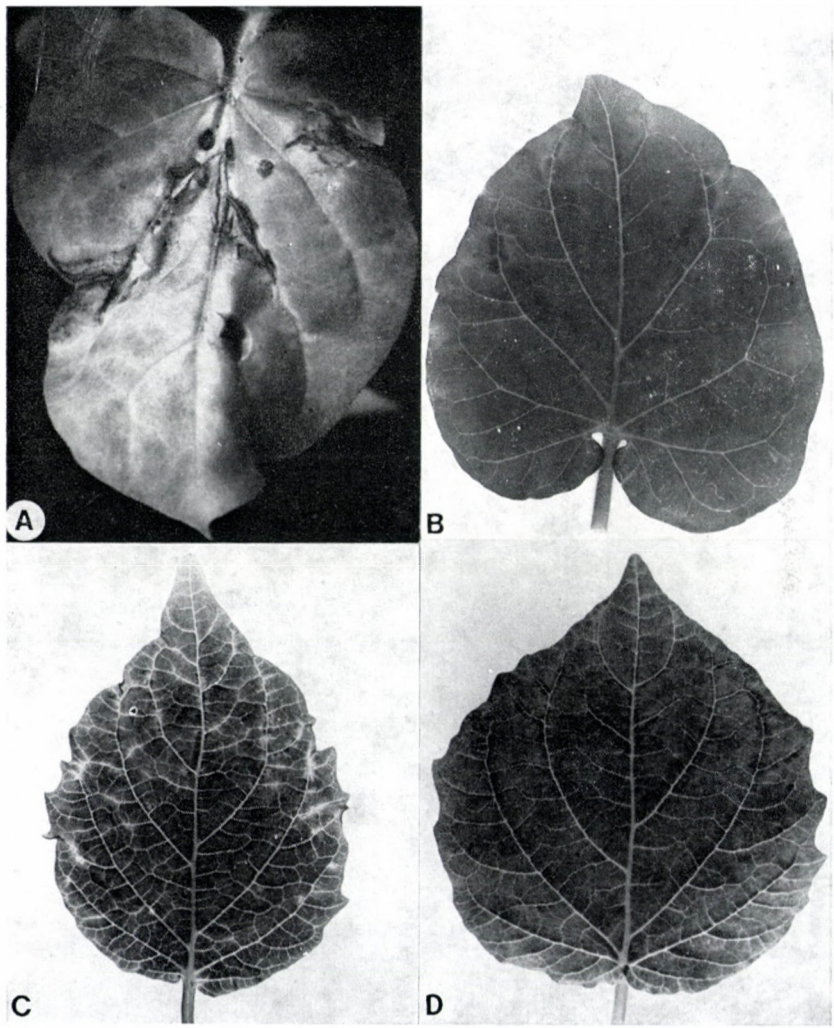

Fig. 3. A = local, B = systemic symptoms on *N. glutinosa*, C = vein clearing and, D = vein banding symptoms on *Physalis floridana* 10 and 25 days after inoculation of HDMV

and 50 ml of carbontetrachloride were added to the homogenate. The homogenate was centrifuged at low speed (6000 rpm) for 20 min. in a Janetzky K 23 centrifuge, the rotor used depending on the quantity of material. To sediment the virus, preparations were centrifuged in a Beckman L3-50 ultracentrifuge at 24 000 g for 1.5 hours (rotor Ti 50). The procedure of centrifuging at low and high speed was repeated and the final sediment (calculating for 100 g leaf material) was resuspended in 1 ml of buffer. For sucrose-gradient centrifuging, 1 ml suspension was layered on a linear gradient of 10–40% sucrose and centrifuging at 25 rpm (rotor SW27) for 1.5 hours. Virus containing zones were isolated from sucrose gradients with a hypodermic syringe and needle. Before concentrating, the sucrose-containing fractions were diluted at least 1 : 1 with distilled water or buffer.

The final 2 ml virus-concentration was used for immunization, serological comparison or electronmicroscopical test, if not immediately, it was mixed with 2 ml of glycerol and stored at – 20 °C.

Antiserum preparation and serology tests. For immunization with HDMV and PVY rabits were given two or three successive intramuscular injections, with 2 ml virus suspension emulsified with 2 ml FREUND's complete adjuvant. Bleedings began 14 days after injection and was repeated at 7–10 days intervals.

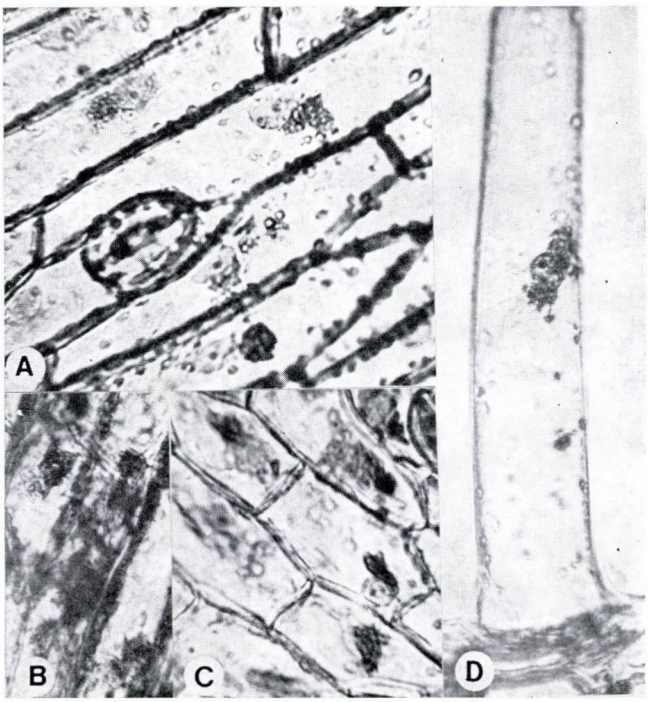

Fig. 4. Cytoplasmic granular inclusions in the epidermic cells of (A) *N. tabacum*, (B, C) *D. innoxia;* (E) granular inclusions in the hair cell of *N. tabacum*

The HDMV serum was tested against preparations of homologous virus and PVY and centrifuged sap of tobacco infected with HDMV, PVY and TEV.

As a serological test method, the micro-precipitin test under paraffin oil was applied. Dilution series of antisera were prepared with saline, containing 0.05% NaN_3. Reactions were recorded after 8–12 hours at room temperature.

Light microscopical test. Epidermal strips taken from leaves of healthy and virus infected plants were stained with 1% phloxine and methylene blue in Christie's solution and examined for the presence of virus-induced inclusions (Bos, 1969).

Electron microscopical test. Electron microscopical investigations were carried out on OPTON EM 9 S-Z. Samples in buffer were placed on carbon-formvar

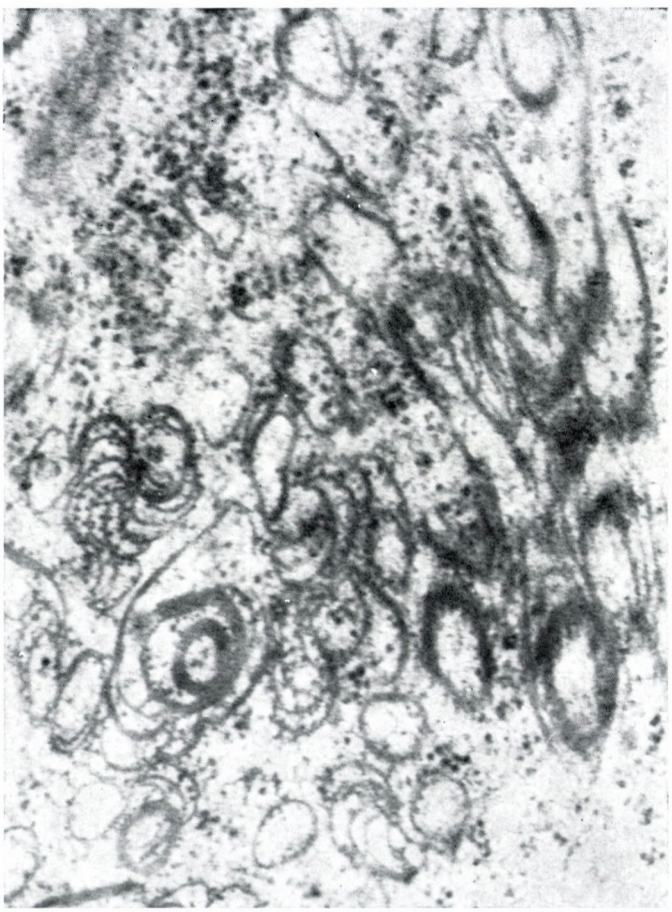

Fig. 5. Electron micrographs of portions of HDMV-infected *D. innoxia*. Pinwheels and scrolls indicate the lamellar inclusions

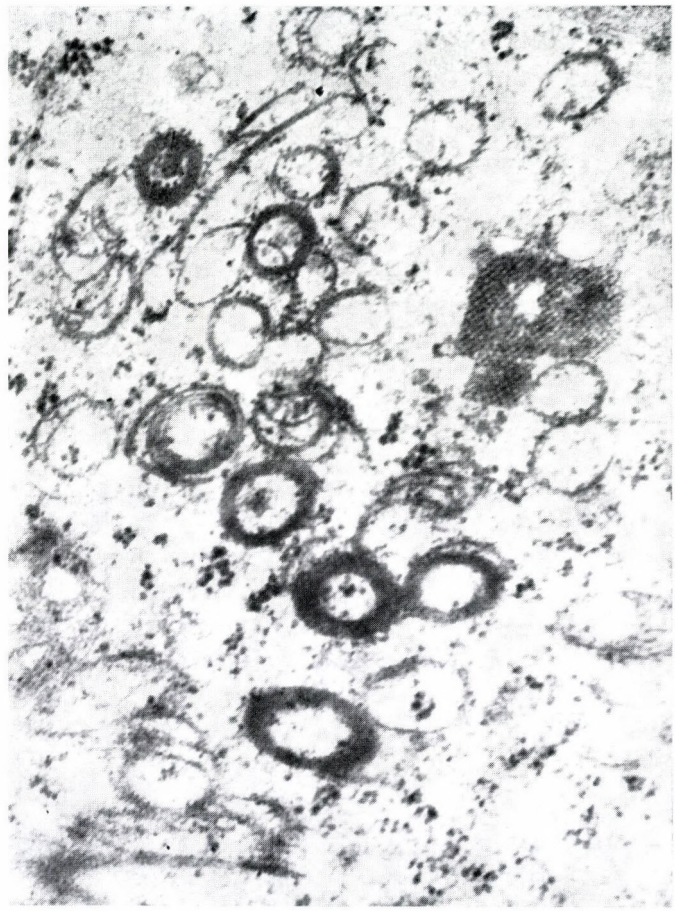

Fig. 6. Electron micrographs of portions of HDMV-infected *D. stramonium*. Planty of scrolls, few pinwheels, laminated aggregates and one honey comb inclusions are shown

coated grids and stained with 2% phosphotungstic acid adjusted to pH 6.5 with KOH. Measurement of the length of the particles was done on crude sap of *D. innoxia* and/or *N. megalosiphon*. TMV was used as standard.

For electron microscopy, samples were taken from HDMV-infected *D. innoxia* and *D. stramonium* leaves having systemic mosaic and malformation symptoms. The standard method of WEINTRAUB *et al.* (1973) was followed. The samples were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 at room temperature for 90 min., rinsed for 30 min. with distilled water, and post fixed for 1 h in 1% OsO_4 solution, pH 7.2. After three washes with distilled water, they were dehydrated in graded dilutions of ethanol, treated with propylene oxide, and embedded in DURCUPAN ACM. Thin sections were cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate.

Results

Symptoms on herbaceous hosts. Results of host range tests are given in Table 2, and Figs 1–3. The experimental host range for HDMV appears to be restricted to the Solanaceae family. The HDMV does not infect *Capsicum annuum*, *C. frutescens*, *Chenopodium quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *Nicotiana glauca*, *Solanum dulcamara*, *S. melongena*, *S. tuberosum* and *S. tuberosum* × S. demissum A6 hybrid.

Stability of HDMV in vitro. HDMV had a thermal inactivation end point between 58 and 60 °C. The dilution end point in Sörensen buffer was between 10^{-4}–10^{-5}, and the crude sap kept at room temperature (20–24 °C) was infective after 3 days. In dry material from infected leaves of *D. innoxia* HDMV remained infective after 7 years.

Cross-protection tests. In an experiment set up to test a possible cross-protection between HDMV, PVY and TEV, *N. tabacum* cv. Xanthi and Bel 61-10 breeding line were inoculated with HDMV, PVY and TEV. 7 days later, when systemic symptoms just started to develop, the plants were re-inoculated with another of these viruses. There was no protection among HDMV, PVY and TEV in the cross-protection test.

Table 2

Host range of Hungarian *Datura innoxia* mosaic virus

Species inoculated	Reaction local / systemic
Datura innoxia Mill.	–/VC, Mo, Ma, Stu
D. stramonium L.	–/Mo, Ma, Stu
Lycopersicon esculentum Mill.	–/Mo
Nicotiana debneyi Domin.	Lc/LR, VC, Mo,
N. glutinosa L.	Ln, W/VC, Mo, SN
N. megalosiphon Heurck et Muell.	Ln/VC, VN, SN, Ma
N. rustica L.	–/Ch, Rec.
N. sylvestris Spegaz. et. Comes	–/VC, Mo
N. tabacum L.	Ln, W/SN, VN, Stu
cv. Bel 61-10	
Samsun	–/VC, Mo, VN, SN.
Xanthi nc	–/VC, Mo, VN, SN
Petunia hybrida Hort. ex. Vilm.	Ln/VC, VN, Stu
Physalis floridana Rydb.	–/Mo, VB

Symbols used for virus-induced symptoms:

– = no symptoms, / / = variable, symptom not always expressed, Ch = chlorosis, Lc = local chlorotic, Ln = local necrotic, Ma = malformation, Mo = mottle or mosaic, Rec. = recovery, SN = systemic necrosis, Stu = stunt, VB = vein banding, VC = vein clearing, VN = vein necrosis, W = wilting; numerator = inoculated leaf; denominator = = systemic reaction

Table 3

Differential host range for HDMV and some potyviruses and their properties in sap

Test plants	HDMV	CDV	Viruses compared				
			DSV	DMV	PVY	TEV	HEMV
Capsicum frutescens cv. Tabasco	—	—	/	/	—	Tn, W	Tn, W
Chenopodium quinoa	—	/	—	—	Lc	Ln	Ln
Datura innoxia	S	(LS)	—	LS	S	S	—
D. stramonium	(S)	(S)	—	LS	—	S	S
Gomphrena globosa	—	L	—	—	—	—	S
Lycopersicon esculentum	S	—	—	—	S	S	S
Solanum tuberosum × S. demissum (= A-6 hybrid)	—	Ln	—	/	Ln	Ln	Ln
TIP (°C)	58—60	55—60	60—65	45—50	55—60	54—58	50—60
DEP	10^{-4}	10^{-5}	10^{-4}	10^{-6}	10^{-3}	10^{-3}	10^{-4}
LIV (days)	3	6	1	1	2	3	4

Abbreviations defined: L = local infection, Lc = chlorotic local lesion, Ln = necrotic local lesion, S = systemic infection, // = infection occasionally, TN = top necrosis, W = = wilting, — = no infection, / = no information. The type of reactions presented are compiled from findings of KAHN and BARTELS (1968, CDV), GIRI and AGRAWAL (1971, DSV), QURESHI and MAHMOOD (1978, DMV), KLINKOWSKI, M. (1977, PVY, TEV and HEMV (= = henbane mosaic virus)) and the present work (HDMV). TIP = thermal inactivation point, DEP = dilution end point, LIV = longevity in vitro.

HDMV could delay the systemic symptom expression of PVY and TEV 2–3 days only in case of Xanthi tobacco, while on Bel 61-10 there was no differences et all. When the HDMV followed the PVY infection, much stronger systemic symptoms were observed than those caused by PVY alone on Xanthi tobacco. This phenomenon was not noticed on Bel tobacco when the appearance of HDMV symptoms delayed and they were less pronounced.

Inclusion bodies. Cytoplasmic granular inclusions resembling those of other potyviruses were seen by light microscope (Fig. 4). In ultrathin section of infected *D. innoxia* and *D. stramonium* leaves pinwheels, scrolls and short laminated aggregates were observed (Fig. 5, 6). According to these results the HDMV belongs into the 3rd subdivision of potyviruses suggested by EDWARDSON (1974).

Purification and serology. The purification method adapted from BECZNER *et al.* (1976), using 0.18 M phosphate-citric acid buffer pH 7.0 gave very good yield of virus, in spite of that there is no good propagating host for HDMV. When the tobacco plants collapse *(N. megalosiphon* or *N. tabacum)* the virus concentration is rather low. The UV-absorbtion spectrum of purified HDMV

showed a minimum at 245 nm and a maximum at 260 nm and a slight shoulder at 290 nm. The 260 : 280 ratio was 1.22, and the 260 : 245 ratio 1.18.

Extracts of plants of *D. innoxia* infected with HDMV and also the purified preparation contained a number of flexous particles which were typical for other potyviruses too, and their normal length was about 750 nm.

Antisera with titers of 64 and 256 varied according to the blood samples. This antisera reacted only with homologous antigens and did not react with PVY and TEV. Also there was no serological reaction when HDMV was tested against the PVY-serum.

Discussion

The mosaic of *Datura innoxia* in Hungary is caused by a virus with flexous particles about 750 nm long, and mechanically transmissible to some Solanaceae plants. The virus has typical cytoplasmic granular inclusions resembling those of other potyviruses, in ultrathin section of infected *D. innoxia* plant pinwheels, scrolls and short laminated aggregates were observed. Some of the viruses reported from Datura species in other countries have similar particle morphology. Of these CDV (KHAN and BARTELS, 1968) infected *Gomphrena globosa* and A6 hybrid, whereas HDMV did not; DSV (GIRI and AGRAVAL, 1971) did not infect *D. innoxia*, *D. stramonium* and *L. esculentum*, whereas our isolate did it. The DMV (QUARESHI and MAHMOOD, 1978) differs also in host range (it does not infect *L. esculentum)* and moreover very much in symptomatology. The reactions of *C. frutescens* and *D. stramonium* differentiated also the HDMV from the PVY, TEV and from HEMV, too.

We therefore consider this virus isolate to be a previously uncharacterized virus, which we tentatively named Hungarian *Datura innoxia* mosaic virus.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 97—108 (1981)

Some Data on Viruses Occurring in Cruciferous Plants in Hungary[1, 2]

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During the last few years the authors have investigated the virus diseases of members of the family *Cruciferae (Brassicaceae)* in Hungary. According to this investigations, cucumber mosaic virus (CMV), radish mosaic virus (RMV), turnip yellow mosaic virus (TYMV), turnip mosaic virus (TuMV) as well as the DNA-containing cauliflower mosaic virus (CaMV) were reported as natural pathogens of members of the concerned family.

The CMV was found to cause serious damages to rape *(Brassica napus)* plants from which the mentioned virus was recovered. On the other hand, the concerned host, as a wintering host, was shown to play an important role in the epidemiology of the CMV.

According to the results approached from agar gel double diffusion and intragel absorption tests, the Hungarian isolates of RMV, which were recovered from turnip plants *(Brassica rapa* var. *rapa)*, were found to be identical with the European isolate but differed from the American type strain of the virus.

The different isolates of TYMV, recovered from turnip plants, were found to be serologically related to but still not identical with the Yugoslavian one which is identical with the type strain of the virus. Among the newly determined artificial hosts of TYMV, *Capsella grandiflora, Diplotaxis viminea* in addition to the perennial *Bunias orientalis* and *Crambe abyssinica* are of a special interest.

Isolates of TuMV which were recovered from *Alliaria petiolata, Brassica rapa* var. *rapa, B. oleracea* convar. *botrytis* and *B. oleracea* convar. *capitata* were all found to be related to the so-called cabbage or ordinary strain of the virus. The antagonistic interaction between TuMV and CMV is concerned to be of an atmost importance from both the theoretical and the practical points of view. It is also worthy to mention that the perennial *Bunias orientalis* plant was shown, for the first time, to be an artificial host of TuMV.

The different isolates of CaMV, which were recovered from cauliflower *(Brassica oleracea* convar. *botrytis)* and turnip plants *(B. rapa* var. *rapa)*, were found to be biologically identical with the original or the so-called cabbage B-strain. On the other

[1] Presented at the Conference on New Endeavours in Plant Protection on the occasion of the 100th anniversary of the founding of the Research Institute for Plant Protection, Budapest, held September 2—5, 1980 at the Hungarian Academy of Sciences, Budapest.

[2] In memoriam of Dr. K. SCHMELZER (1928—1976) on the occasion of the fifth anniversary of his decease.

hand, they deviated from those strains of CaMV which are known with their pathogenesity to *Datura stramonium* and *Nicotiana clevelandii*. Among the newly determined cruciferous artificial hosts of the mentioned virus, the woody *Crambe* species (e.g. *Crambe scaberrima*, *C. strigosa)* are of a special importance. The *Crambe* species are considered to be the only woody perennial hosts of CaMV which are reported for the first time.

It was the standard works of the English BROADBENT (1957) and the German USCHDRAWEIT and VALENTIN (1956, 1957, 1959) that at the end of the fifties called the virologists' attention to the families *Cruciferae (Brassicaceae)*, a group of plants little known for its virological aspects but highly important from an epidemiological point of view. On the basis of prodromal investigations it was ascertained that between the cruciferous plants and cucumber mosaic virus (CMV), tobacco rattle virus (TRV) and turnip mosaic virus (TuMV) there is a theoretically and practically equally important host-parasite relation. Researches in different parts of the world during the past two decades enriched our knowledge with highly valuable scientific results. Among the various lines of research the fundamental work directed by the late Dr. K. SCHMELZER (1928–1976) for several years until his death must be mentioned in the first place. It was his work "*Studies on viruses and virus diseases on cruciferous plants*" written in common with his co-workers between 1970 and 1975 that first gave account of 185 different naturally occurring virus-host relations in 129 plant species (cf. SHUKLA and SCHMELZER 1970a, b, 1972a, b, c, d, 1973a, b, c, d, e, f, 1974a, b, c, 1975; SHUKLA *et al.*, 1972, 1973, 1975). The basic conclusions concerning some 10 viruses – including those new for the science – will remain an indispensable source of knowledge for the plant virologists of the world for a long time.

Materials and Methods

The viruses were identified on the basis of test plant reaction and host range, serology, electron microscopy, inclusion body, aphid transmissibility, physical properties and interference by the conventional methods (cf. HORVÁTH, 1976, 1977).

Results

During the last few years we investigated the virus diseases of members of the family *Cruciferae (Brassicaceae)* in Hungary. In accordance with the results of this investigation CMV, radish mosaic virus (RMV), turnip yellow mosaic virus (TYMV), TuMV as well as the DNA-containing cauliflower mosaic virus (CaMV) were reported as natural pathogens of the members of the families concerned (Table 1).

For the first time CMV was found to cause serious damages to rape *(Brassica napus)* from which the mentioned virus was recovered (HORVÁTH, 1969a). As a

Table 1

Natural occurrence of viruses on cruciferous plants in Hungary

Viruses	Natural cruciferous hosts
Cucumber mosaic virus (CMV)	*Brassica napus* L.
Radish mosaic virus (RMV)	*B. rapa* L. var. *rapa*
Turnip yellow mosaic virus (TYMV)	*B. rapa* L. var. *rapa*
Turnip mosaic virus (TuMV)	*Alliaria petiolata* (M. B.) Cavara et Grande
	B. oleracea L. convar. *botrytis*
	B. oleracea L. convar. *capitata*
	B. rapa L. var. *rapa*
Cauliflower mosaic virus (CaMV)	*B. oleracea* L. convar. *botrytis*
	B. rapa L. var. *rapa*

consequence of the virus infection mosaic symptoms, deformation of leaf, stem and pod appeared on the affected plants (Fig. 1A and B). According to our observations CMV can be regarded as an important pathogen of rape. Namely, we found that the seed yield of the virus infected plants was 50 per cent lower than that of the healthy plants. This remarkable loss of yield can be traced back partly to the smaller number of both seed per pod and pod per plant, partly to the lower thousand-seed-weight. Further, the germinative ability of seed is 25 per cent lower in

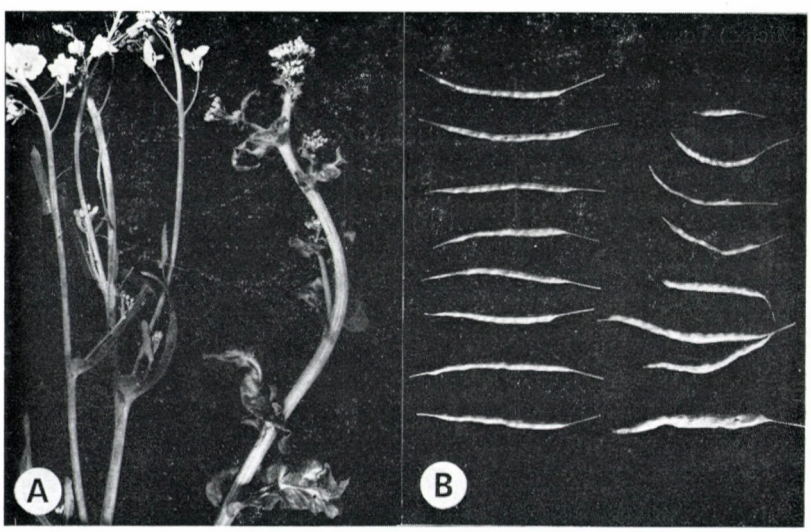

Fig. 1. Naturally infected rape plant (*Brassica napus* L.) with cucumber mosaic virus (A: left healthy, right diseased plant). B: Healthy pods (left) and infected pods (right) of rape plants

the virus infected plants than in the healthy ones. By agar gel double diffusion serological tests we pointed out that the R-isolate of CMV obtained from rape was identical with isolates belonging to the so-called green-strain group, the most wide-spread one in Hungary (Fig. 2). Interference tests proved the existence of partial cross protection between the W-indicator strain and R-isolate of CMV. In the course of studying interference between species of alien viruses we found antagonism between the R-isolate of CMV and the U_1-strain of tobacco mosaic

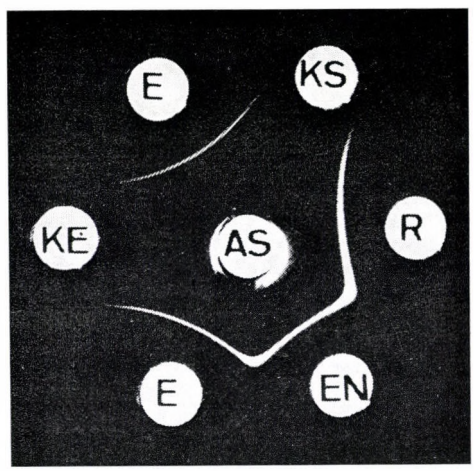

Fig. 2. Agar gel double diffusion serological test between antiserum of cucumber mosaic virus (AS) and isolates (E, EN and R) of cucumber mosaic virus. KE: Healty *Echinocystis lobata* (Michx.) Torr. et Gray plant sap, KS: Healty *Nicotiana tabacum* L. cv. *Samsun* plant sap

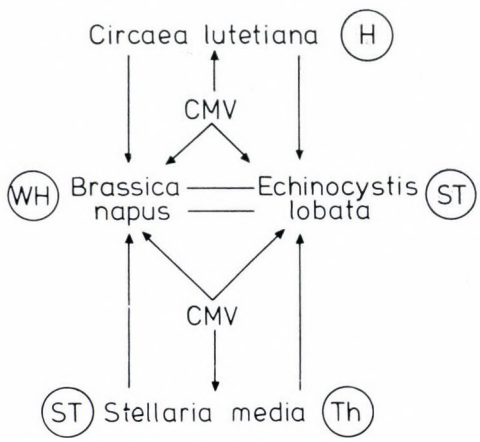

Fig. 3. Some important plants in the epidemiology of cucumber mosaic virus (CMV). (H): Perennial plant, (Th): annual plant, (WH): Wintering host, (ST): Seed-transmissible host

virus (TMV) in the case of inoculation carried out either simultaneously or ta different times. According to our observations rape as a wintering host of CMV plays a highly important role beside the perennial *Circaea lutetiana, Stellaria media* and the *Echinocystis lobata* in the epidemiology of CMV in Hungary (Fig. 3). During investigations into the host range of CMV, a virus equally pathogenic to mono- and dicotyledonous plants, we detected 131 new hosts among which there are many phanerophytic and hemicryptophytic hosts like *Amaranthus deflexus, Commelina tuberosa, Erodium manescavi* as well as *Physalis* species (Fig. 4) and *Lycium* species (cf. HORVÁTH, 1976, 1979a, 1980).

According to the results obtained from agar gel double diffusion and intragel absorption tests the Hungarian isolate of RMV recovered from turnip plants *(Brassica rapa* var. *rapa)* was identical with the European isolate but differed from the American type strain of the virus (MAMULA *et al.*, 1972). On plants infected by RMV chlorotic-necrotic symptoms, leaf deformation, while on the petioles severe necrotic symptoms were found (Fig. 5A). Cytological examinations revealed characteristic spherical or elliptic inclusion bodies occurring beside the nucleus in the epidermis cells of turnip plants. Inclusion bodies were pointed out in the cytoplasm on the third day following the inoculation already, which is important from the point of view of an early diagnosis. Characteristically of the inclusion bodies the chloroplasts did not become vacuolized and did not stick together. As it is known, this is a typical feature of the TYMV. On the basis of this feature characteristic of the inclusion bodies but different in the case of the

Fig. 4. Systemic symptoms on *Physalis curassavica* L. inoculated with cucumber mosaic virus

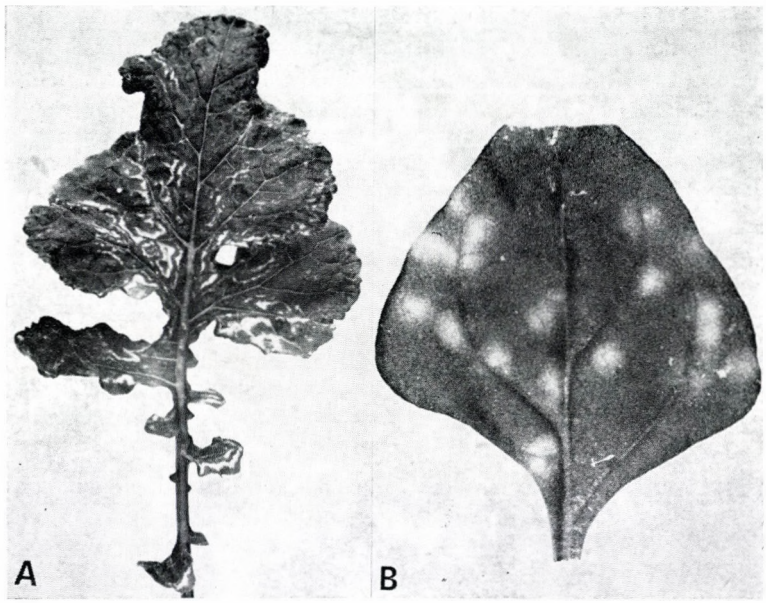

Fig. 5. Systemic (A) and local (B) symptoms on *Brassica rapa* L. var. *rapa* (A) and *Tetragonia crystallina* L'Hérit. (B). A: infected with radish mosaic virus and B: infected with cucumber mosaic virus

two viruses the above two cruciferous viruses can be cytologically differentiated by means of light microscope. In the course of investigations on the artificial host range of RMV we found 25 new host plants of which *Nicotiana occidentalis*, *Tetragonia crystallina* (Fig. 5B) and *T. echinata* are particularly important from a diagnostical point of view, while the perennial *Erodium manescavi*, *Bunias orientalis*, *Crambe abyssinica* and *Diplotaxis tenuifolia* are of importance in virus diagnosis (HORVÁTH, 1979b; HORVÁTH *et al.*, 1973).

The different isolates of TYMV recovered from turnip plants (*Brassica rapa* var. *rapa*) were found to be serologically related to, but not identical with the Yugoslavian one which is identical with the type strain of the virus (JURETIĆ *et al.*, 1973; HORVÁTH *et al.*, 1973). A pronounced vein clearing and yellowing, conspicuous variegation and mosaic with yellow or sometimes almost white areas were observed on leaves of turnip plants infected with TYMV (Fig. 6A). We found the white variety of turnip plants in the field to be more susceptible to the virus than the red variety. In light microscope investigations of the leaf tissue we observed conspicuous alterations of chloroplast in plants infected with TYMV. In cells of healthy plants no chloroplast abnormalities were detected. Natural occurrence of TYMV has been established in several European countries by now (e.g. Austria, Denmark, France, Germany, Portugal, Yugoslavia). Our finding of TYMV in Hungary is a new contribution to the knowledge of the geographical distribution of this virus in Central Europe. During studies on the artificial host

range of TYMV some new host plants belonging to the family *Cruciferae (Brassicaceae)* have been pointed out, of which the perennial *Bunias orientalis* and *Crambe abyssinica* (Fig. 6B) are of particular importance (HORVÁTH, 1976, 1979c). The latter is known to be an increasingly important oil crop in the world (cf. HORVÁTH, 1969b, 1972; HORVÁTH *et al.*, 1973).

The different isolates of TuMV recovered from *Alliaria petiolata* and *Brassica rapa* var. *rapa*, *B. oleracea* convar. *botrytis* as well as *B. oleracea* convar. *capitata* were all found to be related to the so-called cabbage- or ordinary strain of the virus (cf. HORVÁTH *et al.*, 1975; HORVÁTH, 1976). This classification of TuMV isolates was suggested by YOSHII (1963) who placed a great number of TuMV isolates in the above two strains on the basis of the mentioned reaction of *Brassica oleracea* convar. *capitata* and *Nicotiana glutinoa*. The isolates of the ordinary strain produce weak or no symptoms in *Brassica oleracea* convar. *capitata* and *Nicotiana glutinosa*, while the isolates of the cabbage strain produce severe or erratic symptoms. It is interesting that the cabbage strain apparently includes all known TuMV isolates from *Brassica oleracea* convar. *capitata*, while the ordinary strain contains isolates from various cruciferous plants other than *Brassica oleracea* and from non-cruciferous plants, e.g. *Tropaeolum majus* (cf. MAMULA and LJUBESIĆ, 1975). The diseased plants showed ven clearing and mosaic symptoms. There were also remarkable dark green islands on the leaves (Fig. 7A and B). We found a good specific reaction in the microprecipitin serological test between sap of infected turnip plants and TuMV antisera. The isolates of TuMV from various hosts were easily transmitted by *Myzus persicae* aphids in stylet-borne manner to young turnip plants. When studying the interaction between TuMV

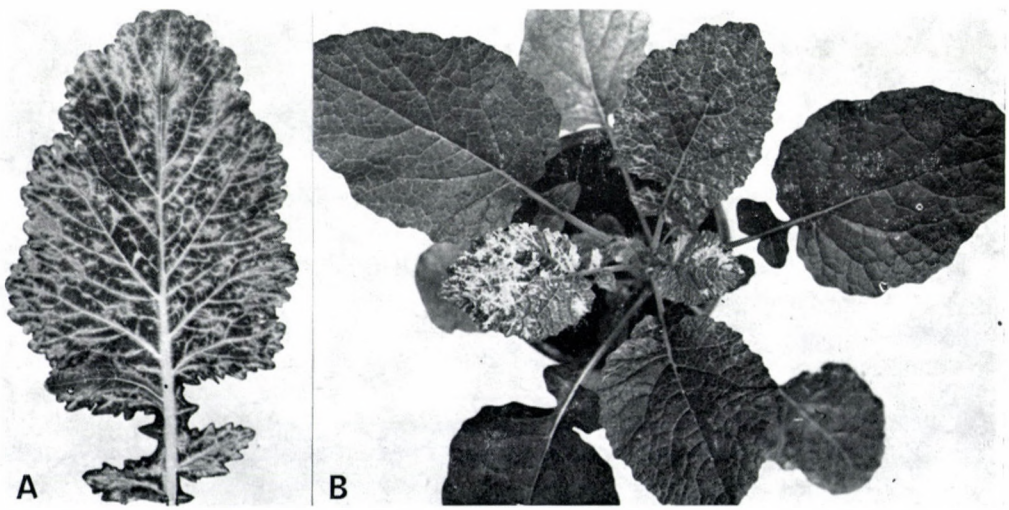

Fig. 6. Systemic symptoms on *Brassica rapa* L. var. *rapa* (A) and *Crambe abyssinica* Hochst. ex R. E. Frees (B) inoculated with turnip yellow mosaic virus

isolates and the W-indicator strain of CMV we found that in the case of a simultaneous inoculation the CMV had an about 60–70 per cent inhibitory effect on the multiplication of the TuMV isolates in *Nicotiana tabacum* cv. *Xanthi-nc*

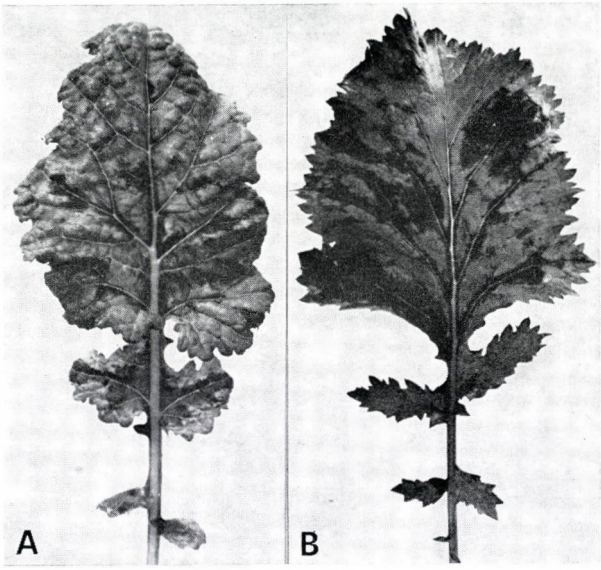

Fig. 7. Systemic symptoms on *Brassica rapa* L. var. *rapa* (A) and *Brassica campestris* L. em. Hartm. (B) infected with turnip mosaic virus

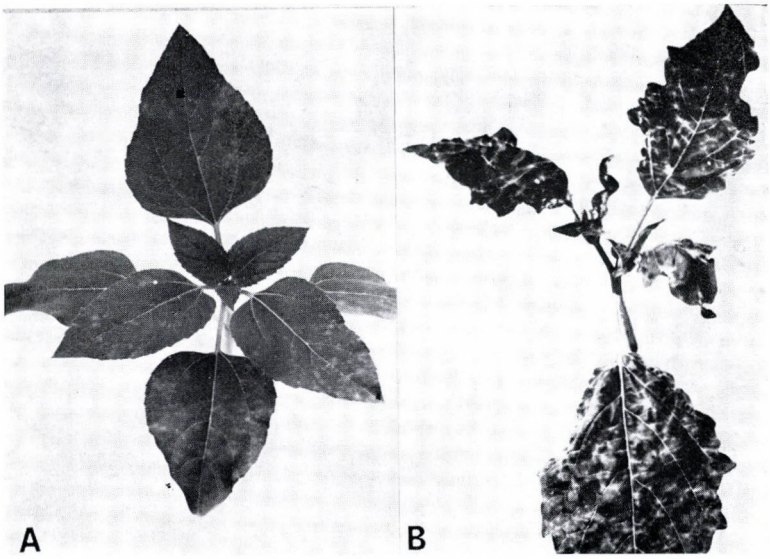

Fig. 8. Systemic symptoms on *Helianthus annuus* L. cv. *Iregi csikos* (A) and *Physalis curassavica* L. (B) inoculated with turnip mosaic virus

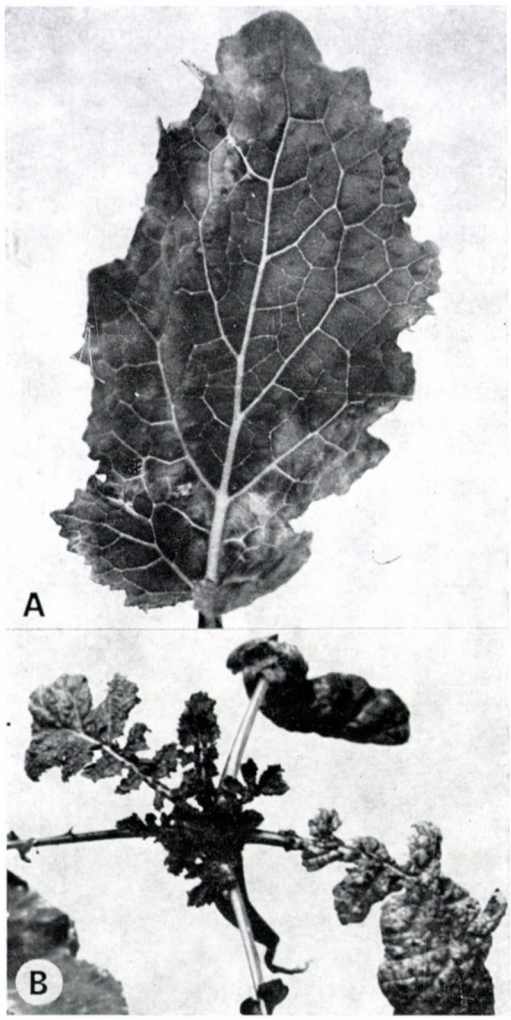

Fig. 9. Systemic symptoms of cauliflower mosaic virus on *Brassica rapa* L. var. ***rapa*** leaves (A and B)

tobacco leaves. Considering that the CMV and TuMV often occur in a complex form in various plants, the antagonistic interaction of the two viruses are of particular interest. In the course of investigating the host range of TuMV isolates we found some new host plants, of which the perennial prognostic *Helianthus mollis*, *H. annuus* cv. *Iregi csíkos* (Fig. 8A), *Bunias orientalis*, *Papaver orientale*, *Physalis curassavica* (Fig. 8B) and *Tinantia* species are particularly important (HORVÁTH, 1979d, 1981).

Among the numerous virus isolates recovered from naturally diseased turnip plants two isolates, the so-called H10 and H11, could be distinguished which proved to be both biologically and cytologically similar to the original isolate of CaMV. Moreover, the two mentioned isolates serologically differed from each of RMV, TYMV, TuMV, turnip crinkle virus (TuCV) and turnip rosette virus (TuRV) (cf. HORVÁTH *et al.*, 1980a). By the spring of the year 1976 our attention was drawn to some cauliflower plants which showed very severe typical symptoms of virus disease. Isolates from diseased cauliflower plants were similar to the isolates H10 and H11 recovered from diseased turnip plants. These turnip and cauliflower plants showed dark green vein banding, vein clearing and mottling symptoms (Fig. 9A and B). Among the examined test plants only the members of the family *Cruciferae (Brassicaceae)* proved to be susceptible to isolates recovered from turnip and cauliflower plants. This fact also indicates that the three isolates are closely related to each other and are almost identical with the original isolate of CaMV. On the other hand, they differed from the strains of CaMV known for being pathogenic to *Datura stramonium* and *Nicotiana clevelandii*. Our purified isolates reacted with antiserum positively to CaMV giving distinct precipitation line. These isolates did not react with antisera to RMV, TYMV, TuMV, TuCV and TuRV. *Myzus persicae* aphids transmitted, in a stylet-borne manner, each of the three isolates from and to turnip plants. Light microscopic examinations of the epidermal cells of leaves of infected turnip and cauliflower plants revealed the presence of amorphous, optically homogenous *X*-bodies, which is considered to be an important characteristic of CaMV. By electron microscope the purified virus is found to consist of isometric particles of a diameter of about 50 nm. Among the newly found artificial cruciferous hosts of CaMV the woody *Crambe* species, e.g. *Crambe scaberrima*, *C. strigosa* are of special importance. The *Crambe* species reported for the first time are considered to be the only woody perennial hosts of CaMV (HORVÁTH *et al.*, 1980; HORVÁTH and BESADA, 1981).

In the course of investigations in the past decade the natural occurrence of five viruses was pointed out in nine cruciferous plants. Four of the five viruses were found in the same period to occur in Hungary. We think that our investigations aimed – *sensu stricto* – at pointing out natural virus infection in new plants, and – *sensu lato* – at supplying data on the survival and circulation of viruses in nature will be useful for all those engaged in studies on the virus-host relations and on the geography of plant viruses.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 109—118 (1981)

Identification of Two Strains of White Clover Mosaic Virus

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White clover mosaic virus (WCMV) strains of different pathological characters were isolated from *Trifolium pratense* (TpVl) and from *T. repens* (TrS2). The TrS2 strain locally and systemically infected Datura stramonium, locally infected *Nicotiana debneyi*, *N. tabacum* cv. Samsun and *Petunia hybrida* while the virus strain TpVl belonging to the European type strain did not infect these test plants. Symptoms occurring on *Vicia faba* after inoculation were basically different. The reactions of the other 24 testplants investigated were similar after infections with both viruses. The *in vitro* physical properties of the two strains were also similar. For TpVl TIP: 60—65 °C, DEP: 10^{-5}, LIV: 35 days; for TrS2 TIP: 62—65 °C, DEP: 5×10^{-4}—10^{-5}, LIV: 41 days. Serologically the two strains were closely related. The average SDI value between TpVl and TrS2 were 3.5 (reciprocal 3.5). Both strains are serologically distantly related to potato virus X (strain PVX-G) but they are serologically unrelated to five other distinct potex-viruses.

In a survey of sap-transmissible viruses infecting Trifolium species in Hungary, in 1975 and 1976, white clover mosaic virus (WCMV) strains of different pathological characters were isolated from *T. pratense* (TpVl) and *T. repens* (TrS2). The WCMV, a typical member of the potex-virus group (HARRISON *et al.*, 1971; LESEMANN and KOENIG, 1977), has been reported to cause diseases on lucerne, red clover, white clover, pea, *Medicago lupulina* and *Trifolium hybridum* (QUANTZ, 1956, 1960; BOS *et al.*, 1959; FRY, 1959; BANCROFT *et al.*, 1960; GIBBS *et al.*, 1966; MUSIL, 1966; PRATT, 1968; MUSIL and LESKOVA, 1970; TAPIO, 1970; BERCKS, 1971; BARNETT and GIBBSON, 1975; BLASZCZAK and MICINSKI, 1977) In Hungary the WCMV was reported from *T. repens* (BECZNER and GÁBORJÁNYI, 1968) and from *T. pratense* (HOLLY, 1968; BECZNER and DEVERGNE, 1979), but in those works the identification of the isolated virus had been based only on symptom expression of test plants.

The present paper reports the identification and characterization of the viruses isolated that we will refer to as WCMV-TpVl and WCMV-TrS2.

Materials and Methods

Isolations and host-range experiments

The WCMV-TpVl and -TrS2 were isolated from *T. pratense* and *T. repens*, respectively. The cultures of WCMV isolates were maintained in an insect proof glasshouse by periodic sap inoculation onto *Vicia faba* and *Phaseolus vulgaris*. The inoculum was prepared in 0.067 M Sörensen buffer pH 7.0.

In this study the common mechanical inoculation method was used, applying carborundum as abrasive. Inoculation was carried out with glass spatulas or with finger. Inoculated plants were rinsed with tap water immediately after rubbing. In each experiment 10 plants of one species or cultivar were used. After inoculation the test plants were examined for symptoms during 4–6 weeks. The reisolation of WCMV isolates was attempted with sap from the rubbed and from the top leaves of each species separately. The surface of rubbed leaves was sterilized with 2 per cent NaOH solution and rinsed with plenty of water before back-inoculation. Assay plants were *Pisum sativum* and *V. faba*.

Physical properties

The source of WCMV-TpV1- and TrS2 for the determination of the *in vitro* physical properties was crude sap extracted from *V. faba* and *Datura stramonium*, respectively, inoculated 2 weeks earlier. *P. sativum* and *V. faba* served as assay hosts in all tests. In the experiments Sörensen buffer was used as diluent. Treatment was carried out according to the methods suggested by Bos *et al.* (1960). Each experiment was repeated three times.

Purification

In the first antisera preparation in 1977 the WCMV isolates were purified as described previously (method A) (Beczner and Vassányi, 1980). In the second case in 1980 the method of Frowd and Tremaine (1979) was applied. The harvested leaves of *P. sativum*, inoculated 7–8 days earlier, were frozen overnight or longer and homogenized in a mixture of 0.5 M sodium citrate buffer (pH 6.5), containing 0.002 M Na-EDTA, 0.1 per cent 2-mercaptoethanol, 0.1 per cent thioglycolic acid, and chloroform in the ratio 1 : 1 : 1 (w/v/v). After centrifugation at low speed (1000 g) for 15 minutes the aqueous phase was further purified by means of 10% polyethylene glycol 6000. The mixture was stirred for 1 hour before centrifugation at 10 000 g for 30 minutes. Pellets of precipitated virus were resuspended in 1/10 volume of clarified sap in 0.1 M sodium borate buffer, pH = = 8.5 and allowed to stand overnight at 4 °C. After centrifugation at 10,000 g for 30 minutes the supernatant was centrifuged for 28,000 rpm for 1.5 hours in a Spinco No. 30 rotor, and pellets were resuspended in the borate buffer. After a low speed centrifugation the suspension was layered onto 10–40% sucrose density gradients in borate buffer. The gradients were centrifuged in a Spinco SW 27 rotor at 25,000 rpm for 120 minutes. The virus zones were collected by means of ISCO fractionator and were pelleted in a Spinco No. 30 rotor at 28,000 rpm for 3 hours. The purified preparation was resuspended in borate buffer finally, for immunization, serology and further work.

Antiserum praparation and serology tests

For immunization with WCMV isolates two successive intramuscular injection were given to rabbits, with about 10 mg virus in 2 ml buffer emulsified with 2 ml FREUND's complete adjuvant. Bleedings began 14 days after injection and were repeated at 7–10 days intervals. The WCMV isolates were tested against sera to the following potexviruses: narcissus mosaic virus (NaMV), potato aucuba mosaic virus (PAMV), Nerine virus X (NeVX), potato virus X (PVX) (kindly provided by Mr. D. Z. MAAT), and PVX, and Boussingaultia mosaic virus (BoMV) from author's collection. As a serological test method the micro-precipitin test under paraffin oil was applied, using purified or partially purified virus preparation. Dilution series of antisera and antigens were prepared with saline, containing 0.05 per cent NaN_3. Reactions were recorded after 8–12 hrs at room temperature.

Electron microscopical test

Electron microscopical investigations were carried out on OPTON EM 9 S-2. Crude sap or purified preparation were placed on carbonformvar coated grids and stained with 2 per cent phosphotungstic acid adjusted to pH = 6.5 with KOH.

Results

Isolation of the virus

The WCMV-TpV1 isolate originated from northern part of Hungary, locality of Visegrád. It was separated from a virus complex infecting a *T. pratense* plant. The WCMV remained infective in sap at laboratory temperature for more than 30 days while peanut stunt virus had relatively short longevity (10–12 days) *in vitro*.

The WCMV-TrS2 was isolated from *T. repens*, collected from southern part of Hungary, locality of Siklós, exhibited symptoms of severe mosaic and stunting. In that case clover yellow vein virus and alfalfa mosaic virus also infected the same plant. This isolate was also easily separated from the mixture using the differences in stability of these viruses.

Mechanical transmission and symptoms

The results of host range test are given in Table 1, and Figs 1 and 2. The experimental host range for WCMV-TpV1 isolate was essentially the same as that reported for WCMV-Wageningen isolate (HAMPTON *et al.*, 1978), but for

Table 1

Host range of white clover mosaic virus isolates

Host plant Genus and species Cultivars or selection	Reaction of isolates	
	TrS2	TpV1
	local / systemic symptoms	
Ammi majus L.	—/—	—/—
Antirrhinum majus L.	—/—	—/—
Chenopodium amaranticolor Coste and Reyn.	—/—	—/—
C. murale L.	—/—	—/—
C. quinoa Willd.	—/—	—/—
Cucumis sativus L.	Lc/s	Lc/s
Datura stramonium L.	l/s	(l)/—
Glycine max (L.) Merr.		
cv. Bragg	l/s	l/Mo
Davis	l/(S)	l/Mo
Gomphrena globosa L.	—/—	—/—
Lycopersicon esculentum Mill.	l/—	l/—
Nicotiana debneyi Domin	l/—	—/—
N. glutinosa L.	—/—	—/—
N. langsdorffii Weinm.	—/—	—/—
N. megalosiphon Heurck et Muell.	l/s	l/s
N. sylvestris Spegaz. et Comes	(Lr)/—	—/—
N. tabacum L. cv. Samsun	(Lr)/—	—/—
Xanthi nc.	—/—	—/—
Petunia hybrida Hort. ex Vilm.	l/(s)	—/—
Phaseolus vulgaris L.		
cv. Bountiful	LLc, VN/Mo, Stu, VN	LLc/Mo
Black Turtle Soup	LLc, VN/Mo, VN, Ma, Stu	LLc/Mo
Pinto III	LLn, W/VC, Mo	LLc/Mo
Pisum sativum L.		
cv. Perfected Wales	LLn, W/VC, Mo, LR	LLc/Mo, LR
Prefection Dark Skin	LLn, VN/Mo, Ma, VN, LR, Stu	LLc/Mo, LR
PetitProvence/(Rajnai törpe)	Lnn, W/VC, Mo, LR	LLc/Mo, LR

Table 1. (contd.)

Host plant Genus and species Cultivars or selection	Reaction of isolates	
	TrS2	TpV1
	local / systemic symptoms	
Spinacea oleracea L.	—/—	—/—
Trifolium pratense L.	l/Mo	l/Mo
T. repens L.	l/Mo	l/Mo
Vicia faba L. cv. Olga	LLn/SNr, Stu, Mo, Ma	LLn/Mo, Ma
Lippói	LLn/SNr, Stu, Mo, Ma	LLn/Mo, Ma
Vigna unguiculata (L;) Walp.	LLc/VC, Mo	LLc/VC, Mo

Key: c = chlorotic, l = latent local infection, n = necrotic, s = latent systemic infection, L = local, LL = local lesion, LR = leaf roll, Ma = malformation, Mo = mosaic, N = necrosis, S = systemic, Stu = stunt, V = vein, VC = vein clearing, W = wilting, — = no symptoms, negativ recovery test, (C) = occasionally; numerator = inoculated leaf reaction; denominator = systemic reaction

TrS2 isolate differed fundamentally. The following species reacted differently to TrS2 than to the inoculation with TpV1: *Antirrhinum majus* (not susceptible to TrS2 and reacted latent local infection for TpV1), *Datura stramonium* (latent and systemic infection in case of TrS2 and only latent local infection occasionally occurred with TpV1), *Nicotiana debneyi*, *N. tabacum* cv. Samsun (both species showed latent local infection on rubbed leaves inoculated with TrS2, and were non-hosts for TpV1), *Petunia hybrida* (it was also latent local and systemic host for TrS2 and non-host for TpV1). *Phaseolus vulgaris* cv. Bountiful, Black Turtle Soup and Pinto, *Pisum sativum* cv. Perfected Wales and Perfection Dark Skin, the three bean and two pea varieties produced much stronger symptoms for infection with TrS2 than with TpV1. The reaction of *Vicia faba* for the two isolates of WCMV was the most pronounced; TpV1 caused diffuse brown local lesions which became very obvious on senescence leaves and appeared slowly 4—6 days after inoculation, the systemic symptoms were generally ring like mosaic which only in the chronic stage produced some diffuse necrotisation. TrS2 caused a very pronounced red brown local lesion which appeared very quickly on the 2nd or 3rd day after inoculation. The secondary symptoms are systemic necrotic rings, vein and stem necrosis, and usually the new shoots show mosaic with strong malformation and necrotic spots.

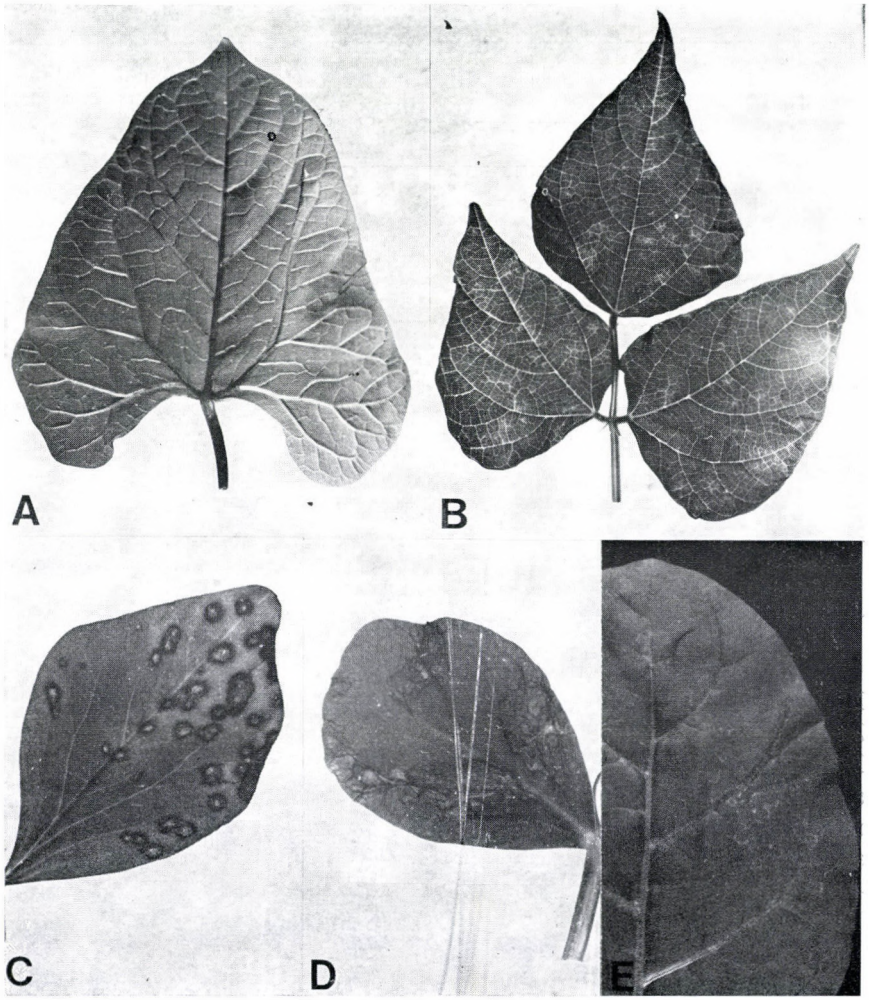

Fig. 1. A) local and B) systemic symptoms on Pinto bean; local symptoms on (C) *Vicia faba* (D) Pisum sativum, and (E) *Nicotiana sylvestris* infected with WCMV TrS2 isolate

Properties in vitro

In crude sap extracted from *V. faba* and *D. stramonium* and bioassayed on *P. sativum* cv. Petit provance (Rajnai törpe) and *V. faba* cv. Lippói, TrS2 and TpV1 were infectious after 10 min at 62 °C and 60 °C, respectively, but not at 65 °C. The dilution end point was for TpV1 $10^{-4}-10^{-5}$ and for TrS2 $5\times10^{-4}-10^{-5}$. The TpV1 was also infectious after 35 days, the TrS2 after 41 days at

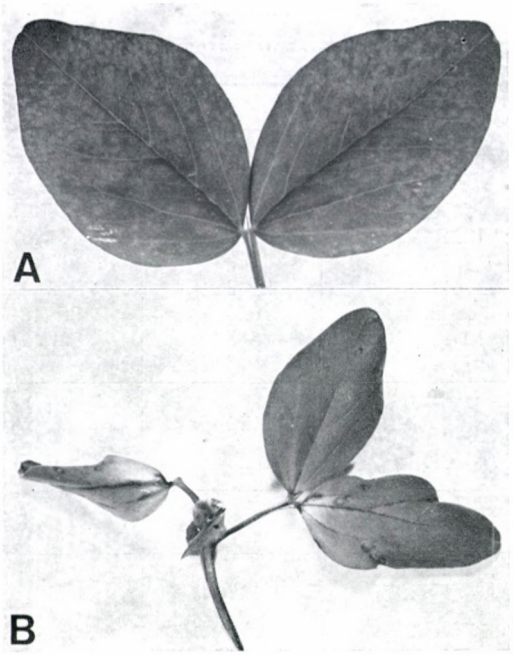

Fig. 2. Systemic symptoms of WCMV-TpV1 (A) and -TrS2 (B) isolates on secondary leaves of *Vicia faba*

laboratory temperature (about 22 °C). In dry material from infected leaves of *P. sativum* both isolates remained infective after 3 years. They also survived for at least 2 years in pea leaves stored at −20 °C.

Serology

The homologous titers of sera TpV1 (1977, 1980), were 1/2048, and that of sera TrS2 (1977, 1980), were 1/2048 and 1/1024, respectively.

The comparison of homologous and heterologous titers (Table 2) showed that there is a definitive differences between the reactivity of both antisera. The average SDI value between TpV1 and TrS2 was 3.5 (reciprocal 3.5). The TpV1 was serologically identical with WCMV-Wageningen strain from which the TrS2 differed also. Both strains are serologically distantly related to PVX (strain PVX-G) but they are unrelated to five other distinct potex-viruses (Table 3).

Discussion

White clover mosaic virus strains were isolated from *T. pratense* and *T. repens*, and characterized by symptoms on a limited host range, in vitro properties and serology.

Table 2

Serological comparison of WCMV-TpV1 and TrS2 isolates

Antisera	Bleedings	Tested antigens*		
		TpV1	TrS2	H
TpV1	5. 10. 1977	1024	128 (3)	2048 (+1)
	12. 10.	512	64 (3)	512 (0)
	18. 11.	2048	512 (2)	2048 (0)
	28. 11.	2048	512 (2)	2048 (0)
	5. 12.	1024	256 (2)	2048 (+1)
	19. 12.	256	128 (1)	1024 (+2)
	5. 05. 1980	1024	16 (6)	1048 (+1)
	23. 05.	2048	16 (7)	2048 (0)
	6. 06.	1024	16 (6)	2048 (+1)
Average	SDI		3.5	+0.6
TrS2	4. 10. 1977	128 (4)	2048	512 (2)
	12. 10.	64 (4)	1024	128 (3)
	18. 11.	128 (4)	2048	512 (2)
	28. 11.	64 (3)	512	128 (2)
	5. 12.	128 (3)	1024	256 (2)
	19. 12.	128 (3)	1024	256 (2)
	28. 04. 1980	128 (3)	1024	32 (5)
	7. 05.	16 (4)	256	32 (3)
Average	SDI	3.5		2.6

Abbreviations see in text. *Titers are reciprocal values. SDI values are indicated in parentheses.

The TpV1 strain was similar to or identical with the WCMV-Wageningen strain and showed also serological identity with the Chechoslovakian WCMV strains described by MUSIL (1966).

The TrS2 strain differed from TpV1 and WCMV-Wageningen strains. First of all TrS2 caused more serious symptoms on Leguminosae hosts, especially more pronounced local and systemic necrotic lesions or rings on *Vicia faba*. It had a remarkable affinity to infect Solanaceae species in which aspect it showed similarity to Indiana strain of WCMV described by BANCROFT *et al.* (1960). MUSIL (1966) also demonstrated strain differences in symptomatology on *Vicia faba*, but in the literature there are no data on serological differentiation of WCMV strains.

Table 3

Heterologous reactions of WCMV-TpV1 and TrS2 isolates*

Antisera	Tested antigens		
	TpV1	TrS2	H
WCMV[1]— M13	1024	128	1024
WCMV[1]— TpM1	2048	1024	2048
WCMV[2]— H	2048	512	2048
BoMV[3]— C	—	2	—
PVX[3]— G	4	4	4
PVX[2]	4	4	4
NaMV[2]	—	—	—
NeXV[2]	2	2	2
PAMV[2]	2	2	2

* Titers are reciprocal values. The antisera of white clover mosaic virus (WCMV), potato virus X (PVX), Narcissus mosaic virus (NaMV), Nerine X virus (NeXV) and potato aucuba mosaic virus (PAMV) were kindly supplied by Drs. MUSIL, M. (1), and MAAT, D. (2), respectively. The antisera (3) of Boussingaultia mosaic virus (BoMV— C) and PVX— G were prepared in our laboratory.

BOS *et al.* (1960) demonstrated the qualitative similarities and relationships among some American, a Dutch, a Canadian and a German isolate of the WCMV, and an exact comparison of two isolates (the Dutch and an American) revealed no quantitative differences between these isolates. According to our identification and characterisation of WCMV isolates we consider that TpV1 belongs to the WCMV-type strain-group and TrS2 is a symptomatilogically and serologically distinguishable deviating strain of the WCMV.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 119–128 (1981)

Grapevine Disease in Hungary Caused by Alfalfa Mosaic Virus Infection

By

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In a ten acres, 7-year-old trellised cv. *Chardonnay/Teleki 5C* vineyard, four vines were diseased with the same symptoms. In spring, the intervenial areas of lower leaves were brilliant yellow discoloured but the veins remained green, while during summer months, on the upper leaves yellow specles, spots, rings or line pattern developed. In summer, symptoms became ivory, but not masked. One of the diseased vines (KeYM-1) was investigated virologically on woody indicators and on herbaceous plants. The results proved that *alfalfa mosaic virus* (AlMV) was the causal agent. The KeYM-1 isolate is a weakly pathogenic one (causing weak symptoms on *tobacco)* and belongs to the AlMV-N strain group (producing necrotic local lesions on *French bean)*. It is serologically identical with AlMV-N and -S strains. Proposed popular name of the disease: "*Yellow spotting and ring pattern of the grapevine*".

Occurrence of alfalfa mosaic virus (AlMV) natural infection on grapevine was observed and proved first in German Federal Republic (BERCKS *et al.*, 1973), then it was found in Switzerland (BOVEY and BRUGGER, 1975; BOVEY and CAZELLES, 1978), in Czechoslovakia (NOVÁK and LANZOVÁ, 1976) and in Bulgaria (JANKULOVA, 1978), too.

Some curious symptoms were observed in Hungary on a single vine of a cv. Chardonnay vineyard near to the Lake Balaton in 1976. The virological investigation gave some evidences that the affected vine with characteristic symptoms was infected by alfalfa mosaic virus. Two previous papers (LEHOCZKY and BECZNER, 1980; BECZNER and LEHOCZKY, 1980) and this one, report on the natural occurrence of AlMV infection on grapevine in Hungary.

Symptoms of the natural infected vine (virus source)

Small and larger bright yellow spots developed scattered on the lower leaves of a 7-year-old trellised cv. Chardonnay vine (its labelled code number: KeYM-1) and then the yellow discolouration extends significantly in the interveinal areas, but the first-, second-, and partially the third-grade veins and furthermore along them a narrow tissue, remained green (Fig. 1). According to the observation the great yellow discolouration develops mainly on lower leaves in spring, while in summer on upper leaves, symptoms are reduced and characterized by yellow specles, spots, yellow rings 3–5 mm in diameter or yellow line pattern (Fig. 2). Symptoms on the leaves do not mask in summer season, merely became ivory.

Fig. 1. Symptoms on naturally infected cv. *Chardonnay* leaves in spring. Brilliant yellow discolouration developed in interveinal areas

Fig. 2. Symptoms on upper leaves of naturally infected cv. *Chardonnay* vine, in summer

So far apparently, there were no other symptoms on shoots and clusters (coulure or millerandage).

In the mentioned cv. Chardonnay vineyard (ten acres) three other vines were found with the same symptoms, in 1979.

Materials and Methods

Source of virus (donor)

Shoot-tips and canes of the cv. Chardonnay/Teleki 5C vine (code number: KeYM-1), with the above-described symptoms, were used for virus-transmission.

Indexing

Short cuttings with two buds made from canes of six woody indicators *FS-4 201-39 (= Siegfriedrebe)*, *V. rupestris* St. George, Chardonnay, Veltliner rouge précoce, Mission and Pinot noir] were used for chip-transmission. A little tongue-shaped tissue (living bark + cambial region + a thin layer of xylem) was excised below the upper bud of the indicator cutting and in the place of it tissue of the same size was transplanted from the donor cane, then it was tied with a plastic band (Fig. 3a). After transplantation the cuttings were placed into turf-pot filled up with Perlite, then they were forced in green-house on 28 °C for ten days and were reared here on 20–25 °C for three weeks (Fig. 3b, c). The forced indicator cuttings were planted in outdoor nursery at the beginning of June and were evaluated here four times over two years.

Isolation and host-range experiments

Transmission experiments in an insect proof green-house took place by mechanical inoculation. The following herbaceous testplants were used: *Ammi majus* L., *Capsicum annuum* L. cv. *Soroksári elit*, *Chenopodium amaranticolor* Coste et Reyn., *Ch. murale* L., *Ch. quinoa* Willd., *Cucumis sativus* L. cv. *Delicates*, *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. megalosiphon* Heurck et Muell., *N. tabacum* L. cv. *Samsun*, *N. tabacum* L. cv. *Xanthi*, *N. tabacum* L. "*Bel 61-10*" *breeding-line*, *Ocimum basilicum* L., *Phaseolus vulgaris* L. cv. *Cheroquee*, *Pisum sativum* L. cv. *Rajnai törpe*, *Tetragonia expansa* Thumb., *Vicia faba* L. és *Vigna sinensis* (Torner) Savi.

Inocula were prepared by grinding shoot-tips and leaves of KeYM-1 vine or infected leaves of *Nicotiana megalosiphon* together with 0.067 M Na-phosphate buffer pH 7.2, in the case of vine, containing 3 per cent PEG 6000. The source of KeYM-1 virus isolate for the determination of the physical properties *in*

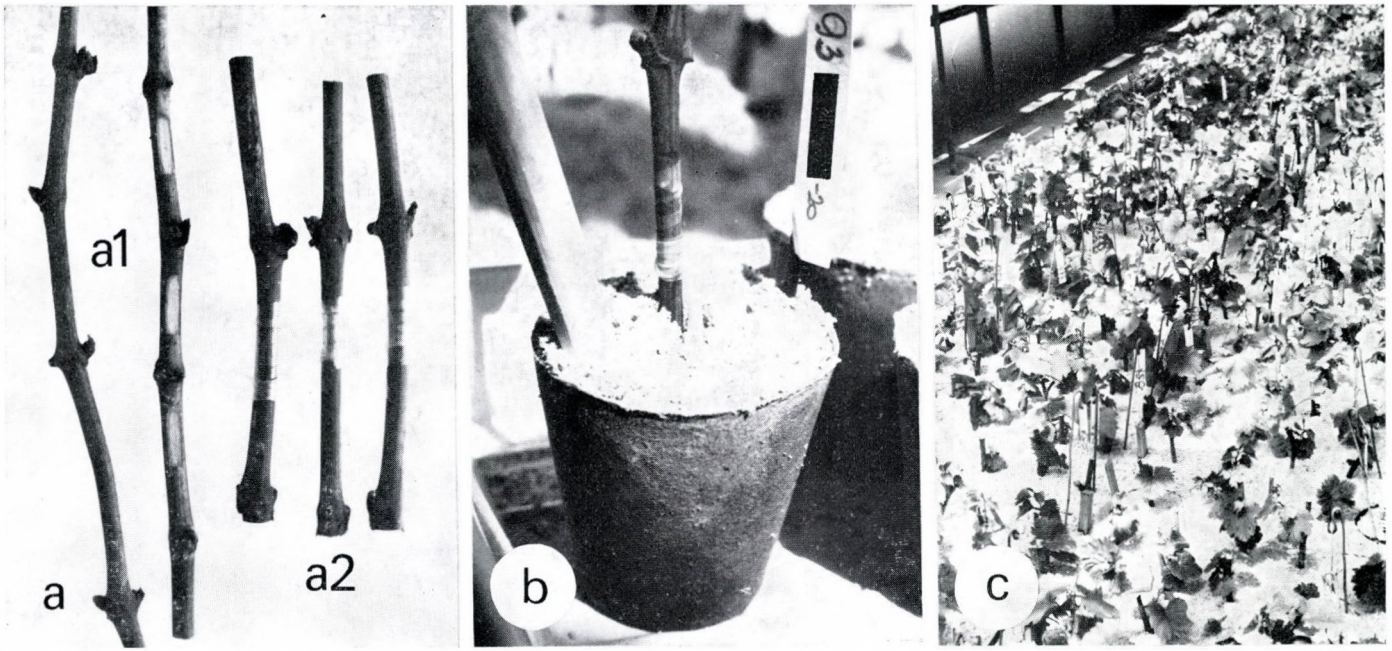

Fig. 3. Method of applied chip-transmission. a. Donor canes and recipient cuttings; a1. Excised chips from donor canes; a2. Recipient indicator cuttings after plastic-band tying of transmissed chips; b. Placing of treated indicator cutting in Perlite for forcing; c. Forced indicator cuttings in green-house

vitro was crude sap extracted from *N. megalosiphon*. The same plant served as assay host. In the experiment phosphate buffer was as diluent. Treatment was carried out as described by BECZNER and LEHOCZKY (1980).

Purification, antiserum preparation and serological test

Virus was purified by the methods of BECZNER (1973), and BECZNER and LEHOCZKY (1980). The antiserum of AlMV-KeYM-1 was obtained from immunization of rabbits. The animals received three intramuscular injection of 2 ml virus purified 250–300 g leaves, emulsified with FREUND's complete adjuvant. Bleeding was began 2 weeks after the last injection and was repeated as one week intervals.

All serological test were performed according to OUCHTERLONY's double diffusion method in petri dishes, the medium used being 0.8% Bacto agar in saline solution containing 0.05 per cent NaN_3. BG4 and 32/1 of AlMV antisera were obtained from our collection.

Electron microscopy

Electron microscopical investigations were carried out on OPTON EM 9 S-2. Samples in SÖRENSEN phosphate buffer were placed on carbon – formvar coated grids and stained with 2 per cent phospho-tungstic acid adjusted to pH 6.5 with KOH.

Results

Indexing

Virus-transmission by indexing gave positive results on all six woody indicators. Yellow specles, rings, or line pattern developed on the leaves of indicator-plants, but the intensity of symptoms changed depending on indicator (Fig. 4). Reaction-symptoms on *St. George* leaves were similar to those BOVEY and BRUGGER (1975) found on the same indicator. Symptoms were the most intensive and conspicuous on cv. *Chardonnay* and cv. *Veltliner rouge précoce*, therefore both are recommended as indicators for detection of AlMV from grapevine. As *St. George* also reacted with the latent fleck and cv. *Pinot noir* with the leafroll symptoms, could consider it certain, that KeYM-1 vine was a carrier of latent fleck and leafroll viruses, too.

Isolation on herbaceous plants

The AlMV was easily transmitted from vine to *Chenopodium quinoa*, *Nicotiana glutinosa*, *N. megalosiphon* with mechanical inoculation. In case of the first transmission most of the plants did not show typical AlMV symptoms as yellow mosaic on *Gomphrena globosa*. The systemic symptoms on *C. quinoa* appeared

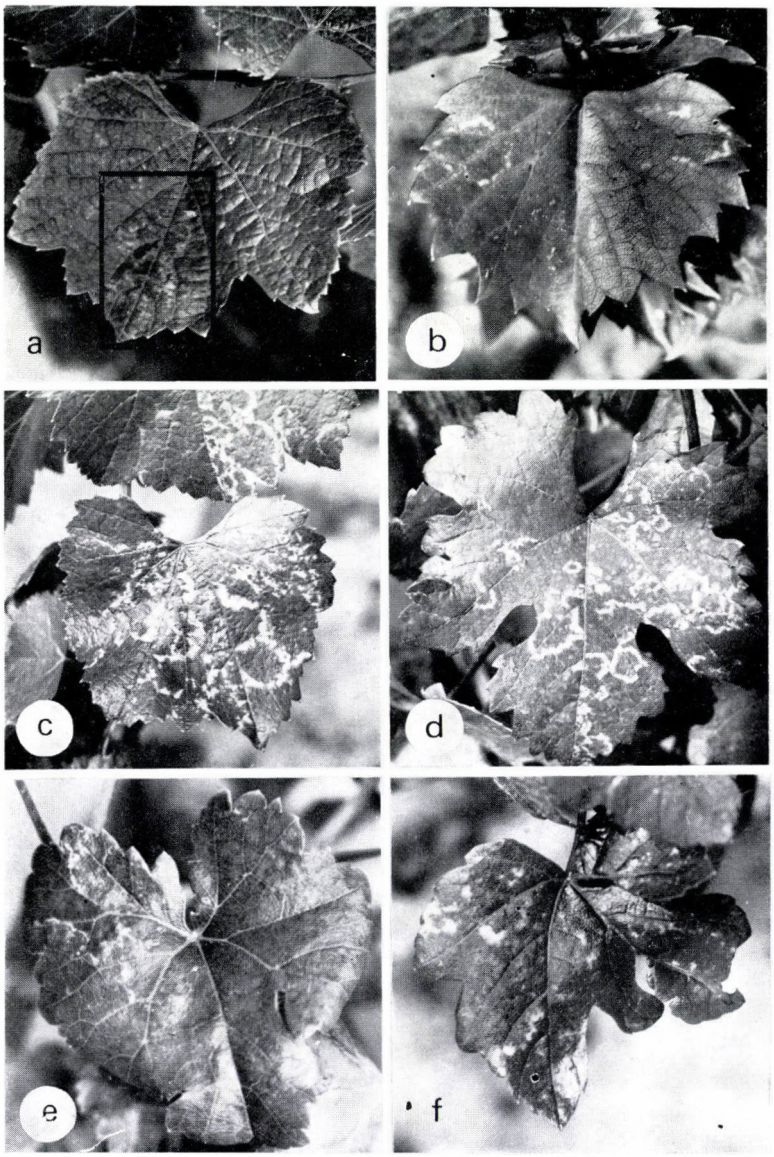

Fig. 4. Reaction-symptoms on woody indicators. a. on *FS-4* leaf; b. on *St. George* leaf; c. on cv. *Chardonnay* leaves; d. on cv. *Veltliner rouge précoce* leaf; e. on cv. *Mission* leaf; f. on cv. *Pinot noir* leaf

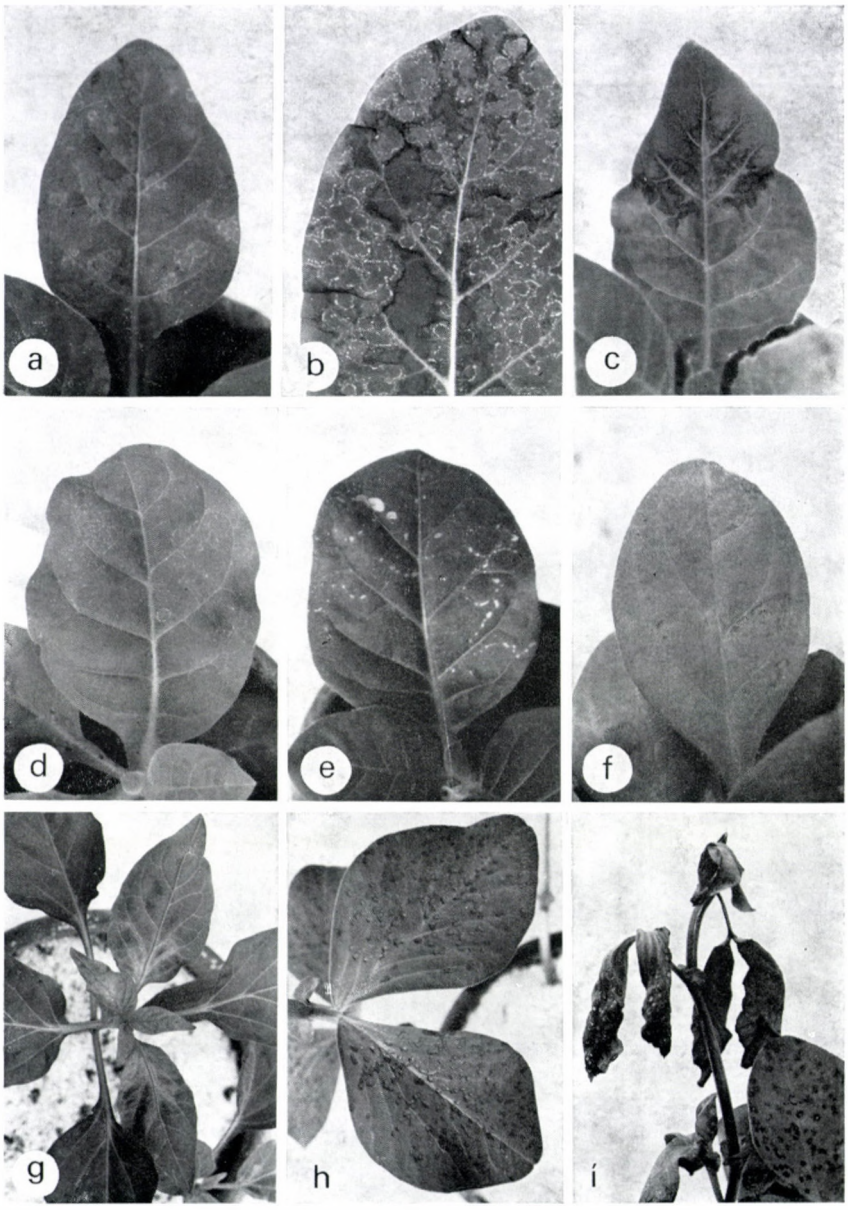

Fig. 5. Symptoms on herbaceous testplants. a—b. necrotic rings on inoculated leaf of *Nicotiana megalosiphon;* c. oak-leaf systemic symptoms on *N. megalosiphon* leaf; d. necrotic rings on inoculated leaf of *N. tabacum "Bel 61-10" breeding-line;* e. necrotic rings and lesions on inoculated *N. tabacum* cv. *Samsun* leaf; f. necrotic rings and lesions on inoculated *Petunia hybrida* cv. *Himmelsröschen* leaf; g. systemic mosaic on the leaves of *Capsicum annuum* cv. *Kecskeméti törpe;* h. necrotic local lesions on the leaf of *Vicia faba;* i. necrotic local lesions and systemic wilting on *Vicia faba*

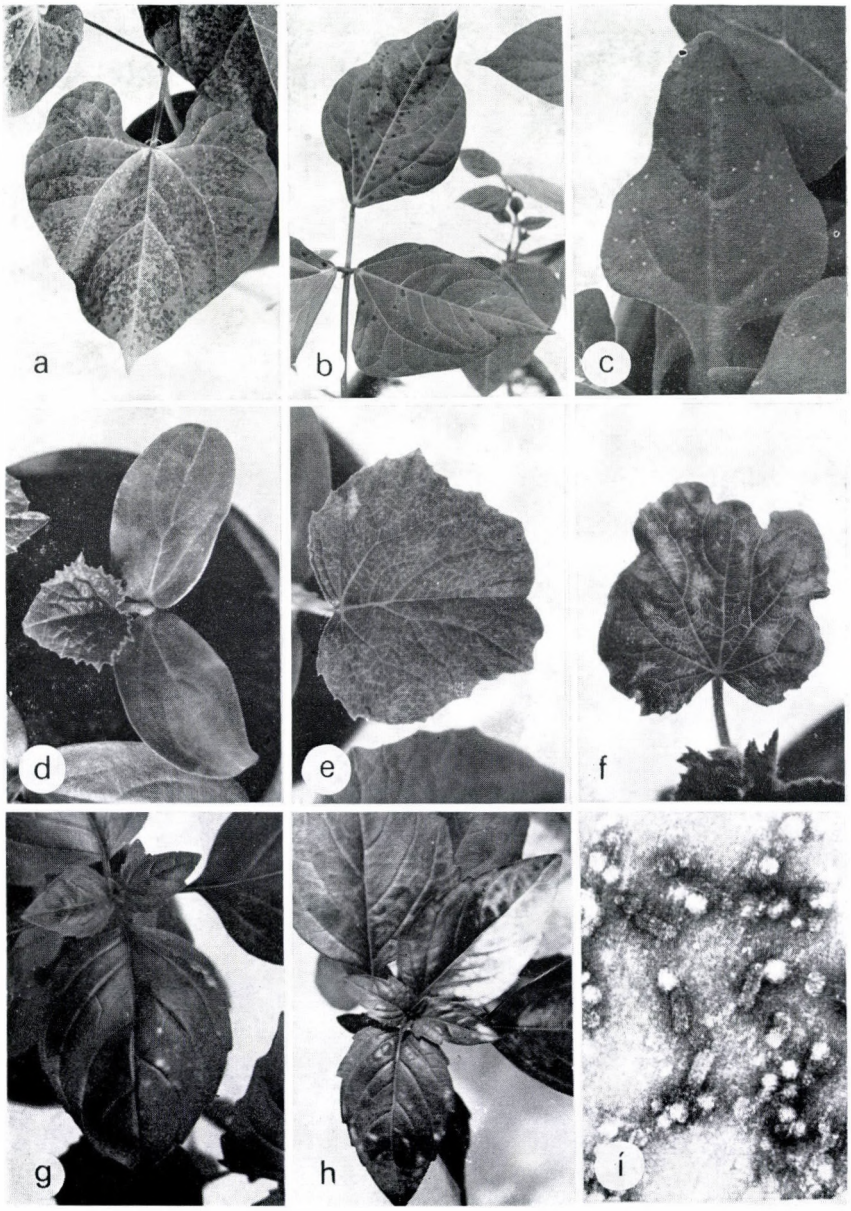

Fig. 6. Symptoms on herbaceous testplants (a—h). a. necrotic local lesions on the leaf of *Phaseolus vulgaris* cv. *Cheroquee;* b. necrotic local lesions on the leaflets of *Vigna sinensis;* c. necrotic lesions on inoculated leaf of *Tetragonia expansa;* d. pale chlorotic local lesions on the cotyledons of *Cucumis sativus* cv. *Delicates;* e.—f. systemic chlorotic veins on the leaf of *Cucumis sativus* cv. *Delicates;* g.—h. systemic "*calico*" symptoms on the leaves of *Ocimum basilicum;* i. electron microscopic picture of bacilli-form particula of AlMV isolated from grapevine (× 154 000)

too late, appearence of local lesion on *N. megalosiphon* was very quick. This slight differences can be due to the strain differences and the altered environmental condition. The clean culture of AlMV was separated from the systemically infected leaves of *Vicia faba* obtained after the second passage.

Host range and symptomatology

AlMV infected all testplants used. 18 out of 20 showed symptoms, on *Ammi majus* and on *Gomphrena globosa* was latent. The experimental host range for AlMV-KeYM-1 was essentially the same as that reported for Hungarian strain of AlMV described by BECZNER (1972). On the basis of necrotic local lesion formation on the primary leaves of *Phaseolus vulgaris* and *Vigna sinensis* and weak symptoms on *Nicotiana tabacum* this isolate belongs to the AlMV-N strain group, and the weakly pathogenic subgroup.

