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PESTICIDE CHEMISTRY

Acta Phytopathologica et Entomologica Hungarica, 23 (1–2), pp. 3–10 (1988)

Variations in Sensitivity to Benzimidazole and Non-Benzimidazole Fungicides of Genetically Different Benomyl Resistant *Fusarium oxysporum* Strains

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Benomyl resistance in *Fusarium oxysporum* is controlled by two genes, *ben-1* and *ben-2*. These genes, if present separately, allow a low level of benomyl resistance, but their synergistic interaction results in a highly resistant phenotype. In cross-resistance tests the two benomyl-resistance genes showed, in general, similar manifestations. However, when less effective benzimidazoles (fuberidazole, thiophanate-methyl) were tested, the resistance controlled by *ben-2* proved to be significantly higher than that controlled by *ben-1*. Furthermore, strains that carried the gene *ben-1* showed increased sensitivity to morpholine compounds (fenpropimorph, tridemorph). This negative cross resistance indicates, that the mechanism by which *ben-1* regulates benzimidazole resistance is not identical with that displayed by *ben-2*. The potential reasons of the negative cross-resistance between benzimidazoles and morpholines are discussed.

Shortly after the worldwide extensive use of systemic benzimidazole fungicides, in the early seventies cases of pathogens' resistance to these chemicals were repeatedly observed. Benzimidazoles are very effective against wilt causing *Fusarium* species, but resistance may also develop in these fungi (*cf.* Magie and Wilfret, 1974). Several experiments were performed on the benzimidazole resistance of different fusaria (Thanassoulopoulos et al., 1971; Bartels-Schooley and MacNeill, 1971; Valásková, 1976; Leski, 1977; Sozzi and Gessler, 1980; Seppänen, 1981; Hornok, 1983) and in some of these studies the cross-resistance to chemically related toxicants was also examined.

Experiments on the cross-resistance relationships are useful for several reasons: genetical background of the resistance can thus be investigated, mode of action studies can be completed and alternative fungicide programmes can be proposed.

In a recent study (Molnár et al., 1985) an interesting genetical control of benomyl resistance was demonstrated in *Fusarium oxysporum* Schlecht. Contrary to other fungi, in this species the high level of benomyl resistance was determined by the synergistic (or additive) interaction of two genes *(ben-1, ben-2)*. We presumed these two genes to be β-tubulin ones, but this is only a probability. In order to obtain further information on the nature of the two benomyl-resistance genes, cross-resistance to benzimidazole and non-benzimidazole fungicides was

investigated. Different strains, which carried *ben-1* or *ben-2* gene, or both or neither of these genes were tested for growth in the presence of benomyl, carbendazim, thiophanate-methyl, thiabendazole, fuberidazole (benzimidazole fungicides), dichlofluanid, dodine, tridemorph, fenpropimorph, triadimenol, and folpet (non-benzimidazole fungicides).

Materials and Methods

The parental strain was *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder et Hansen race 1, IMI 141 140. Auxotrophic mutants, benomyl resistant mutants, and hybrids were produced as described earlier (Molnár et al., 1985). Strains used in the present study are listed in Table 1.

Fungitoxicity tests were carried out on a medium containing salts of the standard Czapek solution plus glucose (20.0 g litre^{-1}), agar (20.0 g litre^{-1}) and amino acid requirements for the auxotrophs (L-methionine and DL-tryptophan,

Table 1

List of *Fusarium oxysporum* strains used in fungitoxicity test

Strain number	Phenotype characters	Genotype symbols	Origin
IMI 141 140	wild-type, benomyl sensitive	—	Dr. C. Booth (CMI, Kew, England)
I-5	methionine dependent, benomyl sensitive	*met*$^-$	obtained from IMI 141 140 after u.v.-irradiation
I-28	tryptophan dependent, benomyl sensitive	*trp*$^-$	obtained from IMI 141 140 after u.v.-irradiation
I-5-14	methionine dependent, moderately benomyl resistant	*met*$^-$, *ben-2*	obtained from I-5 after u.v.-irradiation
I-28-6c	tryptophan dependent, moderately benomyl resistant	*trp*$^-$, *ben-1*	obtained from I-28 after u.v.-irradiation
I-28-11a	tryptophan dependent, moderately benomyl resistant	*trp*$^-$, *ben-2*	obtained from I-28 after u.v.-irradiation
H3	prototrophic hybrid, highly benomyl resistant	*ben-1*, *ben-2*	obtained from hybridization between I-5-14 and I-28-6c
H19	prototrophic hybrid, benomyl sensitive	—	obtained from hybridization between I-5-14 and I-28-6c
H62	prototrophic hybrid, moderately benomyl resistant	*ben-1* or *ben-2*	obtained from hybridization between I-5-14 and I-28-6c

Table 2

Name and formulation of the fungicides used

Common name	Trade name	Formulation	Producer company
Benomyl	Fundazol	50% WP	Chinoin, Budapest
Carbendazim	Kolfugo extra	25% WP	Chinoin, Budapest
Fuberidazole	—	active substance was used	—
Thiabendazole	—	active substance was used	—
Thiophanate-methyl	Topsin-M	70% WP	Nippon Soda Co.
Dichlofluanid	Euparen	50% SP	Bayer AG
Dodine	Carpene	65% WP	American Cyanamid Co.
Fenpropimorph	Corbel	75% EC	BASF AG
Folpet	Folpan	50% WP	Makhteshim, Israel
Triadimenol	Baytan	15% SP	Bayer AG
Tridemorph	Calixin	75% EC	BASF AG

50 and 200 mg litre^{-1}, respectively). Fungicides (Table 2) in different concentrations were added to molten agar medium as a suspension in acetone; the final acetone concentration in the medium never exceeded 1% (v/v). The fungicide activity to different strains was determined by measuring radial colony growth after incubation at 25 °C for 72 h. For comparison, radial growth on different concentrations of the fungicides were expressed as percentages of the growth without fungicides. ED_{50} (i.e., the fungicide concentration causing 50% reduction in radial growth) and MIC values (minimal inhibitory concentration, where no growth occurred) were calculated by means of a curve-fitting method based on log/logistic function, where the proper curve-fitting was used by plotting values on a log/probit graph paper (Oros and Virányi, 1987). Significant differences were calculated by means of F-test (bivariance analysis) at the 0.1% probability level.

Results and Discussion

Table 3 presents the results of tests on cross-resistance of four benomyl sensitive strains (IMI 141 140, I-5, I-28, H19), four moderately benomyl resistant strains (I-5-14, I-28-6c, I-28-11a, H62, which carry *ben-1* or *ben-2* gene), and one highly benomyl resistant strain (H3, which carries both genes) to benzimidazole compounds.

The most effective benzimidazole fungicides were benomyl and carbendazim, with ED_{50} and MIC values for the sensitive strains in the range of 0.38–0.62 and 2–5 μg ml^{-1}, respectively; for the strains with moderate degree of resistance

Table 3

Fungitoxicity of benzimidazole and non-benzimidazole

Fungicides		Strains			
		IMI 141 140	I-5	I-28	I-5-14
Benomyl	ED_{50}	0.50[a]	0.43[a]	0.37[a]	1.48[b]
	MIC	2–5[a]	2–5[a]	2–5[a]	20–50[b]
Carbendazim	ED_{50}	0.42[a]	0.38[a]	0.40[a]	2.59[b]
	MIC	2–5[a]	2–5[a]	2–5[a]	10–20[b]
Fuberidazole	ED_{50}	2.32[a]	3.09[a]	2.28[a]	16.84
	MIC	10–20[a]	10–20[a]	10–20[a]	100–200
Thiabendazole	ED_{50}	1.18[a]	1.11[a]	1.36[a]	4.19[b]
	MIC	4–6[a]	4–6[a]	4–6[a]	20–50[b]
Thiophanate methyl	ED_{50}	10.77[a]	9.51[a]	6.87[a]	975.28
	MIC	50–100[a]	50–100[a]	50–100[a]	10 000–20 000
Dichlofluanid	ED_{50}	1.25[a]	0.82[a]	1.22[a]	1.22[a]
	MIC	500–1 000[a]	500–1 000[a]	500–1 000[a]	500–1 000[a]
Dodine	ED_{50}	52[a]	43[a]	43[a]	91[a]
	MIC	2 000–5 000[a]	5 000–10 000[a]	5 000–10 000[a]	5 000–10 000[a]
Fenpropimorph	ED_{50}	30.86[b]	22.08[b]	32.41[b]	16.82[b]
	MIC	50 000–100 000[b]	50 000–100 000[b]	50 000–100 000[b]	50 000–100 000[b]
Folpet	ED_{50}	67[a]	38[a]	37[a]	34[a]
	MIC	2 000–5 000[a]	2 000–5 000[a]	2 000–5 000[a]	2 000–5 000[a]
Triadimenol	ED_{50}	6.06[a]	6.37[a]	9.51[a]	14.20[a]
	MIC	500–1 000[a]	500–1 000[a]	500–1 000[a]	500–1 000[a]
Tridemorph	ED_{50}	82[b]	27[b]	100[b]	26[b]
	MIC	100 000[a]	100 000[a]	100 000[a]	100 000[a]

ED_{50} and MIC values are expressed as μg active ingredient ml^{-1}.
[a, b, c] Refer to significant differences between the strains.

to benomyl these values were some 4- to 10-times higher and a further 10-fold increase in the resistance level was measured in the highly resistant strain (H3). Benomyl and carbendazim expressed thus the same trends of toxicity towards the strains tested. This finding contrasts with that of Leski (1977), who found that carbendazim was much more effective than benomyl against the sensitive strains of *Fusarium oxysporum* f. sp. *dianthi* (Prill et Del.) Snyder et Hansen, whereas the latter proved significantly more active against the resistant strains. Fuberidazole and thiophanate-methyl were considerably less fungitoxic both to sensitive and tolerant strains, which confirms results of former reports (Valásková, 1976; Leski, 1977). Thiophanate-methyl expressed the poorest effect with MIC values 10- to 500-times higher, than those of benomyl and carbendazim. Thiaben-

fungicides to *Fusarium oxysporum* strains

I-28-6c	I-28-11a	H3	H19	H62
1.36[b] 20–50[b]	4.99[b] 20–50[b]	17.59[c] 500–1 000[c]	0.62[a] 2–5[a]	1.42[b] 20–50[b]
1.49[b] 10–20[b]	2.28[b] 10–20[b]	62.01[c] 500–1 000[c]	0.44[a] 2–5[a]	1.24[b] 10–20[b]
9.57[b] 100–200[b]	40.15 200–500	63.37[c] 500–1 000[c]	2.49[a] 10–20[a]	14.78[b] 100–200[b]
5.94[b] 50–100[b]	18.37[b] 100–200[b]	32.32[c] 500–1 000[c]	1.11[a] 5–10[a]	3.68[b] 50–100[b]
173.43[b] 1 000–2 000[b]	840.87 10 000–20 000	5000.0[c] > 50 000[c]	11.39[a] 100–200[a]	132.63[b] 1 000–2 000[b]
1.22[a] 500–1 000[a]	1.42[a] 500–1 000[a]	1.92[a] 500–1 000[a]	3.50[a] 1 000–2 000[a]	1.57[a] 500–1 000[a]
50[a] 5 000–10 000[a]	29[a] 5 000–10 000[a]	78[a] 10 000–20 000[a]	88[a] 10 000–20 000[a]	78[a] 10 000–20 000[a]
3.50[a] 50 000–100 000[b]	14.50[b] 50 000–100 000[b]	5.00[a] 50 000–100 000[b]	26.26[b] 50 000–100 000[b]	12.22[a] 50 000–100 000[b]
40[a] 2 000–5 000[a]	34[a] 2 000–5 000[a]	50[a] 2 000–5 000[a]	48[a] 5 000–10 000[a]	80[a] 5 000–10 000[a]
9.51[a] 500–1 000[a]	7.98[a] 500–1 000[a]	13.50[a] 500–1 000[a]	10.00[a] 500–1 000[a]	10.51[a] 500–1 000[a]
18[a] 100–000[a]	45[a] 100 000[a]	16[a] 100 000[a]	26[b] 100 000[a]	10[a] 100 000[a]

dazole showed an intermediate level of fungitoxicity. As regards the strains used in this study, no significant effect of the auxotrophy on benzimidazole sensitivity/resistance was observed. The only important fact in this respect was the presence or absence of the benomyl-resistance gene(s) in the given strain.

The results clearly show, that our benomyl resistant mutants (I-5-14, I-28-6c, I-28-11a) obtained after ultraviolet irradiation and the highly resistant or moderately resistant hybrids (H3, H62) obtained by protoplast-fusion of the moderately resistant parental strains were also resistant to all the other four benzimidazoles tested. Complete and clear cross-resistance was demonstrated between benomyl and carbendazim, which is not surprising since the latter is the active breakdown product of the former.

There was also a regular cross-resistance between benomyl and thiabendazole, i.e. the main groups of strains with different degree of resistance to benomyl can also be identified regarding their response to thiabendazole. The negative cross-resistance between benomyl and thiabendazole found by Van Tuyl (1977) in certain fungi *(Aspergillus nidulans, A. niger, Penicillium expansum, Rhodotorula rubra, Ustilago maydis)* was not observed in our experiments.

Benomyl resistance conferred simultaneous tolerance to fuberidazole and thiophanate-methyl, too. The only inconsistency of this cross-resistance occurred in those moderately benomyl resistant strains that carried the gene *ben-2* (I-5-14 and I-28-11a). These strains were significantly more resistant to fuberidazole and thiophanate-methyl than the *ben-1* mutant (I-28-6c) and the degree of resistance of the formers approximated that of the superresistant *(ben-1 + ben-2)* hybrid (H3). (In the hybrid, however, an extremely high degree of resistance could be detected as a result of the additive interaction of the two benomyl-resistance genes.) We explain this phenomenon by a small difference which might exist between the products of the two benomyl-resistance genes. According to literature data (Sheir-Neiss et al., 1978; Neff et al., 1983; Hiraoka et al., 1984) the benomyl-resistance genes encode abnormal β-tubulins with decreased benomyl binding capacity and, because of the poor binding, the fungicid is not able to express its full toxicity. The two benomyl-resistance genes of *F. oxysporum* show similar manifestations in the presence of highly active benzimidazoles (benomyl, carbendazim), but when less effective benzimidazoles (fuberidazole, thiophanate-methyl) were under test the superiority of the *ben-2* gene became obvious.

In the second part of Table 3 results of fungitoxicity tests with non-benzimidazole compounds are summarized. These chemicals showed weak or moderate toxic effect on *Fusarium oxysporum* strains. Their general efficiency decreased in the following order: dichlofluanid > triadimenol > fenpropimorph > folpet > > dodine > tridemorph. The non-benzimidazole fungicides expressed, in general a uniform toxicity on the different strains regardless of their genotypes determining benomyl resistance/sensitivity. However, the morpholine compounds (fenpropimorph and tridemorph) seemed to show an increased toxicity towards those strains that carried the gene *ben-1*.

In order to prove this negative cross-resistance further test series were carried out with four strains of each of the following groups: 1) highly benomyl resistant hybrids that carried both *ben-1* and *ben-2* genes (superresistant recombinants from crosses between strains I-5-14 and I-28-6c); 2) moderately benomyl resistant hybrids that carried the gene *ben-1* only (these were obtained from crosses between strains I-5 and I-28-6c); 3) moderately benomyl resistant hybrids that carried the gene *ben-2* only (from crosses between strains I-28 and I-5-14); 4) benomyl sensitive hybrids containing no benomyl-resistance gene (sensitive recombinants from crosses between strains I-5-14 and I-28-6c). All these strains were tested for sensitivity towards tridemorph, fenpropimorph, triadimenol and dodine. The morpholines are known as multi-site inhibitors (Dekker, 1985) affecting C-14 demethylation or other steps of the sterol biosynthesis pathway (Kerkenaar et al.,

Table 4

ED_{50} values of selected fungicides for genetically different hybrid groups

Fungicides	Hybrid groups*			
	1	2	3	4
Benomyl	13.91[c]	1.24[b]	3.25[b]	0.49[a]
Carbendazim	43.04[c]	1.15[b]	2.59[b]	0.41[a]
Dodine	63.32[a]	46.08[a]	60.12[a]	58.61[a]
Fenpropimorph	4.27[a]	8.19[a]	29.32[b]	27.90[b]
Triadimenol	9.55[a]	8.42[a]	9.81[a]	8.14[a]
Tridemorph	11.43[a]	16.11[a]	54.95[b]	58.87[b]

* For details see the text.
[a, b, c] Refer to significant differences between the groups.

1981; Kato et al., 1980) and inhibiting cell membrane functions (Fischer, 1974). Triadimenol and dodine were involved in this test because they have certain inhibitory functions common with those of morpholines, i.e. triadimenol inhibits sterol biosynthesis (Buchenauer, 1978) and dodine affects mitochondrial membranes (Pressman, 1963; Brown and Sisler, 1960).

Table 4 shows the results of this test. As can be seen, tridemorph and fenpropimorph expresses significantly higher toxicity on strains containing the gene *ben-1*, while triadimenol and dodine affect similarly all groups regardless of benomyl resistance genotypes. From this we conclude, that a negative cross-resistance does exist between benzimidazoles and morpholines but in that case only, if benomyl resistance is controlled by the gene *ben-1*. The mechanism by which *ben-1* regulates benzimidazole resistance is thus not identical with that of displayed by *ben-2*. *Ben-1* causes physiological alterations which result in increased benzimidazole and decreased morpholine resistance, but these alterations affect neither sterol biosynthesis, nor mitochondrial electron transport. If *ben-1* encodes abnormal β-tubulin, resistant to benzimidazoles, this protein might be at the same time responsible for the increased morpholine sensitivity. If so, morpholines, while inhibiting sterol biosynthesis and affecting mitochondrial membranes have an additional site of action related to protein metabolism of the sensitive cells.

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Effect of Tridemorph on *Phytophthora infestans* and *Plasmopara halstedii*

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A comparative study was carried out on the effect of tridemorph on the various developmental stages of *Phytophthora infestans* and *Plasmopara halstedii* within their life cycle. The otherwise potent fungicide tridemorph exhibited a very moderate toxicity to cell wall bearing structures of these fungi. The wall-free zoospores, however, were exclusively sensitive to the compound.

The Oomycetes, which include such fungi of economic importance as *Phytophthora infestans* (Mont.) de Bary, the causative agent of potato late blight and *Plasmopara halstedii* (Farl.) Berlese et de Toni, the downy mildew fungus of sunflower represent a fairly special group among fungi. Unlike the majority of fungi, Oomycetes constitute cellulose in their cell wall and produce wall-free zoospores in their life cycle. Accordingly, the members of this group of fungi exhibit an unusual sensitivity to antibiotics and fungicides. The sensitivity to such compounds may vary according to the various stages within the fungal life cycle (Érsek, 1975). The mycelial stage of certain species of Oomycetes has been reported to be insensitive or weakly sensitive to the otherwise potent fungicides, the morpholine derivatives (Pommer, 1984). On the other hand, cationic detergents having structural similarity to morpholines are toxic to wall-free zoospore (Harris and Dennis, 1977). Thus, although apparently multifunctional poisons, morpholines may also affect the plasmalemma directly.

In order to obtain detailed data on whether and how morpholine derivatives express toxicity to species of Oomycetes, we carried out a comparative study on the sensitivity to tridemorph of *P. infestans* and *P. halstedii* at different developmental stages whithin their life cycle.

Materials and Methods

Chemical

The active ingredient (a.i.) tridemorph (Fig. 1) was vacuum-distilled from CALIXIN 75 EC (BASF, BRD), then dissolved in acetone to obtain a stock solution of 10% w/v.

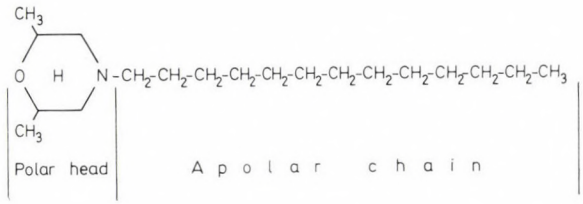

Fig. 1. Chemical structure of tridemorph (2,6-dimethyl-4-tridecilmorpholine)

Test organisms: preparation and sensitivity tests

P. infestans race 1.2.3.4 was maintained on pea agar slants at 18 °C. A piece of mycelial mat was transferred to Petri-plates containing the same medium. Seven- to 9-day-old cultures were used to collect zoosporangia.

The sporangial suspension in glass-distilled water was concentrated on Whatman No. 4 filter paper. Resuspended sporangia were then incubated for direct germination at 20 °C for 16–20 h, and for indirect germination (zoospore release) at 10–12 °C for 4–6 h. Zoospore suspension was obtained by the separation of zoospores from zoosporangia with Whatman No. 4 filter paper (Érsek, 1975).

P. halstedii produced zoosporangia on cotyledons of sunflower (*Helianthus annuus* L. cv. VNIIMK 6540) infected by the WSI method (Cohen and Sackston, 1973). Six to 8 days after planting, cotyledons were excised and placed onto wetted filter paper in Petri-plates. After an incubation at 19 °C for 12–14 h zoosporangia were washed off with glass-distilled water. The suspension obtained was filtered through a double layer of gauze, then subjected to centrifugation at 500 rpm at 4 °C for 5 min. Zoosporangia were then resuspended and the suspension was incubated at 16 °C for 2 h to obtain zoospores that were then separated from zoosporangia by centrifugation at 500 rpm for 5 min (Oros and Virányi, 1986).

Zoospores of *P. infestans* were vigorously vortexed for 30 sec that resulted in synchronous encystment. The zoospores of *P. halstedii* were incubated at 22–24 °C for 4 h to obtain a suspension of cystospores.

The tridemorph sensitivity of the above two fungi was estimated by the determination of mycelial growth, the production and germination of zoosporangia, and the germination and germ-tube elongation of cystospores. In addition, time required for the onset of events in zoospores such as slowing and cease of motility as well as for swelling and disruption was measured. The electrolyte leakage of zoospores was detected by the use of conductometer type Radelkis (Budapest).

Table 1

Effect of tridemorph on *P. infestans* at several developmental stages within its life cycle

Effect of tridemorph on	Sensitivity in		
	MIC	ED_{50}	MEC
	(mg/l)		
Mycelial growth[a]	250–500	20–30	1–5
Formation of zoosporangia[b]	250–500	—	—
Direct germination of zoosporangia[a]	100–500	20–30	1–5
Zoospore release (indirect germination of zoosporangia)[a]	50–100	5–10	0.5–1
Zoospore motility[c]	1–2	—	—
Disruption of zoospores[c]	15–30	—	1–2
Germination of cystospores[c]	30–60	8–16	0.5–1
Germ-tube elongation of cystospores[d]	30–60	4–8	0.2–0.5

[a] = For details in methods see Érsek (1975).

[b] = Infested agar blocks were transferred onto agar plate containing tridemorph. After an incubation at 20 °C for 5–6 days formation of zoosporangia was examined under dissecting microscope.

[c] = Concentration that completely inhibited motion and/or caused disruption 15 min after the addition of tridemorph to zoospore suspension was considered effective.

[d] = Vortexed zoospores were allowed to form germ tube at 16 °C for 18 h. The length of germ tubes was then measured.

Table 2

Effect of tridemorph on *P. halstedii* at several developmental stages within its life cycle

Effect of tridemorph on	Sensitivity in		
	MIC	ED_{50}	MEC
	(mg/l)		
Mycelial extension[a]	2000–4000	250–500	100–200
Formation of zoosporangia[a]	>2000	—	—
Zoospore release[b]	31–62	4–8	0.2–0.5
Zoospore motility[b]	1–2	—	0.05–0.1
Disruption of zoospores[b]	4–8	—	0.2–0.5
Germination of cystospores[b]	4–8	2–4	—
Germ-tube elongation of cystospores[b]	4–8	1–2	—

[a] = The effect of tridemorph was evaluated in sunflower hypocotyls according to the method described elsewhere (Oros and Virányi, 1987).

[b] = For details see Table 1.

Results

The comparison of selective sensitivity to tridemorph of *P. infestans* and *P. halstedii* at the various developmental stages in their life cycle is given in Tables 1 and 2. The mycelium in both species is considerably tolerant (MIC higher than 2000 mg/l). The formation of zoosporangia of *P. infestans* both in vitro and in vivo and that of *P. halstedii* in vivo also takes place at high concentrations of the compound. Markedly lower concentrations are required to inhibit the germination of zoosporangia, either the direct germination of *P.*

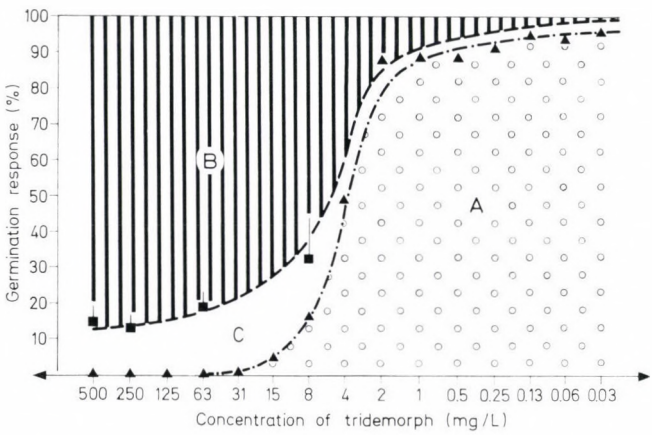

Fig. 2. Direct germination response of *P. infestans* to varying concentrations of tridemorph. Areas *A* (dotted), *B.* (shaded) and *C* represent germinated, killed and retarded (non-germinating, alive) sporangia, respectively

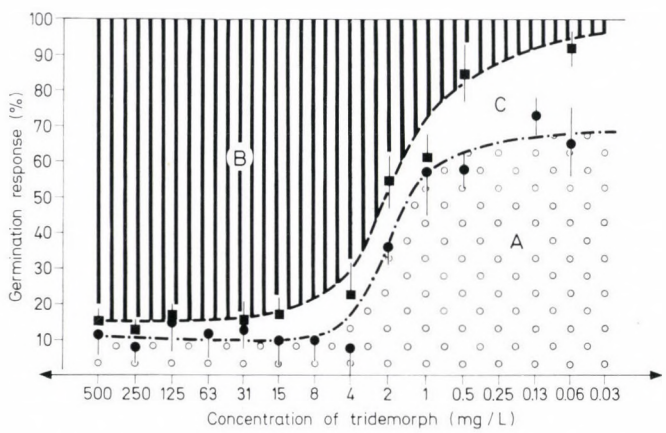

Fig. 3. Indirect germination (zoospore-release) response of *P. infestans* to varying concentrations of tridemorph. Labels correspond to those given in Fig. 2

infestans or the zoospore release (indirect germination) of both *P. infestans* and *P. halstedii* is concerned. However, at higher concentrations (over 500 mg/l) that proved to be phytotoxic and totally inhibited both direct and indirect ger-

Table 3

Time (s) required for the onset of events in zoospores

Tride-morph conc. mg/l	Slowing of motility		Cease of motility		Swelling		Disruption	
	P. i.	*P. h.*	*P. i.*	*P.h.*	*P. i.*	*P. h*	*P. i.*	*P. h.*
500	21	2	22	5	22	11	59	28
250	17	8	17	10	29	14	46	27
125	17	10	21	12	30	27	46	43
62.5	19	12	83	24	42	46	72	56
31.3	23	16	89	32	68	59	128	98
15.6	39	21	87	46	139	85	297	189
7.8	34	24	191	78	188	143	293	199
3.9	38	25	245	68	235	178	350	198
1.95	28	25	310	78	313	182	457	207
0.98	42	30	417	217	347	243	467	262

Table of variance

Variables	SQ	FG	MQ	F	$F_{P=5\%}$
Total	3 352 772.79	231			
Replications	2 749.05	2	1 373.01	1.56	3.07*
Treatment	3 215 450.79	79	40 701.91	45.37	1.37
Concentration (A)	1 488 918.08	9	165 435.34	184.40	1.94
Events (B)	724 154.96	3	241 384.99	269.06	2.68
Species (C)	206 797.80	1	206 797.80	230.50	3.91
Interactions					
A× B	499 533.08	27	18 501.23	20.62	1.59
A× C	150 674.91	9	16 741.66	18.66	1.94
B× C	69 922.54	3	23 307.51	25.98	2.68
A× B× C	75 449.42	27	2 794.42	3.11	1.59
Error	134 572.95	150	897.15		

$LSD^{a}_{5\%} = 17$; $LSD^{b}_{5\%} = 11$; $LSD^{c}_{5\%} = 8$; $LSD^{a,b}_{5\%} = 34$; $LSD^{a,c}_{5\%} = 24$; $LSD^{b,c}_{5\%} = 15$; $LSD^{a,b,c}_{5\%} = 48$ where ^{a} = Concentrations of tridemorph (mg/l), ^{b} = Time (s) required for onset of events (slowing and cease of motility, swelling and disruption), ^{c} = Species (*P. i.* = *Phytophtora infestans*, *P. h.* = *Plasmopara halstedii*).

* $F_{P=10\%} = 2.3$

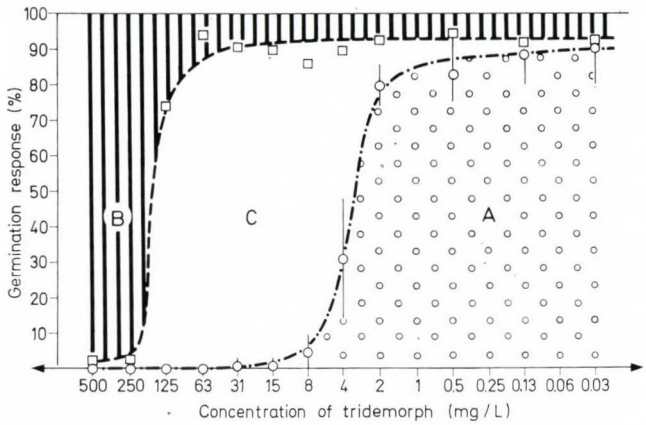

Fig. 4. Germinations (zoospore release) response of *P. halstedii* to varying concentrations of tridemorph. Labels correspond to those given in Fig. 2

mination, some zoosporangia still remained alive (Figs 2, 3 and 4). The most sensitive structure of these fungi is the one lacking cell wall, i.e. the zoospore. The sensitivity to various tridemorph concentrations of zoospores is expressed, as a function of time required for the slowing and the cease of motility, the alteration in membrane permeability and for the occurrence of burst (Table 3). Tridemorph has similar effect on zoospores of *P. infestans* and *P. halstedii*. The comparison of zoospore sensitivity to tridemorph of the two species showed a highly significant linear correlation ($P < 0.1$) (Table 4). The zoospores of *P. halstedii*, notwithstanding, are more sensitive than those of *P. infestans*. That is, lower concentration and/or less time is required for the onset of slowing and loss of motility as well as of swelling and disruption of zoospores. The most accurately

Table 4

Relationship between *P. infestans* and *P. halstedii* in their sensitivity to tridemorph characterized by various events in zoospores

No. Events (i)	Regression constant* (a)	Regression coefficient (b)	Statistical deviation s_b	Correlation coefficient r	F
1. slowing of motility	−4.9374	0.7999	0.171	0.8559	21.9
2. loss of motility	−4.8401	0.4173	0.065	0.9160	41.7
3. swelling	6.5246	0.6530	0.043	0.9830	229.7
4. disruption (burst)	18.6290	0.5060	0.045	0.9702	128.3

* $Y_i = a + bX_i$, where Y_i and X_i are seconds required for the onset of i event in zoospores of *P. halstedii* and *P. infestans*, respectively ($r_{P=0.1\%} = 0.8471$, $F_{P=0.1\%} = 10.37$)

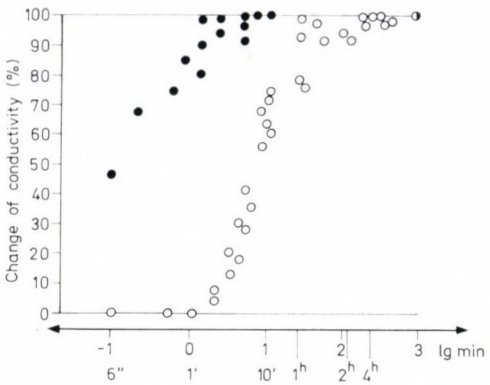

Fig. 5. Electrolyte leakage caused by tridemorph in zoospore suspension of *P. infestans* (•) and *P. halstedii* (○). The change in conductivity is expressed as the percentage of maximum electrolyte loss estimated after 16 h

detectable moments are the ones of swelling and disruption (Table 3). The time elapsing between swelling and disruption increases as a function of decreasing tridemorph concentration. This time interval is longer in the case of *P. infestans*. In contrast, the electrolyte leakage in *Phytophthora* zoospores begins immediately after the addition of tridemorph, whereas it is delayed in *Plasmopara* zooospores (Fig. 5).

Discussion

Cell wall bearing structures as compared to wall-free zoospores of *P. infestans* and *P. halstedii* were found to be rather tolerant to tridemorph. The differential sensitivity to tridemorph in various developmental stages of these fungi thus coincides with the lack or presence of cell wall.

The zoospores after losing their motility encyst by the formation of thin envelop of microfibrils made of β-glucans. The simple architecture of the resulting cyst wall contrasts with the more complex architectural organization of the hyphal wall which has also a microfibrillar skeleton, but this is heavily coated by an other layer of non-fibrillar glucan. The more advanced the architecture of cell wall is the higher tolerance to tridemorph occurs. Thus, the sensitivity of zoospores is higher than that of cysts. So is the sensitivity of cyst *versus* zoosporangia and mycelia. The process of direct germination of sporangia is less affected by tridemorph than the zoospore-release (indirect germination). It is conceivable since, prior to zoospore release, the apical papillum, i.e. the mucilaginous area of the inner sporangial wall dissolves, thereby allowing tridemorph to directly contact the membrane.

The specially unique architecture of cell wall in Oomycetes may thus function as a barrier to certain compounds, e.g. tridemorph. Such a cell wall compo-

sition, on the other hand, can favour the uptake and hence the effectiveness of compounds that are otherwise ineffective against fungi other than Oomycetes (Vörös, 1965, 1966).

Our results suggest that it is likely to be the membrane function tridemorph affects. Whether or not the plasmalemma is the direct target remains to be answered. In microorganisms other than Oomycetes tridemorph and related compounds have been reported to influence mitochondrial electron transport (Müller and Schewe, 1974; Bergmann, 1977), protein synthesis (Fisher, 1974) and nucleic acid synthesis (Fisher, 1974), metabolism of lipids (Polter and Casperson, 1977) and steroids (Kerkenaar et al., 1979; 1981; Kato et al., 1980). However, these metabolic changes take place several hours after the addition of the compound. In contrast, the burst as well as the electrolyte leakage of zoospores occur within minutes. The loss of electrolytes or water in several other organisms was also detected to take place much faster than the above mentioned metabolic changes (Buchenauer, 1974; De Waard, 1977; Oros and Süle, 1980). This would strongly suggest that the receptor site for tridemorph is located in the plasma membrane. In addition, this mode of action and structure of tridemorph are similar to the ones of cationic detergents and the polymyxin B (Érsek, 1975). Both cationic detergents and the polypeptide antibiotic polymyxin B bind to the membrane structure and disrupt its osmotic properties that results in the leakage of metabolites. This somewhat more generalized mode of action undoubtedly serves as a partial explanation of the greater toxicity of such compounds to cells bearing no cell wall.

Beyond the advantages of the use of zoospores regarded as natural protoplasts (Bartincki-García and Hemmes, 1976) in the studies of membrane active agents such compounds apparently are of importance in the control of Oomycetes. The in vitro tests have indicated however, that the total inhibition of sporangial germination does not imply total killing of zoosporangia. That is to say, zoosporangia remained alive may germinate and invade plants when the compound is not available any more. The in vitro effective doses, furthermore, have been shown to be toxic to both potato and sunflower tissues. The phytotoxic doses were 500 and 1000 mg/l, respectively. Therefore, the use of any morpholine derivative *per se*, even in preventive control of Oomycetes, seems to be out of scope.

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Changes in Pigment Constitution of Downy Mildewed Sunflower after Metalaxyl Treatment

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After a foliar spray with metalaxyl, remission of leaf chlorosis, a typical symptom of systemic downy mildew infection occurred in sunflower seedlings. Pigment analysis revealed considerable increases in the chlorophyll content of both chlorotic and green parts of the affected leaves. Carotenoids also increased but to less extent, and there were no changes detectable in the pigments of metalaxyl-treated, healthy plants. Furthermore, metalaxyl resulted in alterations in the ratios between chlorophyll a and b in a direction reminding the pigment constitution of young leaves.

The metalaxyl-induced regeneration process of sunflower leaves seemed to be transient because of a reappearance of chlorotic symptoms 5–6 weeks after metalaxyl spray.

Leaf chlorosis is the most typical symptom of systemic downy mildew infection (SDM) in sunflower caused by *Plasmopara halstedii*. It appears as a distinct light-green pattern along the main veins of affected leaves. In case of severe infection, however, the whole leaf area may become chlorotic, particularly in the upper part of plants. It is also known that, as a result of fungal invasion, some physiological changes occur, such as increased enzyme activity (Venclavovits et al., 1963; Novotel'nova, 1966), altered distribution of assimilates and changes in the constitution of pigments (Virányi and Oros, 1981).

SDM is effectively controlled by metalaxyl, a systemic anti-oomycete fungicide, applied as seed dressing (Iliescu, 1979; Sackston, 1979; Vasas and Csete, 1979), but less so, if the fungicide is used as a foliar spray (Iliescu, 1979; Melero-Vara et al., 1982). Furthermore, it was noticed that, under field conditions, remission of the chlorotic symptoms occurred after metalaxyl treatment. In other words, a "regreening" of the chlorotic leaf tissue could be seen and the affected plants seemed to recover from the disease (Melero-Vara et al., 1982).

The objective of the research reported in this paper was to characterize this "regreening" process by measuring quantitative changes in the pigment constitution of downy mildewed sunflower leaves in relation to metalaxyl-treatment. An effort was also made to determine whether SDM can be eliminated definitely by foliar spray of metalaxyl.

Materials and Methods

Infected plant material

Sunflower seedlings (cv. VNIIMK 6540), preinoculated with a sporangium-suspension of *Plasmopara halstedii* (Farl.) Berl. et de Toni (Peronosporales) by means of a method described elsewhere (Virányi, 1977), were grown in the glasshouse until having two pairs of leaves (approx. 20 days), by the time the typical chlorotic symptoms of SDM had developed. A series of plants with leaf chlorosis were then transferred to field plots and allowed to grow further.

Fungicide treatment

The sunflower seedlings either grown in the glasshouse (four-leaf-stage), or in the field (eight-leaf-stage) were sprayed run-off with an aqueous suspension of Ridomil 25 WP, the metalaxyl concentration being 1000 mg/L.

Sampling for pigment determination

In a preliminary test, samples were taken from sunflower seedlings with four pairs of leaves (three samples from each leaf-pair) to see variations related to leaf age, and to select the best samples for further experiments.

For the effect of metalaxyl treatment on pigment constitution, samples from the first leaf-pairs of downy mildewed seedlings were obtained and both chlorotic and symptomless (green) leaf tissues were considered. As control, leaves of uninoculated plants were taken. Sampling was made one hour before and three days after the foliar spray with metalaxyl.

Determination of leaf pigments

In order to characterize changes in pigment constitution, the amount of chlorophylls and carotenoids, the chlorophyll a/b ratio, as well as the ratio between chlorophylls and carotenoids were determined by using acetonous extracts of samples for spectrophotometric measurements, using the next formulas for calculations:

$$\text{Chlorophyll (a + b)} = 5.224 \times OD_{662} + 20.436 \times OD_{664} \text{ mg/L}$$
$$\text{Carotenoids} = 4.695 \times OD_{440} - 1.348 \times OD_{662} - 5.416 \times OD_{644} \text{ mg/L}$$

The chlorophylls a and b separately were calculated as described earlier (Virányi and Oros, 1981).

Data analysis

Mean comparisons were done following the Student's test at $P = 5\%$.

The coefficient of variation (CV%) used for the characterization of individual variability and expressed in differences of specific pigment content and consti-

tution (i) were calculated as follows:

$$CV\% = \frac{\bar{x}_{ij}}{s_{ij}} \times 100$$

where $\bar{x}_{ij}$ = the average of "i" parameter in the "j" level of leaves,
s_{ij} = the corresponding standard deviation.

Results and Discussion

A remarkable individual variation in the pigment content was found in leaf samples of different age (Table 1). Of the parameters measured the chlorophyll a/b ratio seemed to be rather stable and it was higher in younger leaves. As regards individual variation in pigment content as a whole, it was much more evident in both senescent and newly developing leaves (CV% = 29 and 17, respectively) than in well-developed ones (CV% = 3.8). In view of these results, only the first leaf-pair of the seedlings was considered as sampling material for subsequent experiments.

There was a close correlation found between the amount of leaf pigments and downy mildew infection. Thus, chlorophyll and carotenoid concentrations decreased by 70 and 59%, respectively, in the chlorotic parts of leaves and a

Table 1

Individual variations in the pigment content of sunflower leaves depending on their age

No.	Leaf sample*	Chlorophylls mg/dm²		Chlorophyll a/b		Carotenoids mg/dm²		Chlorophylls/ carotenoids	
		$\bar{x}_a$	CV%	$\bar{x}_b$	CV%	$\bar{x}_c$	CV%	$\bar{x}_d$	CV%
1	Cotyledons	2.546	23.6	2.43	4.2	0.701	24.8	3.44	15.6
2	1. leaf-pair	2.735	4.8	2.71	3.2	0.629	5.6	4.33	1.6
3	2. leaf-pair	2.468	2.9	2.87	5.6	0.596	1.8	4.27	7.0
4	3. leaf-pair	—**		5.00	32.0	—		2.90	24.0

* — Mean comparisons were done following the Student's test at P = 5%.

$t^a_{1,2} = 0.69$ $t^a_{1,3} = 0.29$ $t^a_{2,3} = 4.03$ $t_{P=5\%} = 2.78$
$t^b_{1,2} = 4.89$ $t^b_{1,3} = 5.21$ $t^b_{2,3} = 2.00$ $t^b_{3,4} = 2.96$
$t^c_{1,2} = 0.91$ $t^c_{1,3} = 1.35$ $t^c_{2,3} = 2.03$ $t_{P=10\%} = 2.13$
$t^d_{1,2} = 3.65$ $t^d_{1,3} = 3.00$ $t^d_{2,3} = 0.32$ $t^d_{1,4} = 1.37$

** — Samples consisted of young, still developing leaves and the apical bud; no calculation for unit leaf area was made in this case.

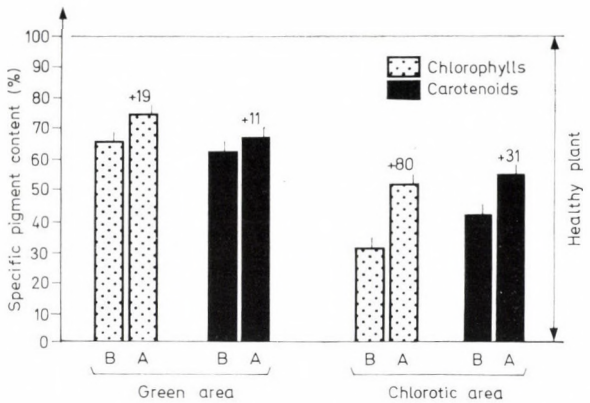

Fig. 1. Changes in specific content of pigments in downy mildewed sunflower leaves following metalaxyl treatment. B — before, A — after the metalaxyl treatment, respectively. Per cent values in all cases refer to healthy control plant

similar but less reduction (35 and 39% in respective samples) occurred in the symptomless, green areas of the same leaves, as compared to healthy plants (Fig. 1). Three days after metalaxyl treatment leaf chlorosis of the seedlings remitted and tended to become similar to healthy tissues. Pigment analysis, however, showed that chlorophyll and carotenoid content increased in both chlorotic and green parts of such leaves and that carotenoids were exceeded by chlorophylls in all cases. By contrast, healthy sunflower seedlings showed no significant changes in pigment content following a spray with metalaxyl.

The alterations in the ratios between chlorophyll a and b were much more evident in the green parts of diseased leaves than in the chlorotic areas, the ratios increasing from 2.69 to 5.63, and from 1.35 to 2.92, in green and chlorotic parts, respectively. No significant changes occurred in the control plants (from 2.77 to 3.12).

Our data suggest that tissue regeneration takes place in the downy mildewed sunflower leaves shortly after metalaxyl treatment. This regeneration could be seen as "regreening" of the chlorotic leaves by Melero-Vara et al. (1982) under field conditions. Remission of leaf chlorosis, however, does not mean a complete recovery of sunflower from SDM. Instead, it may occur that metalaxyl sprays remit chlorotic symptoms only with no killing effect on the fungus. In other words, metalaxyl as foliar spray exhibits a fungistatic activity against *P. halstedii*. In fact, our observations in the field showed the fungus to stop sporulation immediately after a foliar spray with metalaxyl and the newly developed leaves looked like healthy. Five to six weeks later, however, leaf chlorosis appeared on the younger leaves and this was accompanied by a profuse sporulation on them, as well as on older "regreened" leaves. The transient nature of symptom remission was also observed by Melero-Vara et al. (1982) who concluded that the therapy achieved with foliar sprays might be useful in experimental fields and breeding

nurseries but the risk of *P. halstedii* developing resistance to metalaxyl should be considered. Our results fully support this conclusion and indicate that diseases like SDM can be controlled effectively only if a fungicide with eradicant action is used for eliminating fungal propagula within the host plant (Oros and Virányi, 1987). From practical point of view it might be interesting to see whether oospores of *P. halstedii* can develop in sunflower treated with metalaxyl as a foliar spray, and the probability of arising metalaxyl-tolerant strains from such oospores may exceed the value of 10^{-6} to 10^{-7}, reported by Oros and Virányi (1984).

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Zur Unterscheidung von horizontaler und vertikaler Resistenz der Gerste gegen Gelbrost (*Puccinia striiformis* West.)

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A new method was worked out for differentiation between horizontal and and vertical resistance of barley to rust disease.

Eine Reihe von Beobachtungen deutet darauf hin, daß die Wurzel der Gerste eine besondere Rolle bei der Abwehr von Gelbrostinfektionen spielt (Hofferek und Wolffgang, 1975; Hofferek, 1977, 1980, 1981; Schubert, 1979). Werden infizierte Blätter abgeschnitten, ehe die Wirkung der Wurzel einsetzen kann, werden auch Blätter von Pflanzen mit vertikaler Resistenz infiziert (Schlegel und Opel, 1983). Es bleibt aber noch eine gewisse Resistenz zurück, die dann nur horizontal sein könnte. Wenn das so ist, müßte es nicht nur möglich sein, horizontale und vertikale Resistenz nebeneinander nachzuweisen und zu bewerten, sondern auch vertikale und absolute horizontale Resistenz (Robinson, 1976 Vanderplank, 1968) zu unterscheiden.

Material und Methoden

Es wurden die Gerstensorte ‚Xenia' als besonders anfällige, die Sorte ‚Bigo' als vertikal resistente gegen die Gelbrostrasse 24 sowie die Sorten ‚Martonvásári 43', ‚Trumpf' und ‚Nadja' verwendet. Diese drei Sorten hatten bei frühere Arbeiten (Wolffgang und Meister, 1983, 1984) hohe Anteile an Pflanzen ohne Pustelbildung gezeigt, so daß anzunehmen war, daß vertikale Resistenz vorkommt – ohne indes absolute horizontale Resistenz ausschließen zu können. Die Körner wurden in Blumentöpfen mit Komposterde ausgesät, die in Klimazellen mit 24 °C Tagestemperatur und 14 °C Nachttemperatur standen. Die Lichtintensität (Nachroma NC 1000) betrug während des 12-Stunden Tages etwa 23000 Lux in Höhe der Blumentöpfe. Nach der Infektion mit frischen Uredosporen der Gelbrostrasse 24, mit Talkum (3 : 1) verdünnt, mit feinem Pinsel aufgetragen, wurde mit Wasser fein besprüht, dann für drei Tage unter lichtdurchlässigen, mit feuchtem Filtrierpapier teilweise ausgeschlagenen Hauben gehalten. Nach der Inokulation standen Pflanzen und abgeschnittene Blätter in einer Klimazelle, in der ständig 14 °C herrschten, 16 Stunden bei Neon-Mischlicht von etwa 2000 Lux. Die relative Luftfeuchte in allen Klimazellen betrug 70%.

Für den ersten Versuch wurden Gruppen zu je 5 Pflanzen gebildet und die inokulierten Blätter zu verschiedenen Zeiten nach der Infektion abgeschitten und in flachen lichtdurchlässigen Schalen auf Fließpapier (Knopsche Nährlösung plus 60 ppm Benzimidazol) gehalten. Als Kontrollen dienten gleichzeitig und gleichartig inokulierte Blätter an intakten Pflanzen. Täglich wurde zur Ermittlung der Latenzzeiten das Auftreten von Uredolagern notiert, 21 Tage nach der Infektion wurden die Pusteln der isolierten Blätter, 27–28 Tage nach der Infektion die an den intakten Pflanzen ausgezählt und die Blätter vermessen. An intakten Pflanzen treten die Pusteln später auf.

Beim zweiten Versuch wurden 20 Pflanzen am 1. und 2. Blatt wie beschrieben inokuliert. Drei Tage später wurde das erste Blatt jeder Pflanze abgeschnitten und in die erwähnten Schalen überführt. Täglich wurden die Pflanzen notiert, an denen die ersten Pusteln aufgetreten waren. 21 Tage nach der Inokulation wurden die Blätter vermessen und die Pusteln ausgezählt. Das Experiment wurde dreimal wiederholt. Als Kontrolle dienten gleichzeitig und gleichartig infizierte

Tabelle 1

Wirkung der Zeit zwischen Inokulation und Abschneiden der inokulierten Blätter auf Pustelbildung, Latenzzeit, Anteil von Pflanzen ohne Pusteln und Gelbrostanfälligkeit

Sorte	Blätter abgeschnitten — Tage nach Inokulation									Kontrolle
	0	3	4	5	6	7	10	11	12	
Bigo										
P[1]	23,8	26,7	14,2	7,5	3,0	1,1	2,9	0,0	0,0	0,0
L[2]	11,5	15,7	16,8	18,5	20,4	20,9	24,6	—	—	—
O[3]	0,200	0,000	0,160	0,280	0,520	0,550	0,800	1,000	1,000	1,000
$\frac{P}{L}(1-0)$[4]	1,656	1,701	0,710	0,292	0,071	0,024	0,024	0,000	0,000	0,000
Xenia										
P	39,6	49,8	53,4	54,1	52,0	54,1	37,0	28,0	19,5	20,3
L	10,8	12,5	13,4	13,2	13,6	14,4	15,7	16,5	17,4	12,3
O	0,150	0,053	0,000	0,000	0,050	0,000	0,000	0,000	0,333	0,429
$\frac{P}{L}(1-0)$	3,117	3,773	3,985	4,098	3,632	3,757	2,357	1,697	0,747	0,942

[1] Pusten/cm Blattlänge.
[2] Latenzzeit in Tagen.
[3] Anteil (als Dezimalbruch) von Pflanzen ohne Pusteln.
[4] Anfälligkeit
Mittelwerte aus 5 Wiederholungen mit je 5 Pflanzen. In die Werte von P und L gingen nur die Pflanzen ein, die Pusteln bildeten, in die von O nur die Pflanzen, die keine bildeten.

Pflanzen, denen das erste Blatt nicht abgeschnitten wurde. Alle Versuche waren so eingerichtet, daß die Zuordnung der isolierten Blätter zu den Pflanzen, von denen sie entnommen wurden, bekannt blieb.

Ergebnisse

Erster Versuch. Es sollte festgestellt werden, ob und welche Unterschiede zwischen intakten Pflanzen und isolierten Blättern auftreten und wie lange die Blätter nach der Inokulation an der Pflanze verbleiben können, ehe sich die Wirkung der Wurzel bemerkbar macht. Die Ergebnisse enthält die Tabelle 1. Bei ‚Bigo' nimmt die Zahl der Pusteln, wenn nach dem dritten Tag nach der Inokulation die Blätter abgeschnitten werden, zunehmend stark ab, auf den nach dem 10. Tag nach der Inokulation abgeschittenen Blättern treten keine Pusteln mehr auf. Die Latenzzeiten werden bis zum 6. Tag mindestens ständig größer. Sofort nach der Inokulation abgeschnittene Blätter haben nur unwesentlich längere Latenzzeiten als die der sehr anfälligen Sorte ‚Xenia'. Um die Blätter möglichst lange in Schalen halten zu können, wurde deshalb beschlossen, im zweiten Versuch die Blätter 3 Tage nach der Inokulation abzuschneiden. Der Anteil der Blätter ohne Pusteln nahm bei ‚Bigo' etwa vom 5. Tag an stetig zu.

Tabelle 2

Wirkung des Abschneidens der Blätter von 5 Gerstensorten auf Pustelbildung, Latenzzeit, Anteil von Pflanzen ohne Pusteln und Gelbrostanfälligkeit

Sorte	1. Blatt 3 dpi abgeschnitten				2. Blatt				Kontrolle 1. Blatt 2. Blatt			
	P	L	O	$\frac{P}{L}(1-0)$	P	L	O	$\frac{P}{L}(1-0)$	P	L	O	$\frac{P}{L}(1-0)$
Bigo	19,0	17,7	0,017	1,055	0,9	27,4	0,914	0,003	0,0	—	1,00	0,000
									19,6	23,3	0,975	0,046
Xenia	37,2	14,7	0,034	2,445	40,2	14,1	0,069	2,654	11,8	16,8	0,227	0,543
									28,5	14,7	0,024	1,892
Martonvásári 43	29,6	17,0	0,017	1,712	3,4	26,0	0,983	0,002	0,0	—	1,00	0,000
									1,9	25,5	0,849	0,011
Trumpf	24,6	18,5	0,125	1,164	13,4	23,5	0,491	0,290	3,2	25,3	0,882	0,015
									9,6	23,8	0,353	0,261
Nadja	21,2	16,2	0,100	1,178	8,4	19,0	0,383	0,273	1,2	20,0	0,974	0,002
									5,2	19,6	0,194	0,214

Erläuterungen siehe Tabelle 1.
1. Blatt 3 dpi abgeschnitten. 2. Blatt blieb an der Pflanze. Kontrollen: kein Blatt abgeschnitten.

Bei der anfälligen Sorte Xenia stieg die Zahl der Pusteln bis zum 5. Tag nach der Inokulation an und fiel dann allmählich ab. Die Latenzzeit nahm fast stetig zu. Das zeigt wiederum, daß Latenzzeit und Pustelbildung verhältnismäßig unabhängig voneinander sind (Wolffgang und Meister, 1984). Die ‚Anfälligkeit', in der die drei Resistenzfaktoren ‚Pustelzahl', ‚Latenzzeit' und ‚Anteil von Pflanzen

Tabelle 3

Anteil von Pflanzen von 5 Gerstensorten mit a absoluter, horizontaler, Gelbrostresistenz bzw. b sehr hoher
Anteile (in Dezimalbrüchen) der Pflanzen, die — ohne Pusteln auf dem 2. Blatt — bildeten.

Sorte	a	b
Bigo	0,000	0,017
Xenia	0,000	0,034
Martonvásári 43	0,017	0,017
Trumpf	0,125	0,161
Nadja	0,069	0,000

a: keine Pusteln auf dem isolierten 1. Blatt.
b: sehr wenige Pusteln auf dem 1. Blatt (weniger als die Hälfte des Sortendurchschnittes)

ohne Pusteln' zusammengefaßt sind, nahm bei ‚Bigo' bis zum 10. Tag stark und fast stetig ab. Bei ‚Xenia' nahm sie bis zum 5. Tag zu, fiel dann langsam und vom 10. Tag an stark ab. Das deutet darauf hin, daß möglicherweise auch hier noch andere Effekte wirksam werden.

Zweiter Versuch: Die Unterschiede im Infektionserfolg zwischen 1. und 2. Blatt sind deutlich (Tab. 2). Das Zeigt sich, außer bei ‚Xenia', bei allen Sorten. Die Anfälligkeit am 1. Blatt nimmt von ‚Bigo' über ‚Trumpf' und ‚Nadja' über ‚Martonvásári 43' nach ‚Xenia' zu. Die etwas höheren Pustelzahlen bei Trumpf werden durch längere Latenzzeiten und etwas größeren Anteil Pustelloser wettgemacht. Am 2. Blatt, an dem die vertikale Resistenz wirkt, führt das dazu, daß ‚Bigo' und ‚Martonvásári 43' die geringste Anfälligkeiten zeigen — auch hier werden Unterschiede in der Pustelzahl durch die Latenzzeiten sowie durch den Anteil Pustelloser ausgeglichen. Das ist auch bei ‚Trumpf' und ‚Nadja' so, deren Anfälligkeiten sich dadurch nicht unterscheiden. Interessant sind die in Tabelle 3 zusammengefaßten Ergebnisse. Hier wird verglichen, wie sich 1. und 2. Blatt dann unterscheiden, wenn auf dem 2. Blatt keine Pusteln aufgetreten sind. Bei ‚Bigo' und ‚Xenia' sind auf den dazugehörigen 1. Blättern Pusteln aufgetreten, bei einigen sehr wenigen Blättern aber sehr wenige. Bei ‚Martonvásári 43', ‚Nadja' und ‚Trumpf', in dieser Reihenfolge zunehmend, finden sich aber auch Pflanzen, bei denen das Abschneiden der Blätter nicht zur Pustelbildung führte. Auch die Anzahl 1. Blätter, bei denen nur sehr wenige Pusteln auftraten, nimmt, außer bei ‚Nadja', in dieser Reihenfolge zu.

Zu gleichen Ergebnissen führten Experimente (hier nicht dargestellt), bei denen nur die 1. Blätter infiziert und danach etwa 2/3 der Blattspreite abgeschnitten und in Schalen aufbewahrt wurden.

Diskussion

Die Versuchsergebnisse bestätigen die Hypothese, daß – beim Gelbrost der Gerste – die vertikale Resistenz von der Wirkung der Wurzel abhängt. Es wär interessant, Weizen und Gelbrost einer entsprechenden Prüfung zu unterwerfen.

Leider ist unser Wissen über (vertikal wirkende) Gelbrostresistenzgene sehr begrenzt, so daß die Ergebnisse dieser Untersuchung nicht mit resistenzgenetischen Erkenntnissen vergliechen werden können. Unterstellt man die o. a. Hypothese als richtig, dann würde das bedeuten, daß in allen untersuchten Sorten, außer bei ‚Xenia', Individuen mit vertikaler Resistenz gegen die Gelbrostrasse 24 vorkommen, von ‚Nadja' über ‚Trumpf', ‚Bigo' nach ‚Martonvásári 43' zunehmend. Daneben scheinen in allen Sorten, am wenigsten bei ‚Xenia' und ‚Bigo', wenig auch bei ‚Martonvásári 43', häufiger bei ‚Nadja' und am häufigsten bei ‚Trumpf' Individuen vorzukommen, deren horizontale Resistenz so groß ist, daß ihre Wirkung der einer vertikalen Resistenz gleichkommt. Außerdem wurden bei ‚Xenia' einige Erscheinungen beobachtet, die einen Einfluß der Wurzel auch auf die horizontale Resistenz vermuten lassen. Die in dieser Arbeit benutzte Methode dürfte die Suche nach horizontaler Resistenz auch bei Sorten mit vertikaler Resistenz wesentlich erleichtern. Absolute wirkende horizontale Resistenz im Sinne von Robinson und Vanderplank, die auf diese Weise leichter aufzufinden ist, sollte die Züchtung gelbrostresistenter Gerste fördern – obgleich nicht einzusehen ist, weshalb nicht auch vertikale Resistenz verwendet werden sollte.

Zusammenfassung

Es wird eine Methode beschrieben, mit der zwischen horizontaler und vertikaler Gelbrostresistenz der Gerste unterschieden werden kann. Die so geprüften Pflanzen gehen dem Zuchtprozeß nicht verloren. Es wird gezeigt, daß in den geprüften Sorten in unterschiedlicher Häufigkeit – nicht identifizierte – vertikale Resistenz, aber auch absolute horizontale auftritt. Die Sorten mit vertikaler Resistenz besitzen außerdem nicht unbeträchtliche horizontale, die größer ist als die von ‚Xenia' und von ‚Trumpf' und ‚Nadja' über ‚Martonvásári' nach ‚Bigo' zunimmt.

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Nature of Interaction between Immune Varieties of Rice *(Oryza sativa)* and *Pyricularia oryzae*

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Leaves of the varieties of *Oryza sativa* L. reported as resistant to *Pyricularia oryzae* Cav. viz., Te-tep, Tadukan, Co. 4 and Co. 25 reacted as immune — no symptoms — to a culture of *P. oryzae* race IC. 17 (L & O) *in vivo*. Hypersentivity was absent. Browning of epidermal cells of the leaf adjacent to the point of contact of the appressorium with the leaf was noticeable only under mangification after removing the chlorophyll in leaves obtained 48 hours after inoculation with the pathogen. The browning was light in colour in the case of resistant varieties and extended up to 4 cells adjacent to the appressorial point while it was deep in colour extending up to 6 cells in the case of susceptible varieties. While the pathogen could penetrate the leaves of the plants of susceptible varieties through motor cells only it could not penetrate the leaves of plants of resistant varieties *in vivo*. The reaction of the immune varieties of rice to *P. oryzae* denotes a case of dynamic immunity since it involves a pathogenic fungus-plant interaction resulting in the discolouration of the epidermal cells, without presenting visible symptoms.

Host-Pathogen interaction between rice (*Oryza sativa* L.) and *Pyricularia oryzae* Cav. has been reported by a few investigators (Yoshii, 1936; Kawamura and Ono, 1948; Suzuki, 1965; Hashioka et al., 1968) adopting their concept of resistance and their method of investigation. While some of them have studied the interaction between the susceptible cultivars and the pathogen, few others have reported the reaction of resistant rice cultivars without mentioning their concept of resistance. Interaction between plants of *Oryza sativa* L. with *P. oryzae* without symptoms has not been reported so far. The present study was undertaken to obtain information on the interaction between plants of a few varieties of *O.* sativa L. reported as resistant to *P. oryzae* and a particular culture of *Pyricularia oryzae* Cav., race IC.17 (Ling and Ou) *in vivo*.

Materials and Methods

Four blast resistant rice varieties viz., Te-tep, Tadukan, Co. 4 and Co. 25 were taken up for the study. The plants of the first two varieties have been reported to react as resistant to *P. oryzae* race IC. 17 (L & O) by Padmanabhan et al. (1970) and the others have been reported as blast resistant by Padmanabhan and

Ganguly (1959). The rice variety, Co. 25 proved resistant to five races of *P. oryzae* (Mathur, 1971). The rice varieties Dular and Co. 13 which were reported to be susceptible to *P. oryzae* IC. 17 (L & O) by Padmanabhan et al. (1970) were utilized as susceptible checks. The seeds of all the rice varieties were obtained from the Head, Division of Genetics, C. R. R. I., Cuttack. The plants were raised in earthern pots filled with soil containing 0.06% nitrogen, 0.04% phosphorus, 0.18% potassium, 0.16% calcium and 0.15% silicon. Nitrogenous fertilizer was applied at the rate of 8 mg of nitrogen/100 g of soil. One month old plants were utilized for the study. A culture of *P. oryzae* IC. 17 (L & O) was utilized as the pathogen. It was cultured on oat meal agar medium containing thiamine and biotin. Abundant sporulation of the pathogen was obtained by rearing the pathogen on bits of rice culm bearing nodes along with distilled water. Dry conidia produced on sterilized culms were utilized for dust-inoculation of the middle portion of the most expanded leaf from the growing apex of healthy plants *in vivo in situ* held in a moist chamber. Plants of the susceptible varieties Co. 13 and Dular served as controls to assess the success of inoculation and for comparison. Uninoculated check plants of the variety Co. 13 were maintained to detect natural infection, if any. The whole process of successful inoculation and incubation and fixing of the leaves was carried out ten times.

Absence of symptoms involving no damage or limited damage to the tissues of the plants after incubation or presence of tiny brown spots of restricted nature on the leaves revealing absence of sporulation was deemed as resistant reaction of the plants. Presence of lesions involving significant damage to the host-tissue due to necrosis and sporulation *in vivo in situ* was considered as susceptible reaction of the host.

Leaves of the test plants which were successfully inoculated as revealed by infection on plants of susceptible varieties Co. 13 and Dular were utilized in the study. Leaves of inoculated plants were fixed at intervals of 12, 24, 36 and 48 hrs after inoculation in the fixative containing formalin-acetic acid-ethyl alcohol. The leaves were allowed to remain in the fixative for 48 h after which they were transferred to 80% ethanol for storage. The stored material was scanned under the microscope after clearing them in acidified alcohol and portions of the fixed material revealing appressoria were utilized for the study of the host–pathogen interaction on the surface of the leaf blade and anatomical studies. For study of penetration of the leaf only such portions of the lamina which revealed appressoria on the joints between the epidermal cells were utilized since it was observed that browning of the epidermal cells took place only when the appressoria were formed on the joints between the epidermal cells. Portions of the fixed material with appressoria on joints revealing browning of the epidermal cells were utilized for anatomical studies. Camera lucida diagrams were drawn to bring out the host-pathogen interaction on the surface of the leaf blade.

Fixed material was embedded in paraffin-wax and microtome sections were obtained and stained with Sudan IV and Cotton blue. Camera lucida diagrams were drawn to bring out the host-pathogen interaction in the host tissues.

Results and Discussion

The plants of the inoculated resistant varieties Te-tep, Tadukan, Co. 4 and Co. 25 did not reveal any external symptom on inoculation with the pathogen followed by incubation. Immunity which was noticed did not involve hypersensitivity. The plants of the susceptible varieties revealed typical lesions caused by the pathogen at the end of the incubation period.

Conidia of the pathogen were found to germinate on the surface of the leaves of both the resistant and susceptible varieties. Appressoria were found to be formed on the surface of the leaves of both the groups of varieties and there was no site-specificity. Appressoria which were formed on the joints between the epidermal cells were found to produce a brown discolouration of the epidermal cells in materials fixed 24 h after inoculation both in the case of resistant and susceptible varieties. This discolouration of the epidermal cells was visible only under the microscope after clearing off the chlorophyll. Light browning of the epidermal cells was observed in the case of leaves of the resistant varieties whereas the browning was deep in the case of the susceptible varieties. The browning was confined to two and extended rarely to four epidermal cells adjacent to the point of contact of the appressorium with the epidermal cells in leaves of resistant varieties fixed 48 h after inoculation. Deep browning of the epidermal cells extending up to 4–6 epidermal cells adjacent to the point of contact of the appressorium with the epidermal cells was noticed in materials from susceptible varieties fixed 48 h after inoculation (Figs 1 and 2). Since immunity noticed in the immune varieties involved plant-pathogen interaction without visible symptoms the situation is referred to as dynamic immunity.

Anatomical studies revealed absence of penetration of the cuticle by the pathogen in all the four resistant varieties of rice although the appressoria were present on the cuticle above the motor cells which happens to be the site of entry

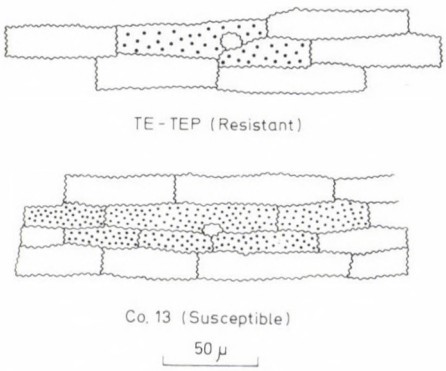

Fig. 1. Interaction between leaves of Te-tep and Co. 13 (*Oryza sativa* L.) and *Pyricularia oryzae* Cav *in vivo* 48 hours after inoculation

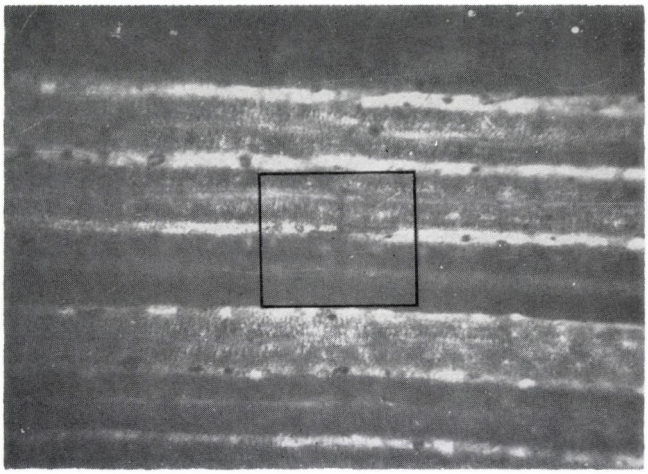

Fig. 2. Interaction between leaves of variety Te-tep (*Oryza sativa* L.) and *Pyricularia oryzae* Cav *in vivo* 48 hours after inoculation

of the pathogen in the case of the susceptible varieties as noticed in the present study. Penetration of the cuticle by the infection hypha was noticed in the case of leaves from the susceptible varieties which was complete by 12 h period following inoculation. The infection thread penetrated the leaf through the cuticle above the motor cell. During the next 12 h period the pathogen established itself in the motor cell. During the next 24 h period the mycelium of the pathogen ramified into the neighbouring cells below the motor cell which turned brown. Direct penetration of the cuticle of leaves in plants of susceptible varieties has been reported by Yoshii (1936). The other investigators have not utilized the same materials or methods in their studies.

The situation revealed that a disturbance of the host-metabolism was brought about by the appressoria of the pathogen on the leaves of plants of resistant varieties *in vivo* although the pathogen is unable to gain ingress into the leaf.

Yoshii (1936) reported penetration of the cuticle of the leaf in the susceptible variety. In the present study penetration of the cuticle was found to be confined to the area above the motor cells only.

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Specificity of *Bremia lactucae* Isolates from *Lactuca sativa* and Some *Asteraceae* Plants

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A study was made of the specificity of relationships between *Bremia lactucae* isolates from *Lactuca sativa* and 21 species of the family *Asteraceae*. Compatibility was confirmed in none of the relationships under study. The investigation of interspecific transmissibility of *B. lactucae* isolates originating from *Lapsana communis*, *Leontodon autumnalis*, *Senecio vulgaris*, *Sonchus asper* and *Sonchus oleraceus* has shown compatibility only with the host species. Interspecific transmission was experimentally proved in *Sonchus asper* and *Sonchus oleraceus* only. It seems evident that the weeds pertaining to the family *Asteraceae* (except for *Lactuca* species) cannot serve a function of *B. lactucae* reservoir plants from the viewpoint of pathogen epidemiology on *Lactuca sativa* cultivars.

Bremia lactucae attacks not only the members of the *Lactuca* genus, but also more than 150 species and about 40 genera of the family *Asteraceae* (Crute and Davis, 1977). It is most often found in the species of the following genera: *Arctium*, *Carduus*, *Carthamus*, *Centaurea*, *Cichorium*, *Cineraria*, *Cirsium*, *Crepis*, *Cynara Gaillardia*, *Gnaphalium*, *Helichrysum*, *Helipterum*, *Hieracium*, *Krigia*, *Lapsana*, *Leontodon*, *Pieris*, *Prenanthes*, *Senecio*, *Sonchus*, *Taraxacum*, *Tragopogon* and some others (Ling and Tai, 1945; Skalický, 1953; Savulescu, 1962; Tao Chia-Feng, 1965; Wittman, 1972; Dange, Jain and Giradhana, 1976; Lucas and Dias, 1976).

It seems important from the epidemiological viewpoint whether some of the above-mentioned species and/or genera may serve a function of a *B. lactucae* reservoir host. Sorauer (1908) was the first to report this epidemiological aspect showing that the disease might be transmitted to lettuce from wild species of the family *Asteraceae*. In the past 70 years numerous works and opinions have appeared either denyeing (Schweizer, 1919; Ling and Tai, 1945; Brooks, 1953; Crute and Dixon, 1981), or confirming this idea (Weber and Foster, 1928; Baudyš, 1935; Butler and Jones, 1949). Crute and Dixon (1981) summarized the up-to-date knowledge of the problem pointing to the lack of experimental data on the importance of the species belonging to the family *Asteraceae* in lettuce downy mildew epidemiology.

The aim of the present paper was to verify specificity of the relationships between *Bremia lactucae* isolates originating from the plants of the family *Aster-*

aceae, and different *Asteracea* species, and to assess their importance in epidemiology of the disease. The preliminary report on the subject has been published recently (Lebeda and Syrovátko, 1984).

Materials and Methods

Specificity of host-pathogen relationships was studied using a) *Bremia lactucae* Regel races and/or isolates (CS9, CS11 and 43/78) with a high degree of virulence originating from *Lactuca sativa* L. (Lebeda, 1979*a*, 1982; Lebeda et al., 1980), b) eight *B. lactucae* isolates obtained from *Lapsana communis* L. (isolate LC 1/81), *Leontodon autumnalis* L. (LA 1/82), *Senecio vulgaris* L. (SV 1/82, 2/82), *Sonchus asper* (L.) Hill (SA 1/82, 3/82) and *Sonchus oleraceus* L. (SO

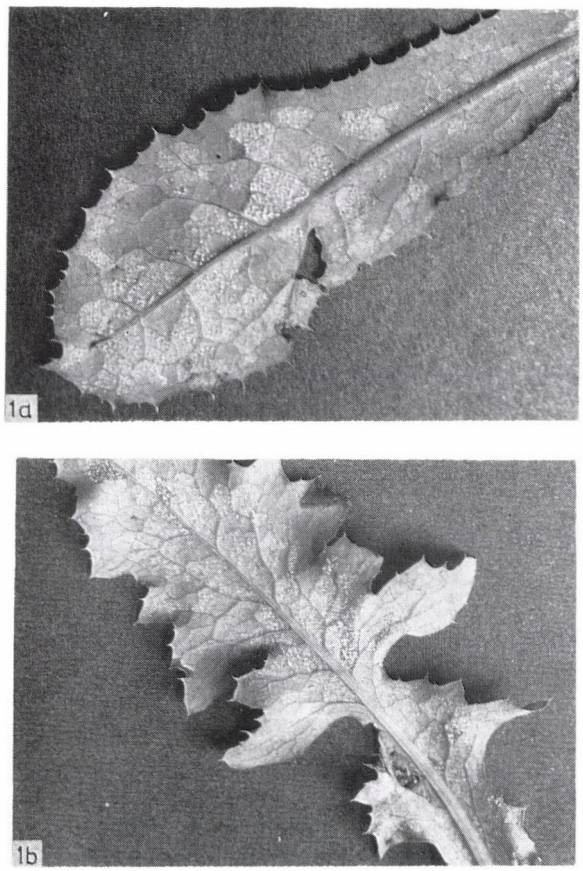

Fig. 1. Sporulation of *B. lactucae* f. sp. *sonchicola* on *Sonchus asper* (1a) and *Sonchus oleraceus* (1b) leaves

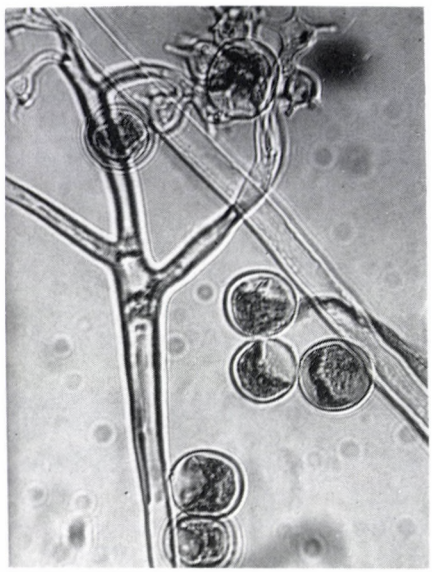

Fig. 2. Sporophores and spores of *B. lactucae* f. sp. *sonchicola*

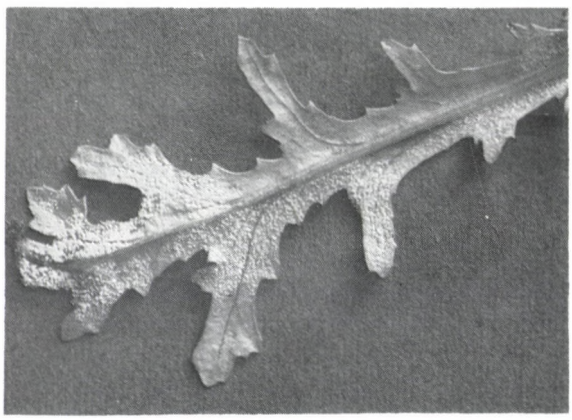

Fig. 3. Sporulation of *B. lactucae* f. sp. *tulasnei* on *Senecio vulgaris* leaves

1/82, 3/82). All isolates from wild *Asteraceae* were obtained in the territory of Czechoslovakia and their precise origin was described by Syrovátko (1983).

Asteraceae species used for the study of interactions with *B. lactucae* isolates from *L. sativa* were kindly provided by University botanical gardens in Pisa (Italy), Liège (Belgium) and Varna (Bulgaria), while *Senecio vulgaris*, *Sonchus asper*, *Sonchus oleraceus*, *Leontodon autumnalis* and *Leontodon hispidus* L. were collected by ourselves.

The experimental plants were grown in a glasshouse at the temperatures of 20–35 °C under natural illumination, in trays filled with garden soil. At the

stage of 3–6 true leaves they were transplanted into 100 mm pots. Leaf blades were removed from adult *Asteraceae* plants (2- to 3-month-old) to excise either discs of 20 mm in diameter or segments of about the same size. Each test for *B. lactucae* isolates from *L. sativa* comprised 5 plants of the respective species with 5 discs and/or segments per variant. When testing *Asteraceae* species isolates, there were 5 leaf segments from one plant only. Discs were placed with their axial side downwards in 100 mm Petri dishes lined with moistened filter paper.

B. lactucae isolates from *L. sativa* were multiplied on lettuce seedlings cv. "Kamýk", while those from *Asteraceae* species were propagated on leaf segments of the respective host plants. Inoculum was prepared by washing off 3- to 5-day-old spores with distilled water. Inoculum concentration was approximately 10^{4-5} spores · ml^{-1}. Inoculation was carried out by means of a glass sprayer under conditions described previously (Lebeda, 1979*b*). Infection degree and/or sporulation intensity was evaluated according to 0–4 score scale (Crute, pers. communication; Lebeda, 1983) on the 6th, 8th, 10th and 13th day after inoculation. The final value of sporulation intensity was expressed in percentages according to Towsend and Heuberger (1943).

Results

Specificity of relationships between B. lactucae isolates from L. sativa and Asteraceae species

Interaction was studied using a total of 21 plant species of the family *Asteraceae*. A survey of results is given in Table 1. The greatest number of the species (21) was inoculated by a highly virulent race CS9. None of the interactions showed

Table 1

Survey of tested *Asteraceae* species with isolates of *Bremia lactucae* from *Lactuca sativa*

Race/isolate of *B. lactucae* (virulence phenotype)	Plant species
CS9 (v1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12)	*Arctium lappa*, *Calendula arvensis*, *Calendula stellata*, *Caranopus squamatus*, *Centaurea scabiosa*, *Cirsium obvallatum*, *Cineraria hybrida*, *Crepis biennis*, *Dimorphotheca pluvialis*, *Dimorphotheca sinnuata*, *Gaillardia lutea*, *Helichrysum bracteatum*, *Hieracium pilosella*, *Hieracium umbellatum*, *Lapsana communis*, *Senecio gallicus*, *Sonchus tenerrimus*, *Tagetes erecta*, *Tagetes patula*, *Zinnia elegans*
CS11 (v1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12)	*Calendula arvensis*, *Calendula stellata*, *Centaurea scabiosa*, *Dimorphotheca pluvialis*, *Dimorphotheca sinnuata*, *Lapsana communis*, *Zinnia elegans*
43/78 (v1, 2, 4, 5, 7, 8, 9, 10, 11, 12)	*Calendula stellata*, *Dimorphotheca pluvialis*, *Dimorphotheca sinnuata*, *Lapsana communis*

sporulation except for *Calendula stellata* where local necroses were observed. The remaining two isolates were also nonpathogenic for the plant species under study.

Interspecific transfer of B. lactucae isolates

The interspecific transmission of *B. lactucae* isolates was studied in 8 wild species and subspecies of the family *Asteraceae*, and 4 universally susceptible

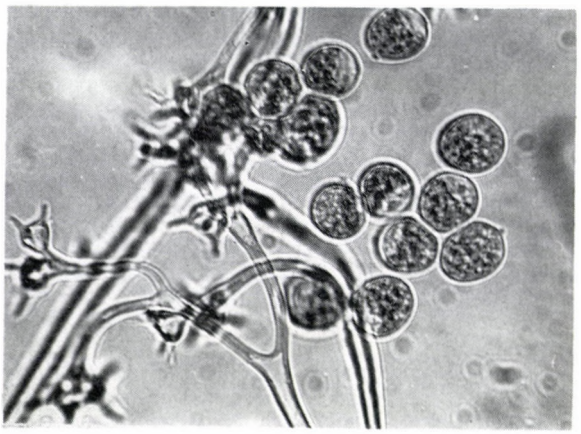

Fig. 4. Sporophores and spores of *B. lactucae* f. sp. *tulasnei*

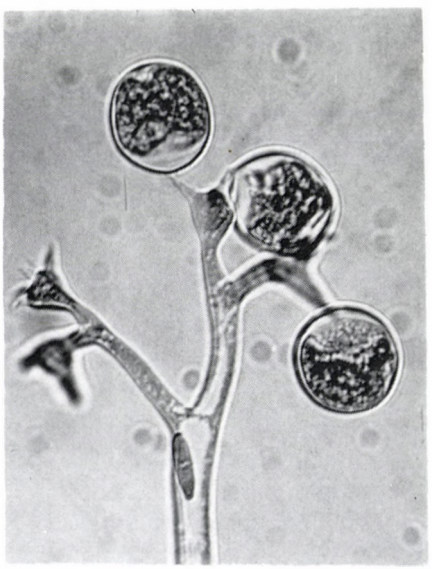

Fig. 5. Sporophores and spores of *B. lactucae* f. sp. *leontodonti*

Table 2

Reaction of adult *Asteraceae* plants to inoculation of *Bremia lactucae* isolates from *Senecio*, *Sonchus*, *Leontodon* and *Lapsana* species

Number of tests	Tested species/cultivar	Isolate of *B. lactucae* (origin)
		Sporulation intensity
1		SV 1/82 *(Senecio vulgaris)*
	Senecio vulgaris	63
	Sonchus asper, *Sonchus oleraceus*, *Lactuca serriola* (CS), *Lactuca serriola* (H), *Lactuca sativa* (cvs. Darka, Resistent)	0
2		SV 2/82 *(Senecio vulgaris)*
	Senecio vulgaris	70
	Sonchus asper, *Sonchus oleraceus*, *Lactuca serriola* (CS), *Lactuca serriola* (H), *Lactuca sativa* (cvs. Cobham Green, Darka, Resistent, Virilde)	0
3		SA 1/82 *(Sonchus asper)*
	Sonchus asper	
	Sonchus oleraceus, *Senecio vulgaris*, *Lactuca serriola* (CS), *Lactuca serriola* (H), *Lactuca sativa* (cvs. Darka, Resistent)	0
4		SA 3/82 *(Sonchus asper)*
	Sonchus asper	45
	Sonchus oleraceus	8
	Senecio vulgaris, *Lactuca sativa* (cvs. Cobham Green, Virilde)	0
5		SO 1/82 *(Sonchus oleraceus)*
	Sonchus oleraceus	61
	Sonchus asper	70
	Senecio vulgaris, *Lactuca serriola* (CS), *Lactuca serriola* (H), *Lactuca sativa* (cvs. Cobham Green, Darka, Resistent, Virilde)	0
6		SO 3/82 *(Sonchus oleraceus)*
	Sonchus oleraceus (Smržice)	53
	Sonchus oleraceus (Nové Město and Metují)	28
	Sonchus asper	33
	Senecio vulgaris, *Lactuca serriola* (CS), *Lactuca serriola* (H), *Lactuca sativa* (cv. Cobham Green)	0

Table 2 cont'd

Number of tests	Tested species/cultivar	Isolate of *B. lactucae* (origin)
		Sporulation intensity
7		LA 1/82 *(Leontodon autumnalis)*
	Leontodon autumnalis	13
	Leontodon hispidus ssp. *hastilis*, *Leontodon hispidus* ssp. *hispidus*, *Senecio vulgaris*, *Sonchus oleraceus*, *Sonchus asper*, *Lactuca serriola* (H), *Lactuca serriola* (CS)	0
8		LC 1/81 *(Lapsana communis)*
	Lapsana communis	nt
	Lactuca sativa (cvs. Capitan, Cobham Green, Virilde), *Lactuca serriola* (LSE/1, LSE/3, LSE/18, LSE/57/15, PI 281876, PI 281877, PIVT 1309), *L. serriola* × L. *sativa* "Brun Hilde" (997376/72), *Lactuca saligna* (LSA/57, LSA/92/1, PI 261653)	0

CS = Czechoslovakia, H = Hungary, nt = not tested.

L. sativa cultivars. Inoculum sources and the isolates used are given in Table 2. Plant species which were used for inoculum preparation, served as controls except for *Lapsana communis* whose leaves were not available at the moment of isolate LC 1/82 removal.

A survey of the results is given in Table 2. In all of the tests infection and pathogen sporulation were recorded only in control plants that were species-identical with the plants which the inoculum was obtained from. There were differences in sporulation intensity among respective positive specific interactions (Table 2). Interspecific transmission of the inoculum was recorded within the scope of the genus *Sonchus* only, i.e. between the species *S. oleraceus* and *S. asper*, and vice versa (tests 4 to 6). When *S. asper* was infected with *S. oleraceus* inoculum, sporulation intensity degree was relatively higher than in the opposite case.

Sporulation intensity of the isolate LA 1/82 on *Leontodon autumnalis* reacted very low values. An attempt to transmit this isolate to both subspecies of *L. hispidus* remained unsuccessful (Table 2, test 7).

Discussion

In spite of the fact that *Bremia lactucae* occurs on numerous host-species of the family *Asteraceae*, the respective isolates seem to be highly host-specific. The previous papers of some authors (Schweizer, 1919; Ling and Tai, 1945; Tao Chia-Feng, 1965) have shown that no infestation occurred during the course of cross infections when lettuce *(L. sativa)* was tested using *B. lactucae* isolates

originating from various species of the family *Asteraceae*. Ling and Tai (1945) for example, worked with *B. lactucae* isolates obtained from *Lactuca sativa*, *L. indica*, *L. chinensis*, *Taraxacum mongolicum*, *Crepis japonicus*, *Saussurea affinis* and *Sonchus oleraceus*. The potentiality of cross transmission was observed only in the isolates from *L. sativa* and *L. indica*. The same results were reported by Tao Chia-Feng (1965) in the similar set of plants. Our work comprised the species (e.g. *Leontodon autumnalis*, *Lapsana communis*) and isolates which have not yet been subject to vast experiments. In these host-pathogen relationships a high, plant species-dependent specificity (concerning e.g. *Sonchus asper*, *S. oleraceus*) of the isolates was confirmed.

An experiment with *B. lactucae* isolate from *Leontodon autumnalis* brought interesting results. The transmission of this isolate to the subspecies of the species *Leontodon hispidus (L. hispidus* subsp. *hispidus* and *L. hispidus* subsp. *hastilis)* appeared to be useless. Our observations accomplished under natural conditions have never revealed *L. hispidus* to be infected, although bunches of both species and subspecies grew in close proximity. It should be admitted, however, that *B. lactucae* sporulated on *L. autumnalis*. Non-transmissibility of the fungus supports the conception of classification of the genus *Leontodon* (Holub, 1977) which comprises *Leontodon* and *Scorzoneroides* Moench.

Some recent data (Lebeda, 1984*a*, *b*, 1986) obtained in the study on variation of relationships between *B. lactucae* isolates from *L. serriola* and *Lactuca* species have shown that rather a more complex system may be involved in many instances.

Experiments oriented on the transmission of *B. lactucae* isolates originating from *L. sativa* to the species of the family *Asteraceae* and some others were made by Crute and Dickinson (1976). A total of 24 species and cultivars were inoculated. Alongside some *Lactuca* species, sporulation was found only in *Cichorium endivia*, *C. intybus* and *Gaillardia aristata*. Sporulation intensity was strongly depressed; the percentage of the plants showing sporulation did not exceed 25%. Other plants exhibited only microscopically detectable hypersensitive response. The plants not pertaining to the family *Asteraceae (Brassica oleracea* var. *capitata*, *B. oleracea* var. *botrytis*, *B. napus* var. *napobrassica*, *Raphanus sativus* and *Beta vulgaris)* also showed hypersensitivity (Crute and Dickinson, 1976; McLean and Tommerup, 1979). Results of our tests supplemented by some of the up-to-now non-tested species are in a full agreement with the above data.

The results obtained prove the existence of formae specialis of *B. lactucae*. *B. lactucae* isolates originating from *L. sativa* are capable of creating a compatible relationship only with the species which are closely allied with the initial host. It is evident from the experiments that weed plants from the family *Asteraceae* (except for *Lactuca* species) take no share in epidemiology of *B. lactucae*, i.e. they do not serve a function of a source of inoculum for *L. sativa* infection. Although strong sporulation of pathogen was often observed on frequently occurring weeds *Sonchus oleraceus*, *S. asper* and *Senecio vulgaris*, they cannot be regarded as reservoir plants of *B. lactucae* from the viewpoint of pathogen transmission to cultivars of *L. sativa* and/or species *L. serriola*.

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HPLC Separation and Germination Inhibition Potential of Inhibitors from Teliospores of *Neovossia indica* (Mitra) Mundkur, the Pathogen of 'Karnal' Bunt of Wheat

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The two endogenous compounds from the teliospores of *Neovossia indica*, the pathogen of 'Karnal' bunt of wheat, were separated by HPLC. The isopropanol extract of the teliospores contained 91 % of the one and methanol extract contained 9 % of the other compound. The compound extracted in isopropanol had a high degree of germination inhibitory property. Its extraction from the teliospores increased their germination by 64 per cent. The teliospores from which the extraction of the compound was done by methanol did not germinate, probably due to the toxic effect of methanol.

'Karnal' bunt of wheat caused by *Neovossia indica* is one of the important diseases of wheat, particularly in North-Western India (Bedi and Dhiman, 1982). The disease causes partial damage to grains, lowers their nutritive value by affecting protein quality, reduces seed germination, adversely affects seedling vigour and their tillering capacity (Bedi et al., 1981; Singh et al., 1983; Singh and Bedi, 1985).

One of the constraints in studying the epidemiology of 'Karnal' bunt is poor and erratic germination of teliospores of *N. indica*. There are many reports which mention that teliospores require longer incubation period and some nutritional stimulus for germination (Munjal, 1970; Krihsna and Singh, 1979; 1982; Dhiman and Bedig, 1984). It is also possible that poor teliospores germination may be due to the presence of endogenous inhibitors or permeability barrier of thick and tough three layered teliospores wall (Khanna and Payak, 1968; Trione, 1973; 1977).

This paper reports the extraction and purification of endogenous inhibitors from teliospores of *N. indica* and their possible role in germination inhibition.

Materials and Methods

Source of teliospores

Teliospores were collected from the samples of 'Karnal' bunt infected wheat grains (cv. WL-711) gathered during 1982 and 1984 crop seasons from

Table 1

Extraction of teliospores of *N. indica* with methanol and isopropanol

Extract	Year of teliospores sampling	Solvent	Stirring (magnetic)
I_a	1982	Methanol	Yes
I_b	1984	Methanol	Yes
II_a	1982	Isopropanol	No
II_b	1984	Isopropanol	No
III_a	1982*	Methanol	No
III_b	1984*	Methanol	No
IV_a	1982	Methanol	No
IV_b	1984	Methanol	No

* Teliospores used were those as obtained after making the exact II_a and II_b respectively.

different agroclimatic areas of Punjab. The teliospores from infected grains were separated by a sterilized needle and passed through standard sieves to remove the extraneous host material.

Extraction of inhibitors

Extracts I_a to IV_b (Table 1) were obtained by the following general method of extraction. 125 mg of teliospores of *N. indica* were taken in 50 ml round bottom flask and 15 ml of "AR" grade solvent was added to it. This mixture was refluxed on water bath for 5 hours. It was filtered and the residual teliospores were washed thoroughly with the respective solvent and the total volume of the combined filtrates was made to 25 ml in a volumetric flask. The extracts were kept in a freezer for subsequent studies.

HPLC-analysis

High pressure liquid chromatography (HPLC) was performed on SHIMADZU-LC-4A (Japan) equipped with C-R2AX data processor/printer plotter and UV/visible variable wavelength Shimadzu SPD-1 detector. Zorbax-ODS reverse phase column (Dupont, 5–7 μ, 250 mm × 4.6 mm) was used at ambient temperatures. Chloroform was used as solvent at 1 ml/min flow rate and the absorbance profile at 254 nm of the eluate was recorded at chart speed of 1 cm/min. Normal pressures at 1 ml/min flow rate of chloroform was in the range of 50 kg/cm^2.

λ_{max} value

The λ_{max} values of the compounds A and B (refer to discussion on HPLC results) were taken from their ultraviolet absorption spectra recorded on Spectronic-2000 Spectrophotometer (Bausch & Lomb) in methanol and isorpopanol respectively.

Thin layer chromatography (TLC)

Silica gel-G TLC plates were prepared in our laboratory. The plates were activated and the blank plates were developed in the respective solvent systems 1 and 2 and reactivated before spotting with compounds A and B. The solvent system 1 constituted chloroform : methanol (97.5% : 2.5%): and the solvent system 2, the acetone (100%) alone. After developing the plates, the spots were examined by keeping them in a chamber saturated with iodine vapours.

Teliospores germination

The teliospore of *N. indica* (extracted and unextracted) were floated (Munjal, 1975) over the surface of 20 ml soil extract (Mathur and Ram, 1963; Meiners and Waldher, 1959; Zscheile, 1965) in Petri-plates (Dia. 10 cm) and incubated at 20 ± 1 °C in a BOD incubator. The soil extract was prepared by extracting field soil with water (1 : 1 W/V). The number of germinated eliospores was counted under a microscope (× 100) and there were at least 400 teliospores in each counting. The time required for the start of germination was determined by examining the incubated teliospores at a regular interval of 24 h. The germination percentage given in Table 2 and 3 is the net as observed on the 30th day of incubation.

Table 2

Germination percentage of teliospores after their extraction with isopropanol and methanol

Crop year of teliospore collection	Germination of unextracted teliospores (%)	Germination of teliospores extracted with Isopropanol (%)	Germination increase over unextracted teliospores (%)	Germination of teliospores extracted with methanol* (directly or after extraction with isopropanol) (%)
1982	18.3	30.0	63.9	0.0
1984	23.4	38.5	64.5	0.0

Figures of teliospore germination are the average of 4 different experiments with 3 replications each.

* Toxic: (Trione, 1973).

Table 3

Effect of addition of isopropanol extracts (II_a and II_b) on germination inhibition of isopropanol extracted and unextracted teliospores*

Volume of respective isopropanol extract (II_a and II_b) added 20 ml soil extract	Percentage germination of teliospores			
	1982		1984	
	Extracted	Unextracted	Extracted	Unextracted
0.0 ml	28.0	17.2	36.0	22.9
0.5 ml	17.0	13.0	29.2	18.2
1.0 ml	11.8	8.4	24.0	14.5
1.5 ml	8.5	2.8	18.5	12.4
2.0 ml	4.0	0.0	15.3	9.1
2.5 ml	<1.0	0.0	11.0	4.0
3.0 ml	0.0	0.0	8.2	<1.0
3.5 ml	0.0	0.0	4.2	0.0
4.0 ml	0.0	0.0	1.0	0.0
4.5 ml	0.0	0.0	0.0	0.0
5.0 ml	0.0	0.0	0.0	0.0
ED_{50}	~0.75 ml	1.0 ml	1.5 ml	1.5 ml
ED_0	3.0 ml	2.0 ml	4.5 ml	3.5 ml

* Each experiment was repeated twice with three replications at each concentration. Average values are given.

Results and Discussion

The germination inhibition of bunt teliospores has been attributed to lipoidal substances present in them (Trione and Ching, 1971; Trione, 1977). The nature of these substances is polar in several species of *Tilletia* (Trione, 1973). The initial extraction of the teliospores of *N. indica* (125 mg) separated from 'Karnal' bunt infected wheat grains from 1982 and 1984 crop seasons was made in methanol and the extracts I_a and I_b respectively were obtained. As indicated by HPLC profile (Fig. 1a), the extract I_a contained only two compounds, marked A and B (9.32% : 90.63%),* with the retention time* of 3.08 and 6.05 min, respectively. HPLC profile of I_b was similar to that of I_a.

The compounds, A and B, were separated successfully in pure form by making use of their partial solubility in different solvents. When teliospores from 1982 and 1984 infected wheat grains were extracted (no stirring) separately with isopropanol, only compound B (99.11% pure) was obtained in each of the extracts II_a (Fig. 1b) and II_b. The compound B from isopropanol extracts II_a and II_b co-chromatographed (HPLC) with compound B present in each of the methanol

* Average values obtained from three injections of extract I_a.

extracts I_a and I_b. The residual teliospores after their extraction with isopropanol (extract II_a and II_b) were air dried and further extracted separately with methanol to obtain extracts III_a and III_b. This procedure enables us to get in each extract III_a (Fig. 1c) and III_b, the compound A in 100% pure form in methanol. The compound A in III_a and III_b extracts also co-chromatographed (HPLC) with compound A present in each of the extracts I_a and I_b. When the teliospores from the crop of 1982 and 1984 were extracted as such with methanol, the extracts IV_a and IV_b were obtained. In each of the extracts IV_a and IV_b only compound

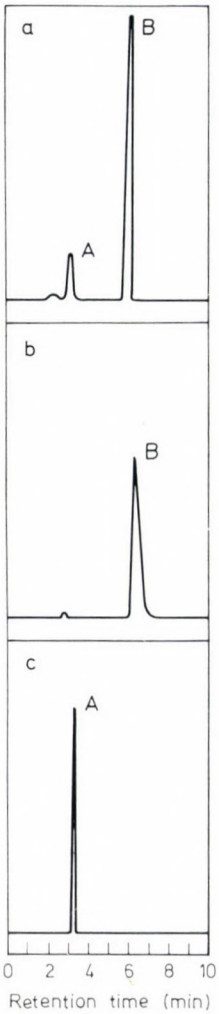

Fig. 1. HPLC elution profile of (a) methanol extract I_a, (b) isopropanol extract II_a and (c) methanol extract III_a

A in 100% pure form was present and its HPLC analysis was similar to that of extracts III_a and III_b. The compounds A and B present in methanol and isopropanol (extracts I_a to IV_b) so obtained were found to be stable for a period of more than one year when these extracts were kept in a freezer.

The U. V. spectrum of compound A showed its $\lambda_{max}^{methanol}$ at 211.1 nm (sharp) whereas compound B showed sharp but overlapping peaks with $\lambda_{max}^{isopropanol}$ 253 and 257.1 nm having small shoulders on either sides at 248 and 265 nm. The thin-layer chromatography of compounds A and B was carried out on silica gel-G plates using solvent systems 1 and 2. The compound B showed its R_f at 0.91 and 0.73 in solvent systems 1 and 2, respectively. However, the compount A in either of the solvent systems could not be detected in iodine. Both the compounds A and B did not absorb U. V. light. It is well known that the fatty acids or their derivatives present in spores inhibit their germination. Some of these have been identified as nonanoic acid and methyl cis-3,4, dimethoxy cinnamate (Trione and Ching, 1971; Macko et al., 1977). The compound A extracted by us does not absorb U. V. light and its λ_{max} being at 211.1 nm which is very close to the λ_{max} of other fatty acids, it is possible that compound A might be fatty acid or its derivative (Scott, 1964). Further work on the structure elucidation of these compounds is in progress and will be reported in a separate communication.

After successful separation of compounds A and B in extracts II_a, II_b and III_a to IV_b (Table 1), it was planned not only to determine the germination potential of the extracted teliospores but also to confirm the inhibitory influence of the extracts on the germination of both, unextracted and extracted teliospores. The germination percentage of teliospores collected during 1982 and 1984 increased by 63.9% and 64.5% respectively when the extraction of inhibitor was done by isopropanol (Table 2). But the germination of methanol extracted teliospores (obtained after making the extracts I_a, I_b and III_a to IV_b) was zero, firstly because of the toxic effects of methanol on the teliospores (Trione, 1973) and secondly because of the broken walls resulting from the stirring of the teliospores. The extraction of the endogenous inhibitor (compound B) from the teliospores with isopropanol not only increased their germination potential but also hastened the initiation of germination process. It was observed that such teliospores started germinating 72 h earlier as compared with the unextracted ones requiring 10 days. Ross et al. (1979) also reported the similar type of results with teliospores of *Tilletia controversa*.

The effect of addition of isopropanol extracts II_a and II_b of the endogenous inhibitor (compound B) on the germination of teliospores is given in Table 3. The data shows that by the addition of increasing volumes of respective isopropanol extracts II_a and II_b to soil extract medium, the per cent germination of teliospores from 1982 and 1984 crops (both extracted and unextracted) decreased gradually and ultimately fell to zero. The addition of these extracts to the extracted teliospores restored their original conditions of low germination. When the respective isopropanol extracts II_a and II_b were added in increasing volume to the unextracted teliospores, a progressive decline in germination percentage was

observed. It confirmed the inhibitory nature of the extracts and their cummulative inhibitory effect on the germination of the teliospores.

It appears that the endogenous compounds present in teliospores of *N. indica* play an important role in determining their dormancy in nature. Further detailed studies of the structure and role of these endogenous inhibitors affecting teliospore germination and post-germination processes, namely promycelial growth and/or formation of sporidia, etc. should aid in enhancing our understanding of the epidemiology of the disease and also in finding the possible focal points for its control.

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Effect of Nitrogen, Phosphorus and Potassium on Sensitivity of Pepper Plants to *Fusarium* Wilt Toxin

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The role of nitrogen, phosphorus, and potassium nutrition in relation to plant growth and development of pepper wilt symptoms caused by culture filtrate of *Fusarium oxysporum* f. sp. *redolens*, and by fusaric acid were studied. The high levels of nitrogen and potassium reduced the susceptibility of pepper plants to culture filtrate and to fusaric acid. Phosphorus in soil could not show such effect. The culture filtrate and fusaric acid exerted similar effect chlorosis, necrosis and epinasty on the plants.

The resistance of certain plants to various pathogenic soil organisms may be affected by subjecting the host plants to nutritional conditions which influence the physiological responses of the plant (Király, 1976; Huber, 1980).

In previous studies with *Fusarium* wilt of tomato it was shown that the extent of disease development decreased with increasing the nitrogen concentration in the nutrient solutions (Sarhan et al., 1982). The fungal metabolite, fusaric acid, (5-n-butyl picolinic acid) is produced by several *Fusarium* species which cause wilt disease in many important crops (Davis, 1969; David et al., 1978; Chakrabarti and Basuchaudhary, 1980; and Barna et al., 1983). However, there is very little information about the effects of nutrition on the resistance of host plants to fungal toxins and especially to those produced by *Fusarium* spp.

The aim of this work was to investigate the effects of nitrogen, phosphorus and potassium nutritions in soil on the resistance of pepper plants to culture filtrate of *Fusarium oxysporum* f. sp. *redolens* and to fusaric acid.

Materials and Methods

Pepper (*Capsicum frutescens*, var. California Wonder) was used for the experiments. Plants were grown in soil in the greenhouse and 2–3 weeks later seedlings were transplanted to a sandy soil in pots of 12 cm in diameter. The plants were supplied with nutrients based on Hogland's solution containing different levels of nitrogen, phosphorus and potassium. The chemical composition of the nutrient solutions is given in Table 1. All pots received 40 ml of appropriate solutions twice a week for 6–8 weeks, the remainder of the water requirement was supplied as tap water. For each treatment 25 plants were used. *Fusarium*

Table 1

Chemical composition of the nutrient solution based on Hogland's solution

Stock solutions	ml of stock solution per liter of nutrient solution*						
	Basal	High N	Low N	High P	Low P	High K	Low K
$Ca(NO_3)_2 \cdot H_2O$	5	5	—	5	5	5	5
KNO_3	5	5	2	5	5	5	—
KH_2PO_4	1	1	1	1	1	1	—
$MgSO_4 \cdot 7H_2O$	2	2	2	2	2	2	2
$CaCl_2 \cdot 2H_2O$	—	—	5	—	—	—	—
$NaNO_3$	—	30	—	—	—	—	5
KCl	—	—	3.5	—	1	12	1
$NaH_2PO_4 \cdot 4H_2O$	—	—	—	2	—	—	—
NaCl	30	—	30	27	30	18	25

* One ml of minor elements stock solution (H_3BO_3, 0.28 g; $CuCl_2 \cdot 2H_2O$, 0.004 g; $ZnCl_2$, 0.003 g; $MnCl_2 \cdot 4H_2O$, 0.18 g; $FeCl_3 \cdot 6H_2O$, 0.05 g; distilled H_2O 100 ml) added to each nutrient solution.

oxysporum f. sp. *redolens* was used for production of toxic material. The fungus was grown in modified Richard's medium (Kuo and Scheffer, 1964). Medium was inoculated with small pieces of mycelial mat and fungus was grown in stillculture at 28 °C. After incubation for 14 days the culture was filtered through cheese cloth and then through membrane filter to obtain a crude stock toxin solution.

To test the effect of fusaric acid, a solution of this toxin (0.5×10^{-3} M) was used. To assess the effects of test solutions on detached leaves of similar positions, pepper leaves were put into small tubes and allowed to take up the toxin solutions or the filtrate or the medium for control experiments. The test tubes were incubated under laboratory conditions. Visual observations were made daily up to 4–6 days.

Results and Discussion

Pepper leaves exposed to the normal atmosphere and treated with culture filtrate obtained from the virulent strain of *Fusarium oxysporum* f. sp. *redolens*, and with 0.5×10^{-3} M fusaric acid produced similar symptoms of grey-green necrotic spots on the leaves besides epinasty as described by Barna et al. (1983). Both culture filtrate and toxin solution exerted similar effects on the treated plants. The most severe symptoms occurred on control plants and on plants treated with the basal solution. The filtrate and toxin solutions caused chlorosis in 50–70 per cent leaves. The high nitrogen and high potassium regimes produced plants which were the most resistant to the culture filtrate as well as to the fusaric acid solutions. However, phosphorus had no significant effect against toxin (Table 2).

Table 2

Effect of nitrogen, phosphorus, and potassium nutrition on the development of symptoms caused by culture filtrate of *F. oxysporum* f. sp. *redolens* and by fusaric acid on pepper

Treatments	Mean height of plants (cm)	Leaves showing chlorosis (%)		Disease intensity*	
		(C.f.)	(F.a.)	(C.f.)	(F.a.)
Control (H_2O)	22.3	53.1	68.3	+++	+++++
Basal	28.1	25.5	32.8	+++	+++
Low N	25.4	17.2	20.0	+++	++
High N	34.9	2.8	5.6	−	+
Low K	26.6	15.1	13.5	+++	++++
High K	29.6	4.2	3.3	−	++
Low P	24.2	18.3	15.4	+++	++++
High P	25.3	8.2	10.0	++	++

* Disease intensity; +++++ = severe symptom; + = very mild symptoms; − = no symptoms.

Pepper plants grew better in basal, high nitrogen and high potassium containing soils. The data obtained in the present investigation strengthen the hypothesis that toxins produced by the pathogen could be involved in pathogenesis and it was primarily responsible for the disease symptoms in wilt of pepper.

Chakrabarti and Chaudhary (1980) have also observed a similar effect in case of *Fusarium oxysporum* f. sp. *carthami* on sunflower plants.

Although in all treatments leaves of the same age were used, their physiological states due to the various treatments were different. Thus leaves of plants grown with high levels of nitrogen and potassium were physiologically more juvenile than those in other treatments. In the previous work (Sarhan et al., 1980) we considered that factors associated with juvenility are involved in the decreased sensitivity of the plants to necrotizing factors (toxins, enzymes) secreated by the pathogen.

These results are consistent with those of Sarhan et al. (1980; 1982) and Barna et al. (1982; 1983) who concluded from experiments with *Fusarium* wilt of tomato that factors which maintained juvenility in plants could increase plant resistance to the pathogen and to its toxins.

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Effect of Fertilizer Treatments on the Incidence of Leaf Blights of Wheat

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The effects of twenty five different treatment combinations of three fertilizers on the occurrence and severity of three leaf blight diseases on the susceptible wheat variety "Balaka" were determined. Five different doses of nitrogen, phosphorus and sulphur in the form of urea. TSP and gypsum respectively were tested. Severity of leaf blight caused by *Alternaria triticina*, *Drechslera sorokiniana* and *Pseudomonas syringae* was scored by measuring the blighted leaf area of 15 leaves randomly selected from 15 plants. Increase in dose of nitrogen and phosphorus fertilizers significantly increased severity of all three blight diseases. In case of sulphur, dose increase had significant effect on *Drechslera* leaf blight. All combinations of two fertilizers among the treatments significantly affected the severity of all diseases except that the combinations of P×S and N×S were found insignificant on bacterial leaf blight diseases. The interaction of three fertilizers significantly favoured the disease increase of *Alternaria* leaf blight and *Drechslera* leaf blight.

Fertilizers have been reported to have marked effects on the severity of plant diseases. Of the three major fertilizers nitrogen has been shown to either increase or decrease the disease severity (Jarvis and Thorpe, 1980). Heavy nitrogenous manuring usually increases disease proneness (Suryanarayana, 1958) and susceptibility of rice varieties to blast has been reported to increase with the increase in the accumulation of nitrogen in the leaf (Ganguly et al., 1954). Dwivedi and Sukla (1981) demonstrated that increase in dose of nitrogen intensified *Drechslera* leaf spot of mung whereas the relation between the dose of phosphorus and potassium to diseases severity was inverse. Therefore, it is seen that host nutrition plays an important role in affecting infection by plant pathogens.

Since a decade ago with the introduction of high yielding cultivars/lines of wheat and their intensive cultivation in this country the use of fertilizers has been increased three to four folds. Diseases of various causes have brought heavy yield loss (Anonymous, 1981) of the crop; among different factors responsible for those diseases, use of fertilizers may be an important one. Recently, three leaf blights popularly known as blight complex of wheat caused by *Drechslera sorokiniana*,

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Alternaria triticina and *Pseudomonas syringae* have drawn attention of both wheat growers and researchers.

The present study was undertaken to see the effect of NPK fertilizers and their interactions on the incidence and severity of leaf blight complex of wheat.

Materials and Methods

The wheat variety 'Balaka' susceptible to the blight complex was used in this study. Nitrogen (N), phosphorus (P) and sulphur (S) in the form of urea, triple superphosphate and gypsium, respectively were applied in the experimental

Table 1

Effect of fertilizer treatments on the incidence of three different blight diseases of wheat at BAU farm, Mymensingh

Treatment, kg/ha			Average disease severity score		
N	P_2O_5	S	Alternaria leaf blight (ALB)	Bacterial leaf blight (BLB)	Drechslera leaf blight (BLB)
0	0	0	0.7	2.5	0.7
40	40	0	0.8	2.7	0.9
40	40	40	1.3	2.4	0.7
40	80	0	0.5	3.0	1.0
40	80	40	1.3	2.4	0.9
40	120	0	1.1	2.7	0.3
40	120	40	0.4	2.8	0.8
80	40	0	0.9	2.6	0.5
80	40	40	0.8	2.7	1.8
80	80	0	0.6	2.2	0.6
80	80	40	0.6	2.9	2.3
80	120	0	0.3	1.9	0.6
80	120	40	0.8	2.0	1.4
120	40	0	1.4	1.9	1.4
120	40	40	0.2	2.7	1.2
120	80	0	0.6	3.0	1.4
120	80	40	0.6	3.1	2.4
120	120	0	0.2	4.0	2.6
120	120	40	0.3	4.0	3.0
160	40	0	0.7	3.2	1.6
160	40	40	0.5	2.5	1.7
160	80	0	0.6	2.2	0.6
160	80	40	1.2	3.0	1.5
160	120	0	0.2	3.0	2.7
160	120	40	1.0	3.4	2.7
Mean			0.7	2.8	1.4
LSD		.05	0.35205	0.83699	0.39532
		.01	0.46978	1.11693	0.52754

field. The experiment was conducted in randomized block design at the Bangladesh Agricultural University (BAU) farm during the rabi (dry) season of 1982–83. Seeds were shown continuously in unit plot of 4 m × 5 m with a spacing of 20 cm between lines. Four levels of N, three levels of P and two levels of S were used as stated below:

Urea (for nitrogen): 40 (N_1), 80 (N_2), 120 (N_3) and 160 (N_4) kg/ha.

Triple superphosphate (for phosphorus): 40 (P_1), 80 (P_2) and 120 (P_3) kg/ha.

Gypsum (for sulphur): 0 (S_0) and 40 (S_1) kg/ha.

