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By

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Reactions of the perennial *Physalis peruviana* L. var. *macrocarpa* and the annual *Physalis pruinosa* L. to virus infection were studied. In artificial inoculation experiments the perennial *Physalis peruviana* L. var. *macrocarpa*, as a new virus host was found to be locally and systemically susceptible to six viruses: alfalfa mosaic virus (R/1 : : 1.3/18 : U/U : S/Ap), cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap), potato aucuba mosaic virus, (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus X (R/1 : ✳/6 : E/E : S/[Fu]), tobacco mosaic virus (R/1 : 2/5 : E/E : S/✳), tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne). The inoculated plants showed immunity from further six viruses (bean [common] mosaic virus, ✳/✳ : ✳/✳ : E/E : S/Ap; potato virus M, ✳/✳ ✳/✳ : E/E : S/Ap; potato virus S, ✳/✳ : ✳/✳ : E/E : S/Ap; radish mosaic virus, R/✳ : ✳/✳ : S/S : S/Cl; tobacco necrosis virus, R/✳ : ✳/✳ : S/S : S/Fu; turnip yellow mosaic virus, R/1 : 1.9/37 : S/S : S/Cl). *Physalis peruviana* L. var. *macrocarpa* plants were systemically susceptible to potato virus Y and locally susceptible to tobacco rattle virus. *Physalis pruinosa* L. responded with local and systemic symptoms to infection by seven viruses (alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco ring spot virus), while showed resistance to six other viruses (bean [common] mosaic virus, potato virus M, potato virus S, radish mosaic virus, tobacco necrosis virus, turnip yellow mosaic virus).

In an earlier paper (HORVÁTH, 1970) we gave account of the susceptibility of 23 *Physalis* species to 67 plant viruses and pointed out that of the species studied so far *Physalis floridana* Rydb., *Physalis pubescens* L. and *Physalis angulata* L. were the best known host plants of various plant viruses. Papers published since the first summarizing work presenting the relevant literary data and our own research results contributed new information to the knowledge of host plants belonging to the *Physalis* species.

In recent investigations LOVISOLO and BARTELS (1970) pointed out spontaneous infection by henbane mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap) in *Physalis alkekengi* L. (bladder cherry, winter cherry, Chinese lantern plant). According to the results of recent experiments carried out by FELDMAN and GRACIA (1972a) *Physalis angulata* L. (tooth leaved winter cherry) showed local susceptibility to infection by turnip mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap). *Physalis mendonica*

[1] First publication: Acta Phytopath. Acad. Sci. Hung. 5, 65—72 (1970).

Table 1

Additional data on the susceptibility of *Physalis* species to plant viruses

Physalis species	Viruses*	Literature
Ph. alkekengi L. (syn.: *Ph. franchetti* Mast.)	Henbane mosaic virus	LOVISOLO and BARTELS (1970)
	Tomato stolbur virus	SCHMELZER and WOLF (1971)
Ph. angulata L.**	Alfalfa mosaic virus	VERHOYEN (1964), HORVÁTH (1971, not published), SCHMELZER *et al.* (1973)
	Potato virus X	SCHMELZER and WOLF (1971)
	Turnip mosaic virus	FELDMAN and GRACIA (1972a)
Ph. elliotti Kunze	Tobacco ring spot virus	ANDERSON (1959)
Ph. floridana Rydb.	Carnation Italian ring spot virus Cymbidium ring spot virus Nasturtium ring spot virus Poplar mosaic virus Potato virus A Tomato bushy stunt virus Turnip crinkle virus White clover mosaic virus	SCHMELZER and WOLF (1971)
Ph. heterophylla Nees	*Physalis* mottle virus***	MOLINE and FRIES (1974)
Ph. ixocarpa Brot.	Potato virus Y Potato witches'broom virus	SCHMELZER and WOLF (1971)
	Beet pseudo-yellows virus	DUFFUS (1973)
Ph. lanceifolia Nees.	Potato leaf roll virus	SCHMELZER and WOLF (1971)
Ph. mendonica Phil.	Potato virus Y	FELDMAN and GRACIA (1972b)
Ph. peruviana L.	Nasturtium ring spot virus Potato leaf roll virus Potato witches'broom virus Tomato aspermy virus	SCHMELZER and WOLF (1971)
	Cherry leaf roll virus	WATERWORTH and LAWSON (1973)
Ph. pruinosa L.	Alfalfa mosaic virus	HORVÁTH (1971, not published), SCHMELZER *et al.* (1973)
	Potato leaf roll virus	SCHMELZER and WOLF (1971)
Ph. pubescens L.	Cabbage black ring spot virus Cuscuta B-virus (flower proliferation disease) Tomato stolbur virus	SCHMELZER and WOLF (1971)
Ph. subglabrata MacKenzie et Bush	Alfalfa mosaic virus	MILBRATH (1963)
Ph. wrightii	Beet pseudo-yellows virus	DUFFUS (1973)

* Tomato stolbur virus, potato witches'broom virus and Cuscuta B-virus diseases are known to be caused by *Mcyoplasma* infections.

** An unidentified virus was recovered from *Physalis angulata* L. with symptoms of yellow mottle and leaf distortion (cf. MOLINE and FRIES, 1972). The new virus was serologically related to belladonna mottle virus.

*** Strain of belladonna mottle virus (turnip yellow mosaic virus group).

Phil. proved to be a further natural host plant of potato virus Y (✳/✳ : ✳/✳ : : E/E : S/Ap) – to our knowledge a new virus host among *Physalis* species (FELDMAN and GRACIA, 1973b). Experimental data recently published by SCHMELZER et al. (1973) on the spontaneous susceptibility of *Physalis angulata* L. and *Physalis pruinosa* L. to alfalfa mosaic virus (R/l : 1.3/18 : U/U : S/Ap) confirm our results obtained in earlier experiments in the course of which the mentioned two *Physalis* species were found to be artificial host plants of alfalfa mosaic virus. In the excellent work summarizing the knowledge of European virus host plants SCHMELZER and WOLF (1971) gave information about new virus–*Physalis* relations. Recent data published after our first paper (HORVÁTH, 1970), are summed up in Table 1.; they complete the earlier observations on the virus susceptibility of *Physalis* species.

The present paper also gives account of the susceptibility and reaction of a *Physalis* species and a *Physalis* variety to further viruses.

Material and Method

The reactions of *Physalis peruviana* L. var. *macrocarpa* and *Physalis pruinosa* L. were studied in artificial inoculation experiments. In the experiments the following viruses were used: alfalfa mosaic virus (strain K_2, R/l : 1.3/18 : U/U : S/Ap; cf. BECZNER, 1972), bean (common) mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap; HORVÁTH, 1973a), cucumber mosaic virus (R/l : 1/18 : S/S : S/Ap; HORVÁTH, 1973a), potato aucuba mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap; HORVÁTH, 1972b), potato virus M (✳/✳ : ✳/✳ : E/E : S/Ap; HORVÁTH and DE BOKX, 1972), potato virus S (✳/✳ : ✳/✳ : E/E : S/Ap; HORVÁTH, 1972), potato virus X (R/l : ✳/6 : E/E : S/(Fu); HORVÁTH and BECZNER, 1968), potato virus Y (✳/✳ : ✳/✳ : E/E : S/Ap; veinal necrosis strain, cf. HORVÁTH, 1967), radish mosaic virus (R/✳ : ✳/✳ : S/S : S/Cl; HORVÁTH *et al.*, 1973), tobacco mosaic virus (strain U_1, R/l : 2/2 : E/E : S/✳; SIEGEL and WILDMAN, 1954), tobacco necrosis virus (strain *f* = TNV*f*, R/✳ : ✳/✳ : S/S : S/Fu; SZIRMAI, 1964), tobacco rattle virus (R/l : 2.3/5 : E/E : S/Ne; HORVÁTH, 1973), tobacco ring spot virus (R/l : 1.8/42 : S/S : S/Ne; HORVÁTH 1973) and turnip yellow mosaic virus (R/l : 1.9/37 : S/S : S/Cl; JURETIĆ, *et al.*, 1973). The pure viruses were kept in *Brassica rapa* L. var. *rapa* (radish mosaic virus, turnip yellow mosaic virus), *Nicotiana glutinosa* L. (tobacco rattle virus, potato aucuba mosaic virus), *Nicotiana tabacum* L. cv. *Samsun* (alfalfa mosaic virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus), *Nicotiana tabacum* L. cv. *Xanthi-nc* (tobacco ring spot virus), *Phaseolus vulgaris* L. cv. *Red Kidney* (bean [common] mosaic virus, tobacco necrosis virus), *Solanum tuberosum* L. cv. *Fortuna* (potato virus S), *Solanum tuberosum* L. cv. *Bintje* (potato virus M). Young *Physalis* plants were mechanically inoculated at the five- and six leaf stage. All plants were grown from seeds in pots and kept in an aphid-free greenhouse. Two or three leaves of each plant were inoculated using carborundum powder (500 mesh) as an abrasive.

The inoculated leaves were sprayed with tap water after inoculation. Symptoms found on the inoculated *Physalis* species were regularly registered. In addition, plants infected with potato virus M and potato virus S were serologically investigated too. The serological investigations were carried out about two or three weeks after virus inoculation. Serological tests were carried out by precipitin test (cf. WETTER, 1965; HORVÁTH, 1972a). With the exception of potato virus M and potato virus S all viruses were re-isolated from the inoculated and non-inoculated, or subsequently developed leaves of *Physalis* plants and tested on the following test plants: *Brassica rapa* L. var. *rapa* (radish mosaic virus, turnip yellow mosaic virus), *Gomphrena globosa* L. (potato virus X), *Nicotiana glutinosa* L. (potato aucuba mosaic virus, tobacco rattle virus), *Nicotiana tabacum* L. cv. *Samsun* and *Nicotiana tabacum* L. cv. *Bel 61—10* (alfalfa mosaic virus, potato virus Y), *Nicotiana tabacum* L. cv. *Xanthi-nc* (tobacco mosaic virus, tobacco ring spot virus), *Phaseolus vulgaris* L. cv. *Red Kidney* (alfalfa mosaic virus, bean [common] mosaic virus, tobacco necrosis virus) and *Tetragonia tetragonoides* (Pall.) O. Ktze and *Cucumis sativus* L. (cucumber mosaic virus). Before the re-isolation of viruses, inoculated *Physalis* leaves were washed with tap water after disinfection with 2 per cent NaOH solution.

Considering that to our best knowledge no data have been reported so far on the virus reactions of *Physalis peruviana* L. var. *macrocarpa*, we inoculated this plant with all the 14 viruses included in our experiments. The reaction of *Physalis pruinosa* L. to tobacco rattle virus is known (cf. SCHMELZER, 1957), therefore this virus was excluded from our experiments.

Results and Discussion

When studying the reactions of *Physalis peruviana* L. var. *macrocarpa* to various viruses we found the inoculated plants to show local and systemic susceptibility to six viruses (Fig. 1A, B and C), systemic susceptibility to one virus, local susceptibility to one virus (tobacco rattle virus), and immunity from further six viruses (Table 2). *Physalis pruinosa* L. (see Fig. 1D) proved to be locally and systemically susceptible to seven viruses and immune from six viruses (Table 3). In the artificial inoculation experiments particularly severe symptoms developed on plants inoculated with alfalfa mosaic virus, cucumber mosaic virus, potato virus Y, tobacco mosaic virus and tobacco ring spot virus.

Since the *Physalis* species and variety studied proved to be resistant to certain plant viruses (e.g. bean [common] mosaic virus, potato virus M, potato virus S, radish mosaic virus, tobacco necrosis virus, turnip yellow mosaic virus) and susceptible to others (e.g. alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tobacco ring spot virus), they can be regarded as satisfactory, and useful indicator and/or screening plants. To our knowledge and according to the results of the above described experiments *Physalis peruviana* L. var. *macrocarpa* is a new impor-

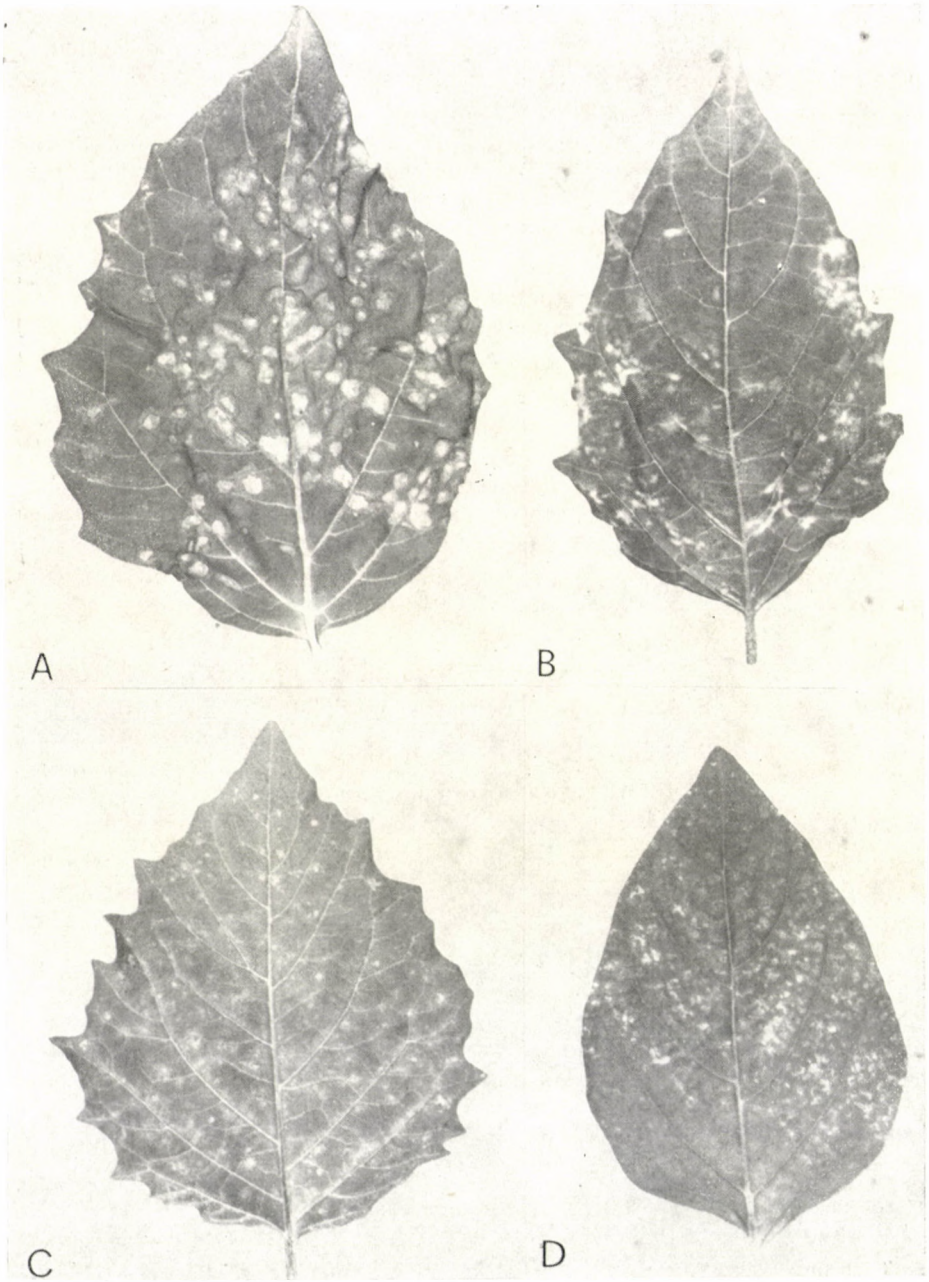

Fig. 1. *Physalis peruviana* L. var. *macrocarpa* leaves locally infected with tobacco mosaic virus (A), alfalfa mosaic virus (B), and potato aucuba mosaic virus (C). *Physalis pruinosa* L. leaf systemically infected with alfalfa mosaic virus (D)

Table 2

Reaction of *Physalis peruviana* L. var. *macrocarpa* to some plant viruses after mechanical inoculation

Viruses	Symptoms*	Results of the re-isolation or serological test of the investigated plant viruses**
Alfalfa mosaic virus	IL: no symptoms NIL: vein clearing, severe mosaic and yellowing	IL: positive NIL: positive
Bean (common) mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: vein clearing, yellowing with green islands NIL: severe mosaic and yellowing	IL: positive NIL: positive
Potato aucuba mosaic virus	IL: no symptoms NIL: chlorotic-necrotic spots, interveinal mosaic	IL: positive NIL: positive
Potato virus M	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Potato virus S	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Potato virus X	IL: chlorotic spots NIL: mosaic	IL: positive NIL: positive
Potato virus Y	IL: no symptoms NIL: severe mosaic	IL: negative NIL: positive
Radish mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco mosaic virus	IL: necrotic spots, leaf dropping NIL: necrotic lesions, top necrosis	IL: positive NIL: positive
Tobacco necrosis virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco rattle virus	IL: necrotic lesions NIL: no symptoms	IL: positive NIL: negative
Tobacco ring spot virus	IL: necrotic spots and rings NIL: chlorotic-necrotic spots and mosaic	IL: positive NIL: positive
Turnip yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

* IL: Inoculated leaves; NIL: non-inoculated leaves or subsequently developed leaves.

** Potato virus M and potato virus S were not re-isolated from the inoculated *Physalis* plants, but were serologically tested in the inoculated and non-inoculated leaves

Table 3

Reaction of *Physalis pruinosa* L. to some plant viruses after mechanical incolutation

Viruses	Symptoms*	Results of the re-isolation or serological test of the investigated plant viruses**
Alfalfa mosaic virus	IL: no symptoms NIL: severe mosaic and leaf deformation	IL: positive NIL: positive
Bean (common) mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: vein clearing, yellowing with green islands NIL: severe mosaic and yellowing	IL: positive NIL: positive
Potato aucuba mosaic virus	IL: no symptoms NIL: chlorotic-necrotic spots, intervenial mosaic	IL: positive NIL: positive
Potato virus M	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Potato virus S	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Potato virus X	IL: chlorotic spots or no symptoms NIL: mosaic	IL: positive NIL: positive
Potato virus Y	IL: no symptoms NIL: severe chlorotic spots, and mosaic	IL: positive NIL: positive
Radish mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco mosaic virus	IL: necrotic spots NIL: mosaic and leaf deformation	IL: positive NIL: positive
Tobacco necrosis virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco ring spot virus	IL: necrotic spots and rings NIL: chlorotic-necrotic spots and mosaic	IL: positive NIL: positive
Turnip yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

* IL: Inoculated leaves; NIL: non-inoculated leaves or subsequently developed leaves.

** Potato virus M and potato virus S were not reisolated from the inoculated *Physalis* plants, but were serological tested in the inoculated and non-incoluated leaves.

tant virus host. Its importance is increased by the fact that – as a perennial plant – it plays the part of a virus reservoir in the virus–host system which deserves special attention from a virus epidemiological point of view.

With our recent experiment results taken into account the *Physalis pruinosa* L. is susceptible to some 13 viruses; its susceptibility to five viruses (alfalfa mosaic virus; potato leaf roll virus, ✳/✳ : ✳/✳ : S/S / S,I/Ap; potato corky ring spot virus [syn.: tobacco rattle virus, R/l : 2.3/5 : E/E : S/Ne]; tobacco etch virus, ✳/✳ : ✳/✳ : E/E : S/Ap; and tobacco rattle virus) was pointed out earlier (cf. SCHMELZER, 1957; HORVÁTH, 1971 not published, SCHMELZER *et al.*, 1973, reviewed by THORNBERRY, 1966 and HORVÁTH, 1970 as well as by SCHMELZER and WOLF, 1971), while in our recent experiments it proved to be susceptible to further seven plant viruses.

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Reaction of *Physalis* Species to Plant Viruses

III. *Physalis floridana* Rydb. and *Physalis peruviana* L. as Immune and Screening Plants to Several Plant Viruses[1]

By

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In the course of artifical infection experiments the reaction of *Physalis floridana* Rydb. and *Physalis peruviana* L. plants to various viruses not yet studied was investigated. Studying the virus relations of the new *Physalis* hosts we found *Physalis floridana* Rydb. and the perennial *Physalis peruviana* L. to be resistant to bean (common) mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus M (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus S (✳/✳ : ✳/✳ : E/E : S/Ap), radish mosaic virus (R/✳ : ✳/✳ : S/S : S/Cl) and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : S/Cl), and *Physalis peruviana* L. — besides the above viruses — to tobacco necrosis virus (R/✳ : ✳/✳ : S/S : S/Fu). In our experiments the symptomatological aspects of reactions shown by the perennial *Physalis peruviana* L. to infections by some polyphagous viruses were also studied. We found that the *Physalis peruviana* L. was a local and systemic host plant to alfalfa mosaic virus (R/1 : 1.3/18 : U/U : S/Ap), cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap), tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne), potato aucuba mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus X (R/1 : ✳/6 : E/E : S/(Fu)), potato virus Y (✳/✳ : ✳/✳ : E/E : S/Ap) and tobacco mosaic virus (R/1 : 2/5 : E/E : S/✳).