This virus produced local lesions only on *C. murale*, *Lycopersicon esculentum*, *Nicotiana tabacum* cv. *Xanthi*, *Ph. vulgaris*, *Tetragonia expansa* and *V. sinensis;* local and systemic symptoms on *Capsicum annuum*, *C. amaranticolor*, *Cucumis sativus*, *Datura stramonium*, *N. glutinosa*, *N. megalosiphon*, *N. tabacum* cv. *Samsun*, "*Bel 61-10*" *breeding line*, *Ocimum basilicum*, *Pisum sativum* cv. *Rajnai törpe (Petit provencal)*, *Vicia faba*.

In vitro properties

The TIP for AlMV-KeYM-1 is between 64 and 70 °C. The DEP is 10^{-4}. The LIV at room temperature is between 4- and 8-days. In dry material from infected leaves of *N. megalosiphon* the virus remained infective after one year.

Purification and density gradient centrifugation

Extracts of plants of *N. tabacum* infected with KeYM-1 contained a number of typical bacilli-form particles. The described purification method yielded reasonable amounts of virus. Purified preparations, centrifuged on sucrose density gradient columns for two hours with SW 27 rotor at 24 000 rev/min formed three zones.

Serology

In serological testing, partially purified preparations of the KeYM-1 from *N. tabacum* cv. *Samsun*, or *N. megalosiphon* formed distinct precipitin lines with AlMV-KeYM-1, BG4 and 32/1 antisera (BECZNER, 1973), whereas extracts from healthy plants of both species processed in the same manner gave no reaction.

In agargel-immundiffusion test, there was coalescence of the lines against AlMV-KeYM-1, BG4 and 32/1, using any antisera of the mentioned strains, indicating the serological identity.

Electron microscopy

Electron microscopy of crude sap of testplants *(tobacco, C. quinoa,* etc.) revealed the presence of bacilliform particles in limited number. Significantly more particles could be seen when the carbon-formvar coated grids were pretreated for 5 minutes by 100 or 1000 times diluted AlMV antiserum of 1 : 1024 titer, and followed by the DERRICK procedure.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 129–132 (1981)

SHORT COMMUNICATION

Occurrence of *Phytophthora* Rot of Soybeans in Hungary

By

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The first occurrence of Phytophthora rot of soybeans in Hungary is reported. The causal fungus *Phytophthora megasperma* var. *sojae* identified by by microscopic studies and pathogenicity tests, is described as well.

The disease caused by *Phytophthora megasperma* (Drechs.) var. *sojae* A. A. Hildebrand (syn. *P. sojae* Kaufmann & Gerdemann) was first observed in the USA in 1948 (SUHOVECKY and SCHMITTHENNER, 1955). More recently KUAN and ERWIN (1980) have re-named the fungus *P. megasperma* f. sp. *glycinea*. To the best of our knowledge, its occurrence has not been reported from other countries than USA and Canada (ATHOW, 1973; SINCLAIR and SHURTLEFF, 1975).

In the spring of 1979, however, symptoms typical of Phytophthora rot were observed in Hungary (village Tápiószele), too. The damage was detected on the soybean cultivars Merit and ISz-13, ISz-14, KZ-237 (Hungarian ones), seeds of which originated from Iregszemcse (Western-Hungary). The causal fungus was found in Hungary as early as 1974 on cv. Merit, but no formal description of it has been given (SZILI, 1975).

Phytophthora rot can be found in soybeans at any stage of development. The infected seeds may die before emergence, especially in wet soil. Post-emergence root and stem rot cause wilting then death of the seedlings (Fig. 1). Older plants are killed more gradually. Leaf wilting is a common symptom of late infected plants. After the plant died, withered leaves remain attached for a week or so.

The fungus was isolated from soybean seeds having been surface sterilized in 0.2% NaOCl and 70% ethanol for 2 min, respectively, then placed into moist chamber. Small pieces of the mycelium developed under the above circumstances were transferred onto potato-dextrose agar (PDA) containing 100 μg/ml chloramphenicol. Cultures (Fig. 2) were kept at 25–27 °C and, illuminated with fluorescent lamp for 12 hrs a day.

The young mycelium was coenocytic, but became septate with age and produced branches with constrictions at their base. Width of hyphae grown on PDA averaged 4–9 μm. Sporangia did not develop on PDA, but can be obtained from culture in pea broth or lima bean extract. The size of the obpyriform non-papillate sporangia is 39–60 × 34–51 μm. Sporangia germinate with ovoid, two-

Fig. 1. Damage caused by *P. megasperma* var. *sojae* on seedling of soybean cultivar ISz-13

Fig. 2. Three-week-old culture of *P. megasperma* var. *sojae* on potato-dextrose agar

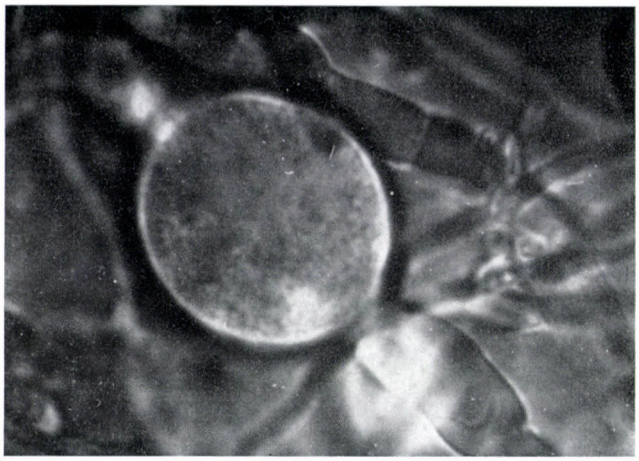

Fig. 3. Sexual process between oogonium and antheridium. × 1350

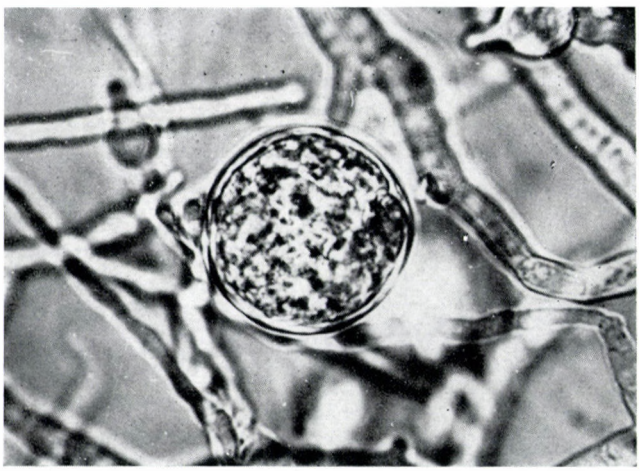

Fig. 4. Cospore of *P. megasperma* var. *sojae* produced on potato-dextrose agar. × 900

flagellate zoospores or directly, functioning as conidia. Sexual organs, the thin walled, spherical oogonia and the paragynous antheridia developed abundantly on PDA. Fertilization of an oogonium by an antheridium results in the development of an oospore (Fig. 3). Oospores have thick, smooth double wall and fine grained cytoplasm (Fig. 4). Their average size was 38.4 (28.8–43.2) μm. The oospores germinate by producing germ tubes from which hyphae then sporangia develop.

Pathogenicity test was carried out on 10-day-old seedlings of cv. Harosoy by using the method 8 of KAUFMANN and GERDEMANN (1958). Introduction of a suspension of minced mycelia into the hypocotyls resulted in the complete death of the plants within 7–10 days. The plant debris put into moist chamber offerred an object for reisolation of the fungus.

The isolates of *P. megasperma* var. *sojae* exhibit various rate of virulence (HILDEBRAND, 1959; HILTY and SCHMITTHENNER, 1962; AVERRE and ATHOW, 1964). Physiological specialization of this fungus is also well known. Nine races as well as soybean cultivars differing in reaction to these races have been reported so far (MORGAN and HARTWIG, 1965; SCHMITTHENNER, 1972; SCHWENK and SIM, 1974; HAAS and BUZZELL, 1976; LAVIOLETTE and ATHOW, 1977). The 9 races are distinguished by using differential soybean cultivars as follows: Harosoy, Harosoy 63, Sanga, Mack, Altona, Tracy, P.I.171.442 and P.I.103.091. In the lack of this series we have not been able to complete race identification work.

Race identification, assessment of incidence, as well as comparative studies on Hungarian cultivars are in progress.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 133—135 (1981)

SHORT COMMUNICATION

Tomatine and Phenol Production Associated with Control of Fusarial Wilt of Tomato by the NO_3-nitrogen, Lime, and Fungicide Integrated Systems

By

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Increased resistance of tomato plants supplied with NO_3-N, lime and benomyl (in the form of Chinoin-Fundazol 50 WP) in an integrated system is in correlation with an increase in the tomatine content of stem tissues. Phenolic compounds do not play any role in resistance of tomato to fusarial wilt.

Antifungal compounds, produced following inoculation, have been implicated in resistance of cotton (BELL, 1969) and possibly tomato (TJAMOS and SMITH, 1974) to vascular wilt caused by *Verticillium albo-atrum*. No convincing evidence is yet available that such compounds are involved in resistance to *Fusarium* wilt. REILLY and KLARMAN (1972) reported that several fungicides stimulated the production of a phytoalexin in soybean and, also, CARTWRIGHT *et al.* (1977) have presented evidence that control of rice blast by systemic fungicides is accompanied with phytoalexin production. There is another evidence that, in some plant species, those cultivars having high level of phenols are more resistant to certain fungi. HARTLEY and HARRIS (1978) suggested that the presence of phenolic groups in the cell wall of the wheat plant, treated with commercial cellulase, may be related to the greater resistance of the plants to the yellow rust. However, KIRÁLY (1964) found that with increased nitrogen fertilization the phenol level decreased in wheat tissues and the susceptibility to rust increased.

This paper reports on the possibility, that treatment of tomato plants with NO_3-nitrogen, lime, and a systemic fungicide in an integrated system produces an inhibitor to *Fusarium oxysporum* f. sp. *lycopersici* R1 in tomato plants.

Details of procedures on growth of tomato seedlings and application of chemicals have been given in detail previously (SARHAN and KIRÁLY, 1981). Seedlings of susceptible cultivar of tomato, Primset, were grown under ordinary greenhouse conditions. Plants, four-week-old, were inoculated by up-rooting and washing them, dipping the roots in the spore suspension of pathogenic strain of the *Fusarium*, and repotting the plants again. Liming the soil has been done by mixing dry $Ca(OH)_2$ with the soil, 2 g/kg soil, one week prior to planting. The fungicide benomyl, in the form of Chinoin-Fundazol 50 WP, was used at the rate

of 300 ppm one day before inoculation. Potted plants were watered with a nutrient solution (Hogland's solution) containing 420 ppm nitrogen in the nitrate form, plus several micronutrients.

Tomato plants inoculated with the pathogen developed typical wilt symptoms. When plants were supplied with the chemicals (nitrate, lime and benomyl) in integrated systems, the development of the symptoms was reduced. Symptoms

Table 1

Estimation of tomatine and phenol content in vascular stem tissues of infected and not infected tomatoes which treated with NO_3-nitrogen, lime, and benomyl in integrated systems

Treatments	Tomatine mg/gm fresh weight		Total phenol mg/gm fresh weight	
	Not infected	Infected	Not infected	Infected
Control (H_2O)	0.85	3.65	3.92	4.36
Nitrate + benomyl	1.45	4.20	1.25	3.83
Nitrate + lime	1.15	3.80	2.75	4.10
Benomyl + lime	1.30	3.95	2.89	3.52
Nitrate + lime + benomyl	1.87	4.62	1.50	2.88

were controlled perfectly in the case when the three chemicals were combined. On the second week after inoculation with the *Fusarium* tomatine and phenol contents were estimated in the vascular stem tissue.

Tomatine was extracted and estimated as described by McCane and Drysdale (1975). Total phenol content was estimated by using the Folin-Ciocalteu reagent according to Spies (1955).

A decrease in total phenol content was found in the not infected stem which was treated with different combinations of nitrate, lime and benomyl (Table 1). The amount of tomatine was positively correlated with the presence of nitrate or benomyl. However, there was a very low increase in other combinations of treatments as compared to the untreated plants (Table 1). The infection of tomato plants resulted in increasing the tomatine in stem tissue, and also, infected tissues showed an accumulation of phenolic compounds as compared to not infected plants. The control of *Fusarium* wilt of tomato by integrated systems may be a consequence of increased tomatine and perhaps this is in a cause-and-effect relationship with suppression of disease symptoms. Király *et al.* (1972) and Érsek *et al.* (1973) observed that application of fungitoxic antibiotics to infection sites changed compatible interactions into incompatible ones. One can hypothesise that integrated measures increase tomatine which cause an incompatible (resistant) reaction of the host. However, Cartwright *et al.* (1977) suggested that they sensitized the plant when treated with systemic fungicides, so that the host responded in a resistant manner to the invading fungus.

Our results suggest that increased resistance of tomato supplied with NO_3-nitrogen, lime and the fungicide benomyl in an integrated system could be related to an increase in the tomatine content of stem tissue. However, phenolic compounds do not play any role in resistance of tomato to fusarial wilt.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 137—138 (1981)

SHORT COMMUNICATION

Isolation of an Agglutination Factor that may Determine Race Specific Resistance of Soybean Leaves to *Pseudomonas glycinea*

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A material obtained from the intercellular fluid of 'Harosoy' soybean leaves having been infiltrated with NaCl solution agglutinated incompatible race (R1) but not the compatible race (R2) and isolate (2c) of *Pseudomonas glycinea*. Host non-pathogen *P. syringae* was agglutinated as well. The roughly purified material exhibited hemagglutinating activity suggesting that lectin-like compound(s) may be involved in the above phenomenon.

Resistance mechanism of plants to phytopathogenic bacteria could be due to selective immobilization of the varietal or species non-pathogens by cell wall constituents of the plant (GOODMAN *et al.*, 1976; SEQUEIRA *et al.*, 1977; CASON *et al.*, 1978). Host cell wall has been shown to contain certain proteins possessing lectin activity (LAMPORT, 1979). These compounds, on the basis of their carbohydrate specificity, can agglutinate bacteria incompatible to host plant (SEQUEIRA and GRAHAM, 1977; EL-BANOBY and RUDOLPH, 1980) and, could thereby be candidates for specific recognition of hypersensitive reaction inducing bacteria.

Since lectin-like compounds are probably located in the host cell wall, it seemed possible that they could be isolated by infiltration of the intercellular spaces of the leaves with solutions of high ionic strength.

Materials and Methods

Trifoliates of 3-week-old soybean cv. Harosoy were harvested. The main veins were removed according to the method of KLEMENT (1965) and the half leaves were vacuum infiltrated with NaCl in sodium phosphate-citrate buffer (1% w/v) or Triton X-100 (0.3% v/v) or distilled water. The surfaces of the leaves were dried and the material was kept at 4 °C for 2 hrs. The infiltrating solution was recovered by centrifugation at 2000 *g*, at 0 °C for 5 min. The supernatant was mixed with ammonium sulfate solution to give a final saturation of 50%. The solution was left to be precipitated overnight at 4 °C and then centrifuged at 20,000 *g* for 1 h. Supernatant was dialysed against distilled water then lyophilized. The dry extracts were taken up either in saline or distilled water up to 10 mg ml^{-1} concentration in order to test their agglutinating activity in Takátsy's microtitre plate.

Races of *P. glycinea* as well as *P. syringae* were cultured in Kolle flasks containing nutrient broth agar at 28 °C. One-day-old cultures were suspended either in distilled water or saline to give a concentration of 10^9 cells ml^{-1}; 25 μl aliquots were added to the same volumes of the plant extract.

Besides bacteria trypsinized human erythrocytes ('0') were also tested as possible targets of lectin activity.

Results and Discussion

Agglutinating activity was exhibited only by the salt-extracted material. At 5 and 10 mg ml^{-1} concentrations it agglutinated the species non-pathogenic *P. syringae* cells as well as the varietal non-pathogenic race (R1) of *P. glycinea*, but not the varietal pathogenic race (R2) and isolate (2c). Within 3 to 4 hours the pathogenic races settled in the bottom of wells forming pin point sediments and the solution became clear. In the presence of varietal and species non-pathogens, however, the samples remained turbid at the same time.

After one day incubation at 28 °C, all the samples cleared, but the sediments of non-pathogens were larger in diameter than those of pathogens.

Trypsinized erythrocytes agglutinated even with the lowest (1.25 mg ml^{-1}) concentration of the extract used.

The crude extract gave positive reactions with anthrone and comassie blue suggesting the presence of carbohydrates and proteins.

Further studies are in progress in order to clarify if the phenomenon can be generalized within this host–parasite interaction and to identify the active material contained by the crude leaf extract.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 139–142 (1981)

Status of Two European Weevils for the Biological Control of *Carduus* Thistles *in the U.S.A.*

By

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Carduus thistles are introduced Eurasian weeds causing major problems in pastures, ranges, croplands, and along state highways in many parts of the U.S.A. Two of the most troublesome species are *Carduus thoermeri* Weinmann[1] (musk thistle) and *Carduus acanthoides* L. (plumeless thistle). They have been able to rapidly take over mismanaged land, especially in overgrazed pastures. In the search for an effective long term control measure, several biological agents were imported into the USA.

Two European weevils which have been released have become well established and have potential as biological control agents of the musk thistle: *Rhinocyllus conicus* Froelich, a thistle head weevil, was first introduced from France and Italy in 1969 into 3 states: California, Montana and Virginia. It has subsequently been relocated to at least 15 other states. Eggs of the weevil are laid on thistle heads and the developing larvae feed on tissues of the receptacle, preventing seed formation. Significant impact has been reported in Virginia, Montana and Missouri.

A second weevil, *Ceuthorhynchidius horridus* (Panzer) was imported for host specificity testing under quarantine in 1970. Based on results of the tests, it was officially approved for field release in Virginia in 1974 and has become established in at least 7 release sites. Several other states have subsequently released this rosette-feeding weevil which is currently being evaluated for efficacy in thistle control. Eggs are laid in leaf mid-ribs, larvae feed towards the crown and kill the growth point.

Compatibility studies of both weevils with 2.4-D, the most commonly used herbicide for thistle control in the U.S.A., revealed that adult survival, fecundity and vitality were not adversely affected. With proper timing the herbicide and both weevils can be compatibly used in an integrated program for control of *Carduus* thistles.

Carduus thistles are introduced Eurasian weeds causing major problems in pastures, ranges, croplands, and along state highways in many parts of the U.S.A. (Kok, 1978). They are especially serious problems in California, Kansas, Montana, Nebraska, West Virginia and Virginia. In Virginia alone, they heavily infest at least 60,000 hectares of pastures. Two of the most troublesome species are *Carduus thoermeri* Weinmann (musk thistle) and *C. acanthoides* L. (plumeless thistle). About $1,000,000.00 per year has been spent by the Virginia Department of Agriculture and Commerce in thistle control during each of the past 12 years. Average cost of thistle control was $25–$30 per hectare. The use of natural enemies for the control of *Carduus* thistles is gaining acceptance within Virginia

[1] Previously published as *Carduus nutans* L.

as well as in many of the other mainland states. Two of the natural enemies which have received considerable attention are *Rhinocyllus conicus* Froelich (head weevil) (HARRIS and ZWÖLFER, 1971; SURLES *et al.*, 1974; HODGSON and REES, 1976; KOK, 1978; PUTTLER *et al.*, 1978; GOEDEN and RICKER, 1978), and *Ceuthorhynchidius horridus* (Panzer) (rosette weevil) (WARD *et al.*, 1974; KOK, 1975; KOK and TRUMBLE, 1979; TRUMBLE and KOK, 1979a). *Rhinocyllus conicus* (Fig. 1) was first introduced from France and Italy in 1969 into three states: California, Montana and Virginia. It has subsequently been relocated to at least 15 other states. Eggs of the weevil are laid on the bracts of thistle heads and first instars on hatching tunnel into the heads, feeding on tissues of the receptacle. Many larvae can develop within a single head and heavily infested heads are prevented from producing seeds. Significant reductions of musk thistle have been attributed to *R. conicus* in Virginia (KOK and SURLES, 1975), Montana (REES, 1977) and Missouri (PUTTLER *et al.*, 1978) and it has become established in the other states. The impact by *R. conicus* is most impressive about 5–6 years after establishment. Reductions of 70–95% have been found in the test (experimental) sites in Virginia. However, *R. conicus* has not been as impressive in the control of the plumeless thistle mainly because of its poor synchronization with the development of *C. acanthoides* (SURLES and KOK, 1977). Thus, a second weevil was introduced to increase the biotic stress on the target weeds.

Ceuthorhynchidius horridus (Fig. 2), the second introduced weevil to be released for *Carduus* thistle control in the U.S.A., attacks the plant at the rosette stage. It was imported from Italy for host specificity testing under quarantine in

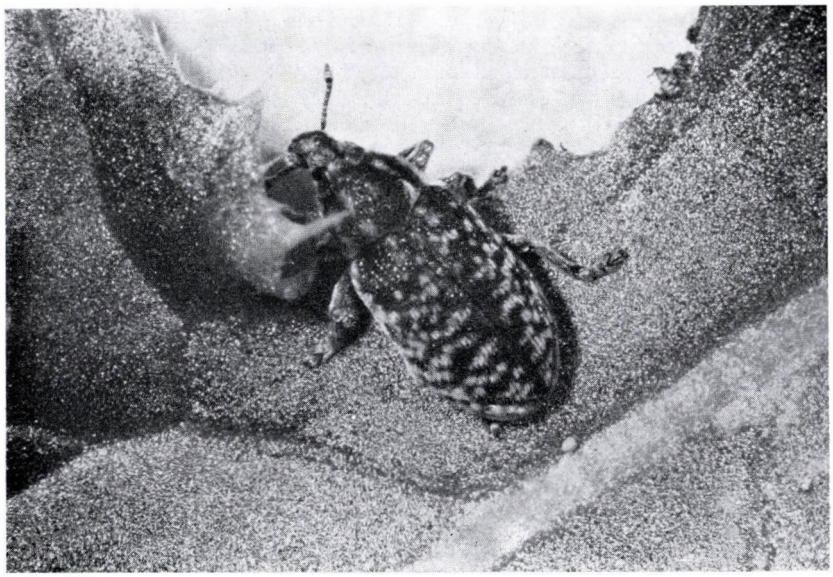

Fig. 1. Adult *Rhinocyllus conicus* Froelich — thistle head weevil

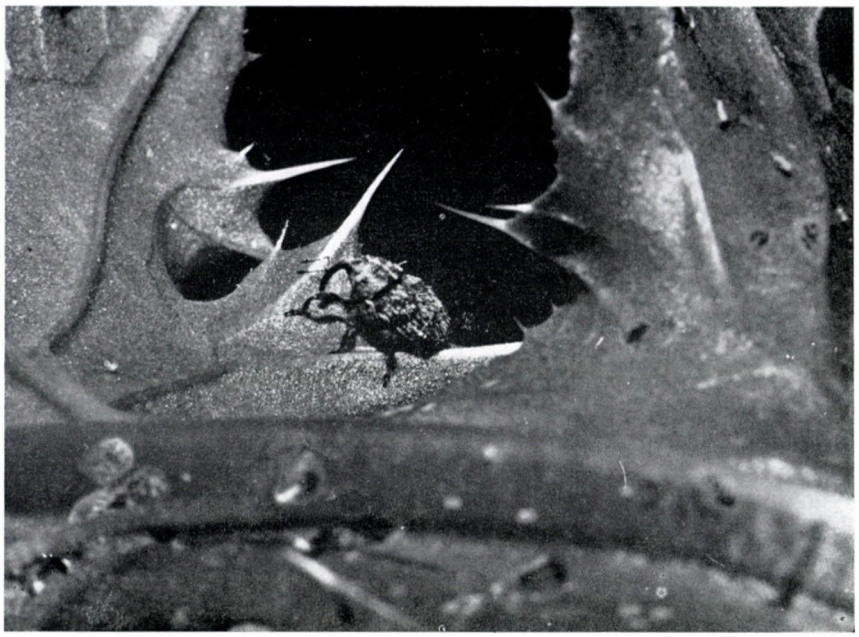

Fig. 2. Adult *Ceuthorhynchidius horridus* (Panzer) — rosette weevil

1970. Based on results of the testing (WARD *et al.*, 1974; KOK, 1975), the USA Biological Weed Control Federal Working Group officially approved it for field release in 1974. Releases made in 1974/1975 became established by 1978 in Virginia (KOK and TRUMBLE, 1979). Several other states have subsequently released this rosette-feeding weevil which is currently being evaluated for efficacy in thistle control. Eggs of this weevil are laid in the mid-ribs of leaves. Upon eclosion, larvae feed toward the crown of the rosette causing a characteristic necrosis. The plants may die if infestation is sufficiently heavy. Larval feeding is completed in 4–8 weeks, and mature larvae move into the soil to pupate (KOK *et al.*, 1975).

Current recommendations for the control of *Carduus* thistle in the U.S.A. are primarily based on the use of herbicides. Most commonly used is 2,4-D at 1.68–2.24 kg/ha sprayed on rosettes in the fall or spring (KATES, 1968). 2,4-D treatment of musk thistle in the late-bud to early-bloom stage of the primary bloom was not detrimental to *R. conicus* larvae (TRUMBLE and KOK, 1979b), and would not prevent survival or reproduction of *R. conicus*. Laboratory tests showed that survival of *C. horridus* weevils treated with 1.68 kg/ha was also not different from the untreated controls, but higher doses (16.8–147.8 kg/ha) caused significantly greater mortality (TRUMBLE and KOK, 1980). Adult vitality was unaffected by the herbicide. When thistle infested fields were sprayed with 1.68 or 2.24 kg/ha of 2,4-D, the host plants died, but survival, reproduction and population increase of *C. horridus* were not affected. Thus 2,4-D application can be manipulated to

have a minimal impact on the use of both biocontrol agents. With proper timing the herbicide and both weevils can be compatibly used in an integrated program for control of *Carduus* thistles.

Acknowledgements

This research project is supported in part by the United States Environmental Protection Agency Grant #R803931-02-0 and in part by USDA/SEA Cooperative Project #12-14-1001-1204.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 143–150 (1981)

Coccinellid Community in an Apple Orchard Bordering a Deciduous Forest*

By

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The composition and diversity of the coccinellid beetle community were studied in an insecticide treated and an untreated block in an apple orchard situated among forests near Budapest from 1977 through 1979. The insecticide-sprayed block contained 11 while the control 10 species. The diversities did not differ significantly ($d = 0.73$ for the sprayed block, $d = 0.69$ for control) but the control plot supported more beetles than the sprayed one. The most abundant species were *Coccinella septempunctata* (71.2% for treated plot vs. 66.5% for the control), *Adalia bipunctata* (9.7 vs. 14.37%) and *Exochomus quadripustulatus* (8.03 vs. 14.08%). More *Adonia variegata* were found in the sprayed block (6.35 vs. 1.44%) which probably was in connection with the neighbouring cereal field. The presence of *Calvia quattuordecimguttata*, *Adalia decempunctata* and *Harmonia quadripunctata* reflected the woody environment. Seasonal dynamics varied widely showing peaks in the spring and summer 1977, in the summer 1978, and in the autumn 1979.

Coccinellids are a major entomophagous group in agroecosystems and often used in biological control programs (DEBACH, 1974). Although many pest insects may be consumed by coccinellids, the individual species are rather specialized, e.g. restricted to aphids living on some plant families (IPERTI, 1978). Different authors found 3–15 coccinellid species in European apple orchards (HODEK, 1973). The most abundant species are *Coccinella septempunctata* L. and *Adalia bipunctata* L. though some species may exceed their numbers in some cases (HODEK, 1973). The effect of insecticides on predatory arthropods in apple orchards was investigated by HUKUSHIMA (1966, 1969, cit. HODEK, 1973) in Japan. The relative abundance of the most common species, *Chilochorus kuwanae* Silvestri was equal in sprayed and unsprayed plots (28 and 26%, respectively). Other species were relatively more abundant in the unsprayed plot except *Stethorus japonicus* Kamiya.

The role of the coccinellids on their prey populations is a controversial topic. It is, however, generally accepted that the overwintered adults are able to reduce prey populations substantially (HODEK, 1973; KACZMAREK, 1973; RABASSE *et al.*, 1978). DIXON and BARLOW (1979) found that the two-spotted ladybird, *A. bipunctata* contributed to the regulation of the lime aphid *(Eucallipterus tiliae)* populations while FRAZER and GILBERT (1976) found no stable equilibrium point

* Ecological Researches on Apple Orchards, No. 12.

in a coccinellid-aphid predator-prey system in a lucerne field in Ontario. The possibility of regulation by a predator is more probable in perennial than annual crops and with a less mobile prey (HASSELL, 1978).

This article reports on the coccinellid community found in an apple orchard with two management practices bordering to a deciduous forest and on the seasonal dynamics of the most abundant species. The data presented here showed that, although the coccinellid faunal density of the insecticide-free block exceeded that of the sprayed one, the diversities did not differ significantly.

Materials and Methods

The study area was a 5.8 ha apple orchard near Budapest at Julianna major Experimental Farm of the Research Institute for Plant Protection. The soil was a heavy clay. The orchard was planted in 1967 and consisted in equal proportions of Jonathan, Golden Delicious and Starking varieties. Trees were 4 m apart in rows 8 m apart. The neighbouring vegetation was mainly the natural climax community of the area, the *Quercetum petreae-cerris* oak forest, of 80-year-old with other orchards, cereal fields and meadows nearby. The orchard was tilled in spring and the subsequently growing weeds tilled between rows and mown around trees during the rest of the growing season. Four-six sprays were applied in each growing season till 1976 when the orchard was divided into an insecticide-free (IF) and a treated (TR) block. Only fungicide sprays were applied to the IF block from 1976 onwards while TR received regular treatments with organophosphate insecticides.

This study was carried out between March 1976 and November 1979. Samples were taken weekly between 900–1200 hours under favourable weather conditions from the canopy and the ground cover. 10 IF + 10 TR (5 constant + 5 random) trees were sampled by beating. When weed cover was present, 100 sweeps were taken with a sweep net both in IF and TR blocks.

Results

During the three years, 1106 specimens of twelve species (995 by beating + 111 by sweeping) were sampled (Table 1). The species numbers in the three successive years were 6, 9 and 5 (IF) and 5, 9 and 7 (TR), respectively. The diversity values of the IF block were slightly higher (Table 1). The average diversities were $d_{IF} = 0.69 \pm 0.07$ and $d_{TR} = 0.73 \pm 0.12$; the difference was nonsignificant ($p > 0.1$, t-test). The two communities differed in three species: *Hippodamia tredecimpunctata* L. and *Harmonia quadripunctata* L. were not present in IF while *Synharmonia conglobata* L. was absent from TR block.

The similarity of the IF and TR communities over three years was $C_N = 0.58$ (BRAVAIS–PEARSON coefficient, see SOUTHWOOD, 1978). The actual com-

Table 1

Coccinellid community composition on the canopy of the insecticide-free (IF) and insecticide-treated (TR) blocks in apple orchard at Juliannamajor, 1977–79

Species	1977		1978		1979		Pooled	
	IF	TR	IF	TR	IF	TR	IF	TR
Coccinella septempunctata L.	122	57	248	53	93	103	463	213
Adalia bipunctata L.	45	2	51	24	4	3	100	29
Exochomus quadripustulatus L.	33	—	54	22	11	2	98	24
Propylaea quattuordecim-punctata L.	6	4	5	1	—	1	11	6
Adonia variegata GOEZE	1	—	3	7	6	12	10	19
Calvia quattuordecimguttata L.	—	—	6	1	—	—	6	1
Adalia decempunctata L.	4	1	—	—	1	—	5	1
Coccinula quattuordecim-pustulata L.	—	1	2	1	—	—	2	2
Thea vigintiduopunctata L.	—	—	1	1	—	1	1	2
Synharmonia conglobata L.	—	—	1	—	1	1	1	—
Hippodamia tredecimpunctata L.	—	—	—	1	—	—	—	1
Harmonia quadripunctata L.	—	—	—	—	—	1	—	1
Pooled	211	66	370	110	115	123	696	299
Dominance index	0.58	0.86	0.67	0.48	0.81	0.84	0.67	0.71

munities showed similarities of 0.46, 0.44 and 0.87, respectively. *C. septempunctata* seemed to tolerate the insecticide pressure, as its relative abundance was 71.2% in the treated plot while 66.5% in the control. *A. bipunctata* (9.7 vs. 14.37%) and *Exochomus quadripustulatus* L. were less common in TR block (8.03 vs. 14.08%), except the 1978 summer immigration. *Adonia variegata* L. was more abundant in TR block (6.35 vs. 1.44%). Other species were too rare to evaluate their tolerance towards TR environment.

The most abundant species were *C. septempunctata*, *A. bipunctata* and *E. quadripustulatus*. *A. variegata* was among the most abundant species in 1979. The difference between the coccinellid numbers in IF and TR blocks resulted from a greater spring peak in 1977 (Fig. 1a), and from the greater number of immigrants in 1978 (Fig. 1b). In both years, the woolly apple aphid, *Eriosoma lanigerum* Hausm. was more numerous in the IF block (KOZÁR *et al.*, 1979) as well as other aphids (MESZLENY and SZALAY-MARZSÓ, 1979). The seasonal dynamics of the most abundant species varied widely (Figs 1–3).

At the spring colonization of *C. septempunctata* the highest numbers were observed in 1977. No serious difference was noted between IF and TR blocks in the summer 1977 (Fig. 1a). In 1978 spring, only minimal numbers of coccinellids were found but a large immigration occurred between mid-July and August (Fig.

1b). The IF block supported more aphids which caused an uneven coccinellid distribution. Practically no coccinellids were found before the midsummer of 1979 (Fig. 1c). The late activity peak showed probably beetles on their way to hibernation sites. The two-spotted ladybird, *A. bipunctata* became active early

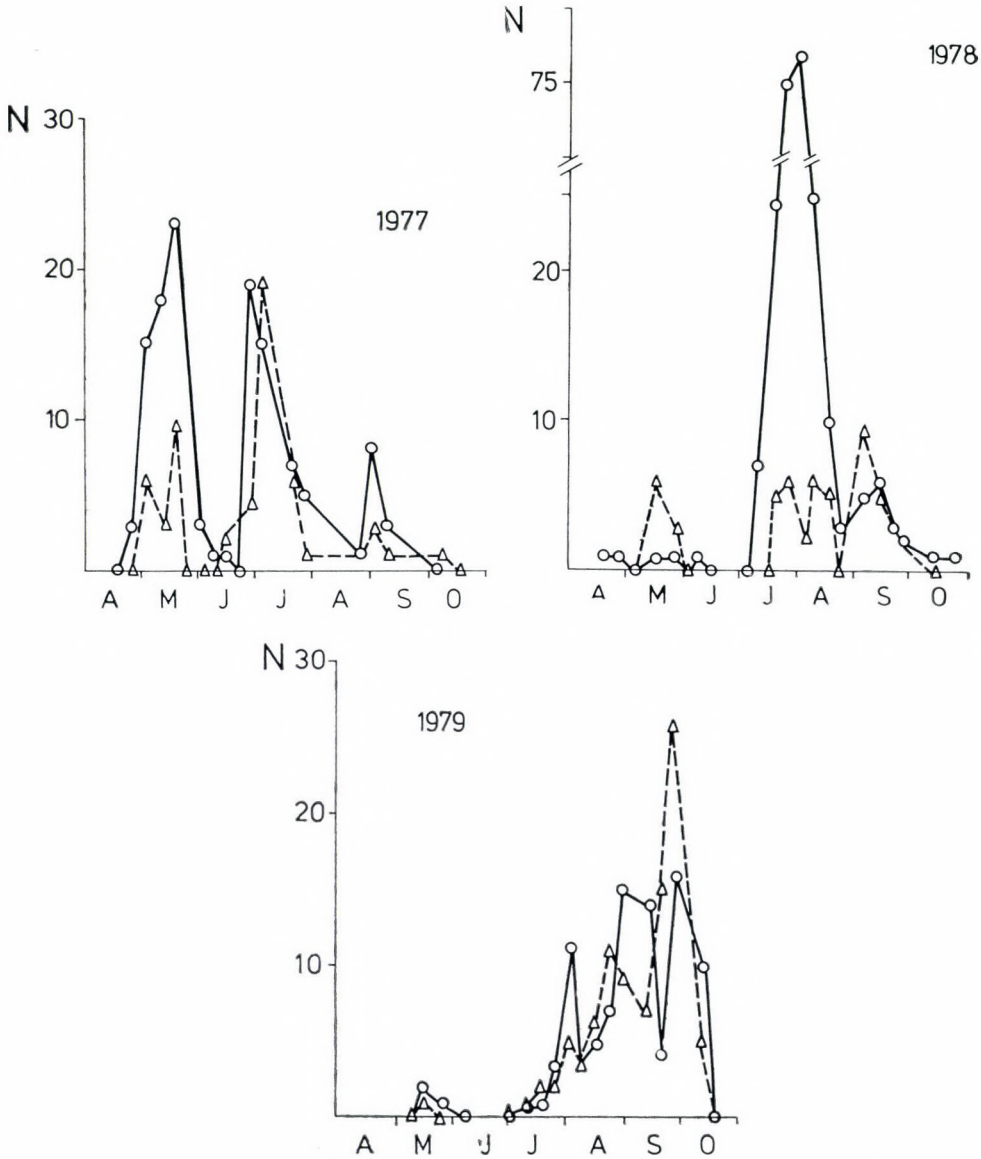

Fig. 1. The seasonal dynamics of *C. septempunctata* at Julianna major, 1977—79 in insecticide-treated (Δ) and control (○) blocks. "N" denotes the numbers sampled from 10 trees

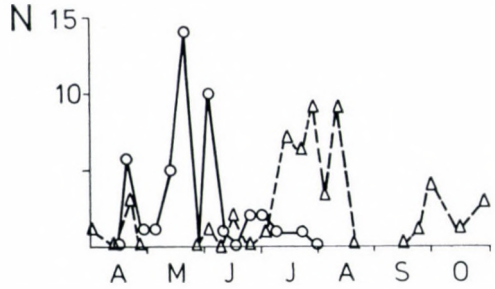

Fig. 2. Seasonal dynamics of *A. bipunctata* in the control block at Julianna major, 1977(○) and 1978 (△)

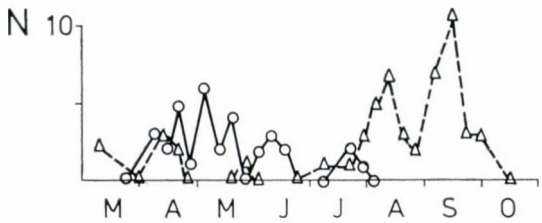

Fig. 3. Seasonal dynamics of *E. quadripustulatus* in the control block at Julianna major, 1977 (○) and 1978 (△). "N" as in Fig. 1

in the spring and colonized the orchard during April (Fig. 2). The species disappeared from the orchard in July and could not been found in the autumn. The more characteristic late activity period (HODEK, 1973) was observed in 1978, when some beetles were found 25 October. In 1979 the species was rare (Table 1). Early colonization was characteristic for *E. quadripustulatus* (Fig. 3): the first beetles were caught 4 April 1977, 8 March 1978, and 11 April 1979. Seasonal dynamics was similar to the other two abundant species. In 1979, only 13 specimens were sampled.

Sweep netting did not reveal additional species. *C. septempunctata*, *A. bipunctata*, *A. decepunctata*, *A. variegata*, *Thea vigintiduopunctata* L., *Propylaea quattuordecimpunctata* L. and *E. quadripustulatus* were found in sweep net samples. The samples did not show the presence of coccinellids on weeds in the summer when aphids emigrated from trees. The activity in sweep net samples coincided with the pattern in canopy samples.

Discussion

A survey of the literature showed that the species richness of this orchard was high: several European apple orchards have 3–12 coccinellid species and the most communities contain less than 10 species (CLAYHILLS and MARKKULA, 1974

HODEK, 1973; HONEK, 1977; NIEMCZYK and OLSZEK, 1975; TALITSKAYA, 1977). However, their average diversity was $d = 0.54 \pm 0.06$ ($n = 8$) which is higher than for our orchard. The following species were most frequently present in 14 European apple orchards: *C. septempunctata* and *A. bipunctata* (present in all the 14 orchards), *P. quattuordecimpunctata* (12), *Calvia quattuordecimguttata* L. (8), *A. decempunctata* (9) and *Coccinella quinquepunctata* L. (7). All but *C. quinquepunctata* were present in the orchard studied. *E. quadripustulatus* was fairly abundant in our orchard (see Table 1) but reported only from three other orchards. NOVAK (unpublished, cit. HODEK, 1973) found that *C. quattuordecimguttata*, *A. bipunctata*, *A. decempunctata* and *C. quinquepunctata* were characteristic for an apple orchard with a forest nearby, in northern Bohemia, Czechoslovakia. *C. quinquepunctata*, which prefers herbaceous vegetation, was present because it hibernates in the litter at forest edges. We did not found the species during the three years of investigation. The presence of *H. quadripunctata* in our samples reflects the proximity of the forest because it occurs mainly in deciduous and pine forests where it overwinters in bark crevices (HODEK, 1973). *A. variegata* and *H. tredecimpunctata* are characteristic to cereal fields (HODEK, 1973) so they may be invaders from the neighbouring fields. *A. variegata* was more abundant in TR than IF plot (6.35 vs. 1.44%, respectively). This was probably because the nearest cereal field was bordering to that plot.

The community was composed of mainly aphidophagous species, distributed on twigs and leaves so beating was a suitable sampling method to detect their relative abundance. The only non-predaceous species, the fungivorous *T. vigintiduopunctata* eats fungi on leaves so its relative density was probably correctly represented. *E. quadripustulatus* might be underrepresented because its feeds also on coccids (HODEK, 1973; TALITSKY and TALITSKAYA, 1977) which are more abundant on tree trunks. Beating underestimated the coccinellid density in the orchard. Counts during the 1978 summer immigration showed that beating sampled about 20–30% of the beetles present in a tree. However, beating was suitable to detect relative densities. Surprisingly, sweep netting did not reveal the presence of coccinellids on the orchard weeds in summer, although such situation was reported by TAMAKI (1974) for peach orchard, and the most abundant species, *C. septempunctata* preferred herbaceous vegetation (IPERTI, 1978).

In controlling pest numbers, the proximity of the forest did not mean a massive source of beetles for colonization in all species. The most abundant species, *C. septempunctata* feeds but does not oviposit near its ovewintering sites (HODEK, 1973). The height of the first peak of coccinellids depends on the overwintering success. The egg-laying of overwintered females and the larval survival depends highly on the climate and food so large number of aphids may support vast numbers of coccinellids (HAGEN, 1962). These beetles may migrate great distances in search for food. Consequently, in some years migrant beetles may destroy or postpone the second aphid peak at the end of the summer. This population fluctuation is restricted to aphidophagous species. The abundance pattern of the most common species was in connection with the abundance of aphids.

The most abundant aphid species in the orchard were the woolly apple aphid, *E. lanigerum*, the green apple aphid, *Aphis pomi* DeGeer and the rosy apple aphid, *Dysaphis plantaginea* Pass. (MESZLENY and SZALAY-MARZSÓ, 1979). Coccinellids preyed upon apple aphids in the spring, but the predation pressure on woolly aphid increased when the other aphids migrated from the trees to their summer hosts and the newly hatched coccinellid adults appeared. However, the coccinellid-aphid relationship cannot be evaluated because the larval stages were not sampled. Beating was not suitable to detect larval abundances. Other aphidophagous predators in the orchard (KOZÁR *et al.*, 1979) also interfered with the coccinellid-aphid system. However, the coccinellids had the most conspicious impact on their prey. In 1978, although many chrysopid and syrphid predators developed, the summer immigration of coccinellid adults had the largest impact on the woolly aphid population (see Tables in KOZÁR *et al.*, 1979).

The coccinellid beetles were not able to keep aphids at low numbers in the orchard studied but formed the most abundant group of predaceous adult insects, and we should not underestimate their role in reducing aphid population growth

Acknowledgements

I thank Dr. T. JERMY and F. SZENTKIRÁLYI for commenting an earlier draft of the manuscript. Thanks are due to Ms. V. BOGDÁNY, Á. TÓTH and Mr. Z. PAPP for assistance with the sampling procedures.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 151–156 (1981)

Recent Advances in the Study of *Coccoidea* with Special Reference to Integrated Pest Management

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Conventional insect control methods are being replaced by integrated pest management (IPM) programs which are based on detailed biological, ecological and systematic information. Some of the new discoveries and techniques applied in IPM programs against scale insects and other related topics are reviewed here.

Host plants of scale insects often show varying degrees of susceptibility to a particular species. For example, the Juniper scale, *Carulaspis juniperi* (Bouché), is usually present in urban environments on *Thuja occidentalis* L. The globose variety of arborvitae *(T. o.* cultivar *globosa)* is usually heavily infested, while the upright growing variety *(T. o.* cultivar *fastigiata)* even when closely surrounded by heavily infested globose shrubs, is free of this insect (Fig. 1). FLANDERS (1970) explained this phenomenon as due to genetic immunity in some plants.

Studies on the physiology of *tree resistance* to insects were reviewed by HANOVER (1975). Apparently, there is a lack of studies on scale insect resistance in cultivated trees. Resistance studies require a sound knowledge of the biology and behavior, especially the feeding habits of the insects involved. Knowledge of feeding habits may contribute to an understanding of the mechanism of resistance (PAINTER, 1968). SMITH (1944) studied the resistance of certain plants or avoidance of others by *Saissetia nigra* (Nietner). Among the several *Pittosporum* species observed in California, *P. tobira* (Thunb.) Ait., *Arctostaphylos manzanita* Parry, and *Eucalyptus ficifolia* F. J. Muell appeared to be immune to infestation. He found *Hibiscus* spp. to be preferred hosts of *S. nigra*, except in California. The latter case tends to indicate the existence of host-specific forms or strains that have not yet become established in California.

KOSZTARAB (1953) found only two of 48 known host plant species of *Quadraspidiotus perniciosus* (Comstock) from Hungary to be accidental carriers of this scale. On these two hosts, *Mahonia aquifolium* (Lindl.) Don, and *Robinia pseudoacacia* L., we might occasionally find scales in a heavily infested environment, but they die during their first or second nymphal stage; never reaching full maturity.

KOZÁR (1972) observed a high degree of resistance to *Q. perniciosus* in 10 varieties of peaches in Hungary, three of which were without any infestation. Such studies on the resistance of cultivars of fruit trees to scale insects should be continued. We lack information on the life cycle of many coccoid species and studies of *host-induced variation.*

Fig. 1. A clump of *Thuja occidentalis* L. Left side of *T. o.* cultivar *globosa*, heavily infested with Juniper scale, *Carulaspis juniperi* (Bouché), while right side of *T. o.* cultivar *fastigiata*, uninfested

McCLURE (1979) found numerical self-regulation in the populations of *Fiorinia externa* Ferris. He noted that "self-restraints" on the populations imposed at high densities are related to changes in the quality and availability of food for the nymphs.

The effect on scale insects of fertilizing their host plants was tested. Nitrogen increased, while potassium reduced, populations of mealybugs and soft scales. McCLURE (1980) proved that the amount of foliar nitrogen can significantly affect the rate of infestation in hemlock scales, *F. externa* Ferris.

When scale insect resistant cultivars are lacking, specialists have to select an ecologically and economically suitable control method. Unfortunately, the *biological control* of San José scale, *Q. perniciosus*, is recently being hindered by the extremely low and variable reproductive potential of *Prospaltella perniciosi* Tower. MILNE and SNOWBALL (1977) reported about 1.5 progeny per female, with a maximum of 7 in their insectary cultures on butternut pumpkins.

Coccoid "*ecotypes*" are often found to be distinct sibling species, and as such have their own species-specific parasites. Recognition of this phenomenon could make the difference between the success or failure of an IPM program.

A new approach, to *directly control males* of San José scale, was suggested by KOZÁR (1971), JENSER and SHETA (1972), and DOWNING and LOGAN (1977). The latter authors used "Penncap E", a 23% microencapsulated formulation of ethyl parathion, and a single application was sufficient to control this pest. Conventional control would have required 2 or 3 additional sprays, when applied against crawlers. Scale insect *male trapping* with the use of sex pheromones could provide needed information for specialists to predict population size and time of appearance of various pest species.

Use of *systemics*, rather than contact insecticides, should be favored in IPM programs. TIPPINS and DUPREE (1975) tested the effect of three granular systemic insecticides on potted azaleas infested with azalea bark scale, *Eriococcus azaleae* Comstock. These were: aldicarb (Temik®) 0.7 g, disulfoton (Di-Syston®) 0.8 g, and carbofuran (Furadan®) 2.0 g for 25 cm pots. All three insecticides provided good controls, but the last gave 100% control.

It was shown by HART and INGLE (1971) that treatments of *sublethal doses* of methyl parathion or dimethoate 2E pesticides increased fecundity and shortened the developmental period in the brown soft scale, *Coccus hesperidum* L. A similar response was reported by MCCLURE (1977) in connection with the hemlock scale, *Fiorinia externa* Ferris, when trees were only partially treated.

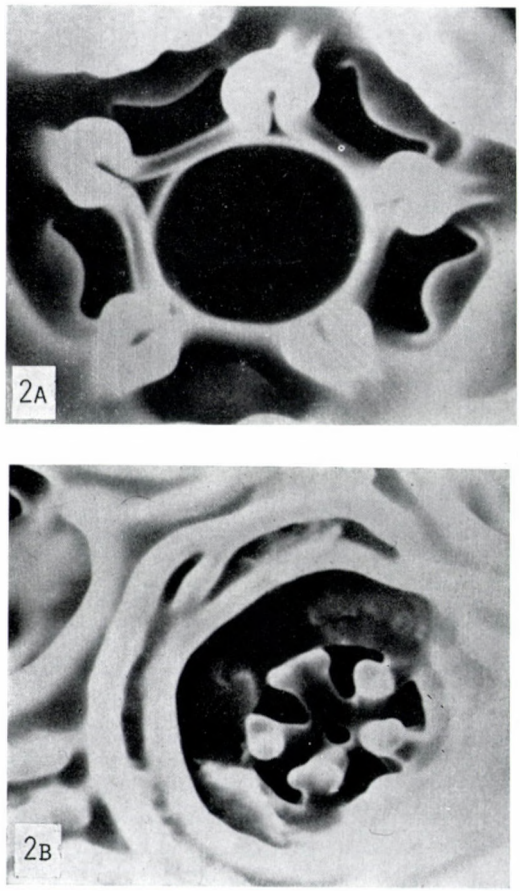

Fig. 2. Quinquelocular disc pores from perivulvar pore groups of armored scale insects: A) *Hemiberlesia lataniae* (Signoret) 24 000× and B) *Lopholeucaspis japonica* (Cockerell) 12 000× ; note obvious structural differences revealed with scanning electron microscope

Kairomones, the chemical substances associated with scales, when applied to infested trees could enhance oviposition by their parasites, thus assist in the manipulation of natural enemies (JONES *et al.*, 1976). *Insect growth regulators* have considerable potential as control agents (STALL, 1975). They may affect mortality, molting, fecundity, as well as production of sex pheromones. Unfortunately, Kinoprene, that was marketed commercially, is too expensive, although effective against Coccidae and Pseudococcidae.

Monitoring populations of both scale insects and their parasites with suction traps (KOZÁR, 1976) and use of predictive models could enhance the effectiveness of IPM programs. Utilization of insecticides, pheromones, insect growth regulators, kairomones, natural enemies, and regulation of nutritional needs of the host plants, are all parts of future IPM systems for the control of scale insects (MILLER and KOSZTARAB, 1979).

IPM programs need to rely on fast and accurate *identification* of insects present in the ecosystem of agricultural crops. Therefore a short summary of the progress made and of the future needs in the systematics of scale insects will be presented.

When closely related or sibling species are impossible to determine with conventional methods, the *scanning electron microscope* can reveal fine structural differences (MILLER *et al.*, 1975) in scale insects as for example, a difference in the quinquelocular pores of two armored scale insects (Figs 2a and 2b).

Systematists need to become more "*commodity oriented*". They should take up such tasks as preparing keys for the identification of insects associated with a certain type of ecosystem or crop habitat (KOSZTARAB, 1975). The museum collections should be expanded to include more material with direct practical application to IPM problems, for example a herbarium of insect and mite damage (KOSZTARAB, 1966).

Coccoid classification has been based almost exclusively on the morphology of adult females, and as such is very inadequate. New species descriptions, generic or family revisions should include all stages of a scale insect, also their tests and other wax products, and the malformations produced; they should also include information on their biology, habitats, host preferences, and associated organisms, such as ants, and on their parasites and predators. A number of such characters were used recently in the analyses of coccoid phylogeny by DANZIG (1980).

Biosystematic studies are needed for many of the common pest genera, such as *Lecanium*, *Lepidosaphes*, *Pseudococcus* and others (KOSZTARAB, 1977). We will also need to clarify types of a large number of coccoid genera.

There is a need for up-to-date keys, including *pictorial keys* for the determination of scale insect species of economic importance. There is an urgent need for species catalogs, since the last comprehensive catalog was published in 1903 (FERNALD, 1903). Only three of 21 coccoid families have up-to-date species catalogs.

These were some of the recent advances made in the study of scale insects and the future needs from the point-of-view of IPM. We are looking forward

to even more rapid progress in the future, and hope that scientists will continue with their traditional international cooperation to insure the free flow of scientific information for the general benefit of all mankind.

Acknowledgements

Appreciation is extended to my colleague, Dr. L. T. KOK, for reviewing this manuscript before publication, and to Dr. D. R. MILLER (USDA-ARS, Beltsville, MD) and Dr. J. A. DAVIDSON (Univ. of MD) for Figures 2a & b.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 157—169 (1981)

Communities of *Chrysopidae* and *Hemerobiidae (Neuroptera)* in Some Apple-Orchards

(Studies in Apple Ecosystems No. 20.)

By

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In 1976—79 communities of *Chrysopidae* and *Hemerobiidae* were investigated in different cultivation systems of apple stands. In the apple-orchards 14 chrysopid and 11 hemerobiid species have been detected among which 7 and 4 species, resp., could be found regularly. Among the quantitative collecting methods, in case of eggs, the observation of 100 leaves per tree, for the larvae, beating proved to be the most suitable means. To follow the flight of adults light-trap, suction-trap, and yellow pan trap, and in the case of hemerobiids the pitfall-trapping also worked well. The most frequent species in the apple-orchards were the following: *Chrysopa carnea*, *C. phyllochroma*, *C. formosa*, *Hemerobius humulinus*, *Wesmaelius subnebulosus*, *Eumicromus angulatus*. In the investigated apple-orchard significant egg parasitization was found. The paper deals with the diversity and similarity of the *Chrysopidae* for years and localities. Further, it describes the phenological characters of the more frequent species as well.

This work has been conducted within the framework of the present population-ecological research project of the Zoology Department of the Research Institute for Plant Protection. On the one hand, it was our aim to specify the chrysopid and hemerobiid communities of apple-orchards under different management practices. On the other hand, however, methods suitable for successful collection of various developmental stages had to be also worked out. In addition, such questions as the phenology and parasitization of the most abundant species, the diversity and similarity of communities, as for different orchard types and years, have also prompted investigations. By looking over the relevant European literature, one can find only few data on chrysopid and hemerobiid fauna of apple-orchards. As far as the authors are aware of the literature, the present paper provides so far the most complete list of chrysopid and hemerobiid species found in the European apple-orchards, based on several and simultaneously applied collecting methods. Only PRINCIPI and CANARD (1976) do mention 8 green and 5 brown lacewing species inhabiting apple plantations, however, without specifying the circumstances of field examinations. In most studies neuropterous species of apple-orchards are differentiated only at family-levels, and emphasis is put almost exclusively on *Chrysopa carnea* (STEINER *et al.*, 1970; NIEMCZIK *et al.*, 1972; MALEVEZ, 1976; BAESCHLIN and TAKSDAL, 1979). The above statement is still valid for works of

MATHYS and BAGGIOLINI (1965) or INJAC *et al.* (1978), albeit there authors applied several collecting methods and carried out more substantially detailed analyses. In our opinion, the inadequacies are due to insufficient systematic evaluations of the collected materials. Both on the basis of our investigations, and that of literature data, one can state with confidence the existence of a rich multi-species community of chrysopids and hemerobiids in European apple-orchards.

The present paper only outlines the results of collections conducted for several years. The evaluation of each collecting method and from the point of view of important species, together with relevant literature data, will be the subject of a series of papers in the future.

Materials and Methods

The investigations were carried out between 1976 and 1979 and they were conducted in the following orchard-types: 5.8 ha experimental orchard at Julianna-major (J) near Budapest, 0.5 ha backyard orchard at Füzesbokor (F), 5.0 ha commercial orchard at Újfehértó (U), 100 ha extra-size commercial orchard at Ilonatanya (I), and finally 0.2 ha abandoned orchard at Sóskút. The last four orchards are to be found on the largest apple-growing area of Hungary, to the north-eastern part of the country, in county Szabolcs-Szatmár. With the exception of a portion of the experimental orchard, all of them received regular sprayings 10 to 15 times a year.

The collecting methods were as follows:

Light trap: The type was the one constructed by Jermy. It was situated at a height of 2 meter (ca. canopy level) and operated with a 100 W incandescent bulb. The collected material was removed daily. Each orchard-type (J, F, U, I) had a light trap operated from 1 April (or in some cases from 1 May) till 31 October.

Suction trap: The same type as described in the paper of MESZLENY and SZALAY-MARZSÓ (1979) was used. The mouth of the suction tunnel was at the level of tree canopy. Suction traps were operated at two localities (J, U), and checked twice weekly to remove collected material. Suction trapping was carried out between 1 May and 31 October.

Yellow pan trap: The yellow pans (24 cm in diameter) were also placed at the level of tree canopy. Their numbers varied according to orchard-type (J, U). They were emptied twice per week, between 1 May and 31 October.

Pitfall trap: Plastic containers of 200 cm^3 and supplied with ethylene glycol were used. Pitfall trapping was conducted at 5 localities (J, F, U, I, S), the number of traps varied. Emptying took place once per week between 1 April and 31 October.

Sweep netting: There were 4×100 strokes made in herb level, once per week, between 1 April and 31 October.

Beating: An open plastic funnel of 1 m diameter collected the material that fell down during beating from 4 sides of a tree. Beatings were done in early morn-

ing hours, and from 1 March till 31 October, once a week. Beatings were carried out at all 5 localities. Number of trees sampled varied according to the size of orchard.

Visual checking of trees: Chrysopid eggs were looked for by using visual control in the experimental orchard (J), once per week from 1 April till 15 October, and only in 1979. On each occasion, there were 10 trees selected, then 100 leaves per tree randomly picked. Larvae and parasites were reared out from the material collected.

Evaluation: There is no detailed analysis carried out as for years and localities, and data were rather selectively used. For instance, in case of phenological events light trap data were used mainly, as they were thought to be the most reliable ones. In case of egg collection and subsequent rearing out of larvae, no details are presented according to species, because of the paucity of material. For a similar reason, no diversity and similarity values were calculated for hemerobiids.

For the computation of diversity of chrysopid community, because of the relatively low number of specimens and species captured, the information-theory function of Shannon – Wiener was used:

$$H = \sum_i p_{ij} \log p_{ij}$$

where p_{ij} is the probability of presence of species i in sample j.

In order to calculate percentage similarity values, the formula of WHITTAKER and FAIRBANKS was used,

$$PS = 1 - 0.5 \sum_i |p_{ij} - p_{ik}|$$

where p_{ij} and p_{ik} are the probability of presence of species i in the samples j and k, respectively.

Results and Discussion

Table 1/a and b present the lists of species found in all types of orchards, during four years and on the basis of all methods applied. Of both families, the most species occured in the experimental orchard. One of the main reasons of this finding should lay in that the orchard is surrounded by a forest. The number of species in the backyard and in the two commercial orchards is nearly the same. The low number of species found in the abandoned orchard is probably erroneus due to the defectively performed collections. Consequently, this place was excluded from further evaluations.

In the table regularly occurring or constant species are separated from rare ones by a dotted line, for each orchard-type and year. There are seven such species among the chrysopids and four among the hemerobiids collected.

Table 1/a

List of species of family Chrysopidae found in different apple orchard-types, during four years and on the basis of all methods applied

Species	Locality				
	J	F	U	I	S
Chrysopa carnea Steph.	×	×	×	×	×
C. formosa Brau.	×	×	×	×	×
C. phyllochroma Wesm.	×	×	×	×	×
C. perla (L.)	×	×	×	×	×
C. 7-punctata Wesm.	×	×	×	×	
C. abbreviata Curt.	×	×	×	×	×
C. ventralis Curt.	×	×	×	×	
C. ciliata (Wesm.)	×	×		×	
C. abdominalis Brau.	×				
C. walkeri McL.	×				
C. dasyptera McL.	×				
C. dorsalis Burm.	×				
Nineta flava (Scop.)	×			×	
N. vittata (Wesm.)	×				
Number of species	14	8	7	9	5

J: experimental orchard, F: backyard orchard, U, I: commercial orchard, S: abandoned orchard.

Table 1/b

List of species of family Hemerobiidae found in different apple orchard-types, during four years and on the basis of all methods applied

Species	Locality				
	J	F	U	I	S
Hemerobius humulinus L.	×	×	×	×	×
Wesmaelius subnebulosus (Steph.)	×	×	×	×	
Eumicromus angulatus (Steph.)	×	×	×	×	
Micromus variegatus (Fabr.)	×	×	×	×	
Wasmaelius betulinus (Strom.)	×	×			
Hemerobius nitidulus Fabr.	×		×		
Hemerobius micans Oliv.		×	×	×	
Hemerobius atrifrons McL.	×				
Hemerobius handschini Tjed.	×				
Drepanepteryx phalaenoides (L.)	×				
Sympherobius elegans (Steph.)	×				
Number of species	10	6	6	5	1

For abbreviations see Table 1/a.

Table 2

Diversity values of chrysopid community as for years and localities, calculated from light trap data

Locality	1977	1978	1979	Average
J	0.415	0.531	0.649	0.532
F	0.463	0.241	0.214	0.306
U	0.586	0.460	0.533	0.526

J: experimental orchard, F: backyard orchard, U: commercial orchard

Comparison of collecting methods:

Figure 1 shows a comparative evaluation of collecting methods. Columns indicate the percentage proportion of specimens caught per year and per each collecting method. Figures above the columns give the number of species collected by that method. In cases of beating, sweep-netting and pitfall trapping the number of larvae was also taken into consideration.

Among chrysopids, the most species and in greatest abundance were collected by the light trap. Almost the same efficiency was gained by yellow pans and suction traps, though yellow pan traps collected a higher proportion of *C. carnea*. Beating, sweep-netting and pitfall trapping should serve as additional data-collecting at most.

Most species of brown lacewings were captured by light traps, followed by pitfall traps and the suction traps. Regarding sample size, most methods – except sweep-netting and beating – provided similar results. The relative inefficiency of sweep-netting and beating must be due to the low sampling frequency per unit time, as compared with the most of methods that collected the material continuously.

For collecting larvae the methods of beating, sweep-netting and pitfall-trapping as well are equally important, because they sample populations at various vegetation levels, in spite of the fact that beating itself produced the overwhelming majority of the collected material (that is, 9 species in 67 percentages).

In order to follow oviposition as it proceeded in time, parasitization of eggs and for the quantitative assessment of species living at the canopy level, the two most effective methods, namely collecting of eggs and the subsequent rearing of hatching larvae and parasites, were used in 1979. By applying such procedures we succeeded in rearing out some 70 percentages of the chrysopid species.

Phenology:

A there were relatively few larvae collected, phenological data in connection with only adults and eggs would be shown. For the analysis of flight of the most frequent and most abundant species only light trap data were used. There were three species, *C. carnea*, *C. formosa* and *C. phyllochroma*, captured in the highest numbers.

C. carnea (Fig. 2)

One could tell apart two flight periods. The peak of first flight is set in June or July, while the second one succeeds in the course of September or October.

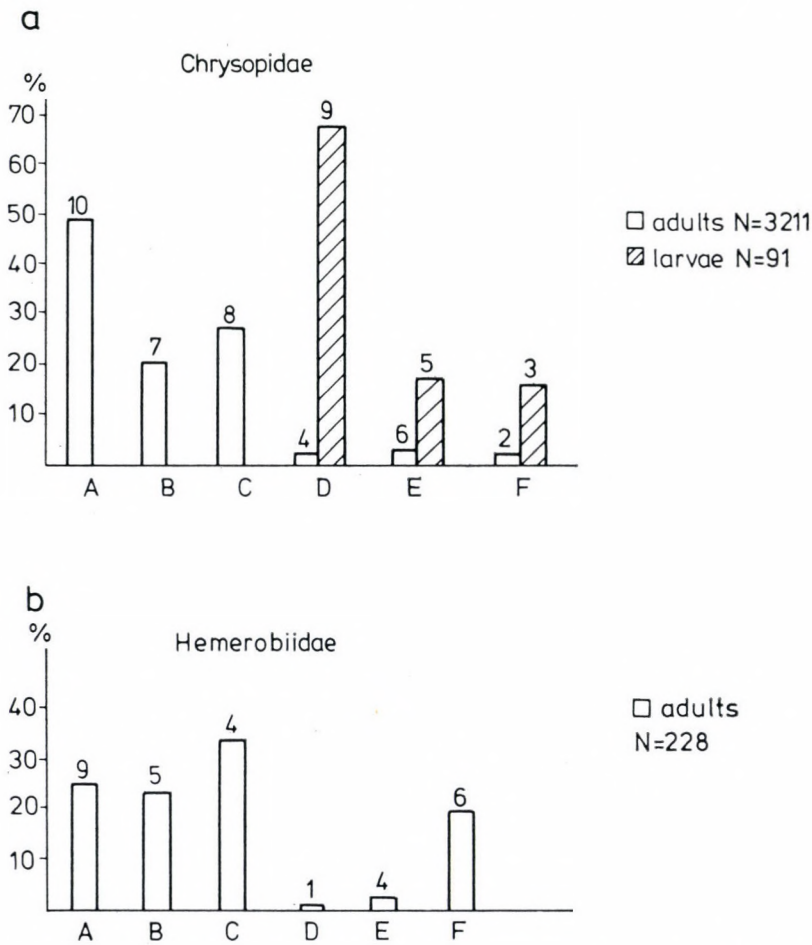

Fig. 1/a, b. Percentages of chrysopid and hemerobiid species according to collecting methods applied. (A: light traps, B: suction traps, C: yellow pan traps, D: beating, E: sweep-netting, F: pitfall traps)

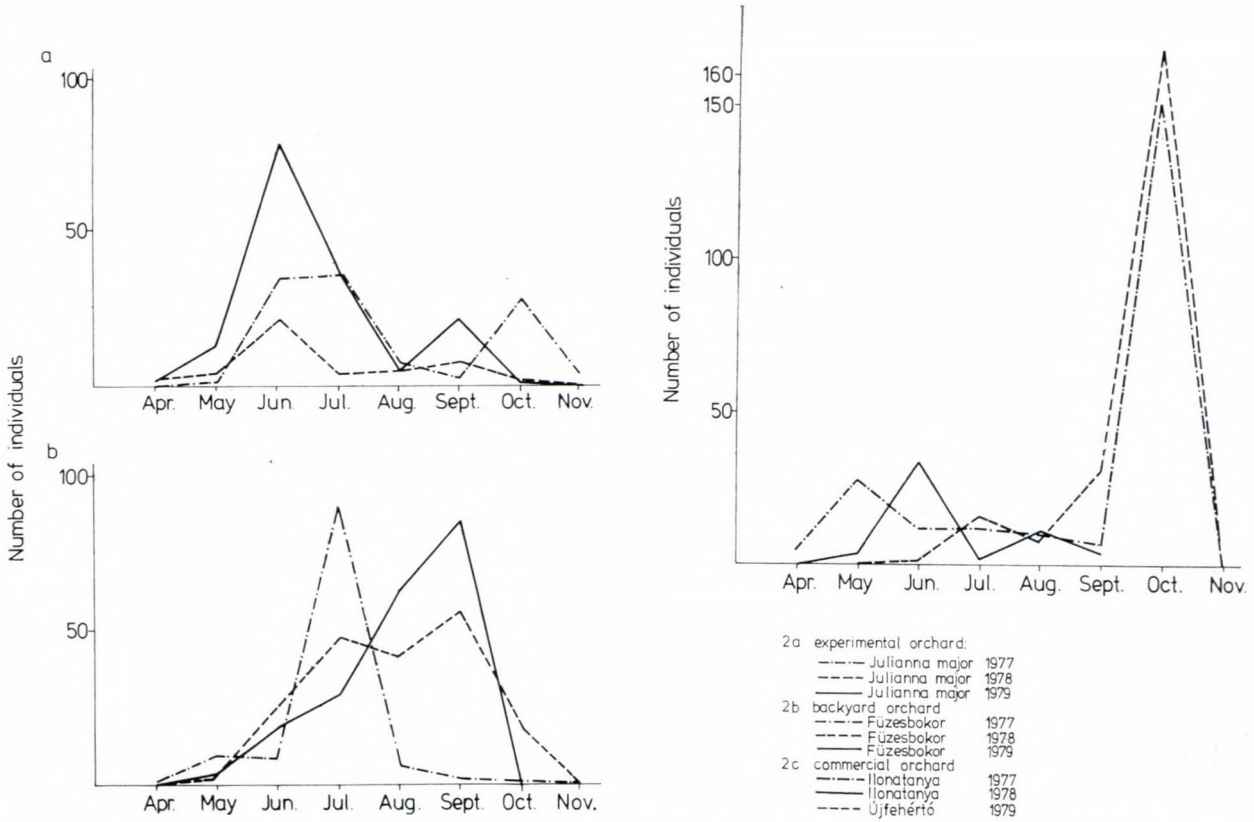

Fig. 2/a, b, c. Flight curves of *C. carnea* recorded in three types of orchards and in different years by light trapping

C. formosa

Figure 3 provides informations about flights of *C. formosa*. The flights vary according to localities and years. There are two flight peaks. The first is in the June and the second in August.

C. phyllochroma

The flight period of the third most frequent species, *C. phyllochroma* (Fig. 4) was from the beginning of June till September. The peak of flight was in all three types of orchards in August.

There were three hemerobiid species occurring in higher numbers in the material of light traps. They were: *H. humulinus*, *W. subnebulosus* and *E. angulatus*.

Figure 5/a demonstrates the flights of *H. humulinus*. It was possible to distinguish two peaks in the experimental orchard. The first period was observed in May and June, while the second one continued from the end of August till October.

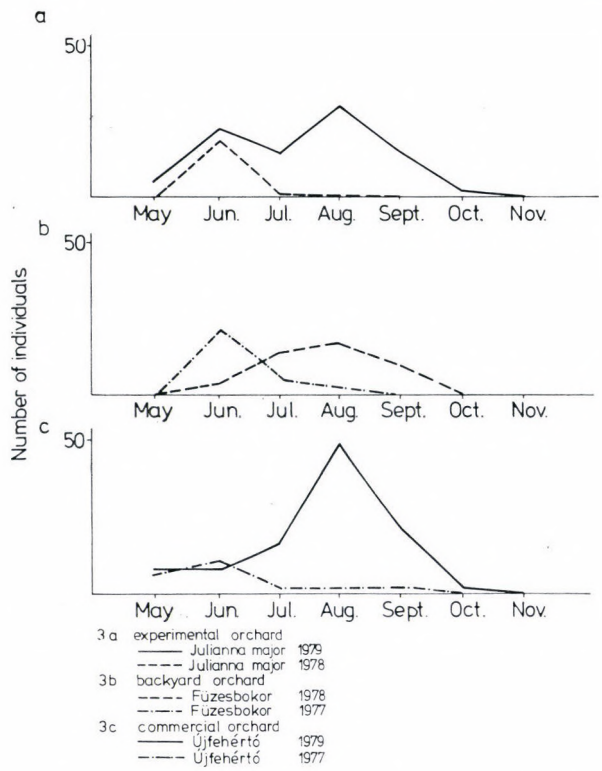

Fig. 3/a, b, c. Flight curves of *C. formosa* in three types of apple orchards based on light-trapping

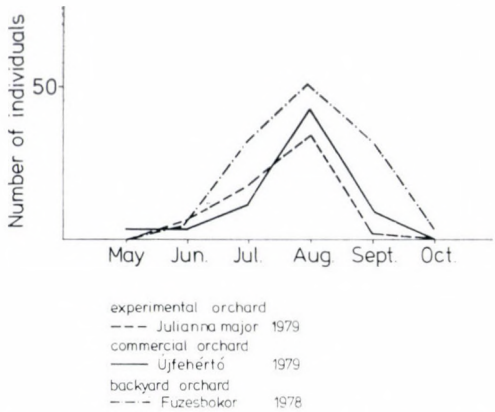

Fig. 4. Flight curves of *C. phyllochroma* in three types of apple orchards based on light-trapping

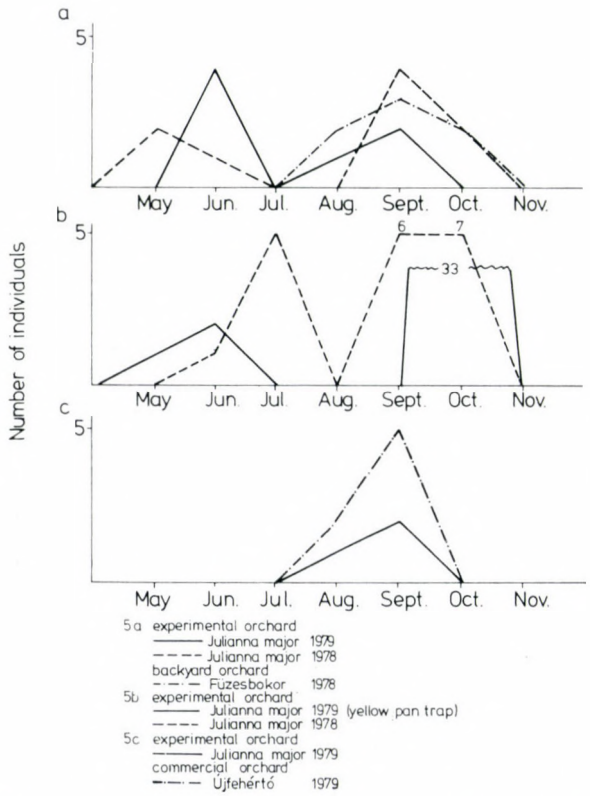

Fig. 5/a, b, c. Flight curve data of *H. humulinus* (a), *W. subnebulosus* (b) and *E. angulatus* (c), obtained by light-trapping

In backyard and commercial apple orchards only the second flight period could be shown out.

In case of *W. subnebulosus* (Fig. 5/b), on the basis of light trap data, the first flight fell in June and July, while taking into consideration catches of yellow pan traps, the first swarming period appeared in May and June. The second flight proceeded during September and October at all localities.

The third hemerobiid species, *E. angulatus* (Fig. 5/c), produced only one long flight period, from the end of July till late September.

It is regretful that brown lacewings can only be collected sporadically and in low numbers even by using light traps. Therefore in future investigations, other means of collecting should also be used, in order to produce a more exact phenological picture of certain species.

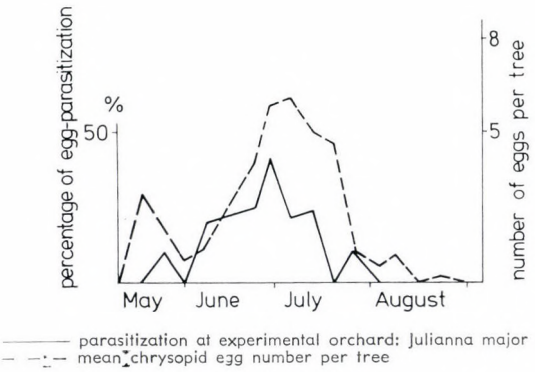

Fig. 6. Percentage parasitization of eggs of chrysopid species

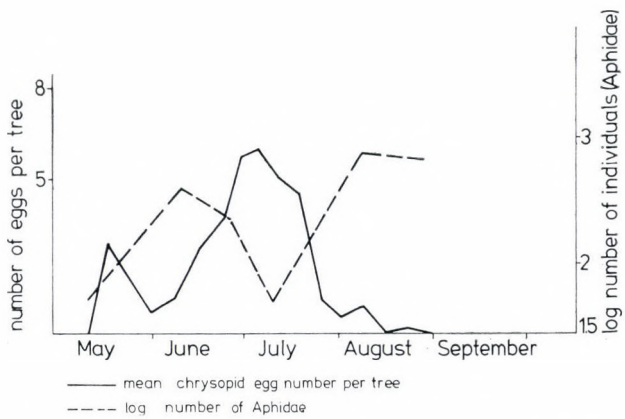

Fig. 7. A comparison of mean number of the chrysopid eggs and aphid species present in the experimental orchard (J). (See text for further explanation.)

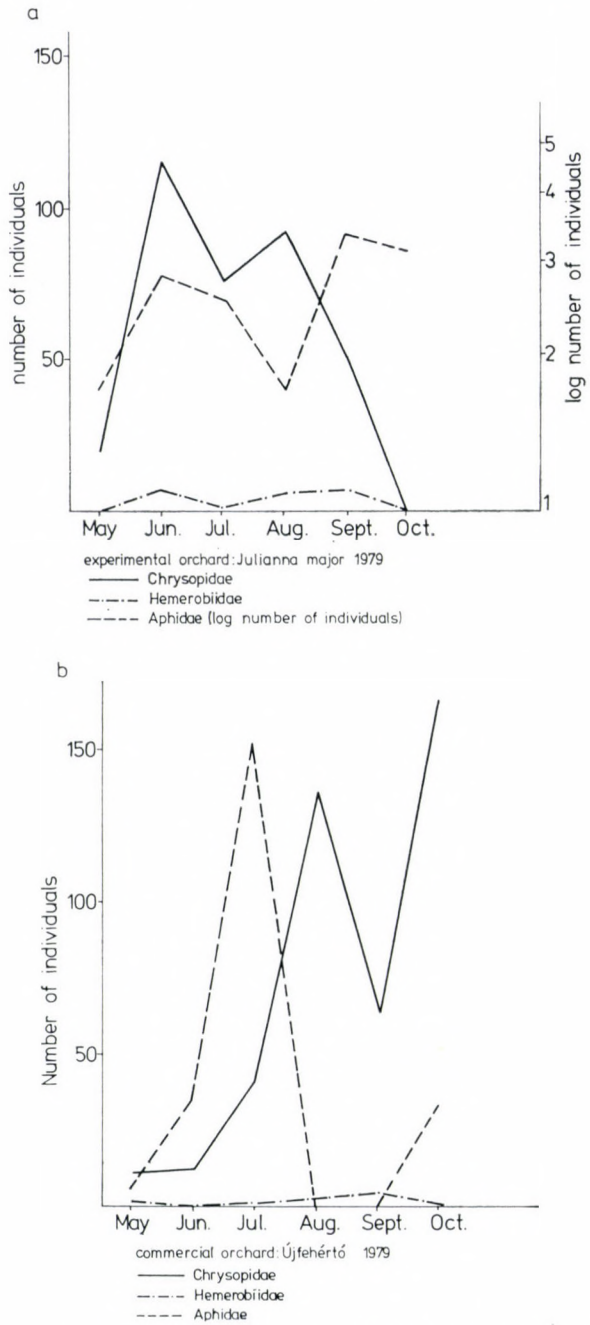

Fig. 8/a, b. Summarized light trap data of all chrysopid, hemerobiid and suction trap data of aphid species present in two orchards. (See text for further explanation.)

The data obtained by the evaluations of egg-collections from the experimental orchard, are presented in Fig. 7.

The main egg-laying period occurred in June and July. It coincided with the mass propagation of aphids. The time course of egg-parasitization is shown in Fig. 6. A mean value of 19 percentages of parasitization was found. There were two species of eggs-parasites identified: *Asolcus phalaenarum* and *Telonomus chrysopae*.

Finally, the summarized flight data of all chrysopid and hemerobiid species were compared with those of the aphid species (Fig. 8). The latters were obtained from suction trappings conducted at the same locality, and were provided kindly by ANDRÁS MESZLENY. It is clearly shown by the graph that the main flight periods of the two neuropterous groups follow the population size changes of aphids, therefore it is probable that both larvae and adults efficiently took their shares in praying upon them (Meszleny and Szalay-Marzsó, 1979).

Diversity and similarity:

On the basis of light traps data, the species diversity and similarity values of chrysopid communities as for localities and years were also computed.

The results are summarized in Table 2 and Table 3. On the one hand, it can be deduced from the table that a uniformly low diversity value is characteristic of all three localities and of each year, and on the other hand, that the average diversity values of the experimental and commercial orchards, respectively, are almost the same.

Table 3 present per cent similarity values. There were considerable similarities of localities in subsequent years, their mean values ranged between 69 and 79

Table 3

Percentage similarity values of chrysopid community as for years and localities, calculated from light trap data

Locality	1977—1978	1978—1979	1977—1979	Average
J	84.5	81.9	71.5	79.3
F	80.5	62.5	88.0	77.0
U	63.5	69.0	73.5	68.7
Locality	**1977**	**1978**	**1979**	**Average**
J—U	73.0	73.0	67.3	71.1
J—F	86.0	69.5	56.0	70.5
F—U	84.5	66.0	64.5	71.7

J: experimental orchard, F: backyard orchard, U: commercial orchard

percentages. The similarity indices of apple orchards with different management practices provided also a uniform picture and the values themselves were very much the same.

Conclusion

For sampling chrysopid populations for faunistical purposes, light traps and suction traps proved to be suitable. In addition pitfall trapping can be successfuly used to collect hemerobiids. For collecting larvae living at various vegetation levels beating, sweep-netting and pitfall trapping are important methods. For species of *Chrysopidae* inhabiting apple trees hand collecting of eggs is one of the most important quantitative method for assessment.

From the point of view of phenology of species, light traps proved to be the most suitable collecting devices, especially if operated through longer period of time.

The swarming and egg-laying periods of the most frequent and abundant chrysopid species fitted satisfactorily to the propagation curve of aphids.

The phenological characters of hemerobiid species can be cleared up only after some years of regular population samplings and collections. An increase in population size of hemerobiid species in late summer or in autumn considerably coincides with a similar growth of aphid populations in the same time period.

Parasitization of eggs is a significant mortality factor for chrysopid species.

In summing up, it can be stated that, in apple orchards under various managament practices, chrysopid and hemerobiid communities exist with constant species members. The communities consist of only few species and can be characterized by low diversity, therefore, by high similarity values.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 171—180 (1981)

Some Factors Affecting Seed Yield Loss of Lucerne Caused by Insect Pests

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The damage caused by the lucerne seed chalcid *(Bruchophagus roddi)* increases along with the erect growth of lucerne, while the ratio of shrivelled seeds evoked by sucking of mirids *(Adelphocoris lineolatus* and *Lygus rugulipennis)* and by nutritional deficiencies has been found to be enhanced along with the prostrate growth. The degrees of the seed chalcid and the seed weevil *(Tychius flavus)* damages show a slight shift from smaller plots of lucerne towards larger ones, whereas the percentages of shrivelled seeds show the opposite tendency. Relationships have been found between the amount and distribution of the August precipitation in the previous year, on the one hand, and seed damages, on the other hand. The ratio of shrivelled seeds increases parallel to the amount and the frequency of precipitation. The peak of *Tychius* damages coincides with 40 to 80 mm precipitation values and 60 to 80 drought indices resp., i.e. *Tychius* damages are favoured by a balanced pattern in the distribution of August precipitation in Hungary. Damages caused by *Bruchophagus* markedly increase with the highest drought indices of the former August. Relationships could also be established between the damages and the precipitations from October to May: increasing amount of precipitation is connected with an increasing ratio of shrivelled seeds and a decreasing damage caused by *Tychius*. *Bruchophagus* seems to be hardly affected by the precipitations from autumn to spring.

Previous estimations have pointed out that the harvested lucerne seed amounts to only one tenth of the potential yield under Hungarian conditions (Manninger, 1978). Mainly the problems of pollination, pests, and certain nutritional deficiencies are responsible for the losses (cf. Kemenesy, 1966; Bula and Massengale, 1972; Bócsa, 1979; Manninger, 1979; Pekáry, 1979). Insect pests cause considerable damages showing a great variety in their appearance. In this paper we focus on seeds attacked by the seed chalcid *(Bruchophagus roddi* Guss.), and the seed weevil *(Tychius flavus* Beck.), as well as on shrivelled seeds. The latter can be caused both by sucking of some mirids, mainly by *Adelphocoris lineolatus* Goeze and *Lygus rugulipennis* Popp., and by nutritional deficiencies.

For achieving an effective control the exact knowledge of factors associated with the different damages is indispensible. We made an attempt to pinpoint some of these factors and to elucidate their relationships with the damages.

Materials and Methods

Investigations concerning the relationships between the damages and the growth habit of lucerne were conducted on plots of a variety collection of the Research Institute at Kompolt (NE-Hungary) on the second crop of 2-year-old stands in 1978. There were chosen 6 varieties of erect and 6 varieties of prostrate growth, each in 3 repetitions. The growth habit of the stands was assessed visually at the full bloom with the use of a scale of 1 to 5, where 1 represents the prostrate growth, and 5 the erect one. In each plot the damages were estimated by examining seeds in 25 to 50 pods. For regression analysis the per cent damages were set against the corresponding values of the growth habit.

The data of a country-wide assessment of the damages caused by seed pests, performed in 1964, served as basis for the analysis of relationships between the damages and the plot sizes, as well as some characteristics of weather conditions. In this former study (unpublished data) pod samples from 183 different lucerne seed growing fields in 16 counties of Hungary were collected, mainly by the network of the Plant Protection Stations. The damages were evaluated by examining the seeds in 25 to 50 pods per plot. The meteorological data were taken from meteorological stations in close vicinity of the sampling sites. The drought indices (= "Dürrezahlen") were calculated according to SCHMIDT's formula (cit. KÉRI, 1941):

$$I = \frac{n(n + 1)}{2},$$

where

I = drought index,

n = the number of the days in the dry period with a precipitation below 1 mm per day.

The index increases progressively with the length of the dry period. The drought index for a month equals the sum of the indices of each dry period within the month in question. The percent values of the damages were plotted against the amounts of the precipitations in some periods of the previous seasons, and the drought indices in August of the former year.

Results

The more erect the lucerne plant, the greater is the damage caused by *Bruchophagus*, and the less is the ratio of shrivelled seeds (Fig. 1). On the contrary, *Tychius* seems to be unaffected by the growth habit of lucerne (r = 0.00).