There were altogether 25 treatments (Table 1) including combination of the levels of fertilizers and one absolute control. Each of the treatments was repeated thrice.

The experimental field received a basal dressing of potash 40 kg K_2O from Muriate of potash.

Under natural field conditions, disease severity was estimated on the upper three leaves of each tiller of the plant. Blighted leaf areas of leaves of randomly selected 15 plants in one-unit plot were measured. Disease intensities of Alternaria leaf blight and Drechslera leaf blight were measured following the scale of Prabhu and Amarsingh (1974), and Bacterial leaf blight was measured following the scale of Sellam and Wilcoxson (1976). The results were subjected to analysis of variance and treatment effects were compared to each other by Duncan's Multiple Range Test.

Results and Discussion

The average disease severity of three blights as scored on wheat variety 'Balaka' for 25 different fertilizer treatments is presented in Table 2. It is evident from the table that the average disease severity for all three blights, in general, was higher in fertilizer treated plots as compared to their corresponding controls. Of the three blights, bacterial leaf blight (BLB) showed highest response to fertilizer treatment and Alternaria leaf blight (ALB) showed the least. The response reaction was found prominent only for nitrogen and phosphorus.

The response of the disease severity to dose of fertilizers for the three blights was different. Increase in dose of nitrogen increased disease severity in all three blights but in case of BLB the disease severity increase was the highest. The same trend was noticed for both phosphorus and sulphur treated plots. The differences in disease severity in case of ALB and BLB did not differ between treated and control plots (Table 2) but these differences were significant in case of Drechslera leaf blight (DLB).

All combinations of two fertilizers among the treatments significantly affected the severity of all diseases except that the combinations of P × S and N × S were found insignificant on BLB. Again the interaction of three fertilizers (N × P × S) significantly favoured the increase of severity of ALB and DLB but not BLB.

Table 2

Single effect of N. P. and S on the incidence of three different blight diseases of wheat

Fertilizer dose kg/ha	Alternaria leaf blight	Bacterial leaf blight	Drechslera leaf blight
Effect of N			
Control	0.7	2.5	0.7
40	0.9a	2.7c	0.8d
80	0.6c	2.4d	1.2c
120	0.5d	3.2a	2.0a
160	0.7b	2.9b	1.8b
Effect of P			
Control	0.7	2.5	0.7
40	0.8a	2.6c	1.2c
80	0.7b	2.7b	1.4b
120	0.5c	3.1a	1.8a
Effect of S			
Control	0.7	2.5	0.7
0	0.6a	2.7a	1.2b
40	0.7a	2.8a	1.7a

In a column figures having same letter(s) do not differ significantly at 5% level of significance.

In the present study, it was found that disease incidence increased with the increase in nitrogen level. This is in agreement with the finding of Krishnaswami (1952), Padmanabhan (1953), Ganguly et al. (1954), Suryanarayana (1958), who reported that heavy nitrogenous manuring increased the proneness of rice plant to blast disease. There is also report that manuring with a high level of nitrogen and low level of phosphorus most effectively reduced the Brown foot rot of wheat in the green house (Onuorah, 1969) and extremely high level of N significantly decreased severity of BLB of *Philodendron selloum* caused by *Erwinia chrysanthemi* (Haygood, 1982). Again it has been found that high dose of phosphorus increased the disease severity which is not in line with the findings of Bolle-Jones and Hilton (1957) who found that intensity of rubber seedling infection by *Helminthosporium heaveae* increased with low dose of phosphorus. On the other hand, Dwivedi and Sukla (1981) have reported that phosphorus in various levels applied to 'mung' crop did not play any role in increase or decrease of Drechslera leaf spot.

The results presented here indicate that fertilizers may have some effects on the incidence and severity of some foliar wheat diseases although the above discussion is not unequivocal.

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Primarily-infected Potato Plants as Sources of Potato Virus Y

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Aphids first transmitted potato virus Y^O (PVY^O) or PVY^N from glasshouse-grown potato plants 12–20 days after having been manually inoculated. The interval between inoculation and first transmission was longer when older plants or plants of more resistant cultivars were tested. Inoculating field potato plants by sap early in the growing season with either PVY^O or PVY^N increased the proportion of infected tubers in the progeny of surrounding plants.

Potato plants grown from infected tubers (secondarily infected plants) are presumed to be the main source of potato virus *Y* (PVY) in British potato crops for the first four weeks following emergence but it is not known whether primarily-infected plants are important additional sources for spread later in crop growth. In the glasshouse aphids may acquire PVY from potato plants as soon as symptoms appear (Kato, 1957). The strain PVY^O is acquired 15–20 days after inoculation (Stevenson, 1959), and PVY^N 10 days after sap inoculation and 14 days after aphid inoculation (Beemster, 1979). However, in a year when aphids were rare, Heathcote and Broadbent (1961), found little spread of PVY from inoculated plants. Field plants may be protected sufficiently by mature plant resistance (Sigvald, 1985), especially against PVY^O (Beemster, 1972, 1976), by the time primarily infected plants can act as virus sources.

We investigated how soon primarily infected potato plants became virus sources and whether in England this occurred early enough in the season to make them important in the epidemiology of PVY^O and PVY^N. In most tests, both cvs King Edward and Record were used because, although PVY causes only a mild mosaic in both, PVY^O induces leaf drop streak in the first and mosaic in the second; plants with mosaic symptoms may be better sources of PVY than those which become necrotic (Bagnall and Bradley, 1958).

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Materials and Methods

Glasshouse experiments

Plants were grown in 15 cm diameter pots of compost from small tubers as single-stemmed plants, were kept at 20–25 °C, and given 16 h day-length using artificial lighting. Plants were sap-inoculated on the youngest fully-expanded leaf using sap from PVY^O- or PVY^N-infected tobacco plants cv. White Burley and carborundum abrasive.

Adult apterae of peach-potato aphid (*Myzus persicae*), reared on Chinese cabbage, were used as vectors, and tobacco seedlings cv. White Burley were used as test plants.

Comparison of plants inoculated at different ages

Batches of four potato plants cv. King Edward were sap-inoculated, 3, 5 or 7 wks after planting with either PVY^O or PVY^N. Twelve and 20 days later, 12 *M. persicae* were given 2 1/2 min access to a fully-expanded uninoculated leaf from each plant and each aphid then caged overnight on a tobacco seedling. The seedlings were then sprayed with the aphicide pirimicarb and kept for 2 wks for virus symptoms to develop. The experiment was replicated three times.

Comparison of different cultivars as virus sources

The cultivars used, arranged in order of increasing resistance to PVY (Anonymous, 1985), were King Edward, Record, Maris, Piper, Desiree and Pentland Crown. Four plants of each cultivar were sap-inoculated with PVY^O or PVY^N 3 wks after planting. Twelve and 20 days after inoculation, two fully expanded uninoculated leaves from each plant were tested by allowing batches of ten aphids 2 1/2 min access. Each batch of aphids was kept overnight on a single tobacco seedling. The seedlings were then sprayed with pirimicarb and kept for two weeks for virus symptoms to develop.

Comparison of different virus isolates

Seven isolates of PVY^O and three of PVY^N from field plants of King Edward (3 PVY^O), (2 PVY^N), Record (1 PVY^O, 1 PVY^N), Cara (1 PVY^O) or Pentland crown (2 PVY^O) were each sap-inoculated to four plants of King Edward 3 wks after planting. Twelve days later, batches of 10 *M. persicae* were allowed 2 1/2 min access to two uninoculated leaves on each plant and each batch was transferred to a single tobacco seedling. Next day, the seedlings were sprayed with pirimicarb and then kept for two weeks for virus symptoms to develop.

On 15 May 1983, 16 plots, each 7.5 m (10 rows) × 10.5 m, were planted with cv. King Edward. The plots were in two blocks of eight plots separated by

14 rows of King Edward but adjacent plots in each block were not separated. In two of the plots in each block, a tuber from the centre of each of the ten rows was removed and replaced with a tuber of cv. King Edward infected with both PVY^O and PVY^N. In three plots, the central tubers were replaced with uninfected tubers cv. Record and in these and the remaining three plots (in which the central King Edward tuber in each row was undisturbed), the plants growing from these central tubers were either uninoculated, sap-inoculated on 18 June with PVY^O, or sap-inoculated with PVY^N. About 0.3% of the King Edward seed tubers planted in this experiment were infected with PVY but these were removed as soon as symptoms were visible.

To compare secondarily and primarily infected plants as sources of PVY, leaves were collected from five primarily and five secondarily infected plants on the 8th and 18th July. Twelve *M. persicae* were given 2 1/2 min access in a leaf and then confined singly overnight on test plants. To assess spread of PVY to neighbouring plants in the field plots, one tuber was taken (15–20 September) from each of the four plants on either side of the ten central plants in each plot. Only tubers >10 g were sampled as small tubers are less subject to infection than large ones (Beemster, 1967). When dormancy had ended, plants were grown in a glasshouse from eyes excised from each tuber and the foliage examined for symptoms of PVY.

The proportions of test plants and tubers infected with PVY were logit-transformed before an analysis of variance was done.

Results

Glasshouse experiments

Comparison of plants inoculated at different ages. Twelve days after the plants had been inoculated, about 7% of aphids transmitted from leaves of plants inoculated when three weeks old with PVY^O or PVY^N, about 1% transmitted from plants inoculated when five weeks old and none transmitted from plants inoculated when seven weeks old. When access was given 20 days after inoculation, 20–50% of aphids transmitted irrespective of whether plants were three, five or seven weeks old and whether they were inoculated with PVY^O or PVY^N.

Comparison of different cultivars as virus source. Leaves of King Edward, Maris Piper and Desiree plants inoculated with PVY became necrotic whereas only mosaic symptoms appeared in Record. Inoculation with PVY^N gave no obvious symptoms in any of the cultivars tested. In some plants of the more resistant Desiree and Pentland Drown, no virus symptoms were seen and no virus was acquired by aphids.

Twelve days after inoculation of the source plants, aphids transmitted PVY^O or PVY^N only from King Edward but eight days later aphids transmitted either strain from each cultivar.

Table 1

Numbers of tobacco plants infected with PVY^O or PVY^N by *M. persicae* allowed access to leaves from field grown plants of King Edward or Record

Days after inoculation of primarily infected plants	Virus strain	Source plants		
		Primarily infected		Secondarily infected King Edward
		Record	King Edward	
20	PVY^O	10/60** (−0.827±0.175)+	10/60** (−0.827±0.175)	35/60 (0.263±0.132)
	PVY^N	9/60** (−0.891±0.183)	12/60** (−0.714±0.163)	
30	PVY^O	31/60 (0.033±0.159)	33/60 (0.101±0.160)	36/60 (0.206±0.162)
	PVY^N	29/60 (−0.035±0.159)	30/60 (−0.001±0.159)	

+ Numbers in parentheses are mean logits ± S.E.

** Transmission rates are significantly different from that from secondarily infected plants at $P = 0.01$.

Table 2

Proportions of PVY-infected tubers in the progeny of plants near to primarily or secondarily infected potato plants in the field

Strain inoculated	Cultivar of central potato plant	
	Record	King Edward
PVY^O	98/160*** (0.229±0.095)+	105/160*** (0.290±0.095)
PVY^N	105/160*** (0.314±0.095)	108/160*** (0.352±0.095)
Uninoculated	34/160 (−0.671±0.095)	36/160 (−0.612±0.095)
Secondarily infected#		299/320 (1.167±0.067)

+ Numbers in parentheses are mean logits ± S.E.

*** Proportion of infected tubers is significantly different from that from plots with secondarily-infected infectors and also from control plots (no infectors) at $P = 0.001$.

$PVY^O + PVY^N$.

Comparison of different virus isolates. All PVY^O isolates induced mosaic symptoms in King Edward followed by leaf drop streak; PVY^N isolates all induced mild mosaic symptoms. Twelve days after inoculation, all isolates were tramsmitted by aphids.

Field grown plants as sources. 20 days after inoculation aphids transmitted PVY^O or PVY^N from leaves of primarily infected field-grown plants inoculation but less frequently than they did from secondarily infected plants. However, 10 days later, rates of transmission from secondarily and primarily infected plants were similar (Table 1).

In plots without deliberate infector plants, 20% of the tubers collected had PVY (Table 2). Infection was about three times more frequent in tubers from plots with primarily infected plants as infectors ($P < 0.001$). Neither the cultivar inoculated nor the PVY strain used affected the amount of virus spread ($P > 0.05$). Infection was even more frequent in tubers from plots with secondarily infected plants.

Discussion

It generally took 20 days before sap-inoculated glasshouse-grown potato became sources of either PVY^O or PVY^N for aphids although transmission from young plants of the susceptible cultivar King Edward occurred infrequently twelve days after inoculation. Transmission from field plants was also recorded 20 days after their inoculation, and after 30 days transmission from such primarily infected plants was as frequent as from secondarily infected plants.

There were more infected tubers in the progeny of field plots where the infector plants were secondarily infected than where they were primarily inoculated. This was to be expected because secondarily infected plants were sources for virus spread from the beginning of the growing season. However, infection was three times more frequent in tubers from plots with inoculated plants as sources than in tubers from plots without infectors, indicating that the primarily infected plants acted as important sources of PVY for late season spread. Although plants inoculated by sap become a source of PVY a few days sooner than plants inoculated by aphids (Beemster, 1979), this is probably counterbalanced by aphids at Rothamsted spreading PVY prior to 18 June (Harrington et al., 1986) the date of sap-inoculation.

Despite the earlier development of mature plant resistance against PVY^O than against PVY^N (Beemster, 1972; 1976), virus spread in plots with plants inoculated with PVY^O and PVY^N was similar, indicating that any differences in the rate of development of resistance to the isolates of PVY^O and PVY^N tested had little epidemiological effect. Furthermore, the proportion of progeny tubers infected in plots containing King Edward plants inoculated with PVY^O as infectors was similar to that in plots where the infectors were King Edward plants inoculated with PVY^N on Record plants inoculated with PVY^O or PVY^N, indicating that leaf drop streak, which occurred only in King Edward inoculated with PVY, also had little epidemiological effect.

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Variability of *Drechslera graminea*, the Causal Fungus of Leaf Stripe of Barley

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The pathogenic variability of 12 isolates of *Drechslera graminea* was apparently recorded on 11 barley varieties. The isolates also varied considerably in their cultural characteristics such as rate of mycelial growth, nature of mycelium, colour of colony, pigmentation and mutation. The majority of isolates showed moderate or high rate of growth. Fluffy mycelium and gray colony were recorded more than velvety mycelium and olivaceous colony, respectively. Pigments and mutants were absent in most isolates. Ability of production of pectolytic and cellulolytic enzymes varied in the tested isolates. Only two isolates were unable to produce pectinmethylesterase in synthetic media, whereas all of them produced polygalacturonase and cellulase with noticeably different amounts.

The cultural filtrates of *D. graminea* isolates displayed distinctly different effects on rate of germination of barley seeds and morphologic characters of seedlings.

Drechslera graminea (Rabh. ex Schlecht.) Shoem., the incitant of leaf stripe of barley is not considered as highly specialized as the rusts and smuts, but this specialization should not be overlooked in testing for varietal reaction (Shands and Arny, 1944). Specialization of this fungus was not clear-cut, in spite of recording two distinct cultures (Arny, 1945).

Pathogenic variability in *Helminthosporium gramineum (Drechslera graminea)* was studied on several barley varieties. Distinct differences were found among 7 groups of *H. gramineum* in their prevalence and severity on barley varieties (Mohammad and Mahmood, 1976).

Isolates of *H. gramineum* were classified into distinguished groups different in their morphological and cultural characteristics (Mohammad and Mahmood, 1973).

Production of pectolytic and cellulolytic enzymes in synthetic media was used also for classification of fungal isolates since a detectable variability was noticed. Polygalacturonase activity was found to be closely correlated with virulence of *Colletotrichum falcatum* (Singh and Hussin, 1964). The virulent strains of *Pseudomonas solanacearum* were superior in producing polygalacturonase, pectinmethylesterase and cellulase in both culture filtrate and infected plant juice (Shalaby, 1975). The culture filtrate of *H. gramineum* was found to cause inhibition

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of germination of barley seeds (Luke and Gracen, 1972). The filtrates also may cause a reduction in length of radicle and plumule of barley seedlings (El-Sherif, 1978).

In the present investigation; pathogenic, morphologic and physiologic characteristics of several isolates of *D. graminea* were compared to provide more criteria for accurate specialization of the fungus.

Materials and Methods

Twelve isolates of *Drechslera graminea* were derived from barley leaves showing typical stripe symptoms. Small pieces of the infected leaves were surface-sterilized in 0.2% mercuric chloride for two minutes, washed several times with sterile distilled water, carefully dried in between two Watman No. 1 papers, transferred to PDA plates and incubated at 25 °C. Pure cultures were obtained by cutting hyphal tip (Brown, 1924).

Varietal reactions

Eleven varieties of barley, showed various reactions against *D. graminea* under different conditions, were inoculated with 12 isolates. Healthy, disinfected seeds were placed on an actively growing 8-day-old mycelial culture of each isolate for 10 days before planting. Forty-five days after planting, reactions were recorded according to the following score; 0: leaves with no disease symptoms (highly resistant); 1: leaves with light brown stripe running through the leaf 1–20% (resistant); 2: leaves with dark brown stripe running 21–40% (moderately resistant); 3: leaves with dark brown stripe running 41–60% (moderately susceptible); 4: leaves beginning to lose normal green colour with rather long stripe running 61–90% (susceptible); 5: leaves drying with stripe running 91–100% (highly susceptible), (Mohammad and Mahmood, 1976).

Cultural characteristics

A disc, 5 mm in diameter, of each tested isolate was placed onto potato dextrose agar (PDA) medium in Petri dish, 9 cm in diameter. Ten days later, the colonies were examined for the following characteristics; rate of mycelial growth, nature of mycelium, colour, pigmentation and mutation.

Production of pectolytic and cellulolytic enzymes

The tested isolates were grown at 25 °C for 15 days on a liquid synthetic medium containing 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001% $FeSO_4 - 7H_2O$, 0.3% L-arginine, 1.8% KH_2PO_4, 2% pectin or carboxymethyl cellulose (CMC) (Talboys and Busch, 1970). Cultures were filtered off in Buchner funnel and then

centrifuged at 3000 rpm for 20 mins. The clear supernatants were used to evaluate enzyme activities.

Polygalacturonase (PG) and cellulase (C_x) activities were measured by estimation of the loss in viscosity of the substrates (10 ml of 1.2% pectin or CMC, respectively, in phosphate buffer solution at pH = 5.6 mixed with 5 ml of culture filtrate), in Ostwald viscometer at 30 °C. Viscosity was measured at five-minute-intervals and percentage of loss in viscosity was recorded.

Assay of pectinmethylesterase (PME) was based on the hydrolytic removal of methyl groups of pectin molecule by the action of the enzyme (Kertész, 1951). A volume of culture filtrate of each isolate (5 ml) was mixed with an equal volume of 1.2% aqueous high methyl pectin buffered at pH 5.5, allowed to react for 4 h at 30 °C, titrated back to pH 5.5 with 0.01 N NaOH, kept for 20 h, then readjusted as before-mentioned. Boiled culture filtrate was also used, and the difference between volumes of NaOH required in the two cases was estimated to give an approximate indicator of relative activity of PME.

Effect of culture filtrate on seed germination and seedling morphology

The isolates under investigation were grown on Czapec Dox broth medium for three weeks at 25 °C, then filtered through Seitz funnel. A medium without fungal growth was used as a control.

Distinfected kernels of Giza 119, a susceptible barley variety, were placed on a layer of sterile cotton inside Petri dishes, 20 kernels/dish. The cotton layers were saturated with 20 ml of culture filtrate. The Petri dishes, containg cotton, kernels and culture filtrate, were incubated at 25 °C for 10 days. Percentage of seed germination, length of both plumule and radicle were recorded.

Results

Variability of varietal reactions

Varietal reactions of 11 barley varieties against 12 Egyptian isolates of *Drechslera graminea* are shown in table 1. Pathogenicity of isolates was apparently different. Isolate No. 4 was found to attack all varieties showing susceptible reactions. Ten varieties were highly susceptible to isolate No. 7, whereas only one variety, Abyssinian, showed resistance. On the contrary, isolate No. 6 was found to attack one variety, Black Hulles, showing moderate susceptibility. Isolates No. 11 and 12 were unable to infect Quin and Abyssinian, respectively. Moreover, Nepal showed resistance against both of them. Isolates No. 2, 5, 10 failed to infect four different varieties. Isolates Nos 9, 8, 1, 3 were unable to infect 3, 5, 6, 9 varieties, respectively. Black Hulles was susceptible to all isolates, and Oderbrucker showed resistance to one isolate, No. 6, only.

Table 1

Reaction of 11 barley varieties against 12 Egyptian isolates of *Drechslera graminea*

No.	Varieties		C.I. No.	Isolates, No.											
				1	2	3	4	5	6	7	8	9	10	11	12
1	Nepal*	H, N	595	0	0	0	5	0	0	5	0	0	0	0	0
2	Hanchen	2	531	0	0	0	3	5	0	5	5	0	0	5	5
3	Abyssinian	A	1243	0	0	0	5	0	0	0	0	0	5	5	0
4	Lion	Bk, S	923	0	5	0	5	5	0	5	0	5	5	5	5
5	Excelsior	N	1248	0	5	0	5	5	0	5	0	5	5	5	5
6	Quinn		1024	5	5	0	5	0	0	5	0	5	5	0	5
7	Gatami	Bk	1413	0	0	0	5	0	0	5	5	5	0	5	5
8	Trebi		936	—	5	0	5	—	0	5	5	5	0	5	5
9	Odessa		934	5	3	0	5	5	0	5	5	5	5	5	5
10	Black Hulles	A, Bk	1277	5	3	5	3	5	3	5	5	5	5	5	5
11	Oderbrucker		940	5	3	5	5	5	0	5	5	5	5	5	5

* All varieties are white, 6-rowed, rough awned and have adhering lemma and palea, except where other characteristics are indicated according to the following Key; 2 : 2 — rowed, N: Naked Kernels, A: Abyssinian intermediate, S: Smooth awned. Bk: Black, H: Hooded

Table 2

Cultural characteristics of *D. graminea* isolates grown on PDA for ten days

Isolate	Rate of mycelial growth	Nature of mycelium	Colour	Pigmentation	Mutation
1	M	Fluffy	Olivaceous	—	+
2	M	Fluffy	Gray	—	—
3	L	Velvety	Olivaceous	+	—
4	H	Fluffy	Gray	+	—
5	M	Fluffy	Gray	—	+
6	L	Fluffy	Gray	+	—
7	H	Fluffy	Gray	—	—
8	M	Velvety	Olivaceous	—	—
9	H	Fluffy	Gray	—	+
10	M	Fluffy	Gray	—	—
11	M	Velvety	Olivaceous	—	+
12	H	Fluffy	Gray	—	—

L, Low = 3–4 cm in diameter. M, Moderate = 5–7 cm. H, High = more than 7 cm (+) Present. (−) Absent.

Variability of cultural characteristics

Five cultural characteristics of 12 isolates of *D. graminea* grown on PDA for 10 days were compared (Table 2). Four isolates showed high rates of mycelial growth, whereas six isolates were moderate, and two isolates only grew slowly. In regard to nature of mycelium, nine isolates were fluffy, whereas three isolates were velvety. Colour of colony was mostly gray except four isolates which were olivaceous. Pigmentation and mutation were recorded only in three and four isolates respectively.

Variability of production of pectolytic and cellulolytic enzymes

Ability of the tested isolates of *D. graminea* to produce cellulase and polygalacturonase was measured by estimating percentage of loss in viscosity.

Pectinmethylesterase production was expressed as the actual values of 0.01 N NaOH required to neutralize the carboxylic groups. Data are presented in Table 3. The isolates were found to show different abilities of producing cellulolytic and pectolytic enzymes.

Table 3

Production of cellulolytic and pectolytic enzymes in culture filtrate of 12 isolates of *D. graminea* grown on special synthetic medium

Isolate	% Loss in viscosity/5 min		NaOH ml required for P.M.E. activity[b]
	Cellulase[a]	Polygalacturonase[a]	
1	17.5[c]	22.5	0.000
2	20.0	23.5	0.050
3	9.0	18.0	0.025
4	25.0	26.5	0.100
5	20.0	20.0	0.050
6	5.0	17.5	0.000
7	24.5	26.5	0.100
8	12.0	22.5	0.025
9	22.5	25.5	0.055
10	16.5	20.5	0.000
11	14.5	20.0	0.025
12	23.5	26.0	0.055
S.E.	0.88	0.44	0.005

[a] Percentage of relative loss in viscosity of 1.2% CMC or pectin solution per five min during 20 min of incubation with culture filtrate at pH = 5.6.

[b] Actual value of 0.01 N. NaOH ml required to neutralize the carboxylic groups produced from 1.2% pectin solution at pH 5.6 after 24 h incubation = volume for normal filtrate-volume for boiled one.

[c] Each figure is the mean of three replicates.

Two isolates, No. 3 and No. 6, showed low activity of C_x (5–9%) and PG (18%). Other two isolates, No. 1 and No. 10, as well as No. 6 were unable to produce PME. Most isolates showed intermediate activity of C_x (10–20%), PG (20–25%) and PME (0.025–0.050 ml). Isolates No. 4, No. 7, No. 9. and No. 12 produced highly considerable amounts of C_x (more than 20%), PG (more than 25%), and PME (more than 0.050 ml).

Effects of culture filtrates on germination of barley seed and morphology of seedlings

A drastic decrease was recorded in percentage of seed germination and length of both plumule and radicle, as a result of application of culture filtrates (Table 4). Rates of seed germination were less than 20% by using filtrates of three isolates, Nos 7, 4 and 12. On the contrary, rates of germination were more than 50% in filtrates of Nos 3 and 6. In the rest isolates, germination in their culture filtrates was 25 to 50%. Rate of seed germination was 83%, when media without fungal growth were used.

Mean length of radicle and plumule was 61.3 and 65.5 mm in the control, whereas it was less than 10 and 5 mm, respectively, in the treatments. Effect of culture filtrate of the isolates und investigation, on length of radicle and plumule, was apparently different.

Table 4

Effect of culture filtrate of 12 isolates of *D. graminea* on seed germination and length of both redicle and plumule of barley seedlings

Isolate	Percentage of germination	Radicle length, mm	Plumule length, mm
1	32.0	4.8	3.4
2	45.8	8.8	3.7
3	52.5	9.0	4.4
4	16.3	1.8	2.3
5	36.5	5.2	3.1
6	55.0	9.0	4.8
7	12.0	1.4	2.5
8	35.0	6.4	2.5
9	20.0	2.1	2.8
10	23.0	3.5	3.2
11	39.0	3.5	3.0
12	18.0	1.8	2.5
Control	83.0	61.3	65.5
LSD (P = 0.05)	10.6	2.7	1.7

Discussion

Physiologic specialization of *Drechslera graminea* was not clearcut (Arny, 1945). This may be referred to dependence only on pathogenic variability using insufficient barley varieties for differentiation. Afterwards, morphological and cultural characteristics of *D. graminea* isolates were used to distinguish different groups of the fungus (Mohammad and Mahmood, 1973). In the present investigation variability of pathogenic, cultural and physiologic characteristics of 12 Egyptian isolates were studied to provide more criteria for accurate specialization of *D. graminea* which should not be overlooked in testing for varietal reactions (Shands and Arny, 1944).

Distinct differences were recorded in varietal reaction of certain barley varieties against several isolates of *D. graminea* (Mohammad and Mahmood, 1976). This result indicates that the isolates under investigation may be related to different pathogenic races.

The cultural characteristics apparently varied in *D. graminea* isolates (Mohammad and Mahmood, 1973). Rate of growth was high or moderate in most isolates, and low in some isolates. Fluffy mycelium and gray colour were recorded more than velvety mycelium and olivaceous colour. Most of the isolates were unable to excrete pigments. Mutation was found to occur rarely in the tested isolates.

Different physiologic activities were used to distinguish different groups of micro-organisms. Production of pectolytic and cellulolytic enzymes was found to vary in isolates of certain pathogenic fungi and bacteria (Singh and Hussing, 1964; Shalaby, 1975). Several isolates of *D. graminea* were able to exhibit intermediate activity of polygalacturonase, pectinmethylesterase and cellulase. Some isolates were unable to produce one enzyme or more, others produced little amounts, and a third group produced highly considerable amounts of PG, PME and C_x, in special synthetic media.

A drastic decrease was recorded in percentage of germination of barley seeds and length of both radicle and plumule when seeds were germinated in culture filtrate of *D. graminea* (Luke and Gracen, 1972; El Sherif, 1978). The effect of culture filtrate was found to vary distinctly in different isolates.

The results of this study indicate that a relationship may be found between pathogenicity and certain characteristic of isolates of *D. graminea* such as rate of growth, production of pectolytic and cellulolytic enzymes in media and effect of culture filtrates on seed germination of barley.

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Conservation des Champignons Phytopathogènes dans L'Azote Liquide après Congelation Progressive Obtenue avec le Minicool

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Mycelium agar discs 5 mm) were taken from Petri-dish cultures of 6 strains (6 *Phytophthora* species) on 1% agar malt media, and *Plasmopara helianthi* conidia were suspended in 15% glycerol-water solution and frozen slowly by minicool. Also, uredospores of flax rust *(Melampsora lini)* were dry-frozen (without protective solution).

After freezing all samples were transferred into liquid nitrogen at −196 °C. Samples were examined after thawing at 38–40 °C. After 17 to 52 days of storage in liquid nitrogen, *Phytophthora* showed no decrease in pathogenicity, no loss of sporangia production, and no change in morphological characteristics. All frozen strains of *Phytophthora* showed a decrease in radiating growth in comparison to controls.

After 100 days of cryogenic storage, *Plasmopara helianthi* conidia displayed germination and pathogenicity. This is very important. We have established that *Melampsora lini* uredospores require dry-freezing without protective solution. After freezing and 6 days of storage in liquid nitrogen, uredospores showed no major decrease in germination and no reduced pathogenicity.

For the first time, minicool was used in a study of the slow freezing of fungi. This method which allows a precise establishment and verification of cooling curves proves to be of great interest. It can be applied to other fungal organisms.

Depuis déjà plusieurs années, on cherche à constituer «des banques de gènes» d'organismes végétaux. On enrichit certaines mycothèques ou on en crée de nouvelles. Rappelons qu'il existe plusieurs types de mycothèques, notamment médicales, industrielles, vétérinaires et agricoles. C'est ce dernier type qui nous intéresse plus spécialement puisque nous sommes agronomes et phytopathologistes.

Bien entendu, la constitution d'une mycothèque nécessite la mise au point préalabale de méthodes de conservation fiables. Nous avons d'abord entrepris l'étude de la préservation de champignons phytopathogènes non cultivables *in vitro (Péronosporacées, Urédinale)*, dont les techniques traditionnelles ne permettent pas la conservation pendant les saisons peu propices à leur reproduction.

Il faut répondre aussi à une autre nécessité, celle de produire une grande quantité d'inoculum pour réaliser des contaminations artificielles. Cette étude est d'autant plus utile que certaines de ces souches sont utilisées comme marqueurs

de gènes dans la recherche de plantes résistantes appartenant à des espèces végétales économiquement importantes.

Nos essais portent aussi sur divers *Phytophthora* qui ne se conservent pas (ou mal) sous huile ou par lyophilisation. D'une façon générale, dans les laboratoires ordinaires, ces souches sont maintenues en vie le plus souvent par des repiquages successifs avec la charge et les risques que cela représente.

En général, on ne s'intéresse dans les mycothèqués classiques qu'à la longévité des souches. Or, une méthode n'a d'intérêt que si l'intégralité des caractères morphologiques, biochimiques (toxines, enzymes, antibiotiques) et génétiques est maintenue après une conservation prolongée.

Pour l'instant, notre but final est l'étude de la congélation progressive (obtenue au Minocool) et du stockage dans l'azote liquide sur le pouvoir pathogène des champignons phytopathogènes.

L'objet de cette courte note[1] est de rapporter les tout premiers résultats obtenus avec cette méthode.

Materiel et Techniques

Matériel

Matériel végétal et fongique. Les champignons utilisés dans nos études et les végétaux à partir desquels ils ont été obtenus sont répertoriés dans le tableau 1.

Tableau 1

Champignons utilisés et leurs origines

Champignons	Provenant de
Phytophthora nicotianae var. parasitica	Saintpaulia
Phytophthora nicotianae var. nicotianae, race 0 et 1	Tabac
Phytophthora cryptogea	Gerbera
Phytophthora cinnamomi	Rhododendron
Phytophthora megasperma var. megasperma	Carotte
Phytophthora cactorum	Pommier
Melampsora lini, race Wiera	Lin
Plasmopora helianthi, race européenne	Tournesol

Les agents cryoprotecteurs. Au cours de cette étude, nous avons testé trois cryoprotecteurs pénétrants: glycérol 15 p. 100; DMSO 15 p. 100 et propanediol-1,2 15 p. 100.

[1] Pour plus amples renseignements se reporter au Mémoire de fin d'études de Jean Konidaris (1985).

Le minicool (Anonyme, 1980)

L'appareil. Le minicool est un congélateur biologique programmable destiné à la congélation de petits échantillons, construit par la Compagnie Française des Produits Oxygénés, filiale de l'Air Liquide (fig. 1 et 2). Il permet la cryopréservation de toute cellule vivante et notamment de cellules sanguines, de cellules de culture en lignée continue, de spermatozoïdes, d'embryons, de micro-organismes, de virus, voire de tissue et d'organes entiers. Il est essentiellement constitué de deux parties: l'enceinte de congélation et le programmateur électronique.

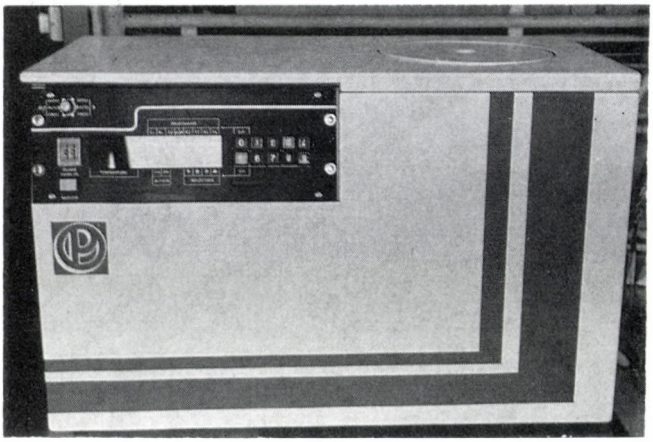

Fig. 1. Minicool (L. 700 mm× H. 450 mm× P. 370 mm)

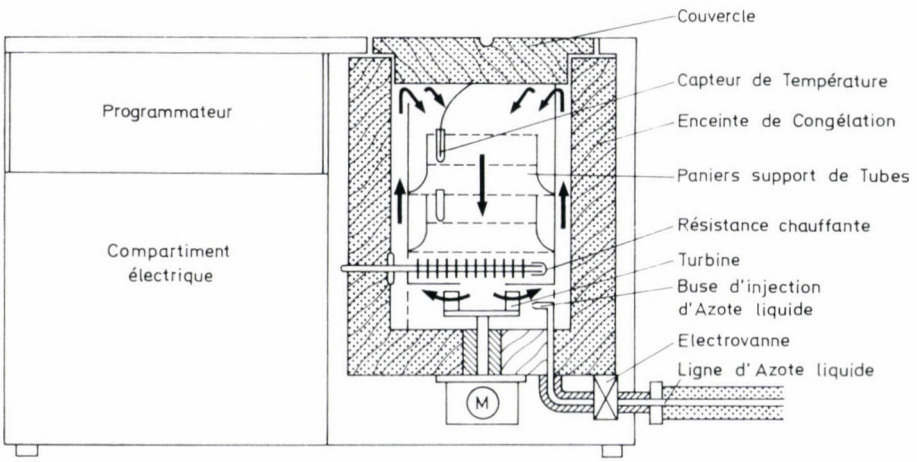

Fig. 2. Composition du minicool (D'après Anonyme, 1980)

Fig. 3. Récipient d'alimentation en azote liquide — TC25

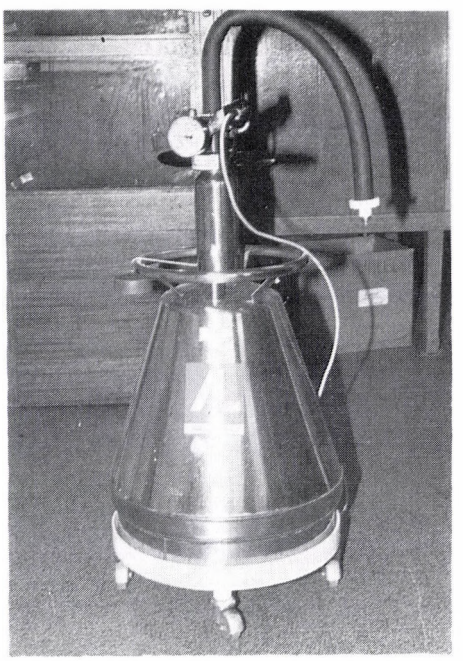

Fig. 4. Ligne de transfert d'azote liquide, adaptée au récipient TC25

En utilisant comme source de froid l'azote liquide, le minicool peut faire baisser la température dans l'enceinte de congélation jusqu'à −180 °C.

Les vitesses de refroidissement pouvant être obtenues vont de 0.1 °C à 50 °C par minute, et ceci dans la gamme de température de +37 °C à −180 °C.

Le refroidissement est obtenu par pulvérisation d'azote liquide à partir d'une buse d'injection placée à proximité de la turbine. Le réchauffement est assuré par une résistance de 250 W placée dans le circuit gazeux.

Les produits à congeler sont placés soit dans des ampoules spéciales (cryotubes), soit dans des paillettes.

L'alimentation en azote liquide du minicool est effectuée à partir d'un récipient inox de 25 litres – TC25 – de l'Air Liquide (fig. 3).

Le transfert d'azote liquide du TC25 au minicool est assuré par une ligne de transfert spéciale, équipée d'une canne de pressurisation électrique – CP25 –, ayant un pressostat taré entre 0.2 et 0.4 bar (fig. 4).

Après congélation, les échantillons sont stockés dans un récipient dans l'azote liquide (fig. 5).

Le principe de fonctionnement. La courbe de descente en température du produit est choisie par l'utilisateur. Cette courbe est décomposée en neuf séquences qui sont définies de la façon suivante:

T1 – Température de l'enceinte de congélation, réglée avant la mise en place des échantillons dans l'enceinte et stabilisée aussi longtemps que le souhaite l'utilisateur.

Fig. 5. Récipient de stockage des échantillons congelés – BT21

R1 – Pente de refroidissement du produit en phase liquide.

T2 – Température de congélation du produit, ou plus précisément température de changement d'état liquide-solide. Cette température est une caractéristique physique du produit considéré et elle est déterminée au préalable.

Δ – Exprimé en degré centigrade, c'est l'écart de température entre le début de l'injection d'azote et la reprise de la régulation. Le passage à R_2 se fait lorsque la température atteint une valeur égale à $T_2 + \Delta$.

W – Puissance frigorifique nécessaire pour éliminer rapidement la chaleur latente de fusion, produite lors de la cristallisation. Elle est exprimée de 1 à 7.

t – Temps affiché à la minuterie: c'est le temps d'injection de la puissance W. Il est liée au conditionnement des échantillons et au volume total de produit à refroidir.

R2 – Vitesse de refroidissement du produit depuis la fin du plateau de congélation jusqu'à une température intermédiaire T3.

T3 – Température négative à laquelle il est possible de changer de vitesse de refroidissement.

R3 – Vitesse de refroidissement de T3 à T4.

T4 – Température finale du refroidissement des échantillons dans l'enceinte avant leur stockage dans l'azote liquide.

Les températures sont exprimées en degrés centigrades. Seule la température finale T4 est affichée en dizaines de degrés, par ex. pour −80 °C, T4 = «08»; pour −120 °C, T4 = «12».

La figure 6 présente un programme et une courbe théorique de congélation.

Le minicool est connecté à un enregistreur afin de visualiser les événement de refroidissement.

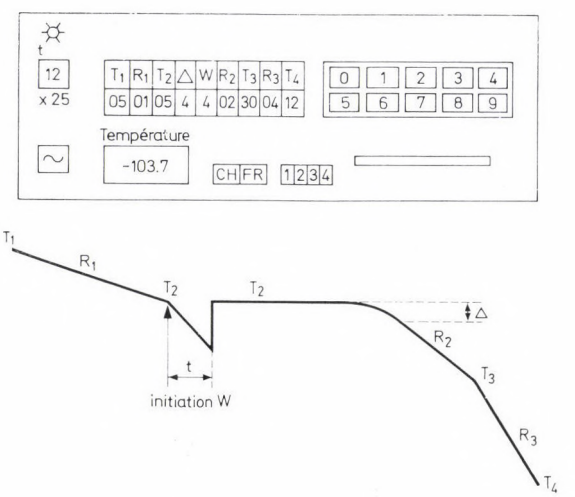

Fig. 6. Courbe et programme théorique de refroidissement (D'après Anonyme, 1980)

Techniques

Test de croissance mycélienne des Phytophthora. De petites rondelles de culture sont mises en contact avec les cryoprotecteurs dans les cryotubes durant une demi-heure. Avant la manipulation, les cryoprotecteurs sont stérilisés à l'autoclave, pendant 30 min à 115 °C. Les rondelles sont ensuite repiquées dans des boîtes de Petri de 90 mm sur un milieu gélosé de malt à 1 p. 100. Les cultures sont mises à incuber à 25 °C à l'obscurité.

Test de germination du Plasmopara helianthi. Ce test a été effectué selon la méthode décrite par Delanoe en 1972.

Les urédospores de *Melampsora lini* doivent être congelées à sec et ne nécessitent donc pas de test de toxicité du cryoprotecteur.

Etude du pouvoir pathogène

Phytophthora spp. Le *P. cactorum* a été testé, après blessure, sur pommes «Golden Delicious» et le *P. megasperma* sur racines de Carotte cv. «Nantaise». Les autres espèces de *Phytophthora* ont été étudiées sur feuilles:

- *P. nicotianae var. parasitica* sur Saintpaulia cv. «Diana 131»
- *P. nicotianae var. nicotianae* sur Tabac «*Xanthi*».
- *P. cryptogea* sur *Gerbera*, clones Fregitane, Peter et Fresamande.
- *P. cinnamomi* sur *Rhododendron «Catambiense Grandiflorum»*.
- *Melampsora lini* sur Lin cv «Antares» (méthode Anonyme, 1982).
- *Plasmapara helianthi* sur Tournesol cv. «Pédérovick» (méthode: Anonyme, 1982).

Resultats

Etude préalable à la congélation progressive: examen de la toxicité des cryoprotecteurs

Le but était d'étudier la toxicité des trois cryoprotecteurs déjà cités sur la croissance mycélienne, la sporulation et la germination du champignon en vue de sélectionner le meilleur produit.

Les essais effectuées ont montré que l'action des cryoprotecteurs dépend de l'organisme fongique. D'une façon générale, c'est le glycérol qui s'est avéré le plus satisfaisant. Par conséquent, c'est cette substance qui a été utilisée avant et pendant la congélation.

Etude pendant la congélation progressive
(Détermination de la température de congélation = T_2)

Pour établir les programmes de congélation du minicool, il est nécessaire de déterminer la température de congélation T2, qui est caractéristique de la suspension étudiée. Dans ce but, on prend comme témoin de la solution à con-

geler, dans un cryotube ou dans une paillette, puis on place le capteur de température – thermosonde – dans la solution, de façon à ce que la partie sensible soit immergée, on met ensuite le tube ou la paillette dans l'enceinte de congélation de l'appareil dont on ferme le couvercle. Nous avons pu ainsi établir par la suite un programme pour chaque champignon étudié. Ces programmes sont les suivants:

Programme pour P. nicotianae var. parasitica

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	04	4	4	02	30	03	12

Le temps d'injection du froid t = 12×2 secondes.

Programme pour P. nicotianae var. nicotianae, souche 184

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	04	4	4	02	30	03	12

t = 12×2 s

Programme pour P. nicotianae var. nicotianae, souche 181

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	04	5	5	01	30	03	12

t = 12×2 s

Programme pour P. cryptogea, souche 52

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	05	3	4	02	30	04	12

t = 11×2 s

Programme pour P. cinnamomi, souche 9079

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	05	5	4	01	30	03	12

t = 12×2 s

Programme pour P. megasperma var. megasperma, souche 230 (fig. 7)

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	05	4	4	01	30	03	12

t = 12×2 s

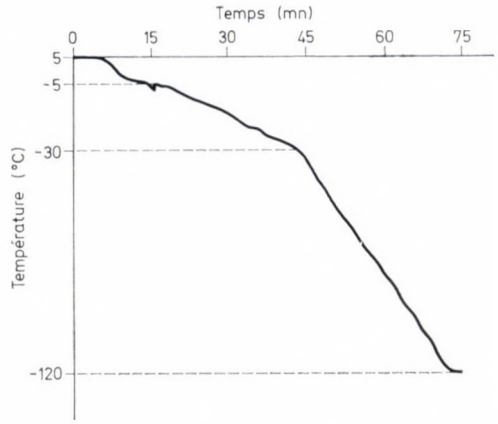

Fig. 7. Courbe de refroidissement de *Phytophthora megasperma*

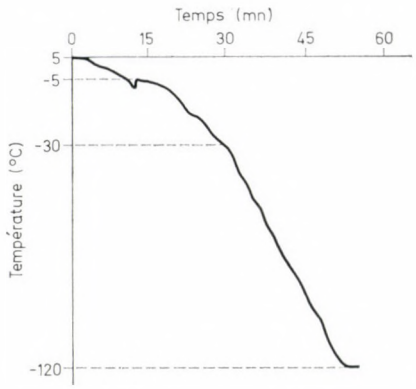

Fig. 8. Courbe de refroidissement de *Plasmopara helianthi*

Programme pour P. cactorum, souche 177

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	04	2	3	01	30	03	12

$t = 12 \times 2$ s

Programme pour des urédospores de Mélampsora lini. Les spores sont congelées sans solution de cryoprotecteur; c'est pourquoi on ne détermine pas la température de congélation et on n'utilise pas de puissance frigorifique pour éliminer la chaleur latente de fusion (W = 0; t = 0).

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	00	0	0	01	40	10	12

$t = 0$

Programme pour des zoosporanges de Plasmopara helianthi (fig. 8). On a choisi une concentration de 80 spores/cellule de Thoma, soit 8×10^4 spores/ml; à ce niveau, la concentration n'influe pas sur la température de congélation.

T_1	R_1	T_2	Δ	W	R_2	T_3	R_3	T_4
05	01	05	3	3	02	30	04	12

$t = 9 \times 2$ s

A l'issue de la congélation progressive, tous les cryotubes et toutes les paillettes sont immergés dans l'azote liquide à -196 °C.

Etude consécutive à la congélation progressive
(Examen de la viabilité des champignons)

Test de croissance mycélienne des Phytophthora. La décongélation des cryotubes de *Phytophthora*, congelés et stockés dans l'azote liquide de 22 à 68 jours, s'opère dans un bain-marie à 38–40 °C et dure 3 minutes environ.

Avant l'ensemencement, les rondelles de cultures congelées sont lavées (à l'aide d'un agitateur) afin d'éliminer le cryoprotecteur resté à l'intérieur et à la surface du mycélium.

Après 6 jours d'incubation, on compare la croissance des cultures décongelées et des cultures témoins.

On constate que l'aspect des colonies de cultures issues des rondelles décongelées est identique à celui des cultures témoins. Par contre, on remarque un effet de retard de croissance important entre les cultures issues des implants décongelés et les cultures témoins. Le glycérol ne provoque pratiquement aucun effet retard, le phénomène semble donc dû à la congélation.

Test de sporocystogénèse des Phytophthora. La méthode utilisée pour la production axénique des sporocystes correspond à celle employée sur la sporocystogénèse du *P. cinnamomi* (Assas M'Bilaut, 1978), à l'exclusion du Fe-EDTA que l'on n'a pas ajouté puisque, d'après les résultats de cet auteur, il affaiblit la sporocystogénèse.

La sporocystogénèse des cultures congelées à la même intensité que celle des cultures non congelées pour toutes les souches examinées. La congélation n'a donc pas d'effet négatif sur la production des sporocystes.

Test de germination des urédospores de Melampsora lini. Après 6 jours de stockage dans l'azote liquide, les cryotubes sont décongelés au bain-marie à 38–40 °C pendant 3 minutes environ.

On ajoute de l'eau aux spores décongelées pour obtenir une suspension d'une concentration de 40 spores/cellule Thoma, soit 40×10^4 spores/ml. A l'aide d'une pipette Pasteur, on étale la suspension de spores sur eau gélosée 1 p. 100, dans des boîtes de Petri de 90 mm de diamètre. On les met à incuber à l'obscurité à 17 °C. Après 48 heures d'incubation, on compare la germination des spores congelées à sec et celle du témoin.

Tableau 2

Nombre de jours entre la congélation et la décongélation suivie d'inoculation

Champignons	Jours
Phytophthora nicotianae var. parasitica	29
P. nicotianae var. nicotianae	17
P. cryptogea	52
P. cinnamomi	34
P. megasperma	29
P. cactorum	28
Melampsora lin	06
Plasmopara helianthi	79

La congélation et la décongélation n'ont pas affecté sensiblement le pouvoir germinatif.

Test de germination des sporocystes de Plasmopara helianthi. Après 100 jours de stockage dans l'azote liquide, on met les paillettes à décongeler dans un bain-marie à 38–40 °C pendant 30 secondes environ. Pour enlever la solution de cryo-protecteur, on centrifuge ensuite les spores pendant 5 minutes à 5500 tr/min.

On ajoute de l'eau additionnée de saccharose 2 p. 100 (Delanoe, 1972) pour obtenir une suspension de 20 à 30 spores/cellule Thoma, soit 20 à 30×10^4 spores/ml. On étale cette suspension sur de l'eau gélosée à 1 p. 100 dans des boîtes de Petri de 90 mm de diamètre. L'incubation a lieu à 17 °C à l'obscurité. Après 48 heures d'incubation, on observe les résultats.

On peut constater que les spores peuvent supporter la congélation et la décongélation dans l'azote liquide, puis la conservation dans l'azote liquide sans perte importante de leur pouvoir germinatif.

Etude du pouvoir pathogène. Les résultats obtenus montrent, d'une façon générale, que le pouvoir pathogène de ces organismes fongiques n'a pas été modifié par rapport aux témoins non congelés, excepté cependant pour *P. helianthi* chez lequel on a observé une légère baisse de la virulence.

Discussion et Conclusion

Les expériences effectuées au cours de cette étude préliminaire conduisent à plusieurs constatations.

Phytophthora spp. Généralement les souches de *Phytophthora* ont bien supporté la congélation et le stockage dans l'azote liquide à −196 °C, ainsi que la décongélation à 38–40 °C.

Malgré la brièveté du stockage dans l'azote liquide (de 17 à 52 jours), la bonne conservation des souches après le changement d'état et le stockage, observée

pendant le test de croissance mycélienne permet, comme l'a remarqué Smith en 1982, de coire à la possibilité d'une longue préservations dans l'azote liquide.

Le retard de croissance des cultures après décongélation peut être attribué soit à l'effet probable de la congélation ou de la décongélation sur celles-ci, soit à l'effet du cryoprotecteur. Pourtant le résultat du test préalable à la congélation, et le lavage des cultures après la congélation, paraissent dégager la responsabilité du cryoprotecteur quant à ce retard important. Cependant on peut se demander si le temps de lavage (5 minutes d'agitation des rondelles mycéliennes dans de l'eau stérile) est suffisant pour garantir la sortie du cryoprotecteur qui a pénétré dans les hyphes mycéliennes.

Les caractères morphologiques des souches n'ont pas été modifiés après la congélation. Les *Phytophthora* congelés, testés également après une période de 17 à 52 jours, forment aussi bien des sporocystes que les cultures non congelées.

Le pouvoir pathogène, testé après 17 à 52 jours de stockage, est resté sensiblement le même qu'avant la congélation progressive.

Melampsora lini. La congélation à sec, en l'absence d'eau et de cryoprotecteur, a donné des résultats satisfaisants.

Avant congélation, la déshydratation partielle des urédospores avec du glycérol (Pope de Vallavieille, 1983) ou dans un courant d'air (Dahmen et al., 1983) n'a pas été effectuée. Cette technique pourrait éventuellement apporter des meilleurs résultats.

Après la congélation, le pouvoir germinatif a subi une diminution, mais reste encore important.

Après 6 jours de stockage, le pouvoir pathogène et la couleur des urédospores n'ont pas été modifiés.

Plasmopara helianthi. Les zoosporanges de *P. helianthi*, race européenne, sont restés beaucoup plus longtemps dans l'azote liquide: 100 jours de stockage pour le dernier test effectué.

On peut dire qu'il supportent bien la congélation et la stockage dans l'azote liquide à -196 °C, ainsi que la décongélation à 38–40 °C, sans perte très importante du pouvoir germinatif et du pouvoir pathogène.

Programme de refroidissement. Une deuxième thermosonde, placée à l'intérieur de l'enceinte du minicool et connectée avec l'enregistreur de température, n'était pas encore montée. Elle aurait permis d'observer la descente de la température dans l'enceinte pour la comparer à celle des échantillons en cours de refroidissement.

Il aurait peut-être fallu diminuer la température intermédiaire T_3 de -30 °C à -35, -40 °C, températures finales le plus souvent utilisées pour la congélation lente avant le stockage dans l'azote liquide.

Le refroidissement au-delà de -35, -40 °C devrait être plus rapide, avec immersion éventuelle dans l'azote liquide; puisque le prolongement de la diminution controlée de la température au-delà de -40 °C s'avère néfaste pour les champignons congelés (McGann, 1978; Augereau, 1985). Il faudrait modifier la courbe de refroidissement après T3 – à noter que le minicool offre une possibilité de refroidissement avec une vitesse maximale de 50 °C/min.

Des études menées dans ce sens pourraient donner pour chaque espèce des courbes de refroidissement améliorées, en fonction du type de cryoprotecteur utilisé et de sa concentration dans la solution considérée.

En conclusion, la vitesse de refroidissement est de 1 °C par minute jusqu'à la surfusion (Δ, W). Ensuite, elle est variable suivant le matériel fongique, la nature du récipient (cryotube: gélose; paillette: spore dans une solution eau + cryoprotecteur).

Les premiers résultats obtenus sont globalement très encourageants. On peut envisager d'utiliser cette méthode de conservation dans l'azote liquide, après congélation progressive, pour la préservation des *Phytophthora*, qu'il est difficile de conserver avec d'autres méthodes, et pour les parasites obligatoires *Melampsora lini* et *Plasmopara helianthi*. Déjà devenue une des méthodes principales de conservation des collections de champignons dans certains pays, notamment aux Etats-Unis et en Grande-Bretagne, elle peut être étendue à d'autres organismes fongiques.

Le minicool, utilisé pour la première fois dans le cadre d'une étude de la congélation progressive des champignons, offre de nombreuses possibilités grâce à l'exactitude de son fonctionnement qui permet un établissement et un contrôle précis des courbes de refroidissement.

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Effect of Trace Elements on Groundnut Root Rot Incited by *Rhizoctonia bataticola*

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B, Cu, Mn and Zn inhibited the growth of *Rhizoctonia bataticola*. Cu, 0.5 mM was most effective in reducing the infection in groundnut plants grown in nutrient solutions while B was least effective. Both Mn and Zn reduced the disease by 76% and about 80% of the plants survived. Combination of metals especially with Cu was effective in reducing the rot. Germination of groundnut seeds sown in artificially infested field plots was substantially improved by Cu. It also checked pre- and post-emergence infection of groundnut seedlings grown in field plots infested with *R. bataticola*.

Frequent germination failure of groundnut and common occurrence of root rot in young seedlings in the field are mainly attributed to the pre-emergence infection and seedling dry rot caused by *Rhizoctonia bataticola* (Taub.) Butl., (*Macrophomina phaseoli*) (Maubl.) Ashby; *M. phaseolina* (Tassi.) Goid.). *R. bataticola* causes root rot and leafspot of seedlings and older plants (Jackson and Bell, 1969).

Soil conditions especially the nutritional status greatly influence the severity of plant diseases in general and soil borne diseases in particular. Garrett (1938) proposed that soil borne pathogens can be effectively controlled by management of the soil especially amendment with trace elements and organic matter. A few plant diseases are controlled by the addition of trace elements (Papavizas and Lumsden, 1980, Dutta and Bremner, 1981). *R. solani* on tobacco (Orellana, 1953), and on potato (Mc Gregor and Wilson, 1966), *F. oxysporum* f. sp. *ciceri* and *F. solani* on *Cicer* sp. (Satyaprasad, 1980).

In this paper we report our results on the effect of trace elements on the pathogen, *R. bataticola* and on the control of rot in groundnut plants.

Materials and Methods

Groundnut seeds Cv. TMV-2 were procured from the State Oil Seeds Research Station, Putlur, Madras. Uniform sized seeds were selected, surface sterilized with 0.1% $HgCl_2$ for 1 to 2 min and washed 5 to 6 times with sterile glass distilled water.

Green House studies

Five seeds were placed in sterile earthenware pots of size 24 × 12 cm, filled with vermiculite. Ten replicates were maintained per treatment.

Trace elements and their combinations which showed maximum inhibition of *R. bataticola* in the laboratory, were used to test the effect of infected plants, grown in a wire mesh house where the temperature fluctuated from 26 to 31 °C. The combinations were B (0.25 mM), Cu (0.5 mM), Mn (1 mM), Zn (1 mM), Cu + Mn (0.5 + 1.0 mM), Cu + Zn (0.5 + 1.0 mM), Cu + Mn + B (0.5 + + 1.0 + 0.25 mM), Cu + Zn + B (0.5 + 1.0 + 0.25 mM); Cu + Mn + Zn (0.5 + 1.0 + 1.0 mM) and B + Cu + Mn + Zn (0.25 + 0.5 + 1.0 + 1.0 mM). B was used as $Na_2B_4O_7$; Cu as $CuSO_4 \cdot 5H_2O$; Mn as $MnSO_4 \cdot H_2O$ and Zn as $ZnSO_4 \cdot 7H_2O$. Stock solutions of 1 mM were prepared and concentrations of 0.25, 0.5 and 1 mM of the respective trace elements were used.

Hoagland's nutrient stock solution modified by Johnson et al. (1957) and suspensions of trace elements were prepared using double glass distilled water. Chemicals used were of 'Analar' and 'Guaranteed' reagents.

Eight-day-old plants were treated with trace elements. Hoagland's nutrient solution without B, Cu, Mn and Zn was used to water the plants. The nutrient solution was added every alternate day to the pots keeping the water holding capacity of the vermiculite at 50 to 55%.

Inoculation of plants and assessment of infection

Ten day old groundnut plants were inoculated with a mixture of inoculum grown on potato dextrose broth and groundnut stubbles with the inoculum: vermiculite ratio of 1 : 2. Inoculation was done by the standard techniques of Kulkarni et al. (1962) and 'unwounded stem inoculation' technique of Chan and Sackston (1969).

The vermiculite around the base of plants was removed so as to expose 1/3rd of the hypocotyl. The inoculum mixture was placed in juxtaposition around the hypocotyl and pressed. The inoculum and the top layer of the pot was covered with sterile vermiculite in quantities equal to the vermiculite removed. Watering was done immediately after inoculation as to just moisten the vermiculite.

All the inoculated plants, both surviving and dead were pulled out and examined. The disease intensity in each plant was rated according to the scale of reaction (Shanmugam, 1971):

(0) No lesion, healthy plant.

(1) Water soaked brown spot on hypocotyl; plants alive.

(2) Necrotic area up to 0.5 to 1 cm in length on lower or upper part of the hypocotyl; plants alive.

(3) Necrosis and rotting of hypocotyl with or without girdling and death of plants.

(4) Extensive necrosis and rotting of hypocotyl spreading to root and stem; black discoloration of roots and death of plants.

(5) Extensive necrosis of roots, shredding of roots, hollowing of stem and death of plants.

The mean root rot index (RRI) was arrived at by rating each plant on the above scale of reaction and dividing the total value by the number of plants rated.

Field studies

Of the 10 combinations tried on healthy and infected plants, Cu at 0.5 mM controlled the disease effectively. Therefore, treatment with Cu was selected for field studies.

The field plots were arranged in a randomized block design with 6 plots per treatment, the plot size being 2.5 sq.m. The gap between each plot was 1 m. Uniform sized seeds were sown in the field amended with 7.245 g as $CuSO_4$ per plot (1), 1280 g (fresh weight) of mycelia of *R. bataticola* to plot (2), another plot having groundnut seeds received only water (3), and combination of $CuSO_4$ and inoculum to plot (4). The space between each row of plants was 30 cm with an interval of 15 cm between each plant. Cu and macerated fungal mat were applied to field soil as suspension. In all, 128 seeds were sown per plot and 768 seedlings for 6 plots constituted one treatment. The per cent germination of seeds per treatment was recorded.

$CuSO_4$ was applied to field soil thrice, once before sowing the seeds and the second and third applications were on 15th and 30th day after sowing. The inoculum was applied onto the field soil twice; first was just before sowing and the next was on the 10th day after sowing. Plants were irrigated once in 15 days and no manure was supplied. From 10th day, post emergence infection was assessed according to the scale of reaction rate from 0 to 5.

Determination of B, Cu, Mn and Zn in soils and plants

Groundnut plants grown in vermiculite amended with trace elements were harvested. Their tops and roots were separated and washed in 0.1 N HCl followed by a wash with deionized water. After drying, they were ashed in a muffle furnace at 900 °C for 25 h, weighed and ground in a blendor. Representative samples were digested in triacid mixture (HNO_3 : $HClO_4$: H_2SO_4 10 : 3 : 1 v/v). Total B, Cu, Mn and Zn were estimated in an atomic absorption spectrophotometer (Prasad and Sinha 1981).

Results

Greenhouse studies

In pots containing vermiculite treated with Cu, plants showed only 19% disease incidence (Table 1). It was 45% in B treated plants. Both Mn and Zn at 1 mM were equally effective in reducing the rot; about 80% of the plants survived.

Table 1

Effect of trace elements on the development of root-rot in peanut plants grown in wire-mesh house

Treatment	Rating of disease intensity* number of plants						RRI	Control
	0	1	2	3	4	5	%	
Control	12	18	11	21	14	24	88	100
B	60	22	2	7	3	6	40	45
Cu	83	10	3	2	2	0	17	19
Mn	80	8	5	4	1	2	20	23
Zn	79	8	6	3	3	1	21	24
Cu+Mn	73	7	9	8	3	0	27	31
Cu+Zn	80	10	5	2	1	2	20	23
Cu+Mn+B	66	9	14	7	2	2	34	39
Cu+Zn+B	69	13	10	6	1	1	31	35
Cu+Mn+Zn	75	10	7	4	3	1	25	28
B+Cu+Mn+Zn	80	9	7	2	2	0	20	23

* The symptoms were rated from 2 to 10 days after inoculation.

Mn along with Cu was not effective in reducing the disease, as only 31% of the plants showed symptoms. But in plants grown in Cu + Zn, the rot was 23%. Plants grown in Cu + Mn + B and Cu + Zn + B, the disease incidence was less and rots occurred in 20 and 35% of the plants, respectively. Rot incidence was still less in plants grown in nutrient solution containing Cu, Mn and Zn. Rot incidence was much less in plants treated with B + Cu + Mn + Zn and about 80% of the plants survived.

Table 2

Effect of $CuSO_4$ and *R. bataticola* on seed germination of groundnut

Treatment	No. of seeds used for germination	No. germinated	Germination (%)
Control	768	599	78
$CuSO_4$*	768	691	90
*R. bataticola***	768	461	60
$CuSO_4$ + *R. bataticola****	768	576	75

* $CuSO_4$ was applied at 7.245 g/2.5 sq. metre plot.

** Macerated fungal mat of *R. bataticola* was applied at 1280 g (fresh weight)/2.5 sq. metre plot.

*** Both $CuSO_4$ + *R. bataticola* were added together.

Field studies

(a) *Germination:* Addition of Cu to field soil increased the seed germination by 15% (Table 2). But seed germination was substantially reduced in plots treated with *R. bataticola.* In presence of Cu and the fungus, seed germination markedly improved.

(b) *Pre-emergence infection:* Pre-emergence infection was only 11 per cent in the plots treated with $CuSO_4$ but in plots receiving the inoculum, it was 33% (Table 3). In treatment with Cu and the fungus, the pre-emergence infection was only 20%.

(c) *Post-emergence infection:* The post-emergence infection of groundnut plants was reduced to 10 per cent in Cu amended soils but it was equal to the

Table 3

Effect of $CuSO_4$ and *R. bataticola* on pre-emergence infection of groundnut seeds

Treatment	No. of seeds used for germination	No. germinated	Germination (%)	Pre-emergence infection (%)
Control	768	630	82	18
$CuSO_4$*	768	684	89	11
*R. bataticola***	768	515	67	33
$CuSO_4$ + *R. bataticola****	768	615	80	20

* $CuSO_4$ was applied at 7.245 g/2.5 sq. metre plot.

** Macerated fungal mat of *R. bataticola* was applied at 1280 g (fresh weight)/2.5 sq. metre plot.

*** Both $CuSO_4$ + *R. bataticola* were added together.

Table 4

Effect of $CuSO_4$ and *R. bataticola* on post-emergence infection of groundnut plants

Treatment	Germination and survival (%)	Post-emergence infection (%)	
		within 20 to 30 days	after 30 to 105 days
Control	14	14	8
$CuSO_4$*	90	7	3
*R. bataticola***	60	29	11
$CuSO_4$ + *R. bataticola****	75	16	9

* $CuSO_4$ was applied at 7.245 g/2.5 sq. metre plot.

** Macerated fungal mat of R. bataticola was applied at 1280 g (fresh weight)/2.5 sq. metre plot.

*** Both $CuSO_4$ + *R. bataticola* were added together.

control (25%) in Cu and fungus treated plots (Table 4). Infection increased to 40% in those plots which received only *R. bataticola.*

Seedling infection and rate of mortality were high (40%) in the plots receiving *R. bataticola* but decreased to 10% in the Cu treated plots.

Estimation of B, Cu, Mn and Zn in soils and plants

Uptake and concn of B were maximum in plants grown in B, Cu + Zn + B and Cu + Mn + B (Table 5). Alternatively, presence of B in the nutrient solution enhanced its availability to plants. However, uptake of B and its concern were less in plants grown in Mn and Cu.

In roots, the uptake of Cu was enhanced by Zn and B more than that caused by Mn and Cu itself; the accumulation of Cu was maximum in root. Combining the metals synergized the uptake and utilization of Cu by plants more than individual metals. Cu + Zn and Cu + Mn were effective combinations. Surprisingly, concentration of Cu was minimum in plants grown in Cu.

Trace elements generally promoted absorption and utilization of Mn by groundnut plants; B being most effective followed by Mn. Combination of metals affected the uptake of Mn more than individual metals. Of the combinations, Cu + Zn was the best followed by Cu + Zn + B.

Table 5

Determination of B, Cu, Mn and Zn in soils and plants

Treatment	Boron		Copper		Manganese		Zinc	
	(μg/g)							
	root	shoot	root	shoot	root	shoot	root	shoot
Control	14.2	6.1	16.3	1.4	81.5	3.1	366	51
B	27.2	10.7	19.5	0.9	77.1	14.0	782	292
Cu	16.7	8.2	17.0	4.0	15.0	17.0	461	449
Mn	15.0	6.5	18.0	1.6	54.3	14.0	586	274
Zn	22.1	10.4	23.8	2.5	33.8	23.8	643	238
Cu+Mn	17.4	8.1	49.0	3.0	68.0	16.7	652	345
Cu+Zn	18.8	9.0	55.1	3.6	99.0	9.6	669	340
Cu+Mn+B	24.8	18.6	27.0	3.6	61.0	31.4	456	290
Cu+Zn+B	26.1	19.2	40.8	2.4	77.8	10.8	418	195
Cu+Mn+Zn	18.5	8.8	22.3	3.5	39.6	17.2	482	256
B+Cu+Mn+Zn	22.3	10.1	23.9	3.5	43.8	22.9	516	341
Field-control (plant)	5.2	2.3	6.5	3.8	46.0	22.3	17	11
Field-copper (plant)	8.4	5.2	10.2	3.8	47.0	22.8	23	17
Soil-control	3.2		0.02		4.5		7.8	
Soil-copper	4.1		0.04		5.0		9.2	

B promoted absorption and accumulation of Zn more than any other metal. Zn, Mn and Cu were least effective. Combination of metals also increased absorption of Zn but not to the extent caused by B. Cu + Zn and Cu + Mn were effective in enhancing the availability of Zn to plants.

Application of $CuSO_4$ to field grown groundnut plants increased the concn of Cu in both root and shoot. In addition, Cu increased the availability of B, Mn and Zn to plants.

Discussion

With soil-borne pathogens, one important albeit deceptive experience is the longevity of inoculum and its recovery. This is especially true with *Rhizoctonia* spp. Therefore, much time and effort were lavished in evolving a suitable technique to ascertain the quantity of inoculum in soil. For, controlling the disease essentially involves reduction of inoculum in soil. Cu reduced the survival and infectivity of *R. bataticola* as determined by the mature groundnut stem segment colonization method of Papavizas (1969) which is very satisfactory. Cu was highly toxic to *R. bataticola* (Murugesan, 1983). Besides direct action on the fungi, trace elements inhibit enzymes: polygalacturonase, polygalacturonate trans eliminase and pectin methyl esterase of *Colletotrichum gloeosporoides* (Agarwal, 1978) and cellulase of *Curvularia geniculata* (Nusrath, 1978).

Baser and Saxena (1967) reported positive correlation between B and available Ca. B increased absorption of cations, especially Ca and anions (Hewitt, 1958). Application of B to groundnut increased the uptake and concn of Ca (Alagarswamy, 1966). Therefore, the increased Ca may alter disease resistance. Resistance of bean and groundnut plants against attack by *Rhizoctonia* sp. was associated with high Ca nutrition (Bateman and Lumsden, 1965, Shanmugam, 1971). Whether trace elements induce resistance of plants through Ca requires definitive evidences. Shanmugam (1971) reported that *M. phaseoli* inoculated groundnut plants produced phytoalexin; it was more in the roots and it increased with increase in the age of plants. Narayanaswamy and Mahadevan (1983) also reported the production of phytoalexins by groundnut pods and leaves. Whether trace elements influence phytoalexin production, besides the direct inhibition of pathogen requires investigation.

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Phenol Metabolism and Plant Disease Resistance*

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Despite the vast literature on the role of phenolics in disease resistance of higher plants against phytopathogenic microorganisms, the picture remains obscure. It has been the aim of the authors to critically evaluate this aspect of study based on the literature available in the last two decades.

The role of phenolic compounds and changes in the enzyme activities of phenolic biosynthesis and oxidation, phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), and polyphenol oxidase (PPO) and peroxidase (PO), respectively, in host resistance against potential pathogens is critically examined and discussed in this review.

The possible involvements of aromatic compounds in disease resistance has been the main theme in a number of reviews (Cruickshank, 1963; Farkas and Király, 1962; Kosuge, 1969; Kuć, 1966, 1972; Overeem, 1976; Rohringer and Samborski, 1967; Schönbeck and Schlösser, 1976). These substances act in the chemical defence of higher plants mainly in three ways. First, they are present in the healthy plants at concentrations sufficient to inhibit growth and sporulation of a pathogen, these compounds are generally referred to as performed resistance factors; secondly, as a response to infection, their concentrations markedly increase imparting resistance to the invading microorganism. Finally, certain post infectional products (phytoalexins), which are not normally found in healthy plants, synthesized or increase in amounts after infection. Investigators concerned with disease resistance have frequently correlated phenolic compounds with the host defence mechanisms because of their ubiquitous occurrence and accumulation in the infected plants (Bhaskaran et al., 1974; Hunter, 1974; Purushothaman, 1974*a*; Rathmell, 1973; Reddy and Sridhar, 1975; Sridhar and Ou, 1974). The fact that certain phenolic compounds and their oxidation products play a role in disease resistance by blocking fungal growth in the process of necrotic lesion development (Kosuge, 1969), or by inhibiting the fungal pectolytic enzymes (Hunter, 1974, 1978) is well known.

Chlorogenic acid was envisaged as an antifungal substance in potato because of its increased accumulation in resistant tubers following infection with *Phytophthora infestans* (Johnson and Schall, 1952; Kuć et al., 1956). This well-known

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case, however, has been confronted by Condon and Kuć (1960), Farkas and Király (1962) and Walker and Stahmann (1955). That increased synthesis of chlorogenic acid is unlikely to be a major contribution of defence mechanism has been suggested (Friend et al., 1973). On the contrary, phenols accumulate in tomato plants both resistant (Matta et al., 1967) and susceptible (Briel, 1967; Davis et al., 1953; Rhoringer et al., 1958) to *Fusarium oxysporum* f. sp. *lycopersici* and also in plants inoculated with nonpathogenic fungi (Matta et al., 1970). There are several instances of marked phenol accumulation in the inoculated susceptible, instead of the expected rise in inoculated resistant plants (Sridhar et al., 1979; Vidhyasekaran et al., 1973) suggesting that a high level of phenols does not always mean resistance. That phenol accumulation is nonspecific and is one of the secondary effects of infection has also been demonstrated (Edreva, 1977; Tanguy and Martin, 1972).

Blast resistant rice varieties are reported to contain more polyphenols than the susceptible cultivars (Suzuki, 1965) whereas a reverse relationship also exists (Ramakrishnan, 1966; Wakimoto et al., 1960). No direct correlation has been found between the resistance of rice cultivars and their phenolic levels (Sridhar, 1972*a*, *b*). In fact, phenolic concentrations fluctuate throughout the growth of rice plants and though phenols are produced as a result of infection, they fluctuate very widely in the sorghum plants inoculated with *Xanthomonas rubrisorghi* (Easwaran, 1971). Contrary to their initial accumulation in systemically infected sunflower and tobacco plants with mildew, the phenols decrease markedly during later stages of infection (Cohen and Ibrahim, 1975; Reuveni and Cohen, 1978) and the reasons for this sharp decline are not known. However, Daly et al. (1971) and Seevers and Daly (1970*a*) have failed to detect significant changes in phenolic components in healthy or stem rust-infected resistant and susceptible wheat cultivars at any stage of disease development. Recently, Manian (1981) reported that N-fertilization increased the level of phenolics as well as activities of oxidative enzymes PO and PPO in *Rhizoctonia solani* infected rice plants. This accumulation however seems to be earlier and faster in the resistant than the susceptible cultivars, commensurate with earlier restriction of lesions in the former.

Reports on decreased levels of phenolic compounds as a result of infection of susceptible varieties are also available in the literature (Agarwal and Bisen, 1977; Ibrahim, 1970; Jalali et al., 1976; Fayachandran-Nair and Chakrabarti, 1980; Rao and Nayudu, 1979*a*; Sempio et al., 1975; Sridhar et al., 1976 and Tomiyama et al., 1957). Thus it is seemingly clear that evidences for the involvement of phenolics in the disease resistance of higher plants are highly ambiguous.

Phenylalanine/Tyrosine ammonia lyases (PAL/TAL)

Changes in the total phenol content may depend upon alterations in the activity of the enzymes responsible for their biosynthesis. PAL is probably important in the synthesis of various phenolic types including the flavonoids, isoflavonoids and hydroxycinnamic acids. The biosynthesis of phenylpropanoids from

phenylalanine and tyrosine is initiated through the action of PAL and TAL, respectively. After the first report of PAL and Koukol and Conn (1961), the occurrence of TAL in various plants has also been demonstrated (Neisch, 1961). The reaction catalysed by PAL is the deamination of phenylalanine to transcinnamic acid and ammonia, while TAL deaminates tyrosine to p-coumaric acid. This deamination mechanism has been consequently linked with disease resistance in veiw of its importance in aromatic compound metabolism (Young et al., 1966). The various aspects of PAL and phenylpropanoid metabolism have been reviewed by Camm and Towers (1973) and Towers (1980), respectively.

A number of investigators have demonstrated increased levels of PAL and TAL activities in many plant tissues as a response to infection (Burrell and Ap Rees, 1974; Friend et al., 1973; Green et al., 1975; Haard and Wasserman, 1976; Maule and Ride, 1976; Partridge and Keen, 1972; Purushothaman, 1974*b*; Rathmell, 1973; Reddy and Rao, 1978; Swinburne and Brown 1975; Vance and Sherwood, 1976; Yamamoto and Nakao, 1976). Causal relation of PAL activation to resistance has been suggested by the observations that administration of PAL synthesis inhibitors diminished plant resistance (Swinburne and Brown, 1975). Contrary to these findings, Grzelinska and Sierakowska (1975) showed that resistant host responses to infection need not always be characterized by increase in PAL activity. Whereas in the susceptible host cutivars no such increases in PAL activity are detected, the enzyme remained at the same level as that of uninoculated controls (Grzelinska and Sierakowska, 1975; Yamamoto and Nakao, 1976). Indeed, lower levels of PAL are recorded in infected-susceptible plants than in the uninfected ones (Friend, 1976; Rao and Nayudu, 1979*b*). Further more it has been shown that N-fertilization decreased the PAL, TAL activities in rice plants (Matsuyama and Dimond, 1973).

The induction of resistance in tobacco is not accompanied by changes in this activity of PAL (Simons and Ross, 1971). Enzyme changes in general preceed the alterations in phenolic levels suggesting that there are temporal and spatial differences between the increases in the amount of PAL that occur during infection and the changes in the levels of phenolic compounds (Rathmell, 1973). Besides, it has been recently indicated that resistance host expression in response to infection cannot be solely attributed to PAL activation (Yamamoto et al., 1977).

There are, thus, numerous reports of increases in enzyme activities of PAL and TAL. Reports of decreased activity, however, are very few (Daly, 1976). That the quantity of these enzymes, likewise is the limiting factor in total phenolic biosynthesis still remains unresolved (Grisebach and Hahlbrock, 1974). Recently, evidence that substrate (phenylalanine) rather than PAL activity is the most likely limiting factor in controlling phenylpropanoid accumulation has been provided (Margna, 1977).

Polyphenol oxidase (PPO) and disease resistance

In general PPO (DOPA – oxidase) catalyses the oxidation of phenolic substances with molecular oxygen. They have differing substrate specificities and vary with species, part and ontogenetic state of the plant.

The presence of phenolic compounds in higher plants, their oxidation following injury, either mechanical or infection and the relatively high toxicity of the oxidation products have long drawn attention. The possible relationship of these properties to hypersensitivity has prompted many research workers to ascribe a role to PPO. This subject has been reviewed from time to time (Farkas and Király, 1958, 1962; Fric, 1976; Kosuge, 1969; Mayer and Harel, 1979). Existence of this enzyme in rice, however, appears to be controversial. Despite several reported failures (Rao and Nayudu, 1979*b*; Sridhar, 1972*b*), Muralidharan (1974) detected the enzyme activity in blast-infected rice leaves.

Further, PPO does increase in activity following infection by viruses, bacteria, fungi and mechanical injury. This increase may be due to activation of latent host enzyme, solubilization of host PPO which is normally particulate or even due to *de novo* synthesis. Enzymic increase mediated by *de novo* synthesis has been demonstrated by Hyodo and Uritani (1966) in sweet potato infected by *Ceratocystis fimbriata*. Release of latent PPO in tomato plants infected by *Fusarium* (Matta et al., 1970) and in *Botrytis*-infected *Vicia* (Balasubramani et al., 1971), has also been demonstrated. An increase in non-particulate enzyme has been shown during *Phytophthora* infection of potatoes (Pitt, 1975). However, both phenolic content and PPO activities are higher in tomato cultivars susceptible to *Verticillium* wilt, compared to resistant ones. Such increases in phenolics and phenol oxidizing enzymes have been correlated with the amount of viable fungus observed in infected plants (Pollock and Drysdale, 1976). Again, in apples *Erwinia* infection induces a delayed increase in enzyme activity, both in virulent and avirulent infections suggesting that mere delayed reaction cannot lead to resistance (Addy and Goodman, 1972). Increased lignification of potatoes following infection by *Phytophthora* did not involve PPO activity. Similarly, increased activity of PPO in infected musk melons has been shown to be due to enzymes originating from the fungus, *Fusarium* (Maraite, 1973). *Helminthosporium oryzae* produces a highly active PPO and contributes to the enhanced enzyme levels of the infected rice plants (Oku, 1967). Though the role of PPO in hypersensitivity has been frequently discussed, it appears that the enzyme has no immediate role in this reaction (Érsek et al., 1973).

Thus, as has been pointed out by Kosuge (1969), the ambiguity about the role of PPO in disease resistance is brought home forcibly (Mayer and Harel, 1979).

Peroxidase (PO) and its involvement in induced resistance

Peroxidase involvement in plant tissues has been implicated to induce disease resistance, but its precise role has remained obscure. Generally PO appears to catalyze a redox reaction between H_2O_2 as electron acceptor and many kinds of substrates (phenolics, aromatic amines, ascorbic acid, etc.). It is composed of a number of isoenzymes and is capable of catalyzing different types of oxidative reactions.

The increased PO activity has often been studied in connection with the oxidation of phenolic substances in diseased plants and resistance of the host was attributed to the toxicity of these oxidation products. Results from studies on the role of PO in resistance or susceptibility have been summarized in a number of reviews (Farkas and Király, 1958, 1962; Frič, 1976; Kosuge, 1969; Stahmann and Demorest, 1973).

The direct participation of this enzyme in the defence reactions of plants may be supported by the findings that PO inhibits the fungal growth (Macko et al., 1968; Lehrer, 1969). Also, this enzyme plays a crucial role in induced resistance in tobacco leaves against wildfire disease (Lovrenkovich et al., 1967).

Increased enzyme levels as a result of infection have been reported in a number of host-pathogen interactions (Bhaskaran et al., 1975; Johnson and Lee, 1978; Reddy and Rao, 1978; Sako and Stahmann, 1972; Simons and Ross, 1971; Vance and Sherwood, 1976; Vance et al., 1976; Van Loon and Geelen, 1971; Vegetti et al., 1975; Yamamoto and Nakao, 1976) and have been implicate in plant disease resistance. However, with severe disease development the enzyme activity decreases markedly over the uninfected controls (Benedict, 1973; Bugbee, 1975; Mitra et al., 1976; Sanden and Moore, 1978; van Lellyveld and Bester, 1978). Increase in PO isoenzymes in blast infected rice leaves due to *de novo* synthesis has been reported (Sridhar, 1978). One of us (Zuber, 1980) studied the electrophoretic analysis of peroxidase isoenzymes and found that PO activity measured *in vitro* of *R. solani* cannot be related directly to resistance or susceptibility among the rice cultivars as measured by differential symptom development. However, the isoenzyme patterns of *R. solani* were compared to differential virulence of the isolates.

Activity of PO is greater in compatible hostparasite combinations than in the incompatible ones (Akutsu and Watanabe, 1978; Cherry et al., 1974; Grzelinska and Sierakowska, 1975; Rao and Nayudu, 1979*b*) suggesting that increased enzyme activity is rather a biochemical symptom of disease and tissue destruction. It is clear, therefore, that the activation of PO in the host-parasite complex need not always be accompanied by the incompatible reaction. A correlation between symptom expression and PO activity has been shown in several cases (Barbara, and Wood, 1972; de Zoeten and Rettig, 1972; Gáborjányi et al., 1973; Grzelinska, 1970; Montalbini, 1972; Retig, 1974; Weststeijn, 1976; Wood, 1971*a*, *b*). However, other investigators have indicated that increased enzyme levels and the appearance of new isoperoxidases are not the cause of resistance; instead they are non-

specific events reflecting the abnormal physiological status of the host plants including ageing (Barna et al., 1975; Daly et al., 1970, 1971; Edreva 1977; Faccioli, 1979; Haard and Marshall, 1976; Hislop and Stahmann, 1971; Marshall et al., 1976; Misawa et al., 1971; Schipper, 1975; Seevers et al., 1971; van Loon, 1976; Wood, 1971*a*; Yamamoto et al., 1978). Furthermore, Nadolny and Sequeira (1980) and Seevers and Daly (1970*b*) concluded that PO was not directly involved in disease resistance. Thus, unequivocal proof for the participation of PO in the symptom expression is still lacking.

Concluding Remarks

From the foregoing account it becomes quite obvious that although much literature is available on the accumulation of phenolics little is known about their role in temporal and spatial relationships in the host-parasite interaction. Reports on the participation of phenolic compounds and enzymes involved in their biosyntheses and oxidation are highly contradictory. It is, imperative to suggest that disease resistance in higher plants is probably mediated by many other physiological or interconnected events which warrant fundamental research right from the entry of the pathogen into the host.

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Occurrence of *Sporidesmium sclerotivorum* Uecker, Ayers and Adams in Hungary. A Hyperparasitic Fungus on Sclerotia of *Sclerotinia sclerotiorum* Lib. de Bary

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Mycoparasitic fungi on sclerotia of *Sclerotinia sclerotiorum* were studied in different soils and seasons. The mycoparasitic fungi of sclerotia could be isolated from overwintered sclerotia. Overwintered sclerotia have been collected continually from different sunflower fields in Hungary since 1984. In 1986 *Sporidesmium sclerotivorum* Uecker, Ayers and Adams was isolated from overwintered sclerotia of a sunflower field at Tác-Gorsium. The morphology of the home isolate is the same as that of the original species. *Sporidesmium sclerotivorum* cannot be cultured on synthetic media but culturing is possible on sclerotia in quartz sand and moist filter paper. It is the first information on the presence of the fungus in Europe, although some American researchers have isolated the fungus from a few soil samples coming from some European countries.

In several countries intensive investigations are carried out on micoparasitic fungi of soilborne fungal plant pathogens. We consider it important to publish the first data about the occurrence of *Sporidesmium sclerotivorum* in Hungary since it is the first entry in Europe as well.

Materials and Methods

The overwintered sclerotia were collected at Tác-Gorsium in May 1986. Ten unsterilized sclerotia were placed in Petri-dishes on wet unsterilised sand (pH 6.5) and these plates were incubated for 7 days at 25 °C. The morphology of the fungus was studied with light microscope.

Results

After incubation of the overwintered sclerotia the following fungus colonies were observed: *Cylindrocarpon* sp., *Fusarium* spp., *Penicillium* spp., *Rhizoctonia* sp., *Gliocladium* spp., *Cephalotrichum* sp., *Trichoderma* spp., *Scopulariopsis* sp., *Coniothyrium minitans*. During the study of the overwintered sclerotia that came from a field at Tác-Gorsium, a dematiaceous hyphomycete: *Sporidesmium sclerotivorum* Uecker, Ayers and Adams was consistently appearant in summer and autumn.

The morphology of the home isolate

Colonies form a very loose mycelia on sclerotia. Mycelium, both superficial and immersed, superficial mycelium pale to mid-brown, cells of immersed mycelium irregular and contorted, dark brown to black. Conidiophores of the same diameter as hyphase or broader, simple or branched, erect, superficial, smooth, pale to dark brown, the conidiophore of its terminal branches usually consisting of two-three cells 15–25 × 4–5 μm, longer when proliferating percurrently, terminal cell of each branch conidiogenous. Conidiogenous cells integ-

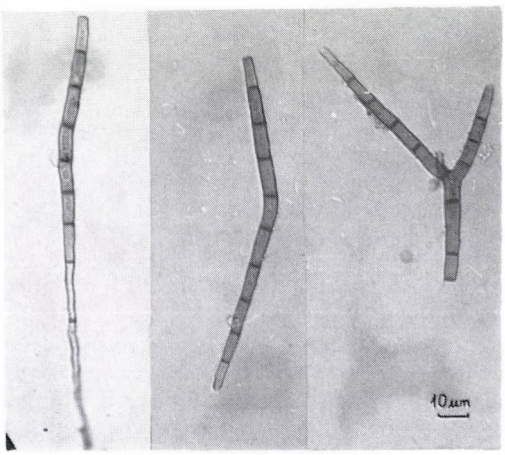

Fig. 1. Different forms of macroconidia of *S. sclerotivorum*

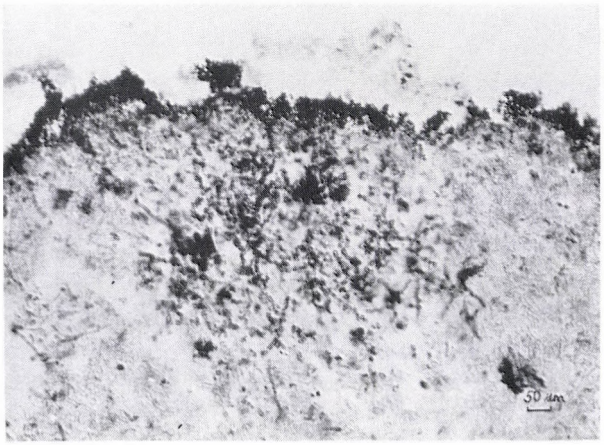

Fig. 2. Disorganized sclerotium. Photo by Dr. L. Vajna

rated. Conidia single, developing holoblastically as blown-out tips of conidiogenous cells and elongating by apical extension, light to mid brown, smoothe, phragmoseptate, tips rounded, tapered gently toward base and apex, base truncate, apical and basal cells paler than intermediate cells, 3–10 septate, septa darker than side walls, 60–90 × 3–8 μm germinating usually from either or both cells (Fig. 1). A Selenopsporella state developing on the same hyphae as the Sporidesmium state or on separate hyphae. Conidiophores of Slenosporella state simple or branched 50–100 μm long and 4–8 μm wide at base. Conidiogenous cells in whorls of two to five on the conidiophore or its branches, 14–33 μm long and 4 μm wide at base, tapered to 1–2 μm at terminus. Conidia hyaline, narrow, banana-shaped to fusiform to cylindric 6–8 × 0.9–1.0 μm.

Sporidesmium sclerotivorum infected and destroyed the sclerotia of *Sclerotinia sclerotiorum* (Fig. 2). The medulla was disorganized and the cortex destroyed and collapsed.

Sporidesmium sclerotivorum cannot be cultured readily on synthetic media.

The home isolate of *Sporidesmium sclerotivorum* can be grown easily on unsterilized sclerotia in Petri dishes on quartz sand.

Discussion

The morphology of home isolate of *Sporidesmium sclerotivorum* is the same as that of the original species. The fungus cannot be grown on synthetic media and this conclusion corresponds with the first American information (Cook and Baker, 1983). *Sporidesmium sclerotivorum* is considered to be an important organism in the development of a new biological control method of *Sclerotinia sclerotiorum*. As it was stated by Papavizas (1984) *Sporidesmium sclerotivorum* displays several extremely unusual properties:

Macroconidia of *Sporidesmium sclerotivorum* germinate within 3 days in soil adjacent to sclerotia of *Sclerotinia* spp. Germination may occur at distances up to 9 mm from a sclerotium and grow through soil from one sclerotium to another, producing many new macroconidia, the mycoparasite originally thought to be an obligate parasite, cultured axenically only on media prepared from sclerotia of host fungi; it attacks and destroys sclerotia of *Sclerotinia spp. S. cepivorum* and *Botrytis* but not those of *Sclerotinia rolfsii*. The new mycoparasite was able to infect and destroy more than 95 per cent of sclerotia of Sclerotinia minor within 10 weeks or less in soil (Papavizas, 1984).

Sporidesmium sclerotivorum grown on sclerotia in a sand culture and added to soil infected with sclerotia of *Sclerotinia sclerotiorum* (1 per cent w/w) infected 97 per cent of the sclerotia in five to six weeks (Baker and Cook, 1984).

Sporidesmium sclerotivorum is now produced experimentally by a private enterprise in the US for biocontrol of *Sclerotinia spp.* (P. B. Adams unpublished).

Sporidesmium sclerotivorum is advancing as a potential biocontrol agent for *Sclerotinia spp.* in Hungary as well.

Further research is being carried out to measure the activity of the mycoparasite on sclerotia of *Sclerotinia sclerotiorum* to obtain more information on the ecology of the fungus, to learn how to apply this mycoparasite most suitably in biological control.

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Occurrence of *Ascochyta fabae* Speg. in Hungary

(Short communication)

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In 1985 a severe ascochytosis was observed on the experimental plots of broad bean. The causal fungus was isolated from lesions of leaves and stems of diseased broad bean (*Vicia faba* L.) plants. On basis of symptoms, morphology and culture characteristics, the fungus was identified as *Ascochyta fabae* Speg.

This is the first report on the occurrence of *A. fabae* in Hungary.

The disease caused by *Ascochyta fabae* Speg. (syn.: *Ascochyta pisi* var. *fabae* Speg.; *A. spragueii* Meln.) is one of the most important diseases of *Vicia faba* L. (Gindrat, 1969). The fungus is wide-spread in Middle- and Southwest-Eurasia (Boerema, 1984). However the *A. boltshauseri* Sacc. was only previously reported from broad bean in Hungary (Békési, 1965). Melnik (1977) described this fungus as a synonym of *A. fabae* Speg., but this synonymous name is not correct (*viz.* Boerema, 1984).

In 1985 a severe ascochytosis was observed on the experimental plots in Hungary (village Tápiószele). The field symptoms were lesions on stems, leaves and pods. The lesions were rounded on the leaves and pods, and were elongated on the stems. The brown pycnidia formed in rather concentric rounds in the mature lesions.

Isolations were made from lesions of stems and leaves. Before and after the surface sterilization (0.5% of NaOCl for 2 min) the prepared pycnidia were macerated in sterile distilled water. The conidia-suspension was incubated on potato-dextrose-agar at 20 °C in dark.

The single-conidial cultures were incubated on oat meal agar and Czapek-Dox media for identification (Ondrej, 1968). Five other media were tested for growing and culture characterization, at 20, 25 and 30 °C.

The mycelium was septated and white in the growing area. The cultures formed thick, yellowish- or pinkish-white aerial mycelia.

The fungus grew and sporulated well on oat meal agar and Czapek-Dox media. We observed good growing and maturation on pea meal agar, and potato dextrose agar. The fungus grew weakly and middling sporulated on 2% malt extract agar, malted Czapek media and Leonian media (Fig. 1). The reverse of cultures became pale yellow to yellowish brown. The media were unpainted or pale yellow. The pycnidia formed near to the surface of media or in the aerial

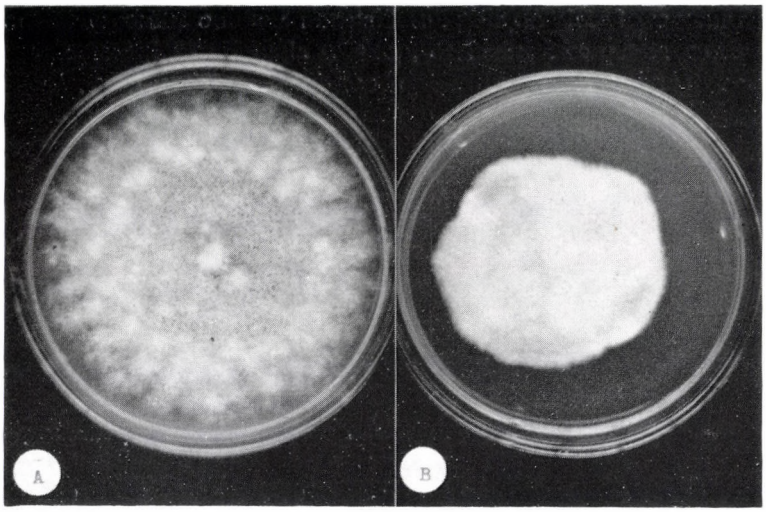

Fig. 1. Typical cultures of *Ascochyta fabae* Speg. on Czapek-Dox (A) and Leonian (B) media

mycelia. The best temperature was 25 °C for growing, but the sporulation started earlier at 20 °C.

The pathogenicity to plant was tested on two week old bean, broad bean and pea plants inoculated with conidia-suspension (7×10^5 conidia ml^{-1}) washed from 14-day-old culture growing on oat meal agar. The conidia-suspension was atomized onto the plants (0.5 ml per plant) and the inoculated plants were covered with a polyethylene tent for 5 days.

The isolated fungus was strongly pathogenic to the broad bean plants (cv. 'Majsi') and was not pathogenic to pea (cv. 'Újmajori középkorai') and bean (cv. 'Cherokee'). The first symptoms developed in the third week on broad beans, which had small reddish-brown spots on the inoculated leaves. Then the spots grew to 6–10 mm diameter and the centre of lesions grew light- or greyish-brown. The first lesions developed on the stems after a month. Pycnidia seldom formed in the lesions of leaves but frequently formed in the lesions of stems in artificial infection.

We tested the effect of the fungus on germinating seeds of broad bean, too. The seeds were germinated on water agar plates containing 3×10^6 conidia in 1 ml water agar. The plates were made in 20 cm diam. Petri dishes, and the conidia were suspended in autoclaved and cooled water agar. The seeds were surface-sterilized (0.1 % $HgCl_2$ for 5 min) and placed into sterile distilled water for a night at 25 °C.

The fungus caused root necrosis in the second week and numerous pycnidia were formed around the germinating seed on the water agar and on the seed-coat.

The microscopic characterization of the fungus was carried out measuring 50 pycnidia and 100 conidia. *Pycnidia* are subepidermal, rather globose, brown

from plants and orange to dark brown from media with a pore. Their diameter was 120–(180)–240 μm from cultures and 120–(144)–156 micrometers from plants. The pores were open to the surface of the epidermis and their diameter was 30–(54)–70 μm (Fig. 2).

Conidia are usually bicellular rarely 3 or 4 celled, hyalin. The oozing conidia-mass is pink to pinkish-orange. The measure is 16.8–(22.6)–24 × 4.8–(5.4)–6.6 μm from plants and 14.4–(16.8)–26.4 × 4.2–(4.8)–5.4 μm from cultures (Fig. 3).

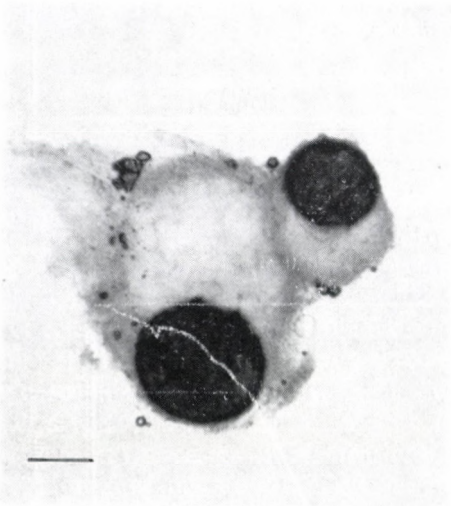

Fig. 2. Pycnidia of *Ascochyta fabae* Speg. from media with the oozing conidia-mass. Bar represents 100 μm

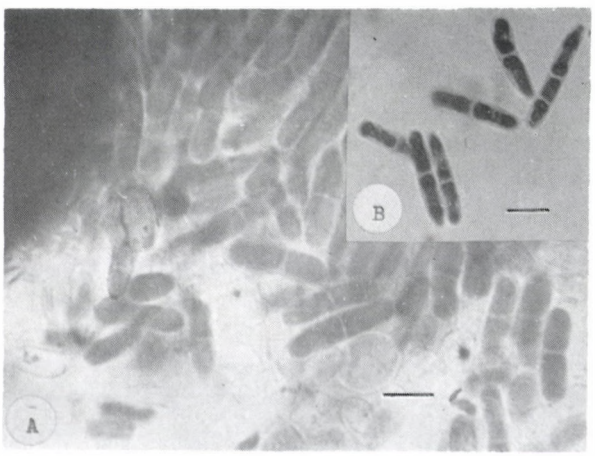

Fig. 3. Conidia of *Ascochyta fabae* Speg. from plant (A); and media (B). Cotton blue staining. Bar represents 10 μm

On the basis of culture characteristics (Ondrej, 1968; 1971), field and artificial symptoms and morphology, the fungus was identified as *Ascochyta fabae* Speg. The investigations on its seed-transmission are in progress.

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Some Filamentous Fungi Associated with Urediniospores of *Uromyces viciae-fabae* (Pers.) Schroet., and their Effect on the Urediniospores

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Several parasited urediniospores were observed in the routin tests in the summer of 1985. Nine filamentous fungi were isolated from pustules of *Uromyces viciae-fabae*. *Acremonium fusidioides* and *Pithomyces chartarum* were first observed in Hungary. The hyperparasitism of *Acremonium strictum*, *Cladosporium herbarium* and *Fusarium concolor* was detected first on this host. The occurrence of *Alternaria alternata*, *Botrytis cinerea*, *Epicoccum purpurascens* and *Phoma herbarum* was also observed.

Microorganisms contaminate the rust urediniopustules rapidly after the erupting through the epidermis. The main components of this flora are bacteria and yeasts (Doherty and Preece, 1978; French et al., 1964; Hevesi and Mashaal, 1975; Last, 1970; Levine et al., 1936; Pady, 1974; Parker and Blakeman, 1984).

Certain filamentous fungi have also been isolated in larger numbers from rust infected plants than from healthy ones (McKenzie and Hudson, 1976). Rudakov (1981) termed those as mycofil fungi, and he is listing some others too occurring in association with rusts. Some of them can parasite the urediniospores or overgrow the pustules (Hawksworth, 1980; McKenzie and Hudson, 1976; Rudakov, 1981).

The aim of this study was to determine the filamentous fungi associated with urediniospores of *Uromyces viciae-fabae* (Pers.) Schroet. and their effect on the rust.

Materials and Methods

Isolation of associated fungi

Rust infected leaves were collected from the experimental plots of our institute (village Tápiószele, Hungary). Mature leaflets were investigated in August 1985.

The urediniospore-mass was taken out from the pustules by sterile needle. The urediniospores were placed into droplets of deionized sterile water and were suspended in them. The suspension of spores was incubated on potato dextrose agar (PDA) in alternating fluorescent light at 25 °C. The light period was 12 h. The primary cultures were also suspended and incubated as termed. 100 pustules were investigated in this way.

Classification of fungi

The isolates of secondary collection were typized and the type isolates were investigated later. These were mass-transferred for maintenance onto PDA slants into tube. Classification was based on culture characteristics and microscopic characterization of conidiogenesis and measurement of 100 conidia. Identification of fungi was carried out according to Arx (1981), Bánhegyi et al. (1985), Booth (1971), Domsch et al. (1980), Ellis (1971, 1976) and Gams (1971). The identification of *Phoma herbarum* was compared to Boerema's (1964, 1970) papers.

Interactions between the urediniospores and the isolated fungi

Observations of interaction beween *U. viciae-fabae* and the isolated fungi were made on slide cultures. Films of 1.5% water agar on sterile glass slides were dusted with fresh urediniospores harvested from artificial infected broad bean plants grown in glasshouse. The dusted films were inoculated with conidia-suspension of each isolated fungi (10^6 conidia ml^{-1}) washing from PDA cultures. 0.1 ml conidia-suspension was dropped onto the dusted films and the treated films were covered with sterile cover-glasses, and the slides were incubated in moist Petri-dishes. The slides were observed daily over a 3-day period by light-microscope.

The germination of urediniospores was determined by recording the number of germinated spores in 20 microscopic fields containing at least 10 spores, and 50 germ tubes were measured for mean length of them on the 4th day. Sterile water treated films were used for control.

Results and Discussion

Filamentous fungi were isolated from 72 usually single infected uredinio-pustules. 79 primer isolates were observed. The most common fungi were the *Alternaria alternata* (Fr.) Keissler, *Cladosporium herbarum* (Pers.) Link et Gray and the *Epicoccum purpurascens* Ehrenb. et Schlecht.

Among the isolated fungi, *Acremonium fusidioides* (Nicot) W. Gams, *A. alternata*, *Botrytis cinerea* Pers., *E. purpurascens*, *Phoma herbarum* Westend., and *Pithomyces chartarum* (Berk. et Curt.) M. B. Ellis were not parasiting the urediniospores of *U. viciae-fabae*. However, a not significant decrease of germination of spores or elongation of germ tubes was observed on films treated by *A. fusidioides*, *A. altermata*, *E. purpurascens* and *P. herbarum*. Urediniospores treated by *Acremonium strictum* W. Gams were germinated in 12% and the length of germ tubes was up to 52 μm. The germ tubes were often lysed and the spores were strongly parasited (Fig. 1). *C. herbarum* treated urediniospores were germinated in 46% and were often parasited through the pores (Fig. 2). The younger spores were lysed (Fig. 3). The mean length of germ tubes was 78 μm.

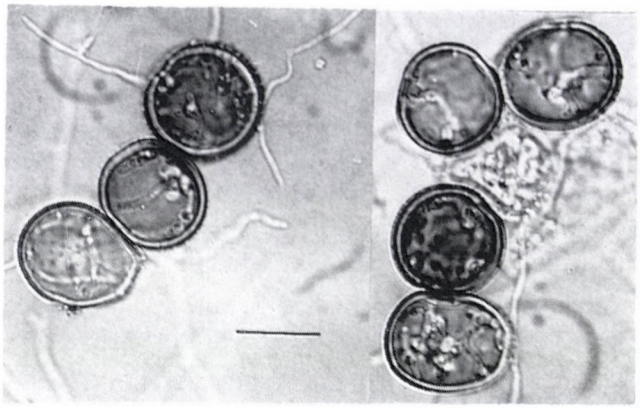

Fig. 1. Parasitized or lysed urediniospores affecting by *Acremonium strictum*. Bar represents 20 μm

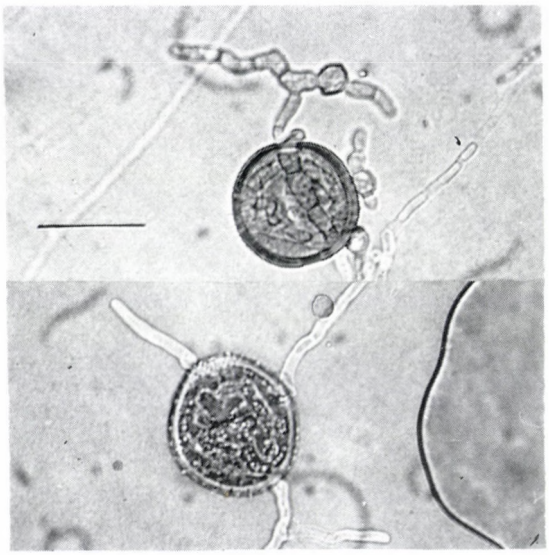

Fig. 2. Parasitism of urediniospores by *Cladosporium herbarum*. Bar represents 20 μm

Parasited germ tubes were not observed. *Fusarium concolor* Reinking reduced the germination of urediniospores to 8%, and the elongation of germ tubes was also depressed. The mean length of them was 36 μm. The urediniospores and the germ tubes were strongly parasited (Fig. 4), but their lysis were not observed. The *water* treated spores were germinated in 97%, and the length of germ tubes 112–138 μm.

Filamentous fungi are observed in association with numerous rust fungi, but only yeasts and some filamentous fungi are known in association with *Uro-*

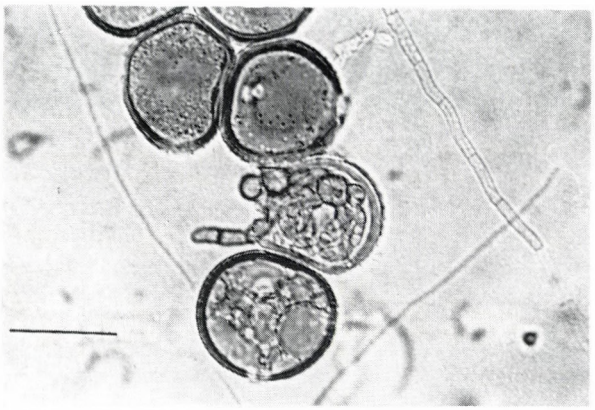

Fig. 3. Lysis of young urediniospore caused by *C. herbarum*. Bar represents 20 μm

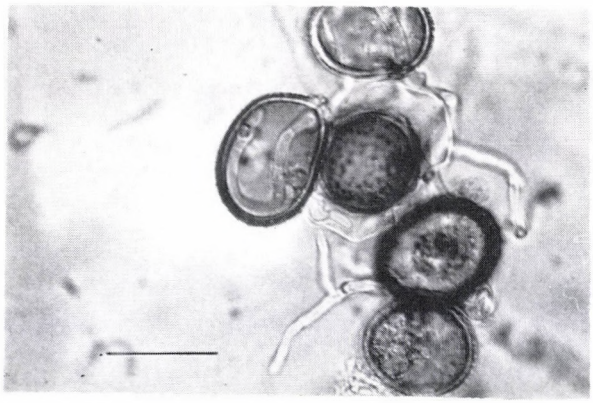

Fig. 4. Parasited group of urediniospores by *Fusarium concolor*. Bar represents 20 μm

myces viciae-fabae (Parker and Blakeman, 1984; Pruszynska-Gondek, 1976). However, their hyperparasitic ability to spores of this rust was not observed.

Among the nine isolated fungi, *Acremonium fusidioides* is a widespread fungus isolated usually from soil and excrements (Domsch et al., 1980), but we have not any other data on its occurrence in Hungary. The other isolated *Acremonium* species, the *A. strictum* is also a widespread fungus. It is well known from different media, including other fungi (Gams, 1971), but this is the first report on its hyperparasitism on any *Uromyces sp.*

Cladosporium herbarum is a very common saprophytic fungus (Ellis, 1971), and occurs in the phylloplane, too (Blakeman and Fokkema, 1982). However, rusts can be inhibited by *C. herbarum* (Kapooria and Sinha, 1969) or it can associate with rust pustules (Baimataeva, 1976; McKenzie and Hudson, 1976; Powell,

1971), its hyperparasitic ability is only known on the rust *Melampsora laricipopulina* Kleb. (Sharma and Heather, 1981) and on the powdery mildew *Phyllactinia corylea* (Pers.) Karst. (Anahosur et al., 1980).

However, numerous *Fusarium* species are known as associated fungi with rusts (Rudakov, 1981), the *F. concolor* is known as a predominantly soil fungus (Booth, 1971). The fungus was earlier known from soil in Hungary (Hornok, 1975). This is the first report on its association with and hyperparasitism on any rust.

Pythomyces chartarum is a rather common saprophyte occurring on different substrates (Ellis, 1971; Domsch et al., 1980). McKenzie and Hudson (1976) report this fungus as early colonizer on no rust-infected leaves, but they did not list it among the rust associating fungi. This is the first report on its occurrence in Hungary.

Alternaria alternata, *Botrytis cinerea*, *Epicoccum purpurascens* and *Phoma herbarum* are very common saprophytes. Among them, *A. alternata*, *B. cinerea* and *E. purpurascens* are known in association with rust and usually cause overgrowing of pustules (Baimataeva, 1976; McKenzie and Hudson, 1976; Rudakov, 1981). *P. herbarum* is known from uninfected leaves (McKenzie and Hudson, 1976). This is the first report on their association with and effect on *Uromyces viciae-fabae*.

The possibilities of application of these fungi as preventive control are discussed.

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Occurrence of *Fusarium ventricosum* Appel and Wollenweber in Hungary

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In the experiments on phylloplane of *Chamacereus silvestrii* and *Foeniculum vulgare* we isolated *Fusarium ventricosum* at first in Hungary. The other also occurring *Fusarium* species were *F. avenaceum*, *F. equiseti*, *F. oxysporum*, *F. redolens*, *F. sacchari* var. *subglutinans* and *F. solani* from both hosts, *F. chlamydosporum* from *Chamacereus* and *F. graminearum* from *Foeniculum*.

Fusarium ventricosum Appel and Wollenweber [syn.: *Fusarium cuneiforme* Sherb.; *F. solani* (Mart.) Appel and Wollenw. var. *ventricosum* (Appel and Wollenw.) Joffe; *Fusisporium argillaceum* Fr.; *?Fusarium argillaceum* (Fr.) Sacc.; *?F. solani* (Mart.) Appel and Wollenw. var. *argillaceum* (Fr.) Bilai] is a rather widespread *Fusarium*-species. The fungus is known from Europe, the USA, the USSR, the Near-East (cf. Booth, 1971; Gerlach and Nirenberg, 1982), and from Cuba (Arnold et al., 1982), but formerly did not occur in Hungary. The aim of this article is to give the formal description of *F. ventricosum* in Hungary.

Materials and Methods

The fungus was isolated from the phylloplane of the cactus *Chamacereus silvestrii* and of fennel (*Foeniculum vulgare*). Eight about 2 cm long branches of the cactus were cut from same plant grown in glasshouse and 5 g leaf material of fennel was cut up to about 1 cm long pieces for the isolation. The micro-organisms of the phylloplane were washed off in 25 ml sterilized physiological salt solution by slow magnetic stirring for 20 min. Then the dish-water was added to 225 ml 2% malt-extract-agar (MEA) medium at 38 °C and the mixture was homogenized by rapid magnetic stirring for 5 min.

The plates were made in 8 cm diam. Petri dishes from 5 ml of mixed agar medium for the isolation of fungus, and were incubated at 25 °C for a week in dark. The pure cultures of the fungus were isolated from microscopic preparates made in a drop steril distilled water by spreading the drops onto potato-dextrose-agar (PDA) plates.