On the ground of the susceptibility of *Physalis floridana* Rydb. to 45 viruses, and of *Physalis peruviana* L. to some 22 viruses (reviewed by HORVÁTH, 1970; 1974; SCHMELZER and WOLF, 1971), as well as their immunity from the viruses discussed in the present paper these plants seem to be suitable — as screening plants — not only for the identification but also for the separation of certain viruses.

In our first paper written on the virus susceptibility of *Physalis* species (cf. HORVÁTH, 1970) we summarized the experimental results obtained for *Physalis floridana* Rydb. and *Physalis peruviana* L. (syn.: *Physalis edulis* L., cape gooseberry). According to the data of the mentioned publication *Physalis floridana* Rydb., one of the best known and thoroughly studied virus hosts recommended for virus tests too, is susceptible to some 37 plant viruses. In a recently published excellent work SCHMELZER and WOLF (1971) completed our earlier published data by eight further viruses which we included in our second paper (HORVÁTH, 1974, see Table 1). According to the available data the *Physalis floridana* Rydb. has been proved to be host plant to some 45 viruses. Our own experiments and those performed by others have shown *Physalis peruviana* L. to be susceptible to 22

[1] Earlier publications: I. Acta Phytopath. Acad. Sci. Hung., *5*, 65—72 (1970); II. Acta Phytopath. Acad. Sci. Hung., *9*, 1—9 (1974)

viruses (HORVÁTH, 1970; SCHMELZER and WOLF, 1971; HORVÁTH, 1974). The importance of this latter plant is increased by the fact that as a perennial plant it can be a virus reservoir.

In our present paper we should like to give account of experiments carried out to study the reactions of the above two plants to further viruses not examined so far.

Material and Method

In our artificial infection experiments young *Physalis floridana* Rydb. and *Physalis peruviana* L. plants were inoculated with viruses that can be found among our virus cultures and to which the reactions of the above plants are not known as yet (cf. HORVÁTH, 1974). The *Physalis floridana* Rydb. plants were inoculated with bean (common) mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus M (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus S (✳/✳ : ✳/✳ : E/E : S/Ap), radish mosaic virus (R/✳ : ✳/✳ : S/S : S/Cl) and turnip yellow mosaic virus (R/l : : 1.9/37 : S/S : S/Cl). In infecting *Physalis peruviana* L. the following viruses were used: bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic, virus, tobacco necrosis virus and turnip yellow mosaic virus. In order to study the reactions of the perennial *Physalis peruviana* L. we carried out further infection experiments with three polyphagous, bacilliform and isomeric viruses, respectively (alfalfa mosaic virus, R/l : 1.3/18 : U/U : S/Ap; cucumber mosaic virus, R/l : : 1/18 : S/S : S/Ap; tobacco ring spot virus, R/l : 1.8/42 : S/S : S/Ne), as well as with potato aucuba mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap), potato virus X (R/l : ✳/6 : E/E : S/(Fu)), potato virus Y (✳/✳ : ✳/✳ : E/E : S/Ap), and with the polyphagous tobacco mosaic virus (R/l : 2/5 : E/E : S/✳). The virus reactions of *Physalis peruviana* L. plants have been known, however, from earlier studies (reviewed by HORVÁTH, 1970; SCHMELZER and WOLF, 1971).

As for the maintenance of the virus cultures – the methods of inoculation, the conditions of virus re-isolation – the same techniques were used as described in detail in our second publication (HORVÁTH, 1974).

Results and Discussion

In the course of studying the new *Physalis* host – virus relations we found *Physalis floridana* Rydb. and the perennial *Physalis peruviana* L. equally resistant to bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus, and *Physalis peruviana* L. – besides the above viruses – to tobacco necrosis virus as well.

In our artificial infection experiments we studied the symptomatological aspects of some important – though already known – susceptibility reactions of *Physalis peruviana* L. too. We found that the *Physalis peruviana* L. was a local

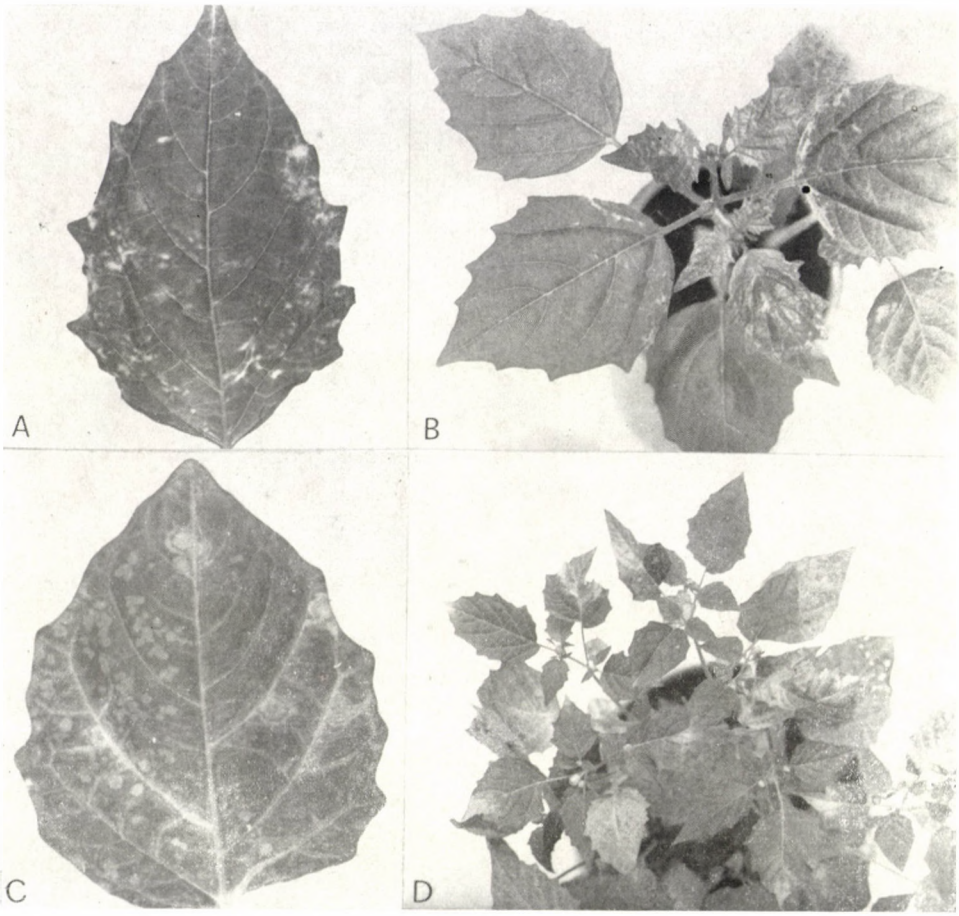

Fig. 1. Local (A and C) and systemic symptoms (B and D) on the leaves of *Physalis peruviana* L. (syn.: *Physalis edulis* L.) to several plant viruses. A and B: alfalfa mosaic virus; C and D: cucumber mosaic virus

and systemic host plant to alfalfa mosaic virus, cucumber mosaic virus (Fig. 1), tobacco ring spot virus, potato aucuba mosaic virus, potato virus X, potato virus Y and tobacco mosaic virus (Fig. 2). However, local symptoms could only be pointed out in plants inoculated with alfalfa mosaic virus (grey, irregular necrotic lesions appearing 2–3 days after inoculation; from the fifth day the inoculated leaves dried up but did not drop), tobacco ring spot virus (ring-shaped necrotic lesions of 3–4 mm diameter) and cucumber mosaic virus (not necrotic but typical mosaic spots of 0.5 cm diameter appearing 5 days after inoculation). Potato aucuba mosaic virus, potato virus X and potato virus Y did not cause symptoms on the leaves of inoculated plants, but the viruses could be re-isolated from the inoculated leaves. It was remarkable that the *Physalis peruviana* L. which had

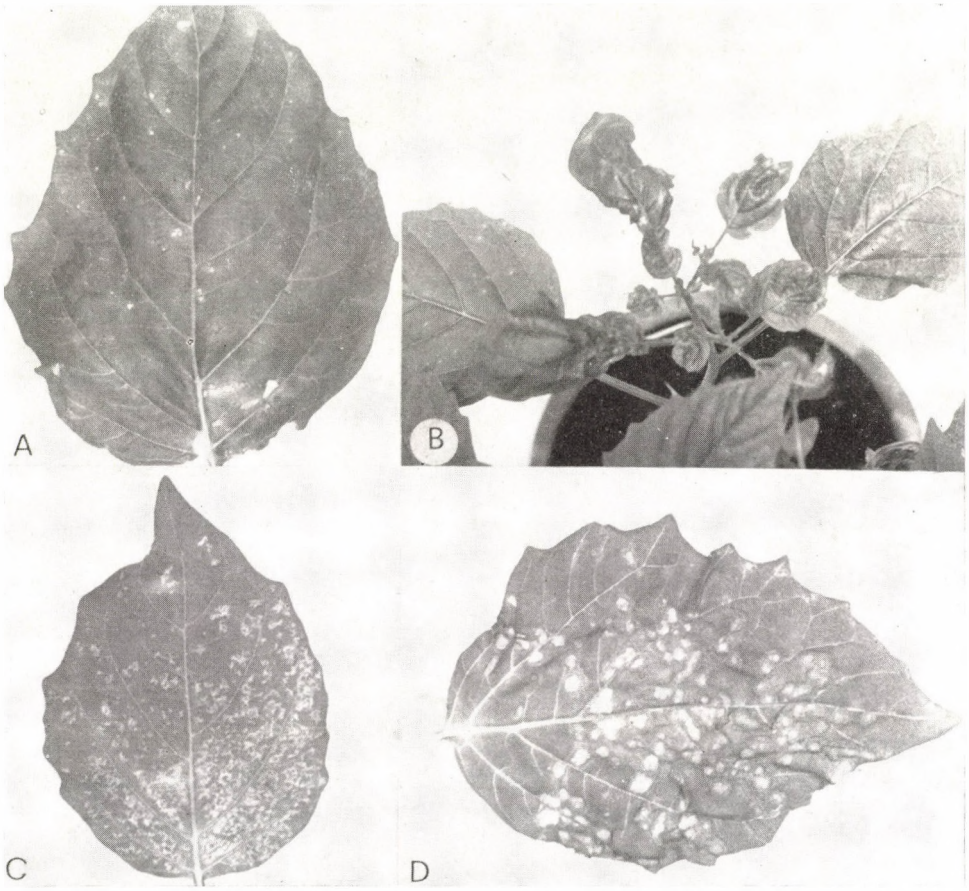

Fig. 2. Local (A and D) and systemic symptoms (B and C) on the leaves of *Physalis peruviana* L. (syn.: *Physalis edulis* L.) to several plant viruses. A and B: tobacco ring spot virus; C: potato aucuba mosaic virus; D: tobacco mosaic virus

proved to be a symptom-free host plant to potato virus X and potato virus Y in our earlier experiments (cf. HORVÁTH, 1970), in recent experiments showed symptoms of systemic intervenial mosaic and yellow vein in some inoculated plants. The above symptoms were, however, found only in a part of the inoculated plants, the rest proved to be latent hosts in our recent experiments too. Besides the local symptoms intensive systemic symptoms were caused by the alfalfa mosaic virus (ochre spots, severe intervenial mosaic), potato aucuba mosaic virus (severe vein clearing, mosaic), tobacco mosaic virus (severe vein clearing after 6 days, leaf blisters, severe leaf deformation, considerable – some 50 per cent – growth inhibition), tobacco ring spot virus (vein clearing, vein banding, leaf deformation, growth reduction, necrotic rings) and cucumber mosaic virus (vein clearing ochre

mosaic, 30 – 40 per cent growth inhibition). The highly intensive symptoms appearing in the artificial inoculation experiments suggested a particularly susceptible host–virus relation. The susceptibility of *Physalis peruviana* L. to alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus and tobacco ring spot virus, as well as its immunity from bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus, tobacco necrosis virus and turnip yellow mosaic virus, calls attention on one hand to a possible natural survival of viruses in the relation of susceptible and perennial hosts to viruses, on the other hand to the possibility of separating the mentioned viruses from susceptible/immune host–virus systems.

Acknowledgements

The author is indebted to the managements of the Botanical Gardens Vácrátót, Budapest (Hungary), Nijmegen (The Netherlands), Bruxelles (Belgium), Kuibyshev (U.S.S.R.), and to the Institut für Gemüsebau, Weihenstephan (German Federal Republic), Institut für Phytopathologie der Friedrich-Schiller Universität, Jena (German Democratic Republic) and Horticultural University, Kiskunhalas (Hungary).

Thanks are also due to Miss M. Bollán and Miss K. Molnár for their valuable technical assistance.

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Effect of Different Ringspot Viruses on the Flowering Period and Fruit Set of Montmorency and Pándy Sour Cherries I.

By

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The effect of different ringspot viruses (chlorotic ringspot virus, chlorotic-necrotic ringspot virus, necrotic ringspot virus) on the flowering period and fruit set of Montmorency and Pándy-48 clone sour cherries was studied in 1972–73.

The flowering period of infected Montmorency trees was longer, that of the Pándy-48 clone took place 3–5 days earlier than the blooming of virus-free trees.

The effect on the fertilization of Montmorency trees appeared in a varying reduction of fruit set, according to the different infecting ringspot viruses involved.

In the case of perfectly self-sterile Pándy-48 sour cherry clone fruit set was influenced in a higher degree by the pollen donor variety, than by virus-free or virus-infected pollen.

Different ringspot viruses have become extremely widespread in stone fruits – especially in cherries – in Hungary. The effect of these viruses on the flowering period and the degree of fertilization has not yet been studied there.

According to SAVIO (1970) bud break, flowering and fruit ripening took place 2–5 days later on trees infected by necrotic ringspot virus.

In 1971, a delay of 3–4 days could be observed in the flowering period of ringspot virus infected Montmorency cherry trees by the authors in Hungary.

The yield-decreasing effect of virus infection was described by RASMUSSEN and CATION as early as 1942, as well as by MOORE in 1946.

KLOS and PARKER (1960) established, that cropping in ringspot virus infected Montmorency sour cherry was lower by 50 per cent.

According to WAY and GILMER (1963) a 10–15 per cent fruit set reduction could be observed on Montmorency, English Morello, Early Richmond sour cherry varieties infected by yellows (ringspot viruses).

POSNETTE *et al.* (1968) studying the effect of necrotic ringspot virus infection on the fruiting behaviour of Early Rivers, Merton Bigarreau and Merton Heart sweet cherry varieties concluded to results similar to those of WAY and GILMER (1963).

Materials and Methods

The observation of the flowering period and fertilization of sour cherries were made on trees situated at the experimental station, Érd-Elvira of the Horticultural Research Institute.

The Montmorency sour cherry trees were planted in 1969; spaced at 1.2×1.5 meters, with 100 trees each, per root-stock species, in a virus nursery. Root-stock species comprehended *Prunus avium*, *Prunus cerasus* and *Prunus mahaleb*. The trees were shaped to shrub trees.

Five trees each of Pándy-48 sour cherry clone, as well as of Germersdorfi-57 sweet cherry and Cigány 7 sour cherry clones – both used as pollen donors – were planted in 1955. The trees were standing on *Prunus mahaleb* root-stocks at a spacing of 8×8 meters and were cut to standard trees. Collection and varietal evaluation of the Pándy, Germersdorfi, and Cigány cherry clones was carried out by BRÓZIK (1969).

Description and identification on ringspot viruses

The Montmorency trees standing on different root-stocks were inoculated simultaneously with budding by NÉMETH (1965) for other experimental purposes with viruses partly isolated and identified by her and partly kindly given by dr. KEGLER (Aschersleben). They were the following:

necrotic ringspot virus (isolate originating from Aschersleben)
chlorotic-necrotic ringspot virus (originating from mahaleb cherry Apagy)
chlorotic ringspot virus (originating from mahaleb cherry, Egervár).

The trees used in the experiment of 1972 were controlled by tests on herbaceous indicators, for there had been some flowering the previous year and natural pollen infection could have had occurred.

It was established, that the trees infected by the viruses mentioned and the control-trees, as well as those used as mother plants were virus-free.

Infected and virus-free trees of Pándy-48, Germersdorfi-57 and Cigány- 7 cherry clones were also tested in a similar way.

In the course of transmissions made with the pollen of infected trees (Cucumber-test) it was established, that the pollen was infected by the different ringspot viruses used.

Observation of flowering

Observations on bloom dynamics of Montmorency sour cherry were carried out on 400 – 1000 blossoms on each 3 trees per ringspot virus; every 3 days the opened blossoms – suitable for pollination – were counted. The period of full bloom was determined by the proportion of opened blossoms per total number of blossoms and represented diagrammatically (Fig. 1).

Systematical phenologic data of the flowering period of the Pándy-48 clone were not recorded, as only one virus-free tree could be found, the others were totally infected by natural spread of the virus. The main periods of flowering were noted.

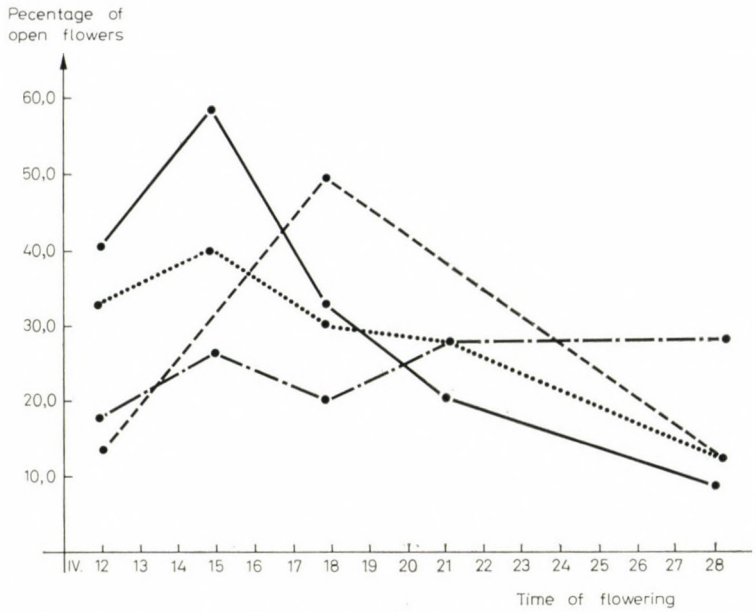

Fig. 1. Trend of full bloom of virus free trees and those infected by different ringspot viruses in the sour cherry variety Montmomercy (Érd-Elvira, 1972). Key to signs used — control; - - - infected by chlorotic ringspot virus; — . — . — infected by chlorotic-necrotic ringspot virus; . . . infected by necrotic ringspot virus

Fertilization experiments

In the sour cherry variety Montmorency the fertilization studies included investigations on free pollination in virus-free trees on one hand, and in trees infected by various ringspot viruses, on the other, as well as in the case of infected pollens of self-pollination and self-fertilization per each ringspot virus.

The flowers were isolated by paper bags and radically castrated. The different pollinating variations were carried out with flowers in full bloom, showing secretion-activity.

Fruit set was evaluated in three development stages: after the first fruit drop, after the June fruit drop as well as at harvesting mature fruits.

The percentage of fruit set was compared to the total number of pollinated stigmae. Frequency of the number of mature fruits per isolators was grouped separately.

The method of fertilization experiments on the Pándy-48 sour cherry clone was identical with the one used in the case of Montmorency cherry, with the exception that — Pándy sour cherry being completely self sterile — castration was omitted, and the stigmae pollinated with collected pollen. Virus-free and infected trees of the sweet cherry clone Germersdorfi-57 and the sour cherry clone Cigány-7 were used as pollen donors.

Results

1. Effect of the different ring spot viruses on flowering period

Observations concerning the time of full bloom of Montmorency cherry are represented in Fig. 1.

Table 1

Trend of fruit set of Montmorency cherry trees infected by different ringspot viruses

(Érd-Elvira, 1971—1972)

♀	♂	Number of pollinated stigmae		Number of mature fruits		Percentage of mature fruits to pollinated stigmae	
Montmorency		1971	1972	1971	1972	1971	1972
Virus-free	Virus-free	—	168	—	28	—	16.7
Virus-free	Chlorotic ringspot virus infected	89	277	16	26	17.9	9.4
Virus-free	Chlorotic–necrotic ringspot virus infected	196	320	21	16	10.7	5.0
Virus-free	Necrotic ringspot virus infected	97	203	1	7	1.0	3.4
Virus-free	Free pollination	—	371		66	—	17.8
Chlorotic ringspot virus infected	Free pollination	—	398		63	—	15.8
Chlorotic–necrotic ringspot virus infected	Free pollination	—	666		20	—	3.0
Necrotic ringspot virus infected	Free pollination	—	322		6	—	2.0

According to the observation data full bloom was differently influenced by the different ringspot viruses. The time of main flowering was longer in the consequence of infection by all the three ringspot viruses.