In Fig. 2 the per cent frequencies of field sizes and those of seed damages, respectively, are plotted against field size intervals. The solid curve shows the frequency distribution of the sizes of the seed growing fields investigated. It can be easily noticed that the most frequent field sizes fall between 11 to 20 hectares. The

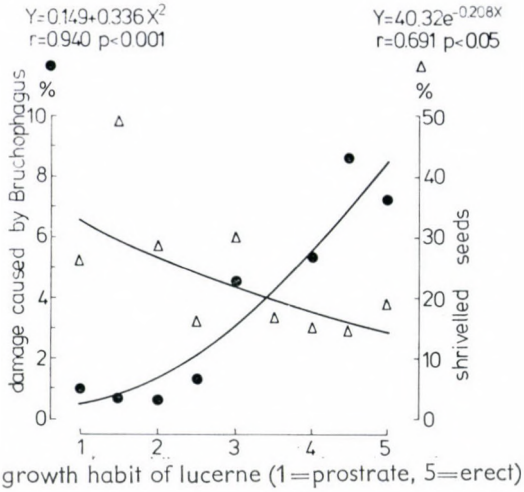

Fig. 1. Relationships between the growth habit of lucerne and the damage caused by seed pests. Kompolt, Hungary, 1978

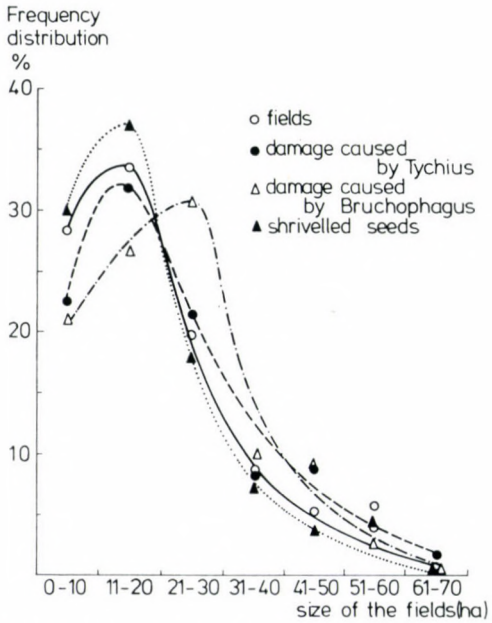

Fig. 2. Relationships between the size of seed producing fields and the damage caused by seed pests. Hungary, 1964 (n = 173)

other curves represent the percentage of the various damages related to each plot size interval. Supposing the damages to be uniform, the curves representing them, should run parallel with the solid line, i.e. the percent distribution of the field sizes. If this is not the case, we should assume the interference of some other factors. It seems that there are some differences between the distributions, although thorough statistical analysis has validated only tendencies. Both in the case of *Bruchophagus* and *Tychius* there is a shift in the position of the curve towards larger fields, though concerning *Bruchophagus* even the peak is displaced, while regarding *Tychius* only the descending part of the curve runs above the standard line. On the contrary, the curve of shrivelled seeds tends to move towards lower classes.

In Fig. 3 per cent damages are plotted against the amount of the August precipitation of the previous year. The proportion of shrivelled seeds increases along with the amount of the precipitation. However, the peak of *Tychius* damages coincides with 40 to 80 mm August precipitation. The damage caused by *Bruchophagus* shows a non-significant decrease with increasing precipitation.

Figure 4 represents an other approach of the same question. Ratios of damaged seeds are plotted against the drought indices, i.e. the distribution pattern of the August precipitation of the former year. The rate of shrivelled seeds reaches its maximum at the least drought index values, i.e. at the most frequent rainfall, while *Tychius* is favoured by a balanced pattern of the distribution of the August precipitation, having its peak between the values 60 and 80. *Bruchophagus* damages show a striking rise with the dryest weather conditions in the former August.

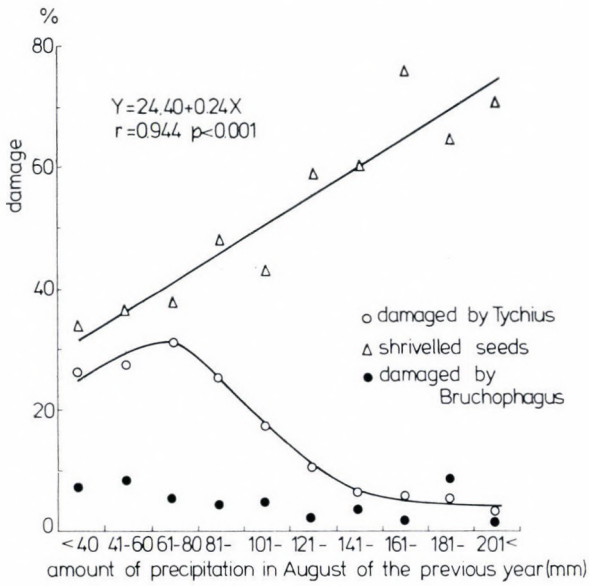

Fig. 3. Relationships between the amount of August precipitation of the previous year and the damage caused by seed pests. Hungary, 1964 (n = 183)

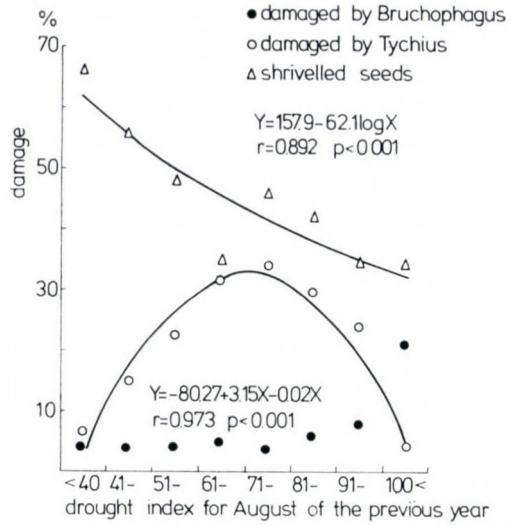

Fig. 4. Relationships between the distribution of August precipitation of the previous year and the damage caused by seed pests. Hungary, 1964 (n = 183)

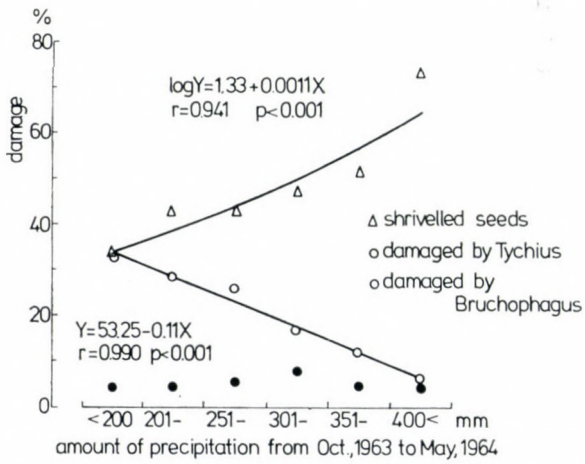

Fig. 5. Relationships between the amount of precipitation from October to May and the damage caused by seed pests. Hungary, 1964 (n = 183)

In Fig. 5 damages are plotted against the amounts of precipitations recorded between October of the previous year and May of the year in question. The relationships between the damages and the precipitations of the same period exhibit an opposite character: *Tychius* damage decreases, while the proportion of shrivelled seeds increases with the increasing precipitation. However, *Bruchophagus* damages do not show much dependence on varying precipitation in this period.

Discussion

We would like to note that our data are recorded within a single year. Therefore, their validity is to be further substantiated in various conditions.

1. The connections between the growth habit and the seed damages (Fig. 1) can be attributed partly to the different microclimatic demands of *Bruchophagus* and the mirids. According to our investigations (unpublished data), confirmed also by literature data (PONOMARENKO, 1956; MANNINGER, 1966), the oviposition of *Bruchophagus* is favoured by warm and dry conditions, similar to those mostly prevailing in erect stands. However, the same conditions inhibit the feeding of mirids: both adults and larvae of *Adelphocoris* escape into the shadow, while larvae of *Lygus* settle into the middle levels of the stand during the warmest period of a summer day. In a cloudy day, however, these insects feed continuously (CHERNOVA, 1958; PUCHKOV, 1966). The prostrate growth habit promotes intensive re-sprouting (MANNINGER *et al.*, 1981). It is probable that the inadequately channelled food supply is the other factor which contributes to the proportion of shrivelled seeds.

The damage caused by the lucerne bud gall midge (*Contarinia medicaginis* KIEFF.) shows the same relationship with the growth habit, as it can be seen in the case of shrivelled seeds (ERDÉLYI *et al.*, 1980).

The pollinating wild bees are more abundant in spaced than in dense stands of flowering lucerne (BENEDEK, 1970). In concordance with this fact, the rate of the opened flowers increases parallel to the erectness, as erect stands provide similar microclimate to that of spaced ones. However, the greatest seed yield does not coincide with the most erect growth, for the number of stems decreases with increasing erectness (MANNINGER *et al.*, 1981).

2. The opposite tendencies in distributions of the damages as functions of field sizes (Fig. 2) may be explained by the differences in the immigration mechanisms of the pests. Both *Bruchophagus* and *Tychius* are practically monophagous seed pests of lucerne, and as such closely tied to a well defined developmental stage, i.e. the seed setting stage of lucerne. It has been demonstrated that *Bruchophagus* shows a marked sensitivity to a special odour, produced by the young pods of lucerne, just suited for egg-laying (TINGEY and NIELSON, 1974). It seems plausible that the odour produced by young pods of larger fields is more effective than that of smaller ones making up the same hectarage. The same mechanism might work in the case of *Tychius*, too. However, there are other possible factors, e.g. the interaction, namely the competitive exclusion being, at least partly, responsible for the difference in the position of the peaks between *Bruchophagus* and *Tychius*.

In contrast to *Bruchophagus* and *Tychius*, *Adelphocoris* is an oligophagous, and *Lygus* is a polyphagous pest. Thus, they are not tied to a particular stage of the lucerne, in fact, are not tied to the lucerne at all. After harvesting of their feeding crops the pests are forced to look for new feeding areas. While doing this, the probability of hitting upon a small field of seed lucerne is greater than of doing a large one, since the relative circumference of a large field is smaller than that of

some small fields, making up the same area. In *Adelphocoris* the realization of this mechanism may be promoted by the fact, that this pest does not fly to large distances (PUCHKOV, 1966).

The relationship we found between the mirid damages and the field sizes of lucerne is quite similar to that one existing between the abundance of some oligophagous species of *Sitona* and the field sizes of some annual leguminous crops, as it was demonstrated by GLUSHCHENKO (1965).

Similar mechanisms work also in the case of wild bees pollinating lucerne. Their total abundance slightly decreases along with increasing field sizes. However, this apparent trend is a sum of two different tendencies of opposite direction and different strength. The most frequent species of short flight period, pollinating lucerne in Hungary, *Rhophitoides canus* EVERSMANN and *Melitta leporina* PANZER feed on lucerne and prepare nests in the soil of lucerne fields. On the contrary, the species of medium and long flight periods are not so closely linked with lucerne. In Hungary, during the past twenty years the sizes of seed growing fields of lucerne have considerably increased. During the same period, the total abundance of wild bees pollinating lucerne, has slightly decreased, the abundance of species of short flight period has slightly increased, while species of medium and long flight periods have shown a marked and moderate, drop in their abundance, respectively (cf. BENEDEK, 1968; 1970; 1979).

3. Mirid damage is favoured by high and frequent August precipitations of the previous year (Figs 3 and 4), because the laying of eggs, which overwinter in the case of *Adelphocoris*, and develop to overwintering adults in the case of *Lygus*, respectively, takes place at this time (BENEDEK *et al.*, 1970), and the eggs can easily dry (cf. MUKHAMEDOV, 1956; PONOMARENKO, 1956; PUCHKOV, 1966). At the same time the fully developed larvae of *Tychius* burrow into the soil, where they pupate. The young adults emerge in some weeks and remain in the soil until the next spring. All of these processes are favoured by a well defined degree of soil humidity which can be provided by a well defined pattern of the amount and distribution of the precipitation (cf. PONOMARENKO, 1956; KRASNOPOL'SKAYA, 1974). As for *Bruchophagus*, the eggs which the bulk of the overwintering larvae developes from, are being laid in August (unpublished data). As it was mentioned above, the oviposition of this species is favoured by warm and dry weather. Whether weather conditions are suitable for oviposition or not, it is characterized well by the drought index.

4. Autumn, winter and spring with large amount of precipitations also contribute to heavy seed losses caused by mirids (Fig. 5). In *Adelphocoris* under conditions of rainy and cool autumn, desiccation of the eggs and precocious hatching of the larvae do not occur. In *Lygus* the same conditions assure prolonged vegetation, therefore, food for adults, enabling them to overwinter. Winter months take a heavy toll of both the overwintering eggs and adults by causing desiccation and freezing, especially in a snowless winter, whereas deep snow-cover provides good protection. As regards spring weather, frequent rains and mild temperature stimulate vegetation, thus providing feeding source for *Lygus* adults on the one hand,

and protects the eggs of *Adelphocoris* from desiccation, as well as promotes hatching and development of its young larvae, on the other hand (cf. KISELEVA, 1948; USHATINSKAYA, 1955; MUKHAMEDOV, 1956; PUCHKOV, 1966). In contrast to mirids, intensive precipitation is dangerous for larvae, pupae, and young adults of *Tychius* because of an increased soil saturation leading to maximal vulnerability to fungal attack (cf. KRASNOPOL'SKAYA, 1974; MEL'NICHUK, 1976). *Bruchophagus* seems to be hardly affected by the amount of precipitations from autumn to spring.

Conclusions

1. There is a clear relationship between the growth habit of seed growing lucerne and the infestations caused by the mirids and *Bruchophagus* (Fig. 1). This fact is worth to be considered by the breeder. Regarding to the pests mentioned, as well as to *Contarinia* (ERDÉLYI *et al.*, 1980), it seems to be advantageous to select lines of growth habit rated at about 3.5. In this type also the re-sprouting in the seed crop is not significant, resulting in little amounts of shrivelled seeds. Fortunately, both the best seed and green mass yielder are stands of similar erectness (rated about 3.5 and 3.2, respectively; MANNINGER *et al.*, 1981).

2. The degrees of damaged seeds show some relationships with the plot sizes. It is advantageous that mirid damage tend to decrease with increasing field sizes, since the field sizes have markedly increased in Hungary during the last years. However, *Bruchophagus* and *Tychius* damages show the opposite tendency. In addition to this fact, also the abundance of pollinating wild bees decreases with increasing plot sizes, moreover, the proportions of the species of short flight period on the one hand, and those of species of medium and long ones, on the other hand, continuously shift in favour of the first group, which results in the gradual decrease of the ecological plasticity of the wild bee fauna (cf. BENEDEK, 1979). Therefore, a limitation of the sizes of the lucerne seed growing fields is needed.

3. Relationships have been found between seed damages on the one hand, and weather conditions of the former August, as well as precipitations from October to May, on the other hand. These relationships give some possibilities for the forecast and prevention of the damages.

a) After a rainy late-summer, autumn, and spring, with a snowy winter, heavy damages caused by mirids are probable in Hungary. In this case it is appropriate to grow seed from the second crop. The majority of the lucerne fields are left to produce seed from this crop in Hungary, the full bloom of which occurs at about the middle of July, and at the same time, a decrease in the abundance of both *Adelphocoris* and *Lygus* adults can be established because of the change of generations (BENEDEK *et al.*, 1970). These circumstances can result in the spreading of adults over large areas.

b) If the weather in late summer of the previous year was balanced, but the autumn and the winter, as well as the spring was dry, we have to count on severe damages caused by *Tychius*. In these conditions the third, or, perhaps, the first

cut should be left for seed, because the abundance of ovipositing adults gradually decreases with the progression of the vegetation period, on the one hand, and the flowering of the first crop, on the other hand, may prevent the mass oviposition (PONOMARENKO, 1956; KRASNOPOL'SKAYA, 1974; MEL'NICHUK, 1976; MANNINGER, 1979).

c) After a warm and dry summer, first of all a late summer, especially when followed by an autumn, winter and spring of balanced weather, mainly *Bruchophagus* damages are to be taken into account. Under such conditions the second crop is to be grown for seed, again. The damage evoked by *Bruchophagus* gradually increases towards the end of the season. However, seed growing from the first crop is very risky, as this crop is generally cut for forage, and the few plots left for seed could promote a high aggregation of chalcids. In Hungary generally the second crop is left for seed production. Thus, the full bloom coincides with a warm and dry period in mid-July, favourable for seed setting. The spreading of oviposition over large areas results in a lower female abundance and damage related to the former situation (cf. URBAHNS, 1914; KRÁL'OVIČ, 1971).

Acknowledgements

We thank Drs. Á. SZENTESI and L. VARJAS for useful suggestions, M. TÜSKE and A. DOBROVOLSZKY for furnishing the statistical analysis. Also thanks are due to all the colleagues who collaborated in sampling, or in assessing the material, especially to Mrs. J. ZSÁMBOKI, Mrs. M. KRISZTIÁN, K. GERGELY, as well as to the heads of the laboratories of the Plant Protection Stations in 1964.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 181—188 (1981)

Phototaxis of the Adult Whitefly, *Bemisia tabaci* Gennadius to the Visible Light

I. Effect of the Exposure Period on the Insect's Response to Different Wavelengths of the Visible Light-spectrum using a Devised Simple Technique

By

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At 35.6 fc light intensity and the median attracting exposure period (AP_{50}) of 17.5 min, mixed populations of the aleyrodid *Bemisia tabaci* Genn. female and male adults showed marked preferandom to the yellow colour of light (520—760 mμ) than any of the other tested colours; the green, white, blue and red, respectively.

Although the morphology, taxonomy, biology and economic importance of whiteflies have been briefly investigated by several whitefly authors, relatively little attention was paid for studying both their physiological and ecological aspects. In this concern, light effect on whiteflies received even fewer attention of a limited number of the authors working on aleyrodids (i.e. LLOYD, 1922; BUTLER, 1938; HUSSAIN and TREHAN, 1940; MOUND, 1962; MACDOWALL, 1972; VAISHAMPAYAN, KOGAN, WALDBAUER and WOOLLEY, 1975 and VAISHAMPAYAN, WALDBAUER and KOGAN, 1975).

Since a long time it is known that light affects the insects by one way or another. Such effects could be morphological, biological, physiological or ecological. Of the latter, the behaviour of the insects in their response to light is important and relatively feasible to be noticed or detected. For example, lamps of all colours were found to attract insects to some degree (GUI *et al.*, 1942), *B. tabaci* Genn. adults were attracted by blue/ultraviolet and by yellow light but not by a mixture of these spectral components (OSSIANNILSSON, 1966); more individuals of *P. pyricola* Forst. were attracted to ultra-violet light traps more than to traps illuminated with colours in the visible spectrum (KALOOSTIAN and WOLF, 1968); it was proved that both the insect species and the distance between the light source and the exposed insects are effective on their response to light (STEWART *et al.*, 1969); the takeoff of eight lines of alate alienicolae of some aphids (DRY and TAYLOR, 1970) flight initiation of the scolytid *B. piniperda* L. (PERTTUNEN and HYARINEN, 1970), and flight inhibition of both whiteflies and aphids (KRING, 1972) were found to be controlled by light of certain wavelengths and intensity.

The positive phototactic behaviour of the adults of *B. tabaci* Genn. which has been observed by some authors (i.e. MOUND, 1962; EL-HELALY *et al.*, 1971/b)

encouraged the achievement of further studies in this respect. Therefore, the present study investigated the effects of both light quality or wavelength of visible light spectrum and the exposure period on the taxis of the mixed groups of both sexes of the whitefly, *B. tabaci* Genn. adults. This was achieved taking into consideration the whitefly phototaxis promising role as an effective tool for its control other than the classical conventional chemical and biological measures which are the most common in use now.

Materials and Methods

The whitefly phototaxis test-kit (WPhTK)

It is a conical plastic cylinder with an opening (11 cm diam), a closed base (9 cm diam) and inner walls darkened with a thick black paper sheet. A circular cork (9 cm diam and 0.6 cm thick) was fixed to the narrow base of the cylinder from outside to fit exactly its auter ridge (0.6 cm hight). Five test tubes (1.5 cm diam and 16 cm hight each) were then fixed in an erect position in five holes (1.5 cm diam each), made through both the circular cork and base of the plastic cylinder, with their openings directed downwards to work as translucent traps for the whitefly adults which were noticed by EL-HELALY *et al.* (1971/b) to be geotropically negative and phototropically positive. When the constructed WPhTK is placed perpendicularly under a suitable source of light the apparatus becomes ready for utilization. For the determination of the colours preferable to the whitefly adults, each of the forementioned five test tubes which were fixed to the WPhTK cylinderical base was enveloped from outside with a certain coloured cellophane sheet. In this way, each of the tested whitefly adults was completely free to choose its most preferable colour. Interference among the tested light-colours was taken into consideration during the designation of such an investigation for representing more or less the more complex conditions that face the insect in nature.

The phototaxis cabinet (Ph.C.) used

It is comprised of a wooden cabinet provided with an observatory glass window which leans downwards for about 45° from the horizontal plane of its ceiling. The cabinet is also provided near its bottom with a vertical anterior rectangular opening used for handling of both the WPhTK and the insects. On the centre of the ceiling of the cabinet, an electrical terminal was fixed. The latter could be supplied with an incandescent lamp emits the required light intensity. In the course of the present study an incandescent lamp of 100 watts illumination power which gave 35.6 fc light intensity was used. On the achievement of the phototaxis experiments, both the glass window and the rectangular opening of the cabinet was covered with a thick black cloth to prevent any probable interacting effect of light rays might penetrate the cabinet from outside.

The cellophane sheets used as light-filters

Sheets of the red, yellow, green, blue and translucent cellophane were used as light-filters in present experiments. As the translucent cellophane sheet transmits the whole visible spectrum of light, the light colour which it produces was given the name "white". Through these cellophane sheets, ninty percent or more of the light waves of 660–760, 520–760, 760, 440 and 720–760 and 380–760 mμ in length were transmitted, respectively.

The green cellophane sheet transmitted, in addition to the 90% or more of the previously mentioned 760 mμ light wave, 50% of the light waves ranged from 380–740 mμ. In other words, the green cellophane transparency is nearly one hal that of the translucent sheets in the region of the 380–740 mμ wavelengthsf

The insects used

A laboratory culture of the whitefly *Bemisia tabaci* Gennadius (Aleyrodidae, Homoptera) which has been first established by EL-HELALY (1966) and still breeds on tobacco plants up to now, was used. The mother culture was identified by two world authorities of white-flies[1] and the identification has been also confirmed by EL-HELALY *et al.*, (1971/a).

Experimental procedure and statistical analysis

After the preparation of the whole units, of both the WPhTK and PhTC, 25 male and female adults collected haphazardly from the stock culture were immediately and as quickly as possible introduced. Then the light was switched on. At the end of the considered tested exposure period, the coloured tubes were then removed and stoppered immediately with pieces of cotton moistened with chlorophorm for anaesthetizing the adults attracted and trapped inside them, in order to facilitate their counting. In the course of this study; 5, 10, 15, 20, 25, 30 and 35 min exposure periods were tested and each of them was replicated five times. The obtained results were then tabulated, represented in illustrating histograms and analyzed by F-test, Duncan's new multiple range test and the regression analysis. The regression equation of the subject whitefly cumulative responses to different exposure periods to light effect were obtained.

Results and Discussion

As a starting point, it was logical to determine first the most suitable exposure period of the whitefly adults to an incandescent lamp with a certain illumination power in order to be used in the achieved investigation. Therefore, a lamp of

[1] Miss LOUISE M. RUSSELL of the US National Museum, USDA and Dr. LAWRENCE A. MOUND of the British Museum (Natural History), London, U.K. (cf. EL-HELALY, 1966).

100 watts which gave 35.6 fc light intensity was used. This lamp has been chosen in order to take into consideration any probable interacting effect for the large amount of heat emitted from such a tested lamp on the response of the whitefly adults.

Table 1 and Fig. 1 show the regression relationship obtained between the tested exposure periods; 5, 10, 15, 20, 25, 30 and 35 min and the percentages of the adults whitefly total cumulative response; 10.4, 25.8, 41.3, 59.5, 74.7, 89.2 and 100%, in respect to the whole visible light spectrum despite its different tested colour components. As in this experiment, the numbers of the adults that were attracted to all the colourless (white), blue, green, yellow and red tubes of the WPhTK were recorded and they were then transformed to percentages of the whole tested numbers of the whitefly adults, and the latters were also transformed to percentages of the cumulative response of the insects to different tested exposure periods. Statistical analysis of the data proved the highly significant regression relationship existed between the two tested variables; exposure periods and adult responses. The steepness of the obtained linear regression curve (the slope value of which was 3.1 ± 0.51) may reflex the potent effect of each change-unit of the independant variable, the exposure period, on the other dependant variable, percentages of the insects cumulative response. The linear regression equation was $y = -4.0 + 3.1x$ and the median attractive exposure period or the exposure

Table 1

Effect of the exposure period on the simple and cumulative response of *B. tabaci* adults to the visible light of 35.6 fc intensity

Statistical parameters determined	Exposure period(x) in minutes	Simple attraction %	Cumulative attraction %(y)	Adjusted cumul. attr. %(y)
	5	54.4	10.4	10.8
	10	80.8	25.8	26.3
	15	81.6	41.3	41.8
	20	95.2	59.5	57.3
	25	80.0	74.7	72.8
	30	76.0	89.2	88.3
	35	56.8	100.0	103.8
Total	140		400.9	
Mean (M)	20		57.3	

Average (M±S. E.)	20±4.1
Regression equation	$y = -4.0 + 3.1x$
The slope value	3.1±0.5**
Median attracting exposure period (AP_{50})	17.5 min

** 99% level of significance.

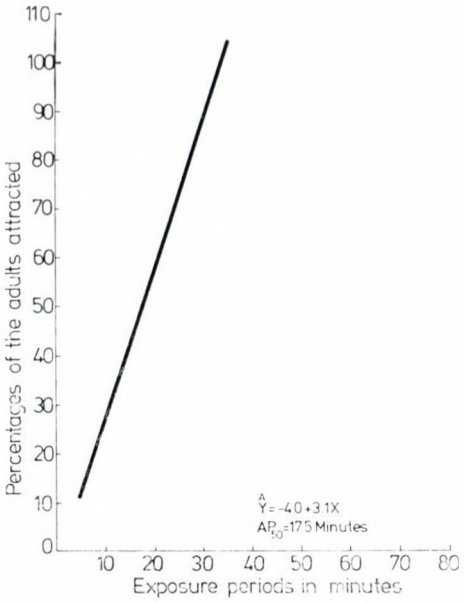

Fig. 1. The regression line of the relation between the exposure period and the percentages of the cumulative positive response of *B. tabaci* Genn. adults to a visible light of 35.6 fc. intensity

period at which 50% of the tested numbers of adults were attracted and which was given the symbol "AP_{50}" to all tested coloured tubes of the WPhTK was 17.5 min. In this concern it is important to draw the attention to the fact that the adoption of the previously mentioned regression equation should be only restricted to the responses ranged from 20–80%, as its accuracy decreased out of this range and the maximum value of the insect adults adjusted cumulative response exceeded 100%, which of course do not represent the actual response of the whitefly adults.

The data present in Table 2 and Fig. 2 show that the percentages of response of the adult insects to different tested colours of light at different exposure periods followed the normal distribution. The data also showed that higher means of simple response percentages of *B. tabaci* male and female adults (in groups) were recorded for the yellow light (520–760 mμ) which was then followed in a descending order by the green, white, blue and red colours of light spectrum, respectively. This trend was recorded for all the investigated exposure periods. On the other hand, the exposure period at which the higher percentages of the simple response of the whitefly adults differed in different colours (Table 2; Fig. 2A), as such maximum percentages of simple response of the whitefly adults which were 40.8, 27.2, 16.0, 11.2 and 7.2% were recorded at 20, 15, 20, 25 and 30 min exposure periods for the yellow, green, white, blue and red colours of the visible light spectrum, respectively (Table 2). This shows that the median attracting exposure period (AP_{50}) for the whitefly adults which gave the highest response to the visible light, despite

of its different colours or wavelengths, lies between the range 15 – 30 min (Table 2), and this range includes the mean value which was obtained before by the regression analysis (AP_{50} = 17.5 min Table 1 and Fig. 1). Also, the forementioned range of exposure periods, within which the maximum values of the percentages of the simple response of the whitefly adults to all tested wavelengths of light are existing, is nearly the same range (15.9 – 24.1 min) obtained by adding or subtracting the standard error (±4.1 min, Table 1) to the calculated mean (20 min, Table 1). In this concern it is important to take into consideration, in addition to the exposure period's effect, the probable interacting effect for the ages of the tested whitefly adults which has been previously reported by OLSON and RINGS (1969) for *Amathes C. nigrum* L.

As the optimum exposure period which has been previously determined by the regression analysis (AP_{50} = 17.5 min, Table 1 and Fig. 1) was not actually tested during the achievement of the experiment, the whitefly adults' responses to the different tested colours or wavelengths of light was statistically compared using Duncan's new multiple range test on the basis of the mean values which have been arithmetically calculated for the responses obtained at all replicates of both the 15 and 20 min exposure periods. At this optimum exposure period (17.5 min), the statistical analysis proved that there were significant differences among all the means of the percentages of attraction of *B. tabaci* adults to different tested colours except in cases of the difference obtained between either the blue (8.76%) and red (6.10%) light colours on one hand, or the white (14.95%) and blue (8.76%) colours of light on the other hand (Table 2). In other words, both the male and female adults of the whitefly could not significantly discriminate between either the blue and red or white and blue colours of light, inspite of its apparent preferandom of the

Table 2

Effect of the exposure period on the mean percentages of attraction of *B. tabaci* Genn. adults to different colours (wave lengths) of a visible light of 35.6 fc intensity

Exposure period in min	Mean percentages of the adults attracted to different tested colours of light				
	blue	green	white*	yellow	red
5	4.0	12.8	9.6	25.6	2.4
10	8.0	23.2	13.6	33.6	2.4
15	8.0	(27.2)	10.4	32.0	4.0
20	7.2	24.8	(16.0)	(40.8)	6.4
25	(11.2)	23.2	15.2	25.6	4.8
30	9.6	20.0	15.2	24.0	(7.2)
35	8.8	12.8	9.6	25.6	0.0

* All the visible light spectrum.

( ) The numbers between brackets are the highest mean percentages of attraction of the whitefly adults to different colours of light at different exposure periods tested.

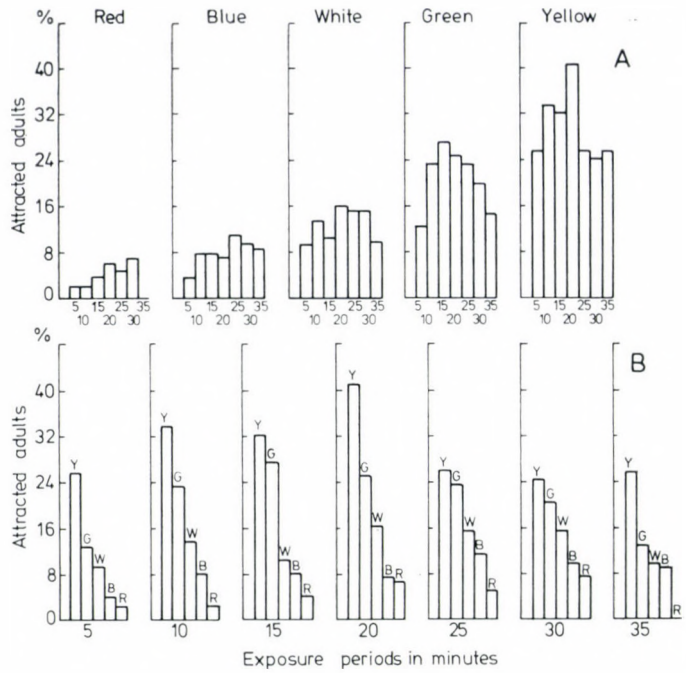

Fig 2. Percentages of attraction k of both the female and male adults, of *B. tabaci* Genn. to different colours (wavelengths) of the visible light at different exposure periods

white light (14.95%) than either the blue (8.76%) or red (6.10%) colours of light. This could be understood if the interaction existing among the spectral ranges transmitted by all these blue, red, and white or colourless cellophane sheets were considered (see materials & methods). At last, it is important to emphasize that at both 35.6 fc light intensity and the most preferable statistically derived exposure period of 17.5 min, females and males of *B. tabaci* Genn. in mixed groups showed marked preference to the yellow colour of light (520–760 mμ*) than any of the other tested colours. The latter findings agree to large extent with those published by VAISHAMPAYAN, KOGAN, WALDBAUER & WOOLLEY (1975) in which they concluded that the greenhouse whitefly, *T. vaporariorum* showed a strongly positive response to surfaces with maximum reflectence or transmittance in the "yellow-green" region (520–610 nm*) and a probable moderately inhibitory response in the red region (610 to ca 700 nm*) of the visible light spectrum.

* The nanometer = the millimicron = 10^{-9} meter.

Acknowledgement

The authors wish to express their deepest gratitudes to both Dr. H. GWIRGUIS and Dr. S. TADROS, Professors of Physics, Department of Physics, Faculty of Science, University of Alexandria, for their generous cooperation by offering several indipensable helps throughout the determination of light properties and measurements needed during the achivement of the present work.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 189–197 (1981)

Microbial Control Experiment Against *Stilpnotia salicis* L., Pest of Poplar Stands in Northwest Hungary

By

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The area of Hanság, situated in Northwest Hungary has been earlier a marshland; in the last decades the region has been transformed and on 6000 hectares poplars planted. Because of stagnant water in the soil, the abundant weed growth *(Solidago gigantea)* and the damage caused by the fungus *Dothichiza populea* the poplar trees show in most places very poor growth; the conditions had been worsened since the early seventies by the attack of *Stilpnotia salicis*. Due to the combined influence of these factors in many areas a dying-off of the poplars has been noticed.

In the region mentioned, the species has only one generation and overwinters in the L_2 stage; as the diapause begins by midsummer, a spring treatment was decided. Beside a chemical pesticide (Fekama AT-25) Thuricide HP, Entobakterin, Dendrobacillin and Gomelin biopreparations were applied on more than 800 hectares. Although the use of Fekama AT-25 proved to be more economical, the authors still recommend the use of the selective *B. thuringiensis* preparations in the future. This would be warranted also by the faunistical data collected in course of the experiment; the light traps operated in the different plots collected more than 250 Macrolepidoptera species indicating a variegated fauna combining ancient and new elements.

The region of Hanság (Northwest Hungary) has changed considerably in course of the last decades when following an intensive canalisation in the former marshland poplars were planted. The poplar stands of about 6000 hectares are extremely important for the cellulose industry so any factors decreasing their vitality (e.g. insect outbreaks) exceed their local importance.

As the poplar planting had to be carried out within a short time it was inevitable that also suboptimal sites became afforested where the development of the young poplars has been inhibited from the begin by weed competition (e.g. *Solidago gigantea)*, followed by the attack of the poplar canker fungus, *Dothichiza populea.*

From among the insect pests since 1972 the satin moth, *Stilpnotia salicis* has been observed in large areas, causing heavy damage. The distribution of this pest has been promoted besides by the presence of extended, continuous stands also by the spacious planting method, presenting thus optimal developmental conditions for this thermophilic species.

The adverse soil conditions, stagnant water, fungus attack and pest damage form a chain of damage factors; the total frass caused by the Stilpnotia salicis larvae prevents the trees from evaporating the stagnant water, the poplar canker

Fig. 1. The poplar stands planted in wide rows present suitable conditions for the thermophilic *S. salicis*

causes extensive bark destruction, which, in turn, opens suitable overwintering sites to the insect larvae.

It was under these conditions that the Észak-Hanság Erdészet (North-Hanság Forest Company) decided to control one of the factors of the extensive dying-off of poplars.

Biology of *Stilpnotia salicis*

The larvae of this pest develop in six larval instars; in course of the fourth, fifth instars the larvae are deep brown or black with lighter dots and covered by dense hairs. The larvae of the last instar are colourful, with large white or yellow spots on their ventral side and with yellow stripes on both sides. The larvae pupate mostly in the crown of the attacked trees; the black pupa, covered by white hairs and white spots rests in a light cocoon woven in branch corners, forks or in leaf rolls. The adult is white with a satin luster-like glistening on its wings; the wing span exceeds on most exemplars 4 cm. Both the male and the female are good flyers. The mass flight occurs in the early evening or after sunset, but sporadic flight can be observed even during the daytime. The light green eggs (1 mm diam-

Fig. 2. Larvae and damage of *S. salicis*

eter) are laid in batches of one or two hundred, enveloped by a white foamlike substance which solidifies after the egglaying.

The overwintering occurs in the L_2 stage. The young larvae form in the second half of summer or in early autumn in the bark crevices small, individual cocoons where they diapause until the April of the next year. In regions with warmer climate (as e. g. also in South Hungary) two generations develop.

The flight of adults lasted in 1978 from the middle of June to mid-July; early July the first larvae of the new generation appeared. By the end of July the larvae were observed in masses and early August most larvae entered their diapause in the L_2 stage.

The first larvae left their overwintering sites in the early spring (March) on spots with a warmer microclimate and fed on the buds as the leaves appeared only by mid-April. Because of the cold and wet spring the majority of larvae appeared by the end of April; after this the development became faster and by the middle of May already the first adults appeared. The females laid 194 eggs in the average (the egg number varied between 93 and 226) mostly in two or three batches. The embryonal development lasted in our observations 15 days and 23% of the eggs was found infertile.

Distribution of *S. salicis* in the Hanság and earlier control measures

The pest was well known earlier in the region, however, no extensive damage was reported. So GRAESER (1938) mentioned the species from the Hanság and also KOVÁCS (1953) reported its presence in Mosonmagyaróvár and Mosonszentjános.

ROKOB (1972) studied the biology of *S. salicis* in the region of Sopron and many of his observations have been confirmed later by present authors.

The mass appearance of *S. salicis* was by no means unexpected as JAHN and SINREICH (1961, 1965) reported one decade earlier its outbreak from the neighbouring Austrian region (Burgenland). In the territory of the North-Hanság Forest Company the damage of *S. salicis* became conspicuous in 1973 (KISS and FITTLER, 1975); the attack spread further in 1974 along the forest clearings and with the main wind direction. In 1974 already some control work has been carried out with Fosfotion aerosol, by the end of June, however, a strong adult flight was observed.

The first large-scale control experiments have been made in 1977 in early August (HALMÁGYI and LENGYEL, 1978) and in 1979 the spring control experiment has been carried out.

Control experiment in 1979

The control experiment was based on the experiences gained in 1977; it has been observed, namely, that in the late summer one part of the larvae had entered their diapause so escaped the treatment. In a spring treatment, however, more larvae were hoped to feed on the foliage together with other lepidopterous larvae.

The experiment was carried out at the begin of intensive larval feeding, on 11th of May. The Forest Company treated 427 hectares with biopreparations and 403 hectares with Fekama AT-25. At the time of the treatment most larvae were in the early instars, based on measurements of 4850 larval head capsules: L_2 = 14.8, L_3 = 52.8, L_4 = 29.7, L_5 = 2.7%.

Materials and methods

a) Preparations used

Fekama AT-25 (2,2,2-trichloro-1-n-butyriloxyethyl phosphoric acid) (Fettchemie, GDR). Dosage: 8.8 kg/hectare;
Thuricide HP (*Bacillus thuringiensis* HD-I strain, serotype No. 3) (Sandoz, Switzerland); 30×10^9 spores/g = 16×10^6 IU/g. Dosage: 1 kg/hectare;
Dendrobacillin (*B. thuringiensis*, serotype No. 4, Soviet Union);
Entobakterin (*B. thuringiensis*, serotype No. 5, Soviet Union);
Gomelin 50 (*B. thuringiensis*, without serotype specification, Soviet Union);

The latter three, Soviet preparations were used on 10 hectares each, in a recommended dosage of 2 kg/hectare.

b) Treatment

The treatment itself has been carried out by a helicopter (typ KA-26) which could treat 17 hectares with one take-off (30 liters per hectare). The helicopter flew 3 meters above the tree canopy with a speed of 40 km/h, treating a swath of 30 meters. During the flight the air temperature was 20 °C with practically no air movement.

Fig. 3. Helicopter treatment of poplar stands. In the left lower corner an apiary illustrates the necessity to use selective insecticides

Fig. 4. Placement of plastic foil stripes on the forest soil

c) Evaluation

The evaluation was carried out by plastic foils, after treatment, soil traps and light traps.

In each treatment 4 plastic foils (4 × 1 m each) were placed on the forest soil and the insect material falling from the trees was collected 48 and 120 hours after the treatment. Near to the above mentioned foils other 4 foils (8 m^2 each) were placed on the soil and on each 1 poplar tree was slowly felled then treated with 0.2% Nuvacron, by using a motor-driven knapsack sprayer. This method was necessary because of the different infestation levels in the plots; so the results of the treatment could be measured on the fauna of the same plot. The abundant fauna collected by the latter method in the untreated plot (300–500 *Lepidoptera larvae*, 170–200 Coleoptera, 18–60 Arachnoidea etc.) justified the method and made possible to judge the results of the treatments.

In each treatment 10 plastic cups (200 ml) were sunk into the soil at 10 m distances and filled to the half with ethylene glycol. The cups were protected against grazing wildlife, rainwater etc. by strong metal covers and emptied 3-weekly.

In the Fekama, Thuricide and check plots light traps were operated (1 on each) and emptied by 48 hours. The light bulbs were 2 m high, fed by 2 accumulators (40 Amp./h each). The light traps were in distances more than 1 km of each other so their light did not disturb the catches.

Fig. 5. Light trap used in the evaluation

Results

The results, as mentioned above, were evaluated mostly on the number of insects collected on the plastic foils.

Fekama AT-25 gave very good results as 48 hours after the treatment 89.6% of the *S. salicis* larvae and 91.3% of the *Geometrid* larvae present were destroyed; these numbers did not change considerably in the subsequent countings. There were, however, heavy losses also in the other faunal elements, so 66.2% of Coleoptera, 62.3% of Hymenoptera (mostly parasites), 62.8% of Heteroptera and 41.1% of Arachnoidea were killed.

The effect of *B. thuringiensis* preparations developed much slower as observed also in earlier similar experiments (HALMÁGYI, LENGYEL and SZALAY-MARZSÓ 1978a, 1978b) so most larvae were found on the plastic foils 120 hours after the treatment. The best results were observed with Thuricide HP, killing 82.1% of

Table 1

Results of control experiment, based on the insects collected on plastic foil stripes, as compared to the "total" number of insects present (Hanság, 1979)

Treatment Species	Per cent mortality of Arthropods after treatment	
	48 hours	120 hours
Fekama AT-25:		
Stilpnotia salicis	89.6	93.7
Geometridae	91.3	92.5
Coleoptera	51.5	66.2
Hymenoptera	50.8	62.3
Diptera	79.3	81.2
Heteroptera	58.4	62.8
Arachnoidea	30.9	41.1
Thuricide HP:		
Stilpnotia salicis	43.1	82.1
Geometridae	23.6	56.8
Others	—	—
Dendrobacillin:		
Stilpnotia salicis	53.9	67.4
Geometridae	42.7	49.8
Others	—	—
Entobakterin:		
Stilpnotia salicis	57.8	63.1
Geometridae	58.1	69.6
Others	—	—
Gomelin:		
Stilpnotia salicis	48.1	53.7
Geometridae	47.3	56.2
Others	—	—

the *S. salicis* larvae while in case of the Geometrids the effect was lower (56.8%). As the latter were not abundant, this did not affect the success of the treatment. The Soviet biopreparations showed different effectivities against *S. salicis*, so Dendrobacillin destroyed 67.4%, Entobakterin 63.1%, Gomelin 53.7%. For a final evaluation of these preparations further tests would be necessary as the effectivities were probably influenced by a 2-year long storage. The results are summarized in Table 1.

Results on other, non-target organisms

The methods used were not extensive enough to draw conclusions regarding the whole ecosystem, some data, however, have to be mentioned.

Regarding the soil trap results, high individual differences were noted among the plots, illustrating the difficulties in finding similar ecological conditions in large surface treatments. The abundant fauna observed in the Fekama treatment showed that the Arachnoidea, Coleoptera, Oniscoidea and Diplopoda active on the soil surface and in the forest litter were not affected by the treatment, maybe due to the Solidago stand already 30 cm high at the time of the treatment.

From the material collected with the light traps the Macrolepidoptera were found to be extremely abundant. Besides *S. salicis* other 13 dangerous poplar pests and other 46 species living on poplar (therefore potential pests) were found in the material, indicating the importance of continuous surveys in the forest protection work. In the traps 253 species were determined; this high number may be explained by the abundant flora of the Hanság, containing not only poplar stands but also cropland, willow and alder stands, clearings and meadows. By planting the poplar stands some forest elements may have migrated into the area. A species new for the Hungarian fauna: *Perisoma taeniata Stph.* and two very rare species: *Perisoma sagittata* F. and *Chariaspilates formosaria* Ev. were collected in the light traps.

The number of species caught in the different plots did not show clear connections with the treatment type: in the Fekama plot 147, in the area treated with B. thuringiensis 166 and in the check 185 species were caught and the differences are by no means significant. There were, however, clear indications on the losses caused by the chemical treatment where 1000 individuals less were caught in the Fekama plot, compared to the biopreparation.

Economical considerations

Some calculations were made regarding the costs and rate of returns. With Thuricide the costs of the preparations amounted to 323.30 Ft, the costs of Helicopter treatment to 178.00 Ft, other additional costs to 4.50 Ft per hectare, 505.80 in total. With Fekama the same costs were found in the same order: 217.00, 178.00, 4.50 Ft, 399.50 in total. If only the losses in tree growth are accounted (the number of destroyed trees disregarded) this amounts to 1200 Ft per hectare. At present, the use of the chemical preparations seems to be more economical, because of the

abundant non-target fauna, honeybees and natural waters (the region contains an intricate network of canals), however, only the use of selective biopreparations seems to be justified.

As the forests have to accomplish more functions than the average agricultural areas (production, landscape protection, recreation) their importance cannot be measured with the agricultural standards. The more complex forest ecosystems increase the responsibility of forest management even in case of a selective treatment.

Acknowledgements

The authors are indebted to the specialists of the Kisalföld Forest Company: István Pechtol, Sándor Balsay and Ottó Fittler for their invaluable help in carrying out the control experiment and Miss Katalin Leskó (Forestry Research Institute) for her help in determining the light trap catches. The photographs were made by I. Michalovszky, Forestry Research Institute, Budapest.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 199—202 (1981)

Influence of Structural Peculiarities of Different Species of Wheat on the Attack by *Haplothrips triciti* and *Trigonotylus coelestialium*

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Structural characters of the ear in some wheat species have been found to correlate with infestations caused by *Haplothrips triciti* and *Trigonotylus coelestialium*.

Haplothrips tritici Kurd. (Thysanoptera, Phloeothripidae) and *Trigonotylus coelestialium* Kirk. (Heteroptera, Miridae) are wide-spread wheat pests practically in all wheat growing areas. These sucking insects reduce grain crops considerably, especially in draughty years. According to our data, the reduction of spring wheat grain weight (of the Harkovskay 46 variety) in a draughty year was 22.4% (usually 11.5%), as a results of ear infestation. *Haplothrips tritici*, according to SCHUROVENKOV (1971; 1975) reduces the weight of 1000 seeds between 7.4% and 13%, and according to other authors e.g. TANSKII (1965) even more, by 18.8%.

H. tritici and *T. coelestialium* infest practically all wheat areas. Their outbreaks occur every 2nd or 3rd year, when the number of *T. coelestialiul* larvae is above 1000 individuals 100 strokes of sweep net, and there may be 100 or even more *H. tritici* larvae on a single ear. One can judge the importance of the two species almost solely by the damage they cause. The chemical control against them is difficult because of the overall infestation of the fields. Two more obstacles also arise, one is the extended period of adult flight and the other is that of larval emergence. In addition, the intensive application of pesticides is undesirable as far as the protection of environment is concerned.

For overcoming the problems outlined above the selection of resistant forms of wheat would be of great importance. The succes of research is based on the fact that the two species, and especially *H. tritici*, are closely connected with the plant, as with a habitat. *H. tritici* females oviposit into the ear, and the larval development takes place also there, then the larvae leave the ear in the stage of waxen ripeness.

The larvae of *T. coelestialium* are not so closely associated with the plant. However, the oviposition of *T. coelestialium* is also specific in that females deposit eggs behind the leaf sheat and spikelet scales.

It seems natural to suppose that structural characters of the host plant certainly influence the population level of species with the above mentioned habits. Therefore, a systematic evaluation of *Triticum* species and varieties possessing

Table 1

Distribution of *Haplothrips tritici* eggs affected by ear structural peculiarities of wheat species

Wheat species	Average number of eggs per ear (±S. D.)	Number of eggs		Structural peculiarities	
		at the ear base	behind the ear scale	Hairiness at ear base	Rim at ear scale
Triticum monococcum	32.3±1.306	23.1	9.2	weak	yes
T. sinskajae	65.8±4.405	39.8	26.0	weak	yes
T. timopheevi	147.7±11.638	138.5	9.2	medium	yes
T. dicoccoides ssp. *pseudojordanicum*	267.8±15.006	249.8	18.0	strong	yes
T. dicoccoides ssp. *spontaneomyum*	299.0±21.518	82.4	216.7	weak	no
T. dicoccum	261.3±7.88	23.9	238.1	weak	no
T. palaeo-colchicum	266.6±21.835	40.8	225.8	weak	no
T. spelta	311.2±8.303	206.7	104.5	strong	no

morphological peculiarities which influence the degree of plant infestation by *H. tritici* and *T. coelestialium*, is of great importance.

In order to compare wheat species and varieties we turned to the rich collection of wild and primitive wheats by academician N. I. VAVILOV (All-Union Plant Growing Institute). The species of wheat are characterized by different combinations of contrasting morphological peculiarities. They differ from selectively cultured varieties of wheat advantageously.

It was found that the intensity of ear attractiveness for a *H. tritici* female during the period of oviposition was associated with the degree of hairiness of the spikelet base and with the formation of spikelet scale.

If the base of the spikelet is hairy, the females oviposit onto the base of spikelets. The more protuberant the spikelet scale is, and the less closely adjoining the top of the scale is to the flowering scale, the more eggs are laid behind the spikelet scale. For example, the number of eggs on the spikelet stem of *T. dicoccoides* (which is a not hairy species) is about 3 times less than that of *T. pseudojordanicum* (a species with rich hairiness, Table 1). In case of *T. dicoccoides* ssp. *spontaneomyum* the spikelet scale at the top does not attach closely, thus it permits *H. triciti* females to freely penetrate behind the scale, where they then oviposit. On the contrary, the spikelet scale of *T. dicoccoides* subsp. speudojordanicum adjoins closely at the top to the flowering scale due to a so-called "rim" and that is why it is difficult for the females to penetrate behind the scale. It was found that the number of eggs was 12 times less behind the spikelet scale of this subspecies than that of the one having another scale structure. *T. timopheevi* possesses the same tightly adjoining scale structure, thus the number of eggs laid behind the spikelet scale is 23 to 25 times less, than on *T. dicoccoides* ssp. *spontaneomyum*, *T. dicoccum* and *T. palaeo-colchicum*, respectively.

Females oviposit the least number of eggs on ears having no or only weak hairiness and flat spikelet scale with a "rim" on the top. On the ears of *T. timopheevi*, *T. sinskajae* and *T. monococcum* there are eggs 2 to 9 times less as compared with the number on ears of *T. palaeo-colchicum*, *T. spelta* and *T. dicoccoides*.

A close correlation was observed between the number of eggs and that of larvae per ear, and the correlation coefficient was 0.86.

The number of larvae on *T. monococcum* and *T. sinskajae* is 2.6 to 10 times less, than on *T. palaeo-colchicum* and *T. spelta*. *T. timopheevi* is intermediate among them (Table 2).

On the basis of the impact of structural peculiarities on oviposition behaviour, it was concluded that there were some cultivated wheat forms with comparatively low infestation levels by *H. tritici*.

Table 2

Number of *Haplothrips tritici* larvae on ears of different kinds of wheat species

Wheat species	Number of larvae per ear (±S. D.)	
	in 1978	in 1979
T. monococcum	0.4±0.063	1.1±0.07
T. sinskajae	1.8±0.112	3.5±0.425
T. timopheevi	3.5±0.229	5.7±0.373
T. palaeo-colchicum	4.7±0.293	9.2±0.615
T. spelta	4.3±0.237	9.8±0.643

Table 3

Number of *Trigonotylus coelestialium* eggs on different wheat species

Wheat species	Frequency of infested stem (%)	Number (±S. D.) of eggs per infested stem	Average number of eggs per stem in the experiment
1977			
T. monococcum	17.6	8.6±0.904	1.5
T. sinskajae	14.6	7.2±0.771	1.0
T. dicoccoides	68.5	13.6±1.405	10.0
T. aestivum	55.7	13.6±1.395	7.6
1979			
T. monococcum	45.8	8.1±0.904	3.7
T. sinskajae	35.8	8.8±0.912	3.0
T. dicoccoides	65.8	18.9±1.623	12.6
T. aestivum	80.2	25.5±2.464	20.0

As mentioned above, the reproduction of *T. coelestialium* is not so strongly influenced by the morphological characters of the plant than *H. triciti*. However, a certain connection between the number of eggs and those characters still can be observed. In 1977, the average number of eggs on *T. monococcum* stems was 1.5 to 1.8 times less, than on *T. dicoccoides* and on the wheat variety Bashkir 9. Since the frequency of egg occurence on *T. monococcum* stems was 3 to 4 times less, the average number of eggs per a single stem was considerably (5 to 10 times) reduced in our experiment (Table 3).

In 1979, the frequency of egg occurence of *T. coelestialium* on the stems of species studied was 1.4 to 2.2 times less. However, the average number of eggs on infested stems differed 2 to 4 times more. The average number of eggs received by a single *T. monococcum* stem, in an experiment, was 3 to 7 times less, than with two other species, so by and large the tendency was the same as in 1977 (Table 3).

T. monococcum and *T. sinskajae* have very compact leaf sheaths adjoining the stem, the ears are also compact, and the spikelets resemble to tiles. Owing to such morphological features, the oviposition of *T. coelestialium* is somewhat hindered on these species.

In the course of the study of the reproduction rate of *T. coelestialium* on the wheat species it was found that the differences in the number of adult were much less than those in the number of eggs. The reproduction rate on *T. monococcum* was 1.5 to 2.6 times less, than on *T. dicoccoides* and on the variety Bashkir 9.

On the basis of results obtained, it was concluded that some morphological characters of inflorescence of certain wheat species proved to be more important in influencing the population size of *T. coelestialium* and *H. tritici*, than other factors, such as the phenology and nutritional values of plants.

By endowing wheat varieties, through the way of selection, with certain useful morphological characters, hopefully we will be able to reduce the population size and damage made by *H. tritici* and *T. coelestialium*, respectively.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 203—209 (1981)

Antifeedant-treated Potato Plants as Egg-laying Traps for the Colorado Beetle *(Leptinotarsa decemlineata* Say, Col., *Chrysomelidae)*

By

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Following an antifeedant (2% Bordeaux mixture) treatment of a field plot, during two consecutive weeks, the number of eggs laid by Colorado beetle females on treated potato plants was significantly higher (P = 1%) compared with that on the untreated control ones. Laboratory choice and no-choice experiments have also strengthened the above. Substance(s) of insect-origin have induced a similar distribution of eggs in choice tests. It is thought that the phenomenon is primarily caused by the differential decrease in the surfaces of the two kinds of foliage and to a lesser extent, by substance(s) accumulated on untreated plants, because of the presence of various developmental stages. The unequal distribution of eggs might bear some effect on the decrease of a local Colorado potato beetle population.

The integrated pest control, besides conventional chemical means, incorporates methods selectively acting on organisms. Such procedures have the similar features in common, to wit, a high efficiency against target species and a low or neglectable harmfulness against beneficial organisms. There is no doubt that chemicals will still dominate in future plant protection practices, though purely biological control measures have already been taking their shares.

One of the selectively acting control procedures is the use of antifeedants (synonymes are: feeding inhibitors, feeding deterrents). It was demonstrated even before the 2nd World War that certain chemicals would inhibit feeding of some insect species (see WRIGHT, 1963, for references).

As it is the usual case in practically oriented research fields, antifeedants sooner became members of the protection arsenal than principles of their modes of action were understood in details. Even though our knowledge about feeding deterrents have developed more or less parallel to the domain of plant/insect relationships, to that of receptor physiology, and so on; and albeit it has also been repeatedly demonstrated by JERMY (1958, 1961, 1965, etc.) and by others, only recently it gains more and more appreciation that most phytophagous insect/host-plant relationships can temporarily be modified, at the behavioural level, by the application of substances rendering host recognition and acceptance depressed or impossible, even in the presence of optimal sensory input from the host.

The deterring effect of copper compounds to the feeding of the Colorado beetle *(Leptinotarsa decemlineata* Say) had long been known and demonstrated by

FEYTAUD (1922), RAUCOURT and BEUGE (1942) and JERMY (1961). Although, there are numerous other chemicals of natural and synthetic origin being potent antifeedants both in laboratory and field conditions, relatively few works discuss impacts of such compounds on pests' population dynamics. Among the sporadically occuring such studies MURBACH and CORBAZ (1963), MURBACH (1967) and ZHEMCHUZHINA (1976) present data about dispersion, damage, egg-laying, feeding ethology, locomotory activity of the Colorado beetle, under the influence of antifeedants containing copper.

For some years, in the Research Institute for Plant Protection, we have been screening chemicals (with or without copper) against the Colorado beetle in the laboratory, in order to develop potent antifeedants. In addition, field tests have also been conducted to study the population dynamics of the pest under antifeedant influence. Efforts are mostly focused on egg-laying responses in presence of antifeedants. Within the scope of the present study I am going to give a *preliminary* account on such field observations and laboratory experiments.

Material and Methods

For field works, the experimental plot was situated near Budapest, at an altitude of about 300 m above sea level, where the Colorado beetle had mostly one and a half or sometimes two generations per year. There was a single plot (0.12 ha) of Desirèe potato variety used, on which there were 6 rows marked in the middle of the plot, each being one hundred plants long. There were 20 plants chosen randomly in each of these rows. Regular population counts were made at approximately one week intervals. All stadia present, the damage done by them, and also the size of the plants were recorded. After the first population count 10 randomly selected plants of the 20 marked in each row received a treatment of 2% Bordeaux mixture (which is a complex chemical containing copper) while the remaining 10 served as control. The spray was renewed whenever it was necessary. The number of surveyed plants per population count ranged between 23 and 60.

In choice tests, under glass-house conditions, 25 to 35 cm long potato shoots were randomly arranged on the bottom of a wire screen (1 × 1 mm)-covered cage (50 × 50 × 100 cm). There was a sheet of black cardboard paper placed on the bottom of the cage having holes through which the stems of shoots were immersed into flasks of fresh water.

For no-choice tests, compound leaves of potato with 5 to 7 leaflets were placed into separate, 15 × 18 cm size glass jars (2 leaves per jar), then were covered with 1 × 1 mm mesh nylon screen. The two leaves in a jar received the same treatment.

Rinses of insect origin were prepared as follows: larvae and adults were collected in the field. They were either kept on paper towels, or were fed potato foliage in cages. After 1 to 2 days both the paper towels and the plant foliage on which the various instars had walked, fed, oviposited, defecated etc. were washed, or sprayed with destilled water until 100 to 150 ml solution was collected, respectively.

The solutions were sifted or centifuged at 6000 rpm for 5 min then sprayed onto fresh potato shoots and offered for gravid females to oviposit in choice tests. For controls either dist. water wash of paper towels or dist. water were sprayed on potato shoots.

In choice tests, depending on the number of choice possibilities and shoots applied, 22 to 250 gravid females were used. After 1 to 5 days the number of eggs laid and area of foliage consumed were recorded. In no-choice test, 4 ovipositing females were applied per jar.

Sprayings were carried out with a "Mistral Triumph N" electric spray machine, using No. 4 or 6 size nozzles. Plants were sprayed either until run-off or glass plates were also placed among shoots to be sprayed both for quantification and to measure uniformity of spray. In the latter case, a mean of 3.09 ($\pm$0.89 S. D.) mg dry material per cm^2 was found on sprayed leaves.

Results and Discussion

The results of population counts show that the number and distribution of eggs and damage (i.e. consumption) suffered by plants were disproportionate, to wit, there were significantly (P = 1 %) more eggs found on treated plants compared to untreated ones (Table 1). Also the damage on unsprayed plants gradually increased during subsequent population counts. A significant difference between eggs laid on sprayed and unsprayed plants did not appear until larval infestation

Table 1

Field oviposition response of a Colorado beetle population to antifeedant (Bordeaux mixture, 2%) spraying on potato plants. (June 13 till August 17, 1978; No. of data used: 23 to 60.)

Population count	Mean no. of eggs ($\pm$S. D.) per plant		Mean no. of larvae ($\pm$S. D. of the mean) per unsprayed plant	Consumption ($\pm$S. D.) of foliage	
	sprayed	unsprayed		sprayed	unsprayed
Before spraying	120$\pm$70		3$\pm$2	20$\pm$9	
After spraying					
1st	227$\pm$89	a — 120$\pm$70	49$\pm$12	13$\pm$5	21$\pm$8
2nd	129$\pm$83	a — 68$\pm$79	71$\pm$24	7$\pm$3	34$\pm$11
3rd	65$\pm$57	NSD — 39$\pm$43	88$\pm$15	0	49$\pm$20
4th	22$\pm$29	NSD — 22$\pm$36	44$\pm$10	0	74$\pm$17
5th	4$\pm$11	NSD — 27$\pm$44	20$\pm$3	0	76$\pm$17
6th	5$\pm$13	NSD — 6$\pm$15	14$\pm$3	0	85$\pm$14

Bordeaux mixture = $CaSO_4 \cdot Cu(OH)_2 \cdot 3\ Ca(OH)_2$ complex

a = Values in same row separated by this symbol significantly differ at 1 % level (Duncan's new multiple range test).

NSD = not significantly different

Table 2

Oviposition response of Colorado beetle females in a *choice* test, to potato shoots sprayed either with 1% Bordeaux mixture or distilled water. (No. or replicates: 9)

Spraying (= treatment)	Total no. of eggs laid/treatment	Consumption % (±S. D.) per shoot
	After 1 day	
Bordeaux mixture	210	0
Dist. water	224	14±11
	After 5 days	
Bordeaux mixture	651	0
Dist. water	374	45±10

Table 3

Oviposition response of Colorado beetle females in a *no-choice* test to potato leaves sprayed either with 1% Bordeaux mixture or distilled water (No. of replicates: 5)

Spraying (= treatment)	Mean (±S. D.) number of eggs laid/treatment	Consumption % (±S. D.) per compound leaf
Bordeaux mixture on the leaves'		
1. upper surface	198±43 a	0
2. lower surface	154±63 ab	0
3. both surfaces	140±59 ab	0
Dist. water	87±29 b	17±7

Means followed by the same letters are not significantly different at 5% probability level (DNMR-test).

level reached about 50 instars per unsprayed plant. The difference still remained, as the number of larvae increased on untreated plants then diminished, since the females of the overwintered generation slowed down and finally ceased laying eggs.

The same result was received in a glass house experiment. In a *choice* test, there were almost twice as many eggs laid on sprayed potato shoots than on the control ones (Table 2). However, the difference did not appear until after 5 days. By then consumption of unsprayed plants reached about 45%.

Treated (i.e. Bordeaux mixture-sprayed) leaf surfaces alone, in a *no-choice* test, received significantly more eggs (P = 5%) as compared with control ones, while consumption was about 17% on the latter (Table 3). The results indicate

that even without any alternative choice possibility, treated foliage by itself can induce a biased egg-laying response.

MURBACH and CORBAZ (1963) did not find any unequal distribution of eggs in the presence of a copper oxychloride spray, in a field experiment. However, they also stated that this compound was repellent to the Colorado beetle, what could explain the lack of a similar response. We think that repellency is not a character of the Bordeaux mixture.

On the basis of field observations it has been supposed that the unequal distribution of eggs might have been caused, partly at least, by (1) the decrease of foliage surface, (2) the presence of various stadia, mostly larvae and/or by (3) the existence of substance(s) on unsprayed plants visited, chewed, defecated, oviposited also both by larvae and beetles.

On the other hand, however, Tables 2 and 3 indicated that the unproportional distribution of eggs could be elicited, in choice tests, (1) merely by the decrease of foliage on unsprayed shoots, (2) by excretions, impacts, as well as other substance(s) left behind by ovipositing females only since no larvae were present in the experiments, and in a no-choice situation, (3) by the Bordeaux mixture alone.

In order to elucidate the situation, various rinses (Table 4, rinses 1 to 4) were prepared and tested on field collected ovipositing females in *choice* experiments. The results obtained merely strengthened the field observations and data of Tables 2 and 3. Various rinses did not produce stronger alterations in egg distribution than that evoked by Bordeaux mixture.

Table 4

Oviposition responses of Colorado beetle females to potato shoots sprayed with freshly prepared solutions of insect origin. (Three choice experiments; No. of replicates: 5 to 6)

Spraying (= treatment)	Total no. of eggs laid/treatment			Consumption % (±S. D.) per shoot		
	I	II	III	I	II	III
Bordeaux mixture	1662	747	910	0	0	0
Rinse no. 1	1075	408	—	23±8	52±37	—
Rinse no. 2	1266	588	—	28±13	37±15	—
Rinse no. 3	—	—	882	—	—	25±17
Rinse no. 4	—	—	601	—	—	23±11
Dist. water	1109	194	699	28±26	77±20	22±17

Rinses: No. 1. Prepared by soaking paper towels in dist. water on which there were 258 gravid females walking for 1 day.
No. 2. Similar as no. 1, except that ca. 2300 larvae of mixed stadia were employed.
No. 3. Potato shoots chewed, defecated, oviposited, etc. by females then sprayed with dist. water.
No. 4. Similar to no. 3, however, gained by washing shoots packed with larvae of mixed stadia.

Explanations of the phenomenon can slightly differ according to the stimulus situations applied. The words "attracted" and "deterred" have not been used deliberately here, as it is thought that the ultimate reason of the unequal distribution of eggs between treated and untreated plants was the decrease of unsprayed foliage (because of consumption), of which degree might have somehow been estimated, together with the egg-load already present, by randomly walking gravid females. However, as some data showed, the influence of other insect-origin factors cannot also entirely be eliminated. Such factors resulted in a similarly distorted partitioning of ovipositing females.

In the field, there were substance(s) of insect-origin present on control plants acquiring a slight or medium level of inhibition on feeding and/or oviposition, and by this way they could produce a more clear cut difference in egg distribution. On the contrary, such substance(s) were absent on distilled water-sprayed control plants in the laboratory tests. Therefore, in the latter stimulus situation plants sprayed with rinses appeared in an intermediate position of preference between plain control and Bordeaux mixture-treated plants.

It can be concluded that Bordeaux mixture treated potato stands, while not being suitable for larval or imaginal consumptions, still can receive large number of eggs from ovipositing Colorado beetle females. This way, antifeedant-treated plants can function as *egg-traps*. Newborn larvae would eventually die on treated foliage (see also MURBACH and CORBAZ, 1963), therefore, a substantial decrease could be expected on a local potato beetle population. There is no doubt, however, that expected changes would considerably depend on actual population size, oviposition drive, dispersial ability, etc.

Acknowledgement

I thank Miss ANDREA MÁNDY for assistance in the field work.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 211—222 (1981)

The Scale Insects *(Homoptera: Coccoidea)* of Deciduous Fruit Orchards in Some European Countries

(Survey of Scale Insect *(Homoptera: Coccoidea)* Infestations in European Orchards No. III)

By

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Twenty five scale insect species were found in the fruit-tree branch and bark samples. The most frequently occuring species, in order of decreasing importance were: *Quadraspidiotus perniciosus*, *Lepidosaphes ulmi*, *Epidiaspis leperii*, *Q. ostreaeformis*, and *Parthenolecanium corni*. The same species were present on a significant proportion of areas studied. The following species were detected on relatively more restricted areas, albeit in very high local abundance: *Parlatoria oleae*, *Pseudaulacaspis pentagona*, *Lopholeucaspis japonica*, *Q. marani*, *Q. pyri*, *Sphaerolecanium prunastri*. The respectiv outbreaks of *P. corni* and *S. prunastri* in about 1976 were registered, too. The former appeared on large areas while the latter one could be detected on smaller isolated localities. Considerable alterations have also been established in respect to the orchards scale insect species composition of various geographical regions.

Integrated control systems require the exact knowledge of the plant pests, including those that appear less important today. This is so, because we do not know which species will become later an important pest due to changes in the cultivation practices, or of other factors. It is also essential to explore the direction and causes of changes in the population dynamics of pest species, so that we can base on these our pest forecasts and control recommendations.

In case of scale insects, we can give an answer to the above-mentioned questions only through the application of standardized techniques simultaneously throughout a large geographical area.

About the distribution of scale insects on fruit trees good informations are given in the monograph of BORCHSENIUS (1966), but the faunistical works do not give detailed picture on the distribution of important species. The distribution of *Quadraspidiotus perniciosus*, *Pseudaulacaspis pentagona*, *Lepidosaphes ulmi*, *Parlatoria oleae* was given in the Distribution maps of Commonwealth Institute of Entomology (ANONYM, 1951—1978). KONSTANTINOVA (1970) published several maps about *Q. perniciosus*, *Q. ostreaeformis*, *Q. pyri*, *Q. marani*, *Epidiaspis leperii*. All these maps, however, do not present informations regarding the population densities on various fruit plants, which could be very important for plant protection. It is also of considerable interest that the expansion of some scale insect spe-

cies as *Q. perniciosus*, *P. pentagona*, *Q. marani*, *P. oleae*, etc., is still in progress. The more detailed works (KOZÁR, 1976; and others) give dates only about restricted areas (mostly about one country).

Some detailed information on the results of this work has been previously published by KOZÁR and VIKTORIN (1978), KONSTANTINOVA, KOZÁR and KOZARZHEVSKAYA (1979), KOZÁR, KONSTANTINOVA, AKMAN, ALTAY and KIROGLU (1979), KOZÁR, JASNOSH and KONSTANTINOVA (1980), KOZÁR and KONSTANTINOVA (1981), KOZÁR, HUBA, MAREK and PIEKARCZYK (1981).

The present work is the first complete summary of investigations on the distribution of fruit pests scale insect species. In this work we will study the distribution of the most important fruit scale insects in some countries of Europe, on the most frequent deciduous fruit species. It is also of interest to determine the degree of density of various scale insect species on various fruit trees in different geographic zones.

Materials and Methods

For the survey in Europe a standardized sample collection method was elaborated. In individual countries 50 samples were collected (in 1976–1977) from each type of fruit orchards from the following countries: Czechoslovakia (217 samples), Greece (89 samples), Hungary (216 samples), Poland (84 samples), Turkey (250 samples), the European part of the USSR (2313 samples), some informations were obtained from Norway, too.

The samples were taken in 1976–1977. Altogether we received 3169 samples, that originated from apple (1159 samples), pear (676 samples), peach (230 samples), plum (594 samples), cherry (155 samples) and sour cherry (355 samples).

The distribution of sampling places is given in Fig. 1, but the next sample places we could not find on the map: *Czechoslovakia:* Nahoretice (Karlovy Vary), Vseruby, Chvalenice, Pnovany (Plzen), Olesná (Rokycany), Javory (Klatovy), Velké Dvorce (Tachov), Obora, Zdirec (Ceská Lipa), Frydstejn (Jablonec), Hrbovice, Lochocice (Usti nad Labem), Vinarice, Ahnikov, Poláky (Chomutov), Ctverin (Liberec), Risuty, Libocany, Vrbicka, Zemechy (Louny), Modlany (Teplice), Markvartice (Ceské Kamenice), Horusice, Svobodna Ves, Semtés (Kutna Hora), Holovousy (Jicin), Kocbere, Koclérov (Trutnov), Placice, Predmerice n. Labem, Vlckovice (Hradec Králové), Dolany Sebuc, Dolany Trebesov (Náchod), Linhartice (Svitavy), Cerná za Bory (Pardubice), Klanecná (Havlickuv Brod), Skály (Pisek), Vrábce (Ceské Budejovice), Budiskovice (Jindr. Hradec), Nezamgolice (?), Morice, Nasedlovice, Zadovice (Kyjov), Detkovice (?), Lipov (?), Velesovice, Nemcany, Koberice (Vyskov), Horny Mesto, Sosnová (Bruntál), Moravicany, Velebor, Hrabová (Sumperk), Opava-Kylesovice, Steborice (Opava), Chotebuz (Tesin), Starojicka Lhota (Novy Jicin), Veselicko, Podhory (Prerov), Hlivice (Olomouc), Lesná (Vsetin), Susany (Rimavská Sobota), Veska (?), Mokrance, Ruzovy Dvor, Nizny Lanec (Kosice), Malé Trakany (Trebisov), Husak

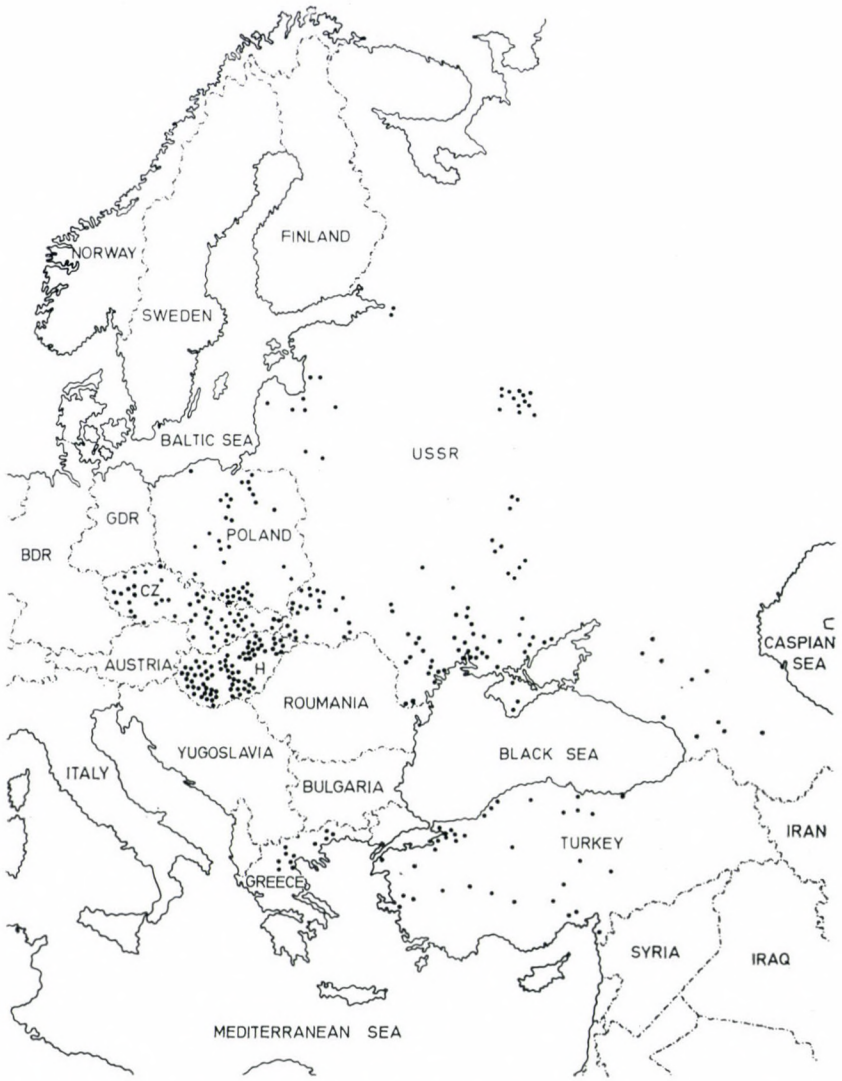

Fig. 1. Localization of all sampling places

(Michalovce), Dulova Ves, Záborské (Presov), Caklov, Dlhé Klcovo, Kamenká Poruba (Vranov nad Toplou), Karha (Humenné);
Greece: Stavros, Platanos, Veria (Hmathias), Naoyssa, Stenimakhos, Monospita, Lazokhori (Hmathia), Kariotissa (Pellis);
Hungary: Donok (?), Bagda, Zalaszádor (Zala);
Poland: Micice, Kwetomino, Sporysz, Cisziec, Rajcza, Meszna, Buczkowice, Bystra, Godziszka, Wapienica, Witanovice, Jaszczurova, Przytkovice, Zaborze, Leki,

Malec, Biedaszki, Uatszyn, Wogstanka, Kajkowo (?), Lachowice, Bienkowka, Jachowka, Lembrzice, Stryszawa, Pewel Wielka (Bielsko Biala), Debowiec, Gumna, Zamarsky Hazlach (Cieszyn), Chetmna (Torun), Jarnoltowo (Maldity); *Turkey:* Karadere, Karamürsel, Ulasli, Halidere, Gebze, Masukiye, Sirin, Hikmetiye, Nusretiye, Aksu, Yarimca, Külfalli (Kocaeli), Soguksu, Degirmen (Hatay), Ekinözü (Elbistan), Cumhuriyet, Vakiflar (Samandagi), Akkuyu (Adana), Karasu (Sakarya), Kerhan (Kahranmaras), Sarikavak (Icel), Tosya, Daday (Kastamonu), Bartin (Zonguldak), Kursunlu (Gemlik), Kirazli Yali (Gebze), Sultandag (Afyon); *USSR:* Kamjanica, Onokovcü, Sztarozsnice, Nevickoe, Goevcü, Gorkij k-z, Ogorodnüj, Szolomonovo, Prihordnik, (Transcarpathia), Bukovina (Csernovcü), Monratna, Panikovica, Bucin, Sezsnje, Ljubickaja, Balica, Szokolov, Brjulovicsi, Szapizsanka, Golubec, Sztriklovicsi, Zaloveszkij, Szorimanka, Bukovka, Zubü (Lvov), Sztaniszlav, Kamüsan, Pervomaevka, Neftjanka, Antonovka, Rubovka, Rozsnovka, Vindisztka (Herszon), Zsovten, Kovalevka, Kolarovka, Dacsnoe, Sevcsenkovo, Palücsan, Timirjazevo, Rodnaja, Csurevka, Komarovka, Ocsakovszk, Odesszk. Gunovka, Rodszad, Kimovka, Galianovka, Cservom. Ukr., Pogorelovo (Nikolajevszkaja), Kutuzovka, Breszt (Harkov), Linarszkoe, Szt. Nekrasz. sz-z, Kogurgan, Pavlovka, Karnagarov, Sztepanovka, Vüpusznoe, Kucsurgan, (Odessza), Szovki sz-z (Kiev), Valnier, Pukumszki, Sztrodenszt (Latvia), Voroncovo, Noszovo, Vnukovo, Timirjazevo, Kablukovo, Seremetevo, Vühino, Trubino (Moszkva), Ozerki, Zavat Lenin k-z, Ucskog, K-Rossia, Sztranosz. k-z (Kurszk), R. Ukrainszkij k-z (Cserkasszk), Petrovka, Mernovka (Krim), Dzsamana, Kolesznoe, Sztrasenszk, Novonevszk, K. Besszarabia, Gerbovcü (Moldavia). All of these places are within the sample collecting area.

The processing of samples has been terminated in 1980. About 2000 microscopic slides were prepared for the exact determination of species. The collecting and the analysis of samples were carried out by the using a special method already described (KOZÁR and VIKTORIN, 1978).

Results

There were 25 scale insect species represented in the samples. These were: *Epidiaspis leperii*, *Lepidosaphes ulmi*, *Quadraspidiotus ostreaeformis*, *Q. perniciosus*, *Q. marani*, *Q. pyri*, *Q. lenticularis*, *Parlatoria oleae*, *P. theae*, *Pseudaulacaspis pentagona*, *Nilotaspis halli*, *Suturaspis archangelskayae*, *Lopholeucaspis japonica*, *Quadraspidiotus* sp., *Eulecanium mali*, *E. nocivum*, *Parthenolecanium corni*, *P. persicae*, *Sphaerolecanium prunastri*, *Palaeolecanium bituberculatum*, *Pulvinaria betulae*, *Ceroplastes japonica*, *Coccidae* indet., *Pulvinaria* sp., *Pseudococcidae* indet. Most of the species (14) belonged to the family *Diaspididae*, while 9 were members of *Coccidae*. The family *Pseudococcidae* was represented through traces only. Although there are seldom pests in orchards in the area studied, the lack of finding specimens was a proof that our sampling technique is inadequate for obtaining intact specimens of this family. Because the data on them are unreliable, we did not consider this group in our present report.

The most frequently occurring species in order of importance were: *Quadraspidiotus perniciosus*, *Lepidosaphes ulmi*, *Epidiaspis leperii*, *Q. ostreaeformis*, and *Parthenolecanium corni*. The same species were present on a significant portion of the area studied. The following species were detected in a relatively more restricted areas, although in very high local abundance: *Parlatoria oleae*, *Pseudaulacaspis pentagona*, *Lopholeucaspis japonica*, *Q. marani*, *Q. pyri*, and *Sphaerolecanium prunastri*.

Some new distribution records have also been obtained on several scale insect species. For example, *Q. marani*, *Q. lenticularis*, *Suturaspis archangelskayae* and *Eulecanium nocivum* were found in Turkey as a new to that fauna.

The number of scale species decreased from south to north in the orchards of the studied geographical area. For example, while in Turkey 18 scale species were present, only 4 species were found in Poland. This was also true for each fruit tree species. It was very obvious in case of peach trees, on which very often 8–9 species were present in the south (Greece and Turkey) while in Czechoslovakia on peach only 3 species were found, and even these were very rare.

As the result of our study we were able to determine the area of their economic importance and the geographical distribution of orchard infecting scale species. We are going to provide below specific distribution records on some economically important species.

The data about *Q. perniciosus* will published in separate work (KOZÁR and KONSTANTINOVA, 1981).

L. ulmi was widely distributed something northward too, than on Commonwealth Institute's map (ANONYM, 1958) and present in high density. It was primarily present in the orchards north of latitude 49, also in the mountainous parts of Turkey, Crimea and Caucasus (Fig. 2). Its frequency and density increased northward. It should be pointed out that its geographical distribution area was larger than the area where it was considered a pest. It became an important orchard pest only in the northern zone of its distribution. It was often present in high population density in Hungary both in humid and semiarid habitats on different host trees (e.g. *Populus*, *Salix*, *Fraxinus*, etc.) but it is uncommon on fruit trees. Thus even with its polyphagous food habits, its host preference varied with the ecological conditions which also influenced its population dynamics.

L. ulmi is most frequent on apples on almost its entire area of distribution (Fig. 3). Its density was also highest on this host. We could point out an increase in frequency of this species on apples toward the northern zone. For example, in Czechoslovakia 41.3% of the apple orchards had 0.53 rate of infestation, while in Poland 72.3% of the apple orchards had a 1.16 rate of infestation. *L. ulmi* was present on pears and plums in a relatively high frequency at latitudes between 49 and 50 (Fig. 4), but its density was low, and only rarely occurred over the 1.0 rate of infestation. Even in this northern zone it was uncommon (8–12%) on cherry and sour cherry.

E. leperii was most frequent in Hungary, also in the southern part of the European USSR and in Turkey in accordance with KONSTANTINOVA's (1970)

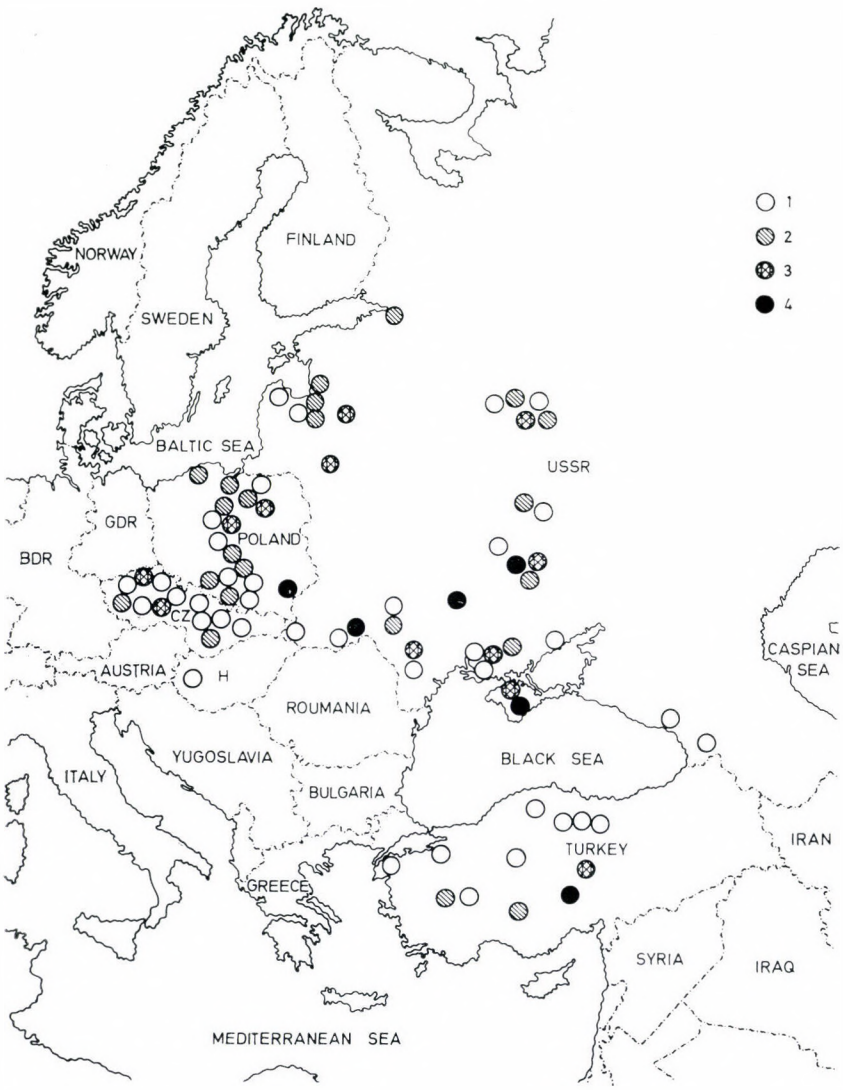

Fig. 2. Distribution of *L. ulmi* in 1976–1977 on six deciduous fruit species and degree of density according to the Borchsenius's scoring system (from 0 to 4). (The map does not include further 41 data from the USSR, 15 from the CSSR, 31 from Poland, and 1 from Turkey, as the collection sites could not be located on maps.)

maps. In the entire area, it infested most often and most heavily pear trees, followed by plums. On those two hosts it was the most important scale pest in this area, and relatively rare on other fruit trees.