Identification was carried out according to Booth (1971) using PDA as standard culture media. The identification was based on Booth (1971) and was

compared with Domsch et al. (1980) and with Gerlach and Nirenberg (1982). 4–4 plates were inoculated from each pure culture and were incubated at room temperature and at normal laboratory light for four days for measuring the growth rate. The culture characters were observed on both PDA and MEA media, the sporogenesis was examined in slide cultures. 100 conidia were measured from each isolate for their dimensions. The characterization was based on two isolates coded as *CH5* (from *Chamacereus silvestrii*) and *FV5* (from *Foeniculum vulgare*).

Results and Discussion

The isolation of *F. ventricosum* was successful among the cultures developed on mixed-agar plates. The other occurring *Fusarium*-species were *F. avenaceum* (Corda ex Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schlecht., *F. redolens* Wollenw., *F. sacchari* (Butler) Gams var. *subglutinans* (Wollenw. and Reinking) Nirenberg and *F. solani* (Mart.) Sacc. from both hosts, and the *F. chlamydosporum* Wollenw. and Reinking from the cactus, and the *F. graminearum* Schwabe from the fennel only.

There was no important difference between the two isolates. *Colonies* are slow growing, reaching 2.8–3.5 cm diam. after four days and 3.6–4.5 cm after ten days. *Aerial mycelium* is white or light greyish with an ochre tinge on MEA medium, rather sparse but more abundant on PDA medium, often showing a distinct zonation. *Conidiophores* are formed abundant in the aerial mycelium, at first unbranched, 90–150 μm long and 4.2–4.8 μm at the base, septate. The apical phyalides are 30–52 × 2.4 μm, collaretted. Then the conidiophores become loosely branched near the base, occasionally densely aggregated, but true sporodochia are not formed (Fig. 1). *Phialides* are monophialidic cylindric, gradually narrowing toward the tip. *Conidia* are micro- and macroconidia, but distinct microconidiophores are absent. The microconidia are straight or slightly curved, 0–1 septate, 9.6–14.8 × 2.8–4.8 μm. The macroconidia are mostly 3 septate, ocassionally 2- or 4- to 6-septate 24–52 × 4.8 μm (Fig. 2). *Chlamydospores* are abundantly formed after a week, at first smooth-walled, then become warted, form singly or in groups of 2–3 at the ends of short lateral branches of hyphal cells or conidia (Fig. 3), measuring 8.4–12 μm diam.

The *F. ventricosum* is known as a secondary invader, particularly rotting potato tubers, but also on beets, melons, groundnuts, tobaccoes, tomatoes (Arnold et al., 1982; Booth, 1960; 1971; Gerlach and Nirenberg, 1982; Lukic, 1967; Rudakov and Alkhovskaya, 1964; Wollenweber and Reinking, 1935). The fungus is occasionally isolated from soils, compost, waters, and Atlantic coast (Domsch, 1960; Domsch et al., 1980; Krehl-Nieffer, 1950; Niethammer, 1939; Ristakovic and Miller, 1969). The teleomorph of the fungus (*Nectria ventricosa* Booth) occurs on discarded decaying potato tubers (Booth, 1971).

This is the first report on the occurrence of *Fusarium ventricosum* Appel and Wollenw. in Hungary, however, the other *Fusarium* species are known from earlier literature (Hornok, 1975; Vörös és Léránth, 1974).

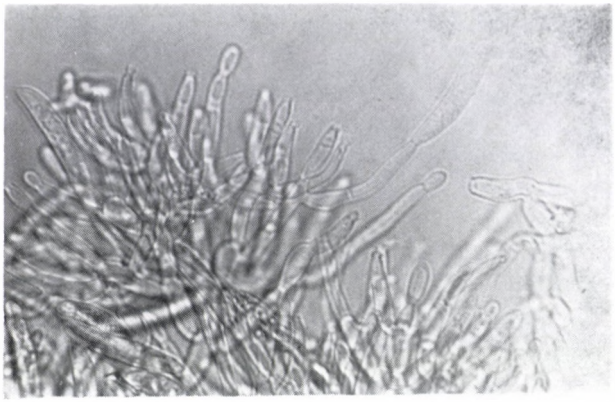

Fig. 1. Densely aggregated conidiophores of *Fusarium ventricosum*

Fig. 2. Macro- and microconidia from potato dextrose agar after a week

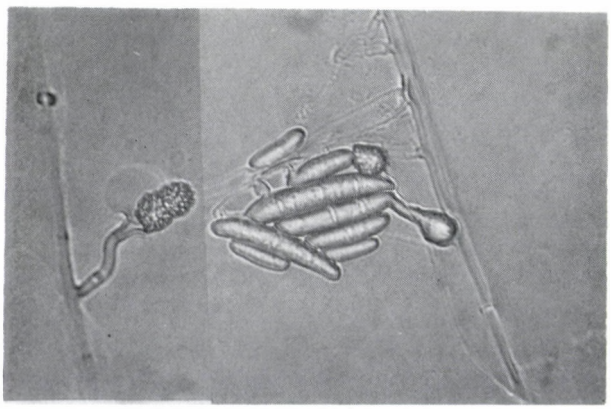

Fig. 3. Chlamydospores of the fungus originated from hyphal cell and conidia

Acknowledgements

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In vivo Occurrence of Hyperparasitism of *Botrytis cinerea* Pers. by *Gliocladium catenulatum* Gilman et Abbott

(Short communication)

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In vivo parasitized hyphae of *Botrytis cinerea* Pers. were observed from broad bean seeds during the seed health testing. *Gliocladium catenulatum* Gilm. et Abbott was isolated from these pieces of hyphae as a contaminating fungus of cultures of *B. cinerea* grown from infected hyphae.

The hyperparasitism of *B. cinerea* by *G. catenulatum* was also investigated *in vitro* by dual cultures carried out on cellophane film. The mycelia and conidiophores of *B. cinerea* became parasited in these cultures and the developed sclerotia were killed by *G. catenulatum*.

Gliocladium Corda spp. are rather common soil fungi (Domsch et al., 1980), and some of them are also possible agents for biological control of plant pathogens (Papavizas, 1985). Among the hyperparasites, *Gliocladium roseum* Bain. is well known as a destructive mycoparasite (Barnett and Lilly, 1962) and it also can penetrate into the host hyphae of some fungi e.g. of *Botrytis allii* Munn. (Walker and Maude, 1975). However, the similar *G. catenulatum* Gilm. et Abbott is only known as a nonpenetrating parasite of sclerotia of *Sclerotinia sclerotiorum* (Lib.) DeBary (Huang, 1978) or an antagonist of it and *Pythium ultimum* Trow. (Teyes and Dirks, 1985; Zazzerini and Tosi, 1985). A mycotoxic metabolite of *G. catenulatum* was also reported (Brian and Hemming, 1947).

In the course of investigations on mycoflora of broad bean seeds some preparates of hyphae of *B. cinerea* were observed containing another hypha, too. Those preparates were placed onto Leonian's media into Petri dishes, because Urbasch (1984) indicates the possible formation of endohyphae in hyphae of *B. cinerea*. The cultures of *B. cinerea* developed quickly and some of them was contaminated with another fungus with pink conidia first and with green ones later. This fungus was identified as *Gliocladium catenulatum* Gilm. et Abbott according to Bánhegyi et al. (1985) and Domsch et al. (1980). Pure cultures were made from both fungi, and the pure cultures were maintained on potato-dexrose-agar (PDA) slant in tube.

The hyperparasitic ability of *G. catenulatum* to *B. cinerea* was investigated in dual cultures growing on cellophane film according to Vajna (1985). The sterilized cellophane films were placed onto surface of Leonian's media in 10 cm diam.

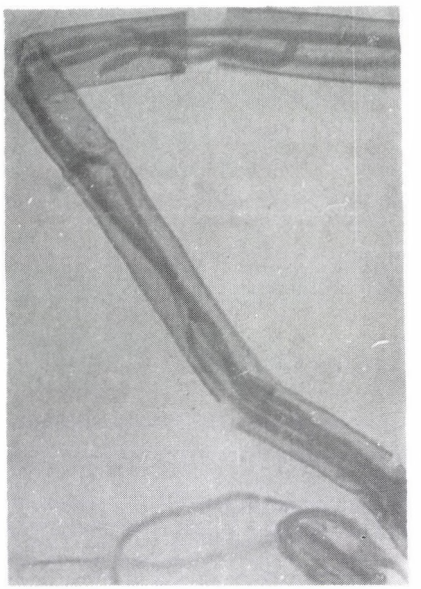

Fig. 1. Parasitized conidiophore of *Botrytis cinerea* from dual culture with *G. catenulatum*. Cotton blue staining

Petri dishes, and were inoculated on the opposite edges with conidia of fungi. Pure cultures of *B. cinerea* under the same conditions were used as control for developing of endohyphae. The cultures were incubated at 22 °C in natural light, and the microscopic examinations were carried out after ten days on cotton blue stained mycelia segments on cellophane.

The hyphae of fungi had grown together after a week. At first the grown of *B. cinerea* was blocked and a sharp line (about 1.0 mm wide) was arizing between the cultures. Then the cultures of *B. cinerea* became overgrown, and the sporulation of *G. catenulatum* was observed on thallus as well as on the developed sclerotia of *B. cinerea*. Hyphae of *G. catenulatum* were observed in hyphae and conidiophores of *B. cinerea* (Fig. 1), and the sclerotia affected by *G. catenulatum* were smaller and were not germinable in fresh media. Endophyae were rarely observed in same old pure cultures of *B. cinerea*.

The process of hyperparasitism was coiling the host hyphae similar to which is reported on *Sclerotinia sclerotiorum* (Huang, 1978), but coloured penetrating points as were observed in *B. allii–G. roseum* relationship were not observed on hyphae of *B. cinerea* affected by *G. catenulatum*. The hyphae of *G. catenulatum* were also not in living host hyphae with densely stained cytoplasm, which may indicate a previous mycotoxic effect before the penetration.

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The author thanks Mrs. J. Molnár and Mrs. A. Boros for their technical assistance.

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Reaction of *Physalis* Species to Plant Viruses XI. Additional Data on the Susceptibility of *Physalis ixocarpa*, *Physalis philadelphica* and *Physalis pruinosa*

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In three *Physalis* species new host-virus relations have been detected. *Physalis ixocarpa* and *P. philadelphica* have been found to be locally and systemically susceptible each to 10, and *P. pruinosa* to 7 viruses. All three *Physalis* species are resistant to bean yellow mosaic virus. Of the plants examined the perennial *Physalis pruinosa* is important from the point of view of virus ecology as well. No less importance is attached to the results obtained for *Physalis ixocarpa* as an edible fruit.

The first publication of the series related to the virus susceptibility of *Physalis* species summed up literary data concerning – among others – *Physalis ixocarpa*, *P. philadelphica* and *P. pruinosa* (Horváth, 1970). *Physalis ixocarpa* and *P. pruinosa* were then described as susceptible each to 3, and *P. philadelphica* to some 9 viruses

Table 1

Three susceptible *Physalis* species to several viruses

Physalis species	Viruses[1]	
	Reviewed by Horváth (1970)	Investigated by Horváth (1974, 1975a, b)
Physalis ixocarpa	AMV, PVX, TEV, TRV	CMV, PAMV, TMV, TNV, TRSV
P. philadelphica	AraMV, PCRV, PLRV, PPV, PVA, PVM, PVS, PVX, PVY	AMV, CMV, PAMV, TRSV
P. pruinosa	PCRV, TEV, TRV	AMV, CMV, PAMV, PVX, PVY, TMV, TRSV

[1] Abbreviations of viruses. AMV = alfalfa mosaic virus, AraMV = *Arabis* mosaic virus, CMV = cucumber mosaic virus, PAMV = potato aucuba mosaic virus, PCRV = potato corky ringspot virus, PLRV = potato leafroll virus, PPV = potato paracrinkle virus, PVA = potato virus A, PVM = potato virus M, PVS = potato virus S, PVX = potato virus X, PVY = potato virus Y, TEV = tobacco etch virus, TMV = tobacco mosaic virus, TNV = tobacco necrosis virus, TRSV = tobacco ringspot virus, TRV = tobacco rattle virus

(Table 1). In subsequent experiments *Physalis ixocarpa* proved to be susceptible to 5 (cucumber mosaic virus, potato aucuba mosaic virus, tobacco mosaic virus, tobacco necrosis virus, tobacco ringspot virus), *P. philadelphica* to 4 (alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, tobacco ringspot virus) and *P. pruinosa* to 7 (alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco ringspot virus) further viruses (Horváth, 1974, 1975*a*, *b*).

The experiments in which the virus susceptibility of the above three *Physalis* species was studied were aimed at detecting new host-virus relations completing thereby our virological knowledge concerning the above species.

Materials and Methods

Young seedlings of the three *Physalis* species were inoculated with 12 viruses. Before inoculation the leaves of the plants were dusted with carborundum (400 mesh), and after inoculation sprayed with water. The inoculated leaves were pierced through with plastic sucker sticks so that when reisolating the viruses local infection could be established for certain. On the origin and maintenance of the viruses and on the method of back-inoculation detailed data are to be found in earlier publications (Horváth, 1974, 1977, 1981, 1983).

Results and Conclusions

In the course of examinations of *Physalis ixocarpa* 10 new local and systemic relations were detected (Table 2). Carnation ringspot virus induced latent infection in inoculated and non-inoculated leaves alike. The results of back-inoculation to *Phaseolus vulgaris* cv. *Red Kidney* plants was indicative of a high concentration of carnation ringspot virus present in the *Physalis* species examined. Lettuce mosaic virus caused latent infection in the inoculated and mosaic symptoms in the non-inoculated leaves. According to the results of our recent experiments the *Physalis ixocarpa* is susceptible to some 19 viruses (see Horváth, 1970, 1974, 1975a, b). *Physalis ixocarpa*—like other *Physalis* species—is resistant to bean yellow mosaic virus (Table 2).

Physalis ixocarpa has recently been introduced in India for its edible fruit (see Deb, 1979), so any information concerning its virus susceptibility is of practical importance, too.

Studies on *Physalis philadelphica* revealed 10 new local and systemic relations (Table 3). The host-virus reactions evoked by inoculation with carnation ringspot virus and lettuce mosaic virus agreed with those described for *Physalis ixocarpa*. To bean yellow mosaic virus it proved resistant. With our results obtained so far taken into consideration it can be established that the *Physalis philadelphica* is a host plant for about 23 viruses (see Tables 1 and 3).

Table 2

Reaction of *Physalis ixocarpa* to some viruses

Viruses	Reaction[1]
Arabis mosaic virus	CoRi/Vc, Mo
Bean yellow mosaic virus	O, Vnr/O, Vnr
Belladonna mottle virus	C–NL/Vc, Vb, Mo, Ld
Broad bean wilt virus	CL/Vc, YM
Carnation ringspot virus	O, Vr/O, Vr
Lettuce mosaic virus	O, Vr/Mo
Melandrium yellow fleck virus	CL/Mo, Y
Tomato aspermy virus	C–NL/Mo, Gi
Tomato mosaic virus	NL/Mo, N
Tomato ringspot virus	NL/NL, Tn
Turnip mosaic virus	CL/Mo, YM, Ld

[1] Local/systemic symptoms. C = chlorotic, chlorosis, C–N = chlorotic-necrotic, Co = concentric patterns, Gi = green islands, L = lesion, lesions, Ld = leaf distortion, Mo = mosaic, N = necrotic, necrosis, O = no manifestation of the disease observed, Ri = rings, ring-like spots, Tn = top necrosis, Vb = vein banding, Vc = vein clearing, Vnr = virus not recoverable, Vr = virus recoverable, Y = yellowing, yellows

Table 3

Reaction of *Physalis philadelphica* to some viruses

Viruses	Reaction[1]
Bean yellow mosaic virus	O, Vnr/O, Vnr
Belladonna mottle virus	NL/Vc, Vb, Mo, Ld
Broad bean wilt virus	CL/Vc, Mo, Y
Carnation ringspot virus	O, Vr/O, Vr
Lettuce mosaic virus	O, Vr/Mo
Melandrium yellow fleck virus	CL/Mo
Tomato aspermy virus	C–NL/Vc, Vb, Mo, Gi
Tomato mosaic virus	NL/NL
Tomato ringspot virus	NL/Vc, Vb, Tn
Tobacco mosaic virus	NL/NL, Tn
Turnip mosaic virus	CL/Mo, Ld

[1] See the abbreviations in Table 2

Table 4

Reaction of *Physalis pruinosa* to some viruses

Viruses	Reaction[1]
Arabis mosaic virus	O, Vr/Mo
Bean yellow mosaic virus	O, Vnr/O, Vnr
Belladonna mottle virus	C–NL/Vc, Mo, Gi, Ld
Broad bean wilt virus	O, Vr/Vc, Mo
Carnation ringspot virus	O, Vr/O, Vr
Melandrium yellow fleck virus	CL/Vc, Vb, Mo
Turnip mosaic virus	O, Vr/Vc, Mo, Ld

[1] See the abbreviations in Table 2

In the course of studies on the host-virus relations of *Physalis pruinosa* 6 new local and systemic relations were discovered (Table 4). Broad bean wilt virus and turnip mosaic virus caused latent local infection in the plants which in this respect differed from *Physalis ixocarpa* and *P. philadelphica*, since the latter responded with manifest symptoms to the viruses in question. Bean yellow mosaic virus—like in the case of *Physalis ixocarpa* and *P. philadelphica*—was apathogenic for *Physalis pruinosa*. On the basis of the data of literature, our earlier experiments and present research results *Physalis pruinosa* can be regarded as host for 16 viruses (see Tables 1 and 4). Data concerning the perennial *Physalis pruinosa* are important from the point of view of virus ecology.

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Reaction of *Physalis* Species to Plant Viruses. XII. *Physalis anisotrichus* and *Physalis filiformis* as New Experimental Plants in Virology[1]

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In the course of studies on the host-virus relations of *Physalis* species new in the literature of plant virology 2 accessions of each of *Physalis anisotrichus* and *P. filiformis* were found to be local and systemic hosts for 11 viruses (alfalfa mosaic virus belladonna mottle virus, cucumber mosaic virus, henbane mosaic virus, *Melandrium* yellow fleck virus, potato virus Y, tobacco rattle virus, tobacco ringspot virus, tomato aspermy virus, tomato mosaic virus, turnip mosaic virus) from 8 virus groups.

In our papers published between 1970 and 1985 on the virus susceptibility of *Physalis* species many new host-virus relations were described for 14 *Physalis* species and one *Physalis* variety (see earlier papers of the author in the series). Of the species examined *Physalis peruviana* var. *macrocarpa* and *P. curassavica* were new experimental plants in the literature of plant virology (Horváth, 1974, 1981). Two *Physalis* species (*Physalis glabripes* and *P. lanceifolia*) mentioned in the literature as susceptible each to a single virus were dealt with by us in detail; *Physalis glabripes* was known to be susceptible to tobacco rattle virus, while *P. lanceifolia* to potato leafroll virus (Schmelzer and Wolf, 1971, 1977; Ambrosaj and Davidchuk, 1978).

In our experiments aimed at studying the virus susceptibility of *Physalis* species unknown so far from a virological point of view *Physalis anisotrichus* and *P. filiformis* were examined.

Materials and Methods

Two accessions of each of young *Physalis anisotrichus* and *P. filiformis* plants were inoculated with various viruses by the carborundum-spatula technique known in virology. The methods of inoculation, origin of viruses and determina-

[1] Earlier papers of the author in the 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), *VI.* Acta Phytopath. Hung. 16, 327–337 (1981), *VII.* Acta Phytopath. Hung. 18, 209–216 (1983), *VIII.* Acta Phytopath. Hung. 19, 303–308 (1984), *IX.* Acta Phytopath. Hung. 20, 97–101 (1985), *X.* Acta Phytopath. Hung. 20, 103–107 (1985), *XI.* Acta Phytopath. et Entomol. Hung. 23, 137–141 (1988).

tion of the infection of inoculated plants were described in earlier publications of this series (cf. Horváth, 1974, 1983, 1985, 1988). The inoculation experiments included henbane mosaic virus too, a virus recently isolated from *Datura stramonium* in Hungary (Horváth et al., 1987, 1988), because *Physalis alkekengi* e.g. was found to be susceptible to henbane mosaic virus (Lovisolo and Bartels, 1970; Salamon, 1988). Henbane mosaic virus was maintained in *Datura stramonium* and/or *Nicotiana tabacum* cv. *Xanthi-nc* plants. From *Physalis anisotrichus* and *P. filiformis* plants inoculated with the tissue sap of the former plants the henbane mosaic virus was back-inoculated into *Datura stramonium* plants in order to find out whether the *Physalis* species examined contained the virus.

Results and Conclusions

In the course of examining two accessions of *Physalis anisotrichus* it was found that they were locally and systemically equally susceptible to 11 viruses of 8 virus groups and showed no symptomatological differences (Table 1). The symptoms appeared both in inoculated and non-inoculated leaves. The inoculated leaves generally showed necrotic lesions, and in the case of certain viruses (e.g. tomato aspermy virus, tomato mosaic virus) dropped. The systemic disease manifested itself in mosaic, chlorotic spots, vein clearing (belladonna mottle virus, henbane mosaic virus, potato virus Y, turnip mosaic virus), red necrotic lesions

Table 1

Reaction of two accessions of *Physalis anisotrichus* to plant viruses[1]

Viruses	Accessions	
	Stock-13268	Stock-13287
Alfalfa mosaic virus	NL/ReL	NL/ReL
Belladonna mottle virus	NL/CSp	NL/CSp
Cucumber mosaic virus	BrNL/Mo, ReL	BrNL/Mo, ReL
Henbane mosaic virus	NL/Mo	NL/Mo
Melandrium yellow fleck virus	NL/NSp	NL/NSp
Potato virus Y	NL/Mo	NL/Mo
Tobacco rattle virus	NL/NL	NL/NL
Tobacco ringspot virus	NL/NL	NL/NL
Tomato aspermy virus	NL, Ld/NL	NL, Ld/NL
Tomato mosaic virus	NL, Ld/NL, D	NL, Ld/NL, D
Turnip mosaic virus	NL/Vc, Mo	NL/Vc, Mo

[1] Local/systemic symptoms. Br = brown, C = chlorotic, chlorosis, D = death of the whole plant, L = lesion, lesions, Ld = leaf drop, Mo = mosaic, N = necrotic, necrosis, Re = reed, Sp = spot, spots, Vc = vein clearing, Y = yellowing, yellows.

Table 2

Reaction of two accessions of *Physalis filiformis* to plant viruses[1]

Viruses	Accessions	
	Stock-02630	Stock-03110
Alfalfa mosaic virus	NL, Y/Vc, Mo	NL, Y/Vc, Mo
Belladonna mottle virus	C–NL/CSp	C–NL/CSp
Cucumber mosaic virus	NL, Ld/NSp	NL, Ld/NSp
Henbane mosaic virus	NL, Y, Ld/Mo	NL, Y, Ld/Mo
Melandrium yellow fleck virus	NL, Ld/NL	NL, Ld/NL
Potato virus Y	NL, Ld/NL	NL, Ld/NL
Tobacco rattle virus	NL/NL	NL/NL
Tobacco ringspot virus	NL/NL	NL/NL
Tomato aspermy virus	NL/NSp	NL/NSp
Tomato mosaic virus	NL, Ld/NL, D	NL, Ld/NL, D
Turnip mosaic virus	NL, Ld/Mo	NL, Ld/Mo

[1] See the abbreviations in Table 1.

(alfalfa mosaic virus, *Melandrium* yellow fleck virus, tobacco rattle virus, tobacco ringspot virus, tomato aspermy virus) or mosaic and red lesions (cucumber mosaic virus). Plants inoculated with tomato mosaic virus ultimately died of total necrosis.

The two accessions of *Physalis filiformis* similarly responded with local and systemic symptoms to 11 viruses of 8 virus groups (Table 2). The local symptoms were chlorotic, chlorotic-necrotic and/or necrotic lesions. The inoculated leaves of plants inoculated with henbane mosaic virus, *Melandrium* yellow fleck virus, potato virus Y, turnip mosaic virus dropped following the infection. However, this hypersensitive reaction did not prevent the virus from causing systemic infection to the plants. The systemic symptoms similarly to those observed in the *Physalis anisotrichus* plants were: chlorotic spots, mosaic or necrotic lesions. *Physalis filiformis* and *P. anisotrichus* gave somewhat different response following inoculation with alfalfa mosaic virus and potato virus Y. The *Physalis anisotrichus* plants inoculated with alfalfa mosaic virus showed systemic red lesions, while in *Physalis filiformis* vein clearing and mosaic were observed. To inoculation with potato virus Y *Physalis anisotrichus* responded with systemic mosaic, while *P. filiformis* with necrotic lesions (see Tables 1 and 2).

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Observations on the Parasitoid *Macrocentrus pallipes* Nees *(Hymenoptera: Braconidae)* in Connection with its Two Hosts *Hedya nubiferana* Haw. and *Pandemis heparana* Den & Schiff. *(Lepidoptera: Tortricidae)**

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Larvae of *Hedya nubiferana* Haw. and *Pandemis heparana* Den. & Schiff. were collected from rolled apple leaves and reared until pupation under laboratory conditions. The pupae were then kept in outdoor isolators until the emergence of adults and their progenies were used in the 2nd larval stage in the parasitization experiments. The *Macrocentrus pallipes* Nees adults were obtained from naturally parasitized Hedya larvae. Both parasitized and normal Hedya larvae underwent diapause as well as the parasitized Pandemis larvae while the unparasitized larvae of the latter continued their development to give a second generation. The keeping of Macrocentrus adults at 5 °C or 15 °C and their feeding with honey or honey-agar lengthened the life-span considerably.

Among the Tortricidae causing considerable damage in Hungarian fruit orchards (Reichart et al., 1975). The species *Hedya nubiferana* Haw. is univoltine and its activity extends to the period April-June. On the other hand, the related species *Pandemis heparana* Den. & Schiff. has two generations yearly. *Macrocentrus pallipes* Nees is one of the most important parasites of the *H. nubiferana* and *P. heparana* larvae in Hungary (Balázs et al., 1983) and was considered by Eady and Clark (1964) as one of the important parasites of Hedya spp. and other lepidipterous larvae. Present paper intends to furnish data on the connections of the parasitoid with its hosts.

Material and Methods

Rolled apple leaves were collected by the end of April, 1985 in the untreated (insecticide-free) plot of the experimental orchard of the Plant Protection Institute (Nagykovácsi, near Budapest). The leaf clusters were carefully opened and the larvae of *H. nubiferana* and *P. heparana* were determined, based on the descrip-

* Apple Ecosystem Research No. 59.

tions of Balázs and Bodor (1969). Special care was taken to differentiate the larvae from those of the very similar *Ptycholoma lecheanum* L. The larvae were then transferred to single rearings (glass cylinders 18 cm high, 15 cm in diameter, covered with cellophane foil). The apple shoots were changed twice a week until pupation. The pupae of the two species were transferred from the rearings singly into small glass vials with cotton plugs and were kept in an outdoor isolator until adult emergence.

Larvae that had transformed into prepupae among rolled leaves were left undisturbed because otherwise they often failed to transform into pupae.

The Hedya larvae that were naturally parasitized under field conditions were daily observed until the parasitoid larvae had left their host. The hymenopterous cocoons were then also transferred into glass vials closed with slightly humidified cotton plugs and were kept in an outdoor isolator until adult emergence.* The adults of *Macrocentrus pallipes* were drinking water from the cotton plug but too much water had to be avoided as the wings of the tiny parasites tended to stick to the walls of the vials and the wasps died.

The males of *Macrocentrus pallipes* showed a tendency to appear earlier than the females and to prevent their premature death different methods were used for their preservation until female emergence:

(a) Storage at 5 °C, 80–100 r.h. and 16/8 hours photophase;

(b) Storage at 15 °C, 70–95 r.h. and 16/8 nours photophase.

The parasitoid adults were fed with diluted (10%) honey or cooked honey-agar. The latter was prepared from 1 gr agar, 100 ml dist. water, 50 g sucrose and 20 g honey.

Rearing of the hosts

The male and female Hedya and Pandemis adults were kept in pairs in rearing cylinders containing some apple shoots and few droplets of diluted (20%) honey, honey-agar and pieces of cardboard. After mating and egglaying the eggs were daily observed until hatching. The larvae were used in the parasitization experiment after attaining their second larval instar. For the latter 100 L_2 larvae of Hedya and Pandemis, respectively, were transferred on apple leaves into rearing cylinders and into each rearing 6 mated Macrocentrus females were added. The rearings were then kept for 48 hours at room temperature (17 – 22 °C). From both host species 10 larvae were fixed in aqueous Bouin solution, imbedded in paraffine wax and sectioned. The sections (thickness: 7 microns) were stained with Mallory triple stain (Mahoney, 1966) to detect the eggs of parasitoids in the host's bodies.

* The authors wish to express their thanks to Dr. J. Papp (Hungarian Natural History Museum) for the determination of their hymenopterous material.

Results and Discussion

By the end of April the Hedya and Pandemis larvae were in different instars. Pupae of Hedya were obtained by mid-May and it took further 11 days until adult emergence. The mated Hedya females laid their eggs for 3–4 days in the first days of June. The eggs are flat, semi-translucent with a diameter of 1 mm and are laid singly mostly on the upper surfaces of leaves but also on the lower leaf surfaces and on the glass walls of the containers. Both for the egglaying process and embryonal development the high humidity seems of utmost importance. The embryonal development lasted 5–6 days in June. The neonate Hedya larvae are white and become yellowish after feeding on apple leaves. The newly hatched larvae moulted to L_2 larvae after 6 days. These latter feed actively on the lower surface of leaves for 4 days, then enter a diapause after hiding near the leaf-rib or under the bark of apple twigs. The diapausing larvae are thinner and shorter than earlier and move sluggish if taken from their hiding place. The diapause lasts until the next spring. On the contrary, the Pandemis larvae continue their development and a second generation appears.

In the rearing containing parasitized larvae of Hedya the cocoons of Macrocentrus were found among the apple leaves after the parasitoid larvae had left the bodies of their mature hosts. The Macrocentrus adults emerged from the cocoons after 9–11 days, by the end of May. In most cases all adults left their group of cocoons on the same day although in some cases their emergence took 2–3 days. The emergencies of both sexes were not synchronized. Similar observations were made by Parker (1931) who reared the related species *Macrocentrus gifuensis* Ashm. In some other cases the emerged Macrocentrus were mixed with Ichneumonidae sp., indicating that superparasitism may occur in parasitized Hedya larvae.

The number of parasitoid adults emerged per cocoon group varied between 8 and 32 with an average of 15. There was a mortality of about 5% established per cocoon group but it seemed to increase (to 20%) in groups containing more cocoons indicating insufficient feeding conditions due to overcrowding.

The life-span of *M. pallipes* at different rearing conditions.

The *M. pallipes* males lived 2–3 days, the females 5 days long in unfed laboratory rearings while in rearings with droplets of 10% honey solution the life-span of males increased to 6 days, that of females to 8 days, although many individuals died because their bodies became entangled into the sticky droplets.

Kept in a thermostat at 15 °C and fed with 10% honey solution or honey-agar, the longevity of males was prolonged to 9–17 days and that of the females to 12–25 days.

At 5 °C and also fed with 10% honey solution or honey-agar the males lived up to 13–20 days, the females to 19–30 days. The honey-agar proved to be more suitable for the rearing purposes than the diluted honey.

The absence of mating seemed to prolong the life of the adults. A similar

observation was made by Osman (1978) on females of the ichneumonid wasp *Pimpla turionellae* L.

The mating took place immediately after the adults emerged from the cocoons. Earlier emerged, non-fed males, however, showed no interest towards the females, fed or drank water from the wet cotton plug, rested on the wall of the rearing flask and then mated. The mating takes place by the male mounting the female, bending its abdominal end under the latter's body and inseminating the female in few seconds. The females allow one copulation but normally decline a second one. Similar observation was reported by Parker (1931) on Macrocentrus gifuensis.

It was observed in the rearing vessels that if the female noticed the presence of host larvae, it began a swinging motion with the ovipositor, bent the latter forward under its body and lunged repeatedly towards the site of the host, even if the host had left its place earlier. This behaviour is explained by the assumption that the smell of the larva and its droppings provide the stimuli for the parasitoid, as supposed by Parker (1931) and Führer and Keja (1976). The female oviposits repeatedly into the same host if they meet again (superparasitism) showing that it does not differentiate the parasitized and unparasitized ones.

The females oviposit into the L_2 instar Hedya and the L_2–L_3 instar Pandemis larvae normally on the lower surface of leaves, where the larvae live. The larvae stung by the ovipositor are greatly disturbed and many jump from their web and fall down to the bottom of the rearing vessel.

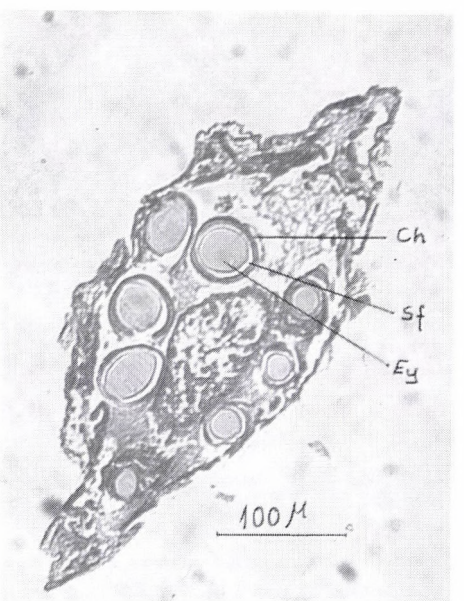

Fig. 1. Transversal section of a L_2 instar larva of Hedya nubiferana Haw. (48 hours after the parasitization). Circular objects are eggs of Macrocentrus. (Photo: Osman)

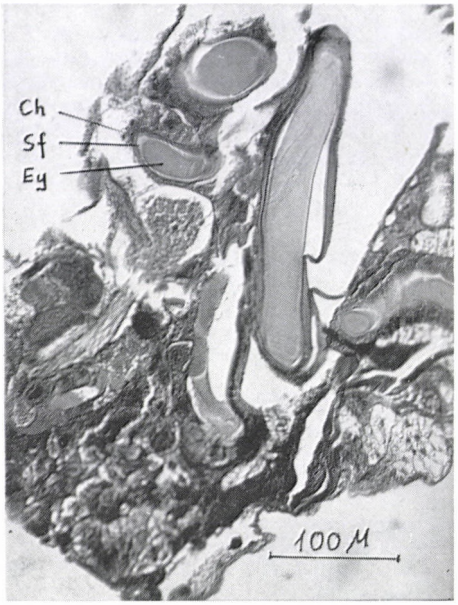

Fig. 2. Longitudinal section of a L_3 instar larva of Pandemis heparana Den. et Schiff. (48 hours after parasitization). (Photo: Osman)

After having been parasitized, the Hedya and Pandemis larvae entered a diapause. Hedya is univoltine but Pandemis has two generations per year, thus the influence of the parasitization on Pandemis is very remarkable. *M. pallipes* retards the development of its host to achieve a synchronization with its own. Vinson and Iwantsch (1980) were of the opinion that this host-regulation by the parasitoid is unique and differs from all other known host-parasite relationships.

The histological study of the parasitized Hedya and Pandemis larvae showed that the eggs of the parasitoid are placed into the body cavity of the host (Figs 1 and 2) and pass the diapause of the host still in egg stage. Fig. 1 shows eight eggs in the haemocoel of a Hedya larva, rendering it dubious that *M. pallipes* developed by polyembryonism. The eggs themselves are elongated with pointed ends and measure 0.4 mm in length, 58 μ in the mid portion and 36 μ at both ends. The chorion (Figs 1 and 2, Ch) is red, measures 5–6 microns, the surface film (Figs 1, 2 Sf) measures 3.5 μ and the egg-yolk (Figs 1, 2 Ey) is yellow.

The results indicated that *Macrocentrus pallipes* may play a significant role in the regulation of tortricid populations however, further studies seem to be necessary to promote its efficiency and to clear the problem of polyembryony.

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First Report of Entomogenous Nematodes of the Families *Steinernematidae* and *Heterorhabditidae* from Hungary

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The incidence of two entomogenous nematode species, *Steinernema feltiae* (Filipjev) and *Heterorhabditis heliothidis* Khan, Brooks & Hirschmann is reported for the first time from Hungary, from localities in the vicinity of Budapest. The number of nematodes of the species *S. feltiae* recovered from samples of experimental orchards of the Experimental Station Érd was found to closely correlate with those of suitable hosts of this nematode. The highest rate of mortality of the experimental hosts (larvae of *Galleria mellonella*) in soil samples from an apple orchard was 100%. Nematodes were present in soil samples in all orchard types of the Experimental Station with the exception of a walnut tree stand. *H. heliothidis* was found in soil samples from an orchard at Szigethalom, in which *Polyphylla fullo*, the host of this nematode, was abundant. Both entomogenous nematode species appear to be highly effective in the control of an outbreak of insect pests and, therefore, show great promise as control agents against insect pests of orchards.

Nematodes of the families Steinernematidae and Heterorhabditidae are obligatory parasites of insects. Their abundance in the field is the highest at the time of outbreaks of host insects, and infect developmental stages living either on or under the soil surface. Many examples of an incidence of these nematodes are known from forestry and agriculture (Poinar, 1976).

Orchards are favourable ecosystems because their ecological conditions favour the outbreaks of insects, mainly of lepidopterous species. The larvae of many of these species pupate either in or on the soil surface where they are frequently attacked by the infective larvae of nematodes. The infected host is killed within two to three days and the nematodes complete their development and reproduction in the dead host. After about two to four weeks, new infective larvae emerge and move to the surrounding soil in search of another host. Thus, nematodes of the families Steinernematidae and Heterorhabditidae play an important role in the natural control of insect pests.

In spite of the fact that steinernematids were found at localities of Czechoslovakia neighbouring Hungary (Mráček, 1980), no data referred to their occurrence in Hungary. Therefore, the area around Budapest was examined between August and September, 1985, in order to disclose the incidence of these entomogenous nematodes in Hungary. The study area selected for this purpose was

an experimental fruit tree plantation, where some orchards were damaged by cockchafer grubs as well as lepidopterous larvae. The control plots were situated in a forest damaged by an outbreak of the moth *Erannis defoliaria* in 1985, in a private orchard, where high infestation of *Polyphylla fullo* was noted in November, 1985.

Materials and Methods

Fruit tree orchards of the experimental plantations of Experimental Station Érd. The soil was composed of calcareous loamy soil overlayed by pseudomiceliar chernozem. The pH of the soil (up to a depth of 60 cm) was between 6.8–7.2, the $CaCO_3$ content of the soil was 5–10%, the humus content 2–2.3%. The orchards were regularly treated with insecticides (mainly organophosphates and pyrethroids) and with fungicides (captan, mancoseb, cupreous fungicides, etc.). Some of the insect pests attacking the trees of the orchards to varying degrees, were suitable hosts for the entomogenous nematodes. For example, apple trees were attacked by *Synanthedon myopaeformis*, *Pandemis spp.*, *Adoxophyes orana*, *Leucoptera scitella* (Lepidoptera), apricot trees by *Grapholitha moleste*, *Enarmonia formosana*, *Anarsia lineatella* (Lepidoptera), walnut trees by *Cydia pomonella* (Lepidoptera), cherry trees by *Rhagoletis cerasi* (Diptera), *Melolontha melolontha* (Coleoptera).

The experimental forest area was situated on the southern slopes of the calcareous montain range Vértes near the village Szár of which some sites were resemblant to a forest steppe. The major tree species were oak and ash. The trees were attacked by larvae of the species *Erannis defoliaria* (Lepidoptera).

The soil of the private orchard at Szigethalom in Csepel island was sandy loam. The species *Polyphylla fullo* (Coleoptera), a suitable host of nematodes, was a frequent insect pest of this orchard.

Using the soil sampling technique suggested by Bedding and Akhurst (1975), the nematodes were isolated from the samples in the laboratory by means of so-called *Galleria*-trap method (Mráček, 1980). The mortality of *Galleria mellonella* larvae was counted on days 4 and 7 p.i. The nematodes recovered from the insect larvae were transferred to a breeding stock belonging to the Department of Insect Pathology, Entomological Institute, České Budějovice. There, they completed their development and started to multiply.

Results

Nematodes of the family Steinernematidae were found in all orchards of the experimental plantation expect the walnut tree orchard in which experimental larvae in the *Galleria*-traps remained uninfected (Table 1). At the forestry experimental site, there was only a single dead larva found in which the reproduction of the nematodes was unsuccessful. Only dead nematodes could be discovered in

Table 1

Mortality of *Galleria mellonella* larvae caused by the nematode *Steinernema feltiae* in soil samples from different orchards (Experimental Station Érd, 1985)

Orchard	Number of *G. mellonella* larvae in *Galleria*-traps	Mortality (%) on days	
		4	7
Apple	10	100	100
Apricot	10	10	10
Cherry	10	70	70
Peach	10	10	20
Pear	10	70	90
Sour cherry	10	60	90
Walnut	10	0	0

its body cavity. In the private orchard we discovered members of another family of entomogenous nematodes namely Heterorhabditidae.

The infection of the experimental *G. mellonella* larvae with the steinernematid nematodes was very frequent. For example, all ten *Galleria* larvae of a soil sample from the apple orchard died on day 4 p.i.. The rate of mortality was similarly high in soil samples from the pear and cherry orchards (Table 1). In a soil sample which was received at a later date, the frequency of infection with heterorhabditid nematodes attained 50%.

The nematode species representing the family of Steinernematidae was determined as *Steinernema feltiae*. The first and the second generation of the species were bisexual in the host. The head was surrounded by two circles bearing 6 papillae each, the spicules' shape were similar to the original description, the body length of infective larvae was 790–880 μm.

The family Heterorhabditidae was represented by the species *Heterorhabditis heliothidis*. The reddish colour of dead *G. mellonella* larvae was typical to the infection caused by this heterorhabditid nematode. Its first generation was parthenogenetic in the host, while the second generation appeared to be bisexual. The excretory pore opens slightly posterior to the nerve ring.

Discussion

The finding of entomogenous nematodes from the families of Steinernematidae and Heterorhabditidae for the first time in Hungary is an interesting piece of data but, nevertheless, it is not surprising, because their presence was expected in the neighbourhood of nematode-infested sites of Czechoslovakia. By contrast to a wealth of information on the distribution of these nematodes in

Western-, Central- and Northern Europe (Poinar, 1979), little is known of their distribution in the southern part of the continent.

The investigation at hand confirmed the concept that the presence of a suitable insect host is a prerequisite for the establishment of entomogenous nematodes. Entomogenous nematode species are abundant at each locality inhabited by high abundance of hosts. The fruit tree orchards (apple, pear, cherry, etc.) were frequently attacked by cockchafer grubs and sometimes by various moth larvae. All these larvae develop, during some of their stage of life either in the soil or on its surface, and these are the sites where they are attacked by the nematodes. Nematode-infected insects are less frequent in apricot and walnut orchards because of the lack of large numbers of suitable hosts. It was stated in earlier papers, for example by Hara & Kaya (1983) that even pesticides applied to trees in standard doses did not affect these nematodes.

For the time being, there is no acceptable explanation available as for the lack of entomogenous nematodes at the forest area, Vértes Mts., specifically at the site of *Erannis defoliaria* outbreak.

The discovery of *H. heliothidis* in a private orchard where larvae of *Polyphylla fullo*, a suitable insect host has been shown to be abundant suggests that entomophagous nematodes are fairly common.

Data obtained so far show that the species of families Steinernematidae and Heterorhabditidae play an important role in the natural protection of an orchard against insect pests. Their preventive use in the form of biological preparations is a method worthy of further field investigations.

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The Swarming Outset of *Macrolepidopterae* Observed by Light-trap as Correlated with the Moon Phases

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The authors worked up a material collected for over a decade at 49 sites of the Hungarian forestry and agricultural light-trap network system. The swarming outset of 31 Macrolepidopteran species was examined as reflected in the light-trap catches in correlation with the moon phases.

It was found that the swarming outset of the species examined was connected to a certain moon phase. With most of the species the outset of swarming was detected most frequently around the last quarter or, with almost the same frequency around the last quarter and new moon.

It was also stated that the normal Jermy-type light-trap detects the outset of the swarming independently from the light conditions changing with the moon phases.

A reliable forecasting of the swarming outset of deleterious insects is extraordinarily important for plant protection practice. The timing of effective protection measures is usually convected with a definite phenophase of the pest, which, from the outset of swarming, in most cases can be calculated with acceptable accuracy.

Phenology phenomena of insects are related to meteorological factors, first of all to temperature. The speed of metabolism of the poikilotherm insects is determined by the temperature of the environment. As a result of observations and experiments, with a number of species a correlation was found between the swarming outset and the heat amount necessary to their development. The time of swarming outset of the insects seems to be dependent, first of all, on the temperature.

However, observations published by some authors indicate that the emergence of certain insects correlate with definite moon phases. Pongrácz (1933) several times observed the mass appearance of *Polymitarcis virgo* Oliv. following directly the fullmoon. According to Csongor and Móczár (1954), the swarming of *Palingenia longicauda* Oliv. onsets when the raising atmospheric pressure and the similarly rising air and water temperature coincide with the changes of moon phases. Hartland-Rowe (1955) observed that the flying activity of some troutflies (Ephemeroptera) is at its height 2–3 days after the fullmoon. According to Fryer (1959) the development rhythm of the African midge, *Chironomus brevibucca* Kief. closely follows the moon cycle. The adults emerge in mass after the

fullmoon. Their lifespan is strikingly short in all cases, some hours or some days at most. Consequently, the outset and culmination of their swarming practically coincides. As for the outset of turnip moth (*Scotia segetum* Schiff.) Nowinszky and Tóth (1982) found that most frequently it emerges during the first quarter and most rarely during fullmoon.

Material and Method

Four our examinations we used the material on *Macrolepidoptera* collected at 49 sites of the Hungarian agricultural and forestry light-trap network. All the traps used were of the Jermy-type with a normal 100 Watt light-bulb. The data collected are predominantly from the years between 1959–1970, but we also had data from the previous years at our disposal supplied by the light-trap network continually developing from 1953 on, while on the other hand, we followed up the catching data of numerous deleterious species up till 1976.

We chose 31 species for our examinations, those flying in mass into light at most observation sites. Thus we wanted to ensure a substantially large sample for the analyses of swarmings. That catching period we considered swarming, in which at one swarming site the number of moths of the same species trapped was over twenty.

Data regarding the moon phases (the lunation, and its respective days) were taken from the astronomy yearbooks of the respective years.

The lunation was approximated to 30 days. Then the days, according to their illumination conditions, were grouped round the four quarters:

fullmoon: 1st 2nd 3rd and 28th 29th 30th days
last quarter: 4th 5th 6th 7th 8th 9th 10th days
newmoon: 11th 12th 13th 14th 15th 16th 17th 18th 19th 20th days
first quarter: 21st 22nd 23rd 24th 25th 26th 27th days

The 30th day occurred only in about 60% of the lunations, as we approximated the 29.53 day mean moonlight period to 30 days. Consequently we calculated the fullmoon phase a 5.6 days period.

We considered the day of trapping the first individual of a generation as the onset of swarming. We determined the number of outsets of each species for the single lunation days. From this we formed five point means then normalized. Following this we calculated for each quarter the one day average of the outsets. By F test we calculated significance level between the daily averages of the outsets referring to the different quarters of the moon.

As the last step, for 10 species taken into account, we compared these present results with those of a previous paper of ours (Nowinszky and Tóth, in press) to determine whether the highest frequency of the outset coincides with the days of the lunation most suitable for light-trap catching.

Results

Table 1 contains the five point normalized moving mean of the outsets of the species examined on the different days of the lunation. The number of swarming considered is also indicated here.

Table 1

Frequency of outsets as the moon phases (normalized values calculated from five-point moving mean)

Lunar day No.	*Eilema complana* L. 60	*Spilosoma lubricipeda* Esp. 166	*Spilosoma menthastri* Esp. 239	*Phragmatobia fuliginosa* L. 245	*Arctia caja* L. 78	*Hyphantria cunea* Drury 210	*Scotia exclamationis* L. 327	*Scotia segetum* Schiff. 240
1	2.57	2.95	2.34	3.44	2.83	3.19	3.12	2.88
2	1.50	3.07	3.72	3.63	3.12	3.62	3.40	2.79
3	2.00	3.49	3.85	3.84	2.82	4.00	4.28	2.75
4	3.00	3.98	4.02	3.67	3.59	3.71	4.83	2.67
5	2.67	3.86	4.69	4.08	4.36	3.81	4.40	2.92
6	4.00	3.73	4.77	4.24	5.13	3.43	4.71	3.33
7	4.33	3.49	4.27	3.84	5.13	3.81	4.59	3.17
8	4.33	3.61	4.44	3.67	5.13	3.81	3.98	3.42
9	4.00	3.86	4.77	3.35	3.85	3.33	3.43	4.00
10	5.33	3.86	3.93	3.18	3.38	2.86	3.67	4.17
11	4.33	3.86	3.93	3.51	2.56	3.14	3.30	3.83
12	4.33	4.10	3.69	3.59	2.05	2.95	3.49	3.67
13	4.33	3.86	3.69	3.51	2.82	3.33	3.43	3.42
14	4.33	3.01	3.35	3.43	3.33	3.62	3.06	3.08
15	3.33	3.37	3.51	3.18	3.07	4.10	3.00	2.83
16	3.33	3.73	3.35	2.78	3.85	4.19	3.06	2.92
17	3.67	3.37	3.01	2.69	3.85	3.81	2.63	3.25
18	3.00	3.25	2.76	2.86	2.56	3.24	2.51	3.42
19	2.67	3.13	3.18	3.35	2.31	3.43	2.63	3.25
20	3.67	3.01	3.18	3.59	3.33	3.43	3.12	3.57
21	3.67	2.29	3.18	3.84	2.05	3.14	3.24	3.50
22	4.00	2.41	3.35	3.76	2.31	3.43	3.91	3.17
23	4.67	2.29	2.85	3.51	3.59	3.62	3.91	3.42
24	4.33	2.65	2.34	3.43	4.10	3.14	3.24	3.75
25	2.67	2.77	2.34	2.78	3.33	2.86	3.12	3.92
26	2.33	3.73	2.68	2.53	3.85	3.24	2.87	3.83
27	2.33	3.86	2.34	2.86	3.85	2.76	2.32	4.25
28	1.83	3.86	2.68	2.90	3.40	2.24	2.11	4.00
29	1.83	3.73	2.59	3.10	3.12	2.76	2.45	3.63
30	2.67	3.49	2.59	3.55	2.83	3.29	2.99	2.79

Table 1 cont'd

Lunar day No.	*Tholera decimalis* Poda 88	*Amathes c-nigrum* L. 314	*Mamestra suasa* Den. et Schiff. 178	*Mam. brassicae* L. 87	*Autographa gamma* L. 118	*Plusia chrysitis* L. 90	*Mythimna pallens* L. 179	*Discestra trifolii* Hufn. 144
1	2.27	2.62	2.47	3.10	1.69	5.43	3.16	2.57
2	3.28	3.68	3.88	4.14	1.36	4.44	3.89	2.57
3	3.41	4.33	4.72	3.91	1.86	4.44	4.36	2.64
4	4.32	4.84	5.84	5.29	2.20	2.89	4.92	3.75
5	5.45	4.78	5.51	4.14	2.71	2.44	4.80	3.75
6	5.00	4.65	6.07	4.41	3.56	2.44	4.80	4.17
7	4.55	4.33	5.73	3.91	3.56	2.44	5.03	3.89
8	4.77	3.69	5.17	4.37	3.39	2.00	4.25	3.33
9	3.86	3.38	4.72	3.91	3.05	2.89	3.58	2.64
10	2.50	3.57	5.06	4.37	2.88	2.89	3.80	2.50
11	2.27	3.38	4.38	5.29	2.71	2.44	3.69	2.92
12	2.05	3.06	4.38	4.60	3.22	2.44	2.91	3.75
13	2.73	2.93	4.72	4.37	3.56	2.44	2.79	4.31
14	2.95	3.57	4.04	4.14	4.58	2.44	2.91	5.00
15	3.86	3.44	3.48	3.68	4.07	3.33	2.35	4.86
16	5.00	3.38	3.26	3.45	4.58	3.78	1.90	5.00
17	4.77	3.82	2.47	3.91	4.24	4.44	1.79	4.03
18	4.09	3.69	2.02	3.91	4.07	4.67	2.12	3.47
19	4.09	3.95	2.02	3.22	3.05	4.67	2.23	2.50
20	4.32	4.20	1.91	3.22	3.73	3.33	3.02	2.64
21	3.18	4.33	2.13	2.07	3.22	3.33	2.91	2.78
22	2.95	4.01	2.58	1.61	3.56	2.44	3.80	2.64
23	2.95	3.57	2.25	1.38	4.24	3.11	4.13	2.78
24	2.95	3.06	2.25	1.38	4.75	3.11	3.91	2.92
25	1.82	2.23	2.36	1.84	4.07	3.33	3.35	3.33
26	2.50	1.91	1.91	2.30	4.41	3.11	3.80	2.78
27	2.73	1.46	1.35	2.30	4.24	4.22	2.91	3.33
28	2.27	1.27	1.24	2.30	3.22	3.70	2.55	3.77
29	2.02	1.34	1.24	2.30	2.63	4.44	2.67	3.47
30	2.27	1.97	1.85	3.10	2.40	5.19	2.92	3.17

Table 1 cont'd

Lunar day No.	*Axylia putris* L. 116	*Caradrina morpheus* L. 90	*Atethis gluteosa* Tr. 170	*Acontia luctuosa* Schiff. 144	*Rivula sericealis* Sc. 163	*Luperina testacea* Schiff. 209	*Unca candidula* Schiff. 165	*Erastria trabealis* Sc. 230
1	3.45	3.92	4.05	3.24	3.27	2.60	3.91	3.57
2	4.79	4.41	4.31	4.17	3.14	2.81	4.04	4.15
4	4.83	4.67	4.24	5.42	3.80	3.35	4.48	4.70
5	5.17	4.44	4.00	6.25	4.05	4.31	4.00	5.22
6	4.48	4.22	4.24	5.97	3.68	4.40	3.39	4.87
7	3.28	3.56	3.88	4.72	3.68	3.64	3.27	4.26
8	3.10	2.89	3.76	4.44	4.05	3.92	2.91	4.09
9	3.28	3.11	3.65	3.47	3.65	4.02	3.15	4.09
10	3.10	2.89	3.53	2.36	3.80	3.16	3.03	3.74
11	2.76	2.89	2.94	2.22	4.66	2.78	3.03	3.57
12	3.45	3.11	3.18	2.64	4.91	3.25	2.91	3.39
13	3.62	2.67	3.29	2.36	4.54	2.87	3.15	3.04
14	4.31	2.67	3.18	2.08	4.54	2.78	4.12	2.61
15	4.14	3.33	3.41	2.78	4.29	2.78	4.61	2.09
16	3.79	3.11	3.53	2.92	3.80	3.06	4.61	2.09
17	3.97	3.11	3.29	4.58	3.80	2.87	4.85	2.78
18	4.66	3.33	2.82	5.14	3.31	3.35	4.12	3.22
19	3.62	3.11	2.59	5.56	3.19	3.25	3.03	3.65
20	3.97	2.89	2.71	5.00	3.07	3.64	3.03	3.91
21	3.79	2.89	3.17	4.86	3.07	3.64	2.91	3.83
22	2.93	2.44	2.94	2.78	2.70	3.64	2.91	3.13
23	2.07	2.44	3.53	2.78	2.70	3.64	3.03	2.70
24	2.07	2.67	3.41	2.50	2.45	3.83	2.67	2.43
25	1.55	2.44	2.59	2.36	1.96	3.54	2.18	2.43
26	2.07	3.33	2.47	2.50	1.84	3.73	2.55	2.52
27	2.07	3.78	2.82	2.92	2.09	4.02	2.42	2.78
28	1.92	4.17	2.48	2.16	2.32	3.64	2.02	2.61
29	2.11	4.17	3.27	2.62	2.59	3.01	2.83	2.90
30	2.30	4.41	4.18	2.93	3.14	2.81	3.50	3.00

Table 1 cont'd

Lunar day No.	*Calothysanis amata* L. 262	*Semiothisa clathrata* L. 223	*Semiothisa alternaria* Hbn. 89	*Xanthorrhoe fluctuata* L. 52	*Tephrina arenacearia* Schiff. 176	*Chlorissa viridata* L. 81	*Ascotis selenaria* Schiff. 195
1	3.98	2.60	2.50	2.96	2.90	3.02	4.33
2	3.73	2.69	3.50	3.40	2.90	2.47	4.33
3	3.74	3.41	4.27	3.08	3.86	2.96	4.92
4	3.66	4.39	3.60	3.08	4.09	2.72	5.03
5	3.59	5.29	4.49	3.08	4.89	2.96	4.82
6	3.28	5.65	4.94	2.69	5.00	2.72	4.31
7	2.98	5.47	4.49	2.31	4.43	2.96	3.90
8	3.59	4.93	3.82	3.46	3.52	2.72	2.87
9	3.28	4.30	4.27	5.38	3.64	3.21	2.87
10	2.90	3.68	4.27	6.15	2.84	2.96	2.87
11	3.21	2.78	3.37	5.77	2.61	3.21	3.08
12	3.59	2.60	2.92	7.69	2.50	2.96	3.28
13	3.13	2.78	3.60	6.15	3.18	3.46	3.49
14	2.98	2.96	3.82	5.38	3.07	2.47	3.90
15	3.13	3.05	3.60	4.23	3.18	2.72	3.79
16	2.98	3.41	3.60	4.23	3.75	3.70	3.69
17	2.75	3.32	3.82	1.92	4.32	3.70	3.59
18	2.98	3.68	3.60	3.08	3.86	3.95	3.38
19	3.21	3.14	2.70	1.54	4.32	3.96	2.56
20	3.28	2.96	2.25	1.92	4.89	3.95	2.67
21	3.51	2.60	2.92	2.31	4.20	3.21	2.77
22	3.82	2.51	2.47	2.31	3.75	3.95	2.36
23	3.66	2.06	2.25	1.54	3.64	3.21	2.36
24	3.44	2.96	2.92	2.31	2.61	3.95	2.56
25	3.28	3.05	3.15	2.69	2.05	3.95	2.26
26	3.36	3.23	2.92	2.31	1.82	4.44	2.26
27	3.44	3.41	3.15	2.69	2.39	4.20	2.97
28	3.15	3.60	2.75	2.96	2.16	4.12	3.30
29	3.73	2.51	2.70	2.54	2.50	4.12	3.42
30	4.14	2.20	2.50	2.12	2.39	3.84	3.99

Table 2

Daily mean of the outset of the examined *Macrolepidopteran* species in the different moon phases

Species	Fullmoon		Last quarter		Newmoon		First quarter	
	outset	n	outset	n	outset	n	outset	n
E. complana	1.43	8	2.14	15	2.20	22	2.14	15
S. lubricipeda Esp.	5.54	31	6.10	43	5.90	59	4.71	33
S. menthastri Esp.	6.25	35	11.43	80	7.70	77	6.71	47
Ph. fuliginosa L.	7.86	44	9.71	68	7.80	78	7.86	55
A. caja L.	2.14	12	3.57	25	2.10	21	2.86	20
H. cunea Drury	6.96	39	8.00	56	6.90	69	6.57	46
S. exclamationis L.	10.00	55	14.14	99	9.70	97	10.86	76
S. segetum Schiff.	6.43	36	8.14	57	8.20	82	9.29	65
Th. decimalis Poda	2.32	13	4.14	29	3.00	30	2.29	16
A. c-nigrum L.	7.45	41	13.57	95	11.40	114	9.14	64
M. suasa Schiff.	3.93	22	10.00	70	6.00	60	3.71	26
M. brassicae L.	2.50	14	3.71	26	3.60	36	1.57	11
A. gamma L.	2.50	14	3.86	27	4.50	45	4.57	32
P. chrysitis L.	4.46	25	2.00	14	3.20	32	2.71	19
M. pallens L.	5.89	33	8.00	56	4.40	44	6.57	46
D. trifolii Hfn.	3.93	22	5.00	35	5.60	56	4.43	31
A. putris L.	3.93	22	4.29	30	4.80	48	2.29	16
C. morpheus Hfn.	3.75	21	3.29	23	2.70	27	2.71	19
A. gluteosa Tr.	6.79	38	6.71	47	5.20	52	4.71	33
A. luctuosa Schiff.	4.46	25	7.14	50	5.40	54	3.57	25
R. sericealis Sc.	5.00	28	6.57	46	6.40	64	3.57	25
L. testacea Schiff.	6.25	35	8.00	56	6.50	65	7.57	53
U. candidula Schiff.	7.14	36	5.57	39	6.00	60	4.29	30
E. trabealis Sc.	7.68	43	10.29	72	7.20	72	6.29	44
C. amata L.	10.36	58	8.86	62	8.00	80	8.86	62
S. clathrata L.	5.71	32	10.86	76	7.20	72	6.14	43
S. alternaria Hbn.	2.50	14	4.00	28	3.20	32	2.14	15
X. fluctuata L.	1.43	8	1.71	12	2.40	24	1.14	8
T. arenacearia Schiff.	4.46	25	7.43	52	6.20	62	5.29	37
Ch. viridata L.	2.68	15	2.57	18	3.00	30	2.57	18
A. selenaria Schiff.	8.04	45	7.43	52	6.70	67	4.23	31

Table 3

Significance levels calculated with F test between the daily means of the outset of sp. *Macrolepidopterae* observed in different moon phases

Species	Fullmoon last quarter	Fullmoon newmoon	Fullmoon first quarter	Last quarter newmoon	Last quarter first quarter	Newmoon first quarter
E. complana L.	75.40	39.10	75.40	53.50	50.00	50.90
S. lubricipeda Esp.	61.90	59.10	67.40	55.20	77.40	75.20
S. menthastri Esp.	97.40	74.10	58.10	95.80	97.40	68.90
Ph. fuliginosa L.	76.90	52.10	50.40	82.40	78.80	51.80
A. caja L.	81.10	53.50	68.30	88.40	68.60	75.10
H. cunea Drury	67.10	52.30	57.60	72.10	75.10	56.40
S. exclamationis L.	91.80	55.90	62.20	96.70	88.40	70.10
S. segetum Schiff.	76.66	78.28	87.22	50.58	69.05	70.22
Th. decimalis Poda	85.46	67.20	51.75	80.32	88.54	70.41
A. c-nigrum L.	98.23	93.70	75.31	81.28	95.24	83.11
M. suasa Schiff.	99.05	85.80	55.91	97.72	99.64	90.66
M. brassicae L.	76.90	75.53	76.68	54.03	92.17	91.84
A. gamma L.	79.24	87.51	87.58	65.62	66.74	52.61
P. chrysitis L.	93.28	80.95	85.92	81.47	70.78	63.76
M. pallens L.	82.27	81.55	62.26	97.81	75.08	90.54
D. trifolii Hfn.	71.58	81.28	60.63	63.28	62.97	75.34
A. putris L.	57.61	68.43	85.65	62.02	89.94	95.07
C. morpheusu Hfn.	61.91	78.66	75.37	68.79	65.89	51.39
A. gluteosa Tr.	51.96	81.32	85.26	81.26	85.24	61.13
A. luctuosa Schiff.	89.36	68.98	70.71	84.03	96.59	86.52
R. sericealis Sc.	77.30	75.74	79.62	54.38	94.48	94.29
L. testacea Schiff.	77.72	53.94	72.05	78.90	57.86	72.07
U. candidula Schiff.	77.31	72.68	91.83	59.08	76.49	83.69
E. trabealis Sc.	84.60	60.16	74.15	93.26	95.80	67.97
C. amata L.	72.61	85.70	72.61	66.76	50.00	66.76
S. clathrata L.	97.98	75.98	57.85	95.89	97.60	70.82
S alternaria Hbn.	81.22	67.31	61.30	72.70	88.90	78.57
X. fluctuata L.	57.97	75.28	61.37	71.51	69.67	84.26
T. arenacearia Schiff.	91.25	81.24	66.51	75.20	85.69	69.21
Ch. viridata L.	53.88	57.40	53.88	62.32	50.00	62.32
A. selenaria Schiff.	60.92	75.22	96.65	65.64	94.97	91.68

Table 4

Comparison of the most frequent outset of the species examined with the days of the lunation most suitable for catching

Species	Days of the lunation most suitable for catching	Most frequent outset of the species
Hyphantria cunea Drury	12th to 20th and 5th to 7th	3rd to 8th and 15th to 17th
Scotia exclamationis L.	10th to 20th	3rd to 7th
Scotia segetum Schiff.	3rd to 10th and 27th to 30th	9th to 12th and 24th to 28th
Amathes c-nigrum L.	9th to 13th and 20th to 23rd	3rd to 7th and 19th to 21st
Mamestra suasa Schiff.	10th to 17th	4th to 10th
Mamestra brassicae L.	16th to 22nd	4th to 14th
Autographa gamma L.	24th to 28th	14th to 18th and 23rd to 27th
Discestra trifolii Hfn.	3rd to 7th	13th to 17th and 4th to 7th
Chiasmia clathrata L.	10th to 22nd	4th to 9th
Tephrina arenacearia Schiff.	8th to 20th	3rd to 9th and 16th to 23rd

The average daily outsets of the different quarters are shown in Table 2; their significance levels calculated with F test in Table 3, respectively.

The most frequent outsets and the days of lunation most suitable for catching are compared in Table 4.

Discussion

The results in Tables 1 and 2 prove that the outsets of the species examined are in relation to a moon phase. In most cases, the frequency of outsets observed at about the last quarter, or the newmoon and the last quarter was nearly identical. About the last and the first quarter the above frequency was strikingly different with most of the species. Despite the high local maximums, the significance levels given here are in a number of cases low. Its cause is that the distribution of the outsets on the days of the lunation does not strictly follow the four moon phase region determined by the similarity of light conditions. In some cases the extraordinarily high frequency lasts 2–3 days all in all.

It is remarkable that at fullmoon and first quarter we found only few species with frequent outsets.

We tried to answer the question: whether the frequency maximum of the outsets coincides with those days of lunation most suitable for light-trap catching, that is whether the light-trap is able to indicate the outset independently from the light conditions connected with the moon phase.

In our previous paper referred to above (Nowinszky and Tóth, in press), in relation to the most important pests, the *Macrolepidopterae*, we pointed out those days of lunation on which, due to the favourable light conditions, catching different species is the most successful. Thus, presumably, on the day of the outset, when the number of the imagos is low, the light-trap detects the presence of a species with the most probability on the days most suitable for catching. In the present and the above mentioned paper the 10 species we dealt with were the same.

As Table 4 shows, the days of lunation most suitable for catching, and those of the most frequent outsets only partly coincide, or do not coincide at all. From this we concluded that the normal Jermy light-trap indicates the outset independently from the light conditions changing with the moon phase. This, of course does not mean that the outset, observed with the light-trap, and the real one necessarily occur on the same day. Light sensitivity of the insects is influenced, beyond the light conditions, by a number of biotic and abiotic factors, so answering this question needs further investigations. The day of the outset observed with light-trap should be compared with results gained by other methods (sex- and lure traps) as well.

On the basis of our examinations we consider the connection of the outsets of different species of moths with a certain moon phase to be proved. But at present we do not know the reason why the outset of certain species is related to different moon phases.

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A Connection between the Illumination of Environment, the Twilight Polarization Phenomena and the Catches of Turnip Moth (*Scotia segetum* Schiff.) by Light-trap

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One of the most important abiotic factors modifying the results of light-trap catches, is the illumination of environment, that consists of illuminations generated by the sun, the moon and the night sky, together with the function of the clouds.

On the basis of the data of fractioned light-trap, the authors—using a particulary elaborated computer method—investigated the influence of the general illumination of the environment, the common and polarized light of the moon upon the results of the catches of turnip moth.

It was concluded that one can observe fundamental differences betwen the same groups of illumination generated by the moon or by twilight with regard of many similarities. When the moon is staying above the horizon, this phenomenon extends the duration of the flying activity. If the intensity of the reflected light from the moon increases, we can observe increasing catches in case of positive polarization, and decreasing one in negative case, respectively. As a new result the authors found that, the individuals of species investigated use the so called Babinet point, on the sky (this point has no polarization) for their spatial orientation. The behaviours can be given mathematically by equations to obtain the expected values of the catches.

From several abiotic factors modifying the results of light-trap catches of insects, the most important is the illumination of environment. The illumination of environment consists of twilight originating from the Sun, the reflected light from the Moon and the usual illumination of night sky. These varies with several magnitudes in a short period of time. The moonlight is also variable in accordance with lunar days (the days counted after fullmoon), its staying time above the horizon, furthermore intensity and polarization are also periodically varied.

It is very important to in vestigate the connection between illumination and the catch in order to increase reliable forecast for plant protecting.

One can find many papers in geophysical and astronomical literature dealing with the general nature of illumination of environment measured on the Earth's surface unit generated by the sun, moon and other celestial bodies. For reference, see for example the papers of Sharonov (1945), Barteneva (1967), Nielsen (1963), Bowden (1973*a* and 1973*b*) and McCarteny (1976). The polarization condition on the sky is also a very important question, the reader is referred to the monographs of Rozenberg (1963) and Megreshvili (1981). It is well known that the light

reflected from the moon is partly polarized. The necessary fundamental information is given by Dollfus (1961).

Several comments are necessary here. All the above mentioned informations are available only in tabular or graphical form without any equations (quantitative treatment) therefore, they are unsuited for data processing by computers. Secondly, some entomological papers also deal with the questions of illumination, for example see the works of Bowden and Church (1973), Nowinszky et al. (1984) and Williams (1936).

The study of the modifying effect of illumination on light-trap catches is available only for a few groups of entomologists because, the wide-spread types of light-traps cannot distinguish the catches for each hour of night. The literature therefore, deals only with the description of catches in a function of lunar phase. The moonlight is only one of the components of illumination of environment, therefore taking into account this light only, one cannot find the general influence. The three main components of illumination are: twilight, moonlight, light of the night sky. We disregard to make an outline of papers which employ the moonlight effect only. Although the published results are contrasted in many cases, according to the most authors (Williams, 1936; Williams and Singh, 1951; Wéber, 1957; Balogh, 1962; Brown et al., 1969; Nemes, 1971; Bowden, 1973*a*; Dzhafarov, 1961; Zlokovity et al., 1958) the intensive moonlight decreases the catch. The opposite view is reported by Day and Reid (1969) and Papp and Vojnits (1976). Chernyshev (1961) investigated the possibility of light-trapping of insects in connection with illumination of environment. He measured simultaneously the illumination too, by this way he determined the optimal regions of daily activity for some species and families. On this basis he divided into four types the behaviours towards light. Bowden and Church (1973) published an analytical regression model for various species of insects trapped in a function of night illumination in Uganda. Heikinheimo (1971) reported on the flying activity of Cydia pomonella L., similarly Skuhravy and Zumr (1981) of *Lymantria monacha* L.

It is well known that the insects receive the polarized light; see the work of Frisch (1949). In the case of moths flying during the night, the authors of this paper verified the role of polarized moonlight in their orientations (Nowinszky et al., 1979). Unfortunately we do not know such papers in literature, which deal with connection between polarization phenomena and light-trap catches.

Materials and Methods

Data for the illumination and polarization of environment

All data which were needed for the illumination and polarization of environment has been calculated on a personal computer of Gothard Observatory. We have separately computed the data for M+ poz. case, when there is an average

polarization of moonlight for M+ neg. case; when the polarization is observable but the plane of polarization has turned, furthermore for the case M−, when the moon does not stay above horizon; resulting data are expressed in units of logarithm of luxes. With the aid of another program, we have calculated the polarization values of moonlight, the polarization measured at zenith and the zenith distance of Babinet point.

Data measured by light-trap

The light-trap data for turnip moth (*Scotia segetum* Schiff.) were collected by Dr. J. Járfás at measuring station of Kecskemét-Katonatelep using fractional technique during the years from 1967 to 1969, to whom we are hereby indebted.

We have processed all data for the first and second generations. The collecting time span was three years, which contains six swarmings, during 190 nights (1900 hours) and 5722 number of individuals were registered. The special light-trap of Dr. Járfás worked with three levels. We have worked with the sum of three collecting levels.

Computing methods to obtain data of illumination and polarization of the moon and the sky

As was mentioned above, all geophysical and astronomical data were available for us only in tabular or graphical form. It was necessary to apply them for computers. We must follow many nonlinear regression and Fourier methods to apply them for computer. In addition, many astronomical calculations were needed, for example fixing the zenith distance of the sun, the moon and the Babinet point, the light intensity and polarization of the bodies, the quantity of illumination of the environment, etc. in the function of trapping time, by programming of the calendar date and trapping time in a computer.

The light-trap worked night, without taking into account the weather but, the meteorological conditions were registered with notices of cloudiness therefore, it must be found a connection between cloudiness and the light absorption of the air at given times. The statistical investigations were successful, resulting an equation, useful to compute the illumination of environment at all weather conditions.

Method of search for connection between the illumination, polarization and the catch

The light-trap working at Kecskemét-Katonatelep consists of three 40 W fluorescent lamps of type F 33 and length of 120 cm, arranged vertically with four horizontal driving plates forming three levels. The collecting vessel is associated with a shifting construction to have a sample in every hour. The locking

time was at 19 h evening and the cut-off was at 5 h morning. The working time was 10 hours daily without taking into account the time of sunset and sunrise. The material caught was processed hourly by levels.

Our following task was to make comparable the catches, collected in different years and in generations. In order to achieve this, we have computed the values of relative catch (RC). The form of RC means a quotient of number of individuals of sampling time and of the average number of individuals to be caught during that time. The catching data of light-traps cannot be compared immediately with one another. It has been shown by Williams (1940) that the favourable and unfavourable environmental influences deform the results of trapping. He removed these influences by geometrical means. In data processing of the Hungarian light-trap network, the so called "museum method" was applied by Benedek and Jászainé (1963), which was suitable according to their opinion. That is true, neglecting the number of individuals to be caught, according to several authors (Wéber, 1959) yields some errors. Járfás (1979) calculated RCs from the number of individuals caught, giving better results compared with setting out from original data.

We have divided into several classes the log lux values of the illumination of environment, of the intensity of moonlight and of the intensity of polarized moonlight separately by M+ poz. and M+ neg. calculated for every hour. Regarding the values of illumination of environment, we divited them to two cases: the classes before and after midnight respectively. After this, we have formed the quantities and means inside each class. With aid of Fourier method, we the expected values of RCs computed for each class.

In order to investigate the modifying effect of moonlight we have computed the values of mean relative catches with ± 1 hour time delay for moonset and moonrise. The differences have been examined by F test.

Similarly, we have divided into several classes the values of zenith polarization expressed in log lux units separately, if the cases of M+ poz., M+ neg. or M− occurred, respectively. Because, the class of M+ neg. contains few data, it is neglected in further treatments. We have formed once again the sums and the means inside each class.

By way of following step, we have searched for a connection between the positions of Babinet point above horizon and the catches.

We have separated the cases of evening and morning twilight hours into M+ poz., M+ neg. and M− classes. As before, we have calculated average of the values of zenith distances and of RCs. After this, we wanted to see, the data of the evening and morning catches in following two cases: 1. The moon is above or below the horizon, 2. M+ poz., M+ neg. and M−. Accordingly, we formed the average of each class relating to illumination and RCs, respectively at log lux values of illumination of -3, -2 and -1, where the Babinet point could take place above and below the horizon. At the end, we have the F test applied for positions of the Babinet point above and below horizont, respectively.

Results

Programs to calculate the behaviours of illumination and polarization for entomological and plant protecting use

The programs referred to in this paperare suitable to calculate of the general illumination of environment, inside this to calculate of moonlight intensity its polarization, to calculate of the position of Babinet point, etc., all these in a function of calendar date and trapping time. The programs can be easly adaptable to use in entomology and plant protecting.

Connection between illumination or polarization and the catch by light-trap

The data for moonlight are given in Tables 1.1 to 1.6 in cases of M+ poz., M+ neg. and M−; the illumination belonging to various classes, the expected catches, the equations and the Fourier coefficients.

The relative catches can be seen in Fig. 1 as a function of illumination for cases M+ poz., M+ neg. and M−. As we have mentioned above, the case of M+ neg. has been neglected because, this case contains few data.

Table 2 contains the RC values belonging to various values of M+ poz., M+ neg. and general moonlight, furthermore the levels of significance. These data have been partly visualized in Fig. 2. In Table 3, one can see the values of RCs at time of moonset and moonrise.

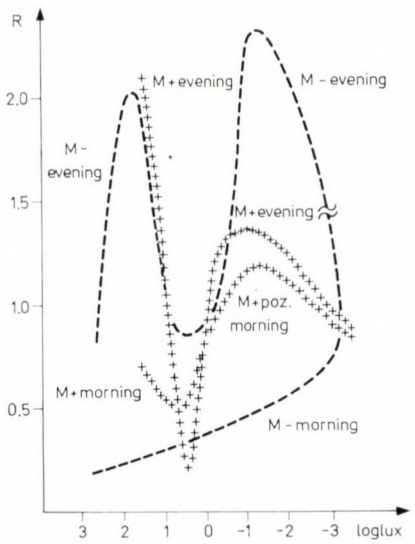

Fig. 1. The relative catch of turnip moth (*Scotia segetum* Schiff.) in a function of illumination of environment

Table 1

The results of the catches of turnip moth (Scotia segetum Schiff.) and its Fourier-analysis at various illuminations

1.1. Case M — at evening hours

log lux	RC observed	Data number	Level of significance
2.5773	0.84	69	
1.7010	2.02	32	
0.6980	0.86	17	
−0.3582	1.13	30	94.4%
−1.3648	2.27	45	
−2.5565	1.77	117	
−3.2103	0.82	450	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
2.5	0.94	1	41.4	
2	1.60	2	70.5	
1.5	1.80	3	79.3	
1	1.19	4	52.4	
0.5	0.91	5	40.1	
0	1.03	6	45.4	
−0.5	1.23	7	54.2	98.88%
−1	1.83	8	80.6	
−1.5	2.19	9	96.5	
−2	1.99	10	87.7	
−2.5	1.80	11	79.3	
−3	1.10	12	48.5	
−3.5	0.40	0	17.6	
Fourier coefficients				
a_0	1.385384615			
a_1	−0.1306504656			
a_2	−0.5423444966			
a_3	−0.1756239064			
a_4	−0.0740911460			
a_5	−0.0340327232			
a_6	−0.0286418777			
b_1	−0.3108491366			98.88%
b_2	0.2008013559			
b_3	−0.0698599974			
b_4	−0.0825572853			
b_5	0.0639898458			
b_6	0.0138417724			
error	0.0922 (calculated for catch)			

Transform. equations: $x = (2\pi/13) \cdot n$

$x = -0.9666438934 \cdot \log \text{lux} + 2.89993168$

$RC = a_0 + \sum_{i=1}^{6} a_i \cdot \cos x + \sum_{i=1}^{6} b_i \cdot \sin x$

1.2. Case M— at morning hours

log lux	RC observed	Data number	Level of significance
−3.2103	0.82	450	
−2.5286	0.52	120	
−1.4802	0.52	42	
−0.4628	0.38	24	
0.5765	0.44	29	92.6%
1.6223	0.21	60	
2.7009	0.29	72	
3.2784	0.15	65	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
−3	0.73	1	89.0	
−2.5	0.52	2	63.4	
−2	0.52	3	62.8	
−1.5	0.51	4	62.2	
−1	0.45	5	54.9	
−0.5	0.39	6	47.6	
0	0.91	7	90.1	99.86%
0.5	0.43	8	52.4	
1	0.35	9	42.7	
1.5	0.23	10	28.0	
2	0.22	11	27.4	
2.5	0.27	12	32.9	
3	0.21	0	25.6	

Fourier coefficients

a_0	0.4415384615
a_1	−0.0949861439
a_2	0.0621505108
a_3	−0.0567185274
a_4	−0.0084405752
a_5	−0.0834304152
a_6	−0.0501133107
b_1	0.1196762823
b_2	0.1289707048
b_3	−0.0218392675
b_4	0.1052367539
b_5	−0.0441624168
b_6	0.0869558997
error	0.0294 (calculated for catch)

Transform. equations: $x = (2\pi/13) \cdot n$

$x = 0.9666438934 \cdot \log \text{ lux} + 3.383253627$

$$RC = a_0 + \sum_{i=1}^{6} a_i \cdot \cos x + \sum_{i=1}^{6} b_i \cdot \sin x$$

1.3. Case M + poz. at evening hours

log lux	RC observed	Data number	Level of significance
1.4277	2.03	15	
0.5099	0.22	19	
−0.4745	1.30	19	
−1.5182	1.34	69	87.2%
−2.2714	1.15	113	
−3.1653	0.92	77	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
1.5	2.12	1	95.7	
1	1.18	2	58.1	
0.5	0.25	3	12.3	
0	0.76	4	37.4	
−0.5	1.30	5	64.0	
−1	1.32	6	64.8	99.82%
−1.5	1.34	7	65.8	
−2	1.22	8	60.1	
−2.5	1.08	9	53.0	
−3	0.95	10	46.8	
−3.5	0.83	0	40.9	

Fourier coefficients		
a_0	1.1213636360	
a_1	0.0471270953	
a_2	0.2036157938	
a_3	−0.2018068742	
a_4	−0.2160958620	
a_5	−0.1242037893	99.82%
b_1	−0.1219723048	
b_2	0.3625746657	
b_3	0.3070268164	
b_4	0.0195495304	
b_5	0.0087225080	
error	0.0813 (calculated for catch)	

Transform. equations: $x = (2\pi/11) \cdot n$

$x = -1.142397329 \cdot \log \text{ lux} + 2.284794657$

$$RC = a_0 + \sum_{i=1}^{5} a_i \cdot \cos x + \sum_{i=1}^{5} b_i \cdot \sin x$$

1.4. Case M + poz. at morning hours

log lux	RC observed	Data number	Level of significance
−3.1653	0.92	77	
−2.3736	1.02	97	
−1.5050	1.19	89	
−0.4619	1.08	21	83.3%
0.5665	0.49	20	
1.2382	0.64	6	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
−3.5	0.87	0	73.1	
−3	0.91	10	76.5	
−2.5	0.99	9	83.2	
−2	1.08	8	90.6	
−1.5	1.19	7	99.6	
−1	1.13	6	94.6	99.68%
−0.5	1.08	5	90.3	
0	0.82	4	68.9	
0.5	0.52	3	43 7	
1	0.58	2	48.2	
1.5	0.70	1	58.4	
Fourier coefficients				
a_0	0.8954545455			
a_1	−0.1429172917			
a_2	0.0993305597			
a_3	0.0086159303			
a_4	−0.0119203374			
a_5	0.0214365936			
b_1	−0.2435234448			99.68%
b_2	0.0071869729			
b_3	0.0317456715			
b_4	−0.0195039530			
b_5	0.0032998504			
error	0.0705 (calculated for catch)			

Transform. equations: $x = (2\pi/11) \cdot n$

$x = -1.142387329 \cdot \log \text{ lux} + 2.284794657$

$RC = a_0 + \sum_{i=1}^{5} a_i \cdot \cos x + \sum_{i=1}^{5} b_i \cdot \sin x$

1.5. Case M + neg. at evening hours

log lux	RC observed	Data number	Level of significance
0.037	0	1	
−0.784	4.6	5	
−1.5406	1.1	59	69.9 %
−2.2635	0.92	32	
−3.1240	0.64	3	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
0	0	1	0	
−0.5	2.81	2	61.10	
−1	3.61	3	78.50	
−1.5	1.18	4	25.70	90.1 %
−2	0.96	5	20.90	
−2.5	0.83	6	18.00	
−3	0.67	7	14.6	

Fourier coefficients

a_0	1.437142857
a_1	−1.133442361
a_2	0.021477469
a_3	0.344822034
b_1	0.631152695
b_2	−1.003350168
b_3	0.160732309
error	0.082 (calculated for catch)

Transform. equations: $x = (2\pi/7) \cdot n$

$x = -1.795195802 \cdot \log \text{ lux} + 0.89759701$

$$RC = a_0 + \sum_{i=1}^{3} a_i \cdot \cos x + \sum_{i=1}^{3} b_i \cdot \sin x$$

1.6. Case M + neg. at morning hours

log lux	RC observed	Data number	Level of significance
−3.1240	0.64	3	
−2.3393	1.36	29	
−1.5010	0.71	46	93.7%
−0.8410	0.22	4	
0.1734	1.15	4	

log lux	RC calculated	Data number	Expected catch, %	Level of significance
−3	0.73	7	53.7	
−2.5	1.20	6	88.2	
−2	1.11	5	81.6	
−1.5	0.73	4	53.7	98.1%
−1	0.35	3	25.7	
−0.5	0.52	2	38.2	
0	1.03	1	75.7	
Fourier coefficients				
a_0	0.81			
a_1	0.2241790738			
a_2	−0.1604091290			
a_3	−0.1437699449			98.1%
b_1	−0.2494270403			
b_2	0.1106713212			
b_3	0.0048707807			
error	0.1002 (calculated for catch)			

Transform equations: $x = (2\pi/7) \cdot n$

$x = -1.795195802 \cdot \log \text{ lux} + 0.897597901$

$$RC = a_0 + \sum_{i=1}^{3} a_i \cdot \cos x + \sum_{i=1}^{3} b_i \cdot \sin x$$

Table 2

The relative catch of turnip moth (Scotia segetum Schiff.) in a function of illumination of general moonlight

Case M + poz.

log lux	n	RC	Relation in significance
−5.2133	18	0.633	4b
−4.2424	203	1.017	5b
−3.4263	270	1.102	6a
−3.0373	101	0.936	1b
−2.8519	54	1.465	—
−2.4292	294	1.028	2b
−1.5933	150	1.318	3a

Case M + neg.

log lux	n	RC	Relation in significance
−5.2647	34	1.470	4a
−4.3203	79	1.040	5a
−3.6650	70	0.880	6b
−3.0257	8	2.910	1a
−2.3370	73	1.118	2a
−1.4780	102	0.872	3b

The results for significance in cases of M+ poz. and M + neg.

Relation	F	Level of significance, %	Note
1a−1b	3.1090	99.22	
2a−2b	1.0875	68.25	no significance
3a−3b	1.5115	98.62	
4a−4b	2.3223	96.16	
5a−5b	1.0226	55.81	no significance
6a−6b	1.2523	86.54	poor significance

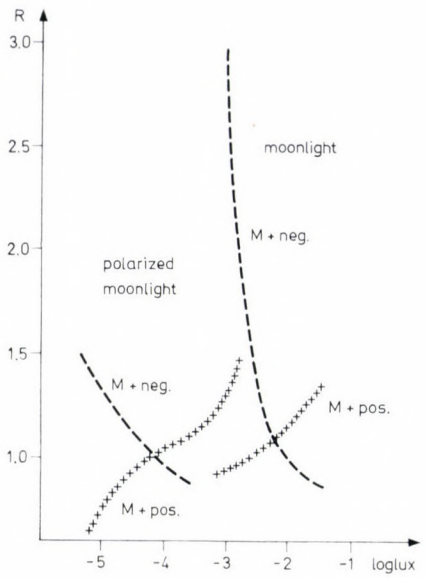

Fig. 2. The relative catch of turnip moth (*Scotia segetum* Schiff.) in a function of moonlight

Table 4 contains the behaviours of the zenith polarization of sky and the RC values. At end, the Table 5 gives the RC in a function of zenith distance of Babinet point.

In Table 6 can be seen, how the relative catch is forming if the Babinet point is above or below the horizon.

Table 3

The relative catch of turnip moth (Scotia segetum Schiff.) at time and ± 1 hour of moonrise moonset

Moonrise

Data number, n	At moonrise, RC	In preceeding, hour	F-test, %
126	1.553	1.391	70.61

Moonset

Data number	At moonset	In preceeding hour	F-test
156	0.555	0.819	99.20

Table 4

The relative catch of turnip moth (Scotia segetum Schiff.) in a function of twilight zenithpolarization, calculated for illumination of the environment. (In case M +, the illumination generated by the Moon is extracted)

Case M−			Case M+		
E (log lux)	RC	n	E (log lux)	RC	n
2.4875	0.85	63	1.0939	1.15	4
1.6523	2.13	44	0.5188	0.28	16
0.6619	0.79	18	−0.3519	1.42	18
−0.5032	1.23	30	−1.3594	1.75	25
−1.4300	2.06	34	−2.4620	1.00	27
−2.4226	1.94	46	−3.3772	0.50	37
−3.3172	1.60	52	−4.3546	1.08	14

Table 5

The relative catch of turnip moth (Scotia segetum Schiff.) in a function of cosine of zenith distance

At evening hours								
M−			M+ poz.			M+ neg.		
$\cos_{BP}$	n	RC	$\cos_{BP}$	n	RC	$\cos_{BP}$	n	RC
0.357	118	1.871	0.361	82	1.405	0.358	23	1.555
0.264	156	1.346	0.261	36	0.799	0.241	8	0.578
0.151	18	1.317	0.139	6	1.073	0.166	4	0.750
0.052	13	1.359	0.053	11	0.398	0.039	4	0.073

At morning hours								
M−			M+ poz.			M+ neg.		
$\cos_{BP}$	n	RC	$\cos_{BP}$	n	RC	$\cos_{BP}$	n	RC
0.349	191	0.326	0.360	73	0.950	0.367	16	0.970
0.260	174	0.331	0.263	44	0.623	0.245	15	0.941
0.145	12	0.104	0.157	5	1.422	0.171	4	0.695
0.057	14	0.401	0.062	5	0.702	—	—	—

Discussion

Reviewing the behaviours in a function of illumination of environment and the forming of relative catch, the cases M+ poz. and M− seem to be of similar character but, with some differences (Fig. 1).

Both curves show local maxima at 50 and 27 luxes, respectively. Higher values do not occur but, in the case of no moonlight, at illumination of 378

Table 6

The relative catch of turnip moth (Scotia segetum Schiff.) in a function of the position of Babinet point

Polarization of moonlight and time	Class of illumination, log lux	BP+		BP−		F	Level of significance, %
		n	RC	n	RC	BP+ − BP−	
M − evening	−3	13	0.666	152	0.993	1.4910	76.50
	−2	90	1.800	21	2.150	1.1944	71.91
M − morning	−3	9	0.268	94	0.535	1.9963	83.16
	−2	91	0.424	17	0.982	2.3160	99.22
M + poz. evening	−3	5	1.121	29	0.418	2.6818	93.33
	−2	50	0.685	27	1.787	2.6088	99.79
	−1	40	1.330	14	1.420	1.0677	58.73
M + poz. morning	−3	3	0.170	13	1.829	10.7590	76.62
	−2	29	1.043	36	0.910	1.1462	65.02
	−1	51	0.829	20	1.465	1.7672	94.14
M + neg. evening	−2	17	0.520	15	1.370	2.6346	96.21
	−1	10	0.180	49	1.080	1.0926	61.49
M + neg. morning	−2	13	1.500	16	1.260	1.1905	62.70
	−1	14	0.480	32	0.810	1.6875	83.08

luxes the catch also can be observed. From the data available, we could not determine the favourable value to which the beginning of the daily flying activity belongs. The data of catches associated with the interval, do not give accurately at what time they were caught. On the other hand, the illumination quite can be varied like several magnitudes during this interval, especially during twilight. Therefore we cannot draw once conclusion from the average value of first interval (i.e. 19 h 30 min) to that illumination suitable to begin the daily activity. The catch, as can be shown on both curves increases up to 50 and 27 luxes. Subsequently, the catch increases once again up to a maxima at 0.04 and 0.03 luxes. Therefore we have two maxima before midnight, which cannot belong to the same modifying effect.

Table 2 and Fig. 2 verify that, the increasing intensity of moonlight and polarization increase the catch. The authors showed in their early papers (Nowinszky et al., 1979; Nowinszky and Tóth, 1983) that, the moonlight intensity and its polarization increase the response to light and the activity. (Under term "response to light" we refer to any capability by which insect orients itself in space.) If we compare the data in Tables 1 and 4, it is concluded that similar values are belonging to illumination and zenith polarization. It goes without saying because, most of the light is polarized during twilight. Therefore the values of illumination and zenith polarization have similar magnitudes. It must be suggested that, the insects have similar response to polarized light at twilight as in case as polarized moonlight. We think that the first maximum of catch occurs at

those conditions when zenith polarization is high enough for flying activity and the illumination is too low for response to light. There is no difference in cases of M+ poz. and M− at first maxima. This latter can be interpreted by the fact that, the only 1% of illumination originates from the moon in this region, therefore it is neglected.

The values of second maxima in catches (at 0.04 and 0.03 luxes) are very near to the illumination observed at fullmoon. We published in a previous paper (Nowinszky and Tóth, 1983) that, the species investigated has high response to light at fullmoon. Therefore it is suggested that, the insect orients itself first of all by stimuli of light, and this is the optimal illumination for it in this region, whether the moon is staying above horizon or not. The second maximum is created by the maximum response to light. This value is higher in case of M− compared with case M+ poz. Referring to cited papers of the authors, we can calculate the catch area for each lunar day from known values of lunar brightness (in a function of lunar phase) and from optical characteristics of the light-trap, by help of theory of probability. Under the term of "catch area" we mean an area of a circle, at the centre of which the light-trap is located and within circumference of it the insect measures as higher the artificial light than the natural one. When the insect is staying inside the catch area, and the orientation by light stimuli is supposed, the insect will fly into the trap with great probability, if the artificial light creates higher level of illumination than the moon. In case of moonless time, the insect can find no other source of light outside the catch area, therefore it will be killed by light-trap from great distance, too.

There appear two minima in catch at 5 and 3 luxes. The probable causes of these are, that the zenithpolarization is lower and the illumination is higher than the optimal value, suitable to active flying.

After the second maxima, a decreasing trend can be seen on both curves, down to $6\text{–}7 \cdot 10^{-4}$ luxes of illumination. This illumination belongs to days in neighbourhood of newmoon. Significant differences occur on both curves: the catch shows strong decrease in case of M−, while this trend is moderate at M+ poz. The same phenomenon can be observed early in the morning, when the illumination increases, the catch in case of M− decreases linearly in large measure up to the morning twilight. Let us now look at case of M+ poz. In this case the catch is up to 0.3 luxes lower than in first half night, measured at the same illumination. Regarding the differences seen in both curves, we refer to Table 3, and to the paper of Tóth, Nowinszky and Járfás (1983), these give some explanations. As you can see in Table 3, at time of moonset the catch is significantly lower than at the hour preceding it. On the contrary, the catch is higher at time of moonrise, than one hour before it. The latter result has poor significance too, because the decreasing trend of the distribution of catches varies during one night. In the above cited paper (Tóth et al., 1983) we showed that the catches of turnip moth (*Scotia segetum* Schiff.) linearly decrease towards early mornings hours. When the moon is staying above horizon, the catch remains smoothed and decreases only towards early morning. It can be established from data of

Table 2 and Fig. 2 that, the increase of M+ neg. develops a decreasing catch. Therefore the moon produces an opposite effect in catches, at the same moonlight, according to its positive or negative polarization. We cannot explain this fact on ground of our present knowledge. The circumstances are the same: the intensity of light, its polarization, the catching area, only the sign of polarization has varied. The negative polarization of the moonlight occurs only in the neighbourhood of fullmoon, when the moon is staying above horizon during whole night, therefore the trapping time also lengthens (see Table 1.3). It is supposed that, the insect is capable to distinguish between the positive and negative planes of natural polarization of light, differentiating the natural and artificial ones, avoiding the trap. In order to clear this question, further investigations are needed but, the difficulty is, that the observations extend only to 2–3 days per lunar month.

We have not been successful in our search for a connection between the zenith distance of Babinet point and the catch (see Table 5). When the position of Babinet point i.e. its staying or not staying above horizon has been compared with catch, significant differences have been found (see Table 6). At evening and at morning twilights: in both cases of M+ and M−, significant differences can be observed in catches, when the Babinet point is down the horizon, the catch increases. This fact perhaps outlines the hypothese that, the individuals of this species use the Babinet point as information, in absence of others. The Babinet point, as known, is a polarizationless "spot" on the continuously polarized sky. Using analogue phenomenon in astronomy, the "black holes" on the sky are the dark areas from which no information can be seen for man, of course. May be insects sees the Babinet point as a "black spot"?

In the latter case the safety of orientation increases because, the insects do not mistake for artificial light source. If the Babinet point is staying above horizon, independently from its zenith distance, perhaps the moth removes out of area of light-trap.

In Tables from 1.1 to 1.3 the equations are given by which the expected RCs can be computed for M−, M+ poz. and M+ neg. cases in a function of various illumination values. The expected value for catch cannot be determined using a single environmental factor. We have outlined the ratios of expected values for catches only, depending on the behaviours of illumination.

We can recognize therefore the probability and limits of the catch originating from various behaviours from one night to the other night, causing modifications in number of trapped insects.

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Peculiarities in the Distribution of Scale-Insects *(Homoptera: Coccoidea)* on Deciduous Fruit Trees in Hungary

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The distribution maps of eleven scale - insect species are given *(Quadraspidiotus perniciosus, Q. ostreaeformis, Q. pyri, Epidiaspis leperii, Lepidosaphes ulmi, Pseudaulacaspis pentagona, Palaeolecanium bituberculatum, Parthenolecanium corni, Sphaerolecanium prunastri, Eulecanium mali, Phenacoccus aceris)* for apple, pear, peach, plum, cherry and sour cherry cultures. Q. perniciosus is distributed in the whole country on all fruit trees mentioned here. *E. leperii* is frequent on pear and plum in the whole country but on the north-western part on apple, as well. *Q. ostreaeformis* is frequent in the Great Hungarian Plain. *L. ulmi* and *P. corni* is distributed in the northern, but *P. pentagona* was known from the southern part of the country, only.

There is new trend in zoogeography in Europe to use grid-mapping systems (Heath, 1971; Scarlato, 1978). These systems will place biogeography on a reliable basis and by help of these techniques data processing could be computerized. For this reason there is a necessity for mapping those old data which seem to be correct, comparable and secured by standard methods. In Hungary the Heaths grid-map was used by Pintér, Richnovszky and Szigethy (1979) for Gastropoda. The standardization of this system for Hungary now is in process by Dévai and his colleagues at the Kossuth Lajos University (Debrecen) (Anonym, 1986). And a simplified grid-map, for forecasting purposes (Anonym, 1975), by the Center for Plant Protection and Agrochemistry (Ministry of Agriculture) was developed, which is widely used in agricultural practice in Hungary. For example, the distribution maps of leafminer moths for Hungary were prepared also by this method (Seprős, 1985).

The main aim for the publication of our data is promoted by the fact that no detailed maps about scale-insects are known in the literature. Data presented here were collected by standard methods and at the same time (Kozár, 1975, 1976), they are comparable with data of other similarly studied groups of insects.

In the distribution of scale-insects in Hungary some significant changes have taken place in the last decade (Kozár and Viktorin, 1978; Kozár and N. Dávid, 1985). For the analysis and comparison of these and future changes, it seems necessary to publish our details.

Materials and Methods

For the survey of the distribution of scale-insects a standardized sample method was elaborated in Hungary. Altogether 1327 samples were taken in 1971. Figure 1 shows the sampling places. The collecting and the analysis of samples were carried out by using a special method already described in detail (Kozár, 1976).

For the data processing we used the Hungarian plant protection grid-map (Anonym, 1975). It will make our data comparable with other data of plant protection in Hungary. However, it is necessary to list the villages' names, because in one quadrate there are often several of them, and in this grid-map (Anonym, 1975) there is no grid-code-village index:

List of sampling places with their grid-code

Abádszalók 55–22, Adásztevel 18–25, Agostyán 28–19, Aklipuszta 22–26, Aparhant 29–43, Aszaló 59–9, Ács 24–18, Ácsteszér 24–23, Babócsa 16–48, Baja 35–46, Balatonhenye 19–32, Balástya 48–41, Bakonyszentlászló 21–24, Barabás 77–8, Baracs 34–33, Bánfa 10–41, Bátaszék 32–46, Beled 13–22, Bercel, 40–15, Beret 60–6, Berettyóújfalu 66–26, Besenyőtelek 51–18, Bezenye 14–13, Békés 61–35, Békéscsaba 61–36, Bicske MgTSz 31–22, Biharkeresztes 68–28, Bikal 27–43, Boconád 50–19, Bodrogszegi 64–9, Boly 30–49, Bonyhád 30–44, Borota 38–44, Borsodszentgyörgy 50–9, Budakalász 36–20, Budapest II. 35–21, 35–22, 36–21, 36–22, Budapest IV. 36–30, 36–21, 37–20, 37–21, Budapest X. 37–22, 38–22, Budapest XI. 35–22, 35–23, 26–22, 36–23, Budapest XIII. 36–21, 36–22, 37–21, Budapest XV. 37–20, 37–21, Budapest XVI. 37–21, 37–22, 38–21, 38–22, Budapest XVII. 38–22, 38–23, 39–22, 39–23, Budapest XVIII. 37–22, 37–23, 38–22, 38–23, Budapest XX. 36–23, 37–23, 37–24, 38–23, 38–24, Bugyi, 37–27, Cegléd 45–28, Celldömölk 13–26, Csanádpalota 56–44, Csapog 11–21, Csaroda 77–9, Csákvár 29–24, Császár 25–22, Csengele 46–39, Csengőd 39–36, Csepreg 8–23, Cserkeszöllő 50–33, Cserszegtomaj 14–34, Csertő 21–47, Csetény 23–25, Csobánka 35–19, Csömör 38–21, Dány 42–21, Debrecen 67–20, 67–21, 68–21, Demecser 71–10, Demjén 52–16, Devecser 17–29, Domaszék 48–44, Dunabogdány 36–17, Dunakeszi 37–19, Ecseg 43–15, Ecséd 45–18, Edelény 56–7, Eger 52–15, Egyed 16–21, Elek 63–39, Emőd 57–14, Esztergom 32–17, Érd 35–24, Farád 14–20, Fegyvernek 54–26, Felsődobsza 60–8, Gagybátor 59–5, Gógánfa 14–30, Göd 37–18, Gödöllő 40–20, Gutorfölde 8–37, Gyálarét 49–45, Gyömrő 40–23, Gyöngyös 47–17, Győrszemere 18–21, Gyulavári 63–37, Hahót 11–37, Hajdúböszörmény 66–18, Hajdúhadház 68–18, Hajdúnánás 65–15, Hajdúszoboszló 64–22, Hajdúsámson 69–20, Halmaj 60–8, Harkány 26–51, Hejő menti Á. G. 58–15, Hetvehely 24–46, Heves 51–20, Homrogd 59–8, Horváthzsidány 7–23, Hosszúhetény 28–46, Hosszúpályi 68–23, Hódmezővásárhely 51–41, Igrici 58–15, Iregszemcse 26–36, Jánosháza 14–28, Jászapáti 49–21, Jászberény 47–22, Józsefpuszta 30–17, Kaba 63–24, Kajárpéc 19–22, Kalocsa 35–40, Kaposvár

21–42, Karcag 59–25, Kecel 38–40, Kecskemét 44–33, Kelebia 43–45, Kemecse 69–11, Kemenesszentmárton 13–25, Kerekegyháza 41–32, Keszthely 15–35, Kisbucsa 11–34, Kiskőrös 39–38, Kisoroszi 36–16, Kistarcsa 39–21, Kisterenye 46–13, Kisújszállás 57–27, Kisvejke 28–42, Kiszombor 53–45, Kokad 71–23, Kondoros 57–35, Kosd 38–16, Kunadacs 39–32, Kup 17–26, Lajtapuszta 14–13, Lengyeltóti 19–37, Léh 59–7, Lovas 23–31, Madocsa 35–36, Maglód 40–23, Makó 53–45, Martonyi 57–4, Mágocs 26–43, Mátészalka 75–13, Mátramindszent 47–13, Mátranovák 47–12, Mende 41–23, Mezőberény 60–34, Mezőtúr 55–31, Mindszent 50–39, Mocsa 26–19, Mogyoród 38–20, Mohács 32–49, Monor 41–25, Monoszló 19–32, Mosonszolnok 14–15, Mozsgó 22–47, Mór 26–24, Mórahalom 46–45, Mucsfa 28–43, Nagyatád 16–45, Nagybajcs 20–17, Nagydobos 75–11, Nagydorog 31–38, Nagykőrös 45–30, Nagylak 56–45, Nagylók 31–31, Nagyszentjános 22-18, Nagyszénás 56-37, Naszály 27–18, Nemesdéd 14–41, Nézsa 39–16, Nova 8–36, Nógrád 36–14, Nógrádkövesd 40–15, Nógrádsáp 40–16, Nyergesújfalu 30–17, Nyírbátor 73–15, Nyírbogát 72–16, Nyírgyulaj 73–14, Nyőgér 11–27, Orosháza 56–39, Oszkó 10–30, Ózd 51–9, Öcsöd 52–32, Őrbottyán 39–19, Őrhalom 40–12, Pacsa 12–35, Pákozd 30–27, Pásztó 44–14, Páty 33–22, Perőcsény 34–13, Petneháza 73–11, Pécsvárad 28–46, Pördefölde 8–38, Püspökladány 61–25, Rácalmás 35–30, Rád 38–17, Rétság 37–14, Ruzsa 44–44, Sajópálfalva 58–10, Salföld 18–34, Sándorfalva 49–42, Sárbogárd 31–33, Sárospatak 66–7, Sárvár 11–26, Siklós 27–51, Simaság 10–23, Siófok 24–32, Soltvadkert 40–39, Sopron 7–18, Sopronkövesd 8–20, Szabadszállás 38–33, Szajol 51–28, Szarvas 54–33, Szarvaskő 51–13, Szatymaz 48–43, Szederkény 29–49, Szekszárd 32–43, Szendehely 37–15, Szendrő Á. G. 56–5, Szentendre 36–19, Szentes 51–37, Szentistván 55–17, Szerencs 62–10, Szécsény 42–12, Székesfehérvár 29–27, Szigetvár 21–48, Szob 34–16, Szőny 25–18, Tahitótfalu 37–17, Taksony 36–25, Tamási 27–37, Tarcal 64–10, Tarnaméra 49–19, Tata 27–19, Tatabánya 29–21, Tákos 77–9, Tereske 38–14, Tés 24–26, Tét 18–21, Tiszabecs 81–10, Tiszaigar 57–21, Tiszajenő 49–30, Tiszaluc 60–12, Tiszanána 54–21, Tokaj 64–10, Tolcsva 65–7, Tornyospálca 74–7, Tófalu 50–17, Törökszentmiklós 53–28, Tura 43–20, Túrkeve 56–29, Turony 26–51, Türje 13–31, Újkér 9–22, Újpuszta 25–19, Urhida 28–28, Uri 42–23, Varsány 41–12, Vác 37–17, Várdomb 32–45, Vecsés 39–23, Veszprém 22–29, Veszprémgalsa 15–29, Vértes 70–23, Zánka 20–33, Zsámbok 43–21.

List of grid-code with their sampling places

7–18 Sopron, 7–23 Horváthzsidány, 8–20 Sopronkövesd, 8–23 Csepreg, 8–36 Nova, 8–37 Gutorfölde, 8–38 Pördefölde, 9–22 Újkér, 10–23 Simaság, 10–30 Oszkó, 10–41 Bánfa, 11–21 Csapod, 11–26 Sárvár, 11–27 Nyőgér, 11–34 Kisbucsa, 11–37 Hahót, 12–35 Pacsa, 13–22 Beled, 13–25 Kemenesszentmárton, 13–26 Celldömölk, 13–31 Türje, 14–13 Bezenye, 14–13 Lajtapuszta, 14–15 Mosonszolnok, 14–20 Farád, 14–28 Jánosháza, 14–30 Gógánfa, 14–34 Cserszegtomaj, 14–41 Nemesdéd, 15–29 Veszprémgalsa, 15–35 Keszthely, 16–21 Egyed, 16–45 Nagyatád, 16–48 Babócsa, 17–26 Kup, 17–29 Devecser, 18–21 Győrszemere,

18–21 Tét, 18–25 Adásztevel, 18–34 Salföld, 19–22 Kajárpéc, 19–32 Balatonhenye, 19–32 Monoszló, 19–37 Lengyeltóti, 20–17 Nagybajcs, 20–33 Zánka, 21–24 Bakonyszentlászló, 21–42 Kaposvár, 21–47 Csertő, 21–48 Szigetvár, 22–18 Nagyszentjános, 22–26 Aklipuszta, 22–29 Veszprém, 22–47 Mozsgó, 23–25 Csetény, 23–31 Lovas, 24–18 Ács, 24–23 Ácsteszér, 24–26 Tés, 24–32 Siófok, 24–46 Hetvehely, 25–18 Szőny, 25–19 Újpuszta, 25–22 Császár, 26–19 Mocsa, 26–24 Mór, 26–36 Iregszemcse, 26–43 Mágocs, 26–51 Harkány, 26–51 Turony, 27–18 Naszály, 27–19 Tata, 27–37 Tamási, 27–43 Bikal, 27–51 Siklós, 28–19 Agostyán, 28–28 Úrhida, 28–42 Kisvejke, 28–43 Mucsfa, 28–46 Hosszúhetény, 28–46 Pécsvárad, 29–21 Tatabánya, 29–24 Csákvár, 29–27 Székesfehérvár, 29–43 Aparhant, 29–49 Szederkény, 30–17 Józsefpuszta, 30–17 Nyergesújfalu, 30–27 Pákozd, 30–44 Bonyhád, 30–49 Boly, 31–22 Bicske MgTSz, 31–31 Nagylók, 31–33 Sárbogárd, 31–38 Nagydorog, 32–17 Esztergom, 32–43 Szekszárd, 32–45 Várdomb, 32–46 Bátaszék, 32–49 Mohács, 33–22 Páty, 34–13 Perőcsény, 34–16 Szob, 34–33 Baracs, 35–19 Csobánka, 35–21 Budapest II., 35–22 Budapest II., 35–22 Budapest XI., 35–23 Budapest XI., 35–24 Érd, 35–30 Rácalmás, 35–36 Madocsa, 35–40 Kalocsa, 35–46 Baja, 36–14 Nógrád, 36–16 Kisoroszi, 36–17 Dunabogdány, 36–19 Szentendre, 36–20 Budakalász, 36–20 Budapest IV., 36–21 Budapest II., 36–21 Budapest IV., 36–21 Budapest XIII., 36–22 Budapest II., 36–22 Budapest XI., 36–22 Budapest XIII., 36–23 Budapest XI., 36–23 Budapest XX., 36–25 Taksony, 37–14 Rétság, 37–15 Szendehely, 37–17 Tahitótfalu, 37–17 Vác, 37–18 Göd, 37–19 Dunakeszi, 37–20 Budapest IV., 37–20 Budapest XV., 37–21 Budapest IV., 37–21 Budapest XIII., 37–21 Budapest XV., 37–21 Budapest XVI., 37–22 Budapest X., 37–22 Budapest XVI., 37–22 Budapest XVIII., 37–23 Budapest XVIII., 37–23 Budapest XX., 37–24 Budapest XX., 37–27 Bugyi, 38–14 Tereske, 38–16 Kosd, 38–17 Rád, 38–20 Mogyoród, 38–21 Budapest XVI., 38–21 Csömör, 38–22 Budapest X., 38–22 Budapest XVI., 38–22 Budapest XVII., 38–22 Budapest XVIII., 38–23 Budapest XVII., 38–23 Budapest XVIII., 38–23 Budapest XX., 38–24 Budapest XX., 38–33 Szabadszállás, 38–40 Kecel, 38–44 Borota, 39–16 Nézsa, 39–19 Őrbottyán, 39–21 Kistarcsa, 39–22 Budapest XVII., 39–23 Budapest XVII., 39–23 Vecsés, 39–32 Kunadacs, 39–36 Csengőd, 39–38 Kiskőrös, 40–12 Őrhalom, 40–15 Bercel, 40–15 Nógrádkövesd, 40–16 Nógrádsáp, 40–20 Gödöllő, 40–23 Gyömrő, 40–23 Maglód, 40–39 Soltvadkert, 41–12 Varsány, 41–23 Mende, 41–25 Monor, 41–32 Kerekegyháza, 42–12 Szécsény, 42–21 Dány, 42–23 Uri, 43–15 Ecseg, 43–20 Tura, 43–21 Zsámbok, 43–45 Kelebia, 44–14 Pásztó, 44–33 Kecskemét, 44–44 Ruzsa, 45–18 Ecséd, 45–28 Cegléd, 45–30 Nagykőrös, 46–13 Kisterenye, 46–39 Csengele, 46–45 Mórahalom, 47–12 Mátranovák, 47–13 Mátramindszent, 47–17 Gyöngyös, 47–22 Jászberény, 48–41 Balástya, 48–43 Szatymaz, 48–44 Domaszék, 49–19 Tarnaméra, 49–21 Jászapáti, 49–30 Tiszajenő, 49–42 Sándorfalva, 49–45 Gyálarét, 50–9 Borsodszentgyörgy, 50–17 Tófalu, 50–19 Boconád, 50–33 Cserkeszőllő, 50–39 Mindszent, 51–9 Ózd, 51–13 Szarvaskő, 51–20 Heves, 51–28 Szajol, 51–37 Szentes, 51–41 Hódmezővásárhely, 52–15 Eger, 52–16 Demjén, 52–32 Öcsöd, 53–18 Besenyőtelek, 53–28 Törökszentmiklós, 53–45 Kiszombor, 53–45 Makó, 54–21 Tiszanána, 54–26 Fegyvernek,

54–33 Szarvas, 55–17 Szentistván, 55–22 Abádszalók, 55–31 Mezőtúr, 56–5 Szendrő Á. G., 56–7 Edelény, 56–29 Túrkeve, 56–37 Nagyszénás, 56–39 Orosháza, 56–44 Csanádpalota, 56–45 Nagylak, 57–4 Martonyi, 57–14 Emőd, 57–21 Tiszaigar, 57–27 Kisújszállás, 57–35 Kondoros, 58–10 Sajópálfalva, 58–15 Hejő menti Á. G., 58–15 Igrici, 59–5 Gagybátor, 59–7 Léh, 59–8 Homrogd, 59–9 Aszaló, 59–25 Karcag, 60–9 Beret, 60–8 Felsődobsza, 60–8 Halmaj, 60–12 Tiszaluc, 60–34 Mezőberény, 61–25 Püspökladány, 61–35 Békés, 61–36 Békéscsaba, 62–10 Szerencs, 63–24 Kaba, 63–37 Gyulavári, 63–39 Elek, 64–9 Borsodszegi, 64–10 Tarcal, 64–10 Tokaj, 64–22 Hajdúszoboszló, 65–7 Tolcsva, 65–15 Hajdúnánás, 66–7 Sárospatak, 66–18 Hajdúböszörmény, 66–26 Berettyóújfalu, 67–20 Debrecen, 67–21 Debrecen, 68–18 Hajdúhadház, 68–21 Debrecen, 68–23 Hosszúpályi, 68–28 Biharkeresztes, 69–11 Kemecse, 69–20 Hajdúsámson, 70–23 Vértes, 71–10 Demecser, 71–23 Kokad, 72–16 Nyírbogát, 73–11 Petneháza, 73–14 Nyírgyulaj, 73–15 Nyírbátor, 74–7 Tornyospálca, 75–11 Nagydobos, 75–13 Mátészalka, 77–8 Barabás, 77–9 Csaroda, 77–9 Tákos, 81–10 Tiszabecs.