The flowering period of ringspot infected Pándy-48 trees was observed — on the contrary — to occur 3—5 days earlier in 1973 on infected than on virus-free trees.

2. Effect of the different ringspot viruses on fruit set

The results of fertilization experiments in the case of Montmorency sour cherry are represented in Table 1, while those of the clone Pándy-48 can be found in Table 2.

Table 2

Fertilization experiments on a virus-free tree of the sour cherry clone Pándy-48

(Érd-Elvira, 1972)

Pollen-donor variety	Number of pollinated stigmae	Number of pollinated mature fruits	Per cent of fruit set	Number of isolators
Germersdorfi-57 virus-free	382	121	31.7	15
Germersdorfi-57 infected by ring spot virus	421	110	28.5	17
Sourcherry Cigány-7 virus-free	301	9	3.0	11
Sour cherry Cigány-7 infected by ringspot virus	568	14	2.5	22
Free pollination of sour cherry Pándy-48	1039	28	2.7	—

Per cents of fruit set of Montmorency variety varied from year to year. The effect of individual ring spot viruses on fruit set was different still fruit set was the poorest in each treatment on trees infected by necrotic ringspot virus. Differences in fertilization had the same tendency without exception in every year and with every root-stock. From the point of view of root-stock effect, trees standing on *Prunus cerasus* were cropping least.

Germersdorfi-57 as pollen donor variety induced better fruit-set on the sour cherry clone Pándy-48, than Cigány-7. Pollination with virus infected pollen of both varieties resulted in poorer fruit set than with virus-free pollen (Tables 1 and 2.).

Discussion

The effect of ringspot viruses on the flowering period of the different sour cherry varieties was likely to vary with the variety according to observations although the fact, that Montmorency and Pándy trees in the experiment were infected by different isolates of Prunus ringspot virus has to be considered. Montmorency trees infected by either of the ringspot viruses bloomed equally later while full bloom of infected Pándy-48 clone set in earlier than that of virus-free trees. Montmorency variety is known to be sensitive to ringspot viruses, it is used as indicator plant. Pándy sour cherry on the contrary as shown by the experiment seemed to be rather tolerant to these same viruses. Thus the observations pointed out that the effect of ringspot viruses on the flowering period had to be studied by varieties resp. even by clones.

The alterations observed in the flowering period caused by ringspot virus infection may be significant in cultivation praxis, as it may influence the simultaneous bloom of certain varieties and may reduce the mutual pollination of self-sterile ones.

Experiments on flowering dynamics – considering the short time of observation and the complex problems involved – are to be continued.

The effect of ringspot viruses on fruit set and cropping capacity varied according to the phase of infection. Trees of the varieties used were in a chronic stage of the disease; Cigány-7 sour cherry trees were even partly declining. The effect varied according to ringspot viruses and differences increased by the varying susceptibility of the varieties. Fruit-set was least in self-fertile varieties, it even approached sterility if necrotic ringspot virus infection occurred. (E.g. mature fruit harvested represented only 2 per cent of pollinated flowers as compared to the 16.7 percentage crop from pollination by virus-free pollen.)

In the case of Pándy-48 clone the effect of the suitability of pollen donor variety was more significant than that of virus infection.

The results of fertilization studies indicated, that a preference given to tolero-resistant clones at selection may be advantegeous, as postinfection of a virus-free plantation would result in minor economic loss.

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Effect of Different Ringspot Viruses on the Physiological and Morphological Properties of Montmorency Sour Cherry Pollen II.

By

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The effect of different ringspot viruses (chlorotic ringspot, chlorotic–necrotic ringspot and necrotic ringspot virus) on Montmorency pollen morphology and physiology was studied in 1971–72. The average diameter of pollen infected by any of the three ringspot viruses was smaller, than that of the virus-free pollen. The proportion of pollens with a diameter below 35 μ was lowest in virus-free pollen, and twice as high in necrotic ringspot virus infected pollen. The proportion of the regular (I-shaped) pollens showed a similar trend. The number of readily stained (viable) pollens was the highest in each case in the virus-free flowers, and lowest in necrotic ringspot virus infected ones.

Percentage of pollen-tube formation varied from year to year and according to the infecting ringspot viruses. It was highest in Montmorency trees growing on mahaleb-cherry root-stocks and lowest on those standing on sour cherry root-stocks.

Proportion of tube formation decreases considerably in pollen infected by ringspot viruses. Lowest percentage of tube formation was found in necrotic ringspot virus infected pollen.

Fertilization experiments of the authors on Montmorency and Pándy-48 sour cherries clearly showed a reduction of cropping caused by ringspot virus infection. WAY and GILMER reported in 1963, that "yellows" infected pollen induced a 10–75 per cent lower fruit set. Effect of ringspot viruses on the pollen of peaches was studied by MARENAUD (1965) and MARENAUD and DESVIGNES (1965). They established that infected pollen was less viable and the proportion of pollen tube growth diminished.

According to SAVIO (1970) morphological changes of pollen might be connected with the disturbing effect of ringspot viruses on meiosis of the mother cells.

The incidence of round shaped pollens was higher and pollen tube growth reduced in the case of plum pox virus infection (MINOIU, 1968; MACOVEI, 1970; PEJKIC and SUTIC, 1970).

LEMOINE (1970) stated that in virus infected pear flowers, less anthers could be counted, than in virus-free ones.

Materials and Methods

Pollen morphological examinations were performed in the Horticultural Research Institute in 1971 and 1972. Pollen used in examinations originated from Montmorency trees, planted in 1969 at a spacing of 1.2 × 1.5 m, standing on three

Table 1

Distribution of Montmorency sour cherry pollen according to size

(Érd-Elvira, 1972)

Treatment	Longest diameter of pollen (μ)																							
	9	13	14	16	18	19	20	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Virus-free	—	1	—	—	—	3	1	1	—	—	10	3	1	1	1	—	10	24	3	1	3	1	2	29
Chlorotic ringspot virus infected	—	—	—	—	1	4	2	1	—	2	11	5	3	2	1	3	10	25	7	2	3	—	5	30
Chlorotic–necrotic ringspot virus infected	1	5	1	2	—	11	—	1	1	—	16	9	1	5	1	5	6	32	6	—	4	—	4	18
Necrotic ringspot virus infected	—	4	—	—	2	11	7	1	—	1	12	7	2	2	—	7	6	45	18	3	3	—	7	26

Treatment	Longest diameter of pollen (μ)																						Average diameter (μ)
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	57	58	60	63	65	
Virus-free	5	11	16	1	19	56	32	14	8	4	10	21	4	1	—	1	—	1	—	1	—	—	42
Chlorotic ringspot virus infected	—	5	6	12	14	54	34	7	4	1	17	22	—	2	1	2	—	1	—	—	1	—	40
Chlorotic–necrotic ringspot virus infected	4	8	7	5	12	42	27	5	17	6	12	16	3	2	—	2	—	2	—	—	—	1	38
Necrotic ringspot virus infected	3	6	8	5	15	31	25	4	9	2	8	14	2	1	—	—	1	1	1	—	—	—	37

root-stock species (*Prunus mahaleb*, *P. cerasus*, *P. avium*) and infected by necrotic ringspot, chlorotic–necrotic ringspot and chlorotic ringspot viruses, 100 trees each per root-stock species and infecting viruses.

Transmission concerning identification of viruses were described in a previous paper (VÉRTESY and NYÉKI, 1974). Each of the ringspot viruses are known to belong to the ILAR viruses and are transmitted by pollen and by seeds. There are data reporting nematode transmission of necrotic ringspot virus.

Pollen used for experimental purposes originated from blossoms in white bud stage. 50 blossoms were used for the examination of pollen tube formation.

Pollen was collected in a similar way for morphological studies. Pollen of 10 flowers per treatment was kept in lactic acid until time was convenient for examination.

Pollen tube growth studies were performed in a 15 per cent saccharose solution over a four hours period, by hanging drop method at 20–25°C. Results were evaluated by counting 1000 pollens per treatment.

Size was established by measuring 300–300 pollens. Size was represented by the longest diameter.

The physiological value of pollen was examined by lactophenol cotton blue staining on 300 pollens per treatment. It was classified according to staining intensity into groups of very well stained, less stained and not stained pollens.

Results

Morphology of pollen

Distribution of Montmorency pollen according to size is shown in Table 1, classification into different size groups in Table 2, and alterations of shape in Figure 1.

Table 2

Classification of Montmorency pollen according to different size groups *(Érd-Elvira, 1972)*

Treatment	No. %	Smaller than 35 μ (small)	Between 35–45 μ (medium size)	Larger than 45 μ (large)	Pollen suitable for pollination in the percentage of total pollen amount (35–65 μ)
Virus-free	No.	60	175	65	
	%	20.0	58.3	21.7	80.0
Chlorotic ring-spot virus infected	No.	79	163	58	
	%	26.3	54.3	19.4	73.7
Chlorotic–necrotic ring-spot virus infected	No.	104	130	66	
	%	34.7	43.3	22.0	65.3
Necrotic ring spot virus infected	No.	128	129	43	
	%	42.7	43.0	14.3	57.3

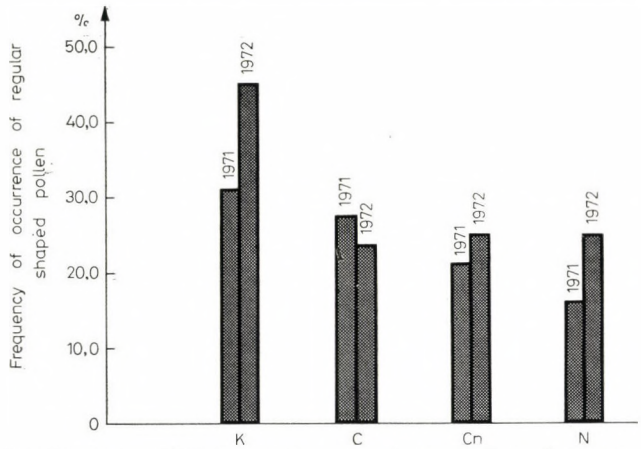

Fig. 1. Alterations in shape of Montmorency sour cherry pollen (Érd-Elvira, 1971—1972). Key to signs K = virusfree; C = chlorotic ringspot virus infected; CN = chlorotic–necrotic ringspot virus infected; N = necrotic ringspot virus infected

Diameter of pollen

The diameter of pollen infected by different ringspot viruses was smaller, than that of virus-free pollen, and smallest in pollen, infected by necrotic ringspot virus.

Pollens were classified into three groups according to the variable length of their diameter: below 35 μ, 35 μ to 45 μ and over 45 μ. According to data of the tables proportion of small pollens (with a diam below 35 μ) was lowest in virus-free

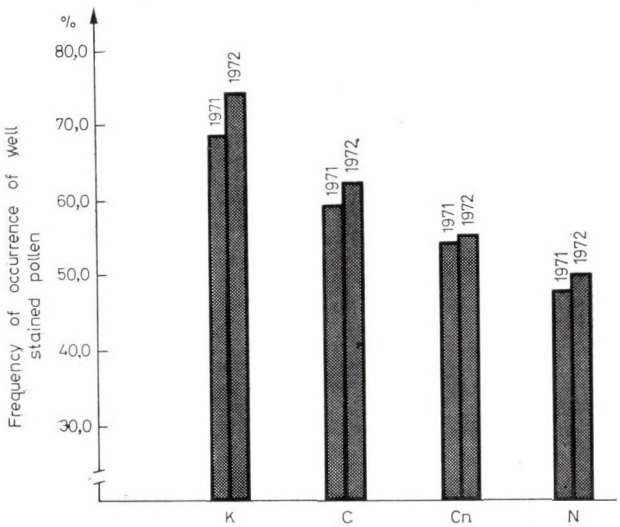

Fig. 2. Alteration in viability of Montmorency sour cherry pollen (Érd-Elvira, 1971—1972). Key to signs K = virusfree; C = chlorotic ringspot virus infected; CN = chlorotic–necrotic ringspot virus infected; N = necrotic ringspot virus infected

pollen. This proportion grew larger in pollen infected by different ringspot viruses, and was largest – twice as large as that of virus-free samples – in necrotic ringspot virus infected ones. Proportion of pollen suitable for fertilization on the contrary showed an inversed trend (Table 2).

Shape of pollen

Proportion of regular shaped pollens was highest in virus-free and lowest in necrotic ringspot virus infected samples. Percentage of regular (Δ-shaped) pollens is shown in Figure 1.

Viability of pollen

Data of pollen-viability are represented in Figure 2. Proportion of viable pollens changed of course from year to year, the percentage of readily stained (viable) pollens, however, was highest in virus-free samples in each case. The different ringspot viruses affected viability of Montmorency pollen in a variable degree; but it was mostly reduced by necrotic ringspot virus infection.

Tube growth of pollen

Proportion of pollen-tube formation is shown in Table 3. It changed according to samples originating from trees on different root-stocks, and was highest in

Table 3

Percentage of pollen-tube formation in virus-free and ringspot virus infected Montmorency sour cherry pollen

(Érd-Elvira, 1972)

Root-stock	Treatment	Percentage of pollen-tube formation	In percentage of virus-free pollen
Prunus cerasus	Virus-free	42.5	100.0
	Chlorotic ringspot virus infected	21.3	50.1
	Chlorotic–necrotic ringspot virus infected	11.9	28.0
	Necrotic ringspot virus infected	5.1	12.0
Prunus mahaleb	Virus-free	68.3	100.0
	Chlorotic ringspot virus infected	35.2	51.5
	Chlorotic–necrotic ringspot virus infected	17.4	25.4
	Necrotic ringspot virus infected	12.3	18.0
Prunus avium	Virus-free	51.8	100.0
	Chlorotic ringspot virus infected	29.7	57.3
	Chlorotic–necrotic ringspot virus infected	15.2	29.3
	Necrotic ringspot virus infected	7.8	15.1

each treatment in the pollen from trees on mahaleb cherry and lowest in pollen from those standing on *Prunus cerasus* root-stocks. The highest percentage of pollen-tube formation, however, was established in virus-free pollen originating from any of the three root-stock species, and lowest in every case of necrotic ringspot virus infected pollen.

Discussion

It seems possible to conclude from the morphological properties (size and shape) of pollen to circumstances of pollen formation (meiosis) and the process of cell division. Pollen quality is determined by the amount of irregular (deformed, elongated, dwarfed) and undeveloped pollen grains as well as by tube forming capacity.

Production of pollen grains, smaller than the average size characteristic of the Montmorency variety is due to disturbed meiosis. These pollen grains generally were shown to be physiologically inactive, their plasma being not or scarcely stained. Medium and large pollens may be considered mostly as physiologically active and are valuable from the point of view of fertilization (NYÉKI: unpublished data).

Our experiments showed similar results: if the percentage of small pollens (below 35 μ) was high, the degree of viability – efficiency of fertilization – decreased.

The examined virus-free pollen contained a low proportion of irregular grains, and pollen-tube forming capacity proved to be over 30 per cent.

The quality of Montmorency pollen was considerably lower and with a constant tendency decreased – as compared to the virus-free control – in every year of the experiment. Fertilization and recent studies showed a great difference between the damaging effect of chlorotic and chlorotic–necrotic ringspot virus, which in some cases reached and even outdid the differences between the most dangerous necrotic ringspot and any of the other two viruses. Pollen was invariably least affected by chlorotic ringspot virus infection. On the basis of these facts interference phenomena between chlorotic and chlorotic–necrotic ringspot viruses are worth being taken into consideration, in order to increase cropping security in infected orchards.

It may be presumed that pistils of ringspot virus infected flowers undergo morphological and physiological changes similar to that of the pollen.

Pistils of virus-free flowers were but partly or not fertilized by infected pollen and early and heavy fruit drop was observed after fruit set.

The main mode of spread of ringspot viruses is known to be pollen and seed transmission; the problem however, at what a degree of fertilization pollen-transmission may occur is yet to be solved.

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Inhibition of the Hypersensitive Reaction of Tobacco Leaves to Bacteria by Foreign Proteins

By

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Bovine serum albumin, human serum albumin and thrombin solutions were injected into tobacco leaves 24 hours prior to inoculation with bacteria. The proteins inhibited the development of the hypersensitive response (HR) induced by *Pseudomonas lachrymans*, *P. mors-prunorum* and *P. syringae*. Treatment of leaves with casein hydrolysate did not alter HR to bacteria injected 24 hours later. From these results is seems that not only protein fractions from the pathogen or host plant suppress HR development, but other foreign proteins are also effective. The effect of albumin and thrombin on HR, induced by bacteria, seems to be aspecific.

The hypersensitive reaction of plants is one of the most widespread defense mechanisms against pathogenic bacteria, fungi and viruses (STAKMAN, 1915; KLEMENT and LOVREKOVICH, 1961; HOLMES, 1931). Tissue necrosis occurs in most incompatible host–parasite relationships and is thought to be involved in localization of the pathogen. Recently the role of tissue necrosis in resistance has been heavily criticized (KIRÁLY *et al.*, 1972). Whether or not necrosis is a consequence or cause of plant disease resistance, the mechanism of tissue necrosis seems to be very similar in all hypersensitive reactions (VISNYOVSZKY *et al.*, 1973).

HR induced by bacteria has been suppressed by pretreatment with heat-killed pathogenic or living saprophytic bacteria (LOVREKOVICH and FARKAS, 1965; LOZANO and SEQUIERA, 1970). This so-called "premunity" is not bound to constituents of bacterial cells, because cell-free extracts of bacteria also inhibited HR to challenge inocula (SEQUIERA and AINSLIE, 1969). The nature of the mechanism involved in suppression of HR is unknown. WACEK and SEQUIRA (1973) examined the possibility that glycoproteins of *Pseudomonas solanaceanum* are involved in protection against HR development. Recent investigations of SÜLE *et al.* (1973) suggest that inhibition of HR is non-specific because choroplast fractions and protein extracts from healthy tobacco and cabbage leaves inhibited development of HR.

Investigations of HR, induced by pathogenic viruses showed that heat-killed pathogenic or living saprophytic bacteria also markedly inhibited HR to viruses (LOVREKOVICH and FARKAS, 1965; KLEMENT *et al.*, 1966). This effect was non-specific because various foreign proteins (albumin, casein, urease) also suppressed

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HR to virus particles. Albumin influenced both the degree of resistance to infection, apparent as a decrease in number of local lesions and also virus multiplication as indicated by a decrease in size of local lesions.

We decided to investigate whether various foreign proteins inhibited HR, induced by plant pathogenic bacteria in a similar manner to that reported with pathogenic viruses.

Materials and Methods

Half leaves of 3 month old intact tobacco plants were infiltrated with 1% (w/v) tap water solutions of bovine serum albumin, thrombin and casein hydrolysate, using the method of KLEMENT (1963). The opposite half leaf served as a control and was infiltrated with tap water which evaporated from the leaves in a few hours. Plants were incubated for 24 hours at 25°C and interveinal leaf panels were then injected with suspensions of *P. lachrymans*, *P. mors-prunorum* or *P. syringae*. Bacteria were suspended in tap water and concentrations of suspensions adjusted using a spectrophotometer (Unicam SP 800) to 10^8, 10^7, 5×10^6 and 10^6 cells/ml. The treated plants were kept in a chamber at 25°C.

Results and Discussion

Rapid necrosis of infected tissues, typical of HR, developed 24 hours after inoculation with suspensions of bacteria at concentrations of 5×10^6 cells/ml or greater. No symptoms appeared following inoculation with inoculum concentrations of 10^6 cells/ml. On half leaves treated with proteins the development of HR to bacteria was affected in various ways.

Treatment with albumin (both human and bovine serum albumin) inhibited tissue necrosis associated with HR induced by *P. lachrymans*, *P. mors-prunonum* or *P. syringae* suspensions at all concentrations. However, treatment with thrombin resulted in inhibition of development of HR only at inoculum concentrations of 5×10^6 cells/ml. In addition, thrombin had a toxic effect on leaf tissues which appeared one day after infiltration. Treatment with casein hydrolysate, containing only polypeptides and amino acids, did not inhibit the development of HR. In some cases, casein promoted the development of HR to *P. syringae* suspensions at low concentrations (10^6 cells/ml).

Protection by foreign proteins was affected by leaf age and was most complete on older, fully expanded leaves. In many cases foreign proteins were unable to inhibit HR at the highest inoculum concentration (10^8 cell/ml) on young, newly developed leaves.