Q. ostreaeformis, although present in the entire area, it attained economic importance only on cherry, sour cherry and plum, and even on these, it was restrict-

ed to the central part of the European USSR, in accordance with KONSTANTINOVA (1970).

It was unexpected to find *Q. marani*, and with it the often associated *Q. pyri*, frequently in very high densities at the northwest coastal area of the Black Sea and in certain areas of Turkey (Fig. 5). The distribution of *Q. pyri* is in accordance with KONSTANTINOVA's (1970) informations, but *Q. marani* now shows a wider distribution indicating an expansion to south and east. It will be very important to study

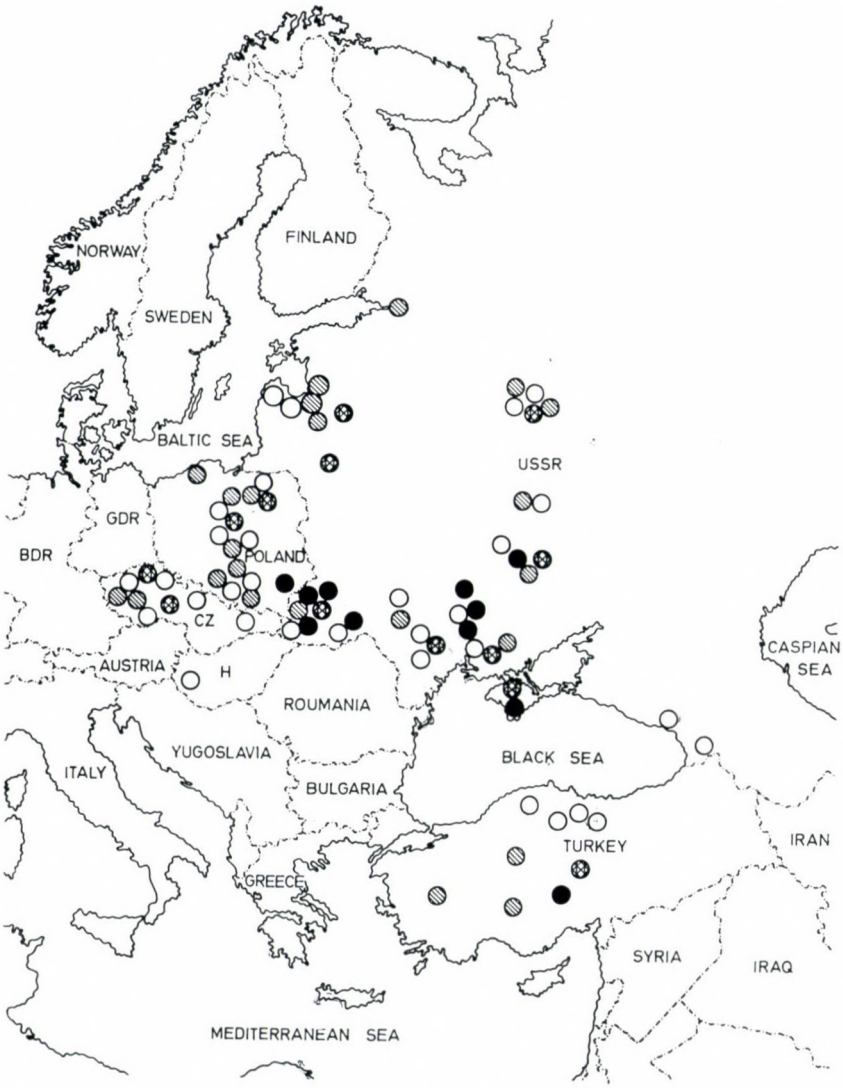

Fig. 3. Distribution and degree of density of *L. ulmi* on apple

Fig. 4. Distribution and degree of density of *L. ulmi* on plum

the distribution of *Q. marani* in fruit orchards in Bulgaria, Roumania an Yugoslavia, too. This species was frequently reported from western Europe from fruit and forest trees, but there it was never reported to cause such a high rate of infestation. We could not find explanation for this phenomenon.

P. pentagona was found in high frequency and density in the southern zone of the studied area (Fig. 6). Area of distribution corresponds with the one given in the Commonwealth Institute's map (ANONYM, 1962). It was frequent in samples

from Greece, Turkey, Georgia (USSR), but also in samples from southern Hungary where it spread north as far as Budapest. This species infested primarily peaches, but in Turkey it was also frequent on cherry. A further northern spread of this species can be expected.

P. oleae was found only in the southern zone (Greece, Turkey and Georgia) corresponding to the Commonwealth Institute's map (ANONYM, 1962). In Greece

Fig. 5. Distribution and degree of density of *Q. marani* on six fruit species. (The map does not include further 14 data from the USSR, as the collection sites could not be located on maps.)

Fig. 6. Distribution and degree of density of *P. pentagona* on six fruit species (The map does not include further 4 data from Turkey and 3 from Greece, as the collection sites could not be located on maps.)

it was very frequent on apples (72%), but appeared on peach only to 22%. In Turkey it occurred on apples only to 14%, while on peach, plum and cherry with frequencies of 36, 55 and 65%. It will be very important to study the distribution of *P. oleae* in fruit orchards in Bulgaria, Roumania and Yugoslavia, too. It was unexpected to find *P. theae* in Georgia more frequently than *P. oleae*.

The outbreak of *P. corni* occurred around 1976 in the studied area, thus it was frequently found in many areas. The outbreak occurred simultaneously in a large adjoining area (Czechoslovakia, Hungary, Turkey and the central and southern parts of European USSR). In USSR it occurs in accordance with the map of SAAKYAN-BARANOVA *et al.* (1971). This observation could be used in the preparation of a continent-wide forecasting. This species was most frequent and in highest density on plum, in lower density on cherry and was found only occasionally on other trees.

As for *P. corni*, the outbreak period of *S. prunastri* coincided with our sampling. But here the population increase was observed only on small isolated areas. The increase was more prevalent in some areas of Hungary and Turkey, but we observed a more sporadic increase of its population in Greece and in the southern parts of the European USSR. Forecasting of outbreaks for this species could be achieved only with data obtained through local observations. Outbreaks were noted primarily on peach, but it was also frequent on plum and cherry.

Among the species studied a few were present almost in the entire area of observation (e.g. *P. bituberculatum* and *E. mali)* but alsays in low frequencies. Some other species were only present in small isolated habitats (e.g. *L. japonica, N. halli, S. archangelskayae, C. japonica)* but there in large density, while some of the species were very rare, such as *Q. lenticularis, P. persicae, P. betulae* and *E. novicum.*

We can summarize from the above that in the southern European countries, e.g. Greece, the dominant species on apples was *P. oleae*, in Turkey *Q. perniciosus.* To the north over the geographical distribution area of *Q. perniciosus* this species was dominant while north of this line the dominance was taken over by *L. ulmi.* On pears, in the southern and central areas, *E. leperii* and *Q. perniciosus* were present to the same frequency. *L. ulmi* appeared here also northward. On peach, in the south the heavy infestation of *P. oleae, P. pentagona* and *Q. perniciosus* was characteristic. In the central area *Q. perniciosus* was very frequent, but in small density, while during the outbreak years *P. corni* and *S. prunastri* became dominant. At the northern limits of peach cultivation only very few scales were present on this plant. For example, only 4% of the Czechoslovakian samples were infested with scale insects. On plums, almost in the entire area of our observation, many scale species were present in high density. In Turkey, besides *E. leperii, Q. perniciosus* and *P. corni*, a dominant role was played also by *P. oleae* and *N. halli.* In Hungary, *E. leperii, Q. perniciosus, Q. ostreaeformis* and *P. corni* were very frequent. In Czechoslovakia, on these fruit trees *L. ulmi* and *P. corni* became dominant. On cherry, in Turkey *P. oleae* was dominant, but several other species were frequently present. In Hungary on cherry *Q. perniciosus* became the most important species, while in Czechoslovakia only rarely were scales present; among them more frequently *L. ulmi* and *P. corni.* Our records for sour cherry were limited to the USSR, where the most frequent species in the south was *Q. perniciosus*, while in the north *Q. ostreaeformis* and *L. ulmi.* Sour cherry was found to be among those with low rate of infestation.

Our scale distribution and frequency records represent the situation at the

time of observations. These findings could be utilized for the improvement of present control techniques. By repeating this survey the direction of changes could be established at a later time, and then may be possible to detect the causes of changes in the population dynamics of these species.

Acknowledgements

The Authors would like to express their sincere thanks to Dr. G. MATHYS, Genera Director of EPPO and to Prof. DR. W. WENGOREK, Head of Coordination Centre for Plan Protection Research of COMECON, as well as to DR. T. JERMY, director emeritus of Research Institute for Plant Protection, Budapest, for their kind help in the organization of sample and data collection in European countries. For the careful work of collecting plant samples we are very much obliged to numerous Czech, Greek, Hungarian, Polish, Turkish, Russian colleagues and to directors of the corresponding institutions. Thanks are also due to our ealier coauthors in this project from various countries, moreover R. A. VIKTORIN and J. HORVÁTH for their careful technical help in the work.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 223—231 (1981)

The Action of Precocene 2 on the Development and Reproduction of the Cotton Stainer, *Dysdercus cingulatus* Following Larval Treatments

By

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Third to 5th instar nymphs of *Dysdercus cingulatus* were treated with precocene 2 using topical or contact application methods. The anti-JH compound evoked specific and unspecific developmental effects as well as disturbances in reproduction. Prolonged development, increased larval mortality, and precocious metamorphosis could be observed. Among adults which survived the morphogenetic action of compound, significant reduction of fecundity and egg hatchability was demonstrated. All the main responses proved to be highly age-dependent. The precocene sensitivity decreased in the consecutive larval instars and parallel with the proceeding age within an instar. Precocious metamorphosis could be induced only before the 2nd day of development in the 4th larval stage.

Two chromenes of plant origin, precocene 1 and 2 have been discovered as agents possessing specific anti-JH action (BOWERS, 1976; BOWERS *et al.*, 1976). They induce precocious metamorphosis in immatures of some *Hemiptera* and locusts (BOWERS *et al.*, 1976; MASNER *et al.*, 1979; PEDERSEN, 1978; NĚMEC *et al.*, 1978; CHÊNEVERT *et al.*, 1978; UNNITHAN *et al.*, 1980). Moreover, significant chemosterilant effects of precocenes have also been reported (BOWERS *et al.*, 1976; BOWERS and MARTINEZ-PARDO, 1977; MASNER *et al.*, 1979; UNNITHAN and NAIR, 1979; JUDSON *et al.*, 1979).

At first, these compounds have been regarded as 'antiallatotropins' since an inhibitory action on the neural control of corpora allata (CA) has been assumed (BOWERS, 1976; BOWERS and MARTINEZ-PARDO, 1976). However, a direct influence on CA function ('anti-allatin' effect) has been subsequently demonstrated (PRATT and BOWERS, 1977; MÜLLER *et al.*, 1979; BOWERS and ALDRICH, 1980). Further studies unequivocally pointed to a selective, relatively rapid, and irreversible action of precocenes on the glandular parenchyma of CA resulting in cell degeneration and atrophy of JH producing tissue (UNNITHAN *et al.*, 1977; PENER *et al.*, 1978; SCHOONEVELD, 1979). More recently the in situ oxidative biotransformation of precocenes into reactive, cytotoxic epoxides has been verified (BROOKS *et al.*, 1979; PRATT *et al.*, 1980).

In the present investigations, we aimed to observe specific morphogenetic as well as chemosterilant effects of precocene 2 concomitantly in 3rd to 5th instar

nymphs of *Dysdercus cingulatus*. These studies represent a continuation of our former experiments performed on young larvae of the same species (FARAG and VARJAS, 1980).

Materials and Methods

In our experiments, 3rd, 4th, and 5th instar larvae of the cotton stainer, *Dysdercus cingulatus* F. were used which were collected from a stock laboratory culture reared at 25 °C, ca. 70% r.h., and 18/6 (L/D) photoperiod and fed with cotton seeds. The nymphs of well fixed ages were treated with synthetic precocene 2 (PRE 2; 6,7-dimethoxy-2,2-dimethyl-chromene) in two ways: 1) in permanent contact with coated glass surface (contact method) and 2) with single topical dosages (topical method).

In the case of contact method, bottom parts of glass petri dishes of 9 cm diameter were internally coated with an acetone solution of PRE 2 providing, after evaporation of solvent, a continuous layer of active material. In this experimental series we used 2.0 and 4.0 $\mu g/cm^2$, resp., surface dosages. From a definite age, *Dysdercus* larvae were confined to develop in these treated dishes where food and drinking water were also ensured. In the topical method, 1 μl amounts of an acetone solution was dropped onto the dorsal body surface of cotton stainer nymphs by means of a micro-applicator. In this case 2.0, 4.0, and 8.0 μg/specimen, resp., topical dosages were applied. These larvae were subsequently kept in untreated petri dishes. Untreated control groups were reared under the same conditions as treated ones. All variants were replicated 3 to 4 times.

The development of individual insect groups was checked in 2-day intervals. The numbers of dead larvae as well as precocious or normal adults were recorded. The morphogenetically unaffected, externally normal adults obtained in different experimental variants were placed in pairs (1 female and 1 male) into petri dishes providing them for drinking water and food (4 replicates). The numbers of eggs deposited per female and the egg hatchability were subsequently determined.

Results

Concerning larval development and metamorphosis, precocene treatments resulted in 3 main types of responses: 1) increased larval mortality, 2) precocious metamorphosis, and 3) prolonged larval development.

In Table 1 results concerning the development and transformation of nymphs treated with the contact method are presented. While in control insects the larval mortality proved to be negligable, in PRE 2 treated nymphs diverse, generally high mortality rates depending well on concentrations and larval ages, were demonstrated. In this respect, a significant decrease of precocene sensitivity could be observed in the consecutive larval stages. The 5th instar larvae tolerated even the 4.0 $\mu g/cm^2$ dose which induced 100% mortality in 3rd instar nymphs. Following

Table 1

Effects of precocene 2 on the development and metamorphosis of *Dysdercus cingulatus* larvae treated with the contact method

Treated larval instar*	Dosage $\mu g/cm^2$	No. of treated larvae	Larval mortality (%)	Precocious adults (% of survivors)	Duration of development in consecutive instars (days)		
					3rd	4th	5th
control		58	5.2	0.0	6.2	8.4	11.9
3rd	2.0	90	77.8	55.0	13.8	12.5	14.0
	4.0	100	100	—	—	—	—
4th	2.0	50	28.0	16.7		10.5	16.7
	4.0	67	46.3	33.3		15.5	17.4
5th	2.0	44	4.5	—			10.5
	4.0	50	12.5	—			13.7

* freshly moulted larvae

Table 2

Effects of precocene 2 on the development and metamorphosis of *Dysdercus cingulatus* larvae treated with the topical method

Treated larval instar*	Dosage μg/spec.	No. of treated larvae	Larval mortality (%)	Precocious adults (% of survivors)	Duration of development in consecutive instars (days)		
					3rd	4th	5th
control		58	5.2	0.0	6.2	8.4	11.9
3rd	2.0	50	50.0	12.0	9.9	15.0	17.9
	4.0		100	—	—	—	—
4th	4.0	40	55.0	11.0		12.0	14.0
	8.0	45	100	—		—	—
5th	4.0	36	22.2	—			15.0
	8.0	40	40.0	—			18.6

* freshly moulted larvae

PRE 2 applications to the 3rd and 4th larval stages, a definite fraction of survivors moulted into precocious adults. These forms appeared always after the 4th larval instar. The induction of precocious transformation was found to be age- and dose-dependent. PRE 2 prolonged larval development significantly. This phenomenon was especially conspicuous after treating 3rd instar nymphs but manifested also in other developmental phases subsequent to treated ones. The development of 5th instar larvae moulting into normal adults was also longer than in the control insects.

Data summarized in Table 2 show the responses of *Dysdercus* larvae treated with the topical method. The main effects of PRE 2 were essentially the same but only a minor fraction of surviving insects developed into precocious adults. The differences in precocene sensitivity between the consecutive larval stages were very obvious again.

By using the contact method, we compared also the efficiencies of precocene treatments at different ages within the 3rd and 4th nymphal instars, respectively (Table 3). Regarding both the larval mortality and the percent occurrence of precocious adults, the highest PRE 2 sensitivity was found in freshly ecdysed nymphs. With the proceeding age (from 0 to 3rd day) within both of these developmental stages, precocene treatments became continuously less effective. Applying 4.0 $\mu g/cm^2$ doses to 4th instar cotton stainer larvae, no specific morphogenetic responses could be induced after the 2nd day. Following this critical point of time, application of still higher precocene dosages increased merely the larval mortality without any effect on the normal course of metamorphosis.

Dysdercus adults having developed from precocene treated nymphs were generally able to reproduce. In most cases they mated and the females layed eggs. However, among these morphologically normal adults various degrees of repro-

Table 3

Effects of precocene 2 on the development and metamorphosis of 3rd and 4th instar *Dysdercus cingulatus* larvae of various ages treated with the contact method

Treated larval instar	Dosage $\mu g/cm^2$	Larval age (days)	No. of treated larvae	Larval mortality (%)	Precocious adults (% of survivors)
3rd	2.0	0	90	77.8	55.0
		1	50	64.0	18.2
		2	50	44.0	7.1
		3	40	30.0	0.0
4th	4.0	0	67	46.3	33.3
		1	45	46.7	25.0
		2	45	40.0	0.0
		3	36	38.9	0.0

Table 4

Effects of precocene 2 on the reproduction of *Dysdercus cingulatus* adults developed from larvae which were treated with the contact method

Treated larval instar+	Dosage µg/cm²	No. of eggs laid per female* $\bar{X} \pm s_{\bar{X}}$	Hatchability of eggs* (%) $\bar{X} \pm s_{\bar{X}}$
3rd	2.0	15.0±2.2 a	0.0±0.0 e
4th	4.0	40.8±3.9 b	32.7±8.2 f
	2.0	39.5±5.6 b	46.1±7.0 f
5th	4.0	61.5±4.8 c	83.5±4.3 g
	2.0	74.3±15.2 c d	89.8±1.3 g h
control		92.0±3.6 d	93.4±1.9 h

+ Both sexes were treated.
* Numbers followed by the same letter do not differ significantly (*t*-test, $N = 4$, $p < 0.1$).

Table 5

Effects of precocene 2 on the reproduction of *Dysdercus cingulatus* adults developed from larvae which were treated with the topical method

Treated larval instar+	Dosage µg/spec.	No. of eggs laid per female $\bar{X} \pm s_{\bar{X}}$	Hatchability of eggs (%) $\bar{X} \pm s_{\bar{X}}$
3rd	2.0	0.0±0.0	—
4th	4.0	48.8±2.0	30.7±5.8
5th	8.0	45.3±3.6	33.1±9.4
control		92.0±3.6	93.4±1.9

+ Both sexes were treated.

ductive disturbances could be observed. In many cases both the fecundity and the egg hatchability were more or less reduced. Results concerning the insects treated with the contact method are summarized in Table 4. The most significant effects were obtained in insects which were exposed to the anti-JH compound in the 3rd larval stage. In this case very low numbers of eggs and 100% reduction of egg

hatchability was detected. Thereafter, in the consecutive nymphal instars, the chemosterilant and ovicidal effect of PRE 2 was less pronounced. Between the effectivenesses of the two dosages applied to the 4th and 5th instar nymphs, resp., no significant differences ($p < 0.1$) could be demonstrated. In the last instar only the 4.0 $\mu g/cm^2$ dose induced slight but significant reduction in reproductive capacity in comparison with the corresponding data of control groups.

Essentially the same reproductive disturbances can be reported for *Dysdercus* adults developed from larvae which were treated with the topical method (Table 5). The precocene applications to 3rd instar larvae proved, as in the former experimental series, highly effective. In elder nymphs even the considerable raise of PRE 2 dosages could not evoke such a perfect chemosterilant effect. Nevertheless, the significant influence of precocene on the reproduction was undoubtedly demonstrated also following treatments in 4th and 5th nymphal stages.

Discussion

In the present investigations the effects of PRE 2 on the development and metamorphosis of the cotton stainer were studied parallel with some observations on the reproduction of adults which escaped the morphogenetic action of the compound.

Concerning the developmental effects that were observed after treatments of 3rd and 4th instar *Dysdercus* larvae, the main types of reactions were the same as in our former studies in which young, 1st and 2nd instar nymphs were exposed to precocene (FARAG and VARJAS, 1980). Similarly to results in those investigations, contact treatments proved morphogenetically more effective than topical ones (see Tables 1 and 2). By using elder nymphs, it was also found that the per cent values of larval mortality ran essentially parallel with the rates of specific morphogenetic response.

Except the first instar nymphs (cf. FARAG and VARJAS, 1980), the effectivenesses of PRE 2 treatments decreased in the consecutive larval instars of the cotton stainer. Similar changes in precocene sensitivity were found in *Oncopeltus fasciatus* nymphs (MASNER *et al.*, 1979) and in *Schistocerca gregaria* larvae, too (UNNITHAN *et al.*, 1980). As to the age-dependent sensitivity alterations within a larval instar, essentially the same tendencies were revealed in the present investigations on 3rd and 4th instar nymphs (see Table 3) as it was observed in 2nd instar larvae (FARAG and VARJAS, 1980): the rate of morphogenetic response decreased with the proceeding age. These data coincide with the results of UNNITHAN and NAIR (1979) on 4th instar nymphs of *Oncopeltus fasciatus*.

Precocious metamorphosis could be induced only until the second day of development within the 4th instar. In elder 4th instar nymphs even higher precocene dosages proved in this respect uneffective. They evoked only high larval mortalities. In *Oncopeltus* larvae precocious metamorphosis was found to occur only after precocene treatments prior to the first day within the 4th larval stage (UNNI-

THAN and NAIR, 1979). A similar morphogenetic effect of precocene in *Locusta migratoria* could be induced in the first 2 days within the 4th larval instar (PEDERSEN, 1978).

Following PRE 2 applications, a considerable prolongation of larval development could be demonstrated which was especially pronounced in the treated instar (see Tables 1 and 2). This phenomenon was found also in 5th instar nymphs which developed subsequently into normal adults. A similar reaction was observed in other *Hemiptera* (UNNITHAN and NAIR, 1979) or in locusts (PENER *et al.*, 1978; UNNITHAN *et al.*, 1980).

Dysdercus adults surviving precocene treatments in previous developmental stages exhibited conspicuous defects in their reproduction which was reflected by a significant reduction in fecundity and egg hatchability (see Tables 4 and 5). These effects, following both contact and topical applications, proved highly age-dependent. The most distinct reduction in reproductive capacity was found after treatments in the 3rd nymphal instar.

The specific chemosterilant action was described in adults of some *Hemiptera*, *Diptera*, and *Coleoptera* still in the first publications on precocenes (BOWERS, 1976; BOWERS *et al.*, 1976). Freshly ecdysed females of *Oncopeltus fasciatus* exposed to contact PRE 2 dosages became permanently sterile in consequence of irreversible degeneration of their CA (MASNER *et al.*, 1979; BOWERS and MARTINEZ-PARDO, 1977). In *Dysdercus cingulatus* (= similis ?) topical applications to young females were also effective (JUDSON *et al.*, 1979).

Most authors remark that precociously metamorphosed females are completely sterile. As to the normal, morphogenetically unaffected andults, MASNER *et al.*, (1979) report them to be fertile without any presentation on detailed studies in this respect. It is merely the paper of UNNITHAN and NAIR (1979) which demonstrates positive reactions subsequent to larval treatments in *Oncopeltus fasciatus*. Adults developed from 4th instar *Oncopeltus* nymphs being treated with precocene reacted fairly well but no reproductive deficiencies could be evoked with applications to freshly moulted 5th instar larvae. Our present data confirm these results on age-dependent differences in precocene susceptibility concerning specific effects on reproduction.

Precocious metamorphosis and chemosterilization are regarded as the most pregnant consequences of the specific inhibitory action of precocenes on CA. UNNITHAN and NAIR (1979) assume that the extents of these responses can be well related to the activity of CA at the time of treatment. The authors explained the differences in PRE 2 sensitivity with this assumption. Our results lead us to the same conclusion. The continuous decrease of precocene susceptibility during larval development can be well compared with a similar tendency in CA activity. The age-dependent changes in PRE 2 susceptibility within an instar, i.e. the higher rate of responses at the beginning and a steady decrease of them thereafter, point to a cyclic function of these endocrine organs in the consecutive nymphal stages. The first instar nymphs (cf. FARAG and VARJAS, 1980) represent presumably an exceptional case: in the first half of this larval instar CA remain possibly inactive.

The direct toxicity of precocenes on larvae of *Hemiptera* and their inhibitory action on the rate of larval development are considered as unspecific reactions which accompany the primary effects. The high larval mortality, in some cases, simply 'masks' the induction of precocious metamorphosis. The similar tendencies in the alterations of larval mortality, on one side, and in the induction rates of precocious metamorphosis, on the other side, (see Tables 1 to 3) indicate a definite involvement of CA intoxication in the production of higher mortality rates.

Acknowledgement

For the critical reading of the manuscript authors want to express their sincere thanks to DR. Á. SZENTESI.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 233—237 (1981)

Philaenus spumarius Linné as a Vector of the Causative Pathogen of Rubus Stunt Disease

By

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In the course of the transmission experiments carried out in the present work, the authors were able to report a new vector *(Philaenus spumarius)* transmitting the mycoplasma causing rubus stunt disease of raspberry. It was also shown that the minimal acquisition period of time is seven days. The incubation period was 4—5 weeks and *Ph. spumarius* was found to be able to maintain its ability to transmit the mentioned mycoplasma up to the end of its life.

Data approached from our observations indicate that Philaenus spumarius is one of the most common leafhoppers inhabiting raspberry plantations in Hungary. Taking into consideration the fact that the mentioned leafhopper species was shown to be the transmitting vector of the causative agent of Pierce's disease (Severin, 1947; 1950), we found it suitable to investigate, the ability of *Ph. spumarius*, to transmit the pathogen causing the rubus stunt disease (mycoplasma) of raspberry. According to Fluiter and Van der Meer (1953, 1958), Murant and Roberts (1971) and Reitzel (1971) *Macropsis fuscula* Zetterstedt and *Macropsis scotti* Edwards, are the only two leafhoppers known to be vectors, of the mycoplasma under consideration.

Materials and Methods

Larvae and adults of *Ph. spumarius*, collected from Budapest-Budatétény and Budaörs-Kamaraerdő, were involved in the transmitting experiments. Before using them, collected larvae and adults, were critically examined, for their freedom from any contaminating pathogen. Diseased raspberry stocks as a source of the pathogen of rubus stunt were forwarded by Dr. Achmet Seljahudin. Celery plants, propagated under well isolated conditions in the greenhouse, were used as indicator hosts.

Micro-isolators, were especially constructed, to insure the continuous stay of the leafhoppers on the host plant throughout the period of acquisition and inoculation feedings. The mentioned isolators, consisted of plastic boxes, each made, of two parts having the following dimensions: 4, 13 and 18 cm. From each side of such a box, a 9 by 15 cm plate was cut off, and replaced by a net, having pores of 1—2 mm in diameter.

Acquisition and inoculation feedings were as follows:

- Feeding of adults on naturally diseased raspberry, and transferring them onto healthy celery.
- Feeding of larvae on naturally diseased raspberry, and transferring the developing adults onto healthy celery.
- Feeding of adults on artificially infected (by *Ph. spumarius)* celery and transferring them onto healthy celery.
- Feeding of larvae on artificially infected (by *Ph. spumarius)* celery and transferring them onto healthy celery.

In case of both acquisition and inoculation feedings, five leafhoppers were placed on each plant.

The acquisition feeding period, had ranged from 3–15 days. On the other hand, the inoculation feeding period had lasted for 70 days, during which, leafhoppers were transferred to five plants one after the other.

To check results, subsequent transmissions by *Ph. spumarius* were carried out from, artificially infected celery plants showing clear symptoms, to healthy ones. Success of transmissions, was also verified, by electron microscopic examinations.

The samples for electrone microscopy examinations were fixed by osmium tetraoxide and glutaric aldehyde. The embedding was done onto Durcupan ACM resin after dehidrating in an alcoholic serie. The ultrathin sections were prepared by LKB type ultramicrotome. The negative staining was done by uranyl acetate and lead citrate. The samples were examined by OPTON EM 9 S 2 type electron microscope.

Results

According to the results approached in the present work, it was determined that 7–15 days was the minimum acquisition feeding period, after which adults of *Ph. spumarius* came to be able to transmit the mycoplasma under consideration. As far as larvae are concerned, it was found that a 3-days long acquisition period was not enough for them to be infectious. Adults developing from larvae which exposed to infected plants for 3 days were not infectious.

On the other hand, adults developing from larvae, which acquisted for 7 days were found to be able to transmit the pathogen. A period of 4–5 weeks, after acquisition was found to be necessary for the adults, to be able to infect indicator plants. Mycoplasma-carrying adults of *Ph. spumarius*, maintain their infectivity for 10 weeks. In other words, they remain infectious up to the end of their lives.

Symptoms started, to be expressed on the inoculated indicator plants, about 6–8 months after transmission experiments. In comparison to their controls, leaves of diseased celery were smaller in size, their number per plant was much greater, petioles were shorter, and they were horizontally positioned. (In addition to the above-mentioned symptoms, infected celery plants, proved to be, unable to flower).

Fig. 1. Infected celery plant with the pathogen of rubus stunt disease (in the right side) and control plant (in the left)

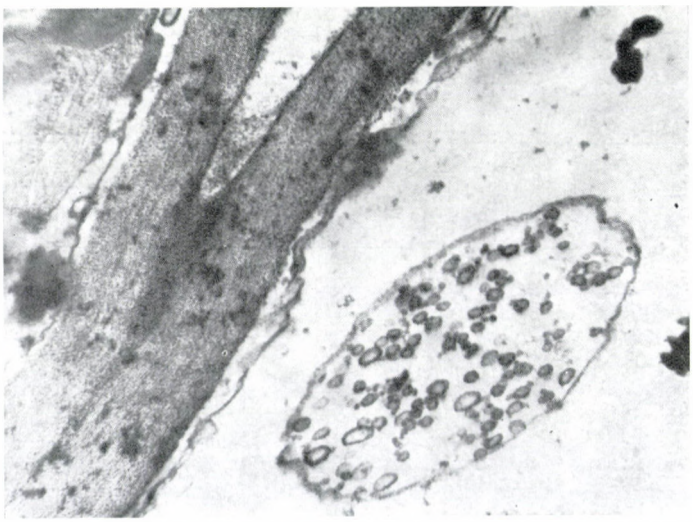

Fig. 2. Mycoplasma like bodies in the cytoplasma of infected celery plant

Table 1

Transmission of the pathogen of rubus stunt from raspberry to celery by *Philaenus spumarius* (L.)
(Budapest, 1978—79)

In 1978

Stage	Acquisition feeding period (days)	Efficiency of transmission within				
		3 weeks	5 weeks	7 weeks	9 weeks	10—11 weeks
Larvae	control	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	0/5	0/5
	21	0/5	0/5	1/5	2/5	2/5
Adult	nonfed	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	0/5	0/5
	5	0/5	0/5	0/5	0/5	0/5
	7	0/5	2/5	2/5	2/5	2/5
	15	0/5	3/5	3/5	3/5	3/5

In 1979

Adult	control	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	0/5	0/5
	5	0/5	0/5	0/5	0/5	0/5
	7	0/5	2/5	2/5	2/5	2/5
	15	0/5	3/5	3/5	3/5	3/5

Table 2

Transmission of the pathogen of rubus stunt from infected celery to celery by *Philaenus spumarius* (L.)
(Budapest, 1979—80)

Stage	Acquisition feeding period (days)	Efficiency of transmission after				
		3 weeks	5 weeks	7 weeks	9 weeks	10—11 weeks
Larvae	control	0/5	0/5	0/5	0/5	0/5
	7	0/5	1/5	1/5	1/5	1/5
	15	0/5	2/5	2/5	2/5	2/5
	21	0/5	3/5	3/5	3/5	3/5
Adult	control	0/5	0/5	0/5	0/5	0/5
	3	0/5	0/5	0/5	0/5	0/5
	5	0/5	0/5	0/5	0/5	0/5
	7	0/5	2/5	2/5	2/5	2/5
	15	0/5	3/5	3/5	3/5	3/5

As a confirmation to the fact that the disease under consideration was caused by mycoplasma, leafhopper, were used in transmission from raspberry (showing clear symptoms of rubus stunt disease) to celery. Transmissions were also successfully repeated from infected celery to healthy ones. Moreover, electron micrographs, of negatively stained ultrathin sections, of leaf blades and petioles of diseased celery, showed clear mycoplasma like bodies.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 239—243 (1981)

An Approach to Integrated Pest Management from the Chemical Industry

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The development of ecologically safe pesticides is a major contribution of the chemical industry to Integrated Pest Management (IPM).

In a first selection process nowadays, only pesticides with acceptable mammalian toxicity are promoted for further development. A further step in our company is the screening at an early stage in the laboratory against the following representative beneficial species: *Anthocoris nemorum* (flower bug), *Chrysopa carnea* (lacewing), *Coccinella punctata* (ladybird beetle), *Coccoygomimus* (Pimpla) *turionellae* (ichneumonid wasp) and *Amblyseius fallacis* (predatory mite). Laboratory selectivity tests are then complemented by field trials against the whole beneficial complex in deciduous fruit and cotton.

New approaches in the chemical syntheses aim at the type of selectivity exemplified by compounds such as chlorodimeform and diflubenzuron. Efforts are also made to synthesize compounds similar to natural plant products, which have hormonal and behavioural effects. One such compound, CGA-29' 170, is actually in development. In the field of more potent toxins a further diversification is derived from *Bacillus thuringiensis*.

The final aim is the development of integrated systems in various crops built around selective pesticides. A first such long-term project has been started in deciduous fruit and will last for several years.

The need for an integrated approach to crop protection is becoming more and more evident as more and more problems of unilateral controls are being discovered. Therefore, a basic requirement for maintaining present levels of crop protection and reducing present losses is the availability of feasible pest management programs for all of our crops. These will require multidisciplinary efforts by scientists in the crop protection, crop production, economics, and related disciplines as well as the cooperation of private industry.

Integrated Pest Management (IPM) is a concept of crop production incorporating effective, stable, long-lasting crop protection components that avoid the negative side effects of current pest control actions. Organic pesticides were initially acclaimed as ultimate solutions to crop protection problems, but experience has shown that for all their advantages with respect to improvements in human health and the conservation of food and fiber, they have many limitations. In particular pest resistance to insecticides and secondary induced pests have resulted in losses. Biological, cultural, genetic and other tactics are all partially effective against specific pests but have limitations that restrict their general usefulness in crop pro-

duction. These developments have led to a general recognition over the past two decades of the need for improved crop protection systems. The IPM concept developed in response to this need.

Present problems and the concern over the use of pesticides for agricultural crops focus on health hazards, environmental solution and the availability and effectiveness of new compounds.

Health problems associated with pesticides involve acute (or subacute) and chronic low-level effects. In developed countries, where medical services are readily available and poison control centres have been established, acute effects are relatively clear-cut and can be identified correctly. The safe use of pesticides has received great emphasis over the past 25 years. The effort has succeeded despite the vast increase in the availability and use of pesticides during this period; the incidence of fatal poisonings directly attributed to pesticides has dropped countinually – e.g. in the US from 152 in 1956 to 31 in 1976 – while total production has more than doubled. The meagre data that are available from developing countries indicate much higher death rates, even though pesticides are not used as extensively as in the US. Although acute toxicity episodes can be minimized through education, they remain a continuing hazard, especially where educational and medical facilities are minimal. Commercial companies nowadays lay great stress on the selection of safer compounds in the development screens. The organochlorine insecticides, which dominated the late forthies and fifthies were much more persistent than modern products. The use of these older products have been discontinued in the developed countries and a steady decline has occurred in the concentrations of these materials in the environment. Nowadays toxicological studies on several experimental species including mammals, birds and fish must be conducted before pesticides can be registered for use in most countries. About 30 toxicological tests are carried out with new developmental products according to the requirements of the Environmental Protection Agency (EPA) in the US. The last step in this procedure are 'bioaccumulation' tests i.e. the process in which low levels of a chemical in organisms, such as algae, at the bottom of the food chain, accumulate through the food chain until high levels occur in such animals such as fish or birds at the top of the chain.

A major overall concern about pesticides is the lack of availability of compounds possessing only the required range of activity against pests. This is particularly the case in developing pest management systems that involve the use of pesticides. For some situations, an insecticide or miticide with a very narrow range or short residual activity is required to reduce a pest species without disrupting biological control agents. In other cases, pesticides may be required that control a broad range of weeds, pathogens, or insects. Sometimes short residual contact materials are required while for others, as for control of soil insects or season-long weed control, residual effectiveness may be required for several months. When no pesticide with appropriate activities is available, substitutes often have to be used at higher rates with repeated applications and sometimes with harmful effects on beneficial species.

To minimize these risks, a Working Group of the International Organization for Biological Control (IOBC) was founded 20 years ago to develop standardized methods to measure the side effects of pesticides on beneficial arthropods. 15 guidelines or drafts for initial toxicity tests have been developed in the meantime. In our own laboratories, the following representative beneficial species are included in the screening process for advanced candidate insecticides and fungicides for contact activity:

Order	Common name	Scientific name
Heteroptery	Flower bug	*Anthocoris nemorum*
Neuroptera	Green lacewing	*Chrysopa carnea*
Coleoptera	Ladybird beetle	*Coccinella 7-punctata*
Hymenoptera	Ichneumonid wasp	*Coccygomimus turionellae*
Acarina	Predatory mite	*Amblyseius fallacis*

At present, there are two crops where selective compounds are most desired – cotton and deciduous fruit. Candidate selective compounds are taken to the field and tested under more practical conditions. A method was developed within our company where the knock-down of beneficial insects on apple trees after a treatment is measured with the help of plastic sheets. The experimental treatment is followed by a 'clean-up' spray with a total poison, which allows the measurement of the whole insect population on the treated trees so that the insect mortality caused by the experimental spray can be expressed as a percentage of the total beneficial population (SECHSER and BATHE, 1978).

Since conventional chemical pesticides remain one of the most powerful and dependable tools available for the management of pest populations, the question is not whether their use should be continued, rather it is a question of how they may be used with the minimum of undesirable side effects and complications. The most appropriate answer to this question is in the selective use of insecticides, and in the use of selective insecticides.

Selectivity, in the broad sense, is accounted for by differences in physiological sensitivity (physiological selectivity) after contact between organisms and toxic chemical and by differences in exposure or behaviour of the fauna (ecological selectivity). Selective physiological toxicity is defined as injury of one kind of living matter without harming some other kind with which the first is in intimate contact. Two striking examples for such a type of compound are chlorodimeform, used in IPM cotton programmes in the US against bollworm and budworm, and diflubenzuron, applied in the same crop against the boll weevil. One might also think back to such compounds as nicotine sulfate, lead arsenate and ryania for codling moth control and their relative innocuousness to natural enemies. There are enough examples of narrowly selective insecticides to justify the belief that chemicals that are toxic to single families, genera, and even species can be developed.

Unlike physiological selectivity which stresses activity of the chemical, ecological selectivity emphasizes application. Ecological selectivity is secured by applying compounds having broad spectra of activity but in a manner that ensures contact of a toxic dose with the target species while avoiding completely, or greatly minimizing, contact of a toxic dose with nontarget species. Selective action of non selective chemicals can be obtained by manipulating dosage, formulations, timing of applications, method of application, and the localization of the area to be treated. Especially by reducing the dosage rate by applying products against the more sensitive egg stage instead of against the harder to kill larval stages, opens a wide field for the more selective use of existing products.

An intensive effort to explore the potential for pest control of the insect growth regulators (IGRs) and their analogues has been underway for almost a decade. Only the Juvenile Hormone analogues have so far been demonstrated to have any promise for use, where the following degrees of activity were exhibited: sterility, reduced reproduction, ovicidal effects, reduced emergence, deformity, mortality, and abnormalities in larval pigmentation. Their greatest weakness appears to be that they have no effect on young larvae or nymphs, are effective only during critically short periods in the life cycle of most species and often require long periods of time between application and expression of effect. The ovicidal action seems to be most promising. We have such a product, CGA-29'170, which is presently tested on a large scale against the winter eggs of the European red mite, *Panonychus ulmi*. With proper timing at temperatures below 22 °C and good coverage, mite suppression up to three months has been achieved. Its high selectivity against predators and parasites on the one side, but the limited possibility with regard to application time on the other side, will allow its use only in carefully evaluated IPM programmes.

A classical method of biological control but similar in its application to insecticides is the use of pathogens, of which Bacillus thuringiensis is the most successful case. It produces a parasporal crystal in its sporangium at sporulation, which is activated when ingested by the gut juices of Lepidoptera. In the past it has not competed well with insecticides, mainly for the reasons of low virulence, high production costs, storage problems, slow production of mortality in the target insect and, especially for industry, limited sale potential because the number of target species is small. By treating them with mutagenic substances we try to obtain strains with a broader spectrum of activity and faster action.

The final aim of our efforts is the development of a concept of crop production incorporating effective, stable, long-lasting crop protection components that minimize the negative side effects of current pest control actions. A long-term study has been started in a Swiss apple orchard, where selective treatments are compared to a conventional programme and no insecticidal treatment at all (SECHSER, 1980). The tools in this IPM programme include the application of selective insecticides for codling moth control, the use of pheromone traps for monitoring purposes, the application of an IGR against winter eggs of the European red mite and of selective acaricides against later generations, the release of a predatory

mite species, *Amblyseius fallacis*, the use of selective fungicides against this predatory mite and the monitoring of the seasonal development of the beneficial insect complex. We finally hope we can offer to apple growers an effective, but safe crop production system.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 245–248 (1981)

CONCEP^R (CGA-43089), a Safening Agent Protecting Sorghum *(Sorghum bicolor)* from Metolachlor Injury

By

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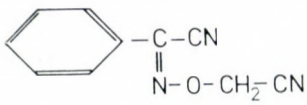
CONCEP^R is another example of a safening agent permitting the extension of a potent herbicide into a crop in which this herbicide alone is not selective. In particular, CONCEP^R protects Grain Sorghum Hybrids from metolachlor injury.

In this paper, which refers to two previous publications, details are given on the biological performance under various laboratory conditions and under field conditions. The application of the safening agent and the extension of the herbicide metolachlor into Grain Sorghum Hybrids is summarized.

CONCEP^R or α-(cyanomethoximino)-benzacetonitrile is a safening agent which protects Sorghum Hybrids *(Sorghum bicotor* (L) Moench) from injuries of metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide. The new safening agent was first described by ELLIS *et al.*, 1), and NYFFELER *et al.*, 2) at the annual Meeting of the Weed Science Society of America, 1978. This paper summarizes these 2 publications, without giving detailed descriptions of methods, experimental conditions and experiments.

In 1980, CONCEP^R was introduced for practical use in the USA. It has proven its qualities as a safening agent under diverse field conditions and will become a standard method in the areas where a strong grasskiller i.e. metolachlor should be used in grain sorghum. Further introductions in other countries are under way.

General Data

Code	:	CGA 43089
Trade Name	:	CONCEP^R
Formulation	:	CONCEP^R 250 SO (in the USA CONCEP^R 2.09 S) –, a liquid containing 250 g/lt. of CGA 43089 active ingredient
Chemical Name	:	α-(cyanomethoximino)-benzacetonitrile
Structural Formula	:	

Empirical Formula	: $C_{10}H_7N_3O$
Molecular Weight	: 185.2
Melting Point	: 55 – 56 °C
Solubility	: 95 ppm in water at 20 °C 55 % benzene 23 % methanol
Toxicity	: CONCEP^R has a moderate oral toxicity to mammals and a low toxicity to fish and birds

Biological results of laboratory and field experiments with CONCEP^R

Placement of the safening agent. CONCEP^R was first used in tankmixture with metolachlor. Considerable safening activity was observed when the rate of CONCEP^R was 4 or more times higher than the rate of the herbicide. A much better ratio safening agent/herbicide is obtained when CONCEP^R is applied on the seed of sorghum hybrids as a dressing: 1.0 – 1.25 g ai of CONCEP^R per 1 kg of seed gives full protection from rates of metolachlor up to 4 kg ai/ha. The exact placement of the safening agent on the seed of the crop has also the advantage that the protection is not extended to weed species, such as *Sorghum halepense.*

Response of sorghum cultivars. Among the germplasms of *Sorghum bicolor* (L) Moench, the subspecies – *subglabrescens, caffrorum, zera-zera, caudatum/kafir* and *roxburghii*, which may be used as a basic material for new grain sorghum hybrids, permit the safe use of metolachlor if they undergo a previous seeddressing with CONCEP^R. In laboratory experiments the 2 subspecies *S. bicolor* ssp. *dochna* and *-sudanense*, both less important as germplasms for grain sorghum hybrids, were less well protected. Cultivars based on these germplasms should be submitted to a careful testing before large practical applications are made.

Effect of temperature. Since grain sorghum hybrids are preferably grown in warm and hot areas, special growth chamber experiments were made, which deg monstrated the following results:

At the highest temperature (38 °C day/22 °C night) the intensity of safenin-activity was higher and phytotoxicity was lower than at any lower temperature. At the temperatures 29 °C day/18 °C night and 22 °C day/14 °C night, respectively, the injury of metolachlor did increase, but CONCEP^R could also compensate this increased risk. Thus the safening potential of CONCEP^R does not depend on the injury potential of metolachlor at a given temperature.

Effect of soil moisture. On many sites planted with grain sorghum hybrids, moisture in the soil may undergo extreme changes within a short time. In a growth chamber experiment with soils maintained at 50 %, 60 % and 90 % of their Field Capacity (FC), it was found that at the high moisture level, both, the safening activity and the herbicidal activity are high and that both decrease when moisture content falls to 60 % FC and 50 % FC. Thus under all soil moisture conditions metolachlor can safely be used on CONCEP^R-treated seed of grain sorghum hybrids.

The investigations with different temperature and moisture levels confirm that the activity of CONCEP^R depends on these 2 important environmental factors in the same way as the herbicidal activity of metolachlor.

Residues, penetration, site of uptake. Results of residue-analyses on and in seeds of sorghum hybrids treated with CONCEP^R demonstrate that the active ingredient is rapidly taken up by the seed. The uptake depends on the form of application: CONCEP^R diluted in an organic solvent is taken up more readily than when applied as a dry seed dust or as a suspension of a wettable powder.

Also, the timing of the application of CONCEP^R has a remarkable influence on its safening activity. When CONCEP^R is put on seeds immediately before planting, a considerable reduction in safening activity is observed. Treatments of the seed of grain sorghum 4 weeks before planting bring full protection.

In laboratory experiments, CONCEP^R was placed respectively in the root and shoot area of the seed of sorghum hybrids, and its vertical movement was inhibited by a thin charcoal layer immediately below or above the seed grains. Protection of the sorghum plants was complete when CONCEP^R could be taken up by the hypocotyls of the germinating plants, whereas protection was almost totally lost when the safening agent was placed in the root zone of the young plants. Since metolachlor is preferably taken up by the shoots of growing grasses (3), it seems to be essential that CONCEP^R and metolachlor should meet at their preferred site of uptake in order to obtain adequate protection, so that only when this immediate confrontation occurs, is a satisfactory safening activity obtained. This encounter of the safening agent with the herbicide is obviously assured by the application of CONCEP^R to the seed at least 4 weeks prior to planting time.

For a good safening activity, as extended experiments have shown, it is important to cover the entire seed grain carefully with CONCEP^R. It follows from this requirement that a liquid treatment of the seed is preferable.

Duration of safening activity in stored seed. Seed dressed with CONCEP^R was stored and tested at intervals up to 18 months after initial treatment. There is evidence that safening activity is not altered during a storage time of 18 months, if the original amount of CONCEP^R was 1 – 1.25 g ai/kg of seed. Under practical conditions, it is possible to carry over seed to another planting season without loss of safening activity.

Application of CONCEP^R under practical conditions

Large-scale use of CONCEP^R can be introduced during the processing of grain sorghum seed. The application is possible after cleaning and grading of the seed in 2 ways:

a) as a sequential treatment after the seed has received the usual fungicide/insecticide protection;

b) as a "one shot treatment" together with the usual fungicide/insecticide sedderessing. Our experience is limited to a captan/methoxychlor seed-dressing

available as a 78% wettable powder, containing 75% captan and 3% methoxychlor (Trade Name Ortho 75/3).

For treatments a) and b) a solution with an organic solvent, containing 250 g CONCEP^R active ingredient per liter is prepared. For the sequential treatment a), 5 ml/kg of seed are applied (1.25 g ai/kg of seed) using the usual dressing equipment, which should have reliably adjusted dosage installation. The dressing process must produce a grain which is entirely covered by the CONCEP^R solution.

For the treatment b), 400 g of Ortho 75/3 (or corresponding rates of similar WP's) are suspended in the CONCEP^R SO 250. The volume of such a mixture is 6.5 ml per 1 kg of seed.

It is important to apply CONCEP^R only to high-class selected seed with a good germination faculty, a high degree of purity, and no broken grains. Germination tests of seed samples should be executed prior to and after the seed-dressing.

Detailed instructions are available on request.

Improved weed control in grain sorghum hybrids

Different sources (1) report an increase of grass infestations in many sorghum growing areas, showing that the current standard herbicides often do not lead to the required elimination of harmful grasses. With the introduction of CONCEP^R as a safening agent, the application of metolachlor as a potent grasskiller opens the way to a considerable and reliable improvement of grass control in grain sorghum hybrids.

The rate of metolachlor (Trade Name Dual EC 720 or Dual EC 960) depends on the locally important grass problems and the prevailing quality of soil structure and texture. The most frequently applied rates are between 1.5–2.5 kg ai/ha.

Whenever grasses appear in association with broadleaved weeds, combinations of metolachlor with triazines are to be preferred as broadspectrum solutions.

The preference is given to PRIMEXTRA^R comprising various combinations of metolachlor + atrazine with ratios adapted to local conditions. In the USA, BICEP^R (metolachlor + atrazine, ratio 1.25 : 1) and MILOCEP^R (metolachlor + propazine, ratio 1 : 1) are in extended use. For some areas, it is also proposed to combine metolachlor + terbuthylazine for full spectrum control. The selection of the appropriate triazine depends on the local infestation and soil conditions.

One has to appreciate that while CONCEP^R protects sorghum from metolachlor injury, it does not offer protection from excessive rates of triazines.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1—2), pp. 249—258 (1981)

Heterocyclic Quaternary Ammonium Salts as Plant Growth Retardants

By

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Some tertiary amines together with their quaternary ammonium salts were prepared from some cycloaliphatic amines and have been tested for plant growth retardant activity. Most of the compounds revealed an activity lower than chlorocholine chloride (CCC), but some of them were more selective. Relationships between structure and activity are briefly discussed.

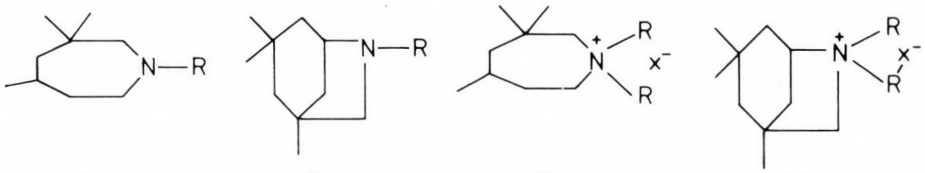
The ability of certain quaternary ammonium compounds to reduce stem elongation of bean plants without causing abnormal, formative changes was first reported by MITCHELL *et al.* (1949). The biological activity of quaternary ammonium salts as fungicides, insecticides, and bactericides has been reported extensively in the literature (DE BENNEVILLE, 1956). One of the most active compounds was a quaternary ammonium carbamate, designated Amo-1618. Many works have been done on the synthesis and testing of derivatives of this compound (KREWSON *et al.*, 1959). (2-chloroethyl)trimethyl ammonium chloride (chlormequat) was also reported to be active in this respect (TOLBERT, 1959).

KNIGHT *et al.* (1969) have studied the influence on plant growth retardant activity of various substituents of aryl quaternary ammonium, phosphonium, and sulphonium compounds. The plant growth retardant activity of some benzyl quaternary ammonium derivatives synthesized from the terpene (+)-limonene has been reported also (NEWHALL and PIERINGER, 1966; 1969; and 1970). Studies on the relationship between chemical structure and plant regulator activity had led to the synthesis of nonterpenoid 2-hydroxycyclohexy ammonium compounds which were studied as plant growth retardants by NEWHALL in 1974.

In 1975, NEWHALL *et al.* demonstrated the direct correlation between enzyme inhibition and plant growth retardation for some n-alkyl derivatives of 2-hydroxycyclohexyldimethyl ammonium bromide and reported that the inhibition of bean root cholinesterase was competitive and reversible. These correlation between growth regulation and enzyme inhibtion suggest that these quaternary ammonium derivatives may act on an enzyme-mediated step essential to plant growth.

Recently, KWANG *et al.* (1978), synthesized and examined some new trimethyl ammonium iodides containing cyclohexane or cyclohexene ring as plant growth retardants.

These results prompted us to synthesize and test for plant growth retardant activity heterocyclic tertiary amines and quaternary ammonium derivatives prepared from (3,5,5) trimethyl-1-azacycloheptane(TMD-imine) and 1,3,3-trimethyl-6-azabicyclo-(3,2,1)-octane (IPD-imine). These derivatives are characterized by the chemical formulae I–IV.



I II III IV

Materials and Methods

Chemical

a) Preparation of N-alkyl imines (I and II)

To a mixture of (0.2 mole) imine and 88 ml (0.22 mole) 10% aqueous NaOH solution is added (0.22 mole) of the corresponding alkyl halide with stirring. After addition, stirring is continued for further one hour at room temperature (the reaction mixture must be alkaline at the end). The reaction mixture separates into two layers, the upper one is separated and the aqueous layer is extracted with ether, the combined ethereal solution is washed with water and dried over anhydrous sodium sulphate. The solvent is removed under reduced pressure and the organic residue is distilled in vacuum.

b) Preparation of N-alkylammonium salts (III and IV)

To (0.03 mole) imine dissolved in 30 ml petrol ether (B. P. 100–120 °C) is added (0.03 mole) of the corresponding alkyl halide. The reaction mixture is allowed to stand at room temperature for one day. The salt formed is filtered, washed with petrol ether (B. P. 100–120 °C) twice, dried in vacuum dissicator and collected.

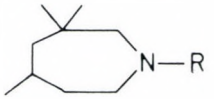
c) Preparation of N,N-dialkylammonium salts (III and IV)

To (0.015 mole) of the N-alkyl imine dissolved in 25 ml petrol ether (B. P. 100–120 °C) is added (0.015 mole) of the corresponding alkyl halide. The reaction mixture is refluxed for 30 minutes, allowed to stand over night. The product is filtered, washed with petrol ether (B. P. 100–120°C) twice, dried and kept in a vacuum dissicator.

The structures of these compounds were identified by methods of elementary, IR and NMR analysis. Physical and analytical data are listed in tables 1, 2, 3, and 4. Melting points were uncorrected. The infrared and nuclear magnetic resonance spectrum of representatives of the tertiary amines and quaternary ammo-

Table 1

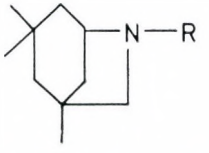
Physical properties of N-alkyl-TMD-imines



Compound No	R	Reaction temperature	M. P. or B. P. °C	Formula	Mol. Wt.	Yield %	C, %	H, %	N, %
1	CH_3	below 20 °C	181+1/760 mm	$C_{10}H_{21}N$	155	71	c, 77.41 f, 77.35	13.54 13.40	9.03 9.02
2	CH_3CH_2	50–60 °C	83+2/20 mm	$C_{11}H_{23}N$	169	73	c, 78.10 f, 78.01	13.06 13.21	8.28 7.92
3	$CH_2=CHCH_2$	below 20 °C	90+2/20 mm	$C_{12}H_{23}N$	181	73	c, 79.55 f, 79.26	12.70 12.51	7.63 7.64
4	$CH_3(CH_2)_3$	60–70 °C	108+2/20 mm	$C_{13}H_{27}N$	197	70	c, 79.18 f, 79.01	13.70 13.62	7.10 7.01
5	$C_{10}H_{19}$ Mentyl	60–70 °C	147+5/20 mm	$C_{19}H_{37}N$	279	75	c, 81.72 f, 81.61	13.26 13.19	5.01 4.97

Table 2

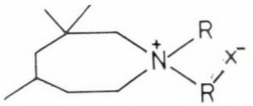
Physical properties of N-alkyl-IPD-imines



Compound No.	R	Reaction temperature	M. P. or B. P. °C	Formula	Mol. Wt.	Yield %	C, %	H, %	N, %
6	CH_3	below 20 °C	189+1/760 mm	$C_{11}H_{21}N$	167	83	c, 79.04 f, 79.00	12.57 12.49	8.38 8.11
7	CH_3CH_2	below 20 °C	110+2/20 mm	$C_{12}H_{23}N$	181	85	c, 79.55 f, 79.29	12.70 12.48	7.73 7.61
8	$CH_2=CHCH_2$	below 20 °C	114+4/20 mm	$C_{13}H_{23}N$	193	87	c, 80.82 f, 80.53	11.91 11.72	7.25 7.01
9	$CH_3(CH_2)_3$	60–70 °C	121+1/20 mm	$C_{14}H_{27}N$	209	82	c, 80.38 f, 80.12	12.91 12.82	6.69 6.65

Table 3

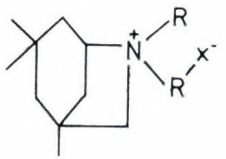
Physical properties of N-alkyl and N,N-dialkyl-TMD-ammonium salts



Compound No.	R	R′	X	M. P. °C	Formula	Mol. Wt.	Yield %		C, %	H, %	N, %	X, %
10	CH_3	H	I	280	$C_{10}H_{22}NI$	283	85	c,	42.40	7.77	4.94	44.87
								f,	42.03	7.54	4.76	44.49
11	CH_3CH_2	H	I	206+2	$C_{11}H_{24}NI$	297	82	c,	44.44	8.08	4.71	42.76
								f,	44.13	7.88	4.62	42.24
12	$CH_3(CH_2)_3$	H	Br	238+2	$C_{13}H_{28}NBr$	278	65	c,	56.11	10.07	5.03	28.77
								f,	56.02	9.97	4.97	28.58
13	$C_6H_5CH_2$	H	Cl	243+3	$C_{16}H_{26}NCl$	268	75	c,	71.64	9.70	5.22	13.43
								f,	71.35	9.59	4.96	13.19
14	CH_3	CH_3	I	284+4	$C_{11}H_{23}NI$	296	89	c,	44.59	7.77	4.72	42.90
								f,	44.21	7.53	4.61	42.63
15	CH_3CH_2	CH_3	I	279+1	$C_{12}H_{26}NI$	311	86	c,	46.30	8.36	4.50	40.83
								f,	46.11	8.04	4.40	40.51
16	$CH_2=CHCH_2$	CH_3	Br	246+2	$C_{13}H_{26}NBr$	276	85	c,	56.52	9.42	4.93	28.98
								f,	56.31	9.20	4.42	28.71
17	$C_{10}H_{17}$ Geranyl	CH_3	Br	271	$C_{20}H_{38}NBr$	372	85	c,	64.51	10.21	3.76	21.50
								f,	64.30	9.98	3.43	21.39
18	2,4-$(NO_2)_2C_6H_3$	CH_3	Cl	148+2	$C_{16}H_{25}O_4N_3Cl$	359	78	c,	53.48	6.96	11.70	10.01
								f,	53.21	6.72	11.64	9.88
19	$CH_2=CHCH_2$	$CH_3(CH_2)_3$	Br	156+1	$C_{16}H_{32}NBr$	318	80	c,	60.37	10.06	4.40	25.15
								f,	60.09	9.86	4.13	24.91
20	CH_3CH_2	CH_3CH_2	I	192	$C_{13}H_{28}NI$	325	78	c,	48.00	8.61	4.30	39.07
								f,	47.61	8.39	4.13	39.01
21	CH_3CH_2	$CH_3=CHCH_2$	I	171+3	$C_{14}H_{28}NI$	337	20	c,	49.85	8.30	4.15	37.68
								f,	49.52	8.11	4.10	37.43
22	CH_3CH_2	$CH_3(CH_2)_3$	I	159+3	$C_{15}H_{32}NI$	353	82	c,	50.99	9.06	3.96	35.97
								f,	50.71	8.96	3.72	35.51

Table 4

Physical properties of N-alkyl and N,N-dialkyl-IPD-ammonium salts



Compound No.	R	R′	X	M. P. °C	Formula	Mol. Wt.	Yield %	C, %	H, %	N, %	X, %
23	CH_3	H	I	129	$C_{11}H_{22}NI$	295	86	c, 44.74 f, 44.62	7.45 7.19	4.74 4.70	43.05 42.82
24	CH_3CH_2	H	I	182+2	$C_{12}H_{24}NI$	309	81	c, 46.60 f, 46.41	7.76 7.55	4.53 4.28	41.10 40.80
25	$CH_2=CHCH_2$	H	Br	118+2	$C_{13}H_{24}NBr$	274	87	c, 56.93 f, 56.69	8.87 8.53	5.01 4.87	29.19 28.99
26	$CH_3(CH_2)_3$	H	Br	162+1	$C_{14}H_{28}NBr$	290	82	c, 57.93 f, 57.71	9.65 9.42	8.82 8.57	27.58 27.39
27	$C_2H_5OOCCH_2$	H	Cl	202+2	$C_{14}H_{26}O_2NCl$	276	85	c, 60.86 f, 60.43	9.42 9.21	5.07 4.87	13.04 12.80
28	$C_6H_5CH_2$	H	Cl	208	$C_{17}H_{26}NCl$	208	85	c, 72.85 f, 72.62	9.28 9.02	5.00 4.73	12.85 12.51
29	CH_3	CH_3	I	165+2	$C_{12}H_{23}NI$	308	89	c, 46.75 f, 46.68	7.46 7.21	4.54 4.19	41.23 40.92
30	$C_{10}H_{17}$ Geranyl	CH_3CH_2	Br	161+4	$C_{22}H_{40}NBr$	398	78	c, 66.33 f, 66.12	10.05 9.88	3.51 3.29	20.10 20.03

Table 5

Physical and spectral data

Compd. No.	Spectral data
	(a) TMD-imine derivs.
2	IR Typical paraffin spectra; nmr δ 0.90 and 0.95 2× s, t(3× 3H), 0.90, d(3H)[CH_3], 2,6,3 overlap m(6H)[CH]; n_D^{20} 1.4621.
3	IR 3080(=CH), 1645(C=C), 920(=CH); nmr δ 0.90 and 0.95 2× s (2× 3H), 0.90 d(3H)[CH_3], 3.00 m(2H)[CH_3N allyl], 5.80 m(1H)[=CH], 5.00 m(2H)[=CH_2]; n_D^{20}1.4692.
11	IR 3200-2300(NH_2), 1580(NH); nmr δ 0.90 and 0.95 2× s, t(3× 3H), 0.90 d(3H)[CH_3], 2.60 m(6H)[CH].
21	IR Typical paraffin spectra; nmr δ 0.90 and 0.95 2× s(2× 3H), 0.90 d(3H)[CH_3], 2,6,3 overlap m(6H)[CH], 3.00 m(2H)[NCH_2 allyl], 5.80 m(1H)[=CH], 5.00 m(2H)[=CH_2].
22	IR 3200-2300(NH_2), 1580(NH); nmr δ 1.05, 1.20 and 1.30 3× s(3× 3H) [CH_3], 3.00 and 3.35(J_{AB} = 12 Hz) [NCH_2], 4.04 m(1H)[NCH], 8.20 s(2H)[NH_2].
	(b) IPD-imine derivs.
8	IR 3080(=CH), 1645(C=C), 920(=CH); nmr δ 0.90, 1.05 and 1.30 3× s(3× 3H) [CH_3], 2.05 and 2.90(J_{AB} = 10 Hz) [CH_2N ring], 3.10 m(3H)[CH_2N allyl], 280–320 Hz, m(2H)[= CH_2], 325–370 Hz, m(1H)[=CH]; n_D^{20}1.4790.
9	IR Typical paraffin spectra; nmr δ 0.90, 1.65 and 1.30 3× s(3× 3H) [CH_3], 1.00 t(3H)[CH_3], 2.05 and 3.85(J_{AB} = 10 Hz)[CH_2N ring], 2.50 m(2H)[CH_2N chain], 3.00 m(1H)[CHN]; n_D^{20} 1.4697.
26	IR 3200-2300(NH_2), 1580(NH); nmr δ 1.05, 1.20 and 1.30 3× s(3× 3H) [CH_3], 3.00 and 3.35(J_{AB} = 12 Hz) [NCH_2], 4.05 m(1H)[NCH], 8.20 s(2H)[NH_2].
29	IR 3200-2300(NH_2), 1580(NH); nmr δ 0.90, 1.05 and 1.30 3× s(3× 3H) [CH_3], 2.40 s(3H)[NCH_3], 2.00 and 2.95, AB-quaternary(2H), (J_{AB} = 10 Hz) [NCH_2], 2.90 m(1H)[NCH].
30	IR Typical paraffin spectra; nmr δ 0.90, 1.05 and 1.30 3× s(3× 3H) [CH_3], 1.63 and 1.68(CH_3–7,8), 1.74(CH_3–3), 2.05 and 2.12(CH_2–4,5), 1.00 t(3H)[CH_3 ethyl], 2.50 m(2H)[NCH_2 ethyl], 2.65 and 2.85 (J_{AB} = 10 Hz) [NCH_2 ring], 3.00 m(1H)[NCH].

nium salts are given in Table 5. The IR spectra were recorded on a Perkin-Elmer 577 grating spectrometer in KBr pellets. The ^1H NMR spectra were recorded on a JEOL 60-HL instrument at 60 MHz in $CDCl_3$ at room temperature, using TMS as internal standard.

Biological

Bioassay for plant growth retarding activities

Beans *(Phaseolus vulgaris* cv. Pinto) was planted into soil, four plants in each pot. The experiments were carried out in the greenhouse. The plants were sprayed at the two leaf stage with the solution or suspension of compounds tested (0.1 % active ingradient, 0.001 % Cytowett as a wetting agent) to complete covering. After two weeks, when the plant has five leaf-floor, the plants were cut off above the soil level and the length of each internodia "floor" was measured on a millimetre scale.

Eight plants were used in one treatment. The mean and standard error were calculated for each floor separately. The results were expressed as percentile growth compared to that of the untreated control. The common standard deviation of all experiments was ±20 %. The common coefficient of variation was ±8 %.

Chlorocholine chloride (CCC), and N,N-dimethylpiperidinium chloride (Pix) were used as standards.

The results of plant growth retarding activity are shown in Table 6.

Results and Discussion

Some of the compounds tested were found to be effective on the growth of bean plant at 0.1 % concentration. In addition to the growth suppression observed, most of the compounds prepared brought about an appreciable dark green coloration and thickening of the leaves.

As for structure-activity relationships, the compounds containing only the tertiary amino group (1 – 9) showed, as expected, no retardation of the growth of bean plant indicating that quaternarization of these amines derivatives is necessary for such a type of action. On the other hand, the quaternary ammonium derivatives derived from these amines showed a measurable growth retardation of bean plant. These quaternary ammonium derivatives varied in their degree of activity depending on the structure of the molecule.

The quaternary ammonium salts prepared from TMD-imine showed appreciable activities on the growth of bean plant, while those derived from IPD-imine proved to be inactive. This, may be due to the sterochemistry associated with the IPD-imine derivatives containing the methylene bridge which may reduce the possibility of interaction between the substrate and the enzyme responsible for the activity.

Table 6

Growth retarding activities of tertiary amines and quaternary ammonium derivatives

Compound No.	Relative length of internodia				
	1st	2nd	3rd	4th	5th
Control*	15.5	16.6	14.4	18.2	10.9
CCC**	93	38	25	—	—
PIX	88	60	52	28	—
1	105	105	100	103	140
2	105	98	81	86	58
3	108	98	89	91	129
4	103	97	88	91	87
5	97	96	90	88	76
6	107	92	91	94	148
7	93	107	99	92	107
8	94	107	92	51	—
9	91	103	86	83	84
10	112	117	131	112	146
11	95	99	73	62	83
12	95	113	83	108	126
13	110	101	96	97	95
14	89	86	68	43	—
15	98	88	69	63	99
16	103	93	75	62	73
17	88	79	70	65	127
18	85	32	50	35	26
19	113	82	59	63	77
20	105	73	45	50	93
21	99	109	123	104	125
22	96	92	78	87	100
23	103	97	67	49	36
24	90	74	74	51	38
25	104	103	100	107	82
26	88	100	83	62	—
27	97	107	106	111	100
28	108	114	122	130	100
29	88	102	80	94	79
30	102	106	145	117	98

* Calculated from 7 experiments (n = 53) in cm.
** Calculated from 7 experiments (n = 46).

N,N-dialkyl TMD ammonium derivatives (14–20) 23, 24, and 26 showed an appreciable activity especially those containing one N-methyl group. Those compounds revealed also a selective inhibition on the growth of bean plant particularly on the third and fourth internodia. This finding suggests that these derivatives are, in contrast to CCC, selectively active at certain spicific stage of growth.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (1–2), pp. 259–261 (1981)

The Effect of Titanium on Plants Damaged by Herbicides

By

E. FARKAS, Á. TÓTH and I. PAIS

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Titanium in a chelated form can protect some plants against damages of herbicides by influencing enzyme activities in treated plants.

Titanium is one of the most common elements in the earth's crust and as a consequence of this, the soils contain on an average 3000–4000 ppm from this metal. Titanium is bound mostly in silicates and therefore the uptake for the plants is nearly impossible. The textbooks and the literature regard the titanium as an unnecessary element which has not any role in the biosphere.

Because the essential trace elements are mostly in the first line of the transition metals in the periodical system, we supposed more than 10 years ago that titanium, if it is in watersoluble form, may play also an important role in the biochemical processes.

After some preliminaries we organised first in the year 1974 field-experiments: we sprayed a very diluted solution of titanium(IV) on the leaves of winter-apples, grape-vines and tomatoes. The chlorophyl-content of the sprayed plants was significantly greater as compared with the unsprayed samples. In consequence of this the carbohydrate-content is also increased (PAIS *et al.*, 1979).

Having the very favourable experiences, in the last 6 years we have extended our experiments to new test-plants, such as to wheat, alfalfa, sugar beet, sugar corn, sunflower, fodder grasses, etc. Giving a comprehensive survey, we should mention, that the yield increased on an average by 10–25 per cent and the sugar-content, or the protein-content of the products was also greater by 10–20 per cent (FEHÉR *et al.*, 1980; PAIS *et al.*, 1979a; PAIS *et al.*, 1979b).

Looking into the background of this favourable effect of titanium, we measured the activities of different enzymes. According to our experiences, the titanium has a promotive effect on the activity of catalase, nitrate reductase and also of nitrogenase (PAIS, 1979).

It is well-known, that the protective effect of herbicides is very important for having a great yield in the agricultural production. Spraying the different plants from the air with herbicide solutions, it is often the case, that the solution is carried away to grape-vines or to other sensitive cultures and they can be heavily damaged. Being well aware of the fact, that titanium is very favourable for the plants, we organized experiments to have information about the possible protective effect against the herbicide-damage.

In our preliminary experiments we used the sodium-salt of dichloro-phenoxy-acetic acid as herbicide. Two week old tomato plants were sprayed with 2 ml 0.01 per cent herbicide solution and a proportionable part of plants received 1, 3, 6 and 8 days after the herbicide-spraying a treatment with 0.001 per cent titanium-chelate solution. Our experiments showed (FARKAS *et al.*, unpublished), that the rapid application of titanium gave about the same result as the case of control. If the application is too late, the protective effect is much less; the plants are damaged: no flowering occurs and the stems become bent.

In the next experiment we used two grape-varieties: Pannónia kincse and Olasz rizling as test-plants. The vine-stocks received 200 cm^3 herbicide-solution (5, 15 and 50 ppm) at the end of April 1979 and half of the plants was sprayed 6 and 12 days later with 5 ppm titanium-solution. The results can be seen in Table 1.

Table 1

The protective effect of titanium on vine-grapes against herbicides

Variety	Treatment	Reducing sugar %	Sugar-acid ratio
Pannónia kincse	Control	8.0	13.2
	5 ppm Dikonirt	8.1	16.5
	5 ppm Dikonirt + Ti	9.4	19.2
	15 ppm Dikonirt	7.8	12.9
	15 ppm Dikonirt + Ti	8.1	14.3
	50 ppm Dikonirt	8.3	14.2
	50 ppm Dikonirt + Ti	8.0	14.7
Olasz rizling	Control	12.6	15.1
	5 ppm Dikonirt	15.9	20.3
	5 ppm Dikonirt + Ti	15.6	23.2
	15 ppm Dikonirt	13.6	21.1
	15 ppm Dikonirt + Ti	17.6	28.3
	50 ppm Dikonirt	16.2	24.7
	50 ppm Dikonirt + Ti	17.9	29.6

Table 2

The protective effect of titanium on tomato plants against herbicides

Treatment	Average yield in kg	In the percentage of control
Control	0.79	100.0
1.4 kg/ha Hungazin PK	0.40	50.6
1.4 kg/ha Hungazin PK + Ti	0.64	81.0
2.8 kg/ha Hungazin PK	0.40	50.6
2.8 kg/ha Hungazin PK + Ti	0.83	105.1
4.0 kg/ha Hungazin PK	0.47	59.5
4.0 kg/ha Hungazin PK + Ti	1.45	183.5

You can observe, that vine-stocks treated with titanium-solution after herbicide-spray recover from the herbicide effect and the sugar-content of the must is mostly much greater than in the control or in the herbicide samples.

In the year 1980 we examined the after-effect of the Hungazin PK herbicide on tomato-plants. In May 1979 we gave to the experimental field 1.4 kg, 2.8 kg and 4.0 kg Hungazin PK in solved form pro hectar, respectively. The tomato-seedlings died out after the planting. In the same plots we planted tomato-seedlings in the following year: in May 1980. Half of the stand were sprayed 12, 22 and 32 days later with a 5 ppm titanium-solution. The plants sprayed gave more yield than the others, as we can see in Table 2.

According to our experiences the patented titanium-chelate can be applied to protect some plant cultures against herbicide-damage. The protective effect can be interpreted as a help to different enzymes to counteract the herbicides.

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Book Review

D. GOTTLIEB: *The Germination of Fungus Spores.* Meadowfield Press Ltd., I. S. A. Building, Shindon, Co. Durham, England. 1978. 166 pp. Price: $12.60

The publication of the book "The Germination of Fungus Spores" by Professor David Gottlieb (Dep. Plant Path. Univ. Illinois) gives a great help to understand the complex phenomenon of fungus spore germination. The book is the thirteenth member of the series "Patterns of Progress (Microbiology)".

Germination is the process whereby the spore shifts from its low metabolic state to a rate approximating that of the normal vegetative phase of the fungus. This dramatic shift common to all germination. The morphological and biochemical changes accompanying this development are described in detail. Spore morphology and cytology, developmental and environmental factors are discussed. One can find special chapters dealing with nutrition, respiration, energy production, the biosynthetic mechanisms of the germinating spore. Especially the synthesis and sequence of formation of the macromolecules are discussed in their relationship to the different phases in the development of the germinated spore.

The book contains a large number of references and the easy-to-read text is illustrated with 34 figures and 5 tables. On the last pages addendum contains the last up-to-date informations on this area.

B. BARNA

GEORGE N. AGRIOS: *Plant Pathology.* Second Edition. Academic Press, New York, San Francisco, and London. 1978. 703 pages.

The second edition of "Plant Pathology" by Agrios is an excellent book for all plant pathologists and graduate students working on different fields of plant pathology. The book is divided into two parts. The first one deals with general aspects which includes eight chapters: Introduction, Parasitism and disease development, How pathogen attack plants, Pathogen effects on plant physiological functions, How plants defend themselves against pathogens, Genetics and plant diseases, Effect of environment on development of infectious plant diseases, and Control of plant diseases. The second part deals with specific plant diseases, which include, also, eight chapters. Three of them are new chapters which have been added to this edition. These are: Plant diseases caused by mycoplasmalike organisms, Plant diseases caused by protozoa, and Plant diseases caused by viroids. The other chapters are as follows: Environmental factors that cause plant diseases, Plant diseases caused by fungi, Plant diseases caused by bacteria, Plant diseases caused by parasitic higher plants, Plant diseases caused by viruses, Plant diseases caused by nematodes. One of the most important new information is about the isolation of the pathogens from infected hosts.

Many new diagrams and high quality photographs have been added to make the book more stimulating and more effective. The good design and the high standard of the contents are making this edition very useful for all the experts and students working in plant pathology.

A. R. T. SARHAN

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A kézirat nyomdába érkezett: 1981. II. 12. — Terjedelem: 23,25 (A/5) ív, 104 ábra

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14. XII. 1981.

HU ISSN 0001—6780
Index: 26.032

VOLUME 16 • NUMBERS 3—4 • 1981

acta Phytopathologica

ACADEMIAE SCIENTIARUM HUNGARICAE

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Acta Phytopathologica

Academiae Scientiarum Hungaricae

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Megjelenik negyedévenként, évi egy kötetben.

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Megrendelhető a belföld számára az Akadémiai Kiadónál (1363 Budapest Pf. 24.), a külföld számára pedig a „Kultura" Külkereskedelmi Vállalatnál (1389 Bp. 62, P.O.B. 149).

Acta Phytopathologica Academiae Scientiarum Hungaricae. A quarterly review of the Hungarian Academy of Sciences.

Papers are published mainly in English.

Editorial Office: 1525 Budapest, P.O.B. 102.

Distributor: "Kultura" Foreign Company (1389 Budapest 62, P.O.B. 149) or its representatives abroad.

Acta Phytopathologica Academiae Scientiarum Hungaricae. Vierteljahresschrift der Ungarischen Akademie der Wissenschaften.

Aufsätze erscheinen in englischer bzw. in deutscher, französischer oder russischer Sprache.

Redaktion: 1525 Budapest, P.O.B. 102

Bestellbar bei »Kultura« Außenhandelsunternehmen (1389 Budapest 62, P.O.B. 149) oder seinen Auslandsvertretungen.

Acta Phytopathologica Academiae Scientiarum Hungaricae. Журнал Академии наук Венгрии. Выходит ежеквартально (один том в год). Статьи публикуются на английском или на русском, немецком и французском языках. Адрес редакции: 1525 Budapest P.O.B. 102.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 265—267 (1981)

Evaluation of Sunflowers for the Degree of Resistance to Downy Mildew

By

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Pregerminated seeds of sunflowers, susceptible and resistant to downy mildew, were inoculated by the whole seedling immersion method, and were grown either in the greenhouse, or in a controlled cabinet. At cotyledonary and four-leaf stage the seedlings were assessed for the occurrence and intensity of downy mildew infection caused by *Plasmopara halstedii*.

According to the disease symptoms obtained, all the plants of the sunflower cultivars tested were separated into four groups each representing different degrees of downy mildew resistance, as susceptible, moderately resistant, resistant and highly resistant.

Though members of the groups 2 to 4 in these experiments are generally accepted by plant breeders as resistant sunflowers, from the results it is assumed that at least two different levels of resistance to *P. halstedii* exist among and within the cultivars tested.

Some 20 years ago GOOSSEN and SACKSTON (1968) have found a sunflower line which showed resistance to *Plasmopara halstedii*, the causal agent of the downy mildew disease of sunflower. Subsequent genetic studies carried out in several countries, in Roumania (VRANCEANU and STOENESCU, 1970), in France (VEAR and LECLERCQ, 1971), and in the USA (ZIMMER and KINMAN, 1972) revealed that three or four genes (Pl_1, Pl_2, Pl_3 and Pl_4) are responsible for downy mildew resistance.

Nevertheless, the fact that *P. halstedii* has two races of different aggressivity (ZIMMER and KINMAN, 1972), and the recent finding of incomplete resistance in sunflowers possessing one or two resistance genes (VEAR, 1978; VIRÁNYI, 1978, 1980), indicate that further efforts have to be made to find out new sources of resistance to downy mildew.

The selecting work, as a rule, is based on artificial inoculation followed by counting the number of plants with fungal sporulation on their cotyledons. However, recent studies in Spain (SACKSTON *et al.*, 1976), in France (VEAR, 1978), and also in Hungary (VIRÁNYI, 1978) made this evaluation questionable.

For this reason an attempt was made to find out a better evaluation technique which, on the basis of various disease symptoms, allows to determine the different levels of resistance to downy mildew of sunflower.

Materials and Methods

Pregerminated seeds of sunflowers known to be susceptible and resistant to *P. halstedii*, respectively, were inoculated by the WSI method and the plants were grown either in the greenhouse or in a controlled cabinet. The assessment for resistance was made two times after inoculation, at cotyledonary and four-leaf stage of the seedlings by registering the number of plants per each cultivar showing sporulation, leaf chlorosis, damping off, as well as hypocotyl lesions.

Results and Discussion

According to the disease symptoms obtained in our experiments all sunflower plants could be arranged in one of the four groups shown in Table 1. Each group represents a host-parasite interaction different from the others, as it is demonstrated on the right of this table. It is easy to recognize that the degree of downy mildew resistance is closely associated with the extent of fungal invasion within the seedlings. Although, most of the plant breeders consider the sunflowers belonging to groups 2 to 4 practically resistant to *P. halstedii*, the great number of such plants, infected to some extent by the pathogen, is of practical importance.

When compared various sunflower cultivars in respect of their response to *P. halstedii* infection, it was found that susceptibility is less uniform than it was expected, which may probably due to individual diversity rather than any

Table 1

Classification of sunflowers for resistance to *Plasmopara halstedii*

Group	Response to downy mildew	Disease symptoms	Extent of fungal invasion
I.	Susceptible	damping off, sporulation on hypocotyl and true leaves leaf chlorosis sporulation on epicotyl pieces	entire plant
II.	Moderately resistant	sporulation on hypocotyls and cotyledons	roots, hypocotyl and cotyledons
III.	Resistant	lesions and/or sporulation on hypocotyls	roots and hypocotyl
IV.	Highly resistant	no symptoms	no fungal invasion

Table 2

Segregation of sunflower cultivars for the degree of downy mildew resistance

Cultivar	Genes for resistance	% of plants			
		Susceptible	Moderately resistant	Resistant	Highly resistant
Chakinskii 269	—	94	3	3	0
GK-70	—	89	0	11	0
Sorem-80	Pl_1	2	41	26	31
Sorem-82	Pl_1	2	31	39	28
Remil	Pl_2, Pl_3	13	3	67	17
Luciole	Pl_2, Pl_3	5	0	61	34
NK-212	Pl_2, Pl_3	23	0	31	46
NSH-26	Pl_2, Pl_3	12	0	34	54

resistance factor (Table 2). A very remarkable ratio of the seedlings containing the gene Pl_1 against *P. halstedii* showed sporulation on the cotyledons of the inoculated plants. It means that the gene Pl_1 seems to be unable to control effectively this type of infection. However, the sunflower cultivars with the genes Pl_2 and Pl_3, proved to be much more resistant to downy mildew, especially the cultivars NK-212 and NSH-26, where half of the seedlings escaped the infection.

From our results it is clear that there is a complex relationship between sunflower and *P. halstedii* which involves susceptible, highly resistant, and some intermediate forms. To determine the practical value of these, further experiments in the field are needed.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3–4), pp. 269–272 (1981)

Systemic Acquired Resistance of Cucumber to *Pseudomonas lachrymans* as Expressed in Suppression of Symptoms, but Not in Multiplication of Bacteria

By

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Infection of the first true leaves of cucumber with *Colletotrichum lagenarium* or treated with $HgCl_2$ induced necroses on the leaves. Acquired (induced) resistance was developed in the upper leaves of cucumber against the symptoms caused by *Pseudomonas lachrymans* used as a challenge. Only the symptoms of disease was suppressed in the resistant leaves, bacterial multiplication was not altered.

It has been shown that the systemic acquired resistance (induced protection) suppresses symptoms of diseases caused by viruses, bacteria and fungi (Bozarth and Ross, 1964; Van Loon, 1976; Ross, 1961; Leben, 1964; Kuć *et al.* 1975; Sziráki *et al.*, 1980). In the case of virus infections it was also shown that multiplication of the virus was not influenced by the acquired resistance, only the necrotic symptoms have been inhibited (Balázs, Sziráki and Király, 1977). On the other hand, Caruso and Kuć (1979) claimed that both the symptoms and the bacterial multiplication of *Pseudomonas lachrymans* were suppressed in cucumber leaves in which the systemic acquired resistance have been developed.

In this paper we are reporting on experiments which clearly demonstrate that multiplication of *P. lachrymans* is not affected in resistant leaves of cucumber, only the symptoms of disease is inhibited as a consequence of acquired resistance.

Materials and Methods

Pseudomonas lachrymans (Sm. and Bryan) Carsner, L9 (isolated in Hungary, in 1972 by Éva Visnyovszky) was used in the experiments. Cucumber plants (*Cucumis sativum* L., Hungarian cultivar Francia fürtös) susceptible to anthracnose and angular leaf spot were grown from seed in pots under greenhouse conditions and inoculated with *P. lachrymans*. Twenty-four-hour log phase cultures of bacteria (3×10^5 cell ml^{-1}) were sprayed onto the abaxial surface of cucumber leaves using an atomiser from a distance of 5 cm to produce mechanical water-soaking of leaves. This technique permitted to introduce a large number of bacteria into the intercellulars, and, thereby it was possible to detect significant differences in bacterial multiplication during a considerable short time.

Numbers of bacteria in infected cucumber tissues were estimated using the conventional (10 fold) dilution plate methods. Results are expressed as the logarithm of viable colony numbers per 1 cm^2 of leaf area.

In some experiments we wanted to determine the extent of necrotization in control as well as in resistant leaves. In order to avoid mechanical water-soaking or injuries of leaves, a suspension of 10^8 cell ml^{-1} of isolate L9 was sprayed on the abaxial surface of cucumber leaves with an atomizer from a distance of 20 cm to get angular leaf spot symptoms.