Every infested place for every fruit tree was marked on the maps. For all data we gave a score of density of the species in the samples according to Borchsenius's (1950) scoring system, which is a 5 grade system (from 0 to 4). If we had more data for a square we would use the higher density data only. If there were no big differences in density then one map would be used, for plotting all the data of the cultures (Figs 13–15). For *Q. perniciosus* sampling places for every culture were given separately (Figs 2–7) showing non-infested places, too. Marks presented on the maps show all sampling places for every culture.

Results and Discussion

Figure 1 shows all the sampling places, which seem to represent quite well every region of the country. In the following the scale-insect species will be listed in decreasing order of frequency.

Quadraspidiotus perniciosus (Comstock, 1881)

This species is widely distributed in Hungary, almost in all the six cultures studied here (Figs 2–7). The highest density was found on apple, less on sour cherry. There are not big differences in density among the various parts of the country. On the bases of these data this species, at present, is the most important scale-insect pest of the fruit cultures in Hungary. The frequency and the density of this species decreased from 1971 to 1976 (Kozár and Viktorin, 1978).

Epidiaspis leperii (Signoret, 1869)

This species is widely distributed in Hungary, but, as related to occurrence there are big differences among cultures and different regions of the country

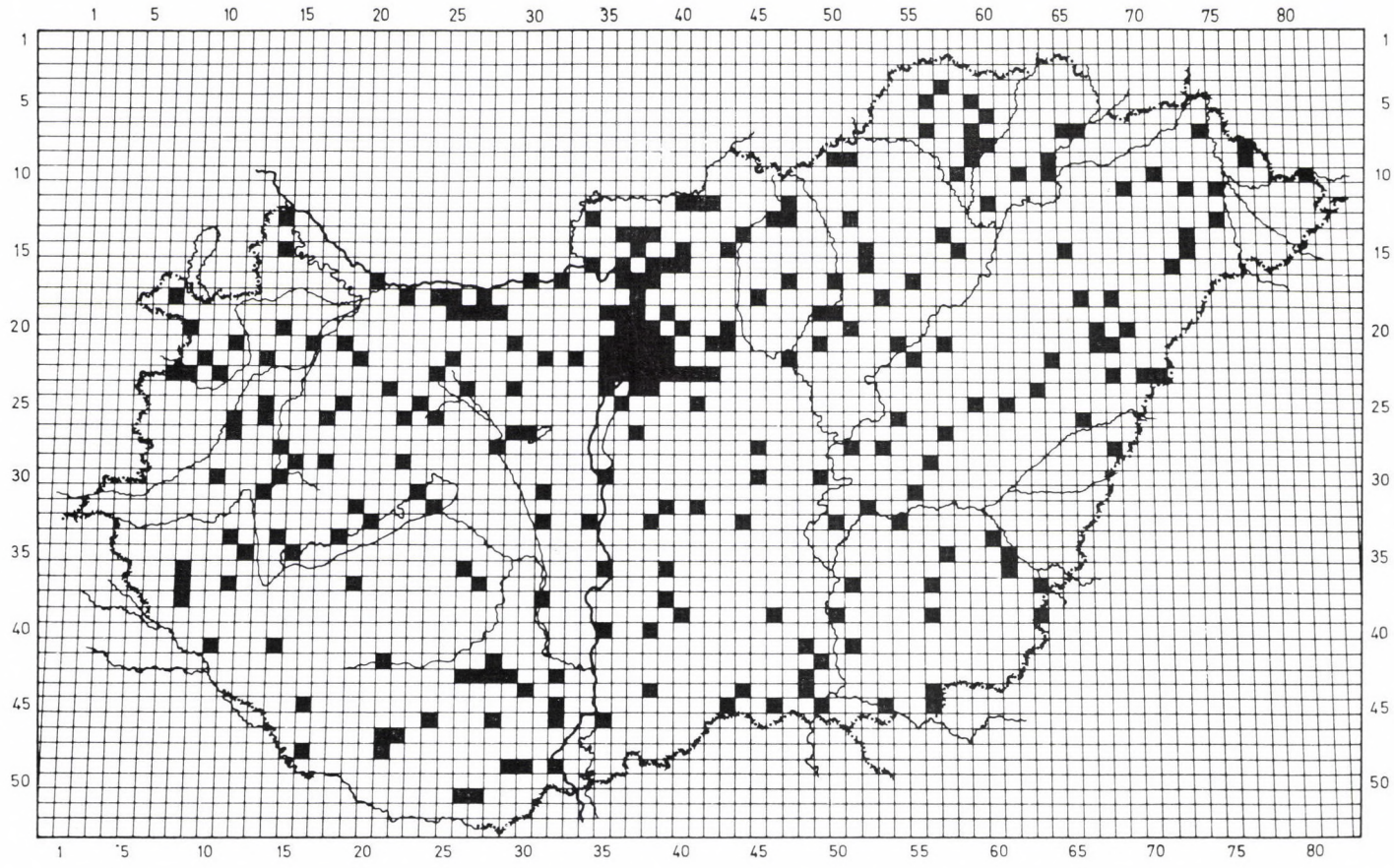

Fig. 1. Distribution of sampling places in Hungary

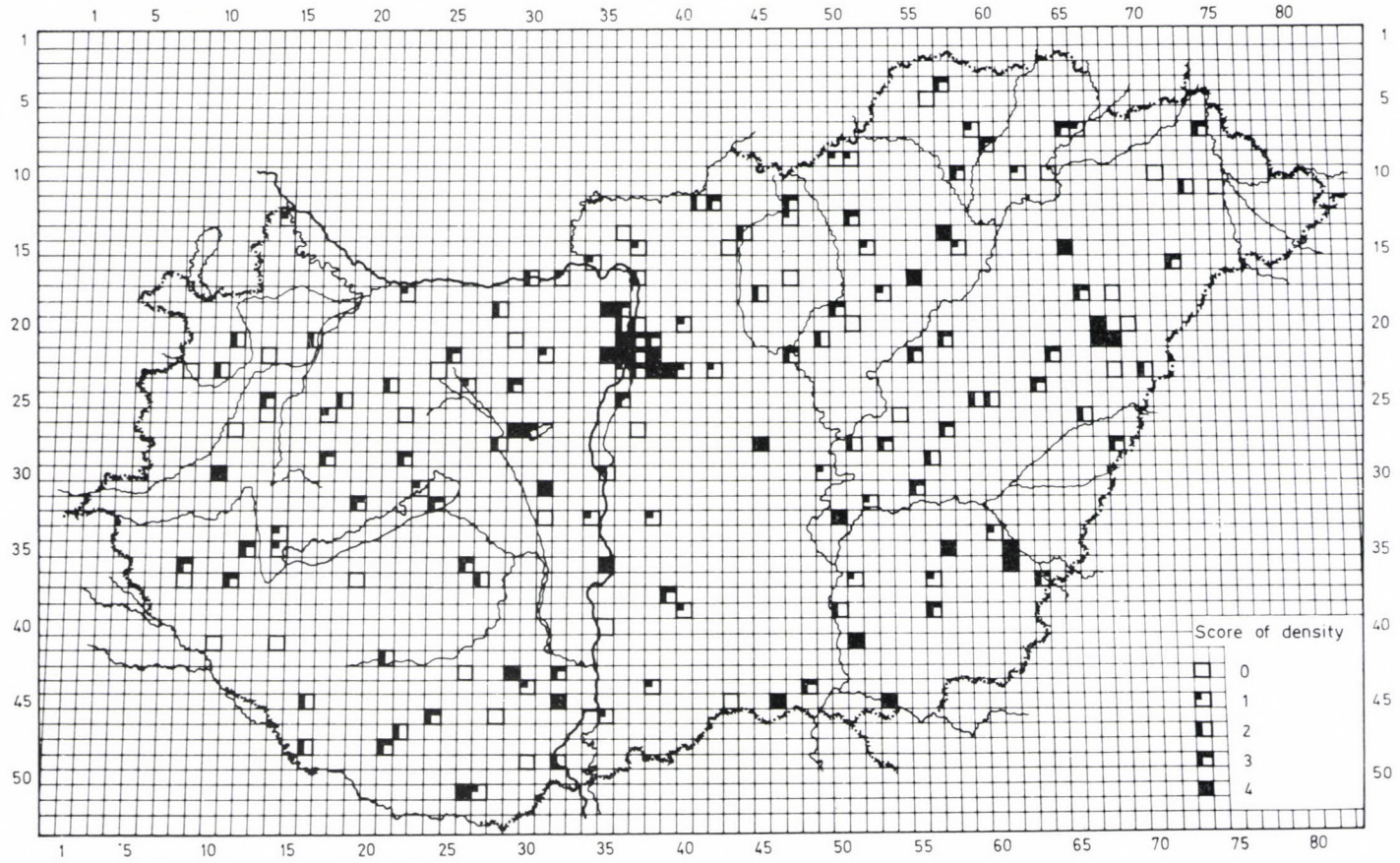

Fig. 2. Distribution and density of *Q. perniciosus* on apple in Hungary (On the figures 2–12 the marks are the same!)

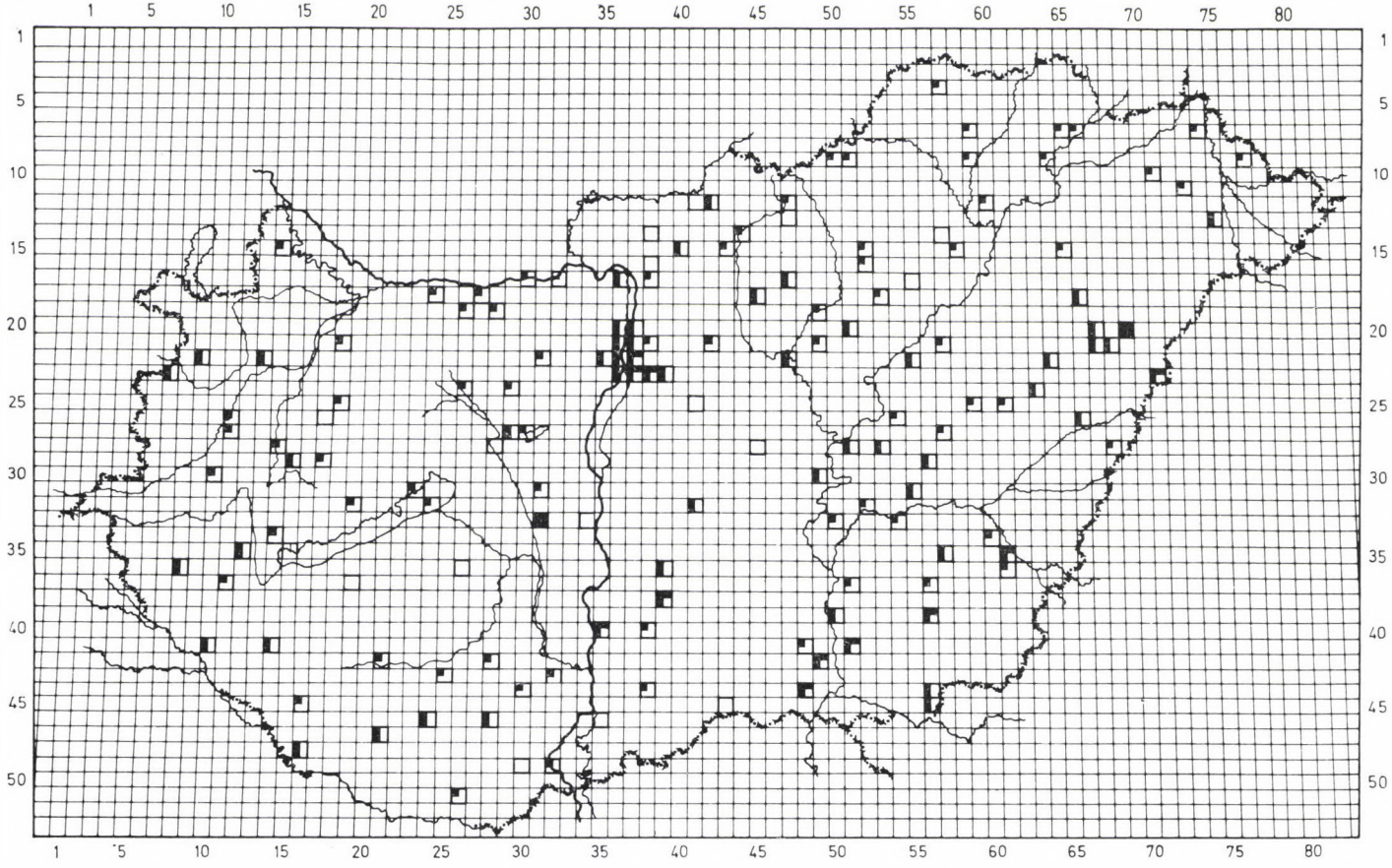

Fig. 3. Distribution and density of *Q. perniciosus* on cherry in Hungary

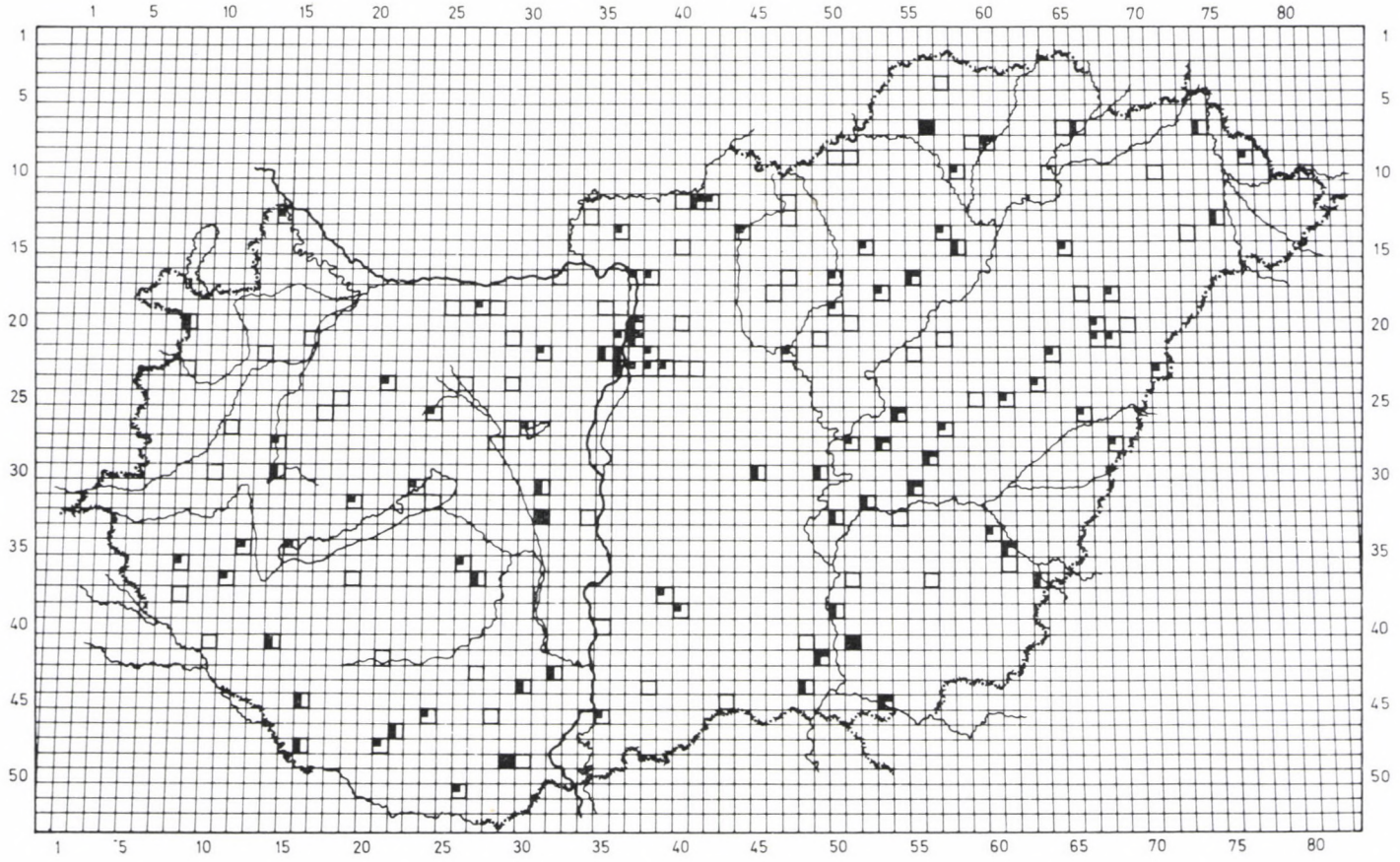

Fig. 4. Distribution and density of *Q. perniciosus* on pear in Hungary

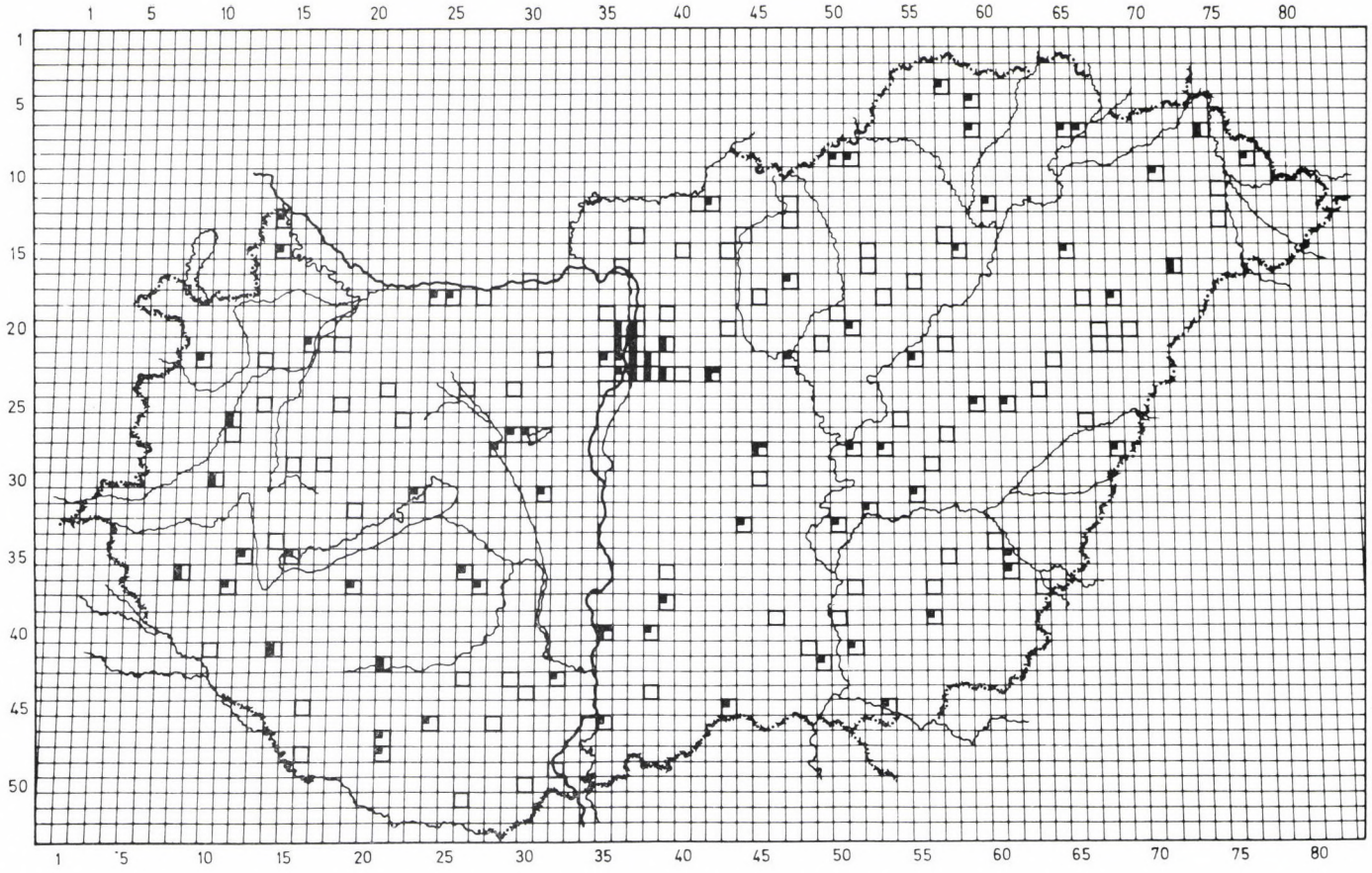

Fig. 5. Distribution and density of *Q. perniciosus* on sour cherry in Hungary

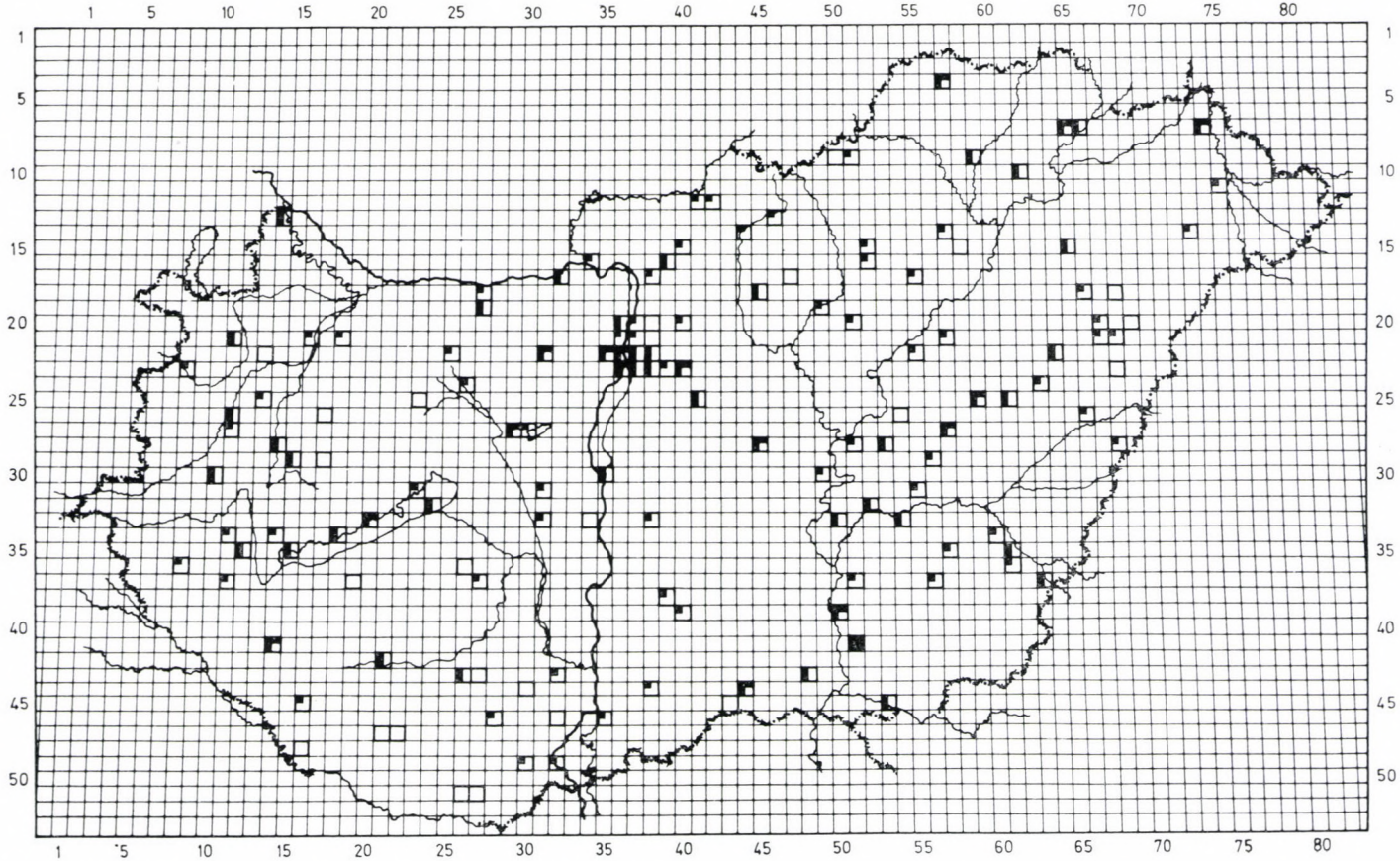

Fig. 6. Distribution and density of *Q. perniciosus* on peach in Hungary

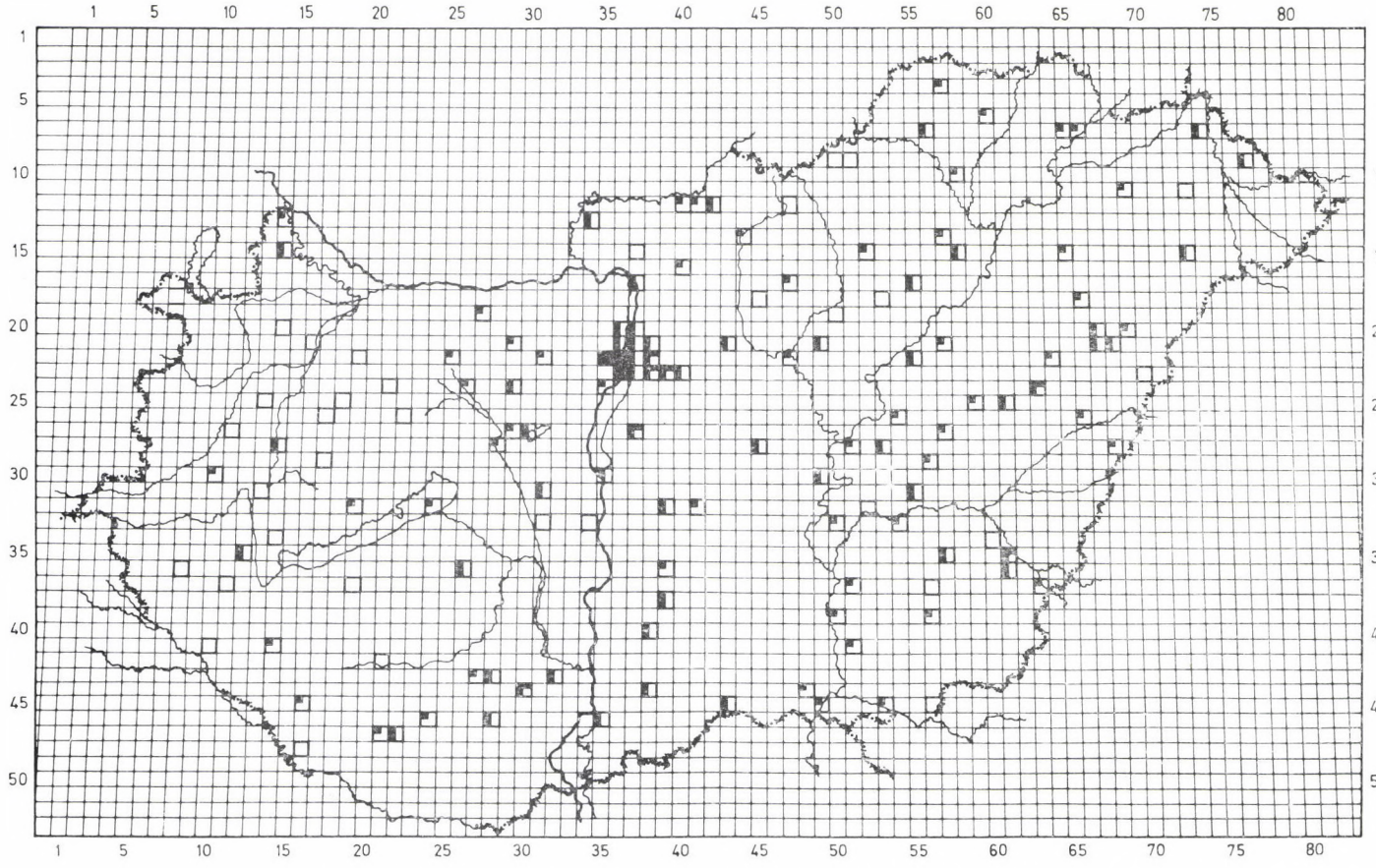

Fig. 7. Distribution and density of *Q. perniciosus* on plum in Hungary

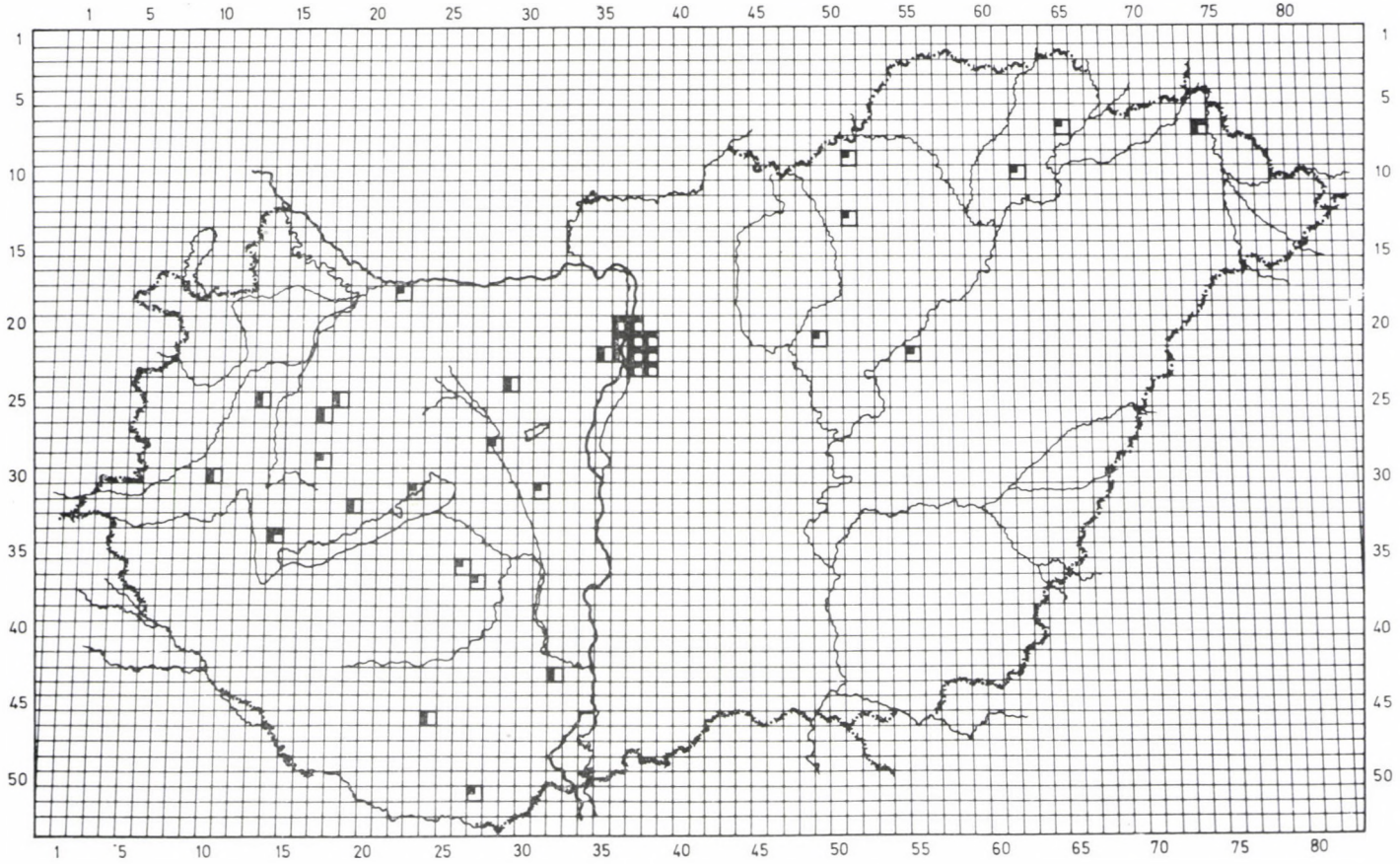

Fig. 8. Distribution and density of *E. leperii* on apple in Hungary

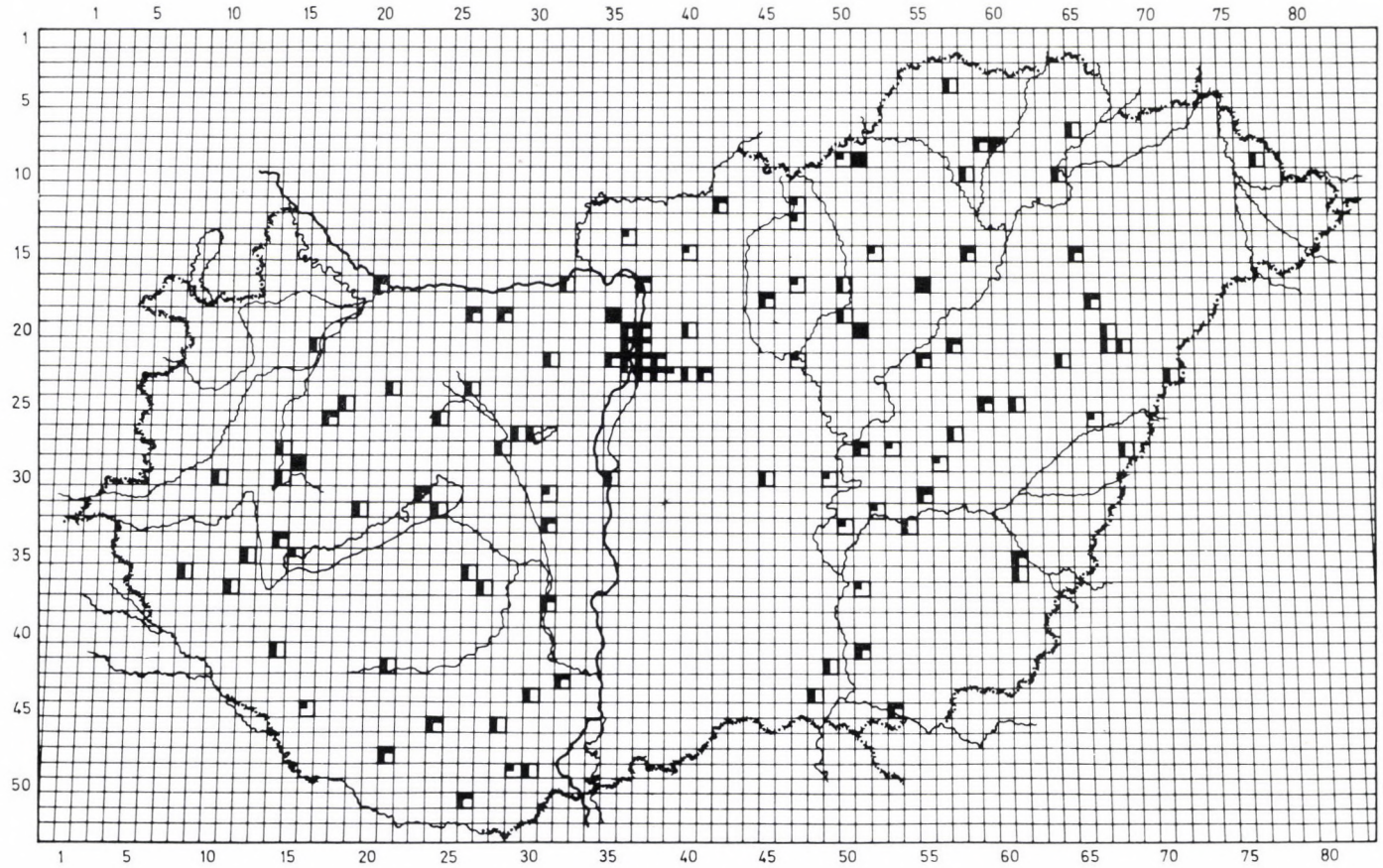

Fig. 9. Distribution and density of *E. leperii* on pear in Hungary

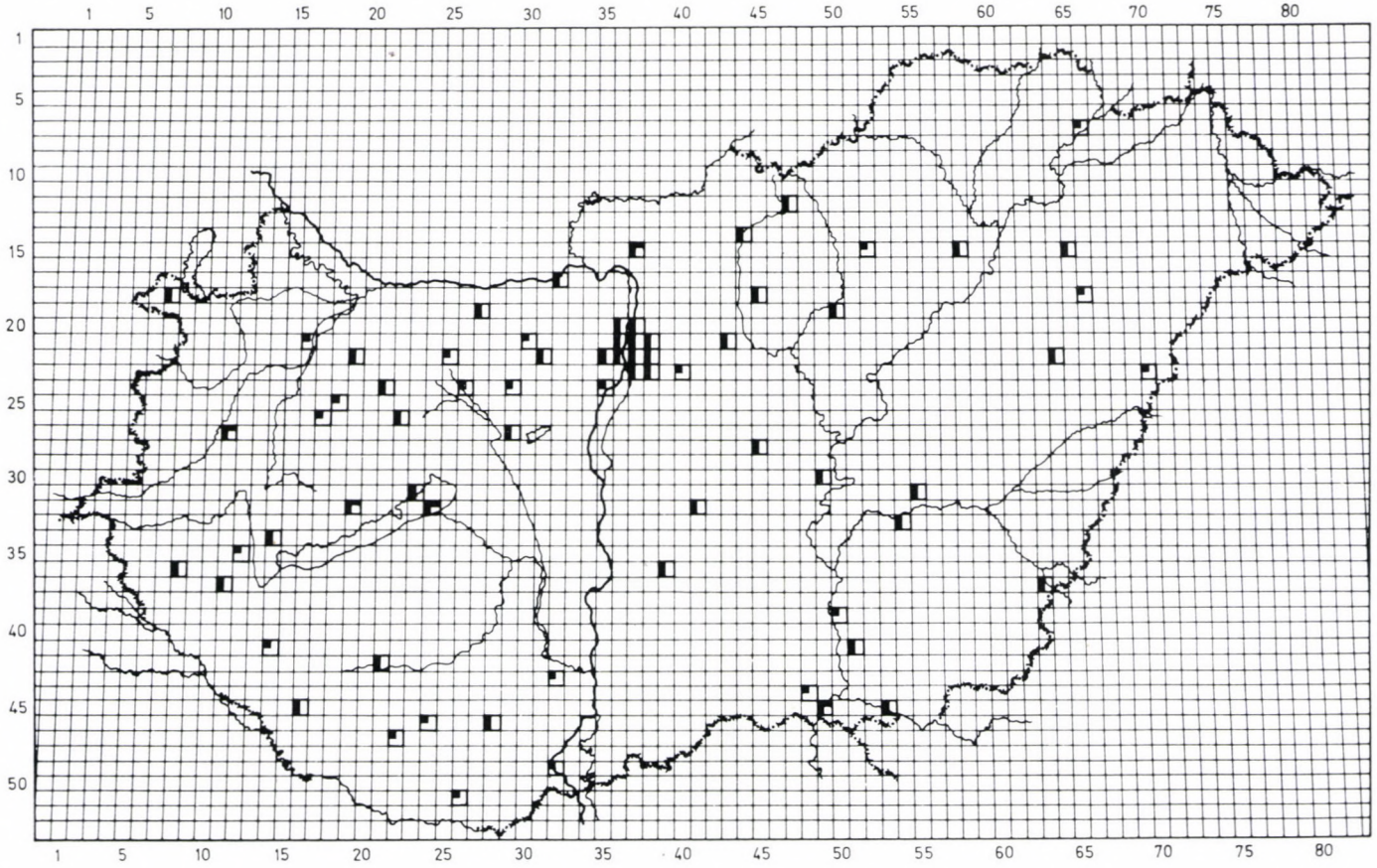

Fig. 10. Distribution and density of *E. leperii* on plum in Hungary

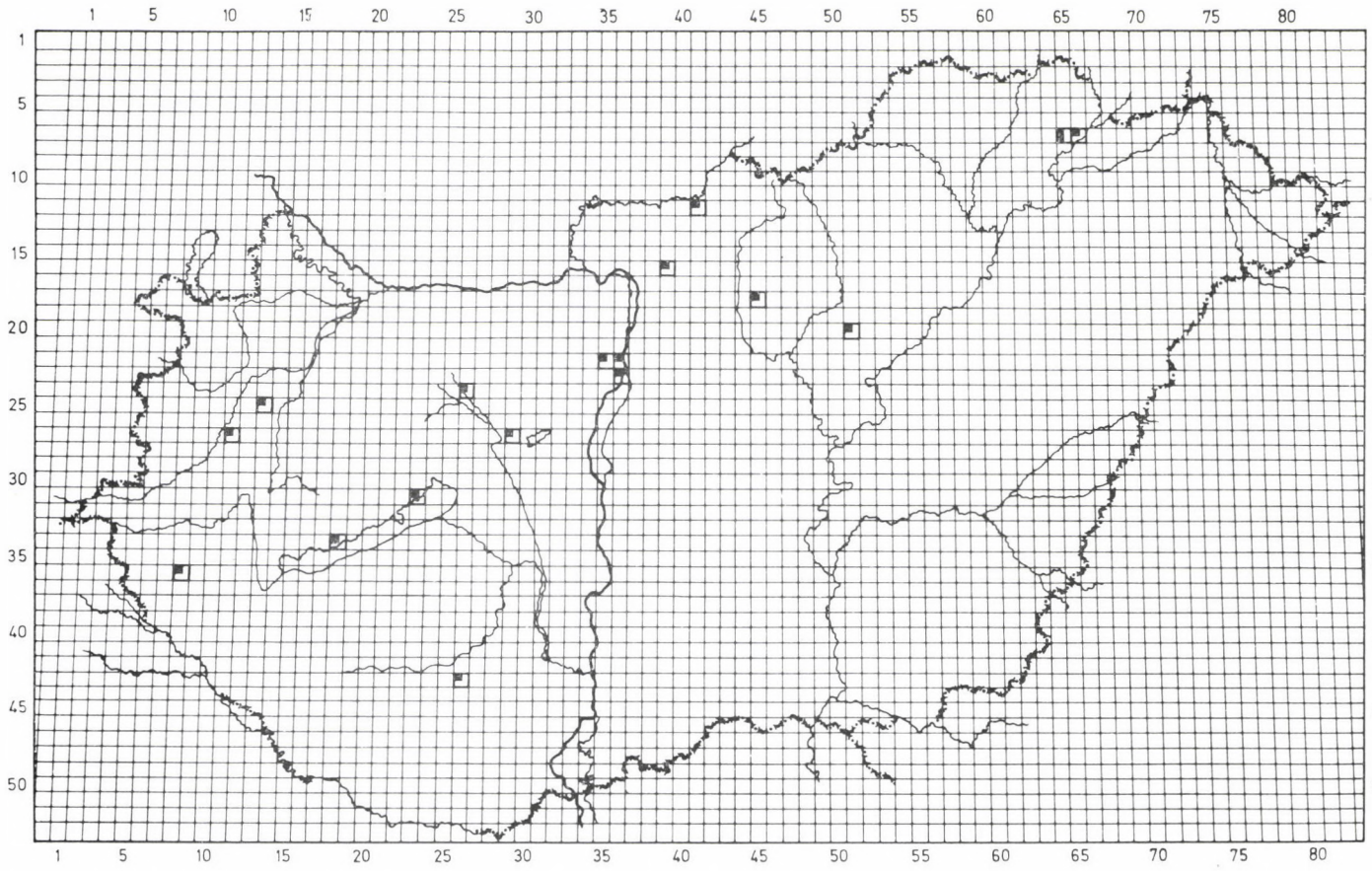

Fig. 11. Distribution and density of *P. corni* on peach in Hungary

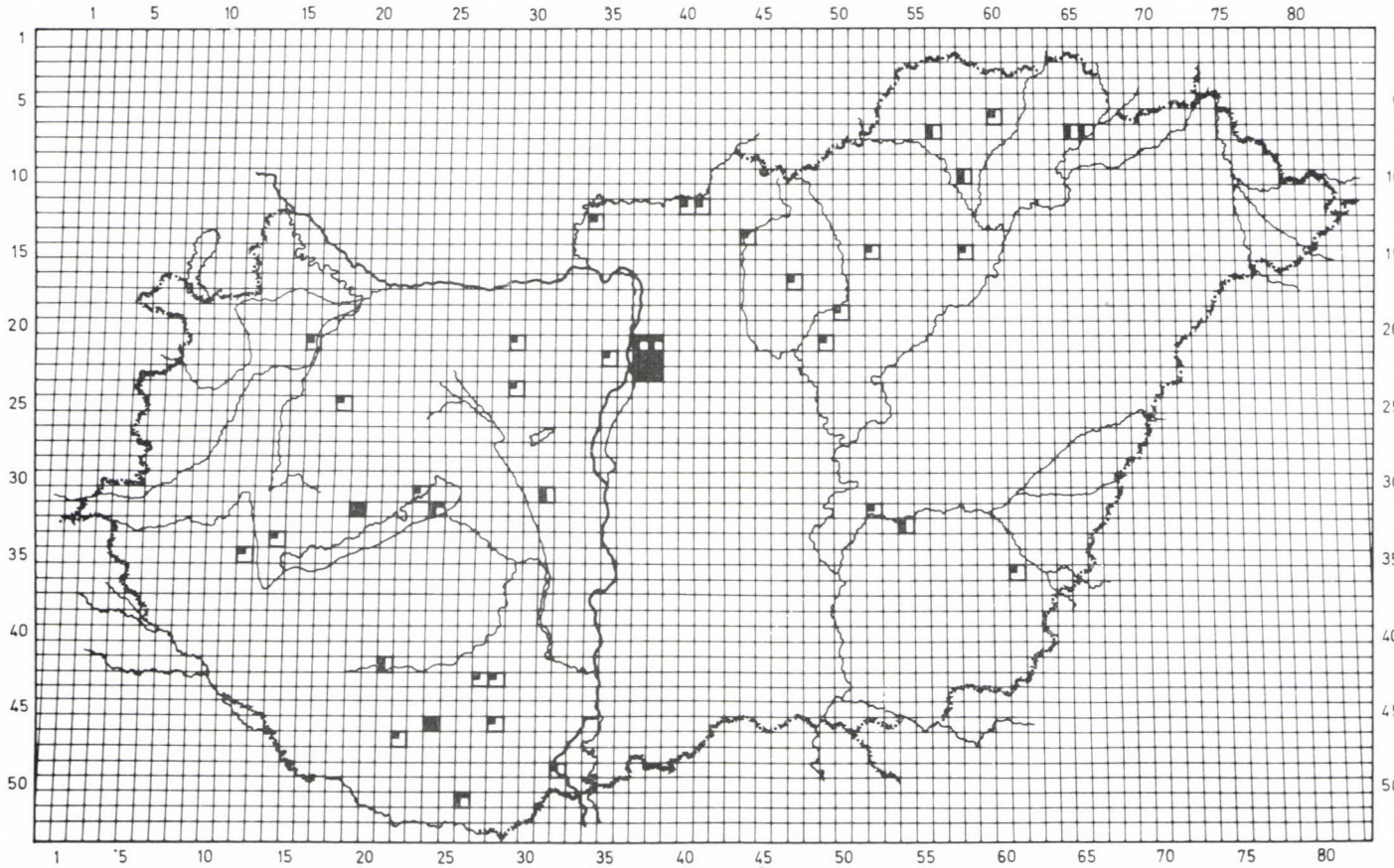

Fig. 12. Distribution and density of *P. corni* on plum in Hungary

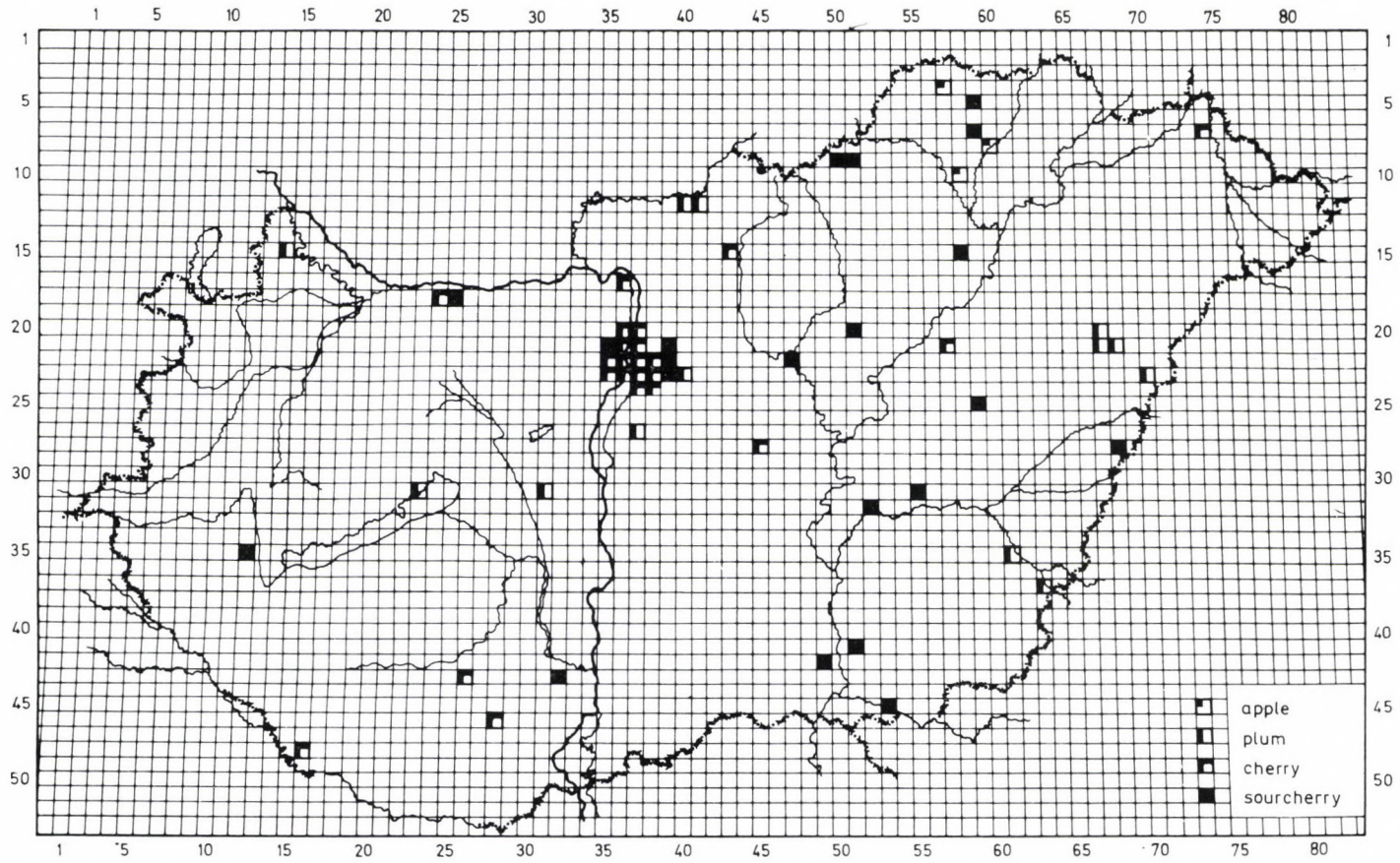

Fig. 13. Distribution of *Q. ostreaeformis* on various fruit cultures in Hungary (On the figures 13–15 the marks are the same!)

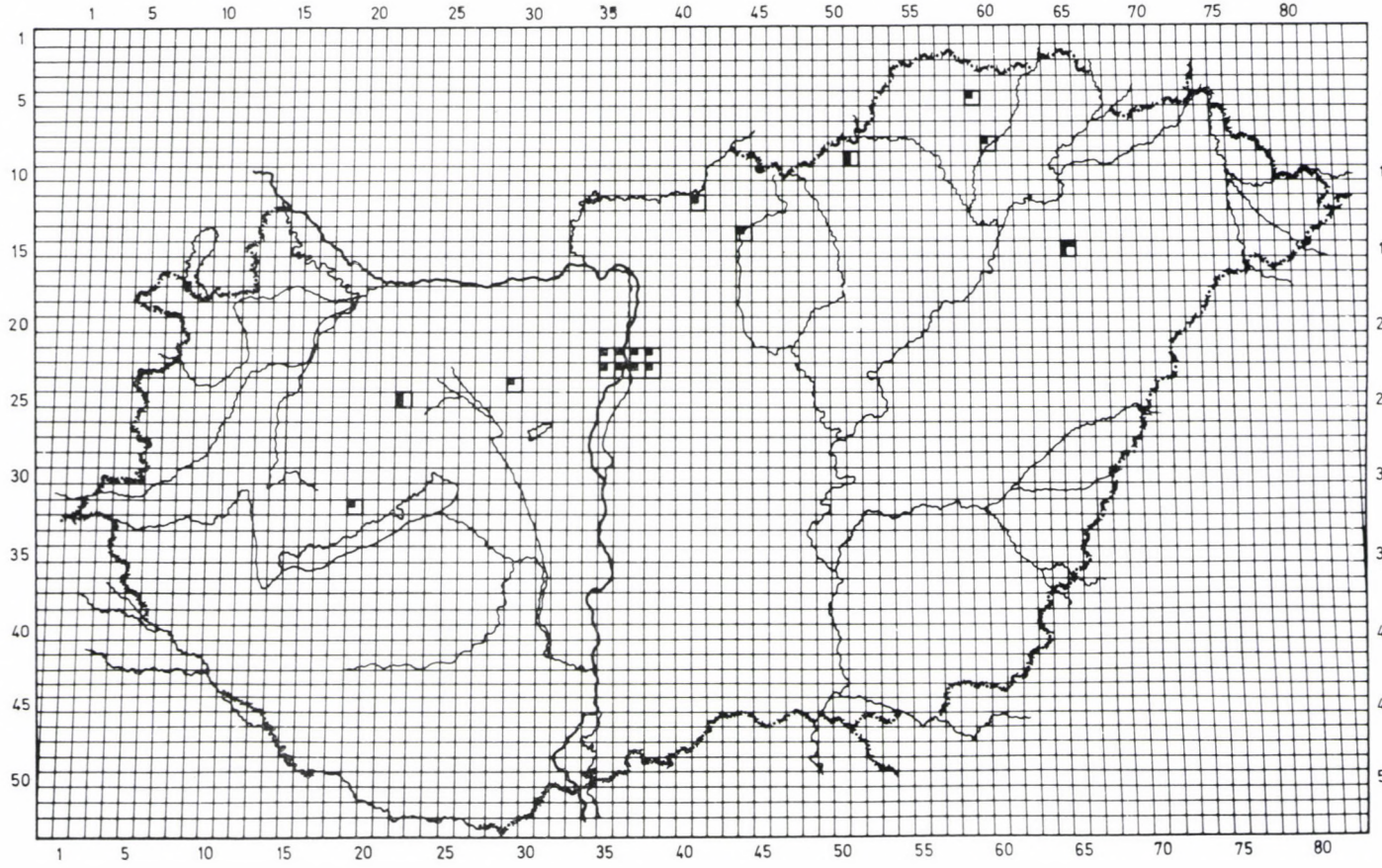

Fig. 14. Distribution of *L. ulmi* on various fruit cultures in Hungary

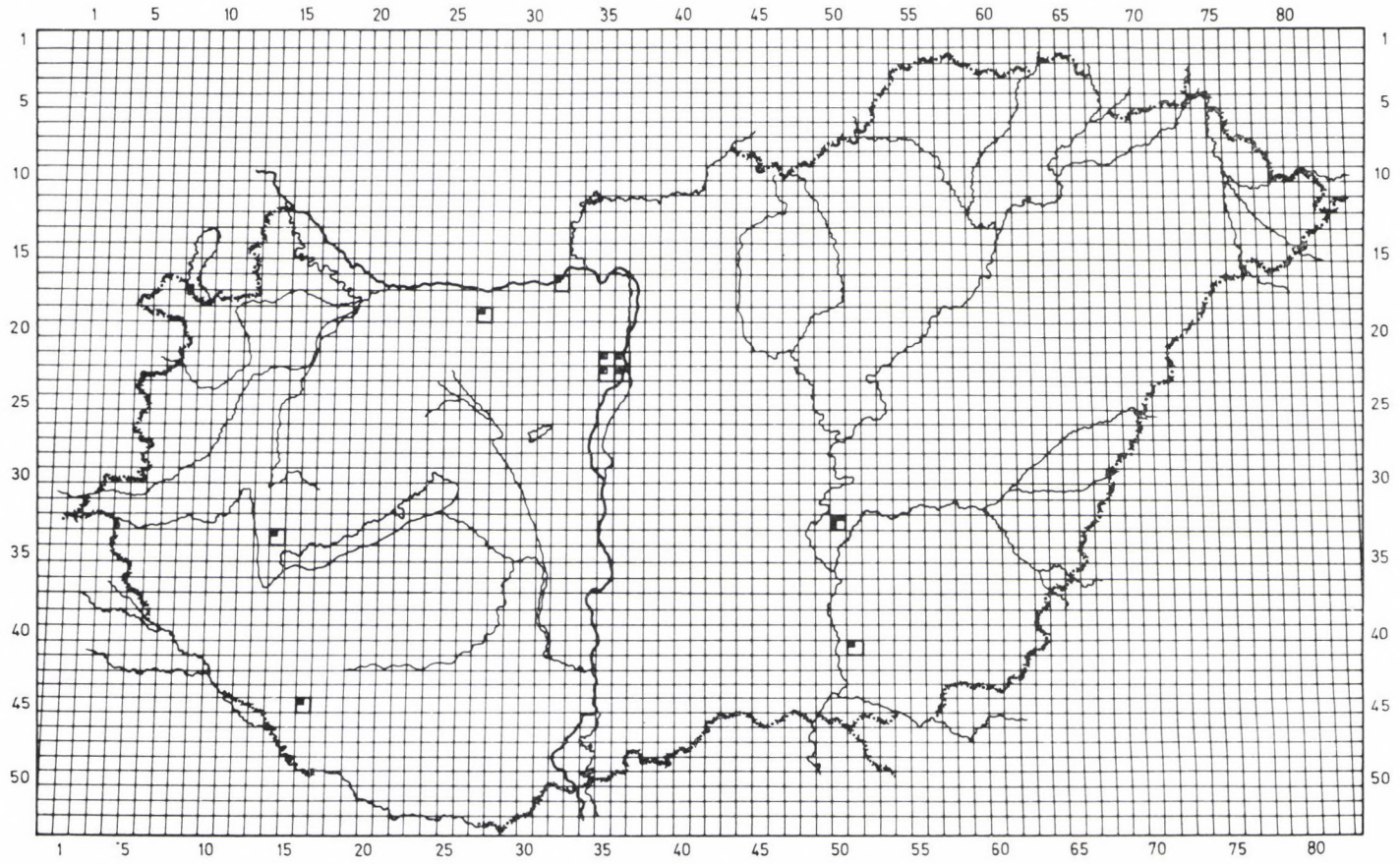

Fig. 15. Distribution of *S. prunastri* on various fruit cultures in Hungary

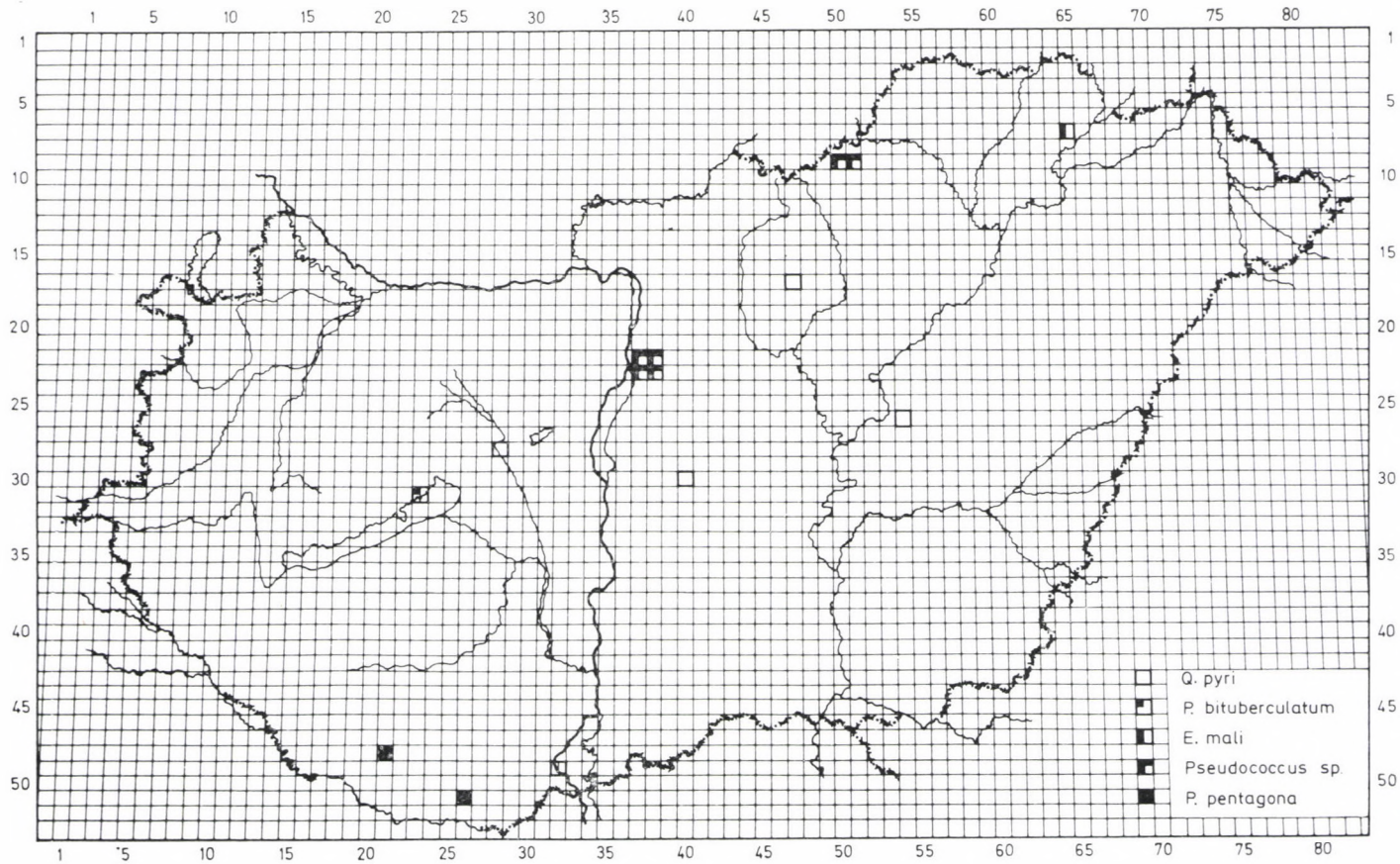

Fig. 16. Distribution of some rare scale-insect species on various fruit cultures in Hungary

(Figs 8–10). It is distributed on apple mostly in northern and western part of the country (Fig. 8). It is the most frequent species on pears, distributed almost all over the country, but the region between Danube and Tisza rivers is less infested (Fig. 9). This phenomenon cannot be explained, consequently itneeds further invertigations. The plum was infested somewhat less than the pear in the whole country (Fig. 10). This species was found at low density on peach only in three places and on cherry in Budapest only, where the infestation was very high on pear, apple and plum. No infested sample was found on sour cherry. The frequency of this species increased somewhat in recent years, especially on plum (Kozár and Viktorin, 1978).

Parthenolecanium corni (Bouché, 1844)

This species was distributed in some peach orchards in the northern and western parts of the country, but at very low density (Fig. 11). It was more widely distributed on plum, except in the Great Hungarian Plain (Fig. 12). In other cultures (apple, cherry, pear, sour cherry) some infested samples were recorded. The frequency and the density of this species highly increased from 1971 to 1976 due to an outbreak under natural conditions (Kozár and Viktorin, 1978).

Quadraspidiotus ostreaeformis (Curtis, 1834)

The density of this species at the time of sampling was very low everywhere and in all cultures (Fig. 13). It was more frequent on sour cherry in Great Hungarian Plain, but, in other parts of the country, it was more frequent on cherry, especially in Budapest. It was not found on pear and peach. The density and frequency of this species is on the increase, too (Kozár and Viktorin, 1978).

Lepidosaphes ulmi (Linnaeus, 1758)

This species was found only in the northern part of the country, mostly on apple (Fig. 14). We have no data from pear and peach.

Sphaerolecanium prunastri (Fonscolombe, 1836)

This species was very rare in 1971. It was found in some places only and usually at a very low density. It was found more often on plum (Fig. 15). We have no data from apple and pear. But some years later this species became wide-spread, especially in peach orchards treated with insecticides (Kozár and Viktorin, 1978).

Pseudaulacaspis pentagona (Targioni-Tozzetti, 1886)

This species was found only in some places in the southern part of Hungary (Fig. 16), but later became wide-spread in our country (Kozár and N. Dávid, 1985).

Quadraspidiotus pyri (Lichtenstein, 1881)

This species was found on stone fruit trees, in some places. (Fig. 16).

Palaeolecanium bituberculatum (Targioni-Tozzetti, 1868)

This species has been rarely found on apples in this region (Fig. 16).

Eulecanium mali (Schrank, 1781)

It is a rare species on apples, however, it was found at a high density in one case (Kozár, 1970) (Fig. 16).

Phenacoccus aceris (Signoret, 1875) and *Phenacoccus mespili (Geoffroy, 1762)*

Some larval specimens of mealybug which could not be determined exactly were also found (Fig. 16). Presumably it is *P. aceris*, which is widely distributed in Hungary on forest trees. It was mostly found in the northern part of the country on apple and cherry. The presence of *P. mespili* in Hungary is not fully verified.

Concluding Remarks

According to Kozár's (1976) data 21 scale-insect species were found on all fruit species in Hungarian orchards. However, on the six fruit species studied in this survey, we could find only 11 scale-insect species. The other 10 species were found on other fruit species, as walnut, currant, raspberry, etc. They indicate only potential risk to the studied six fruit species. The species form four main groups, as the widely distributed *Q. perniciosus*, the southernly distributed *P. pentagona*, the northernly distributed *L. ulmi* and members whose frequency and density are so low as to be unsuitable for the determination of the type of their distribution.

Owing to host specialization the species form the subsequent groups. *Q. perniciosus* is frequent on all fruit tree species. *E. leperii* was found mostly on pear and on plum but no other stone-fruit species. *P. corni*, *S. prunastri* and *Q. pyri* are found mostly on stone-fruit trees, while *L. ulmi*, *P. bituberculatum* and *E. mali* mostly on pome fruits.

For a better understanding of the distribution processes and changes of density it is necessary to repeat the survey in the future.

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Experimental Ecology and Its Role in Agricultural Entomology*

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In his study the author surveys the development of experimental insect ecology in Hungary and its role in agricultural entomology.

The study throws light upon the importance of ecology beside molecular biology. The concept of ecology is shown in its historical development from Haeckel (1866), the creator of the concept, to these days. According to the author the best definition is: "the ecology is a synbiological science concerned in general with interactions between living organisms and their environment, more exactly with the pressure and rules of existential conditions operating in the environment and acting on the populations of organisms. Its task is to find out what the pressing circumstances acting on the organisms (or biological populations) and on the complex systems formed by them (e.g. communities, ecosystems) are, and how these circumstances determine statistically the behaviour and spatial distribution of different groups of organisms, their function and material- and energy production in the ecosystem" (Biológiai Lexikon /Biological Encyclopaedia/ 3., ed.: Straub, 1977).

"The experimental insect ecology is concerned with the effect of coercive abiotic conditions produced artificially or existing in nature in a given place on different development forms and -stages of the insect species examined in definite populations with special experiment methods" (Sáringer, 1972).

The paper lists the major species examined (mostly in diapause) in Hungary with experimental insect ecological methods. These species belong to the following orders: Homoptera (2 species), Coleoptera (16 species), Lepidoptera (9 species), Diptera (1 species), Hymenoptera (3 species), Acarina (2 species).

As an example, results obtained with Grapholita funebrana Tr. (Lepidoptera) are described in the paper. Finally, in autumn (short-day period), with half an hour of illumination in each of various periods of the scotophase the author broke the facultative diapause of Athalia rosae L. (Hymenoptera). This new, insecticide-free control method, as a possibility of reducing the individual number, is called ecotechnical control by the author.

Nothing proves better the importance of ecology than the extraordinary interest shown in this science for the last two decades all over the world. While a quarter of a century ago it was the results of molecular biology that made the world gape, today ecology is expected to solve the most pressing problems of the human race, e.g. to supply the world with sufficient amounts of food and protect the human environment from contaminations caused by the technical civilization.

* This work is dedicated to the 70 years old academician Tibor Jermy.

No one of those engaged in one branch or another of the natural sciences, e.g. in plant protection, can dispense with a knowledge of the results of this science any longer.

Every professional plant protectionist must be aware of the fact that when carrying on disease- or pest- or weed control he may influence the abiotic and biotic factors of the environment to an extent harmful to the functions of the ecological systems surrounding us. Today the principles of modern ecology must by all means be known if our plant protection activity is to serve the purpose of surplus production with the least possible danger to the environment.

The concept of ecology and its development from Haeckel to this day

Let us examine briefly and in outlines what in fact ecology is. The word is of Greek origin; the meaning of "oikos" is: house or home, while "logos" means science; accordingly, ecology when translated word for word means domestic science. However, in the special literature it is used in the sense of environment science.

The science of ecology, like other biological sciences, is the result of a long historical progress. Though the word ecology goes back to a 100 years only, the concept was already written about by the Greek Theophrastus, a pupil and friend of Aristotle (B. C. 384–322), when he discussed plants and animals usually found in so-called "communities". Of the Roman writers Pliny (A. D. 23–79] and others also wrote about what we now call ecological phenomena without knowing the word "ecology". After the Renaissance Réaumur (1663–1757), the great French natural scientist and Buffon (1707–1788) also dealt with ecological problems (in: Andrewartha, 1963). A prominent scientist in the 18th century Antoni van Leeuwenhoek, known for his microscope, was the first to write about the nutrition chain and the population dynamics, these two important fields of research in today's ecology. (In: Odum, 1971.)

The use of the word ecology (Oekologie) and the definition of the concept came from the German Ernst Haeckel who – first in 1866 – wrote the following: "Unter Oekologie verstehen wir die gesamte Wissenschaft von den Beziehungen des Organismus zur umgebenden Aussenwelt, wohin wir im weiteren Sinne alle Existenzbedingungen rechnen können." Later, in 1870, he gave the following definition of ecology: ". . . hat die gesamten Beziehungen des Tieres sowohl zu seiner anorganischen als zu seiner organischen Umgebung zu untersuchen, vor allem die freundlichen und feindlichen Beziehungen zu denjenige Tieren und Pflanzen, mit denen es in direkte oder indirekte Berührung kommt, oder mit einem Worte alle diejenigen verwickelten Wechselbeziehungen, welche Darwin als die Bedingungen des Kampfes um's Dasein bezeichnet." Still later, in 1879, he formulated the subject of ecology as follows: ". . . die Wechselbeziehungen aller Organismen, welche an einem und demselben Orte mit einander leben."

Ecology, as a special branch of the biological sciences can be spoken of only since the beginning of this century.

The development of modern ecology was greatly influenced by the book "Animal ecology" published by Elton (1927) in London, which can just as well be regarded as a protestation against the branches of science established in biology after Darwin's book "The origin of species" had appeared in 1859 (the last publication in Hungarian was in 1973). These branches were: taxonomy, comparative anatomy, evolution and phylogenesis. In Elton's afore cited book, on the other hand, the question is put in that way: "In solving ecological problems we are concerned with what animals do . . . We have next to study the circumstances under which they do these things, and, most important of all, the limiting factors which prevent them from doing certain other things. By solving these questions it is possible to discover the reasons for distribution and numbers of animals in nature."

Afterwards ecology was naturally given various definitions. In general, it was defined as a science concerned with the changing relation of organisms or groups of organisms to their surroundings. According to a definition by Odum (1963) ecology is a science which examines the structure and function of nature, in the framework of which first of all the biology and function of organisms in uncultivated and cultivated lands as well as in fresh waters and seas are dealt with. By a later definition from the same author (Odum, 1971) ecology is a science concerned with the economy of the Earth including all elements which take part in the interactions of this economy: animals, plants, microorganisms and the human population. According to the definition of Webster's Unabridged Dictionary (cit. Odum, 1971): ". . .the totality or pattern of relations between organisms and their environment." By a quite short definition: ecology is equal to environment biology (Odum, 1971; Széky, 1977, 1979).

According to Jakucs et al. (1984) ". . .ecology should only be spoken of when the investigations are concentrated on actual reasons (pressure of circumstances) for the mass distribution (in the simplest case occurrence and non-occurrence) of organisms in space and time, and for the behaviour manifested in its change. And that so, that the direct linkage of the external factors of environment and the internal factors of tolerance are regarded as primary and determinative."

According to Précsényi (1984) ". . . the ecology deals with the regulation and control of populations and cenoses; it studies the control on a supraindividual level".

Juhász-Nagy (1984, 1986) discusses at length the subject of ecology in its historical development, but without giving exact definition of the concept.

Other definitions for ecology are found in works by Price (1975), Huffaker and Rabb (1984) and Schubert (1986).

The most exact definition of ecology—that we too accept—is: "The ecology is a synbiological science concerned in general with interactions between living organisms and their environment, more exactly with the pressure and rules of existential conditions operating in the environment and acting on the populations of organisms. Its task is to find out what the pressing circumstances acting on the organisms (or biological populations) and on the complex systems formed

by them (e.g. communities, ecosystems) are, and how these circumstances determine statistically the behaviour and spatial distribution of different groups of organisms, their function and material- and energy production in the ecosystems" (Biológiai Lexikon /Biological Encyclopedia/ 3., ed.: Straub, 1977).

Out of the levels of biological organization the *individual* level, in other words the organization level of organisms, and the *supraindividual* levels, that is the organization level of populations, communities (biocenoses) and of the biosphere fall within the scope of ecological researches. They are not concerned with the infraindividual levels, such as e.g. the organization levels of molecules, cells, tissues and organs, respectively.

The science of ecology can be divided by the object examined, so we can speak of phyto- and zooecology, or according to the degree of their complexity of *auto-* and *synecology*. The latter two concepts were introduced by Schröter and Kirchner (1896).

The autoecology or individual ecology is that field of ecology where – in opposition to the synecology which deals with the ecological conditions of the community as a whole – the objects to be studied are comparatively simple, e.g. the relations of environment-tolerance on individual level or the way of adaptation are subjected to examination. The autoecology tries to find answer to two questions: first, why does the object examined occur in one place and why is it absent in the other, that is, what determines e.g. the distribution area of a species? The other question is: why does the individual number change in space and time on a given area?

Although the *autoecological investigations* – as far as they concern the object and the method – are in many cases closely related with physiological problems, their purpose is always exclusively ecological. Investigations which while carried out for ecological purposes are concerned with physiological problems are generally called *ecophysiological* investigations. Other aspects of ecology are again considered when speaking of ecogenetics and ecoethology. Ecophysiology is a branch of ecology of French origin. At many a university in France, e.g. at the Université Paris-Sud a department called Laboratoire d'Entomologie et d'Ecophysiologie Experimentales (Laboratory for Experimental Entomology and Ecophysiology) has been functioning for nearly 25 years.

The synecology i.e. community ecology is that field of ecology where the rules of environment-tolerance relations observed for organisms of various degrees of organization, e.g. populations, communities, biocenoses, are studied. The synecological investigations always take a holistic view of the subject concerned.

The auto- and synecology – rather than expressing opposite conceptions – approach the phenomena from different points of view. In a number of cases the results of synecological researches can in many respects be correctly interpreted only on the basis of autoecological research results. This naturally does not mean that the higher organization levels, the supraindividual systems cannot be understood without the details – in our case the autoecological results summed up. They have their own peculiar rules which as a whole is more than the sum of

the parts they are composed of. (By the way, even the antique Greek philosopher knew that the whole is more than the sum of the parts.)

The ecology of animals was divided by two famous authors of the sixties in three parts (Schwerdtfeger, 1963; Tischler 1965). In their conception the individuals and the populations consisting of them are dealt with by the *autoecology*, the biocenoses by the *synecology*, and the relation of various populations to their environment is the subject of the so-called *demcology*. This division into three was pointed out as unacceptable by Jermy (1971) already.

Schwenke (1979) suggested to eliminate the concept of autoecology and return to the English interpretation of ecology according to Elton (1927), Andrewartha and Birch (1954) and Odum (1963, 1971), who by the single concept of "ecology" understand studies of either populations or communities, or even productivity.

Experimental insect ecology and its development in Hungary

It is a well-known fact that every period of the human history has had its *ideal of science*. In the period of revolutionary technico-scientific development after World War II. *mathematics* was chosen for the ideal of science. Biomathematics, the branch of mathematics entering the field of biology, has not naturally left the ecology unaffected either, particularly not the ecosystem research which falls within the scope of synecology; but it has gained special importance in population biology, a science concerned with the movement of population.

Ecological researches – carried out either with auto- or with synecological purposes – are in each case based on the description of interactions between the life conditions offered by the environment on the one hand, and the individual or population, on the other. It is on the facts thus described that ecology builds the general rules for the relations between environment and animal, now with its experimental methods substituted for the earlier quantitative (statistical) methods. The *experimental insect ecology* as a part science has developed from the *descriptive phase* of ecology; we no longer are satisfied with a mere registration of phenomena but carry out laboratory-, small plot- or farm-scale experiments to solve the problems.

Thus, the experimental insect ecology is concerned with the effect of coercive abiotic and biotic conditions produced artificially or existing in nature in a given place on different development forms and stages of the insect species examined in definite populations, using special experiment methods (Sáringer, 1972).

The experimental insect ecology requires up-to-date technical equipment. In our opinion the absence of an adequate technical equipment has long delayed the development of experimental insect ecology both in Hungary and abroad.

Researches in experimental insect ecology are mostly carried out in laboratories. The development and behaviour of insects are followed in an instrumentally conditioned milieu. The results obtained under artificial conditions are checked under semi-operative and operative conditions, so as to give the truest possible

explanation of the development of insects observed in nature. Experimental insect ecology is thus a hundred per cent pragmatic science.

The essence of this field of science can perhaps be best understood from the historical development of the working method of entomology in plant protection.

Some fifty years ago the working method of applied entomology was the following: identifying the species concerned, observing the phenology of the successive development stages, then using various methods to disclose relations with populations of other insect species living together with the pest, and finally, experimenting with a number of pesticides in order to find the most suitable one and establish the optimum dosis. That is, the whole process of investigation was largely of descriptive character. Some forty years ago a radical change of view took place. We began to search for the reasons of phenomena observed and described so far. With the aid of our technical equipment we put questions to certain semaphoront populations of the pest organisms and registered the extent of response, then in possession of the new information we "queried" again the animal until arriving in the logical process at a satisfactory explanation of the given phenomenon. Up to this point this is a problem of experimental insect ecology.

Ecological experiments with some major insect pest species and their importance in agricultural entomology

Experimental insect ecological researches with plant protection in view were started in Hungary in the middle of the thirties at the Research Institute for Plant Protection of that time. The research work began under the guidance of professor Gusztáv Szelényi D. (1904–1982), and the first work of this character was also written by him (see Table 1). In the years following World War II the work was taken up again resumed at the Institute, but with much more intensity. A radical change took place in 1947 when the colorado potato beetle (*Leptinotarsa decemlineata* Say) settled at Hédervár. The mass reproduction and rapid spread of this dangerous pest brought the Ministry of Agriculture to establish a laboratory for the special purpose of colorado potato beetle researches. The laboratory was built up at Keszthely, by Lake Balaton, on the basis of Tibor Jermy's plan. Between 1952 and 1958, up to the time the laboratory was completed, the investigations were carried out in a provisional glasshouse at Keszthely. The new working place was given the name: Research Institute for Plant Protection, Budapest, Keszthely Laboratory. The Laboratory had 4 work-rooms, 3 thermostat chambers and two glass houses west-east orientated lengthwise, half sunk in the ground, each 150 m^2 in groundspace. Besides, the Laboratory contained three guest-rooms and a workshop. The building was surrounded by a 5.5 ha mixed orchard (apple, pear, plum, cherry). Its address was: Felszabadulás út 1/a, where it functioned up to 31 December 1977, when the City Council expropriated the area for housing purposes. In that Laboratory opportunity was given to carry out modern experimental insect ecological researches. Within a short time the researchers working

Table 1

Order and species	References
Homoptera	
Quadraspidiotus (= Aspidiotus) *pyri* Lichtenstein	Szelényi (1936)
Coccoidea spp.	Sheta (1968) Kozár (1975, 1977)
Coleoptera	
Zabrus tenebrioides Goeze	Kadocsa (1944)
Psylliodes chrysocephala Linné	Sáringer (1984a)
Subcoccinella vigintiquatuorpunctata Linné	Szelényi (1944) Csehi (1964) Ali (1979) Ali et al. (1974a, b) Ali and Sáringer (1975)
Leptinotarsa decemlineata Say	Jermy (1951, 1958, 1961a, b, 1972) Jermy and Sáringer (1954, 1955a, b, c, 1957, 1959)
Phytodecta fornicata Brüggemann	Rakk (1974)
Crioceris spp.	Szabolcs (1975)
Colaphellus sophiae Schaller	Sáringer (1960a, b, c. d, 1961a)
Bruchus pisorum Linné	Baranyovits (1944)
Acanthoscelides obtectus Say	Jermy (1952) Szentesi (1972) Szentesi and Jermy (1973)
Tanymecus dilaticollis Gyllenhal	Sáringer (1954) Sáringer and Móritz (1966) Takács (1973)
Sitona humeralis Stephens	Nádasy (1984) Nádasy and Sáringer (1985, 1986a, b)
Phytonomus (= Hypera) variabilis Herbst	Sáringer (1967a) Sáringer and Deseő (1966) Boraei (1984) Boraei and Sáringer (1981, 1982, 1983)
Ceuthorrhynchus quadridens Panzer	Sáringer (1967b, 1978)
Ceuthorrhynchus assimilis Paykull	Sáringer (1967b)
Ceuthorrhynchus macula-alba Herbst	Sáringer (1970a, b, 1976a, 1979)
Curculio elephas Gyllenhal	Bürgés (1972) Bürgés and Gál (1981a, b)
Lepidoptera	
Leucoptera malifoliella Costa	Sáringer et al. (1985, 1986a, b, c)
Plutella maculipennis Curtis	Czencz (1973a, b)
Scrobipalpa ocellatella Boyd	Bognár (1952) Huzián (1962)
Grapholitha delineana Walker	Sáringer and Nagy (1971, 1975)
Grapholitha molesta Busck	Deseő and Sáringer (1973, 1975a, b, c)

Table 1 (continued)

Order and species	References
Grapholitha funebrana Treitschke	Sáringer (1967c, 1970c, d, 1971a, 1972a, 1982a, 1984b) Sáringer and Deseő (1968, 1972) Sáringer, Wégh and Rada (1968) Sáringer and Szentkirályi (1980) Deseő and Sáringer (1970, 1975a) Deseő et al. (1971)
Laspeyresia (= Cydia) pomonella Linné	Szelényi et al. (1953) Bognár (1960, 1962) Jermy (1964, 1967) Sáringer (1971b, 1977, 1982b) Deseő and Sáringer (1975a, b, c)
Ostrinia nubilalis Hübner	Csou-Jü-Csön (1961) Nagy (1961, 1970) Mészáros (1965, 1969) Sáringer (1956, 1976b, 1980a)
Hyphantria cunea Drury	Nagy (1952, 1953) Nagy et al. (1953) Szalay—Marzsó (1955, 1957) Jermy and Sáringer (1955a) Kozár (1971) Sáringer (1974a)
Diptera	
Rhagoletis cerasi Linné	Sáringer (1972b)
Hymenoptera	
Athalia rosae Linné	Sáringer (1957, 1961b, 1964, 1967b, d, 1974b, 1980b, 1983a, b, 1984c) Sáringer and Kacsó (1977)
Athalia glabricollis Thomson	Sáringer (1958, 1961c, 1966)
Hoplocampa spp.	Nagy (1954, 1957, 1960)
Acarina	
Panonychus ulmi Koch	Jenser (1969) Kozár (1974)
Tetranychus urticae Koch	Bognár (1978)

there acquired international fame and their results attracted visitors: researchers from France, USA, Egypt, Soviet Union, German Federal Republic, German Democratic Republic, Lebanon, etc. Almost all leading insect ecologists of Europe spent some time in the Laboratory.

The experimental insect ecology, as a new experimental science born in the Keszthely Laboratory, has grown to full proportions in Hungary, and the results

achieved there have had an effect on all institutions (Universities of Agricultural Sciences, County Stations of Plant Protection) concerned with problems of applied entomology. The methods elaborated in the Keszthely Laboratory are known and applied all over the country. These methods and the results attained have had a decisive influence on the way the questions are put in the entomological research of plant protection, on its work hypothesis, etc. As a result of our work information on the achievements of the experimental insect ecology has been included in the curriculum of agricultural higher education. The general rules of insect ecology are now taught in a considerable number of lectures.

Of insect ecological experiments with the major pests carried out in the past nearly 40 years. Table 1 gives a survey without any claim to completeness. To show the working style and results of experimental insect ecology in Hungary several experiments related with the diapause of insects – an important ecological subject – are described below in some detail; then through the interpretation of these results the importance of experimental insect ecology in agricultural entomology is pointed out.

Most of the experiments were carried out with *Grapholita funebrana* Tr. The results can be briefly summarized as follows:

1. In South-West Hungary (Keszthely, latitude of 46° 46′, longitude of 17° 14′) the *G. funebrana* Tr. flies three times a year, in other words, it has an overwintering and two summer generations. The overwintering generation begins flying at the end of April or in the first days of May. Fifty per cent of the overwintering generation fly out between 10 and 15 May. The flight ends at the end of June – beginning of July. The flight of the first summer generation begins in the last days of July and lasts to the end of September. Under laboratory conditions four generations could be raised.

2. Data obtained in the course of evaluating the results of experiments with larvae raised at a steady temperature (18 ± 1.4 °C, 23 ± 1.2 °C and 28 ± 0.9 °C) with varying photoperiods (12/12, 13/11, 14/10, 15/9, 16/8 and 17/7 hours of light/dark (LD)) show that the diapause of *G. funebrana* Tr. larvae setting in at a mature stage is determined by the light conditions prevailing during the larval development. The so-called critical time of illumination, decisive for the induction of the diapause is between 14 and 15 hours. Days shorter than 14 hours induce a 100 per cent diapause. Day-lengths between 15 and 17 hours, on the other hand, greatly inhibit the development of larvae in diapause. It is remarkable, that with 17 hours of light (considered under Hungarian conditions an extremely long day) the diapause still ranges between 25 and 55 per cent. This relatively high diapause percentage indicates that the species originally had a single generation; even now a considerable part of the population has but one generation a year.

Of larvae raised under constant illumination and at laboratory temperature 57.3 per cent ramined in diapause.

Changing temperatures somewhat modify the photoperiod conditions decisive for the diapause.

3. The length of the light period important for the induction of the diapause is perceived by the larvae in the second and third development stage.

4. Temperature shortens to some extent the diapause induced by the photoperiod, but this effect compared to that of the photoperiod is negligible.

5. The diapause is not affected by the number of generations, but the ripening stage of the fruit in the critical period of light has some influence on it.

6. The qualitative differences (ripe, unripe) of various fruits (Besztercei plum, greengage, Jonathan apple, cherry, sour-cherry, apricot, peach, summer pear) had no demonstrable effect on the diapause of the plum piercer (in laboratory).

7. According to our investigations in the field the mass diapause of *G. funebrana* Tr. started in the last week of July. After 15 August 100 per cent of the population was in diapause. Some difference could be found between larvae developing in greengage and in Besztercei and Olaszkék plum varieties, respectively, as to when and in what numbers they went into diapause. Larvae developing in greengages ripening early in August began diapausing earlier and in a higher percentage than those growing in the two plum varieties.

8. The appearance of the adults in spring has nothing to do with the generation to which the larvae belonged in the previous year nor with the month in which they went into diapause.

9. If we subject the diapause percentage and the change in day-length to examination we shall arrive at the conclusion that the larva population which develops from the last decade of June will gradually go into diapause due to the decreasing day-length. Larvae developing from 21 June with an effective day-length of 16 hours and 59 minutes will receive less light from day to day. This day-length steadily decreasing in tendency results in a 100 per cent diapause of larvae developing after 15 August. On the area examined the time of illumination critical for the diapause reaches its upper limit on 20 August (Sáringer, 1967, 1970).

The laboratory observation that a short light impulse given in the scotophase more or less prevents the diapause from setting in can be put down as the most recent achievement of the photoperiod research (Bünning and Joerrens, 1960; Bünning, 1969; Adkisson, 1963, 1965, 1966; Barker et al., 1964; Aschoff, 1966; Ankersmit, 1968; Sullivan et al., 1970; Pittendrigh and Minis, 1971; Bonnemaison, 1973; Saunders, 1976; Beck, 1980; Zaslavsky, 1984; Tauber et al., 1986). On this line we carried out investigations with three species: *Grapholita funebrana* Tr., *Laspeyresia pomonella* L. and *Athalia rosae* L.

Our investigations aimed at determining the stage of scotophase in which the diapause percentage can be reduced by illumination for a shorter or longer time. The practical aspect of these investigations is that in the nature, at the end of summer, during the larval development the diapause of larvae could be prevented by illumination in certain periods at night (that is in the scotophase) whereby for the non-diapausing population unfavourable conditions would be produced (e.g. in autumn a lack of place for egg laying, nutrient deficiency, or temperatures catastrophic for the animal, etc.), which would reduce the individual, number of the next generation.

The result of our investigations made so far can be outlined as follows:

1. The diapause percentage of *Grapholita funebrana* Tr. larvae decreased by 25 and 20 per cent, respectively, in response to one hour of illumination 3 and 7 hours after the beginning of the scotophase (Deseő et al., 1971).

2. In the case of *Laspeyresia pomonella* L. one hour of illumination in each of the 4th, 5th and 8th hour from the beginning of the scotophase reduced the number of diapausing larvae to a particularly great extent (in the 5th hour by 72 per cent). And a one hour illumination one or two hours before the beginning of the photophase hindered in nearly 30 per cent the onset of the diapause (Sáringer, 1982).

L. pomonella L. larvae raised at 28 ± 0.9 °C were exposed to half an hour of illumination in the scotophase. The decrease in diapause percentage showed a tendency similar to that with the one-hour illumination, though its values were lower (maximum 26 per cent).

3. The diapause percentage of *Athalia rosae* L. larvae exposed to a half-hour illumination between 11.30 and 12 p.m. as well as between 3.30 and 4 a.m. decreased by 47–56 per cent. This practically meant that an average of 50 per cent of the adult population of *A. rosae* L. flying out in October no longer found rape plants *(Brassica napus* L.) suitable for egg laying. And even if they found suitable host plants, the embryos or the possibly hatched larvae were destroyed by the low temperature. In any case, a considerable reduction of individual number took place (Sáringer, 1983a, b).

According to the results of the above described experiments there is a possibility of influencing the diapause in the species examined by controlling the photoperiod conditions during the larval development. The application in practice requires, however, further series of experiments.

The results listed briefly in the foregoing can perhaps make it perceptible how many important questions are answered by an experimental insect ecological investigation carried out with up-to-date work hypothesis and methods, which through a mere observation in the field could never be solved.

And not only statements of local validity can be made; by getting acquainted with the ecological demands of the different populations we may be given explanation for the geographical distribution of species as well. We shall know e.g. whether it was from the south or from the north that the population of a species started. Also, we shall receive an answer to the question whether in certain places the given species will find living. In the case of quarantine animals this is an extremely important information from the point of view of plant protection. And we may enumerate further conclusions that can be drawn from the results of insect ecological experiments.

Perhaps in the framework of this paper we have succeeded in making the readers acquainted with the methods and some results of the experimental insect ecology, a new experimental science developed and introduced by us in Hungary. We believe that this branch of biology, – and entomology within – it must not be missing from the scientific repertory of a country where famous predecessors

haven been working—and well-known contemporaries are working at present too. In the age of molecular biology, ecology—among others—has as good a reason for existing as any of the other biological sciences—as pointed out by Kendrew (1966), an English pioneer of molecular biology.

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Effect of Mosquito Control by Helicopter on the Fauna of the Ecosystem Treated in Hungary (Short communication)

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A successful chemical method has been developed whereby the number of mosquitos has been reduced to a tolerable level. The arthropodous population of the untreated adjacent areas can make up for the loss of animals forming the ecosystem.

In Hungary mosquito control by helicopter is carried out in a number of resort districts. Present paper gives account of the mosquito control around Lake Balaton, the most important resort district of Hungary, and of its influence on the ecosystem.

Today everybody in Hungary is fully aware of the fact that the earlier practice of mosquito control with "ground machines", which as a matter of course was restricted to small areas, no longer fulfils the demands of civilized recreation. That is why in 1976 the most up-to-date way of control: to distribute the mosquitocide with helicopter, was put on the agenda.

Up to 1981 the material used for mosquito control was an insecticide named Cytion ULV, containing malathion as active agent. Its structural formula is: 0,0-dimethyl-S-(1,2 dicarbetoxy-etil)-dithiophosphate. It is a mosquitocide recommended by the World Health Organization of the United Nations Organization (WHO), with a very wide range of action, equally efficient against sucking and chewing insects. Similarly to parathion it is a compound of deep action, that is sprayed on the leaf surface it penetrates into the leaf tissues, but since it is not further translocated it has no systemic effect. Of all phosphoric acid insecticides known so far it is the least poisoning. Its acute oral LD_{50} value is 940 mg/kg for male, and 1200 mg/kg for female rats. Its half-period is 8 ± 2 h. The amount to be used is 0.6 l per hectare.

In 1982 the treatments were carried out with K-Othrin 1 ULV, a compound containing deltametrin, a synthetic pyrethroid. The composition of the material as expressed in weight percentage is 0.12 per cent deltametrin, 0.06 per cent butyl-hydroxy-toluol, 11.74 per cent isophorone and 88.08 per cent liquid petrolatum. Its half-period is about 4 days. Its acute toxicity for male rats is 129 mg/kg.

The compound was distributed by a KA-26 type helicopter furnished with ULV spray heads. Spraying took place in the small hours and late in the afternoon. The amount distributed was 0.6 litres per hectare. The falling drops of spray were about 30–40 micron in diameter.

The pilots were given precise maps of the areas to be treated. According

to the prescriptions the helicopter must not have come nearer than 100 m to the shore in order to prevent the chemical from getting into the water. Reeds—if wider than 50 metres—could be sprayed, but a 100 metre zone from the water had to be left untreated. From 1982 onwards a total area of some 11,800 hectares has been treated on each of the five occasions a year between the end of June and the end of August. The Lake Balaton resort area was divided in 18 sectors; a single treatment for the total area took 3 days.

After the above few data of control technics we now are giving a brief account of the results obtained in the course of surveying the biological effects of the treatments.

According to the results of mosquito collecting regularly carried out since the early seventies 16–20 species occur on the shores of Lake Balaton. In 1982, for example, we collected 19 species. The species flying with the largest individual numbers, making 71.7 per cent of the total population was *Aëdes vexans* Meig., followed by *Coquillettidia (Mansonia) richiardii* Fic. with a 17.74 per cent occurrence. *Aëdes annulipes* Meig. had a 1.80 per cent—, *Aë. sticticus* Meig. a 3.08 per cent—and *Aë. cinereus* Meig. a 2.77 per cent share in the population. The percentage frequency of further 14 species was below 1 per cent. The mentioned 5 species represented 98.6 per cent of the mosquitos collected while biting (Datas of Dr. S. Tóth).

The average decrease in the individual number of mosquitoes on the southern shore, for example, showed in 1982 the following trend: treatment I. 88.0 per cent, treatment II. 86.3 per cent, treatment III. 87.7 per cent, treatment IV. 86.9 per cent, treatment V. 65.8 per cent. As seen from the data, on the southern shore the fifth treatment (between 20 and 22 August) had the poorest effect (65.8 per cent), while after the previous four treatments the destruction of the mosquito population was nearly 90 per cent. This value commes close to those observed earlier on the southern shore.

In consequence of the poor results of treatment V the daily press expressed disapproval of the helicopter control of mosquitoes at Lake Balaton. However, the newspaper articles left it out of consideration that the relatively poor effect proved advantageous to the ecosystem of the lake, because the *Chironomidae* population whose swarming period coincides with that of the mosquitoes "escaped" destruction. The *Chironomidae* are extremely useful, partly as a food for fish, partly because the larvae of certain species develop in the mud where they take up large quantities of phosphorus decreasing thereby the phosphorus load of the lake when swarming out.

Before the treatments and 24 hours after 50–60 thousand specimens of *Arthropoda* were collected at eleven sites. In this way the harmful effect of spraying could be approximately determined. The material was sorted according to order. In general 60 per cent of the collected population consisted of flies *(Diptera)* followed by leaf aphids *(Aphidinae)* with 10 per cent. Hymenoptera represented 8 per cent of the population, and the individual number of all the other orders: beetles *(Coleoptera)*, moths *(Lepidoptera)*, etc. remained below 5 per cent. In

1982 65–74 per cent of the arthropodal population living in the sprayed zone perished. In earlier years when spraying was carried out with insecticides containing malathion as active agent this value ranged between 85 and 95 per cent. From this point of view K-Othrin proved to have a protective effect. In our estimation on killing one mosquito destruction of 100–150 arthropodous specimens should be reckoned with. At first sight it seems to be a rather negative effect, but there is a chance that the fauna will be regenerated in a short time from the areas left out of the treatment. The danger of extinction only threatens the rare species, but considering that at the time of spraying only a definite number of specimens of one or another development stage (eggs, larvae of different development stage, pupae, adults) of the species are present in the stratum (air or vegetation) possibly reached by the spray, the probability of eradicating all individuals at every development stage of the species is very low.

To follow up the regeneration of the fauna after the treatments we have observed since 1976 three frequent cicada species: *Macrosteles laevis* Rib., *Psammotettix alienus* Dhlbm. and Philaenus spumarius L. in the same place. According to the observations the individual numbers though considerably reduced after the treatment, almost reach the original value by the time of the next treatment.

On two occasions during the five treatments in 1978 malathion carried into the water by the wind considerably reduced the individual number of the crab plankton. According to the investigations the regeneration of the crab plankton takes 10–14 days. Regeneration may, however, be due not only to the self-reproduction of the populations of the different species, but also to immigration from the open water.

In 1982 destruction of zooplankton organisms could not be proved.

To sum up, we can safely say that the helicopter control of mosquitoes at Lake Balaton hit the target, since the 5–8 million people visiting the lake a year could spend their holidays undisturbed by mosquitoes. However, the effect exercised on other insect populations of the ecosystem of Lake Balaton cannot be judged as positively as that. Still, on the basis of a continuous biological check-up made parallel with the treatments we must say that we have succeeded in developing a chemical control method whereby the number of mosquitoes has been reduced to a level where they no longer vex the visitors, and at the same time we have every reason to suppose that the arthropodous population of the untreated adjacent areas can make up for the loss of animals forming the ecosystem.

On the basis of experiences gained in the summers of the past ten years it can be established that for the time being no undisturbed recreation on the shores of Lake Balaton can be imagined without a successful mosquito control. But it has also been proved that its dangerous effect on the environment cannot be neglected. It follows that further areas must be made free from chemization and new, less drastic methods of control searched after. Such attempts are experiments in process with juvenile hormone preparations (Dr. L. Varjas) and with the *Bacillus thüringiensis* var. *israelensis* (H-14 serotype) (Dr. L. Szalay-Marzsó).

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Synthesis and Bioactivity of Cyclic Analogues of the Sex Pheromone of the European Grapevine Moth, *Lobesia botrana* (Den. et Schiff.)

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The synthesis and biological activity of a series of cyclic analogues of (7*E*, 9*Z*)-7,9-dodecadien-1-yl acetate, the sex pheromone of the European grapevine moth, *Lobesia botrana*, are reported. Field experiments, conducted in different vine-districts in Hungary, revealed that none of the compounds were either attractants or inhibitors. One of the compounds: methyl (*Z*)-8-(1-cyclopentenyl)-7-octenoate, however, showed synergistic activity on the attractiveness of traps baited with either virgin females or synthetic sex pheromone compositions.

Insect sex pheromones have been widely used in monitoring and detection as well as for mating disruption of economically important insect pests. The sex pheromone components of moth and butterflies are usually mono- or polyunsaturated, straight-chain aliphatic alcohols, acetates and aldehydes. Parallel with the isolation and identification of substances in sex pheromone blends, works to find pheromone analogues which would either mimic or inhibit the effect of the natural chemical have also begun. Among the various pheromone analogues devised so far, cyclic (Chapman et al., 1978; Schmidt et al., 1980), aromatic (Prestwich et al., 1984), alkyl-branched (Bestmann et al., 1979), chain-elongated analogues (Liljefors et al., 1985) formate (Mitchell et al., 1976), and alkene (Tatsuki and Kanno, 1981) derivatives are such examples.

The sex pheromone of the European grapevine moth, *Lobesia botrana* (Den. and Schiff.), a serious pest of vineyars in Europe and some parts of Africa and Asia, was isolated and identified as (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate (*1*) (Roelofs et al., 1973; Buser et al., 1974; Descoins et al., 1974). In Hungary, the moth has generally two or three generations per year (Voigt, 1980; Voigt, 1983; Voigt and Újváry, 1984) and frequent insecticide treatments are needed throughout the season to control it. Pheromone-baited traps have been used in IPM programs for monitoring populations of this insect and the use of special formulations of the synthetic pheromone for mating suppression experiments has also been reported (Roehrich et al., 1977; Roehrich and Carles, 1979).

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During our search for analogues of (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate *(1)* (Fig. 1), we prepared and tested a series of cyclopentene derivatives. Here we

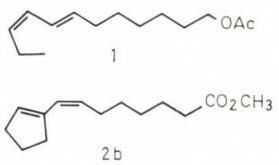

Fig. 1. Structures of (7*E*,9*Z*)–7,9-dodecadien-1-yl acetate (*1*), the sex pheromone of the European grapevine moth, *Lobesia botrana* and its cycloalkenyl analogue *2b*

report the synthesis of the compounds and the results of field experiments which have revealed that some of the compounds show synergistic activity on the attractiveness of the pheromone.

Materials and Methods

Synthesis

IR spectra were obtained with a Pye Unicam SP-3-200 spectrometer. ^{1}H-NMR spectra were determined in $CDCl_3$ at 60 MHz on a Hitachi Perkin–Elmer R-24/A spectrometer. Chemical shifts were measured against TMS as an internal standard. Mass spectra were measured at 74 eV with a JEOL-20K and JMS-01SG-2 combined GC-MS system. The crude products obtained after conventional work-up procedures were purified by preparative column chromatography on Kieselgel 60 (0.063–0.2 mm; REANAL) using benzene or benzene/ethyl acetate (10 : 1) as eluent. Gas-liquid chromatography was performed on a Chrom 4 chromatograph fitted with 3% OV-1 on Chromosorb WHP column (3 mm ID × 1.2 m), 140 °C, using nitrogen carrier gas.

The synthesis of the cyclic analogues of the pheromone *1* was accomplished as shown in Figure 2. The key reaction is the stereoselective Wittig reaction of 1-cyclopentenecarbaldehyde (*3*) and the phosphonium salt of heptanoic acid esters *4a* or *4b*.

(6-Ethoxycarbonyl)hexyl-triphenylphosphonium bromide (4a). This compound was obtained by reacting triphenylphosphine and ethyl 7-bromoheptanoate, prepared by esterification of 7-bromoheptanoic acid (Ames et al., 1950). IR($CHCl_3$): 1730, 1440, 1100, 995 cm^{-1}; ^{1}H-NMR: δ 1.1–2.0 (11H,m), 2.3 (2H, t, J = 6 Hz), 3.4–4.4 (4 H, m), 7.5 (15 H, bs).

(6-Methoxycarbonyl)hexyl-triphenylphosphonium bromide (4b). This phosphonium salt was prepared as above from triphenylphosphine and methyl 7-bromoheptanoate. IR($CHCl_3$): 1725, 1590, 1485, 1440, 1240, 1110, 1000 cm^{-1}; ^{1}H-NMR: δ 1.3–2.0 (8 H, m), 2.3 (2H, t, J = 6 Hz), 3.4–3.9 and 3.5 (5 H, m + s), 7.5 (15 H, bs).

(Z)-8-(1-Cyclopentenyl)-7-octenoic acid ethyl ester (2a). This substance was obtained as a 94 : 6 mixture of the corresponding *Z* : *E* isomers by Witting reaction as the methyl ester *2b*. IR(neat): 2930, 2850, 1740, 1175, 1030, 960, 815 cm^{-1}; ^{1}H-NMR: δ 1.0–2.7 (16 H, m), 4.0 (2 H, q, J = 6 Hz), 4.9–5.8 (3 H, m); MS: M^+ 236 *m/e*.

(Z)-8-(1-Cyclopentenyl)-7-octenal (5). Partial reduction of the ethyl ester *2a* by diisobutylaluminium hydride at −78 °C in hexane gave the corresponding aldehyde *5*. IR(neat): 3005, 2930, 2850, 2710, 1730, 960, 820 cm^{-1}; ^{1}H-NMR: δ 1.2–2.7 (16 H, m), 4.9–6.0 (3 H, m), 9.45 (1 H, t, J = 6 Hz). MS: M^+ 192 *m/e*.

(Z)-8-(1-Cyclopentenyl)-7-octenoic acid methyl ester (2b). Wittig reaction of 1 cyclopentenecarbaldehyde (*3*) with the phosphorane generated from phosphonium salt *4b* by potassium *tert*-butoxide in tetrahydrofuran at −78 °C gave a crude product which was shown to be by glc as a 90 : 10 mixture of the *Z* and *E* isomer *2b* and *2c* (retention times are 5.52 min for the *Z*, and 6.60 min for the *E isomer*). Pure *2b* was obtained in 40% yield. IR(neat): 3005, 2940, 2850, 1740, 1640, 1250, 1150, 965, 820 cm^{-1}; ^{1}H-NMR: δ 1.2–1.6 (16 H, m), 3.55 (3 H, s), 4.85–5.85 (3 H, m). MS: M^+ 222 *m/e*.

(E)-8-(1-Cyclopentenyl)-7-octenoic acid methyl ester (2c). This compound was prepared from the *Z* isomeric ester *2b* by thiophenol catalyzed isomerization (Henrick et al., 1975). The pure ester *2c* contained less than 2% of the corresponding *Z* isomer as shown by glc. IR(neat): 1740, 1640, 1250, 1150, 960, 820 cm^{-1}; ^{1}H-NMR: δ 1.1–2.3 (16 H, m), 3.55 (3 H, s), 5.0–6.3 (3 H, m). MS: M^+ 222 *m/e*.

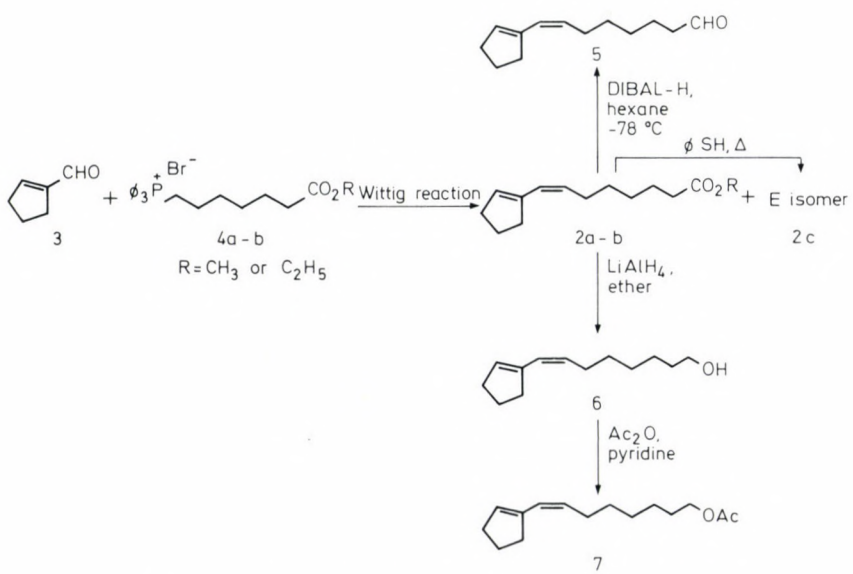

Fig. 2. Synthesis of 8-(1-cyclopentenyl)-7-octenoic acid derivatives

(Z)-8-(1-Cyclopentenyl)-7-octen-1-ol (6). Reduction of ester *2b* by $LiAlH_4$ in diethyl ether gave the alcohol *6*. IR(neat): 3400, 2930, 2860, 1640, 1060, 1040, 960, 820 cm^{-1}; ^{1}H-NMR: δ 1.0–2.4 (17 H, m), 3.48 (2 H, t, J = 6 Hz), 5.1–5.9 (3 H, m).

(Z)-8-(1-Cyclopentenyl)-7-octen-1-yl acetate (7). Alcohol *6* was acetylated by acetic anhydride in pyridine to give the acetate *7*. IR(neat): 1740, 1640, 1240, 960, 820 cm^{-1}; ^{1}H-NMR: δ 1.4–2.5 (16 H, m), 2.1 (3 H, s), 4.0 (2 H, t, J = 6 Hz), 5.1–5.9 (3 H, m).

Field experiments

Field trials were conducted in vineyards at two different wine-growing areas in Hungary (Etyek and Kecskemét) during the 1979–1982 flight seasons. White, plastic-board, sticky traps (REANAL, Budapest) were baited with two natural rubber septa. One of them was impregnated with the test chemical (1.0 mg) and the other with the synthetic pheromone formulation. The traps were placed at a height of 120–130 cm and approx. 30 m apart in a randomized complete block design with 3–5 replicates. Captures were recorded weekly. The following synthetic pheromones were used:

(1) ZOECON EGM (commercial formulation);
(2) INRA formulation (Saint-Remy-Les-Chevreuse; 1.0 mg of *1*);
(3) EGYLURE (EGIS, Budapest; 1.0 mg of *1* (98% *7E,9Z* isomer content) (Újváry et al., 1985).

In the initial experiments, traps baited with five virgin females, held within small wire screen cages and changed every 3–5 days, were also used.

Results and Discussion

Recently, there has been a growing interest in the synthesis and bioassay of analogues of pheromones which serve as useful tools in structure-activity studies and also hold out promises of practical application. Among studies aimed at understanding the underlying principles of the unique sensitivity and selectivity of insect olfaction, the method of chain-branching has been used most extensively (Bestmann et al., 1979; Bestmann et al., 1980). Though conformation-fixing of flexible aliphatic compounds by insertion of a ring into a carbon chain has proven to be useful in structure-activity studies of biologically active compounds, there have been only a few reports on its application in pheromone chemistry (Chapman et al., 1978; Schmidt et al., 1980; Prestwich et al., 1984).