These results indicate a similarity in HR, induced by bacteria and viruses because both are prevented by treatment with foreign protein. The "premunity" is possibly induced in different ways by proteins of host or parasite, and by foreign

proteins. Because of the similar effect of the proteins it seems that inhibition of HR is an aspecific response. It is likely that proteins do not produce true "premunity" but merely prevent the development of necrosis in plant tissues.

In our previous investigations we found that albumin inhibited the multiplication of compatible bacteria *in vivo* and suppressed the development of disease symptoms. Similar results were found in this investigation of the incompatible host–parasite relationship. There is therefore an interesting similarity between the inhibition of development of the susceptible reaction and tissue necrosis associated with the hypersensitive response.

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Apoplexy of Apricots

III. Relationship of Winter Frost and the Bacterial Canker and Die-back of Apricots

By

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As it has been shown earlier (ROZSNYAY and KLEMENT, 1973) the death of phloem and cambium tissues was responsible for the symptoms of apoplexy disease of apricots. *Pseudomonas syringae* can attack these tissues only after leaf-fall during the winter period. Therefore, the role of the dormant stage of apricots as well as the effect of winter frost in disease development were investigated under laboratory and orchards conditions.

Before the leaf-fall we could not induce damage in phloem and cambium by the bacterium. This was possible only during the dormant stage of trees.

Both orchard and laboratory experiments showed that the frost was an important factor in the development of the symptoms of apoplexy. Artificial inoculations with the bacterium were carried out in different climatic zones of Hungary and Yugoslavia, namely at the mediterranean area of Yugoslavia where the winter temperature does not fall below $-5°C$ and in the northern part of Yugoslavia and in Hungary where the winter frost $-15°C$ is generally common. The cankers and die-back on the artificially inoculated trees developed only at the localities where the winter frost was colder.

Laboratory experiments were conducted using two-year-old cut branches. The bacterium was able to invade the phloem and cambium, however typical phloem and cambium necrosis formed if the bacterium had enough time to multiply in these tissues before the frost. Neither the frost nor the bacterium alone caused tissue necrosis of phloem and cambium. Xylem infection was independent of the frost effect.

The most sensitive organ of apricot to phloem infection was the stem, and less sensitive were the branches, especially, the crotches.

As it seems, in Central Europe where the winter frost is usual and heavy, the appearance of cankers and die-back symptoms on apricot trees are more frequen than at the mediterranean and southern parts of Europe.

CROSSE (1953), DYE (1954), HEYNS (1960), VASILKOVA (1964), ARSENIJEVIC (1968) and KLEMENT *et al.* (1972) demonstrated that the bacterial canker and die-back caused by *Pseudomonas syringae* van Hall play an important role in the apoplexy disease of apricots. The symptoms of apoplexy, however, only develop if the bacterial infection occurs after the leaf-fall, in the winter months (KLEMENT *et al.*, 1972). Then as a consequence of infection, the phloem and cambium die in the course of winter by spring. If the phloem and cambium necrosis is not of such an extent that it engirdles the branch or the trunk, then in summer following the

infection, the surrounding healthy tissues try to overgrow the necrotized area resulting in cankerous wounds. When cambial tissue necrosis completely engirdles the branch or the trunk, the healthy parts, above the infected area suddenly die in spring or in the course of summer. If the cambial necrosis only girdles one or two branches, then partial apoplexy occurs, but if the trunk is engirdled, the result is complete apoplexy, that is complete destruction of the tree.

When the inoculation experiments were carried out in greenhouse conditions during winter period, the destruction of cortical tissues did not take place.

Numerous observations and statistical surveys prove that the apoplexy disease is characteristically much higher after colder winters. The role of winter injury in the formation of apricot apoplexy has been proved also by NYUJTÓ and TOMCSÁNYI (1959). Following the hard winter of 1956, the per cent of the apoplexy diseases markedly increased;

	1954	1955	1956	1957	1958
complete apoplexy	4.62	3.87	8.30	4.80	5.71
partial apoplexy	2.63	2.31	13.10	1.12	2.68

The importance of the role of winter frost is demonstrated by the observation that in Yugoslavia along the Adriatic coast where winter frost is only mild, die-back of apricots occurs rarely. This is confirmed by the fact that in warmer mediterranean-like regions such as Spain or California, the occurrence of die-back is very limited in contrast with the more northern apricot growing areas, where the damage is rather high.

From these observations many research workers and fruit growers have come to the conclusion that one the most important causes of apricot apoplexy is the winter frost. That is why we found it advisable to study the role of winter frost in bacterial die-back and their interaction.

Methods

Experiments were carried out under field and laboratory conditions. The experiments in orchards were carried out in Hungary and Yugoslavia.

In Hungary the experiments were conducted in two co-operative orchards. 8 year-old apricot trees of very good condition and 10-year-old ones of good condition were inoculated at Dunavecse and Kiskunlacháza, respectively.

The inoculations were done from the middle of October, 1970 to the middle of December (shown in Figs 2 and 3) at two-week intervals. The suspension of *Pseudomonas syringae* AP_7 strain isolated from apricot trees was applied to a wounded surface with a brush. The inoculations were done on different parts of the trees:

a) on the trunk, 10–15 cm above soil level
b) on the trunk, 10–15 cm below the crown

c) in the crotches

d) in the lateral main branches

The inoculations on the trunk were carried out as follows: the bark was cut 0.5 – 1 cm deep till the xylem with a saw at three different sides at the same level. In the crotches the wound was cut with a saw. The inoculation of thicker branches was achieved through V-shaped cuts. These wounds were immediately brushed with the bacterial suspension after wounding. The wounds on the control trees were treated with water only. For every treatment, 3 trees at a time were inoculated at Dunavecse and 2 at Kiskunlacháza. Altogether, 125 trees including the controls were used in the experiment. The evaluation of the experiment took place in the following year in spring and summer. The extension of the phloem necrosis caused by the bacterium, that is the length of the cankers from the site of inoculation was measured in cm.

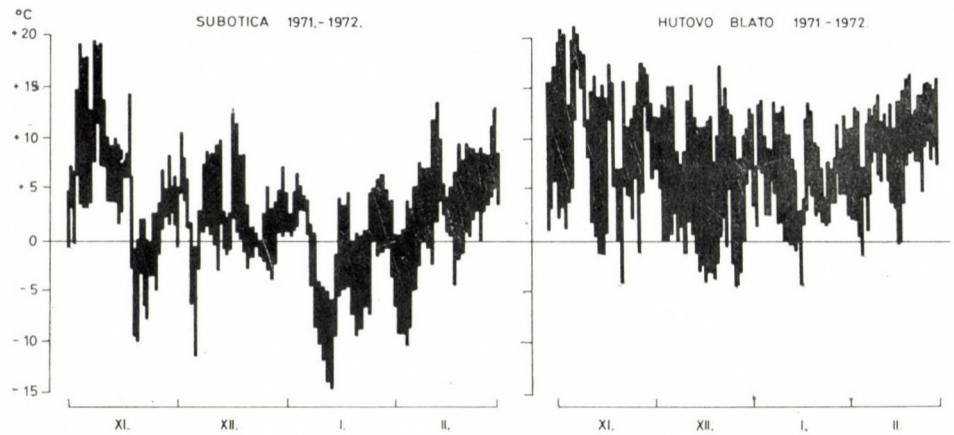

Fig. 1. Fluctuation in temperature in winter of 1971 – 72 at Subotica and Hutovo Blato (Yugoslavia)

In Yugoslavia the inoculations were conducted in two different climatic zones. One location was in the southern part of the country, close to the Adriatic sea, in the region of Hutovo Blato under mediterranean climate where winter temperature did not go below $-5°C$. The other experimental orchard was in North Yugoslavia close to the Hungarian border, in Subotica where the winter temperature went below $-15°C$. The inoculations were conducted in 1971 at two periods of time. In Hutovo Blato on the 19th November and 17th December. Subotica on 9th November and 14th December. The winter temperature changes in the regions close to the experimental areas were registered by meteorological stations (Fig. 1). The inoculation of the trees on the trunk was carried out 10 cm below the crown with two bacterial strains, one Hungarian isolate (AP_7) and one Yugoslavian strain (96). The 10^7 cell/ml bacterial suspension prepared from a 48 hr old bacterial culture brushed on a horizontal 5 – 8 cm long, V-shaped freshly made wound.

Evaluation of the experiments was carried out one year later, in November 1972. The length of the necrotized phloem was measured in cm.

Laboratory experiments: In laboratory experiments after leaf-fall, 30 cm long, 2-year-old freshly gathered cut branches were put into tap water or sterilized sand. The upper part of cut branches was inoculated on the freshly cut surface with a 10^7 cell/ml suspension of *Pseudomonas syringae* AP_7 strain. The control branches were brushed only with water. The branches before or after inoculation, depending on the experimental purpose, were placed in a chamber at $5-8°C$ for 3 days. After the cold treatment cut branches were again put back to a laboratory environment at $+15°C$ (Fig. 7). Three weeks after the inoculation the branches were cut longitudinally, the damage of phloem and xylem was established from the brown discolouration of these tissues.

Table 1

The size of necrotized phloem on the trunk of apricot trees inoculated with *Pseudomonas syringae*

Locality	Minimal temperature in 1971–72	The data of the inoculation	The strain used for inoculation	The size of the necrotized phloem in cm*
Hutovo Blato	−4°C	November 19	AP_7	0.2
			96	10.2
			Control	0.0
		December 17	AP_7	0.4
			96	5.4
			Control	0.0
Subotica	−15°C	November 9	AP_7	24.5
			96	92.4
			Control	0.0
		December 14	AP_7	7.0
			96	17.0
			Control	0.0

* Average of 10 inoculated sites.

Results

Experiments in orchards: Figures 2 and 3 demonstrate the results of the experiments carried out in Hungary. The two experimental orchards at Dunavecse and Kiskunlacháza gave the same results. When the bacterial inoculation was carried out before the leaf-fall, cortical tissue necrosis did not occur. On the contrary, the inoculations, carried out after the leaf-fall, caused destruction of phloem and cambium followed by the formation of large cankers, at the inoculated

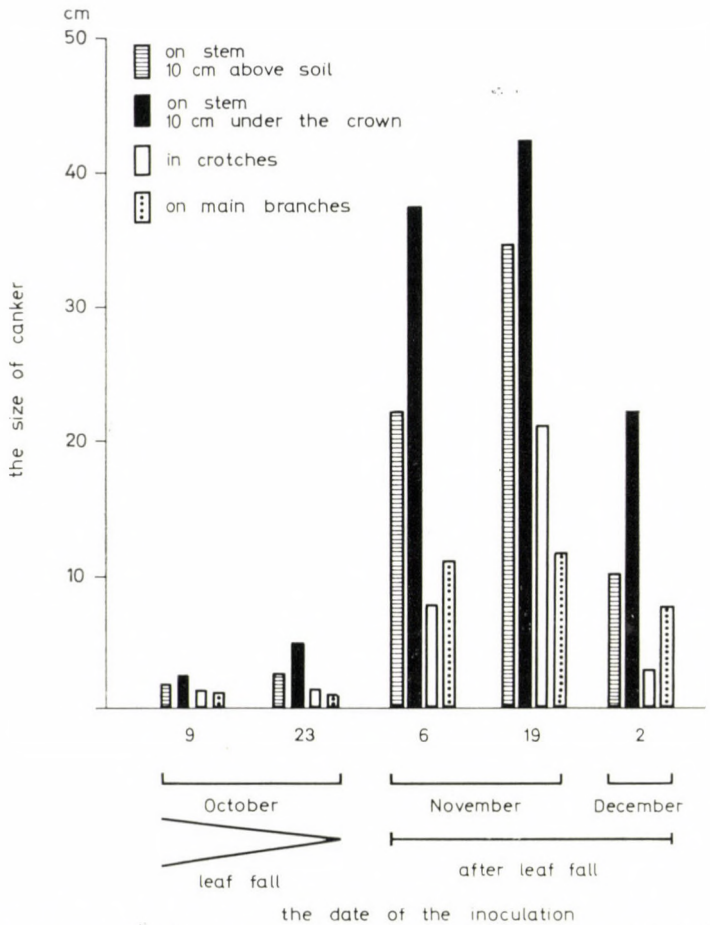

Fig. 2. The size of the cortical tissue necrosis on 8-year-old apricot tree inoculated with *Pseudomonas syringae* at Dunavecse (Hungary)

sites the following summer or in more serious cases, the partial or complete die-back of the trees. The extent of destruction strongly depends on the date of inoculations and the temperature during the infection. After the inoculations the first frosty days began after December 4 (0° −2°C) and the temperatures on December 24 and 25 were −8°C and −12°C, respectively.

The more the opportunity of bacteria to increase in the tissues after the leaf-fall and before the stronger frosts, the larger the destruction. In the trunks inoculated in November the destruction was so serious, that 50−90% of each tree died at the beginning of the following summer (Fig. 4). In the later inoculations (in December) the length of the cankers was not so extensive as in the November inoculations.

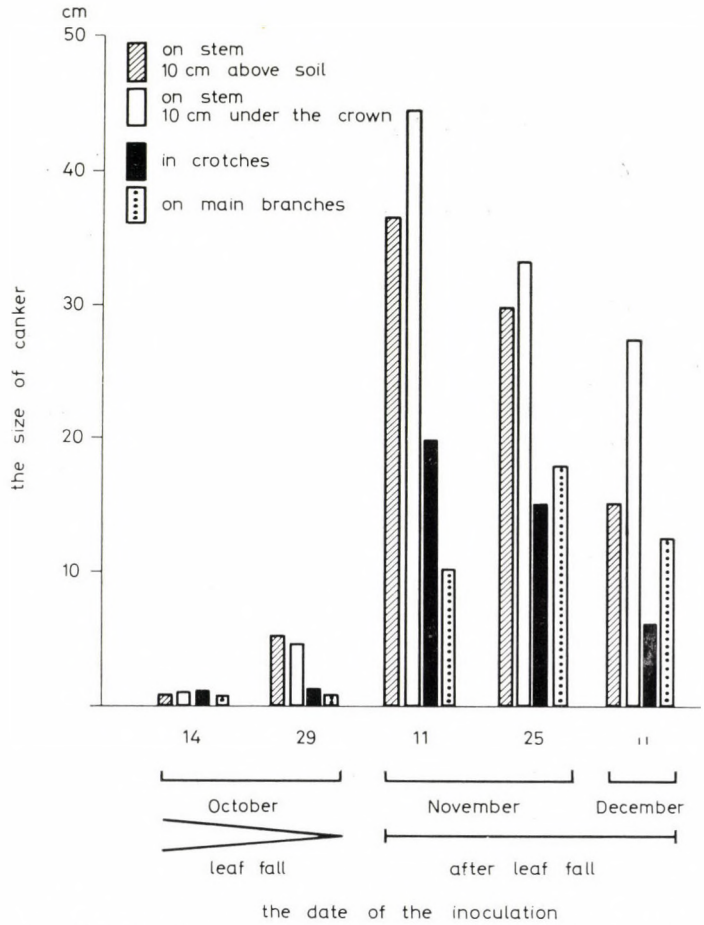

Fig. 3. The size of the cortical tissue necrosis on 10-year-old apricot trees inoculated with *Pseudomonas syringae* at Kiskunlacháza (Hungary)

The susceptibility of certain parts of the apricot trees varied (Figs 2 and 3), and accordingly, most susceptible was the trunk. Trunk inoculation just below the crown had more serious symptoms than those close to the soil level. The crotch was the least susceptible. Compared to this, larger cankerous necrosis formed on the main lateral branches but they did not reach the susceptibility of the trunk. The experiments in Hungary led to the following conclusions. 1. As a consequence of bacterial infection, necrosis of the cortical tissues occurs only during the winter period after the leaf-fall. The experiments supported our earlier investigations according to which the phloem tissue and cambium are not damaged during the vegetation period (KLEMENT *et al.*, 1972). 2. The most sensitive part of the tree to bacterial infection is the trunk.

Fig. 4 8-year-old 2 apricot trees inoculated on 19th November, 1971 with the bacterium photographed the following August.

Fig. 5. Phloem necrosis does not occur or it is minimal at the inoculated site of trunk Hutovo Blato (Southern Yugoslavia)

The inoculations in Yugoslavia were carried out in a warmer and a colder climatic zone. In the Adriatic coast of a warmer area Hutovo Blato, the lowest winter temperature rarely was below 0°C but never went below −4°C. In the northern Yugoslavian orchards at Subotica, the cold winter reached even −15°C

(Fig. 1). The inoculations were carried out on the trunk below the crown. The extent of cankers is shown in Table 1. The data refer to the bark destruction on 10 trees. Where the winter temperature did not go below $-5°C$, the phloem necrosis did not occur or it was only minimal (Fig. 5). In inoculations carried out in colder areas, the phloem necrosis spread characteristically (Fig. 6).

In different climatic zones the results of inoculations prove that 1. the extent of the bacterial canker depends on the strength of winter frost; 2. without bacterial infection phloem necrosis does not develop.

Fig. 6. Extensive cortical tissue necrosis developed on the trunk inoculated with *Pseudomonas syringae* at Subotica (Northern Yugoslavia)

Laboratory experiments: In laboratory experiments we wanted to find out whether the frost creates susceptibility for development of the disease or the effect of frost injury is a consequence of the bacterial infection. The experiments were, therefore, conducted under controlled conditions.

On the effect of the different treatments the phloem, cambium and xylem tissues of cut apricot branches did not become infected similarly (Fig. 7).

a) When the apricot cut branches afters inoculation were kept at $+15°C$ until the end of the experiment, only the xylem became infected but the cortical tissues remained healthy.

b) If the cut branches, immediately after inoculation, were put into a chamber at $-5°C$ for 3 days and thereafter at a normal temperature ($+15°C$) for 20 days, again only the xylem became damaged by the bacteria in a length of 10–15 cm. The phloem and cambium remained healthy.

c) Before inoculation the cut branches were stored at $-5°C$ for 3 days and following inoculation were kept under normal temperature ($+15°C$) until the end of the experiment. In this case, too, only the xylem tissue became necrotic.

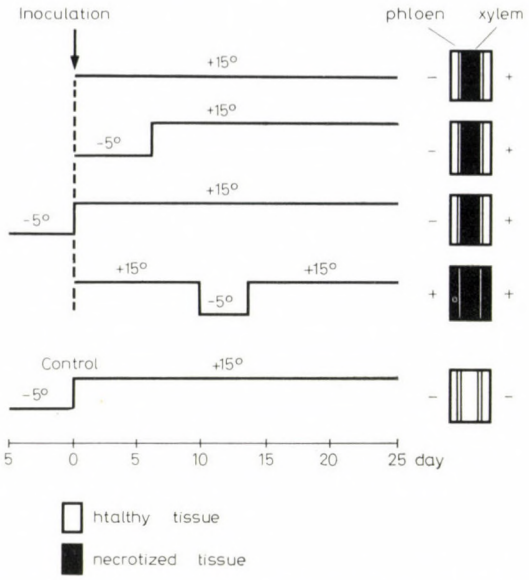

Fig. 7. Effect of frost on the phloem and xylem of apricot branches inoculated with *Pseudomonas syringae*

d) Branches were kept under a temperature of +15°C for 10 days after the inoculation and then for 3 days under −5°C. After this frost effect they were kept again under normal conditions for 8 days. On the effect of treatment, the phloem and cambium became brown 5−10 cm long from the inoculation site. In the xylem, the brown discolouration spread 10−12 cm.

The uninoculated control branches were placed under the same temperature effects and in this case there was no discolouration in the tissues.

Summarizing these experiments it can be concluded that 1. the necrosis both of the phloem and the cambium occurred only when the bacterium had enough time to increase at a warmer temperature before the frost. 2. When the *Pseudomonas syringae* could not multiply before the frost effect, the phloem and cambium necrosis did not appear and only the xylem tissue was damaged. 3. Frost injury did not arise without bacterial infection.

The same experiments were repeated at the end of August. We wanted to determine whether the frost or the physiological state of the tree after the leaf-fall plays the decisive role in the development of phloem and cambium necrosis.

These experiment were carried out on leafless shoots or branches. The results prove that if the inoculation is carried out during the vegetative period, the bacterium cannot damage the cortical tissues even if later are exposed to the frost effect. The results pointed out the fact that the phloem and cambial necrosis, as a consequence of bacterial infection, can only take place in the period after the leaf-fall. The laboratory experiments prove that 1. the physiological state after the leaf-fall is suitable for phloem and cambium infection 2. the winter frost does not create

a susceptibility to the disease, but quite the contrary, the cortical tissues, infected with *Pseudomonas syringae*, become sensitive to the frost.

Discussion

In the experiments conducted in previous years, it was definitely established that the appearance of the apoplexy symptoms of apricots is followed by the phloem and cambium necrosis (ROZSNYAY and KLEMENT, 1973). Therefore, in our experiments we studied the factors, necessary for the development of the necrosis and the role of these facors in apoplexy.