Colletotrichum lagenarium (Pass.) Ell. and Hals race 1 was provided by Prof. J. Kuć of the University of Kentucky, Kentucky. The inoculum consisted of 10^6 conidia ml^{-1} of the fungus. The inoculation was made as follows: The first true leaves of cucumber were sprayed on the upper sides with the suspension of the fungus, and then plants were placed into a moist chamber for 1 day at 26 °C. $HgCl_2$ (0.1 %) was dropped to induce necrotic spots, 20 per leaf, on the first true leaves of cucumber similar to necroses induced by the fungus. The

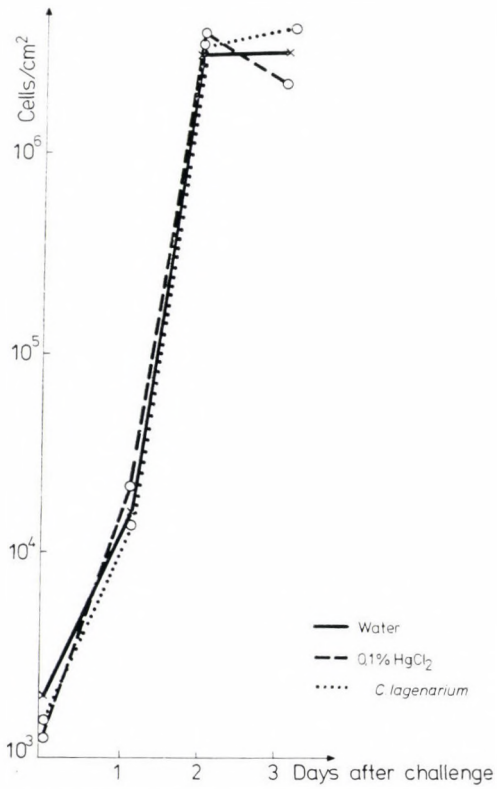

Fig. 1. Multiplication of *Pseudomonas lachrymans* in tissues of upper cucumber leaves challenged 7 days after the inoculation of the lower leaves with *Colletotrichum lagenarium* or treated with $HgCl_2$ (0.1 %). Inoculum contained 3×10^5 cell ml^{-1} bacteria

acquired resistance in the upper leaves developed 7 days after the inoculation or treatment of the first leaves mentioned above. Accordingly, the upper second leaves were inoculated with the bacterium 7 days after the first inoculation or treatment with $HgCl_2$. In every case the effectiveness of systemic acquired resistances was checked by inoculation one part of plants on the second leaves by *C. lagenarium* and *P. lachrymans*.

Results and Discussion

We wanted to determine the number of bacteria in the upper (second) leaves 7 days after the challenge inoculation. The first (lower) leaves were treated with water (control), with $HgCl_2$ or inoculated with *Colletotrichum lagenarium*. As is seen on Fig. 1, there was no difference in the number of bacteria in the upper leaves during 3 days after the challenge whether there was induced a systemic acquired resistance in those leaves or not (water control). Regarding the development of symptoms Table 1 shows the results of another experiment. One can see that necrotic symptoms caused by *P. lachrymans* in the upper leaves are suppressed when the systemic acquired resistance was induced either by *C. lagenarium* or by treatment with $HgCl_2$. Also in this experiment we checked the bacterial multiplication, however, in this case after the appearance of the symptoms (on the seventh day after the challenge). The bacterial number in the upper leaves was not significantly altered by the acquired resistance. In other words, it turned out that resistance in the upper leaves was effective against the necrotic symptoms but not against multiplication of bacteria.

Table 1

Changes in the number of necrotic spots, in the diameter of spots and in number of bacteria of the whole cucumber leaves caused by *Pseudomonas lachrymans* in the upper leaves 7 days after the first treatments

First treatment or inoculation on the 1st leaf[1]	Number of necrotic spots on the upper leaves after challenge[2]	Diameter of necrotic spots[2] (mm)	Bacterial number in the upper leaves after challenge[2]
Water[1]	49.0	3.0	6.4×10^8
$HgCl_2$	18.0	2.0	8×10^8
C. lagenarium	5.3	1.0	2.1×10^8

[1] The first true leaves of cucumber were infected with the fungus, 10^6 conidia ml^{-1}, treated with water or $HgCl_2$ (0.1 %).

[2] Challenge inoculation was made by spraying the upper leaves (2nd leaves) with a suspension of *P. lachrymans* (10^8 cell ml^{-1}), 7 days after the first treatments. Necrotic were counted or measured. The number of bacteria was estimated in the whole leaf 7 days after the challenge.

These results contradict the paper of CARUSO and KUĆ (1979) in which they came to the conclusion that both symptoms of disease and multiplication of bacteria would be suppressed by acquired resistance. We used the very same strain of *C. lagenarium* as the above-mentioned authors, however, the variety of cucumber and the strain of *P. lachrymans* were different.

It is worth to mention that systemic acquired resistance does not suppress virus infectivity only the necrotic symptoms of TMV (BALÁZS *et al.*, 1977).

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 273—279 (1981)

Changes in the Development and Metabolism of Sunflowers Infected by *Plasmopara halstedii*

By

F. VIRÁNYI and G. OROS

The sunflower cultivars, VNIIMK 6540 and Remil, susceptible and resistant to downy mildew, respectively, were inoculated with *Plasmopara halstedii* at the germination stage, and were grown in the greenhouse until they developed four true leaves. The plants for analysis were classified as noninfected, infected symptomless and diseased ones, the latter showing retarded plant growth (resistant cultivar) or severe stunting with leaf chlorosis (susceptible cultivar).

Depending on the intensity of infection and the cultivar tested significant differences have been found in plant weight, stem to leaf ratio, chlorophyll content, photosynthetic productivity, as well as distribution of ^{14}C labelled photosynthetic assimilates among the plant organs. It was concluded that the photosynthetic activity and the distribution of assimilates in both kinds of sunflowers is closely associated with internal development of the pathogen.

The downy mildew of sunflower, caused by *Plasmopara halstedii* (Farlow) Berlese et de Toni, is a widespread and serious disease. The fungus usually attacks the plants very early in the season, i.e. at a period between seed germination and emergence, mostly resulting in systemic infection of such plants. The most typical symptoms include stunting, leaf chlorosis and degeneration of the underground tissues. Although all these symptoms occur on sunflowers susceptible to downy mildew, the pathogen is able to induce retarded plant growth and tissue degeneration in a number of resistant sunflower plants as well (VIRÁNYI, 1978).

There are only a few data in the literature concerning changes in the metabolism of the downy mildewed sunflowers. So, NOVOTELNOVA (1966) and VENCLAVOVITS *et al.* (1963) reported about a higher respiration rate, an increased enzyme activity, and a lower carbohydrate content due to downy mildew infection.

The aim of this study was to determine the changes in the photosynthetic activity and distribution of photosynthetically fixed radiocarbon between leaves and stem of susceptible and resistant sunflowers infected by *P. halstedii*.

Materials and Methods

The sunflower cultivars VNIIMK 6540 and Remil, susceptible and resistant to downy mildew, respectively, were inoculated by the whole seedling immersion method (COHEN and SACKSTON, 1973). The plants were then grown in the greenhouse until they reached the four leaf stage, when the typical symptoms of the disease appeared.

The seedlings of both cultivars for analysis were classified as non-infected, healthy (H), infected symptomless or latent infected (L) and diseased with symptoms (D), the latter showing retarded plant growth (resistant cultivar), or severe stunting with leaf chlorosis (susceptible cultivar). In every plant either non-infected, or latent infected, the presence or abscence of the fungus was checked microscopically.

Measuring the photosynthetic activity. The soil was washed off from the roots with tap water, and the plants were put into Hoagland'–Arnon's nutrient solution (half strength). After two hours they were put into an isolator supplied with artificial light of 10 000 lux, where they were incubated in $^{14}CO_2$ atmosphere for 1 hour. The fixed ^{14}C was measured in the tissues of leaves and stem, respectively. The results were calculated on the basis of both fresh weight and chlorophyll content.

The chlorophyll content was determined according to GRODZINSKY (1973) in acetonous solution, using the following formules:

chlorophyll *a:* $\pm$ 9.784 D_{662} – 0.990 D_{664} mg/l
chlorophyll *b:* $\pm$ 21.426 D_{644} – 4.650 D_{662} mg/l.

The values obtained were calculated to one plant, unit of leaf area, as well as crude plant weight. In addition, the chlorophyll *a*/*b* ratio was also calculated.

The surface of the leaves was determined by using the planimetric method, and the result are given in cm^2/plant.

In previous experiments we have found that, at least in our conditions, 96–98% of the chlorophyll of sunflower seedlings occurred in the leaves, and furthermore 99.5% of the total radioactivity incorporated was found in the leaves and stem. Thus, the chlorophyll content of the stem, as well as the radioactivity obtained in the roots were omitted throughout this experiment.

Results

By the time the sunflower plants were taken for analysis they were colonized by *P. halstedii* in a different way. So in case of latent infection the fungus invaded the hypocotyls of both cultivars to a very limited extent, the diseased resistant plants, however showed extended fungal growth in their roots and hypocotyls. Of course, entire plants of the diseased susceptible cultivar were invaded.

Depending on the infection rate and the cultivar tested significant differences were found in weight of the seedlings. In Fig. 1 it is demonstrated that the total weight of the infected symptomless plants of Remil was less than that of the control, while no such difference could be detected in the susceptible plants. Nevertheless, when compared the diseased plants of both cultivars which showed typical symptoms, a remarkable decrease of plant weight at each was found. In addition, there was also a significant difference between the stem to leaf ratio of the susceptible diseased, as well as symptomless or healthy plants.

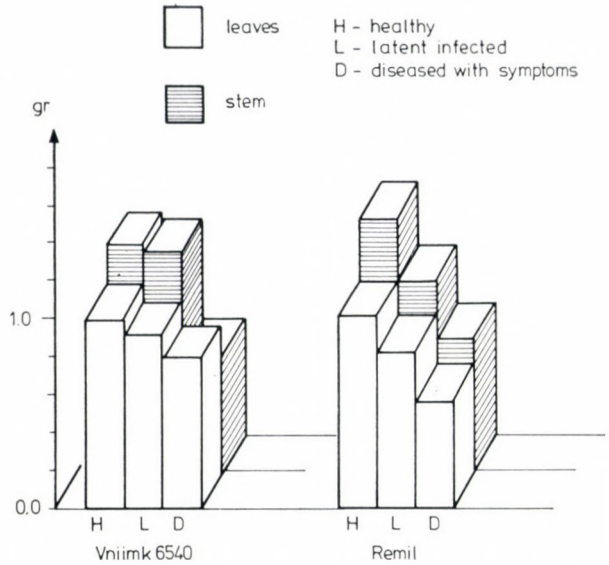

Fig. 1. Effect of disease rate of sunflower downy mildew on the weight of leaves and stem associated with disease resistance

Similar result were obtained when differences in leaf surface were examined (Table 1). While a decrease of 14% of the total leaf area was found in case of symptomless resistant plants, this reduction was much more less in the susceptible latent infected seedlings. However, as a result of pronounced development of *P. halstedii* completed with disease symptoms the surface of leaves of the cultivar VNIIMK 6540 was reduced, but no such influence on the leaves of Remil was found.

In relation to weight and area of the leaves it was concluded that, as a result of severe infection, the specific weight of the susceptible leaves proved to be higher compared to symptomless or healthy ones, while no difference could be detected in case of resistant variety.

Investigations on the chlorophyll content of sunflowers revealed that downy mildew infection and the disease rate are closely associated with a lower amount of chlorophyll in the leaves (Table 2), which was more evident in the resistant plants. Furthermore the diseased plants of both types of cultivars differed greatly in the chlorophyll *a*/*b* ratio. The higher amount of chlorophyll *a* in the susceptible seedlings was in contrast with an increase of chlorophyll *b* in the cultivar Remil.

In Table 3 changes in the photosynthetic activity of sunflowers due to *P. halstedii* infection are demonstrated. Although a close correlation was found between the increased infection rate and the lower amount of photosynthetic productivity in both cultivars, this decrease was more evident in the resistant

Table 1

The areas and specific weight of leaves of sunflower plants diseased at different levels by *P. halstedii*

	Variety	Healthy	Latent infected	Diseased
Area of leaves cm^2/plant	VNIIMK 6540	55	52	36
	Remil	45	38	38
Specific weight of leaf tissue mg/cm^2	VNIIMK 6540	18.7	17.9	22.4
	Remil	22.8	21.4	15.3

Table 2

The chlorophyll content of sunflower plants diseased at different levels by *P. halstedii*

	Variety	Healthy	Latent infected	Diseased
mg chlorophyll/plant	VNIIMK 6540	0.36	0.26	0.15
	Remil	0.78	0.41	0.26
mg chlorophyll/dm^2 of leaf area	VNIIMK 6540	0.70	0.50	0.42
	Remil	1.74	1.07	0.70
chlorophyll a/b	VNIIMK 6540	1.21	1.54	1.27
	Remil	1.47	1.20	0.79

Table 3

Differences in the photosynthetic ^{14}C assimilation of susceptible and resistant sunflowers induced by *P. halstedii*

	Variety	Healthy	Latent infected	Diseased
Productivity cpm/plant	VNIIMK 6540	117 377	102 007	46 075
	Remil	130 553	91 001	50 934
Specific activity of photosynthesis cpm/mg of chlorophyll	VNIIMK 6540	325 775	395 223	308 657
	Remil	166 437	220 876	193 298

Significance of differences in specific photosynthetic activities:
VNIIMK 6540 $L > H = D$
Remil $L > D \sim H$

plants. In addition, the specific photosynthetic activity of both cultivars proved to be higher than that of the control, when the plants were latent infected, and this increased activity remained in the diseased resistant plants (Table 3).

The distribution of assimilates labelled with ^{14}C are illustrated in Fig. 2. With the increased disease rate the amount of incorporated radioactivity de-

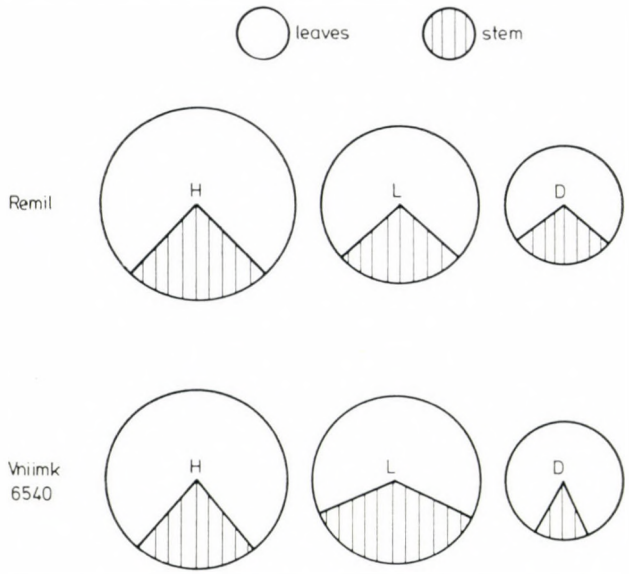

Fig. 2. Productivity of photosynthesis and distribution of assimilates (labelled with ^{14}C) between leaves and stem after one hour of exposition. (Area of each circle is proportionated to incorporated radioactivity.) H — healthy; L — latent infected; D — diseased with symptoms

creased in both cultivars, and there was a positive correlation between the severity of infection and the sites of accumulation of the photosynthetic products in the host.

Thus, the amount of incorporated ^{14}C was found to be higher in hypocotyls of the latent infected plants of both cultivars, than in hypocotyls of the healthy sunflowers. However, the majority of these assimilates always occurred in the leaves of the diseased susceptible plants.

These results mentioned above are also shown in Fig. 3, where plant tissues colonized by the pathogen are also indicated. Each circles are proportional to the corresponding radioactivity, and the ratio of their distribution is given by presenting the K values of each. For instance, in case of Remil there was no difference at all between the K values of the latent and diseased plants, but in contrast, plants of the susceptible VNIIMK 6540 showed significant differences in this respect.

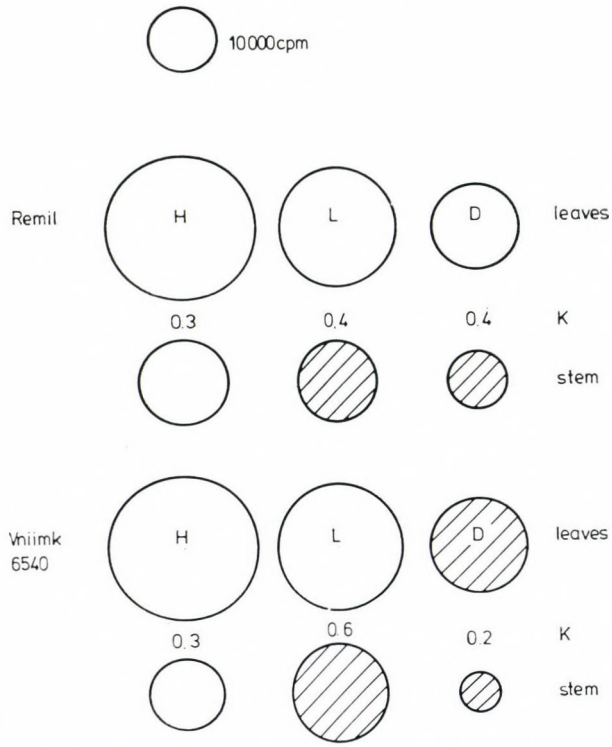

Fig. 3. Distribution of ^{14}C between leaves and stem after one hour of exposition. (Lined areas mean the occurrence of the fungus.)

$$K_{value} = \frac{\text{radioactivity in the stem}}{\text{radioactivity in the leaves}}$$

H — healthy; L — latent infected; D — diseased with symptoms

Discussion

The downy mildew pathogen, *P. halstedii* has a very remarkable influence on the development of sunflower plants. Similarly to NOVOTELNOVA (1966) it was found that the downy mildew infection causes significant reduction in size and weight of the susceptible sunflower plants, but no such indications of the infected resistant sunflowers are available in the literature.

From our experiments it was concluded that the sunflower cultivars either susceptible or resistant to downy mildew react to infection in a different way. While in case of susceptibility a positive correlation was found between the extent of fungal colonization and the relative weight of stems, no such difference could be detected in the resistant plants.

Furthermore, there were significant alterations in the following parameters: chlorophyll content and concentration, and the chlorophyll *a*/*b* ratio. As it is demonstrated in Table 3, the healthy resistant sunflowers contain double amount of chlorophyll than susceptible ones, but this difference does not imply significant increase of photosynthetic productivity of the former. Nevertheless, the chlorophyll content and photosynthetic productivity of both cultivars were lower in the infected and diseased plants compared to healthy ones. The differences in these parameters correlated with the disease rate, but in different manner. It means that the specific photosynthetic activity of latent infected sunflowers increased.

Apart from changes in the chlorophyll content mentioned above a very remarkable but contrasting alteration was evident in the chlorophyll *a*/*b* ratio of the cultivars, the cause of which is still unknown.

From our results it was also pointed out that the downy mildew infection has a major influence on the distribution and accumulation of photosynthetic assimilates within the host. Thus, there was a very close correlation between the amount of incorporated ^{14}C and the site of fungal invasion in the sunflower plants. While in the healthy sunflowers a more or less uniform distribution of these assimilates was found, in case of latent infection they tended to be attracted to hypocotyl tissues of both cultivars. In the diseased susceptible plants, however, most of the photosynthetic products were located in the leaves, i.e. at the site of intensive fungal development.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 281—292 (1981)

The Role of Aggressiveness of *Fusarium graminearum* Isolates in the Inoculation Tests on Wheat in Seedling State

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The variety reaction to *F. graminearum* infection depends heavily on the aggressiveness of inocula used. Therefore a nearly stable aggressiveness level should be secured to decrease the risk of using inocula with uncontrolled aggressiveness.

No vertical races were verified, actually similar results can be achieved with any isolate of *F. graminearum*. So the use of mixture of different isolates or inocula has no practical value. Only differences in aggressiveness were found, every isolate can be considered as a horizontal race of the species.

The reproduction of the experimental results is not perfect, even with improved methods remain problems. The source of this is the immanent character of the host-pathogen relationship, where no host, no pathogen has the stability in aggressiveness or resistance, respectively. With improved knowledge on factors resulting inconsistency the experiments can be made more exact, but the correctness of the results with obligate pathogens can not be achieved. If the same variety sortiment in two parallel tests gives a correlation above $r = 0.70$, the results are considered to be valuable for further use.

One of the main problems of resistance tests is to reproduce properly the experimental results. This is especially valid for diseases caused by pathogens of high variability. The seedling and head blight caused by different *Fusarium* species in wheat belongs to this group.

Extensive studies (EIDE, 1935, GODDARD, 1939, ULLSTRUP, 1935) proved that the species *F. graminearum* is a population of individuals with very large differences in morphological and pathological characteristics. Similar conclusions were drawn recently by MESTERHÁZY (1978a).

The poor replicability of *Fusarium* tests (ANDERSON, 1948; CAPETTI, 1974; pers. communication, HANSON *et al.*, 1950; MESDAG, 1976; pers. communication, SCOTT, 1927) is probably caused by methodical problems of inoculation in addition to the variability of the fungus and host plant (MESTERHÁZY, 1977a, 1978a).

In this paper the effect of variability within *F. graminearum* is studied on the expression of resistance of wheat to seedling blight.

Materials and Methods

For inoculation isolates of *F. graminearum* identified by the author according to BOOTH's (1971) *Fusarium* manual were used.

To raise the inoculum the bubble breeding method of MESTERHÁZY (1977a) was applied. The isolates and their conidium concentrations for the 1st, 2nd and 3rd experiments are listed in Tables 1, 7, and 8.

Table 1

Conidium concentrations of the inocula used in the 1st experiment

Isolate	Conidium concentration $\times 10^6$/ml	
	1st test	2nd test
648	1.01	0.00*
3 689	0.53	0.00
4 530	0.83	0.00
6 967	1.11	0.00
7 783	0.75	0.25
10 897	0.50	0.00

* Only mycelium was present

In the 1st experiment eight wheat lines with different reaction to *F. graminearum* were choosen based on three years testing procedure.

The inoculations were made with the Petri dish method (MESTERHÁZY, 1978a). Five replicates were set for each treatment, every replicate contained 25 surface disinfected seeds (1% NaOCl solution, for 20 minutes). The number of isolates were six. The number of healthy germs was counted from the 2nd to the 6th day daily after sowing. The experiment was repeated with new inoculums of the same isolates in order to obtain data about the aggressiveness of the isolates.

In the 2nd experiment the reaction of four wheat lines to 26 isolates of *F. graminearum* was studied. Every treatment had four replicates, the method of evaluation was the same as performed in the 1st test. To decrease the high number of entries into the biostatistical evaluation, the means of five readings were used.

In the 3rd experiment the variability of three of the 26 isolates was studied on four genotypes of winter wheat. Six parallel inocula were produced from every isolates and utilized for inoculation. The treatments were set in five replicates.

The biostatistical evaluation included variance and correlation analyses.

Results

1st experiment

The variance analysis is shown in Table 2. The more complex interactions are subordinated to the less complex ones and all of the interactions differ significantly from the main sources of variance, e.g. wheat line, isolate, time of reading

Table 2

Isolatum specific reaction of wheat genotypes to *F. graminearum*. Variance analysis

Source of variance	SQ	FG	MQ	F_1	F_2
Genotype (A)	78 191.99	7	11 667.43	111.04***	46.94***
Isolate (B)	67 398.11	5	13 479.62	128.29***	54.23***
Date of reading	1 635 092.71	4	408 773.18	3898.48***	1644.69***
Biological repetition	84 973.67	1	84 973.67	808.73***	341.89***
A×B	18 858.67	35	538.62	5.12***	2.16***
A×C	30 818.21	28	1 100.65	10.47***	4.42***
A×D	13 239.78	7	1 891.40	18.00***	7.61***
B×C	56 621.01	20	2 831.05	26.94***	11.39***
B×D	164 189.08	5	32 837.82	312.53***	132.12***
C×D	16 648.62	4	4 162.41	39.61***	16.74***
A×B×C	21 941.11	140	156.72	1.49***	0.63
A×B×D	14 866.86	35	427.77	4.07***	1.72***
A×C×D	10 866.72	28	388.10	3.69***	1.56***
B×C×D	33 522.64	20	1 676.13	15.95***	8.74***
A×B×C×D	34 796.17	140	248.54	2.36***	
Error	201 783.25	1920	105.07		
Total	2 483 744.60	2399			

*** $P = 0.001$
F_1 = F values based on the error
F_2 = F values based on the A×B×C×D interaction

and biological replication. These effects do not overcome independently of each other, the significant interactions demonstrate this.

As the explanation of the three- and four-way interactions is subjective, so to the main effects and double interactions were paid attention.

Main effects

The response of winter *wheat genotypes* (Table 3) is very similar in both tests. This agrees well with the results of the earlier experiments.

The aggressiveness of the *isolates* (Table 4) shows significant differences, the two partial experiments did not give the same results. On this small basis the

Table 3

Reaction of winter wheat genotypes to *F. graminearum* measured by the number of living germs related to the control

Genotype	Germination of the check %	Test		
		1	2	Mean
		in % of the control		
74-2	95.20	44.56	32.63	38.60
74-10	99.20	35.25	29.43	32.34
74-14	96.80	34.72	28.69	31.70
74-127	95.20	46.26	27.90	37.08
74-146	95.20	41.24	22.21	31.72
74-158	94.40	29.76	20.64	25.20
74-333	92.80	25.22	14.46	19.84
74-394	98.40	40.37	26.36	33.31
LSD 0.1%		4.27	3.45	2.75

Table 4

Aggressiveness of the isolates of *F. graminearum*, measured by the number of living germs related to the check

Isolates No.	Test		
	1	2	Mean
	in % of the check		
648	37.81	17.27	27.54
3 689	34.61	39.82	37.22
4 530	23.67	19.47	21.57
6 967	36.65	29.59	33.12
7 783	51.24	13.66	35.87
10 897	32.15	31.90	32.03
LSD 0.1%	3.70	3.01	2.37

Table 5

Number of living germs according to the reading dates due to *F. graminearum* infection Data are related to the not infected control

Date of reading	Test		
	1	2	Mean
2nd day	78.90	71.56	75.23
3rd day	56.29	34.43	45.36
4th day	26.85	15.64	21.14
5th day	15.21	3.72	9.46
6th day	8.61	1.04	4.84
LSD 0.1%	3.38	2.75	2.15

identification of horizontal races was not possible, therefore this problem was studied in more detail in the 3rd experiment.

The development of the *infection process* (Table 5) is different in the two partial experiments. A higher aggressiveness always results in a higher decreasing ratio of living germs.

Interactions

From our point of view the analysis of the genotype x isolate interaction is the most important. The variance table (Table 2) clearly shows that the level of interaction is relatively low, it just over the limit of significance. The results are presented in Fig. 1.

The wheat lines do not react by the same way to different isolates. This specificity is, however, not a stable characteristic of the host-pathogen relationship, the different inocula of the same isolates behave themselves as they would belong to entirely different isolates. Therefore the specificity is not an immanent

Table 6

Correlation coefficients between genotype performances to different *F. graminearum* isolates

No. of test	Isolates	10 897	4530	3689	6967	648	7783
1	10 897	—	0.78***	0.83***	0.71*	0.66°	0.85***
	4 530	—	—	0.94***	0.83***	0.89***	0.76*
	3 689	—	—	—	0.88***	0.86***	0.83***
	6 967	—	—	—	—	0.79**	0.74*
	648	—	—	—	—	—	0.63°
				Mean r = 0.80			
2	10 897	—	0.84***	0.70*	0.51	0.76*	0.87***
	4 530	—	—	0.62°	0.74*	0.93***	0.96***
	3 689	—	—	—	0.40	0.38	0.81***
	6 967	—	—	—	—	0.73*	0.70*
	648	—	—	—	—	—	0.85***
				Mean r = 0.72			
Mean of 1 + 2	10 897	—	0.85***	0.77*	0.56	0.84***	0.85***
	4 530	—	—	0.88***	0.79**	0.94***	0.80**
	3 689	—	—	—	0.68°	0.76*	0.93***
	6 967	—	—	—	—	0.81**	0.53
	648	—	—	—	—	—	0.71*
				Mean r = 0.78			

*** P = 0.001 ** P = 0.01 * P = 0.05 °P = 0.11

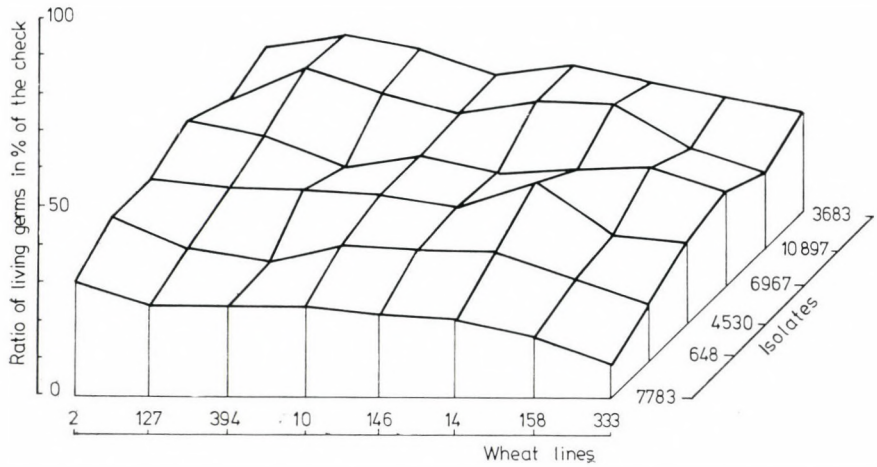

Fig. 1. Interaction between winter wheat genotypes and isolates of *Fusarium graminearum*

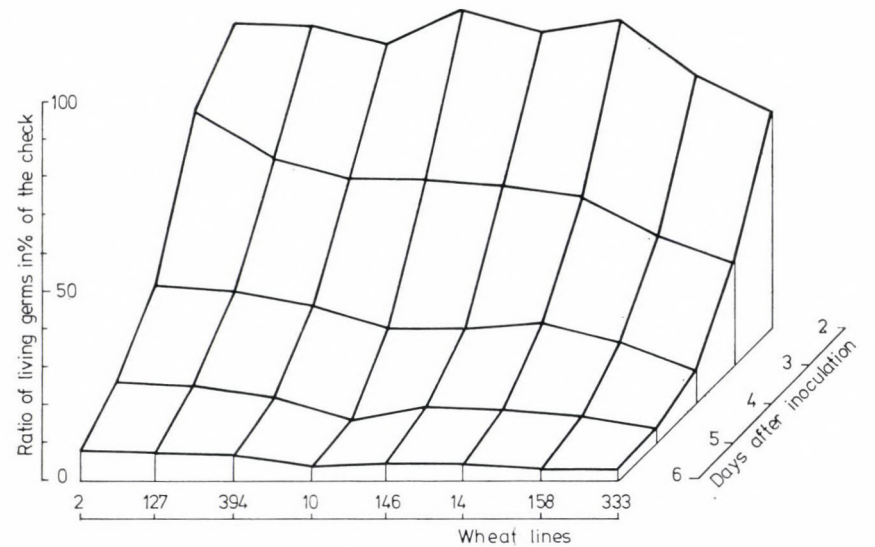

Fig. 2. Interaction between winter wheat genotypes and days after inoculation with *Fusarium graminearum*

characteristic of the isolates, like it is in the case of rust races, but a steadily changing phenomenon.

In spite of the differences in range orders, the genotype reactions are very similar to each isolates. The close correlations between variety reactions to different isolates support this insight (Table 7).

Table 7

Effect of 26 isolates of *F. graminearum* on four genotypes of winter wheat in seedling stage test. Data are means of four readings in % of the control

No.	Isolate conidium concentration $\times 10^6$/ml	Genotypes				
		74-2-3	Bzt-1	74-160-10	74-2-5	Mean
Germination of the control in %		95,32	96,00	98,00	100,00	97,32
648/1	0.01	42.18	16.38	45.35	88.00	47.98
648/2	1.58	24.60	20.55	50.23	54.50	36.44
2 856	0.27	17.25	10.20	43.30	59.25	32.50
3 682	0.68	3.35	2.58	22.13	39.00	16.77
3 711	1.22	83.88	86.93	97.65	89.50	89.49
3 715	0.47	10.95	3.35	16.30	30.43	15.26
3 905	0.27	0.00	0.00	0.00	1.25	0.31
4 041	0.01	12.00	9.58	11.68	52.25	21.38
4 059	0.25	40.63	20.53	42.16	65.00	42.24
4 366	0.30	11.23	2.03	13.23	56.00	20.26
4 502	0.83	97.03	86.68	98.68	84.75	91.79
4 530	0.90	10.15	3.10	22.40	45.25	20.23
7 073	0.00[1]	33.50	27.83	60.15	80.75	50.56
7 252	0.63	29.33	15.85	35.18	41.00	30.34
7 558	0.00	16.20	33.80	40.00	68.25	39.56
7 874	1.73	16.20	10.38	37.20	63.25	31.76
7 905	0.01	21.73	11.95	38.23	69.75	35.42
8 179	0.00	28.28	18.95	60.90	66.00	43.53
8 760	0.45	4.13	0.50	2.25	20.50	6.85
9 800	0.88	0.00	0.00	0.00	1.75	0.44
10 758	1.32	28.00	18.98	65.55	74.00	46.63
10 791	1.47	17.00	12.98	27.25	61.50	29.68
10 885	1.63	4.15	1.50	9.33	15.72	7.83
10 887	1.17	15.68	17.15	42.25	67.60	35.65
10 897/1	0.00	19.63	15.05	53.18	66.00	38.47
10 897/2	0.01	3.33	2.83	8.13	36.50	12.70
	Mean	22.71	17.29	36.31	59.76	32.84
		LSD		P=0.001	P=0.01	P=0.05
		Genotypes		7.96	6.21	4.72
		Isolates		20.29	15.83	12.04
		Combinations		40.59	31.66	24.08

[1] only mycelium occurs

Correlation coefficients Parameters		Bzt-1	74-160-10	74-2-5	
74-2-3		0.94***	0.89***	0.70***	
Bzt-1		—	0.87***	0.64***	
74-160-10		—	—	0.83***	

*** P = 0.001

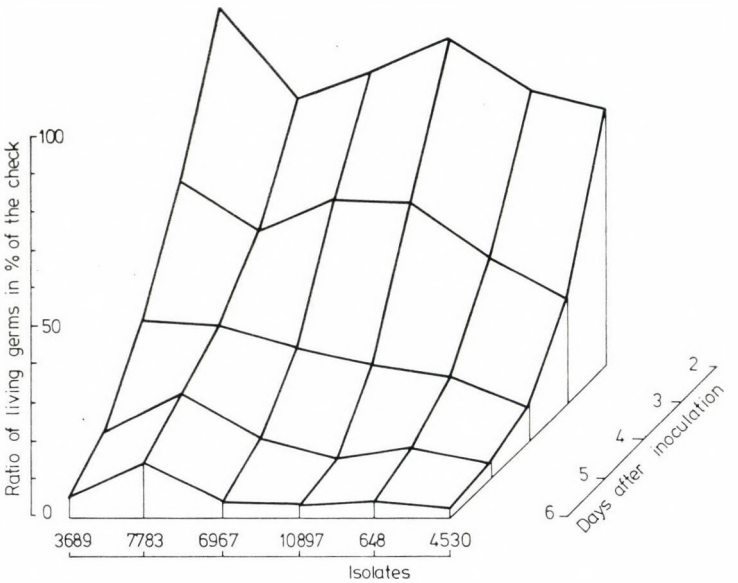

Fig. 3. Aggressiveness of *Fusarium graminearum* isolates on successive days after inoculation

There are significant interactions between varieties and reading data (Fig. 2). On the second day only the most susceptible lines differ significantly from the others, the differences between lines occur first on the third day after inoculation. The ratio of decrease of living germs differs from genotype to genotype. Hence the difference between the tolerant and susceptible lines is of quantitative nature, the decrease of living germs is more rapid in susceptible lines. This makes understandable also why the plant response depends on aggressiveness of the inoculum used. The data show furthermore that a longer observation period is necessary to obtain more exact data about the performance of genotypes investigated.

Similarly significant interactions were calculated between isolates and reading data (Fig. 3). This is a factor which influences the replicability of the results and helps to clarify some of the background of the problem.

For the other double interactions where one factor is the biological replication show the incompleteness to reproduce perfectly the results. The tendency of ranks is, however, the same to any isolates investigated, the best materials are always the same.

I suppose that the isolates belong to the same vertical race of *F. graminearum* and differences in host ranks are accidental only. This means that one experiment is enough to screen a large amount of material, but another test is necessary to identify smaller differences. Earlier results (MESTERHÁZY, 1977b) and these findings show that mixing of different isolates or using single isolates is an arbitrary problem.

How can we secure the nearly uniform aggressiveness in the experiments? Conidium concentration does not fit for this purpose (MESTERHÁZY, 1978a). The answer is a biological test, where the same seed stock is used to control aggressiveness. By this way the necessary level of dilution can be identified for very aggressive inocula and the weak inocula can be discarded. By this way the use of inoculums with unidentified aggressiveness can be avoided. As the results show even in this case occur deviations of smaller importance. Therefore, part of experiments or the whole should be tested again to control the reliability of the data. If the correlation between tests is above $r = 0.70$, the results can be accepted. In other case methodical problems are responsible for the deviations.

2nd experiment

The results are shown in Table 7. The isolates differ significantly from each other in aggressiveness, where between minimum and maximum of disease many intermediates occurred. The differences in disease reactions are clearly bounded to the aggressiveness of isolates used. For example, isolates 3711 and 4502 are very weak of aggressiveness, no significant reaction differences were observed. On the contrary, isolates 9800 and 3905 are very aggressive, again without significant differences in disease reaction. Significant genotype differences can be observed at isolates with intermediate aggressiveness. The variety reactions show very close correlations (Table 7) which support the results of the previous experiment: with any of the isolates similar results can be achieved except very weak and very aggressive inocula. The dependence of the variety ranks on the aggressiveness is expressed in the significant variety × isolate interaction.

3rd experiment

Results are presented in Table 9. There were significant differences between parallels of the same isolates. But the amplitudo of the deviations was small, so the isolates could be identified as horizontal races of the fungus.

Table 8

Conidium concentrations of inocula used in the 3rd experiment

Inocula	Isolates		
	4059	4502	10 897
1	0.01	0.26	0.00*
2	0.45	0.31	0.00
3	0.01	0.00	0.00
4	0.61	0.26	0.00
5	0.36	0.63	0.00
6	0.56	0.00	0.00

* Only mycelium was present

Table 9

Aggressiveness of six parallel inocula of three isolates of *F. graminearum* on four wheat genotypes. Data are numbers of living germs in % of the not inoculated control

Isolate	Genotype	Inocula						
		1	2	3	4	5	6	Mean
4 502	Bzt-1	100.34	92.44	94.96	94.72	96.18	96.82	95.91
	74-2-8	96.24	98.08	95.04	93.60	98.98	98.50	96.59
	75-I-144	91.86	90.18	89.54	85.14	97.12	101.54	92.56
	74-182-12	86.30	85.06	87.52	79.78	92.80	96.88	88.06
	Mean	93.69	91.44	91.77	88.31	96.01	98.44	93.28
4 059	Bzt-1	5.12	15.68	10.72	6.36	1.02	0.00	6.48
	74-2-8	4.42	12.30	19.80	4.84	0.00	1.40	7.13
	75-I-144	0.40	9.42	6.44	2.88	0.40	1.00	3.42
	74-184-12	0.20	2.00	2.62	2.42	0.00	0.00	1.21
	Mean	2.54	9.85	9.90	4.13	0.36	0.60	4.56
10 897	Bzt-1	2.66	0.00	0.00	4.10	0.82	0.62	1.37
	74-2-8	1.00	0.00	0.40	2.80	0.60	1.40	1.03
	75-I-144	0.62	0.00	0.20	4.12	0.60	0.60	1.62
	74-184-12	0.20	0.00	0.00	2.20	1.42	0.00	0.64
	Mean	1.12	0.00	0.15	3.31	0.86	0.66	1.02
Means for inocula		32.33	33.76	33.94	31.91	32.42	33.23	32.95
Means for genotypes		Bzt-1			34.59			
		74-2-8			34.91			
		75-I-144			32.33			
		74-184-12			30.30			

Source of variance	P = 0.001	P = 0.01	P = 0.05
Genotypes (A)	3.31	2.58	1.96
Isolates (B)	2.86	2.23	1.69
Inocula (C)	—	3.15	2.40
A × B	5.71	4.46	3.39
B × C	6.99	5.45	4.15
A × C	—	—	4.79
A × B × C	—	10.91	8.30

These results support the statements of the two previous experiments. For example the very aggressive isolate 10 897/2 killed every genotype without difference. On the contrary, isolate 4502/6 did not cause any significant harm to seedlings. Other inocula like isolate 4502/1 or 4059/3 produced significant variety differences. The variety ranks for the three isolates are the same (see average data).

As in the previous experiments the significant variety × isolate interaction was shown, here, too, the background of this is considered the same discussed earlier.

Discussion

The species *F. graminearum* is a species consisting of innumerous horizontal races with different aggressiveness. The disease causing capacity of individual inocula of the same isolate may show differences, even major deviations may occur. Generally, a weak isolate can not give inoculum of good quality, therefore, such isolates should not be utilized for serial tests. Isolates with high aggressiveness regularly give inocula of good quality, but exceptions often occur. Therefore, tests are necessary for pathogenicity.

According to BURGESS (1975, pers. comm.) and FRANCIS and BURGESS (1977) two vertical races are existing within *F. graminearum*. They are connected to the sexual stages of the fungus. Our isolates were identified as members of the second vertical race, where the fungus is homothalic (BURGESS, 1978, pers. comm.).

The disease reaction of wheat genotypes depends on the aggressiveness of horizontal race used for inoculation. No genotype difference occur at very low and very high aggressiveness level, because in the first case no disease develops or its amount is very limited, or every genotype is killed, when too aggressive isolates are used. Therefore, inocula with intermediate aggressiveness should be used for resistance tests. Inocula with too strong aggressiveness can be used after dilution.

The isolates for the first experiments were controlled for toxins produced by *F. graminearum*. In this test none of the isolates produced F-2 and T-2 toxins, other metabolites with smaller effect were, however, detected. But this was not connected to the varietal reaction, every isolate with different toxic background gave similar results (MESTERHÁZY, 1978b). Therefore, these results and variety differences are not a consequence of the toxin production, but that of the different fungal activity.

There are significant genotype × isolate interactions in all experiments analysed. This is, however, not connected to races with stable differential interactions. Here the reaction is not stable, so there is no prove that a special isolate would prefer a genotype as it occurs in obligate pathogens. Therefore, earlier data about vertical races (TU 1930) or genetical results (LARSON and ATKINSON) or environmental variance (NAKAGAWA *et al.*, 1966) should be used with care, because at least a part of this variability is due to the incomplete ability to reproduce results. The source of this is the instability of the fungus and instability of the host plant. It is important to conduct such experiments several times and this helps to decrease the accidental deviations.

In this paper the effect of variability within the pathogen was studied on the expression of the host performance. On the other hand, the variable reaction

of the host is similarly important. The resistance or tolerance of the genotypes is considered genetically to be stable. But actual response may be very different depending on physiological and ecological factors. Therefore the identification of the genetic background needs more detailed studies. In one experiment, should it be as complete as possible, statements can be made only on phenotypical reaction of genotypes.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3–4), pp. 293–297 (1981)

Fusarium Wilt of Gladiolus with Reference to Varietal Response and Chemical Control in Iraq

By

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Wilt of gladiolus observed for the last two years in Mosul appears to be the first record for Iraq. The disease is incited by *F. oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen. The disease is corm-transmitted in imported and locally produced corms, as the fungus was consistently and considerably isolated from them. Gladiolus wilt is more severe in spring than in autumn planting. It could be controlled by pre-planting dips of corms in 0.05% a.i. Benomyl or Thiocur for 15 minutes, followed by a drench of the same concentration after 15 days.

In Iraq gladioli are beautiful cut flowers usually grown in autumn and spring plantings. In late autumn 1978 and 1979, wilted gladiolus plants were observed in the Experimental Station of the College of Agriculture and Forestry, Hammam Al-Alil, Mosul, and in special gardens in the vicinity. Wilted plants showed bending of the young leaf stalk, with a gradual yellowing and drying beginning with the oldest leaves. It is believed that the disease was introduced into Iraq in imported corms from Europe especially from the Netherlands.

Wilt is the worst disease of gladiolus. It was reported from USA (Mc CULLOCH, 1944). It caused an estimated loss of $1^1/_2$ to 2 million dollars per year in Florida alone (FORSBURG, 1955). The losses in gladiolus crop were 30% in Germany (BRUHN, 1955) and 60 – 80% in USSR (PROTSENKO, 1958). The disease was reported from Italy (PETRI, 1940), England (BUXTON and ROBERTSON, 1953), New South Wales and Australia (ANON., 1955), Greece (DEMETRIADES *et al.*, 1957), Egypt (EL-ZARKA, 1961), India (SINGH, 1969) and Lebanon (SAAD and NIENHAUS, 1969). The present work was carried out in order to study the causal organism, varietal response, along with the chemical control.

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Materials and Methods

Samples of imported and local gladiolus corms, each comprising 9 corms showing rotting, were used for isolation. Infected parts were washed thoroughly, surface-sterilized by dipping in 1% sodium hypochlorite solution for 5 minutes, plated on PDA and incubated at 25 °C.

Gladiolus (cv. Herman van der Mark) was planted in the Experimental Station of the College of Agriculture and Forestry, Hammam Al-Alil, Mosul during autumn 1978 and spring 1979. Three hundred and eighty corms were planted in each season. Gladiolus plants with wilt symptoms were collected and the corms of which were used for isolation as described earlier.

Pots, 15 cm diameter, filled with sifted loam soil, were inoculated with the isolated fungus at the rate of half petri dish PDA culture/pot. One week later, apparently disease-free gladiolus corms of five cultivars, namely, Woodpecker, Herman van der Mark, Lilac Wonder, True Love and White Friendship, pre-treated with 1% sodium hypochlorite solution for 5 minutes, were planted at the rate of two corms/pot. Similarly treated corms planted in non-inoculated pots served as check. Each treatment comprised four pots. Pots were kept in the greenhouse (30 °C) and were examined periodically for eight weeks to determine the percentage of infection.

For controlling the disease, Benlate 50, Captan 50, Thiocur 50 and Vitavax-Thiram were used. The effect of different concentrations of the tested fungicides on the isolated fungus was studied on PDA. The concentrations used were 0.025%, 0.05%, 0.15% and 0.20% a.i. Check treatment was carried out on PDA without fungicide. Inocula, 5 cm in diameter, were taken from the growing margin of 5-day-old culture of the isolated fungus. Three replicates in each treatment were used, and incubated at 25 °C.

The tested fungicides were used at the rate of 0.05% a.i. for treating the corms (cv. Herman van der Mark) which were divided into two lots. Corms of the first lot were dipped in the fungicide for 15 minutes and then planted in methyl bromide-sterile soil contained in plastic bags, 10 cm diameter at the rate of two corms/bag. Corms of the other lot were treated similarly with the exception that they received a fungicidal drench (100 ml of 0.05% a.i./pot) after 15 days of planting.

Results

Isolations. Isolations carried out from the imported (cv. Herman van der Mark) and local gladiolus corm samples, and from corms of wilted plants, consistently yielded *Fusarium oxysporum*. Infection percentages were 22.2% and 100% in the imported and local corms respectively. The infection percentage of the naturally-wilted gladiolus plants in the Experimental Station were 11% and 80% in the autumn and spring plantings respectively. Pure culture of the isolated fungus was maintained on PDA slants.

Pathogenicity tests. Pathogenicity tests using an isolate of *F. oxysporum* obtained from corms of wilted plants cv. Herman van der Mark resulted in 100% infection after two months in the same cultivar. The other four cultivars showed moderate infection. Symptoms were a gradual yellowing, stunting, and then drying of the foliage. Roots showed reduced growth with clear rotting. Corm rot started from the parent corm and extended towards the core of the new corm, forming a brown-colored triangle with a wide base contacting the parent corm and passing into the roots. The disease extended from the top of the brown core towards the leaves.

Histopathological studies. Histopathological studies in which free hand sections were made in leaf bases of artificially-inoculated wilted plant (cv. Herman van der Mark) and stained with safranin and fast green, showed the presence of hyphae in the wood vessels with no such hyphae in the healthy plants.

Table 1

Effect of temperature on the linear growth of *F. oxysporum* f. sp. *gladioli*, grown on PDA

Temperature °C	Mean daily increase in diameter (mm)
10	0
15	5.4
20	6.7
25	12.3
30	10.7
35	4.8
40	0

Table 2

Effect of certain fungicides on *Fusarium* wilt incidence of gladiolus, applied as pre-plant dips of corms and pre-plant dips + drenching after 15 days

Fungicides	Total number of healthy plants after 2 months of planting	
	Pre-plant dips** for 15 minutes	Pre-plant dips for 15 minutes** + drenching after 15 days**
Check	0	0
Benomyl	6	8
Captan 50	2	4
Thiocur 50	6	8
Vitavax-Thiram	4	8

* Eight corms/treatment

** Each fungicide concentration was 0.05% a.i.

Effect of temperature on fungal growth. The effect of temperature on mycelial growth of the isolated pathogen grown on PDA medium at temperatures ranging from 10 to 40 °C at 5 °C intervals (Table 1) show that the fungus grew from above 10 °C to above 35 °C with an optimum at 25 °C.

Effect of the tested fungicides on the pathogen. In vitro studies of the tested systemic and non-systemic fungicides on *F. oxysporum* f. sp. *gladioli* at 25 °C, showed that Benomyl, Thiocur and Vitavax-Thirm, all at 0.025% a.i. were effective in checking the growth of the fungus. On the other hand, Captan 50 at 0.2% a.i. did not inhibit completely the growth of the fungus.

In vivo studies showed that Benomyl and Thiocur at 0.05% a.i. were the best fungicides in supporting better stand, especially when corms were at first dipped in the fungicide followed by drenching with the same fungicide two weeks later. Although Vitavax-Thiram gave also an equal stand-in the combined treatment – yet the plants were shorter than those treated with Benomyl or Thiram.

Discussion

Wilt of gladiolus which was observed for the last two years in Mosul appears to represent the first record of the disease in Iraq (MATHUR, 1968 and MOSTAFA, 1974). The disease is incited by *F. oxysporum* f. sp. *gladioli*, as the fungus was consistently isolated from the imported and local corms at considerably high rates, i.e., 22% and 100% respectively. This may support the findings of HENIS and ZILBERSTEIN (1973) who detected latent infection with *F. oxysporum* f. sp. *gladioli* in apparently rot-free gladiolus corms. The disease was more severe in the spring than in autumn planting, as it was exhibited by 80% and 11% wilted plants, respectively. This may be due to the fact that the corms used in the spring were taken from the autumn planting. So such corms are produced locally and are not imported as those used in the autumn planting. Moreover, the causal fungus is prevailing during spring in Mosul (22–25 °C), while in autumn the temperature is still rather high (23–38 °C). VLASOVA and SHTAIN (1974) found that some gladiolus cultivars exhibited 60–70% wilt due to *F. oxysporum* f. sp. *gladioli*. Corm rot started from the parent corm and extended towards the core of the new corm, forming a brown-colored triangle with a wide base contacting the parent corm and passing into the roots. The disease extended from the top of the brown core towards the leaves. This mode of infection agrees with that found by MICHAIL and ELAROSI (1965).

The present work revealed the effectiveness of using Benomyl or Thiocur as pre-plant dip at the rate of 0.05% a.i. for 15 minutes followed by drenching with the same concentration after 15 days. Such combined treatment showed to be better than the pre-plant dip alone, as it gave higher and more healthy plants. These results are in accord with those of FORSBERG (1970) and PAULUS *et al.* (1970 and 1971) who found that Benomyl was the most effective against the disease as it reduced the number of corms infected and increased the number of flower harvested.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 299—305 (1981)

Reaction of Onion Cultivars to Scald Disease Incited by *Alternaria porri*

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Bulbs of three onion cultivars differed in their reaction to scald disease incited by *A. porri*. Giza 6 and Behairi cultivars were susceptible and Taliani Red was resistant. The differential reaction to the disease is attributed to the higher content of the phenolic compounds and the higher oxidases activity in the resistant cultivar which inhibit the macerating enzymes excreted by two isolates of *A. porri* (differing in their virulence) that induce the scald disease.

Onion is one of the main export crops in Egypt. Exported onions are extensively grown in upper Egypt. In 1972, the total cultivated area with onions was 31 672 feddans (13 302 hectars) which yielded 269 747 tons of onion bulbs (ANON., 1972).

In Egypt, scald disease of onion bulbs, incited by *Alternaria porri* was repeatedly reported (EL-HELALY *et al.*, 1966 and WASFY *et al.*, 1977). No work has been achieved on the varietal resistance and the enzymatic activities of different isolates of the causal pathogen as far as the available literature is concerned. This stimulated to determine the response of certain onion cultivars to the disease, and to determine the oxidative and macerating enzymes which might be involved in disease response.

Materials and Methods

Medium-size onion bulbs of certain cultivars, namely: Behairi, Giza 6 and Taliani Red were used. Onion bulbs were surface-sterilized by dipping in 0.1% mercuric chloride solution for 3 minutes, then rinsed in sterile water. Isolate no. 1 (avirulent) and isolate no. 6 (virulent) of *A. porri* (WASFY, 1977) were used. Six mm discs of the advancing edge of the isolate was inserted 3 mm deep near the disc stem of apparently onion bulbs. Bulbs were kept in perforated polyethylene bags at room temperature (24—31 °C) until symptoms developed. Bulbs served as checks were similarly treated with the exception of using — free PDA discs. Four bulbs were used in each replicate (bag) and four replicates consisted a treatment.

Phenolic compounds were determined in the *A. porri* isolates artificially-infected and healthy onion bulbs of the three cultivars by adopting the method of KHALIFA *et al.* (1968).

Determination of enzymes

Cellulase, pectolytic (polymethyl-galacturonase (PMG), pectinmethylesterase (PME) and pectinmethyl-transeliminase (PMTE)) and oxidative (polyphenol-oxidase, peroxidase and catalase) enzymes were assayed in tissues of healthy and *A. porri* (isolate no. 1 and isolate no. 6) artificially – inoculated onion bulbs of the cultivars tested. The assay of the pectolytic enzymes and the cellulase were determined according to the method used previously (WASFY *et al.* 1977).

Polyphenoloxidase assay

For determination of polyphenoloxidase, the method described by BRAESCH (1954) was used. Polyphenoloxidase activity was determined in inoculated and non-inoculated onion bulbs after 30 days. Enzyme activity was measured by grinding 10 g of inoculated and non-inoculated tissues in a mortar with 14 ml borate buffer (pH 9). Extracts were centrifuged for 15 minutes ar 4000 rpm. The supernatant was diluted by adding 8 ml distilled water to 2 ml supernatant. Aliquots of diluted supernatants were assayed for polyphenoloxidase activity using Spekol colorimeter at 575.5 nm. The reaction mixture consisted of 2 ml borate buffer (pH 9), 1 ml of 1% P-aminobenzoic acid, 2 ml of 1% catechol and 0.2 ml extract. After mixing for 45 minutes, a red colour was formed. The activity of the enzyme was measured as relative deepness in red colour, by optical density. Control treatment was done similarly except that extracts were previously boiled. Four replicates were used in each treatment.

Peroxidase assay

For determination of peroxidase, the method described by Fehrmann and Dimond (1967) was used. Peroxidase activity was determined in inoculated and non-inoculated onion bulbs after 30 days. Enzyme activity was measured by grinding 10 g of inoculated and non-inoculated tissues in a mortar with 14 ml phosphate buffer (pH 6). Extracts were centrifuged for 15 minutes at 4000 rpm. The supernatant was diluted by adding 8 ml distilled water to 2 ml supernatant. Aliquots of diluted supernatant were assayed for peroxidase activity using Spekol colorimeter at 470 nm. The reaction mixture consisted of 1.5 ml of 0.04 M catechol solution; 1.5 ml H_2O_2 (20 volume), 1.5 ml phosphate buffer (pH 6) and 0.2 ml of extract. The control treatment was done similarly except the extract was previously boiled. Four replicates were used in each treatment. The difference in optical density between the reaction mixture and that of the control was taken as a measure of the activity of the reaction. Enzyme activities were expressed as the increase in optical density from 60 – 120 seconds after the substrate was added.

Catalase assay

For determination of catalase activity, the method described by COLOWICK and KAPLAN (1955) was used. Catalase activity was determined in inoculated and non-inoculated onion bulbs after 30 days. Enzyme activity as measured by grinding 10 g of tissues in a mortar with 14 ml phosphate buffer (pH 7). Extracts were centrifuged for 15 minutes at 4000 rpm. Aliquots of the supernatants were assayed for catalase activity, by the addition of 4 ml of the extract to 60 ml of 0.01 N H_2O_2 solution. The mixture was incubated at 25 °C for 55 min. The decomposition of H_2O_2 was measured by titrating the remaining substrate with 0.0052 N potassium permanganate solution after stopping the enzymatic reaction with 5 ml 2% (V/V) sulfuric acid. A sample of 5 ml was taken from each assay mixture at 2-min at first and 5-min intervals, and the remaining H_2O_2 was titrated. Four replicates were used in each treatment.

Results

Pathogenicity

Scald symptoms of *A. porri* appeared on the susceptible cultivar after 30 days of inoculation, with no symptoms on the check. Symptoms of artificially-inoculated bulbs were similar to those obtained naturally. Onion cultivars tested differed in their response to *A. porri*. Giza 6 and Behairi were susceptible and Taliani Red was resistant as it showed no disease symptoms after the same period of inoculation (Fig. 1).

Phenolic compounds

Determination of phenolic compounds in *A. porri*-artificially-infected (two isolates) and healthy onion bulbs of the three cultivars showed higher polyphenol-

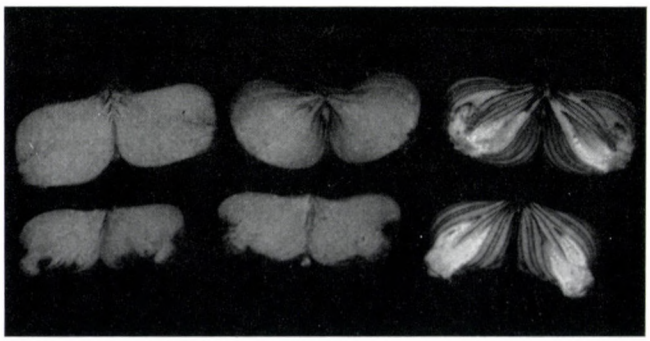

Fig. 1. Onion bulbs of three cultivars artificially-inoculated (lower set) near the disc stem with *A. porri* and cut lengthwise after 30 days, showing scald disease symptoms on Giza 6 (left) and Behairi (middle) with no symptoms on Taliani Red (right). Check upper set

Table 1

Phenolic compounds in onion bulbs of three cultivars artificially-inoculated with two isolates of *A. porri* after 30 days at room temperature (24—31 °C)

Cultivar	Treatment	mg/100 g dry weight
Giza 6	Non-inoculated (check)	12.9
	Inoculated with isolate no. 1	13.1
	Inoculated with isolate no. 6	18.2
Behairi	Non-inoculated (check)	12.7
	Inoculated with isolate no. 1	12.9
	Inoculated with isolate no. 6	18.4
Taliani Red	Non-inoculated (check)	20.4
	Inoculated with isolate no. 1	20.9
	Inoculated with isolate no. 6	22.1

ic content in the Taliani Red than in Behairi and Giza 6. The polyphenols content were invariably higher with isolate no. 6 as compared with isolate no. 1 in the three cultivars tested (Table 1).

Enzyme activities

Determination of the macerating (PMG, PME and cellulase) and oxidative enzymes in healthy and *A. porri* artificially – inoculated onion bulbs showed that the activities of such enzymes were higher in the inoculated than in healthy bulbs.

Table 2

Pectolytic and cellulolytic enzyme activities in onion bulbs of three cultivars artificially-inoculated with two isolates of *A. porri* after 30 days at room temperature (24—31 °C)

Cultivar	Treatment	PMG[1]	PME[2]	PMTE[3]	Cellulase[1]
Giza 6	Non-inoculated (check)	2.97	3.13	—	2.45
	Inoculated with isolate no. 1	3.11	3.65	—	2.66
	Inoculated with isolate no. 6	59.99	19.77	—	82.12
Behairi	Non-inoculated (check)	2.59	3.00	—	2.36
	Inoculated with isolate no. 1	2.99	3.41	—	2.45
	Inoculated with isolate no. 6	53.31	15.61	—	79.46
Taliani Red	Non-inoculated (check)	2.12	2.36	—	1.78
	Inoculated with isolate no. 1	2.15	2.67	—	1.99
	Inoculated with isolate no. 6	2.64	3.41	—	2.13

[1] Reduction in viscosity %; [2] μ equivalent/min/ml; [3] Transmission (%)

Table 3

Polyphenoloxidase and peroxidase activities in onion bulbs of three cultivars artificially-inoculated with two isolates of *A. porri* after 30 days at room temperature (24—31 °C)

Cultivar	Treatment	Optical density	
		Polyphenol-oxidase	Peroxidase
Giza 6	Non-inoculated (check)	0.120	0.050
	Inoculated with isolate no. 1	0.160	0.055
	Inoculated with isolate no. 6	0.250	0.095
Behairi	Non-inoculated (check)	0.140	0.055
	Inoculated with isolate no. 1	0.150	0.085
	Inoculated with isolate no. 6	0.280	0.120
Taliani Red	Non-inoculated (check)	0.230	0.140
	Inoculated with isolate no. 1	0.280	0.150
	Inoculated with isolate no. 6	0.340	0.155

The macerating enzymes (PMG, PME and Cellulase) were much higher in Giza 6 and Behairi than in Taliani Red cultivar (Table 2). Tests for "PMTE" enzyme in all tests showed negative results which suggested that the enzyme was lacking. On the other hand, the activity of the oxidative enzymes (polyphenoloxidase, peroxidase and catalase) was more marked in Taliani Red than in either Giza 6 or Behairi cultivars (Tables 3 and 4).

Discussion

The differential reaction of the three onion cultivars to the scald disease incited by *A. porri* (two isolates) could be explained in the light of the polyphenol content and the oxidative enzymes activities. Pathogenicity tests showed that Giza 6 and Behairi cultivars were susceptible, whereas Taliani Red was resistant. The macerating enzymes, i.e. PMG, PME and cellulase were higher in the *A. porri*-inoculated bulbs of Giza 6 and Behairi than in those of Taliani Red. On the other hand, the polyphenol content, oxidative enzymes, i.e., polyphenoloxidase, peroxidase and catalase were higher in the *A. porri*-inoculated bulbs of Taliani Red than those of Giza 6 and Behairi cultivars (especially with isolate no. 6). Greater accumulation of polyphenols in the resistant than in the susceptible cultivars is supported by HAMPTON (1962), KIRÁLY and FARKAS (1962), PATIL *et al.* (1962) and ABD-ELRAZIK *et al.* (1972). Meantime, such polyphenols were most likely inhibitory to the macerating enzymes excreted by the fungus as such enzymes decreased markedly in the resistant as compared with the susceptible cultivars. These results agree with those obtained by BYRDE (1957 and 1963) and BYRHE *et al.* (1960),

Table 4

Catalase activity in onion bulbs of three cultivars artificially-inoculated

Cultivar	Treatment	MgH_2O_2 Decomposed		
		2	5	10
Giza 6	Non-inoculated (check)	0.441	0.592	0.931
	Inoculated with isolate no. 1	0.450	0.601	0.944
	Inoculated with isolate no. 6	0.601	1.199	1.311
Behairi	Non-inoculated (check)	0.552	0.891	1.331
	Inoculated with isolate no. 1	0.574	0.898	1.384
	Inoculated with isolate no. 6	0.632	1.224	1.521
Taliani Red	Non-inoculated (check)	0.640	1.226	1.500
	Inoculated with isolate no. 1	0.695	1.301	1.621
	Inoculated with isolate no. 6	0.717	1.613	1.623

DEESE and STAHMANN (1963) and WILLIAMS (1965) who stated that the oxidized phenols are strong inhibitors of extracellular pectolytic enzymes of fungi causing wilt and soft rot diseases.

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with two isolates of *A. porri* after 30 days at room temperature (24—31 °C)

after (min)								
15	20	25	30	35	40	45	50	55
1.069	1.188	1.462	1.711	1.823	1.910	2.110	2.220	2.301
1.091	1.221	1.489	1.723	1.846	1.951	2.133	2.262	2.308
1.512	2.144	2.198	2.201	2.211	2.230	2.311	2.320	2.331
1.494	1.611	1.822	1.886	1.998	2.123	2.251	2.282	2.311
1.515	1.626	1.871	1.911	2.022	2.222	2.255	2.295	2.333
1.648	2.246	2.266	2.278	2.299	2.344	2.365	2.381	2.599
1.801	1.913	2.011	2.096	2.188	2.204	2.296	2.326	2.344
1.829	2.032	2.101	2.155	2.213	2.241	2.299	2.327	2.500
1.930	2.261	2.312	2.378	2.382	2.462	2.466	2.468	2.692

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 307—313 (1981)

Biological Control of Crown-gall Tested on Bean Leaves

By

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Crown-gall tumors caused by *Agrobacterium tumefaciens* can be controlled biologically on primary bean leaves by a related non-pathogenic bacterium, *Agrobacterium radiobacter* (strain K84). Two pathogenic strains of *Agrobacterium tumefaciens* an agrocin-sensitive wild-type strain (0) and an agrocin-resistant derivative of strain 0 (103) were tested with K84 on primary bean leaves at different times of inoculation. In the case of agrocin-sensitive strain no galls developed on leaves treated with strain K84 30 minutes after inoculation of the pathogenic strain, but galls appeared on leaves treated 24 hours after inoculation. Tumor formation was also reduced when the agrocin-resistant strain was inoculated simultaneously with or after of K84. This type of inhibition seems to be independent from agrocin sensitivity. Further investigations are needed to elucidate its mechanism.

Crown-gall, caused by *Agrobacterium tumefaciens* (Smith and Townsend) Conn affects a wide range of host plants and causes serious economic losses especially of fruit trees in nursery stock. Attempts to control the disease by chemical means have met with limited success. In 1972, NEW and KERR (1972) published the first report of biological control of crown-gall by using strain K84 of *Agrobacterium radiobacter* which produces a bacteriocin that inhibits sensitive strains of *Agrobacterium tumefaciens* (KERR and HTAY, 1974). The method has given excellent control of the disease on stone fruits (HTAY and KERR, 1974). Tumor induction by *Agrobacterium tumefaciens* occurs only after the bacterium attaches to a specific sites in a plant wound (LIPPINCOTT and LIPPINCOTT, 1969). To understand the nature of this attachment and its role in tumor induction, both the bacterial and host attachment site components were isolated and characterized by WHATLEY *et al.* (1976). They found the bacterial site component was in the lipopolysaccharid moiety of the cell enveloppe. LIPPINCOTT and HEBERLEIN (1965) have shown that tumors may be initiated on primary bean leaves and that a general relationship exists between number of tumors per leaf and number of bacteria in the suspension of inoculum. The number of tumors initiated by strain B6 on bean leaves (LIPPINCOTT and LIPPINCOTT, 1969) or potato discs (GLOGOWSKI and GALSKY, 1978) was decreased when cells of an avirulent strain were included in the inoculum. Plant cell walls but not membranes contain surfaces to which pathogen bacte-

ria adhere and these exhibit the specificity of the host site to which virulent bacteria attach to induce tumors (LIPPINCOTT *et al.*, 1977). In biological control the suitable time for adding strain K84 to roots should be within 2 h after lifting (HTAY and KERR, 1974). The present study was designed to determine: 1. The timing of application of the controlling organism (K84) on bean leaves; 2. To determine the number of sensitive and resistant strains at various times after the inoculation of the antagonistic strain on bean leaves.

Materials and Methods

Bacterial strains

Strain K84 of *Agrobacterium radiobacter* was kindly supplied by Professor A. KERR, Australia. Strain 0 is a wildtype agrocin sensitive strain isolated from grapevine in Hungary. Strain 103 is an agrocin resistant virulent derivative of strain 0 (SÜLE and KADO, 1980).

Media

All strain were grown routinely in medium containing per litre: 10 g peptone, 1 g yeast extract (Oxoid), 2 g glucose and 15 g agar. The medium was sterilized at 121 °C for 20 min.

Plants

Bean plants (*Phaseolus vulgaris* L. cv. Pinto) were sown in steril sand in glasshouse and at 7–9 days after sowing were selected for uniformity and transplanted to 60 mm pots on the day previous to use. Selection of plants proved to be more practical on size basis than on an exact age basis. Leaves falling within the size range of 8–10 square cm in area at the time of inoculation are near their peak of sensitivity (LIPPINCOTT and HEBERLEIN, 1965b).

Inoculation

The method of LIPPINCOTT and HEBERLEIN (1965a) was used. Primary bean leaves were lightly dusted with No. 500 grift carborundum and placing 0.05 ml of a suspension (10^9 cells/ml) of pathogenic strain. The suspension was then spread over on the leaf surface with a glass-spatula. Application of the suspension of K84 (0.05 ml, 10^9 cells/ml) followed in different intervals of the inoculation of pathogen. Ten replicates were used for each treatment.

Counting of bacteria on leaves

Antibiotic resistant strains were obtained by cultivating bacteria on media containing increasing concentrations of chloramphenicol and streptomycin. Strains resistant to 500 ppm of antibiotic were inoculated simultaneously with strain K84.

After various times five discs from five leaves were taken with cork borer (9 mm diameter) and crushed in mortar with 1 ml water. After serial dilutions, the plating method with media containing 50 ppm antibiotics was used for accounting colonies.

Results

Timing of application of K84

The results obtained in Fig. 1 and Table 1 show that no tumors developed on leaves when strain K84 was applied in the first hour after inoculation of strain 0 (sensitive), but tumors developed on all other plants. Mean numbers of tumors

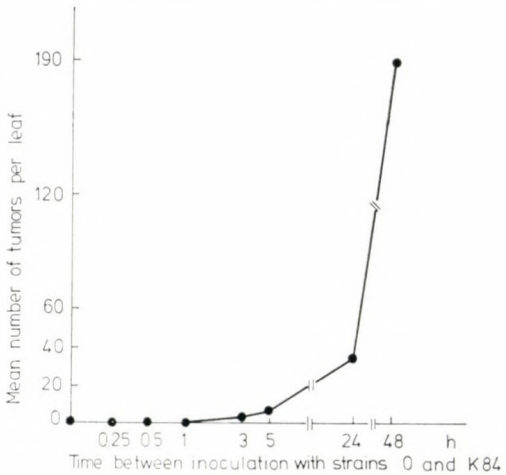

Fig. 1. Effect of K84 on tumors number when added to leaves at various times after inoculation of strain 0 (agrocin sensitive)

Table 1

The effect of application of K84 at various times after inoculation of *A. tumefaciens* on tumor numbers

Time between inoculation and application of K84	Number of tumors per leaf							
	0	15 min	30 min	1 h	3 h	5 h	24 h	48 h
Str.0	181.5[a]							
Str.0 + K84	0	0	0	0	2.5	4.5	34.3	190
Str.103	174							
Str.103 + K84	14	21.6	24.8	40	50	73.5	162	193

[a] Mean values from ten leaves from each experiment

per leaf were 2.5, 4.5, 34.3 and 190 at 3, 5, 24 and 48 hrs respectively after inoculation of strain 0. The control number in the same experiment was 181.5 tumor per leaf. If results of this experiment can be extrapolated to fruit trees, than strain K84 should be applied to transplants within 1 h after lifting. Tumors developed on the leaves inoculated with strain 103 (agrocin resistant) and followed in different times with K84 (Fig. 2 and Table 1). It was very interesting that tumor numbers were much less if K84 was applied at 0, 15 and 30 minutes after inoculation of strain 103, than at 1, 3, 5 and 48 hours. According to the time of the application

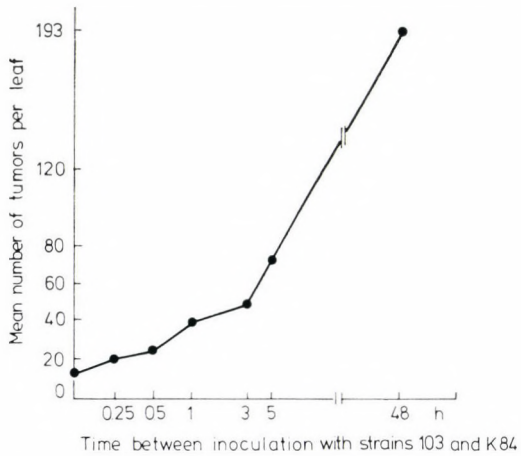

Fig. 2. Effect of K84 on tumors number when added to leaves at various times after inoculation of strain 103 (agrocin resistant)

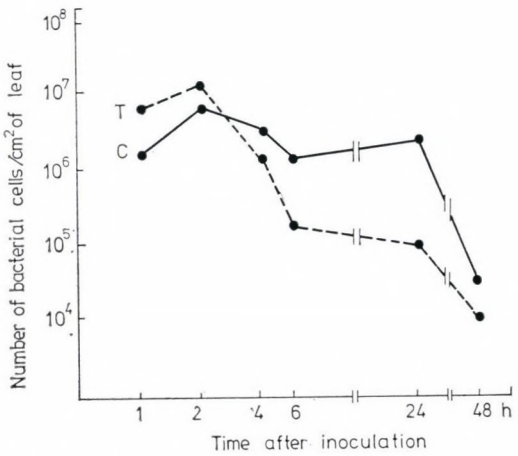

Fig. 3. Number of bacterial cells on bean leaves at various times after inoculation. C = strain 0 alone; T = strain 0 + K84

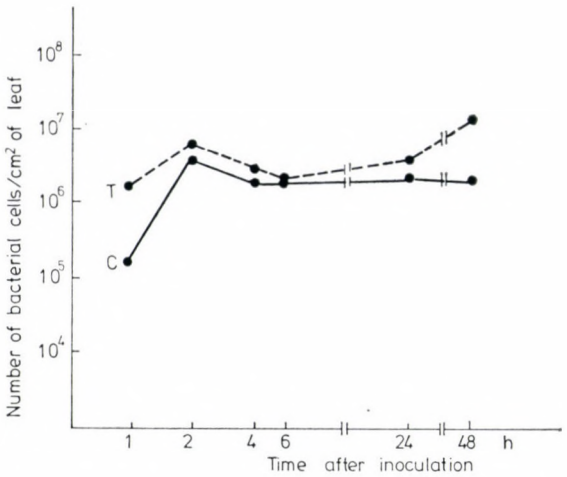

Fig. 4. Number of bacterial cells on bean leaves at various times after inoculation. C = strain 103 alone; T = strain 103 + K84

of K84 the mean numbers of tumors were 14, 21 and 25 at 0, 15 and 30 minutes and 40, 50, 73.5 and 193 at 1, 3, 5 and 48 hrs, respectively. The control was 174.5 tumors per leaf. The inhibition was most effective when K84 was inoculated previously (not shown in Fig. 2) or together with strain 103. On the other side, the maximum number of tumors developed when K84 was applied after 48 h of pathogens.

Number of bacterial cells on leaves

The agrocin sensitive (0) and resistant (103) strains were inoculated simultaneously with K84 onto bean leaves. Control leaves were inoculated only with the strain 0 or 103. After inoculation at various times, samples were taken and the cell numbers were evaluated on antibiotic containing media. The results are

Table 2

Number of bacterial cells after various times of inoculation

Time after inoculation (hours)	Number of bacteria ($\times 10^6$) per ml leaf extract[a]					
	1	2	4	6	24	48
Str.0	2.2	8.2	5.3	1.4	4	0.05
Str.0 + K84	8.5	15.5	1.9	0.3	0.1	0.01
Str.103	0.23	6	2.7	2.85	3.2	2.9
Str.103 + K84	2.3	8	5	3.2	6	14

[a] Figures are mean of two different determinations.

shown on Figs 3–4 and Table 2. It is clear that after inoculation both strain 0 and 103 multiplied for 1–2 hrs. After 2 h the cell number of strain 0 gradually decreased however strain 103 remained on the same level. Cell number of control (strain 0 alone) also decreased after 24 h. In the case of strain 103 after 2 h there was also a slight decreasing in cell number, but after 6 h it increased gradually to the end of the experiment (48 h). Cell number of the control (103 alone) remained on the same level.

Discussion

Tumor formation on bean leaves can be controlled effectively with strain K84. The protective effect of K84 was much higher against agrocin sensitive strain (0) than against resistant strain (103). The mechanism by which K84 prevents infection has been attributed to a bacteriocin (its special name is agrocin) by KERR and HTAY (1974), rather than to exclusion of the pathogen from the attachment sites (LIPPINCOTT and LIPPINCOTT, 1969). Our results with strain 0 support the first proposition and underline that agrocin plays a basic role in biological control of crown-gall. On the other side, investigations with strain 103 show that beside agrocin the theory of attachment sites also has some merit. K84 inoculated before or together with strain 103, also decreased the number of tumors, however this type of inhibition was much weaker than inhibition of agrocin. This suggests that inhibition of agrocin resistant strains might be due to a slightly mode of attachment inhibition or to secretion of some other substance than agrocin. The number of cells of agrocin sensitive strain on bean leaves was only reduced after 2 h of the application of K84, indicating that agrocin was not bactericid but bacteriostatic *in vivo*. This results is in accordance with in vitro observations (SÜLE, 1978). Cell number of agrocin resistant strain increased after 48 h of the inoculation which means that agrocin had no effect on this strain.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 315—326 (1981)

New Artificial Hosts and Non-Hosts of Plant Viruses and their Role in the Identification and Separation of Viruses. XV. Monotypic (Almovirus) Group: Alfalfa Mosaic Virus

By

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In the course of experiments carried out in the last ten years we found many new alfalfa mosaic virus host plants. Of the 139 new virus susceptible plants — belonging to the families *Amaranthaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Commelinaceae*, *Fabaceae (Leguminosae, Papilionaceae)*, *Labiatae (Lamiaceae)*, *Malvaceae*, *Scrophulariaceae*, *Solanaceae*, *Umbelliferae (Apiaceae)* — 64 showed local, 29 systemic and 46 plants local and systemic susceptibility. Special attention is worth being paid to a number of *Amaranthus* species, -varieties, -forms, -cultivars, and *Obione-*, *Vigna-*, *Cucubalus-*, *Datura-*, *Galega-*, *Lycium-*, *Melandrium-*, *Ocimum-*, *Pentstemon-*, *Petunia* species as well as to *Solanum tuberosum* varieties which as host plants are new to the science.

In artificial inoculation experiments nine plants belonging to the families *Aizoaceae*, *Cucurbitaceae*, *Scrophulariaceae* and *Solanaceae* proved resistant to alfalfa mosaic virus. Dichotomous separators and semiseparators used in the experiments play an important role in differentiating the virus pathogens of plants.

In our experiments we were able to differentiate 22 viruses with 148 plants in 340 combinations of which 320 variations have become known through our recent investigations. Considering that the alfalfa mosaic virus and potato aucuba mosaic virus cause similar, often deceptive symptoms in potato, and their joint occurrence is not impossible either, the disjunction of the two viruses is of extreme practical importance. In the course of our investigations we found that the dichotomous separators systemically susceptible to alfalfa mosaic virus and locally susceptible to potato aucuba mosaic virus (e.g. *Lycium horridum*, *L. ruthenicum*, *L. turcomanicum*, *Ocimum canum*, *O. sanctum*), and the semiseparators resistant to alfalfa mosaic virus while locally and systemically *(Browallia cordata*, *Br. grandiflora*, *Br. roezli*, *Br. viscosa)*, or only locally *(Paulownia fargesii)* susceptible to potato aucuba mosaic virus were extremely useful in differentiating the two above-mentioned viruses.

The alfalfa mosaic virus (R/1 : 1.3 + 1.1 + 0.9/18 : U/U : S/Ap; BOS and JASPARS, 1971; JASPARS and BOS, 1980), a polyphagous virus transmissible mechanically and in a styletborne manner by aphids was discovered some five decades ago in California (cf. WEIMER, 1931). The occurrence of the world-wide distributed virus in Hungary were first published by SZIRMAI (1952). Details of the various isolates and strains of the virus as well as of their biological and physical properties have become known in Hungary through the investigations of BECZNER (1966, 1972, 1973a, b, 1974) and HORVÁTH (1976).

The first detailed account of its host range was given by the American virologist PRICE (1940). The rapid spread and economic importance of the virus as well as its role in the theoretical virus research rendered it necessary to acquire a thorougher and fuller knowledge of its host range. According to the papers published since the beginning of the sixties (CERVANTES and LARSON, 1961; SCHMELZER, 1962; BECZNER, 1966; HULL, 1969; CRILL *et al.*, 1970; BECZNER and SCHMELZER, 1972a, b; BECZNER, 1973a, b; SCHMELZER *et al.*, 1973; BECZNER, 1974; SCHMIDT, 1977) the natural and artificial hosts of the alfalfa mosaic virus include some 600 mono- and dicotyledonous plants distributed all over the world. On the basis of the literary data available it can be established that the alfalfa mosaic virus has the third widest range of host plants after the cucumber mosaic virus and tobacco mosaic virus. Among the natural hosts there are plants – like e.g. potato – important from an economic point of view. It is remarkable that the so-called calico disease induced in potato by the alfalfa mosaic virus was first described in the United States of America, the native country of potato (HUNGERFORD, 1922). At that time, however, the calico disease of potato was considered to be a genetic abnormality. Nine years later PORTER (1931) pointed out the infectious nature of the calico disease, then through the investigations of BLACK and PRICE (1940) in New Jersey State it become known that the disease was caused by the alfalfa mosaic virus. The description of two essentially different alfalfa mosaic virus strains (calico strain and tuber necrosis strain) occurring in potato was made possible by the discovery of the phenomenon cross protection at the end of the twenties and its wide application in the thirties (CERVANTES and LARSON, 1961; CRILL *et al.*, 1970). According to our present knowledge outside the United States of America alfalfa mosaic virus has been pointed out in potato – in a chronological order – only in the following countries: Spain (FERNOW and GARCES, 1949), Peru (SILBERSCHMIDT, 1954; BAZAN DE SEGURA, 1955), Italy (GRANCINI, 1956), England (RICHARDSON and TINSLEY, 1956), German Federal Republic (RAMSON and JANKE, 1958), then following its discovery in Hungary (HORVÁTH, 1963) in Japan (KOMURO *et al.*, 1964), Bulgaria (KOVACHEVSKY, 1965), India (NAGAICH and GIRI, 1968), Jugoslavia (KUS, 1977), Poland (CHRZANOWSKA and ZAGÓRSKA, 1977) and Argentina (BUTZONITCH, 1978). The increasing frequency of alfalfa mosaic virus appearing in potato in Hungary is – according to our observations – closely related with the growing area of lucerne, one of its most important perennial host plants, and of various virus susceptible leguminous fodder crops as well as with the rapid spreading of weed plants susceptible to it (e.g. *Solanum nigrum*).

In our opinion it is just possible that the appearance of the alfalfa mosaic virus in Europe was due to the potato as its original host plant carried in the 16th century from the New World first supposedly to Spain, then gradually introduced in other European countries. This supposition is backed up by the fact that Spain was the very place where the disease of potato caused by alfalfa mosaic virus was first described in Europe (FERNOW and GARCES, 1949). It is not naturally excluded either that the alfalfa mosaic virus – which is easily transmitted with seeds of certain plants – was introduced in Europe by the seeds of diseased potato plants.

The possible role played by the potato seed in the transmission of the alfalfa mosaic virus is a mere supposition which – according to our knowledge – has not been experimentally proved so far. The polyphagous, aphid transmissible alfalfa mosaic virus is supposed to have become pathogenic through gradual adaptation and mainly with aphid vectors specialized to lucerne as feed plant which owing to its perennial nature and wide distribution in Europe is a primary source of the alfalfa mosaic virus. The ever closer host-virus relation between potato and alfalfa mosaic virus can possibly be traced back to the increasing production area of the leguminous fodder crops and the large number – some 600 plants in 70 families – of virus susceptible host plants and aphid vectors.

In the course of investigations carried out in the last several years in which we had dealt with the host range of alfalfa mosaic virus many new hosts and resistant plants were pointed out (cf. HORVÁTH, 1966; 1967; 1969a, b; 1970; 1973a, b; 1974a, b, c; 1975a, b, c, d; HORVÁTH and BECZNER, 1968; 1972; 1973; BECZNER and HORVÁTH, 1968; 1972; 1973; HORVÁTH, 1980). In this paper we list the plants examined while studying the host range of alfalfa mosaic virus.

Materials, Methods and Results

As to the Materials and Methods of experiments information was given in the second publication of the series (cf. HORVÁTH, 1977). In this paper the results are presented.