Accordingly, cycloalkenyl derivatives *2a–c* and *5–7*, as analogues of *1*, the sex pheromone of *L. botrana* were synthesized and evaluated for potential pheromonal activity. Although these compounds cannot be regarded as the

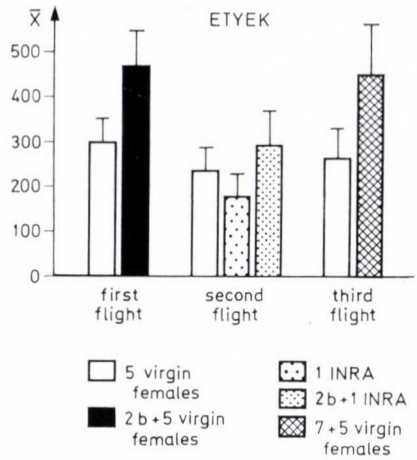

Fig. 3. Effect of cyclic pheromone analogues *2b* and *7* on attractancy of traps baited with virgin females or synthetic pheromones of *Lobesia botrana* (Etyek, April–September 1979)

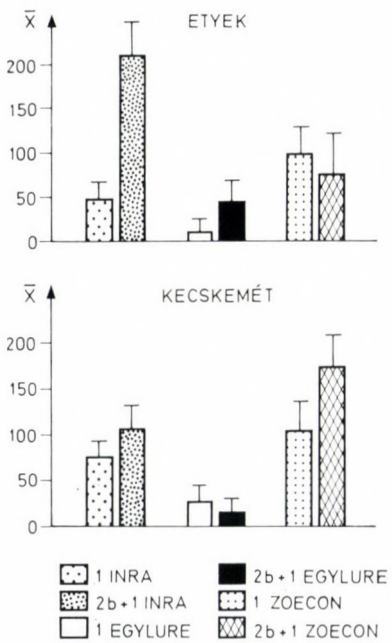

Fig. 4. Effect of cyclic pheromone analogue *2b* on attractancy of traps baited with synthetic pheromones of *Lobesia botrana* (Etyek and Kecskemét, first flight, April–May 1981)

"strictest" analogues of *1*, interaction of pheromone receptors was speculated. In field tests, compounds *2a*, *2c*, *5* and *6* showed neither inhibitory nor synergistic effect on the attractiveness of traps baited with five virgin females. However, the presence of either *2b* or *7* in traps baited with virgin females increased trap captures significantly (Fig. 3). None of the compounds attracted *L. botrana* males by themselves alone (dose: 1–10 mg; data not shown). Compound *2b* was selected for further detailed studies. The results of these experiments are shown in Fig. 4. While markedly increased catches (about 5-fold) could be observed in traps containing the INRA or EGYLURE pheromone preparations and the synergist, compound *2b* did not enhance the attractivity of the ZOECON formulation in Etyek. In Kecskemét, however, only the ZOECON pheromone could be synergized.

In 1982, similar experiments were conducted with the EGYLURE formulation and two different doses (1.0 and 10.0 mg) of compound *2b* (Table 1). Again, maximal synergistic effect, i.e. a catch increase of up to 6 times was observed in Etyek. In Kecskemét, the low infestation does not allow any meaningful comparison of the catches.

Interestingly, only the 7*Z* isomers were active, the 7*E* isomer (*2c*) with its exocyclic double bond having the same configuration as the one in *1* had no activity. (Discussing the genetic heterogenicity of the wild *L. botrana* population in Italy, Arsura et al. reported (1979–80) that the (7*Z*,9*Z*) isomer of *1* attracted the males of this species, but its possible isomerization to the (7*E*,9*Z*) isomer could not be ruled out. The pheromonal activity of the (7*Z*,9*Z*) isomer of *1* has not been studied in Hungary.) The most pronounced synergistic effect was observed for traps baited with either virgin females or the INRA pheromone. In the case of pheromones obtained from other sources the results were not so consistent.

Although the effect of the active compounds cannot be explained as yet, our results suggest that interaction of a pheromone receptor protein with both

Table 1

Mean captures of *Lobesia botrana* males using traps baited with dispensers containing synthetic pheromone *(1)* formulation (EGYLURE) and different doses of compound *2b* (first flight, April 29–May 20, 1982)

Composition of attractant (mg)		Mean number of males per trap*	
1	*2b*	Etyek	Kecskemét
1.0	—	25a	2c
1.0	1.0	158b	7c
1.0	10.0	133b	18d

* Means followed by the same letter are not significantly different at the 95% probability level according to the Student-Neuman-Keuls test.

the planar, electron-rich conjugated double bond and the polar ester group of the natural pheromone and its cyclic analogues *2b* and *7* is important for binding. However, the fact that neither the acetate *7* nor the "reverse" ester *2b* show any activity by themselves, points to a more complex problem of interpreting the synergistic action. (It is worth mentioning that the sex pheromone blend was shown to be composed of one main component *(1)* only with a minor amount (<3%) of other (isomeric) compounds (Buser et al., 1974).) According to our knowledge, no other substances which enhance the attractiveness of the pheromone *1* are known. This finding, while confirming the feasibility of designing rigid, cyclic pheromone analogues, provides a new tool for the investigation of insect olfaction and might find practical application as well.

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Application of Modified Silica Gels in the Pesticide Analysis II. Thin-Layer Chromatography of Benzonitrile and Triphenylmethane Derivatives*

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Retention behaviour of four modified silicas was tested in adsorptive and in reversed-phase chromatography applying 4-cyanophenylesters and triphenylmethane derivatives as solutes. The data were evaluated by principal component analysis. The retention characteristics of silicas depended both on the quality of eluents and solutes, however, the mode of separation (adsorptive or reversed-phase) had the highest impact on the retention behaviour of silicas.

Modified silicas have found growing acceptance and application not only in high performace liquid chromatography (El Rassi and Horváth, 1984) but also in thin-layer chromatography (Brinkman, 1985). Silicas are generally modified by covalently bonded hydrocarbons (Bien-Vogelsang et al., 1984), by hydrothermal treatment (Ohmacht and Matus, 1984) or by pyrolizing macromolecules on silica surface (Juvancz et al., 1984). As the quantity and quality of modifying agents and the original adsorptive character of supports influence the retention also after modification (Gonnet and Marichy, 1985) it is reasonable to expect that the retention of various solutes will be different on different modified silicas.

The objectives of our work were to prepare some new silica derivatives and to evaluate their retention characteristics.

Materials and Methods

The sorbent to be modified was Kieselgel HF_{254} (Merck, FRG). Two pyrolized (silicas 1 and 2) and two chemically bonded variants were prepared as described in references Juvancz et al., 1984 and Cserháti et al., 1983 respectively. The silylating agents were:

N-aminoethyl-aminopropyl-triethoxi-silane: $NH_2C_2H_4NHC_3H_6Si(OC_2H_5)$ for silica 4 and
dimethyl-silane-propionic-acid-lactone: $(CH_3)_2Si(CH_2)_2CO$ for silica 5.

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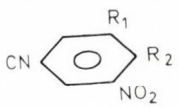
Untreated Kieselgel HF_{254} served as control (silica 3).
Thin-layer chromatographic plates of 0.25 mm thickness were prepared on 20×20 cm glass supports.

The chemical structure of solutes are compiled in Figs 1 and 2. The 4-cyanophenylesters and triphenylmethane derivatives were dissolved at 2 mg/cm^3 concentration in acetone and ethanol respectively, 5 mm^3 of each solution was spotted on the plates. The 4-cyanophenylesters and triphenylmethane derivatives were developed in acetone: benzene 1 : 1 vol. and in CCl_4 respectively (adsorption chromatography), the triphenylmethane derivatives were also developed in ethanol : water 7 : 3 vol. (reversed-phase chromatography). After development the plates were dried and the spots were detected by their visible or UV adsorption.

To extract maximal information from the retention data, each data matrix was evaluated by principal component analysis: PCA (Mardia et al., 1979).

General structure

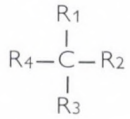


No. of compounds	R_1	R_2
1	H	O—CO—CH_3
2	I	2,4-D
3	Br	O-2,4-D
4	I	O—CO—CH_3
5	I	O—CO—O—C_2H_5
6	Br	O—CO—O—C_2H_5
7	H	O—CO—OCH_3
8	Cl	O—CO—OCH_3
9	Cl	2,4-D
10	Br	O—CO—O—CH_2—OCH_3
11	Br	O—CO—CH_2—CH=CH_2
12	Br	O—CO—O—C_6H_5
13	Br	O—CO—OCH_3
14	Cl	O—CO—CH_3
15	Cl	2,4-D
16	Br	O—CO—CH_3

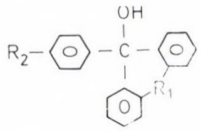
Fig. 1. Chemical structure of 4-cyanophenylesters 2,4-D =CO-CH_2-O- 

General structures

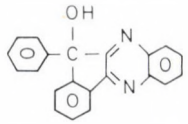
for compounds 1–15.



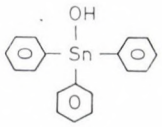
for compounds 16–21.



for compound 22.



for compound 23.



No. of compounds	R_1	R_2	R_3	R_4
1	phenyl	phenyl	phenyl	H
2	phenyl	phenyl	phenyl	OH
3	phenyl	phenyl	phenyl	2-imidazole
4	phenyl	phenyl	phenyl	1-triazole
5	phenyl	phenyl	2-pyridine	OH
6	phenyl	phenyl	3-pyridine	OH
7	phenyl	2,4-dichlorophenyl	5-pyrimidine	OH
8	phenyl	phenyl	4-chlorophenyl	OH
9	phenyl	4-chlorophenyl	2-pyridine	OH
10	phenyl	phenyl	2-chlorophenyl	2-imidazole
11	phenyl	phenyl	2-chlorophenyl	OH
12	phenyl	4-methoxyphenyl	3-pyridine	OH
13	phenyl	phenyl-SO_2-phenyl	phenyl	OH
14	phenyl	phenyl	phenyl-SO_2-phenyl	OH
15	4-methoxyphenyl	4-methoxyphenyl	3-pyridine	OH
16	$-C_2H_4-$	H		
17	$-C_2H_4-$	Cl		
18	$-C_2H_4-$	OCH_3		
19	$-O-CH_2-$	H		
20	$-O-CH_2-$	OCH_3		
21	$-CH_2-S-CH_2-$	Cl		

Fig. 2. Chemical structure of triphenylmethane derivates

The principal component loadings and variables were visualized by the nonlinear mapping technique (Sammon, 1969).

To evaluate the role of various solutes in the determination of retention characteristics of various modified silicas linear correlations were calculated between the coordinates of the nonlinear maps of PC loadings.

Results and Discussion

The R_f values of 4-cyanophenylesters are shown in Table 1. The highest retention was observed on the untreated silica proving that the modifications decreased considerably the number and/or the adsorptive strength of free silanol groups. The pyrolized silicas showed higher retention than the chemically modified ones suggesting that chemical bonding is a more effective procedure to influence the retention characteristics of silica surface. The correlation matrix of PCA (Table 2) clearly shows that the various surface treatments of silica modify considerably not only the retention strength but also the selectivity. Only the two pyrolized silicas show similar retention orders, the others differ from each other in their retention behaviour. The results of principal component analysis (Table 3) indicate the existence of common background variables responsible for the 40, 29 and 17% of the change of R_f values on all the five silicas. The PC loadings

Table 1

R_f values of 4-cyanophenylesters on various modified silicas

Eluent: acetone : benzene 1 : 1

No. of compound	No. of silica				
	1	2	3	4	5
1	0.61	0.74	0.89	0.98	1.00
2	0.55	0.65	0.44	0.97	0.91
3	0.64	0.68	0.40	0.93	0.83
4	0.59	0.66	0.44	0.96	1.00
5	0.57	0.64	0.44	0.96	1.00
6	0.30	0.32	0.38	0.98	1.00
7	0.47	0.55	0.90	0.94	1.00
8	0.52	0.60	0.33	0.93	1.00
9	0.55	0.62	0.33	0.94	0.72
10	0.51	0.58	0.37	0.95	1.00
11	0.53	0.58	0.37	0.97	1.00
12	0.49	0.52	0.36	0.97	1.00
13	0.49	0.56	0.37	0.95	0.60
14	0.66	0.54	0.34	0.98	0.82
15	0.48	0.54	0.33	0.96	0.80
16	0.49	0.55	0.34	0.95	0.67

Table 2

Correlation matrix of R_f values of 4-cyanophenylesters

Eluent: acetone : benzene 1 : 1. Numbers indicate modified silicas

	2	3	4	5
1	0.822	0.090	−0.168	−0.077
2		0.319	−0.326	0.020
3			0.077	0.365
4				0.158

Table 3

Results of principal component analysis

4-cyanophenylesters, eluent: acetone : benzene 1 : 1

No. of principal components	Eigenvalues	Sum of variance explained, %
1	2.01	40.29
2	1.44	69.02
3	0.83	85.54
4	0.60	97.56

Table 4

Principal component loadings

4-cyanophenylesters, eluent: acetone : benzene 1 : 1

No. of silica	No of principal components			
	1	2	3	4
1	0.88	−0.12	0 36	0.18
2	0.96	0.02	0.08	0.02
3	0.35	0.75	−0.15	−0.54
4	−0.43	0.45	0.78	−0.02
5	0.01	0.81	0.25	0.53

(Table 4) prove again the similarity of pyrolized silicas (high loadings in the first principal component) and the weaker similarity of silicas 3 and 5 (high loadings in the second principal component). The two dimensional nonlinear map of PC loadings (Fig. 3) supports our previous conclusions, the pyrolized silicas are similar in their retention behaviour, the others deviate considerably from each other indicating different retention behaviours. Considering simultaneously the retention of 4-cyanophenylesters on all the five silicas (Fig. 4) they do not form

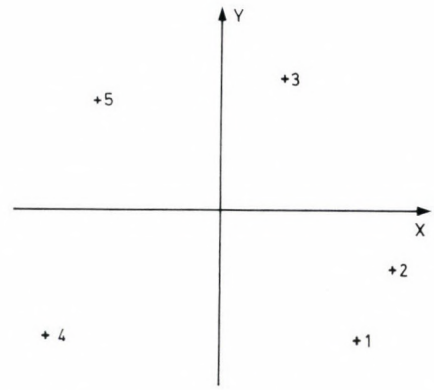


Fig. 3. Two-dimensional nonlinear map of PC loadings. Numbers indicate silicas. 4-cyanophenylesters, eluent: acetone : benzene 1 : 1

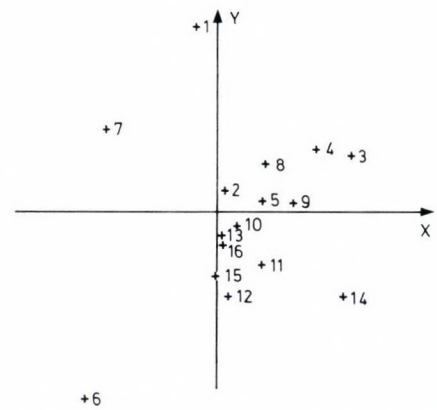


Fig. 4. Two-dimensional nonlinear map of PC variables Numbers indicate 4-cyanophenylesters in Table 1

clusters neither according to the quality of halogen substituent (iodo derivatives: comps 2, 4 and 5, bromo derivatives: comps 3, 6, 10, 11, 12, 13 and 16, chloro derivatives: comps 8, 9, 14 and 15) nor according to the quality of organic substituents. This finding makes it probable that the halogen and organic substituents have similar impact on the retention therefore their effect cannot be separated. Only comps 1 and 7 containing hydrogen in position R_1 show different retention behaviour that is in our case the number of substitution exerts a higher impact on the retention than the type of substituent.

The R_f values of triphenylmethane derivatives in adsorptive thin-layer chromatography are compiled in Table 5. The components remained on the start are excluded from the Table. Similarly to the retention behaviour of 4-cyano-

Table 5

R_f values of triphenylmethane derivatives on various silicas
Eluent: CCl_4

No. of compound	No. of silica				
	1	2	3	4	5
1	0.80	0.81	0.67	1.00	0.82
2	0.05	0.07	0.04	0.28	0.90
3	0.03	0.03	0.06	0.15	0.83
4	0.03	0.04	0.07	0.45	0.11
5	0.02	0.03	0.02	0.55	0.61
8	0.07	0.10	0.06	0.29	0.80
9	0.03	0.05	0.02	0.62	0.62
11	0.21	0.30	0.17	0.78	0.85
16	0.09	0.16	0.08	0.34	0.83
17	0.10	0.17	0.08	0.28	0.82
18	0.03	0.05	0.02	0.27	0.83
19	0.06	0.09	0.05	0.20	0.82
20	0.02	0.03	0.02	0.17	0.81
21	0.04	0.06	0.03	0.18	0.82

Table 6

Correlation matrix of R_f values of triphenylmethane derivatives
Eluent: CCl_4. Numbers indicate silicas

	2	3	4	5
1	0.986	0.996	0.754	0.172
2		0.982	0.758	0.217
3			0.745	0.115
4				−0.169

Table 7

Results of principal component analysis
Triphenylmethane derivatives, eluent: CCl_4

No. of principal components	Eigenvalues	Sum of variance explained, %
1	3.64	72.74
2	1.10	94.67
3	0.25	99.70

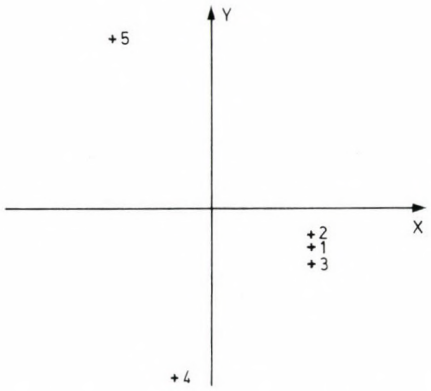


Fig. 5. Two-dimensional nonlinear map of PC loadings. Numbers indicate silicas. Triphenylmethane derivatives, eluent: CCl_4

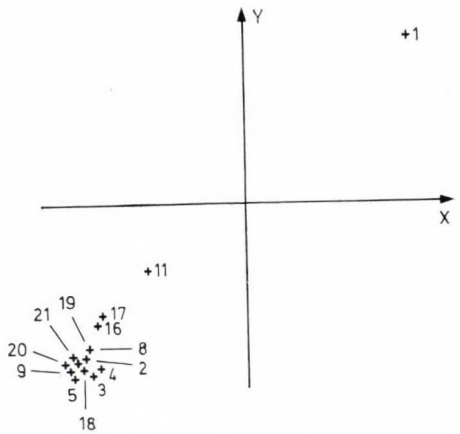


Fig. 6. Two-dimensional nonlinear map of PC variables. Eluent: CCl_4. Numbers indicate triphenylmethane derivatives in Table 2

phenylesters the triphenylmethane derivatives were more strongly retained by the untreated and pyrolized silicas proving again that the chemical bonding modifies more effectively the silica surface than the pyrolitic treatment. The correlation matrix of R_f values of triphenylmethane derivatives (Table 6) shows that – oppositely to 4-cyaniphenylesters – the retention orders are highly similar on untreated and pyrolized silicas, they show acceptable similarity with the retention order on silica 4, but they differ considerably from that of silica 5. The stronger relationship between the retention behaviour of various silicas is also manifested in the results of PCA (Table 7). In this case the first two principal components explain the overwhelming majority of variance. The principal component loadings prove again

the similar retention of silicas 1, 2, 3 and 4 (Table 8). The two dimensional non-linear map of PC loadings differs from that of 4-cyanophenylesters (Fig. 5), the retention characteristics of pyrolized silicas are nearer to that of untreated control. This finding indicates that the similarities and differences in the retention behaviour

Table 8

Principal component loadings

Triphenylmethane derivatives, eluent: CCl_4

No. of silica	No. of principal components		
	1	2	3
1	0.99	0.06	−0.12
2	0.99	0.10	−0.07
3	0.98	0.01	−0.18
4	0.84	−0.36	0.41
5	0.14	−0.98	0.17

Table 9

R_f values of triphenylmethane derivatives on various modified silicas

Eluent: ethanol : water 7 : 3

No. of compounds	No. of silica				
	1	2	3	4	5
2	0.93	0.72	0.93	0.08	0.80
3	0.95	0.65	0.94	0.09	0.78
5	0.95	0.75	0.94	0.09	0.63
6	0.94	0.76	0.94	0.09	0.67
7	0.98	0.73	0.97	0.08	0.75
8	0.95	0.72	0.93	0.09	0.79
9	0.97	0.72	0.96	0.08	0.64
10	0.94	0.76	0.95	0.08	0.25
11	0.98	0.70	0.96	0.09	0.79
12	0.98	0.76	0.96	0.10	0.67
13	0.93	0.65	0.95	0.08	0.80
14	0.95	0.77	0.93	0.10	0.81
15	0.95	0.65	0.93	0.08	0.62
16	0.94	0.72	0.94	0.08	0.78
17	0.98	0.68	0.93	0.09	0.78
18	0.97	0.73	0.96	0.10	0.78
19	0.93	0.77	0.95	0.08	0.79
20	0.97	0.77	0.95	0.08	0.78
21	0.93	0.72	0.97	0.08	0.78
22	0.94	0.77	0.94	0.08	0.77
23	0.93	0.72	0.93	0.08	0.80

of modified silicas depend considerably on the test conditions (types of solutes and eluents). The two dimensional nonlinear map of PC variables (Fig. 6) shows that the non substituted (comp. 1) or with —OH substituted derivatives (comp 2) differ the most strongly in their retention behaviour. Compared with them the other differences are negligible.

Table 10

Correlation matrix of R_f values of triphenylmethane derivatives

Eluent: ethanol : water 7 : 3. Numbers indicate silicas

	2	3	4	5
1	0.103	0.191	0.365	0.040
2		−0.061	0.195	−0.162
3			0.186	−0.061
4				−0.076

Table 11

Results of principal component analysis

Triphenylmethane derivatives, eluent: ethanol : water 7 : 3

No. of principal components	Eigenvalues	Sum of variance explained, %
1	1.57	34.41
2	1.15	54.32
3	0.97	73.76
4	0.70	87.72
5	0.61	100.00

Table 12

Principal component loadings

Triphenylmethane derivatives, eluent: ethanol : water 7 : 3

No. of silica	No. of principal components				
	1	2	3	4	5
1	0.72	0.29	0.26	−0.64	−0.51
2	0.41	−0.68	0.33	0.50	−0.10
3	0.48	0.42	−0.62	0.46	−0.02
4	0.78	0.02	0.14	−0.20	0.57
5	−0.22	0.65	0.62	0.35	0.12

Th R_f values of triphenylmethane derivatives under reversed-phase conditions are compiled in Table 9. As comp. 1 was in each case on the start it was not included in the calculations. The lowest retentions were observed on silicas 1 and 3, the highest one on silica 4. This order of retention strength deviates considerably from that observed in adsorptive TLC. This finding is easily understandable when we take into consideration the opposite retention mechanism of the two chromatographic systems. The retention orders on different silicas do not show any correlation that is under reversed-phase conditions the differences between the modified silicas are higher than in adsorptive chromatography (Table 10). The high discrepancies between the retention characteristics of modified silicas under reversed-phase conditions is reflected in the results of PCA, the first

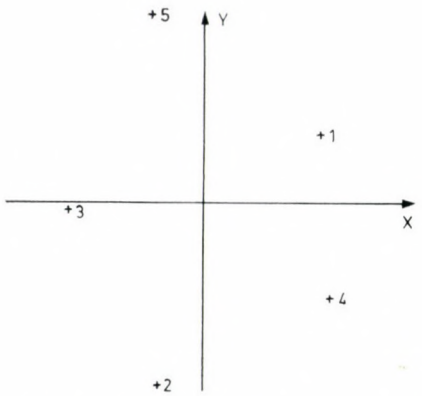


Fig. 7. Two-dimensional nonlinear map of PC loadings. Numbers indicate silicas. Triphenylmethane derivatives, eluent: ethanol : water 7 : 3

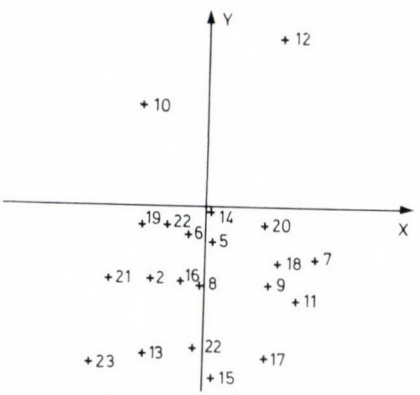


Fig. 8. Two-dimensional nonlinear map of PC variables. Eluent: ethanol : water 7 : 3. Numbers indicate triphenylmethane derivatives in Table 2

Table 13

Coordinates of the two dimensional nonlinear maps of PC loadings x_1 and y_1 for Fig. 5., x_2 and y_2 for Fig. 3., x_3 and y_3 for Fig. 1

No. of silica	x_1	x_2	x_3	y_1	y_2	y_3
1	0.65	0.45	0.62	0.35	−0.18	−0,60
2	−0.28	0.43	0.79	−0.95	−0.12	−0.28
3	−0.67	0.44	0.30	−0.03	−0.24	0.60
4	0.67	−0.16	−0.79	−0.48	−0.75	−0.58
5	0.26	−0.45	−0.56	0.95	0.75	0.50

Table 14

Correlation matrix of coordinates in Table 13

	x_2	x_3	y_1	y_2	y_3
x_1	−0.436	−0.420	0.237	−0.128	−0.696
x_2		0.908	−0.480	−0.409	−0.206
x_3			−0.328	−0.046	−0.129
y_1				0.701	0.457
y_2					0.609

principal component explains only 34% of the total variance (Table 11). These values were in adsorptive chromatography 40 and 72% for 4-cyanophenylester and triphenylmethane derivatives respectively. The principal component loadings (Table 12) indicate some similarities between silicas 1 and 2 (first principal component) and somewhat opposite retention behaviour of silicas 2–5 and 3–5 (second and third principal components). The two-dimensional nonlinear map of PC loadings (Fig. 7) supports entirely our previous conclusions, the silicas are situated far from each other. The two dimensional nonlinear map of PC variables (Fig. 8) shows that the triphenylmethane derivatives do not form clusters that means that the various substituents influence commensurably the retention behaviour and their effect counterbalance each other.

The coordinates of the two-dimensional nonlinear maps of the 3 sets of PC loadings and the coefficients of correlation between them are compiled in Tables 13 and 14 respectively. In most cases no linear correlations were found between the coordinates indicating that—taking into consideration simultaneously the retention behaviour of each compound—the silicas really differ from each other. The one significant correlation between the first coordinates of the two adsorptive systems suggests that the type of chromatographic separation (adsorptive or reversed-phase) has a prevalent role in the determination of retention characteristics of various modified silicas.

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Prediction of High Performance Liquid Chromatographic Behaviour with Thin-layer Chromatography

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The HPLC and TLC behaviour of 16 symmetric trisubstituted triazine derivatives was studied in ionic and in ion free eluent systems. The retention data were correlated with each other and with the molecular structure of triazine derivatives. Multilinear regression analysis proved that TLC had a considerable predictive power to calculate retention in HPLC.

Trisubstituted symmetric triazine derivatives are widely used in agricultural practice therefore extensive investigations have been carried out to separate them from their contaminations. Gas chromatographic (Tindle et al., 1968; Hörman et al., 1972; Greenhalgh and Cochrane, 1972; Thompson et al., 1975; Hofberg et al., 1975; Ramsteiner et al., 1974; Matisova and Krupcik 1977; Pacakova and Nemec, 1978; Pacakova and Kozakova, 1978; Matisova et al., 1979), high performance liquid chromatographic (Byast and Cotterill, 1975; Byast, 1975; Vitali et al., Byast, 1977; Jork and Roth, 1977; Dufek and Pacakova, 1980; Dufek et al., 1980) and thin-layer chromatographic methods (Stammbach et al., 1964; Abbott et al., 1965; Kondela, 1970; Henkel, 1964; Fishbein, 1967; Frei and Namura, 1967; Smith and Fitzpatrick, 1971) have been equally applied for their separation. In many cases the retention data were successfully correlated with the molecular parameters mainly with the lipophilicity of the substituents. As ions modify the solvate shell of substituents, decrease the adsorptivity of active sites and modify also the distribution of solvent components between the mobile and stationary phases, ion environments have been extensively applied to facilitate the separation of various solutes (Ragazzi and Veronese, 1977; Grippo et al., 1965; Chiang et al., 1969).

The objectives of our work were to investigate the effect of ion concentration on the chromatographic behaviour of some triazine derivatives and to study the correlation between the retention data and molecular structure under the influence of ion environment.

Materials and Methods

The chemical structure of trisubstituted symmetric triazine derivatives is shown in Table 1.

HPLC measurements were carried out on a Hewlett Packard 1084-A chromatograph at 255 nm wavelength. Stainless-steel columns (25 cm × 4.6 mm i.d.)

Table 1

Chemical structure of trisubstituted-s-triazines investigated

Number of compounds	Name	R_1	R_2	R_3
1	Ametryn	$-SCH_3$	-ethyl	-isopropyl
2	Atraton	$-OCH_3$	-ethyl	-isopropyl
3	Atrazine	$-Cl$	-ethyl	-isopropyl
4	Hydroxy-atrazine	$-OH$	-ethyl	-isopropyl
5	Prometryn	$-SCH_3$	-isopropyl	-isopropyl
6	Prometon	$-OCH_3$	-isopropyl	-isopropyl
7	Propazine	$-Cl$	-isopropyl	-isopropyl
8	Hydroxy-propazine	$-OH$	-isopropyl	-isopropyl
9	Terbutryn	$-SCH_3$	-ethyl	-tert.butyl
10	Terbumeton	$-OCH_3$	-ethyl	-tert.butyl
11	Terbuthylazine	$-Cl$	-ethyl	-tert.butyl
12	Hydroxy-terbuthylazine	$-OH$	-ethyl	-tert.butyl
13	Etazine	$-OCH_3$	-ethyl	-isobutyl
14	Hydroxy-etazine	$-OH$	-ethyl	-isobutyl
15	Simazine	$-Cl$	-ethyl	-ethyl
16	Hydroxy-simazine	$-OH$	-ethyl	-ethyl

Table 2

Parameters of the HPLC investigation of trisubstituted-s-triazines

Column	Number of systems	Mobile phase	
		Flow rate ml/h	Composition
RP-8	1	120	methanol : water 1 : 1
RP-8	2	120	methanol : 1N NaCl 4 : 1
Silicagel	3	90	ethyl acetate
Silicagel	4	90	ethyl acetate saturated with LiCl

Table 3

TLC conditions for the investigation of s-triazine derivatives

Layer	Eluent composition	Number of systems
Reversed phase	methanol : water 1 : 1	1
Reversed phase	methanol : 1N NaCl 1 : 1	2
Cellulose	methanol : water 1 : 3	3
Cellulose	methanol : 2N NaCl 1 : 3	4
Silicagel	chloroform	5
Silicagel prepared with 0.2N NaCl	chloroform	6
Aluminum oxide	toluene	7
Aluminum oxide prepared with 0.2N NaCl	toluene	8

were packed with LiChrosorb RP-8 (particle size 10 μm) and LiChrosorb SI 100 (particle size 10 μm) respectively. The other parameters are compiled in Table 2. The TLC conditions are listed in Table 3. The spots were visualized by the chlorotolidine reaction.

Results and Discussion

The retention data of triazine derivatives measured by HPLC are compiled in Table 4. On the RP-8 as on the RP-18 reversed phase the thiomethyl derivatives are the most strongly, the hydroxy derivatives the most weakly retained. The retention order is $-SCH_3 > -Cl > -OCH_3 > -OH$. The retention increases at the growing number of carbon atoms in the alkyl groups. The retention of the terc.-butyl group is greater than that of the i-butyl group.

The ion concentration increases the differences between the retention times (the slope of the linear correlation deviates from the theoretical $b = 1$ at the significance level of 95%) but the retention order is the same. The effect of the ion environment is the highest for the thiomethyl and the lowest for the hydroxy groups it increases with the growing number of the carbon atoms in the alkyl chain. Under adsorptive conditions the situation is somewhat different. The retention order of the polar groups is $-OH > -OCH_3 > -SCH_3 > -Cl$. Derivatives with highest number of carbon atoms in the alkyl chain are less

Table 4

Retention data of s-triazine derivatives in HPLC
(Retention time in minutes, averages of three parallel determinations)

Number of s-triazine	Number of mobile phase			
	1	2	3	4
1	2.37	2.55	2.05	2.08
2	2.14	2.26	2.51	2.63
3	2.17	2.03	2.01	2.01
4	1.82	1.85	✳	2.67
5	2.67	2.89	1.98	2.00
6	2.34	2.51	2.38	2.47
7	2.39	2.55	1.95	1.96
8	1.91	1.96	✳	2.77
9	2.75	2.96	2.02	2.04
10	2.42	2.59	2.46	2.53
11	2.44	2.60	1.98	1.98
12	1.94	1.97	✳	2.64
13	2.32	2.46	2.50	2.57
14	1.90	1.94	✳	2.63
15	1.99	2.08	2.11	2.10
16	1.71	1.74	✳	2.66

✳ : Very strongly adsorbed

Table 5

Linear correlations between the HPLC retention times (R_t) of s-triazine derivatives measured on different columns and with different mobile phases (1–4)
($y = a + bx$)

y	x	a	b	s_b	n	$r_{calc.}$	$r_{tabl.}$	$t_{calc.}$	$t_{tabl.}$	Number of functions
R_{t2}	R_{t1}	0.82	0.702	0.1341	16	0.8136	$r_{99.9\%} = 0.7420$	2.22	95% 2.15	I
R_{t1}	R_{t3}	3.29	−0.353	0.3482	11	0.3152	$r_{90\%} = 0.5214$			II
R_{t2}	R_{t4}	4.45	−1.027	0.3655	16	0.6004	$r_{98\%} = 0.5742$			III
R_{t3}	R_{t4}	0.28	0.8549	0.0208	11	0.9974	$r_{99.9\%} = 0.8471$	6.98	99.9% 4.78	IV

strongly retained reversely to the retention behaviour observed on RP-8 phase. Ion concentration increases the retention expect for hydroxy derivatives. This finding can be explained by the fact that ions decrease the neutralizing solvate shell around the solutes making them more accessible to the adsorptive sites. The effect is more expressed for methoxy group and it is negligible in the case of chloro group.

Table 6

R_f values of s-triazine derivatives in different TLC systems
(Averages of 5 parallel determinations)

Number of s-triazines	Number of systems							
	1	2	3	4	5	6	7	8
1	0.41	0.34	0.51	0.57	0.21	0.34	0.41	0.53
2	0.51	0.49	0.76	0.88	0.14	0.27	0.11	0.38
3	0.48	0.42	0.68	0.53	0.20	0.34	0.32	0.52
4	0.53	0.74	—	—	—	—	—	—
5	0.35	0.29	0.34	0.52	0.22	0.42	0.52	0.64
6	0.46	0.42	0.61	0.72	0.16	0.22	0.17	0.30
7	0.42	0.34	0.49	0.33	0.20	0.33	0.43	0.56
8	0.62	0.73	—	—	—	—	—	—
9	0.33	0.29	0.23	0.38	0.26	0.49	0.48	0.63
10	0.42	0.41	0.47	0.80	0.14	0.23	0.18	0.30
11	0.40	0.33	0.43	0.26	0.21	0.39	0.42	0.56
12	0.60	0.70	0.53	—	—	—	—	—
13	0.44	0.43	0.63	0.83	0.13	0.24	0.14	0.25
14	0.71	0.80	—	—	—	—	—	—
15	0.55	0.50	0.94	0.78	0.16	0.28	0.21	0.44
16	0.60	0.79	—	—	—	—	—	—

Table 7

Parameters of the linear correlations between the R_f values of s-triazine derivatives measured in different TLC systems
(R_f values are the averages of five parallel determinations)

$y = a + bx$
$n = 16$ $r_{99.9\%} = 0.7420$
$n = 11$ $r_{99.9\%} = 0.8471$ $r_{99\%} = 0.7348$ $r_{95\%} = 0.6021$

Number of systems							Number of functions
y	x	n	a	b	$r_{calc.}$	s_b	
1	2	16	0.23	0.523	0.9315	0.055	I
3	4	11	0.20	0.588	0.6396	0.236	II
5	6	11	0.04	0.453	0.9435	0.053	III
7	8	11	−0.18	1.043	0.9431	0.123	IV
1	3	11	0.26	0.322	0.9858	0.018	V
2	4	11	0.22	0.274	0.8030	0.068	VI
1	5	11	0.51	−0.409	0.2869	0.4556	VII
1	7	11	0.53	−0.321	0.7430	0.096	VIII
2	6	11	0.61	−0.476	0.7840	0.178	IX
2	8	11	0.57	−0.387	0.7262	0.122	X
3	5	11	1.16	−3.306	0.6829	1.179	XI
3	7	11	0.85	−1.101	0.7247	0.321	XII
4	6	11	1.20	−1.881	0.7884	0.489	XIII
4	8	11	1.19	−1.28	0.8174	0.300	XIV

The parameters of linear correlations between the retention times measured in different columns and with different mobile phases are compiled in Table 5. The correlation between the retentions in ion environment on RP-8 and on silica phase is a reverse one (significance level over 98%, Function III). It means that the most hydrophobic substituents are responsible for the retention on the RP-8 phase and they decrease the adsorption of the molecule on silica. The same correlation was not observed in ion free systems (Funct. II).

The R_f values and the parameters of linear functions between the R_f values of triazine derivatives in different TLC systems are given in Tables 6 and 7 respectively. On reversed phase the ions decrease the R_f value of methoxy, thiomethyl and chloro derivatives and increase that of hydroxy derivatives improving this way the separation of hydroxy derivatives from the other triazines. The retention order is the same for both systems (Table 7, funct. I).

On cellulose layer the retention order is different in ionic and in ion free systems, the ion concentration increases the R_f value of methoxy and thiomethyl derivatives but it decreases that of chloro derivatives. Positive correlations were found between the R_f values measured on the reversed phase and on the cellulose layer (Functs V and VI) and negative ones between the R_f values measured on

Table 8

Multilinear correlations between the HPLC (y) and TLC (x_1; x_2; x_3) behaviour of s-triazine derivatives

($y = a + b_1x_1 + b_2x_2 + b_3x_3$)
y: retention time (minutes)
x_1, x_2, x_3; R_f values
$n = 11$ $F_{99.9\%} = 15.83$ $F_{99\%} = 7.59$

Number of systems	Functions			
	I	II	III	IV
y (HPLC)	1	2	3	4
x_1(TLC)	1	2	1	2
x_2(TLC)	3	4	3	4
x_3(TLC)	5	6	5	6
b_1	−2.56	−4.46	0.83	−1.26
b_2	−0.29	0.65	−0.73	1.01
b_3	−0.24	0.22	−6.33	−1.26
a	3.68	3.79	3.39	2.50
r	0.9839	0.9563	0.9045	0.8788
F	70.9	25 02	10.49	7.91
b_1'%	71.28	67.43	11.81	22.10
b_2'%	24.51	28.66	31.66	52.29
b_3'%	4.21	3.91	56.53	25.61

inorganic adsorptive layers and on cellulose (Functs XI–XIV). It can be concluded that the adsorption of triazines on cellulose are governed mainly by hydrophobic interactions where the role of hydrophilic interactions is of minor importance. Our assumption is supported by the fact that similar correlations were found between the R_f values measured on reversed phase and on adsorptive layers (Functs VII–X). Ions adsorbed on silica and on alumina decrease the retention of all triazine derivatives but do not change the retention order (Functs III–IV).

The results of our calculations concerning the correlation between HPLC and TLC methods are compiled in Table 8. The behaviour of triazine derivatives in different TLC systems determines their behaviour also in HPLC (RP-8 phase: signficance level over 99.9%, silica: significance level over 99%).

The retention time of triazine derivatives on RP-8 phase depended mainly on the retention characteristics of reversed phase TLC (path coefficient 71%), moderately on the behaviour on cellulose layer (24.5%) and negligibly on the adsorptivity of the compound (4.2%). These results suggest that in our case the RP-8 phase can be considered to be a reversed phase with very low adsorptive side effects. Ions did not change markedly the situation outlined above. In ion free adsorptive HPLC the relative importance of the retention behaviours in various TLC systems is reversely related to that observed on RP-8 phase (adsorptivity on silica 56.5%, adsorptivity on cellulose 31.7%, lipophilicity 11.8%). Ion environment changes the relative importance of TLC data: adsorptivity on cellulose becomes the most important (52.3%) the other two factors have similar impact on the retention. This finding can be explained by the assumption that ions adsorbed on silica decrease its adsorptivity approximating it to that of cellulose.

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Book Reviews

Mihályi, F.: *Tachinidae–Rhinophoridae*. Fauna Hungarica 161. XV. Vol. Diptera II., Akadémiai Kiadó, Budapest, 1986. 425 p., 150 figs (Distributors: KULTURA, H-1389 Budapest, P O. Box 149)

The introduction of integrated control methods makes it imperative to know the parasites of species harmful to plants. Among the parasites the members of the family Tachinidae play an important role. The collapse of the gradation of Lymantria dispar L., which in certain years causes particularly great damages to forests, is due to several species of the family Tachinidae. The species of Tachinidae are parasites for the larvae and adults of lepidopterous pests as well as of those belonging to the orders *Coleoptera*, *Hemiptera*, (Tenthred.), etc.

The well-known dipterologist's book based on examinations of more than 26 000 specimens sums up the results of 15 years work.

The author identified 425 species on the present area of Hungary; further 54 species are known in the Carpathian basin. Taxonomic keys are given for 598 species including those expected to appear in Hungary from neighbouring and nearby countries. Data of distribution, gradation, and the name of the host animal can also be found with each species. The rich material of line drawings greatly facilitates the otherwise difficult task of identifying the species of this family.

The last 18 pages of the book contain taxonomic keys for the members of the family Rhinophoridae. The author has identified 13 species of this family in Hungary so far. As further 15 species are expected to appear, the book describes 28 species.

Gy. Sáringer

Zaslavsky, V. A.: Photoperiodic and Temperature Control of Insect Development. Leningrad: Nauka Verlag 1984. 188 S., 91 Abb., 12 Tab. Kart. (Russisch).

Der in Leningrad tätige Professor Dr. Victor A. Zaslavsky hat ein Aufmerksamkeit erregendes Buch geschrieben In englischer Sprache lautet der Titel: "Photoperiodic and temperature control of insect development".

In der Sowjetunion haben Forschungen auf dem Gebiet der Beziehungen zwischen Insektenentwicklung und Photoperiode sowie Temperatur große Traditionen. In der Weltliteratur war die Arbeit des Engländers A. D. Lees: "The physiology of diapause in Arthropods" (Cambridge monogr. exper. biol. 1955, 151 pp.) die erste, die die Ergebnisse auf diesem Gebiet zusammenfaßte. Dieser folgte die Arbeit von A. S. Danilewskii (Sowjetunion): "Photoperiodism and seasonal development of insects" (Oliver and Boyd, Edinburg and London, 1965, 283 pp.). Die russischsprachige Ausgabe erschien 1961 in Leningrad. Später schrieben der Amerikaner S. D. Beck: "Insect photoperiodism" (Academic Press, 1968, 288

pp.; 2. Auflage 1980, 378 pp.) und der Engländer D. S. Saunders: "Insect clocks" (Pergamon Press, 1976, 279 pp.; 2. Auflage 1979, 279 pp.) zusammenfassende Werke.

Die Arbeit von Herrn Zaslavsky besteht aus drei größeren Kapiteln. Es handelt sich dabei um die folgenden: 1. Reaktionen und Prozesse, die die jahreszeitliche Entwicklung der Insekten beeinflussen. 2. Allgemeine Charakterisierung des Kontrollmechanismus von Photoperiode und Temperatur bei der Entwicklung. 3. Modell physiologischer Mechanismen, Innerhalb der drei großen Kapitel werden die diesbezüglichen Kenntnisse in zahlreichen Unterkapiteln behandelt. In Kenntnis der Weltliteratur wird eine individuelle Konzeption angestrebt.

Ich bin davon überzeugt, daß dieses Buch bei Erscheinen in englischer Übersetzung ein genauso großer Erfolg wird wie seinerzeit das Werk von Professor Danilewskii und möchte daher dem Springer-Verlag empfehlen, das Buch von Professor Zaslavsky möglichst bald herauszugeben.

Gy. Sáringer

Tauber, M. J., Tauber, C. A. and Masaki, S.: Seasonal Adaptations of Insects. Oxford University Press, New York—Oxford, 1986. 411 pp. 16 figs, 3 tabs. ISBN 0-19-503635-2.

Seasonal changes is the development of insects were already observed by Aristotle. Yet, it is only since the first half of the 1920s that up-to-date scientific information on the subject has been available in the literature of entomology. The results of investigations into the subject up to the early fifties were summarized by Andrewartha (1952). The highly intensive research started in the subsequent period subjected to examination not only the ecological but also the physiological aspects of seasonal changes in insect development.

Papers on the seasonal development of insects have been published by Masaki, from the mid-fifties and by Tauber, M. J. and Tauber, C. A. from the end of the sixties. The present work of the three authors known all over the world gives a critical synthesis of almost the full material of world literature concerning the seasonal adaptations of insects. The book consists of 10 chapters covering 5 larger scopes of subject. Initially they deal with the long- and short-term environmental changes that insects encounter, and with how insects adapt themselves to these changes (Chapter 2). Then they focus on the types of seasonal changes that insects undergo, and discuss diapause as the major physiological adaptation underlying the seasonal expressions of dormancy, migration and polyphenism (Chapters 3 and 4). The diverse mechanism through which environmental and genetic factors influence seasonal cycles form the topic of the next part of the book (Chapters 5, 6 and 7). Subsequently they delve into the evolutionary implications of seasonal cycles (Chapters 8 and 9), and consider such topics as the evolution of diapause, the influence of seasonality on the evolution of life histories, and the importance of seasonal cycles in speciation. Finally, the last chapter (Chapter 10) deals with the use of phenological knowledge in applied ecology, specifically in insect pest management.

The bibliography contains 1899 literary references. Then the author index, a species index, and finally a subject index follows. The latter greatly help in finding the data and topics looked for. The list of references contains the papers published until 1985. Several clear tables and figures are inserted in the text.

It can be said that no summarizing work comparable to the authors' present book has so far been published in the world literature. University teachers, research workers, and last but not least university students can make much use of it.

Gy. Sáringer

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The Spermatozoa of *Thysanoptera* and their Relevance for Systematics

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The spermatozoa of Phlaeothripidae *(Cryptothrips, Haplothrips)*, Thripidae *(Thrips, Parthenothrips)*, Aeolothripidae *(Aeolothrips)*, and Merothripidae *(Merothrips)* have been examined by transmission electron microscopy, most of them also by phase contrast microscopy. The phlaeothripid spermatozoa exhibit some kind of modified biflagellarity, which is considered to be the plesiomorphic condition within Thysanoptera. The modified triflagellarity observed in the sperm of all other species examined is supposed to be a synapomorphic character of the terebrantian families. So the recent hypothesis that the traditional suborder Terebrantia should be abolished as paraphyletic is not supported by the results on sperm ultrastructure.

The cytological structure of metazoan sperm cells is not only complicated, but also variable. That is why their fine structure sometimes includes characters which are suited for the analysis of systematic relationships, following the rules of Hennig's phylogenetic systematics. Of course the analysis of sperm characters, which usually implies the use of electron microscopical techniques, is especially appropriate when the evaluation of other, easier accessible characters does not allow a clear decision for one of several phylogenetic trees.

This appears to be the situation in the systematics of Thysanoptera. Hennig (1969) had the suspicion that the traditional division into the suborders Terebrantia and Tubulifera might not reflect the true phylogenetic relationships. He suspected the Terebrantia to be a paraphyletic group in his terminology, i.e. the rest of a monophyletic group after exclusion of a part of it. Also Stannard (1968) and Schliephake (1975) – the latter after an analysis of morphological characters according to the rules of phylogenetic systematics – contended that the sister group of the Phlaeothripidae or Tubulifera should not be the Terebrantia as a whole, but instead only the Thripidae (Schliephake) or even only their subfamily Panchaetothripinae (Stannard). However, the most recent and most comprehensive cladistic analysis by Mound, Heming and Palmer in 1980 did not result in a clear decision, but instead in two alternative cladograms (Fig. 1). The first alternative, scheme A, returns to the traditional view of a basal division between the tubuliferan family Phlaeothripidae and all the rest of the families – seven in their classification – united in the suborder Terebrantia. The second alternative, scheme B, postulates a sister group relationship between

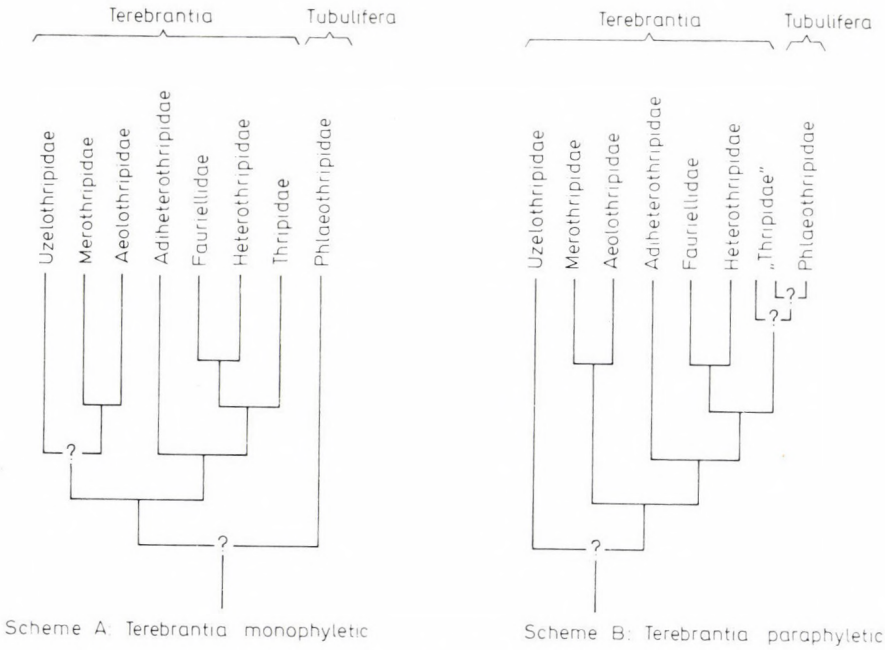

Fig. 1. Two alternatives for the phylogenetic relationships between the families of recent Thysanoptera according to Mound, Heming and Palmer 1980

the Phlaeothripidae and the Panchaetothripinae, that means the taxon Terebrantia ought to be abolished.

I don't want to comment in detail on the arguments for both hypotheses. Instead, in search for an exit from this dilemma, let us have a look at the sperm ultrastructure in Thysanoptera. The first study of this kind was made by Baccetti and co-workers in 1969, in the phlaeothripid species *Crytothrips nigripes*. The structure of Cryptothrips sperm is rather different from the so-called "typical" insect spermatozoa. Nucleus, acrosomal vesicle, mitochondrial derivative and the highly modified flagellar complex are situated more or less side by side, not one behind the other as usual. The elements are twisted in helicoid fashion. In the hexapodan ground plan, the sperm cells have a flagellum or axonema with a so-called "9 + 9 + 2" pattern, i.e. there are 9 additional peripheral singlet microtubules besides the familiar 9 + 2 arrangement of generalized cilia and flagella (Kristensen 1981). Instead, in *Cryptothrips*, we see a chaotically arranged bundle of 18 doublet microtubules without dynein arms and 4 singlet microtubules. The authors postulated that these sperm cells ought to be immotile, although they had no direct evidence for that.

It's not surprising that Baccetti and co-workers explained the 18 + 4 configuration in *Cryptothrips* sperm by addition of two 9 + 2 cilia with subsequent

loss of the normal arrangement, of the dynein arms and of the motility. They interpreted this as a particular form of biflagellarity. Sperm cells with two flagella are also observed in many Psocodea and at least in one species of Hemiptera; probably, they are a character in the ground plan of rhynchotoid insects or Acercaria (Kristensen 1981).

Electron microscopical investigation of the sperm cells in five other species of Thysanoptera has revealed structural differences whose knowledge appears to be useful for phylogenetical considerations.

Material and Methods

Phase contrast microscopy

Adult specimen of *Haplothrips*, *Thrips*, *Parthenothrips*, and *Aeolothrips* were dissected on a glass slide in a drop of insect Ringer solution or Grace's Medium. When the testes or spermathecae were isolated, a cover slip was placed upon them, and the organs were ruptured by slight pressure, so the living sperm cells were set free. They were observed in phase contrast with high magnification.

Transmission electron microscopy

The praeterminal abdominal segments of males (in *Thrips*, also of females and male nymphs) were fixed either in glutaraldehyde (mostly 3.5%, phosphate buffer) or in 3.5% osmium tetroxide in veronal acetate buffer. One *Merothrips* ♂ was kindly provided by Miss A. K. Walker (DSIR), Auckland/New Zealand. It was fixed in the field in 2% formaldehyde in phosphate buffer and sent to Bielefeld by air mail in this solution. The fixed tissue was dehydrated in graded series of ethanol and embedded in Araldite. The sections were cut with Reichert Om U2 or Ultracut ultramicrotomes, stained with uranyl acetate and/or lead citrate and examined with Siemens Ia, Zeiss EM 9A or Hitachi H-500 electron microscopes. For further details, see Bode 1983.

Results

Haplothrips aculeatus

In order to test the occurrence of spermatozoa with modified biflagellarity of the *Cryptothrips* type (cf. "Introduction") within the subfamily Phlaeothripinae of Phlaeothripidae, I studied the sperm ultrastructure in *Haplothrips aculeatus* (Fig. 3a). There is much resemblance to *Cryptothrips* sperm, but many repeated counts gave a total of 24 tubules in the axonemal complex; it probably consists of 18 doublets and 6 singlets (the precise determination of doublets and singlets is often difficult, because the tubules are not quite parallel and, above all, many

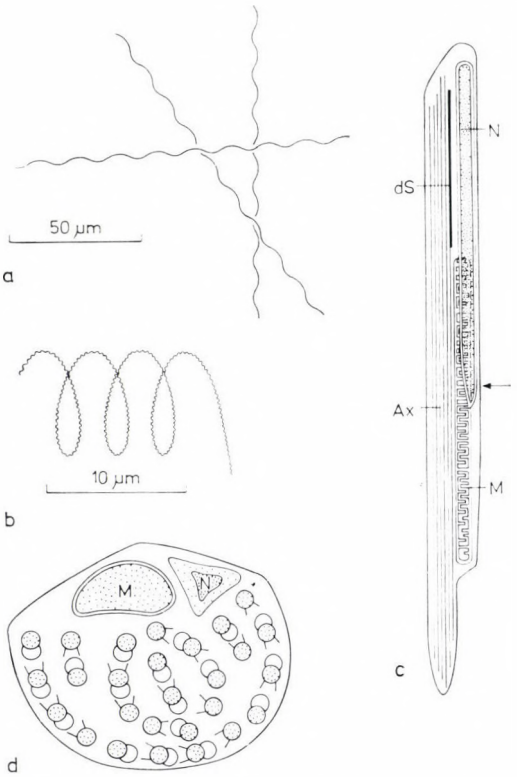

Fig. 2. a, b: Living sperm cells of *Haplothrips aculeatus* (a) and *Thrips validus* (b) as shown by phase contrast microscopy. The waveline in (b) points to the motility in *Thrips* sperm

c : Scheme of the relative position of organelles in *Thrips validus* spermatozoa, irrespective of the actual shape of the cell. N — nucleus, M — mitochondrial derivative, Ax — axonemal complex, dS — electron dense structure of unknown origin Arrow: approximate position of the cross section (d)

d : Scheme of a cross section of *Thrips validus* sperm. (cf. fig. 3b). Note the correlation of adjacent B-tubules and dynein arms

of the doublets are incomplete). That means not 2× (9 + 2), but 2× (9 + 3); so the starting point for this configuration of tubules should be two cilia with three central singlets instead of two. The corresponding 9 + 3 flagellum is very rare among insects, although common among arachnids. Whether the occurrence of 18 + 4 and 18 + 6 sperm in Phlaeothripids corresponds to the range of the subfamilies Idolothripinae and Phlaeothripinae, has still to be tested in other species. Living *Haplothrips* sperm cells show a strange cork-screw shape (Fig. 2a). They appear to be completely immotile indeed, as to be expected because of the disordered axonemal complex and the lack of dynein arms on the doublet microtubules.

Thrips validus

Sperm ultrastructure and the development of spermatids in *Thrips validus* has already been described in detail (Bode 1983). In contrast to the cork-screw rods of *Haplothrips* sperm, the spermatozoa of *Thrips* form a wide helix (Fig. 2b). They exhibit a certain kind of motility: small amplitude transversal waves run along the filamentous cell body, but don't result in conspicuous locomotion of the cell as a whole. The relative position of nucleus, mitochondrial derivative and axonemal complex (Fig. 2c) is reminiscent of the side by side arrangement of the organelles in phlaeothripid sperm, but in *Thrips* the acrosomal vesicle is entirely lacking (the rodlike electron dense structure (ds in Fig. 2c) is obviously not the acrosome, but a different structure of unknown significance).

Cross sections show clearly (Figs 2d, 3b) that the axonemal complex does not correspond to that in Phlaeothripidae. There is a characteristic arrangement of 18 doublets and 9 "singlets". Like half the doublets, the "singlet" microtubules

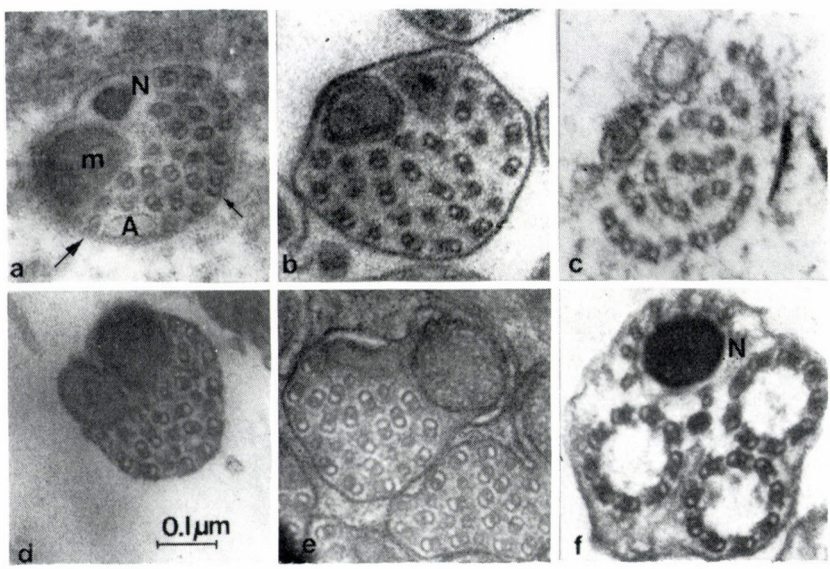

Fig. 3. Electron micrographs of cross-sectioned spermatozoa (f: spermatid) in several species of Thysanoptera

a : *Haplothrips aculeatus*
b : *Thrips validus* (cf. fig. 2d)
c : *Aeolothrips intermedius*
d : *Merothrips spec. (brunneus?)*
e : *Parthenothrips dracaenae*
f : *Thrips validus*, late spermatid in the late nymphal stage

The sections have roughly the same orientation: Mitochondrial derivative (m) at the left side of the nucleus (N). In *Haplothrips* spermatozoa (a), the doublets are sometimes incomplete (e.g. small arrow) or additional microtubules are simulated by small vesicles close to the acrosomal vesicle (A)

have dynein arms, so they must be residual A-tubules of a former doublet. By ultrahistochemistry, ATPase activity was detected in the region of the arms. This is consistent with the observed motility of the *Thrips* sperm cells.

We have to conclude that the basic pattern does not consist of 18, but of 27 doublets, without true singlets, which does not correspond to two, but instead to three cilia, in this case of the "9 + 0" type, i.e. without central singlets. This hypothetical mode of origin was confirmed by investigation of the structure of Thrips spermatids in various stages (for details and Figs, see Bode 1983). The spermatids in the early nymphal stage are endowed with three separate, partially free flagella. Then the development does not take the straight way: The spermatids merge into huge syncytial complexes with up to 42 nuclei and mitochondria and 126 cilia. They divide again in steps; in the late nymphal stage, we have mononucleate single spermatids again (Fig. 3f). The typical pattern of the axonemal complex is formed by a rearrangement of the doublet microtubules which proceeds from the posterior to the anterior end of the cell.

Aeolothrips intermedius

It is decisive for the question of monophyly of Terebrantia whether the sperm structure in other families corresponds to the modified biflagellarity of phlaeothripid sperm or to the modified triflagellarity seen in *Thrips*. A cross section of a spermatozoon of *Aeolothrips intermedius* (Fig. 3c) shows clearly (in spite of rather poor general fixation) that the same specific tubule pattern as in *Thrips* is present. Also the shape of living sperm cells is quite similar.

Merothrips spec. (brunneus?)

The cross sections of *Merothrips* sperm cells show again the same pattern of microtubules as in *Thrips* or *Aeolothrips* (Fig. 3d). Accidentally, I also detected a residual immature stage of spermatogenesis in the *Merothrips* testis, which reminds of the syncytial stages in *Thrips* nymphs. So the spermatid development in both genera appears to proceed in a similar way.

Parthenothrips dracaenae

As mentioned before, several cladograms have been published which are characterized by the hypothesis of a sister-group relationship of Phlaeothripidae and the subfamily Panchaetothripinae of Thripidae. For this reason, the ultrastructure of the panchaetothripine spermatozoa is particularly important. The electron micrographs of *Parthenothrips dracaenae* show that the number and arrangement of tubules in the axonemal complex correspond to the pattern of modified triflagellarity in other Terebrantia (Fig. 3e). Also the shape and motility of living *Parthenothrips* spermatozoa are much alike those in *Thrips*.

Discussion

In the opinion of the author, the known facts on sperm structure in Thysanoptera are only consistent with the hypothesis of monophyly of Terebrantia, for example with scheme A of Mound, Heming and Palmer (1980). In spite of several specialized characters, the sperm cells of Phlaeothripidae with their modified biflagellarity and the presence of an acrosome must be considered as plesiomorphic, as these characters are probably herited from the ground plan of the "rhynchotoid" insects or Acercaria. The very specific tubule pattern derived from three flagella observed in both subfamilies of Thripidae and in representatives of Aeolothripidae and Merothripidae must be considered as synapomorphy of the terebrantian families, in spite of the fact that the sperm of some families has not yet been studied. If we try to construct an alternative hypothesis, we have to postulate secondary loss of triflagellarity in phlaeothripids and subsequent return to a tubule pattern which corresponds at least numerically to a biflagellate condition. It is very unlikely that the evolution took this way.

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Comparative Examination of Thrips Populations on Perennial Grasses in Hungary

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The species spectra of thrips inhabiting different perennial grasses show great similarity to one another. However, when each grass genus was studied individually, ratios of thrips species and their individual numbers were found to be different from genus to genus. According to the results of the present work, differences in preference, in the case of perennial grasses can be attributed to a strong relationship between thrips feeding ethological, biological and morphological characters as well as the nutritive value, morphological and biological indices of the host plant.

The well-defined group of thrips, feeding and multiplying on grasses, was reviewed — under the name of grass thrips — by Wetzel (1964). In spite of the great similarity among grass thrips species spectra, remarkable differences are noticed when each grass genus is separately investigated. The observations about the conditions of thrips population dominancy of important fodder grasses — published by Mühle (1953), Schober (1959) and Franssen and Mantel (1964) — serve a good comparative base for the Hungarian investigations.

The population of phytophagous insects of a host plant is determined by its vegetative or generative characters, the ratio between its stem and leaves, the hairiness of its surface, nature of its internal contents, its digestibility, its taste, etc. In this respect, and as far as perennial grasses are concerned, the work of Klapp et al. (1953) is worth mentioning.

Grass thrips find their way to their host plant by the aid of their sensitivity to green colour (Kirk 1984; Czencz 1986b). Among the species, having different preference to vegetative or generative plant organs, thrips inhabiting flowers are helped by plant scents (Wetzel 1964; Czencz 1983 and Kirk 1985). The mechanical and chemical receptors play an important role in determining whether a plant is a host or non-host. Because of the claim of thrips to tigmotatic stimuli, hairiness of the plant surface is of a general favourable effect on their life activities (Putman 1965, in: Lewis 1973; Mikhailova 1981). Among the primary feeding stimulating plant materials, thrips respond to sugar content (Bailey 1941, in: Lewis 1973). The role of tasting and smelling in the choice of host plant was well demonstrated by Holtmann (1963) in his olfactometric experiments.

In the present work, a trial was made to determine the host plant orientations of already known Gramineae inhabiting thrips species (Jenser 1979) of the Hungarian fauna. This orientation was determined in ten perennial grasses which differ in their internal contents, flowering time and morphology.

Materials and Methods

Investigations of the present work were carried out in the western part of Hungary, where the annual mean temperature is 10.8 °C and the annual average amount of rainfall is 679 mm. Comparisons were made among thrips populations of ten perennial grasses. The experimental field consisted of closely situated grass-seed propagating plots. The area of each plot was 0.5 ha. By the time of investigations, (1985 and 1986) the tested perennial grasses were three and four years old. Thrips populations were investigated by weekly netting from April until harvesting time. Each netting consisted of ten net strokes. The morphological characters as well as the degree of individual growth of each tested grass was continuously registered. The ten investigated grasses were: Kentucky bluegrass, timothy, fiorin, gigant fiorin, English ryegrass, cock's foot, reed fescue, tall fescue, Hungarian brome, reed canary grass.

Results

Apart from the complete table of main data, the approached results will be reviewed in a suitable grouping according to the principal aim of the work.

The first column of Fig. 1 shows the distribution of the 9325 thrips individuals (collected and identified during the two experimental years) on the tested

Grass species	Total ind. number of thrips	Klapp index	Leaf		Grain length
			Transverse section	width (mm)	
Poa pratensis L.	1487	8		6-8	2-3
Phleum pratense L.	1423	8		7-10	2-3
Agrostis a. var. gig.	1269	7		7-10	2-3
Agrostis alba L.	1179	7		4	2
Dactylis glomerata L.	1056	7		6-9	5-7
Lolium perenne L.	819	8		4	5-7
Festuca rubra L.	687	4-5		1.5	5-6
Bromus inermis LEYS.	542	4		8	10-13
Festuca arundinacea SCHREB.	487	4		8-10	6-7
Phalaris arundinacea L.	412	5		12	3-3.5
Total:	9352				

Klapp index: 1-8 degrees of quality of grass plants

Fig. 1. Order of thrips populations of the examined perennial grasses (Keszthely, Hungary, 1985 and 1986)

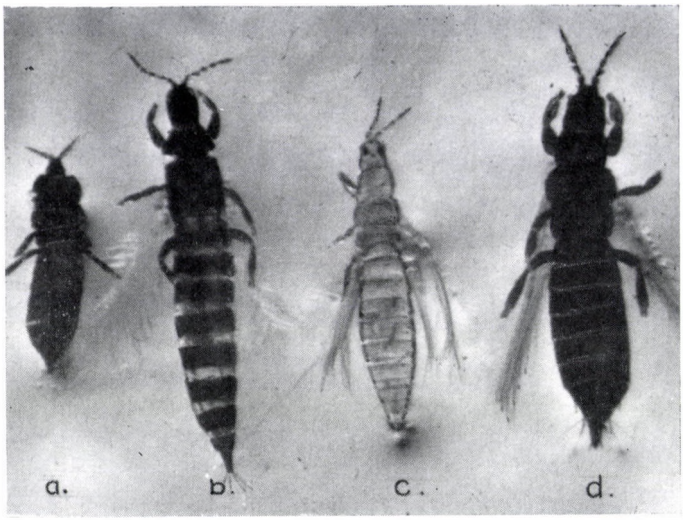

Fig. 2. Adults of Chirothrips manicatus (a), Haplothrips aculeatus (b), Anaphothrips obscurus (c), Limothrips denticornis (d)

Table 1

Order of absolute and relative individual numbers of thrips populations of the examined perennial grasses (Keszthely, Hungary, 1985 and 1986)

Thrips species	Total occurrence number		number of collect. (grass sp.)	Signif. degree
	absolute	%		
Chirothrips manicatus Hal.	5789	61.90	10	***
Haplothrips aculeatus Fabr.	1735	18.54	10	*
Anaphothrips obscurus Müll.	887	9.50	10	***
Limothrips denticornis Hal.	247	2.64	10	**
Aptinothrips rufus Hal.	185	1.98	7	*
Limothrips consimilis Priesn.	101	1.08	1	**
Frankliniella tenuicornis Uz.	56	0.60	7	ns
Frankliniella intonsa Tryb.	53	0.57	8	ns
Aeolothrips intermedius Bagn.	53	0.57	10	ns
Bolacothrips jordani Uz.	48	0.51	5	*
Stenothrips graminum Uz.	34	0.36	7	ns
Thrips angusticeps Uz.	33	0.36	7	ns
Thrips minutissimus Linn.	32	0.34	10	ns
Aeolothrips albicinctus Hal.	23	0.24	7	ns
Chirothrips hamatus Priesn.	19	0.20	4	ns
Thrips conferticornis Priesn.	12	0.13	4	ns
Rare grass thrips sp. (3)	12	0.13	—	ns
Other species (9)	33	0.35	—	ns
	9352	100.00		

*** P: 0.1%, ** P: 1%; * P: 5%; ns: not significant.

grass species. As a primary step for the explanation of similarities as well as differences in the total individual members of thrips, the tested grasses were analysed as fodder plants. We noticed a definite coincidence between the total thrips number and the value numbers established by Klapp and his co-workers (1953), by which they decreasingly (from 8 to 3) qualified the grass species. Their qualification was based on the internal contents, digestibility, taste, etc. of the grass. It is clear that the thrips individual number was higher on those grasses with high value number than that on e.g. the Hungarian brome which quickly turns woody and the Reed fescue having a bad ratio between its stem and leaves.

For understanding the differences in preference, surface area of leaf, its anatomical structure and width as well as the length of grain of each tested grass were investigated. The minimal differences among hairiness of the tested grass species proved to be of no effect on the individual number of thrips. On the other hand, the number and width of the conducting vessels and of the mechanically supporting elements was found to have a remarkable effect. In the case of grasses having big numbers of fibre bundles (e.g. Reed canary grass) or when the bundles are wide (e.g. Reed fescue), the spongy parenchyma tissue – suitable for thrips growth – becomes restricted into unfavourable narrow stripes. Accord-

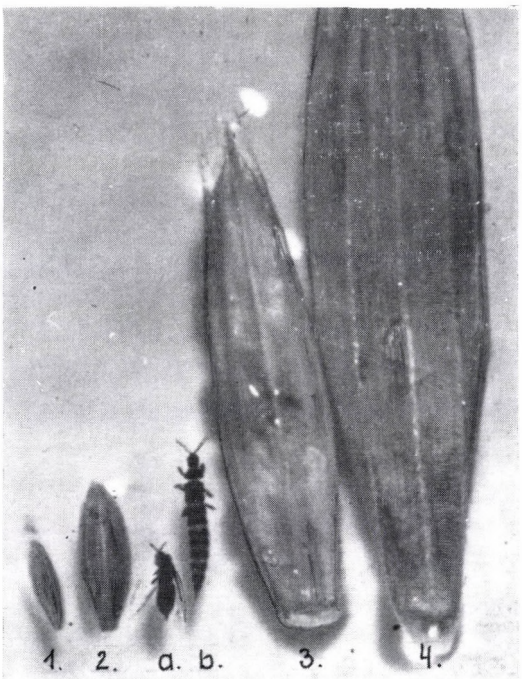

Fig. 3. Adults of Chirothrips manicatus (a), Haplothrips aculeatus (b) beside grains of its most favourable grass species (1. fiorin, 2. Kentucky bluegrass, 3. English bluegrass, 4. Hungarian brome)

Table 2

Preference of each examined thrips species to the tested grass species

Chirothrips manicatus Hal.			Haplothrips aculeatus Fabr.		
Grass species	ind. no.	%	Grass species	ind. no.	%
A.					
Poa pratensis	1211	20.9	Bromus inermis	277	16.0
Agrostis a. var. gig.	1033	17.8	Lolium perenne	235	13.5
Agrostis alba	993	17.2	Festuca arundinacea	220	12.7
Dactylis glomerata	886	15.3	Phalaris arundinacea	205	11.8
Phleum pratense	686	11.8	Phleum pratense	182	10.6
Festuca rubra	432	7.5	Festuca rubra	165	9.6
Lolium perenne	212	3.7	Poa pratensis	138	8.0
Phalaris arundinacea	137	2.4	Agrostis a. var. gig.	129	7.2
Festuca arundinacea	116	2.0	Dactylis glomerata	108	6.2
Bromus inermis	83	1.4	Agrostis alba	76	4.4
	5789	100.0		1735	100.0
		P: 0.1%			P: 5%

B. Dispersion of thrips species on the host plant

on generative organ = 99% (84) G = 95% (94)
on vegetative organ = 1% (16) V = 5% (6)

Wetzel, 1964 (Czencz, 1983)

ing to the width of their leaves and consequently diameters of sheathing leaf base as well as the length of their grains, the tested grasses were grouped into four size groups. When the examined grass species were arranged according to the total thrips population, the mentioned groups scattered. As far as the morphological characters are concerned, they were found to have a role only in the case when a single thrips species was concerned.

The absolute and relative individual numbers of thrips species, reported in the area of investigations, showed great differences in their values (Table 1). The dominant species, *Chirothrips manicatus* represented about 62% of the total number of the collected individuals. Considering the number of grass species as thrips collecting sites, it was noticed that some thrips species had occurred on all the ten examined grass species while others were reported on a fewer number of host plants. However, differences in attraction towards certain grass species was statistically verified only in case of thrips species having big numbers. The only exception was *Limothrips consimilis* which during the 2-year investigation period was reported on only one host, the Hungarian brome. The preference of this thrips to the mentioned host was statistically verified on the statistical level of 1%.

For further analysis of reasons of preference, four thrips species – which occurred in high or at least satisfactory individual numbers and in some cases showed remarkable differences in their mode of life and morphology – were chosen. Adults of the four chosen species are shown in Fig. 2. Among them, the dominant *Chirotrhips manicatus* is the smallest in size. According to earlier investigations, dealing with cereal plants, of the author, and those of Wetzel (1964) dealing with grasses, it is known that the individual development of the mentioned species takes place on the generative organs of the host plants (Table 2). From our examinations, it seems that this small species (0.8–1.1 mm body length), sensitive to the external influences, prefers grasses with small (2–3 mm) but numerous grains and having dense fine panicles, namely, the Kentucky bluegrass and species of Agrostis.

In the case of *Haplothrips aculeatus*, with its bigger body size and less sensitivity to injury and also developing on the generative plant organs, the order of preference is different. For its individual development, grass species with rough glumellae and bigger grains (6–13 mm) insure suitable conditions for its life. Because of the small differences in preference were only verified on the statistical level 5%. Adults of the two examined seed-pest thrips species are

Table 3

Preference of each examined thrips species to the grass species

Anaphothrips obscurus Müll.			Limothrips denticornis Hal.		
Grass species	ind. no.	%	Grass species	ind. no.	%
A.					
Phelium pratense	428	48.3	Phelium pratense	89	36.0
Lolium perenne	226	25.0	Lolium perenne	77	31.2
Poa pratensis	71	8.1	Bromus inermis	22	8.9
Agrostis a. var. gig.	54	6.2	Phalaris arundinacea	13	5.3
Agrostis alba	40	4.6	Dactylis glomerata	13	5.3
Dactylis glomerata	29	3.3	Festuca arundinacea	11	4.5
Festuca rubra	22	2.5	Agrostis alba	9	3.6
Festuca arundinacea	9	1.1	Poa pratensis	8	3.2
Bromus inermis	5	0.6	Agrostis a. var. gig.	4	1.6
Phalaris arundinacea	3	0.3	Festuca rubra	1	0.4
	887	100.0		247	100.0
		P: 0.1%			P: 1%

B. Dispersion of thrips species on the host plant

on generative organ	= 46% (36)	G = 2% (11)
on vegetative organ	= 54% (64)	V = 98% (89)

Wetzel, 1964 (Czencz, 1983)

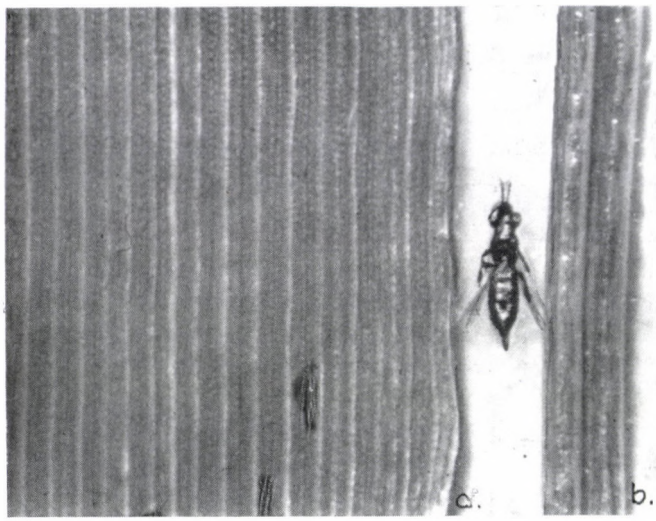

Fig. 4. Adult of Limothrips denticornis between a leaf of the favourable timothy grass (a) and another of the unfavourable reed fescue (b)

shown in Fig. 3 beside grains of their most favourable grasses (Fiorin, Kentucky bluegrass, English ryegrass and Hungarian brome).

The third one is the adults of *Anaphothrips obscurus* with its long wings (f. *macroptera*). In spite of the fact that individuals of this species can be collected from the generative organs of its hosts (Table 3), yet its development is mainly bound to vegetative organs. According to our results, it can be stated that, for egg-laying and embryo development of *Anaphothrips obscurus*, grasses having leaves of wider mezophyll and more valuable internal contents (e.g. timothy grass and English ryegrass) are more suitable than densely ribbed early turning woody ones.

In addition to the above mentioned reasons, the high thrips individual number, recorded for Timothy grass, can be attributed to its late flowering which coincides with the time of appearance of the new generation of *Anaphothrips obscurus* whose long winged adults fly in crowds from the nearby plots of Kentucky bluegrass which had already finished its flowering.

A *Limothrips denticornis* (Fig. 2d) is strongly bound to the vegetative organs of grasses. Accordingly, leaf morphology and its internal contents are important in the determination of the host plant. Considering that this species lays its eggs and grows its larvae deep in the leaf sheath, it is clear that the diameter of the leaf sheath as well as the sitting of the stem to this sheath are decisive characters in the choice of host plant. In Fig. 4, an adult of *Limothrips denticornis* is shown between the broad leaf of Timothy grass and that of Reed fescue having too narrow sheath to allow the penetration of the flat but big body of the mentioned adult.

Discussion

In the present work, pure populations of ten perennial grasses were comparatively investigated for their thrips populations. Investigations were carried out in the moderately warm, rainy western part of Hungary during 1985 and 1986. Grass species, as thrips nutritive plants, were evaluated according to the thrips spectrum and individual numbers living on them.

It was proved that all species of typical grass thrips which occur in big individual numbers were recorded on all the ten examined species with remarkably varying individual numbers. It is probable that by increasing the number of sampling would have resulted in a better balance between the thrips species spectrum and host grasses as well as in a further increase in differences among individual numbers.

The knowledge of life mode, ethology and morphology of thrips species occurring in statistically analysable numbers is of a big help in understanding the phenomenon of preference between the most and the least favourable nutritive plant.

Structure of panicle (loose or compact), glumellae and habit of grain play decisive roles in the choice of host plant of thrips species laying their eggs and the development of their larvae on the generative plant organs *(Chirothrips manicatus, Haplothrips aculeatus)*. On the other hand, for those primarily inhabiting leaves e.g. *Limothrips denticornis and Anaphothrips obscurus* which are bound – in their nutrition, eggs laying and larval development – to the vegetative plant organs, width of the mesophyll tissue and the nutritive value of the green parts are of vital importance.

From our investigations, it can be concluded that the differences in thrips species orientations, within the plants of the family Gramineae, may be due to morphological and biological reasons and, furthermore, to differences in insect ethology and plant internal contents.

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Thysanoptera Species Occurring Frequently on Cultivated Plants in Hungary

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The authors summarize the literature references and the results of their own observations concerning the economical significance of 22 frequently occurring Thysanoptera species associated with cultivated plants in Hungary.

Our present contribution is an endeavour to give a survey of those phytophagous species that occur frequently on cultivated plants in Hungary. Thysanoptera species occurring on cultivated plants in Hungary and the evaluation of their significance have been discussed by Jablonowski (1893, 1894, 1926), Priesner (1928), Fábián (1938), Jenser (1955, 1958, 1959, 1982), Jenser and Voigt (1968), Czencz (1982, 1983, 1985).

Materials and Methods

In order to establish the occurrence and the ratio of Thysanoptera species associated with cultivated cereals the samples were taken from different species at Keszthely (West Hungary) in 1976–78 and subsequently in different areas of the country.

The abundance and dominance of the Thysanoptera species living on cultivated grasses have been studied by examining the vegetative and generative parts of the plants and by sweeping of the cereal stand from the beginning of the vegetation period till harvesting.

Thysanoptera species living on flax was collected by singling and by sweeping the flax stand.

Thysanoptera species occurring on ornamentals were also collected by singling.

With a view to establish the distribution and the host range of some Thysanoptera species we have considered the results of collecting in the different areas of the country including the Hortobágy National Park and Kiskunság National Park, too.

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Results

Anaphothrips obscurus (Müller) was regularly observed on cereals especially on oat *(Avena sativa)*. Among cultivated grasses it occurs in great individual number on timothy *(Phleum pratense)* and on rye-grass *(Lolium perenne)* as recorded by Czencz and Török (1987).

Aptinothrips rufus Haliday is a frequent species on various Gramineae under Hungarian conditions. It may regularly increase in number in 3–4 years old stand of seed-grass producing areas.

Ceratothrips dianthi Priesner is a serious pest on pink *(Dianthus caryophyllus)*, known since 1952 in Hungary (Jenser, 1955, 1959). The data of its introduction to our country is unknown. As far as we are aware of its occurrence it was recorded only from cultivated pink. So far not a single reference is known regarding its presence on wild pink in Hungary (Jenser, 1983, 1986).

Chirothrips manicatus (Haliday) is a well spread species in Hungary, being frequent especially on various Gramineae. Its mass occurrence was recorded by methodical investigations (Keszthely 1979–1981) on seed-grass producing areas. The specimens especially favour the small, many-seeded, fine panicled meadow-grass *(Poa pratensis)* and the *Agrostis alba*. We have often found the different instars and the adult of this species on underdeveloped grain or on the place the missing grain in the ears of the above-mentioned species of the cultivated grasses. Their sucking may cause infertility and "partial white ears".

Dendrothrips ornatus (Jablonowski) has been known to occur in Hungary since its description in 1894). In Hungary it occurs as a pest on privet *(Ligustrum vulgare)* and to a smaller degree on lilac *(Syringa vulgaris)* (Jenser, 1959). Occasionally it is serious pest on privet significantly decreasing the ornamental effect. It frequently occurs in great numbers on the leaves of privet in wild state, especially in mixed oak woods. Even in the latter case the signs of sucking of the larvae and adults are conspicuous.

Drepanothrips reuteri Uzel caused damage on vine which was first recorded in 1967 in the environs of Kecskemét on the Danube–Tisza Mid-Region (Jenser and Voigt, 1968). The subsequent references suggest that only sporadically does the species occur in masses causing damage to vine.

Frankliniella intonsa (Trybom) frequently occurs in Hungary in large quantities. Its mass occurrence was regularly recorded on lucerne among our cultivated plants (Jenser, 1983). On the summer of 1958, near Nyíregyháza, we observed it as a pest on *Phaseolus coccinea*, which was unknown before. The flowers of the attacked plants were deformed and could not reach full bloom. The damage was recorded only on the flowers (Jenser, 1958).

Frankliniella tenuicornis (Uzel) frequently develops on various *Gramineae* species. Among the cultivated cereals the following percentual values were calculated during a series of investigations carried out in the neighbourhood of Keszthely (1979–1981): winter wheat 20%, oat 18%, barley 12% (Czencz, 1983).

The species was also found both in the leaf sheath and the developing cobs of maize, but neither stadia seemed to cause any damage to the corn.

Haplothrips aculeatus Fabricius occurs all over Hungary on various cereals and on cultivated grasses. In the cooler parts of West Hungary where extensive examinations were carried out between 1978 and 1981 on different cereals, the species proved to be dominant. The dominance value fluctuated between 57.6 and 73.1% (Czencz, 1982). Nevertheless the population density of this species was very low on all occasions. The lack of the damage may also be explained by the low individual number per spike (0.1–1 individual/spike).

Haplothrips niger Osborn occurs in great individual number in cultivated *Trifolium pratense* quite regularly. We have no data to its damage in Hungary.

Haplothrips tritici Kurdjumov samplings prove that the species occurs only in the warmer region under our climatic conditions, like the Great Hungarian Plain. The individual number of the species on winter wheat may reach 30–40 specimens per spike (Czencz, 1978). The ripening seeds are sucked by larvae causing brown spots by destroying the pericarpial cells. According to Bournier and Bernaux (1971) this damage may be dangerous primarily to durum wheat.

Hercinothrips femoralis (O. M. Reuter). According to our home references (Jablonowski, 1893; Fábián, 1938) the following species have introduced to Hungary from tropical areas: *Heliothrips haemorrhoidalis* (Bouché), *Hercinothrips femoralis* (Q. M. Reuter), *H. bicinctus* (Bagnall) and *Parthenothrips dracaenae* (Heeger). Green-house investigations in Hungary carried out in 1954 and 1957 provided the presence of only *H. femoralis* and *P. dracaenae* (Jenser, 1959). Since then only these species occur as pests on green-houses and on indoor ornamental plants.

Kakothrips robustus Uzel causes damage to pea since 1937 (Baranyovits, 1937). Its mass occurrence has been reported only sporadically in small plots of land, mainly in cultivated pea of vegetable gardens.

Limothrips consimilis Priesner has so far been reported only once on Bromus inermis (Jenser, 1958). Recently its mass outbreak was observed at Keszthely in 1986–87 on Bromus inermis cultivated for seed (Czencz and Török, 1987). According to the results of our comparative study on the Thyasanoptera species associated with grasses (10 varieties) *L. consimilis* occurred exclusively on Bromus inermis.

Limothrips denticornis Haliday has been regularly observed since 1893 on wheat (Jablonowski, 1893). Wetzel (1964) reported both the larvae and imagos on the vegetative parts of the plant (80–90%) which was confirmed also by Czencz (1983). Their sucking causes the death of mesophyll cells, thereby decreasing the surface area of assimilation. We mention here that Jablonowski (1926) established according to the results of his investigations that the Thysanoptera specimens associated with wheat do not induce white ears under the conditions of Hungary. Our own observations are in agreement with the opinion of Jablonowski.

Odontothrips confusus Priesner regularly occurs in the flowers of lucerne in Hungary. According to Bournier and Kochbev (1963) it may be a significant pest on the seed on lucerne. So far we have no references as to its damage to seed in Hungary.

Partenothrips dracaenae (Heeger) see *Hercinothrips femoralis.*

Taeniothrips simplex Morison was most likely introduced into Hungary following the years of the Second World War, where when established became one of the most serious pests of cultivated gladiolus (Jenser, 1955, 1959). Its persistence in Hungary and its occurrence as a pest are most likely due to its ability to overwinter on the tubers of the stored Gladiolus (Jenser, 1959).

Thrips angusticeps Uzel occurs on various wild and cultivated plants. The mass occurrence of the species in Hungary caused damage on flax (Czencz, 1985) and on various cereals (Czencz, 1983).

Thrips lini Ladureau for a long time has been known from Hungary in one locality only (Priesner, 1928; Jenser, 1982). According to data of investigations carried out between 1977 and 1981 in Hungary this species is a pest of flax, besides *T. angusticeps.* Its damage mainly affects the seed production of oil flax (Czencz, 1985).

Thrips nigropilosus Uzel has so far caused damage in Hungary only in green-houses, besides the numerous host-plants (Priesner, 1928; Stannard,1968) it caused heavy losses on Sinningia speciosa (Jenser, 1959).

Thrips tabaci Lindeman is also wide-spread in Hungary frequently damaging cultivated plant species. Under our conditions it may be a pest of onion on which it occurs quite regularly. Its damage was also recorded on the cultivated medicinal plant Digitalis purpurea, where it attacks the leaves. Under green-house conditions cyclamen specimens were attacked by *T. tabaci.* In 1986 its damage was recorded on the flowers of Saintpaulia ionantha in the green-houses of Budapest (Tasnádi, 1986).

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Economic Damage Caused by Cereal Thrips in Winter Rye in Sweden

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The thrips population in the leaf sheathes and ears of winter rye was measured over a 3-year period. The efficiency of the turpentine extraction method used to estimate thrips populations was evaluated. The most common species were found to be *Limothrips denticornis*, *Limothrips cerealium* and *Haplothrips aculeatus*. A fungus, *Verticillium lecanii*, was found to be an important mortality factor. Field trials with insecticides were made to estimate the economic injury level and the economic threshold of thrips in cereals. The economic injury level is indicated to be more than 20 thrips/straw.

Although thrips have long been known to be capable of causing serious damage in cereal crops, there are very few recent reports quantifying such damage in economic terms.

Thus, it is difficult to find figures for the economic injury level or economic threshold for the various thrips species on different crops. Holtmann (1962) found that thrips-infested rye, oats and barley had lower 1000 kernel weights and less kernels/head than uninfested material. In oats the panicles and the last internodium were shorter. Mantel, (1969) increased his yield by 10–18% in oats when he treated a thrips population (15–20 thrips/panicle) with an insecticide. Hinz, Daebeler and Giessmann (1971) demonstrated the serious effect of *Limothrips cerealium* and *Haplothrips aculeatus* on oats. Early infestation before heading resulted in shorter straw, less grains/panicle and lower 1000 kernel weight. The number of thrips/panicle ranged between 22–40. The economic impact of one species, *Haplothrips tritici*, which causes serious damage to wheat in eastern Europe, has been very well investigated. The economic injury level was found to be 80 thrips/head by Kamenchenko (1982) and 40–50 thrips larvae/head by Rumyantseva (1981). Seidel (1983) recommended spraying when the number of *Limothrips* spp. or *Haplothrips* spp. (irregardless of development stage) reached 5–10/ear in cereals. In contrast, Freier, Volkmar, Lübke and Wetzel (1982) estimated 30 larvae and adults/head to be the economic injury level of thrips in cereals.

To get more exact information on the economic impact of thrips in cereals in Sweden, our department started a project in rye 1983 aimed at determining the economic injury level and economic threshold. The economic injury threshold is defined as the lowest population density that will cause economic damage and

the economic threshold is the pest density at which control measures should be determined to prevent an increasing pest population from reaching the economic injury threshold. The results will be published in details elsewhere.

Materials and Methods

Most of the work was done in field trials with natural infestations of thrips and the crops were treated with insecticides at various stages in their development. Every plot was 60 m^2 and there were four replications in each trial. The tests were conducted on private farms in southern Sweden. We measured the thrips population in the ears with a turpentine extractor modified after Lewis (1960). The leaf sheathes were frozen and the thrips population counted later. The population was measured at heading to get an estimate of the number of egg-laying females. Four weeks after heading we took samples of leaf sheathes and 6 weeks after heading we took samples of the ears. In some trials the population was measured once or twice per week from late April to August.

Results

Species diversity in rye

In Sweden, rye is normally infested by three thrips species: *Limothrips denticornis* in the leaf sheathes and *Limothrips cerealium* and *Haplothrips aculeatus* in the ears. Other species found were *Frankliniella tenuicornis Chirothrips manicatus* and *Thrips angusticeps. Limothrips denticornis* is the first species flying into the crop, often 2–3 weeks before heading which normally starts 18–20 May in southern Sweden. *Limothrips cerealium* also start flying into the crop before heading but *Haplothrips aculeatus* does not arrive until after heading. Spring 1985 was exceptionally late and heading started 28 May. Thrips immigration during 1985 was also later than normal.

Mortality factors

In early 1984 we found thrips-eggs from leaf sheathes infected with a fungus. Later, we found this fungus on all stages of *Limothrips denticornis* and it seemed to be an important mortality factor. The fungus was identified by the Commonwealth Mycological Institute and found to be *Verticillium lecanii* Viégas.

Increase in yield with insecticides

In the trials with cereal aphids in wheat and barley we also tested the effect of different insecticides on cereal thrips. One of the most effective was cypermetrin 80 g ai/ha (Tradename Ripcord or Cymbush) which we have in our trials

in winter rye. To efficiently reduce *Limothrips denticornis* populations in the leaf sheathes, the rye must be treated before heading since females enter and start egg-laying when the last leaf sheath is opening. Once the eggs have been laid, they cannot be killed with a contact insecticide. With this early treatment we have had good results with the thrips in the ears (mainly *Limothrips cerealium*). *Haplothrips* immigrates after heading; thus it is not affected in the same way at an early treatment. In 1983 we started with four trials and the average increase in yield was 13% or 430 kg/ha (yield in untreated, 34 dt/ha). In 1984, we had 20 trials and the average increase in yield was 3% or 150 kg. In 1984 the yield of all cereals was exceptionally high and the crop was able to effectively compensate for the thrips damage. In 1983 we had a more normal year with a dry summer and it is often stated that thrips damage is greatest in dry years. The average number of thrips/straw in the trials was 15.4 in 1983 and 13.9 in 1984.

Economic threshold

It is too early to recommend an economic threshold for thrips in rye but I will give a very short summary of our reasoning. The economic injury level is determined by four variables: the control cost, the crop market value, the proportionate injury per individual pest and the crop response to injury. We have found that control costs about 250 kg of rye/ha. In our trials the correlation between the number of thrips/straw and the yield response with insecticides shows that with more than 20 thrips/straw the yield response will be near 250 kg/ha.

The correlation between the number of egg-laying females at heading and the final thrips-population shows that with more than 1 female/straw you will probably get more than 20 thrips/straw later in the season.

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Assessing the Effects of Flower Thrips

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Flower thrips can have many effects on plants. Pollen damage and pollination are particularly likely to have been overlooked. Some floral characteristics of thripsophily, a thrips pollination syndrome, are proposed and an *in vitro* technique for measuring the number of pollen grains destroyed per thrips per day is described and illustrated. Some future research objectives are suggested.

In order to explore the relationship between thrips and flowers, it is important to be aware of the time-scale of the relationship. When flowering plants appeared about 100 million years ago, thrips were already present (see Thien, 1980). Honeybees, however, did not appear until about 60 million years later (Seeley, 1985). Thrips have had a long time to adapt to flowers. Some evidence that this opportunity was exploited rapidly comes from the close association between flower thrips and some plants in families which have retained primitive floral characteristics (Thien, 1980). Close adaptation of thrips to flowers should be expected. Responses to floral colours (Kirk, 1984a) and floral scents (Kirk, 1985a) and choice of oviposition site (Kirk, 1984b) appear to be adaptations. The discovery that many species of flower thrips feed on large quantities of pollen, depend on pollen for reproduction and can recognize host pollen (Kirk, 1985b, 1987) is not surprising when the time-scale of the relationship between thrips and flowers is considered, but despite this the possible harmful effects of pollen damage by thrips do not seem to have been considered before.

Flower thrips feed on individual pollen grains. The mandible pierces a hole in the wall of the grain and the paired maxillary stylets suck out the liquid contents through the hole. The empty shell of the grain is left behind. Figure 1 shows an adult female *Thrips vulgatissimus* Haliday feeding on a pollen grain of Brassica napus L. (oilseed rape). This skilful control of pollen grains does not need narrowly specialized mouthparts; the same mouthaprts can be used for other food types. For example, *Aeolothrips intermedius* Bagnall can feed on petal and leaf cells, pollen, thrips larvae and mites (Bournier et al., 1979).

The fact that thrips destroy pollen does not mean they cannot be pollinators. Indeed the association with pollen makes pollination more likely. Bees collect

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large quantities of pollen that they destroy by feeding it to their larvae, but they are still recognized as pollinators. Often, pollinators are identified by matching a flower with the floral characteristics described in a pollination syndrome (Faegri and van der Pijl, 1979). Since no thrips pollination syndrome has been described, thrips pollination cannot be identified in this way! Thrips pollination is likely to

Fig. 1. An adult female *T. vulgatissimus* feeding on a pollen grain of *B. napus*. The view is of the underside of the thrips as seen through a glass cover slip on which the thrips is standing. One of the four grains by the mouthparts is dark in the centre where the labral pad in contact with the grain shows through

have been overlooked in the past. I propose that a syndrome of thripsophily will include white, sweet-scented flowers, since these characters are found in two groups of plants pollinated in their natural habitats, by thrips (Thien, 1980; Appanah and Chan, 1981) and the visual and olfactory stimuli of such flowers would greatly increase the number of thrips landing in the area (Kirk, 1984a, 1985a).

The net effect of thrips in flowers can only be assessed from a full knowledge of the benefit and the harm that thrips do. Possible benefits to consider are: self-pollination; cross-pollination; and predation on mite pests (Bailey and Caon, 1986). Possible harmful effects are: oviposition damage to flower stems, filaments and styles; floral abscission; damage to floral tissue; stunting and scarring of fruit; repulsion of pollinators; and reduced pollination through destruction of pollen in anthers or on stigmas. This long list of effects demonstrates the complexity of the system. Pollen damage and pollination, which are closely linked, are

likely to have been overlooked because they are difficult to observe and measure. A technique for measuring pollen damage *in vitro* is described below and the statistical analysis is illustrated by an example.

Measurement of pollen-feeding rate

The method, in outline, is to confine thrips in a "cell" with some pollen for 24 h, then stain the pollen to see, under a microscope, how many grains are empty. Some of the grains would have been empty before the experiment, so control cells are used to measure the "background" proportion of empty grains and hence estimate the background number of empty grains in each treatment cell.

The cell was made by removing a 15 mm diameter disc from a piece of dental wax (30 mm long, 23 mm wide, 1.5 mm thick). The hole formed the cell. A warm microscope slide was pressed against the wax to seal it in place and form the floor of the cell. A single thickness of dialysis tubing, which is permeable to water vapour, formed the roof; it was held in place by a thin layer of clear petroleum jelly brushed onto the surface of the wax. The dialysis tubing was attached after the thrips had been placed in the cells.

Pollen from as few as possible newly-opened flowers was thoroughly mixed and a few thousand grains were sprinkled over the floor of each cell with a fine paint brush. A 3–5 mm length of stamen filament from the flowers was placed

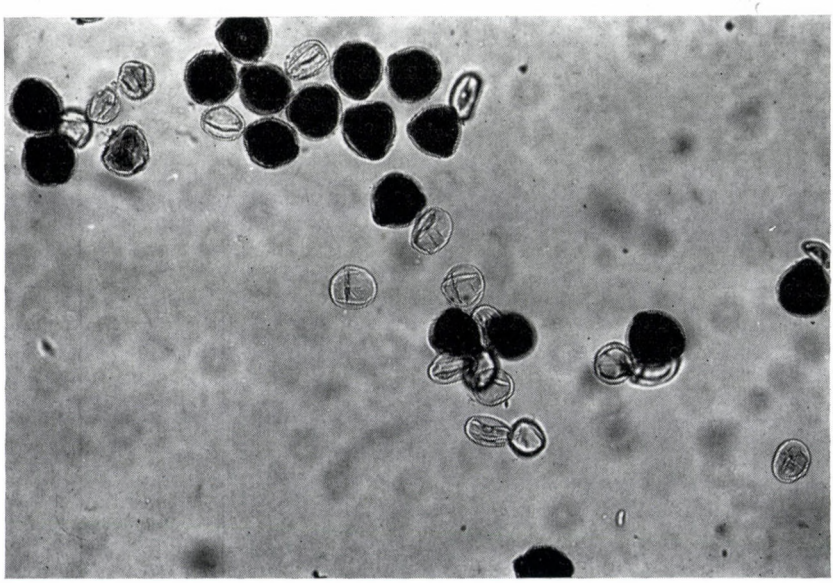

Fig. 2. Pollen grains of *Medicago sativa L.* (lucerne) stained after expousre to adult female *T. imaginis* to distinguish full and empty grains. Full grains are about 30 μm across

Table 1

Results of measurements of pollen-feeding rate for *T. imaginis* adult females on *B. napus*. The background proportion of unstained grains for the two control cells (p) was 0.0613

Cell	Number of grains				
	Total (G)	Unstained (U)	Stained (G-U)	Background ($G.p$)	Consumed (U-$G.p$)
C1	1229	82	1147	–	–
C2	1987	115	1872	–	–
T1	2454	560	1894	150.3	409.7
T2	2077	887	1190	127.2	759.8
T3	2225	866	1359	136.3	729.7
T4	2199	606	1593	134.7	471.3
T5	1796	782	1014	110.0	672.0
T6	2396	674	1722	146.8	527.2

at the side of each cell as an alternative food source. Two control cells with no thrips and six treatment cells, each with two thrips, were set up. The cells were left in a constant temperature room at 20 °C with 12 h light: 12 h dark for 24 h. A high relative humidity of 76%, necessary for survival of the thrips, was maintained by keeping the cells above a saturated solution of sodium chloride in a small chamber.

After 24 h, the microscope slide was removed from the wax. A thin layer of 0.1% cotton blue in lactophenol was brushed onto a cover slip which was lowered onto the pollen. The stain acts on the pollen grain contents, so full grains are dark blue and empty grains are clear (Fig. 2). The numbers of full and empty grains were counted by systematically scanning across the slide. A grid graticule is essential to give a parallel-sided field of view for scanning.

This technique has been used successfully for adults and larvae on several species of pollen (Kirk, 1987). Results for adult female *Thrips imaginis* Bagnall (taken from rose flowers) on pollen of *B. napus* in South Australia in November 1985 are in Table 1. The detailed theoretical treatment of such data is given elsewhere (Kirk, 1987). When the background proportion of unstained grains is low, the mean and standard error of the mean of the number of grains consumed can be calculated very simply. The proportion of unstained grains (p) in the two control cells (C1, C2) combined was 0.0613. This proportion multiplied by the total in each treatment cell (T1–T6) estimated the background number of unstained grains in each cell. Subtraction of the background number from the number of unstained grains gave the estimated number of grains consumed in a particular cell (last column of Table 1). The mean and standard error of the number of grains consumed per cell can be calculated directly from the figures in the last column of Table 1. The mean 595 $\pm$ 59 grains per cell per day has to be divided by two to give a result per thrips i.e. 298 $\pm$ 30 grains per thrips per day. This is 0.2% of the pollen in the flower per thrips per day.

Future research objectives

Now that the potential level of pollen damage has been shown to be high, the next step is to develop the above technique for field use on plants with many thrips in the flowers. It will be necessary to know whether grains can remain unstained for other reasons, such as failure to develop in the anther.

If the level of pollen damage is found to be high in the field, it will be necessary to know whether this is reducing yield. This will require detailed studies of when pollination occurs in relation to the pollen damage, because there might be no effect if flowers were pollinated before the pollen was damaged. Thrips might also reduce yield by feeding on grains germinating on the stigma, but reduced pollination could still have little effect if compensation occurred at the plant level. It will not be sufficient to show a yield increase in the absence of thrips, because thrips could be decreasing yield by ways other than damaging pollen.

Rigorous field techniques for measuring the harmful effects of pollen damage need to be developed. Rigorous investigations of thrips pollination are also needed so that a syndrome of thripsophily can appear in the literature and make it less easy for the beneficial effects of thrips to be overlooked.

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A Contribution to the Knowledge of *Thysanoptera* in Ornamental and Bulbous Crops in the Netherlands

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A survey is given of the *Thysanoptera* which were observed to infest ornamental and bulbous crops in glasshouses or in the open in the Netherlands. Faunistic data are supplemented with remarks on the occurrence of the thrips species and the damage symptoms they cause.

Characteristic for ornamental production is the vast number of plant species belonging to many families and orders. Usually a minority of these plants is endemic; the majority is imported from different geographical areas. Another characteristic in ornamental production is that both leaves and flowers may have decorative value; even the slightest damage may decrease the commercial value. Control of pest organisms that deteriorate parts of the plants or decrease their performance is of much importance.

Most of the floriculture is in glasshouses. There the plants grow under predominantly warm conditions, while relative humidity varies usually between 60–90%, resulting in rapid and luxuriant growth. To this may be added the absence of biotic reducing factors as competition for food, while predators and parasites usually are absent. This provides ideal conditions for rapid multiplication of pest organisms once they have successfully entered this environment.

Pest organisms are introduced most frequently on vegetatively propagated perennials. The world-wide trade in plant material, the short period of transport of this material and the occurrence of polyphagous insects result in transmittance of pest organisms over long distances.

In spite of quarantine and plant health procedures, several pest organisms have been transported from continent to continent. This explains why, for instance, several species of thrips have become established pests in glasshouses all over the world.

In this review we list the thrips species collected in glasshouses and in the open on ornamentals in the Netherlands. Among these species various tropical and subtropical species were found. In bulbous crops, however, mainly indigenous thrips species were found. Several imported thrips species, damaging bulbous

crops, however, apparently were imported in the past from widely different geographical areas and have become established in the Netherlands.

Thrips species marked with an asterisk (*) are new for the fauna of the Netherlands.

Ornamentals

Chaetanaphothrips orchidii (Moulton)

The banana rust or orchid thrips, *Chaetanaphothrips orchidii*, was collected in the Netherlands first from *Amaranthus* at Wageningen (van Eecke, 1921). After that it was collected from Peperomia at Aalsmeer (van Rossem et al., 1963), on *Saintpaulia ionantha* at Kudelstaart (van Rossem et al., 1975) and by us on *Rhododendron simsii* (Azalea indica), *Monstera*, *Philodendon* and *Saintpaulia ionantha;* all cases at Aalsmeer.

On all plants females and larvae were found, so they are hostplants for C. orchidii. This species can be found all year round in glasshouses on the leaves of the plants concerned.

According to zur Strassen (1986) C. orchidii is also known from *Begonia*, *Cyclamen*, *Dracaena* and *Tradescantia*.

C. orchidii is originally described from California, USA (Moulton, 1907).

**Cranothrips emersoni Girault*

Cranothrips emersoni was collected from imported Banksia prionotes (van Rossem et al., 1981a). According to Jacot-Guillarmod (1970) *C. emersoni* is only known from Australia where it was observed on a Boronia-species. *C. emersoni* occurs probably on the leaves and in the flowers.

Dendrothrips ornatus (Jablonowski)

The privet thrips, *Dendrothrips ornatus*, was found by us causing damage to buds and flowers of *Syringa* at Aalsmeer in 1976 during the period when the shrubs were in the glasshouse for forcing. This damage is caused by the activity of the hibernating population. From the flowers only females were collected.

During the period May till September *D. ornatus* breeds on the leaves of *Syringa* in the open (van de Vrie, unpublished). In that period we observed females and larvae on the leaves, causing local discolouration of the foliage.

**Dichromothrips corbetti (Priesner)*

On *Phalaenopsis Dichromothrips corbetti* caused heavy damage on the flowers, Aalsmeer, 24. V. 1982. Besides females and a male also larvae were collected from the flowers. D. corbetti originates from S E Asia where it lives on orchids (Mound, 1976). We have found it once in a glasshouse in the Netherlands.

**Dichromothrips phalaenopsidis Sakimura*

This orchid thrips was collected from *Paphiopedilum* at Pijnacker, 18. IV. 1975, (leg. J. Woets) where it damaged the flowers. Females, males and larvae were present on the plants which were imported from Cambodia. The identification is verified by Mr. K. Sakimura (Hawaii, USA).

Frankliniella bondari Hood

The specimen published as *Frankliniella* spec. (Franssen and Mantel, 1962a) was identified as *Frankliniella bondari* by Mrs. K. O'Neill (USA). *F. bondari* was collected at Wageningen in 1940 on Asclepias tuberosa, (leg. J. Doeksen). *F. bondari* is originally described from Brazil (Hood, 1942).

Frankliniella intonsa (Trybom)

The flower thrips, *Frankliniella intonsa*, is common in the open in the Netherlands. Faunistic data are given by Franssen and Mantel (1962a). New hostplants are *Dianthus* (Sint Annaland, Biezelinge and Stavenisse) and *Dahlia* (Aalsmeer). On these plants F. intonsa was found in July and August, primarily in the flowers.

In glasshouses F. intonsa females and larvae damaged the buds and flowers of *Saintpaulia ionantha* (Aalsmeer, July 1976; Lent, October 1983). It infested the anthers and contaminated the petals with pollen. The symptoms cannot be distinguished from those caused by the western flower thrips, which is described by Strauss and Schickedanz (1986).

**Frankliniella occidentalis (Pergande)*

The western flower thrips, *Frankliniella occidentalis*, has been a pest of economic importance in ornamental and vegetable crops in the Netherlands since 1983, especially on *Chrysanthemum*, potplants, cucumber and sweetpepper in glasshouses (van de Vrie, 1987). A historical account concerning distribution, hostplants in glasshouses, problems in the biological and chemical control is in press (Mantel and van de Vrie, 1988). *F. occidentalis* also was observed during the months July till October on *Aconitum napellus*, *Aster novi-belgii* and *Liatris*-cultivars growing in the open near glasshouses. As from these plants only females and males were collected, it is not certain whether they are host plants for *F. occidentalis*. The populations in glasshouses and the open mostly belong to the pale or intermediate colour form. The dark form is observed on *Aster novi-belgii* in the open, Aalsmeer, 16. X. 1986 and consisted only of females.

F. occidentalis is originally described from California, USA (Pergande, 1895) and is extremely polyphagous (Bryan and Smith, 1956; Yudin et al., 1986).

**Gynaikothrips ficorum (Marchal)*

From curled leaves of *Ficus retusa* which was imported from Taiwan, *Gynaikothrips ficorum* was collected (van Rossem et al., 1981b). According to these authors *F. retusa* often is heavily infested while other *Ficus*-species seem to be less sensitive.

Hercinothrips femoralis (O. M. Reuter)

The banded greenhouse thrips, *Hercinothrips femoralis*, is a polyphagous species in glasshouses where it lives on the leaves of the hostplants. According to Franssen and Mantel (1962a) it is found on *Araceae* by van Rossem et al. (1975). This species has been found on *Dieffenbachia*, *Monstera*, *Peperomia*, *Phaseolus vulgaris*, *Pseuderanthemum reticulatum* and *Vriesea splendens*, at different localities. Other hostplants are mentioned by zur Strassen (1986). *H. femoralis* can be present all year round in glasshouses.

On the plants mentioned here only females were observed; males were absent. On *Dieffenbachia*, *Monstera*, *Peperomia* and *Vriesea splendens*, also larvae were observed, so these plants are considered as hostplants.

In a meristem culture in *Gerbera* at Roelofarendsveen, larvae of *H. femoralis* in January 1978 infested the meristems. The explanation for this infestation is probably the neglect of hygienic rules in the room where the vials with meristems were handled.

Parthenothrips dracaenae (Heeger)

The palm thrips, *Parthenothrips dracaenae*, is a common thrips in glasshouses and on potplants in houses. It is a very polyphagous species and lives on the leaves where it has preference for the underside. Often it lives in colonies.

Since the account of Franssen and Mantel (1962a) we have found *P. dracaenae* inclusive all immature stages on *Anigozanthos*, *Anthurium andreanum*-hybride, *Chamaedorea*, *Codiaeum variegatum* (Croton), *Cyperus diffusus*, *Dieffenbachia*, *Dracaena*, *Hedera*, *Hoya bella*, *Microcoelum weddelianum Plumbago*, *Pseuderantheum reticulatum*, *Ruscus aculeatus*, and *Vriesea splendens*. On *Peperomia* we observed only females. Infested plants came from commercial growers and private homes from many localities. *P. dracaenae* can be found on the plants all year round.

Scirtothrips longipennis (Bagnall)

The begonia thrips, *Scirtothrips longipennis*, was collected for the first time from the leaves of *Amaranthus* in a glasshouse at Wageningen (van Eecke, 1921). Later on *S. longipennis* was found on *Cyclamen* at Aalsmeer (van Eecke, 1931). Since a few years it is known from *Begonia* at Aalsmeer. *S. longipennis*,

is also known from *Anthurium*, *Dieffenbachia* and *Philodendron* (zur Strassen, 1986).

S. longipennis occurs exclusively on the leaves. On *Begonia* leaves it causes characteristic symptoms, namely discolouration near the main veins (van de Vrie and Boogaard, 1985).

**Synaptothrips gezinae (Faure)*

Synaptothrips gezinae was collected from flowers of imported Protea-flowers from South Africa (van Rossem et al., 1981a). According to Jacot-Guillarmod (1974) *S. gezinae* is only known from South Africa, where it lives in flowers of *Protea*-species.

Taeniothrips atratus (Haliday)

The carnation thrips, *Taeniothrips atratus*, lives on many plant species (Franssen and Mantel, 1962a). We have found *T. atratus* on *Aconitum napellus*, *Aster*, *Dahlia*, *Dianthus barbatus*, *Dianthus-cultivars* (carnation) and *Gypsophila paniculata* at different localities during the months July till September in the open.

In most cases only females were found; males are rare. However, on *Dianthus barbatus* and *Dianthus*-cultivars (carnation) adults and immatures of *T. atratus* were observed.