Many research workers consider the winter frost as the significant factor. According to this hypothesis, the south-side of the trunk and the branches warm up on sunny days and, as a consequence of the sharp night temperature drop, they become damaged. This type of necrosis was called "frost injury".

In Hungary NYUJTÓ and TOMCSÁNYI (1959) examined 40 000 apricot trees. On the trees with complete apoplexy the "frost injury" was 78%, on partially apoplectic trees 84%, whereas on healthy trees it was 47%. This also added to the fact, why apricot apoplexy was attributed to the frost effect.

These observations proved to be partially true in our experiments, too. True, because the winter frost evidently has a considerable role in bacterial die-back of apricot. The frost alone, without bacterial infection, does not cause tissue necrosis. Therefore, the pathogen has a primary role, the frost only takes part in forming the final symptoms. It is necessary to remark that on the trees, inoculated with *P. syringae* the phloem necrosis could not be distinguished from the "frost necrosis". The phloem necrosis formed only on the sites of bacterial inoculation. So, it is not known whether the so-called frost necrosis is really the consequence of frost.

However the pathogen and frost together caused cortical tissue destruction only in that case if the bacterium had had time to multiply at a higher temperature before the frost. There are many possibilities in nature since in winter the frost days are followed by $+5°C - +10°C$ temperatures. At such times the pathogen can multiply and on the effect of a later frost the cortical tissue necrosis can begin.

Practical observations indicate that cankerous wounds on the trunk are more common on the south-side of trees.

In our experiments, on artificially inoculated trees this observation proved true. This can probably be explained by the fact that on the sunny side the trunk warms up much better which is favourable for the multiplication of the pathogen. These experimental evidences likewise show that our opinion about "frost necrosis" have to be changed to a certain extent.

Our conclusions were proved by the 1971–72 and 1972–73 mild winters. In Hungary as a consequence of the mild winters there was practically no bacterial die-back in 1972 and 1973 and in this way apoplexy disease of apricot trees was characteristically less than in the earlier years. Apoplexy which occurred could be caused by *Cytospora cincta* (ROZSNYAY and KLEMENT, 1973).

The physiological state of the tree after the leaf-fall similarly has a decisive role in apricot apoplexy. With *Ps. syringae* we could only reproduce the symptoms of apoplexy disease in the inoculations after the leaf-fall. The physiological basis of this phenomenon is not known yet.

PANAGOPOULOS and CROSSE (1964) studied the blossom blight of pear trees which is also caused by *P. syringae*. They established that the flowers, which were exposed to $-1-2°C$ frost became more susceptible to the disease.

Partial or total die-back is very common in apricots. Our experiments have proved that the trunk is the most susceptible to the disease (Figs 2, 3). On the trunk, the necroses are 2 or 3 times larger than, for example, on the branches. This also makes us realize that after the fall the trunk should be protected from all kinds of injury in the dormancy period. Injury by mechanical means may be dangerous too. Moreover it may be possible that rabbits have a part in spreading the disease with their bites too.

Apricot trees require regular pruning. In our earlier work (KLEMENT *et al.*, 1972 and ROZSNYAY and KLEMENT, 1973) we proved that pruning has important role in apoplexy disease of apricots. Usually, in apricot orchards the pruning is carried out after the fall in the winter months just when experimental evidence shows that the trees are in most danger. At this time apricot trees are most susceptible and the environmental factors are also most favourable for developing the disease. Therefore, according to our investigation evidence so far, the winter pruning should be replaced by spring pruning.

The physiological role of frost effect in the case of bacterial infection is under investigation at present.

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Sur Une Nouvelle Espèce de *Curvularia*

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Dans le cadre des recherches effectuées par l'un d'entre nous sur les organismes fongiques transmis par les semences, il a été isolé an 1970 un *Curvularia* sur les graines de *Coriandrum sativum* L. en provenance de l'Inde. Ce champignon, suivant les critères pris en considération par plusieurs auteurs (BOEDIJN, 1933; ELLIS,[2] 1966, 1971), apparaît morphologiquement différent des espèces de ce genre jusqu'à maintenant décrites. Nous proposons d'en faire un espèce nouvelle dont le diagnose latine est la suivante:

Curvularia hexamera VEGH et BENOIT *spec. nov.*

Coloniae in substrato ficticio gelosato (P. D. A.) effusae, olivaceae deinde nigro-olivaceae (sensu SACCARDO, in *Chromotaxia*) et cinereae.

Hyphae ramosae, septatae, leves vel aliquot locibus tenuiter verruculosae, hyalinae deinde melleae vel umbrinae, 2–8 μ in diametro.

Stromata cylindrata, simplicia, atra, usque ad 3 mm alta.

Conidiophorae solitariae vel paucae-fasciculatae, interdum moderate ramosae at plerumque simplices, rectae vel flexuosae, rarius geniculatae, septatae, ortae ab apice lateribusque hypharum, umbrinae, in apicem pallidiores, 3–7 μ latae, usque ad 220 μ longae, plerumque leves, interdum tenuiter verruculosae in basim.

Conidia levia, acropleurogena (id est orta ab apice lateribusque conidiophororum), recta vel paulum curvata, obovoidea vel late fusiformia-ellipsoidea, 2–7-septata, plerumque 5-septata, cellulis extimis subhyalinis, cellulis intermediis plus minus pallide umbrinis, 2 (interdum 1) cellulis mediis inflatioribus et saepe crassius umbrinis, hilo incluso vel perraro leviteter eminenti; 20–47 × 10–17 μ (mediet. 33 × 12). Germinatio conidiorum bipolaris, raro unipolaris.

Hab. in seminibus *Coriandri sativi* L. incubatis in aere humido, India, an. 1969.

Typus servatus in cultura viva in Comm. Mycol. Instit. ad Kew in Britannia, sub numero accessionis C. M. I. 136 795.

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[2] Nos remerciements vont au Dr. ELLIS qui a bien voulu examiner notre souche et confirmer qu'elle semble n'appartenir à aucune espèce précédemment décrite et à Monsieur C. LAMARQUE, professeur agrégé, pour l'aide apportée à la rédaction de la diagnose latine.

Aspect cultural

L'incubation est faite à 25°C et, sauf indication contraire, en éclairement continu sur milieu P. D. A.

Cultures de 2 7 3 jours

Le champignon produit un mycélium aérien blanc qui prend une teinte gris-olivâtre au centre de la colonie.

Cultures de 7 jours

La colonie (fig. 1), arrondie à contour entier ou très faiblement lobé par endroits, présente une mince marge blanche, une zone intermédiaire olivacé foncé et une partie centrale grise d'étendue variable, généralement plus cotonneuse et parfois épaisse. La sporulation est déjà assez importante mais, contrairement à ce que l'on observe sur milieu gélosé à 1% de malt, aucune formation d'ébauches stromatiques n'est encore visible macroscopiquement. Le diamètre de la culture est en moyenne de 7 cm.

Sur malt et sur Czapek gélosés, la croissance linéaire du mycélium est respectivement un peu plus faible et un peu plus rapide.

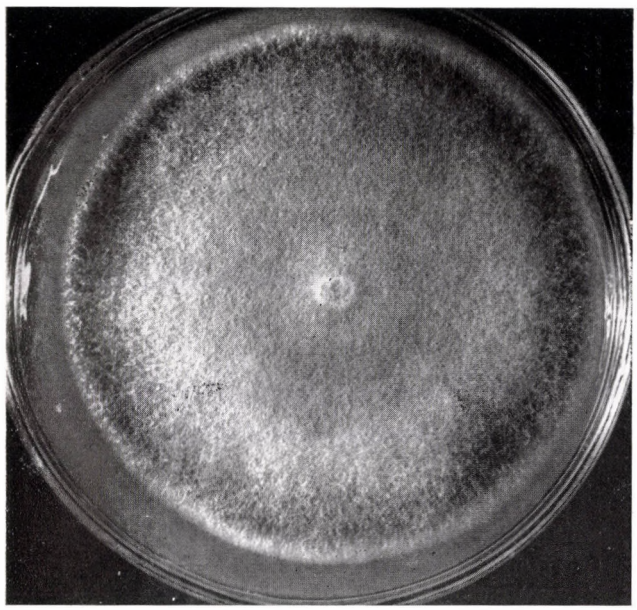


Fig. 1. Culture du *Curvularia hexamera* VEGH et BENOIT sur milieu P.D.A. en éclairement continu à 25°C après 7 jours d'incubation

En éclairement alterné, la colonie présente des zones concentriques régulières.

Vue par-dessous, la culture est noir olivacé et entourée d'un mince bord blanc.

Cultures de 25 jours

La face supérieure de la colonie est noir olivacé avec une grande zone centrale grise et des cristaux allongés sont présents dans la gélose.

La formation des stromas et la sporulation sont abondantes et il en est de même sur malt à 1% et sur Czapek.

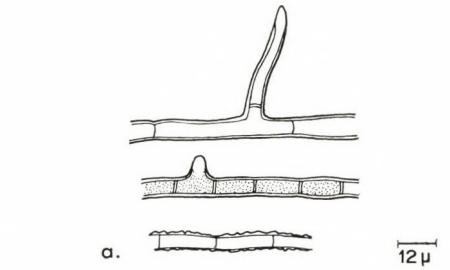
Caractères microscopiques

Le mycélium végétatif est composé de filaments cylindriques de 2 à 8 μ de diamètre, ramifiés, abondamment cloisonnés, à paroi généralement lisse mais qui peut être finement verruqueuse par endroits (fig. 2, a). Les filaments jeunes sont hyalins, puis ils deviennent subhyalins, de couleur miel ou brune, sauf à leurs parties apicales qui restent claires.

Les colonettes stromatiques sont érigées, droites ou arquées, cylindriques, simples, noires (fig. 2, b); elles mesurent jusqu'à 3 mm de haut et environ 300 μ de large, mais leur base présente parfois un renflement qui peut atteindre 500 μ. Sur malt à 1%, elles sont égales ou supérieures à 5 mm. Sur Czapek, elles ont à peu près la même hauteur mais se ramifient généralement avec l'âge, souvent dichotomiquement (fig. 2, b et 3). Elles portent, en particulier sur Czapek, de nombreux conidiophores et des conidies. Ces stromas ont été aussi observés sur grains de riz.

Les conidiophores, solitaires ou groupés, le plus souvent simples mais parfois faiblement ramifiés, abondamment septés, sont droits ou flexueux ou plus rarement géniculés (fig. 2, c). Ils naissent soit à l'extrémité du rameau mycélien (position terminale), soit sur le long de celui-ci (position latérale). Ils s'amincissent souvent vers l'apex et leur base est parfois munie d'un renflement vésiculeux d'un diamètre de 10 μ environ. Ils sont bruns, avec une extrémité plus claire (hyaline, subhyaline ou brun pâle) et ils mesurent en largeur $3-7$ μ. Leur longueur, variable, est égale ou supérieure à 200 μ. Leur paroi est parfois finement verruqueuse vers la base (en particulier sur stroma produit sur Czapek).

Les conidies à paroi lisse sont acropleurogènes et se séparent du conidiophore en perçant un petit pore dans sa paroi. Elles sont obovoïdes, largement fusiformes ou parfois piriformes, droites, faiblement courbes ou plus rarement géniculées (fig. 2, d). Le point d'insertion des spores (hile) est inclus ou exceptionnellement un peu saillant. Elles sont pourvues de 2 à 7 cloisons brun foncé, mais 80% d'entre elles en possèdent 5. Les cellules intermédiaires sont brun pâle à brun foncé. Par contre, celles des extrémités ont une teinte hyaline, subhyaline (principalement


a.
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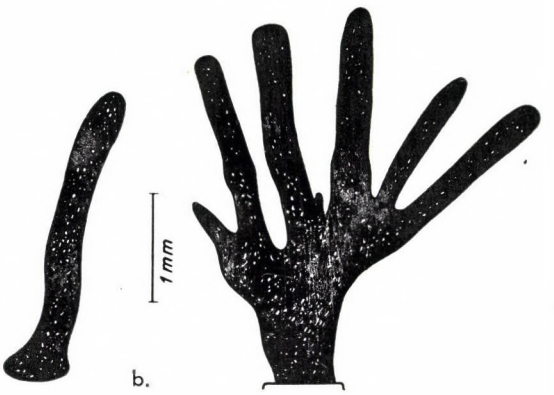
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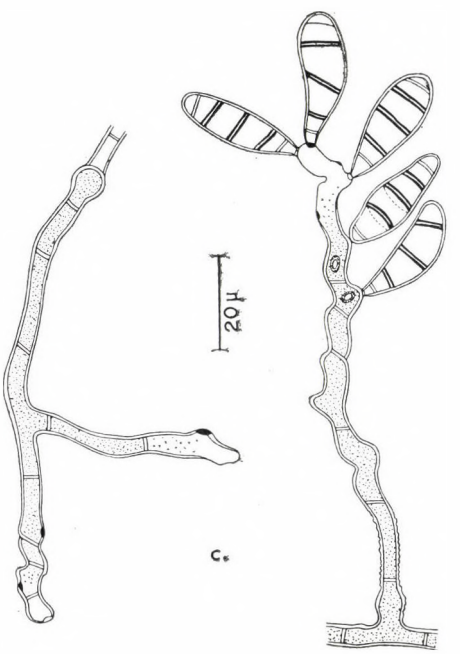
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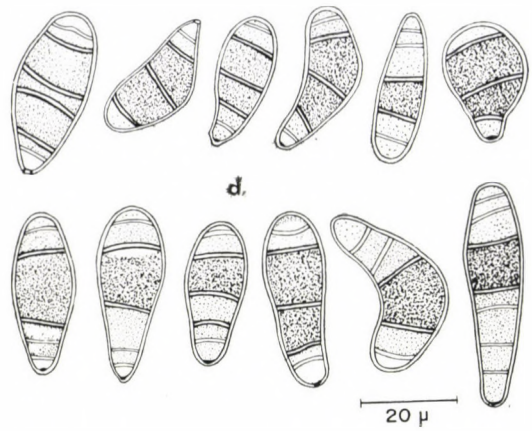

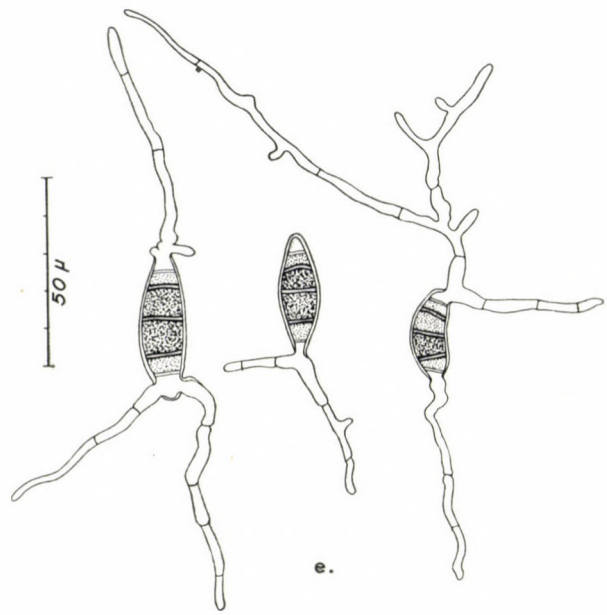

Fig. 2. Morphologie du *Curvularia hexamera* VEGH et BENOIT. a — Mycélium végétatif produi sur milieu P.D.A. après 30 jours d'incubation; b — Stromas obtenus sur P.D.A. (à gauche et Czapek gélosé (à droite); c — Conidiophores et conidies formés sur P.D.A.; d — Conidies produites sur P.D.A.; e — Germination des conidies dans l'eau permutée stérile à 22°C après 20 heures d'incubation en lumière continue

Fig. 3. Stromas du *Curvularia hexamera* VEGH et BENOIT formés sur milieu Czapeck gélosé après 35 jours de culture

la loge portant le hile) ou brun pâle. Les deux cellules centrales, ou plus rarement une seule, sont plus renflées que les autres et, dans certains cas, légèrement plus foncées. Les spores mesurent $20-47 \times 10-17$ μ (m: 33×12).

La germination des conidies s'accomplit aisément à 22°C en éclairement alterné aussi bien en eau permutée stérile (goutte déposée sur une lame plane) que sur eau gélosée à 2%. Après 20 heures d'incubation, le taux de germination atteint 97%. Elle est bipolaire ou plus rarement unipolaire (fig. 2, e). Les premiers filaments germinatifs apparaissent déjà au bout de quelques heures d'incubation. Leur position est terminale, subterminale au latérale. Les tubes germinatifs développés sont cylindriques ($1.7-8$ μ de diamètre), abondamment cloisonnés, parfois droits, le plus souvent flexueux. Ils présentent souvent à leur base des portions vésiculeuses, se ramifient rapidement et s'amincissent vers l'apex. Chaque loge en produit 1 à 3, le plus souvent 1.

Afin de préciser le rôle du champignon isolé et identifié, nous avons réalisé des essais préliminaires d'inoculation sur feuilles de plantules à l'aide d'un broyat comprenant mycélium, spores et milieu nutritif gélosé, mais sans aucun résultat. D'autres expériences devraient être encore effectuées pour pouvoir conclure sur ce point.

Dans le cadre de recherches sur les Dématiacées, F. RAPILLY et Michèle SKAJENNIKOFF (communication personnelle) ont constaté que le *C. hexamera* se distingue sérologiquement des *Helminthosporium sensu lato*. En effet, ces auteurs ont pu obtenir un immunsérum réagissant vis-à-vis de protéines cellulaires de divers groupes d'*Helminthosporium* appartenant, selon SHOEMAKER (1959), aux genres *Drechslera* et *Bipolaris (H. rostratum, H. sativum, H. spiciferum, H. teres, H. turcicum)*. Confronté suivant la méthode d'OUCHTERLONY aux divers antigènes correspondants, il donne des lignes de précipitation caractéristiques des *Helminthosporium*. Cet immunsérum, mis en présence d'antigènes protéiques obtenus avec un *Curvularia sp.* et *C. hexamera*, n'a jamais donné de ligne de précipitation. Ce fait permet de penser que la nouvelle espèce proposée n'appartient pas sérologiquement au genre *Helminthosporium* LINK.

Par la taille de ses conidies, le *C. hexamera* ressemble au *Curvularia inaequalis* (SHEAR) BOEDIJN et au *C. fallax* BOEDIJN; mais il s'en distingue par la forme des spores et, surtout, par le nombre presque constant de 5 cloisons, au lieu de 4 en majorité chez les 2 espèces citées. De plus, l'aspect cultural du *C. inaequalis* est différent sur P. D. A. tandis que le *C. fallax* produit, sur le même milieu nutritif, des stromas souvent ramifiés pouvant atteindre 7 mm (ELLIS, *loc. cit.*).

Le *C. hexamera* peut être placé dans le groupe "*geniculata*" de BOEDIJN *(loc. cit.)*.

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Studies on the Nutrition of *Xanthomonas malvacearum* and its Relationship to the Free Amino Acids, Organic Acids and Sugars of Resistant and Susceptible Cotton Cultivars

By

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Amino acid and organic acid nutrition of three races of *Xanthomonas malvacearum* (E. F. Smith) Dowson, the incitant of bacterial blight of cotton has been investigated. It has been concluded that races could not be differentiated on the basis of amino acid or organic acid nutrition. Some correlation has been obtained between the nutrition of the pathogen and the free amino acids, sugars and organic acids of the host and its susceptibility. It has been shown that the susceptibility of a cultivar depends on several nutritional factors, and resistance in cotton to *X. malvacearum* appears to be associated with low amount of citric acid, high amounts of carbohydrate and amino acids, and especially the presence of such amino acids like serine, which on one hand inhibit the growth of the pathogen while on the other hand interfere with the utilization of essential amino acids like glutamic acid and alanine.

Nutritional requirements of pathogens, especially obligate parasites can be very exacting. In this context such studies on *Xanthomonas malvacearum* (E. F. Smith) Dowson the incitant of bacterial blight of cotton becomes very important because this pathogen is very near to obligate parasites and, therefore, cannot live for long in soil, but can survive in association with the host for long periods of 17 years in trash under laboratory conditions (SCHNATHORST, 1969). The pathogen cannot be cultured but only on very complex media. Further, this pathogen instead of living in balance with the host causes severe damage to the host and has become a limiting factor in the cultivation of cotton, particularly the *G. hirsutum* and *G. barbadense*, which are highly susceptible to the bacterium (VERMA and SINGH, 1971). Although some investigations on *X. malvacearum* have been made in the past and it has been shown that it requires a particular proportion of a large number of amino acids for its luxuriant growth (NAYUDU, 1970), and that the pathogen could not grow in media with unfavourable carbohydrate-soluble nitrogen ratios (BIRD, 1954) yet a detailed knowledge on this aspect is lacking, specially on the newly discovered races (VERMA and SINGH, 1970). The biochemical basis of resistance is also not clear (PERRY, 1964) A detailed investigation on these aspects was, therefore, carried out including the newly discovered races of the pathogen. One idea was to determine if the isolates could be differentiated into distinct types on the basis of nutritional requirements, because such a possibility has been shown

to exist (BLACKMON, 1958). Further, the free amino acids, organic acids and sugars of resistant and susceptible cultivars were studied with a view to ascertain the availability of specific nutrition to the individual races and if it could be related to their virulence.