New Hosts and Non-Hosts of Alfalfa Mosaic Virus and their Role in the Separation of Viruses

Local susceptible hosts

Amaranthus aureus (A. paniculatus var. *flavus):* Ama, Th // M // / + // ⊟ –
A. caracu (A. hypochondriacus): Ama, Th // M // / + // ⊟ –
A. caudatus cv. *Atripurpureus (A. caudatus* var. *sanguineus)°:* Ama, Th // M // / + // ⊟ –
A. chlorostachys (A. hybridus)°: Ama, Th // M // / + // ⊟ –
A. chlorostachys f. *strictus (A. chlorostachys* f. *leucocarpus)°:* Ama, Th // M // / + // ⊟ –
A. chlorostachys var. *powelli°:* Ama, Th // M // / + // ⊟ –
A. emarginatus (A. lividus var. *ascendens)°:* Ama, Th // M // / + // ⊟ PVS18
A. gangeticus var. *multicolor (A. tricolor)°:* Ama, Th // M // / + // ⊟ –
A. graecizans (A. graecizans var. *sylvestris, A. sylvestris):* Ama, Th // M // / + // ⊟ –
A. mantegazzianus°: Ama, Th // M // / + // ⊟ –
A. spinosus: Ama, Th // M // / + // ⊟ –

A. sylvestris (A. graecizans var. *sylvestris)°:* Ama, Th // M /// + // ⊟ −
A. tricolor cv. *Malten Fire°:* Ama, Th // M /// + // ⊟ −
Obione sibirica (Atriplex sibirica)°: Chen, Th // M /// + // ⊟ BCMV, CLRV/, TRSV/, TYMV, CeMV
Phaseolus ricciardianus°: Fab = Legu = Pap, ? // M /// + // ⊟ −
P. vulgaris cv. *Aladin:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Andrásbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Annelise:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Barnabab°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Cardinal:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Caroline:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Cukorbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Fehér gyöngy°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Fertődi 5.°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Fertődi 23.°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *GN 59.:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *GN 123.:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Harkovszkaja:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Icar Fundulea 51.:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Icar Fundulea 416.:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Japan gyöngybab°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Kanizsai csikosbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Kentucky Wonder:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Kereskedelmi hosszú fürjbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Kinghorn Wax:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Kompolti gyöngybab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Korai fürj°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS, TMV
P. vulgaris cv. *Kőbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Középbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Májbab°:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Michelite:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Michigan:* Fab = Legu = Pap, Th // M /// + // ⊟ −
P. vulgaris cv. *Moldovszkaja:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Olomucka Zelenoluska:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Őrségi cseresznyebab°:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Perlicska:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Prinzess:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS
P. vulgaris cv. *Processor:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Red Kidney:* Fab = Legu = Pap, Th // M /// + // ⊟ BCMV/, BYMV53/, BMV, PAMV, PVS, CLRV/, TMV, ToMV, RMV, CMV
P. vulgaris cv. *Refugee:* Fab = Legu = Pap, Th // M /// + // ⊟ TMV
P. vulgaris cv. *Robust:* Fab = Legu = Pap, Th // M /// + // ⊟ PVS

P. vulgaris cv. *Soproni lapos°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Szegedi fehér°:* Fab = Legu = Pap, Th // M // / + // ⊟ –
P. vulgaris cv. *Tápiószelei barna°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Tápiószelei fürj°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS, TMV
P. vulgaris cv. *Tápláni fekete "cirádás" fürjbab°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS, TMV
P. vulgaris cv. *Tápláni nagyszemű cseresznyebab°:* Fab = Legu = Pap, Th // M // / + // ⊟ TMV
P. vulgaris cv. *Tétényi cukorbab°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Tétényi gyöngybab°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Tétényi fehér középbab°:* Fab = Legu = Pap, Th // M // / + // ⊟ –
P. vulgaris cv. *Tétényi középbab°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Tétényi nagyszemű fehér°:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
P. vulgaris cv. *Wade:* Fab = Legu = Pap, Th // M // / + // ⊟ PVS
Vigna catjang (V. cylindrica)°: Fab = Legu = Pap, Th // M // / + // ⊟ BCMV/, TRSV/, ToMV, BBWV 45/

Systemic susceptible hosts

Amorpha fruticosa: Fab = Legu = Pap, Ph // M // + / // ⊟ – (Fig. 1A)
Cucubalus baccifer°: Cary, H // M // + / // ⊟ – (Fig. 1B)
Datura carolinianum°: Sol, ? // M // + / // ⊟ /BMV, /TMV
D. ceratocaula°: Sol, Th // M // + / // ⊟ /BMV50, /TRSV, /TMV, /CMV
D. chlorantha (D. humilis): Sol, Th // M // + / // ⊟ /BMV, /TRSV, /TMV, /TRV, /ToMV, /CMV

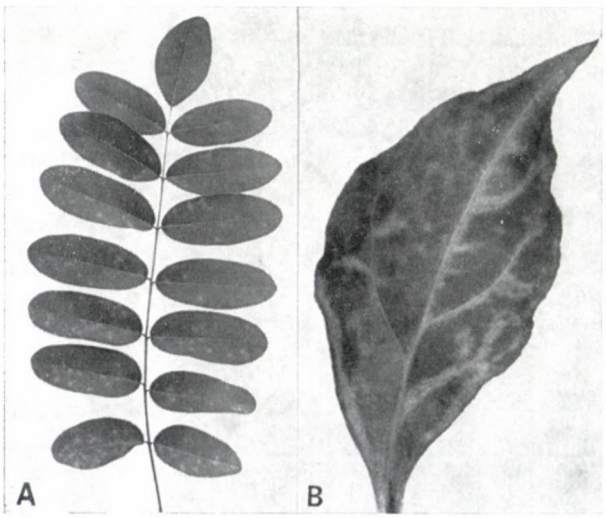

Fig. 1. Systemic symptoms on the leaves of *Amorpha fruticosa* (A) and *Cucubalus baccifer* (B) plants inoculated with alfalfa mosaic virus

D. fastuosa cv. *Alba*°: Sol, Th // M // +/ // ⊟ /BMV, PVY, /TRSV, /TMV, /TRV, /ToMV, /CMV
D. gigantea (D. tatula): Sol, Th // M // +/ // ⊟ /BMV, /TRV, /ToMV, /CMV
D. godronii cv. *Minka*°: Sol, ? // M // +/ // ⊟ BCMV, BYMV, /BMV, PVY, CLRV, /TRSV, /TMV, /TNV, /TRV, WMV, /ToMV, RMV, TYMV, /CMV, CeMV
D. inermis (D. stramonium f. *inermis)*: Sol, Th // M // +/ // ⊟ /BMV50, /TRSV, /TMV, /TNV, /TRV, /ToMV, /CMV
D. leichardtii: Sol, ? // M // +/ // ⊟ /BMV, /TRSV, /TMV, /TNV, /TRV, /ToMV, /CMV
D. metel var. *muricata*°: Sol, ? // M // +/ // ⊟ /BMV, /TMV, /TNV
D. rosei°: Sol, ? // M // +/ // ⊟ /BMV, /TRSV, /TMV, /TNV, /TRV, WMV, /ToMV, /CMV, CeMV
Galega bicolor°: Fab=Legu=Pap, H // M // +/ // ⊟ –
G. hartlandii°: Fab=Legu=Pap, H // M // +/ // ⊟ PVM, PVS
Hibiscus manihot: Mal, H // M // +/ // ⊟ –
Lycium carolinianum°: Sol, ? // M // +/ // ⊟ /BMV, /PVX, /PVY, /TRSV, /CMV
L. flexicaule°: Sol, Ph // M // +/ // ⊟ /BMV, /PVX, /PVY, /TRSV, /TMV, /CMV
L. horridum°: Sol, Ph // M // +/ // ⊟ /BMV, /PAMV, /PVX, /PVY, /TRSV, /TMV, /CMV
L. ruthenicum: Sol, Ph // M // +/ // ⊟ /BMV, /PAMV, /PVX, /PVY, /TRSV, /TMV, /ToMV, /BBWV, /CMV
L. turcomanicum°: Sol, H // M // +/ // ⊟ /BMV, /PAMV, /PVX, /PVY, /TRSV, /TMV, /CMV
Malva pusilla: Mal, Th, TH // M // +/ // ⊟ /TRSV, /WMV, /ToMV
M. verticillata: Mal, Th, H // M // +/ // ◫ /CLRV58, /TRV56
Melandrium album: Cary, TH, H // M // +/ // ⊟ –
M. rubrum (M. silvestre)°: Cary, H // M // +/ // ⊟ –
M. silvestre (M. rubrum)°: Cary, H // M // +/ // ⊟ –
Ocimum canum: Lab=Lami, Th // M // +/ // ⊟ BCMV, /PAMV, PVM, PVS, /PVX, PVY, CLRV, /TRSV, /TMV, /TRV, WMV, /ToMV, RMV, TYMV, /CMV
O. sanctum°: Lab=Lami, Th // M // +/ // ⊟ /PAMV, PVM, PVS, /PVX, PVY, CLRV, /TRSV, /TMV, /TRV, WMV, /ToMV, RMV, TYMV, /CMV, CeMV
Silene dichotoma: Cary, Th // M // +/ // ⊟ BMV
S. pendula: Cary, Th, TH // M // +/ // BMV, /TRV56

Local and systemic susceptible hosts

Amaranthus angustifolius (A. graecizans)°: Ama, Th // M // +/+ // ⊟ –
A. ascendens (A. lividus var. *ascendens)*°: Ama, Th // M // +/+ // ⊟ –
A. atropurpureus (A. hybridus)°: Ama, Th // M // +/+ // ⊟ –
A. bouchoni°: Ama, Th // M // +/+ // ⊟ –
A. cruentus (A. paniculatus): Ama, Th // M // +/+ // ⊟ –

A. dubius: Ama, Th // M // +/+ // ⊟ –
A. hypochondriacus°*:* Ama, Th // M // +/+ // ⊟ PVS18
A. hypochondriacus cv. *Monstrosus*°*:* Ama, Th // M // +/+ // ⊟ –
A. oleraceus (A. lividus var. *oleraceus)*°*:* Ama, Th // M // +/+ // ⊟ –
A. paniculatus cv. *Roter Dom*° *(A. cruentus):* Ama, Th // M // +/+ // ⊟ –
A. paniculatus cv. *Roter Paris*°*:* Ama, Th // M // +/+ // ⊟ –
A. paniculatus cv. *Sanguineus nanus*°*:* Ama, Th // M // +/+ // ⊟ –
A. paniculatus var. *flavus (A. aureus)*°*:* Ama, Th // M // +/+ // ⊟ –
A. speciosus (A. paniculatus f. *speciosus)*°*:* Ama, Th // M // +/+ // ⊟ –
A. viridis (A. lividus var. *ascendens)*°*:* Ama, Th // M // +/+ / ⊟ –
Ammi visnaga°*:* Umb=Api, Th // M // +/+ // ⊟ BCMV, BMV, PVY, RMV, TuMV
Beta macrocarpa (B. vulgaris ssp. *macrocarpa):* Chen, Th // M // –/+ //⊟ BCMV, BYMV, PVY, WMV, TuMV, TYMV
Chenopodium botrys: Chen, Th // M // +/+ // ⊟ PAMV, PVS18
Ch. capitatum: Chen, Th // M // +/+ // ⊟ PVS18, TRV56
Ch. foetidum (Ch. schraderianum): Chen, Th // M // +/+ // ⊟ PVM, PVS18
Nicotiana attenuata: Sol, Th // M // +/+ // ⊟ – (Fig. 2A)
N. auriculata°*:* Sol, Th // M // +/+ // ⊟ – (Fig. 2B)
Penstemon alpinus°*:* Scrop, H // M // –/– // ⊟ PVY, CMV
Petunia atkinsiana°*:* Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
P. axillaris: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV

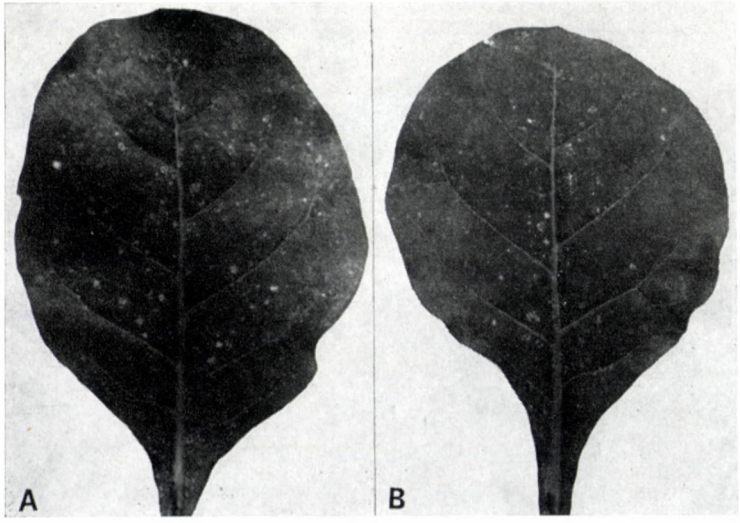

Fig. 2. Local lesions on the leaves of *Nicotiana attenuata* (A) and *Nicotiana auriculata* (B) plants inoculated with alfalfa mosaic virus

P. hybrida cv. *Rose de Haven amélioré*°: Sol, Th // M // +/+ // ⊟ PVM, PVS, CLRV, RMV, CeMV
P. parviflora°: Sol, Th // M // +/+ // ⊟ BMV, PVS, CLRV, WMV, CeMV
P. violacea: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV
Physalis peruviana: Sol, H // M // +/+ // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
P. peruviana var. *macrocarpa*°: Sol, H // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
P. pruinosa: Sol, Th // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Scopolia lurida°: Sol, ? // M // +/+ // ⊟ −
Solanum tuberosum cv. *Aranyalma*°: Sol, Th // M // +/+ // ⊟ −
S. tuberosum cv. *Auriga:* Sol, Th // M // +/+ // ⊟ −
S. tuberosum cv. *Axilia:* Sol, Th // M // +/+ // ⊟ −

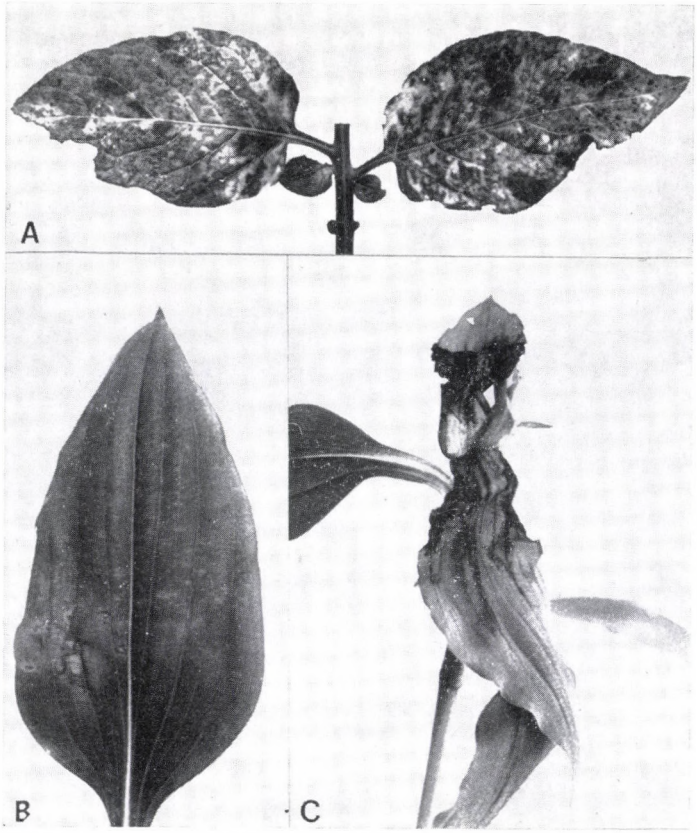

Fig. 3. Systemic symptoms on the leaves of *Solanum tuberosum* cv. *Somogyi kifli* (A) infected with alfalfa mosaic virus. Necrotic lesions (B) and top necrosis (C) on the leaves of *Tinantia erecta* inoculated with alfalfa mosaic virus

S. tuberosum cv. *Früka:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Gülbaba°:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Jowisz:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Kisvárdai rózsa°:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Korai rózsa°:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Opal:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Osa:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Somogyi kifli°:* Sol, Th // M // +/+ // ⊟ – (Fig. 3A)
S. tuberosum cv. *Somogyi korai°:* Sol, Th // M // +/+ // ⊟ –
S. tuberosum cv. *Somogyi sárga°:* Sol, Th // M // +/+ // ⊟ –
Tinantia erecta (T. fugax)°: Com, H // M // +/+ // ⊟ BCMV, PAMV, PVM, PVS, RMV, TYMV, CMV (Fig. 3B and C)

Resistant plants

Browallia cordata°: Sol, ? // M // –/– // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
B. grandiflora: Sol, Th // M // –/– // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, PVY/PVY, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, BBWV59/, CMV/CMV
B. roezli°: Sol, ? // M // –/ // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, CLRV/, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
B. viscosa: Sol, ? // M // –/– // ⊟ BMV/BMV, PAMV/PAMV, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
Bryonia alba: Cuc, H // M // –/– // ⊟ /TNV, /TRV56, /ToMV, CMV65/
B. dioica (B. cretica ssp. *dioica):* Cuc, H // M // –/– // ⊟ /TNV, /TRV56, /ToMV, TuMV60/, TYMV66/, CMV60/
Cucurbita pepo convar. *patissonina* f. *radiata°:* Cuc, Th // M // –/– // ⊟ TRSV/TRSV, /TMV, TNV/TNV, WMV/, /ToMV, CMV/
Paulownia fargesii°: Scrop, Ph // M // –/– // ⊟ /PAMV, /PVX, TRSV/TRSV, /TMV, /TRV, /ToMV, RMV, TYMV, CMV/CMV
Tetragonia crystallina°: Aiz, Th // M // –/– // ⊟ /BYMV, /PVX, CLRV/CLRV, TRSV/TRSV, /TMV, /TNV, TRV/TRV, /WMV, /ToMV, /RMV, TuMV/TuMV, CMV/CMV

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 327—337 (1981)

Reaction of *Physalis* Species to Plant Viruses. VI. *Physalis curassavica* L. as New Experimental Plant in Plant Virology

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In the course of investigations concerning the virus susceptibility of *Physalis curassavica* L., a plant so far unknown in plant virology, we have found it to be locally and systemically susceptible to 13 viruses (alfalfa mosaic virus, *Arabis* mosaic virus, belladonna mottle virus, cucumber mosaic virus, *Melandrium* yellow fleck virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco ring spot virus, tomato aspermy virus, tomato mosaic virus and turnip mosaic virus) and a systemic host of broad bean wilt virus. The same experimental plant has proved resistant to 12 viruses (bean common mosaic virus, bean yellow mosaic virus, beet mosaic virus, carnation ring spot virus, cauliflower mosaic virus, celery mosaic virus, cucumber green mottle mosaic virus, lettuce mosaic virus, radish mosaic virus, tobacco necrosis virus, turnip yellow mosaic virus and watermelon mosaic virus).

The knowledge of compatible and incompatible new host-virus relations plays an important role in differentiating viruses often occurring together in the nature, like e.g. cucumber mosaic virus and lettuce mosaic virus, celery mosaic virus and cucumber mosaic virus, cucumber mosaic virus and watermelon mosaic virus, turnip mosaic virus and turnip yellow mosaic virus or turnip mosaic virus and cucumber mosaic virus.

In the first part of a series of papers published eleven years ago (cf. HORVÁTH, 1970) we gave a general survey of the susceptibility of 23 *Physalis* species to 76 viruses. Since then further two *Physalis* species and one variety, formerly unknown in plant virology, have been found to be susceptible to viruses. The susceptibility of *Physalis lanceifolia* Nees to potato leaf roll virus became known from the report of SCHMELZER and WOLF (1971, 1977). FELDMAN and GRACIA (1972) demonstrated the susceptibility of *Physalis mendonica* Phil. to potato virus Y. It was in our own experiments that *Physalis peruviana* L. var. *macrocarpa* proved susceptible to alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco rattle virus and tobacco ring spot virus (cf. HORVÁTH, 1974a), and *P. lanceifolia* susceptible

[1] Earlier papers in these series: I. *Acta Phytopath. Hung. 5*, 65—72 (1970), II. *Acta Phytopath. Hung. 9*, 1—9 (1974), III. *Acta Phytopath. Hung. 9*, 11—15 (1974), IV. *Acta Phytopath. Hung. 10*, 67—75 (1975), V. *Acta Phytopath. Hung. 10*, 247—256 (1975).

to alfalfa mosaic virus and resistant to bean common mosaic virus (HORVÁTH, unpublished results).

According to our present knowledge responses of about 25 *Physalis* species and one variety to 90 viruses have been disclosed so far. In earlier publications 49 new host-virus relations and some 55 cases of resistance revealed in our experiments for eleven *Physalis* species and a variety were described (HORVÁTH, 1974a, b, 1975a, b).

Host plant experiments performed in past years threw light upon the local, latent susceptibility of e.g. *Physalis floridana* Rydb., one of the best known virus indicator, to pea leaf roll mosaic virus, bean yellow mosaic virus and cowpea aphid-borne mosaic virus (cf. BOS, 1970). *Physalis minima* – so far known to be susceptible to potato virus A, potato virus X, potato virus Y and tobacco mosaic virus only (cf. HORVÁTH, 1970) – has recently proved to be an important host plant of cucumber mosaic virus too (JOSHI and DUBEY, 1976). Several years ago a virus serologically similar to the European belladonna mottle virus was isolated from *Physalis heterophylla* Nees (cf. MOLINE and FRIES, 1972, 1974; MOLINE, 1973), and not much later another one, the *Physalis* mosaic virus from *Physalis subglabrata* MacKenzi et Bush., which is closely related to the Andean potato latent, belladonna mottle, dulcamara mottle and egg-plant mosaic viruses (cf. PETERS and DERKS, 1974). This *Physalis* mosaic virus is a member of the Andean potato latent virus, subgroup of the turnip yellow mosaic virus group.

In the present, sixth paper of our publication series we give account of recent experiment results concerning the virus susceptibility of *Physalis curassavica*, a *Physalis* species unknown so far in the literature of plant virology.

Materials and Methods

Young seedlings raised from the seeds of the annual *Physalis curassavica* plants placed at our disposal by the Botanical Garden, Nijgemen, the Netherlands, were inoculated with 32 strains or isolates of 26 viruses (Table 1) by means of the so-called carborundum-spatula technique, a conventional method in plant virology.

As to the maintenance of the viruses, the methods of inoculation, the re-isolation of viruses from the inoculated plants and the test or indicator plants detailed data can be found in our earlier publications (cf. HORVÁTH, 1974a, 1977). In these papers newly investigated viruses were kept in *Ammi majus* L. (celery mosaic virus, watermelon mosaic virus), *Beta vulgaris* L. (beet mosaic virus), *Brassica rapa* L. var. *rapa* (cauliflower mosaic virus, turnip mosaic virus), *Cucumis sativus* L. (Arabis mosaic virus, cucumber green mottle mosaic virus), *Datura stramonium* L. (broad bean wilt virus), *Gomphrena globosa* L. (*Melandrium* yellow fleck virus), *Lactuca sativa* L. (lettuce mosaic virus), *Nicotiana glutinosa* L. (belladonna mottle virus, tomato aspermy virus), *N. tabacum* L. cv. *Samsun* (tomato mosaic virus) and *Phaseolus vulgaris* L. cv. *Red Kidney* (bean yellow mosaic virus, carnation ring spot virus). These viruses

Table 1

Viruses inoculated in the experiments

Viruses, virus groups and cryptograms	Strains or isolates	Literature
Alfalfa mosaic virus (monotypic group), R/1 : 1.3/18 : U/U : S/Ap	K_2	BECZNER (1972)
Arabis mosaic virus (nepovirus group), R/1 : */41 : S/S : S/Ne	Phil.2	SCHMELZER (1968, 1974)
Bean common mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	F23	HORVÁTH (1973), KOVÁCS and HORVÁTH (1973)
Bean yellow mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	S and RM	HORVÁTH (1976)
Beet mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	IPA[1]	SCHMELZER (1959)
Belladonna mottle virus (tymovirus group) R/1 : 2.0/37 : S/S : S/Cl	H	HORVÁTH *et. al.* (1976)
Broad bean wilt virus (ungrouped virus) R/1 : */33 : S/S : S/Ap	Tm and HZ	HORVÁTH and SZIRMAI (1975), HORVÁTH (1976)
Carnation ring spot virus (?tombusvirus group) R/1 : 1.4/20 : S/S : S/Ne	IPA	FRITZSCHE and SCHMELZER (1967)
Cauliflower mosaic virus (caulimovirus group) D/2 : 4.5/16 : S/S : S/Ap	DH	HORVÁTH *et al.* (1979, 1980a)
Celery mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	P	HORVÁTH *et al.* (1976)
Cucumber green mottle mosaic virus (tobamovirus group) R/1 : */6 : E/E : S/*	IPA	SCHMELZER (1967)
Cucumber mosaic virus (cucumovirus group) R/1 : 1/18 : S/S : S/Ap	W and EN	HORVÁTH and SZIRMAI (1973), HORVÁTH (1976)
Lettuce mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	Mk-4	HORVÁTH (1979, 1980), HORVÁTH *et al.* (1981)
Melandrium yellow fleck virus (bromovirus group) $R/1 : \frac{1.97 + 0.8}{20} : S/S : S/*$	LB	HOLLINGS *et al.* (1978), HOLLINGS and HORVÁTH (1981)
Potato aucuba mosaic virus (potexvirus group) R/1 : */5 : E/E : S/Ap	SS	HORVÁTH (1972a, b)
Potato virus X (potexvirus group) R/1 : 2.1/6 : E/E : S/(Fu)	G	HORVÁTH and BECZNER (1968), HORVÁTH (1976)
Potato virus Y (potyvirus group) R/1 : */* : E/E : S/Ap	C, N, R, An	HORVÁTH (1966a, b, 1967 a, b)

Table 1 (continued)

Viruses, virus groups and cryptograms	Strains or isolates	Literature
Radish mosaic virus (comovirus group) R/1 : (1.3/26 + 2.2/34) : S/S : S/Cl	HS7	HORVÁTH *et al.* (1973)
Tobacco mosaic virus (tobamovirus group) R/1 : 2/5 : E/E : S/*	U_1	SIEGEL and WILDMAN (1954)
Tobacco necrosis virus (monotypic group) R/1 : 1.5/19 : S/S : S/Fu	f	SZIRMAI (1964)
Tomato aspermy virus (cucumovirus group) R/1 : */* : S/S : S/Ap	Tm 12	HORVÁTH *et al.* (1980b)
Tomato mosaic virus (tobamovirus group) R/1 : 2/5 : E/E : S/*	H	HORVÁTH and BECZNER (1973), MAMULA *et al.* (1974), JURETIĆ *et al.* (1977)
Tobacco ring spot virus (nepvirus group) R/1 : 2.2/40 : S/S : S/Ne	D	HORVÁTH (1976)
Turnip mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	A11	HORVÁTH *et al.* (1975a)
Turnip yellow mosaic virus (tymovirus group) R/1 : 1.9/34 : S/S : S/Cl	HB	HORVÁTH *et al.* (1973)
Watermelon mosaic virus (potyvirus group) */* : */* : E/E : S/Ap	PW	HORVÁTH *et al.* (1975b)

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were also re-isolated from the inoculated and non-inoculated or subsequently developed leaves of inoculated *Physalis curassavica* plants and tested on the following test or indicator plants: *Ammi majus* (celery mosaic virus, watermelon mosaic virus), *Brassica rapa* var. *rapa* (cauliflower mosaic virus), *Chenopodium amaranticolor* Coste et Reyn., *Ch. quinoa* Willd. (*Arabis* mosaic virus, beet mosaic virus, broad bean wilt virus, lettuce mosaic virus), *Cucumis sativus* (cucumber green mottle mosaic virus), *Gomphrena globosa* (*Melandrium* yellow fleck virus), *Nicotiana glutinosa* (belladonna mottle virus, tomato aspermy virus), *N. tabacum* cv. *Samsun* (turnip mosaic virus), *N. tabacum* cv. *Xanthi-nc* (tomato mosaic virus) and *Phaseolus vulgaris* cv. *Red Kidney* (bean yellow mosaic virus, carnation ring spot virus).

Results and Discussion

In our experiments *Physalis curassavica* was found to be locally and systemically susceptible to 13 viruses, systemically susceptible to one virus, and resistant to further 12 viruses (Table 2).

Table 2

Reaction of *Physalis curassavica* L. to some plant viruses

Viruses	Symptoms[1]	Results of the re-isolation of the investigated plant viruses
Alfalfa mosaic virus	IL: necrotic rings NIL: vein clearing, mosaic, leaf deformations	IL: positive NIL: positive
Arabis mosaic virus	IL: chlorotic or necrotic spots NIL: vein clearing and severe mosaic	IL: positive NIL: positive
Bean common mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Bean yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Beet mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Belladonna mottle virus	IL: necrotic rings NIL: chlorotic and/or necrotic rings, mosaic, leaf deformation	IL: positive NIL: positive
Broad bean wilt virus	IL: no symptoms NIL: vein clearing and severe mosaic, recovery	IL: negative NIL: positive
Carnation ring spot virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cauliflower mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Celery mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber green mottle mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: vein clearing, yellowing, interveinal mosaic NIL: severe mosaic, dark green islands, leaf deformations	IL: positive NIL: positive
Lettuce mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Melandrium yellow fleck virus	IL: necrotic lesions NIL: no symptoms	IL: positive NIL: positive
Potato aucuba mosaic virus	IL: no symptoms NIL: chlorotic and/or necrotic spots, interveinal mosaic	IL: positive NIL: positive
Potato virus X	IL: necrotic rings NIL: chlorotic spots	IL: positive NIL: positive

Table 2 (continued)

Viruses	Symptoms[1]	Results of the re-isolation of the investigated plant viruses
Potato virus Y	IL: no symptoms NIL: severe chlorotic spots, leaf deformations	IL: positive NIL: positive
Radish mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco mosaic virus	IL: necrotic lesions NIL: necrotic lesions, top necrosis	IL: positive NIL: positive
Tobacco necrosis virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tomato aspermy virus	IL: chlorotic lesions NIL: mosaic, dark green islands, leaf deformations	IL: positive NIL: positive
Tomato mosaic virus	IL: necrotic lesions NIL: necrotic lesions, top necrosis	IL: positive NIL: positive
Tobacco ring spot virus	IL: necrotic rings NIL: vein clearing, chlorotic and/or necrotic rings	IL: positive NIL: positive
Turnip mosaic virus	IL: chlorotic and/or necrotic lesions NIL: mosaic, leaf deformations	IL: positive NIL: positive
Turnip yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Watermelon mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

[1] IL: Inoculated leaves; NIL: Non-inoculated leaves, or subsequently developed leaves.

In the local and systemic host-virus relation 10 viruses caused manifest local and manifest systemic diseases in *Physalis curassavica* plants. *Melandrium* yellow fleck virus, a virus known to have a wide host range (cf. HOLLINGS *et al.*, 1978; HOLLINGS and HORVÁTH, 1981), induced necrotic local lesions in *Physalis curassavica* plants. Systemic symptoms did not develop in the inoculated plants although the re-isolation of the virus from non-inoculated plants was also successful. The experimental plant proved to be a visible local and latent systemic host of *Melandrium* yellow fleck virus.

Physalis curassavica, like *P. peruviana*, a plant tested in earlier years (cf. HORVÁTH, 1974b), turned out to be a latent local and visible systemic host of potato virus Y. SINGH *et al.* (1979) reported that *Physalis angulata* L. and *P.*

pubescens L. reacted systemic symptoms without inducing local lesions against potato virus Y, while potato virus Y induces distinct local lesions on open leaves of *P. floridana*. Potato aucuba mosaic virus caused a visible systemic disease in *Physalis curassavica* in agreement with earlier observations made in various *Physalis* species (HORVÁTH, 1974a, b, 1975a, b). The rubbed leaves of the inoculated plants never showed symptoms although the virus could be re-isolated from these leaves in every case. Similar observations were made in earlier years of the relation between potato aucuba mosaic virus and various *Physalis* species. Of the local and systemic host-virus relations the one between *Physalis curassavica* and the belladonna mottle virus deserves special attention. As far as we know, only the relation between *Physalis heterophylla* and the Iowa strain (*Physalis* mottle strain) of the belladonna mottle virus (cf. MOLINE and FRIES, 1972, 1974; MOLINE, 1973), and that between *Physalis subglabrata* and the *Physalis* mosaic virus (closely related to belladonna mottle virus, cf. PETERS and DERKS, 1974), further the relation between the Kansas strain of belladonna mottle virus – a virus isolated from *Capsicum frutescens* L. '*Hybrid Tokyo Bell*' – on the one hand, *Physalis angulata* and *P. floridana* on the other, have been described so far (LEE *et al.*, 1979).

According to our own experiment results *Physalis curassavica* is important not only as a diagnostical, but also as a prognostical host of the belladonna mottle virus. Here we note that the local (chlorotic and necrotic lesions or rings) and systemic symptoms (mosaic and leaf deformation) caused by belladonna mottle virus in *Physalis curassavica* are similar or equal to those found by MOLINE and FRIES (1974) in *Physalis angulata* and *P. heterophylla* plants artificially inoculated with the Iowa strain of belladonna mottle virus, as well as to the symptoms induced by PETERS and DERKS (1974) in *Physalis floridana*, *P. ixocarpa*, *P. peruviana* and *P. subglabrata* with the isolate of the *Physalis* mosaic virus, and in *P. alkekengi* and *P. floridana* with the Kansas strain of belladonna mottle virus (LEE *et al.*, 1979).

Beside the tomato aspermy virus susceptible *Physalis angulata* and *P. floridana*, the turnip mosaic virus susceptible *P. floridana* and *P. pubescens*, and the *Arabis* mosaic virus susceptible *P. angulata*, *P. floridana*, *P. phyladelphica* Lam. and *P. curassavica* plays an important role in the epidemiology of the belladonna mottle virus.

In the course of our investigations we pointed out a systemic host-virus relation between *Physalis curassavica* and the broad bean wilt virus (see Table 2). After 8 days the examined plant showed intensive vein clearing and mosaic symptoms and plays therefore an important role in the diagnosis of broad bean wilt virus. Considering, however, that recovery is a rather frequent phenomenon in the inoculated plants, the diagnostical reliability of the host-virus relation is disputable. We mention here that SCHMELZER (1960) also observed the phenomenon of recovery in the host-virus relation of *Physalis floridana* and *P. peruviana* to broad bean wilt virus. It seems to be that recovery is a general symptomatological phenomenon in the host-parasite relation between *Physalis* species and broad bean wilt virus.

During investigations into the host-virus relations of *Physalis curassavica* we disclosed 12 incompatible relations too in the case of the mentioned plant (see Table 2). These results correspond to those obtained in earlier studies made with other *Physalis* species. Exceptions are the *Physalis aequata* Jacq., *P. viscosa* L. and *P. ixocarpa* Brot. which unlike the so far tested species of *P. angulata*, *P. peruviana*, *P. peruviana* var. *macrocarpa* and *P. pruinosa* L. proved susceptible to tobacco necrosis virus (cf. HORVÁTH, 1975b). *Physalis curassavica*, the experimental plant of our present study proved resistant to bean yellow mosaic virus as well. Here we should like to point out that according to the investigations of BOS (1970) *Physalis floridana* is a symptomless (latent) local host of the normal strain (B 25) of bean yellow mosaic virus isolated from bean and at the same time a systemic host of the same strain showing a striking stunting symptom. It would be desirable to find out whether the divergent reactions of the *Physalis* species are due to the different strains of bean yellow mosaic virus and/or to the different *Physalis* species.

Acknowledgements

I am indebted to Dr. G. W. M. BARENDSE, Botanical Garden, Nijmegen, The Netherlands for his valuable information about the nomenclature of *Physalis curassavica* L. The author wishes to express his appreciation to Miss K. MOLNÁR and Miss E. SZIGETI for their valuable technical assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 339—348 (1981)

Two Viruses Isolated from Some Legume Plants in Kosovo (Yugoslavia)

By

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The paper deals with investigations of virus diseases of alfalfa, white clover and bean bred in the Province of Kosovo (Southeastern region of Yugoslavia). Virus diseases of legume plants have not been studied yet in this region. A total of 6 isolates found in various parts of Kosovo were studied; 3 isolates originated from alfalfa (isolates L1, L2 and L3), 1 isolate from white clover (isolate BD) and 2 isolates from bean (isolates G2 and G3).

On the basis of the test plant reactions, light and electron microscope investigations, aphid and seed transmission, and serological tests, it has been established that isolates L1, L2, L3 and BD belong to alfalfa mosaic virus (AMV) and isolates G2 and G3 to bean common mosaic virus (BCMV). AMV isolates showed serological reactions of identity when compared with one another. They did not differ serologically from 425 strain of AMV supplied by Dr. L. Bos. AMV is rather frequent on alfalfa and clover in Kosovo. The percent of alfalfa and clover plants infected with AMV often amounts to 85%.

Alfalfa, white clover, bean and some other legume plants are very important crops in the Province of Kosovo (southeastern part of Yugoslavia). Although virus diseases have been noticed in this region (PERIŠIĆ, 1951), until now, however, virus diseases in Kosovo have rarely been the subject of any research; nor have virus diseases of legume plants in this region been studied in general.

In this paper some properties of six virus isolates found on alfalfa, white clover and bean are presented.

Material and Methods

A total of 6 virus isolates found on alfalfa, white clover and bean were investigated. Infected plants were collected in various localities in the Province of Kosovo. The isolates marked L1, L2, L3 were found in alfalfa (near Djakovica, Priština and Peć, respectively), the isolate BD in white clover (near Djakovica) and the isolates G2 and G3 in bean (near Prizren and Uroševac, respectively). Some of these isolates were compared with AMV-425 strain of alfalfa mosaic virus kindly supplied by Dr. L. Bos (Institute of Phytopathological Research, Wageningen, The Netherlands).

In serological tests carried out by agar gel immunodiffusion method 3 antisera to alfalfa mosaic virus (AMV) were used. One antiserum (titre 1/16) was supplied by Dr. D. Z. MAAT (Institute of Phytopathological Research, Wageningen, The Netherlands), another (titre 1/16) by Dr. M. BABOVIĆ (Faculty of Agriculture, Beograd) and the third antiserum was prepared during this work.

At the beginning of the investigations the isolates were cleaned by local lesion transmission. The determination of some properties *in vitro* (thermal inactivation point, dilution end point and longevity *in vitro*) was made in the standard way. Aphid transmission tests were performed by *Myzus persicae*. Acquisition feeding was 30 min, starving 120 min and infection feeding 120 min, too. To find out whether our isolates were transmissible by soil, the healthy plants were planted into soil in which the plants previously infected with the isolates had been grown.

The isolate L1, which belongs to AMV, was purified with minor adjustments after the method of GIBBS *et al.* (1963). The amount of purified virus in solutions was determined by absorbance at 260 nm (A260) in a spectrophotometer.

The purified virus (isolate L1) was used for immunization. Each rabbit received 3 intravenous injections, one injection per day. Every injection contained 1 ml of virus suspension of concentration at 1 mg/ml. Nine days after the last intravenous injection, the animals received booster injections using FREUND's incomplete adjuvant. The produced serum had titre 1/32.

Particle types were separated by centrifugation at 25 000 rpm for 3 hours in a Spinco SW 25.1 rotor on gradients of 10–40% sucrose. The centrifugation of virus samples in analytical centrifuge was performed at 44 000 rpm (20 °C, bar angle 60°) in a Spinco model E analytical centrifuge.

The purified virus suspension (isolate L1) was analysed by electron microscope using negative staining with phosphotungstate. The inclusion bodies of the isolates G2 and G3 were observed in live cells by light microscope. For electron microscope analysis of ultrathin sections of the infected tissue, strips of leaves were prepared in the way described earlier (TARAKU *et al.*, 1977).

Results

Since the experiments showed that isolates L1, L2 L3 and BD belonged to one virus and the isolates G2 and G3 to another virus the results obtained will be presented separately.

Investigations of isolates L1, L2 L3 and BD

Test plant reactions

The four isolates were mechanically transmitted to 14 test plants. These plants were mainly chosen from the host range of AMV because preliminary tests

Table 1

Reactions of plants infected with isolates L1

Hosts	Symptoms*
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	L chlorotic lesions; S mild variegations
C. quinoa Willd.	L chlorotic lesions; S mild variegation
CUCURBITACEAE	
Cucumis sativus L.	0
FABACEAE	
Phaseolus vulgaris L. cv. Trešnjevác	L necrotic lesions
Pisum sativum L.	0
Vicia faba L.	L necrotic rings; S necrotic variegation
Vigna sinensis (L.) Endl.	L necrotic lesions
SOLANACEAE	
Datura stramonium L.	0
Capsicum annuum L.	0
Lycopersicum esculentum Mill.	0
Nicotiana clevelandii Gray.	S mosaic
N. glutinosa L.	L chlorotic and necrotic spots S chlorotic and necrotic spots
N. megalosiphon Heurck et Muell.	L necrotic rings S necrotic zones
N. tabacum L. cv. Samsun	L necrotic rings S necrotic variegation

* L symptoms in inoculated leaves; S symptoms in non-inoculated leaves; 0 unsusceptible.

indicated that the investigated isolates could belong to AMV. Symptoms on the test plants are shown in Table 1. Since all four isolates produced very similar symptoms, only symptoms caused by isolate L1 are presented in the Table.

Properties in vitro

Thermal inactivation point (TIP) of the four isolates is between 65 and 70 °C, diluted end point (DEP) about 10^{-4} and longevity *in vitro* (LIV) at room temperature about 4 days.

Aphid and soil transmission

The isolates L1, L2, L3 and BD are transmissible by *Myzus persicae*. When young healthy plants of *Nicotiana clevelandii* were planted into soil in which plants infected with L1 had been grown, it was established that all of the 15 plants involved in experiments remained healthy. This was checked by back inoculation experiments.

Purification, ultraviolet absorption and electron microscopy

Isolate L1 was purified after the method of GIBBS *et al.* (1963) with minor adjustments. Spectrophotometric analyses of purified virus solutions showed that A260/280 of the isolate L1 was between 1.42 and 1.64. Electron microscope analyses revealed that the isolate L1 had several types of virus particles; some of them were spherical in shape and others were bacilliform (Fig. 1). The size of the particles corresponded to the ones of AMV. The isolate is obviously multicomponent. This was confirmed by centrifugation in sucrose density gradient and by analysis in an analytical ultracentrifuge. As can be seen in Fig. 2 the virus has 5 types of virus particles.

Serological properties

An immune serum was prepared against isolate L1. It had the titre 1/32. In addition, two other sera to AMV were involved in the experiments. One of them was supplied by Dr. D. Z. MAAT and the other by Dr. M. BABOVIĆ (see Material and Methods). The experiments were performed in agar gel double diffusion tests. The four isolates reacted positively with all three immune sera. The

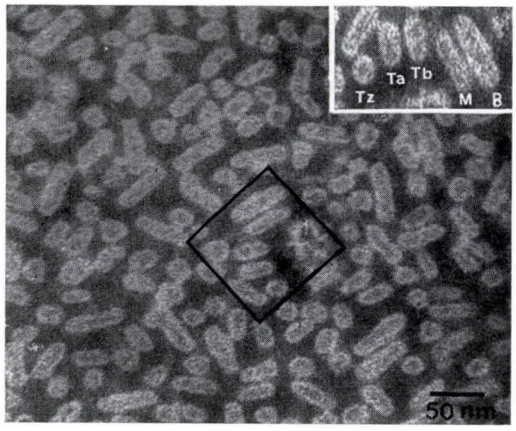

Fig. 1. Virus particles of isolate L1: five types of virus particles can be observed; the particles bordered in the centre of the Figure are shown above right as well

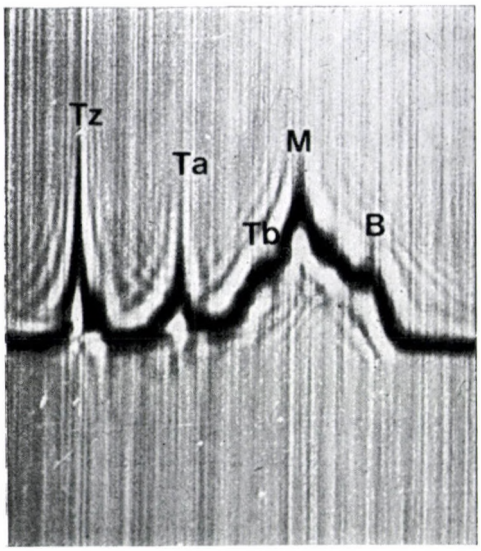

Fig. 2. Schlieren diagram of isolate L1 photographed 23 min after reaching 44 000 rpm at 20 °C (bar angle 60°) in Spinco model E analytical centrifuge

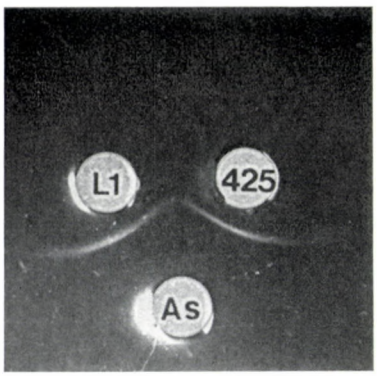

Fig. 3. Gel diffusion precipitin patterns of isolate L1 and isolate 425 of alfalfa mosaic virus (AMV); As — antiserum to AMV

isolates showed serological reactions of identity when compared with one another by using these sera. They do not differ serologically from Bos's 425 strain of AMV (Fig. 3).

Inclusion bodies

The isolates L1, L2, L3 and BD form x-bodies in the infected tissue. They were observed in tobacco hair cells. However, these inclusions occurred very rarely.

Investigations of isolates G2 and G3

Bean is also affected in Kosovo by virus diseases. Three virus isolates were found on this plant (cv. Tetovac). Two isolates, G2 and G3, were similar and the third one (isolate G1) differed from them. The last isolate is not the subject of this paper. Identification of isolates G2 and G3 on the basis of differential hosts, properties *in vitro* and light and electron microscopic inclusion bodies is presented here.

Table 2

Reactions of test plants infected with isolate G2

Hosts	Symptoms*
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	L chlorotic lesions with necrotic center
C. quinoa Willd.	L chlorotic lesions
FABACEAE	
Phaseolus vulgaris L. cv. Trešnjevac	S mosaic, dark green banding along veins (Fig. 4a)
Pisum sativum L.	0
Vicia faba L.	L necrotic lesions S necrotic ringspotting
SOLANACEAE	
Nicotiana tabacum L. cv. Samsun	L chlorotic spots S variegation
N. megalosiphon Heurck et Muell.	L chlorotic and necrotic bands along veins S variegation, stunting

* L symptoms in inoculated leaves; S symptoms in inoculated leaves; 0 unsusceptible.

Test plant reactions

The isolates G2 and G3 were found on two different localities (see Material and Methods). On naturally infected plants isolates G2 and G3 caused different symptoms; G2 isolate provoked a green variegation and isolate G3 a yellow variegation. However, it was shown later that both isolates belonged to the same virus. Since both isolates produced very similar reactions on test plants, only plant reactions to isolate G2 are presented (Table 2).

Symptoms in Table 2 indicate that isolates G2 and G3 can belong to bean common virus (BCMV).

Properties in vitro

The following properties of isolates G2 and G3 were established: thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV). They have TIP between 60 and 65 °C, DEP between 10^{-2} and 10^{-3} and LIV at room temperature about 4 days. According to these data isolates G2 and G3 do not differ from most BCMV isolates described earlier (Bos, 1971; Drijfhout and Bos, 1977).

Aphid and seed transmission

Both isolates were easily transmitted by *Myzus persicae*. The seed-borne tests were performed by isolate G2 using seeds of infected bean plants. The experiments were carried out in greenhouse and in field. The bean plants were infected by mechanical inoculation. The seeds of infected plants were sown in pots in greenhouse. From 30 seeds collected on infected plants bred in greenhouse 7 germinated plants were infected. On the other hand, from 30 seeds collected on infected plants bred in field 8 germinated plants were infected. The symptoms on these plants were usually mild. Efficiency of seed transmission was checked by mechanical inoculation of sap of each seedling in *Chenopodium amaranticolor* and *C. quinoa*.

Light and electron microscopy

Both isolates produced amorphous x-bodies in infected leaf hair cells of *Nicotiana megalosiphon*. These inclusions were oval or irregular and they were usually in contact with the nucleus (Fig. 4b).

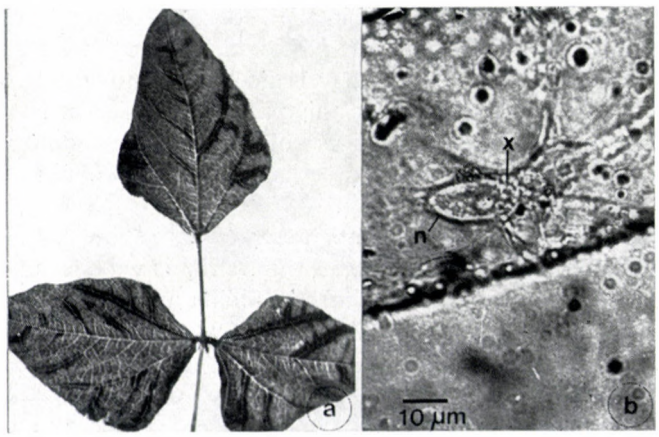

Fig. 4. *a: Phaseolus vulgaris* cv. Trešnjevac artificially infected with isolate G2; *b:* x-body (x) in hair cell of *Nicotiana megalosiphon* (n — nucleus)

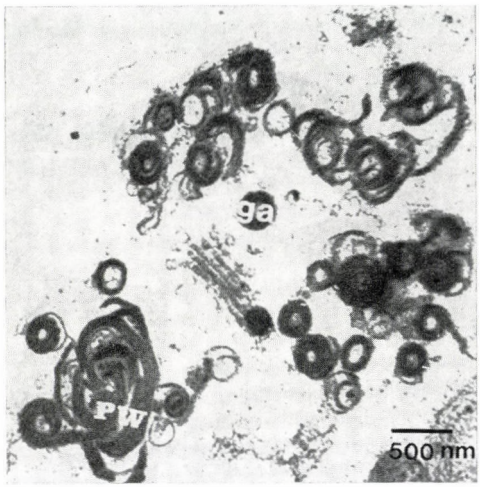

Fig. 5. Submicroscopical view of bean leaf cell infected with isolate G2: *Pw* pin-wheel structure, *ga* scroll

The isolates G2 and G3 were studied in respect to their submicroscopic inclusions. Both isolates produced pin-wheel structures with scrolls (Fig. 5). On the basis of these inclusions it could be concluded that isolates G2 and G3 belonged to the first subgroup of potyviruses, i. e. to the same subgroup in which BCMV is included (comp. EDWARDSON, 1974).

Discussion

Investigations of virus diseases in the Provinde Kosovo (southeastern part of Yugoslavia) began rather late. The first data on virus diseases in this region were reported by PERIŠIĆ (1951). Many years later ZEKOVIĆ and ŠOŠKIĆ (1969) investigated plum pox virus found in this part of Yugoslavia. However, a more comprehensive investigation of one virus isolated in Kosovo was done by MILIČIĆ and PLAKOLLI (1974). These authors described alfalfa mosaic virus found on wild legume plant *Ballota nigra.*

Data obtained by the experiments presented here show that isolates L1, L2, L3 and BD belong to AMV. This means that AMV is widespread on alfalfa and clover in Kosovo. The per cent of infected alfalfa and clover with AMV often amount to 85%. The spread of AMV on alfalfa was established in other parts of Serbia as well (BABOVIĆ, 1965).

The isolates investigated in this paper caused only local necrotic lesions on bean and cowpea. The same type of symptoms are caused by most extensively described AMV isolates (MILBRATH and MCWHORTER, 1954; ZAUMEYER, 1963; HAGEDORN and HANSON, 1963). On the basis of the type symptoms on bean,

AMV isolates are grouped in different classes (HULL, 1969; HAMPTON *et al.*, 1978). However, whether one isolate causes local or systemic symptoms in bean depends on bean cultivar. Since various authors often use various cultivars of bean, a confusion in this respect has arisen. Because of that HAMPTON *et al.* (1978) consider with good reason that in identification of virus isolates from legume plants accurately defined plants (cultivars) have to be used.

The purified virus solution of isolate L1 had to be fixed in 1% solution of formaldehyde because unfixed virus particles were usually destroyed. During centrifugation in sucrose density gradient our isolates usually yielded only three centrifugal components, but five virus components were obtained when the virus was analysed by analytical centrifugation. All components could not be visualized in sucrose density gradient probably because of their low concentration.

The isolates G2 and G3 belonging to bean common mosaic virus (BCMV) cause in bean and *C. quinoa* symptoms which are very similar to the ones caused by BOS's (1971) and ALEKSIĆ's (1967) isolates. Generally, BCMV is transmissible in a high percent through seeds (CRISPIN and GROGAN, 1961; SCOTLAND and BURKE, 1961). Our isolates G2 and G3 of BCMV are also seed-borne but only in about 23%. However, CRISPIN and GROGAN (1961) have reported that one of their BCMV isolate was not seed-borne through bean seeds. According to this data BCMV isolates vary in respect of transmission by bean seeds as well.

On the basis of symptoms it seems that bean yellow mosaic virus (BYMV) also occurs in Kosovo. We observed numerous bean plants with pronounced yellow symptoms in a few past years. During this work we also tried to isolate which provoked typical BYMV symptoms on bean did not produced pin-wheel structures with laminated aggregates, i.e. inclusions which are characteristic of that virus. In addition, that isolate caused symptoms which were more similar to symptoms of BCMV than to symptoms of BYMV.

In the course of our investigations of legume viruses in Kosovo cucumber mosaic virus (CMV) was also isolated from bean. Thus, it has been found that AMV, BCMV and CMV occur on legume plants in this region of Yugoslavia. Of course, this does not mean that some other viruses may not occur in legume plants in that region (comp. MUSIL, 1969; MUSIL, 1975). Further investigations of virus diseases in Kosovo which are in progress will surely confirm this opinion.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 349—354 (1981)

Occurrence of Grapevine Bulgarian Latent Virus in Hungary

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Grapevine Bulgarian latent virus was serologically detected from different naturally infected grapevine varieties in Hungary. 25 varieties were tested in May of 1980. Virus infection was found on the following grapevine varieties: Piros szlanka, Merlot, Fűszeres kadarka, Fekete muskotály, Cirfandli, Siroka melniska and Guzal kara.

GBLV was mechanically transmitted from naturally infected grapevines to *Chenopodium quinoa* which reacted with chlorotic lesions on inoculated leaves and systemic symptoms consisting of leaf mottle and necrotic flecks. Virus infection was serologically detected both from the sap of grapevine and *Chenopodium quinoa* leaves.

A Bulgarian isolate was used for production of antiserum. The virus was partially purified from *Chenopodium quinoa* leaves by clarifying leaf extract with ether and carbontetrachloride followed by one cycle of differential centrifugation. The antisera were obtained from rabbit immunised with partially purified antigen. Serological tests were done using sap of grapevine and *Chenopodium quinoa* applying the double diffusion agar-gel method described by Ouchterlony.

According to results approached from serology, symptoms on herbaceous plants and the electron microscopical investigation, this virus was identified as grapevine Bulgarian latent virus. So far, there were no data about the occurrence of this virus in Hungary. Therefore, it is a new virus in the Hungarian vinegrowing areas.

Grapevine Bulgarian latent virus was first described by MARTELLI *et al.* (1976, 1977). This virus is latent is some cultivars of *Vitis vinifera*, but it has also been isolated from grapevine varieties with reduced growth and fanleaf-like symptoms (MARTELLI *et al.*, 1976). The virus causes delayed budbreak, irregular elongation of the shoots and straggly fruit clusters (UYEMOTO *et al.*, 1977), in *Vitis labrusca* cv. Concord.

This mechanically transmissible virus was occurred up to the present in Bulgaria and New York State, USA only.

In Bulgaria two strains were distinguished, serological relationship between them was very close (MARTELLI *et al.*, 1976). A more distant relationship was found between Bulgarian and New York strains (MARTELLI *et al.*, 1978).

The present paper reports the occurence of grapevine Bulgarian latent virus in southern part of Hungary.

Materials and Methods

Serological investigations have been carried out in a grapevine variety-collection of Hungary. 25 varieties were tested in May of 1980. The Ouchterlony double immunodiffusion test was used for the virus detection.

Antiserum was produced to a Bulgarian isolate, which was forwarded by Dr. M. JANKULOVA.

The virus was propagated for purification in *Chenopodium quinoa* in greenhouse under normal greenhouse conditions. For preparing of inoculum, 0.1 M phosphate buffer pH 7.2 was used containing 0.2% ascorbic acid, and 0.2% sodium sulfite. Locally and systemically infected leaves were harvested 8 days after inoculation and homogenized together with the above-mentioned buffer in the ratio 1 : 1 (weight per volume) in a Waring blendor.

The juice was expressed through nylon-cloth. The extracts were clarified by ether and carbontetrachloride.

The resulting emulsion was separated by low-speed centrifugation. The clear yellow supernatant aqueous phase was subjected to one cycle of differential centrifugation (MOM. 3170/b) ultracentrifuge P-40 rotor, 2 hours 30 000 rpm, (97 000 g).

The pellets were resuspended in 1.5 ml 0.02 M phosphate buffer pH 7.2. Insoluble material was removed by a low speed centrifugation at 5600 g for 15 minutes.

This partially purified virus suspension was used for immunization. The rabbit was immunized three times by intramuscular injections over a period of three weeks.

Bleedings began 7 days after the last injection and repeated four times 3–4 days intervals.

At the time of the serological testing the grapevine shoots were 5–8 cm long.

Five pieces of shoots were collected from each plant in a nylon-sack.

Leaves of grapevines were homogenised in mortars in 0.02 M phosphate buffer, pH 8.0, (1 ml buffer/g leaf tissue), containing 0.2% ascorbic acid, 0.2% sodium sulphite and 0.6% caffeine (POCSAI *et al.*, 1979).

For serological tests, gel plates in petri dishes were prepared using 0.8 g Difco-Bacto agar in 100 ml 0.85% saline, containing 0.06% sodium azide.

Wells were cut 7 mm in diameter and 4 mm apart by a gel-cutter according to SCHMELZER and GOMM (1971).

The virus from the infected samples was mechanically transmitted to *Chenopodium quinoa*, using 0.1 M phosphate buffer, pH 7.2. This buffer contained 0.2% ascorbic acid, and 0.2% sodium sulfite.

Chenopodium quinoa plants were grown in greenhouse where the temperature ranged between 20 °C and 25 °C.

Test plants were inoculated, when they had developed eight to ten leaves, by gentle rubbing the inoculum onto, leaves lightly dusted with carborundum (600 mesh).

Serological tests were also carried out using sap of infected *Chenopodium quinoa* leaves.

For electron microscopical investigation, the virus preparation was purified by two cycles of differential centrifugation. Electron microscopical investigations were carried out on by OPTON EM 9 S-2. Samples in 0.02 M phosphate buffer were placed on carbon-formvar coated grids and stained with 2% phosphotungstic acid.

Results

The results of serological test from different grapevine cultivars are shown in Tables 1 and 2.

Seven of the 25 examined grapevine cultivars gave positive serological reaction with prepared antiserum.

Table 1

Grapevine cultivars in which grapevine Bulgarian latent virus has been tested by serology

Cultivars	Number of tested plants	Number of infected plants
Tzimlianski belli	2	—
Triomubbe	2	—
Ai Izum belli	3	—
Nebide nero	2	—
Fuorno	1	—
Rizamat	3	—
Boulouth usumu	2	—
Vereia	2	—
Oba belli	2	—
Guzal kara	3	1
Pinot noir P-1	10	—
Siroka melniska	4	1
Vavrud	7	—
Cirfandli	10	2
Piros szlanka	10	2
Cabernet Sauvignon	10	—
Kövidinka	10	—
Merlot noir	10	1
Olaszrizling P-2	10	—
Fűszeres kadarka	10	2
Csabagyöngye P-1	10	—
Pinot blanche P-9	10	—
Pinot blanche P-24	10	—
Fekete muskotály	9	1
Rizling szilváni	10	—

Table 2

Results of serological tests from the crude sap of grapevine naturally infected with grapevine Bulgarian latent virus

Cultivars	Antiserum dilution			
	1 : 8	1 : 32	1 : 64	1 : 128
Cirfandli 5.	+	+	+	−
Cirfandli 6.	+	+	+	−
Guzal kara 2.	+	+	−	−
Fekete muskotály 13.	+	+	+	−
Fűszeres kadarka 10.	+	+	+	−
Fűszeres kadarka 3.	+	+	+	−
Merlot noir 9.	+	+	+	−
Piros szlanka 2.	−	−	+	−
Piros szlanka 11.	−	+	+	−
Siroka melniska 16.	+	+	+	+

Table 3

Results of serological tests from the sap of *Chenopodium quinoa* infected with grapevine Bulgarian latent virus

Sap samples of infected *Ch. quinoa*	Antiserum dilution			
	1 : 8	1 : 32	1 : 64	1 : 128
Cirfandli 5.	+	+	−	−
Cirfandli 6.	+	+	+	−
Guzal kara 2.	+	+	+	−
Fekete muskotály 13.	+	+	+	−
Fűszeres kadarka 10.	+	+	−	−
Fűszeres kadarka 3.	+	+	+	−
Merlot noir 9.	+	+	−	−
Piros szlanka 2.	+	+	−	−
Piros szlanka 11.	−	+	+	−
Siroka melniska 16.	+	+	−	−

These were the following: Piros szlanka, Merlot noir, Fűszeres kadarka, Fekete muskotály, Cirfandli, Siroka melniska and Guzal kara.

At the time when testing was carried out (end of May) infected grapevine shoots contained enough virus to react serologically. The sharpest precipitation-lines were observed at antiserum dilution of 1 : 64.

GBLV was mechanically transmitted from the naturally infected grapevine to *Chenopodium quinoa.*

Test plants showed chlorotic lesions on inoculated leaves, and systemic symptoms consisting of leaf mottle and necrotic flecks about 10–15 days after inoculation.

Results of serological tests from the sap of *Chenopodium quinoa* are given in Table 3.

These results show that the antiserum reacts in the same manner both with the sap of infected *Chenopodium quinoa* and with the crude leaf-extracts of grapevine.

The most beautiful precipitation lines were also observed at antiserum dilution of 1 : 64.

We obtained antisera by injecting partially purified virus preparation from *Chenopodium quinoa* leaves.

The titer of the prepared antiserum was 1 : 256 as determined with sap of *Chenopodium quinoa.*

With sap of healthy plants, it reacted at dilutions 1 : 8. The shape and dimension of virus particles were determined by electron microscopy. Shape of particles was found to be polyhedral and the average diameter of virus particles was more than 30 nm.

Discussion

The results of this paper confirm that grapevine Bulgarian latent virus occurs in Hungary. So far, there were no data at all about the occurrence of this virus. According to the results approached from serology, symptoms on herbaceous plant and the electron microscopical investigation, this virus was identified as grapevine Bulgarian latent virus.

We did not examine whether the infected grapevine cultivars showed fanleaf-like and other syndromes or not.

The well-spreading of the virus in the Hungarian vine-growing areas is not yet determined. Therefore, the susceptibility of grapevine cultivars to the virus disease, and the influence of GBLV on growth and yield of grapevine will require additional investigation.

Acknowledgements

Thanks are due to Dr. M. JANKULOVA (Institute of Plant Protection Kostinbrod, Bulgaria) for providing of GBLV isolate, also to Mr. M. DEZSÉRI who made the electron microscopical examinations, and Mrs. I. CZINKA, Miss I. FARKAS for excellent their technical assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 355—364 (1981)

Suppression of Challenge Bacteria in Tobacco Leaves in the Early and Late Period of Induced (Acquired) Resistance Caused by *Pseudomonas fluorescens*

By

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The existence of an early induced defence mechanism of tobacco plant which developes as early as 3—6 hours after induction with *Pseudomonas fluorescens* has been published recently (BURGYÁN and KLEMENT, 1979). This type of induced resistance was compared with the already known induced resistance that develops following injection of plants with heat killed bacteria. We have shown that the early induced resistance is light independent, but the other type i.e. the late induced resistance is a light dependent reaction of tobacco. The early defence mechanism strongly protects against the development of incompatible symptoms but weakly against the compatible one, whereas the late induced resistance completely blocks both types of symptom in the light.

It was shown that the early induced resistance inhibits not only the development of necrotic symptoms but also inhibits the multiplication of challenge bacteria.

Induced resistance (in other words acquired resistance) against bacterial infections occurs following injection of plant with living or heat-killed bacteria (LOVREKOVICH and FARKAS, 1965; LOZANO and SEQUEIRA, 1970; and NOVACKY, ACEDO and GOODMAN, 1972). Recently, an early induced selective protection (ESP) of tobacco leaves was published by KLEMENT and BURGYÁN, 1978; and BURGYÁN and KLEMENT, 1979. The two types differ from each other in some respects. In this paper acquired resistance will be noted as late induced resistance and the early selective protection as early induced resistance.

Early induced resistance inhibits or delays the symptom development of challenge bacteria in protected leaves as early as 3—6 hours until 21 hours. Late induced resistance develops much later and persists for several days. The early host response is independent from the light but the late one is a light-dependent reaction of plants.

The inhibition of bacterial multiplication in the later period of acquired resistance has been investigated (LOZANO and SEQUEIRA, 1970), however, in the early period determination of bacterial multiplication was not possible because of methodical difficulties (BURGYÁN and KLEMENT, 1979). In the case of early induced resistance only an indirect evidence indicated the inhibition of bacterial multiplication. This indirect evidence was based on the absence of the hypersen-

sitive reaction (HR) induced by challenge bacteria. It has been shown earlier the HR does not appear when the multiplication of bacterial cells was inhibited (DURBIN and KLEMENT, 1977). Consequently, it was supposed (BURGYÁN and KLEMENT, 1979) that the absence of the HR in the early protected leaves demonstrates the inhibition of the bacterial multiplication, too.

In this paper, we give direct evidence for the inhibitory effect of the early induced resistance on the multiplication of challenge bacteria and we compare the early and late induced resistance on the basis of inhibition of symptom development on tobacco leaves caused by compatible and incompatible pathogens.

Materials and Methods

Nicotiana tabacum L. cv. Xanthi *nc* plants were grown in greenhouse. Plants of the 5–10 leaf stage were used for experiment. *Pseudomonas fluorescens* Migula (ATCC. No. 13 525) was used for induction of the early and late induced resistance. This saprophytic bacterium was grown on nutrient-agar culture for 24 h. the cell concentration of washed bacteria was adjusted to 5×10^8 cells ml^{-1}. For comparison of the protective effect of the early and late induced resistance leaves were injected either with the suspension of P. fluorescens or with steril water that served as control. After infiltration, plants were kept in growth chamber at 25 °C either in continuous light (3000 cd) or in the dark. Compatible *P. tabaci* (Wolf and Foster) Stevens (ATCC No. 11528) pathogenic to tobacco and an incompatible *P. pisi* Saccket (ATCC No. 11055) pathogenic to pea as challenge bacteria were used for detection of host responses. Challenge bacteria were injected into about 1 cm^2 large areas of pretreated tobacco leaves in 10^7, 10^8 and 10^9 cells ml^{-1} concentrations.

To detect the early host response plants were injected with challenge bacteria in most of the experiments 6 h after pretreatment. In the case of late induced resistance the second injection was applied 48 h after the first injection. The appearance of symptoms were recorded during 3 days. Desintegrated cells of *P. fluorescens* were used for pretreatment of tobacco leaves when the multiplication rate of the challenge bacteria was measured. Forty-eight-hour-old nutrient-agar culture of *P. fluorescens* were washed in distilled water and the number of cells was adjusted to 10^9 cells ml^{-1}. Cells of bacteria were collected from 50 ml suspension by centrifugation at a rotor speed of 6×10^3 r min^{-1} for 20 minutes and were then resuspended in 10 ml distilled water. Desintegration of cells was carried out with ultrasonic energy (MSE type MT 20) for 45 min. The sonicated cells were adjusted to the original value.

It is known, that tissue necrosis itself as a consequence of the HR also suppresses bacterial multiplication. In the earlier experiments detection of multiplication of the HR inducing challenge bacteria in the protected leaves was not possible because the time of the development of the early protection coincided with the appearance of the HR. To solve this question, an avirulent strain of *P. syringae*

(received from DeVay, No. 761–6) which does not induce HR but is able to multiply in tobacco was used for observation of changes in bacterial population. This strain of *P. syringae* isolated from *Setaria sp.* is identical with other common hypersensitive necrosis-inducing strains of *P. syringae* in syringomycin production and in antigenic structure as well. The number of bacteria in leaf tissue were followed by counting the number of bacterial colonies developed from discs of infiltrated leaf areas. Five discs (1 cm^2 each) were removed in 24 h intervals for 72 h. The number of bacterial cells from macerated leaf tissues were calculated by the platecount method using the standard serial dilution technique. Each reported cell number is the average of at least three replicate plating.

Results and Discussion

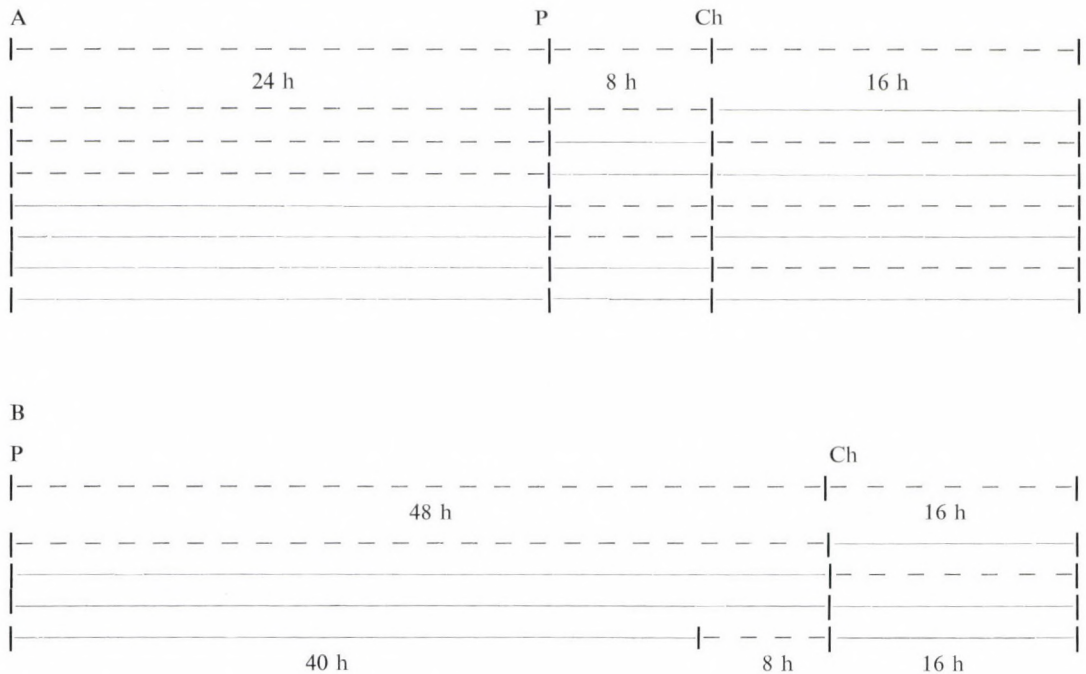
Effect of light on the development of the early and the late induced resistance

Light may be an important distinctive factor for the separation of the two defence reaction, because the early induced resistance is light independent (BURGYÁN and KLEMENT, 1978) but the late one is a light dependent reaction of plants (SEQUEIRA, 1976). In earlier works (KLEMENT and BURGYÁN, 1978; BURGYÁN and KLEMENT, 1979), during and after the injection of *P. fluorescens* or of the challenge bacterium, plants were kept under light for about one hour to help evaporation of water from the water-soaked intercellular spaces of the injected areas before they were put back into the dark chamber. The objection that this short illumination may disturb the continuous dark effect seemed to be warranted. To avoid this source of error, tobacco plants were put into a dark room throughout the experiments and *P. fluorescens* as well as the challenge *P. pisi* were injected under subdued green light for a few minutes. Water was evaporated from the injected leaves in complete dark.

It has been shown earlier that the early induced resistance may be demonstrated when the time between the pretreatment and challenge injection is about 6 hour. One may think of that this time is too short for the manifestation of the effect of the darkness. To solve this supposition in some experiments tobacco plants were kept in continuous dark for 24 hours before the pretreatment. To test the role of the light before the induction or during and after the development of the early induced resistance, tobacco plants were kept either in light or in dark in various combinations (Table 1). It was shown that the illumination before the induction and during the development of plant reaction had no influence on the early induced resistance (Table 1/A and Fig. 1 and Fig. 2). These investigations clearly verified that the early induced resistance is light independent but the late one is a light dependent reaction of tobacco plants. When plants were illuminated for a short period during the development of the late induced resistance, the inhibitory effect was recovered (Table 1/B). It is also proved by experiments

Table 1

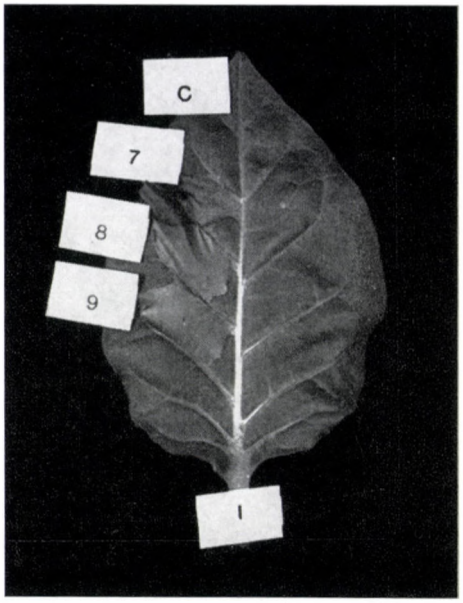
Effect of light on development of the early (A) and the late (B) induced resistance in tobacco induced by *P. fluorescens* and challenged by *P. pisi*



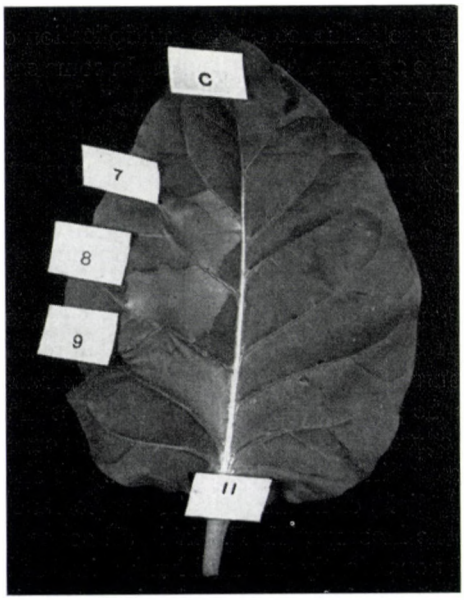
HR*		
10^7	10^8	10^9
–	–	–
–	–	–
–	–	–
–	–	+–
–	–	–
–	–	–
–	–	–
–	–	–

HR*		
10^7	10^8	10^9
–	–	–
–	–	–
++	++	++
++	++	++
–	–	–

P = pretreatment with *P. fluorescens* 5×10^8 cells ml^{-1}; Ch = challenged with *P. pisi* 10^7, 10^8, 10^9 cells ml^{-1}; ——— in dark; – – – – in light; * appearance of the HR induced by 10^7, 10^8, 10^9 cells ml^{-1}; – no HR; +– weak HR; ++ strong HR



1



2

Figs 1—2. Inhibition of tissue necrosis induced by *P. pisi* in various concentrations (10^7, 10^8, 10^9 ml^{-1}). Tobacco plants were pretreated either with *P. fluorescens* 5×10^8 cells ml^{-1} (on the right half leaf) or with water (C) (on the left half leaf). 6 hours before the challenge. Plants were held under dark (I) or under light (II) conditions. The tissue necrosis appeared only on the control leaf part but it was inhibited on the opposite half both in the light and dark

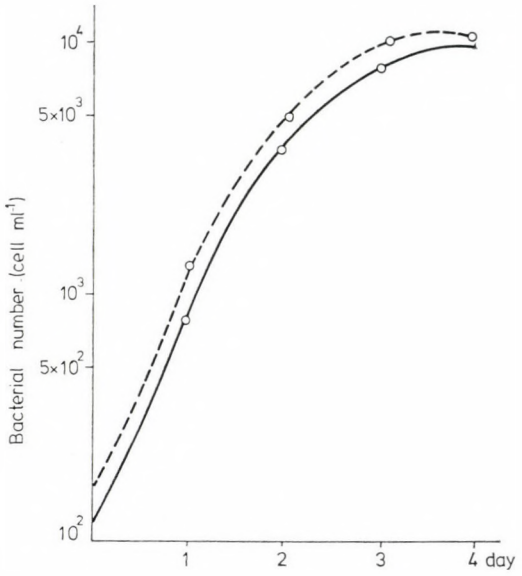


Fig. 3. Multiplication rate of *P. syringae* in tobacco leaf in light (----) and dark (——) conditions

that darkness itself has no influence on the multiplication of the challenge bacteria (Figs. 3, 4) or on the appearance of the HR. In some experiments the development of the HR somewhat delayed in the dark.

Comparison to the early and the late induced resistance regarding symptom development

Tobacco leaves pretreated with living cells of the saprophytic *P. fluorescens* were challenged with different concentrations of either the compatible *P. tabaci* or the incompatible *P. pisi* (Table 2). The early induced resistance was investigated 6 hours after the pretreatment. In the case of the late reaction the challenge bacteria were used 48 hours after the injection of *P. fluorescens.*

Six hours after the pretreatment of tobacco leaves the development of wildfire disease symptom caused by *P. tabaci* was delayed in comparison to the control. There was no significant difference in the wildfire symptom development between tobacco plants kept in light or in the dark. The appearance of the hypersensitive necrosis induced by the incompatible *P. pisi* in all concentrations was, however, strongly protected independently of the illumination of the pretreated plants. It was concluded that the early defence mechanism strongly protects the development of incompatible symptom but only weakly suppresses the compatible one, and this type of host response is light independent.

Forty-eight hours after the pretreatment both the wildfire disease symptom and the HR were strongly protected in light but not in the dark. Confirming the re-

Table 2

Protection of disease symptoms in tobacco leaf pretreated with *P. fluorescens*

Challenge		6 h after pretreatment											
		Light						Dark					
		Control (water)			*P. fluorescens*[a]			Control (water)			*P. fluorescens*		
		24[c]	48	72	24	48	72	24	48	72	24	48	72
P. tabaci (normosensitive necrosis)	10^{9a}	+[d]	+	+	−	+	+	+	+	+	−	+	+
	10^{8}	+	+	+	−	±	±	±	±	+	−	+	+
	10^{7}	−	+	+	−	−	±	−	+	+	−	−	±
P. pisi (hypersensitive necrosis)	10^{9}	+	+	+	−	−	−	+	+	+	−	−	−
	10^{8}	+	+	+	−	−	−	+	+	+	−	−	−
	10^{7}	−	+	+	−	−	−	−	+	+	−	−	−

Challenge		48 h after pretreatment											
		Light						Dark					
		Control (water)			*P. fluorescens*			Control (water)			*P. fluorescens*		
		24	48	72	24	48	72	24	48	72	24	48	72
P. tabaci (normosensitive necrosis)	10^{9b}	+	+	+	−	−	−	+	+	+	±	±	±
	10^{8}	+	+	+	−	−	−	+	+	+	−	±	+
	10^{7}	±	+	+	−	−	−	±	+	+	−	−	±
P. pisi (hypersensitive necrosis)	10^{9}	+	+	+	−	−	−	+	+	+	+	+	+
	10^{8}	+	+	+	−	−	−	+	+	+	+	+	+
	10^{7}	±	±	±	−	−	−	−	±	±	−	−	±

[a] 5×10^8 cell ml^{-1}; [b] cells ml^{-1}; [c] symptom development after the challenge inoculations in hours; [d] + tissue necrosis; ± partial tissue necrosis; − no tissue necrosis

ports of LOSANO and SEQUEIRA (1970) and SEQUEIRA (1976) the late induced resistance completely blocked the development of compatible disease symptom and the visible HR, however, this type of host response is a light dependent reaction of tobacco plants. A comparison of the early and the late induced resistance suggests that their actions on the compatible and incompatible pathogens are different and the environment influences their developments in different ways.

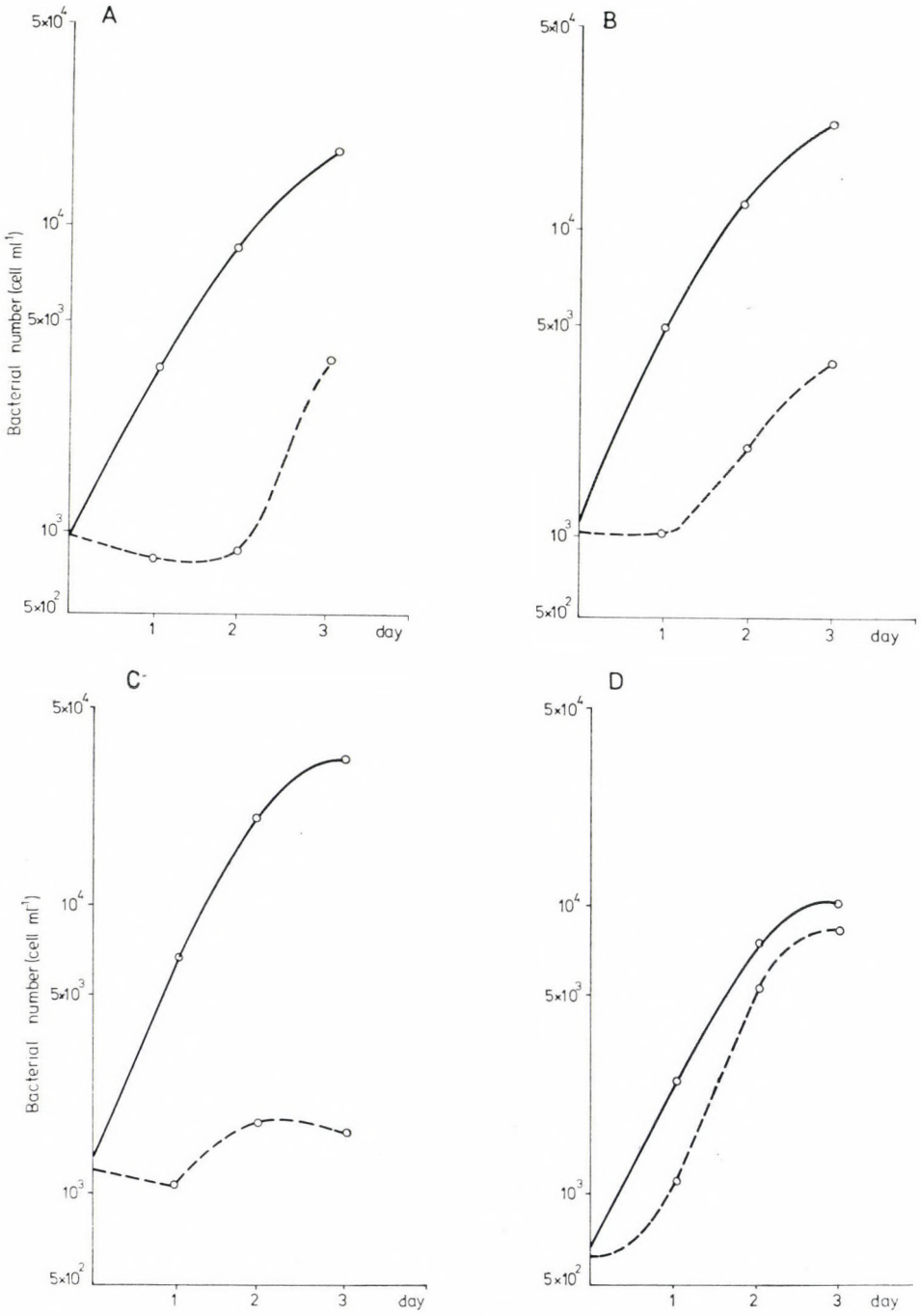


Fig. 4. Multiplication rate of the challenge *P. syringae* in tobacco leaf pretreated by sonicated cells of *P. fluorescens* (– – –) or by water (——). The time between the pretreatment and the challenge was 6 h, plants were kept in the light (A) and in the dark (B). The time between the pretreatment and the challenge was 48 h, plants were kept in the light (C) and in the dark (D)

Inhibition of multiplication of the challenge bacteria by the early and the late induced reaction

In our earlier works inhibition of bacterial multiplication in protected tissue was indicated by the absence of the HR or that of the normosensitive necrosis. This hypothesis was supported by experiments of DURBIN and KLEMENT (1977), SASSER (1978) and KLEMENT *et al.* (1978). They have shown that the necrosis in hypersensitive resistant plants or in susceptible hosts is induced only by multiplying bacteria. Consequently, if the necrosis did not develop presumably bacterial multiplication was inhibited in host tissue. We used this hypothesis because the direct detection of multiplication of challenge bacteria in the protected leaves was not possible because the time of the early protection coincided with the HR. It is also well known, that the hypersensitive necrosis also suppresses bacterial multiplication, so the separation of inhibitory effect of the early induced resistance from the effect of HR was methodically impossible. To solve this difficulty a no HR inducing strain of *P. syringae* was used as challenge and desintegrated cells of *P. fluorescens* were used for the induction of induced resistances.

Figure 3 shows the multiplication rate of *P. syringae* in the non-protected control tobacco leaves kept in the light and in the dark. As is seen, light did not influence the multiplication of *P. syringae*.

We compared the multiplication of the challenge bacterium in the pretreated tobacco plants kept in either light or in the dark (Fig. 4). *P. syringae* could equally multiply in control (only water pretreated tobacco leaf) under both light and dark conditions (Fig. 3). However, the multiplication was inhibited in comparison to control as early as 6 hours after the pretreatment (early induced resistance) in plants kept either in light or in the dark (Fig. 4/A and 4/B). Bacterial growth was also delayed in the leaves pretreated 48 hours before (late induced resistance) only in light (Fig. 4/C), but in dark condition this inhibitory effect disappeared (Fig. 4/D). These results are similar to the results of the experiment on symptom protection (Table 2). We have shown that there is a correlation between the protection of necrosis and the inhibition of bacterial multiplication. In other words the absence of symptoms caused by the challenge bacteria may be a consequence of inhibition of bacterial multiplication and not of the necrosis *per se*.

These results confirmed the conclusions of our previous paper that either two types of induced resistance or an early and a late period of induced resistance exist in tobacco plant, and they differ from each other in some important respects.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3–4), pp. 365–369 (1981)

Components of *Pseudomonas fluorescens* Causing the Early and the Late Induced Resistance of Tobacco to Challenge Infection

By

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We tried to separate the components of cells of *Pseudomonas fluorescens* to find the responsible component(s) that has a role in induction of the early and late induced resistance of tobacco plant. Complete and desintegrated cells of *P. fluorescens* can induce both type of host responses against challenge bacteria. The dialyzed and ethanol precipitated fractions of supernatant of desintegrated cells were more effective to induce the early than the late induced resistance. In contrast, the sedimented cell wall fraction caused a stronger host reaction in the case of the late induced resistance. These results indicate that the bacterial cell contains two kind of inducers at least.

LOVREKOVICH and FARKAS (1965) injected tobacco leaves with heat-killed cells of *Pseudomonas tabaci* and suppressed a challenge inoculation with living cells of *P. tabaci*. This, so called induced resistance or in other words acquired resistance is a time and light dependent reaction of plant (SEQUEIRA, 1976). The protection developed by 12 – 48 hours in light but not in dark. A similar defence mechanism of tobacco plant induced by saprophytic *Pseudomonas fluorescens*, which developed as early as 3–6 hours after infection was published by us (KLEMENT and BURGYÁN, 1978; BURGYÁN and KLEMENT, 1979). This latter "early induced selective protection" (ESP) differs from the former one in some respects.

In our recent work (HEVESI *et al.*, 1981) we tried to compare the influence of these two types of induced resistances of tobacco leaves provoked by *P. fluorescens* on the suppression of development of tissue necrosis and on the inhibition of multiplication of the challenge bacterium. We believe that the phenomenon of induced resistance (acquired resistance) is not a unique reaction of plant. The living and sonicated cells of *P. fluorescens* could induce two different host responses, namely the so called early and the late induced resistance. The former occurs as early as 6 hours after the first inoculation by *P. fluorescens*. It strongly protects the development of the hypersensitive reaction (HR) but does not protect or only delays the appearance of disease symptom (necrosis) caused by the compatible pathogen. The early protection of plant is independent from light. The late form which was rather time-dependent reaction of tobacco plant inhibits equally both the compatible and incompatible symptom development in the light. In contrast to the early protection, the late one does not occur in the dark.