Taeniothrips simplex (Morison)

The gladiolus thrips, *Taeniothrips simplex*, was found on a *Dianthus*-cultivar at Kruiningen in the open. Probably the female was a passer-by as in this area gladiolus growing is very common.

More information on *T. simplex* will be given in the part on bulbous crops.

Taeniothrips vulgatissimus (Haliday)

Taeniothrips vulgatissimus, a common and polyphagous thrips in flowers is since the account by Franssen and Mantel (1962a) recorded from *Aconitum napellus*, *Aster*, *Dahlia*, *Echinops* and *Gypsophila paniculata* at different localities. We observed only females during the months July till September in the open.

Thrips major Uzel

Thrips major is a very common thrips with preference for the flowers of many plants. Since the account by Franssen and Mantel (1962a) we found *T. major* on *Aconitum napellus*, *Dahlia*, *Echinops*, *Erynchium* and *Phaseolus vulgaris* at Aalsmeer and Middelburg.

On the plants mentioned here in the open we observed only females during the months June till August with exception of *P. vulgaris* on which also larvae of *T. major* were present.

Thrips nigropilosus Uzel

The chrysanthemum thrips, *Thrips nigropilosus*, was found on *Aster* at Aalsmeer, 29. VIII. 1985, on which it caused heavy damage to the flowers. The identification was performed by Dr. R. zur Strassen (BRD). *T. nigropilosus* prefers *Asteraceae* (zur Strassen, pers. comm.) and seems to prefer the flowers.

Thrips tabaci Lindeman

The onion thrips, *Thrips tabaci*, is one of the most common species in the Netherlands (Franssen and Mantel, 1962a). To their data can be added that *T. tabaci* females and larvae were found on *Chrysanthemum leucanthemum*, *Dahlia*, *Dianthus barbatus* and *Dianthus*-cultivars (carnation) in the open at different localities. In glasshouses *T. tabaci* and its larvae were found on *Alstroemeria*, *Cucumis sativus*, *Cyclamen*, *Gladiolus* and *Schefflera* at different localities. Prepupa and pupa were observed on *Dahlia* and *Dianthus*-cultivars in the open and on *Cyclamen* in glasshouses.

In the open *T. tabaci* reaches the highest population densities in summer and autumn. However, we have observed that the adults damaged different Gramineae in winter at 2 °C. In that period neither oviposition occurs, nor the eggs hatch. *T. tabaci* can enter into the glasshouses during the whole year through open ventilators, with infested plant material, or carried on clothes.

T. tabaci causes unusual symptoms on *Cymbidium*. As a result of an incision by the ovipositor of the thrips females the plant tissue is damaged, which results in dark spots with a light margin on the leaves, stems and flowers. The spots are 2–4 mm in diameter and irregularly distributed on all parts of the plant. The eggs hatch, however, the larvae cannot survive. It means that *T. tabaci* accepts *Cymbidium* for oviposition, but populations cannot develop on this plant. Infestation takes place in an early developmental stage of *Cymbidium*-flowers, the symptoms are visible much later, namely after the development of the flowers. The same applies to the damage caused to the leaves. At this stage the thrips have disappeared already for a long time. Measures to prevent damage consist of spraying at the time when the first thrips are observed (Timmerman and van de Vrie, 1982).

Other thrips species

For completeness of the records on the thrips-fauna from ornamentals in glasshouses the reader is referred to Franssen and Mantel (1962a). We have not observed the species mentioned below during our inventories. It concerns the

grape thrips, *Drepanothrips reuteri*, the glasshouse thrips, *Heliothrips haemorrhoidalis*, the smilax thrips, *Hercinothrips bicinctus*, and the fern thrips, *Leucothrips nigripennis*.

Bulbous crops

**Frankliniella fusca (Hinds)*

The polyphagous tobacco thrips, *Frankliniella fusca*, infested bulbs of a *Hippeastrum*-cultivar stored at 17 °C and 60–70% R. H. The infested bulb scales discolour to red-brown. Heavily infested bulbs internally rot away partly, consequently the stalk does not develop. On the leaves, buds and flowers silvery spots can be found. Infestation is observed during winter months on bulbs in a storeroom; on leaves, flower-buds and flowers in spring in glasshouses.

Infested material is known from Anna Paulowna (van Rossem et al., 1975), Baarn, 's Gravezande, Hillegon and Heemstede. In storerooms, or glasshouses, *F. fusca* was present in different years, but was never found in the open. According to Jacot-Guillarmod (1974) F. fusca is a pest on cotton, peanuts and tobacco in the USA. *F. fusca* is originally described from Massachusetts, USA (Hinds, 1902).

**Frankliniella lilivora Kurosawa*

Frankliniella lilivora was collected from bulbs of a *Lilium*-cultivar imported from Japan (van Rossem et al., 1980). According to Jacot-Guillarmod (1974) *F. lilivora* is known from Japan, China and Korea where it occurs of different *Lilium*-cultivars.

Frankliniella schultzei (Trybom)

The cotton bud thrips, *Frankliniella schultzei*, has been found in the Netherlands infesting hyacinth bulbs in propagating rooms (Mantel, 1968). Infestation takes place only on bulbs used for vegetative propagating. For that purpose the basal parts of the bulbs are removed, mentioned hollowed bulbs, or incised, mentioned incised bulbs, through which daughter bulbs are produced. Hollowed bulbs treated in this way are stored in propagating rooms during the first two months at 20 °C, followed by one month at 23 °C, one month at 25.5 °C and one till two weeks at 20–15 °C, all with 80–85% R. H. Incised bulbs are stored the same periods, but the temperature is at that time some degrees higher (Krabbendam, 1968).

Bulbs infested in an early stage produce no or only a few daughter bulbs. If infestation takes place when daughter bulbs already are present, most young bulbs rot away after planting or do not develop to normal plants. The daughter bulbs discolour from yellow till brown. The infested bulbs desiccate much and are at planting time lighter in weight.

F. schultzei, which is originally described from South Africa, (Trybom, 1910), is known in the Netherlands only on hyacinth bulbs of different cultivars in these rooms and not in the open. Infested bulbs are found from July till November in the propagating rooms in the bulb centre in west and north west of the Netherlands.

Liothrips vaneeckei Priesner

The lily thrips, *Liothrips vaneeckei*, occurs between the bulb scales on different *Lilium*-cultivars. Heavy infestation destroys the bulbs totally. Lightly infested bulbs show reduced growth and develop no or only a small flower which has no commercial value.

Although *L. vaneeckei* was observed also on the leaves, stem and flowers, it apparently does not infest these parts of the plants. It leaves the plant probably in search of other lily bulbs (Franssen and Mantel, 1962b). *L. vaneeckei* only occurs in glasshouses.

In Amstelveen *L. vaneeckei* is observed on a *Dactylorchis*-species in a glasshouse where it was feeding on the roots.

L. vaneeckei is described originally from the Netherlands (Priesner, 1920)

Taeniothrips atratus (Haliday)

The carnation thrips, *Taeniothrips atratus*, was found infesting *Freesia*- and *Vallota*-cultivars at different localities in glasshouses in autumn. It also infested leaves and flowers of *Iris*-cultivars (Franssen and Mantel, 1962a). On these crops the immature stages and imagines were observed. Infestation on an *Iris*-cultivar by *Taeniothrips simplex* has been found recently. The damage symptoms on *Iris*-cultivars caused by T. simplex are very similar to those caused by *T. atratus*.

Taeniothrips simplex (Morison)

The gladiolus thrips, *Taeniothrips simplex*, is a cosmopolite which occurs on different crops in the open, storerooms and in glasshouses. Here we give only short notes on damage symptoms on *Gladiolus*- and *Crocus*-cultivars.

On Gladiolus *T. simplex* causes typical silvery spots on the leaves, flower-buds and flowers. Severely infested buds come out poorly and flowers are misformed. Especially in the dark coloured cultivars the silvery spots are conspicuous. On the corms superficial brown discolouration occurs. *T. simplex* is present during summer months in the field only on the leaves, flower-buds and flowers, but not on the corms. Corms are infested probably during the harvest when they are removed from the infested leaves.

On *Crocus T. simplex* occurs only on the corms. There it causes a light brown discolouration, limited by the implantation of the skin. Damage is visible on root parts of the corms and on the young sprouts which develop irregularly.

After planting lightly to moderately infested corms show a delay in growth but nevertheless a practically normal crop is produced. The cause of the infestation was the fact that the corms were stored for some days after harvest in a room where infested *Gladiolus*-flowers were prepared for sale (pers. comm. P. J. Muller, Lisse). That means that infestation of the corms could be prevented by effective hygienic measures.

Thrips tabaci Lindeman

The onion thrips, *Thrips tabaci*, infests hyacinth bulbs which are stored in propagating rooms. The damage symptoms caused by *T. tabaci* on the bulbs are exactly the same as described for *Frankliniella schultzei*. The period of infestation and localities are also identical (Franssen and Mantel, 1965). Males of *T. tabaci* are very rare, however, they were found once in infested bulbs in these propagating rooms.

T. tabaci damaged *Freesia*-corms which were stored at 30 °C. The corms showed in the beginning of the infestation silvery spots which changed to light brown; heavily infested corms are brown. The infested corms were planted in a glasshouse at 13–15 °C. After five months the lightly infested corms gave normal flowers. Heavily infested corms produced no flowers.

Xylaplothrips subterraneus Crawford

Xylaplothrips subterraneus occurs on bulbs of a *Lilium*-cultivar. It is observed in a glasshouse in company with *Liothrips vaneeckei* and probably it causes identical damage symptoms on lily bulbs as *L. vaneeckei* does (Franssen and Mantel, 1962b).

X. subterraneus is originally described from specimens which were taken at New York, N. Y., USA. They were found in company with many *L. vaneeckei* in lily bulbs which were imported from England (Crawford, 1938).

Acknowledgement

We are much indebted to Mrs. K. O'Neill (Washington DC, USA), Mr. K. Sakimura (Honolulu, Hawaii, USA) and Dr. R. zur Strassen, Frankfurt am Main, BRD, for their kind help with various identifications, to Mr. P. J. Muller, Bulb Research Centre, Lisse, the Netherlands, for sending infested bulbs and to Mr. J. Bakker, Aalsmeer, the Netherlands, for correcting the plant names. Dr. J. Price, University of Florida, Bradenton (USA), was so kind to check the English text.

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On the Genesis of the Wings and the Wing Musculature of *Hercinothrips femoralis* (O. M. Reuter) *(Thysanoptera, Insecta)*

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The morphogenetic processes involved in the formation of the wing system are characterized by wing annexes being developed during metamorphosis as well as by specifically differentiating oscillatory wing muscles. The quantitative distribution of bundles of myofibrils in the raisers and descenders of the wings just as the wing size of various species will be discussed with the help of several statistics.

The existence of prepupal- and pupal stages during ontogenesis as well as the wing and genital annexes which become morphologically observable at these stages led to the classification of Thysanoptera into the Neometabola group, and, again, within this group into Remetabola (Takahashi, 1921). The morphogenetic processes involved in the formation of the wing system can be subdivided into the development of the forewings and the hindwings and the development of a motor necessary for the wing stroke.

Material and Methods

Stock cultures of *Hercinothrips femoralis* were maintained on *Zanredeschia aethiopica* L. at 24 °C ± 1K, 60 ± 10% relative humidity and a photoperiod L : D = 12 : 12. For the collection of prepupae and pupae the females were caged for 12 hours on a barley-stalk within a glasstube closed with a tampon. In this way it was able to time complete ontogenesis accurately and the oviposition is taken as time zero. The majority of the pupae hatches between 18 and 20 days and the pupae between 21 and 24 days after oviposition. The animals were fixed in Carnoy's fluid and processed for routine microtomy. 6 μm thick paraffin sections were stained by Azan-technique (Smith and Bruton, 1979) and by silver impregnation (Rowell, 1963). The data were statistically analysed with a personal computer by the help of the basic-listings of Tassel (1984) and Müller and Kick (1985).

Results and Discussion

Wings

The imaginal disks of the wings start working already at the end of the last larva stage. This leads to the development of an independent wing system underneath the epidermal cuticle which is later on covered by a cuticular sheath of the pupa. The basal membrane of the two epidermal cell layers are separated from each other until the pupal moulting takes place by the body cavity. At the pupal stage, however, they communicate with one another, thus forming a central lamella. A number of lacunae situated between stilt-like epidermal cells indicate an ancestrally stronger subdivision with alongside veines in the wings of Thysanoptera. At an advanced pupal stage the cells become shorter and the wings become more and more flat.

The construction of the wings of insects whose size does not exceed 1 to 2 millimetres shows a convergent modification of the wings resulting from the following physical conditions: small body and wing sizes, slow locomotion speed combined with a high frequency of wing strokes. Hence, Thysanoptera can be expected to reveal Reynolds numbers between 1 and 20 *(Hercinothrips femoralis:*

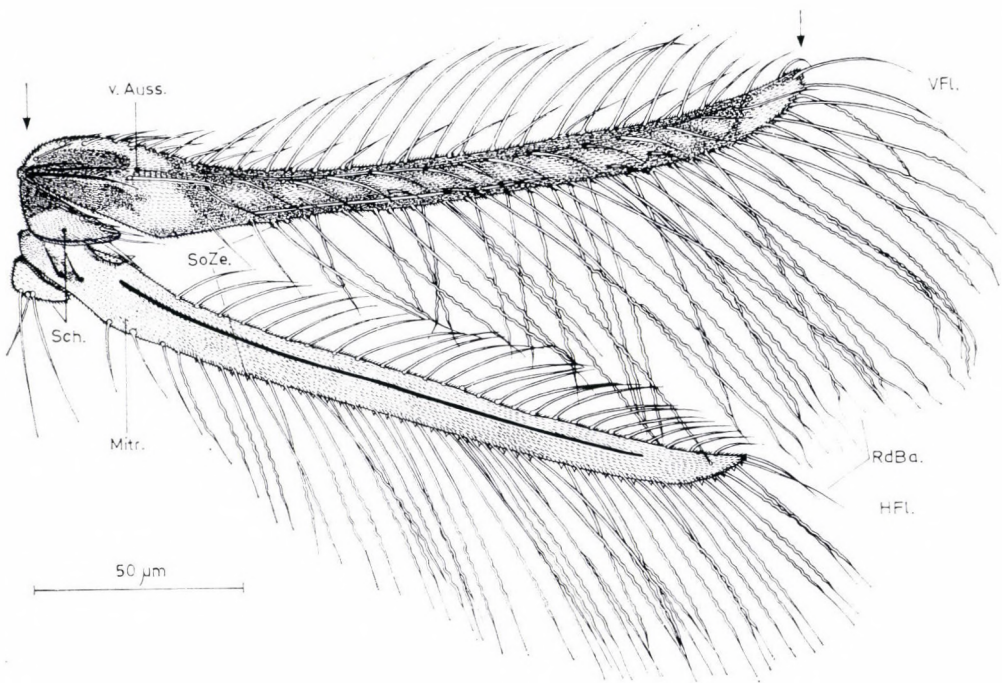

Fig. 1. *Hercinothrips femoralis:* Right forewing and hindwing of a female imago. (v. Auss. = vein sacks, SoZe. = fringe socket cell, VFl. = forewing, Sch. = scale, Mitr. = microtrichia, RdBo. = fringe cilia, HFl. = hindwing)

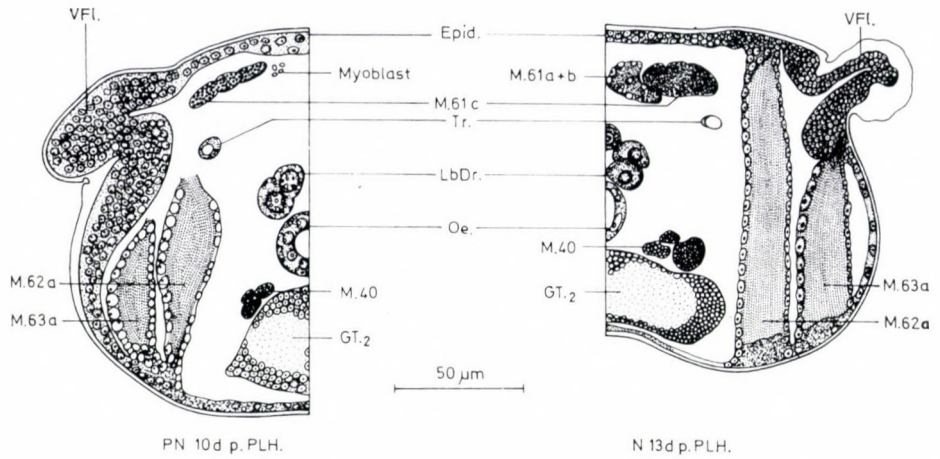

Fig. 2. *Hercinothrips femoralis:* Development of the M. sternonotalis, the M. praecoxo-basalaris and the oscillatory part of the M. dorsalis rectus in prepupal and pupal stage. (Epid. = epidermis, GT. = thoracic ganglion, Lbdr. = labial gland, M. = muscle, Oe. = esophagus, PLH. = moulting first instar, PN. = prepupal, Tr. = trachee, VFl. = forewing, N. = pupal)

body length $1.5 \cdot 10^{-3}$ m; locomotion speed of about $1 \cdot 10^{-1}$ m $\cdot$ s^{-1}; R_e = = 10.07). This means that air for Thysanoptera is a viscous medium in which they paddle like water beetles in water. Adaptation to this medium is achieved via dissolution of the membranous planes of the wings into a long and narrow ribbon whose front and back edges are equipped with long bristles (= fringes) (Fig. 1).

Each wing base is equipped with a caudal scale (alula) linking itself to the respective wing at every wing joint. The formation of the imaginal chaetotaxis already takes place at the pupal stage whereby the long bristles in the wing sheaths have a distal position and the ones at the back edge a proximal position. The different kinds of the fringe bases for the two suborders becomes remarkably evident. Thus, Terebrantia can stretch out their fringes at an angle between 65 and 70° from the wing edge. At rest they are tightly fitted onto the membranous wing planes due to the asymmetric socket cells. On the contrary, the fringe base of Tubulifera is deeply embedded between upper and lower surfaces, thus determining the angle between fringes and membranous wing planes. Presumably, the relatively long abdomen ensures tilting-stability during flight. The alulae of the wings guarantee both the coupling of fore- and hind wings (functional single-wingness) and the fixation of the wings at rest. Thus, the alulae of the fore wings have two bristles turned in itself, folding distally. The opening between these two allows coupling a trichobothrian-like bristle of the hindwing. This link is supported by a dense row of bristles as well as by a microtrichia-free ribbed sclerid

region of the hind wing which can be crossed with the lower surface of the alula of the forewing. The alula of the hindwing bears on the caudal edge four cranially bended sharp teeth supporting the fixation of the wings at rest.

Musculature (flight motor)

The function of the wings is strongly connected with the sclereid-membrane system of the thorax because increased mobility of the imagines through wings needs higher stability of the pterothoracic structures. Kinematics of the wings allows a subdivision into levators, depressors, promotors and remotors as well as regulation of the wing plane. Raising the wings is exclusively the task of sterno-notal groups of muscles (m. 62), whereas depressing movements are performed

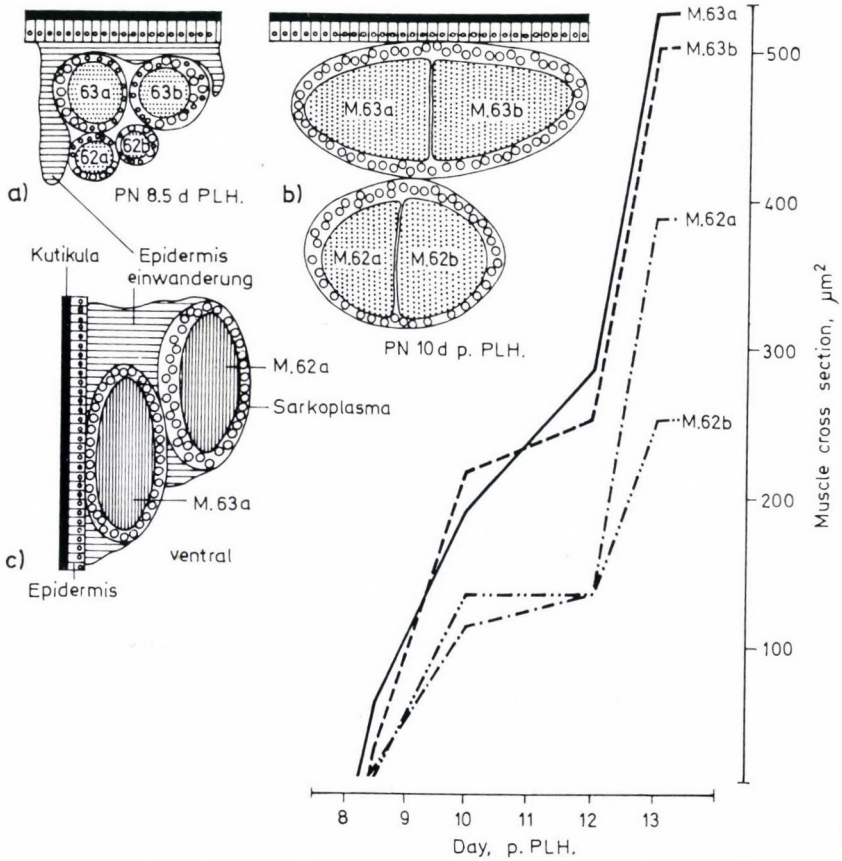

Fig. 3. *Hercinothrips femoralis:* Development of the dorsoventral muscles in prepupal and pupal stage. a) and b) transversal selections of the muscle 62 and 63, c) number of myofibril bundles dependent on the age of the stage (M. = muscle, PLH. = moulting first instar, PN. = prepupal)

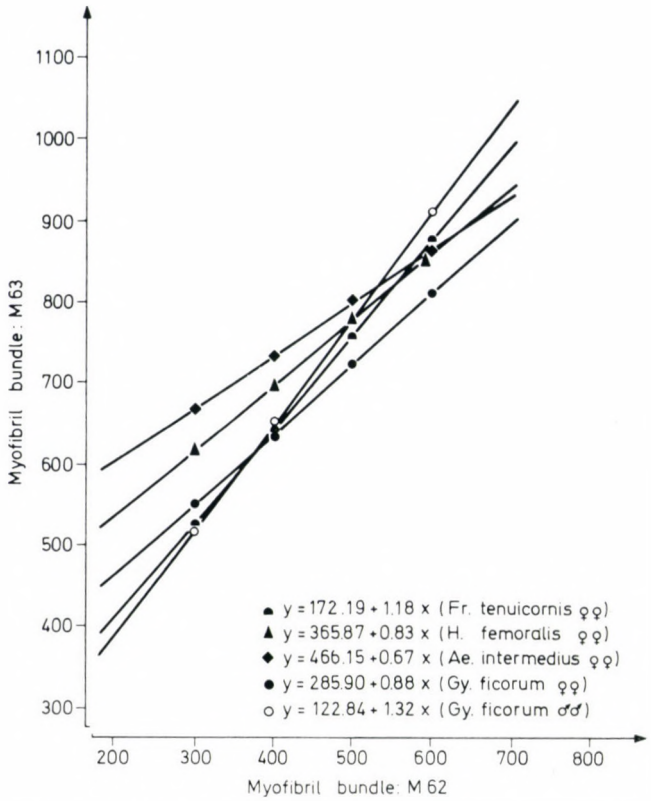

Fig. 4. Relation of myofibril bundles of the M. 62 and M. 63 in some species of the *Thysanoptera*

by both indirect (m. 61) and direct muscles (m. 63) together (Fig. 2). The development of the groups of muscles begins immediately after the prepupal moulting. Thus, at the prepupal stage, aggregation of myoblasts in the pleural and the sternal areas become visible in close connection to the hypertrophic epidermis. The aggregations will enclose a basophile central space as muscular nuclei during the further stages of development. The origin of the myoblasts is still uncertain and could be explained either by a secondary separation from the epidermis or by independent precells floating in the myxocoel. Within this central space bundles of myofibrils are formed whose number and diameter increases periodically during the stages of metamorphosis. A dorsoventral parallel position of the bundles of myofibrils already becomes visible at the prepupal stage. The birefringence develop only immediately before the imaginal moulting.

Their formation is accompanied by an acidosis of the cytoplasm situated between the fibrils (Fig. 3).

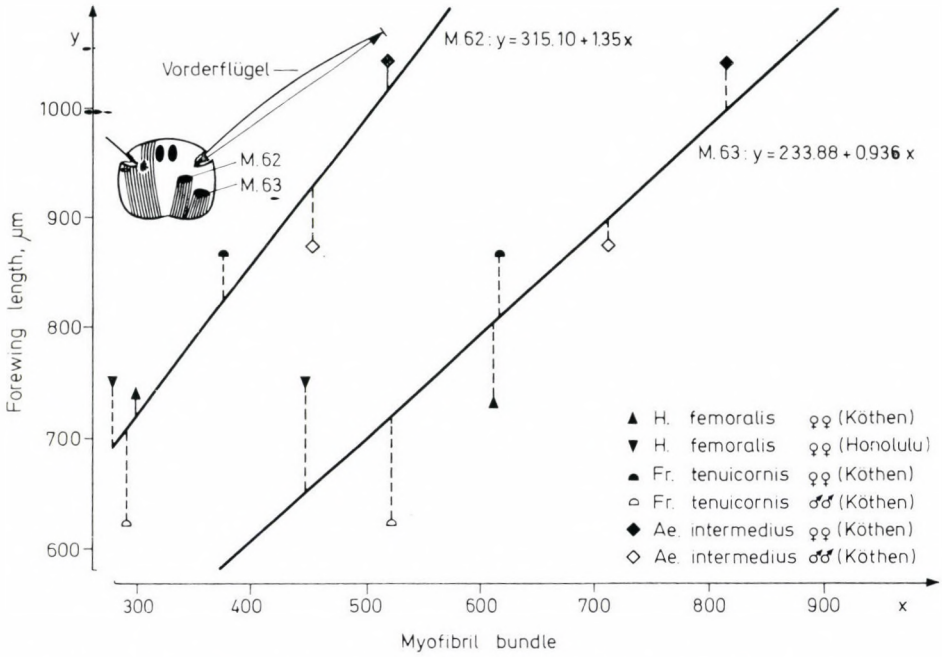

Fig. 5. Relations between the length of forewing and the flight muscles in some species of the *Thysanoptera*

The close relationship between pre-muscles and epidermis at the beginning which is still strengthened by epidermal curves disappears successively through medial dislocation of muscle groups. At the same time the dorsal and the ventral fixations of the indirect wing muscle move towards the centre. At these very points epidermal cells and musculature have direct contact to each other whereby the epidermal part provides for the connection with the cuticle with the help of tonofibrils. The dorsal longitudinal wing musculature consists of the M. dorsalis rectus (M. 61) which – like the dorso-ventral wing muscles – stems from spindle-shaped structures whose proportion of myofibrils increases in length and breadth. However, the syncytial combination of myoblast aggregations of this muscle which strive for partition is carried out only at the end of the prepupal stage. This means that the temporal differentiation of the wing musculature starts off with the M. sterno-notalis (M. 62) followed by M. praecoxo-basalaris (M. 63), and ends with the M. dorsalis rectus (M. 61).

Due to its particular features at the adult stage the wing musculature can relatively easily be distinguished. It is significantly different from normal skeleton muscle tissue with respect to remarkably shorter lengths of the sarkomers (wing muscles: 1.5 µm; other muscles: 5 µm). Such contractile elements, here referred to as oscillatory muscles cause vibrations of high frequency in the pterothorax resonance system. These vibrations are intensified by a bistable clicking-mechanism

of the wing muscles which cannot be started off with the help of neurons the starting mechanism must be of a different kind. With *Hercinothrips femoralis*, this function is carried out by very strong metafurcal trochanteric depressor in connection with other groups of muscles. Its contraction makes the basalar oscillatory trochanteric depressor oscillate which in turn causes the entire system of asynchronous wing muscles to work. Apart from that, contraction of the trochanteric depressors including the stretching of the hind legs has a supporting effect on the starting phase.

The quantity of bundles of myofibrils in the wing-raiser compared to the quantity in the wing-descendor reveals a proportion of 1 : 2 in *Hercinothrips femoralis*. Investigations of other species confirm this fact. From that it can be concluded that these microinsects require more power for the downward wing movements. The comparison of bundles of myofibrils of geographically interesting populations (*Hercinothrips femoralis:* Köthen/Hawaii) shows a difference coefficient for the mesothoracic wing descendor of 1.13 (cf. Mey and Botosaneanu 1985). The absolute figures of the bundles of myofibrils of the island population reveal significant differences (U-test, according to Mann and Whitney) compared with the Köthen population. In addition to this it could be proved that the species of Terebrantia with parthenogenetic arrhenotoky show a distribution of bundles of myofibrils the differs significantly according to sex. All species under investigation reveal a certain interplay of wing raisers and descendors. Given an error rate of 5 per cent for *Hercinothrips femoralis* a correlative interaction was found for females of *Frankliniella tenuicornis* and *Aeolothrips intermedius* just as with the males and females of Tubuliferan *Gynaikothrips ficorum* (Fig. 4).

If one takes the length of the membranous wing plane it becomes obvious that species with a higher number of bundles of myofibrils also have longer wings. Determining the straight regression line for the wing raisers and descendors only makes sense with Terebrantia. Tubulifera have a completely different wing structure (Fig. 5).

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Preliminary Study of Facetal Pigmentation in The Compound Eyes of *Terebrantia (Thysanoptera)*

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Facetal pigmentation of the compound eyes has not been used in the systematic studies of the Thysanoptera. Representatives of 500 species in 7 families of the suborder Terebrantia were examined from various areas of the world. Pigmentation was found in the ventral facets of 306 species. The number of pigmented facets and distribution pattern were constant for each species, usually for both sexes, and usually in each genus. Pigmented facets are absent from the compound eyes of Adiheterothripidae, Faurielli-dae, Merothripidae, Uzelothripidae and Aeolothripidae, except for three species. Six pigmented facets were characteristic of most Heterothripidae, whereas, in Thripidae, pigmentation, when present, was found in 2–7 facets. Facetal pigmentation is princi-pally a generic character and only occasionally a specific character.

The compound eyes have been used only superfically in the taxonomy of Thysanoptera, principally because the eyes are often inadequately cleared, and their details are difficult to observe. The compound eyes of unmounted thrips normally have red pigment granules in the ommatidial pigment cells and those of uncleared specimens usually appear black when mounted on slides. While studying slide mounted specimens of *Frankliniella occidentalis* (Pergande) and related species with cleared eyes, I noticed that some facets of the ventral omma-tidia were pigmented orangebrown. I originally considered this pigment to be an artifact; but after further study, facetal pigment was consistently found in the same number of ventral facets and in an identical pattern on all specimens of the same species. Pigment was not observed in the dorsal facets. Examination of many other species in different genera revealed that the ventral facets of many species were also pigmented. Although Peterson (1915) illustrated facetal pigments for *Caliothrips phaseoli* (Pergande), this character has not been studied compre-hensively nor used previously in thrips taxonomy. This study was initiated to determine the distribution of pigmented facets among species, genera and families and to assess the significance of this character in the systematic study of the terebrantians.

Material and Methods

Macerated, slide mounted specimens with adquately cleared eyes were examined for each species whenever available. Although some species with facetal pigmentation did not have any representative specimens with completely cleared eyes, facetal pigmentation was observed at least in two adjoining anteromarginal facets. In specimens of some species, the cuticular pigment apparently was bleached when the eyes were cleared, and a series of specimens had to be examined to ascertain whether pigmented facts were present. Inadequately cleared eyes may appear to have additional, pigmented facets or a different distribution pattern. In members of some species groups, the presence or absence of pigmented facets or the number of pigmented facts were difficult to ascertain.

Five hundred species in 102 genera in 7 families of the suborder Terebrantia from various areas of the world deposited in the Thysanoptera Collection of the U. S. National Museum were studied (Table 1). The examined taxa are listed by family and genus in alphabetical order. The family Thripidae is further categorized into subfamily and tribe and the tribe Thripini into subtribes. The seven families listed were proposed by Mound et al. (1980), and the higher categories for Thripidae are those of Jacot-Guillarmod (1971, 1974, 1975).

The number of pigmented facets is given for each species. "Yes" indicates that the species has pigmented facets but that exact numbers could not be ascertained. A question mark (?) is used in uncertain situations.

Figures 1–10 are drawn to different scales and are diagrammatic to show the number of pigmented facets and their distribution pattern.

Results and Discussion

Each compound eye of terebrantian thrips either lacks or has normally two to seven pigmented facets located ventrally. Pigmented facets often are larger than others on the venter, and usually occur in a constant position in relation to each other for a species and often for a genus. The same number and distribution pattern usually occur in both sexes of the same species; however, males with fewer ommatidia than females may have fewer pigmented facets and have them in a different pattern. The six ommatidia that normally have pigmented facets are numbered from 1–6 (Fig. 1); this pattern usually occurs in the heterothripids and thripids. Species with pigmented facets normally have two adjoining ones on the anterolateral margin (numbers 1 and 2), and one mesad of 2 (number 3). When four or five pigmented facets are present, number 6 is always unpigmented. Species with four pigmented facets lack pigmentation in either facet 4 or 5. The designated numbers for the pigmented facets are used in the discussion of distribution patterns.

Adiheterothripids, fauriellids, merothripids and uzelothripids apparently lack pigmented facets. Fifty-nine species of aeolothripids lack pigmented facets

Table 1

Distribution of Pigmented Facets in the Compound Eyes of Terebrantian Thrips

ADIHETEROTHRIPIDAE

Oligothrips oreios Moulton 0.

AEOLOTHRIPIDAE

(Examined: 62 species in 18 genera)

Aeolothrips auricestus Treherne 0; *bicolor* Hinds 0; *brevicornis* Bagnall 0; *bucheti* Bagnall 0; *carpobrotus* Hartwig 0; *cursor* Priesner 0; *deserticola* Priesner 0; *duvali* Moulton 0; *ericae* Bagnall 0; *fallax* zur Strassen 0; "*fasciatus*" (L.) 0; *hartleyi* Moulton 0; *intermedius* Bagnall 0; *melaleucus* Haliday 0; *mexicanus* Priesner 0; *quercicola* Bournier 0; *scabiosatibae* Moulton 0; *tenuicornis* Bagnall 0. *Allelothrips cincticornis* Bagnall 0. *Andrewthaia kellyana* (Bagnall) 6. *Ankothrips aequalis* Moulton 0; *diffractis* Hood 0; *gracilis* Moulton 0; *niezabitowskii* (Schille) 0; *notablis* Bailey 0; *yuccae* Moulton 0. *Corynothripoides marginipennis* Bagnall 0. *Cranothrips karrooensis* Jacot-Guillarmod 0; *ravidus* Mound 0; *sititor* Mound 6; *vesper* Mound 6. *Dactuliothrips boharti* Bailey 0; *spinosus* Moulton 0; *xerophilus* Bailey 0. *Desmidothrips walkerae* Mound 0. *Desmothrips australis* (Bagnall) 0; *reedi* Mound 0; *tenuicornis* (Bagnall) 0. *Dorythrips chilensis* Hood 0. *Ekplectothrips priesneri* Bournier 0. *Erythrothrips arizonae* Moulton 0; *asiaticus* Ramakrishna & Margabandhu 0; *bishoppi* Moulton 0. *Franklinothrips atlas* Hood 0; *fulgidus* Hood 0; *tenuicornis* Hood 0; *vespiformis* (Crawford) 0. *Melanthrips fuscus* (Sulzer) 0; *libycus* Priesner 0; *pallidior* Priesner 0; *setarieae* Hartwig 0. *Mymarothrips ritchianus* Bagnall 0. *Rhipidothripiella turneri* (Moulton) 0. *Rhipidothrips brunneus* Williams 0; *cinctus* Hood 0; *gratiosus* Uzel 0. *Stomatothrips angustipennis* Hood 0; *atratus* Hood 0; *flavus* Hood 0; *rotundus* Hood 0; *septenarius* Hood 0.

FAURIELLIDAE

Fauriella natalensis Hood 0.

HETEROTHRIPIDAE

(Examined: 37 species in 2 genera)

Aulacothrips dictyotus Hood 0. *Heterothrips aesculi* Watson 6; *analis* Hood 0; *angusticeps* Hood 6; *arisaemae* Hood 6; *auranticornis* Watson 6; *azaleae* Hood 6; *bicolor* Hood 6; *borinquen* Hood 6; *cacti* Hood 6; *clusiae* Hood 0; *cuernavacae* Watson 6; *decacornis* Crawford 6; *decoratus* Hood 6; *fimbriatus* Hood 6; *flavicruris* Hood 6; *flavidus* Hood 6; *lasquerellae* Hood 6; *limbatus* Hood 6; *lyoniae* Hood 6; *marginatus* Hood 6; *mexicanus* Watson 6; *miconiae* Hood 6; *minor* Hood 6; *nudus* Moulton 6; *ornatus* Hood 6; *pectinifer* Hood 6; *peruvianus* Hood 6; *prosopidis* Crawford 6; *quercicola* Crawford 6; *salicis* Shull 6; *sericatus* Hood 0; *trinidadensis* Hood 6; *vernus* Hood 6; *vitis* Hood 6; *watsoni* Bailey & Cott 6; *xolismae* Hood 6.

MEROTHRIPIDAE

(Examined: 11 species in 3 genera)

Damerothrips gemmatus Hood 0. *Erotidothrips mirabilis* Priesner 0. *Merothrips brunneus* Ward 0; *floridensis* Watson 0; *mirus* Crawford 0; *morgani* Hood 0; *nigricornis* Hood 0; *ottonis* zur Strassen 0; *productus* Hood 0; *tympanis* Hood 0; *williamsi* Priesner 0.

Table 1 (continued)

THRIPIDAE

(Examined: 388 species in 78 genera)

Subfamily Panchaetothripinae

Anisopilothrips venustulus Priesner 6. *Astrothrips aucubae* (Kurosawa) 6; *bhatti* Mound 6; *roboris* (Bagnall) 6. *Australothrips bicolor* Bagnall 6. *Brachyurothrips anomalus* Bagnall 0. *Caliothrips braziliensis* (Morgan) 6; *cintipennis* (Hood) 6; *fasciapennis* (Hinds) 6; *fasciatus* (Pergande) 6; *graminicola* Bagnall & Cameron 6; *helini* Hood 6; *impurus* (Priesner) 6; *indicus* (Bagnall) 6; *insularis* Hood 6; *marginipennis* (Hood) 6; *phaseoli* (Hood) 6; *striatus* Hood 6; *sudanensis* (Bagnall & Cameron) 6. *Chaeturothrips machadoi* Hood 6. *Dinurothrips hookeri* Hood 6; *vezenyii* Bagnall 6. *Elixothrips brevisetis* (Bagnall) 6. *Helionothrips cephalicus* Hood 6; *compressus* Hood 6; *errans* (Williams) 6; *funebris* Hood 0; *kadaliphilus* (Ramakrishna & Margabandhu) 6; *stephaniae* Hood 6. *Heliothrips haemorrhoidalis* (Bouche) 6. *Hercothrips bicinctus* (Bagnall) 6; *brunneus* Hood 6; *dimidiatus* Hood 6; *femoralis* (Reuter) 6; *pattersoni* (Bagnall) 6; *tenuis* Hartwig 6. *Hoodothripoides lineatus* (Hood) 6. *Hoodothrips constrictus* (Hood) 6. *Monilothrips kempi* Moulton 6. *Palleucothrips musae* Hood 0. *Panchaetothrips indicus* Bagnall 6. *Parthenothrips dracaenae* Heeger 6; *Retithrips syriacus* (Mayet) 6. *Selenothrips rubrocinctus* (Giard) 0.

Subfamily Thripinae

Tribe Dendrothripini

Dendrothrips degeeri Uzel 0; *ornatus* (Jablonowski) 0; *saltatrix* Uzel 6. *Leucothrips furcatus* Hood Yes; *nigripennis* Reuter 6; *theobromae* Priesner Yes.

Tribe Sericothripini

Charassothrips urospathae Hood 0. *Hydatothrips abdominalis* (Kurosawa) 0; *adolfifriderici* Karny 0; *rutilus* (Hartwig) 0; *spadix* (Hartwig) 0. *Neohydatothrips abatitus* (Hartwig) 5; *baptisiae* (Hood) 5; *circumfusus* (Priesner) 5; *gracilicornis* Williams 5; *mundus* (Hartwig) 5; *variabilis* (Beach) 5. *Scirtothrips aceris* Moulton Yes; *brevipennis* Hood 4; *citri* (Moulton) 4; *clivicola* Hood Yes; *ewarti* Bailey Yes; *inermis* Priesner 0; *kenyensis* Mound 0; *manihoti* Bondar 4; *multistriatus* Hood Yes; *pan* Mound & Walker 0; *prosopis* Hood Yes; *ruthveni* Shull Yes; *techachapi* Bailey Yes. *Sericothrips abnormis* (Karny) 5; *baileyi* Hood 5; *bicornis* (Karny) 5; *chrysothami* Hood 5; *collaris* Hood 5; *ctenogastris* Hood 5; *ephedrae* Hood 5; *fimbriatus* Hood Yes; *flavicollis* Hood 5; *gracilipes* Hood 5; *hemileucus* Hood 5; *macullicollis* Hood 5; *nubilipennis* Hood 5; *opuntiae* Hood 5; *paraensis* Hood Yes; *pubescens* Hood 5; *pulchellus* Hood 5; *sambuci* Hood 5; *sativus* Hartwig 5; *setosus* Hood 5; *sidae* Crawford 5; *staphylinus* Haliday 5; *vicenarius* Hood 5.

Tribe Chirothripini

Agrostothrips guillarmodi Hood 0; *Chirothrips aculeatus* Bagnall 5, (male 3–4); *alexanderae* Stannard 5; *crassicellis* zur Strassen 5; *crenulatus* Hood 5; *cuneiceps* Hood 5; *cypriotes* Hood 5; *egregius* zur Strassen 5; *falsus* Priesner 5; *faurei* zur Strassen 5; *fulvus* Moulton 5; *hamatus* Trybom 5; *hoodi* Jacot-Guillarmod 5; *insolitus* Hood 5; *kurdistanus* zur Strassen 5; *manicatus* Haliday 5; *mexicanus* Crawford 5, (male 3–4); *molestus* Priesner 5; *meridionalis* Bagnall 5; *mongolicus* zur Strassen 5; *patruelis* Hood 5; *praeocularis* Andre 5; *pretorianus* Hood 5; *priesneri* Hood 5; *ruptipennis* Priesner 5; *similis* Bagnall 5; *simplex* Hood 5; *spiniceps* Hood 5; *spinulosus* Andre 5; *takahashii* Moulton 5; *talpoides* Hood 5; *tenuicauda* zur Strassen 5; *texanus* Andre 5; *tuttlei* zur Strassen 5; *vestis* Hood 5. *Limothrips angulicornis* Jablonowsky 6; *cerealium* (Haliday) 6; *consimilis* Priesner 6; *denticornis* Haliday 6.

Table 1 (continued)

Tribe Thripini

Subtribe Aptinothripina

Anaphothrips amoenus Hood Yes; *articulosus* Priesner Yes; *atroapterus* Priesner Yes; *bicinctus* Hood 6?; *bicolor* Morgan 6; *cameroni* (Bagnall) 6; *catawba* Hood 6; *decolor* Hood Yes; *euphorbiae* Uzel 6; *eversi* zur Strassen Yes; *figuratus* zur Strassen 6?; *flavicinctus* (Karny) 6?; *gracillimus* Priesner 6?; *graminum* Priesner Yes; *launaeae* zur Strassen Yes; *limbatus* Hood 6; *mohelensis* Pelikan Yes; *nanus* Hood 4; *obscurus* (Mueller) 6; *occidentalis* Pitkin 6; *orchidaceus* Bagnall 0; *pannonicus* Priesner Yes; *retamae* Priesner Yes; *ripicola* Hood 6; *sakimura* Ananthakrishnan Yes; *silvarum* Priesner 6; *sordidus* Priesner 6; *spartina* Hood 6; *speciosus* Hood Yes; *sudanensis* Trybom 6; *transvaalensis* Faure Yes; *tenebrosus* Hood Yes; *validus* Karny Yes; *vitalbae* Bagnall 6?; *zelandicus* Mound 0; *zizania* Hood 6. *Apterothrips secticornis* (Trybom) 6. *Aptinothrips rufus* (Haliday) 5; *stylifer* Trybom 3. *Arpediothrips mojave* Hood 4. *Ascirtothrips antilope* (Priesner) 5; *arya* zur Strassen Yes; *brunneus* zur Strassen Yes; *docas* zur Strassen Yes; *tamaricis* zur Strassen Yes. *Baileyothrips arizonensis* (Morgan) 6. *Belothrips acuminatus* Haliday 6; *morio* Reuter 6; *pillichi* Priesner 6?. *Bregmatothrips gracilis* Hood & Williams 5; *sonorensis* Stannard 5; *venustus* Hood 5. *Caprithrips decorus* Faure 2; *insularis* Beshear 5. *Chaetanaphothrips clarus* (Moulton) 0; *machili* Hood 0; *orchidii* (Moulton) 0; *signipennis* (Bagnall) 0. *Chilothrips pini* Hood 6. *Dendrothripoides innoxius* (Karny) 5. *Oxythrips ajugae* Uzel 6; *bicolor* Reuter 6; *cannabensis* Knechtel 6; *coloradensis* Hood 6; *nickelae* zur Strassen Yes; *pinicola* Hood 6; *priesneri* Pelikan 6; *ulmifoliorum* (Haliday) 6. *Proscirtothrips zeae* (Moulton) 6. *Pseudanaphothrips achaetus* (Bagnall) 0.

Subtribe Thripina

Adelphithrips nothofagi Mound & Palmer 0. *Baliothrips dispar* Haliday 0; *kroli* (Schille) 0. *Bolacidothrips oryzae* Moulton 0. *Bolacothrips jordani* Uzel 0. *Ceratothripoides brunneus* (Bagnall) 0; *cameroni* (Priesner) 5. *Ceratothrips discolor* (Karny) 5; *ericae* (Haliday) 0; *frici* (Uzel) 5: *hilarius* zur Strassen 5; *pallidivestis* Priesner Yes. *Coremothrips nubilans* Hood 0; *pallidus* Hood 0. *Ctenothrips bridwelli* (Franklin) 0. *Dichromothrips corbetti* (Priesner) 0; *indicus* Mound 0; *maori* Mound 0; *nakahari* Mound 0; *viatorus* Mound 0. *Dorcadothrips caespitis* Priesner 0; *walteri* (Crawford) 0; *xanthius* (Williams) 0. *Echinothrips americanus* Morgan 7; *asperatus* Hood 0; *caribeanus* Hood Yes; *mexicanus* Moulton 7; *subflavus* Hood 7. *Frankliniella annulipes* Hood 3; *auripes* Hood 3; *bratleyi* (Watson) 3; *brunneri* (Watson) 4; *cephalica* (Crawford) Yes; *citripes* Hood 3; *exigua* Hood 4; *extremitata* Hood 4; *fusca* (Hinds) 4; *fuscipennis* Moulton Yes; *georgiensis* Beshear 3; *gossypiana* Hood Yes; *hawksworthi* O'Neill Yes; *hemerocallis* Crawford 4; *inutilis* Priesner 4; *intonsa* (Trybom) 3–4; *invasor* Sakimura 3; *iridis* (Watson) 4; *kelliae* Sakimura 3; *lilivora* (Kurosawa) 5; *minuta* (Moulton) Yes; *nigricauda* Hood Yes; *occidentalis* (Pergande) 4; *pallida* (Uzel) 3; *panamensis* Hood 4; *parvula* Hood 0?; *phaener* Hood 5; *regia* Hood 3; *schultzei* (Trybom) 5; *simplex* Priesner 4; *sulfuripes* Hood 3; *tenuicornis* (Uzel) 3; *tritici* (Fitch) 4; *tuberosi* Moulton 5; *vaccini* Morgan 4; *williamsi* Hood 4; *xanthomelaena* Hood 3. *Fulmekiola serrata* Kobus 0. *Glaucothrips glaucus* (Bagnall) 6. *Isochaetothrips setipennis* (Bagnall) 0. *Megalurothrips distalis* (Karny) 5?; *kellyanus* (Bagnall) 0; *sjoestedti* (Trybom) 5; *usitatus* (Bagnall) 5. *Microcephalothrips abdominalis* (Crawford) 0. *Mycterothrips consociatus* (Targioni-Tozzetti) Yes; *glycines* (Okamoto) 5; *latus* (Bagnall) 5. *Neurisothrips antennatus* (Moulton) 5; *fullawayi* (Moulton) 5. *Odontothripiella australis* (Bagnall) 5; *compta* Pitkin 5; *concolorata* Pitkin 5; *fasciatipennis* (Bagnall) 5; *passalaina* Pitkin 5. *Odontothrips loti* (Haliday) 5; *pictipennis* Hood 5; *retamae* Priesner 5; *biuncus* John 5. *Organothrips bianchi* Hood 4. *Pezothrips dianthi* (Priesner) 5. *Plesiothrips ayarsi* Stannard 0; *brunneus* Hood 0; *perplexus* (Beach) 0; *setivestris* Hood 0. *Pseudothrips beckhami* Beshear 6; *inequalis* (Beach) 6; *interruptus* Hood Yes; *spadix* Hood 6. *Rhamphothrips pandens* Sakimu-

Table 1 (continued)

ra 5. *Rhaphidothrips longistylosus* Uzel 0. *Scolothrips pallidus* (Beach) 4; *priesneri* Sakimura 4; *sexmaculatus* (Pergande) 4. *Stenchaetothrips bambusae* (Shumsher Singh) Yes; *brasiliensis* (Hood) Yes; *melanurus* (Bagnall) 5. *Stenothrips graminum* Uzel 0. *Synaptothrips distinctus* (Bagnall) 4; *geyeri* Faure 4; *gezinae* (Faure) 4; *psoraleae* Jacot-Guillarmod 4; *rhynchosiae* (Faure) 4. *Taeniothrips eucharii* (Whetzel) 0; *inconsequens* (Uzel) 0; *mexicanus* Priesner 0; *orionis* Treherne 0; *picipes* Zetterstedt 0; *croceicollis* Costa 0; *funestus* Hood 0; *silvestris* Hood 0. *Thrips addendus* Priesner 0; *albopilosus* Uzel 0; *alliorum* (Priesner) 0; *alysii* Hood 0; *annulatus* (Karny) 0; *angusticeps* Uzel 0; *aureus* Hood 5; *brevicornis* Priesner 0; *brevipilosus* Moulton 0; *calcaratus* Uzel 0; *discolor* Haliday 0; *fascicornis* zur Strassen 0; *flavus* Schrank 0; *flavidulus* Bagnall 0; *floreus* Kurosawa 0; *florum* Schmutz 0; *frosti* Moulton 0; *fuscipennis* Haliday 0; *gracilis* Moulton 0; *hawaiiensis* (Morgan) 0; *helianthi* Morgan 0; *heraclei* Moulton 0; *herricki* Bagnall 0; *illicis* Hood 0; *imaginis* Bagnall 0; *impar* Hood 0; *inopinatus* zur Strassen 0; *juniperinus* L. 0; *madronii* Moulton 0; *magnus* Moulton 0; *major* Uzel 0; *meridionalis* (Priesner) 0; *minutissimus* L. 0; *monotropae* Hood 0; *nigropilosus* Uzel 5; *obscuratus* (Crawford) 0; *orientalis* (Bagnall) 0; *origani* Priesner 0; *pallidicollis* Hood 0; *palmi* Karny 0; *physapus* L. 0; *pillichi* Priesner 0; *quinciensis* Morgan 0; *rapaensis* (Moulton) 0; *rhabdotus* Sakimura 0; *robustus* Priesner 0; *setosus* Moulton 0; *sierrensis* Gentile & Bailey 0; *simplex* (Morison) 0; *sumatrensis* Priesner 0; *sieversiae* Hood 0; *spinosus* Morgan 0; *tenellus* Trybom 0; *thalictri* Hood 0; *tripartitus* Hood 0; *urticae* F. 0; *validus* Uzel 0; *varipes* Hood 0; *vulgatissimus* Haliday 0; *winnemanae* Hood 0.

UZELOTHRIPIDAE

Uzelothrips scabrosus Hood 0

and only *Andrewarthaia kellyana* (Bagnall) (Fig. 2) and two *Cranothrips* spp. have 6 pigmented facets. Pigmented facets of the *Cranothrips sititor* Mound and *C. vesper* Mound follow the distribution pattern found in other families but *A. kellyana* has a distinct pattern that is different from other terebrantians. Thirty-three species of *Heterothrips* (Heterothripidae) have six pigmented facets per eye (Fig. 3) and four species lack pigmentation.

In the Thripidae, 36 species of Panchaetothripinae have six pigmented facets, five species have unknown number of pigmented facets, four species lack pigmentation and *Dendrothripoides innoxius* (Karny) (= *ipomoeae* Bagnall), included in the Panchaetothripinae by Jacot-Guillarmod (1971), has five pigmented facets per eye (ex. Fig. 4). According to Wilson (1975), *D. innoxius* does not belong in this subfamily but in the Anaphothripina (Thripinae). In the Thripinae, the number of pigmented facets varies, usually along generic lines. In the Chirothripini, four species of *Limothrips* spp. have six pigmented facets per eye, and 34 *Chirothrips* spp. have five pigmented facets in females and some males have fewer pigmented facets; *C. aculeatus* Bagnall and *C. mexicanus* Crawford males apparently have three pigmented facets (Fig. 5), whereas females have facets 1–5 pigmented (Fig. 6). Sexual differences evidently are related to the fewer ommatidia in male eyes. In the Sericothripini most species of *Sericothrips* have five pigmented facets per eye and endemic, New World *Scirtothrips* species apparently have four pigmented facets.

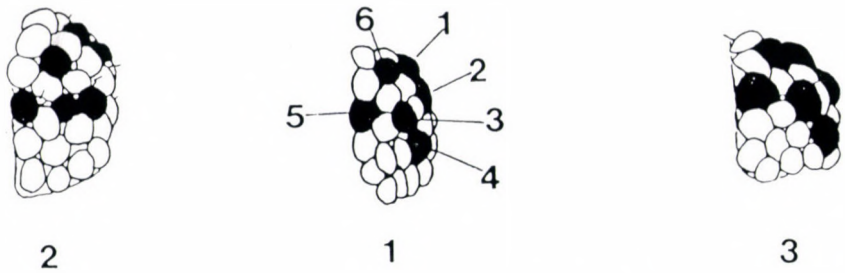

Fig. 1. Ventral aspect of the compound eye *Anaphothrips obscurus* (Mueller), female right eye

Fig. 2. *Andrewarthaia kellayna* (Bagnall), male right eye

Fig. 3. *Heterothrips aesculi* Watson, female right eye

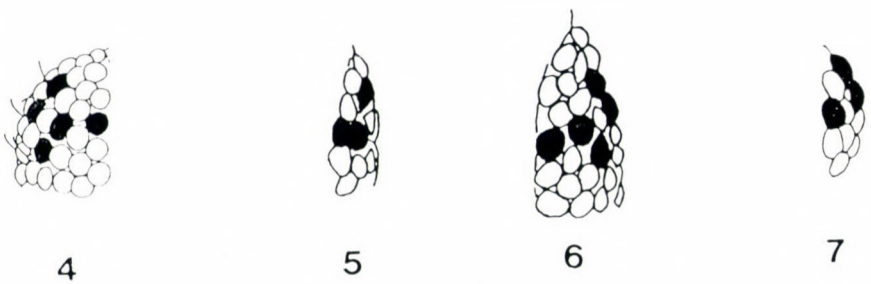

Fig. 4. *Thrips nigropilosus* Uzel, female left eye

Fig. 5. *Chirothrips mexicanus* (Crawford), male right eye

Fig. 6. *Chirothrips mexicanus* (Crawford), female right eye

Fig. 7. *Aptinothrips stylifer* Trybom, female right eye

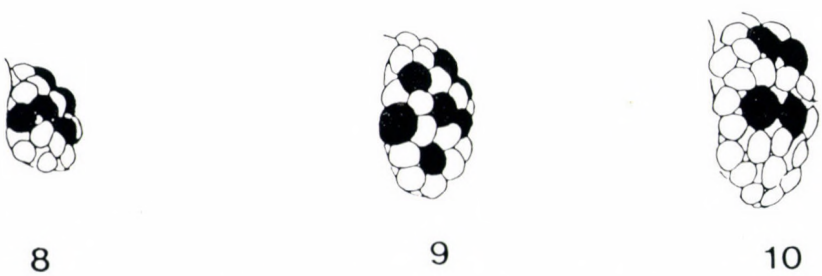

Fig. 8. *Aptinothrips rufus* (Haliday), femal right eye

Fig. 9. *Echinothrips subflavus* Hood, female right eye

Fig. 10. *Scolothrips sexmaculatus* (Pergande), female right eye

In the Thripini, 34 species of *Anaphothrips* have pigmented facets (18 species with six in 1–6 pattern (Fig. 1), one species with four, and 15 species with unknown number of pigmented facets) and two species lack pigmentation. *Aptinothrips stylifer* Trybom has apparently three pigmented facets (Fig. 7) in contrast to five for *A. rufus* (Haliday) (Fig. 8). *Caprithrips decorus* Faure has two pigmented facets in 1–2 pattern, whereas, *C. insularis* Beshear has five pigmented facets in 1–5 pattern. Most *Echinothrips* spp. have seven pigmented facets (Fig. 9), and some species of *Heterothrips* occasionally appear to have a seventh pigmented facet anterior to 6. In the large genus, *Frankliniella*, which evolved principally in the New World, the number of pigmented facets is more variable than in other groups studied. Most species have either three or four pigmented facets in a 1–3 or 1–3, 5 pattern. Four pigmented facets in a 1–4 pattern occur in *F. hemerocallis* Crawford and *F. iridis* (Watson). These two species are palearctic origin. Some species have five pigmented facets in a 1–5 pattern. Pigmentation in the *minuta* and *tritici* groups is varied and some species appear to have more than five pigmented facets. The number and distribution pattern are difficult to ascertain in these species. Five species of *Synaptothrips* have four pigmented facets in a 1–4 pattern. *Scolothrips* spp. (Fig. 10) have four pigmented facets that are positioned transversely and differ from the normal pattern. The eyes of *Taeniothrips* and *Dichromothrips* species do not have pigmented facets. Fifty-eight members of *Thrips* do not have pigmented facets and only *T. aureus* Hood and *T. nigropilosus* Uzel (Fig. 4) have five pigmented facets in a 1–5 pattern.

Although distribution pattern normally is consistent within a genus, one or more species may have a pattern differing from that of the majority or may lack pigment completely. Often these species have one or more correlated morphological character(s) that differ from the corresponding character(s) of the other species. *Ceratothrips ericae* (Haliday) lacks pigmented facets and differs from four congeners with five pigmented facets in general coloration and in placement of median, posteromarginal setae on abdominal sternite VII. *Anaphothrips nanus* Hood, with four pigmented facets per eye in contrast to six for 18 congeners, differs in having fewer ommatidia in the eyes and the microtrichia of the posteromarginal comb on abdominal tergite VIII fused at their bases. Four members of *Echinothrips* have pigmented facets except for *E. asperatus* Hood with none. The latter species differs from others in having fringed major setae on the pronotum and forewings instead of apically expanded setae.

Thus, the absence or presence of pigmentation is often a generic characteristic. When a species differs in this character from other members in the genus, other significant related difference(s) will often be found; thus indicating that the taxon is incorrectly assigned to that genus or is aberrant. However, when significant differences cannot be found (ex. *Thrips* spp.), the presence or absence of facetal pigments should be considered as a specific character.

Pigmented facets have arisen relatively recently in the evolution of Terebrantia. They are absent from members of the more primitive families, Adiheterothripidae, Aeolothripidae, Merothripidae and Uzelothripidae, but are well devel-

oped in the Heterothripidae and most highly derived family, Thripidae. In the Thripidae, pigmented facets occur with few exceptions in the panchaetothripines, chirothripines and aptinothripines, which are considered to be more primitive groups. In the more specialized subtribe Thripina, (Mound et al. 1980), most species in *Thrips*, which is considered the most evolutionarily advanced group, do not have pigmented facets.

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A Comparative Study of the Chorion of the DDT-resistant and Susceptible Strains of *Thrips palmi* Karny

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A comparative study has been conducted on the structure and chemical nature of the chorion of the pesticide-susceptible and resistant strains of *Thrips palmi*. The structure of the chorion in both strains is similar. There is a thin superficial layer, a middle broad zone overlying an inner membrane. The outer thin layer has lipid and acid mucopolysaccharides. In the susceptible strain the middle and the inner layers have a protein rich in sulphydryl groups and a protein containing aromatic substances, respectively. The middle layer in the resistant strain has disulphide groups while the inner layer has both aromatic as well as disulphide groups. It is suggested that this existence of -R-S-R- groups may be responsible for the resistance of the eggs to pesticides.

Seasamum indicum is a commercial crop in South India; it is cultivated in several hundreds of hectares during the last several decades in private agricultural farms.

During the period from 1960 to 1963 sporadic instances have come to the surface that the flower heads are being eaten by some insect pests and the problem became grave around 1965 crippling the agricultural activities.

The pest which caused the damage was identified as thrips of the species *Thrips palmi* Karny whose occurrence was already reported by Ananthakrishnan (1955) on *Sesamum indicum*.

Pest control measures were geared up in full swing empolying various insecticides and as a result of these operations it was found that DDT (0.5–1.0 lb a.i./ac), Dieldrin (0.3 lb a.i./ac) and Malathion (0.5–2.0 lb a.i./ac) successfully controlled the pest. It was observed that the adults succumbed to the toxicant to various extent and the eggs were more vulnerable.

Within a span of another 5 years (*i.e.* by 1970) it was found that the pest in the southern-most parts of the peninsula was no more affected by these poisons, denoting the development of resistance as in many cases of insect pests and vectors (Ascher, 1955; Brown, 1956; Busvine, 1956; Livadas, 1957). It was found that the same pest in the northern parts of this region however continued to succumb to the poison.

Glasshouse studies revealed that the adults of the thrips from the southern-most region were resistant to the toxicants at the concentrations already mentioned but died when it is increased (DDT 1.0–2.0 lb a.i./ac, Dieldrin 0.5 lb

a.i./ac and Malathion 2.0–3.0 lb a.i./ac). Interestingly it was noted that the eggs of the southernmost region strain are unaffected even by the increased concentration of the pesticides.

Since the method of application of the insecticides is topical it was presumed that the chorion of the eggs of the southern strain of *Thrips palmi* would have developed some mechanism either physical or chemical or both to prevent the entry of the toxicants employed into the egg.

The present investigation was undertaken to find out the exact mechanism involved in rendering the eggshell impregnable by the pesticides in the resistant strain of the thrips.

Materials and Methods

The materials used in the present investigation were the eggs of the resistant strain of *Thrips palmi* collected from the southern region and those of the susceptible strain obtained from the northern zone of South India.

The thrips lays the eggs in short rows beneath the veins in both the strains. The eggs of the resistant and strains were collected and studied separately. They were embedded in epoxy resin, after appropriate processing, and sectioned by ultramicrotome at 2 to 4 μ.

The stains employed were Mallory's triple stain and Heidenhain's iron haematoxylin. The histochemical tests performed are given in the text together with references.

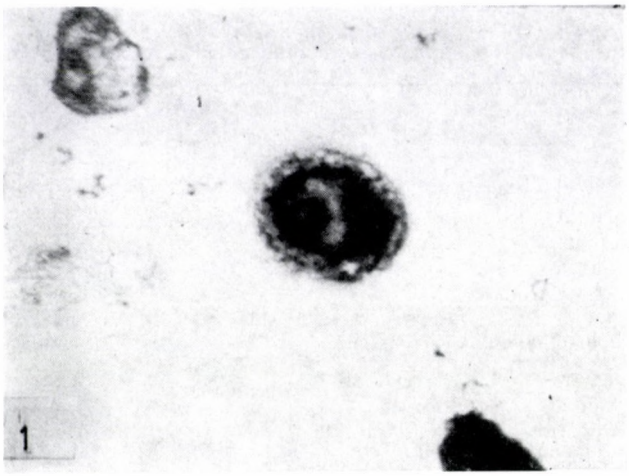
For the study of the amino acid composition of the chorion, the eggs were subjected to ultrasonic breaking in a medium of phosphate buffer (pH 7.0) removal of the soft parts the embryonic body which is emulsified during the process. The solid flakes of the chorion were separated off by centrifugation.

The solid materials thus separated were hydrolyzed in 6N hydrochloric acid under reflux and the hydrolysate analyzed by 2-dimensional paper-partition chromatography on Whatman No. 1 paper. Ninhydrin (0.1% W/V) was used as spray and the amino acids on the chromatogram were identified by comparison with standard chromatogram prepared in the same manner using authentic samples of amino acids.

Results

Structure and staining reactions

Figure 1 is the photomicrograph of a transverse section of an egg of *Thrips palmi* and the structure of a portion of the chorion is semidiagrammatically presented in Figure 2. The chorion is 5 to 7 μ in thickness and comprised of 3 distinctly distinguishable layers. The outermost layer (OCL) is only about a μ in thickness. The middle layer (MCL) forms the bulk of the structure and it is 3 to 4 μ in width. The inner layer (ICL) forms the rest of the thickness of the chorionic membrane.



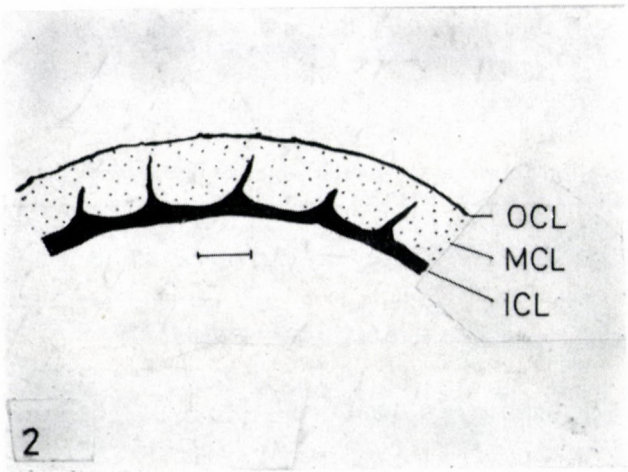


Fig. 1. Transverse section through an egg of the pesticide-susceptible strain of *Thrips palmi*, stained in Heidenhain's haematoxylin

Fig. 2. Semidiagrammatic representation of a part of the chorion of *Thrips palmi*. Scale lin 4 μm. OCL, outer chorionic layer; MCL, middle chorionic layer; ICL, inner chorionic layer

The above histological features are common for the chorion of the pesticide-susceptible and -resistant strains of the thrips. When stained in Heidenhain's haemotoxylin the outer chorionic layer of the pesticide-susceptible strain took a black colour while the middle layer turned grey. The inner layer in contrast stained blue-black. With the Mallory's triple dye these layers stained deep blue, light blue and red, respectively (Table 1).

The staining properties of the layers of the chorion of the pesticide-resistant strain are different from the above. The outer layer alone is reactive to the stains

Table 1

Staining reactions of the layers of the chorion of the pesticide-susceptible strain of *Thrips palmi*

Stains/Tests	References	OCL	MCL	ICL
Mallory's triple stain	Mallory, 1938	Deep Blue	Light Blue	Red
-do- after Diaphanol	Kennaugh, 1957	-do-	-do-	Blue
-do- after alk. stannite	Brown, 1950	-do-	-do-	-do-
-do- after alk. Sod. Sulphide	-do-	-do-	-do-	Red
-do- after Sod. thioglycollate	-do-	-do-	-do-	-do-
Heidenhain's haematoxyline	Lillie, 1965	Black	Grey	Blue Black
-do- after Diaphanol	Kennaugh, 1957	-do-	-do-	Grey
-do- after alk. stannite	Brown, 1950	-do-	-do-	-do-
-do- after alk. Sod. sulphide	-do-	-do-	-do-	Blue Black
-do- after sod. thioglycollate	-do-	-do-	-do-	-do-

OCL, outer layer of the chorion; MCL, middle layer of the chorion; ICL, inner layer of the chorion.

Table 2

Staining reactions of the layers of the chorion of the pesticide resistant strain of *Thrips palmi*

Stains/Tests	References	OCL	MCL	ICL
Mallory's triple stain	Mallory, 1938	Deep Blue	Refractive	Refractive
-do- after Diaphanol	Kennaugh, 1957	-do-	-do-	Red
-do- after alk. stannite	Brown, 1950	-do-	-do-	Red
-do- after sod. thioglycollate	-do-	-do-	Blue	Red
-do- after alk. sod. sulphide	-do-	-do-	Blue	Red
Heidenhain's haematoxylin	Lillie, 1965	Black	Refractive	Refractive
-do- after Diaphanol	Kennaugh, 1957	-do-	Grey	Blue Black
-do- after alk. stannite	Brown, 1950	-do-	-do-	-do-
-do- after sod. thioglycollate	-do-	-do-	-do-	-do-
-do- after alk. sol. sulphide	-do-	-do-	-do-	-do-

employed; it took deep blue with Mallory's triple stain and black with the other stain. The middle and the inner chorionic layers are unreactive to these stains (Table 2).

Kennaugh (1957), Brown (1950) and others used certain chemical reagents to have a preliminary information on the nature of the different layers of the cuticle of several insects and molluscs. These chemicals are of two categories, one employed to detect the presence of phenolic tanning (*i.e.* diaphanol and

alkaline stannite) and the other used to find out the occurrence of disulphide linkages (*Viz:* sodium thioglycollate and alkaline sodium sulphide).

Following treatment with diaphanol the refractive inner chorionic layer of the pesticide-resistant strain becomes reactive and stained red with Mallory and blueblack in Heidenhain's haematoxylin, respectively. The middle layer of the chorion continues to remain unreactive to the stains even after treatment with diaphanol. The reactions of the three layers of the chorion to the stains after treatment with alkaline stannite is similar to those noted after treatment with diaphanol.

The inert middle chorionic layer of the pesticide-resistant strain is rendered stainable by the sodium thioglycellate and alkaline sodium sulphide reagents; after treatment with these two keratinolytic chemicals, the middle layer took a blue colour in Mallory and became grey in Heidenhain's haematoxylin.

A feature that deserves special attention at this juncture is the behaviour of the inner chorionic layer with the two keratinolytic reagents. This layer was normally refractive but became reactive to the stains after treatment with diaphanol and alkaline stannite as already stated. The two keratinolytic reagents also rendered this layer stainable, the staining reactions being similar to those noted after treatment with diaphanol.

In the case of pesticide-susceptible strain also the first category of chemicals modified the staining reactions of the inner layer of the chorion. The layer normally stained red and blue black with Mallory and Heidenhain's haematoxylin, as already given. After treatment with diaphanol and alkaline stannite the same layer became blue and grey with the two stains, respectively.

It may be inferred in the light of the foregoing results that the middle chorionic layer in the pesticide-resistant stain is stabilized by disulphide bonds while the inner layer is hardened with aromatic tanning and –s–s– bonds; the inner layer in the pesticide-susceptible strain has aromatic linkages alone.

Table 3

Histochemical reactions shown by the different layers of the chorion of the pesticide susceptible and resistant strains of *Thrips palmi*, for lipid and mucopolysaccharides

Tests	References	Susceptible strain			Resistant strain		
		OCL	MCL	ICL	OCL	MCL	ICL
Sudan Black-B	Baker, 1946	++	+	–	++	+	–
Sudan IV	-do-	++	+	–	++	+	–
Nile Blue	Cain, 1947	++	+	–	++	+	–
Oil red-O	Lillie, 1965	++	+	–	++	+	–
PAS	Gomori, 1952	+	–	–	+	–	–
Alcian Blue	Steedman, 1950	+	–	–	+	–	–
Toluidine Blue-O	Glick, 1949	β	–	–	β	–	–

Histochemical reactions

Sections of the chorion of both the susceptible and resistant strains were subjected to histochemical tests in order to verify the inference made out of the staining and chemical reagents. Tables 3 to 5 present summaries of the data recorded.

Table 4

Histochemical reactions shown by the layers of the chorion of the pesticide-susceptible strain of *Thrips palmi*, for proteins

Tests	References	OCL	MCL	ICL
Acrolein-Schiff Test	Pearse, 1968	−	+	+
Bromophenol Blue	Mazia *et al*, 1953	−	+	+
Millon's Test	Pearse, 1968	−	−	+
Morner's Test	Lillie, 1965	−	−	+
Hg/nitrite Test	Baker, 1956	−	−	+
Argentaffin Test	Feigl, 1960	−	−	+
Dimethylparaphenylene-diamine	Lison, 1936	−	−	−
Nitroprusside Test	Pearse, 1968	−	+	−
DDD Test	Barnett and Seligman, 1952	−	−	−
Lead Acetate Test	Pearse, 1968	−	−	−
Alk. Tetrazolium	Pearse, 1968	−	−	−
Performic acid-Alcian Blue	Adams and Sloper, 1951	−	−	−
Ferric-ferricyanide Test	Pearse, 1968	−	+	−

Table 5

Histochemical reactions shown by the layers of the chorion of the pesticide-resistant strain of *Thrips palmi*, for proteins

Tests	References	OCL	MCL	ICL
Acrolein-Schiff Test	Pearse, 1968	−	+	+
Bromophenol Blue	Mazia *et al*, 1953	−	+	+
Millon's Test	Pearse, 1968	−	−	+
Morner's Test	Lillie, 1965	−	−	+
Hg/nitrite Test	Baker, 1956	−	−	+
Argentaffin Test	Feigl, 1960	−	−	+
Dimethyl paraphenylene diamine	Lison, 1936	−	−	+
Nitroprusside Test	Pearse, 1968	−	−	−
DDD Test	Bernett and Seligman, 1952	−	+	+
Lead Acetate Test	Pearse, 1968	−	+	+
Alk. Tetrazolium Test	Pearse, 1968	−	+	+
PFA-Alcian Blue	Adams and Sloper, 1951	−	+	+
Ferric-ferricyanide Test	Pearse, 1968	−	−	−

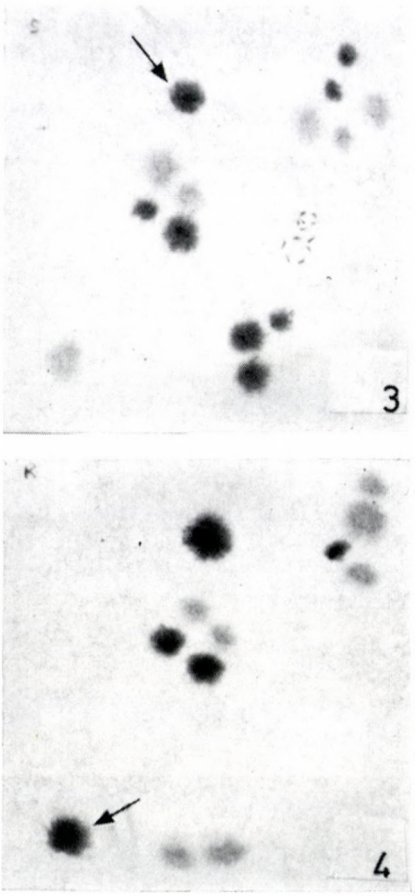


Fig. 3. Two-dimensional chromatogram of the amino acid constituents of the hydrolysate of the pesticide-susceptible strain of *Thrips palmi*. The arrow denotes the position of tyrosine
Fig. 4. Two-dimensional chromatogram of the amino acid constituents of the hydrolysate of the pesticide-resistant strain of *Thrips palmi*. The arrow denotes the position of cystine

The outer layer in both the strains is intensely positive to Sudan Black-B suggesting the lipoidal nature of the layer. This is substantiated by the positive reactions yielded by the layer for the Sudan IV, Nile Blue and Oil red-0.

The same layer is positive to PAS and alcian blue reagents indicating the occurrence of acid mucopolysaccharides. That the acidity of these substances is fairly high is inferable by the β-metachromatic reaction yielded by the layer with the Toluidine Blue-0 reagent (Table 3).

Apart from the outer layer, lipid is present in the middle layer of the chorion also in both the strains as evident from the positive reactions given by the layer

Table 6

Amino acid constitution of the chorion of the pesticide-susceptible and resistant strains of *Thrips palmi*

Sl. No.	Amino acids	Susceptible strain	Resistant strain
	Aliphatic amino acids:		
1.	Alanine	+	+
2.	Glycine	+	+
3.	Isoleucine	+	+
4.	Leucine	+	+
5.	Serine	+	++
6.	Threonine	++	++
7.	Valine	+	+
	Aromatic amino acids:		
8.	Phenylalanine	+	+
9.	Tyrosine	++	++
	Sulphur-containing amino acids:		
10.	Cysteine	+	−
11.	Cystine	−	++
12.	Methionine	+	−
	Heterocyclic amino acids:		
13.	Hydroxyproline	+	−
14.	Proline	+	−
	Acidic amino acids:		
15.	Aspartic acid	−	+
16.	Glutamic acid	−	+
	Basic amino acids:		
17.	Arginine	++	−
18.	Histidine	++	−
19.	Lysine	++	−

to lipophilic stains employed. This layer is however negative to the tests for acid mucopolysaccharides.

The inner layer is unreactive the tests for lipid as well as acid mucopolysaccharides. The outer layer is positive to none of the tests for proteins (Tables 4 and 5). The remaining 2 layers are however distinctly positive to the acrolein-Schiff and bromophenol blue tests, in both the strains, connoting their proteinoceous nature.

The tests for organic sulphur brought out differences in the nature of the protein component of the middle chorionic layer between the two strains. In the

susceptible strain the layer is positive only to nitroprusside and ferric-ferricyanide tests. Its homologue in the resistant strain is negative to these 2 tests but positive to DDD, lead acetate, alkaline tetrazolium and PFA-Alcian blue reactions. These results denote that the middle layer in the susceptible strain contains organic sulphur in the form of SH groups while its counterpart in the resistant strain has –S–S– groups.

Differences are seen also in the precise nature of the constitutents of the inner chorionic layer in the two strains. In the susceptible strain the layer is positive to Millon and Morner's tests. To the Hg/nitrite and the argentaffin tests also the layer yields positive reaction suggesting the presence of aromatic substances. That these substances are simple and not quinones is denoted by the negative dimethyl paraphenylene-diamine test shown by the same layer.

The inner chorionic layer in the resistant strain presents a complex picture as evident from the results recorded in Table 5. The layer is positive to all the tests for aromatic substances including the dimethyl paraphenylene-diamine test. The same layer responded positively to tests for disulphide bonds like DDD, lead acetate, alkaline tetrazolium and PFA-alcian blue.

Amino acid analyses

The combined occurrence of both the aromatic and sulphur groups in the same structure is rare (Hughes, 1959). Hence hydrolysates of the chorion of both the strains were subjected to analyses of their amino acid constitution.

The susceptible strain contains 16 amino acids such as the full compliments of the aliphatic, aromatic, heterocyclic and basic amino acids. Among sulphur-containing amino acids cysteine and methionine are found (Table 6 and Fig. 3).

The hydrolysate of the chorion of the resistant strain has only 12 amino acids which includes both the aromatic amino acids in addition to the sulphur-containing cystine. Heterocyclic and basic amino acids are unrepresented but both the acidic ones are found (Table 6 and Fig. 4).

Discussion

The previous literature on the eggs of thrips deals with the shape, colour, shell sculpture and size (Russell, 1912; Morison, 1947; Loan and Holdaway, 1955). Hall (1930) reports an interesting phenomenon with the eggs of *Scirtothrips aurantii;* the eggs when fleshly laid are kidney-shaped but later when inserted into the citrus fruits absorb the juice and swell into oval cylinders. No information is available on the histology and chemistry of the chorion. This in contrast to the availability of precise details of the egg membranes in other groups of insects (see Edney, 1977).

Beament (1946), Slifer (1948) and Takahashi (1959) have recorded in the chorion of several species of insects like *Rhodnius prolixus* and *Melanoplus differ-*

entialis, that there is a thin superficial layer containing wax overlying a broad membrane made of a tanned lipoprotein.

In the present investigation it is found that there are 3 layers in the chorion of *Thrips palmi*. The outer layer is lipoidal recalling the superficial layer of the chorion of *Rhodnius prolixus* and *Melanoplus differentialis*.

The inner chorionic layer in the pesticide-susceptible strain of *Thrips palmi* contains tanned protein. Hence it is homologus to the inner layer of the chorion of *R. prolixus* and *M. differentialis*. The middle chorionic layer containing organic sulphur in *Thrips palmi* is therefore unrepresented in the other insects.

It is further observed that in the pesticide-susceptible strain the organic sulphur in the middle chorionic layer is in the form of sulphydryl groups while the same in the resistant strain exists as disulphide groups.

Disulphide groups are found also in the inner chorionic layer in the resistant strain of the thrips investigated; the layer also contains tanned protein.

The combined occurrence of tanned protein and disulphide groups in one and the same layer has been recorded in the cuticle of a mite *Acarus siro* by Hughes (1959) who represents it as –R–S–R– groups. The significance of this combination is not worked out.

In a study of the mechanism of DDT-resistance in some strain of the mosquitoes of the species *Anopheles fluviatilis* Vasudevan (1985) found that the barrier for the entry of the toxicant into the cuticle is made of –R–S–R groups.

In the light of the above observation it is suggested that the coexistance of the –S–S– groups as well as aromatic groups in the inner chorionic layer in the resistant strain of *Thrips palmi* may be responsible for conferring the pesticide resistant property.

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Biology of the Predatory Thrips, *Scolothrips longicornis* Priesner *(Thysanoptera: Thripidae)*

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In the present study the development, longevity, feeding activity, fecundity as well as some ecological aspects of the predatory thrips, *Scolothrips longicornis* Priesner were investigated in the laboratory using eggs of *Tetranychus cinnabarinus* Boisd. as prey. *S. longicornis* females and males required a mean of 13.1 and 12.5 days respectively, to complete development from egg to adult. The adult thrips females and males lived for mean of 57 and 47 days. Both larvae and adults showed a high feeding activity. Female thrips killed a mean of 63 mite eggs during development and a mean of 3070 during adulthood, males only 26 and 711, respectively. Mating was not necessary to induce oviposition. Unmated females only produced male progeny, but did not differ in fecundity. Mated females produced a mean of 285 eggs during an oviposition period of 45 days. The percentage of females in the progeny was a mean of 75% during the first week and strongly decreased with increasing age of the females. Leaves of apple and grapes were least suitable to oviposition by *S. longicornis*, whereas on cotton and the ornamental plant the highest oviposition occurred. It was also revealed that *S. longicornis* overwinters in the adult stage.

The predatory thrips, *Scolothrips longicornis* Priesner has been recognized as a promising predator of spider mites since the beginning of this century (Priesner 1950). However, besides some information on its distribution and occurrence (Stellwaag 1928) and on its effectiveness on *Tetranychus urticae* Koch (Fritzsche 1958) only little information is available on this species, which has not become well and widely known.

In the present study development, longevity, feeding activity, fecundity as well as some ecological aspects of *S. longicornis* were investigated in the laboratory to further elucidate its biological properties and effectiveness as a biological control agent of spider mites.

Material and Methods

S. longicornis was taken from the stock culture, which originated from a few specimens that were collected in southern Turkey (Kilikien plain). The carmine spider mite, *Tetranychus cinnabarinus* Boisd. and the two-spotted spider mite, *T. urticae* were used as prey in the stockculture and the experiments.

All studies were conducted in a climatic chamber maintaining a temperature of 25 ± 1 °C and relative humidity of 50 ± 10%. A 16h photophase (6–22h) was maintained with a light intensity of approximately 4000 Lux. In the experiments the thrips were held singly on leaf discs cut from cotton leaves *(Gossypium hirsutum*, "Caroline Queen" Variety) according to the modified leaf-island method (Sengonca and Gerlach 1983, Gerlach and Sengonca 1987).

To establish the duration of egg development 5 adult *S. longicornis* females were placed each on a leaf disc infested with *T. cinnabarinus* for 4 hours. Larval developmental time was determined by placing freshly hatched larvae singly on leaf discs with prey eggs and transferring them daily to new leaf discs. The stage of development was recorded 4 times per day.

For recording longevity pupae were taken from the stockculture and after adult eclosion transferred daily to infested leaf discs until death.

To determine the feeding activity of the immature stages 1st-stage larvae were placed each on a leaf disc infested with 70 eggs of *T. cinnabarinus* and transferred daily until the prepupal molt. Four times a day the remaining noncollapsed eggs were counted and the number of eggs killed determined. The feeding activity of adults was investigated using the same method, except that the leaf discs were infested with 130 mite eggs.

To establish the duration of the oviposition period and the number of total progeny, after adult eclosion females were transferred daily to infested leaf discs, on which after some days the hatching larvae were counted.

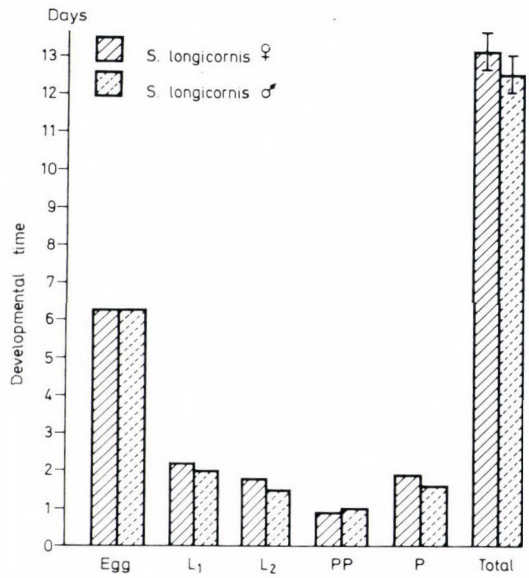

Fig. 1. Mean developmental times per immature stage of *Scolothrips longicornis* females and males using *Tetranychus cinnabarinus* eggs as prey (Mean of 9 females and 10 males)

Progeny sex ratios of *S. longicornis* were determined for the first 4 weeks by incubating, hatching and rearing the progeny of 5 females to adults.

To study the suitability of 9 different plants which are all infested by spider mites to *S. longicornis* the oviposition of 5 thrips females on the leaves was recorded over 7 days.

To determine whether and in which stage *S. longicornis* overwinters several potted strawberry plants were infested with *T. urticae* and *S. longicornis* and left outside from October to March.

Results

The developmental times for immature stages of *S. longicornis* are shown in Fig. 1. The egg stage lasted longest and required a mean of 6.3 days. The first larval stage required a mean of 2.2 days in females and 2.0 days in males. The mean duration of the second larval stage was slightly shorter and 1.8 days for females and 1.5 days for males. Both females and males remained about 1 day in the prepupal stage. The pupal stage lasted a mean of 1.9 and 1.6 days for females and males, respectively. Adult eclosion occurred after a mean of 6.8 (females) and 6.2 days (males) of larval development, whereby females required significantly longer than males. The mean duration of the life cycle from egg to adult was 13.1 and 12.5 days for females and males.

The adult thrips females lived for a mean of 57.1 days, whereas the mean adult longevity of *S. longicornis* males was only 47.7 days, but not significantly different (Fig. 2).

S. longicornis larvae initiated feeding shortly after eclosion.A mean number of 12.9 and 10.3 eggs of *T. cinnabarinus* were killed by 1st-stage female and male larvae respectively (Table 1). In the 2nd-larval stage feeding highly increased, especially in females which consumed a mean of 50.3 eggs and thereby twice as

Table 1

Mean number of eggs of *Tetranychus cinnabarinus* killed by *Scolothrips longicornis* females and males during immature development and adulthood

Sex	Number of mite eggs killed during					
	Development			Adulthood		
	Number of replicates	L_1	L_2	Number of replicates	Total	Per day
♀	9	12.9 a	50.3 a	13	3070.9 a	53.8 a
♂	10	10.3 a	26.1 b	4	711.5 b	14.9 b

Means followed by the same letter on a vertical line are not significantly different at P = 5% (Duncan's Multiple Range Test)

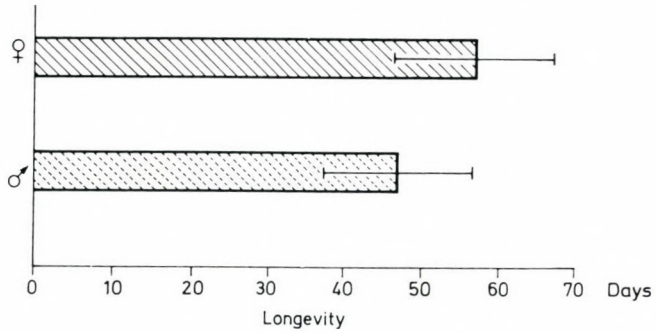

Fig. 2. Mean longevity of *Scolothrips longicornis* females and males using Tetranychus cinnabarinus eggs as prey (Mean of 13 females and 4 males)

many as the males (26.1 eggs). Prior to the prepupal molt feeding decreased and both the prepupa and pupa were nonfeeding. During adulthood a mean of 3070 and 711 mite eggs were killed by one female and male *S. longicornis*, respectively. On the average females and males consumed 53.8 and 14.9 eggs per day of their lifetime.

The fecundity of mated and unmated females is shown in Table 2. A mean total progeny of 285.2 and 301.0 eggs were produced by one mated and unmated female during a mean oviposition period of 45.6 and 40.5 days, respectively. Thus mated and unmated females laid a mean of 6.3 and 7.4 eggs per day which was not significantly different.

The percentage of females in the total progeny of 5 *S. longicornis* females was 75.3% in the 1st week (Fig. 3). As the parent females aged the percentage of females in the progeny strongly decreased to 51.7% in the 2nd week, 30.5% in the 3rd week and in the 4th week only 11.1% females were produced.

Since the eggs of *S. longicornis* are inserted into the leaf tissue the suitability of different plants to oviposition of the thrips was studied. On all plants oviposition took place, but in the number of total progeny produced significant differences

Table 2

Mean oviposition period, total and daily progeny per mated and unmated *Scolothrips longicornis* female using *Tetranychus cinnabarinus* eggs as prey

Female	Number of replicates	Oviposition period (days)	Total progeny		Daily progeny
			Mean	Range	
Mated	13	45.6±8.8 a	285.2 a	98–432	6.3 a
Unmated	5	40.5±15.5 a	301.0 a	174–447	7.4 a

Means followed by the same letter on a vertical line are not significantly different at P = 5% (Duncan's Multiple Range Test)

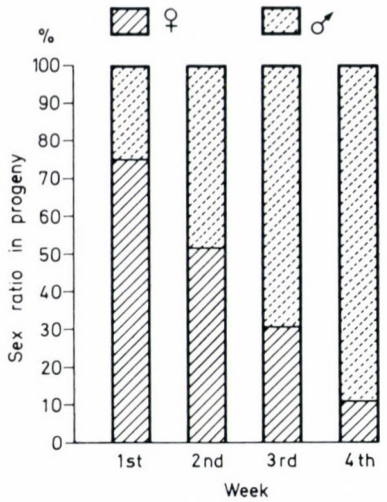

Fig. 3. Sex ratio of progeny produced during the first 4 weeks by 5 *Scolothrips longicornis* females using *Tetranychus cinnabarinus* eggs as prey

were found (Table 3). On apple leaves oviposition with only 7.4 eggs per female was lowest, on grape leaves significantly more eggs than on apple, but significantly less than on the other plants were laid. A mean total progeny of 43 to 50 eggs were produced on potato, cucumber and ivy leaves, whereas on strawberry and green beans a mean number of 55 and 58 eggs were laid. Oviposition was highest on cotton (67 eggs) and the ornamental plant (65 eggs) and significantly different from potato, cucumber and ivy.

Table 3

Mean number of eggs laid per *Scolothrips longicornis* female during 7 days on different host plants using *Tetranychus urticae* as prey

Host Plant	Number of eggs laid
Apple	7.4± 5.6 a
Grape	26.6± 5.9 a
Strawberry	55.0± 4.5 cde
Cotton	67.6± 8.1 e
Potato	45.0± 13.8 cd
Cucumber	50.0± 7.1 cd
Green beans	57.8± 16.3 de
Ivy	43.0± 13.8 c
Ornamental	65.6± 10.6 e

Means followed by the same letter are not significantly different at P = 5% (Duncan's Multiple Range Test)

The study on overwintering revealed that *S. longicornis* overwinters in the adult stage. One week after the strawberry plants had been put inside several adult females were found on the plants.

Discussion

The present study revealed that *S. longicornis* females and males required 13.1 and 12.5 days to complete the life cycle from egg to adult. In contrast Gilstrap and Oatman (1976) reported a considerably longer development time of 22.1 days at 24 °C for the related species *Scolothrips sexmaculatus* (Pergande) which occurs in California. In *S. longicornis* the development of the females took significantly longer than of the males, whereas in *S. sexmaculatus* no differences between females and males were found (Gilstrap and Oatman 1976).

The mean longevity of *S. longicornis* females was about 57 days and thereby slightly longer than that of the males. In *S. sexmaculatus* males lived about one third longer than the females (Gilstrap and Oatman 1976). Fritzsche (1958) reported a very short longevity of only 14 to 21 days for *S. longicornis*, which is not in accordance with the present results. In studies on *Leptothrips mali* (Fitch), another predator of spider mites, Parella *et al.* (1982) also found a high longevity of about 50 days.

Both in female and male *S. longicornis* larvae feeding activity greatly increased in the 2nd-larval stage. Male 1st-stage larvae showed about the same feeding activity as females, whereas in the 2nd-larval stage egg consumption by females was significantly higher. The same tendency was observed for *S. sexmaculatus* by Gilstrap and Oatman (1976). The feeding activity of the adult males also was considerably lower than of the females. The males daily consumption of about 10 to 20 mite eggs was equivalent to the feeding activity of the females after oviposition had ceased.

Mating was not necessary to induce oviposition, but unmated females only produced male progeny. The studies revealed that no differences exist in fecundity between mated and unmated females, since the oviposition period as well as the total progeny were not significantly different.

The sex ratio in the progeny of *S. longicornis* was shown to greatly depend upon the age of the parent female, as the percentage of females decreased as the parent females aged. After 4 weeks only male progeny was produced. The same observation, although not as extreme, was made by Gilstrap and Oatman (1976) for *S. sexmaculatus*. A remaining question is, whether in a natural population *S. longicornis* females are mated several times and thereby provided with a higher amount of sperm, so that female progeny could be produced for a longer period. In *S. sexmaculatus* Gilstrap and Oatman (1976) found a sex ratio of 89%, Coville and Allen (1977) of 67%.

Predatory insects will always choose plants infested with their prey. According to the wide range of host plants of spider mites, *S. longicornis* was found on

many plants, like *Euphorbia* sp., *Coronilla* sp., *Stachys* sp., *Carpinus* sp. and green beans in Europe (Priesner 1950, Fritzsche 1958). The study on the oviposition of *S. longicornis* on different plant leaves revealed some clear differences, which to a great extent can be attributed to leaf surface features. The extremely low oviposition on apple leaves probably is due to the felted hairiness of these leaves. On the smooth and non-hairy leaves of cotton and the ornamental plant, the highest number of eggs was laid. However, in ivy leaves oviposition was low despite their smoothness. It is suspected that the leaves were too hard and the few leaf veins present not enough pronounced, which, however, are used for shelter and protection during oviposition by *S. longicornis*.

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The Biogeographical Character of the *Thysanoptera* Fauna (*Insecta*) of Andalusia, Spain

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The distribution patterns of 145 species of *Thysanoptera* occurring in Andalusia are briefly characterized. About 45.5% of the species are of Mediterranean origin, and 27 species (= 18.6%) are ascertained only from the westernmost third of the Mediterranean basin. As many as 76 species are introduced as new members to the fauna of Andalusia, 26 of these species have not been recorded from the Iberian Peninsula before.

The territory of Andalusia in southern Spain is since long a very attractive region to biologists, particularly with regard to its fascinating indigenous flora and fauna. A pretty good number of both plant and animal species are endemical to that area. One of the significant results of the analysis of the distribution patterns of Andalusian organisms reveals the exclusive occurrence of many species in Andalusia and the countries of northwestern Africa, but not in the remaining part of the Iberian Peninsula.

Among the zoologists, many entomologists from the more temperate European countries have visited Andalusia in order to study there that insect group they are specialized in. This observation applies chiefly to the favoured insects orders such as Coleoptera, Hymenoptera, Lepidoptera, Orthoptera (in the old sense), and some others. Some of the small orders like that of the Thysanoptera, however, have in Andalusia not yet been explored as thoroughly as it happened to be with the big ones being rich in species.

Nevertheless, in the course of the years 64 thysanopterous species have to date (July 1987) been recorded in literature from Andalusia. This figure is adequate to 36% of the total of 177 species at present known to occur in Spain. Del Canizo (1932) was the first who gave some faunistic data of a few thrips from Andalusia. Titschak (1976) in his list of the Spanish tubuliferous Thysanoptera has enumerated detailed Andalusian records of the species treated. A number of single reports on thrips from localities of the area in question have been published in the past by Lacasa (1983), Pelikán (1977), Priesner (1957), Titschack (1970), and zur Strassen (1966; 1968; 1973).

The present writer had in spring 1985 joined a team of botanical and entomological taxonomists of the Research Institute Senckenberg at Frankfurt (Main) [SMF] who have toured some parts of Andalusia. One of the aims of the expedi-

Table 1

Check list of the Thysanoptera of Andalusia (as per July 1987) with indications of provincial records, and of the distribution patterns. ● = First record for the Iberian Peninsula; × = recorded according to literature; O = collected by the writer in 1985; * = published record confirmed by the writer in 1985

		Murica	Almería	Granada	Jaén	Cordoba	Malaga	Sevilla	Cadiz	Huelva	A = Algeria	M = Morocco	Distribution
Aeolo. andalusiacus z. Str.				O			×						Andalusian
balati Pel.	●			O									S-European
bournieri Bgn.		×		O			O						Andalusian
bucheti Bgn.		O		O	O		*	O	O	O		M	Andal.-Moroccan
citricinctus Bgn.							O	O				M	W-Mediterranean
collaris Prs.					O				O		A	M	Indo-Mediterranean
ericae Bgn.								O	O	O			W-Palaearctic
gloriosus Bgn.							O		O			M	Mediterranean
intermedius Bgn.				×								M	Palaearctic
melisi Prs.		×		O					O	O	A		W-Mediterranean
pelikani Tit.				×									Andalusian
quercicola Brn.							O						W-Mediterranean
tenuicornis Bgn.		O	O	*	O		O	O	O	O			SW-Palaearctic
versicolor Uzel				O			O		O				Euro-Siberian
Allo. pillichellus bournieri Mnd.			×						O				SW-European
Amphibolo. grassii Bff.									O	O		M	Mediterranean
marginatus (Brn.)	●								O				SW-European
Anapho. obscurus (Mll.)										O		M	Semi-Cosmopolitan
Apterygo. hispanicus (Bgn.)				O	O		×						Ibero-Canarian
longiceps z. Str.	●								O				Ibero-Canarian
piceatus z. Str.				O			×						Andalusian
priesneri z. Str.	●			O									Mediterranean
Aptino. rufus Hal.		O		×	O		O	O	O	O		M	Cosmopolitan
stylifer Trb.				×									Holarctic

Bacillo. longiceps (O. M. Rtr.)			O		×		×			O			Mediterranean
Bebelo. latus Bff.									O				W-Mediterranean
dentipes (O. M. Rtr.)		×											European
insularis (Bgn.)		*		O			*		*	O			Mediterranean
Bregmato. dimorphus (Prs.)	●									O			S-European
Capri. melanophthalmus (Bgn.)	●								O				Indo-Mediterranean
Cephalo. albostriatus z. Str.	●				O							M	Andal.-Moroccan
coxalis Bgn.			O		O		*		O	O		M	Mediterranean
monilicornis (O. M. Rtr.)				O									Holarctic
Cerato. discolor (Krn.)		O	O	O			O		O	O		M	Turano-Mediterranean
ericae (Hal.)									O	O			Euro-Siberian
frici (Uzel)					O		O			O		M	W-Palaearctic
pallidivestis (Prs.)		O											Ponto-Mediterranean
Chiraplo. graminellus Prs.	●								O			M	Indo-Mediterranean
Chiro. aculeatus Bgn.			O				×		O	O			W-Palaearctic
ammophilae Bgn.										O			SW-European
manicatus Hal.		O	O	O	O		×		O	O		M	Holarctic
medius z. Str.	●						O						Ibero-Canarian
meridionalis Bgn.	●	O							O			M	Indo-Medit.-Ethiop.
spinulosus Andre	●				O								(?Europ., ?Andalus.)
Compso. albosignatus (O. M. Rtr.)				*	×	×	*	O			A	M	Ponto-Mediterranean
maroccanus Prs.			×	O	O							M	Andal.-Moroccan
Crypto. nigripes (O. M. Rtr.)									O	O			Palaearctic
Dendro. degeeri Uzel					O				O				European
phyllireae Bgn.	●							O				M	Turano-Mediterranean
saltator Uzel							O						Euro-Siberian
Drepano. reuteri Uzel					O						A		Palaearctic
Frankliniella tenuicornis (Uzel)			×										Holarctic
Gynaiko. ficorum (Mrch.)		×	×								A	M	Circ.-Subtropical
Halo. salicorniae Brn.	●									O			Mediterranean
Haplo. acanthoscelis (Krn.)				×			×						S-Eurosiberian
andresi Prs.		O	*	*	*		*		O			M	Indo-Mediterranean
anthemidinus Prs.						×						M	Mediterranean
arenarius Prs.		O								O			Euro-Siberian
crassicornis (John)				×									Euro-Siberian
distinguendus (Uzel)			×	×					O				W-Palaearctic

Table 1 (continued)

		Murcia	Almeria	Granada	Jaén	Cordoba	Malaga	Sevilla	Cadiz	Huelva	A = Algeria	M = Morocco	Distribution
frustrator z. Str.		O	*										Andalusian
gallarum Prs.			×				×		*	O		M	Andal.-Moroccan
gowdeyi (Frkl.)							×						Circ.-Subtropical
hispanicus Prs.				×									Mediterranean
janetscheki Prs.				×									Andalusian
juncorum Bgn.				*			×			O		M	W-Palaearctic
kurdjumovi Krn.						×							Palaearctic
longipes Bgn.					O	×			O			M	SW-Mediterranean
marrubiicola Bgn.							×		O				S-European
minutus Uzel							×						European
niger (Osb.)					O				O				W-Eurosiberian
reuteri (Krn.)				*	O		*	O		O		M	Turano-Mediterranean
salloumensis Prs.		O	O							O		M	S-Mediterraneam
setiger Prs.		O	*	*	×	×	*	O	O	O		M	W-Palaearctic
setigeriformis Fab.							×						Turano-Mediterranean
teucrii Brn.				O			×						SW-European
tritici (Krdj.)			×	*		×	×				A	M	W-Palaearctic
vuilleti Prs.		O	*	*			×		O				S-European
Hoplandro. bidens (Bgn.)							×						W-Palaearctic
Idio. maghrebinus z. Str.	●	O										M	Andal.-Moroccan
Isoneuro. australis Bgn.							×			O		M	Circ.-Subtropical
Kako. firmoides Prs.				O									W-Mediterranean
Karnyo. flavipes (Jones)									O				Circ.-Subtropical
Limo. angulicornis Jabl.			O	O				O					Holarctic
cerealium Hal.		O	O				O		O	O		M	Semi-Cosmopolitan
Lio. amabilis Bgn.									O			M	W-Mediterranean
leucopus Tit.							×			O		M	SW-Mediterranean

oleae (Costa)				×	×				O			M	Mediterranean
reuteri (Bgn.)				×			×				A	M	Turano-Saharo-Medit.
Mega. lativentris (Hgr.)									×				Euro-Siberian
Melan. acetosellae John							O		O				Turano-Mediterranean
areolatus Prs.	●									O		M	S-Mediterranean
ficalbii Bff.				O	O		O	O				M	SW-Palaearctic
fuscus (Slz.)					×		*	O	O	O	A	M	W-Palaearctic
hispanicus Pel.			×										Andalusian
libycus Prs.							×				A	M	S-Mediterranean
nigricornis Bgn.									O			M	W-Mediterranean
pallidior Prs.		O								O			Turano-European
rivnayi Prs.	●								O				Mediterranean
sinaiticus Prs.	●			O								M	S-Mediterranean
tristis Prs.	●			O					O				S-Mediterranean
Neoheegeria dalmatica Schm.			×	O		×							Turano-Mediterranean
verbasci (Osb.)				O					O				European
Neohydato. gracilicornis (Wlls.)				O			O					M	Palaearctic
Odonto. cytisi Mor.				O									W-European
ignobilis Bgn.		O		O			×	O	O	O		M	SW-Palaearctic
karnyi Prs.		O					×		*	O	A	M	Turano-Mediterranean
retamae Prs.		O	O				*						SW-Mediterranean
Oro. priesneri (Tit.)				O			×		O				Mediterranean
Oxy. ajugae Uzel							O		O	O			W-Palaearctic
bicolor (O. M. Rtr.)				O									Turano-European
claripennis Pts.	●	O										M	S-Mediterranean
ulmifoliorum (Hal.)				O	O		O		O	O			European
Phlaeo. coriaceus Hal.								×					European
Physo. albidicornis (Knch.)				O					O				European
Priesneriella mavromoustakisi (J. C. Crf.)	●		O									M	Mediterranean
Prosopo. capitatus z. Str.				O			*		O	O		M	SW-Mediterranean
Pseudocrypto. meridionalis Prs.		×											Mediterranean
Rhipido. brunneus Wlls.					O			O		O		M	Holarctic
gratiosus Uzel							O	O	O	O			W-Palaearctic
unicolor z. Str.	●	O	O					O				M	Mediterranean
Ropotamo. buresi Pel.							×						S-European

Table 1 (continued)

		Murcia	Almería	Granada	Jaén	Cordoba	Malaga	Sevilla	Cadiz	Huelva	A = Algeria	M = Morocco	Distribution
Rubio. sordidus (Uzel)				O	O								European
vitalbae (Bgn.)					O				O				S-European
Scirto. canizoi Tit.							×		O				Andalusian
inermis Prs.									O				Circ.-Subtropical
Sito. almargensis Tit.							×						Andalusian
lindbergi z. Str.	●				O				O			M	SW-Mediterranean
Sophio. terminalis (Bgn.)	●									O			W-Mediterranean
Steno. graminum Uzel				O	O		O	O	O	O		M	W-Palaearctic
Taenio. atratus (Hal.)				O									Palaearctic
meridionalis Prs.		O		O			*		O				Turano-Mediterranean
vulgatissimus (Hal.)				×									Holarctic
Tameo. tamicola (Bgn.)				O									W-European
Thrips angusticeps Uzel		O		*	×		O	O	O	O		M	W-Palaearctic
asparagi z. Str.	●		O									M	Andal.-Moroccan
brevicornis Prs.				O									Euro-Siberian
fuscipennis Hal.					O								Palaearctic
major Uzel					O				O	O	A	M	Holarctic
mareoticus (Prs.)				×					O	O		M	Mediterranean
mediterraneus Prs.	●	O							O				S-Mediterranean
minutissimus Linn.				O			O	O	O				European
physapus Linn.							×		O			M	Euro-Siberian
tabaci Ldm.		*	×	O	O		*	O	O	O		M	Cosmopolitan
Treherniella inferna (Prs.)	●	O											Indo-European

tion was the collection of reference material to be included in biogeographical studies. On this occasion, thrips samples have been taken from several places in each of seven of the eight provinces of the territory mentioned. A few days have been spent also in the southernmost part of the province Murcia.

Material and Methods

Sites especially looked up for fieldwork were situated in lowlands as well as in mountainous regions, in dry areas along the sea shore as well as in wet marshes near the coast, in dense forests as well as in open savanna-like countrysides, and at the margins of both pine forests and cor-oak woods, in grasslands, macchia-like areas, at road sides and at edges of agricultural plantations. Exact data on localities and host plants will be specified elsewhere.

During the journey some 130 species of *Thysanoptera* have been collected of which about a dozen ones are not yet identified to species level. The distribution patterns of the species taken into account as discussed below are based on material preserved in the SMF and on records published in literature. The same applies to those species which are known to the present writer only from literature to occur in Andalusia.

The writer is particularly grateful to the Paul Ungerer Stiftung (Frankfurt/Main) for granting the travel expenses, and to Prof. Dr. W. Ziegler, Director of the Research Institute Senckenberg, for facilitating the field work. Advices regarding specific collecting sites and other kinds of help are to be indebted to Dr. J. Berzosa (Madrid), and to Dr. A. Lacasa (La Alberca, Murcia).

Results, Discussions

The material of *Thysanoptera* collected in 1985 in Andalusia and the southernmost part of Murcia consists of more than 130 species. Out of this lot, as many as 119 species have so far been identified, and are listed alphabetically in Table 1. The species concerned are marked in the table by the symbol "O" being placed in the clefts of the respective provinces where they have been taken. The province of Murcia has been considered in the present reflection too because of its close neighbourhood to the Andalusian provinces Granada and Almería, and because of the similar ecological situations characterizing both the southern part of Murcia and wide parts of the adjoining territories of Almería. A black dot (●) behind a species name (outside the clefts) stresses the first record for the Iberian Peninsula.

The Table comprises also the 64 species hitherto been recorded in literature from Andalusia, and additionally four species from the province of Murcia. All these species are marked in table by the symbol "X", indicating in the respective clefts the provinces from which they were reported. Out of this group, 24 species have not been found during the trip in 1985. The symbol "*" means that

a species has in the respective province been recorded earlier as well as been collected newly (by the writer).

The Table below may serve as a preliminary check list of the Thysanoptera of Andalusia. It enumerates 145 species of which 76 are new to this region, and not less than 26 ones are even new to Spain, and the Iberian Peninsula as well. By all means, *Chirothrips spinulosus* Andre 1941 has been discovered already previously by J. Berzosa (unpublished) in the province Jaén where he has obtained in April 1975 a few specimens from litter under *Pinus* trees; I have seen the specimens, – and have collected the same species myself in the same province.

With regard to the distribution patterns of the species under discussion, the presently applicable biogeographical terms are also to be seen from the table. Furtheron, it has in one of the clefts been indicated by the letters "A" and "M", respectively, whether a species is known to occur also in Algeria and/or Morocco. By that, one has to bear in mind that the total figure of thrips species so far recorded from Algeria amounts to only 23, where as that from Morocco to as many as 118.

A large portion of the species occurring in Andalusia is indigenous purely to the Mediterranean basin, or to a bigger, or smaller, part of it. This refers to 66 of 145 species (= 45.5%), as specified below:

Holo-Mediterranean	16 species	SW-European	4 species
W-Mediterranean	9 species	Ibero-Canarian	3 species
S-Mediterranean	7 species	Andal.-Moroccan	6 species
SW-Mediterranean	5 species	Andalusian	9 species
S-European	7 species		

The remaining species are of a wider distribution, particularly within the western Palaearctic region (a total of 108 species).

Eight of those species which are less widely distributed within the Mediterranean are recorded for the first time from the continental Europe. Considering the westernmost third of the Mediterranean basin, 27 species seem to have originated inside that limited area. Not less than nine species of the latter group are endemics of Andalusia.

The real chorological state of *Chirothrips spinulosus* has at present to be regarded as vague. The species has been described from 6♀♀ taken in 1934 in plant quarantine at Washington, D. C., from grass seeds *(Melica ciliata, Isopyrum anemonoides)* imported from Russia into the United States. Since the description of the species in 1941, no further specimen of *spinulosus* has ever been collected until 1975 when Berzosa found some ones in Andalusia (s. above). It is therefore not deviously to presume that the statement "Russia" as the country of origin of that material could be given erroneously by some reason or other.

The seven species marked in the table as circum-subtropical are all, or certainly to the greater part, been introduced to Andalusia.

To summarize, the thysanopterous fauna of Andalusia is composed of 145 species, of which a big majority of 108 species (= 74.5%) is distributed in

the western Palaearctic. Mediterranean elements amount to 66 species (= 45%), and the Andalusian endemics to nine species (= 6.2%). The number of species showing the Andalusian-Moroccan distribution pattern is low and figures out to only six species.

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Some New Bionomical and Morphological Studies on *Pelikanothrips kratochvili* Pelikan, 1947 *(Thysanoptera: Thripidae)*

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During studies on the Thysanoptera fauna of northwestern Lower Saxony, *Pelikanothrips kratochvili* was found for the first time in the Federal Republic of Germany in 1985. In 1986, Carex gracilis Curt., Carex elata All. and Carex nigra (L.) Reich. were identified to be the host plants of *P. kratochvili*. This species lives in the leaf sheaths of Carex spp. and has two generations per year. More then 500 female individuals were collected from June 1986 until June 1987, but no males could be found. A wing polymorphism was discovered, which is characterized by a continuous reduction of wing-length in the course of the year. The metanotum craniale was investigated by light- and scanning electron microscopy. Individuals with sensillae campaniformiae and sensillae trichodeae could be identified just as individuals without any sensillae. The frequency of occurrence is described.

Pelikanothrips kratchvili belongs to one of those species in Thysanoptera on which knowledge is very scarce. Up to now only a small number of female individuals was found in Czechoslovakia (Pelikán, 1947; 1951); one female individual could be collected in The Netherlands by Franssen and Mantel (1963). The male, the larval stages and the host plant remained unknown.