Materials and Methods

Cotton varieties: Two varieties of *Gossypium hirsutum* namely 101 – 102B and Acala-44 were selected because the former possesses a gene combination $B_2 + B_3$ and is resistant to all the known races of *X. malvacearum*, while the latter is susceptible to all the races (VERMA and SINGH, 1970).

Bacterial cultures: Three isolates of *X. malvacearum* namely XM-28 (race 16), XM-33 (race 8) and XM-72 (race 15) were used.

Utilization of amino acids and sugars: The basal medium consisted of KH_2PO_4 6.4 g, Na_2HPO_4 3.2 g, KCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g per litre. Amino acids were incorporated at a concentration of 70 μg/ml of nitrogen when they were used either as N or C source.

Utilization of organic acids: Medium employed was YS-agar (ammonium dihydrogen phosphate 0.5 g, dipotassium hydrogen phosphate 0.5 g, magnesium sulphate 0.2 g, sodium chloride 5 g, yeast extract 0.08 g, agar 20 g, distilled water 1000 ml) containing organic acid (0.2%) and bromothymol blue (0.0016%). The organic acids used were sodium salts of succinic, citric, propionic, lactic and tartaric acids. Actively growing cultures (48 hrs old) were used for inoculation purposes. Observations were recorded after an incubation of 21 days for growth and alkaline reaction, which was indicated by the change in the colour of the medium from green to blue.

Extraction of plant parts for free amino acids, sugars and organic acids: 10 g fresh material of different parts of 16-week-old cotton plants was taken viz. seeds (*delinted*), stem (alternate pieces selected from base to top), leaf (of all ages mixed randomly) and boll (unopened). The material was frozen and ground at low temperature and extracted in 80% ethanol for 8 hrs at 60°C. The extract was filtered. The whole process of extraction was repeated twice and the collection concentrated by evaporation under vacuum. The residue was dissolved in a known amount of 80% ethanol and the insoluble residue was removed by centrifugation at 5000 rpm for 30 minutes. The supernatant was collected and evaporated to dryness. The residue was finally dissolved in 10% isopropyl alcohol and kept in refrigerator until used.

Estimation of amino acids, sugars and organic acids: Amino acids and sugars were separated by two or one dimentional chromatography using phenol-water-ammonia (160 : 40 : 1) and/or butanol-acetic acid-water (4 : 1 : 1) and estimated by the standard procedures (PRIMOSIGH *et al.*, 1961; HANCOCK, 1967; VERMA and MARTIN, 1967). The organic acids were separated in a solvent system of butanol-acetic acid-water (4 : 1 : 1) and identified by spraying a 0.05% solution of bromocresol green in 95% ethanol at pH 7.0.

Results

Utilization of organic acids: Succinic and citric acids were utilized rather rapidly while tartaric acid was not utilized at all (Table 1). Abundant growth was observed on succinic and citric acids; moderate growth on propionic and lactic acids and no growth on tartaric acid. There was variation in the utilization of succinic, propionic and lactic acids, and 88% of the isolates utilized succinic acid within 24 hours.

Table 1

Utilization of organic acids by the isolates of *X. malvacearum*

Organic acids	Alkaline reaction (in days)	Growth (in 1 week)
Succinic acid	1–3	++++
Citric acid	1	++++
Propionic acid	3–7	++
Lactic acid	3–7	++
Tartaric acid	§	0

§, incubated for 21 days; 0, no growth; ++, moderate growth; ++++, [a]bundant growth.

Utilization of amino acids: A preliminary experiment was run to study the suitability of a few representative amino acids as a source of either C or N or both. The results (Table 2) show that: (i) organic N sources (alanine and glutamic acid)

Table 2

Growth of *Xanthomonas malvacearum*

Carbon source	Nitrogen source	O.D. at 620 nm		
		Isolate number		
		XM-72	XM-33	XM-28
Sucrose	DL-alanine[1]	0.71	0.52	0.70
Sucrose	L-glutamic acid[1]	0.52	0.59	0.81
DL-alanine[2]	ammonium sulphate	0.15	0.10	0.01
L-glutamic acid[2]	ammonium sulphate	0.22	0.11	0.02
Nil	DL-alanine[3]	0.14	0.04	0.00
Nil	L-glutamic acid[3]	0.14	0.09	0.01
Sucrose	ammonium sulphate[4]	0.40	0.32	0.21
Sucrose	peptone[5]	0.23	0.27	0.25
Sucrose	peptone[6]	1.13	1.09	1.10

1, as a sole source of N; 2, as a sole source of C; 3, as a sole source of C and N; 4, control, containing amino N and sucrose; 5, peptone with 70 μg N/ml; 6, peptone with 1000 μg N/ml; concentration of sucrose 1%, ammonium sulphate 70 μg N/ml, amino acids 70 μg N/ml; incubation time 4 days at 28°C.

were superior to inorganic N source (ammonium sulphate), (ii) amino acids were more useful as an N source but not as a C source, (iii) some of the amino acids, however, could serve as the sole source of C and N, (iv) growth in peptone at the same concentration as that of individual amino acids (i.e. 70 μg N/ml) was less, further when the concentration of peptone was increased a very good growth, much better than any individual amino acid was obtained. Experiments were further conducted to study the role of amino acids as the sole source of C and N, where some variation in their utilization by various isolates was expected. The relative role of amino acids as an N-source was also studied and the results are given in Table 3. It is concluded that alanine, glutamic acid and to some extent proline,

Table 3

Growth of *Xanthomonas malvacearum* on amino acids

Amino acids	O.D. at 620 nm					
	Sole source of N: sole source of C and N					
	XM-28	XM-33	XM-72	XM-28	XM-33	XM-72
Neutral						
DL-alanine	0.70	0.52	0.71	0	0.14	0.13
DL-2-amino-n-butyric acid	0	0	0	0	0	0
L-cysteine.HCl	0	0	0	0	0	0
L-Cystine	0.30	0.17	0.16	0	0	0
Glycine	0.06	0.80	0.92	0	0	0
L-leucine	0.32	0.40	0.33	0.03	0.03	0.01
DL-nor-leucine	—	—	—	0—	0—	0—
DL-iso-leucine	0.10	0.29	0.31	0	0	0
DL-methionine	0.09	0.23	0.26	0	0	0
DL-phenylalanine	0.10	0.03	0.15	0	0	0
L-proline	0.46	0.39	0.43	0.05	0.05	0.19
DL-serine	0	0	0	0	0	0
DL-threonine	0	0.05	0.25	0	0	0
DL-tryptophan	0.19	0.13	0.12	0	0	0
L-tyrosine	0.20	0.27	0.31	0	0	0
DL-valine	0.25	0.62	0.89	0	0	0
Acidic						
DL-aspartic acid	0.53	0.39	0.52	0.03	0.09	0.09
L-glutamic acid	0.81	0.68	0.52	0.04	0.22	0.25
Basic						
L-asparagine	0.65	0.60	0.71	0	0	0
L-arginine.HCl	0.30	0.51	0.55	0	0	0.03
L-histidine	0.20	0.34	0.39	0.02	0.04	0.03
L-lysine	0.36	0.13	0.28	0	0	0
DL-ornithine	0.11	0.18	0.13	0.03	0.03	0.05

Concentration of amino acids, 70 μg N/ml; incubation period, 4 days at 28°C.

aspartic acid, histidine, L-leucine and ornithine could serve as a sole source of C and N. As a source of N, the best sources were glycine, alanine, valine and glutamic acid. No growth was noticed on butyric acid, cysteine and serine. All the isolates behaved more or less in a similar fashion and strain characterization was not possible on the basis of amino acid utilization.

Free organic acids of cotton cultivars: Only malic and citric acids were present in detectable amounts (Table 4). Malic acid was absent from seeds and stem while citric acid was present in traces or less amounts in resistant seeds, stem, leaf and bolls. The maximum amount of citric acid was in boll and generally the amount was greater in susceptible tissues.

Table 4

Free organic acids in cotton cultivars

	Resistant (R)/ Susceptible (S)	Organic acids	
		Citric acid	Malic acid
Seed	R	traces	—
	S	traces	—
Stem	R	traces	—
	S	+	—
Leaf	R	traces	traces
	S	++	+
Boll	R	+++	traces
	S	+++++	traces

—, nil; +, little amount; +++, moderate amount; +++++, good amount; R, 101—102B; S, Acala-44.

Free amino acids of cotton cultivars: Generally the amount of amino acids was greater both quantitatively and qualitatively in resistant cultivars, the maximum being in bolls and the least in leaves (Table 5). The amino acids present in almost all the tissues were aspartic acid, glutamic acid, butyric acid, alanine, asparagine and glutamine. The distribution of serine was extremely interesting because it was present mainly only in resistant cultivars and absent from susceptible cultivar's seed, stem and leaf, and present only in traces in bolls. Asparagin was absent from susceptible cultivar's seeds and present in traces or extremely reduced amounts in susceptible cultivar's stem, leaf and bolls. Butyric acid was also generally more in resistant tissues and was absent from susceptible seeds and present only in traces in susceptible leaves.

Free sugars of cotton cultivars: The sugars generally present were arabinose, glucose, galactose, sucrose and raffinose, and the quantity was more in resistant tissues. The maximum amount of sugars was in bolls while leaves contained the minimum amount. Arabinose was present in traces or absent in seed and leaf;

Table 5

Free amino acids of cotton cultivars

Amino acids	Micrograms/100 mg fresh weight of tissue							
	Seed		Stem		Leaf		Boll	
	R	S	R	S	R	S	R	S
Aspartic acid	16	14	Tr	F	4.6	4.6	4	0
Glutamic acid	14	10	Tr	F	1.4	2.2	5	1
Glycine	0	0	0	0	0	0	8	0
Threonine	0	0	0	0	0	0	8	0
Arginine	0	0	0	0	0	0	62	5
Valine	Tr	0	Tr	0	0	0	Tr	0
γ-amino-N-butyric acid	7	0	27	28	3.4	2.2	25	18
Alanine	8	6	7	5	1	Tr	9	4
Glutamine	Tr	11	29	24	0	Tr	48	36
Asparagine	13	0	34	15	3.6	1.6	61	5
Serine	13	0	Tr	0	Tr	F	11	Tr

Tr, traces; F, faint spot.

glucose was absent from resistant seeds; galactose was present in traces in leaves; and raffinose was present only in resistant seeds and bolls, and to a lesser degree in susceptible seeds. The major sugar of leaves was glucose (Table 6).

Table 6

Free sugars in cotton cultivars

Plant parts	Resistant (R)/ Susceptible (S)	Micrograms of sugar in term of glucose/ 100 mg fresh weight of tissue					Total
		Arabinose	Galactose	Glucose	Sucrose	Raffinose	
Seed	R	Tr	51	0	109	74	234
	S	Tr	51	54	83	46	234
Stem	R	119	160	100	67	0	446
	S	52	59	50	51	0	212
Leaf	R	Tr	Tr	75	20	0	95
	S	0	Tr	75	Tr	0	75
Boll	R	202	150	106	130	20	608
	S	171	110	100	78	0	459

R, 101—102B; S, Acala-44.

Discussion

The availability in a host of particular nutrients like amino acids, organic acids, sugars etc. has been emphasized in the past for a successful host–parasite interaction (GARBER, 1954; LEWIS, 1953) and the concepts of definitive nutrition

(LEWIS, 1953; NAYUDU and WALKER, 1961), carbohydrate-soluble nitrogen ratios (BIRD, 1954), competitive inhibition (MAGASANIK, 1957; NAYUDU, 1972) have been developed.

In the present investigations the nutrition, especially organic acids, sugars and amino acids of *X. malvacearum* has been studied. The growth of the pathogen was supported very well by both citric and succinic acids. Of these citric acid was present in all parts of susceptible cultivar and the amounts were always less in the resistant tissues. It is, therefore, concluded that the presence of citric acid is, at least one factor which provides favourable condition for the development of the pathogen and establishment of disease.

X. malvacearum is known to grow well on sucrose and glucose. The present results show somewhat higher sucrose in resistant seeds and bolls. But this could not be a resistance factor because sucrose was absent from stem and leaf. Glucose was absent only from resistant seeds. The total amount of sugars in terms of glucose was generally higher in resistant tissues except seeds. A high glucose content of leaves with Knight's B_2B_3 factors associated positively with resistance for the first 90 days after planting (HUGHES and FOWLER, 1953). Increased resistance was correlated with the rise in level of reducing sugars (SABET and HASSAN, 1961). BIRD (1954), however, found that resistant lines had higher carbohydrates; but the carbohydrate level of resistant Stoneville 2B was lower than the susceptible Deltapine. Thus the general conclusion appears to be that the level of sugars could be one of the factors contributing to the susceptibility of a cultivar. However, there appears to be no direct correlation between the sugar content and the degree of susceptibility of the cultivars, and generally either good amount of sucrose or glucose was present to provide good nutrition to the pathogen. It appears more probable that it is the nitrogen sources which become the limiting factor and have a greater role than carbon sources in controlling the growth of the pathogen.

Several amino acids could serve as the source of N in presence of sucrose as the C-source. Alanine, glutamic acid and to some extent proline could supply both C and N adequately. That alanine and glutamic acid could serve as the sole source of C and N has already been demonstrated (LEWIS, 1930). BLACKMON (1958) obtained results more or less similar to those of present studies. Serine and cysteine were shown to be not utilized by either of the races studied by him. However, aspartic acid and glutamic acid, which were found to be not utilized by his isolate served generally as good sources of N in the present studies. Threonine and glycine, similar to this results, were utilized to different extents by various isolates, e.g. XM-28 did not utilize threonine; glycine and threonine were sparingly utilized, respectively, by XM-28 and XM-33. In contrast to the results of KOTASTHANE *et al.* (1965) various isolates could utilize valine, tryptophan and glycine as a N-source to varying degrees. NAYAUDU (1972) also found that glutamic acid supported growth of *X. malvacearum*. Alanine and glutamic acids were shown to be essential to *X. vesicatoria* (NAYUDU and WALKER, 1961). It is interesting that in the present studies both alanine and glutamic acid were found to be present in more or less similar amounts in the free pool in both resistant and susceptible

cultivars of cotton. Therefore, it appears that their utilization is inhibited and the pathogen is starved to death in the resistant cultivars due to the presence of other amino acids like serine, which is known to inhibit the growth of *X. malvacearum* and antagonize the utilization of glutamic acid (BLACKMON, 1958; NAYUDU, 1972). LIPKE (1968) also showed a relationship between disease severity and some free ninhydrin positive compounds in F_2 and F_3 progenies of cotton with gene B_4 and minor genes; however, no single amino acid could be held responsible for resistance.

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Phenolics Changes in Rice Varieties Infected by *Xanthomonas oryzae*

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Rice varieties differing in resistance to *Xanthomonas oryzae* were analysed for changes in *ortho*-dihydroxy and total phenols. The resistant TKM. 6 plants inoculated with *X. oryzae* contained larger quantities of total and *ortho*-dihydroxy phenols than both Co. 13 and IR. 8. Inoculation with *X. oryzae* in general caused the accumulation of phenols in all the three cultivars; however, the net increase was strikingly high in the resistant TKM. 6 compared with the less resistant varieties. In the inoculated TKM. 6 tissues, appeared two DSA reacting substances which were not found in Co. 13 and IR. 8.

Xanthomonas oryzae causes blight disease of rice *(Oryza sativa)*. At times the disease assumes epiphytotic proportion in the rice growing countries since most of the high yielding varieties are susceptible to the bacterium. There are however, a few resistant varieties (ANONYMOUS, 1969; MIZUKAMI and WAKIMOTO, 1969). The cultivar TKM. 6 is fairly resistant but IR. 8 is highly susceptible while Co. 13 is moderately susceptible to the disease (PURUSHOTHAMAN and PRASAD, 1972). What is the physiological basis of differences in the relative resistance of these varieties to *X. oryzae?* Is it due to phenols or some other factors? Since in the recent years phenols have gained much attention (FARKAS and KIRÁLY, 1962; KOSUGE, 1969), in such studies, the changes in the phenolic constituents of the three varieties were investigated and reported in this communication.

Materials and Methods

Rice seedlings were grown in wet land soil kept in cement pots. Fifteen days old seedlings were gently rubbed with a surface sterilized emery paper and spray inoculated with a heavy suspension of (10^6 cells/0.1 ml) a virulent isolate of *X. oryzae*. Control plants received distilled water spray. To ensure maximum infection, optimum temperature and humidity conditions were provided. Plants were removed immediately after inoculation (0 hr) and at suitable intervals for analysis.

Extraction of plant material: Shoot portions of plants alone were analysed for phenols. Chopped tissue, 4 g, was extracted in 16 ml of boiling 80 per cent ethyl alcohol (CHANDRAMOHAN *et al.*, 1967). The material was homogenized in a pestle with mortar, filtered and the residue reextracted with 5 ml of alcohol. The alcohol extract was pooled, and filtered through Whatman No. 1 filter paper.

Estimation of ortho-dihydroxy phenols: Ortho-dihydroxy phenols were measured by using Arnow's reagent (JOHNSON and SCHAAL, 1952). The flesh red colour was read in Spectronic-20 colorimeter at 522 mμ.

Estimation of total phenols: The total phenols were estimated by employing Folin–Ciocalteu reagent (BRAY and THORPE, 1954). The intensity of the blue colour was read in a Spectronic-20 colorimeter at 725 mμ.

Qualitative analysis of phenols: Chopped tissue, 5 g, was extracted in 80 per cent boiling methyl alcohol as detailed earlier. The combined and clarified methanol extract was evaporated to near dryness at 40°C and suspended in 3 ml of distilled water acidified to pH 4.0 with 0.5 N HCl and extracted three times with 10 ml portions of ethyl acetate for 12 hr. The ethyl acetate fractions were pooled and evaporated to near dryness. The residue was dissolved in 3 ml of ethyl acetate and washed twice with 5 ml portions of acidified water. The water washed ethyl acetate fractions were evaporated to dryness and the final residue was dissolved in 2 ml of 80 per cent ethanol. All evaporations were carried out at reduced pressure and at ca. 40°C (BIEHN *et al.*, 1967). Plant samples collected on the last stage (15 days after inoculation) alone were analyzed.

Chromatographic separation of phenols: Fifty μl of the final fraction was spotted on Whatman No. 1 chromatographic paper (28 × 23 cm) and developed ascendingly in a solvent system of n-butanol : acetic acid : water: 4 : 1 : 5 (v/v) for the first direction and 2 per cent acetic acid for the second run. The papers were air dried and sprayed with 0.1 per cent alkaline diazolized sulphanilic acid (DSA) (KUĆ, 1959).

Results

Ortho-dihydroxy phenols: Although TKM. 6 contained the lowest quantity of *ortho* dihydroxy phenols subsequent samples possessed large amounts. Inoculation with *X. oryzae* increased the *ortho*-dihydroxy phenols in the three varieties; however, maximum accumulation occurred in TKM. 6 (Table 1).