The bacterial components responsible for the induction of induced resistance were investigated in several laboratories. Some investigations indicated that the bacterial protein-lipopolysaccharide complex acts as an inducer of plant responses. SEQUEIRA and his co-workers (1972) have the opinion that the protein component of protein-lipopolysaccharide complex is responsible for the induction of host response. However, according to MAZZUCCHI *et al.* (1979), the active part of the protein-lipopolysaccharide complex is the lipopolysaccharide part of the cell wall.

In our present work we tried to separate the components of cells of *P. fluorescens* to find the responsible component(s) that has a role in induction of the early and the late induced resistance of tobacco plant.

Materials and Methods

Forty-eight hours old nutrient-agar-culture of *P. fluorescens* Migula (ATCC No. 13525) was washed with steril distilled water and the cell number was adjusted to 10^9 cell ml^{-1}. The cells were collected from 50 ml suspension by centrifugation at a rotor speed of 6×10^3 r min^{-1} for 20 minutes and were then resuspended in 10 ml distilled water. Desintegration of bacterial cells was carried out with ultrasonic energy for 45 minutes. When the suspension became transparent the broken cell parts were removed by centrifugation at 6×10^3 r min^{-1} for 60 minutes, and the supernatant fluid was filtered through Seitz-filters. Both supernatant and the pellet were adjusted to the original volume and tried their biological activities. The supernatant of sonicated bacterial cells was dialysed for 24 hours against bidistilled water. When the ethanol content of the dialysed supernatant was adjusted to 80% a fine precipitation appeared. This was allowed to stand overnight. The precipitate was centrifuged for 20 minutes at the upper speed and dissolved in 50 ml distilled water. Concentration of each fraction was adjusted to the initial cell number.

Each fraction of *P. fluorescens* was separately injected into the intercellular spaces of whole tobacco leaves with a hypodermic needle. Only the middle, fully-expanded leaves of plants in the 8–10 leaf stage were used. Thus pretreated tobacco plants *Nicotiana tabacum* L. cv. Xanthi *nc* were kept partly in light (3000 cd), partly in dark chamber at 25 °C and were challenged with either *P. tabaci* (Wolf and Foster) Stevens (ATCC No. 11528) pathogenic to tobacco or with *P. pisi* (Saccket) (ATCC No. 13535) pathogenic to peas, either 6 hours or 48 hours after the pretreatment. All experiments were performed at least three times with representative data presented here.

Results and Discussion

The effectivity of fractions of *P. fluorescens* on the *early induced resistance* was investigated 6 hours after the pretreatment (Table 1). The development of wildfire disease caused by *P. tabaci* in the pretreated tobacco leaves was delayed

and the symptom was only partially protected by each fraction of cells of *P. fluorescens*, both in the light and in the dark. The sediment of the sonicated cells was still less effective than the supernatant. – Development of the hypersensitive necrosis induced by *P. pisi* was significantly protected by most of the fractions in the light and in the dark. The effectivity of the sedimented cell wall parts, however, was lower than the supernatant of the desintegrated cells. It was concluded, that both soluble and insoluble fractions of the bacterial cell contain active materials, however, the quantity of the inductive material was less in the sedimented cell wall parts. – There was no difference observed under light and dark condition in the protection of symptom development on tobacco plants in the case of early protection.

Effectivity of the cell fractions on the induction of the *late induced resistance* was investigated by 48 hours after the pretreatment because it has been proved that the early reaction persists until about 21 hours (BURGYÁN and KLEMENT, 1979) (Table 2). Both wildfire symptom and hypersensitive reaction were strongly inhibited by the complete and the desintegrated bacterial cells as well as by the supernatant of desintegrated cells of *P. fluorescens* in light. No or only partial protection was induced by the sediment, by the dialysed or the ethanol precipitated fractions. This results agrees with the data of SEQUIERA (1976) who also has shown

Table 1

Development of symptoms caused by challenge bacteria 6 h after the pretreatment of tobacco leaves with *Pseudomonas fluorescens*

Condition	Challenge		Fractions of *P. fluorescens*						
			Water control	Complete cells	Desintegrated cells	Supernatant of sonicated cells	Sediment of sonicated cells	Dialysed fraction of supernatant	Precipitate of dialysed fraction
In light	*P. tabaci*	10^{9a}	+++[b]	−++	−++	−±±	±++	−±±	−±+
		10^{8}	+++	−±±	−±±	−±±	−±±	−−±	−−±
		10^{7}	−++	−−±	−−−	−−−	−−±	−−−	−−−
	P. pisi	10^{9}	+++	−−−	−−−	±±±	+++	−±±	−±±
		10^{8}	+++	−−−	−−−	−−−	±++	−−−	−−−
		10^{7}	−++	−−−	−−−	−−−	−−−	−−−	−−−
In dark	*P. tabaci*	10^{9}	+++	−++	−++	−±±	±++	−±±	−++
		10^{8}	+++	−±±	−±±	−±±	−++	−±±	−±±
		10^{7}	−++	−−±	−−±	−−−	−−±	−−−	−−−
	P. pisi	10^{9}	+++	−−−	−−−	±±±	+++	−±±	−±±
		10^{8}	+++	−−−	−−−	−−−	±±+	−−−	−−−
		10^{7}	−++	−−−	−−−	−−−	−−−	−−−	−−−

[a] Cells ml^{-1}; [b] The symbols indicate the symptom development of the challenge by 24, 48 and 72 hours; + strong, ± weak, − no symptom

Table 2

Development of symptoms caused by challenge bacteria 48 h after the pretreatment of tobacco leaves with the components of *Pseudomonas fluorescens*

Condition	Challenge		Water control	Complete cells	Fractions of *P. fluorescens*				
					Desintegrated cells	Supernatant of sonicated cells	Sediment of sonicated cells	Dialysed fraction of supernatant	Precipitate of dialysed fraction
In light	*P. tabaci*	10^{9a}	+++[b]	−−−	−−−	−−−	−++	±++	±++
		10^{8}	+++	−−−	−−−	−−−	−±+	−++	−±±
		10^{7}	±++	−−−	−−−	−−−	−−±	−−−	−−−
	P. pisi	10^{9}	+++	−−−	−−−	−−−	−±+	+++	+++
		10^{8}	+++	−−−	−−−	−−−	−±±	−±±	−±±
		10^{7}	±±±	−−−	−−−	−−−	−−−	−−−	−−−
In dark	*P. tabaci*	10^{9}	+++	±±+	±±+	+++	+++	+++	+++
		10^{8}	+++	−±+	±++	±±±	±±±	±++	±±+
		10^{7}	±++	−−±	−±±	−−±	−−±	−−±	−−±
	P. pisi	10^{9}	+++	+++	+++	−±+	+++	+++	+++
		10^{8}	+++	±++	±++	±±±	±±+	±±+	±±+
		10^{7}	−±±	−−±	±±+	−−−	−−−	−−−	−−−

[a] Cells ml^{-1}; [b] The symbols indicate the symptom development of the challenge by 24, 48 and 72 hours; + strong; ± weak; − no symptom

a partial protection with ethanol precipitate fraction of *P. solanacearum* at 24 hours. It seems from these data that mainly the dialysed and ethanol precipitated fractions of bacterial cell lost their effectivity on the induction of the late protection as compared to the early one. Under dark condition no or only a slight protection appeared in tobacco leaves in all cases. No significant differences exist in the inhibitory effect of the fractions of *P. fluorescens* on tobacco leaves challenged either with the compatible *P. tabaci* or with the incompatible *P. pisi*. Therefore the late reaction equally inhibits both type of pathogens contrary to the early reaction which mainly inhibits the incompatible one.

These results confirmed the idea that the acquired resistance phenomenon induced by bacteria is not a unique reaction of plant and that the complete cells of *P. fluorescens* can induce an early and a late induced resistance in tobacco plant against challenge bacteria. Our results indicate that the bacterial cell contains two kind of inducers at least. The dialysed and ethanol precipitated fractions of supernatant of desintegrated cells were more effective to induce the early induced resistance than the sedimented cell wall fractions. In contrast, the sediment was more effective in the case of the late induced resistance. Because we were unable to separate pure active material neither from the sedimented cell wall parts nor

from the supernatant of desintegrated cell, it is suggested that the inductive factor may involve labil, soluble and insoluble constituents of bacterial cell wall, which is found in both fractions in the supernatant and in the sediment of sonicated *P. fluorescens* cell. SEQUEIRA and his co-workers (1972) and later MAZZUCCHI *et al.* (1979) separated two different constituents of bacterial cells which were responsible for induced resistance. At present it is difficult to establish the bearings of results of SEQUEIRA *et al.* (1972). It is clear from our investigations that the time between the pretreatment and challenge is an important factor. For instance, when the challenge is used 24 hours after pretreatment as in the experiments of SEQUEIRA *et al.* both host responses may exist at the same time. Additional investigations are necessary to determine and separate the two inducers particularly the one which can induce the early induced resistance in plants.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 371—374 (1981)

Bacterial Blight of Soybean in Hungary (Short communication)

By

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Pseudomonas glycinea was isolated from soybean leaves and identified by comparative biochemical methods and pathogenicity test. The isolates suggested two respective races of the bacterium.

Bacterial blight caused by *Pseudomonas glycinea* Coerper* (Syn.: *P. syringae* van Hall) was first reported in USA (COERPER, 1919). The disease has widely spread and is one of the most common bacterial diseases of soybean. In Hungary bacterial blight has also been found several times (KURNIK, 1962; KLEMENT, 1965; SZILI, 1975; KOCSISNÉ, 1979), but no exact identification of the bacterium has been given so far.

We observed symptoms characteristic of bacterial blight *(viz.* SINCLAIR and SHURTLEFF, 1975) in south-west Hungary (village Bóly) on leaves of the soybean cultivars Okuhara, Merit and ISz-14 in July, 1978 (Fig. 1), as well as on pods of the above cultivars in August.

Isolations were made from necrotic area of leaves onto nutrient broth agar, then incubated at 28 °C. Single colonies were further maintained and used for identification work. In order to select phytopathogenic bacteria that induce hypersensitive reaction in tobacco leaves, KLEMENT's method (1963) was used. To test pathogenicity on soybean, the primary leaves of 2-week-old plants were infiltrated with bacterial suspension (10^6 or 10^8 cells/ml) using a hand-atomizer. Soybean pathogenic strains were furthermore subjected to comparative biochemical testing (LELLIOT *et al.*, 1966; BUCHANAN and GIBBONS, 1974; GROSS and DEVAY, 1977). The data corresponded to those of the literature on *P. glycinea.* The isolates produced nitrites from nitrates, peptonized litmus milk and reduced litmus as well. The production of fluorescent pigment was also observed. The bacterium exhibited tyrosinase activity but failed to have either oxidase or lipolytic activity. It did not form acid from either glucose or tartarate. It did not produce syringomycin and did not liquefy gelatin either. The isolates agglutinated with *P. syringae* serum.

In each study *P. glycinea* races 1 and 2 obtained from B. W. Kennedy (University of Minnesota) through the courtesy of N. T. Keen (University of California, Riverside, USA) were compared to our isolates.

* Recently proposed as *P. syringae* pv. *glycinea* (Coerper) Young, Dye and Wilkie.

Fig. 1. Leaf symptoms of bacterial blight caused by *Pseudomonas glycinea*

Fig. 2. Resistant, intermediate, and susceptible reaction of soybean leaves to *Pseudomonas glycinea*, 6 days after inoculation (left to right). Reactions are shown both on the upper and lower surface of leaves (first and second row, respectively)

Several soybean cultivars were inoculated as above to make attempt to identify our isolates with the recently known 8 races of *P. glycinea* on the basis of plant response. Resistant, susceptible and intermediate reactions, as reported by CROSS *et al.* (1966) and THOMAS and LEARY (1980), were observed (Fig. 2). Environmental factors caused variability in pathogenic response. Controlled conditions (12 000 lux light intensity, 22 °C, 70% relative humidity for 12 hours, then 18 °C, 90% relative humidity in the dark) resulted in more stable plant reactions than glass house circumstances. In the lack of the complete series of differential soybean cul-

Table 1

Xilose utilization and pathogenic response of Hungarian isolates 1e and 2c in comparison with races 1 and 2 of *Pseudomonas glycinea*

P. glycinea	Xilose utilization	Soybean cultivars										
		Harosoy	Merit	Chippewa	Ewans	Wilkin	Express Green	Hakucko Early	Beltskaya 25	ISz-10	ISz-13	ISz-14
1e	−	S	I	I	I	S	R	S	R	S	S	S
2c	+	S	S	I	S	S	R	S	R	S	S	S
R1	−	R	R	R	R	R	I	R	R	R	R	R
R2	−	S	I	I	I	I	I	I	I	S	I	I

R: resistant; S: susceptible; I: intermediate reactions

tivars offered by CROSS *et al.* (1966) we could not draw conclusions simply from direct evidences. The results obtained had to be compared with those of the above literature.

Two isolates suggesting two respective races were found. Isolate *Pg*1e might correspond to race 3 according to the pathogenic response, unlike to race 3 however, did not utilize xilose in minimal medium. *Pg*2c, which might be identical with either race 4 or race 5 in pathogenicity test, utilized xilose like race 5, but unlike race 4 (THOMAS and LEARY, 1980) (Table 1). Despite the distinguishing trait among races, xilose utilization gives no or little selective advantage to *P. glycinea* because it is easily lost.

Cultivar Chippewa on which races 4 and 5 could have been differentiated, interestingly enough, gave an intermediary reaction to race 2, instead of the reported resistant one. The pathogenic response of this cultivar to *Pg*1e and *Pg*2c, therefore, was not taken into account.

We failed to obtain exact race determination of isolates *Pg*1e and *Pg*2c. The pathogenicity test, however, focused attention to the Russian cultivar Beltskaya-25, which proved to be resistant to both isolates found.

*

Thanks are due to Miss Á. NAGYIDAI for her conscientious assistance.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 375—387 (1981)

A Comparative Study on the *Macrolepidoptera* Fauna of Apple Orchards in Hungary (Research on Apple Ecosystems. No. 18)

By

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A comparative survey of the *Macrolepidoptera* fauna occurring in apple orchards of different types (abandoned orchard, backyard garden, traditionally and intensively treated large-scale orchards) is presented on the basis of investigations made in 1977—1979. In 3 types of apple orchards light traps have been operated and the insect material captured by them has been evaluated in detail. The species have been categorized according to the life form of caterpillars. Some conclusions concerning the formation and structure of the *Macrolepidoptera* fauna in apple orchards of Hungary have been drawn.

The Zoological Department of the Research Institute for Plant Protection, in cooperation with some other institutions, has undertaken a 10-year project of agroecological research in 1976. These investigations are conducted in two economically important cultures, in apple orchards and maize fields, respectively. In the present paper the results of lepidopterological studies carried out in apple orchards of the County Szabolcs-Szatmár, will be reported. This area ("Nyírség") belongs to the most significant winter-apple growing areas of Hungary, providing the highest amount of production for both the home market and export purposes.

The results of our former lepidopterological studies have already been published (BALÁZS *et al.*, 1978; MÉSZÁROS, 1978). Earlier data obtained with light traps in apple orchards of Hungary have been reported by NAGY (1975).

In our investigations we aimed at a comparison, the composition and structure of some parts of the agroecosystem in some characteristic types of apple orchards.

Materials and Methods

For our comparative investigations, 4 apple orchards of divers types concerning chemical treatments were selected as follows:

1. Abandoned orchard: Nyíregyháza-Sóskút. Size: ca. 0.2 ha. No chemical treatments. Surroundings: woody-bushy fields as well as meadows and arable lands.

2. Backyard garden: Nyíregyháza-Füzesbokor. Size: 0.5 ha. Regularly treated with insecticides (10 to 12 spraying per year). Surroundings: other backyard gardens, meadows and arable lands.

3. Traditionally treated large-scale orchard: Újfehértó. Size: ca. 5 ha. Regularly treated with insecticides (10 to 12 sprayings per year). Surroundings: similar large-scale orchards, arable lands and – slightly removed – meadows and pastures.

4. Intensively treated large-scale orchard: Nyíregyháza-Ilona-tanya. Size: ca. 100 ha. Regularly treated during whole season (10 to 12 sprayings per year, occasionally even more). Surroundings: similar large-scale orchards, in some places woody-bushy fields and arable lands.

The soil in each orchard is "brown sandy soil". The apple variety produced here generally is Jonathan (in large-scale orchards along with Starking as pollinator variety).

In each orchard type, regular surveys were conducted with the use of different collection methods. Light traps were operated in orchards 2., 3., and 4. In the orchard type 1. no light trap could be used owing to the lack of electricity. Irrespectively to the weather conditions, our light traps were in continuous operation during the whole vegetation period (from April 1 to October 31). By way of trial, the light traps were operated also during the winter season 1977–1978. As a consequence of some organizational difficulties, the functioning of the light trap in orchard 3. was not continuous in 1978.

Caterpillars were collected from the canopy of the apple trees and from the undergrowth of experimental orchards, in regular intervals, during the whole vegetation period. The caterpillars were reared to adults in the laboratory. The larvae of Lymantria dispar were not preserved.

All adults of Macrolepidoptera caught by light traps were determined and classified as Geometridae, Noctuidae, and Bombyces + Sphinges. Into the latter taxon, there were classed not only the families placed here according to the modern taxonomy, but in agreement with the traditional usage, also the families Cossidae and Hepialidae.

In the questions of nomenclature and systematics we followed HERBULOT (1961 – 1963) in the Geometridae, HARTIG and HEINICKE (1975) for the Noctuidae, and FORSTER and WOHLFAHRT (1960) for the Bombyces and Sphinges.

Results and Discussion

The lists of *Macrolepidoptera* captured by light traps are presented in Tables 1, 2 and 3. The larval life forms (see categorization in Table 5) are given beside the scientific names.

A summary of results, with respect to the 3-year catches of light traps which were operated in 3 various types of apple orchards is given in Table 4. One can easily notice that the light trap used in the backyard garden (orchard type 2)

Table 1

List of Geometridae caught in 1977 to 1979 years

Species	Life-form groups	2. Backyard garden				3. Traditionally treated large-scale orchard				4. Intensively treated large-scale orchard			
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
Operophtera brumata	1.1.							+	+				+
Pelurga comitata	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Perizoma flavofasciata	2.1.	+	+	+	+								
— *alchemillata*	2.1.			+	+						+		+
Orthonama vittata	2.1.			+	+								
— *obstipata*	2.1.										+		+
Xanthorhoe spadicearia	2.1.		+	+	+					+		+	+
— *ferrugata*	2.1.	+	+	+	+			+				+	+
— *fluctuata*	2.1.	+	+	+	+								
Catarhoe rubidata	2.1.			+	+								
Epirhoe alternata	2.1.	+	+	+	+		+		+		+		+
— *rivata*	2.1.									+	+		+
Costaconv. polygrammata	2.1.	+	+		+					+			+
Mesotype virgata	2.1.		+		+								
Lithostege farinata	2.1.	+	+	+	+	+	+	+	+	+	+		+
— *griseata*	2.1.	+	+	+	+	+	+	+	+	+	+		+
Eupithecia centaureata	2.1.	+	+	+	+			+	+	+	+	+	+
— *absinthiata*	2.1.	+	+	+	+						+		+
— *linariata*	2.1.		+		+			+	+		+		+
— *innotata*	2.1.(?)		+		+			+	+				
— *millefoliata*	2.1.		+		+								
— *ochridata*	2.1.(?)	+			+								
Calliclys. rectangulata	1.2.		+		+								
Chloroclys. v-ata	2.1.(?)		+		+								
Idaea muricata	2.1.	+	+	+	+			+	+	+		+	+
— *slyvestraria*	2.1.	+	+	+	+								
— *fuscovenosa*	5.		+	+	+								
— *dimidiata*	5.	+	+	+	+			+	+	+	+	+	+
— *aversata*	5.(?)	+	+	+	+		+	+	+	+	+		+
— *nitidata*	2.1.(?)	+	+		+	+	+	+	+				
— *serpentata*	2.1.		+		+								
— *rusticata*	5.		+		+								
— *seriata*	5.	+			+								
— *inornata*	5.	+			+								
Calothysanis amata	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Scopula immorata	2.1.	+	+	+	+			+	+		+		+
— *corrivalaria*	2.1.		+	+	+		+		+			+	+
— *virgulata*	2.1.	+	+	+	+								
— *rubiginata*	2.1.	+	+	+	+			+	+	+	+	+	+
— *marginepunctata*	2.1.			+	+								
— *immutata*	2.1.	+	+	+	+		+	+	+	+	+	+	+
— *flaccidaria*	2.1.	+	+	+	+			+	+				

Table 1 (continued)

Species	Life-form groups	2. Backyard garden				3. Traditionally treated				4. Intensively treated			
						large-scale orchard							
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
— *subpunctaria*	2.1.			+	+			+	+	+		+	+
— *nigropunctata*	2.1.		+		+								
Cosymbia punctaria	1.2.		+		+			+	+				
— *linearia*	1.2.		+	+	+								
— *annulata*	1.2. (?)		+		+								
— *quercimontaria*	1.2.		+		+								
— *ruficiliaria*	1.2.	+			+								
Lomaspilis marginata	1.1.	+	+	+	+		+	+	+			+	+
Ligdia adustata	1.1.	+		+	+								
Stegania dilectaria	1.3.			+	+			+	+				
Semiothisa alternaria	1.1.	+	+	+	+		+	+	+	+	+	+	+
— *notata*	1.1.		+		+								
— *clathrata*	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Narraga tessularia	2.1.		+	+	+		+	+	+				
Tephrina arenacearia	2.1.	+	+	+	+	+		+	+	+	+		+
— *murinaria*	2.1.	+			+								
Epione repandaria	1.3.		+		+								
Therapis flavicaria	2.1.	+	+	+	+								
Ennomos autumnaria	1.1.		+		+								
Selenia lunularia	1.1.		+		+								
Angerona prunaria	1.1.	+	+	+	+			+	+		+		+
Lycia hirtaria	1.1.	+		+	+								
Erannis defoliaria	1.1.							+	+				
Biston betularia	1.1.	+			+								
Peribatodes rhomboidaria	1.1.	+	+		+			+	+	+	+		+
B. punctinalis-danieli	1.1.	+	+	+	+		+	+	+	+	+	+	+
Ascotis selenaria	1.1.	+	+	+	+		+	+	+	+	+		+
Ectropis bistortata	1.1.(2.1.)	+	+	+	+	+	+	+	+	+	+	+	+
— *extersaria*	1.1.		+	+	+			+	+	+		+	+
Ematurga atomaria	2.1.	+	+	+	+			+	+	+		+	+
Cabera exanthemata	1.1.(?)			+	+								
Lomographa bimaculata	1.1.	+	+		+								
Aplasta ononaria	2.1.		+		+								
Thetidia smaragdaria	2.1.	+			+					+	+	+	+
Thalera fimbrialis	2.1.	+	+	+	+								
Chlorissa cloraria	2.1.(1.3.)			+	+								
— *viridata*	2.1.(1.3.)	+	+		+								

collected prominently high numbers of species and specimens of *Macrolepidoptera* (208 species and 5827 specimens in the 3-year period). The light trap placed into the large-scale orchards (types 3 and 4) yielded in the same period 985 specimens of 117 species and 945 specimens of 123 species, respectively.

Table 2

List of Noctuidae caught in 1977 to 1979 years

Species	Life-form groups	2. Backyard garden				3. Traditionally treated large-scale orchard				4. Intensively treated			
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
Euxoa tritici eruta	2.2.					+	+	+	+				
Agrotis cinerea	2.1.											+	+
— *vestigialis*	2.1.						+	+	+	+	+	+	+
— *segetum*	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
— *exclamationis*	2.1.	+	+	+	+	+		+	+		+		+
— *ipsilon*	2.1.		+	+	+								
— *crassa*	2.1.	+			+								
Ochropleura praecox	2.1.					+			+				
— *plecta*	2.1.		+	+	+					+		+	+
Axylia putris	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Noctua pronuba	2.1.		+		+			+	+	+			+
Spaelotis ravida	2.1.(5.)						+		+				
Diarsia rubi	2.1.	+	+	+	+					+	+	+	+
Xestia c-nigrum	2.1.	+	+		+	+	+	+	+	+	+	+	+
— *xanthographa*	2.1.	+	+		+						+	+	+
Discestra trifolii	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Hada nana	2.1.	+	+	+	+								
Heliophobus reticulata	2.1.	+	+	+	+								
Mamestra brassicae	2.1.(1.1.)	+	+	+	+		+	+	+	+	+	+	+
— *thalassina*	2.1.(1.1.)		+	+	+							+	+
— *suasa*	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
— *oleracea*	2.1.	+	+	+	+		+		+	+	+	+	+
— *pisi*	2.1.(1.3.)	+			+								
— *bicolorata*	2.1.							+	+				
Hadena rivularis	2.1.		+		+						+		+
— *perplexa (lepida)*	2.1.	+			+								
— *luteago*	2.1.		+		+	+			+				
— *bicruris*	2.1.		+		+								
Cerapteryx graminis	2.2.	+			+			+	+				
Tholera cespitis	2.2.	+	+	+	+		+	+	+				
— *decimalis*	2.1. (2.2., 1.1.)	+	+	+	+	+		+	+	+		+	+
Orthosia incerta	1.1.	+	+	+	+					+	+		+
— *gothica*	1.1.(2.1.)	+	+	+	+						+		+
Hyssia c. gozmányi	2.1.	+	+	+	+		+	+	+			+	+
Mythimna turca	2.2.	+	+		+	+			+	+	+		+
— *albipuncta*	2.2.	+	+	+	+	+		+	+	+	+	+	+
— *pudorina*	3.2.	+	+	+	+					+			+

Table 2 (continued)

Species	Life-form groups	2. Backyard garden				3. Traditionally treated				4. Intensively treated			
						large-scale orchard							
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
— *pqllens*	2.2.	+	+	+	+	+		+	+	+	+	+	+
— *l-album*	2.2.	+	+		+	+			+	+	+		+
Leucania obsoleta	3.2.	+			+								
Senta flammea	3.2.		+	+	+								
Cuculia fraudatrix	2.1.	+	+	+									
— *artemisiae*	2.1.											+	+
— *umbratica*	2.1.	+	+	+	+			+	+				
— *scopariae*	2.1.	+			+								
Episema glaucina	2.1.											+	+
Xylena vetusta	2.1.			+	+						+		+
Eupsilia transversa	1.1.		+	+	+						+		+
Agroch. circellaris	1.1.(?)							+	+				
— *helvola*	1.1.(?)							+	+				
— *litura*	1.1.(?)											+	+
— *lychnidis*	1.1.(?)	+			+			+	+				
Parastichtis suspecta	2.1. (1.3.)	+			+	+			+				
Cirrhia gilvago	1.3.(2.1.)	+			+								
— *ocellaris*	1.3.(2.1.)	+			+								
Simyra albovenosa	2.2.	+	+	+	+	+		+	+	+			+
Subacr. megacephala	1.3.					+		+	+				
Apatele tridens	1.1.(1.3.)		+	+	+								
— *psi*	1.1.(1.3.)			+	+								
Pharetra rumicis	2.1.			+	+					+		+	+
Cryphia fraudatricula	4.	+	+	+	+								
Amphipyra livida	2.1.(1.1.)											+	+
— *tragopogonis*	2.1.					+			+			+	+
Dypteryg. scabriuscula	2.1.					+			+			+	+
Rusina ferruginea	2.1.		+		+						+		+
Thalpophila matura	3.1.										+		+
Trachea atriplicis	2.1.			+	+						+		+
Euplexia lucipara	2.1.		+	+	+		+	+	+			+	+
Phlogophora meticulosa	2.1.		+		+							+	+
Eucarta virgo	2.1.	+	+	+	+			+	+			+	+
— *amethystina*	2.1.			+	+								
Ipimorpha subtusa	1.3.			+	+								
Calymnia trapesina	1.1.			+	+			+	+				
Apamea monoglypha	3.1.		+		+								
— *sordens (basilinea)*	3.1.	+	+	+	+					+	+		+
Oligia strigilis	3.1.	+	+	+	+								
— *latruncula*	3.1.		+	+	+					+			+
— *furuncula*	3.1.		+	+	+								

Table 2 (continued)

Species	Life-form groups	2. Backyard garden				3. Traditionally treated large-scale orchard				4. Intensively treated large-scale orchard			
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
Mesapamea secalis	3.1.			+	+								
Photedes extrema	3.1.										+		+
— *fluxa*	3.1.		+		+								
— *pygmina*	3.1.		+		+							+	+
Luperina testacea	3.1.	+	+	+	+	+	+	+	+	+	+	+	+
Hydraecia micacea	3.1.		+		+								
Gortyna flavago	2.1.	+			+								
Calamia tridens	3.1.	+			+								
Archanara algae	3.2.	+		+	+								
— *geminipuncta*	3.2.	+	+	+	+			+	+				
— *sparganii*	3.2.	+		+	+					+			+
Rhizedra lutosa	3.2.	+			+								
Sedina buettneri	3.2.							+	+				
Hoplodrina alsines	2.1.		+	+	+			+	+			+	+
— *blanda*	2.1.			+	+								
— *ambigua*	2.1.	+	+	+	+		+		+	+			+
Caradrina morpheus	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Chilodes maritima	3.2.			+	+					+			+
Athetis gluteosa	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
— *pallustris*	2.1.	+	+		+						+	+	+
— *furvula*	2.1.	+	+		+	+			+	+	+		+
— *lepigone*	2.1.	+	+	+	+	+	+		+	+	+	+	+
Hapalotis venustula	2.1.	+			+								
Pyrrhia umbra	2.1.	+		+	+								
Heliothis maritima	2.1.							+	+				
Jaspidia pygarga	2.1.	+		+	+		+		+				
Eustrotia uncula	2.2.							+	+		+	+	+
— *bankiana (olivana)*	2.2.	+	+	+	+			+	+		+		+
— *candidula*	2.1.	+	+	+	+		+		+	+			+
Emmelia trabealis	2.1.	+	+	+	+	+	+	+	+			+	+
Acontia luctuosa	2.1.	+	+	+	+		+	+	+				
Nycteola asiatica	1.3.			+	+								
Earias chlorana	1.3.	+	+		+	+			+			+	+
Bena prasinana	1.1.										+		+
Colocasia coryli	1.1.			+	+								
Abrostola triplasia	2.1.						+		+				
— *trigemina*	2.1.		+		+								
Diachrysia chrysitis	2.1.	+	+	+	+			+	+		+		+
Macdunnoughia confusa	2.1.	+		+	+	+	+	+	+				
Autographa gamma	2.1.	+	+	+	+	+		+	+	+	+		+
Scoliopteryx libatrix	1.3.		+	+	+								

Table 2 (continued)

Species	Life-form groups	2. Backyard garden				3. Traditionally treated large-scale orchard				4. Intensively treated large-scale orchard			
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
Parascotia fuliginaria	4.	+			+					+			+
Rivula sericealis	2.1.	+	+	+	+		+	+	+	+	+	+	+
Macrochilo tentacularia	2.1.	+	+	+	+								
Zanclognatha lunalis	5.		+	+	+			+	+				
— *grisealis*	5.	+			+								
Hypena proboscidalis	2.1.		+	+	+					+			+
Schrankia costaestigalis	2.1.	+			+								
Paracolax glaucinalis	5.	+		+	+			+	+				

The backyard garden is small, therefore a light trap placed anywhere in it, collects practically the fauna of the surrounding too. Thus, these data characterize mostly the *Macrolepidoptera* occurring in the neighbourhood of the garden. In the two large-scale orchards the light traps were located centrally. Their lights were not visible from the edges of the orchards. The traps caught also the adults which flew, due to their dispersion, even into an orchard where the continuous presence of toxic chemicals prevents the respective species to live there.

The differences between the light trap catches of individual orchards are the most significant in the case of Geometridae owing to their weak flight ability: while the light trap of the backyard garden collected 2143 specimens of 75 geometric species, in the large-scale orchards 492 specimens of 35 species and 370 specimens of 35 species, respectively, were captured. On the contrary, the differences between the numbers of species collected in various orchard types proved rather small if "Bombyces" with excellent flight ability were taken into account: in the backyard garden 28 species (629 specimens), in the large-scale orchards 23 species (92 specimens) and 23 species (167 specimens), respectively, were detected by the light traps.

The proportion of species caught in the 3-year period per season is also characteristic. Regarding the main taxa or Macrolepidoptera as a whole, the highest ratios were found in the backyard garden (type 2.). In the three taxonomic groups, 36.2 to 42.8% of the species and 38.5% of the total number of species were collected every year. Concerning the traditionally treated large-scale orchard (type 3.) the values for the main taxa ranged between 18.6 and 30.4%, and it amounted to 21.4% in case of all species. The corresponding percentages for the

Table 3

List of Bombyces caught in 1977 to 1979 years

Species	Life-form groups	2. Backyard garden				3. Traditionally treated				4. Intensively treated			
						large-scale orchard							
		77.	78.	79.	77-79.	77.	78.	79.	77-79.	77.	78.	79.	77-79.
Nola cuculatella	1.1.		+		+						+		+
Roeselia albula	2.1.	+	+	+	+								
Celama centonalis	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Comacla senex	4.			+	+			+	+			+	+
Cybosia mesomella	4.									+			+
Eilema complana	4.							+	+	+			+
Pelosia muscerda	4.		+		+			+	+	+	+	+	+
Phragmatobia fuliginosa	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Spilarctia lutea	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
Spilosoma menthastri	2.1.	+	+	+	+	+	+	+	+	+	+	+	+
— urticae	2.1.	+	+	+	+								
Hyphantria cunea	1.1.(2.1.)	+	+	+	+	+	+	+	+	+	+		+
Arctinia caesarea	2.1.	+	+	+	+	+			+	+	+		+
Diacrisia sannio	2.1.		+	+	+								
Arctia caja	2.1.	+		+	+			+	+	+			+
Dysauxes ancilla	2.1.(4.?)	+	+	+	+	+	+	+	+	+	+	+	+
Gluphisia crenata	1.3.			+	+								
Pheosia tremula	1.3.					+			+				
Notodonta ziczac	1.3.	+			+								
Pterostoma palpinum	1.3.	+	+	+	+	+		+	+				
Clostera pigra	1.3.(1.1.)						+		+				
— anastomosis	1.3.							+	+				
— curtula	1.3.	+	+		+	+		+	+				
Orgyia antiqua	1.1.									+		+	+
Lymantria dispar	1.1.	+	+	+	+					+		+	+
Euproctis chrysorrhoea	1.1.	+			+							+	+
Habrosyne pyritoides	1.2.		+	+	+		+		+		+		+
Thyatira batis	1.2.		+		+								
Tethea or	1.3.			+	+			+	+				
— ocularis	1.3.					+			+				
Cilix glaucatus	1.1.	+			+					+			+
Malacosoma neustrium	1.1.(2.1.)							+	+			+	+
Lasiocampa quercus	1.1.(1.3.)									+			+
Smerintus ocellata	1.3.	+			+								
Amorpha populi	1.3.	+			+	+			+				
Phragmataecia castaneae	3.2.	+	+	+	+	+	+	+	+	+	+	+	+
Dyspessa ulula	3.3.									+			+
Triodia sylvina	3.1.	+	+		+			+	+		+		+

Table 4

Number of species and specimens caught by light traps

Years	Species and specimens	2. Backyard garden			
		Geom.	Noc.	Bomb.	*Macrolep.* (sum)
1977	species	44	70	20	134
	specimens	821	1211	119	2151
1978	species	58	69	19	146
	specimens	718	1042	313	2073
1979	species	46	69	18	133
	specimens	604	802	197	1603
1977 to 1979	species	75	105	28	208
	specimens	2143	3055	629	5827
In all 3 years	species	30	38	12	80
	%	40.0	36.2	42.8	38.5

intensively treated large-scale orchard (type 4.) were 24.6 to 31.4%, and 27.6%, respectively. The latter data reflect that it is not a definite portion of the fauna living in the surrounding which regularly flies into such an orchard, the immigration of species happens randomly. These orchards do not possess a *Macrolepidoptera* fauna of their own.

For this reason, the fauna of the small backyard garden proved much more constant, and the lepidopterous species of the neighbouring fields – in consequence also of the higher number of flying individuals – will appear in the light trap with an increased probability. In the large-scale orchards the numbers of specimens caught by light traps were found to be significantly lower. Owing to the bigger extension of these orchards, the *Macrolepidoptera* of the surroundings have fewer chances for regular immigrations. Therefore, the species spectrum of a large-scale orchard exhibits higher fluctuations in the consecutive years.

In Table 5 categorization of species according to the habitats of caterpillars is presented. As is to be seen, only 17 to 25% of the species live on woody plants and only a small portion of them, the polyphagous species and an oligophagous one *(Chlorocystis rectangulata)*, is to be considered as potential pests of apple. Larvae infesting *Salix* and *Populus* plants can not feed on Rosaceae species. These *Lepidoptera* having no foodplant in an apple orchard, are dealt with separately.

The species living in the harbecaous undergrowth occurred in the highest ratios (58 to 66% of the total number of species). The immigration of some faunal elements from the surrounding, mostly steppe ("puszta") regions deserves more attention, since the fauna of the undergrowth in apple orchards proved

and summarized regarding taxonomic groups and orchard types

3. Traditionally treated				4. Intensively treated			
large-scale orchard							
Geom.	Noc.	Bomb.	*Macrolep.* (sum)	Geom.	Noc.	Bomb.	*Macrolep.* (sum)
8	28	13	49	25	35	17	77
118	77	22	217	142	184	66	392
16	25	9	50	25	37	12	74
39	43	15	97	101	129	65	295
33	43	17	93	19	38	12	69
335	281	55	671	127	95	36	258
35	59	23	117	35	65	23	123
492	401	92	985	370	408	167	945
7	11	7	25	11	16	7	34
20.0	18.6	30.4	21.4	31.4	24.6	30.4	27.6

rather poor and strongy disturbed. During the 3-year period – in spite of thorough investigations – we could not find any caterpillars on the herbaceous plants.

Concerning the endophagous species, Lepidoptera feeding in woody plants – among them also some apple pests *(Cossus cossus, Zeuzera pyrina, Synanthedon myopaeformis)* – were not found at all. The adults of these insects are not caught by light traps, or only exceptionally.

The low numbers of species feeding on lichens and mosses conformed with our expectations, as in chemically treated apple orchards the lichen and moss flora disappears and the herbaceous vegetation of neighbouring fields hardly provide habitats for these Lepidoptera. The insignificant ratios of saprophagous species can be explained in a similar way.

During the 3-year period of our investigations the caterpillars of the following species could be collected (and reared until the adult stage) on apple trees:

1. Abandoned orchard (8 species): Geometridae: *Lycia hirtaria* (1977, 1979), *Boarmia punctinalis* (1977, 1978), *Ectropis bistortata* (1979); Noctuidae: *Calymnia trapezina* (1979), *Colocasia coryli* (1977, 1978); Bombyces: *Orgyia antiqua* (1977, 1979), *Lymantria dispar* (1977, 1979).

2. Backyard garden (1 species): Noctuidae: *Orthosia incerta* (1979).

3. Traditionally treated large-scale orchard (4 species): Geometridae: *Lycia hirtaria* (1979); Noctuidae: *Calymnia trapezina* (1979); Bombyces: *Orgyia antiqua* (1979), *Lymantria dispar* (1979).

4. Intensively treated large-scale orchard: no species.

Table 5

Numbers and percent ratios of species caught by light traps in 1977 to 1979 years, summarized regarding the life-forms of larvae

		2. Backyard garden		3. Traditionally treated large-scale orchard		4. Intensively treated large-scale orchard	
		numbers	per cent ratios	numbers	per cent ratios	numbers	per cent ratios
		of species		of species		of species	
1. Species of canopy and bush levels	1.1. Polyphagous spp.	29	13.94	16	13.68	21	17.07
	1.2. Oligophagous spp.	8	3.85	2	1.71	1	0.81
	1.3. Species feeding on *Populus* and *Salix*	15	7.21	11	9.40	1	0.81
2. Species of herbaceous undergrowth	2.1. Oligo- and polyphagous species feeding mostly on dicotyledonous plants	114	54.81	66	56.41	74	60.16
	2.2. Polyphagous species feeding on graminaceous plants	8	3.85	10	8.55	7	5.69
3. Endophagous species	3.1. Species feeding in grass stems and roots	12	5.77	2	1.71	7	5.69
	3.2. Species feeding in cane stems and in other marshland *Gramineae*	9	4.33	3	2.56	4	3.25
	3.3. Species feeding in bulbs of *Liliaceae*	0	0	0	0	1	0.81
	3.4. Species feeding in tree stems	0	0	0	0	0	0
4. Species feeding on lichens and mosses		4	1.92	3	2.56	5	4.07
5. Saprophagous species		9	4.33	4	3.42	2	1.63
Sum		208	100.00	117	100.00	123	100.00

Conclusions

The chemically treated orchards have no *Macrolepidoptera* fauna of their own. The regular treatments with insecticides raise difficulties for the development of caterpillars both on apple trees and in the herbaceous undergrowth of an apple orchard.

In apple orchards, only a small portion of *Macrolepidoptera* detected by light traps can be found in the larval stage. The specific spectrum is determined mostly by the fauna of the surrounding fields and the ability dispersion of a given species.

The composition of the *Macrolepidoptera* fauna in large-scale orchards with considerable surface does not differ essentially from that of backyard gardens which are much smaller. However, in large-scale orchards the numbers of species and specimens are highly reduced.

The species spectrum in a small backyard garden proved to be more constant because of the much higher probability of immigration from the surroundings. On the contrary, in large-scale orchards the spectrum of *Macrolepidoptera* showed significant fluctuations.

Concerning the endophagous species, the presence of potential apple pests could not be demonstrated by means of light trap.

Species feeding on lichens and mosses, similarly to saprophagous Lepidoptera, occurred in negligible numbers.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 389—398 (1981)

Phototaxis of the Adult Whitefly, *Bemisia tabaci* Gennadius to the Visible Light

II. Effects of Both Light Intensity and Sex of the Whitefly Adults on the Insect's Response to Different Wavelengths of Light Spectrum

By

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The present investigation proved that there was a significant regression relationship between the percentages of the cumulative response of *B. tabaci* adults and the light intensity irrespect of both the sex ratio and the sex of the whitefly adults. The most significantly preferred colour or wavelength of the visible light for the females was the yellow followed in a descending order by the green, white, blue and red respectively. The males were found responsible for the irregularities recorded in the pooled simple responses of female and male mixed groups to the different tested light colours at different tested light intensities. Also, the males were responsible for shifting the behaviour of mixed populations of both sexes far from the aforementioned colours' preferandom trend which has been noticed by the authors either in their earlier investigation (1976) on the effect of exposure period on mixed populations of both sexes or in the course of the present study on the effect of light intensity on the response of the female whitefly adults. When each segregated sex was tested, the median attracting light intensities (AI_{50}s) of both female and male whitefly adults, recorded at 20 min exposure period, were 11.0 and 12.5 fc, in respect.

AUCLAIR (1967) reported that in multiple choice tests, aphids preferentially settled on and colonized diets receiving no or only weak light of 5—50 fc, while they generally avoided diets exposed to more intense light. On the other hand, HALGREN (1970) stated that *Schizaphis graminum* aphidids stopped flying when the light intensity was decreased to less than 18 fc. But this hazard of the responses of different species of insects to light could be understood in the light of the study published by STEWART *et al.* (1969) in which they proved that both the insect species and the distance between the light source and the exposed insects (which represents the light intensity, the authors) are effective on their response. Also, the effect of sex on the insect response to different light intensities and wavelengths or colours was reported by several authors in several species of insects; i.e. TREHAN (1941) on the whiteflies *Aleyrodes proletella* and *Trialeurodes vaporariorum;* OSSIANNILSSON (1966) on *Bemisia tabaci;* AMOS and WATERHOUSE (1967) on *C. dimidiatus;* ARBOGAST and FLAHERTY (1973) on *T. castaneum* and *T. confusum;* VAISHAMPAYAN, KOGAN, WALDBAUER and WOOLLEY (1975); and VAI-

SHAMPAYAN, WALDBAUER and KOGAN (1975) on the greenhouse whitefly *T. vaporariorum*.

The present investigation along with that published by the authors (1977) was achieved in the sake of better understanding of the whitefly *Bemisia tabaci* adults' phototaxis in the visible light spectrum with a hope of making use of such a behaviour in the insect's control in the field.

Materials and Methods

The same insect culture, whitefly phototaxis test kit, whitefly phototaxis cabinet, coloured cellophane sheets and statistical analysis tests established or used by the authors in their earlier investigation on the effect of exposure period on *B. tabaci* adults' response to different colours of light, were also used here. Only in case of the 1 : 1 sex ratio experiment which is carried out here, the numbers of the adult insects used in each replicate were 24 (12 males and 12 females) instead of being 25 as in all the other experiments. In the course of the present study, the effects of light intensity and insect's sex on *B. tabaci* adults' responses to different colours (= wavelengths) of the visible light spectrum were investigated. The adult whiteflies were exposed to the effects of the following light intensities: 1.8, 5.3, 8.9, 14.2, 21.3 and 35.6 fc.

Results and Discussion

In this work, responses of *B. tabaci* male and female adults, whether segregated by sex or in mixed groups, to different light intensities and light colours (wavelengths) were investigated. Therefore, four different sorts of experiments were conducted; in the first, such responses were recorded for mixed groups of the insect male and female adults of unknown sex ratios (as these adults were haphazardly collected from the stock culture and they were used in the experiments without determining their sex ratio which represents its value in nature at the time of the achievement of these experiments); in the second, mixed groups of 1 : 1 sex ratio were tested in order to give each sex the same chance to express its own effect on the pooled response; while in the third and fourth experiments, segregated female or male adults were studied, respectively, to know the behaviour of each sex alone when kept apart from any probable interacting effect of the other opposite sex.

Responses of mixed groups of male and female adults of B. tabaci collected haphazardly from the stock culture and exposed to the effects of different wavelengths and intensities of the visible light

It was thought logical to investigate first whether there is a regression relationship between light intensity and the percentages of the cumulative response

of the whitefly adults, as the percentages of the simple response of the insects followed nearly the normal distribution relationship. The nearst tested exposure period (20 min) to the statistically determined median attractive exposure period (AP_{50} = 17.5 min) which has been derived by the authors in the course of an earlier investigation (1977) was used in this experiment as well as in all the next ones. It was found that there was a positive significant regression relationship between the light intensity and the percentages of the cumulative response of the whitefly adults to the visible light and that this relation is controlled by the regression equation: $\hat{y} = 22.6 + 2.4\,X$. The data also showed that the slope's average value of the adjusted regression line was 2.4 ± 1.0. As a good estimate for the most preferable light intensity by the whitefly adults, the median attracting light intensity (AI_{50}) which attracted 50% of the tested populations which represents the mean, mode and median percentages of the simple responses of *B. tabaci* adults; was found to be 11.5 fc.

Figure 1(A) shows the percentages of the simple positive responses (attractions) of the whitefly adults to different colours (wavelengths) of the visible light spectrum obtained at each tested light intensity. These histograms showed no clear or steady trend for the insect's preferandom among the different tested colours of light at any of the tested light intensities except in cases of both the 8.9 and 35.6 fc light intensities, at which the yellow colour was the most attractive followed in a descending order by the green, white, blue and red colours, respectively.

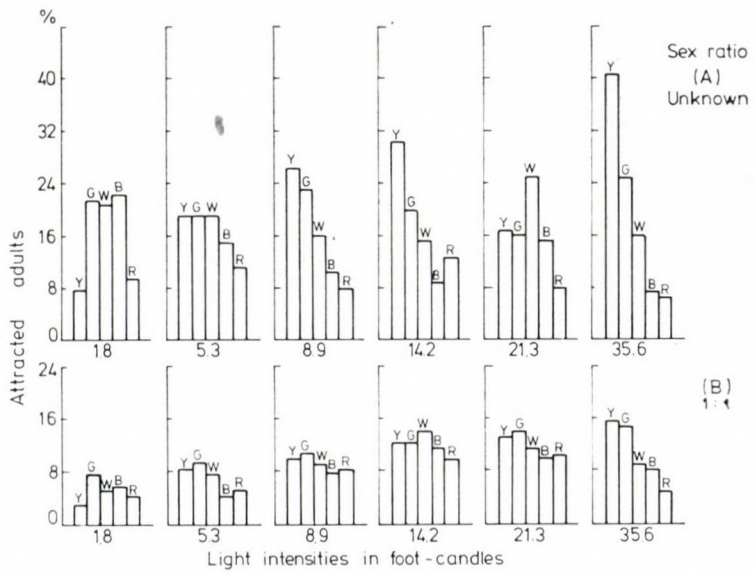

Fig. 1. Percentages of attraction of both the female and male adults of *B. tabaci* Genn. to different colours (wavelengths) of the visible light-intensities (sex-ratio; unknown and 1 : 1)

On the other hand, Fig. 2(A) shows the percentages of the simple positive response of the whitefly adults to different tested light intensities at each studied colour or wavelength of the visible light spectrum. However, the obtained histograms showed no clear pattern or constant trend in this concern. These irregular histograms (Figs 1A and 2A) along with the relatively wide range of confidence intervals of the calculated slope value of the previously mentioned regression line (1.4 – 3.4) may declare that the heterogeneous responses of *B. tabaci* adults to different light colours and light intensities might be due to; the interaction effect of the change of either of the two latter variables in each treatment, the probable interacting effect among the tested light colours, the probable variations in the sex ratio at each treatment and replicate, or to the pooled effect of either some or all of these factors together.

In the light of the latter discussion and taking into consideration the fact that the aforementioned arithmetically derived median attracting light intensity (AI_{50}; 11.5 fc) was not experimentally investigated, the mean values of the percentages of simple responses of the whitefly adults that were actually obtained to different tested colours of light at both the two light intensities of 8.9 and 14.2 fc (the mean of which is 11.6 fc) were used for the statistical comparisons among the obtained mean responses of the whitefly adults in all cases. The results proved that there were significant differences among all probable comparisons of these means except in case of the red-blue-comparison.

The 1 : 1 sex ratio experiment

The same steps carried out during the achievement of the previous experiment have been also followed here, but with female and male mixtures of the whitefly adults grouped together with 1 : 1 sex ratio. This sex ratio was chosen to give each sex the same chance to express its effect on the total response of both sexes together. The statistical analysis results proved that there was a highly significant regression relationship, in contrast to only the significance level observed in case of the aforementioned unknown sex ratio experiment, between the different tested light intensities and the cumulative response percentages of the whitefly adults. The AI_{50} value was found to be 14.2 fc, the regression equation was $\hat{y} = 10.9 + 2.8\,X$ and the average slope value of the regression line was 2.8 ± 0.8. This declared that equal representation of each sex of the whitefly adults in all treatments and replicates increased the significance level of the regression relationship between the total cumulative response of the insects and the visible light intensity at 20 min exposure period.

It was observed that the percentages of response of the whitefly adults to different light intensities followed the normal distribution relationship in case of the red, blue and white colours of light, but it followed nearly a regression relationship in cases of both the yellow and green colours, as the percentages response of the whitefly adults obtained for these two colours increased by the increase of light intensity (Fig. 1B). It was also observed that the percentages of

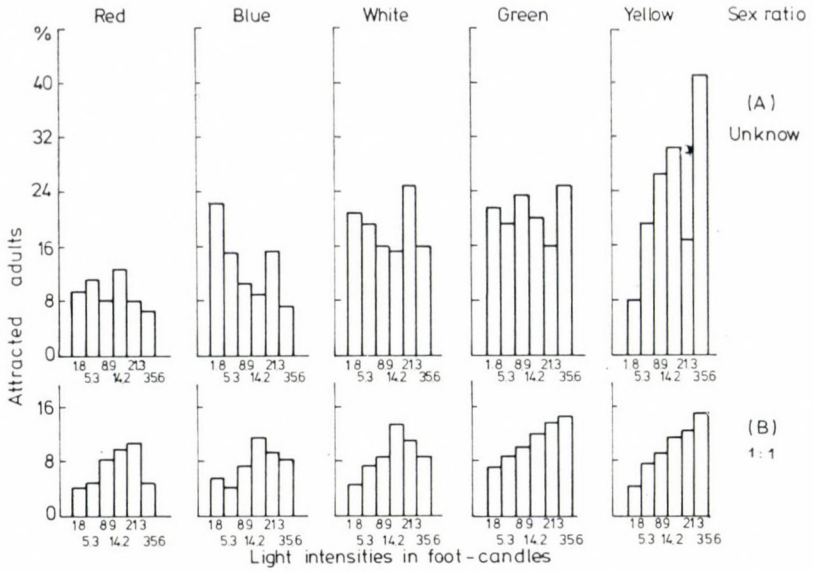

Fig. 2. Percentages of attraction of both the female and male adults of *B. tabaci* Genn to different light intensities at each tested colour (wavelength) of the visible light (sex ratio; unknown and 1 : 1)

the whitefly adults to different light intensities followed the normal distribution relationship in cases of the red, blue, and white colours of light, but it followed a regression relationship in cases of both the yellow and green light (Fig. 2B). This may emphasize that equal representation of each sex of the whitefly adults in the tested populations minimized, to somewhat extent, the heterogeneity of the percentages of the whitefly adults' responses to different light intensities and different colours or wavelengths of light which were also noticed before in the unknown sex ratio experiment. It was also clear that the 14.2 fc light intensity, at which the maximum responses were obtained, was nearly identical with that derived from the regression analysis results. This seems logical as the white light comprises all the other tested colours and in return it represents their pooled effect. Therefore, this 14.2 fc light intensity was taken as the most suitable light intensity at which the actual percentages of the whitefly adults' responses obtained in the present experiment could be statistically compared. It was proved that there were no significant differences among all the compared means of responses at different tested colours of light. This could be better understood by reviewing the aforementioned discussion of the results present on Figures 1(A) and (B) which emphasized that irrespect of whether the whitefly female adults had the same chance of effect as their partner males or not in their mixed groups, they preferred the yellow colour of light more than the green, white, blue and red colours, respectively. This was only true when they were exposed to the highest tested light

intensity (35.6 fc; Figs 1A and B) which is too far from the light intensity used here (14.2 fc).

As all the above-mentioned trials and experiments did not clearly explain the heterogeneous simple responses of *B. tabaci* adults to different colours of light at different light intensities (Figs 1A and B), investigating the response of each segregated sex deemed necessary.

Responses of segregated female adults

It was proved that there was a significant regression relationship between the percentages of the cumulative responses of the whitefly female adults and the intensity of the visible light. The average slope value of the regression line which represents this relation was 2.4 ± 0.9, the regression equation was $\hat{y} = 23.6 + 2.4\,X$, and the AI_{50} value was 11.0 f.

The histograms shown on figure 3(A) declared that at all the tested light intensities, the female adults of *B. tabaci* preferred the yellow more than the green, white, blue and red colours of light, respectively. On the other hand, no clear trend was observed for the females' responses to different light intensities at each tested light colour or wavelength (Fig. 4A). Differences among the female adult responses were always significant except only in cases of the green-white, white-blue, and blue-red comparisons. This may declare that the females failed to discriminate significantly between either couple of the aforementioned light colours or ranges of wavelengths.

Responses of segregated male adults

The results proved the significant regression relationship between the male adult cumulative positive responses and the different tested light intensities. The regression equation which represents this relation is $\hat{y} = 18.3 + 2.6\,X$, the slope value of the drawn regression line averaged 2.6 ± 1.1 and the AI_{50} value was 12.5 fc which is more than that determined before for the female adults (11.0 fc).

The histograms depicted in figures 3(B) and 4(B) represent the percentages of the simple positive responses of the male whitefly adults. They showed no clear trend whether the responses to different light colours were compared with each other at each tested light intensity (Fig. 3B) or vice versa (Fig. 4B). Only in the former case, the males differed from the females which showed a very clear constant trend in their preferandom for the tested colours of the visible light spectrum, in which they preferred the yellow colour more than the green, white, blue and red colours, respectively (Fig. 3A).

It was proved that there were no significant differences among all probable comparisons of the mean responses of males to different tested light colours at 12.5 fc light intensity (males AI_{50} value) except in case of the white-green comparison. Taking into consideration the regular responses of the female whitefly adults to different colours or wavelengths of light, it could be concluded that

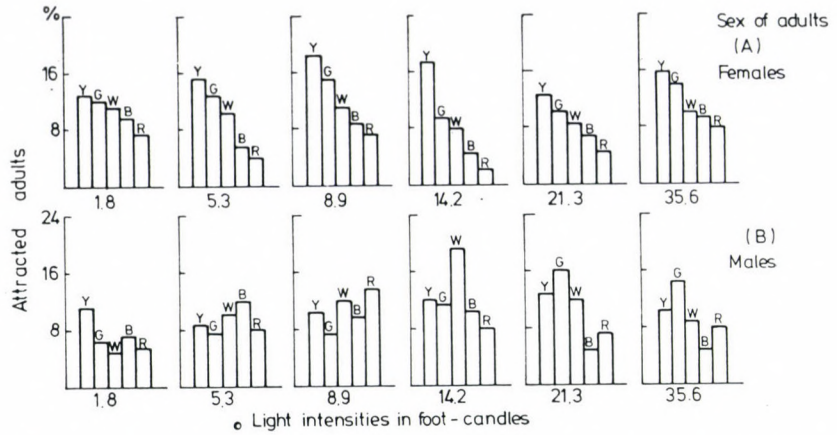

Fig. 3. Percentages of attraction of either the female or male adults of *B. tabaci* Genn. to different colours (wavelengths) of the visible light at each tested light intensity

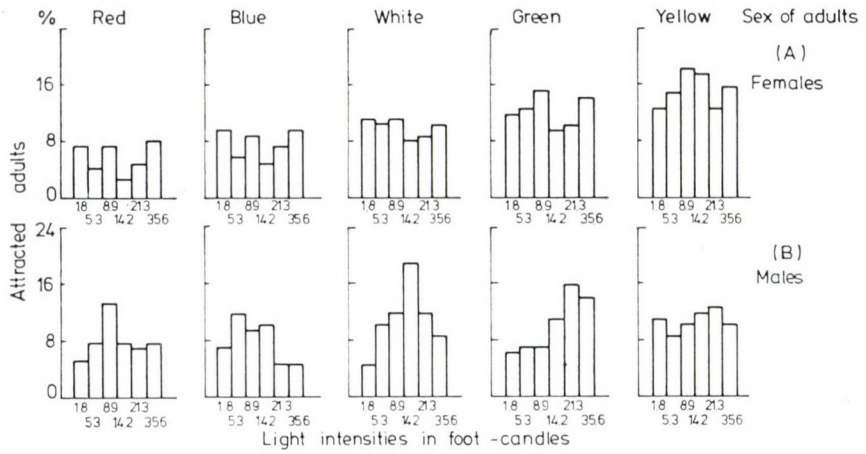

Fig. 4. Percentages of attraction of either the female or male adults of *B. tabaci* Genn. to different light intensities at each tested colour (wavelength) of the visible light

male adults of *B. tabaci* were responsible for the irregular responses which were recorded in the pooled simple responses of male and female mixtures to different tested colours of light at different tested light intensities. Also, the males were responsible for shifting the behaviour of mixed-sex populations far from the trend obtained either for them in the exposure period's experiment (the authors, 1976) or for the segregated females, response obtained in the present study (Figs 3A and 4A).

At the end of the present investigation it may be profitable to summarize all the obtained results and conclusions as follows: 1. Both the whitefly adult exposure period to the light source (the authors, 1976) and the light intensity at which the insects are exposed affected significantly their responses, 2. equal representation of each sex of *B. tabaci* adults in their mixed populations slightly reduced the heterogeneity of their responses, 3. male adults proved to be responsible for such heterogeneity, 4. the general trend for *B. tabaci* adults' preferandom to different colours or wavelengths of the visible light was as follows: the yellow colour was more preferable than the green, white, blue and red colours, in respect; and this trend could be primarily attributed to the females, 5. statistical analysis proved that females were more sensitive in their discrimination among both the different tested colours and intensities of the visible light in comparison with males; and this may indicate that the whitefly sexes are different in their visual capabilities in the visible spectrum of light, and 6. in addition to the probable heterogeneous effect of sex (the males, mentioned in the 3rd point), exposure period, light intensity, and the number of each sex in mixed populations on the responses of the whitefly mixed populations to the visible light; similar effect for the adult ages should not be neglected.

Sensitivity of male and female adults of *Bemisia tabaci* Genn, whether segregated by sex or in mixed groups, to weak tested light intensities (ranged from 1.8 to 35.6 fc) is in agreement with AUCLAIR's findings on aphids (1967). On the other hand, HALGREN (1970) contradicted these findings with *Schizaphis graminum*. Such effects of both insect species and light intensity were also recorded by STEWART *et al.* (1969). Sex effect on the whitefly adults' responses to different light intensities and light colours which is observed here was also noticed by several authors working on different species of insects; i.e. AMOS and WATERHOUSE (1967) on *C. dimidiatus* and ARBOGAST and FLAHERTY (1973) on *T. castaneum* and *T. confusum*.

The present findings proved that *B. tabaci* female adults' phototactic preferandom of light colours was as the same as that of the two allied species of whiteflies; *Aleyrodes proletella* and *Trialourodes vaporariorum* in which these two species preferred, on biological basis, the yellow light more than any of the white, red, green or blue colours of light (TREHAN, 1941). Also, OSSIANNILSSON (1966) reported that *B. tabaci* adults were attracted to yellow light and he suggested that this light colour induces the whitefly adults' return to the vegetation. On the other hand, the authors did not notice KRING's conclusion (1972) which stated that there was inhibition of flight by yellow reflected light for both whiteflies and aphids.

In their invaluable investigation was published (1975), VAISHAMPAYAN, KOGAN, WALDBAUER and WOOLLEY concluded that the greenhouse whitefly *Trialeurodes vaporariorum* adults showed a strongly positive response to the surfaces with maximum reflectance or transmittance in the "yellow-green" region of the spectrum (520–610 nm). The latter findings goes without saying with the present results of *B. tabaci* adults, which showed that their general trend of responses to different colours or wavelengths of light decreased gradually from

the yellow to the green, white, blue and red, respectively. Also, the range of both the yellow and green spectra tested here (520–760 mμ*; see materials and methods of the authors, 1980) were not so far from the aforementioned range of the same "yellow-green" region of the spectrum (520–610 nm*) which was obtained by VAISHAMPAYAN, KOGAN, WALDBAUER and WOOLLEY (1975). Also, the latter authors' statement that red light (610 to ca 700 nm*) might be moderately inhibitory for the greenhouse whitefly adults, is not so far from the present findings which showed that the tested red colour of light (660–760 mμ*) was the least preferable, in comparison with all the other tested colours of light, by *B. tabaci* adults. The same previous authors reported too, that the spectral composition of green leaves show a peak of reflectance in the 500 to 600 nm* region which coincides with the region of maximum positive stimulus (520–610 nm*) for the greenhouse whitefly adults. The authors also said that this supports the assumption that visual orientation is an important factor in the host finding behaviour of whiteflies. They also concluded that the strong attraction of *T. vaporariorum* adults towards the yellow-green region of the spectrum and their strong inhibitory response to blue, suggest that phototaxis could be employed in integrated control programs for the whitefly.

According to VAISHAMPAYAN, WALDBAUER and KOGAN (1975) findings, the first steps in host plant selection, orientation and landing, are mediated in the greenhouse whitefly, largely if not exclusively by a response to colour and not to odour, which may play only a minor role in this concern.

In the light of the present findings, it could be concluded that almost all the above-mentioned *T. vaporariorum* phototaxis discussions and conclusions could be adopted for *Bemisia tabaci* Gennadius whitefly adults.

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* The nanometer (nm) = the millimicron = 10^{-9} meter.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 399—404 (1981)

The Impact of Meteorological Factors onto the Light-attraction of Codling Moth

By

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The number of insects caught by a light trap depends not only on the size of the population living on a given area, but it can also be largely modified by dominant meteorological conditions during the time of trapping. Therefore, the number of insect caught can not be taken into consideration without correction. In this study we have examined the modifying effect of individual meteorological factors to the light attraction.

We have estimated the number of moths caught between 1967—69 in Kecskemét by the use of fractionated light trapping and analysed them by mathematical regression in correlation with air-temperature, relative humidity, and wind-velocity and also by empirical way in correlation with cloud covering, weather fronts and moonlight. The codling moth does not fly to the light trap under 11 °C but an air temperature over 25 °C inhibits it markedly. The number proportionally decreases with the rise of relative air humidity. The impeding effect of the velocity of wind begins at the value of 3 km per hour and the full impediment ensues at 14 km per hour. Following a 10—15 mm precipitation the flight begins after 3—4 hours, in the case of 10 mm, after 1—1.5 hours. With the decrease of cloud covering the number of caught increases. During clear nights when there is moonlight, no increased flight activity can be observed.

In our country the light trap is one of the most widely used methods for plant-protecting forecast. During the time of its establishment it caused great care that beyond the failures of technical origin in most of the cases the values, indicated by the light trap did not show any connection to the number of the free living population. Great damages occurred, however, light-trapping did not let to make any conclusions for them. Naturally it could have a lot of reasons that there were deviations from reality but it was sure that the permanently changing meteorological factors had a decisive part.

In our country Dr. Tibor JERMY called the attention to this important symptom.

In the interest of discovering the effect of the meteorological factors from 1967 to 1969 we have operated a collecting light trap in Kecskemét with an hourly separation. It collected the insects until 360 cms height from the ground. We have compared the datas of the hourly collecting with the values of the hourly deter-

mined meteorological factors. We have revealed the modifying effect of air temperature, relative air humidity and velocity of wind with the help of regression analysis while we dared to undertake a comparison between the other factors (moisture, clouding, weather front and moonlight) and the number of insects flying to light only by a simple empirical way.

We expound the effect of the examined meteorological factors separately.

The temperature of air

We have not observed the flight to light in the case of the codling moth under the temperature of 11 °C. By our function from 11 to 15 °C the flight to light is strongly impeded; it gradually increases between 16 and 25 °C and over that it decreases (Fig. 1).

Relative air humidity

We cannot unambiguously decide the effect of this factor because the magnetic effect decreases because of the increasing of light absorption of the more humid air as well as because of increasing of the activity, following the change of relative air humidity and these factors cannot be separated. In spite of all these facts our function shows that with the increase of relative air humidity the number of codling moth, flying to light proportionally decreases (Fig. 2).

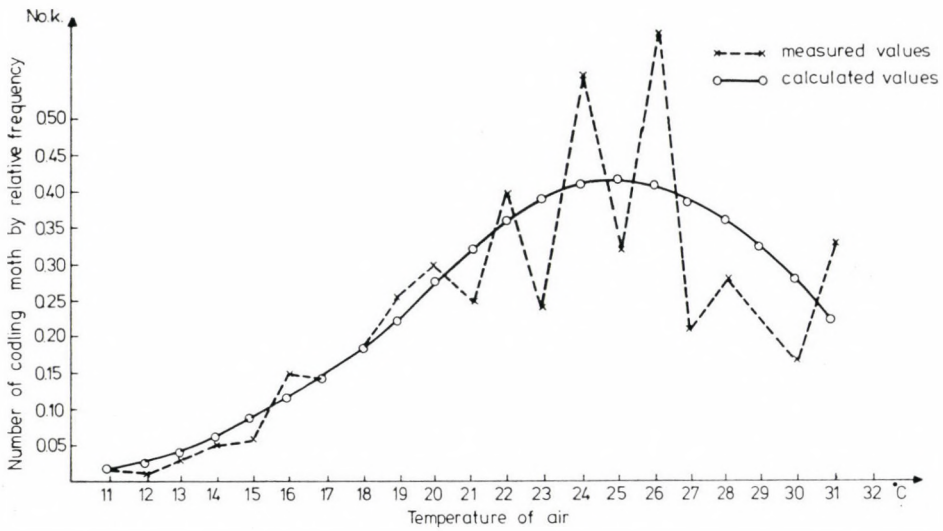

Fig. 1. The connection of the quantity of codling moth (*Laspeyresia pomonella* L.) flying to light and temperature of air

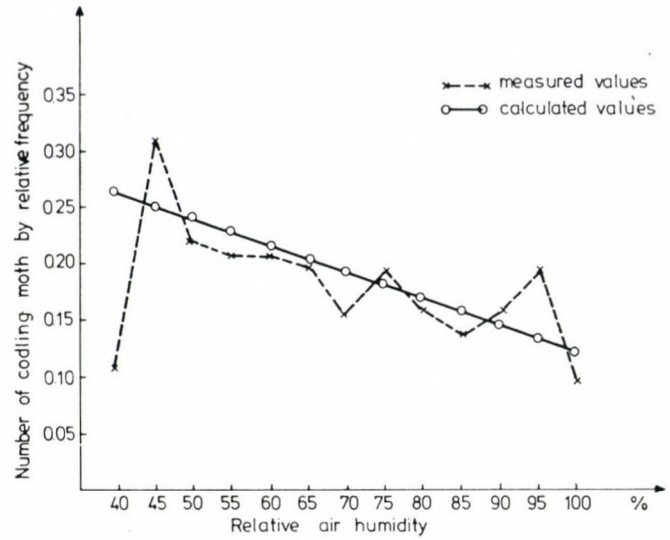

Fig. 2. The connection of the quantity of codling moth (*Laspeyresia pomonella* L.) flying to light and the relative air humidity

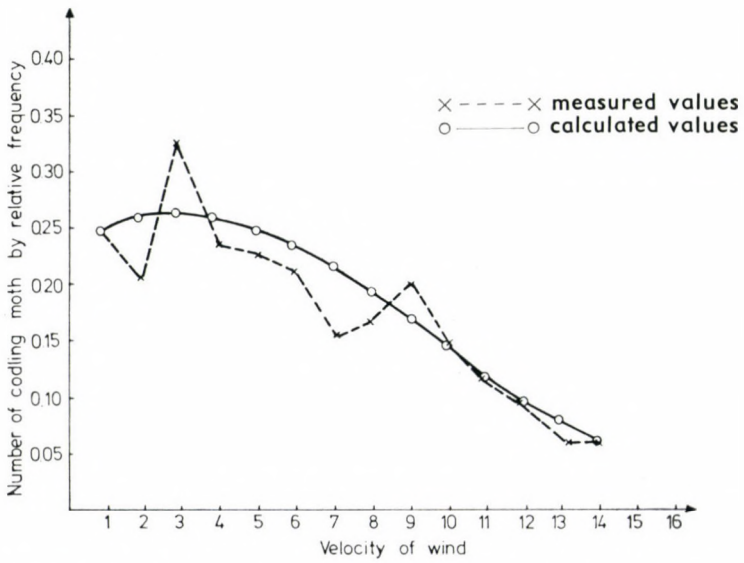

Fig. 3. The connection of the quantity of codling moth (*Laspeyresia pomonella* L.) flying to light and the velocity of wind

The velocity of wind

The impeding effect of the velocity of wind – comparing to other insects – begins at a strongely low value of 3 km/hour. The 14 km/hour value fully places the flight to light (Fig. 3).

We have also numerically decided the effect of these three meteorological factors (air temperature, relative air humidity, velocity of wind) that is shown in the Table 1.

Moisture

During the wet days – according to the time and amount of moisture the catch of the codling moth was very different. At a 10–15 mm daily moisture a very strong impeding effect could be observed. After this amount of moisture 3–4 hours had to pass that the flight to light could start. In the case of a tenth mms amount of moisture 1–1.5 hours are enough after the rain had stopped and the flight to light starts again. The impeding effect of a moisture fallen only in traces or in an amount that cannot be measured, is almost fully negligible.

Cloud covering

With the decrease of cloud covering the number of codling moth, flying to light increases. Most of the imagos were caught by the light trap at a sky without any clouds, that was 57% of the whole catch. We could observe similarly high values in the nights when there was moonlight. The more increased flight to light that could be observed on moonlighted nights without any clouds was perhaps a symptom that was connected with the orientation of the insects.

Weather fronts and mass of air

It is very hard to estimate how the weather fronts modifying effect of flight to light, therefore it seems a feasible arrangement to examine the effect of the single meteorological factors (Table 2).

During the experiments in three-quarters of the cases the absence of catch can always be explained by being over or under the threshold of the meteorological factors. Therefore, it is not advisable to examine the modifying effect of the weather fronts' limits to flight towards light in itself.

In our case our conclusion about the single weather fronts must be concretely taken into consideration as the limits of the single meteorological factors that are essentially modifying (Table 3). In the case of air temperature, relative air humidity and velocity of wind we could determine the optimum (saturated) zones and also the preceding and following zones in respect of the flight to light of the codling moth. In the preceding and following zones the less of saturation could be experienced.

Table 1

Temperature of air		Relative air humidity		Velocity of wind	
values	saturated	values	saturated	values	saturated
11	4.0	45	100.0	1	85.1
12	6.2	50	95.2	2	98.8
13	9.5	55	88.5	3	100.0
14	13.8	60	85.7	4	98.8
15	19.5	65	81.0	5	95.1
16	28.0	70	76.2	6	89.4
17	35.2	75	71.4	7	81.9
18	44.8	80	66.6	8	73.6
19	55.5	85	62.0	9	64.2
20	66.4	90	57.5	10	54.7
21	77.0	95	52.7	11	45.3
22	86.4	100	48.0	12	36.9
23	93.5			13	29.3
24	98.0			14	22.6
25	100.0				
26	98.0				
27	93.5				
28	86.4				
29	77.0				
30	66.4				
31	55.5				

Table 2

The kind of air mass	The number of imagos, flying to light/hour *L. pomonella*
Arctic continental	—
Arctic marine	—
Temperature continental	0.17
Temperature marine	0.31
Subtropical continental	0.42
Subtropical marine	0.42

Table 3

The kind of air mass	Imagos, flying to light	
	altogether	number/hour
Arctic continental	—	—
Arctic marine	—	—
Temperature continental	6	0.11
Temperature marine	27	0.17
Subtropical continental	10	0.33
Subtropical marine	3	0.30

During the light-trapping of the codling moth – if we take into consideration the data of the saturation table and the statements of the other meteorological factors – we could get more accurate facts about the real mass relations of the codling moth as earlier, too, when we had not still taken into consideration the facts at preparing the prediction.

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3–4), pp. 405–431 (1981)

Observations on the Biology and Diseases of *Lobesia botrana* Den. and Schiff. *(Lepidoptera, Tortricidae)* in Central-North Italy*

By

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Observations were carried out on *Lobesia botrana* in vineyards, in laboratory and in the laboratory garden in Bologna in a period of 5 years.

Although in the laboratory the diapause inducing short photoperiod was 13 hours long, the first diapausing pupae were observed already from the end of July (second generation), on the first fortnight of September (third generation) and by the end of October (partial fourth generation?).

Sensibility to short photoperiod was observed in the first 15 days after the oviposition. This period seems to consist of two parts: (1) In the first 8 days (a) the continuous development becomes irreversible if confirmed by long-day illumination; (b) or the supposed earlier "program" of continuous development becomes cancelled by short-day effect. (2) During the successive 7 days the short-day effect becomes irreversible and the diapause becomes induced.

The sensibility to the photoperiod is the highest on the 6–8th day after egg-laying. However, changes from long-day to short-day illumination during embryogenesis influenced the sensitive period. During further developmental stages the change of LD to SD increased the fecundity, whereas at the emergence of the adults it disturbed the mating behaviour.

The food quality of the larvae influenced the fecundity; mature berries increased it very much. During oviposition the temperature could affect the number of eggs laid; in our *L. botrana* population the fecundity decreased significantly already at 22 °C.

60% of the females mated once, 24% twice, 3% three times and in 13% there were no spermatophores at all.

The population dynamics of *L. botrana* in the vineyards were strongly influenced by pathogens: cytoplasmic polyhedrosis virus and Microsporidia were found. However, the population collapsed after two years only in one grape variety. In the same vineyards but in other varieties and in other vineyards in the neighbourhood where the pathogens were found as well, in spite of the same weather and topographic conditions, the *L. botrana* populations became reduced, but did not collapse. The mortality was highest in the eggs at L_1 and L_2 stages in August. Further studies are needed to clear the circumstances of the outbreak of the epidemic and to establish the individual and combined effect of the two pathogens found.

* This work was partly supported by the Consiglio Nationale delle Ricerche, Rome, Italy.

The European grape berry moth: *Lobesia (Polychrosis, Eudemis) botrana* Den. and Schiff. *(Lepidopt.; Tortricidae)* is one of the most harmful lepidopterous species in the vineyards of the palearctic area. Its importance increases towards Mediterranean basin. In Central North Italy, in the area of Bologna, it is the only insect species to be controlled in the vineyards every year. Particularly the damage of the third generation can be very heavy (sometimes 100%) because, beyond the direct damage, the larvae predispose the infection of the grey mould *(Botrytis cinerea)*.

The observations on the biology of *L. botrana* started about one hundred years ago, so a vast literature is available today. All authors agree that the number of adults fluctuates considerably not only from one year to another but also from generation to generation. The population can completely disappear from a vineyard to begin a new infestation after a shorter or longer period.

Entomologists were looking for the reasons of this fluctuation from the last century on and had tried to find them in the biological factors as birds, parasites, predators and fungus diseases. Many of the parasites of *L. botrana* were described and a big importance was attributed to their action or to that of fungus diseases during winter (CATONI, 1910; SILVESTRI, 1912; SCHWANGART, 1929; VOUKASSOVITCH, 1922).

From the early 1920, with the knowledge of influence of meteorological factors on the insects, the motive of the strong fluctuation in the number of the population from a generation to another was attributed to meteorological factors. High temperature (22–28 °C) and low humidity increase the number of swarming adults and enhance oviposition, whereas rainy weather and low temperature reduce the meeting possibility of the sexes and so the egglaying as well. Generally the adults and the egg stage are considered to be sensitive to the environmental factors (see reviewed by STELLWAAG, 1928 and by BALACHOWSKI, 1972).

However, the field observations with *L. botrana*, and those made in laboratory seem somewhat contradictory. JANCKE and ROESLER (1940) found that high humidity (90%) prolonges the lifespan of adults and so the number of eggs laid. The enhancing effect of high relative humidity on the egghatch was put in evidence by GÖTZ (1941). A four years' field study made by VOIGT (1970) on the relationship between weather conditions and the number of adults (and larvae) suggests that larvae may be sensitive to chilly and rainy weather as well; the number of adults was reduced not only during, but also after a cold period. When the suppression of the population could not be explained by the general weather conditions, microclimatic differences due to the topographic conditions were supposed (REICHART, 1968).

All these discrepancies in literature suggest that other factors than the pure ecological preference or ecological plasticity of *L. botrana* adults might play a role in its population dynamics.

Furthermore, in Central-North Italy an unusual phenomenon was observed at the time of the third swarming in August 1976 and 1977: the sex-pheromone traps caught a high number of males and on the basis of the following intensive

egglaying (1 – 3 eggs/bunch) a heavy damage was foreseen. In spite of that a very low larval population developed even though weather conditions were favourable. (Similar observations were made by BARBIERI and BECCHI [1977] in Modena.) The eggs, collected in August near Ferrara, hatched in our laboratory but failed to develop neither on artificial diet nor on grape berries. Larvae died at the age of the first and second larval-instars, although temperature and humidity were at optimal level. On the basis of the mentioned general opinion concerning the influence of bad weather and on the basis of our earlier observations with other Tortricids (DESEŐ, 1973; DESEŐ and SÁRINGER, 1975) we supposed that diapause inducing factors are responsible for the phenomenon described above and reproduction processes are influenced in some way. So, the first observations were carried out in these directions.

Materials and Methods

Field and laboratory observations were made in order to understand the biology of *L. botrana.*

Field observations

Observations were made continuously in two vineyards. The distance between them was approximately 20 km. In vineyard No. 1 there were many varieties and in 1976 and 1977 the sudden disappearance of the larvae was observed here in cv. Riesling. In vineyard No. 2 there was only one variety: cv. Riesling.

Biological observations in cages, monitoring with sex-pheromone traps and treatments with different insecticides were made in 1976, 1977 and 1978 as well (BRUNELLI *et al.*, 1978).

In 1979 observations on the biology of *L. botrana* were continued only in vineyard No. 2, because vineyard No. 1 was uprooted. In 1980 some observations were made in a vineyard near Modena.*

Figures 1a and b show the catches of males by sex-pheromone traps (type Zoecon). These data with the infestation values and treatments give some information about the population dynamics of the grape berry moth in the vineyard No. 1. By evaluating the data on swarming, the protandria and the decreasing male–female rate during the flight should be taken in consideration as well (GÖTZ and STELLWAG, 1940; GÖTZ, 1942); furthermore the period between the first catch and first egg: 8 – 12 days in the first flight and 4 – 5 days in the second, third one (TOUZEAU, 1975).

* The authors are indebted to Dr. R. BARBIERI and R. BECCHI for their help in collecting the material in the vineyard near Modena.

In the vineyard No. 2 the fluctuation of the population dynamics was similar to that shown in Fig. 1 b. E.

Observations were carried out on *L. botrana* brought in from the vineyard, also in the garden of our Institute.

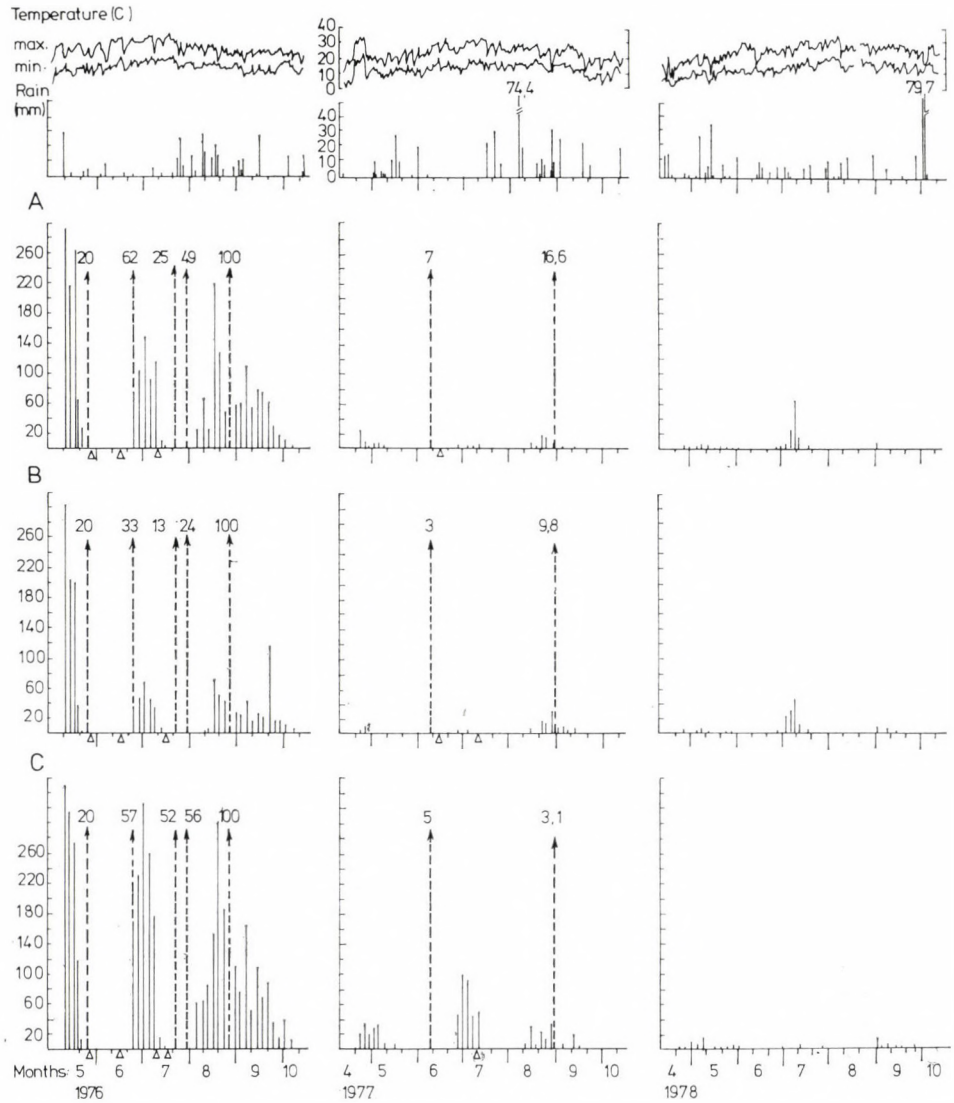


Fig. 1. a. Number of *L. botrana* males caught in the untreated plots by sex-pheromone traps in vineyard No. 1 and the meteorological data. a) in 1976, 1977, 1978 in cv. Riesling (A.B.C.). b) in 1977, 1978 in cv. Merlot and Uva d'oro (D.E.F.C.). Δ = Date of insecticide treatments. ° = % of 500—1000 grapes infected by *L. botrana*

Laboratory observations

The population observed in the laboratory originated from the vineyard No. 1 cv. Riesling and was collected in July 1975. Some observations were made in the laboratory also on the population of vineyard No. 2.

For rearing the artificial diet of SENDER (1970) was used. After two years the diet was slightly modified: 5 ml of glacial acetic acid was added to 1 l diet, to prevent the disturbing mould infection. However, this amount was too high so it was later reduced to 2.5 ml.

The eggs were always disinfected in 4% formaldehyde for 1–2 minutes. Before putting the eggs on the diet, the surface of the small pieces of the diet was dried with warm air, because the larvae used to be very sensitive to condensed water. For the rearing 0.5 l glass-jars covered with linen cloth were used. For pupation paper bands were offered.

In the mass rearing for oviposition a $25 \times 30 \times 50$ cage made of cheesecloth was used. A strip of plastic was offered for the females as egg laying place. For individual pairs (usually 1 ♀ + 2 ♂♂, or 2 ♀♀ + 1 ♂) small plastic glasses covered with cheesecloth were employed. In both methods 5–10% honey-water was added, soaked in cotton to feed the adults. One generation developed in 24 days, but a part of the larvae finished the cycle sometimes in 40 days.

The effect of constant and changing environmental factors on the diapause and the reproduction were studied in thermostate chambers with different temperatures and photoperiods.

The number of the individuals and the developmental stages besides the detailed methods used in the different observations are indicated at the results.

Results

Effect of temperature and photoperiod on the diapause of L. botrana

100–300 eggs of the 3rd and 4th laboratory generations of *L. botrana* in the age of 24–48 hours were put on artificial diet and held under constant changing temperature and photoperiodic conditions. The number of diapausing pupae and that of the swarming adults were observed (Table 1).

As the data of Table 1 show, the diapause inducing photoperiod is about 13 hours at 25–26 °C (see No. 2); at a photoperiod of 13.30 hours no diapause occurs (No. 19), even at 22 °C.