A first description of his species was given by Pelikán (1947), who characterized it to be hemi-macropterous. Based on another finding of a greater number of females in a single catch in 1949, Pelikán (1951) gave some detailed supplementary informations to his first description, concerning the colouration and a continual variability in wing-length. Further on he supposed that *P. kratochvili* is probably living in the sheaths of various gran species or in the grass-sed directly.

Additional to the morphological descriptions given by Pelikán (1947; 1951), Schliephake and Klimt (1979) noted that on the metanotum craniale no "Haplopores" can be found.

During studies on the Thysanoptera fauna of north-western Lower Saxony, *P. kratochvili* could be found for the first time in the Federal Republic of Germany in 1985. By the identification of the host plant in June 1986 more detailed studies on this species became possible. In this paper, first bionomical data from current studies are presented. The occurrence of various wing-lengths and different types of sensillae on the metanotum craniale are described.

Origin of Material and Methods

P. kratochvili could be found in a small landscape protective area about 10 km to the south-west of Bremen in Lower Saxony. This area, named "Steller Heide", is a former wind blown sand area, which has conserved its origin to some extent. The plains are covered with dry meadows and in some parts with *Calluna vulgaris* (L.) Hull. The dunes are mainly grown with clear stands of *Pinus sylvestris* L., whereas for the rest of stands *Quercus robur* L., *Betula pendula* Roth or mixed stands of both can be found. As a particularity there are also heathponds and moisty sites between the dunes and the plains.

At two of those moisty sites *P. kratochvili* could be found. The first area is formed like a flat hollow. Within this area there is a continuous growing of *Carex elata* All. and *Carex nigra* (L.) Reich. At the borders of the hollow *Juncus effusus* L., *Molinia coerulea* (L.) Moench and also a few bushes of *Salix caprea* L. can be found. The second area is of artificial square shape, possibly a result of sand-working in former times. This place has the character of a meadow, in whose centre vegetation is formed by *Carex elata* All., *Carex nigra* (L.) Reich, *Carex gracilis* Curt, and *Calamagrostis canescens* (Web.) Roth. In contrast to the first area, this place is very interspersed in its middle with *Betula pendula* Roth and *Salix caprea* L. In both areas normal ground water level is high, and due to this, these areas are partly flooded in late winter or early spring. The soil type in the underground is a gley. Further on both areas are sheltered sites and they are warmer than their surroundings.

Studies on *P. kratochvili* have been started in June 1986 and are still current. Within this time, methods of collecting had to be adjusted to the seasonal behaviour of this thrips. In summer 1986 the adults were beaten from the plants. During late autumn and winter a Tullgren funnel was used to extract the specimens from the litter. In early spring in 1987 dry plants of last year and the new young shoots were cut off and beaten into a dish. From the end of April until June plants were controlled singly. In 1986 and from April 1987 to the last decade of May 1987, observing and collecting was done in intervals of two weeks. In June 1987 plants were controlled in intervals of 2–3 days.

The collected specimens were singly mounted in Balsam. To prevent deviation in measuring results, all wings were measured at a magnification $\times 250$. In general only the right fore-wing was measured. Studies on the metanozum were carried out using a light-microscope. Further on a number of individuals was examined by scanning electron microscopy. For this the specimens were treated with acetone. After air-drying they were sputtered with gold.

Results

From June 1986 until June 1987 more than 500 individuals of *P. kratochvili* were collected from *Carex gracilis* Curt, *Carex elata* All., and *Carex nigra* (L.) Reich. All these individuals were of female sex and no males were found. During hibernation only female adults were found, but no second-stage larvae. After

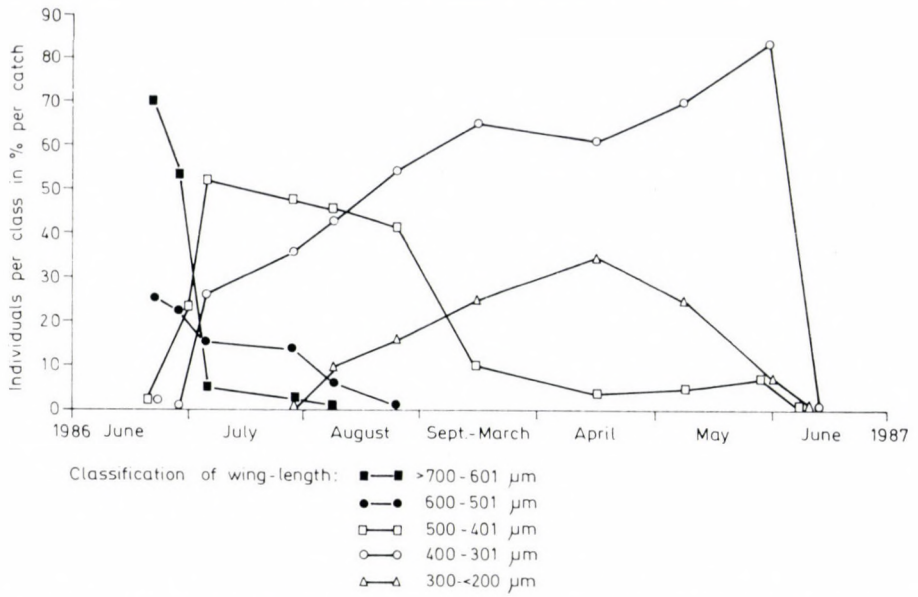

Fig. 1. Seasonal appearance of different wing-lengths in *Pelikanothrips kratochvili* (♀)

hibernation in litter of last year plants, *P. kratochvili* females stayed at the basis of the young shoots in early spring. In the last decade of April 1987 first females with well developed ovaries were registered. The developmental stage of the ovaries can be described as "late maturing" or "laying" (after Lewis, 1973). At the same time the young shoots of *Carex* spp. reached a height of 10–15 cm and the first foliage leaf appeared. Within a few days the overwintering adult individuals now left the basis of the shoots and wandered into the first leaf sheath. Here they feed and lay eggs about 2 cm below the border of the leaf sheath. From the last decade of May until the middle of June the number of females in leaf sheaths decreased to one and then to null.

At the end of the first decade of May first eggs of *P. kratochvili* were found. First-stage larvae were found 8 days later and another 7 days later second-stage larvae could be observed. From that day until the first appearance of the prenymphe and nymphe more than three weeks passed. In the last decade of June first just emerged and long-winged female individuals could be found. From the first finding of eggs until the first appearance of just emerged adults about 46 days passed.

Two generations could be established in the annual cycle in *P. kratochvili* in 1986.

As a predator on *P. kratochvili* an undetermined red mite could be observed. In all cases this mite attacked the adult females from the ventral side, pearcing them at the membrane between the last two coxae. Although being pearced individuals were able to run around trying to free themselves. This mite also lives on

the leafs of *Carex spp.*, but she never could be observed attacking larvae in the leaf sheaths.

Measurements in 462 individuals, collected from June 1986 until the end of May 1987, showed a continual variability in length of wings, which was about 175–720 μm. In individuals with a maximal length of wings the VIIIth abdominal segment is reached only. As it can be seen in Fig. 1., there is a seasonal appearance of different wing-lengths in *P. kratochvili.* Long-winged individuals only appear in June. In 469 female individuals the metanotum craniale was examined light-microscopically. Individuals with sensillae campaniformiae (Fig. 3 (b), (c), (e)) and sensillae trichodeae (Fig. 3 (b), (d), (f)) could be identified just as individuals without any sensillae.

If sensillae are developed, they occur as a couple or singly (Figs 2, 3). Insertion points of sensillae campaniformiae and sensillae trichodeae show no differences in their positions on the metanotum and both types of sensillae can be found on the metanotum of the same individual at the same time (Figs 2, 3b). The sensory hair of the sensillae trichodeae has a length of about 9–12 μm. The frequency of the development of both types of sensillae shows remarkable differences which are figured in Fig. 2.

Reviewing field observations in June 1987 confirmed that the long-winged individuals are those, which emerge from the first nymphes of the first generation. In all individuals which later emerge, length of wings decreases continually. The continual reduction of wing-length in the course of one year is shown in Fig. 2.

Further on in 29 individuals the metanotum craniale was examined by scanning electron microscopy to review the light-microcopically identified structures. The results could be confirmed, but in one individual also a sensilla basiconica could be identified.

Types of sensillae and arrangements	No. of individuals	%
Without any sensillae	100	21.3
1 Sensilla campaniformia	141	30.0
2 Sensillae campaniformiae	138	29.5
1 Sensilla trichodea	46	9.8
1 Sensilla campaniformia and 1 Sensilla trichodea	37	7.9
2 Sensillae trichodeae	7	1.5
Total	469	100.0

Fig. 2. The occurrence of various sensillae on the metanotum craniale in *Pelikanothrips kratochvili* (♀)

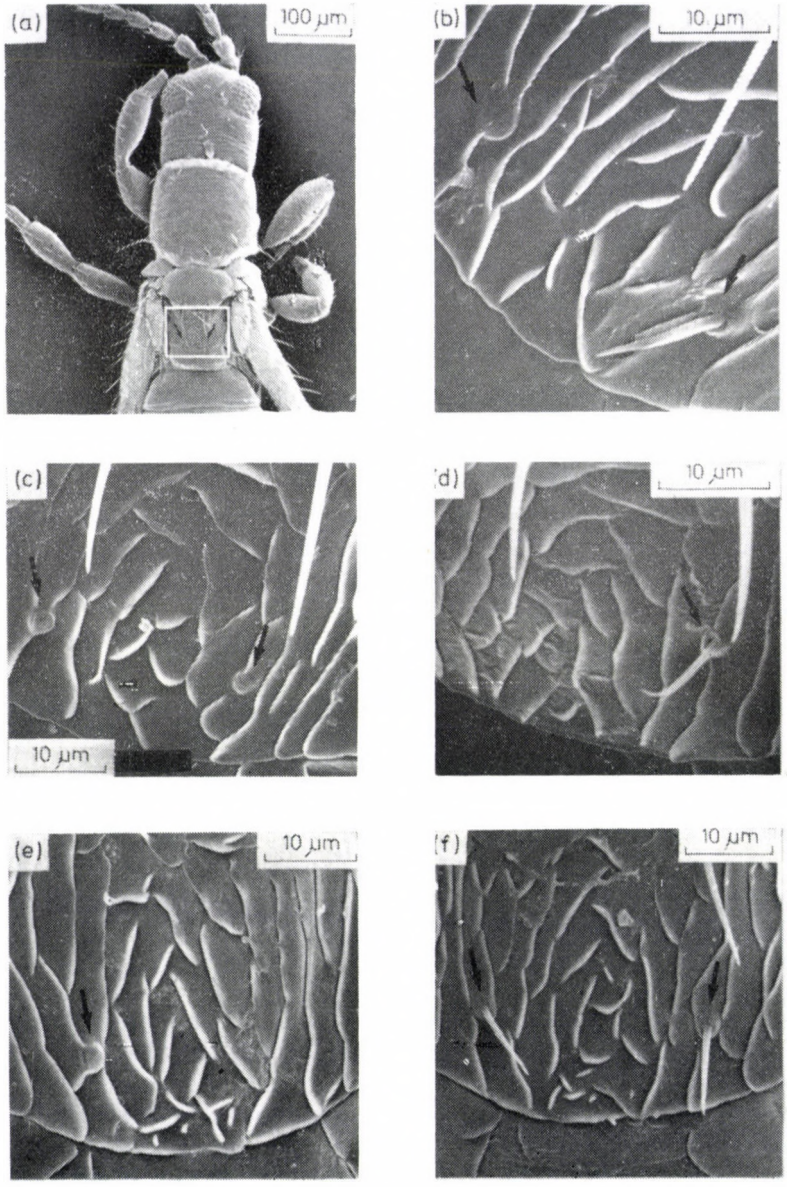

Fig. 3. Scanning electron micrographs of the metanotum craniale of *Pelikanothrips kratochvili* (♀). (a) survey, ↘↙ insertion points sensillae. (b) metanotum with one sensilla campaniformia and one sensilla trichodea. (c) metanotum with two sensillae campaniformiae. (d) metanotum with one sensilla trichodea. (e) metanotum with one sensilla campaniformia. (f) metanotum with two sensillae trichodeae

Discussion

P. kratochvili was found in Czechoslovakia (Pelikan, 1947; 1951), in The Netherlands (Franssen and Mantel, 1963) and now also in Lower Saxony in the Federal Republic of Germany. A description of the locality of this new finding is given above. *Carex gracilis* Curt., *Carex elata* All. and *Carex nigra* (L.) Reich. could be identified to be the host plants of *P. kratochvili*. Unfortunately there are no detailed data on the vegetation from the finding in The Netherlands. In Czechoslovakia *P. kratochvili* was found in peat-bogs and swampy meadows in the High Jesenik mountains in Silesia at a height of about 870 m above sea-level. Although there are great differences in the geographic position between the localities in Czechoslovakia and in the Federal Republic of Germany, several communities are obvious. In all investigated localities *Carex spp.* is the predominating plant, the sites are sheltered and humidity and temperature are higher than in the surrounding areas. Further findings will show whether the enumerated abiotic factors can be comprehended as belonging to the habitat requirements of this species. It seems to be very probable that the distribution of *P. kratochvili* is much more extensive than it is known up to now.

As males of *P. kratochvili* could not be found in the investigated population, reproduction is supposed to be of thelotokous type. By means of hitherto existing results, two generations per year could be established definitely, but the occurrence of a third generation can not be excluded.

The reason for the long duration of larval development in 1987 can be attributed to very unfavourable weather conditions with low temperatures in May and June.

The decreasing number of females in leaf sheaths, which could be observed from the last decade of May until the disappearance of these individuals in the middle of June 1987, gives a hint on the duration of life after hibernation. But as it can be seen in Fig. 1., there are differences in the longevity of these individuals. Individuals with a wing-length of about 200–300 μm appeared in August. After hibernation their proportion per catch decreased constantly until they disappeared in June. Possibly the remaining time between emergence and hibernation or the quality of food is not sufficient to build up the necessary reserves for a longer survival. In contrast to this, a decreasing number of individuals with a length of wings of about 301–400 μm could not be observed. The sudden die off in individuals with a wing-length of about 301–400 μm in June probably was caused by heavy rainfalls close to the end of their longevity. The reasons for the extremely decreasing number of long-winged (>700–601 μm) individuals at the end of June 1986 are not known, but it might be possible that these individuals are able to fly of their own accord, although wings reach the VIIIth abdominal segment only.

Wing polymorphism in *P. kratochvili* females is characterized by a continual reduction of wing-length in the course of the year. In female adults, collected in July 1949, Pelikan (1951) found a variability in wing-length from about 310–680 μm, which is corresponding with the results shown in Fig. 1.

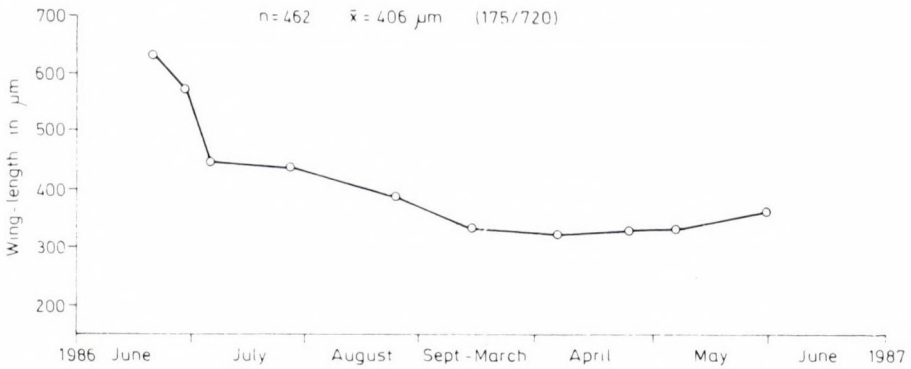

Fig. 4. Average wing-length in *Pelikanothrips kratochvili* (♀) in the course of one year

Alternating generations of long-winged and short-winged adults are known for example in *Thrips angusticeps* Uzel, 1895 (Franssen and Huismann, 1958) and in the grass-living species *Hemianaphothrips obscurus* (O. F. Müller, 1776) (Kamm, 1972; Koppa, 1970), but in contrast to *P. kratochvili* there is no significant variability in wing-length within the generations of these species. In studies on the development of macropterous and brachyopterous progeny in *H. obscurus*, Kamm (1972) and Köppä (1970) found out that for the development of macropterous progeny a long photoperiod is far more important than temperature. The causes of wing polymorphism in *P. kratochvili* are still not known, but it is striking that long-winged individuals only hatch at the time of greatest daylength, and parallel with the decreasing of daylength also the length of wings decreases. This also might be accidental and further investigations are necessary to find out whether there is a correlation between photoperiod and length of wings in this species.

Presence and absence of sensillae campaniformiae, also named "Haplopores" or "Micropores", on the metanotum craniale are known in a number of species, for instance in *Frankliniella tenuicornis* (Uzel, 1895), *Kakothrips robustus* (Uzel 1895) or *Thrips major* Uzel, 1895 (Schliephake, G. and Klimt, K. 1979), whereas the occurrence of sensillae trichodeae has not been described before. Although the function of these sensory receptors is still unknown, their occurrence and absence in one population at the same time shows that they obviously can not be of essential importance for this species. The frequency in the development of both types of sensillae, their occurrence in the same individual at the same time and complete absence of sensillae might point to a reduction of these sensory receptors in *P. kratochvili*. Further investigations in other ecological and geographical populations will be necessary to review this idea.

Acknowledgements

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Thrips as Crop Pests in Korea

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This study was undertaken to determine the relative importance of the thrips affecting the fruit and seasoning vegetables grown in Korea. Eight species of thrips: *Thrips tabaci*, *T. setosus*, *Taeniothrips alliorum*, *Frankliniella intonsa*, *F. tenuicornis*, *Aeolothrips kurosawaii*, *Haplothrips chinensis and H. aculeatus* were collected from eleven host plants. Onion thrips, *Thrips tabaci* and flower thrips *Frankliniella intonsa* were the most damaging species.

Thrips are comparatively small-sized insects of considerable economic importance and are mostly phytophagus, extending over a wide range of habitats mostly in the tropical, subtropical and temperate regions, which quickly exploit suitable circumstances resulting in their increased damage potential (Ananthakrishnan, 1984). Thrips are harmful to cereal crops, commercial crops, fruit and berry orchards, and plants growing under glasshouse conditions.

Adult thrips damage cereal crops mainly in the phase of emergence, initially they suck plant juices from the sheath of the apical leaf and, after it ruptures, pierce through into the young ear (Dydechko, 1977). Studies on the Korean thrips were recorded only by foreign entomologists until 1963, e.g. in Kurosawa (1938), Kuwayama (1962) as taxonomic in nature.

During the years of 1971 to 1974 Woo and Paik added some species new to the Korean fauna. All these works, however, deal with adult thrips, and taxonomic information on larvae and biology of the Korean thrips is extremely limited.

In this paper, studies on the distribution, damage and host plants of thrips on seasoning and fruit vegetables grown in Korean peninsula have been made.

Materials and Methods

The study was conducted during the growing seasons of 1985 and 1986. Fourteen fields in four survey sites, e.g. Site I (Suwon, Yongin), Site II (Taejon, Yeosung), Site III (Changweon, Jinju, Euisung, Sinryeung, Pohang) and Site IV (Muan, Hampyung, Naju, Kwangju) were monitored. Each was sampled approximately every two weeks between May and early September.

The eleven tested crops were cucumber, pumpkin, watermelon, tomato, melon and eggplant in fruit vegetables and red pepper, garlic, onion, Welsh onion and sesame in seasoning vegetable.

The collected specimens from the fields were preserved in 70% ethanol and mounted by the Hemmings method.

Results and Discussion

Species composition of fruit vegetables is showed as Table 1. There was no significant difference in composition. Whereas only *Frankliniella intonsa* was collected from watermelon and *Haplothrips chinensis* was collected from pumpkin, except in Site II.

Particularly severe damage was caused by *Thrips tabaci* Lindeman both in cucumber and garlic.

The leaves of the garlic plant lost their water-retention capacity, wilted rigidly and nonuniformly, and became highly fragile. It also damaged onion crops. The pest damages the leaves of the first year onion crop and the turgor of such leaves is reduced. The leaves turn yellow, wilt and finally twist.

Table 1

Species of thrips colleted from different sites on fruit vegetables during May–October in 1986

Cucumber	Pumpkin	Watermelon	Tomato	Melon	Eggplant
SITE I					
T. tabaci T. setosus F. intonsa	T. tabaci F. intonsa H. chinensis	F. intonsa	T. tabaci F. intonsa	T. tabaci F. intonsa	T. tabaci T. setosus F. intonsa
SITE II					
T. tabaci F. intonsa	T. tabaci F. intonsa	F. intonsa	T. tabaci F. intonsa	T. tabaci F. intonsa	T. tabaci F. intonsa
SITE III					
T. tabaci F. intonsa	T. tabaci H. chinensis	F. intonsa	F. intonsa T. tabaci	T. tabaci F. intonsa	T. tabaci F. intonsa
SITE IV					
T. tabaci H. chinensis	T. tabaci F. intonsa H. chinensis	F. intonsa	T. tabaci F. intonsa	F. intonsa	T. tabaci F. intonsa

SITE I: Suwon, Yongin
SITE II: Taejon, Yeosung
SITE III: Changweon, Jinju, Euisung, Sinryeung, Pohang
SITE IV: Muan, Hampyung, Naju, Kwangju

Table 2

Species of thrips collected from different sites on seasoning vegetable during May–October in 1986

Red pepper	Garlic	Onion	Welsh onion	Sesame
SITE I				
T. tabaci	T. tabaci	T. tabaci	T. tabaci	T. tabaci
F. intonsa	F. intonsa	Tae. alliorum	F. intonsa	T. setosus
H. chinensis	F. tenuicornis		F. tenuicornis	H. aculeatus
H. aculeatus	Ac. kurosawaii		H. chinensis	
SITE II				
T. tabaci	T. tabaci	T. tabaci	T. tabaci	T. tabaci
F. intonsa	F. intonsa		F. tenuicornis	F. intonsa
			H. chinensis	H. aculeatus
SITE III				
T. tabaci	T. tabaci	T. tabaci	T. tabaci	
F. intonsa	F. intonsa	Tae. alliorum	F. intonsa	
H. chinensis			H. chinensis	
SITE IV				
T. tabaci	T. tabaci	T. tabaci	T. tabaci	
F. intonsa	F. intonsa		F. intonsa	
			F. tenuicornis	
			H. chinensis	

SITE I: Suwon, Yongin
SITE II: Taejon, Yeosung
SITE III: Changweon, Jinju, Euisung, Sinryeung, Pohang
SITE IV: Muan, Hampyung, Naju, Kwangju

Table 3

Thrips composition in fruit and seasoning vegetables from different sites

	Fruit Vegetable						Seasoning vegetable					
	Cu	Pu	Wm	To	Me	Ep	Rp	Ga	On	Wo	Se	
Thrips tabaci	×	×		×	×	×	×	×	×	×	×	10
T. selosus	×					×					×	3
Taeniothrips alliorum									×			1
F. intonsa	×	×	×	×	×	×	×	×		×	×	10
F. tenuicornis								×	×	×		3
A. kurosawaii								×				1
H. chinensis	×	×					×			×		4
H. aculeatus							×				×	2
8	4	3	1	2	2	3	4	4	3	4	4	

Cu: Cucumber, Pu: Pupkin, Wm: Watermelon, To: Tomato, Me: Melon, Ep: Eggplant
Rp: Red pepper, Ga: Garlic, On: Onion, Wo: Welsh onion, Se: Sesame.

A great deal of damage to garlic and onion is caused by the larve of tobacco thrips. After emerging from their eggs they feed upon the plant juice of the bracts of the spike and floral sheaths and cause discoloration. In seasoning vegetables of which *Thrips tabaci* and *Frankliniella intonsa*, *Haplothrips chinensis*, *Haplothrips aculeatus* were collected from red pepper and garlic plants from Site I (Table 2).

Among the eight identified thrips *Thrips tabaci* and *Frankliniella intonsa* were dominant both in population density and distribution.

This means that the relation abundance and species composition of thrips populations were significantly different in relation to the crops sampled and their phenology North and Shelton, 1986.

The results of the present study indicate that eight species of thrips were collected from the eleven host plants of fruit and seasoning vegetables (Table 3).

Taeniothrips alliorum was collected from onion for the first time in Korea.

Until now, *Thrips palmi* was not established in Korea, though it had recently been established in the southern parts of Japan (Miyazaki et al. 1986).

According to the observations made in the southern parts of Korea, the commonest species from test crops were *Thrips tabaci* Lindeman and *Frankliniella intonsa* (Trybom).

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Ceratothrips friči (Uzel) and *Ceratothrips reichardti* (John) — A Comparison of Morphology

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Diagnostic features of *Ceratothrips friči* (Uzel) and *Ceratothrips reichardti* (John) are discussed. Features were selected based on the study of 70 females and 55 males of *C. friči* and 65 females and 55 males of *C. reichardti*. Both species differed in:

- colour and surface sculpture of some parts of the body,
- size proportion of certain sclerites,
- size and location of certain bristles,
- size of male's areae porosae.

The species of the genus *Ceratothrips* Reuter which belong to the *C. friči* species-group, namely *C. friči* (Uzel), *C. hispanicus* (Bagnall), *C. pallidivestis* (Priesner) and *C. reichardti* (John), are morphologically very similar both in the imaginal as well as in the larval stage. The most conspicuous differences between them appear in the size and shape of areae porosae on the abdominal sternites of males. One of these species *(C. hispanicus)* differs from the others in the shape of the apices of parameters. The morphological differences among the females are not so clear: the differences in the size of the body, the colour of some of its parts and the development of some features. They are mostly rather small, and the extreme values of the measurable characters in which the individual species differ from one another overlap in all or at least in some species belonging to this species-group. Besides, the species *C. hispanicus* occurs in various forms differing from each other in the colour as well as in the size of the body. Therefore, I have decided to compare the species belonging to the *C. friči* group on the basis of their detailed morphological analysis and tried to determine a set of characters typical of each of them which would make it possible to identify properly even a single specimen.

Now I am presenting the first part of this study, namely, a comparison of morphology of the two species occurring in Poland: *C. friči* (Uzel) and *C. reichardti* (John). I mentioned already some morphological differences between them in my paper on new Thysanoptera species in Poland at the symposium at Smolenice in 1985 (Zawirska, 1987).

Material and Method

The present paper is based on a comparison of the colour, surface sculpture and chaetotaxy as well as the size of the selected parts of the body and setae in 70 females and 55 males of *C. friči*, and 65 females and 55 males of *C. reichardti*. Most of the specimens were taken from samples collected from *Hieracium pilosella*, *Leontodon* spp. and *Hypochoeris radicata* flowers at several localities near Warsaw in different seasons of three years. Some specimens of *C. friči* were collected in the environs of Poznań and some specimens of *C. reichardti* near Biłgoraj (in south-eastern Poland). In the study the dimensions of some parts of the body and the length of the characteristic setae were determined, and the ratios of various pairs of features were computed for each of the specimens examined. The distance between setae as well as those between the campaniform sensilla (discale pores) were measured between the centres of their insertations.

From a large number of measurements and the computed ratios of various characters those have been chosen which were different in the specimens of *C. friči* and *C. reichardti*.

It should be emphasized that the following discussion of both species contains no morphological description at all, but only a description of the characters in which they differ from each other.

Results

The results of measurements are given in Table 1. It contains the maximum, minimum and mean values of each feature.

Females of *C. friči* are larger than those of *C. reichardti*. In *C. friči* the body length (distended) ranges from 1450 to 1900 μm, in *C. reichardti* – from 1230 to 1550 μm.

Colour. In both species the body is uniformly brown, in *C. friči* dark brown, in *C. reichardti* usually somewhat paler. In both the antennae are similar in colour, though in *C. reichardti* mostly a little darker. Particularly in autumn some specimens of *C. reichardti* have conspicuously dark antennae – their antennal segments III–VIII are almost uniformly pale brown.

The species differ in the colour of wings as well as in the mid and hind tibiae. In *C. friči* the wings are fairly shaded, uniformly grey, seldom with a yellowish tint, in *C. reichardti* they are pale yellow to dark yellow. In autumn the wings of *C. reichardti* turn somewhat darker, but they are always paler than those of *C. friči*.

In *C. friči* the mid and hind tibiae are dark brown, the same colour as that of femora, paler basally and apically or only apically. In *C. reichardti* the mid tibiae are always, and the hind ones mostly paler than femora; the hind tibiae are dark yellow to pale brown, medially more or less shaded. The specimens of *C. reichardti* with dark hind tibiae paler only basally and apically are seldom found, usually in autumn.

Table 1

Comparison of some measurements (in μm) and their mutual ratios in *Ceratothrips friči* (Uzel) and *Ceratothrips reichardti* (John)

Females	*C. friči* (70 specimens)			*C. reichardti* (65 specimens)		
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$
Total body length (distended)	1450	1900		1230	1550	
Head						
length	102	123	110	92	100	97
width	145	173	158	127	150	140
width to length ratio	1.33	1.55	1.45	1.31	1.54	1.45
length of mouth cone	92	107	101	97	110	103
width of head to length of mouth cone ratio	1.38	1.80	1.57	1.27	1.49	1.39
length of palpus maxillaris	33	41	37	35	40	37
length of eye (dorsal)	70	88	77	58	66	62
length of vertex behind eye	30	40	34	32	38	35
length of head occupied by eye (in %)	68	74	69	60	67	64
length of eye to length of vertex behind eye ratio	2.07	2.73	2.30	1.60	1.95	1.80
length of interocellar seta	32	43	37	30	43	36
distance between hind ocelli	27	33	30	20	28	24
length of interocellar seta to distance between hind ocelli ratio	1.08	1.42	1.25	1.20	1.89	1.50
distance between postocellar setae	15	30	mostly 20–25	10	20	mostly 13–15
distance between postocular setae S_1 and S_2	5	10	7.5	6	15	10
distance between postocular setae S_2 and S_3	17	30	24.5	11	22	15
Pronotum						
median length	117	150	132	105	133	120
number of transverse sculptural lines on the median axis of pronotum	23	33	27	24	37	33
number of sculptural lines per 25 μm of the median axis of pronotum	4.4	5.8	5.1	5.6	8.0	6.8
Fore wing						
length	710	896	807	550	678	628
width across scale	80	93	85	58	75	68
width across middle	50	63	56	38	50	45
number of distal setae on upper vein	3	4	mostly 3	2	4	mostly 3
number of setae on lower vein	10	14	mostly 11	6	10	mostly 8
length of fore wing to sum of length of abdominal tergites ratio	1.05	1.16	1.10	0.87	0.97	0.90

Table 1 (coninued)

Females	*C. friči* (70 specimens)			*C. reichardti* (65 specimens)		
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$
Postmetanotum						
median length	40	52	46	30	38	33
length of anterior margin	68	95	83	65	83	73
length of anterior margin to median length of postmetanotum ratio	1.42	2.12	1.80	2.00	2.46	2.20
Abdomen						
sum of length of abdominal tergites	657	846	736	615	752	628
abdominal tergite III						
width	238	306	274	225	270	249
distance between discal setae:						
$-S_1-S_1$	57	82	70	35	63	50
$-S_1-S_2$	55	73	62	47	73	61
distance between S_1-S_1 in relation to width of tergite (in %)	21.0	29.0	25.7	14.0	25.0	20.0
distances S_1-S_1 to S_1-S_2 ratio	0.88	1.36	1.13	0.53	1.09	0.83
abdominal tergite VII						
width	250	305	276	235	273	251
distance between discal setae:						
$-S_1-S_1$	72	100	87	57	83	69
$-S_1-S_2$	35	53	44	35	53	42
distance between S_1-S_1 in relation to width of tergite (in %)	29.3	36.0	32.0	23.2	31.4	27.5
distances S_1-S_1 to S_1-S_2 ratio	1.43	2.64	2.00	1.19	2.20	1.60
abdominal tergite VIII						
partial width (between stigmata)	180	223	199	167	205	187
distance between discal setae:						
$-S_1-S_1$	57	75	67	35	60	50
$-S_1-S_2$	30	43	35	27	40	33
distance between S_1-S_1 in relation to partial width of tergite (in %)	28.6	36.7	33.7	19.4	31.6	26.6
distances S_1-S_1 to S_1-S_2	1.47	2.23	1.92	1.06	2.09	1.51
length of abdominal tergite V	70	96	80	62	80	70
abdominal tergite IX length	52	68	62	60	73	65
length of dorsal seta	45	60	53	40	73	58
length of dorsolateral seta	20	33	26	30	43	34
length of marginal seta S_1	103	130	118	95	128	111
length of marginal seta S_2	108	138	123	102	133	116
length of seta S_1 to length of tergite IX ratio	1.61	2.28	1.90	1.41	2.00	1.73
length of seta S_1 to length of dorsal seta ratio	1.91	2.56	2.23	1.57	2.71	1.90
lengths of abdominal tergites V to IX ratio	1.16	1.46	1.29	1.00	1.18	1.09
length of pronotum to length of abdom. tergite IX ratio	1.93	2.48	2.13	1.68	2.04	1.86
length of abdominal segment X	60	78	68	63	78	72

Table 1 (coninued)

Females	*C. friči* (70 specimens)			*C. reichardti* (65 specimens)		
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$
Total body length (distended)	1120	1340		970	1180	
Head						
length	92	110	101	80	95	88
width	122	157	141	112	138	126
width to length ratio	1.34	1.46	1.40	1.27	1.59	1.40
length of mouth cone	80	93	86	77	93	86
width of head to length of mouth cone ratio	1.53	1.76	1.64	1.31	1.61	1.46
length of eye (dorsal)	67	80	73	55	63	60
length of vertex behind eye	25	35	30	25	35	30
length of head occupied by eye (in %)	69	76	72.4	62	75	67.6
length of eye to length of vertex behind eye ratio	2.07	2.73	2.44	1.64	2.27	1.96
length of interocellar seta	27	35	31	22	39	32
distance between hind ocelli	22	30	27	17	26	22
length of interocellar seta to distance between hind ocelli ratio	1.00	1.30	1.14	1.06	1.69	1.42
distance between postocellar setae	12	25	mostly 19–22	8	18	mostly 12–14
distance between postocular setae S_1 and S_2	5	10	7.5	6	13	9.8
distance between postocular setae S_2 and S_3	15	27	20.5	10	18	13.8
Pronotum						
median length	100	120	112	92	113	102
number of transverse sculptural lines on median axis of pronotum	21	33	27	29	38	33
number of sculptural lines per 25 μm of median axis of pronotum	4.9	7.2	5.9	7.0	9.5	8.2
Fore wing						
length	608	723	660	480	582	541
width across scale	62	80	74	52	65	61
width across middle	42	50	47	37	43	40
number of distal setae on upper vein	2	4	mostly 3	1	4	mostly 3
number of setae on lower vein	8	12	10	5	9	8
Postmetanotum						
median length	32	45	39	25	33	29
length of anterior margin	47	70	60	47	65	56
length of anterior margin to median length of postmetanotum ratio	1.33	1.87	1.56	1.27	2.27	1.95

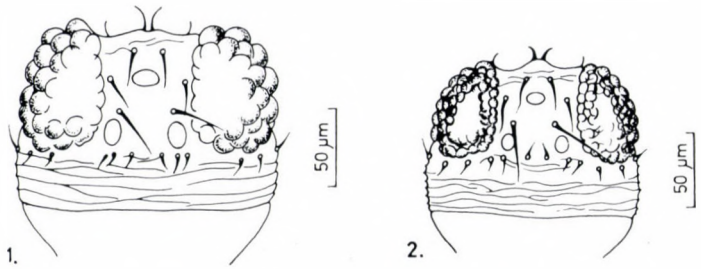
Table 1 (continued)

Females	*C. friči* (70 specimens)			*C. reichardti* (65 specimens		
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$
Abdomen						
sternite III						
length	47	63	55	42	58	51
width	135	175	157	137	170	153
length of area porosa	10	30	21	25	33	28
width of area porosa	22	53	40	50	85	62.5
sternite IV						
length	45	63	54	45	60	51
width	130	175	147	130	168	150
length of area porosa	15	30	23	23	35	29
width of area porosa	32	60	46	53	78	65
sternite V						
length	45	63	54	43	58	51
width	128	165	147	128	163	145
length of area porosa	18	30	23	23	35	28
width of area porosa	30	60	47	50	90	65
sternite VI						
length	44	58	53	45	60	51
width	123	160	141	125	160	140
length of area porosa	15	30	23	23	33	28
width of area porosa	30	63	45	50	83	64
sternite VII						
length	45	58	53	45	60	51
width	118	163	134	118	145	132
length of area porosa	13	30	21	23	33	27
width of area porosa	23	58	40	48	83	60
tergite IX						
length	42	58	50	47	60	51
length of discal seta S_1	30	45	38	21	48	35
length of discal seta S_2	20	38	27	21	44	32
lengths of S_1 seta to S_2 seta ratio	1.10	1.87	1.45	0.62	1.60	1.13
length of penis	120	130	125	107	115	110

The head (Figs 1 and 2) in *C. friči* is always conspicuously larger than in *C. reichardti*, but in both species the ratio of the head width to its length is similar equalling 1.45 on the average. The head in *C. friči* is characterized by the large, somewhat bulging eyes and straight cheeks, converging only near at the base. *C. reichardti* has smaller and slightly less protruding eyes; its cheeks are also

parallel sided, but somewhat converging at the base. The length of the mouth cone is similar in both species. In *C. friči* the mouth cone is always a little shorter, in *C. reichardti* – generally a little longer than the dorsal length of the head.

The compound eyes in *C. friči* are both absolutely and relatively (in relation to head length) distinctly larger than those in *C. reichardti*. The dorsal length



Figs 1–2 Head dorsal: 1. *Ceratothrips friči* (Uzel) ♀, 2. *Ceratothrips reichardti* (John) ♀

of the eye in *C. friči* ranges from 70 to 80 µm, occupying about 70% of the head length. In *C. reichardti* the length of the eye ranges from 58 to 66 µm occupying about 65% of the head length. The eye is always more than twice (2.3 times on the average) in *C. friči* and less than twice (1.8 times on the average) longer than the vertex behind the eye.

The species studied differ significantly in the size of ommatidia of the compound eyes. Diameters of the largest ommatidia on the lateral part of the eye measure 15–17 µm in all the examined specimens of *C. friči* and 12 µm in all the specimens of *C. reichardti*.

Both pairs of anteocellar setae are similar in length (20–25 µm) in both species. Neither is there a significant difference in the length of their interocellar setae. In *C. friči* the latter are 37 µm and in *C. reichardti* 36 µm long on the average. The interocellar setae are 1.1–1.4 (mean 1.25) times in *C. friči* and 1.2–1.9 (mean 1.50) times in *C. reichardti* longer than the distance between inner margin of the posterior ocelli.

Such diagnostic feature, as the distance between postocellar setae cannot be used in the identification, because many specimens may lack either both setae or one of them. Both postocellar setae were developed in only 33 from among the 70 examined females of *C. friči*. Their distance ranged from 15 to 30 µm, mostly 22–25 µm. From among the 65 examined females of *C. reichardti*, only 34 had both setae developed. Their distance was distinctly smaller and ranged from 10 to 20, mostly 13–15 µm.

In *C. friči* setae S_1 and S_2 of the postocular row are placed very close together, 5–10 µm, mostly 7–8 µm apart, whereas the distance between setae S_2 and S_3 ranges from 17 to 27, mostly 25 µm. In *C. reichardti* postocular setae are spaced more regularly. The distance between setae S_1 and S_2 ranges from 7 to 15, mostly

10 μm, whereas that between setae S_2 and S_3 ranges from 11 to 22 μm, mostly 14–16 μm. The distance between setae S_1 and S_2 is often only insignificantly greater than or even as great as the distance between setae S_2 and S_3.

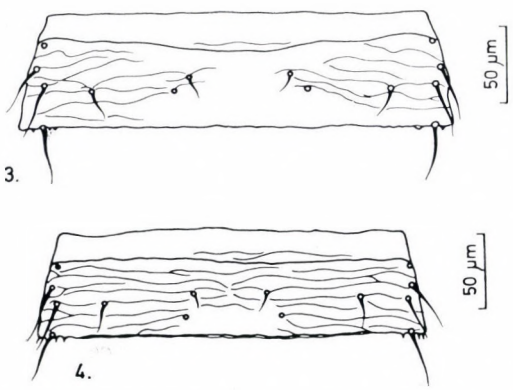
The transverse lines of sculpture on the pronotum in *C. reichardti* are closer placed than in *C. friči*. The mean number of these lines on the median axis of the pronotum is 27 in *C. friči* and 33 in *C. reichardti*, i.e. 5.1 and 6.8 lines per 25 μm of the median length of pronotum, respectively.

The form of the mesonotum and metanotum is similar in both species, whereas the postmetanotum is always distinctly shorter in *C. reichardti* than the same in *C. friči*. In *C. reichardti* the postmetanotal width across the anterior margin is generally more than twice its length, whereas in *C. friči* the width is usually smaller than the double length. The sculpture lines on the postmetanotum in *C. reichardti* are mostly better developed and occupy a greater part of the surface of this sclerite than those in *C. friči*.

The species studied differ significantly in the size of the wings. In *C. friči* the fore wings are much longer and wider than in *C. reichardti*. In order to prove that the wings are longer in *C. friči*, irrespective of specimen size, I compared the length of the fore wing with the sum of length of abdominal tergites in all specimens. In *C. friči* the wing was always longer (1.03 to 1.16 times) than the total length of abdominal tergites. In *C. reichadti* the fore wing was always shorter than the sum of length of abdominal tergites (0.87–0.97 of the latter). Accordingly, the mean number of setae on the lower vein of the fore wing is 11 in *C. friči* and 8 in *C. reichardti*.

From among the abdominal characters I discuss the sculpture and the chaetotaxy of tergites and the development of some segments.

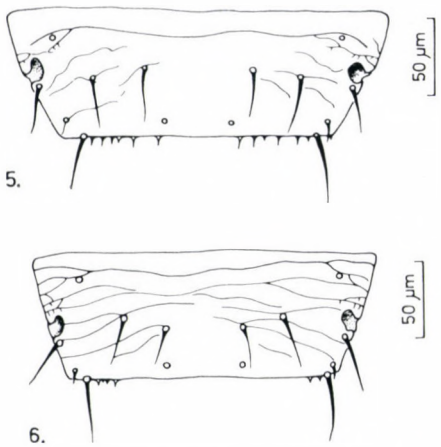
In *C. friči* the sculpture lines on tergites III–VIII are developed laterally and extend to the medial setae. There is no sculpture on the area between these setae behind the subbasal line. In *C. reichardti* the transverse sculptural lines run across the width of tergite III on the area from the subbasal line to at least the



Figs 3–4 Abdominal-Tergit III: 3. *Ceratothrips friči* (Uzel) ♀, 4. *Ceratothrips reichardti* (John) ♀

median setae, and – sometimes – to the distal campaniform sensilla (discal pores) (Figs 3 and 4). Also on tergite VIII in *C. reichardti* delicate sculptural striae occur between and stometimes behind the median setae.

In *C. reichardti* the distance between the median discal setae on the abdominal tergites are, not only absolutely but also in relation to tergal width, shorter



Figs 5–6 Abdominal-Tergit VIII: 5. *Ceratothrips friči* (Uzel) ♀, 6. *Ceratothrips reichardti* (John) ♀

than those of *C. friči*. Accordingly also the unsculptured part of the tergal area is smaller in *C. Reichardti*. Data concerning the position of discal setae on abdominal tergites III, VII and VIII are given in Table 1.

In both species the comb on the posterior margin of tergite VIII is developed only laterally, its microtrichia vary in size and are scatted. In *C. friči*, however, the comb is better developed and extends usually to the discal campaniform senilla on both sides of the posterior margin. Microtrichia of the comb are up to 12 µm long. In *C. reichardti* the comb is rudimentary and consists mostly of 2–3 pale, irregular and short microtrichia both with narrow and broad base (Figs 5 and 6). Some specimens of *C. reichardti* lack the comb, but in one of the females examined it was better developed and reached the campaniform sensilla.

In *C. friči* tergite IX, unlike the other sclerites, is shorter than of *C. reinchardti*. Its mean length is 62 µm in *C. friči* and 65 µm in *C. reichardti*. A composition of the length of tergites II to VII with the length of tergite IX has shown that in *C. reichardti* tergite IX is equal in length to or only slightly shorter than each of the tergites compared, whereas in *C. friči* it is distinctly shorter. For example, tergite V on an average is 1.08 times in *C. reichardti* and 1.29 times in *C. friči* longer than tergite IX. The pronotum on an average is 2.13 times in *C. friči* and 1.86 times in *C. reichardti* longer than tergite IX.

Among the setae on tergite IX (Figs 7 and 8) the dorsolateral one is strikingly developed in *C. reichardti*. It is generally longer, darker and distinctly thicker

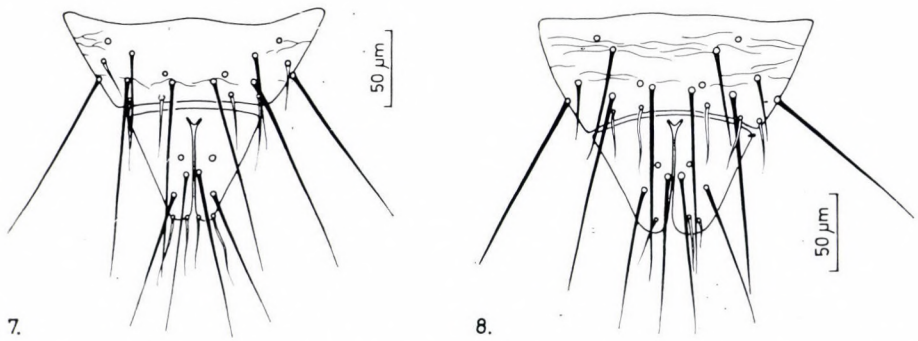
in *C. reichardti* than that of *C. friči*. Its mean length is 34 μm in *C. reichardti* and 26 μm in *C. friči*. In *C. reichardti*, also the dorsal seta is thicker and mostly somewhat longer than that of *C. friči*. Its mean length is 58 μm in *C. reichardti* and 53 μm in *C. friči*. The major posteromarginal setae on tergite IX are, however, somewhat longer in *C. friči* than those of *C. reichardti* (Figs 11 and 12).

In *C. reichardti* segment X is also somewhat longer than that of *C. friči*, its mean length is 72 μm in *C. reichardti* and 68 μm in *C. friči*.

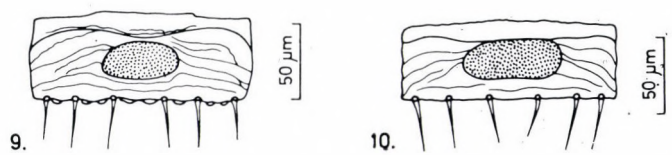
The males of both species are similar to the females in form, colour, surface sculpture and chaetotaxy of the individual parts of the body. They are, however, mostly somewhat paler than in the females. In some specimens of *C. reichardti*, the thorax is distinctly paler than the head and abdomen. In *C. friči* the mid tibiae are frequently paler than femora, sometimes even yellow or yellowish brown and more or less shaded only medially. In males of *C. friči* the hind tibiae are clearly broaded basally and apically than those in females.

An important feature in which the species studied differ from each other is the size and shape of areae porosae on sternites in males (Figs 9 and 10). In *C. reichardti* the areae porosae are generally larger than those of *C. friči*.

Within each of these species the size of the areae porosae is about the same on all sternites; sometimes the areae porosae on sternites III and VII are a little smaller than those on median sternites.



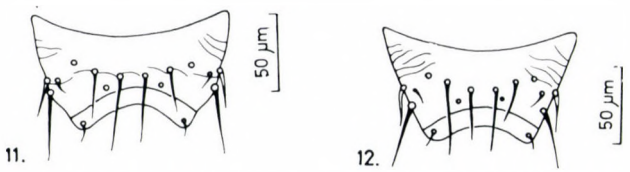
Figs 7–8 Abdominal-Segments IX and X: 7. *Ceratothrips friči* (Uzel) ♀, 8. *Ceratothrips reichardti* (John) ♀



Figs 9–10 Abdominal-Sternit V: 9. *Ceratothrips friči* (Uzel) ♂, 10. *Ceratothrips reichardti* (John) ♂

In the examined specimens of *C. friči* the smallest area porosa measured 10 μm in length and 22 μm in width, the largest one – 30 μm and 60 μm, respectively. Mostly, the length of area porosa ranged from 20 to 23 μm, and its width from 35 to 43 μm.

In *C. reichardti* the smallest area porosa was 22 μm long and 50 μm wide, the largest one 33 μm and 90 μm, respectively. Mostly, the length of area porosa ranged from 25 to 30 μm, and its width from 55 to 60 μm.



Figs 11–12 Abdominal-Tergit IX: 11. *Ceratothrips friči* (Uzel) ♂, 12. *Ceratothrips reichardti* (John) ♂

In *C. reichardti* the anterior margin of area porosa touches along almost its entire width the subbasal sculptural line on each sternite. In *C. friči* the area porosa and the subbasal sculptural line do not adjoin on any of the sternites. Only in very few cases the area porosa touches the subbasal line along a small part of its anterior margin or in one point. On sternite III the subbasal line and the area porosa did not adjoin in any of the 55 examined specimens of *C. friči*.

In *C. reichardti* the subbasal line is straight and undivided. The part of sclerite in front of it is not sculptured. In *C. friči* the line is generally more or less curved and divided into many delicate, curved and irregular striae on both sides.

It is proper to add, that in *C. friči* the areae porosae are mostly dark grey, while in *C. reichardti* mostly yellowish.

Priesner (1928) mentions the length of setae S_1 and S_2 on tergite IX as a feature differing in the males of *C. friči* and *C. reichardti*. According to Priesner, in *C. friči* seta S_1 is about 1.5 times longer than seta S_2, while in *C. reichardti* setae S_1 and S_2 are about equal in length. In the examined males of *C. friči* the length of seta S_1 ranged from 30 to 45 μm (mean 38 μm) and that of seta S_2 varied from 20 to 38 μm (mean 27 μm). In all the specimens of *C. friči* setae S_1 were 1.10–1.87 (mean 1.45) times longer and distinctly thicker than setae S_2.

In *C. reichardti* both pairs of setae varied greatly in length and thickness. In many specimens the setae in one of the pairs considerably differed from each other in length. The length of seta S_1 as well as S_2 ranged from 21 to 45 μm. The mean length of seta S_1 was 35 μm, of seta S_2 32 μm. Seta S_1 was on an average 1.13 times longer than seta S_2. In 21 out of the 50 specimens examined both setae S_2 were longer than or equal in length to setae S_1, in 6 specimens both setae S_1 were slightly longer (i.e. 1.1–1.2 times) than setae S_2, in 9 specimens only one seta S_1 was distinctly longer (sometimes even more than twice) than setae S_2; in 14 specimens both the setae S_1 were 1.3–1.6 times longer than setae S_2.

Discussion

The described morphological differences were sufficient to identify all the specimens of *C. reichardti* and *C. friči* which I collected in Poland. It should be, however, emphasized that the material examined was collected only in a small area of central Europe, whereas the geographical range of both species is much wider. *Ceratothrips friči* is widespread in the northern part of the Palearctic Region, and has been also introduced into other parts of the world, e.g. North and South America, India, Australia and New Zealand (Jacot-Guillarmod, 1974). *Ceratothrips reichardti*, which was described from Siberia, has been subsequently in Mongolia (Pelikan, 1972) and in the Kursk district of the USSR (Schliephake, 1977). The range of variability of the individual morphological characters of both species may be different in the specimens of the populations living under different climatic and environment conditions. Consequently, the diagnostic rank of some features yet may change. This question has to remain open until more specimens of *C. friči* and *C. reichardti* from different areas of their distribution are examined.

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On the Question of the Relationship between Free Zeatin Content and Resistance of Wheat to Biotrophic Fungi*

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Wheat cultivars Little Club and Grekum that are susceptible to *Puccinia graminis* f. sp. *tritici* and *Erysiphe graminis* f. sp. *tritici*, have low content of free endogenous zeatin in young healthy leaves and in dry grains as well. In diseased leaves 2-isopentenyladenine (2iP) occurs to accumulate and the total cytokinin content is also enhanced. The resistant cultivar Khapli has higher amount of free zeatin in primary leaves. Free zeatin and its conjugates are also present in infected tissue where, however, neither qualitative nor quantitative changes in cytokinin content are detected. These results may imply the importance of hormone metabolism, i.e. of free zeatin in the resistance of wheat to the obligate fungi above.

Many sorts of biochemical events following fungal infections have thoroughly been investigated in cereal plants (e.g. Frić, 1976; Hare, 1966; Uritani, 1976). Of those metabolic changes that, in certain cases, highly correlate with disease resistance less attention has been paid to cytokinins as far as their relationship with plant's resistance is concerned. Recent findings have stated, that grains and young leaves of barley resistant to biotrophic fungi contain high level of free zeatin (Vizárová, 1975; 1979; 1986; Vizárová and Vozár, 1984: 1985; Vizárová et al., 1986), whereas susceptible cultivars have low zeatin content. Upon infection by biotrophic fungi cytokinins in the susceptible cultivars undergo considerable qualitative and quantitative changes (Mills and Van Staden, 1978; Sziráki et al., 1976; Vizárová et al., 1986).

This paper deals with the correlation found between zeatin content and the resistance of wheat cultivars to biotrophic parasites such as stem rust and powdery mildew.

Materials and Methods

Grains of wheat (*Triticum* spp.) cvs. Little Club, Grekum and Khapli were ground then extracted with 75% (v/v) ethanol. Further methodological steps were performed as previously described (Vizárová and Vozár, 1984). Cytokinin activity was calculated in μg cytokinin as reference unit of zeatin.

* Devoted to the 100th anniversary of Vavilov's birth.

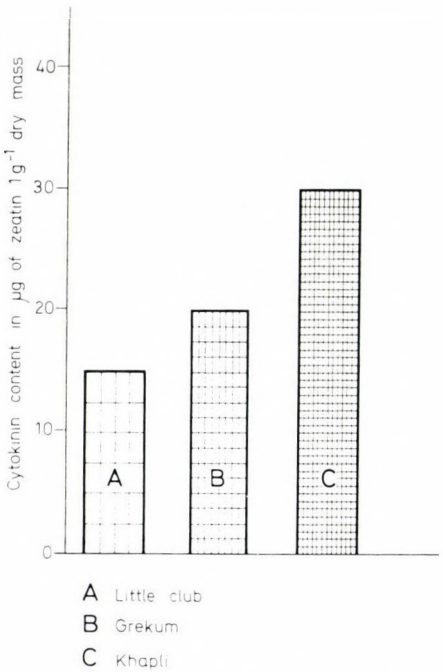

Fig. 1. Content of free endogenous zeatin in dry grains of wheat cultivars with different degree of resistance to stem rust and powdery mildew

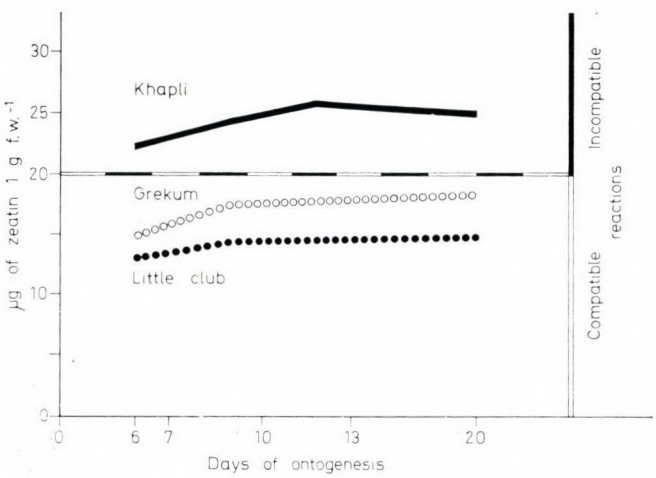

Fig. 2. Content of free endogenous zeatin at various developmental stages of the primary leaves of wheat cultivars with different degree of resistance to stem rust and powdery mildew

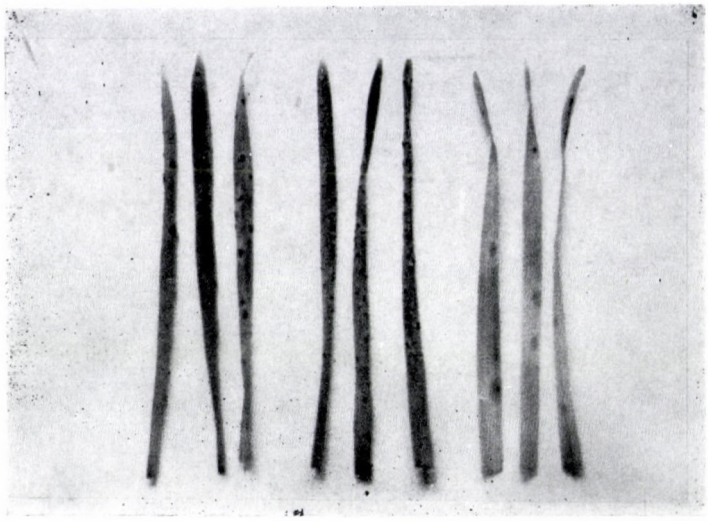

Fig. 3. Reaction of wheat leaves to *P. graminis* f. sp. *tritici*, race 21. A: cv. Little Club, B: cv. Grekum, C: cv. Khapli

Plants grown in climatic chamber and in the greenhouse were inoculated with stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn., race 21) by the method of Mazin et al. (1982) and with powdery mildew (*Erysiphe graminis* f. sp. *tritici* Marchal, race C_5), respectively, on the 10th day of ontogenesis. In order to determine cytokinin content, primary leaves at various stages of ontogenesis and 10 days after inoculation were harvested and extracted with 96% boiling ethanol. The extract was evaporated to dryness in vacuum (Vizárová, 1974). Thin-layer chromatography was used to separate cytokinins (Vizárová and Kováćová, 1980). Cytokinins were identified on the basis of R_f values, UV absorption and, as trimethylsilyl (TMS) derivates, on the basis of retention time in gas-liquid chromatography (Vozár and Vizárová, 1982). Cytokinin activity was determined by the use of radish cotyledon assay (Letham, 1968).

Results and Discussion

Dry grains of wheat cv. Khapli contain more free zeatin than cvs. Little Club and Grekum (Fig. 1). In the first-leaf stage of ontogenesis the leaf tissue of cv. Khapli as compared to that of cvs. Little Club and Grekum also exhibits higher level of free zeatin (Fig. 2). It is noteworthy that cv. Khapli is resistant, whereas cvs. Little Club and Grekum are susceptible to the majority of stem rust and powdery mildew races (Figs 3, 4).

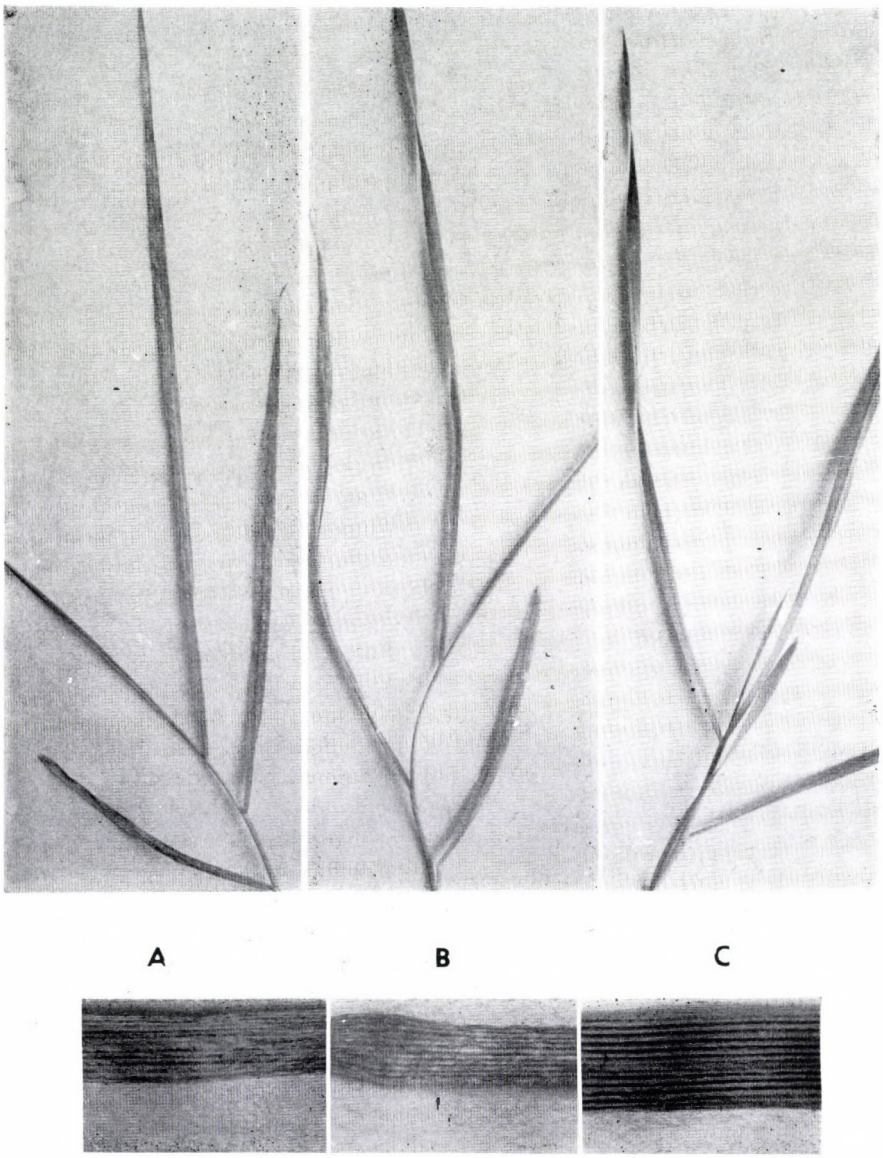

Fig. 4. Reaction of wheat leaves to *Erysiphe graminis* f. sp. *tritici*, race C_5. A: cv. Little Club, B: cv. Grekum, C: cv. Khapli

Ten days after inoculation with either rust or powdery mildew considerable qualitative and quantitative changes in cytokinin content can be detected in the suseptible cvs. Little Club and Grekum. The most striking change is the occurrence of 2iP, which is not quantifiable in either the non-inoculated or the inocul-

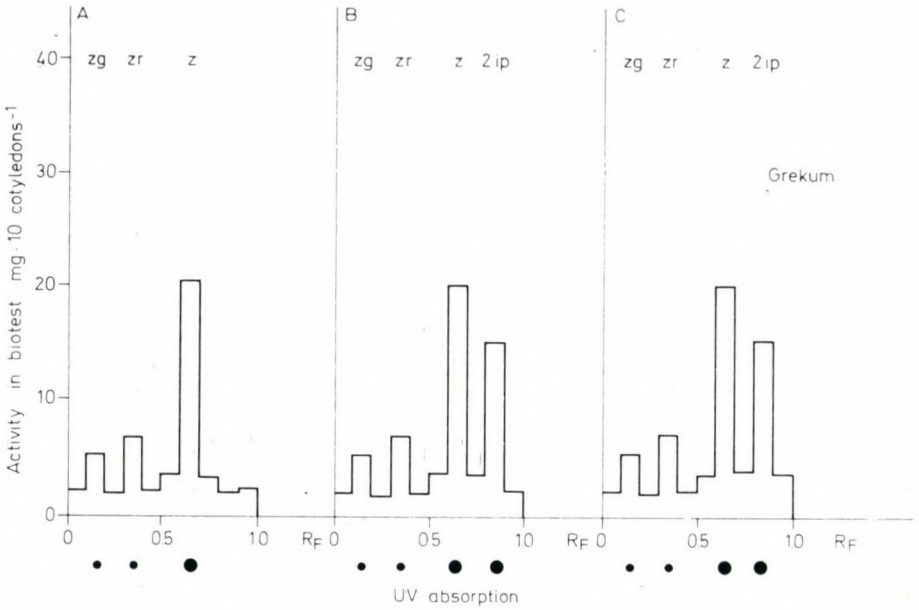

Fig. 5. TLC histogram of free endogenous cytokinins from the primary leaves of 20-day-old wheat cv. Little Club non-inoculated (A), 10 days after inoculation with stem rust (B) and 10 days after inoculation with powdery mildew (C); z, zg, zr and 2iP abbreviate zeatin, zeatin glucoside, zeatin riboside and 2-isopentenyladenine, respectively

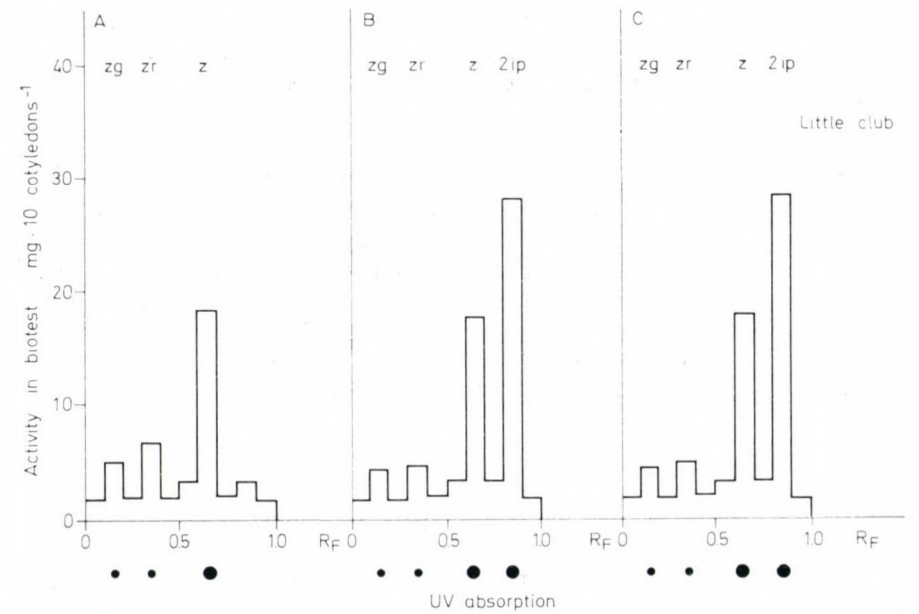

Fig. 6. TLC histogram of free endogenous cytokinins from the primary leaves of 20-day-old wheat cv. Grekum (Details as in fig. 5)

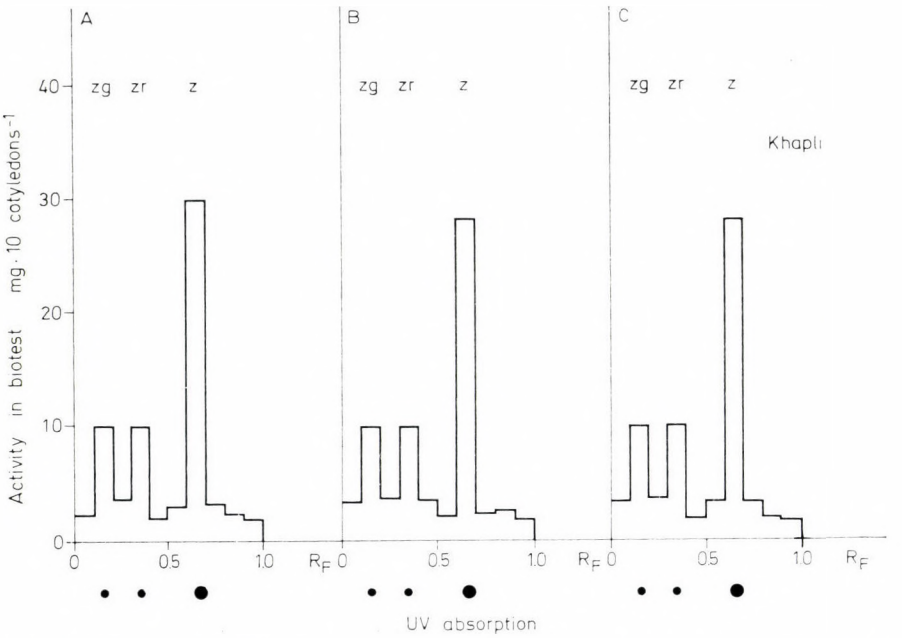

Fig. 7. TLC histogram of free endogenous cytokinins from the primary leaves of 20-day-old wheat cv. Khapli (Details as in fig. 5)

ated resistant tissue (*cf.* Figs 5, 6 and 7). The amounts of other cytokinins do not change considerably. As TMS derivates, the cytokinins zeatin glucoside, zeatin, 2iP and zeatin riboside have the retention time 5, 7, 10 and 12 min, respectively.

Our results presented here give further support to previous findings, that is, the biotrophic fungi dramatically alter the cytokinin metabolism of their host plant (Chan and Trower, 1980; Király et al., 1967; Mills and Van Standen, 1978; Sziráki et al., 1976; Vizárová, 1974; 1979; 1986). In accordance with other results (Vizárová and Paulech, 1979; Vizárová, 1983; Kern, 1986), a close correlation between free zeatin content and the degree of resistance in wheat to stem rust and powdery mildew seems also to be proven at least in the race-cultivar relationship studied. Additional combinations need to be investigated in order to obtain definite proof. Based on the hypothesis of Haberland (1921) zeatin and its conjugates may have something to do with resistance of cereal plants to obligate fungus parasites.

As early as 1919, Vavilov (1919, 1936) pointed out that wheat cultivars resistant to stem rust are, at the same time, resistant to powdery mildew and, that the resistance response is controlled by intimate relations of plant cells with parasitic microorganisms. This delicate relationship may partly be due to cytokinins.

Acknowledgement

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Total Phenols and their Oxidative Enzymes in Sorghum Leaves Resistant and Susceptible to *Ramulispora sorghicola* Harris

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The levels of total phenols and specific activities of polyphenol oxidase, peroxidase and catalase in Sorghum leaves resistant and susceptible to *Ramulispora sorghicola* (Harris) were measured at 15 days interval after 25 days of sowing. Resistant varieties exhibited high polyphenol oxidase, peroxidase specific activity along with high phenol content in comparison to susceptible ones at all stages of growth. Peroxidase activity was several times more as compared to polyphenol oxidase activity and increased markedly in response to infection in both the resistant and susceptible varieties. Healthy and diseased leaves of susceptible varieties showed high catalase activity up to 40 days after sowing in comparison to resistant ones and this trend was reversed at 55 days after sowing. Role of phenolics and oxidative enzymes particularly the peroxidase in defense mechanism against oval leaf spot disease is suggested.

Sorghum (*Sorghum bicolor* (L.) Moench) foliage is subjected to a number of fungal diseases. Among these, oval leaf spot caused by *Ramulispora sorghicola* (Harris) is a major foliage disease of Sorghum in Northern India. This disease is of great economic importance because it affects palatability and digestibility of the crop (Gandhi et al., 1980).

Phenolic compounds are generally believed to be important in plant resistance mechanisms and their accumulation is much higher in resistant cultivars than in susceptible ones (Arora and Wagle, 1985; Bashan, 1986). Oxidative enzymes viz. polyphenol oxidase and peroxidase are also known to confer resistance by oxidation of phenolic compounds to quinones which are toxic to the invading fungi. A positive correlation between the activities of these enzymes and disease resistance has also been observed in different crop plants (Fehrmann and Dimond, 1967; Jennings et al., 1969; Sempio et al., 1975). As sorghum cultivars differ in their susceptibility to oval leaf spot pathogen, it was thought necessary to investigate differences during growth in the levels of total phenols and specific activities of polyphenol oxidase, peroxidase and catalase in leaves of resistant and susceptible varieties.

Materials and Methods

Healthy and diseased sorghum leaves (lower and middle) of two resistant (S-171 and S-260) and susceptible (JS-20 and JS-263) varieties were collected at 25, 40 and 55 days after sowing. Disease severity varied between 40–50 per cent in susceptible varieties and 5–10 per cent in resistant ones. These leaves were freed of foreign material by washing thoroughly with running tap water followed by distilled water and then dried in four layers of filter paper.

To 1.0 g fr. wt. of finely chopped leaves was added 4–5 ml of 0.01 M phosphate buffer, pH 7.0 and extract prepared by hand homogenization at 0–4 °C (Arora and Bajaj, 1981). It was passed through four layers of cheese cloth and then centrifuged for 20 min at 10,000 g(0–4 °C). The resulting supernatant was used as enzyme extract. In each case, three separate extractions were made. Polyphenol oxidase and peroxidase were assayed according to Horwitz et al. (1960) and Seevers et al. (1971), respectively. Enzyme units were expressed as change in 0.01 absorbance min^{-1} mg $protein^{-1}$. Catalase activity was measured by adopting the procedure of Sinha (1972) and expressed as μmoles of H_2O_2 utilized min^{-1} mg $protein^{-1}$. Suitable blanks in each case were also run simultaneously. Soluble protein in the enzyme extracts was determined with folin phenol reagent using BSA as standard (Lowry et al., 1951).

Table 1

Total Phenols in Healthy and Diseased Sorghum leaves of Resistant and Susceptible varieties at different stages of growth on dry weight basis

Varieties	Days after sowing	Total Phenols* (%)	
		Healthy	Diseased
Resistant			
	25	4.00±0.03	4.28±0.06
S-171	40	4.29±0.07	4.90±0.08
	55	3.80±0.03	4.67±0.02
	25	3.13±0.03	4.80±0.02
S-260	40	3.74±0.06	5.13±0.04
	55	3.86±0.02	4.47±0.03
Susceptible			
	25	2.87±0.03	5.71±0.05
JS-20	40	3.10±0.04	4.72±0.04
	55	3.37±0.05	4.22±0.03
	25	2.88±0.04	5.88±0.07
JS-263	40	3.37±0.03	4.85±0.04
	55	3.35±0.04	4.10±0.04

* Mean values of triplicate determinations.

Leaf samples left after extraction of enzymes, were dried at 60 °C in a hot air oven and ground in a micro-Willey mill. Total phenols were then extracted and estimated by the method of Swain and Hills (1959).

Results

Total phenols

Phenol content was higher in resistant varieties throughout the growth period in comparison to susceptible ones and increased after infection in both the resistant and susceptible varieties (Table 1). But with the growth of the crop, the concentration of total phenols decreased continuously in diseased leaves of susceptible varieties, whereas its level increased in diseased leaves of resistant varieties up to 40 days after sowing (DAS) followed by decline at 55 DAS.

Polyphenol oxidase, peroxidase and catalase activity

Resistant varieties exhibited high polyphenol oxidase (PPO) specific activity in their leaves as compared to susceptible ones (Table 2). The specific activity

Table 2

Specific activity of Polyphenol oxidase in Healthy and Diseased Sorghum leaves of Resistant and Susceptible varieties at different stages of growth

Varieties	Days after sowing	Polyphenol oxidase*	
		Healthy	Diseased
Resistant			
	25	9.45±0.29	12.12±0.33
S-171	40	11.21±0.37	10.06±0.25
	55	10.78±0.53	7.97±0.53
	25	12.46±0.71	12.45±1.19
S-260	40	14.23±0.84	10.97±0.41
	55	11.21±0.18	10.47±0.38
Susceptible			
	25	10.15±0.69	12.82±0.61
JS-20	40	6.89±0.06	9.13±0.29
	55	7.49±0.26	9.41±0.20
	25	9.44±0.44	14.71±0.50
JS-263	40	8.33±0.09	10.03±0.15
	55	8.84±0.46	9.34±0.67

* Enzymes Units – Change in 0.01 absorbance min^{-1} mg $protein^{-1}$

Table 3

Specific activity of Peroxidase in Healthy and Diseased Sorghum leaves of Resistant and Susceptible varieties at different stages of growth

Varieties	Days after sowing	Peroxidase*	
		Healthy	Diseased
Resistant			
	25	149.58± 10.21	392.11± 6.19
S-171	40	2056.82± 58.42	3736.39± 59.97
	55	3014.84± 55.81	3063.05± 170.24
	25	230.12± 13.49	359.89± 35.00
S-260	40	3764.91± 385.27	4790.78± 146.26
	55	3201.89± 160.47	4510.23± 54.23
Susceptible			
	25	130.44± 11.74	386.41± 54.12
JS-20	40	1661.04± 45.21	3747.52± 68.07
	55	2362.41± 65.55	3544.11± 281.70
	25	140.09± 10.61	561.03± 7.74
JS-263	40	1982.24± 119.74	3639.16± 66.37
	55	4289.50± 138.64	3677.38± 287.90

* Enzyme Units — Change in 0.01 absorbance min^{-1} mg $protein^{-1}$

increased after infection in susceptible varieties but decreased in resistant varieties at all stages of growth except at 25 DAS, where it increased in S-171 and remained almost constant in S-260.

Peroxidase (PO) specific activity was several times more than PPO specific activity in resistant as well as in susceptible varieties. Like PPO, the peroxidase was higher throughout the growth period in resistant varieties and increased markedly in response to infection in both the resistant and susceptible varieties (Table 3). In the beginning, the enzyme activity in diseased leaves of resistant as well as susceptible varieties was low, but increased sharply to reach maximum at 40 DAS and decreased thereafter. However, in healthy leaves it was found to increase with growth and attained maximum value at 55 DAS except in S-260 where it was maximum at 40 DAS.

Healthy and diseased leaves of susceptible varieties exhibited high catalase activity up to 40 DAS in comparison to resistant varieties and this trend was reversed at 55 DAS whereas healthy leaves of resistant varieties developed high catalase activity (Table 4). No catalase specific activity could be detected at 55 DAS in diseased leaves of resistant and susceptible varieties except in JS 263 where it was present in considerable amount.

Table 4

Specific activity of Catalase in Healthy and Diseased Sorghum leaves of Resistant and Susceptible varieties at different stages of growth

Varieties	Days after sowing	Catalase*	
		Healthy	Diseased
Resistant			
	25	430.20± 19.07	219.08± 13.11
S-171	40	122.63± 2.81	4.86± 0.13
	55	240.07± 29.62	—
	25	305.63± 39.70	256.45± 30.58
S-260	40	146.03± 24.98	21.47± 0.69
	55	164.03± 9.54	—
Susceptible			
	25	652.47± 14.48	442.59± 22.66
JS-20	40	325.80± 6.63	61.24± 4.42
	55	71.57± 4.79	—
	25	377.03± 13.37	206.09± 10.48
JS-263	40	362.43± 7.42	118.23± 18.90
	55	98.63± 8.18	102.80± 8.81

* Enzyme Units — μmoles of H_2O_2 utilised min^{-1} mg $protein^{-1}$.

Discussion

After phytoalexins, phenolic compounds and related oxidative enzymes are generally considered as most responsible parameters for disease resistance. Phenol accumulation is usually higher in resistant genotypes than in susceptible ones (Arora and Wagle, 1985; Patil et al., 1985; Bashnan, 1986). Presence of more phenolics in leaves of resistant varieties and their increase in response to infection in these studies also suggests a positive role of phenolics in resistance of Sorghum plants to oval leaf spot disease. The post infection increase in total phenols is either due to its increased synthesis or translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosidase to yield free phenols. Similar observations have also been made in maize blight (Sharma et al., 1983).

It is now well known that PPO and PO contribute to resistance by the oxidation of phenolic compounds. Moreover, PO activity is associated with development of symptoms and is also known to be involved in lignification (Hammerschmidt and Kuć, 1982). The presence of high specific activities of oxidative enzymes particularly the PO in resistant varieties and their increase after

infection indicates their involvement in resistance. Quinones, which are produced as a result of oxidation of phenolic compounds by these enzymes undergo condensation and polymerization thereby producing dark red to brown lesions on Sorghum leaves. High PPO and PO specific activity in resistant genotypes has been observed in case of beans (Deverall and Walker, 1963), cotton (Bhaskaran et al., 1975) and wheat (Arora and Wagle, 1985). Increase in PPO and PO activity in tissues of both resistant and susceptible plants following infection by fungal pathogens has also been reported (Fehrmann and Dimond, 1967; Jennings et al., 1969) and this increase is either due to *de novo* enzyme protein synthesis or due to degenerative processes of tissues induced by pathogen (Novacky and Hampton, 1968; Maxwell and Batemann, 1967). The reason for the observed decrease in PPO specific activity in response to infection in resistant varieties at 40 and 55 DAS is not known to us and warrants further study.

The role of catalase in disease reaction still remains obscure. The presence of this enzyme in healthy and diseased leaves of both the varieties in the present case indicated its role in reducing the level of H_2O_2 which may otherwise accumulate to the toxic level in diseased tissues. Moreover, the enzyme catalase may also oxidise the compounds which are not being affected by peroxidases (Rudolph and Stahmann, 1964).

The present study suggests that phenolics and their oxidative enzymes particularly the PO are involved in defense mechanism in Sorghum leaves against oval leaf spot disease. However, similar studies in other resistant and susceptible cultivars of sorghum are necessary in order to use peroxidase as a marker for disease resistance mechanism.

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Effect of Various Fungicides on Mycelial Growth, Sporangial Production, Enzyme Activity and Control of *Phytophthora* Leaf Blight of *Colocasia esculenta* L.

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The studies on the effect of twenty three fungicides on mycelial growth of *Phytophthora colocasiae* revealed that Apron 350 FW, Blitox, Blimix, Cuman-L, Demosan 65W, Dexon, Difolatan 80W, Fytolan, Hexaferb, Kitazin, Milton, Ridomil 25 WP and Syllit, showed 100% inhibition at different concentrations. The data on the effect of different fungicides on sporangial production showed that all the fungicides had some effect on sporangial formation. The effect of eight fungicides on pectolytic and cellulolytic enzyme activity revealed that Ridomil-25WP inhibited the activity of these enzymes maximum followed by Apron-350FW, Demosan-65W, Difolatan-80W, Phytoalexin 84, Blimix, Fytolan and Topsin-M, respectively. Out of 23 systemic and non-systemic fungicides, eight were tried in the field conditions and Ridomil 25WP at 200 ppm was found to be most effective followed by Apron 350FW (500 ppm), Demosan 65W (20 ppm), Difolatan 80W (50 ppm), Phytoalexin-84 (500 ppm), Blimix (100 ppm), Fytolan (200 ppm) and Topsin-M (500 ppm).

Phytophthora leaf blight of *Colocasia esculenta* Linn. caused by *Phytophthora colocasiae* Racib. is a serious foliar disease at Kurukshetra and adjoining areas in India (Narula, 1981 and Aggarwal, 1986). Trujillo (1965) reported that disease occurs in regions having high relative humidity and frequent rainfall, whereas warmer areas that have little rainfall and low humidity are relatively free of the disease.

Pectolytic and cellulolytic enzymes play an important role in cell wall degrading process during pathogenesis (Wood, 1960; Albersheim *et al.*, 1969 and Aggarwal and Mehrotra, 1986). Several compounds including antibiotics and fungicides have been found to play an important role in inhibiting the production and activity of cell wall degrading enzymes (Manldes and Reese, 1965; Grover, 1964; Wood, 1959 and Mehta, 1977). In the present communication, the effect of several fungicides on mycelial growth, sporangial production, enzyme activity of the pathogen has been studied with a view to controlling leaf blight of *Colocasia* caused by *P. colocasiae*.

Materials and Methods

The following fungicides were selected for experimentation:

(1) Apron 350 FW — Metalaxyl compound (Methyl D,L-N-(2-6-dimethyl phenyl)-N-(2'methoxy acetyl) alaninate
(2) Benlate — (50% Methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate)
(3) Bavistin — (50% methyl-1H-benzimidazole-2-yl carbamate)
(4) Blitox — (50% Copper oxychloride)
(5) Brassicol — (75% Pentachloronitrobenzene (PCNB))
(6) Blimix — (6% Copper oxychloride)
(7) Cuman-L — (Zinc dimethyldithio carbamate)
(8) Calixin — (80% N-tridecyl-2, 6-dimethyl morpholine)
(9) Demosan 65 W — 65% Chloroneb (1,4-dichloro 2,5-dimethoxybenzene)
(10) Dexon — (Dimethyl amino benzenediazo-sodium sulphate)
(11) Dithane Z-78 — (75% Zinc ethylenebisdithio carbamate)
(12) Dithane M-45 — (75% Manganese ethylenebisdithio carbamate)
(13) Difolatan 80 W — N-(1,1,2,2-Tetrachloroethyl)sulphenyl-cis-4-cyclohexene-1,2-dicarboximide
(14) Fytolan — (88% Copper oxychloride)
(15) Hexacap — (50% Captan (N-trichloromethyl thio-4-cyclohexene-1-2-dicarboxymide)
(16) Hexaferb — (75% Ferric dimethyl-dithiocarbamate)
(17) Kitazin — (Organophosphororus compound (0,0, diisopropyl S-benzyl thiophosphate)
(18) Monosan — (1% Phenyl mercury acetate)
(19) Miltox — (50% Copper oxychloride)
(20) Phytoalexin-84 —
(21) Ridomil 25 WP — Metalaxyl compound (Methyl D-L-N (2-6-dimethyl phenyl)N (2' methoxy acetyl)-alaninate
(22) Syllit — (n-dodecylguanadine acetate (65%))
(23) Topsin-M — (1,2-bis(3-methoxy carbonyl-2-thioureido) benzene)

Effects on mycelial growth in vitro

To evaluate the efficacy of different fungicides against *P. colocasiae*, poisoned food technique (Nene, 1971) was used. The fungicides were incorporated at 1, 10, 20, 50, 100, 200 and 500 ppm concentrations in the potato dextrose agar (PDA) medium after autoclaving. These plates were centrally inoculated with the disc of the pathogen, 8 mm in diameter taken from the margin of six day-old colony on PDA plates. The diameter of the fungal colony was measured after 7 days after inoculation. Inhibition of the mycelial growth by fungicides was calculated using the formula suggested by Vincent (1947).

Effects on formation of sporangia (in vitro)

The fungus was grown on PDA at 28 °C in the dark for 7 days to give colonies approximately 9.0 cm diameter. They were flooded with 15 ml fungicides preparation or with sterile distilled water (control) for one hour. The liquid was removed and the treatments repeated. After liquid had been removed the second time, colonies were slightly scraped with a glass rod to dislodge sporangia and rinsed several times with sterile distilled water to remove sporangia before incubating at 25 °C in the light for 1 week. Sporangia were then removed in 10 ml sterile distilled water per plate and concentrations were measured with a haemocytometer.

Effects on pectolytic and cellulolytic enzyme activity (in vitro)

To assess the effect of different fungicides on the activity of pectolytic and cellulolytic enzymes by *P. colocasiae*, the pathogen was grown in a liquid medium (Garg and Mehrotra, 1975) supplemented with different concentrations (10, 50, 100 ppm) of fungicides, having pectin as a carbon source. Pectolytic and cellulolytic enzyme activity was estimated following the standard viscometric method. The data are presented as per cent loss in viscosity.

Chemical control of Colocasia plants (in vivo)

Out of 23 fungicides used in *in vitro* conditions only 8 fungicides were tested in the field conditions. The different fungicides used were: Apron 350 FW, Blimix, Difolatan 80W, Demosan 65W, Fytolan, Phytoalexin-84, Ridomil 25 WP and Topsin-M.

The above mentioned eight, systemic and non-systemic fungicides were found to be most effective in *in vitro* condition. There were other fungicides, which were also found to be effective against *P. colocasiae* in laboratory conditions but could not be tried in the field conditions because of practical difficulties.

Inoculation of plants

To prepare the inoculum of *P. colocasiae*, 10 ml distilled water was added to each 10 day-old culture grown on Potato dextrose agar medium in Petri dishes and zoosporangia were dislodged from the mycelium by shaking. The resulting sporangial suspension was strained through a single layer of cheese-cloth and incubated for 2 hours at 25 °C to induce liberation of zoospores. The zoospores suspension was diluted with distilled water to provide 2500 zoospores per ml and then sprayed on the leaves with an atomizer. In another experiment, plants of *Colocasia* bearing several sporulating blight lesions, were so placed in each plot of 48 plants (four rows of 12) that each pot was surrounded by 16 plants.

Table 1

Relative efficacy of different fungicides against *P. colocasiae (In vitro)*

Fungicides	Percentage Inhibition* Fungicidal concentrations (ppm)							ED_{50} level
	1 ppm	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm	500 ppm	
1	2	3	4	5	6	7	8	9
1. Apron 350 FW	46.33 (42.89)	52.22 (46.27)	56.77 (48.89)	73.33 (58.90)	81.33 (64.39)	88.88 (70.52)	100 (90.00)	9.57 (18.02)
2. Benlate	4.28 (11.93)	6.13 (14.33)	10.70 (19.09)	12.64 (20.82)	15.08 (22.85)	20.16 (26.67)	67.59 (55.29)	370.04
3. Bavistin	2.62 (9.31)	5.63 (13.72)	9.89 (18.32)	40.82 (39.71)	50.50 (45.28)	63.33 (52.73)	79.28 (62.92)	99.00
4. Blitox	14.38 (22.28)	18.47 (25.45)	31.57 (34.18)	61.63 (51.72)	73.45 (58.98)	100 (90.00)	100 (90.00)	40.56
5. Blimix	20.75 (27.09)	27.23 (31.45)	32.03 (34.46)	38.39 (38.28)	100 (90.00)	100 (90.00)	100 00 (90.00)	65.12
6. Cuman-L	20.74 (27.09)	31.57 (34.18)	37.77 (37.92)	66.56 (54.67)	85.13 (67.31)	100 (90.00)	100 (90.00)	37.56
7. Calixin	5.64 (13.73)	11.63 (19.93)	15.51 (23.19)	18.91 (25.24)	23.38 (28.91)	26.55 (31.01)	31.25 (33.98)	800.00
8. Demosan 65 W	38.22 (38.18)	77.11 (61.41)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	1.30
9. Dexon	26.98 (31.29)	35.20 (36.39)	46.67 (43.09)	56.58 (48.78)	81.87 (64.79)	100 (90.00)	100 (90.00)	21.42
10. Dithane Z-78	2.44 (8.98)	6.33 (14.57)	17.11 (24.43)	17.11 (24.43)	22.22 (28.12)	30.00 (33.21)	41.11 (39.87)	608.12
11. Dithane M-45	3.89 (11.37)	26.94 (31.26)	31.18 (33.94)	41.09 (39.86)	51.36 (45.77)	61.46 (51.62)	75.61 (60.40)	97.35
12. Difolatan 80 W	22.88 (28.57)	45.44 (42.38)	66.88 (54.86)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	11.00

13. Fytolan	6.25 (14.47)	13.35 (21.43)	20.00 (26.56)	37.40 (37.70)	57.34 (49.22)	100 (90.00)	100 (90.00)	87.15
14. Hexacap	3.44 (10.68)	6.99 (15.33)	9.98 (18.41)	11.54 (19.85)	14.42 (22.31)	21.86 (27.87)	34.73 (36.10)	719.83
15. Hexaferb	16.11 (23.66)	22.55 (28.35)	27.44 (31.58)	33.66 (35.46)	39.33 (38.83)	65.33 (53.92)	100 (90.00)	127.12
16. Kitazin	20.93 (27.22)	49.33 (44.61)	55.23 (48.00)	63.80 (53.01)	69.26 (56.32)	83.96 (66.39)	100 (90.00)	10.13
17. Monosan	32.22 (34.58)	34.44 (35.93)	35.88 (36.79)	38.22 (38.18)	43.22 (41.10)	54.33 (47.48)	85.55 (67.65)	184.06
18. Miltox	6.31 (14.54)	15.65 (23.30)	18.94 (25.79)	33.02 (35.07)	60.92 (51.30)	100 (90.00)	100 (90.00)	82.07
19. Phytoalexin 84	3.50 (10.78)	5.60 (13.68)	12.00 (20.26)	20.40 (26.85)	24.35 (29.56)	27.30 (31.49)	35.75 (36.72)	699.30
20. Ridomil 25 WP	47.88 (43.78)	53.33 (46.90)	57.77 (49.46)	75.55 (60.36)	83.33 (65.90)	100 (90.00)	100 (90.00)	9.37
21. Syllit	10.88 (19.25)	22.22 (28.12)	44.11 (41.61)	71.33 (57.62)	82.66 (65.39)	100 (90.00)	100 (90.00)	22.67
22. Topsin-M	31.11 (33.90)	40.22 (39.36)	50.55 (45.31)	55.11 (47.93)	60.22 (50.89)	65.55 (54.05)	67.66 (55.34)	19.78
23. Brassicol	2.99 (9.95)	12.11 (20.36)	17.64 (24.83)	24.68 (29.78)	30.91 (33.77)	34.48 (35.95)	42.67 (40.78)	585.89

* Mean of 5 replicates; Figures in parantheses indicate the transformed angular value.

The plotted plants remained in the plots for the specified period. Within 10 days of introducing the fungus, all plants in the control plots showed symptoms of the disease.

Parameter of disease control

Five major parameters were taken to see the effect of various fungicides in field conditions:

1. Number of plants infected in treated plots.
2. Number of leaves infected in each plant in treated plot.
3. Number of blight lesion in each leaf.
4. Size of lesion in each leaf.
5. Corm yield in each plot.

The incidence of *Phytophthora* leaf blight in the *in vivo* tests was based on observations of five replicates. The *Phytophthora* incidence of *P. colocasiae* was rated as 0, 1, 2, 3 and 4 for zero, 1–25, 26–50, 51–75 and 76–100 per cent of leaf infection, respectively.

Results

Effects on mycelial growth

It is evident from the Table 1 that all the fungicides were effective in inhibiting the growth of *P. colocasiae* but the degree of inhibition varies with the fungicides. It is found that systemic fungicides especially Matalaxyl fungicide were more effective in inhibiting the growth of the pathogen as compared to non-systemic fungicides.

Higher concentrations of metalaxyl compound proved to be toxic to the pathogen. Apron 350 FW gave 100% inhibition at 500 ppm while in case of Ridomil, 100% inhibition of the pathogen was found at 200 ppm. Amongst other fungicides, Benlate showed 67.59% inhibition at 500 ppm, Bavistin showed 79.28% inhibition at 500 ppm. Blitox showed 100% inhibition at 200 ppm, Blimix showed 100% inhibition at 100 ppm, Fytolan and Miltox showed 100% inhibition at 200 ppm. Out of four copper fungicides tested against *P. colocasiae*, Blimix was found to be most effective as it brings about 100% inhibition at 100 ppm while other three, Blitox, Fytolan and Miltox showed inhibition at 200 ppm.

Three carbamate compounds were tested viz., Dithane M-45, Dithane Z-78 and Cuman-L. Cuman-L was found to be most effective at 200 ppm showing 100% inhibition of the pathogen while Dithane M-45 and Dithane Z-78 showed 75.61% and 41.11% inhibition at 500 ppm. Calixin showed 31.25% inhibition at 500 ppm, Dexon showed 100% inhibition at 200 ppm, Demosan 65 W showed 100% inhibition at 20 ppm and Difolatan 80 W showed 100% inhibition at 50 ppm and Kitazin showed 100% inhibition at 500 ppm.

Amongst other non-systemic fungicides tested, Hexacap showed 34.73% inhibition at 500 ppm, Hexaferb 100% inhibition at 500 ppm, Monosan 85.55% inhibition at 500 ppm, Syllit 100% inhibition at 200 ppm, Topsin-M 67.66% inhibition at 500 ppm and Brassicol showed 42.67% inhibition at 500 ppm.

Table 2

Effect of fungicides on sporangial production of *P. colocasiae (in vitro)*

Fungicides	Concentrations of Fungicides* (ppm)						
	1 ppm	10 ppm	20 ppm	50 ppm	100 ppm	200 ppm	500 ppm
1	2	3	4	5	6	7	8
1. Apron 350 FW	++	+	+	−	−	−	−
2. Benlate	++	++	++	++	++	++	+
3. Bavistin	+++	++	++	++	++	++	+
4. Blitox	++	++	++	+	+	−	−
5. Blimix	++	++	++	++	−	−	−
6. Brassicol	+++	++	++	++	++	++	+
7. Cuman L	+++	++	++	+	+	−	−
8. Calixin	++	++	++	++	+	+	+
9. Demosan 65 W	+	+	+	+	−	−	−
10. Dexon	++	++	++	++	+	−	−
11. Dithane Z-78	++	++	++	++	+	+	−
12. Dithane M-45	++	++	++	+	+	+	−
13. Difolatan 80 W	++	++	+	+	+	−	−
14. Fytolan	+	+	+	+	−	−	−
15. Hexacap	+++	+++	+++	++	++	++	+
16. Hexaferb	++	++	++	++	+	+	−
17. Kitazin	+++	++	++	++	++	++	++
18. Monosan	+++	++	++	++	++	++	+
19. Miltox	++	++	++	+	+	−	−
20. Phytoalexin-84	+++	+++	++	++	+	−	−
21. Ridomil 25 WP	++	++	++	+	+	+	−
22. Syllit	++	++	++	++	+	−	−
23. Topsin-M	+++	++	++	++	++	+	−
Control	++++						

* Mean of five replicates.
++++ = Excellent (75–100)
+++ = Good (50–75)
++ = Fair (25–50)
+ = Poor (1–25)
− = Nil (0)

A newly discovered fungicide, Phytoalexin 84 (supplied by West Coast International, Bombay) was also tested against *P. colocasiae* and it was found that Phytoalexin-84 was able to inhibit the growth of the pathogen 35.75% at 500 ppm.

Effects on sporangial production

It is evident from Table 2 that all the fungicides had some effect on sporangial formation of *P. colocasiae*. Out of 23 fungicides tested, Matalaxyl fungicides were found to be most effective. Apron 350 FW reduced the formation of sporangia

Table 3

Effect of different fungicides on the pectolytic and cellulolytic enzyme activity

Sr. No.	Fungicides	Concentration (ppm)	PG[b]	PMG[b]	PMTE[b]	PGTE[b]	C_x[b]
			Percent reduction in viscosity after 1 hr.				
1.	Control	—	80.00	129.40	75.00	42.45	52.00
2.	Ridomil-25 WP	10	20.63	40.00	15.36	15.42	20.00
		50	13.89	20.40	8.98	8.10	12.50
		100	9.25	10.10	4.00	5.00	9.75
3.	Apron-350 FW	10	25.40	58.00	20.40	20.43	23.40
		50	15.20	27.70	10.00	10.78	14.00
		100	10.20	13.10	5.25	5.18	2.50
4.	Demosan-65 W	10	28.50	75.10	23.00	23.40	25.00
		50	17.53	38.00	12.35	13.44	19.50
		100	8.94	25.00	8.49	7.76	13.50
5.	Difolatan-80 W	10	22.25	80.40	25.00	25.00	27.10
		50	19.50	75.18	14.40	15.49	20.50
		100	15.83	70.85	13.83	9.00	15.00
6.	Phytoalexin-84	10	32.55	85.25	28.00	26.23	28.75
		50	22.63	70.00	16.36	17.48	20.10
		100	19.00	65.75	14.50	11.00	15.79
7.	Blimix	10	33.50	87.75	29.50	29.43	30.00
		50	23.00	80.10	18.73	19.00	22.75
		100	20.74	75.00	16.47	13.43	18.92
8.	Fytolan	10	35.50	89.00	30.50	30.00	30.50
		50	22.00	85.00	19.00	19.75	22.90
		100	19.00	75.00	15.40	14.00	19.00
9.	Topsin-M	10	36.30	89.50	35.00	33.10	32.40
		50	24.00	86.80	19.75	20.30	23.73
		100	20.00	75.50	17.30	15.40	20.71

[a] — The data represented are average of five replicates. C_x = Cellulase.

[b] — The enzymatic activity have been expressed as relative activity/ml filtrate PG = Polygalacturonase; PMG = Poly methyl galacturonase; PMTE = Pectin methyl transeliminase; PGTE = Poly galacturonase transeliminase.

Table 4

Relative efficacy of different fungicides against *P. colocasiae (In vivo)*

Sr. No.	Fungicides	Conc. (ppm)	No. of plants infected in treated pots	Per cent inhibition over control	No. of leaves infected in each plant in treated plots.	No. of blight lesion in each leaf	Size of lesion in leaf (in cm)	Corm yield in each plot kg/plot	Rating[1]
1.	Apron 350 FW	500	5	80	2.0±0.317	1.8±0.491	1.4±0.245	12.5	0.80
2.	Blimix	100	15	40	3.4±0.401	3.0±0.549	2.2±0.584	7.8	2.40
3.	Demosan 65 W	20	7	72	2.4±0.245	2.2±0.375	1.8±0.200	11.6	1.12
4.	Difolatan 80 W	50	9	64	2.4±0.511	2.2±0.491	1.6±0.401	9.5	1.44
5.	Fytolan	200	18	28	3.8±0.375	3.6±0.401	2.8±0.375	4.0	2.88
6.	Ridomil 25 WP	200	4	84	1.2±0.200	1	0.6±0.245	13.1	0.64
7.	Phytoalexin 84	500	10	60	3.0±0.317	2.8±0.375	1.6±0.401	9.0	1.60
8.	Topsin M	500	22	12	5.4±0.245	4.8±0.200	3.0±0.448	2.0	3.52
9.	Control	—	25	—	5.8±0.337	5.4±0.245	3.4±0.401	1.5	4.00

[1] Rating of 0, 1, 2, 3 and 4 for zero, 1–25, 26–50, 51–75, 76–100 per cent of plant infected respectively.

at 20 ppm and at 50 ppm onwards, no sporangia are formed. Other fungicides decreased production of sporangia to different degrees with different concentrations. At 1 ppm, the sporangia were not much affected but further sporangial formation was inhibited while in the control excellent production of sporangia was observed.

Effects on enzyme activity

Out of 23 fungicides, eight were observed to be more effective i.e. Ridomil-25 WP, Apron 350 FW, Demosan-65 W, Difolatan-80 W, Phytoalexin-84, Blimix, Fytolan and Topsin-M and these were tried further on enzyme activity of *P. colocasiae in vitro* conditions.

All the eight fungicides used in the present study showed their variable effects on both pectolytic and cellulolytic enzyme activity of *P. colocasiae* (Table 3). The fungicides showed various degrees of inhibition at various concentrations. The maximum decrease of pectolytic and cellulolytic enzyme activity was shown by Ridomil 25 WP followed by Apron-350 FW, Demosan 65 W, Difolatan-80 W, Phytoalexin-84, Blimix, Fytolan and Topsin-M respectively. Maximum inhibition was observed at higher concentration while minimum at lower concentrations.

Chemical control (in vivo)

Out of 23 systemic and non-systemic fungicides, eight were tried in the field condition (Table 4). Ridomil 25 WP at 200 ppm was found to be most effective followed by Apron 350 FW (500 ppm), Demosan 65 W (20 ppm), Difolatan-80 W (50 ppm), Phytoalexin-84 (500 ppm), Blimix (100 ppm), Fytolan (200 ppm), and Topsin-M (500 ppm). It was found that Metalaxyl compound were quite effective as compared to other systemic and non-systemic fungicides.

Discussion

In vitro tests with twenty three fungicides showed that Metalaxyl and some of non-systemic fungicides were active at low concentration against *P. colocasiae* in all stages of development. Metalaxyl was toxic to mycelial growth and greatly decreased formation of sporangia, but it was not very active against sporangia as assessed by effects on germination of sporangia. Bruck *et al.* (1980) noted that the *in vitro* toxicity of Metalaxyl differed significantly with different races of *P. infestans*. Metalaxyl showed similar activity towards mycelial growth of many *Phytophthora* spp., with ED_{50} of < 1 ppm, for *P. cinnamomi* (Benson, 1979), *P. parasitica* and *P. citrophthora* (Farih *et al.*, 1981a, b) and *P. cactorum* (Crute *et al.*, 1981) although some isolates of *P. capsici* were less sensitive to it (Papavizas and Bower, 1981). In the present investigation, both Apron 350 FW and Ridomil 25 WP showed inhibitory activity towards mycelial growth of *P. colocasiae* with ED_{50} of <10 ppm.

Production of sporangia of *P. colocasiae* was also greatly suppressed by Metalaxyl. Similar results have been reported for *P. capsici* (Papavizas and Bower, 1981), *P. cinnamomi* (Benson, 1979), *P. parasitica* var. *nicotianae* (Staub and Young, 1980), *P. parasitica* and *P. citrophthora* (Farih *et. al.*, 1981b), *P. parasitica* var. *piperina* and *P. palmivora* (Aggarwal, 1982) and *P. palmivora* (Tey and Wood, 1983).

Control by the acylalanines of diseases caused by soil borne *Phytophthora* spp. has been reported by several authors (Benson, 1979; Englander *et al.*, 1980; Farih *et al.*, 1981a). *In vitro*, Metalaxyl inhibits mycelial growth, chlamydospores and sporangium formation. The concentration required for inhibition of sporangium formations is 25 times lower and that for inhibition of chlamydospore formation is 100 times lower than that required for inhibition of mycelial growth.

Some of the non-systemic and systemic fungicides were found quite promising *in vitro* studies. Complete inhibition of the pathogen was noted at 200 ppm of Blitox, 100 ppm of Blimix, 200 ppm of Cuman-L, 20 ppm of Demosan 65 W, 200 ppm of Dexon, 50 ppm of Difolatan 80 W, 200 ppm of Fytolan, 500 ppm of Hexaferb, Kitazin, 200 ppm of Miltox, Syllit. Hexaferb has also been reported to be effective in controlling rot of some fruits (Singh and Bhargava, 1974). Gupta (1980) reported Hexaferb effective against *P. nicotianae* at 500 ppm *in vitro*. The present results are in agreement with that of Gupta (1980).

Generally, copper fungicides were effectvie *in vitro* but Blitox, Blimix and Fytolan were more effective against *P. colocasiae*. Complete inhibition of the colony was found at 200 ppm when treated with Dexon *in vitro*. Kuhalman *et al.* (1963) found that Dexon reduced losses of root rot of Fraser fir, caused by *P. cinnamomi* but did not provide a practical control in nursery beds. Demosan 65 W was found to be effective at 20 ppm. Sharma and Tiwari (1975) reported that Demosan 65 W is highly fungistatic to *Rhizoctonia* spp., moderately so as to *Pythium* spp., and slightly effective to *Fusarium* spp. At 10 ppm, it inhibited the sporangial development of *P. parasitica* var. *piperina*, whereas in *Pythium aphanidermatum* zoospore production was not inhibited even at 200 ppm.

Bergquist (1972) reported that the dithiocarbamate fungicides (Dithane M-45 at 5 μg/ml) provide very good control of *P. colocasiae*, causing leaf blight of taro. In the present investigation, Dithane M-45, showed 75% inhibition at 500 ppm and Dithane Z-78 showed 41.11% inhibition at 500 ppm. The dithiocarbamates are capable of inactivating a number of enzymes, some of which are not dependent on metals (Owens, 1963). Ramarao and Umbala (1982) tested three dithiocarbamates viz., Ferbam, Thiram and Zineb and reported that all the three are quite effective at 100 ppm concentration and no sporangia are formed on 100 ppm and 1000 ppm concentration when used against *P. colocasiae (in vitro)*.

In contrast with Metalaxyl, the *in vitro* activity of a systemic fungicide, Topsin-M was less pronounced. Substantial effect on mycelia and sporangia were obtained only at concentrations greater than 500 ppm but it suppressed formation of sporangia at much lower concentration. The mode of action of Topsin-M may be by interference with DNA synthesis or cell division (Lyr, 1977).

The effect of eight fungicides was studied on enzyme activity, and it was observed that all the fungicides inhibited the pectolytic and cellulolytic activity. Ridomil-25 WP and Apron-350 FW reduced the cellulolytic and pectolytic enzymes activity to the minimum. Mehta (1977) has also observed the suppression of PG activity by *Alternaria solani* and *Alternaria tenuis*. A correlation based on these results can be established where a fungicide inhibiting enzyme activity is also found to cause suppression in fungal growth and sporangial formation. It is likely that they directly or indirectly act on oxidative metabolism of the organism. In the present investigations, the activity of pectolytic and cellulolytic enzyme was also found to be reduced by Demosan-65 W, Difolatan-80 W, and Phytoalexin-84. Blimix and Fytolan, both being copper compounds, but Blimix showed more inhibitory effect on enzyme activity than Fytolan. The common explanation of the toxic action is based upon the properties of this ion of precipitating or denaturing proteins. Similar results have been schown by Garg and Mehrotra (1975) in case of *Fusarium solani*, Singh and Mehrotra (1978) in case of *Helminthospirium sativum*.

All the eight fungicides tested checked the pectolytic and cellulolytic enzyme activity but none could completely check the enzyme activity in *P. colocasiae*. Grover (1964) reported that addition of Phaltan and Difolatan to culture filtrate of *Sclerotinia sclerotiorum* and *Botrytis alli* considerably reduced pectolytic enzyme activity in filtrates. The effect of fungicides on enzyme activity in culture filtrate may be either due to inhibition of fungal growth or direct inhibition of enzyme production. On the other hand the fungicides may not inhibit the enzyme production, but may inactivate the enzyme after its production (Mehta, 1977).

In vivo tests with eight fungicides showed that Metalaxyl compounds i.e. Apron 350 FW and Ridomil 25 WP were more effective than systemic and non-systemic fungicides. The activity of Metalaxyl against soil borne fungi was analysed using *P. parasitica* var. *nicotianae* as a test fungus (Staub and Young, 1983). It was found that the first signs of inhibition during the infection process were seen after the fungus had penetrated the host tissue. In addition, sporangial formation from chlamydospores and on infected root tissue was strongly inhibited by Metalaxyl compounds (Staub and Young, 1983). The Demosan 65 W and Difolatan 80 W fungicides provided good control of *P. colocasiae* while copper fungicides (Blimix and Fytolan) resulted in fair control. Bergquist (1972) while working on the leaf blight of taro reported that Difolatan and copper fungicides provided excellent control of *Phytophthora* blight of taro. Topsin-M was not much effective than other fungicides. A newly discovered 'Phytoalexin-84' was quite effective than Topsin-M. Gollifer and Brown (1974) failed to get good results with Bordeaux mixture and a range of dithiocarbamate fungicides.

In fungicide trials, there was a correlation between ultimate corm yield and the reduction in disease, when the plants were exposed to *P. colocasiae* inoculum. In treatments where leaf blight was inadequately controlled there was rapid destruction of leaf tissue and a reduction in leaf number and plant height.

The increase in corm yield due to blight control in our trial agrees well with the results obtained by different workers (Trujillo and Aragaki, 1964; Bergquist, 1974 and Jackson *et al.*, 1980).

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Non-Fungicidal Control of Certain Post-harvest Diseases of Banana Fruits

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For protecting banana fruits cv. Hindi and Paradica against certain post-harvest pathogens viz. *Botryodiplodia theobromae* and *Gloeosporium musarum* which cause fruit rotting, several chemicals and systemic fungicides were tested *in vitro* and *in vivo*. Effective control measures were achieved by dipping banana fruits for 15 minutes in either 1% acetic, propionic acids, in 10% H_2O_2 or in 200 or 400 ppm benlate and TBZ respectively. As acetic and propionic acids and H_2O_2 showed to be promising against such pathogens and they are non-toxic and volatile leaving no chemical residues as compared with the systemic fungicides, the results obtained might suggest the possibility of their applicability as post-harvest protectants for banana fruits. Although the present attempts were performed only in laboratory on a limited number of banana fruits, yet application on a commercial scale needs more detailed evaluation.

Banana fruits are harvested while still green and then artificially ripened. During transit, storage and ripening, fruits are invaded by different fungi which cause serious deterioration to fruits. Two pathogenic fungi, viz. *Botryodiplodia theobromae* Pat. and *Gloeosporium musarum* Cooke and Massee are most important in causing storage rots of banana in Egypt (El-Helaly *et al.*, 1955, Elarosi, 1960, Abdel Sattar, 1978 and Shoeib, 1981). *B. theobromae* causes watery soft rot of the pulp of banana fruits, and *G. musarum* causes spotting and finger rot. Both pathogens infect bruised mature, pre-mature and immature fruits and subsequently invade the pulp (Tsai, 1973 and Slabaugh and Grove, 1982).

For controlling such fruit rots of banana, systemic fungicides viz. Benlate and thiabendazole (TBZ) were used as dip treatment (Ogawa *et al.*, 1968, Tsai, 1969 and 1971 and Slabaugh and Grove, 1982). As systemic fungicides are toxic, this stimulated to test certain non-fungicidal chemicals for protecting banana fruits against *B. theobromae* and *G. musarum* rots.

Materials and Methods

Partially rotted banana fruits cv. Hindi were collected from market during 1984 season and were used for isolation. Small pieces of peel taken from the intermediate tissues between healthy and rotted ones were cut, dipped in 1% sodium

hypochlorite solution for 5 minutes, rinsed in sterile water, plated on PDA medium and incubated at room temperature (20–26 C). Single spore cultures were obtained and maintained on PDA slants.

Effect of chemicals and fungicides on mycelial growth and disease development of the isolated fungi

I. *In vitro* tests

Concentrations of acetic, propionic and lactic acids at 5, 10, 20 and 50%, H_2O_2 at 10, 20, 25 and 30%, sodium hypochlorite (Chlorox) at 1, 3, 4 and 5%, benlate and thiabendazole (TBZ) at 5, 10, 20 and 50 ppm were used.