Table 1

Changes in *ortho*-dihydroxy phenols* in the three rice varieties as influenced by *X. oryzae* inoculation

Sampling time after inoculation	TKM. 6			Co. 13			IR. 8		
	Healthy	Inoculated	Per cent change	Healthy	Inoculated	Per cent change	Healthy	Inoculated	Per cent change
0 hr	0.85	0.92	+ 8.2	0.90	0.81	−10.6	0.86	0.95	+10.7
24th hr	1.06	1.05	− 1.2	0.94	1.02	+ 8.5	0.95	1.09	+13.7
3rd day	1.27	1.32	+ 3.6	1.03	1.07	+ 3.6	0.94	1.13	+20.6
5th day	1.69	1.38	−18.0	1.26	1.29	+ 2.5	1.23	1.09	−11.3
10th day	0.97	1.45	+48.7	1.31	1.06	−18.3	1.23	1.09	−11.4
15th day	0.78	1.35	+ 7.1	1.03	1.02	− 1.0	0.88	0.90	− 2.3

* mg of *ortho*-dihydroxy phenols/g of oven dry tissue in catechol equivalents; +: increase; −: decrease

Table 2

Changes in total phenols* in the three rice varieties as influenced by *X. oryzae* inoculation

Sampling time after inoculation	TKM. 6			Co. 13			IR. 8		
	Healthy	Inoculated	Per cent change	Healthy	Inoculated	Per cent change	Healthy	Inoculated	Per cent change
0 hr	4.68	4.62	− 0.13	4.46	4.50	+ 0.9	4.16	4.20	+ 0.8
24 hrs	4.66	4.65	− 0.02	4.16	4.52	+ 8.4	4.10	4.32	+ 5.1
3rd day	4.15	4.69	+12.8	4.18	4.61	+10.2	4.12	4.38	+ 6.2
5th day	3.17	5.21	+64.2	3.43	4.69	+35.2	4.01	4.75	+ 8.4
10th day	3.07	4.38	+42.3	3.31	3.96	+18.4	3.88	4.06	+ 4.6
15th day	3.58	3.63	+14.5	2.89	3.07	+ 6.3	3.64	3.75	+ 2.8

* mg of total phenols/g of oven dry tissue in catechol equivalents;
+ : increase; − : decrease

Table 3

Changes in phenolic constituents in the rice varieties as influenced by *X. oryzae* inoculation

Spot No.	Rf value	TKM. 6		Co. 13		IR. 8	
		Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
1	0.92	+	++	+	+	+	+
2	0.86	+	++	+	++	+	++
3	0.52	+	++	+	++	+	+
4	0.55	+	++	+	+	+	+
5	0.63	+	+++	+	++	+	++
6	0.72	+	++	−	−	+	+
7	0.95	+	+++	++	+++	+	+
8	0.80	+	++	−	−	+	++
9	0.63	−	+	−	−	+	++
10	0.52	−	+	−	−	−	−

\+ to +++ colour intensity in increasing order;
− absent

Total phenols: Table 2 presents the changes in total phenols of the rice varieties inoculated with *X. oryzae*. Generally, the resistant cultivar, TKM. 6, contained more total phenols than either Co. 13 or IR. 8, the susceptible cultivars. Inoculation with the pathogen increased the level of total phenols in the three varieties; however, TKM. 6 displayed a maximum increase compared with the less resistant ones.

Chromatographic separation of phenols: Table 3 presents the qualitative changes in phenols in the three rice varieties. For want of authentic compounds, identification of individual phenol was not made.

The healthy tissue of TKM. 6 and the most susceptible IR. 8 contained only 8 DSA positive substances; but Co. 13 contained 6 substances. However, the chromatograms spotted with extract of TKM. 6 exhibited a higher quantity of phenols as visualized by colour intensity and area of the spots. Inoculation clearly favoured an increase in phenols. Moreover, in TKM. 6, two new DSA positive substances appeared in addition to the 8 that were present. The variation in phenols in the other two varieties was only quantitative.

Discussion

Rice cultivars TKM. 6, Co. 13 and IR. 8 differ in resistance to *X. oryzae* and these also differ markedly in their phenolic content. In the resistant variety TKM. 6 *ortho*-dihydroxy and total phenols were more than in the other two varieties. SRIDHAR (1970) also reported that rice cultivar, Co. 29 resistant to *Pyricularia oryzae* contained more total phenols than the susceptible Co. 13. Admittedly this relationship conforms to the general view that plants resistant to diseases contain higher amounts of phenols than the susceptible ones (FARKAS and KIRÁLY, 1962; KOSUGE, 1969).

The rate of increase of phenols in the three rice varieties was not uniform; in the resistant TKM. 6 the increase was up to 64.2 per cent while in Co. 13 and IR. 8 it was 35.0 and 8.4 per cent respectively. This indicates that the resistant plants are characteristic of rapidly synthesizing phenolic substances.

Apart from high phenol level in the *X. oryzae* inoculated TKM. 6 plants two new DSA reacting substances presumably phenols appeared. But in the other two varieties, only quantitative variations in phenolics were discernible. Nother is presently known about the two DSA + spots. I can not rule them out as phytoalexins since UEHARA (1960) had claimed that rice plants upon inoculation which *X. oryzae* produced phytoalexin-like substances. Therefore the identity of these compounds and their biological properties need further work.

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Cultural Variation in Potato Isolates of *Rhizoctonia solani* Kühn

By

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Fifty isolated of *Rhizoctonia solani*, cultured from affected potato material showed that the vegetative stage of the pathogen occurs in a wide range of forms differing in cultural characters. Variations were observed in the rate of growth of colonies, colour of substrate, intensity of sclerotia, type of sclerotia and pattern of formation of sclerotia. It was observed that isolates classed into a group on the basis of one character may or may not be similar in other characters. The pattern of formation of sclerotia was found to be the best character for classifying the different isolates. Accordingly, the isolates were grouped into five main types and four sub-types.

Rhizoctonia solani Kühn (*Pellicularia filamentosa* (Pat.) Rogers) is a widely distributed soil-inhabiting organism that causes diseases of various crops and produces various types of symptoms. The pathogen affects potato tubers causing the black scurf disease, kills the potato sprouts resulting in gappy germination and causes stem canker responsible for wilting of plants. The complex nature of the organism and its adaptability to varied ecological and climatic conditions may probably be due to the existence of its many recognizable strains. Several studies have been made on variation and variability in *R. solani* affecting different crops (EXNER and CHILTON, 1943; HOUSTON, 1945; KOTILA, 1947; HAWN and VANTERPOOL, 1953; WHITNEY and PARMETER, 1963; FLENTJE and STRETTON, 1964; PAPAVIZAS, 1964 and GARZA-CHAPA and ANDERSON, 1966). The studies of KERNKAMP *et al.* (1952), FLENTJE (1956) and LUTTERELL (1962) have shown that there is an indefinite number of cultural races of the organism. Variation in potato isolates of *R. solani* collected from Punjab were reported by SINGH (1964). In this paper, studies on the cultural variations in 50 isolates of *R. solani* collected from a number of potato varieties in the hills and plains of India are reported. Different isolates were classified mainly on the basis of the pattern of formation of sclerotia in culture plates.

Materials and Methods

Fifty isolates of *R. solani* were cultured from black scurf affected tubers and roots of different potato varieties collected from various localities in the States of Himachal Pradesh, Punjab and Uttar Pradesh. The isolations were made from

the diseased materials on 2 per cent water agar. Subsequently, single hyphal tip cultures of each isolate were maintained in potato dextrose agar (PDA) tubes. To study the cultural characters, each isolate was inoculated in five Petri plates (100 mm diameter) each containing 25 ml PDA. The inoculated plates were incubated at 25° ± 1°C and observations were recorded periodically for different characters. Each test was repeated four times for confirmation of results.

Results

Observations on cultural characters showed variation in the rate of growth, colour of substrate and characters of sclerotia in different isolates of *R. solani*. The results of the studies on cultural characters of each isolate are given under the respective character.

Rate of growth

Measurement of diametric growth of each colony was started 24 hours after inoculation and recorded daily for four days. Variations in rate of growth were observed and on that basis, the isolates were classified into three groups–fast, medium and slow growing.

Fast growing: Diameter of colony ranged from 71 – 100 mm in 96 hours after inoclation. Isolate numbers R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R12, R15, R18, R23, R32, R34, R37 and R39 were found to be fast growing. The maximum growth of 93.5 mm was recorded in isolate R10.

Medium growing: Diameter of colony ranged from 51 – 70 mm. Isolate numbers R11, R13, R22, R27, R30, R35, R36, R41, R47, R48 and R50 represented this group.

Slow growing: Diameter of colony was less than 50 mm. Isolate numbers R14, R16, R17, R19, R20, R21, R24, R25, R26, R28, R29, R31, R33, R38, R40, R42, R43, R44, R45, R46 and R49 showed slow growth rate. Isolate R43 was found to be the slowest (28 mm diameter).

The average growth of each isolate was found to remain fairly consistent in repeated tests. In case of the fast-growing isolates it was observed to be about 77 mm and in slow-growing about 37 mm (Table 1).

Table 1

Average total growth in different isolates of *Rhizoctonia solani*

Isolates	Average growth (mm) after			
	24hr	48 hr	72 hr	96 hr
Fast growing	8.8	25.5	52.7	76.9
Medium growing	5.5	16.2	39.4	60.5
Slow growing	3.2	7.5	20.8	37.5

The daily rate of growth in the different groups also remained almost uniform and in each group it was observed to be the lowest during the first day and highest during the third and fourth days. However, the growth was more during each day in the fast-growing isolates as compared to that in the other two groups (Table 2).

Table 2

Average daily growth of different isolates of *Rhizoctonia solani*

Isolates	Average growth of colony (mm) during			
	1st day	2nd day	3rd day	4th day
Fast growing	8.8	16.6	23.2	23.7
Medium growing	5.5	11.2	22.5	22.3
Slow growing	3.2	6.4	13.3	16.7

Substrate colour

Variation in the substrate colour of the cultures of different isolates was not observed during the first three days of the growth of the colony in PDA plates. The colour starts appearing on the fourth day and the characteristic colour of the isolates develops by the seventh day. Thereafter, no appreciable change in colour develops. There was variation in the density of brown colouration of the substrate of different isolates. The colour varied from light brown to dark brown and remained consistent for an isolate which helped in classifying the isolates into the following three groups:

Light brown: The substrate colour in culture of these isolates was very light with a brownish tinge. Isolate numbers R4, R7, R10, R11, R15, R16, R18, R20, R21, R23, R24, R25, R30, R31, R34, R36, R37, R40, R41, R45, R46, R47, R48 and R50 were always observed to develop light brown colour in the culture.

Medium brown: Substrate colour medium brown. The colour was darker than the light brown and markedly lighter than the dark brown. Isolate numbers R3, R5, R6, R12, R22, R26, R27, R28, R29, R32, R35, R38, R43, R44 and R49 consistently showed medium brown colouration in the PDA plates.

Dark brown: The substrate colour was darker as compared to that in the isolates of the other two groups. Isolate numbers R1, R2, R8, R9, R13, R14, R17, R19, R33, R39 and R42 showed dark brown colouration in the culture substrate.

Characters of sclerotia

Sclerotia showed great variation as regards their intensity, type and pattern of formation in PDA plates. The observations recorded on each of the three characters are given here.

Intensity of sclerotia: Observations on size and number of sclerotia were recorded 18 days after inoculation. Variations were observed in the percentage of colony area covered by the sclerotia. In some isolates, the sclerotia were few and small and these did not cover more than 5 per cent of the colony while in others these covered more than 50 per cent of the colony. Percentage area covered by the sclerotia was calculated on the basis of the size and number of the sclerotia and the isolates were grouped into three categories:

Light intensity: Up to 5 per cent area of the colony covered by the sclerotia. Isolate numbers R3, R5, R10, R13, R16, R20, R21, R23, R24, R27, R31, R34, R36, R42, R44, R45, R49 and R50 developed this type of sclerotia.

Moderate intensity: More than 5 and up to 25 per cent area covered by the sclerotia. Isolate numbers R1, R2, R4, R6, R7, R11, R14, R15, R17, R18, R19, R22, R25, R26, R29, R30, R33, R35, R37, R38, R40, R41, R43, R46, R47 and R48 develop sclerotia of moderate intensity.

Heavy intensity: More than 25 per cent are covered by the sclerotia. Isolate numbers R8, R9, R12, R28, R32 and R39 included in this group, were found to cover about 50 per cent of the colony.

Type of sclerotia: Observations on the type of sclerotia were recorded on 18-day-old cultures. Three types of sclerotia (minute, crustaceous and large) were observed in different isolates.

Minute sclerotia: The sclerotia vary from pin-point to 2 mm in size but the majority are about 1 mm in diameter. These sclerotia generally do not coalesce and were observed in isolate numbers R1, R3, R4, R5, R6, R7, R10, R11, R13, R18, R20, R21, R22, R24, R25, R26, R27, R28, R29, R31, R33, R37, R39, R40, R41, R42, R47, R48 and R50.

Crustaceous sclerotia: The size of sclerotia ranged from pin-point to 3 mm in diameter and these coalesced to form large flat crusts. Isolate numbers R2, R8, R9, R12, R14, R17, R32, R34, R36, R38, R43 and R46 were found to have crustaceous sclerotia.

Large sclerotia: Sclerotia 1 mm to 4 mm in diameter. These are mostly globular as compared to somewhat flat form in the other two groups. The sclerotia may coalesce to form large, raised areas. Isolate numbers R15, R16, R19, R23, R30, R35, R44, R45 and R49 develop this type of sclerotia.

Pattern of formation of sclerotia

The sclerotia of an isolate developed in a particular pattern in culture plates. The sclerotia may be scattered in the whole plate or may be restricted to certain areas. The pattern was characteristic of each isolate, remained consistent and was found most suitable for determination and classification of the cultural races. The studies with 50 isolates showed that these could be grouped into five main types and four sub-types (Fig. 1). The characters of each type are described and the isolates representing each type are listed in the respective group.

Type A (Sclerotia scattered) – Sclerotia scattered all over the colony. These

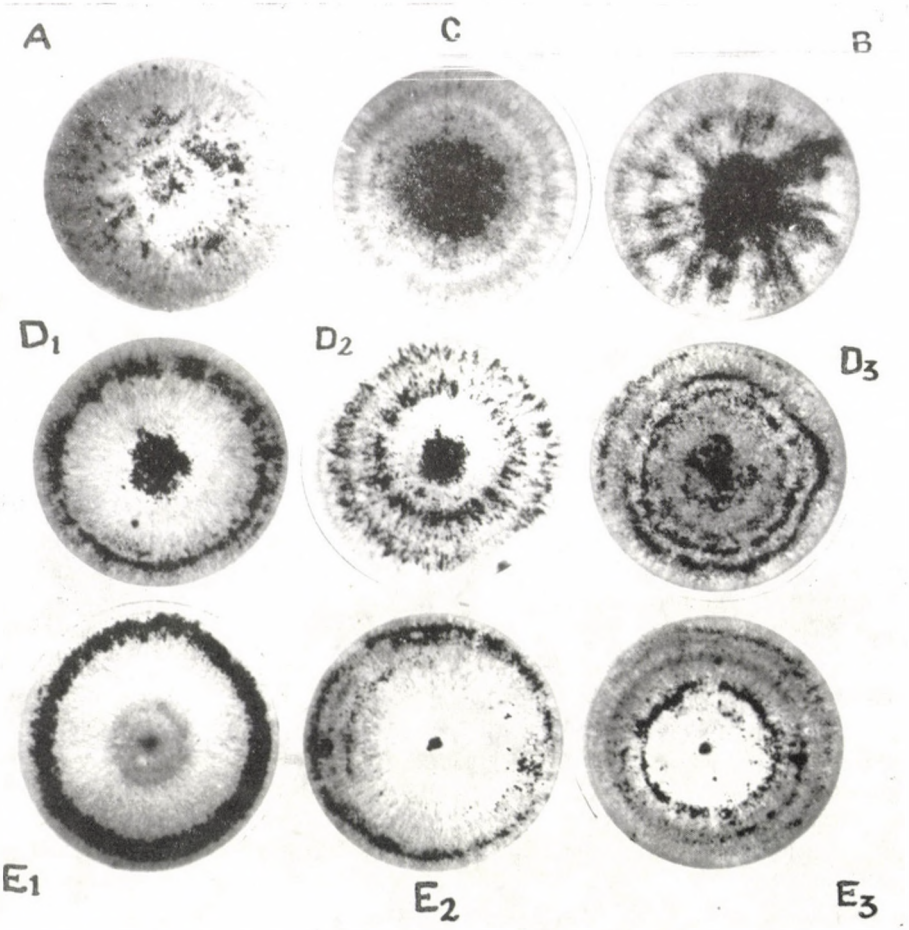

Fig. 1. Pattern of formation of sclerotia in cultures of *Rhizoctonia solani*. A = scattered sclerotia, B = sclerotia in radiating rays, C = sclerotia in central core, D_1 = sclerotia in central core and one ring, D_2 = sclerotia in central core and two rings, D_3 = sclerotia in central core and three rings, E_1 = sclerotia in one ring, E_2 = sclerotia in two rings and E_3 = sclerotia in three rings

may be minute to large in size. Isolate numbers R1, R2, R5, R7, R9, R10, R12, R13, R15, R18, R20, R21, R22, R23, R24, R27, R28, R31, R32, R36, R44, R46, R47 and R50 show scattered sclerotia in culture.

Type B (Sclerotia in radiating rays) – Sclerotia arranged in rays radiating from the centre towards perpiphery of the colony. This arrangement of sclerotia was observed in isolate numbers R16 and R19.

Type C (Sclerotia in a central core) – Sclerotia mainly arranged at the centre of the colony. In this type, the sclerotia are crowded and coalesce to form a central

core. A few sclerotia may also be scattered. The isolate numbers R17, R25, R29, R33, R37, R39, R43, R48 and R49 represent this type of the pattern of formation of sclerotia.

Type D (Sclerotia in central core and rings) – The sclerotia developed at the centre of the colony and coalesced to form a central core. Sclerotia also developed in rings around the central core. The rings may be one, two or more in number and on this basis three sub-types were recognized:

Sub-type D1: Sclerotia arranged in central core and one ring–isolate numbers R11 and R34.

Sub-type D2: Sclerotia arranged in central core and two rings–isolate numbers R4, R14 and R30.

Sub-type D3: Sclerotia arranged in central core and 3 or more rings–isolate numbers R26 and R45.

Type E (Sclerotia in rings only) – Sclerotia developed only in rings. There were no sclerotia to form a central core. In this type also the number of rings may be one, two or more, on the basis of which the isolates were grouped into 3 sub-types.

Sub-type E1 – Sclerotia arranged in only one ring–isolate numbers R3, R8 and R40.

Sub-type E2 – Sclerotia arranged in two rings–isolate numbers R6, R35 and R41.

Sub-type E3 – Sclerotia arranged in three or more rings–isolate numbers R8 and R42.

It is observed from the above results that the scattered type of sclerotia (Type A) is the most common as this was observed in almost 50 per cent of the isolates studied. Formation of sclerotia in radiating rays was rare.

Other characters

Besides the main characters already described, a few isolates also showed other characteristic features. The mycelium in most cases comprised of very fine thin hyphae but in four of the isolates (R5, R16, R19 and R49) the hyphae were observed to be prominently rough, thick and thread-like. Normally, the sclerotia develop on the agar medium only. However, isolate numbers R3 and R5 showed the development of sclerotia on the walls of the Petri-plate besides the agar medium. The mycelial hyphae, generally, grow horizontally along agar surface but in two of the isolates (R10 and R44) the hyphae also developed vertically upwards reaching the upper lid of the plate, where the sclerotia were formed.

The main cultural characters of 50 isolates are summarized in Table 3 to show their interrelationship. It is observed that different isolates may be similar in some characters and may differ in others. Taking "pattern of formation of sclerotia" as the main character for differentiating the cultural types, it is observed that the isolates with the same pattern of sclerotia may or may not be similar in other characters. For example, isolates R1 and R2 both have scattered sclerotia

Table 3

Cultural characters of fifty isolates of *Rhizoctonia solani*

Isolate No.	Growth rate			Substrate colour			Intensity of sclerotia			Type of sclerotia			Pattern of formation of sclerotia				
	Fast	Medium	Slow	Light	Medium	Dark	Light	Moderate	Heavy	Minute	Crustaceous	Large	Scattered	Radiating	Central core	Central core rings	Rings only
R1	+	−	−	−	−	+	−	+	−	+	−	−	+	−	−	−	−
R2	+	−	−	−	−	+	−	+	−	−	+	−	+	−	−	−	−
R3	+	−	−	−	+	−	+	−	−	+	−	−	−	−	−	−	+
R4	+	−	−	+	−	−	−	+	−	+	−	−	−	−	−	+	−
R5	+	−	−	−	+	−	+	−	−	+	−	−	+	−	−	−	−
R6	+	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	+
R7	+	−	−	+	−	−	−	+	−	+	−	−	+	−	−	−	−
R8	+	−	−	−	−	+	−	−	+	−	+	−	−	−	−	−	+
R9	+	−	−	−	−	+	−	−	+	−	+	−	+	−	−	−	−
R10	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	−	−
R11	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	+	−
R12	+	−	−	−	+	−	−	−	+	−	+	−	+	−	−	−	−
R13	−	+	−	−	−	+	+	−	−	+	−	−	+	−	−	−	−
R14	−	−	+	−	−	+	−	+	−	−	+	−	−	−	−	+	−
R15	+	−	−	+	−	−	−	+	−	−	−	+	+	−	−	−	−
R16	−	−	+	+	−	−	+	−	−	−	−	+	−	+	−	−	−
R17	−	−	+	−	−	+	−	+	−	−	+	−	−	−	+	−	−
R18	+	−	−	+	−	−	−	+	−	+	−	−	+	−	−	−	−
R19	−	−	+	−	−	+	−	+	−	−	−	+	−	+	−	−	−
R20	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−
R21	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−
R22	−	+	−	−	+	−	−	+	−	+	−	−	+	−	−	−	−
R23	+	−	−	+	−	−	+	−	−	−	−	+	+	−	−	−	−
R24	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−
R25	−	−	+	+	−	−	−	+	−	+	−	−	−	−	+	−	−
R26	−	−	+	−	+	−	−	+	−	+	−	−	−	−	−	+	−
R27	−	+	−	−	+	−	+	−	−	+	−	−	+	−	−	−	−
R28	−	−	+	−	+	−	−	−	+	+	−	−	+	−	−	−	−
R29	−	−	+	−	+	−	−	+	−	+	−	−	−	−	+	−	−
R30	−	+	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−
R31	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−
R32	+	−	−	−	+	−	−	−	+	−	+	−	+	−	−	−	−
R33	−	−	+	−	−	+	−	+	−	+	−	−	−	−	+	−	−
R34	+	−	−	+	−	−	+	−	−	−	+	−	−	−	−	+	−
R35	−	+	−	−	+	−	−	+	−	−	−	+	−	−	−	−	+
R36	−	+	−	+	−	−	+	−	−	−	+	−	+	−	−	−	−
R37	+	−	−	+	−	−	−	+	−	+	−	−	−	−	+	−	−
R38	−	−	+	−	+	−	−	+	−	−	+	−	−	−	−	−	+
R39	+	−	−	−	−	+	−	−	+	+	−	−	−	−	+	−	−
R40	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	−	+
R41	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	+
R42	−	−	+	−	−	+	+	−	−	+	−	−	−	−	−	−	+
R43	−	−	+	−	+	−	−	+	−	−	+	−	−	−	+	−	−
R44	−	−	+	−	+	−	+	−	−	−	−	+	+	−	−	−	−
R45	−	−	+	+	−	−	+	−	−	−	−	+	+	−	−	+	−
R46	−	−	+	+	−	−	−	+	−	−	+	−	+	−	−	−	−
R47	−	+	−	+	−	−	−	+	−	+	−	−	+	−	−	−	−
R48	−	+	−	+	−	−	−	+	−	+	−	−	−	−	+	−	−
R49	−	−	+	−	+	−	+	−	−	−	−	+	−	−	+	−	−
R50	−	+	−	+	−	−	+	−	−	+	−	−	+	−	−	−	−

(Type A) and are also similar in growth rate, substrate colour and intensity of sclerotia, but differ in the type of sclerotia which are minute in R1 and crustaceous in R2. Similarly, it is observed that isolates in a group may be similar in four of the characters. However, these groups differ among themselves. Isolates R20 and R21 are the only two isolates which are similar in all the five characters even though these were obtained from two different potato varieties and from far apart localities.