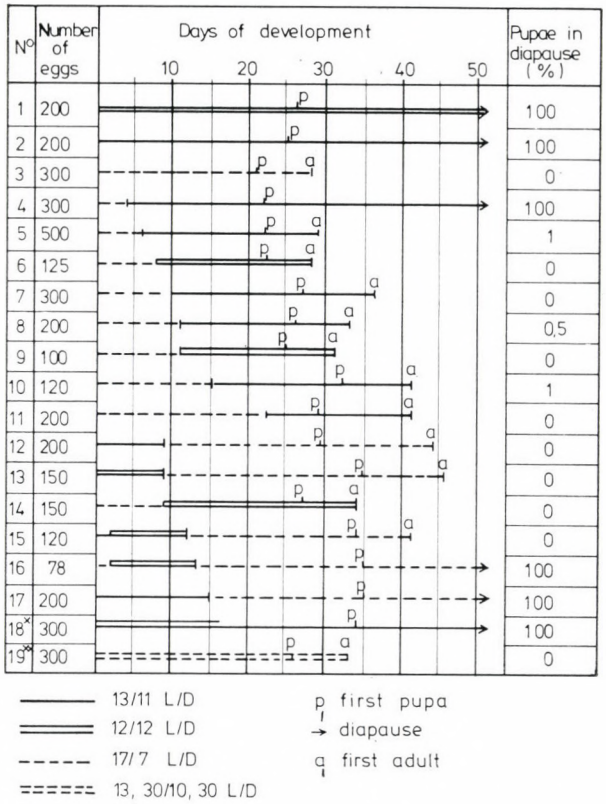
No. 1 and 18 indicate that photoperiods shorter than the critical one did not affect the direction of the development: even at naturally lengthening spring photoperiod.

As experiment No. 4 shows, short day (SD) on the 5th and 6th* day begins to induce diapause whereas long-day (LD) conditions on these days elicite a con-

* Eggs were 24–48 hours old, so 1–2 days should be added.

Table 1

The sensitivity of *L. botrana* Den. and Schiff. egg/larvae to the action of short-days at different periods of development. Temperature: 25—26 °C. (3rd and 4th generation in the laboratory)



* No. 18: naturally increasing photoperiod in March (12 h 40′—13 h 15′)
** No. 19: repeated at 22 °C with the 6th generation in the laboratory

tinuous development (No. 5). So the diapause sensitive age seems to be at the end of the embryogenesis, in the first larval instar and perhaps in the first part of the second larval stage.

The effect of the SD illumination on the physiological processes seems to be weaker than that of the long-day photoperiod. Only after 15 SD-s the diapause development (No. 17) becomes irreversible. In cases Nos 12, 13, 15 when the number of short-days was inferior to 15, no diapause occurred.

No. 16 suggests that in *L. botrana* not only the numbers of the short or of the long-days are critical for the diapause induction, but the change itself to the opposite photoperiod. The change from LD to SD in the first part of the

embryogenesis and SD in the sensitive period (5–6th days) induces diapause, even if afterwards long-day conditions continue. When this change fails (No. 15) no diapause occurs.

From the experiments Nos 5–11 and 14 it can be seen that short photoperiods after the 6th day of long-day development did not influence the physiological processes basically anymore, but it can increase the developmental time. However, the differences between the lengths of the developmental periods are not significant.

We have no data concerning the factors responsible for the reactivation of the diapausing pupae. In spite of different long and intensive cold treatments with about 300 pupae, the diapause lasted always about 5.5–6 months. When diapausing pupae were held in laboratory at constant 25 °C the swarming began after a similarly long period; however, the adults were very weak.

Field observations on the 2nd generation of *L. botrana* in the vineyard No. 1 showed that from the larvae collected on July 22, 1976 (about 14 hours' long photoperiod) 10% went to diapause.

On August 30 and Sept. 26, 1976 infested grapes were collected in the vineyard No. 1 and were held during the winter in large cages in the laboratory garden. In next spring, the swarming began on 5th and 26th April and finished on 2nd and 16th May respectively. The development of *L. botrana* population in the laboratory garden preceded the developments in the vineyard by two-four days. Four generations were developed in the laboratory garden in 1976.

The corrugated paperbands, fixed at different parts of the vine-stocks in the vineyard No. 1 at the end of July with the aim to collect the diapausing pupae, did not contain any of them in next spring. Although when the infested grapes were suspended in the cages at outdoor conditions by Sept. 20, 1977 adults swarmed between 27th April and 15th May, 1978.

The results in 1980 were better with CATONI's method (1910) i.e. textile bands fixed on the vine at 30–50 cm heigth. The number of the diapausing pupae proved to be already high in the first fortnight of September 1980: 5 pupae/plant and by the end of October about 10 pupae/plant.

Influence of (1) temperature and photoperiod, (2) food and (3) group effect on the fecundity of L. botrana

(1) For this observation adults were taken partly from the diapause experiments and partly reared separately with the usual method. Results are summarized in Table 2. The differences are significant between the average fecundities of the low and high temperature-reared populations.

When the photoperiod was changed (during the development) from long-day to short-day from the 8th day on, the fecundities of the populations increased. These differences were significant also in comparison with the "optimal" conditions (25–26 °C and 16 hours' long photoperiods). The influence of the change of the long-day photoperiod to shorter day during larval development

Table 2

Number of eggs laid by females of *L. botrana* Schiff. and Den. developed under standard/changing temperature conditions and photoperiods. (Egglaying at 24—26 °C and 16 hours long photoperiod.)

Temperature (°C) Photoperiod (hour)	24—26 16	24—26 14	24—26 16→14*	24—26 16→13*	20—22 14
	70	40	135	71	38
	164	20	149	151	42
	106	91	75	123	50
	129	91	158	242	56
	98	140	123	162	40
	133	52	143	159	130
	78	111	186	79	135
	113	148	138	120	40
	96	114	126	120	44
	63	70	120	135	124
	113	104	132	149	20
	69	82	173	141	45
	88	71	173	137	26
Average*	102**(a)	87(a)	141(b)	138(b)	61***(c)

* Photoperiod changed in different ages from 8 days on.

** The mean fecundity of the population (♀♀ with or without egglaying) was 75 eggs/♀.

*** The mean fecundity of the population was 43 eggs/♀.

+ Same small letters indicate Duncan's multiple range grouping that do not differ significantly. Significant difference at the 0.05 level is only between the groups signed by different letters.

seems to increase the fecundity of the swarming adults; independently whether the change occurred towards an illumination inducing a diapause or only to smaller, but sudden change from 16 to 14 hours.

A photoperiod (14 hours) during the development of the larvae shorter than optimal seems to influence somewhat the fecundity of the adults, however, the difference is not significant.

From the population's mean-fecundity data (Table 2) it follows that not all the females laid eggs since the population's mean fecundities of the lots were lower than those in egglaying females.

(2) The mean fecundity of the population reared on artificial diet under optimal egglaying conditions was 75 eggs/day; however, the mean fecundity became nearly twice as much (135 eggs/day) under the same environmental conditions when *L. botrana* had been reared on mature grapes in autumn.

(3) The group-effect on the fecundity was observed as follows. Females were held alone with two males, or two females with one male and the number of eggs laid was compared to those jars, where 5—8 females were put together

with 4–12 males. No differences were observed between the average fecundities of the two groups (75.8 [22 ♀♀], 79.5 [27 ♀♀] and 70.0 [95 ♀♀] eggs respectively at 25 °C and 16/8 L/D photoperiod).

Field observations

Two females and eight males were found on a warm day (May, 7, 1976) in the shadow on the trunks of trees in the vineyard No. 1. These females laid 140 eggs.

In a population collected in autumn in the vineyard No. 1 and left in outdoor conditions over the winter the fecundity was very low (6–19 eggs/female). These 35 ♂♂ and 21 ♀♀ were held partly under chilly and rainy outdoor conditions and partly in laboratory. Even these latter females held under optimal conditions laid few eggs as well, and the larvae died at the first and second larval instars. As Fig. 1 (a, b) shows also the number of males caught was very low in 1977.

In the spring of 1978 the fecundities of females proved to be between 38–85 eggs, the adults emerged under dry and warm weather conditions.

In the vineyard No. 2 in 1978 the fecundities of the females of the 1st, 2nd and 3rd swarmings varied from 75 to 97, from 70 to 100 and from 100 to 180 eggs, respectively. (Mean fecundities: 75, 87 and 140 eggs/female.)

Effect of the photoperiod on the reproduction activity of L. botrana

Adults from the 10th laboratory generation, reared on artificial diet at 25–26 °C and 16 hours long photoperiod were observed. In the jars 1 ♀ with 2 ♂♂ or 2 ♀♀ with 1 ♂ were put together. When the adults were held under the same conditions as their development had been: 84% of the 44 females laid eggs. The dissection of the females showed that 13.3% did not mate, 60% contained one, 23.4% two and only 3.3% three spermatophores.

The mating behaviour was disturbed of adults exposed to 13/11 L/D photoperiod immediately after emerging. Some pairs were stuck together, other males had the spermatophores on the outside of their genitalia. Only 28% of the 25 females laid eggs. The egg hatch was normal.

In an other experiment the population was reared at 14/10 L/D photoperiod and the adults were exposed immediately after emerging to the 13/11 L/D illumination. Neither their mating behaviour, nor the oviposition were influenced.

Diseases of L. botrana

By low temperature (about 18–20 °C) not only the fecundity was influenced but morphological deformations could be seen as well; adults with swollen greenish abdomens, males with prolonged genitalia (Fig. 2) and pupae with unperfect ecdysis (Fig. 3).

Other symptoms of supposed diseases were observed as well; many white pellets in the excrements, white midgut in the transparent larvae, unusual differences in the development rate (5–80%), a big difference between the developmental time of larvae originating from the same female, etc.

In an experiment the deformed insects mentioned above were washed in 4% formaldehyde, homogenised with distilled water and smeared on equal pieces

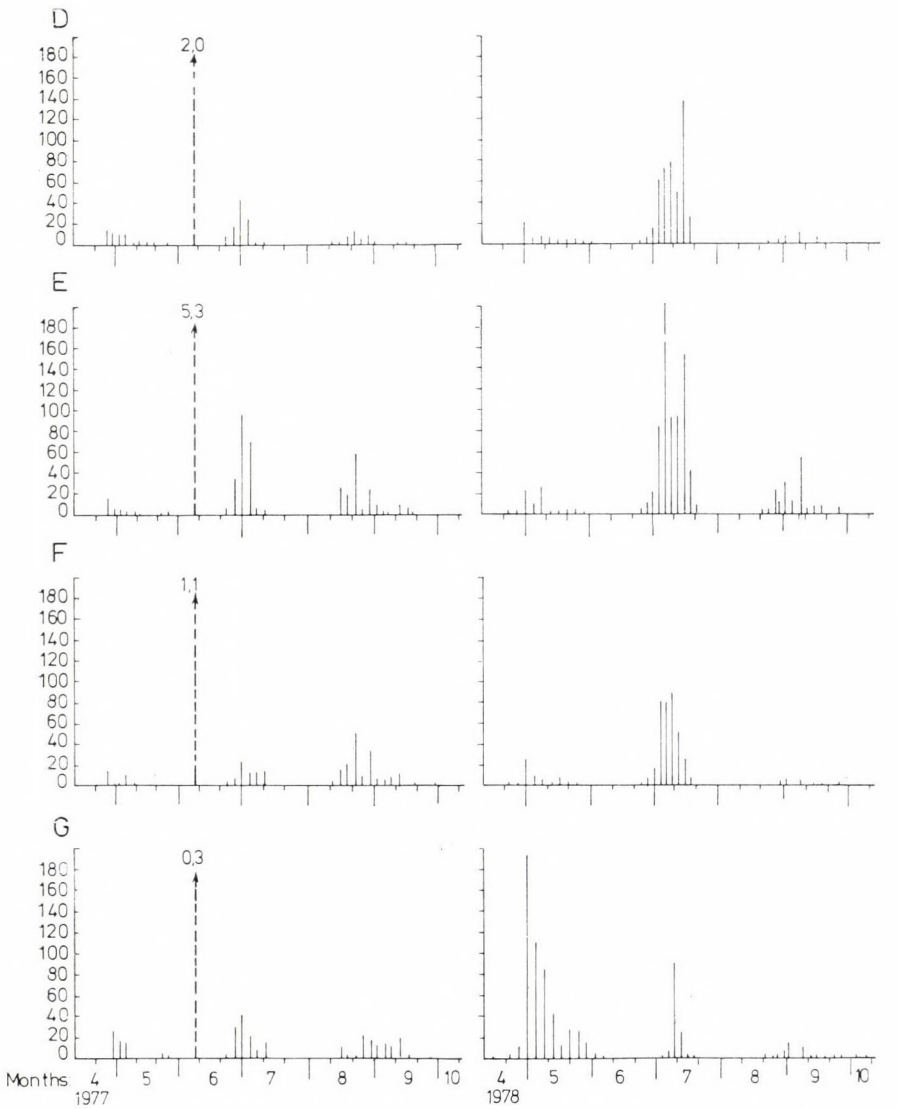


Fig. 1. b. Diseased *L. botrana* male with swollen abdomen and protruded genitalia (Foto: Laffi)

Fig. 2. Diseased pupa with unperfect ecdysis (Foto: Laffi)

of artificial diet. All pieces were covered completely with the homogenate, so the newly hatched larvae were forced to feed the smear when boring into the diet. Results are shown in Table 3.

The data in Table 3 suggest the presence of pathogen(s). The mortality rate in the treated replications, independently from temperature and photoperiod, was significantly higher (92.5 and 95%) than in the check (71 and 82.5%).

The mortality at the lower temperature was higher; it is probable that this factor did enhance a chronic illness in this 22nd laboratory population.

Lower pH value seems to reduce somewhat the number of the developing adults, although the differences were not significant.

In the treated repetitions many dead L_1 and L_2 had been found, partly sticking to the diet with the posterior end of the body. Some L_2 showed empty cuticles with white colour in their immediate neighbourhood. Some of the developing larvae had, in older instars, white midguts and developed more slowly

Table 3

Effect of non-purified virus treated artificial diet on the development of *L. botrana* Den. and Schiff

Temperature °C	Photoperiod L/D (hours)	Artificial diet	PH	Number of adults developed						
				1.	2.	3.	4.	Total ♀	Total ♂	%
23—26	16/8	Treated	4.5	1	1	2	0	1	3	
			5.5	2	5	0	4	4	7	
		(Total)		3	6	2	4	5	10a+	7.5
		Check	4.5	11	3	2	5	11	10	
			5.5	7	3	9	18	21	16	
		(Total)		18	6	11	23	32	26b	29
22—24	13/11	Treated	4.5	2	1	1	2	6		
			5.5	1	1	2	0	4		
		(Total)		3	2	3	2	10a		5
		Check	4.5	3	2	3	2	10		
			5.5	8	4	5	7	24		
		(Total)		11	6	8	9	34b		17.5

+ Same small letters indicate Duncan's multiple range grouping that do not differ significantly. Significant difference at the 0.05 level is only between the groups signed by different letters.

than the others. Dead pupae showed the well-known case of half-ecdysis (see Fig. 3) and between the fully developed adults swollen ones could be observed with long, protruded genitalia (see Fig. 2). Adults from the short-day photoperiod experiment after the diapause were very weak and did not reproduce.

At the time of the 23rd generation the rearing seemed to be heavily infected by pathogens, and changes could be observed in the reproduction activity as well (Table 4).

When more females and males were held together, increasing thus the competition between the males, the reproduction activity improved and the number of inviable eggs was lower. However, in an experiment with the 27th generation, all stages died earlier than the third larval instar.

The rearing at this time was weak and ill, despite the egg disinfection from the first generation on with 4% formaldehyde. This circumstance might indicate that the transmission of the pathogen is transovarial. This hypothesis was supported by the following trial: 168 eggs were put after disinfection with 4% formaldehyde in Petri dishes one by one on artificial diet. 37% of the eggs proved to be inviable, 25% developed into adults (30% died as L_1). Even in the case of finished development white pellets were present in the excrements.

The following pathogens were found in the larvae/adults:

1. Cytoplasmic polyhedrosis virus (Figs 4, 5, 6)
2. Microsporidia (Figs 4, 5, 7)
3. The presence of a baculovirus is to be confirmed; the interpretation of the data is not yet clear (Fig. 8)

Figures 4, 5, 6 show the CPV and microsporidia in L_3 of *L. botrana.* The midgut is infested very heavily by CPV accompanied by microsporidia. The latter were observed in the fat body and in the silk glands too. In the heavily infested larvae the midgut becomes white from the polyhedra (inclusion bodies) with sizes of 60–70 nm. The inclusion bodies in the excrements are the main source of infection (Fig. 8).

Both CPV and microsporidia are known as agents of chronic diseases. Unfortunately we have no data about the symptoms caused either by CPV or by microsporidia separately.

Figure 9 shows particles which may be baculovirus. These formations were found in the dead and swollen L_1 and in the ovariole of a swollen female.

Field observations made in vineyard No. 1 on the population dynamics of males are reported in Fig. 1. In 1976 the number of the males was very high in the cv. Riesling, it decreased in 1977 and both number of males caught and the level of infestation were so low in 1978 that not even one treatment was needed.

In 1976 and 1977 in August, the number of eggs was very high (1–3 eggs/grape) in cv. Riesling, but the damage did not correspond to that level, indeed in 1977 not even one treatment was necessary. The eggs, from which supposedly diseased larvae hatched and soon died, were collected in this vineyard by the end of the summer. Symptoms of CPV could be noticed on a few larvae found on cv. Riesling already in 1977 and on all those observed in 1978.

In the same vineyard but on other varieties the traps caught a great number of males in 1978 and the infestation of the grapes of the 3rd generation was about 20%. In these varieties, few diseased larvae and only in the 3rd generation in 1978 were observed. The most remarkable symptoms proved to be the white midgut and white pellets in the excrements. Swollen males were caught by the traps as well, but only seldom.

In the vineyard No. 2 in 1978 (cv. Riesling also) the infestation was low, probably because of the treatments. In spite of this, at the time of the third swarming the egglaying proved to be more abundant, and until the first days of September the infestation of the grapes reached 100% level. On 10th September, hundreds of grapes were collected with the eggs and suspended in a big cage in the vineyard, one hundred of them were put in the thermostat chamber under constant temperature (25 °C) and photoperiodic (16/8 L/D) conditions. In the laboratory, where of course diapause did not take place, after three weeks 63 ♀♀ and 67 ♂♂ emerged. Of these females 25% laid eggs, the average was 135 eggs/female. From the following two generations, at the same temperature level 25% developed again. Holding the rearing under 20 °C they developed the symptoms of the CPV, and the eggs laid by these females did not hatch.

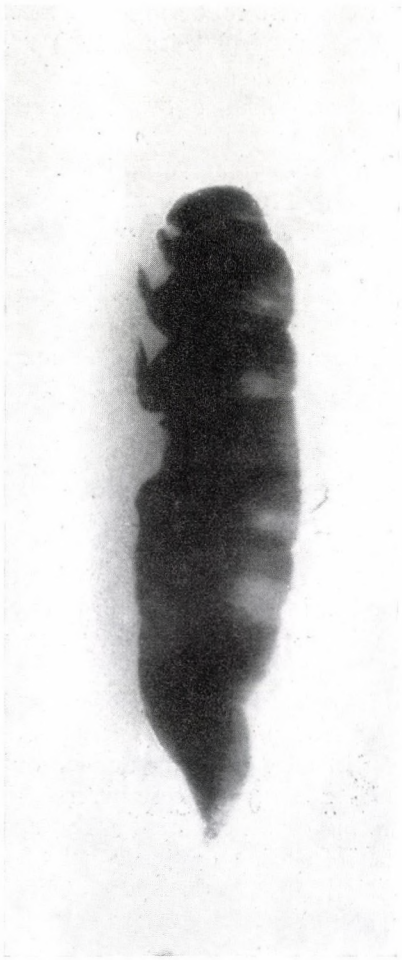


Fig. 3. Dead pupae

In the cage left in the vineyard 2 ♀♀ and 3 ♂♂ swarmed between 24th April and 2nd May in next spring. Of the two females only one laid 75 eggs in the laboratory. The progeny has been reared on artificial diet, 2 ♀♀ and 5 ♂♂ developed having greenish swollen abdomens with abnormal genitalia, i.e. the symptoms of diseases. In this vineyard (No. 2) the infection of the grapes by the 1st generation was 0.5%, that of the 2nd one was 2%. In 1979, without any treatment, at the time of the 3rd generation the infestation reached only a 15% level.

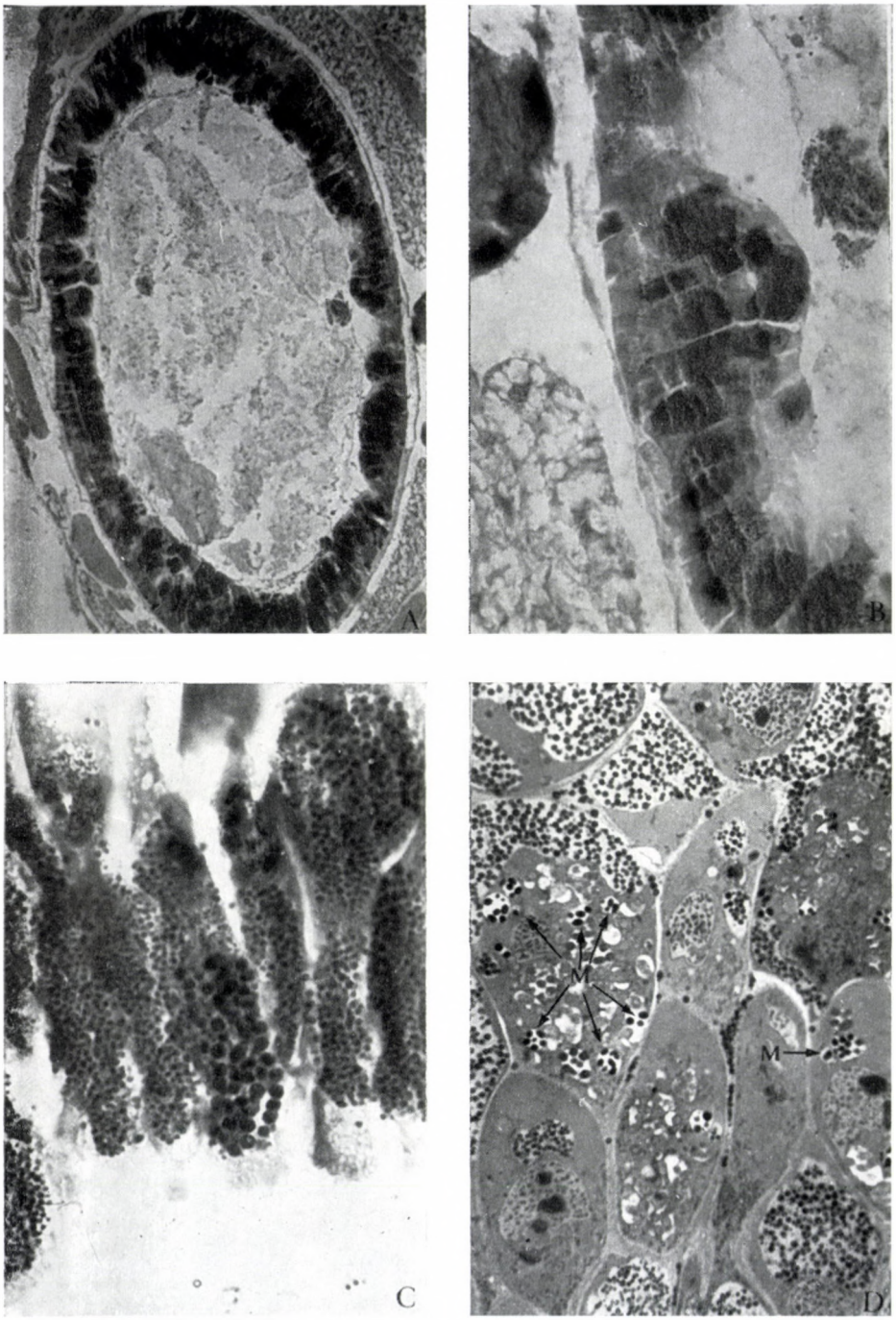


Fig. 4. A) Midgut section of L_3 with CPV (120×). B) Midgut fragment of L_3 with CPV (480×). C) The same (1200×). D) Fat tissue with CPV and microsporidia (M) (1200×). (Courtesy of Dr. Meynadier, Lab. INRA, Saint Christol les Alés-France)

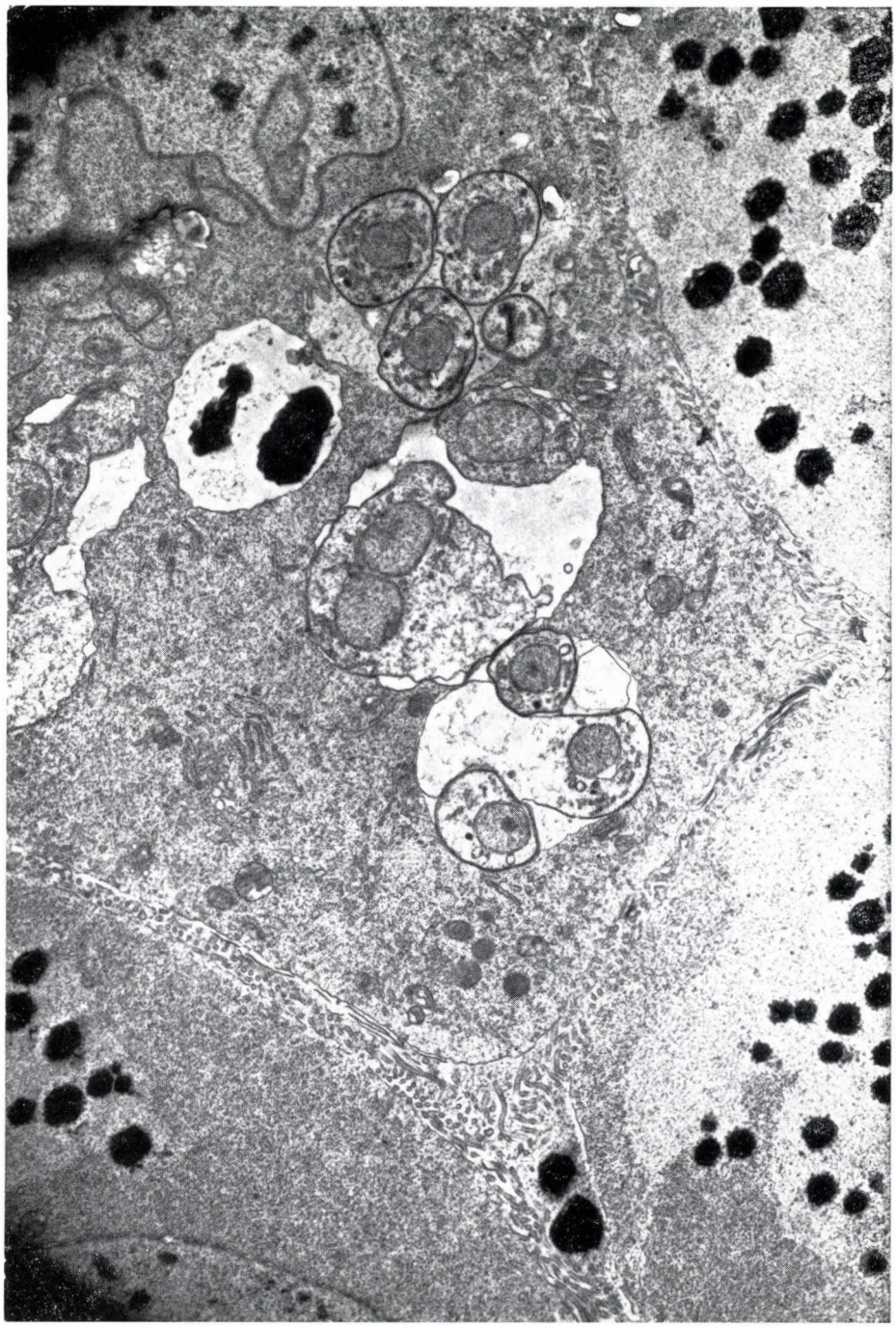


Fig. 5. Thin section of midgut cells of L_3 with CPV and microsporidia. (×11 300) (Courtesy of Dr. MEYNADIER)

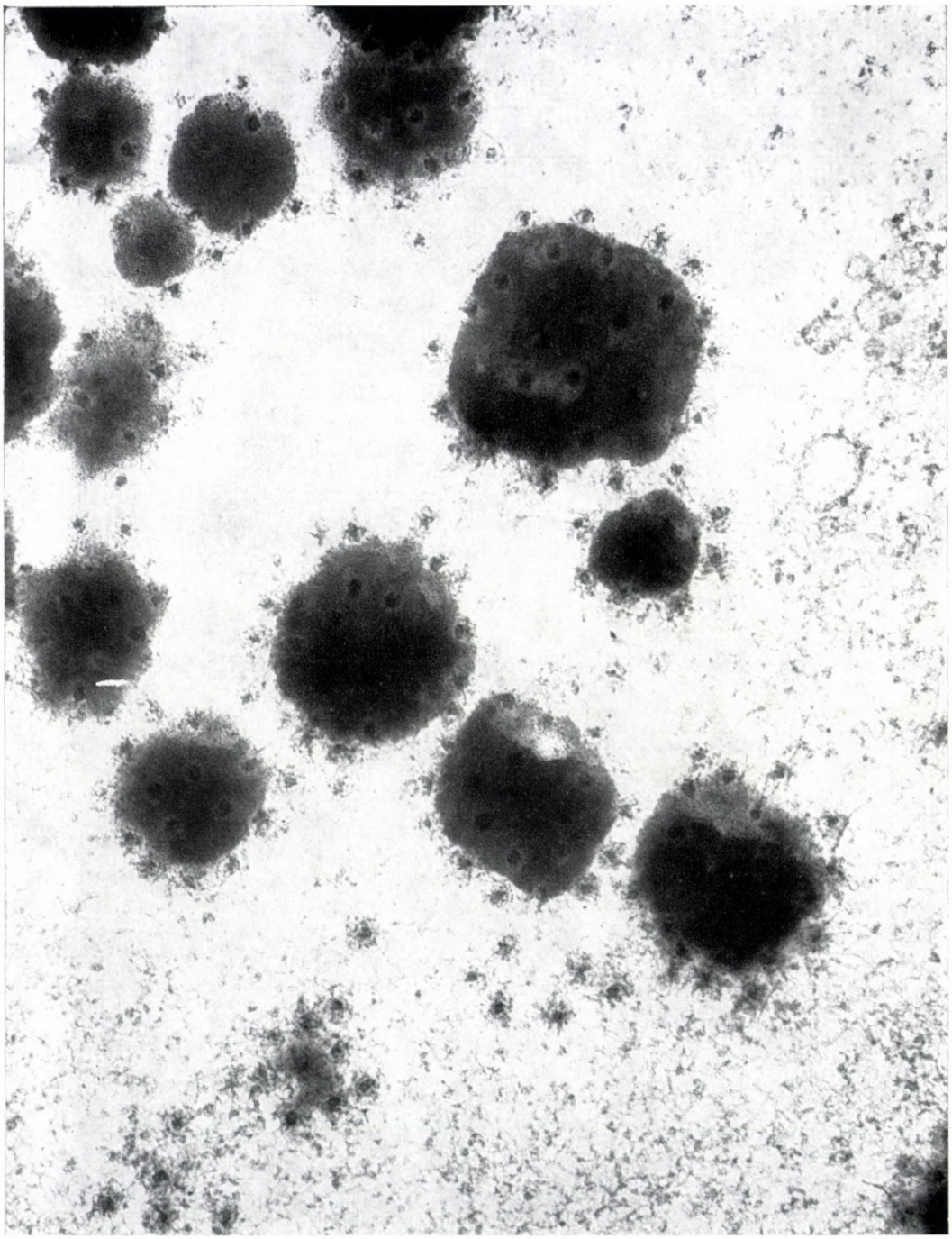


Fig. 6. Thin section of midgut cell with CPV inclusion bodies and virions (×91 200). (Courtesy of Dr. MEYNADIER)

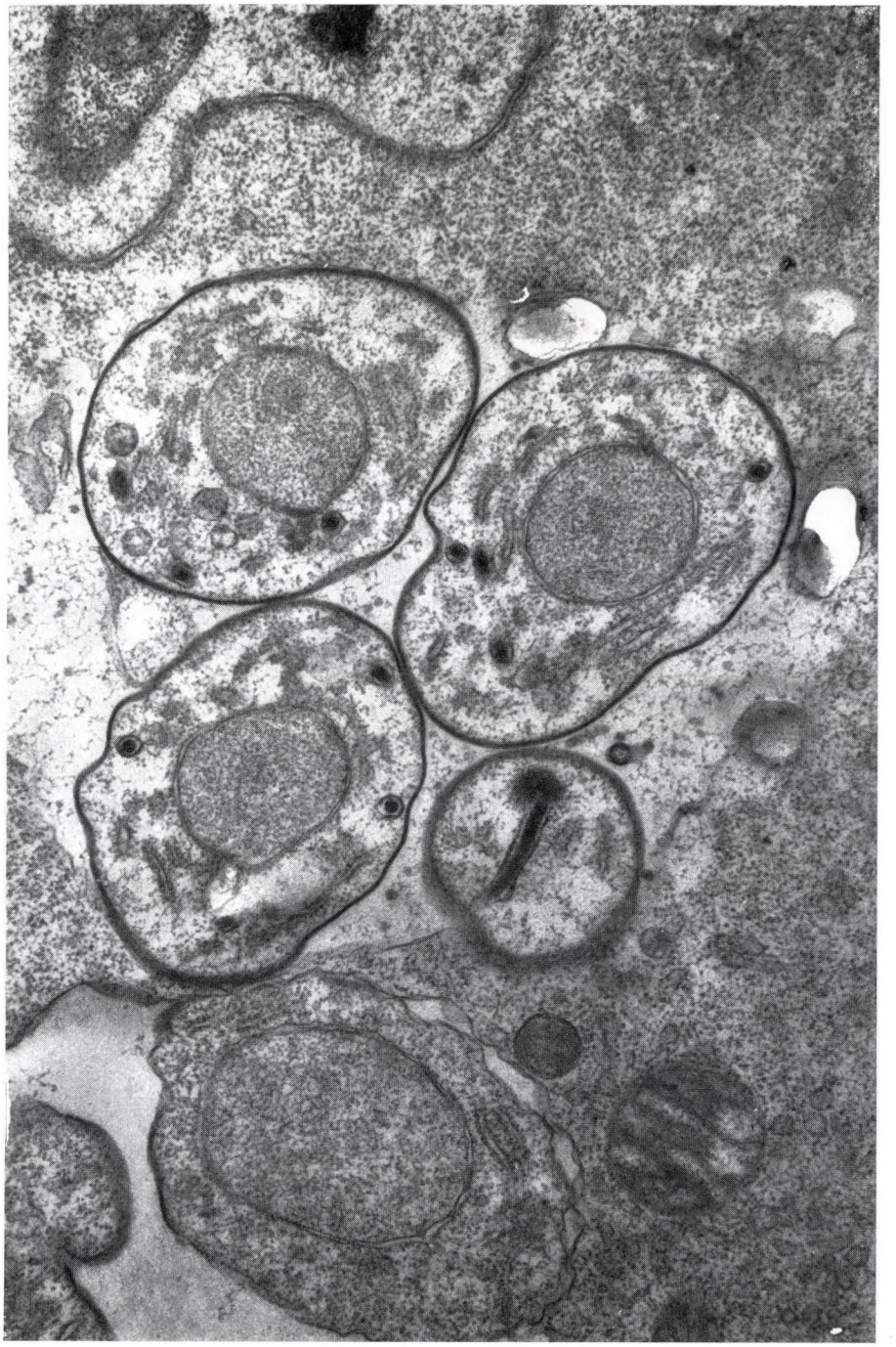
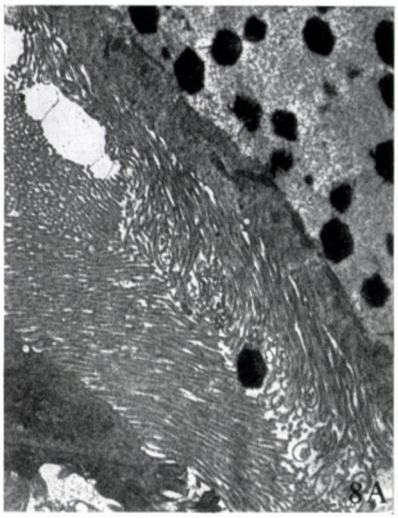
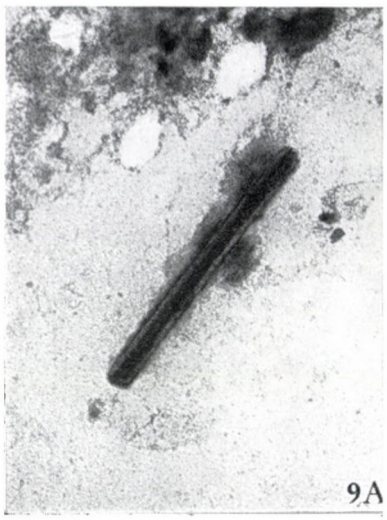
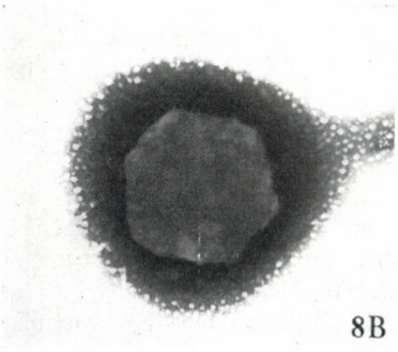


Fig. 7. Thin section of microsporidia in the midgut cell (×36 800).
(Courtesy of Dr. MEYNADIER)







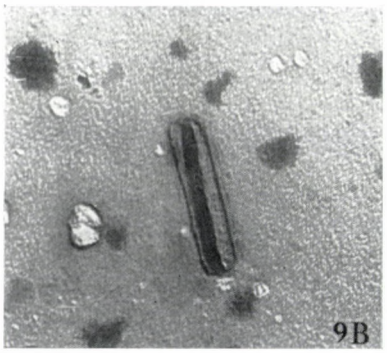


Fig. 8. A) Thin section of L_2 with CPV in the midgut lumen (×6200). (Fixed in glutaraldehyde 5% in 0.1M phosphate buffer pH 7.2, postfixed in osmium tetroxid in the same buffer, dehydrated in aceton series and embedded in Spurr's low viscosity plastic. Ultramicrotome Reichert OM U3 was used for cutting, double stained in uranyl acetate and lead citrate, examined in a Hitachi U-12 A electron microscope. B) Negative staining of CPV inclusion body from the excrement of an ill larva (×31 000). (PTK droplet on Formvar-film covered grid.)

Fig. 9. A) Thin section of an eggcell with unknown particle (×47 000). B) Negative staining of unknown particle in the smear of a diseased L_1 (×14 000)

Discussion

Observations carried out in Italy (CATONI, 1910; SILVESTRI, 1912; GRANDI, 1951; RANGHERI, 1959; CELLI *et al.*, 1977; 1980; TRANFAGLIA and VIGGIANI, 1976; VALLI, 1977; MOLEAS, 1979) showed that four swarmings of *L. botrana* developed in the South in warm years only (VIVONA, 1955).

In our observations in Bologna emerged the possibility of a fourth flight also in this area. In the laboratory garden, where the development preceeded of some days the events in the vineyard we reared four generations from the first eggs of the first females. The long swarming in the vineyard in autumn and the eggs found even in October support this possibility, even though probably only a small percentage of the population gives origin to a fourth swarming. Other authors (VOUKASSOVITCH, 1914), reported also the development of a fourth generation from the same geographical latitude in warm year with hot August.

Critical daylength and sensitive stage

For collecting the diapausing pupae in the vineyard textil-bands, fixed on the stocks were suitable. The efficacy of these textil-band traps was well known already at the beginning of our century: they were recommended as control method against the grape berry moths (CATONI, 1910). The textile traps in South Tyrol had to be fixed on the stock from the middle of August on, when the larvae were observed while seeking hidden places for diapause.

In our observations near Ferrara about 10% of the larvae of the 2nd generation, collected in the vineyard, went into diapause at the end of July. The progeny of the third swarming began to pupate from the middle of August, so in the textile traps between the last week of August and the middle of September about 5 pupae/stock were found. The number of the diapausing pupae increased until the end of October (10 pupae/vine approx.). Considering the heavy infestation (about 3 larvae/bunch) it seems that only a small percentage of the population were caught by the textile traps.

At the date of the appearance of the diapausing pupae in the vineyard and that in the laboratory, a discrepancy can be stated. In the laboratory, at constant temperature of 22, or 25 °C, the critical daylength was about 13 hours. Furthermore the developmental stages on the 6th–8th days after oviposition are mostly sensitive to the daylength; the photoperiodic experience in the first part of the embryogenesis does not determine irreversibly the direction of development. However, the 13 hours' long photoperiod occurs in our area only from the 1st of September i.e. theoretically only in these days could happen the diapause induction and only in the young stages. In spite of that in this period already a huge number of diapausing pupae were in the vineyard.

The data given by other authors do not explain the discrepancy found in our circumstances. So, VOUKASSOVITCH (1914, in STELLWAAG, 1928) reported that 18% of the larvae collected in the vineyard was swarming at the end of August/be-

ginning of September in 1912 at practically the same geographical latitude (44°) as ours (45°). That means that the eggs must have been laid in the first days of August at a photoperiod of about 15 hours. KOMAROVA (1949, in DANILEWSKII, 1965), however, stated that the diapause inducing photoperiod was 15.40 hours in Azerbaijan at a geographical latitude of 41°. According to her data, all the eggs laid after 20th July developed into diapausing pupae and she concluded: "thus the number in diapause mainly depends on the calendar dates of egglaying." The sensitivity to the photoperiod proved to be in her population in the first eight days after the oviposition.

The difference between KOMAROVA's observations and ours are 1. in the daylength: the critical photoperiod found in our laboratory was 2.40 hours shorter than that observed in Azerbaijan and 2. in the Italian population short day during the first 8 days from the oviposition on, does not induce diapause, but predispose only the following photoperiodic effects. There are 7 more SDs needed for an irreversibile diapause induction. It seems that the 8 SDs are necessary to cancel an earlier "program" for the continuous development, for which 6 DSs are not sufficient. However, big and sudden changes in the length of the photoperiod during embryogenesis increase the sensibility to SD effect. The existence of an earlier "program" may also explain that already with 8 LDs the "program" of a development without diapause becomes confirmed irreversibly.

In the outdoor conditions it is possible that the shortening daylength induces the diapause from July on, at about 15 hours' daylength. However, it cannot be excluded that the diapause in July, observed in the second generation, was the result of the heterogenity in the species, observed in a Hungarian population by REICHART (1968).

To clear this discrepancy, exact observations are needed on the effect of the shortening daylength in diapause induction, on the role of the quality of food, on the role of possible diseases.

Observations on the reproduction

In outdoor conditions the fecundity of the females vary from generation to generation and from one year to another.

Our observations on the fecundity of *L. botrana* show that the number of eggs laid are influenced by many factors. We observed in the 1st, 2nd and 3rd swarmings mean fecundities as 78, 87 and 140 eggs/female, respectively. REICHART (1968) also found differences between the mean fecundities of the three swarmings: 76.7, 139.6 and 91.1, respectively. In the case of the Italian population we think to find the explanation for the different mean fecundity-values in the changing quality of the grape. We have shown that on the artificial diet used, the mean fecundity resulted to be about 75 eggs/female, meanwhile the lot reared on mature berries at the same time under the same conditions produced 135 eggs/female. In case of the Hungarian population we suggested an other explanation (DESEŐ and SÁRINGER, 1975). The differences in the mean fecundities were particularly

big after winter between the first swarmings from one year to another e.g. 78 eggs/female in 1976, 11 eggs/female in 1977. JANCKE and ROESLER (1940) observed too variable fecundities from one year to another; furthermore the data reported demonstrate also the differences between that of the successive generations.

The very important role of the temperature during the egglaying is well known with *L. botrana*. Under laboratory conditions the effect of temperature on the number of eggs laid was demonstrated by JANCKE and ROESLER (1940): under 15 °C the fecundity decreases, although females can lay eggs even at 13 °C and 34.5 °C. The regime between 21–25 °C was observed as optimal for the egglaying, however the difference in the fecundities at different temperatures do not seem to be significant. According to GÖTZ (1943) oviposition takes place between 18–26 °C, with 45–85% relative humidity and mostly between 17–22 hours. Many authors agree with the above-mentioned opinions, however the lower limit of the optimum seems to be flexible as reviewed and discussed by BALACHOWSKY (1972).

In our observations under laboratory conditions, 21 °C proved to be under the lower limit of the temperature optimum for egglaying because at 25 °C the average number of eggs laid was significantly bigger. This observation under our circumstances can be interesting in the spring when lower temperature than 22 °C can occur even for a longer time.

On the contrary to the effect of different temperature values, the different but constant photoperiods during the development of the larvae did not influence the number of eggs laid.

The change from long-day to short-day during the development (even in the prepupae) from the 8th day on, significantly enhanced the oviposition of the females. Similar results were seen earlier in *Laspeyresia pomonella* l. *(Lepidopt. Tortr.)* (DESEŐ, 1973). We have no explanation for this observation by the time being; any supposition should be proved by experiments.

On the contrary, of the fecundity-promoting effect of the change from LD to SD during larval development, a similar event experienced by the newly emerged adults disturbs the mating behaviour, decreases the number of matings and elicits aberrations when mating does occur. When the adults are exposed to day-length not very much different from the photoperiod on which the rearing had been held, the mating behaviour is not disturbed. This phenomenon suggests that not the photoperiod itself, but the sudden change is responsible for the altered reproduction activity. It is highly probable that the disturbed circadian rhytm elicits the inappropriate mating behaviour.

The role of diseases

In the population dynamics of *L. botrana* three remarkable changes have been observed: 1. Big differences between the individual numbers of the autumn and of next spring, furthermore between the spring and the following spring

populations. These differences were mostly attributed to the action of parasites and fungal diseases, but "unknown reason" of this winter mortality was mentioned as well (CATONI, 1914; VOUKASSOVITCH, 1922). 2. The sudden changes in the population number in August, mostly in the form of an abundant swarming followed by low infestation, were explained by CATONI (1910) and SILVESTRI (1912) as "not yet known pathogens", whereas FEYTAUD (1913) stated that the high mortality of eggs in August was due to the high temperature and bright sunshine. 3. Later on, as suggested by STELLWAAG (1938, 1940), the change type 2 and the observed slow, 1–2 years' long suppression of the grape berry moth in a vineyard was attributed to the solely effect of climatic factors as: low temperature, high humidity and much rain (reviewed in BALACHOWSKI, 1972; VOIGT, 1970, 1972; SCHMID *et al.*, 1977; RÖHRICH, 1978, etc.). In vineyards, where the population did not decrease on the effect of the unfavourable weather conditions, topographical differences were suggested.

Without questioning the important role of the climatic factors we have shown in our area the decisive influence of pathogens in the population dynamics of *L. botrana* in the vineyard No. 1, where the population collapsed in three years. In vineyard No. 2 the fluctuation had more or less the same rhythm in the three years' long observations; however, in the third autumn the number of the larvae was high and in the laboratory the diseases were present. In the following spring the population was weak and the infestation of *L. botrana* was very low; it became sowhat higher in autumn.

From the pathogens found the cytoplasmic polyhedrosis virus was new for *L. botrana* (DESEŐ *et al.*, 1979a, b) and it had been found until now only in the area of Ferrara and in 1980 near Modena.

Microsporidia, however, had already been reported earlier in France by LIPA (oral communication of Dr. MEYNADIER of INRA, Saint Christol les Alès) and in 1979 in the Italian population by Dr. MEYNADIER.

The existence of a third pathogen, a *Baculovirus* which was suggested earlier (DESEŐ *et al.*, 1979) is not confirmed. The elongated particles found in the L_1 and in the ovary of females seem to be identical to the particles doubted and discussed by SUMMERS and PASCHKE (1968) and GREGORY *et al.* (1969).

As we have seen in the Italian populations CPV and *Microsporidia* were found to occur together. It would be important to know the role of a single pathogen in the population dynamics, but, by the time being, no data are available. The observations of other authors can give some suggestions. So, the fact that *Microsporidia* were found in France in *L. botrana*, but the sudden disappearance of the third generation larvae were not observed, could give more importance to the effect of the virus. Furthermore, as we have shown, at low temperature the symptoms of a CPV disease become well visible; symptoms similar to that described in other lepidopterous species by VAIL *et al.* (1969). Thus it seems highly probable that CPV is in the first line responsible for the decrease of the population. However, it is not clear if the low temperature makes the CPV virulent directly or indirectly, making acute the microsporidia first, as it was observed

by WEISER (1961). In any case the result of this action is the suppression of *L. botrana* in a vineyard.

In Fig. 1 the spread of the disease can be followed according to the varieties, thus demonstrating at the same time a generation by generation effect of the pathogen. However, when the action of the pathogens is the strongest in the diapausing population, the symptoms of the disease are hardly noticeable.

From the diseased larvae the inclusion bodies (polyhedra) and/or mature spores are voided with the excrements. Rainy weather and high humidity help the diffusion of the pathogens on the grapes: larvae feeding on these berries become infected orally. This type of "horizontal" diffusion of these pathogens is well known. However, the possibility of the vertical diffusion i.e. transovum transmission, cannot be excluded as it was already mentioned with the laboratory strain. Combined transovum transmission of CPV and microsporidia was reported already in other Lepidopterous species (ADAMS *et al.*, 1967).

Returning to the difference in the population dynamics of *L. botrana* in the two compared vineyards, it is to emphasize that the conditions of the outbreak of the epidemics are unknown and for this further studies are required.

The possibility of a fourth swarming for a small percentage of *L. botrana* population cannot be excluded in the area of Bologna in years with warm spring and hot summer.

Conclusions

The diapause inducing photoperiod proved to be in the laboratory 13/11 LD period which did not correspond to the outdoor conditions. In the vineyard a huge number of diapausing pupae was found at the time of such photoperiod. So further observations are required to understand the reasons of the diapause of *L. botrana* in field conditions; e.g. to study the eventual effect of the shortening daylength, of the food and of the diseases in the diapause induction.

The sensitivity to the photoperiod was the highest on the 6 – 8th days at the age of the fully developed embryo and the first, partly the second larval instars. Long-day in this period elicits continuous development, whereas the effect of the short-day during the sensitive period becomes irreversible only after further 7 SD-s (sum 15). Therefore, the photoperiod sensitive period seems to consist of two parts: 1. during the first 8 days only an earlier "program" of the continuous development becomes cancelled by short-days. 2. The second part of the sensitive period arrives when the effect of SD becomes irreversible and so the diapause becomes induced. The first part of the embryogenesis is sensitive to the photoperiod only if the change in the length of the illumination is big.

In the observed *L. botrana* population 22 °C was already under the optimal temperature limit of the oviposition. The number of eggs laid was significantly lower than that at 25 °C.

The fecundity was strongly influenced by the food quality; larvae developed on mature grapes laid as adults nearly twice as much eggs as those developed

on immature grapes or on artificial diet. The egglaying was not influenced by the "group effect".

As to the number of spermatophores in the females it was stated that 60% mated once; 40% twice or three times or mating did not occur at all.

Whilst constant photoperiodic conditions during development did not influence the fecundity of the developing females, changes in LD periods did. Therefore, the change from the long to the short-day illumination at any time after the first part of the photoperiod sensitive developmental period (8th day), increases significantly the number of eggs laid. Even the pupae resulted to be sensitive to this kind of change.

However, by exposing the newly emerged adults to short-day illumination after having reared them under long-day conditions, they show disturbed mating behaviour. If the difference between the two photoperiods is not very big, the mating behaviour will be "normal".

In the population dynamics of the European grape berry moth the diseases play a very important role: they can suppress the population in the vineyard and in the laboratory as well.

Two pathogens were found: Cytoplasmic polyhedrosis virus and microsporidia. The separate effect of the pathogens is unknown, but literature data and observations on the symptoms suggest that CPV is more effective in destroying a population. By the time being we consider the observed epidemics as the joint action of the two pathogens. To the build-up of the epidemics at least one year is required and the disease in the diapausing population is hardly noticeable.

The influence of pathogens can explain the diversity in the fluctuation of *L. botrana* populations in vineyards and varieties under the same weather and topographic conditions.

Acknowledgements

The authors are deeply indepted to Dr. C. VAGO for discussion and advices and to Dr. C. MEYNADIER for confirming the presence of CPV and for finding the Microsporidia in the sectioned materials (both authors: INRA Lab. of Comparative Pathology, St. Christol les Alés, France). Acknowledgement is made also to Dr. J. WEISER (Inst. of Insect Pathology of ČSAV, Prague) for his valuable discussion and criticism.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 16 (3—4), pp. 433—445 (1981)

Observations on Aphid Flight in Hungarian Orchards in 1978—1979

By

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The authors compared data gained on aphid flight in 1978 and 1979, in three fruit growing regions (Budapest, Nyírség, Kecskemét), by using yellow pan and suction traps.

Data are presented on the species composition and flight dynamics of aphids (mostly species known as vectors of plum pox) and also on other aphid populations observed in the orchards on weeds.

In course of the investigations 56 066 aphids were determined to species, 33 453 of which belonged to species known as virus vectors. The paper reports on the individual numbers of the most common 14 species, according to localities, trapping methods and years. Detailed graphs are given on the flight of *Aphis fabae* Scopoli, *Brachycaudus helichrysi* Kaltenbach, *Brevicoryne brassicae* Linnaeus, *Hyalopterus pruni* Geoffroy, *Myzus persicae* Sulzer, *Phorodon humuli* Schrank and *Rhopalosiphum padi* Linnaeus. A list is given also on the aphids collected by trapping, new for the Hungarian fauna.

Present studies aimed to yield data on the flight dynamics and species composition of aphids transmitting plum pox virus, well known as one of the most dangerous pathogens of plum, apricot and peach. This type of study was necessitated also by the fact that besides the general taxonomy and occurrence of aphids living in orchards and gardens (SZALAY-MARZSÓ, 1972) practically no, or only very few data were available on their flight activity either in Hungary or in the neighbouring countries.

The studies undertaken constituted a part of the 10-year complex research of apple ecosystems, carried out in the Research Institute of Plant Protection and some papers were already published or are at present in press on some results (MESZLENY, 1980; MESZLENY, in press; JENSER *et al.*, in press).

Material and Methods

Localities

The trapping studies were carried out in the following localities:

a) Experimental orchard of the Research Institute of Plant Protection (Nagykovácsi-Julianna major) in a 14-year-old, 5 hectare apple stand and in

neighbouring 3 hectare apricot and peach stand; the whole orchard was divided into a chemically treated and an untreated half. The suction trap and some yellow traps operated in the untreated plot. Both in and around the orchard variegated weed flora existed.

b) Intensively treated, 15–21-year-old, 100 hectare apple orchard of the Research Institute of Fruit- and Ornamental Plant Production, Újfehértó–Nyíregyháza. Both in the vicinity of the traps in the middle of the orchard and in the surrounding area the weed growth was very sparse, due to intensive treatment.

c) Intensively treated, 15–20-year-old, 50 hectare apricot orchard of the Research Institute of Fruit- and Ornamental Plant Production, Kecskemét-Szarkás. The orchard was surrounded by untreated orchards and cropland with abundant weed growth, while the soil of the experimental orchard was practically free of weeds.

Equipment and methods

The studies were carried out in all localities mentioned in the crown height of the trees, by using suction traps and yellow pans, described in an earlier paper (MESZLENY and SZALAY-MARZSÓ, 1979). So the method based on the colour perception of aphids (MOERICKE, 1950; SZALAY-MARZSÓ, 1964) was compared with suction trapping (TAYLOR, 1951). The advantage of the latter, non-selective method is obvious: the amount of insects caught in a trap filtering constant air masses may be directly related to the density of the aeroplancton moving in the air space of the given orchard.

The number of insects (aphids) caught and also their species composition are further determined by the height of the trap. While the 12.2 m high Rothamsted-type suction traps collected data on the aphids flying in greater heights over relatively large areas (TAYLOR and FRENCH, 1970), the trapping in the crown height aimed to collect data more closely related to the given orchard's fauna.

The suction traps were placed into open places between the tree rows by the end of April and operated until begin of November. The trap in Kecskemét-Szarkás could operate from technical reasons only from 1st August 1978. In the three localities mentioned 1–1 suction traps and 2–2 yellow pan traps were operated with the exception of Julianna major, where 8 yellow traps functioned.

In case of the yellow pan traps the average catch data per trap per day were used, the numbers brought up to round figures. The determination of the collected aphid material was carried out by stereomicroscope (25×). In case of injured specimens and rare species slide preparations were made. The determinations included 26 species (Table 3,) which are recorded as virus vectors (KENNEDY *et al.*, 1962) or which seemed to be important due to their high density. For determination the works of MÜLLER (1975, 1976), TAYLOR (manuscript) and SZELEGIEWICZ (1977, 1978) were used.

Meteorological data

In the evaluation of trapping data also the weather factors were considered. Table 1 shows the yearly amounts of precipitation in the three localities and the deviations of the 100 year's average. In the evaluations also the daily temperatures

Table 1

Precipitation total in 1978 and 1979 and deviations from the 100 year's average (Data of the Hung. Meteorological Institute)

Meteorological station	Precipitation total per year		Deviation of the 100 year's average, mm	
	1978	1979	1978	1979
Budapest-Szabadsághegy	597	568	+33	+62
Kecskemét	446	469	+142	+161
Nyíregyháza	564	552	+66	+78

and 5 day's precipitation data were considered, based on the data of the Hungarian Meteorological Institute.

Results

The summarized catch data of suction traps and yellow pan traps are shown in Table 2 and Fig. 1. The species recorded as virus vectors comprised 60–80 per cent of the total catch in all localities and years (with the exception of the catch of the trap in Kecskemét-Szarkás in 1979). In some cases the high individual number of virus vectors indicated also the possibility of direct damage, besides virus transmission.

The traps operated in 1978 and 1979 caught 56 066 aphids in total, 52 885 by the suction traps and 3191 by the yellow traps. The number of aphids determined to species was 31 289 in case of suction traps and 2164 specimen in yellow traps, indicating that the latter caught virus vectors to a higher ratio.

The summarized catch data for the most common species are shown in Table 3 and the flight dynamics of the most important ones are shown in Figs 2–8. The selection of the "most common" species was based on the occurrence data shown in Tables 4 and 5.

No detailed data were presented on the following species, which were nevertheless considered in the evaluation, but their individual number was low:

Acyrtosiphon pisum Harris
Amphorophora rubi Kaltenbach
Aulacorthum solani Kaltenbach
Brachycaudus cardui Linnaeus
Metopolophium dirhodum Walker

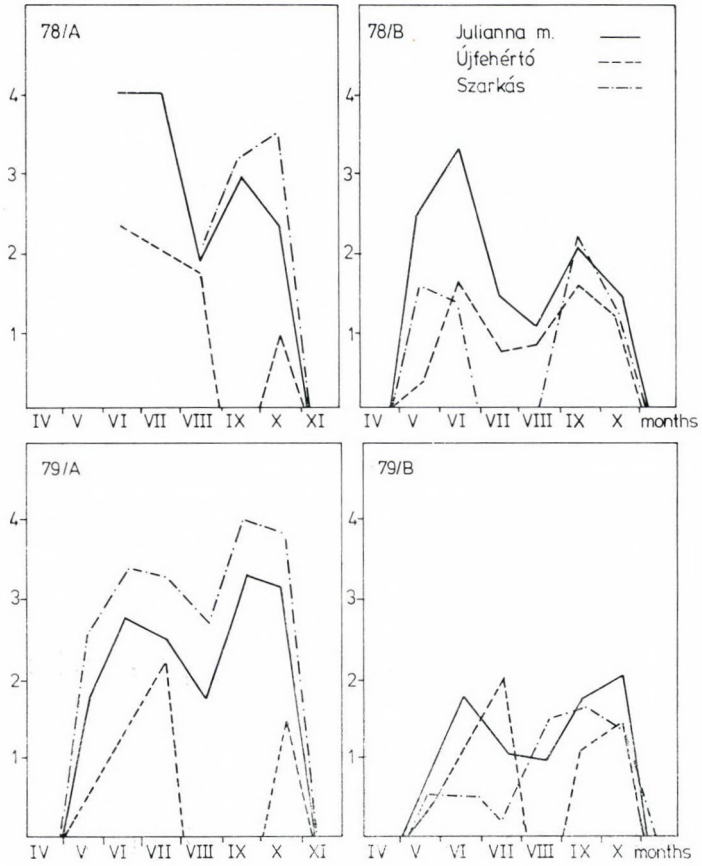

Fig. 1. Total individual number of aphids caught in (A) suction traps and (B) yellow pan traps, in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

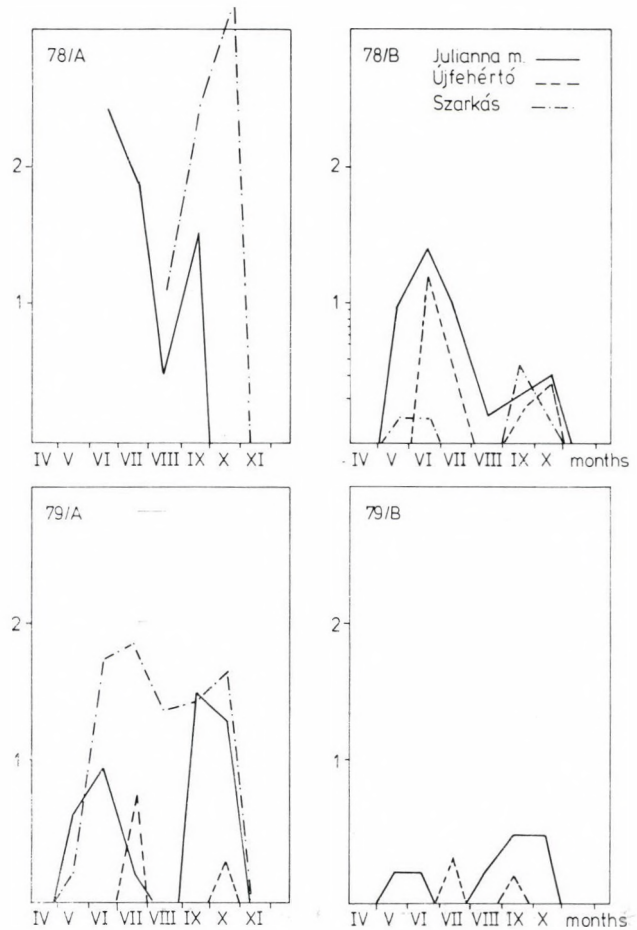

Fig. 2. Monthly individual numbers of *Aphis fabae* caught in (A) suction traps and (B) yellow traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

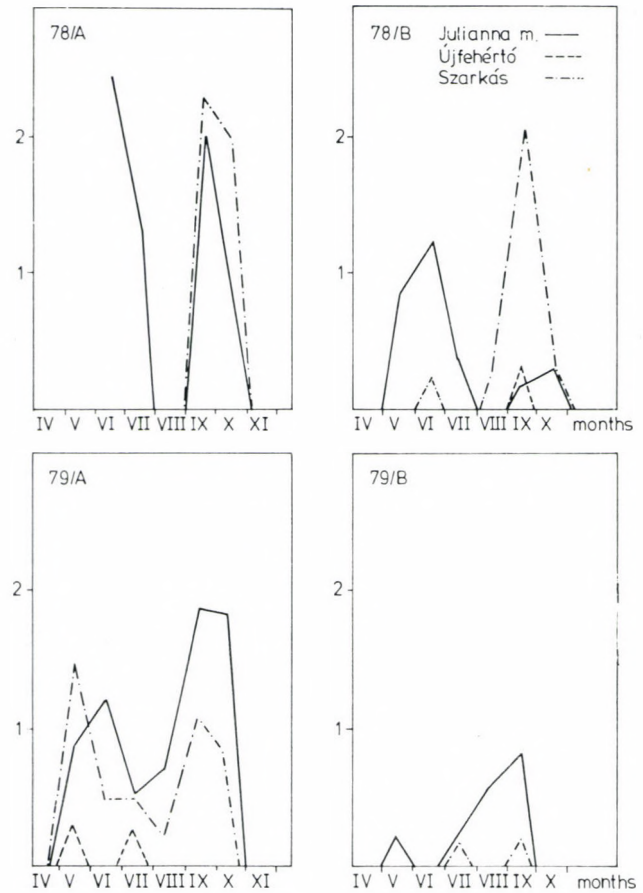

Fig. 3. Monthly individual numbers of *Brachycaudus helichrysi* caught in (A) suction traps,(B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

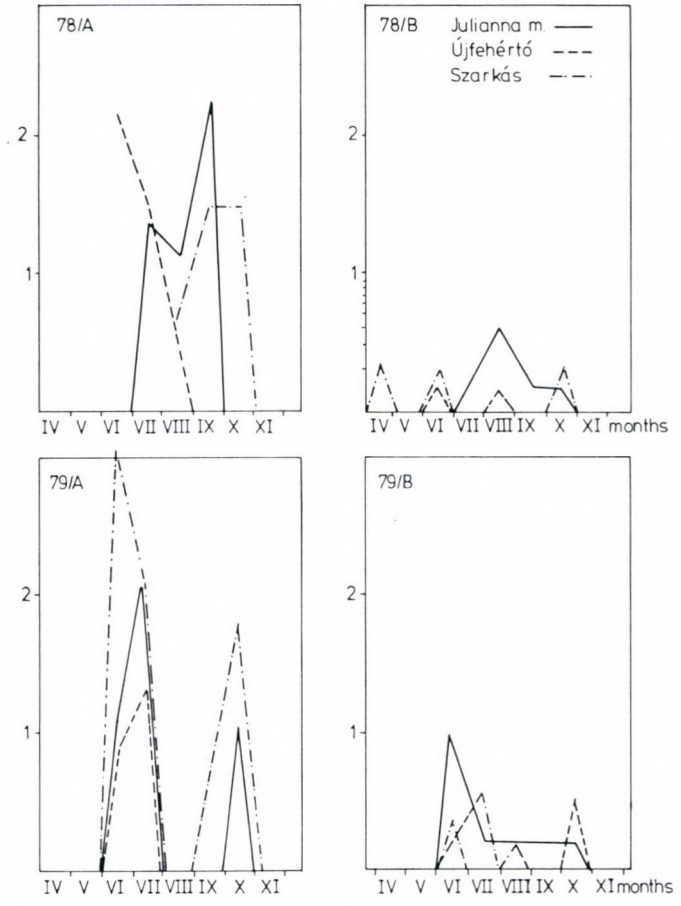

Fig. 4. Monthly individual numbers of *Brevicoryne brassicae* caught in (A) suction traps, (B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

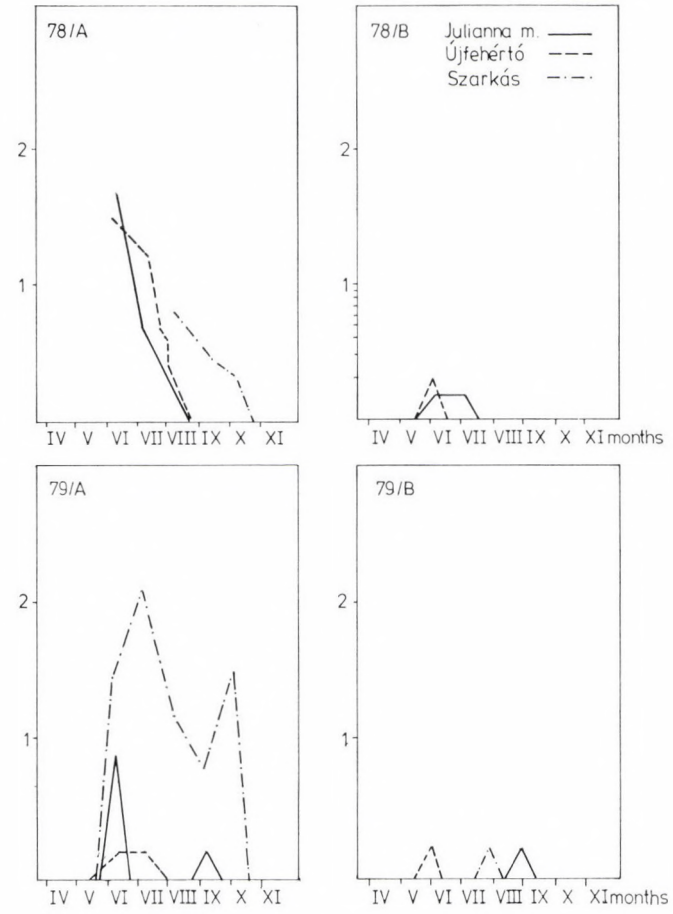

Fig. 5. Monthly individual numbers of *Hyalopterus pruni* caught in (A) suction traps, (B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

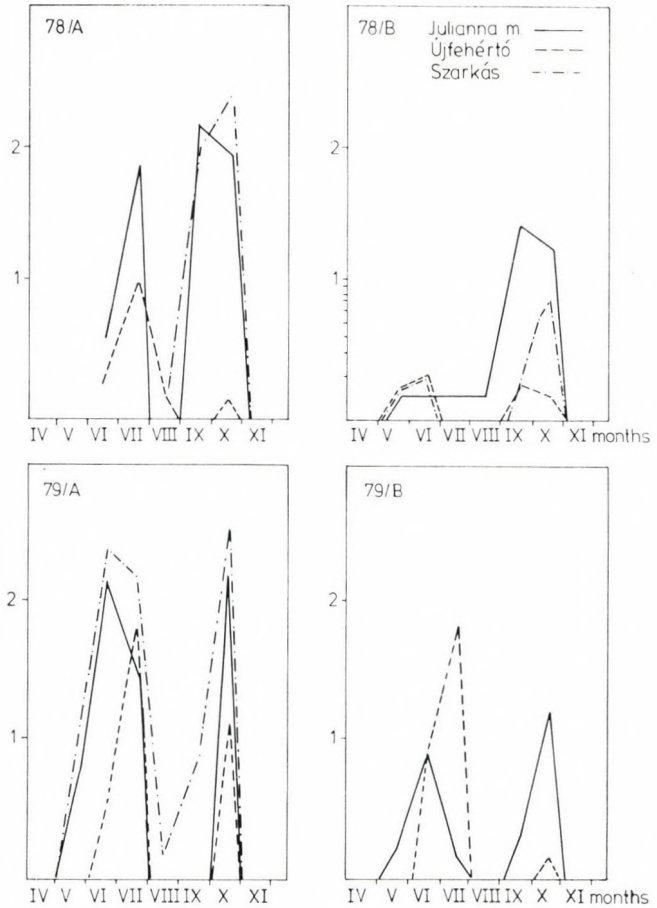

Fig. 6. Monthly individual numbers of *Myzus persicae* caught in (A) suction traps, (B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

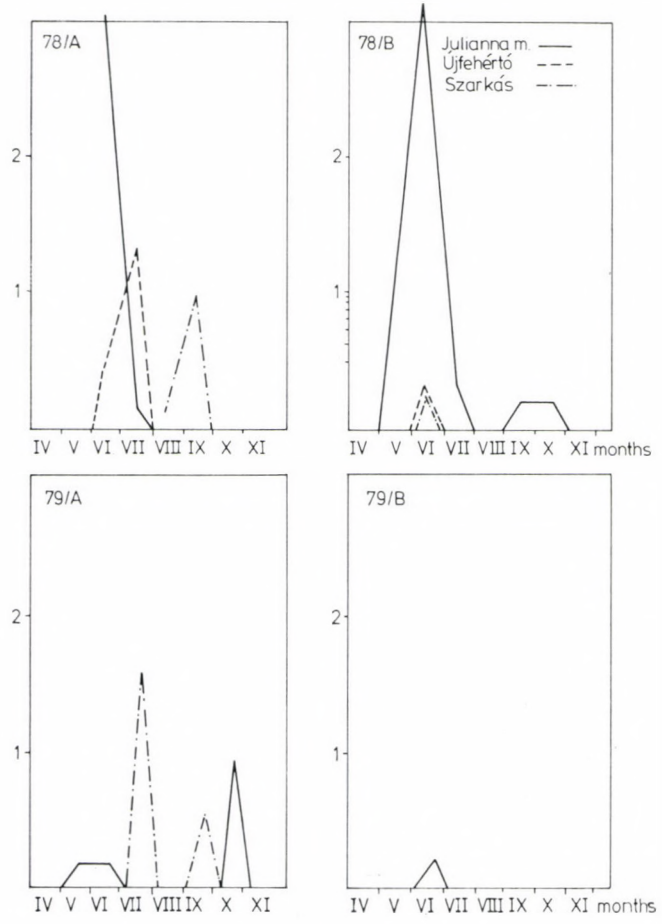

Fig. 7. Monthly individual numbers of *Phorodon humuli* caught in (A) suction traps, (B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

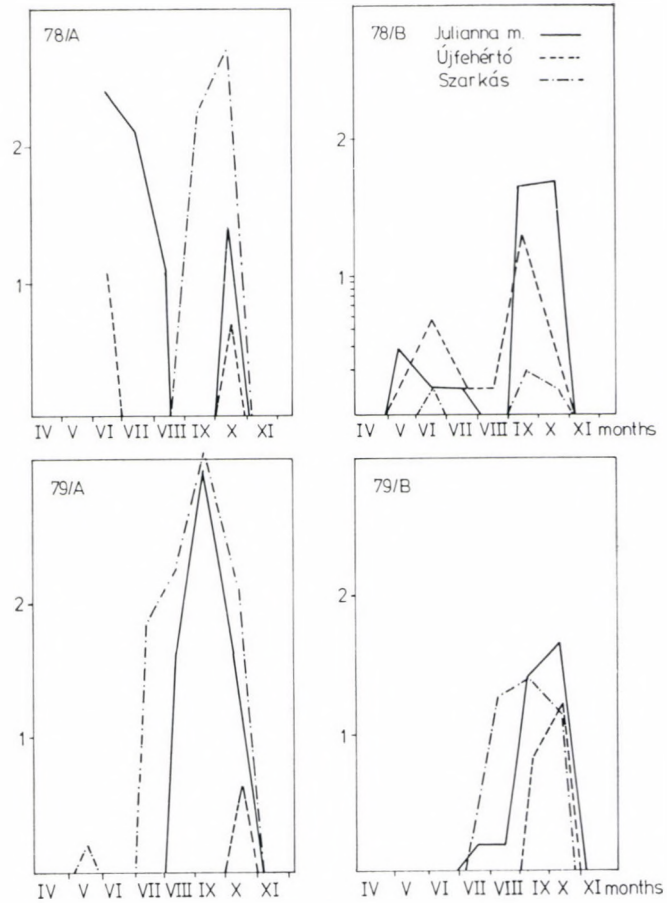

Fig. 8. Monthly individual numbers of *Rhopalosiphum padi* caught in (A) suction traps, (B) in yellow pan traps in the years 1978 and 1979. Ordinate: logarithms of individual numbers per month

Table 2

Totals of aphid numbers caught in suction traps and yellow traps in 1978 and 1979 (the traps operated from June to November in 1978, in Szarkás only from August to November)

		1978		1979	
		Suction trap	Yellow trap	Suction trap	Yellow trap
Julianna major	Total catch	22 273	2385	4 690	239
	Determined to species	16 446	1606	3 122	198
	Others %	26.2%	32.7%	33.4%	17.1%
Újfehértó	Total catch	379	100	224	151
	Determined to species	338	83	199	15
	Others	10%	17%	11.1%	10%
Kecskemét-Szarkás	Total catch	4 382	203	20 937	103
	Determined to species	3 179	172	8 005	70
	Others %	27.5%	15.2%	61.8%	32%

Myzus cerasi Fabricius
Myzus varians Davidson
Nasonovia ribisnigri Mosley
Rhopalosiphum insertum Walker
Rhopalosiphum maidis Fitch
Schizaphis graminum Rondani

As regards the flight dynamics of the different species, the following can be stated:

a) It could be observed that the species *Rhopalosiphum padi* Linnaeus and *Myzus persicae* Sulzer belonged nearly in all cases to the most frequently caught aphids and as shown in Figures 6 and 8 their presence in the air space of the orchards was practically continuous. This fact is most important for the latter, especially dangerous virus vector.

The occurrence of *Rh. padi* is therefore especially interesting, as the species is not recorded among the ones attracted by the yellow colour (EASTOP, 1955; SZELEGIEWICZ, personal communication, 1980). In spite of that, in Újfehértó in 1978, in Julianna major and Kecskemét-Szarkás in 1979 this species was caught in the highest numbers in the yellow traps (Tables 4 and 5).

These high numbers were most likely connected with the cereals grown in the vicinity of the orchards and may be also with the trapping method, as accord-

ing to ROBERT *et al.* (1974) the species was caught in high numbers in yellow pan traps in France, operated 70 cm high above soil level.

b) The suction trap collected in Julianna major in June–July of 1978 high numbers of *Aphis* sp. (very likely belonging to *Aphis pomi* Degeer and *Dysaphis plantaginea* Passerini (Table 3). This could be easily explained by the high indi-

Table 3

Total catches of most common aphid species in suction traps and yellow pan traps in 1978, 1979

Species	1978					
	Suction trap			Yellow trap		
	Julianna major	Újfehértó	Szarkás	Julianna major	Újfehértó	Szarkás
Aphis spp.	11 335	71	250	44	1	3
A. fabae	373	—	1363	46	20	5
B. helichrysi	357	—	290	15	2	89
B. brassicae	149	141	67	7	1	1
C. eleagni	52	13	—	8	—	—
C. aegopodii	198	—	—	38	—	20
D. plantaginea	1 305	—	—	3	—	1
H. pruni	50	49	11	1	2	—
H. lactucae	—	—	12	5	—	1
M. persicae	305	14	337	45	5	13
P. humuli	1 721	29	11	1257	2	1
T. trifolii	—	—	42	8	—	—
R. padi	432	17	770	97	31	1
S. avenae	—	—	—	—	—	—

Species	1979					
	Suction trap			Yellow trap		
	Julianna major	Újfehértó	Szarkás	Julianna major	Újfehértó	Szarkás
Aphis spp.	12	10	200	3	1	3
A. fabae	66	8	231	13	3	—
B. helichrysi	170	3	61	10	—	1
B. brassicae	155	31	1290	13	7	2
C. eleagni	—	5	476	1	—	—
C. aegopodii	17	—	164	1	—	—
D. plantaginea	2	—	—	2	—	—
H. pruni	9	2	228	1	2	1
H. lactucae	74	1	23	2	1	—
M. persicae	567	93	930	26	62	1
P. humuli	12	—	43	1	—	—
T. trifolii	85	2	857	3	2	—
R. padi	1412	5	2319	60	24	60
S. avenae	388	24	966	3	2	1

vidual densities of these two species, causing economic damage in the given period (MESZLENY and SZALAY-MARZSÓ, 1979). It is still without explanation, why these species were not caught by yellow traps operated nearby.

c) Table 4 and Fig. 7 show the spring flight of *Phorodon humuli* Schrank in Julianna major in 1978, which may have been connected with the high population density of this species in the neighbouring plum orchard. Later never occurred these high densities, in accordance with the personal communication of H. SZELEGIEWICZ (1980), who characterized both *P. humuli* and *Metopolophium dirhodum* as migrating high and to long distances, therefore caught only in high suction traps.

d) As shown in Tables 4 and 5 and in Figs 3 and 4, the traps caught *Brachycaudus helichrysi* Kaltenbach and *Brevicoryne brassicae* Linnaeus in considerable numbers in 1978 and 1979, with the exception of Újfehértó where *B. helichrysi* was caught in both years in very low numbers.

e) It was remarkable that the traps caught practically no alatae of *Brachycaudus cardui* and *Myzus varians*, although their colonies have been found in Julianna major both on plum and on peach. The absence of *Sitobion (Macro-*

Table 4

Order of sequence of most common aphid species, as ranged according to their frequencies in the trap catches in 1978

	No.	Julianna major	Individual number	No.	Újfehértó	Individual number	No.	Szarkás	Individual number
Suction trap	1.	*Aphis* spp.	11 335	1.	*B. brassicae*	141	1.	*A. fabae*	1663
	2.	*P. humuli*	1 721	2.	*A. fabae*	71	2.	*R. padi*	770
	3.	*D. plantaginea*	1 305	3.	*H. pruni*	49	3.	*M. persicae*	337
	4.	*R. padi*	432	4.	*P. humuli*	29	4.	*B. helichrysi*	290
	5.	*A. fabae*	373	5.	*R. padi*	17	5.	*Aphis* spp.	250
	6.	*B. helichrysi*	357	6.	*M. persicae*	14	6.	*B. brassicae*	67
	7.	*M. persicae*	305						
	8.	*C. aegopodii*	198						
	9.	*B. brassicae*	149						
Yellow trap	1.	*P. humuli*	1 247	1.	*R. padi*	33	1.	*B. helichrysi*	112
	2.	*R. padi*	99	2.	*A. fabae*	22	2.	*C. aegopodii*	20
	3.	*M. persicae*	47	3.	*A. solani*	9	3.	*M. persicae*	13
	4.	*Aphis* spp.	46	4.	*M. persicae*	6	4.	*A. fabae*	7
	5.	*A. fabae*	46				5.	*B. brassicae*	6
	6.	*C. aegopodii*	39				6.	*Aphis* spp.	5
	7.	*B. helichrysi*	29						

Species not mentioned in the text: *Dysaphis plantaginea* PASSERINI, *Cavariella aegopodii* SCOPOLI, *Aulacorthum solani* KALTENBACH

Table 5

Order of sequence of most common aphid species, as ranged according to their frequencies in the trap catches in 1978

	No.	Julianna major	Individual number	No.	Újfehértó	Individual number	No.	Szarkás	Individual number
Suction trap	1.	*R. padi*	1412	1.	*M. persicae*	93	1.	*R. padi*	2319
	2.	*M. persicae*	567	2.	*B. brassicae*	31	2.	*B. brassicae*	1290
	3.	*S. avenae*	388	3.	*S. avenae*	24	3.	*S. avenae*	966
	4.	*B. helichrysi*	170	4.	*Aphis* spp.	10	4.	*M. persicae*	930
	5.	*B. brassicae*	155	5.	*A. fabae*	8	5.	*T. trifolii*	857
	6.	*H. lactucae*	74	6.	*A. craccivora*	7	6.	*C. eleagni*	476
							7.	*H. pruni*	228
							8.	*A. fabae*	231
Yellow trap	1.	*R. padi*	69	1.	*M. persicae*	79	1.	*R. padi*	57
	2.	*M. persicae*	29	2.	*R. padi*	24	2.	*Aphis* spp.	5
	3.	*B. brassicae*	14	3.	*B. brassicae*	8	3.	*B. brassicae*	3
	4.	*B. helichrysi*	13	4.	*R. insertum*	5	4.	*B. helichrysi*	2
	5.	*A. fabae*	9	5.	*S. avenae*	4			
	6.	*S. avenae*	9	6.	*A. fabae*	4			

Species not mentioned in the text: *Hyperomyzus lactucae* LINNAEUS, *Aphis craccivora* KOCH, *Therioaphis trifolii* MONELL, *Capitophorus eleagni* DEL GUERCIO, *Rhopalosiphum insertum* WALKER

siphum) avenae Fabricius from the catches in 1978 was also remarkable, as its host plants were grown in the area of orchards.

For the latter the literature data may give some explanation. In the wheat-growing region of France (Bretagne) ROBERT *et al.* (1976) caught the species in low numbers in the yellow pan traps placed 70 cm high, whereas in the same period in England it has been caught in series of many thousands in the 12.2 m high suction towers (TAYLOR and FRENCH, 1977). So it is quite possible that this species belongs also to the ones migrating in greater height.

Conclusion

By representing the monthly total catches, typical curves with maxima in the early summer (end of May-middle of June) and in the autumn (between 1 and 15 of September) were drawn (Fig. 1). It has to be mentioned that the years studied showed considerable differences from the average ones, being cooler and with amounts of precipitation deviating from the 100 year's mean (Table 1). The aphids flying in the first period mentioned belonged mostly to the emigrantes of the fundatrigenae, while in the autumn maximum mostly gynopara and males par-

ticipated, returning to the winter hosts. This corresponded to earlier data (SZALAY-MARZSÓ, 1964; TAYLOR, 1973).

The comparison of the two trap types is only relative, as they differ considerably both in their principle and selectivity. It could be observed that both types caught the alatae of *Myzus persicae* but the yellow traps collected nearly no or very few *Brevicoryne brassicae*, *Hyalopterus pruni* and *Phorodon humuli* (with the exception of the latter in Julianna major in 1978), so it may be concluded that these are hardly attracted by the yellow colour.

The use of yellow traps can be nevertheless recommended, due to their advantages (they are cheap, easy to handle and collect important virus vectors). It is, however, advisable to operate simultaneously suction traps to control the catch data.

Our data showed differences from the literature data on the collection of some virus vectors (e.g. *Myzus persicae*) which flew in higher numbers in the crown height of the fruit trees than indicated by the Rothamsted-type traps. According to other data (LECLANT, 1978), the numbers of *Myzus persicae* were inferior to other species in vegetable plots, trapped at soil level. The species yielding the highest numbers, *Rhopalosiphum padi*, was predominant both in our suction traps and in the traps of 12.2 m (TAYLOR, 1973; RUSZKOWSKA and ZLOKOWSKI, 1977).

As a conclusion we may venture that the study of virus vectors of a given plant species yields the best results with trapping carried out in a height corresponding to the plant. To draw general conclusions on the aphid flight of larger geographical areas, however, the higher, Rothamsted-type traps seem to be necessary. The data furnished by the latter give more reliable basis for comparison, utilizable even in neighbouring territories and countries.

Further, it has to be mentioned that even the best trapping method has to be completed by surveys carried out both in the cultivated plant stand (the virus vectors of which necessitated the study) and in the neighbouring region, with special regard to the weeds harbouring sometimes populations of high individual numbers. So in the seemingly inactive periods (mid-summer) when only few alatae were caught in the traps, the dangerous virus vector *Myzus persicae* occurred in high densities in the orchards studied, on young *Convolvulus arvensis* plants.

As an appendix it may be mentioned also that besides the virus vectors many uncommon species or even ones new for the Hungarian fauna were collected in the traps (for their determination the authors are indebted to Dr. F. P. MÜLLER (Rostock) and Dr. H. SZELEGIEWICZ (Warsaw):

Cavariella pastinacae Linnaeus
Cryptomyzus galeopsidis Kaltenbach
Drepanosiphum dixoni Hille Ris Lambers
Hyperomyzus pallidus Hille Ris Lambers
Macrosiphoniella oblonga Mordvilko
Microlophium carnosum Buckton

Myzus ascalonicus Doncaster
Myzus lythri Schrank
Thelaxes dryophila Westwood
Uroleucon cichorii Koch
Uroleucon tussilaginis Walker

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HU ISSN 0001—6780
Index: 26.032