Plugs (6-mm diam.) were taken from surface-sterilized peel of green banana fruits, using sterilized cork borer. The plugs were immersed in the chemicals mentioned earlier for 15, 30, 60 and 120 minutes, then transfered to PDA plates previously inoculated at the centre by 6-mm diam. discs of the isolated fungi which were taken from the growing margin of those fungi. Two fruit plugs receiving the same chemical treatment, were placed equidistantly on both sides of the fungal inoculum disc. Similar treatment but using fruit plugs previously immersed in sterile water served as check. Plates were incubated at room temperature (20–26 C). Data were recorded after the isolated fungi filled out the check plates.

II. *In vivo* tests

Bunches of mature banana fruits cv. Hindi and cv. Paradica were divided into hands. Any wounded or bruised fruits were discarded. Selected hands with medium-sized fruits were divided into small clusters. Clusters were washed under running tap water, dipped in a detergent solution for 10 minutes, rinsed in water, then kept to air dry.

Standard amount of inocula of the isolated fungi were introduced singly through a bore 2–3 mm deep made by a cork borer in the fruit half-way along the fruit length. After inoculation, the bore was plugged with the removed piece of peel. Four hours later, inoculated fruits were immersed for 15 minutes in the concentrations of the tested chemicals. The required concentrations of the non-fungicidal and fungicidal chemicals used were freshly prepared prior to performance of the experiment. Similarly treated fruits but immersed in sterile water served as check. Each treatment comprised four replicates. Each replicate (hand) consisted of four fruits. Inoculated and uninoculated fruits were kept for ten days at room temperature (20–26 C). The amount of the decay was estimated by measuring the length of the rotted portion.

Results

Isolation

Isolations from banana fruits showing soft, black, wrinkled skin and soft watery pulp yielded *Botryodiplodia theobromae* Pat. Isolations from banana fruits showing sunken spots with bright salmon colour yielded *Gloeosporium musarum* Cooke and Massee. Artificial inoculation of healthy green mature banana fruits cv. Hindi and cv. Paradica, with the isolated fungi produced similar symptoms as those of naturally diseased fruits after ten days of incubation at room temperature (20–26 C) (Fig. 1).

In vitro effect of certain non-fungicidal chemicals and systemic fungicides on B. theobromae and G. musarum

Data in *in vitro* effect of certain non-fungicidal chemicals and systemic fungicides on mycelial growth of *B. theobromae* and *G. musarum* are presented in Table 1. Data show the following:

1. Steeping fungal inoculum of *G. musarum* for 120 minutes in 10% acetic or propionic acid or 20% H_2O_2 completely inhibited the mycelial growth of the fungus.

2. Immersing fungal inoculum of *B. theobromae* for 120 minutes in 20% acetic acid or 10% propionic acid or 30% H_2O_2 completely inhibited the mycelial growth of the fungus.

3. Immersing fungal inocula of the two fungi in 15 ppm a.i. Benlate and TBZ respectively resulted in complete inhibition of mycelial growth of the two fungi.

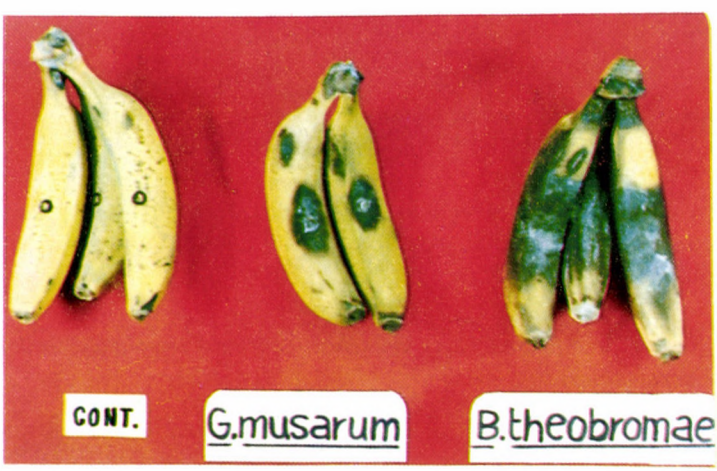

Fig. 1. Hindi banana fruits, artificially inoculated with *B. theobromae* and *G. musarum*

Fig. 2. Effect of acetic acid, propionic acid and H_2O_2 on protecting banana fruits against rotting caused by *B. theobromae* and *G. musarum*

The other chemicals tested as lactic acid and sodium hypochlorite were ineffective in checking mycelial growth of both fungi even up to the concentration of 50% and 5% respectively.

In vivo effect of certain non-fungicidal chemicals and certain systemic fungicides on disease development

Data on the *in vivo* effect of certain non-fungicidal chemicals and systemic fungicides in protecting banana fruits against *B. theobromae* and *G. musarum* infection show the following:

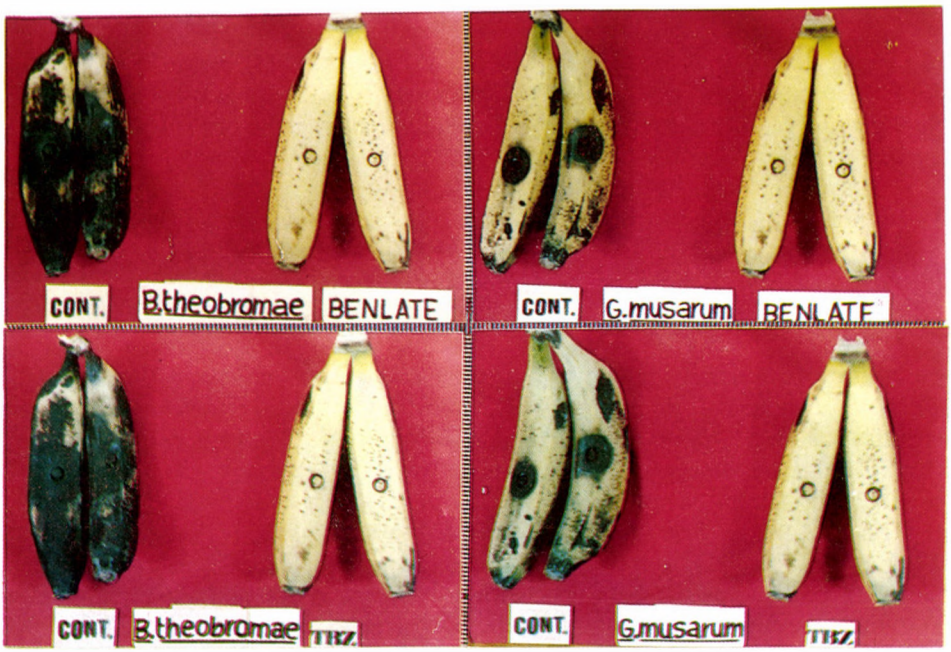

Fig. 3. Effect of Benlate and TBZ on protecting banana fruits against rotting caused by *B. theobromae* and *G. musarum*

1. Steeping artificially-inoculated banana fruits in 1% acetic or propionic acid for 15 minutes completely protected the fruits against *B. theobromae* and *G. musarum* infection (Fig. 2).

Table 1

Effect of certain non-fungicidal chemicals and systemic fungicides on mycelial growth of *B. theobromae* and *G. musarum*

Chemical	Fungus	Least inhibitory conc.	Period (min) of inoculum immersion
Acetic acid	*B. theobromae*	20%	120
	G. musarum	10%	120
Propionic acid	*B. theobromae*	10%	120
	G. musarum	10%	120
H_2O_2	*B. theobromae*	30%	120
	G. musarum	20%	120
Benlate	*B. theobromae*	15 ppm	10
	G. musarum	15 ppm	10
TBZ	*B. theobromae*	15 ppm	50
	G. musarum	15 ppm	50

2. Immersing artificially-inoculated banana fruits in 10% H_2O_2 for 15 minutes gave good protection of fruits against both the test fungi (Fig. 2).

3. Dipping artificially-inoculated banana fruits in 200 and 400 ppm a.i. Benlate and TBZ respectively for 15 minutes completely protected the fruits against rot caused by the two fungi (Fig. 3).

Lactic acid and sodium hypochlorite up to 50% and 5% respectively for 15 minutes were ineffective in checking fruit rotting caused by any of the test fungi.

Discussion

For protecting banana fruits against certain postharvest pathogens, viz. *B. theobromae* and *G. musarum* which cause fruit rotting, certain non-fungicidal chemicals showed to be effective and comparable with systemic fungicides in checking disease development. Steeping artificially-inoculated banana fruits by each fungus for 15 minutes in either 1% acetic or propionic acid or in 10% H_2O_2 could protect fruits from rotting. Chakrabarti (1979) in India, found that 20 ppm propionic acid completely inhibited *B. theobromae* isolated from decayed banana fruits. Dipping banana fruits for 15 minutes in 200 and 400 ppm benlate and TBZ respectively gave the same result. The effectiveness of benlate and TBZ in protecting banana fruits against post-harvest diseases caused by *B. theobromae* and/or *G. musarum* substantiates the work of Slabough and Grove (1982) who found that benlate and TBZ gave good protection of banana fruits against *B. theobromae*, *Cephalosporium* sp., *Colletotrichum musae*, *Dieghtoniella torulosae* and *Fusarium roseum*.

As acetic and propionic acids and H_2O_2 showed to be promising against such pathogens and these chemicals are non-toxic and volatile leaving no residues as compared with the systemic fungicides, results obtained in the present work suggest the possibility of their application as post-harvest protectants for banana fruits. Although the present attempts were performed only in laboratory on a limited number of banana fruits, yet application on a commercial scale needs more detailed evaluation.

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Potato Gene Centres, Wild *Solanum* Species, Viruses and Aphid Vectors[1]

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In his paper the author gives a comprehensive view of the importance of the potato gene centres, and of the virus susceptibility and resistance of wild *Solanum* species as well as of their aphid resistance. After a discussion of the gene centres and the genetic basis some problems of resistance breeding are analysed. The author lists the wild *Solanum* species important from a breeding point of view and point to their virus susceptibility. By showing the relations of viruses occurring and described in wild *Solanum* species and varieties the author inspires thoughts and emphasizes responsibilities in using the breeding materials.

Gene centres and the genetic basis

The original home of potatoes is in the tropical heights of Central- and South America. The greatest centre of diversity for potatoes is near Lake Titicaca on the boundary of Peru and Bolivia. This region is believed to be the centre of origin for cultivated potatoes. In the Latin-American gene centres the *Solanum* species are very rich in number. According to Brush (1977) there are more than 2000 named potato cultivars in Peru. The potato collection of the International Potato Center, Lima, Peru consists of two major components of genetic resources; wild species and primitive cultivated species. More than 150 wild tuber-bearing *Solanum* species and more than 12–15 000 clonal samples of primitive cultivated species are known; this has now been reduced to about 5 000 because of duplicate identification (Thurston, 1980; Mendoza, 1983; Huaman 1986). In 1984 the International Potato Center distributed more than half a million units – clones tuber families, in vitro plantlets and true potato seed – to 51 countries. All export material was tested for freedom from 12 different viruses and a viroid as well as from other pathogens and pests. The pathogen-free in vitro collection has been increased to include 214 varieties, advanced clones, primitive cultivars and wild species (Anonymous, 1985).

The second most important country richest in tuber-bearing species after Peru is Bolivia. In spite of this, in the present collections Bolivia plays a sub-

[1] Dedicated to the late Dr. K. Schmelzer on the occasion of his 60th birthday.

ordinate role, although its genetic material has long since been known to be important from the point of view of breeding (van Soest, 1980; van Soest et al., 1980; Hondelmann and van Soest, 1980; Hawkes and Hjerting, 1989). It is due to these expeditions that many known wild *Solanum* species have been collected and examined for resistance, and so far unknown *Solanum* species identified and studied (van Soest and Hondelmann, 1983; van Soest, 1983).

The wild *Solanum* species of Mexico and adjacent countries are also interesting to the potato breeder with their wide range of genes conferring resistance to many potato diseases. According to Hawkes (1966) 32 species are recognized for this area, 29 occur in Mexico, 5 in Guatemala, 3 in the USA and Costa Rica, 2 in Panamá and one in Honduras. Mexico is therefore an important centre of variability of this group, and is probably also their centre of origin (Hawkes, 1966).

Systematic maintenance of *Solanum* germplasm in sexually propagated form has been achieved in a number of countries. The United States Potato Collection, known as the Inter-Regional Potato Project or IR-1, maintained at Sturgeon Bay, Wisconsin, is the most extensive one consisting of about 3000 accessions (see Rowe, 1966). Of extremely high importance is the German-Netherland potato collection at Braunschweig-Völkenrode (German Federal Republic), which includes some 2900 accessions of 91 wild *Solanum* species and 5 primitive cultivars (van Soest, 1983).

Another important collection is the *Solanaceae* gene bank of the Birmingham University (Birmingham, England), which contains a special collection of *Solanum* species (R. N. Lester, personal communication). In the course of studies on some *Solanum* species of this gene centre it was found that e.g. *Solanum hermanni* (BIRM/S.0210) was immune of potato virus Y (Horváth, 1984b, Horváth et al., 1988b).

In addition there are yet existing three large collections today: Commonwealth Potato Collection (CPC) at the Scottish Crop Research Institute in Pentlandfield near Edinburgh (Scotland), Argentine Potato Collection of the Instituto Nacional de Technologia Agropecuaria (INTA), Balcarce (Argentina), Chilean Potato Genebank in Valdivia (Chile).

Some years ago, Burton (1966) predicted "The future will see greatly expanded programs directed toward the collection, biosystematic study, and preservation of germplasm. World collections will be increased in size. Wild progenitors and distantly related species will be added to these collections, and germplasm collections of all crops, including so-called minor ones, will be made."

In the gene centres the occurrence of native pathogens (e.g. viruses) is extremely wide and diversified. In consequence of a very strong selective pressure of various pathogens acting on plants found for a long time in the gene centres resistant plants have come into existence (Leppik, 1968, 1970; Knott and Dvorák, 1976; Sprague, 1980). As an example of how plants become reistant, the importance of a geographic relation between potatoes and the fungal pathogen *Phytophthora infestans* could only develop in the gene centre of the fungal pathogen

(Central-Mexico; Niederhauser and Cobb, 1959; Niederhauser et al., 1954) while the cultivated potato grown in the Andes is highly susceptible to the fungus.

Owing to the diverse biological characteristics of the viruses the relations between *Solanum* species in gene centres or other alternative hosts and viruses are different from other host-parasite relations. The host-range of viruses is known to cover almost all living organisms (Gibbs and Harrison, 1976; Horváth, 1977a, 1982b). Nevertheless, it should be emphasized that the host species to which the viruses cause severe diseases are smaller in number then those which they infect without series consequences. In other words, the plant viruses are pathogens not always dangerous for the systems they are able to infect. This is the reason why owing to the diversity of host-virus relations the plant viruses occurring in the gene centres may for a long time show a harmonious coexistence with plants (being latent they cannot even be recognised), then in the case of a favourable change of host or vector, or through satellitism or mutation they suddenly become dangerous pathogens. Further, the above facts explain why the gene centres are sources not only of resistance genes but of pathogens (e.g. viruses) too. Thus, the genetic bases of the gene centres possess both favourable characteristics, such as resistance, and susceptibility to pathogens.

Investigations concerning the genetic bases of potato from the 19th century up to now can be divided into three phases (Ross, 1978). The botanical phase (*a*) consisted of the hybridization of wild *Solanum demissum* species and cultivated potatoes for botanical purposes. The *Phytophthora* phase (*b*) began in the 1890s, and covered, in essentials, investigations related with the *Phytophthora* resistance of *Solanum demissum*. As it is known, the *Phytophthora* resistance of the current potato varieties is genetically traced back to the *Solanum demissum* hybrids. The virological phase (*c*) started in the 1930s, following the expeditions of Vavilov and his fellow researchers (1916–1931) to South-America and Mexico, the gene centre of potato. The about 250 thousand wild- and cultivated plant accessions (Leppik, 1969; Smith, 1969) collected by Vavilov's expeditions up to 1940 are of high scientific importance, though a large part of them were unfortunately destroyed during World War II. Vavilov's pioneer expeditions were followed by German, American, Swedish, English and Dutch expeditions. It is due to them that the number of the wild *Solanum* species collected exceeds today two thousand, and some 200 tuberous *Solanum* can also be found among them (Vavilov, 1926, 1928; Schick, 1931; Bukasov, 1933, 1936; Juzepczuk and Bukasov, 1936; Balls, 1940; Hawkes, 1941, 1944, 1947, 1956, 1958a; Hammarlund, 1943; Ochoa, 1955, 1980a,b, 1981, 1984a,b; Correll, 1952, 1962; Toxopeus, 1956; Cockerham et al., 1958; Rudorf, 1959; Ross, 1960a; Rothacker, 1961; Hawkes et al., 1979; van Soest, 1980, 1983; van Soest et al., 1980; Hondelmann and van Soest, 1980; van Soest and Hondelmann, 1983). The same expeditions deserve credit for the fundamental knowledge we now possess of the resistance genes occurring in the wild *Solanum* species of the gene centres (Ross and Köhler, 1953; Ross, 1957, 1958a, b, c, 1960a, b, c, 1961, 1962; Ross and Rowe, 1965, 1966; Cockerham, 1970; Russel, 1978; Hooker, 1978; Radcliffe et al., 1981; Jones, 1982a, b). There is one more thing these

expeditions should be thanked for. According to Hawkes (1958a) they opened a new era in the history of potato breeding. In the opinion of Ross (1978) the expeditions while widening the genetic basis of potato had a dramatic effect. It is evident the establishment of germplasm collections carries with it the risk of long distance dissemination of viruses that may be harbored in or on the plant material that is transported. The growing development of worldwide exchanges involves an important risk of worldwide spread of a great number of viruses (see Stace-Smith, 1985).

Problems of breeding for resistance

Though the intensive work of plant breeding and virus resistance breeding has produced undeniable successes there are still many fundamental problems that make the research work difficult. The most important of them is perhaps the appearance of new host-virus relations. The history of plant breeding gives evidence of once existing varieties and local forms grown for a long time without any systematic breeding- or maintenance work, until with a more intensive plant breeding work started after the 1940s – which consisted of (*a*) seeking out resistant initial forms, (*b*) determining the types of resistance, (*c*) clearing the question of the transmissibility and inheritance of resistance genes, and (*d*) producing resistant varieties – an unfavourable process began. First of all, it should be emphasized that plants and viruses have to meet two requirements totally opposed from the point of view of genetic diversity. According to the demand of breeding raised on the plant varieties maximum yield must be ensured in a homogenous stand. This is naturally a very difficult task, since plant breeding can only achieve its aim with a considerable genetic heterogeneity present. To homogenous plant varieties, on the other hand, various viruses may become specialized, which sooner or later renders it necessary to produce new homogenous and virus resistant varieties. And this again has the precondition of high diversity plant species to be found in the gene centres which meet the new requirements. For this very reason the gene centres, the natural gene banks as "irreplaceable resources" (Vida, 1979) are very important from the point of view of genetic diversity and resistance (Sprague, 1980; Mooney, 1981).

Solanum species of high genetic importance

The expeditions to South-America and Mexico started in the second half of the 1910s had a great impact on the work of potato breeding. The expeditions had the aim of collecting wild *Solanum* species to serve as the basis of potato breeding. Characteristically of the extensive work related with the collection and systematization of *Solanum* species the number of species known at present is above two thousand, while at the beginning of the century this number was only 30. In our present knowledge more than hundred wild *Solanum* species are now

known to cross with potato *(Solanum tuberosum)*, and with nearly half of them the conditions of resistance are also known.

According to Hawkes (1963) the potatoes native in the Andes, the gene centre for potato, can be traced back to four diploid *(Solanum ajanhuiri, S. goniocalyx, S. phureja, S. stenotonum)*, two triploid *(S. caucha, S. juzepczuki)*, one tetraploid *(S. tuberosum* ssp. *andigena)* and one pentaploid *(S. curtilobum)* species. The current potato varieties in Europe (England, Holland, German Democratic Republic, German Federal Republic, Soviet Union) have developed genetically from the following eight major wild *Solanum* species: *Solanum demissum, S. tuberosum* spp. *andigena, S. acaule, S. tuberosum* spp. *tuberosum* (Chile), *S. spegazzini, S. stoloniferum, S. vernei, S. phureja* (Ross, 1978). *Solanum demissum* and *S. tuberosum* ssp. *andigena* are undoubtedly the most important of them, as they have a share of 42 and 31 per cent, respectively, in the varieties. In the crossing of wild species *Solanum demissum* × *S. tuberosum* ssp. *andigena* lead with 99 per cent (Ross, 1978). More than 83% of cultivars of the Federal Republic of Germany carry genes from *Solanum demissum* and 26% genes of other wild species, almost always in addition to those from *Solanum demissum* (Ross, 1986). An examination of the genetic bases of the European potato varieties for virus susceptibility and virus resistance reveals a very strong affinity between the species and the best known viruses, such as e.g. potato leaf roll virus, potato virus X,

Table 1

Virus susceptibility and virus resistance of the genetic basis[1]

Solanum species	Susceptibility	Resistance to infection	Hypersensitive reaction	Immunity
Solanum acaule	PLRV, PVY, PVA, PVX	PLRV	PVX, PVY, ToMV	PVX
S. demissum	PLRV, PVX	PLRV, PVY	PVA, PVX, PVY, PVV	
S. phureja[2]			PVY	
S. spegazzinii[3]	PVM			
S. stoloniferum	PLRV, PVX		PVY, PVA	PVY, PVA
S. tuberosum ssp. *andigena*	PVA, PVX, PVS		PVS	PVX, PVY
S. tuberosum ssp. *tuberosum*	PVV			PVX
S. vernei	PLRV, PVX	PVY	PVY, TMV	PVY, PVA

[1] PLRV: potato leafroll virus, PVA: potato virus A, PVM: potato virus M, PVS: potato virus S, PVX: potato virus X, PVY: potato virus Y, PVV: potato virus V, TMV: tobacco mosaic virus, ToMV: tomato mosaic virus

[2] Resistant to *Pseudomonas solanacearum*

[3] Resistant to *Globodera rostochiensis, Fusarium coeruleum* and *Streptomyces scabies*

potato virus M (Table 1). From the point of view of virus resistance those species are naturally the most important (e.g. *Solanum acaule*, *S. stoloniferum*, *S. tuberosum* ssp. *andigena*, *S. tuberosum* ssp. *tuberosum*, *S. vernei)* in which so-called immunity genes occur (Easton et al., 1958; Hawkes, 1958b; Ross, 1960b, c, 1961, 1966, 1978, 1986; Horváth, 1968a, 1976; Cockerham, 1970; Howard, 1970; Munoz et al., 1975 etc.). Recently a major dominant gene for resistance to potato virus M was found in the species *Solanum gourlayi* (Świezyński et al., 1981). Among the genetic donors listed in Table 1 there are two *Solanum* species which – so to say – are of not much use for virology. They are: *Solanum phureja* and *S. spegazzinii*. The reason why these species were included in the genetic basis of the European potato varieties is that *Solanum phureja* was found to be resistant to *Pseudomonas solanacearum*, while *S. spegazzinii* showed resistance to *Fusarium coeruleum*, *Streptomyces scabies* and *Globodera rostochiensis* (Ross, 1962; Rowe and Sequeira, 1972).

Wild *Solanum* species and viruses

Besides the virus resistant gene sources there are such viruses and virus strains in the gene centres of plants whose presence has created new tasks not only for the plant breeding programmes but for the plant virus research too. We know a number of virus, viroid and virus-like diseases can be transmitted in the course of breeding plants, so that the viruses are incorporated into the breeding stock with which the breeder is working (Weintraub, 1985).

Due to an extensive research work in the recent years many known and so far unknown viruses and viroids have been detected in the genetic bases, which – and it should be emphasized – are the bases of the European potato varieties as well (Table 2). Remarkable are the results of the experiments in which the *Solanum* species proved to be not only natural hosts for those viruses, but also susceptible to artificial infection by many further viruses occurring in the gene centres of potatoes, whereby these *Solanum* species are at the same time prognostic virus hosts too (see Table 2). Recently the henbane mosaic virus has been found to be pathogenic for wild *Solanum* species and potato plants (Horváth et al., 1987, 1988a). It should be mentioned as an example the now established fact that the potato leafroll virus has been introduced from Mexico to the United States of America with *Solanum brachycarpum*, the potato virus A with *S. bulbocastanum* and the potato virus X with *S. brachycarpum*, *S. bulbocastanum*, *S. sambucinum* and *S. stoloniferum* plants (Kahn et al., 1963). Highly remarkable are those research results which pointed out that 66 per cent of 551 accessions of cultivated tuberous *Solanum* species collected in eight South- and Central American countries (Columbia, Ecuador, Peru, Bolivia, Chile, Mexico, Guatemala, Haiti) and introduced to North-America were infected by viruses (Kahn and Monroe, 1970). Considering that the indicator plant tests have only covered mechanically transmissible and known viruses, the virus infection is supposed

Table 2

Virus and viroid susceptibility of the genetic basis[1]

Solanum species	Naturally occurring pathogens	Artificially transmissible pathogens	Literature
Solanum acaule	PSTV	CMV, TMV, ToMV	Diener and Raymer (1967), Horváth (1968*b*), Schmelzer and Spaar (1975)
S. demissum	PVT	APLV, APMV, CMV, TMV, ToMV, PepMV, PVV	Horváth (1968*b*), Schmelzer and Spaar (1975), Salazar (1977*b*), Fribourg et al. (1977*a,b*), Jones and Fribourg (1978), Jones et al. (1980), Fribourg and Nakashima (1984)
S. phureja		CMV	Schmelzer and Spaar (1975)
S. spegazzini		PVM	Horváth (1982*a*)
S. stoloniferum	PSTV, PVT	APLV, CMV, PepMV, PTV	Schmelzer and Spaar (1975), Salazar (1977*b*), Salazar and Harrison (1978*c*), Jones and Fribourg (1978), Diener (1979), Fribourg (1979), Jones et al. (1980)
S. tuberosum ssp. *andigena*	PBRV, PMTV, PVX-HB, PVT, TRSV-APCS, PVV	APLV, APMV, CMV TRSV-APCS, PTV	Schmelzer and Spaar (1975), Salazar and Jones (1975), Fribourg (1977), Fribourg et al. (1977*a,b*), Salazar and Harrison (1979), Fribourg (1979), Moreira et al. (1980), Fribourg and Nakashima (1984)
S. tuberosum ssp. *tuberosum*	PBRV, PMTV, PVT, TRSV-APCS	APLV, PBRV, PTV, PSTV, TRSV-APCS TPMV, PVV	Jones and Harrison (1972), Salazar and Harrison (1977), Fribourg (1977), Fribourg et al. (1977*a*), Salazar and Harrison (1979), Diener (1979), Fribourg (1979), Jones et al. (1980), Galindo et al. (1982) Fribourg and Nakashima (1984)
S. vernei		CMV, PSTV, PVX-HB, TMV, ToMV, WPMV	Horváth (1968*b*), Schmelzer and Spaar (1975), Diener (1979), Jones and Fribourg (1979), Moreira et al. (1980)

[1] APLV: Andean potato latent virus, APMV: Andean potato mottle virus, CMV: cucumber mosaic virus, PBRV: potato black ring virus, PepMV: pepino mosaic virus, PMTV: potato mop-top virus, PSTV: potato spindle tuber viroid, PTV: Peru tomato virus, PVX-HB: resistance-breaking strain of potato virus X, PVT: potato virus T, PVV: potato virus V, TMV: tobacco mosaic virus, ToMV: tomato mosaic virus, TPMV: tomato planta macho viroid, TRSV-APCS: Andean potato calico strain of tobacco ringspot virus, WPMV: wild potato mosaic virus

to be actually much more serious than that. In the course of examinations the cultivated *Solanum* plants generally showed higher virus infections than the wild *Solanum* species, but – in a particularly remarkable way – the necrotic strain of potato virus Y could be more frequently detected in the wild *Solanum* species than in the cultivated *S. tuberosum* plants. It should be mentioned here that the strain of potato virus Y which has recently appeared in Hungary and causes the so-called tuber necrotic ringspot disease shows frequent occurrence in various potato varieties coming from abroad (Beczner et al., 1984). Attention should be called to the risks involved with the introduction of the exotic potato viruses and viroid in Europe (see Howell, 1981). Of these viruses those are of extremely great importance which spread by true seed transmission (e.g. Andean potato latent virus, potato black ringspot virus, potato virus T, Andean potato mottle virus, *Arracacha* virus B, tomato black ring virus, potato virus U, tobacco streak virus, pepino mosaic virus, potato yellow vein virus, Andean potato calico strain of tobacco ringspot virus, potato spindle tuber viroid). The above research results make one consider that the genetic bases bear not only favourable features, such as resistance, but show unfavourable susceptibility to pathogens as well. For this very reason an unconsidered use of the genetic bases may lead to a dramatic situation in breeding.

Three serious consequences of earlier breeding activities should be mentioned here, although it is not sure whether or not the European potato breeding built on the genetic basis of the gene centres has actually more virological problems to overcome.

The first example is the introduction of the tobacco veinal necrosis strain of potato virus Y from South-America to Europe with wild *Solanum* species (Klinkowski and Schmelzer, 1957, 1960; Ross, 1959; Horváth, 1967, 1969; Brücher, 1969). The new virus strain having got into Europe infected the earlier resistant varieties, and actually destroyed the highly virus resistant variety *Ackersegen*. The virus strain occurs at present all over Europe and causes extremely serious damages (Horváth, 1967; Oertel et al., 1980; De Bokx and Huttinga, 1981).

The second example is the introduction of the tobacco mosaic virus with the species *Solanum commersonii* (Hansen, 1960). It is supposedly due to this species that the tobacco mosaic virus, and the tomato mosaic virus respectively, can be detected in India and Hungary under natural conditions in potatoes (Phatak and Verma, 1967; Horváth, 1977*b*; Juretić et al., 1977; Horváth et al., 1978). During the detailed examination of the genetic bases of potato it was first in our experiments that a very strong affinity between wild *Solanum* species and tobacco mosaic virus was demonstrated (Horváth, 1968*b*). Many latent host-virus relations as well as the transmissibility of tobacco mosaic virus with tuberous *Solanum* species deserve special attention. In artificial inoculation experiments Boyle (1969) found that not only the tobacco mosaic virus – which with potato tuber was transmissible even after six tuber generations – but the tomato mosaic virus too caused systemic infection to potatoes.

The third example concerns the relation of potato and cucumber mosaic virus. The earlier described spontaneous host-virus relation between potato and cucumber mosaic virus (MacArthur, 1958) became clear and evident when in the course of artificially infecting 200 accessions of 46 *Solanum* species Schmelzer and Spaar (1975) found the species examined to be equally susceptible to the virus. It is not surprising that *Solanum acaule* and *S. demissum*, the two best known genetic bases proved to be the most cucumber mosaic virus susceptible species (Schmelzer and Spaar, 1975). The cucumber mosaic virus susceptibility of the potato varieties known at present can in all probability be traced back to the above two *Solanum* species as genetic basic material. Among the most recent research results concerning the potato and cucumber mosaic virus relations highly remarkable is Bode's (1975) statement, namely, that in the European potato varieties a deviating cucumber mosaic virus showing new characteristics has appeared. The biologically and serologically different strains of the cosmopolitan cucumber mosaic virus – which has spread all over the world through some 60 aphid species, and its range of host covers more than thousand plant species (Horváth, 1972*b*, 1976, 1979, 1980*a*; Douine et al., 1979; Francki et al., 1979) – as well as the virulence-increasing satellite ribonucleic acid pointed out in it (Gould et al., 1978; Kaper and Tousignant, 1978; Mossop and Francki, 1978) call attention to the growing importance of the virus. In a work by Bagnall (1977) the following is written of the cucumber mosaic virus: "CMV (cucumber mosaic virus) is a problem that potato people do not want – and, apparently, neither does the potato!". During the 1984--1985 season, Somerville et al. (1987) identified the natural infection of potato cultivars *White Rose* and *Red La Soda* by a legume strain of cucumber mosaic virus in California.

Table 3

Occurring of different viruses in wild *Solanum* species
(After Bode, 1977)

Viruses[1]	Occurrence (%)
APLV	18.7–21.6
APMV	8.7–9.5
PLRV	5.8
PVY	49.0
PVX	61.0
PVM	3.9
PVS	40.2
Virus free plants	4.6

[1] APLV: Andean potato latent virus, APMV: Andean pota to mottle virus, PLRV: potato leafroll virus, PVY: potato virus Y, PVX: potato virus X, PVM: potato virus M, PVS: potato virus S

Considering that apart from a few exceptions (Silberschmidt, 1954, 1960; Pontis and Feldman, 1963; McKee, 1964; De la Torre, 1966; Mendoza, 1967) hardly any research activity concerning the occurrence of viruses had earlier been carried on in the gene centres, it is not surprising that investigations on viruses occurring in the gene centres necessarily were started in countries outside the centres. The wild *Solanum* species of Central- and South-America were first examined for viruses between 1939 and 1944 in Germany (Stelzner, 1950). Subsequent studies by Ross and Baerecke (1950, 1951), Ross (1957, 1958*c*), Rothacker and Witt (1959), Kahn et al. (1963, 1967), Horváth (1968*a*, *b*, 1984*a*, *b*, 1983, 1985), Kahn and Monroe (1970), Schmelzer and Spaar (1975), Schmelzer (1976), Koenig and Bode (1977, 1978), Kahn et al. (1979) and Horváth et al. (1987, 1988*a*, *b*) have supplied important data concerning the responses of various *Solanum* species to viruses. These prodromal examinations were completed by Bode (1977), Koenig and Bode (1977, 1978) and Koenig et al. (1979) who found some further viruses, e.g. Andean potato virus, Andean potato mottle virus, to occur in the South-American wild *Solanum* species (Table 3). It is still more interesting that in the great bulk of plant material examined only 4.6 per cent virus-free plants were found. No wonder then, that the genetic bases – as basic material for breeding, genetic donors – represent a great danger in the work of breeding for virus resistance.

From plant virological and genetic points of view great progress was made with the establishment of the International Potato Center in Lima (Peru), the gene centre of potato, in 1972, where with an excellent professional guidance and co-operation the occurrence of various virus pathogens and many surprising new host-virus relations have been revealed (Fribourg and Salazar, 1972; Salazar and Jones, 1975; Jones, 1975, 1981*a*; Fribourg, 1977, 1979, 1980; Salazar and Harrison, 1977; Jones and Kenten, 1978; Jones and Fribourg, 1979; Jones et al., 1980; Jones and Kenten, 1981). In the genetic bases and potato varieties many viruses, viroids and mycoplasmas have been detected (see Table 4; Gibbs et al., 1966; Calvert, 1968; Harrison and Jones, 1970; Bode, 1975; Salazar and Jones, 1975; Salazar and Harrison, 1977, 1978*a*, *b*; Fribourg, 1977; Jones and Fribourg, 1978; Rodriguez and Jones, 1978; Jones and Kenten, 1981).

In the diploid, triploid and tetraploid groups of *Solanum tuberosum* and in the species *S. verrucosum* Andean potato latent virus was pointed out. Many potato varieties showed infections by Andean potato mottle virus, potato black ringspot virus and potato mop-top virus. Infection by potato mop-top virus was also established in the case of *Solanum curtilobum* and *S. juzepczuki*. Out of the new viruses attention is called to the so-called *Oxalis* strain of the recently identified *Arracacha* virus B, which has remained so far unnoticed by the researchers because the *Chenopodium murale* as a systemic virus diagnostical plant has not been included in the identification list of potato pathogenic viruses. This example is to call attention to the importance of diagnostical plants on the one hand, and to the role played in the circulation of the virus by the *Oxalis tuberosa (Oxalidaceae)*, the alternative host of the virus occurring in the Andes of Peru (Jones,

1981*b*), on the other hand. According to the latest literary data in Bolivia a mycoplasma was also found in the phloem cells of the *Oxalis tuberosa* plant (Atkey and Brunt, 1982).

A survey of the reports related with the spread of the viruses mentioned (Table 4) will show that some of them are transmissible by aphids, beetles, leaf hoppers, nematodes, whiteflies, and fungus vectors as well as by mechanically, true seeds and tubers. These manners of transmission and spreading are of very great importance and dangers of virus transmission by seed and tuber in the international trade of genetic basic materials and in breeding work must be specially emphasized (Benson and Singh, 1964; Gibbs et al., 1966; Kahn et al., 1967, 1979; Lister and Murant, 1967; Horváth, 1968*c*, 1972*b*; Kahn and Monroe, 1970; Bos, 1977; Salazar and Harrison, 1977; Kaiser et al., 1978; Mandahar, 1981; Salazar et al., 1981; Waterworth and White, 1981).

Out of the recently identified viruses and viroids no less importance should be attached to the potato spindle tuber viroid, potato virus S, the resistance-breaking strain of potato virus X, the deviant strain of potato virus Y, the aphid transmitted strain of potato virus Y^C tomato black ring virus, potato virus U, potato virus V, tobacco streak virus etc. (see Table 4; Phatak and Verma, 1967; Diener and Raymer, 1967; Smith, 1972; Hinostroza-Orihuela, 1973; Fribourg, 1977, 1980; Horváth, 1977*b*; Juretić et al., 1977; Salazar, 1977*b*; Horváth et al., 1978; Kaiser et al., 1978; Salazar and Harrisons, 1978*b*, *c*; Diener, 1979; Jones and Fribourg, 1979; Calvert et al., 1980; Thompson et al., 1987; Dolby and Jones, 1987, 1988, etc.). Out of them the resistance-breaking strain of potato virus X deserves particular attention, since it was found to infect all *Solanum* species, hibrids and varieties so far known to be immune of the virus *(Solanum acaule* P. I. 230554, USDA 41956, 44/1016/10, G. 4298.69; Moreira, 1978; Moreira et al., 1978*a*, *b*, 1980; Jones, 1982*a*, 1985). The so-called resistance-breaking strains seldom occur in plant virology. The potato virus X, the strawberry ringspot virus and the tobacco mosaic virus are exceptions. The resistance-breaking faculty is known to be inconsistent with characteristics related with the transmission and survival of the virus. In spite of this, in the course of breeding for virus resistance the appearance of so-called resistance-breaking strains must be reckoned with.

Some years ago in Peru a new virus identified as a member of the potexvirus group was found in the *Solanum muricatum* plant (Jones et al., 1980). It can be transmitted to many wild *Solanum* species some of which are important as genetic donors as well (e.g. *Solanum demissum*, *S. stoloniferum)*. Considering that the *Solanum muricatum*, a plant of vegetative reproduction in the subtropical regions of Central- and South-America, plays an important role in human nutrition, the pepino mosaic virus isolated from it represents a considerable danger not only for the potato but also for various *Solanum* species as genetic bases. Its importance is increased by the fact that it is transmissible and spreads by potato tuber. The latter viruses are also wide-spread. Some further ways of spreading are: the transmission of the potato spindle tuber viroid by pollen, of the potato yellow vein virus by whiteflies, of the potato virus U and tobacco ringspot

Table 4

Newly detected viruses, virus strains, viroid and mycoplasma in wild *Solanum* species, potato cultivars and their transmission modes

Viruses, viroid and mycoplasma[1]	*Solanum* species and potato cultivars	Mode of transmission[2]	Literature
Viruses:			
AMV	Different potato cultivars (e.g. *Bolona*, *Maria Tropical*, *Red La Soda*, *Somogyi Kifli*)	A, Me, S, T	Cervantes and Larson (1961), McLeod (1962), Horváth (1980*b*), Anonymous (1985)
APLV	Different potato cultivars (e.g. *Participation*, *Ranrahirca*, *Revolucion*)	B, Me, S, T	Gibbs et al. (1966), Jones and Fribourg (1977), Fribourg et al. (1977*a*), Jones and Fribourg (1978)
APMV	Different potato cultivars (e.g. *Delta*, *Renacimiento*, *Chunqui*, *Revolucion*)	B?, Me, T	Fribourg et al. (1977*b*), Salazar and Harrison (1978*d*), Fribourg et al. (1979), Availa et al. (1984)
ArVB-O	Potato cultivars (e.g. *Cara*, *Mi Peru*)	P, S, T	Jones and Kenten (1978, 1981), Kenten and Jones (1979), Jones (1982*b*)
CMV	Different potato cultivars (e.g. *Hansa*, *Maryke*, *Sieglinde*, *White Rose*, *Red La Soda*)	A, Me, T	Bode (1975), Sangar and Agrawal (1986), Somerville et al. (1987)
MATV	Potato cultivars (e.g. Kennbec, *Sebago*)	WF	Debrot (1981)
PepMV	*Solanum muricatum*	Me, T	Jones et al. (1980)
PBRV	*Solanum tuberosum* ssp. *tuberosum* x *Solanum tuberosum* ssp. *andigena* cv. *Atarqui*	Me, T	Salazar and Harrison (1977, 1979)
PMTV	*Solanum tuberosum* ssp. *tuberosum*, *Solanum tuberosum* ssp. *andigena*, *Solanum curtilobum*, *Solanum Juzepczuki*	F, Me, T	Calvert and Harrison (1966), Calvert (1968), Jones and Harrison (1969), Harrison and Jones (1970), Jones (1970), Jones and Harrison (1972) Hinostroza-Orihuela and French (1972), Cooper and Harrison (1973), Harrison (1974), Jones (1975), Salazar and Jones (1975)
PV14R	*Solanum tuberosum* ssp. *andigena* x ssp. *tuberosum* cv. *Aleli*	Me	Salazar (1977*a*)
PVS	Potato cultivars (e.g. *Montaro*, *Ocolla*)	A, Me, T	Hinostroza-Orihuela (1973), Rose (1983), Slack (1983), Kobayashi et al. (1985), Dolby and Jones (1987, 1988)
PVT	*Solanum tuberosum* ssp. *tuberosum* x *Solanum tuberosum* ssp. *andigena* cv. *Antarqui*	Me, P, S, T	Salazar and Harrison (1977), Salazar (1977*b*), Salazar and Harrison (1978*c*), Jones (1982*b*)
PVU	Potato cultivar (unknown)	Me, N, S	Jones et al. (1983)

Table 4 (continued)

Viruses, viroid and mycoplasma[1]	*Solanum* species and potato cultivars	Mode of transmission[2]	Literature
PVV	*Solanum tuberosum* ssp. *andigena*	A, Me	Fribourg and Nakashima (1984) Jones and Fuller (1984)
PVX-HB	*Solanum tuberosum* ssp. *andigena* cv. *Suta*	Me, T	Moreira (1978), Moreira et al. (1978*a*, *b*, 1980), Jones (1982*a*, 1985), Adams et al. (1984)
	Solanum tuberosum cultivars (e.g. *Cara*, *Pentland Dell*)		
PYVV	*Solanum andigenum*, *Solanum tuberosom* cvs.	Me, T, WF	Smith (1972), Fribourg (1980), Anonymous (1988)
SB-22V	*Solanum tuberosum* cvs.	S	Anonymous (1988)
TMV	*Solanum commersonii*	Me, T	Hansen (1960), Phatak and Verma (1967)
TRSV-APCS	*Solanum tuberosum* ssp. *tuberosum x Solanum tuberosum* ssp. *andigena* cv. *Ticahuasi*	Me, N, S, T	Fribourg (1977), Jones (1982*b*)
	Potato cultivar *(Cara)*		
	Potato clone [*G* 5998 (6)]		
TSV	*Solanum phureja* x *Solanum tuberosum*	Me, S, T	Salazar et al. (1981)
TBRV	Potato cultivar *(Anett)*	Me, S	Kaiser et al. (1978)
ToMV	Potato cultivar *(Astilla)*	Me, S, T	Horváth et al. (1978), Juretić et al. (1977), Horváth (1977*b*)
ToYMV	*Solanum tuberosum* cv. *Sebago*	WF	Debrot and Centeno (1985)
WPMV	*Solanum chancayense*	A, Me	Jones and Fribourg (1978)
Viroid:			
PSTV	Different potato cultivars (e.g. *Irish Cobbler*)	Me, P, S, T	Diener and Raymer (1967), Diener (1979), Hunter et al. (1969), Singh (1970), Fernow et al. (1970), Főglein and Nechay (1982), Jones (1983)
	Breeding lines		
Mycoplasma:			
AYM	Different potato cultivars	Le	Hooker (1981), Jones (1983)

[1] AMV: alfalfa mosaic virus, APLV: Andean potato latent virus, APMV: Andean potato mottle virus ArVB-O: *Oxalis* strain of *Arracacha* virus B, CMV: cucumber mosaic virus, MATV: Mosaico amarilo tomato virus, PepMV: pepino mosaic virus, PBRV: potato black ringspot virus, PMTV: potato mop-top virus, PV14R: potato 14R virus (similar to a tobamovirus), PVS: potato virus S, PVT: potato virus T, PVU: potato virus U, PVV: potato virus V, PVX-HB: resistance breaking strain of potato virus X, PYVV: potato yellow vein virus, SB-22V: SB-22 virus, TMV: tobacco mosaic virus, TRSV-APCS: Andean potato calico strain of tobacco ringspot virus, TSV: tobacco streak virus, TBRV: tomato black ring virus, ToMV: tomato mosaic virus, ToYMV: tomato yellow mosaic virus, WPMV: wild potato mosaic virus, PSTV: potato spindle tuber viroid, AYM: *Aster* yellows mycoplasma

[2] A: aphids, B: beetles, F: fungus, Le: leaf hoppers, Me: mechanically, N: nematodes, P: pollen, S: true seeds, T: tubers, WF: whiteflies

virus by nematodes (see Table 4; Hunter et al., 1969; Fernow et al., 1970; Singh, 1970; Smith, 1972; Fribourg, 1977, 1980; Jones et al., 1983). Similarly important is the tomato planta macho viroid, a pathogen of tomato in Mexico, which is artificially transmissible to *Solanum melongena* and *S. tuberosum* plants. The danger of infection is increased by the fact that in potato the viroid is latent (Belalcazar and Galindo, 1974; Galindo et al., 1982).

We think that the examples shown and the literary data listed duly emphasize the importance of the genetic bases occurring in the gene centres, as well as the problems encountered when using them in breeding for virus resistance. We hope that we have been able to make a useful contribution to the latest comprehensive works on gene centres (Sowell, 1967; Leppik, 1968, 1970; Knott and Dvořák, 1976; Sprague, 1980) which have so far been discussed in the world literatue mainly as sources of disease resistance – by pointing out that the plants found in the gene centres carry not only resistance genes but also pathogens, including viruses, which play an important and dangerous role in the international trade and use of plant materials (Bos, 1977; Hewitt and Chiarappa, 1977; Shephard, 1977).

Wild *Solanum* species and aphid vectors of viruses

The wild *Solanum* species as sources of resistance play a highly important role not only in breeding for virus resistance, they are of priceless value in resisting to the aphids which transmit the viruses. The first research results related with the aphid resistance of the *Solanum* species are dated from the 1940s, when Cockerham (1943), Howey and Simpson (1944) and Adams (1946) pointed out the resistance (immunity) of *Solanum polyadenium* to the green peach aphid *(Myzus persicae)*. The resistance was attributed to the action of an "oil" that accumulated on the tarsi of aphids (Stringer, 1946). Much later it was determined that grandular hairs on the surface of *Solanum polyadenium* were the source of an adhesive exudate that restricted aphid mobility (see Gibson, 1971*a*). The discovery of an aphid resistance in *Solanum stoloniferum* is of particular importance (see Ross and Rowe, 1965). Resistance to *Myzus persicae* in *Solanum antipoviczii*, *S. ajuscoense* and *S. neoantipoviczi* was first pointed out by Adams (1946) though it is known that the three species names are synonyms for *Solanum stoloniferum*. According to the recent results of investigations the *Solanum stoloniferum* is resistant to *Myzus persicae*, potato aphid *(Macrosiphum euphorbiae)* and foxglove aphid *(Dysaulacorthum antirrhinii)*, but susceptible to *Aphis fabae* (Gibson, 1971*b*). An extensive field survey of the Potato Introduction Station collection for aphid resistance was first undertaken by Radcliffe and Lauer (1966, 1968, 1970, 1971*a*, *b*). The highest levels of resistance to *Myzus persicae* were found in certain diploid species of Mexican origin including *Solanum bulbocastanum*, *S. brachistotrichum*, *S. michoacanum* and *S. trifidum*. Resistance to *Macrosiphum euphorbiae* was characteristic of *Solanum bulbocastanum* and of the Mexican tetraploid *Solanum hjertingii*, *S. polytrichon* and *S. stoloniferum*. The

aphid resistance of *Solanum stoloniferum* is very important, since this species is one of those most important in the programme of breeding for virus resistance (cf. Ross, 1966). Of the latest research results particularly valuable are those of Radcliffe et al. (1981) who studied the resistance of 1600 tuber-bearing *Solanum* entries to *Myzus persicae* and 1218 *Solanum* entries to *Macrosiphum euphorbiae* under field conditions at the University of Minnesota (USA) between 1966 and 1979. They found 34 *Solanum* entries highly resistant, 100 resistant, 307 intermediate, 520 susceptibe and 639 highly susceptible to *Myzus persicae;* as for *Macrosiphum euphorbiae*, 72 *Solanum* entries were rated highly resistant, 151 resistant, 366 intermediate, 416 susceptible and 213 highly susceptible (Radcliffe et al., 1981). Since the experiments of Hermsen and Taylor (1979) – in which non-tuberous species were successfully crossed with tuber-bearing ones – the importance of *Solanum brevidens*, a non-tuberous species being resistant to *Myzus persicae* has grown (Gibson, cit. Jones, 1979). Recent potato breeding experiments have produced aphid-resistant interspecific hybrids of *Solanum berthaultii* × *Solanum tuberosum* bearing both types of grandular trichomes (type A and B), there are associated with aphid resistance in wild *Solanum* species (Tingey et al., 1982; Wright et al., 1985). In the latest experiments of greenhouse and field studies *Lapointe* et al. (1987) reported that potato virus Y transmission was reduced in the aphid-resistant species, *Solanum berthaultii.*

Out of the more than 30 potato viruses known so far about 12 viruses are transmissible by aphids (see Table 4), therefore the aphid resistant wild *Solanum* species are of extremely great importance in the complex virus resistance breeding programme.

Conclusions

With an active domestic and international co-ordination assumed, the perspective of breeding for virus resistance depends on how the fundamental questions related with the (*a*) interactions of resistance genes, (*b*) pathogenicity of virus genes and (*c*) inheritance of resistance genes can be settled, further, to what extent, (*d*) the methods of resistance can be improved and (*e*) with the establishment and maintance of a virus-gene bank, and (*f*) preservation of unselected plant gene banks i.e. with the genetic polymorphism and diversity of species the survival of populations ensured. The interfering activity of the expeditions – which undoubtedly played a very important role in exploring the gene centres – has not perhaps led to a serious deterioration of the gene bank yet, and it is only to be hoped that the genetic polymorphism of the gene banks of ecosystems preserved perhaps without selection by man will for a long time ensure the survival of the populations of species and of the gene forms capable of adapting themselves to the changing conditions. Here it must be noted, though, that for the last four decades a gradual impoverishment, gene erosion has taken place in the potato varieties native in the Andean region; in Chile this process is undoubtedly due to the European imports. It is not for nothing that in one of his works Hawkes

(1973) considers the present situation dangerous from the point of view of gene preservation. Therefore we should like to emphasize, here too, the highly important role and ever growing responsibility of man in taking care of the germ-plasm collections (Frankel, 1973; Sterbetz, 1979; Vida, 1979; Sprague, 1980; Mooney, 1981; Horváth, 1984*a*).

The historical events of the genetic transformation of viruses in the biological evolution taking place in the highly diversified plant gene centres under the influence of the reciprocal selective factors of plants and viruses astonish us, even if our knowledge of the motive forces of the processes are very poor. That much can undoubtedly be established that in possession of their genetic faculties the viruses have been becoming more and more independent. Their dependence left, that is their parasitism, is of pure feeding character, and therefore they are in any case able to adapt themselves to their environment. The persistent infection the different range of hosts, the adaption and variability ensure considerable evolutionary advantages for the viruses. For this very reason virus ecology and virus geography – as a part of the science of geopathology (Weltzien, 1967, 1972, 1978) – which are concerned with the gene centres too, play an ever increasing attention to diclosing the rules of the host-virus relations (Duffus, 1971; Horváth, 1972*a*, 1976, 1982*a*; Gibbs and Harrison, 1976; Schmelzer, 1970; Harris, 1981; Bos, 1981; Horváth et al., 1981). The genetic bases of the gene centres, which with the words of Ross (1978) have created a dramatic situation in plant breeding are at the disposal of the creative man as "intellectual toys", but it is a question, whether they really are always in safe hands, and whether everyone can do good with them.

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Experimental Host Range of *Melandrium* Yellow Fleck Virus[1]

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In the course of investigations into the host-range of *Melandrium* yellow fleck virus (MYFV, Bromovirus group) we found that out of 228 plants belonging to 20 families and 69 genera 78 showed local, and 141 local and systemic susceptibility. Nine plants proved resistant to infection by the virus. *Aizoaceae*, *Aristolochiaceae*, *Caryophyllaceae*, *Compositae*, *Labiatae*, *Malvaceae*, *Nolanaceae*, *Papaveraceae*, *Polygonaceae*, *Scrophulariaceae*, *Tropeolaceae* and *Umbelliferae* can be regarded as families susceptible to the new bromovirus. Of the plant families recently examined, *Cruciferae* and *Turneraceae* have so far been found resistant to the MYFV.

In 1974, in the wildlife reserve of Lake Balaton, Hungary, the senior author isolated a virus from *Melandrium album*, which differed from previously reported viruses of this plant (viz. cucumber mosaic virus, CMV; turnip mosaic virus, TuMV; lettuce bing vein virus, LBVV; tobacco rattle virus, TRV; tobacco mosaic virus, TMV; tobacco ringspot virus, TRSV; cf. Schmelzer and Wolf, 1971). In naturally infected *Melandrium album* the virus induces systemic vein clearing and yellow flecks (Fig. 1B and C), hence its name: *Melandrium* yellow fleck virus (MYFV). Preliminary results obtained with the virus were given by Hollings and Horváth (1978), and Hollings et al. (1978). On the biological, physical and biochemical characteristics of the MYFV Hollings and Horváth (1981) as well as Barton and Hollings (1981) published data. On the basis of its *in vitro* characteristics, MYFV belongs to the bromovirus group. Lane (1974, 1981), Bancroft and Horne (1977) and Matthews (1982) list three members of the bromovirus group (brome mosaic virus, BMV; broad bean mottle virus, BBMV; cowpea chlorotic mottle virus, CCMV), MYFV has joined them as the fourth member of the group (Hollings and Horváth, 1982; Francki et al., 1985). According to the results of earlier investigations the BMV, BBMV and CCMV though differing widely in host range are all spherical with anhydrous diameters of about 25 nm, they all contain about a million daltons of ribonucleic acid (RNA) and 180 protein subunits weighing about 20,000 daltons each, and they have not caused serious crop losses (Lane, 1974, 1979, 1981; Boswell and Gibbs, 1983; Francki et al., 1985). To our knowledge the three members of the bromovirus group infect 26 species of three plant families (*Fabaceae*, *Gramineae*, *Rosaceae*) in nature. The

[1] Dedicated to Dr. L. Bos on the occasion of his 60th birthday.

Table 1

Results of experimental host range of *Melandrium* yellow fleck virus (MYFV)

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
AIZOACEAE		
Tetragonia amplexicoma	NL	Mo
T. echinata	NL	NSp, Rg, D
T. eremaea	NL	NSp, Rg, D
T. tetragonoides	NL	Vc, Mo, Ld
AMARANTHACEAE		
Gomphrena decumbens	NL	C-NSp
G. diffusa	NL	C-NSp
G. dispersa	NL	C-NSp
G. globosa (Fig. 1A)	NL	YM, Stu
ARISTOLOCHIACEAE		
Aristolochia brasiliensis	NL, Vn	O, Vnr
CARYOPHYLLACEAE		
Agrostemma githago	NL, Ab	Mo
Cucubalus baccifer	NRi	Mo, Ld
Cerastium semidecandrum	NL	ISp
C. biebersteinnii	NL	Mo
C. tomentosum	NL	Mo
Dianthus barbatus	CL	O, Vnr
D. caryophyllus cv. *Joker*	O, Vnr	O, Vnr
D. caryophyllus cv. *William Sim*	O, Vnr	O, Vnr
Gypsophila acutifolia	NL	Mo
G. altissima	CL	Vc, Mo, (?Ry)
G. paniculata	CL	Vc, Mo
G. perfoliata	CL	Vc, Mo
G. repens	CL	Vc, Mo
Lychnis chalcedonica	C-NL	Mo, (?Ry)
L. coronaria	NL	CSp
L. flos-jovis	NL	CSp
L. githago	NL	Mo
L. haageana	NL	Mo
L. viscaria	NL	Mo, Ld
L. vulgare	NL	Mo
Melandrium album (Fig. 1B and C)	NRi	Vc, YSp
M. apetalum	C-NL	Vc, Mo, Rg
M. dichotama	C-NL	YSp
M. diurnum	C-NL	Vc, Mo, Ld
M. noctiflorum	C-NL	Vc, Mo
M. rubrum	NL	Vc, Mo, Ld
M. silvestre	NL	Mo, YSp, Ld
M. zawadsky	C-NL	Vc, Mo
Saponaria cerastioides	NL	Mo, Rg
S. officinalis	O, Vr	O, Vnr
S. vaccaria	CL	Mo

Table 1 (continue d

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
Silene alba	NL	M, Ld
S. altaica (Fig. 2A)	C-NL	Vc, Mo, Ld
S. armeria	NL	Mo, Ld
S. coeli-rosa	C-NL	Mo, Ld
S. cucubalus	CL	Mo, Ld
S. dichotoma	NL	Mo, Ld
S. dioica	NL	Mo, Ld
S. fridvalszkyana	C-NL	Mo
S. gallica	NL	Mo, Ld
S. italica	C-NL	Vc, Mo, Ld
S. lamilaconica	C-NL	Mo, Ld
S. otites	C-NL	Mo, Ld
S. pendula (Fig. 2D)	NL	Vc, Vb, Mo, Ld
S. saxatilis	C-NL	Mo, Ld
S. sendtneri	C-NL	Mo, Ld
S. tatarica	C-NL	Mo
S. vulgaris	C-NL	Vc, Mo
Stellaria media	O, Vr	Mo, Ld
Tunica prolifera	O, Vr	Mo
Vaccaria segetalis	NL	Mo
V. grandiflora	NL, NRi	Mo
V. hispanica	NL, NRi	Mo
V. pyramidata	NL, NRi	Mo
Viscaria viscosa	O, Vr	Mo
CHENOPODIACEAE		
Acroglochin chenopoides	NL, LeAb	StN, Tn, D
Atriplex hortensis	CL	O, Vnr
Axyris amaranthoides	C-NL	Mo, Rg
Beta orientalis (Fig. 2B)	CL	Vc, Mo, Ld
B. patellaris (Fig. 2C)	CL	Vc, Mo, Ld
Chenopodium album	NL	O, Vnr
C. album var. *centrorubrum*	C-NL	O, Vnr
C. amaranticolor (Fig. 3A)	NL	O, Vnr
C. hybridum	C-NL	O, Vnr
C. capitatum	C-NL	O, Vnr
C. multifidum	NL	O, Vnr
C. murale	NL	O, (?LeC, LeDw)
C. pumilio	C-NL	O, Vnr
C. rigidum	NL	O, Vnr
C. sandwicheum	NL	O, Vnr
C. schraderianum var. *schraderianum*	NL	O, Vnr
C. suecicum	NL	O, Vnr
C. quinoa	NL	O, Vnr
Rhagodia nutans	C-NL	Mo
Spinacia oleracea	NL, LeAb	Mo, Rg
S. tetrandra	C-NL	Mo, Rg
S. turkestanica	NL	Mo, Ld, Rg

Table 1 (continued)

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
COMMELINACEAE		
Commelina coelestis (Fig. 3B)	BrNL,PeN, Vn	Mo, Ld, Rg
Commelina communis	BrNL, PeN, Vn	Mo, Ld, Rg
COMPOSITAE		
Calendula officinalis	O, Vr	O, Vnr
Emilia flammea	C-NL	O, Vnr
E. sagittata	C-NL	O, Vnr
Helianthus annuus	NL	CM, Rg
H. annuus cv. *Iregi csikos*	BrNL	CM, Rg
H. annuus cv. *Peredovik*	BrNL	CM, Rg
H. annuus cv. *Csakinszkij 269*	BrNL	CM, Rg
H. californicus	NL	(?Mo)
H. cernuus	NL	(?Mo)
H. decapetalus	NL	(?Mo)
H. giganteus	NL	O, (?Mo)
H. grosse-serratus	NL	O, (?Mo)
H. maximiliani	NL	(?Mo)
H. occidentalis	C-NL	O, (?Mo)
H. organophyllus	NL	O, (?Mo)
H. salicifolius	NL	O, (?Mo)
H. tomentosus	NL	O, (?Mo)
H. trachelifolius	NL	O, (?Mo)
Zinnia elegans	BrNL	Vc, Mo, (?Ry)
Z. elegans cv. *Thumbelina varié*	BrNL	Vc, Mo, (?Ry)
Z. haageana	BrNL	Vc, Mo, (?Ry)
Z. pauciflora	BrNL	Vc, Mo, (?Ry)
Z. pumila	BrNL	Vc, Mo, (?Ry)
Z. verticillata	BrNL	Vc, Mo, (?Ry)
CRUCIFERAE		
Brassica pekinensis	O, Vnr	O, Vnr
B. oleracea	O, Vnr	O, Vnr
B. rapa var. *rapa*	O, Vnr	O, Vnr
Crambe abyssinica	O, Vnr	O, Vnr
CUCURBITACEAE		
Benincasa hispida	O, Vr	Mo
Bryonia alba	NSp	O, Vnr
B. dioica	NSp	O, Vnr
Bryonopsis laciniosa	O, Vr	Mo
Citrullus colocynthis	O, Vr	Mo
Cucumis sativus cv. *Delicatess* (Fig. 3C)	YL	(?M)
C. sativus cv. *Butcher's Disease Resistenter*	YL	CSp
Cucurbita ficifolia	C-NL	Mo
C. maxima convar. *hubbardina*	C-NL	Mo
C. maxima convar. *maxima*	C-NL	Mo

Table 1 (continued

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
C. moschata	C-NL	Mo
C. pepo convar. *patissonina* f. *radiata* (Fig. 3D)	YL	(?M)
Cyclanthera pedata	C-NL	Mo
Lagenaria siceraria	C-NL	Mo
FABACEAE		
Phaseolus aborigineus	NL	O, Vnr
P. acutifolius	NL	O, Vnr
P. coccineus	NL	O, Vnr
P. gonospermus	NL	O, Vnr
P. hysterinus	NL	O, Vnr
P. lunatus	NL	O, Vnr
P. mungo	NL	O, Vnr
P. tuberosus	NL	O, Vnr
P. vulgaris cv. *Gordon*	NL	O, Vnr
P. vulgaris cv. *The Prince*	NL	O, Vnr
P. vulgaris cv. *Red Kidney*	NL	O, Vnr
Pisum sativum cv. *Onward*	O, Vr	M, Ld, Stu, N, D
Trifolium incarnatum	O, Vnr	O, Vnr
Vicia faba (Fig. 4A)	NL	O, Vnr
Vigna sinensis	NL	O, Vnr
LABIATAE		
Ocimum basilicum	NL	O, Vnr
O. carnosum	NL	O, Vnr
O. gratissimum	NL, Ri	O, Vnr
MALVACEAE		
Althaea officinalis	O, Vr	Mo
Hibiscus abelmoschus	CL	O, Vr
H. manihot	CL	Mo
Lavatera arborea	O, Vr	Mo
Malva crispa	YSp	O, (?Vr)
M. pusilla (Fig. 5A)	O, Vr	Mo, Ld
M. silvestris	O, Vr	Vc, Mo
M. verticillata	O, Vr	C, Vc, Mo
NOLANACEAE		
Nolana paradoxa	NL	Vc, Mo
N. prostrata (Fig. 4B)	CoNL	Vc, Mo
PAPAVERACEAE		
Papaver somniferum (Fig. 6A)	NL	Mo, Ld, Rg
POLYGONACEAE		
Emex australis	NL	O, Vr
E. spinosa	NL	O, Vr

Table 1 (continued)

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
SCROPHULARIACEAE		
Pentstemon hartwegii	O, Vr	O, Vr
Torenia fournieri	NL	Mo
SOLANACEAE		
Datura ceratocaula	CSp, Ri	O, Vnr
D. chlorantha	CSp, Ri	O, Vnr
D. fastuosa	CSp, Ri	O, Vnr
D. ferox	CSp, Ri	O, Vnr
D. gigantea	CSp, Ri	O, Vnr
D. inermis	CSp, Ri	O, Vnr
D. innoxia (Fig. 5B)	CSp, Ri	O, Vnr
Datura leichardtii	CSp, Ri	O, Vnr
D. metel	CSp, Ri	O, Vnr
D. meteloides	CSp, Ri	O, Vnr
D. quercifolia	CSp, Ri	O, Vnr
D. rosei	CSp, Ri	O, Vnr
D. stramonium (Fig. 5C)	CSp, Ri	O, Vnr
Hyoscyamus niger	O, Vnr	O, Vnr
Lycium australe	NL	O, Vnr
L. barbarum	C-NL, LeAb	O, Vnr
L. carolinianum	C-NL, LeAb	O, Vnr
L. cestroides	C-NL	O, Vnr
L. chinense	C-NL, LeAb	O, Vnr
L. europaeum	C-NL, LeAb	O, Vnr
L. flexicaule (Fig. 5D)	C-NL, LeAb	O, Vnr
L. halimifolium	C-NL, LeAb	O, Vnr
L. mediterraneum	C-NL	O, Vnr
L. pallidum	C-NL	O, Vnr
L. ruthenicum	C-NL, LeAb	O, Vnr
L. turcomanicum	C-NL, LeAb	O, Vnr
Lycopersicon esculentum cv. *Moneymaker*	CL	O, Vnr
Nicandra physaloides (Fig. 6C)	BrNL	O, Vr
Nicotiana benthamiana	C-NRi	Mo
N. clevelandii	C-NL	Mo, Bli, Pu, N
N. glutinosa	C-NL	O, Vnr
N. glutinosa x *N. clevelandii* hybrid	CL	Y-GMo
N. tabacum cv. *Bel 61–10*	C-NRi	O, Vnr
N. tabacum cv. *Samsun*	C-NRi	O, Vnr
N. tabacum cv. *White Burley*	C-NRi	O, Vnr
N. tabacum cv. *Xanthi-nc*	C-NL	O, Vnr
Petunia hybrida cv. *Fire Chief*	CL	O, Vnr
P. inflata	CL	O, Vnr
P. nyctaginiflora	CL	O, Vnr
P. violacea	CL	O, Vnr
Physalis aequata	C-NL	Vc, Mo
P. anisotrichus (Stock 13287)	NL	NSp
P. alkekengi	NL	Vc, Mo

Table 1 (continued)

Family and species (varieties and cultivars)	Results of inoculation[1]	
	Locally	Systemically
P. curassavica	NL	O, Vr
P. filiformis	NL, LeAb	NL
P. floridana	C-NL	Mo
P. franchetti	C-NL	Vc, Mo
P. glabripes	C-NL	Mo
P. ixocarpa	CL	Mo, Y
P. lanceifolia	YSp	Mo
P. phyladelphica	C-NL	Mo
P. pruinosa	C-NL	Vc, Mo
Saracha edulis	NRi	Mo
Solanum americanum	C-NL	O, Vnr
S. auriculatum	CoNL	E, Vr
S. canadense	BrNL	O, Vnr
S. decurrens	NL	O, Vnr
S. gibberulosum	C-NL, Co	O, Vnr
S. macrocarpum	C-NL	O, Vnr
TROPEOLACEAE		
Tropaeolum majus (Fig. 6B)	CL	YVnt, LeDw
TURNERACEAE		
Turnera ulmifolia	O, Vnr	O, Vnr
UMBELLIFERAE		
Ammi majus (Fig. 6D)	CL	M, Ld
A. visnaga	CL	M, Ld
Apium graveolens	O, Vnr	O, Vnr
Coriandrum sativum	O, Vr	Mo

[1] Abbreviations used in the Table indicate following:

Ab, abcission
Bli, blistering
Br, brown
C, chlorotic; chlorosis
C-N, chlorotic-necrotic
Co, concentric patterns
M, mottling
Mo, mosaic
N, necrotic; necrosis
O, no visible symptoms
Pe, petiole; petioles
Pu, puckered
Rg, retardation of growth
Ri, rings; ringspots
Ry, recovery
Sp, spots
St, stem; stems
D, death of plant
Dw, dwarfing
E, etched patterns
L, lesion; lesions
Ld, leaf distortion
Le, leaf; leaves
Stu, stunting
Tn, top necrosis
Vb, vein banding
Vc, vein clearing
Vn, vein necrosis
Vnt, vein netting
Vnr, virus not recoverable
Vr, virus recoverable
Y, yellowing; yellows
Y-G, yellow-green
(?) occasionally

most widespread of them is BMV (22 species) followed by CCMV (4 species) and BBMV (1 species). The experimental host range of the bromoviruses, on the other hand, is much wider. Virus susceptible species have so far been found in 8 plant families; 164 virus susceptible species have been described for BMV, 39 for CCMV and 24 for BBMV (reviewed by Lane, 1974, 1981; Edwardson and Christie, 1986). The present paper reports on the experimental host range of MYFV.

Materials and Methods

MYFV was maintained and increased in *Gomphrena globosa* and/or *Nicotiana clevelandii*. All mechanical inoculations were made by the carborundum (400 mesh) gauze-pad method. The plant extracts were diluted with distilled

Fig. 1. Symptoms of *Melandrium* yellow fleck virus. A: systemic yellow mosaic in *Gomphrena globosa*. B and C: natural infection in *Melandrium album*

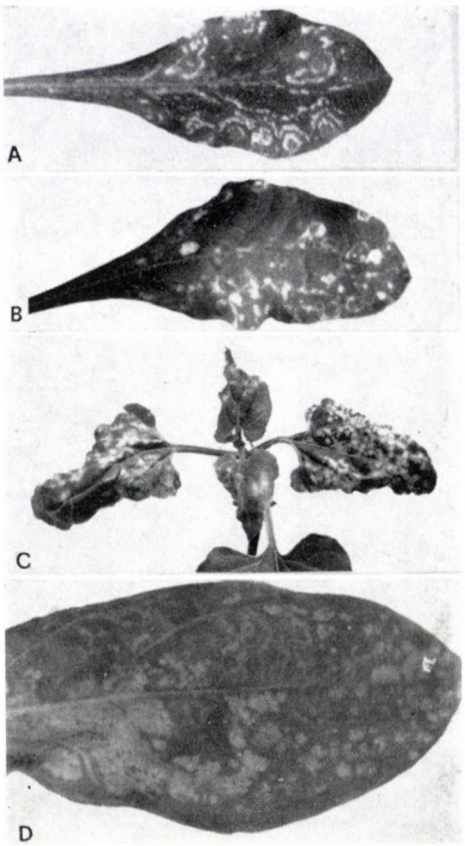

Fig. 2. Symptoms of *Melandrium* yellow fleck virus. Chlorotic-necrotic local lesions on leaves of *Silene altaica* (A), and *Beta orientalis* (B). Systemic vein clearing and mosaic in *Beta patellaris* (C) and *Silene pendula* (D)

water (1 : 5) and were used without additives as inoculum. To detect latent infections back-inoculation tests were made to *Chenopodium quinoa* or *Phaseolus vulgaris*. Tissue extracts were prepared both from inoculated labelled leaves and from non-inoculated or subsequently developed ones of the inoculated plants – previously surface disinfected in a 2 per cent solution of NaOH than washed with a jet of water –, diluted with distilled water (1 : 5) and inoculated by the carborundum gauze-pad method onto assay species (*Chenopodium quinoa* and/or *Phaseolus vulgaris*). In the course of the host-range studies 228 species (varieties, cultivars) of 20 plant families were inoculated with MYFV. A total of some 2000 plants were inoculated and about the same number of plants used for back-inoculation.

In describing the host-virus reactions, the symptoms, symbols were used (see at the end of Table 1).

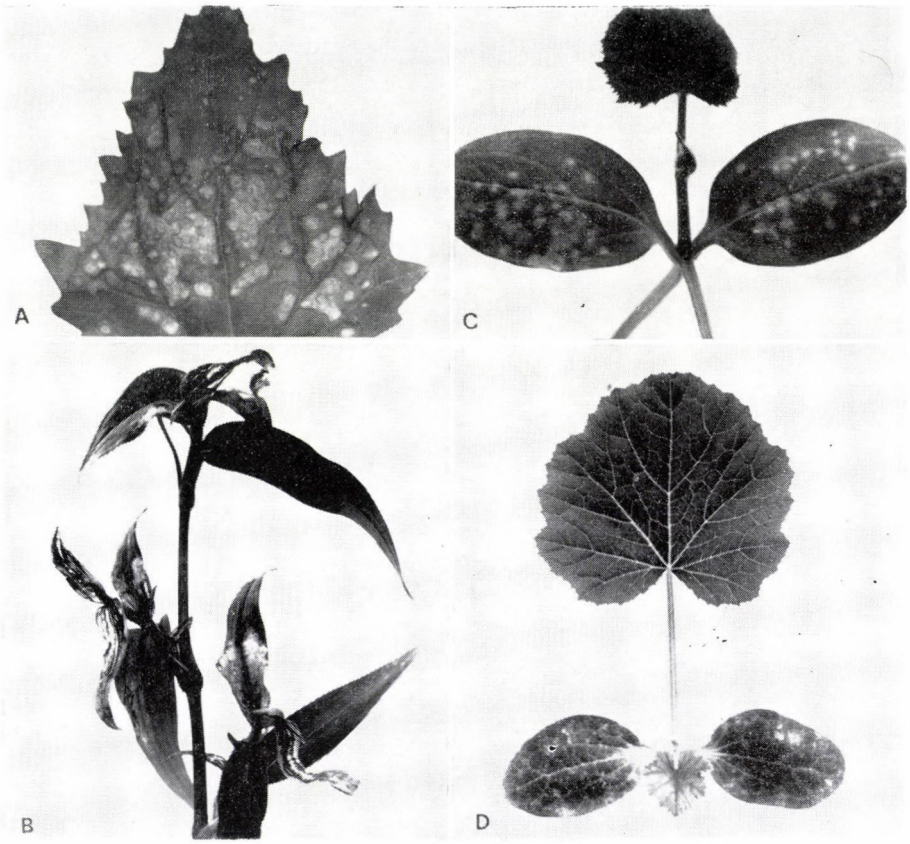

Fig. 3. Symptoms of *Melandrium* yellow fleck virus. Chlorotic-necrotic local lesions on leaves of *Chenopodium amaranticolor* (A), *Cucumis sativus* (C) and *Cucurbita pepo* convar. *patissonina* f. *radiata* (D). Systemic mosaic and leaf distortion in *Commelina coelestis* plant (B)

Results and Conclusions

The results of our experiments are summarized in Table 1. Accordingly, of the 228 plants belonging to 20 plant families and 69 genera 78 (34 per cent) showed local susceptibility, including 3 plants which showed latent local susceptibility. Of the plants examined 141 (62 per cent) were locally and systemically susceptible to MYFV. Within this group latent local and manifest systemic susceptibility was established for 13, and manifest local and latent systemic susceptibility for 6 plants. Seven *Melandrium* species gave very similar responses to that of the *Melandrium album* from which MYFV was originally isolated. Nine plant species (4 per cent) proved resistant to the virus.

The host plants belong to various plant families. The following plant families can be regarded as new bromovirus susceptible families: *Aizoaceae*, *Aristolochia-*

ceae, *Caryophyllaceae*, *Compositae*, *Labiatae*, *Malvaceae*, *Nolanaceae*, *Papaveraceae*, *Polygonaceae*, *Scrophulariaceae*, *Tropeolaceae* and *Umbelliferae*. The species of *Cruciferae* and *Turneraceae*, which were also among the newly examined plant families, were resistant to MYFV.

It is remarkable that in some families all (or nearly all) species examined proved locally and systemically susceptible to MYFV. Such families are: *Aizoaceae*, *Amaranthaceae* and *Caryophyllaceae*. Each of the bromoviruses seems to be restricted to different plant families. E.g. BMV is largely restricted to *Gramineae*, BBMV and CCMV to *Leguminosae* and MYFV to *Caryophyllaceae*. Besides the families *Cruciferae* and *Turneraceae* the families *Labiatae*, *Fabaceae* and *Solanaceae* included some non-susceptible species. MYFV e.g. systemically infected 52 of 56 species in the family *Caryophyllaceae*, but induced only local lesions in *Dianthus barbatus* and did not infect virusfree carnations (*Dianthus caryophyllus* cv. *William Sim.*). *Saponaria officinalis* in the family *Caryophyllaceae* showed latent local susceptibility to MYFV. As it has been mentioned already, the number of latent virus-susceptible species is relatively small (e.g. *Emex* spp., *Hibiscus abelmoschus*, *Malva crispa*, *Pentstemon hartwegii*, *Physalis curassavica*, *P. minima*). Recovery is also an interesting phenomenon in some species (e.g. *Lychnis chalcedonica*, *Zinnia* spp.).

The various *Chenopodium* species (e.g. *Chenopodium hybridum*, *C. quinoa*) have been used as assay species for known members of the bromovirus group, and proved equally good for the biological assay of MYFV. The *Phaseolus*

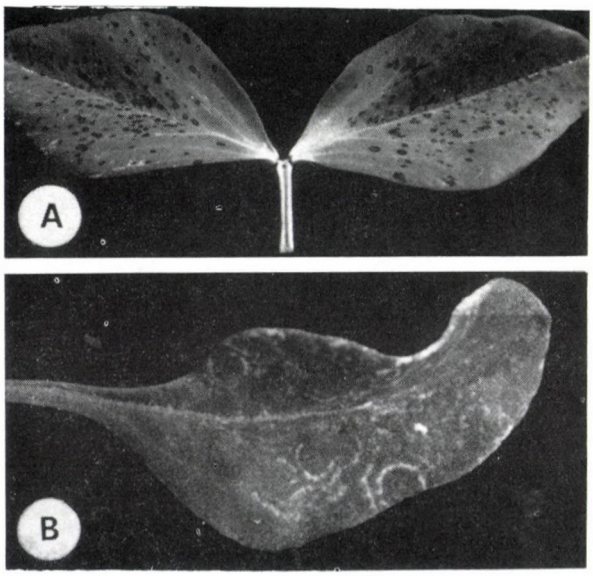

Fig. 4. Symptoms of *Melandrium* yellow fleck virus. A: necrotic local lesions on leaf of *Vicia faba*. B: local concentric patterns on leaf of *Nolana prostrata*

species included in our study proved satisfactory assay species for MYFV, though it is also known that e.g. in the case of BMV the *Phaseolus vulgaris* is a non-host plant (McKinney, 1944), while for other bromoviruses (BBMV, CCMV) it is a suitable assay plant (Bawden et al., 1951; Kuhn, 1964). Of the *Datura* species *D. bernhardii*, *D. inermis* and *D. metel* are known as bromovirus (BMV) local lesion hosts (Chiu and Sill, 1963; Stoner et al., 1967; Richter et al., 1966). Further,

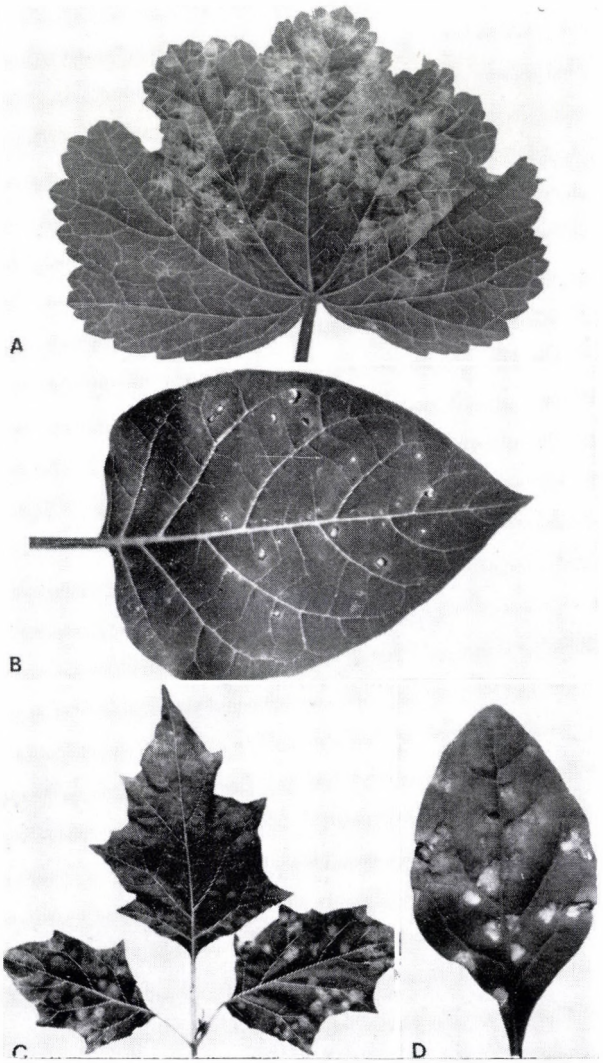

Fig. 5. Symptoms of *Melandrium* yellow fleck virus. (A): systemic mosaic on leaf of *Malva pusilla*. Chlorotic-necrotic local lesions on leaves of *Datura innoxia* (B), *Datura stramonium* (C) and *Lycium flexicaule* (D)

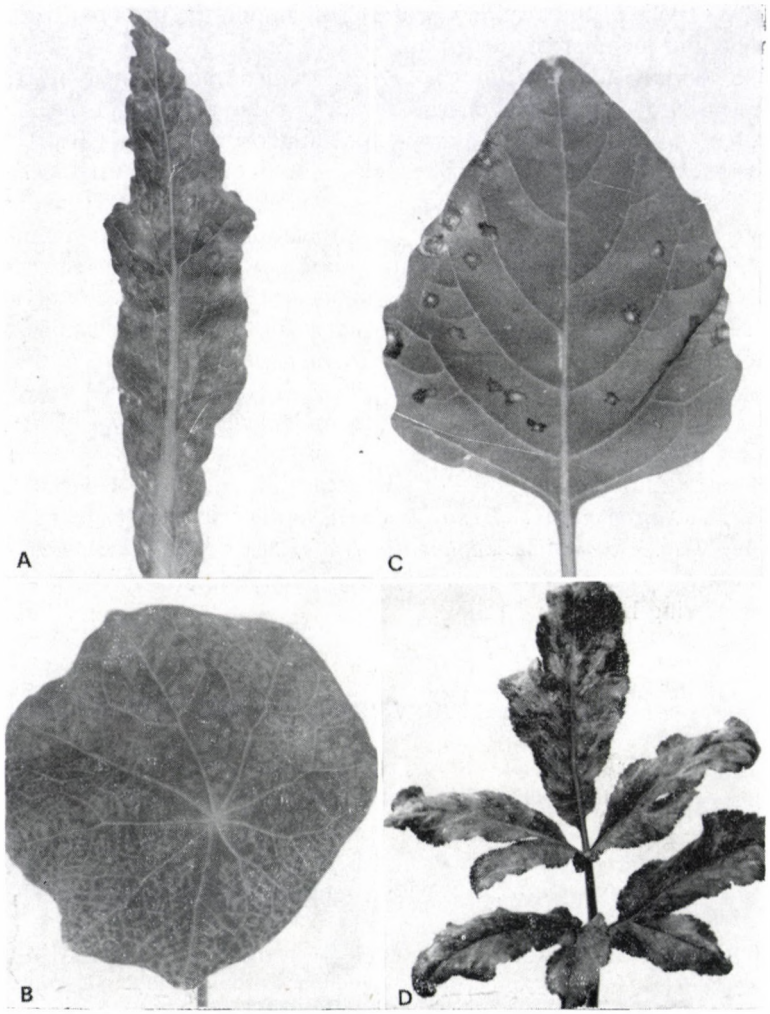

Fig. 6. Symptoms of *Melandrium* yellow fleck virus. A: systemic mosaic, and leaf distortion in *Papaver somniferum*. B: systemic yellow vein netting and mosaic in *Tropaeolum majus*. C: brown necrotic local lesions on leaf of *Nicandra physaloides*. D: systemic mosaic and leaf distortion in *Ammi majus*

9 *Datura* species (*D. ceratocaula*, *D. chlorantha*, *D. fastuosa*, *D. ferox*, *D. gigantea*, *D. innoxia*, *D. leichardtii*, *D. meteloides*, *D. quercifolia*) were found to be local-lesion hosts for MYFV.

On the basis of our experiments with the host-range of MYFV the restricted host-range characteristic of the other bromoviruses (see Lane, 1981; Boswell and Gibbs, 1983; Francki et al., 1985) is not the case with MYFV. We think

that the MYFV is of the broadest distribution among the bromoviruses, possibly a cosmopolitan member of the group.

The bromoviruses are known to reach high levels in their hosts, and are therefore easily diagnosed and characterized. It is due to this fact that the bromoviruses as model viruses play a highly important role in theoretical virus research. As far as we know the bromoviruses have not frequently caused serious losses in crop plants, their resistance studies have therefore been neglected. However, the MYFV isolated from biennial (hemithereophyton) and perennial (hemicryptophyton) *Melandrium album* plants to be found in waste lands, arable fields, weed communities, groves, meadows is important even from the practical point of view since is pathogenic for such species of 17 of the 69 genera examined among cultivated plants (e.g. *Beta*, *Spinacia*, *Helianthus*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria*, *Pisum*, *Vicia*, *Papaver*.)

No natural host for MYFV apart from *Melandrium album* has so far been detected. Nevertheless, our experimental host-range studies suggest the possibility of natural infection in some of the susceptible perennial *Melandrium* species (e.g. *Melandrium rubum*, *M. silvestre*) as well as other perennial species susceptible to MYFV (e.g. *Commelina communis*, *Helianthus* spp., *Pentstemon hartwegii*, *Physalis alkekengi* and *Silene tatarica*), such plants might play an important role as overwintering hosts for MYFV.

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Reactions of Wild *Solanum* Species to Potato Virus X and Potato Virus Y[1]

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Reactions of forty-four accessions from twenty-seven wild *Solanum* species to potato virus X (PVX) and potato virus Y (PVY) have been studied. Out of them *Solanum lesteri* P. I. 442694, *S. marinasense* P. I. 458380 and *S. tuberosum* ssp. *andigena* P. I. 243450 possess hypersensitive resistance; the other wild *Solanum* species examined are locally and systemically susceptible to PVX. As to PVY, certain species (*Solanum brevidens* P. I. 218228, P. I. 245763, P. I. 245764, P. I. 273401; *S. fernandezianum* IS/C--1663; *S. hermanni* BIRM/S. 0210 and *S. trifidum* P. I. 255536) are immune.

The potato virus X (PVX, potexvirus group) and potato virus Y (PVY, potyvirus group) have spread all over the world in potatoes. In Hungary it is the most frequent virus after potato leaf roll virus (PLRV, luteovirus group), and in a great many cases it occurs in mixed form (Horváth, 1963, 1967). The fact that the course of inheritance and the occurrence of resistance genes in many wild *Solanum* species have been cleared for both viruses is of very great importance in producing virus resistant potato varieties (Ross, 1958, 1960, 1961, 1966, 1978; Horváth, 1968, 1984a; Cockerham, 1970; Munoz et al., 1975; Hooker, 1978; Oertel et al., 1980 etc.). Due to the investigations, there are today several virus resistant potato varieties known in the world. Characteristically of the variability of PVX and PVY strains with different properties have recently appeared of them (e.g. Klinkowski and Schmelzer, 1960; Rozendaal et al., 1971; De Bokx et al., 1975; Koenig and Bode, 1977; Moreira et al., 1978, 1980; Calvert et al., 1980; Jones, 1985), which at the same time have made the work of breeding more difficult. This fact explains the great attention paid for some years to studies of relationships between wild *Solanum* species in gene centres and viruses, and to descriptions of further *Solanum* species (Schmelzer and Spaar, 1975; Jones and Fribourg, 1978; van Soest, 1980, 1983; van Soest and Hondelmann, 1983; van Soest et al., 1980; Horváth, 1984b, 1987, 1988). Seeking out virus resistant wild *Solanum* species and detecting relationships between them and various viruses are equally important for breeding and from point of view of virus ecology and virus diagnosis.

[1] Dedicated to the late S. Barsy, given Kossuth-Prize for her potato breeding work, on the occasion of her 85th birthday.

Materials and Methods

Forty-four accessions of twenty-seven wild *Solanum* species were inoculated by carborundum-spatula technique with water diluted (1 : 1, v/v) tissue sap from *Nicotiana tabacum* cv. *Xanthi-nc* plants previously infected with PVX and PVY. The inoculated plants were symptomatologically checked for infection every 7 days, then at the end of the 3rd and 6th week serological examinations were performed for both viruses. To check the susceptibility to PVX and PVY of the wild *Solanum* species double sandwich ELISA method was applied using horse-radish peroxidase conjugate (see Clark and Adams, 1976). The PVX and PVY antiserum was conjugated with horse-radish peroxidase enzyme. The color reaction was measured at 492 nm wavelength on Dynatech ELISA reader. In latent host-virus relations back-inoculation was also carried out to removed leaves of indicator plants: *Gomphrena globosa* for PVX and *Solanum demissum* A6-hybrid for PVY (Köhler, 1953; Paul, 1964; Zschüttig and Horváth, 1968).

Results and Discussion

1. Experiments with potato virus X

In the course of experiments with PVX various host-virus relations were detected. As seen from Table 1 the host-virus relations were essentially characterized by the following symptoms: chlorotic and/or necrotic local lesions with leaf drop or without, and absence of systemic symptoms (A); chlorotic and/or necrotic local lesions with or without leaf drop, and systemic vein clearing, mosaic, in some cases necrotic symptoms (B); necrotic local lesions and necrotic systemic lesions (C); symptomless local susceptibility and symptomless systemic susceptibility (D); symptomless local susceptibility and systemic vein clearing and mosaic (E); necrotic local lesions with leaf drop (F). According to the diversity of the symptoms the individual wild *Solanum* species and their accessions showed local and systemic susceptibility (A-E), or with a mere local susceptibility hypersensitive resistance (F) to PVX (see Table 1). The P. I. 243390 and P. I. 243453 accessions of *Solanum tuberosum* ssp. *andigena* developed to necrosis too in response to infection by PVX. Particularly remarkable are certain latent host-virus relations (e.g. *Solanum abancayense* P. I. 458404; *S. brevidens* P. I. 218228, P. I. 245763, P. I. 473401; *S. canasense* P. I. 265863; *S. fernandezianum* IS/C – 1663/1981). Out of the latter plants only *Solanum canasense* P. I. 265863 is mentioned for virus susceptibility by Hanneman and Bamberg (1986) as susceptible to PVX and PVY. These data have been confirmed in our own experiments. Resistance based on hypersensitivity was established for *Solanum lesteri* P. I. 442694; *S. marinasense* P. I. 458380; *S. tubersoum* ssp. *andigena* P. I. 243450.

Table 1

Origin of the investigated wild *Solanum* species and their reaction to potato virus X (PVX) and potato virus Y (PVY)

Solanum species	Accession number[1]	Type of reaction[2]	
		PVX	PVY
Solanum abancayense	P. I. 458403, Peru	A	B
S. abancayense	P. I. 458404, Peru	D	B
S. ambosinum	P. I. 365316, Peru	NT	B
S. ambosinum	P. I. 365362, Peru	B	B
S. brachistotrichum	P. I. 283095, Mexico	NT	A
S. brachistotrichum	WRF 1271	B	A
S. brevidens	P. I. 218228	D	G
S. brevidens	P. I. 245763, Chile	D	G
S. brevidens	P. I. 245764, Chile	A	G
S. brevidens	P. I. 473401, Argentina	D	G
S. canasense	P. I. 265863, Peru	D	B
S. fernandezianum	IS/C-1662/1981	D	G
S. hermanni	BIRM/S.0210	E	G
S. khasianum	IS/C-673/1982	B	B
S. lesteri	P. I. 442694, Mexico	F	B
S. marinasense	P. I. 365333, Peru	A	B
S. marinasense	P. I. 458380, Peru	F	NT
S. olgae	IS/C-1013/1981	B	B
S. oplocense	P. I. 442682, Argentina	A	B
S. oplocense	P. I. 473192, Argentina	A	B
S. ottonis	IS/C-1672/1982	B	B
S. pampasense	P. I. 442697, Peru	B	B
S. panduriforme	BIRM/S.1398	B	NT
S. papita	P. I. 275227	B	B
S. papita	P. I. 275228, Mexico	B	B
S. platense	BIRM/S.0738	NT	B
S. polytrichon	P. I. 186545, Mexico	A	F
S. polytrichon	P. I. 25546, Mexico	A	B
S. polytrichon	P. I. 275241, Mexico	B	B
S. quitoense	IS/C-578/1981	B	B
S. rigescens	IS/C-1024/1981	B	F
S. saponaceum	IS/C-1026/1981	B	B
S. scabrum	BIRM/S. 0246	B	E
S. sodomeum	IS/C-1031/1981	B	B
S. symonii	BIRM/S.0797	B	B
S. trifidum	P. I. 255536, Mexico	B	G
S. trifidum	P. I. 255542, Mexico	B	NT

Table 1 (continued)

Solanum species	Accession number[1]	Type of reaction[2]	
		PVX	PVY
S. tuberosum ssp. *andigena*	P. I. 243390, Colombia	B	E
S. tuberosum ssp. *andigena*	P. I. 243400, Colombia	NT	E
S. tuberosum ssp. *andigena*	P. I. 243436, Colombia	B	B
S. tuberosum ssp. *andigena*	P. I. 243450, Colombia	E	B
S. tuberosum ssp. *andigena*	P. I. 243453, Colombia	B	E
S. tuberosum ssp. *andigena*	WRF 1758 (279291 × 306302)	C	B
S. weberbaueri	P. I. 442703, Peru	A	B

[1] BIRM/S. — Birmingham *Solanaceae* Gene Bank, Birmingham, England; IS/C — Index Seminum of the Botanic Garden, Copenhagen, Denmark; P. I. and WRF — Potato Introduction Station, Sturgeon Bay, Wisconsin, USA.

[2] A, chlorotic and/or necrotic local lesions with leaf drop or without, and absence of systemic symptoms; B, chlorotic and/or necrotic local lesions with or without leaf drop, and systemic vein clearing, mosaic, in some cases necrotic symptoms; C, necrotic local lesions and necrotic systemic lesions; D, symptomless local susceptibility and symptomless systemic susceptibility; E, symptomless local susceptibility and systemic vein clearing and mosaic; F, necrotic local lesions with leaf drop (hypersensitive reaction); G, immunity; NT, not tested.

2. *Experiments with potato virus Y*

The types of host-virus relations between the *Solanum* species and accessions examined and the PVY partly agreed with those described for PVX (see A, B, E and F). It is remarkable that some *Solanum* species and accessions showed immunity of PVY (G), namely, neither the inoculated nor the non-inoculated leaves of *Solanum brevidens* P. I. 218228, P. I. 245763, P. I. 245764, P. I. 273401; *S. fernandezianum* IS/C–1662/1981; *S. hermanni* BIRM/S.0210; *S. trifidum* P. I. 255536 showed infection by PVY, and the virus could not even be detected in them by serological and biological tests (see Table 1). These latest experiment results are important because — to our best knowledge — literary data concerning the *Solanum* species and accessions mentioned are not available (Hanneman and Bamberg, 1986). The only exception is *Solanum brevidens* P. I. 245764, which according to the data of Hanneman and Bamberg (loc. cit.) is hypersensitive to PVY.

Some of the wild *Solanum* species discussed in present paper (e.g. *Solanum abancayense* P. I. 458404; *S. ambosinum* P. I. 365362; *S. canasense* P. I. 265863; *S. lesteri* P. I. 442694; *S. oplocense* P. I. 442682; *S. papita* P. I. 275227; *S. polytrichon* P. I. 255546; *S. weberbaueri* P. I. 442703) were also found to be susceptible to henbane mosaic virus (HeMV, potyvirus group); the pathogeneity of the latter virus for potato has recently been demonstrated (cf. Horváth et al., 1988).

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Noninvolvement of Glyceollin in Acquired Resistance of Soybean Leaves to Bacterial Blight

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Pre-inoculation of soybean half-leaves with a varietal non-pathogenic race of *Pseudomonas syringae* pv. *glycinea* elicits protection in the opposite half-leaves against challenge with the same race or with a varietal pathogenic race. Phytoalexin glyceollin was supposed to contribute to this type of defence. The half-leaves opposite to those pre-inoculated with varietal non-pathogenic race do not accumulate glyceollin before challenge. After challenge with varietal pathogenic and varietal non-pathogenic races, glyceollin content in challenged half-leaves is almost the same as in normally compatible and incompatible combinations, respectively. The multiplication of bacteria is somewhat slower in the protected than in the nonprotected control half-leaves but the trends of multiplication do not greatly differ.

This type of acquired resistance seems to be due to the suppression of symptoms rather than that of bacterial multiplication which glyceollin does not contribute to.

Resistant reactions of soybean leaves to varietal non-pathogenic races of *Pseudomonas syringae* pv. *glycinea* are accompanied by the accumulation of isoflavonoid phytoalexins. Among these glyceollin has been most extensively studied and stated as a putative warding-off compound (Keen and Kennedy, 1974). Glyceollin production is associated with tissue browning, i.e. host cell death. On the other hand, browning, in response to inducing agents, often results in induction of protection against damage by causal microorganisms. In some cases only localized protection was detected (e.g. Carroll and Lukezic, 1972; Érsek, 1973; Lovrekovich and Farkas, 1965; McIntyre and Miller, 1978; Ross, 1961a) but in others systemic resistance developed in plant tissues remote from those treated with the inducing agent (e.g. Kuć, 1982; Lozano and Sequeira, 1970; Ross, 1961b).

A number of studies has been concerned with the mechanism involved in this type of resistance termed acquired or induced resistance or cross-protection. The literature indicates some uncertainty as to whether the acquired resistance is associated with phytoalexins.

We report here on the induction of opposite half-leaf protection of soybean against varietal pathogenic (compatible) and non-pathogenic (incompatible) races of *P. syringae* pv. *glycinea* by infection with the varietal non-pathogenic race of the bacterium inducing the hypersensitive reaction. The mode of expression of this phenomenon in relation to glyceollin production is also described.

Materials and Methods

Soybean (Glycine max [L.] Merr.) cv. Harosoy plants were grown under greenhouse conditions. Seeds were planted in pots containing 1 : 1 mixture of sandy loam and peat overlaid with perlite.

Races 1 and 2 of the bacterium *Pseudomonas syringae* pv. *glycinea* (= *P. glycinea* Coerper) kindly supplied by N. T. Keen (University of California, Riverside, USA) were grown in semi-synthetic liquid medium (Bruegger and Keen, 1979) by continuous shaking at 28 °C for 18h. To prepare inoculum, twice washed bacterium cells were resuspended in water. Water suspensions of 10^8 bacteria per ml were used for inoculation, unless otherwise indicated. One half of ca. half-expanded primary leaves was infiltrated at a time with either water (control) or bacterium suspension by the use of a hand atomizer. During infiltration the opposite half-leaves were carefully covered with aluminum foil, then 0, 48 or 96 h later challenge-inoculated with race 1 or race 2; this time the pre-treated half-leaves were covered. Infiltrated plants were kept in growth chamber at 24 °C daily and 18 °C night temperatures on 14 h photoperiod and 70% relative humidity.

Bacterial populations in half-leaves were estimated by grinding 10 leaf disks of 5 mm diam. in distilled water. The homogenate was serially diluted and plated onto nutrient broth agar medium.

Glyceollin was extracted from leaves by facilitated diffusion technique and quantified by TLC-UV spectrometry (Keen, 1978).

In order to observe single cell death in tissue, leaf sections were vacuum-infiltrated with sodium fluorescein and viewed with fluorescence microscope for epifluorescence as described previously by Holliday et al., 1981 and Érsek and Hevesi, 1983.

Results

A substantial amount of glyceollin accumulates in leaves of soybean cv. Harosoy following inoculation with varietal non-pathogenic but not with varietal pathogenic races of *P. syringae* pv. *glycinea* (Fig. 1).

Pre-inoculation of one half of the primary leaf with the varietal non-pathogenic race 1 prevents the opposite half from responding hypersensitively to a subsequent inoculation with the same race 2 days later (Fig. 2). The rate of early occurring single cell death in the protected half-leaves, however, does not differ from that in the non-protected ones (Fig. 3). The multiplication of the challenge race 1 in protected half-leaf as compared to that in the non-protected half-leaf is restricted and slower (Fig. 4). As for phytoalexin, preinoculation *per se* with race 1 does not induce glyceollin production in the opposite half-leaf before challenge. Glyceollin contents in protected and nonprotected half-leaf do not considerably differ from one another (Fig. 5).

The development of symptoms caused by the varietal pathogenic race 2 is also restricted in half-leaf opposite to that pre-inoculated with race 1 (Fig. 6).

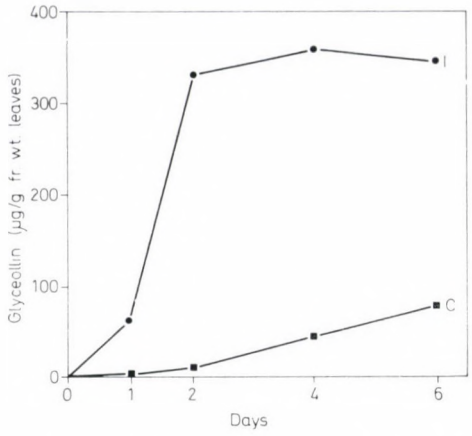

Fig. 1. Accumulation of glyceollin in primary leaves of soybean cv. Harosoy inoculated with varietal pathogenic (C = compatible) race 2 and with varietal non-pathogenic (I = incompatible) race 1 of *P. syringae* pv. *glycinea*

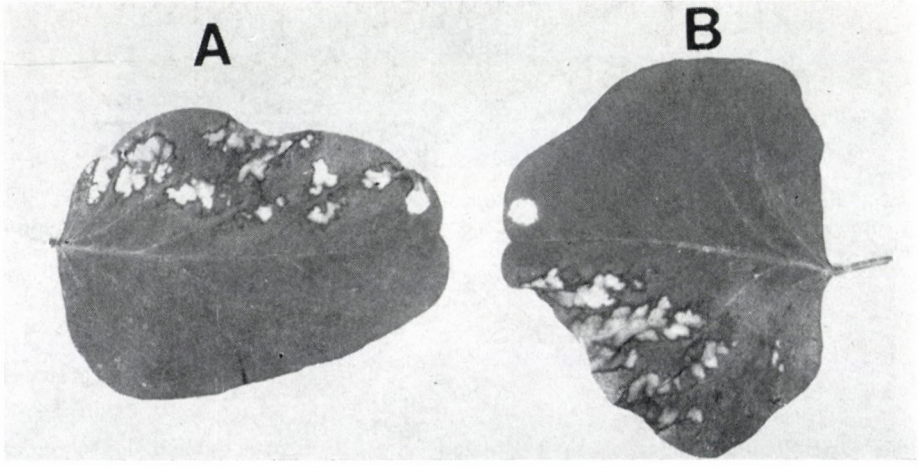

Fig. 2. Suppression (A) and lack of suppression (B) of the hypersensitive reaction in cv. Harosoy soybean half-leaves challenged with race 1 of *P. syringae* pv. *glycinea* 2 days after pre-treatment of opposite (punched) half-leaves with the same race and water (control), respectively. Photograph was taken 6 days after challenge inoculation

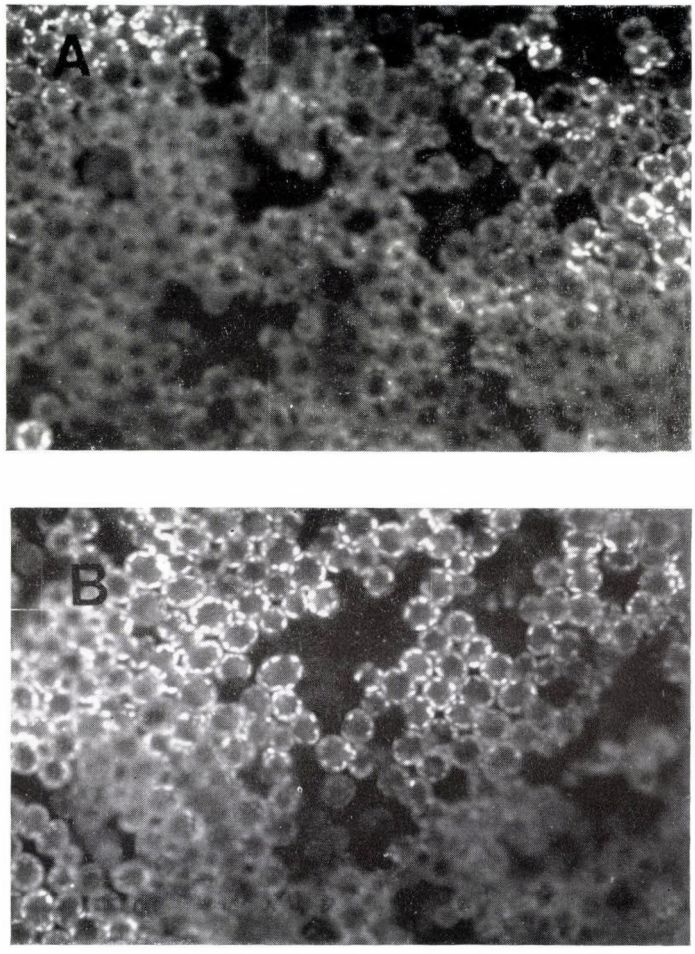

Fig. 3. Fifteen-hour-old pattern of cell death caused by *P. syringae* pv. *glycinea* race 1 in mesophyll tissue of cv. Harosoy soybean half-leaves that were protected (A) and nonprotected (b) by pre-treatment of the opposite half-leaves with the same race and with water, respectively

The multiplication of race 2 in protected *versus* nonprotected half-leaves delays but is alike by the third day after challenge (Fig. 4). The amount of glyceollin is not remarkably higher in protected than in nonprotected half-leaves (Fig. 5).

The time elapsing from pre-inoculation till challenge does not effectively influence glyceollin production in challenged half-leaf (Table 1). The protection,

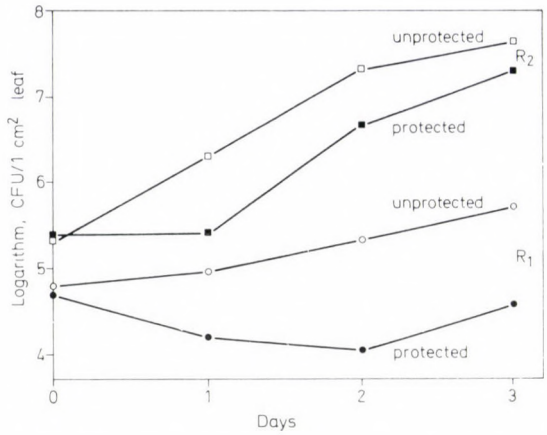

Fig. 4. Multiplication of the challenge bacterium *P. syringae* pv. *gylcinea* race 1 (R_1) or race 2 (R_2) in cv. Harosoy soybean half-leaves that were protected and non-protected by pre-treatment of the opposite half-leaves with race 1 and water, respectively

however, is more remarkable if the second inoculation is carried out 48 h rather than 0 or 24 h after pre-inoculation. Longer than 48 h incubation after the primary inoculation, though results in valuable protection, extends the experimental period to no purpose.

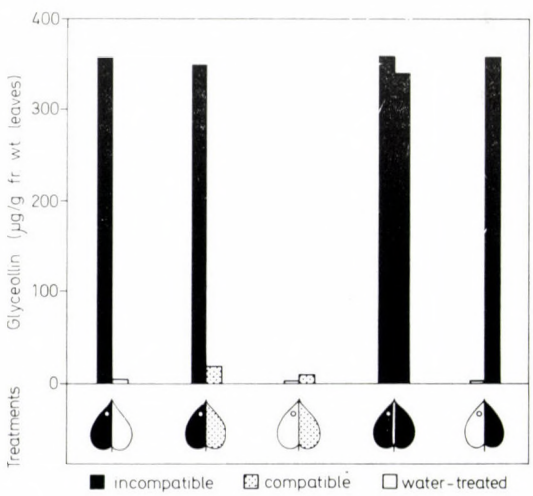

Fig. 5. Glyceollin content of cv. Harosoy soybean leaves 2 days after challenge following pre-treatment after 2 days. Left (punched) half-leaves were pre-treated with water or race 1 of *P. syringae* pv. *glycinea* (incompatible combination), and right half-leaves were challenged with race 1 and 2 (compatible combination)

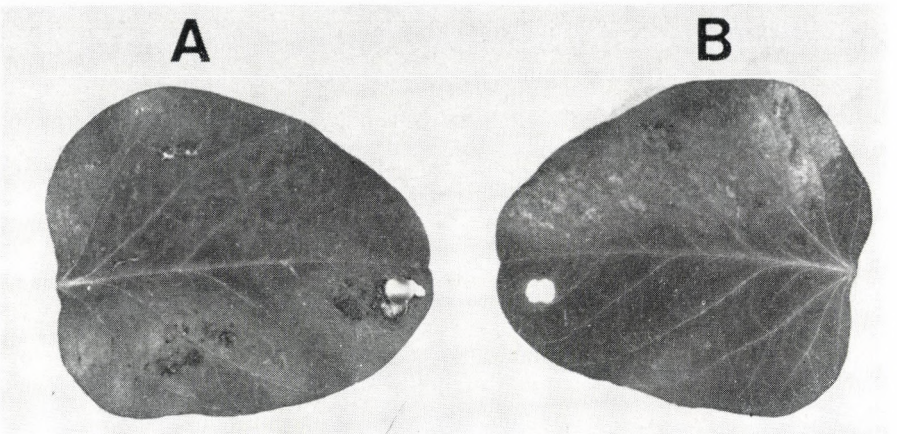

Fig. 6. Suppression (A) and lack of suppression (B) of disease symptom in cv. Harosoy soybean half-leaves challenged with race 2 of *P. syringae* pv. *glycinea* 2 days after pre-treatment of the opposite (punched) half-leaves with race 1 and water (control), respectivelv. Photograph was taken 6 days after challenge inoculation

Table 1

Glyceollin content* 2 days after challenge with races 1 and 2 of *P. syringae* pv. *glycinea* in cv. Harosoy soybean half-leaves opposite of those pre-inoculated with race 1

Challenge	Days after pre-inoculation		
	0	2	4
Race 1	335	340	340
Race 2	18	21	33

* In μg/g fresh weight of leaves

Conclusion

The original phytoalexin theory (Müller and Börger, 1941) is based upon the phenomenon of acquired resistance, concerning its local type. In many cases, there is a close correlation between phytoalexin accumulation induced by an avirulent pathogen (or by abiotic inducers) and the local protection against a virulent pathogen, as challenge, applied to the same site as the initial inoculum (cf. Kuć and Caruso, 1977). Vanderplank (1975) argues that phytoalexins may be of economical importance as resistance factors against secondary infections under natural conditions that accumulate as a consequence of the primary infections, to which plants in the field are continuously exposed. Even if phytoalexin accumulates at the site of local protection, it is hard to prove experimentally that phytoalexin can be in contact with the secondary inoculum.

As far as the systemic acquired resistance is concerned phytoalexins have not been shown to accumulate in unchallenged tissues remote from those treated with the inducer (e.g. Elliston et al., 1977). This is also the case with the 'opposite half-leaf' system we used. Not even the challenge with varietal pathogenic race 2 induced glyceollin production. Glyceollin in great amount accumulated only after challenge with varietal non-pathogenic race 1.

The protection aginst hypersensitive reaction by race 1 could be due to the restriction (ca. 1 log) of bacterial multiplication. The suppression of symptom caused by race 2, however, is not associated with restricted multiplication rate. Therefore, it seems that the suppression of symptom rather than that of bacterial multiplication is involved in this type of defence. Unlike Caruso and Kuć (1979), Doss and Hevesi (1981) claimed that systemic acquired resistance of cucumber to *P. lachrymans* was expressed in the suppression of symptoms and not in that of bacterial multiplication. Similarly, the size of necrotic lesions and not the number of them (i.e. infectivity of TMV) was inhibited in viral cross-protection (Balázs et al., 1977).

Such sorts of protection are obviously associated with necrotic cell death caused by the inducer. In general, the stronger and faster the necrotization the more valuable the protection. Inoculum concentrations of *P. syringae* pv. *glycinea* lower than 10^8 cells per ml (10^4, 10^6) induced valuable protection only if the concentration of challenge inoculum was alike (Hevesi and Érsek, unpublished). In other words, the stronger the stress, i.e. the hypersensitive reaction, the stronger the anti-stress elicited. This protection phenomenon cannot be attributed to either phytoalexin or to another single factor; there must be a series of processes (cf. Kuć, 1982).

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