Discussion

Studies on the cultural characters of *R. solani* have shown that it is a complex species. Various isolates of the pathogen differed widely in their cultural characters. The isolates grouped together on the basis of one character differed in other characters. Similar variations in *R. solani* have been recorded by several workers as regards growth rate, presence or absence of sclerotia, type of sclerotia, amount of aerial hyphae, etc. (KOTILA, 1947; KERNKAMP *et al.*, 1951; EXNER and CHILTON, 1943; HAWN and VNATERPOOL, 1953; WHITNEY and PARMETER, 1963; FLENTJE and STRETTON, 1964; PAPAVIZAS, 1965; GARZA-CHAPA and ANDERSON, 1966). In this study the isolates were classified into different groups on the basis of the rate of growth of colony, substrate colour, intensity of sclerotia, type of sclerotia and pattern of formation of sclerotia. Though these characters were consistent for a group, yet slight variations were observed in the grade of a character of an isolate depending upon various factors of media, temperature and humidity. The pattern of formation of sclerotia was always well defined and was found most suitable for classification of the isolates.

SINGH (1964) indentified four races of *R. solani* on the basis of characters of sclerotia. However, in this study the isolates were obtained only from Punjab which may explain the limited range in the cultura variations. The cultural characters of different isolates were found to remain stable even when sub-cultured frequently for more than two years. This was in comformity with observations made by KERNKAMP *et al.* (1952), WHITNEY and PARMETER (1963) and FLENTJE and STRETTON (1964).

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Development and Morphology of Synnema in *Trichurus spiralis* Hasselbring

By

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The development and morphology of synnema in *Trichurus spiralis* are studied. The structure was found to be initiated from a prostrate hypha as a single cell. The sporulation apparatus of the terminal part of the synnema was also described. The tip of the synnema does not keep growing, but it is sooner or later occupied by phialides abstricting conidia. Phialide apices show no annellations and thus the authors do not recommend the classification of the genus *Trichurus* with the series Annellosporae of the Hypomycetes.

In the last few decades comprehensive studies have been carried out on initiation, development and structure of the fasciculate spore-bearing bodies (synnemata or coremia) in fungi. SWART (1964) briefly described the development of coremia in *Trichurus terrophilus* Swift and Povah, *T. gorgonifer* Bain and *T. spiralis* Hasselbring. With respect to *T. spiralis*, his results should be taken with much reservation, as the studied isolate of this species had been kept under culture for about 50 years and was in a poor condition. His description of coremium development lacks details and precision. Certain species of *Penicillium* as *P. isariiforme* (STOLK and MEYER, 1957), *P. clavigerum* and *P. expansum* (CARLILE *et al.*, 1961; 1962a, b) constitute the major moulds studied that possess synnemata. Of the entomogenous organisms investigated are species of *Akanthomyces*, *Gibellula*, *Hirsutella*, *Hymenostilbe* and *Insecticola* (MAINS, 1949; 1950a, b; LOUGHHEED, 1963). BESADA and YUSEF (1968) briefly described the mature synnema of *T. spiralis* Hasselbring but they did not follow up its development.

Although the terms "coremium" and "synnema" are commonly interchangeable, yet the coremium may be used for a bundle of closely appressed conidiophores that consistently come out from different initials. On the other hand, the synnema is applied for both vegetative hyphal strand and columnar sporiferous structures which may originate from a single initial cell (LANGERON and VANBREUSEGHEM, 1965).

It is thus thought that it would be worthy to study the initiation, development and structure of the synnema in *T. spiralis* Hasselbring in a more extensive manner.

Material and Methods

The organism under investigation was isolated from an agricultural soil at Tanta, Egypt. Stock and plate cultures are maintained on glucose-nitrate agar (as g/l: glucose 10, $NaNO_3$ 2, KH_2PO_4 1.5, $MgSO_4$ $7H_2O$ 0.5, agar 15). Hyphae were removed from the advancing border of a plate culture, cleared in lactophenol (2 volumes of glycerine, one volume of each of melted phenol crystals, lactic acid and distilled water) and stained with cotton blue (1% in lactophenol) and finally mounted in lactophenol.

Results and Discussion

Synnemata of different stages of development were examined, and formation of synnema can be described as follows: It appears that this structure is initiated from the prostrate mycelium as an erect and short filament (Fig. 1). The most

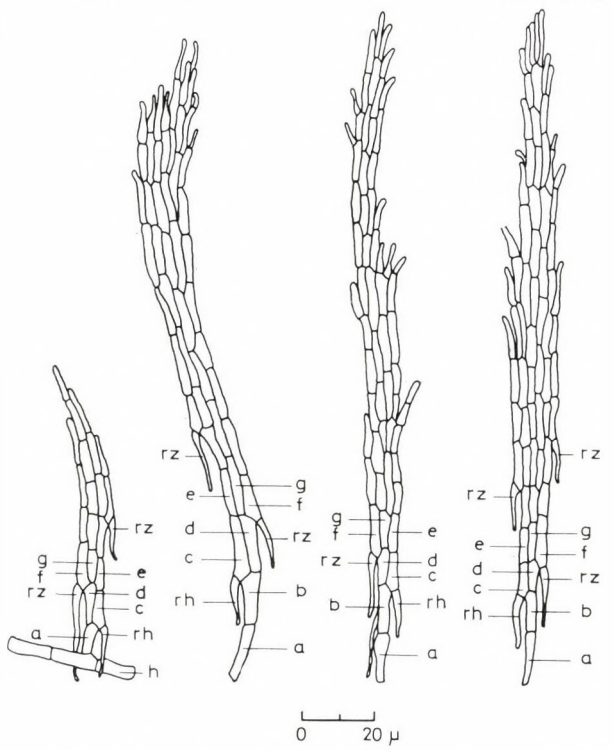

Fig. 1. Development of synnema from a prostrate hypha (h). The synnema is initiated as a single basal cell (a) that forms at its tip either another cell (b) or two prallel cells (c) and (d) which are united by their adjacent longitudinal walls. A rhizoidal appendage (rh) may come out from the base of one of the new parallel cells. Cell (c) forms a single cell (e), while cell (d) usually gives rise at its apex, to two parallel cells (f) and (g). The synnema primordium adds more rows of cells in the manner formerly described and new rhizoidal appendage (rz) may emerge from the bases of the newly formed rows

basal (a) or the next upper cell (b) of this filament usually gives at the upper end two parallel cells (c) and (d) which remain adnate through the whole length of their adjacent longitudinal walls, thus producing a two-celled thick filament. An appendage, rhizoidal cell (rh) which grows downwards can be produced from one of the newly formed cells. Cell (c) forms at a higher level a single cell (e), while cell (d) usually gives rise to two parallel and united cells (f) and (g). The filament then continues to produce other cells upwards in the manner formerly described and more rhizoidal appendages (rz) come out. With this process going

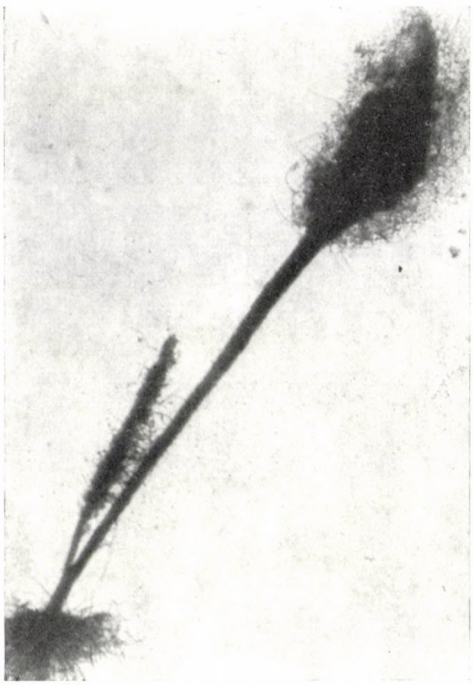

Fig. 2. A branched synnema. The basal part represents a bulbous body formed by aggregation of rhizoidal appendages

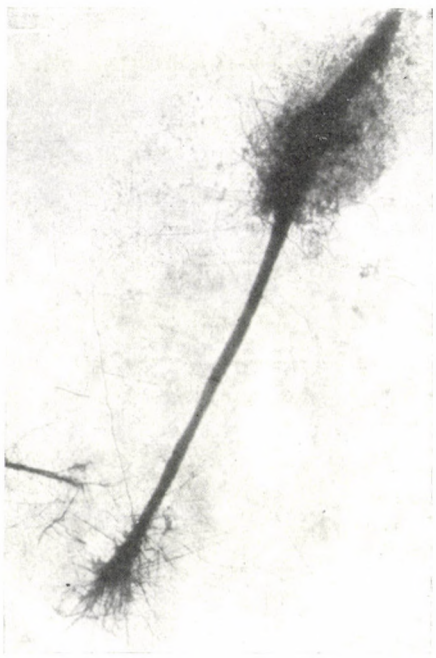

Fig. 3. A fully mature synnema showing a basal part, a stipe and a spear-shaped fertile head

on, the developing synnema adds more cells in diameter. The cells of the developing synnema are usually $12-22\ \mu$ in length, and $3-4\ \mu$ in width. The rhizoidal appendages grow downwards and become multicellular. They usually aggregate around the basal part of the synnema to form a swollen structure (Fig. 2) referred to by BESADA and YUSEF (1968) as bulbous body.

The fully mature synnema can be differentiated into a basal region, a median sterile stipe and a terminal sporulating region (Fig. 3). This latter part has a central column of closely packed and parallel hyphae and an outer mantle developing from external branches of the column and carries phialides rarely as terminal

cells but usually as lateral ones developing at acute angles to their subtending hyphae (Fig. 4). The mantle thus forms a compact to loose hymenium or palisade layer over the surface of the terminal sporulating region. Phialides can be seen at the tip of the upper fertile part indicating that the growth of synnema in *Trichurus* is of the determinate type as suggested by TABER (1961) for *Graphium ulmi*

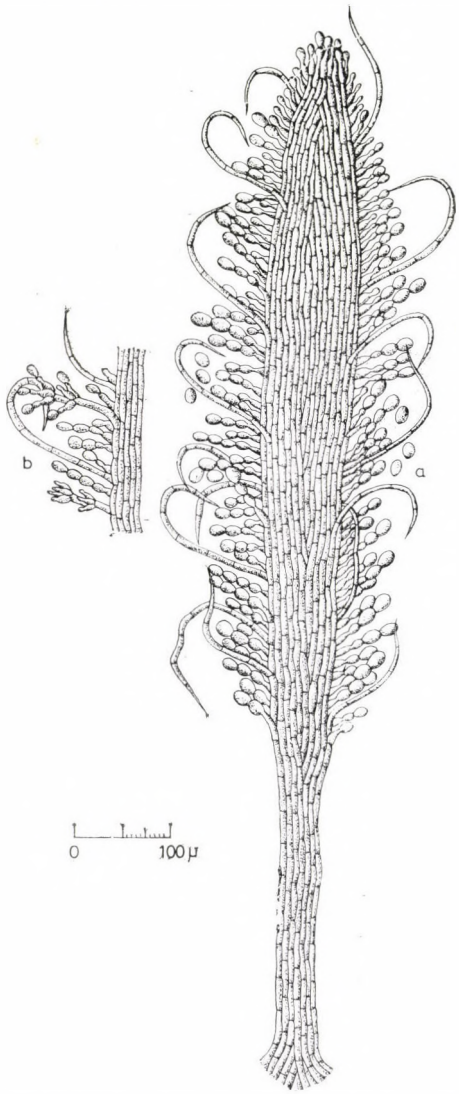

Fig. 4. A fully mature synnema with a terminal fertile part consisting of a central sterile column covered by a mantle of hyphae that subtend phialides abstricting conidia. Curved, uniseriate and multicellular setae are also observed. After Besada (A study of UAR soil Mycoflora. M. Sc. Thesis, University of Alexandria)

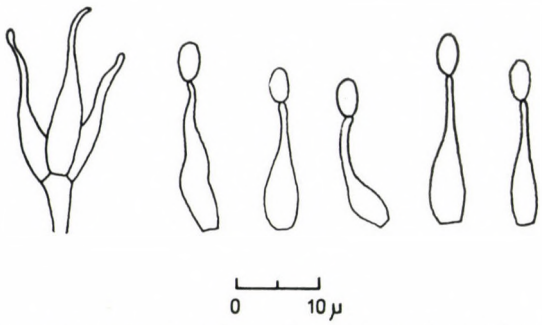

Fig. 5. Phialides or sporogenous cells, each with a sterigma subtending a conidium

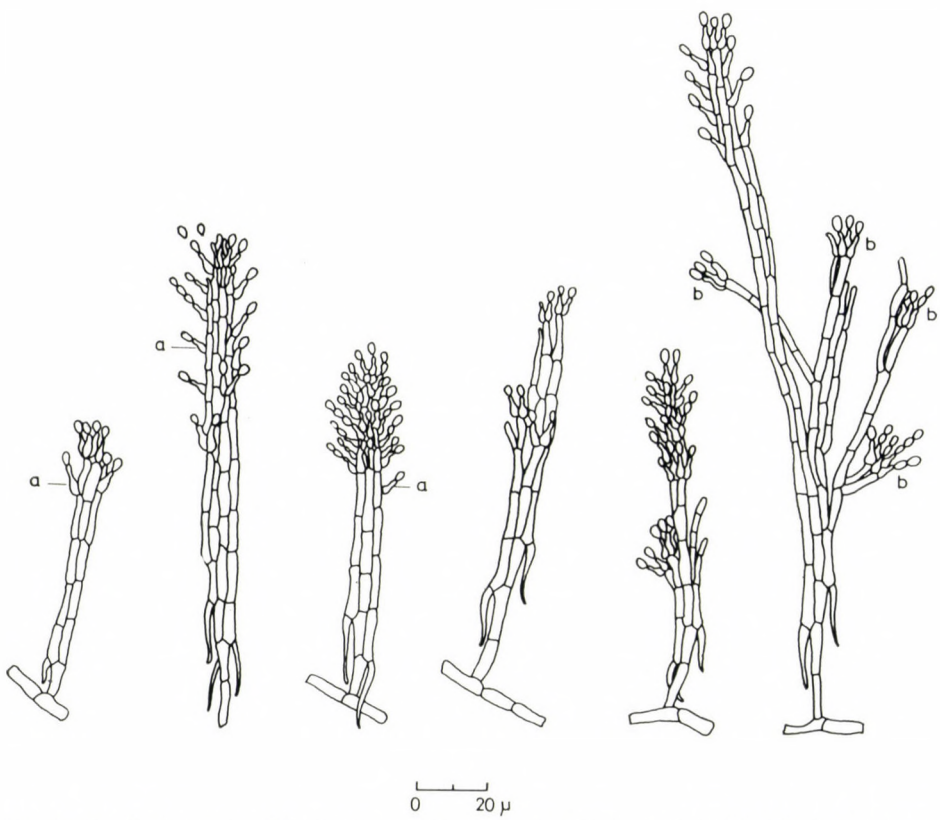

Fig. 6. The development of the synnema proceeds as previously explained in Fig. 1. It appears that sporulation could take place early before the synnema attains its full length and mature form. Phialides abstricting conidia arise solitary (a) or in fascicles (b). The illustration to the extreme left shosw a synnema primordium that keeps two-celled thick for a considerable length. The illustration to the extreme right represents a synnema primordium that gives ultimately a branched synnema

and *Stysanus* and not of the indeterminate type as represented by *Isaria cretacea* where a growing apex persists and sporulation occurs only at some distance from this apex. Phialides are smooth-walled, fuliginous, almost flask-shaped, 6×2 μ, slightly inflated below and narrowing at the apex to carry directly a chain of conidia or a slender sterigma that subtends the conidia (Fig. 5). Phialides arise either solitary (Fig. 6a) or in groups at the ends of metulae (Fig. 6b) which may be carried

Fig. 7. A long synnema with a tail-like fertile terminal part

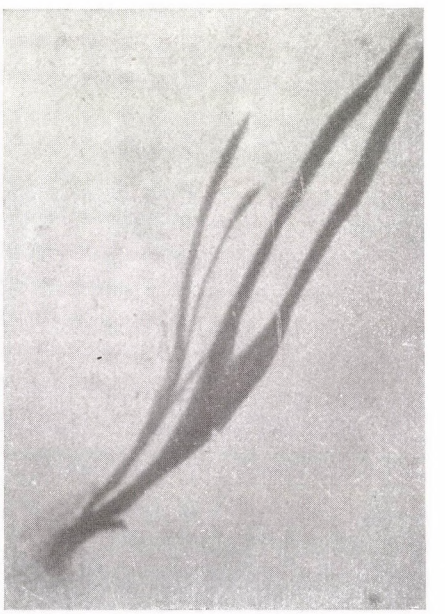

Fig. 8. A repeatedly forked synnema with comparatively low load of conidia on the branches, upper parts and thus these latters look at this stage tail or feather-like and not spear-headed

on more or less dichotomously branched hyphae. The terminal sporulating region of the synnema is tail-like in comparatively young synnemata where none or a few branches subtending the metuale carrying the phialides are present (Fig. 7), or it may be spear-shaped in older synnemata where the branches carrying the metulae are more extensive (Fig. 3). On examining the phialides under the oil-immersion lens no annellations were observed at their apices, contrary to what was reported before by SWART (1964) in *Trichurus terrophilus* and by MORTON and SMITH (1963) in *Doratomyces*. Consequently the present authors are not inclined to group the genus *Trichurus* with the series *Annellosporae*. Conidia catenulate in a basipetal succession, one-celled, ovate, with truncate base, approximately 4×3 μ, smooth-walled, and without a mucilage coat. They are hyaline, fuliginous or in mass black-

ish metallic grey. Curved, multicellular, uniseriate, fuliginous setae are observed in the fertile part of the synnema (Fig. 4). Synnemata arise usually solitary and unbranched (Figs 3, 7). In some cases, however, the stalk of the synnema may branch producing two to several stipes, each ending in a fetile part (Figs 2, 8).

Acknowledgment

The authors wish to thank their colleague W. H. BESADA Esq. for the supply of a pure culture of the organism used in this study and for his sincere help with the photographic work. They gratefully acknowledge the kind help of Abdullah Zein EDDEAN Esq. with the line drawings.

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A Study on Certain Factors Affecting Synnema Length in *Trichurus spiralis* Hasselbring

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The effect of temperature, pH, light, carbon source, nitrogen source, osmotic pressure and the antibiotic actidione (cycloheximide) was tested on synnema length and frequency in cultures of *T. spiralis*.

Long synnemata were noticed at 20° and 25°C; at 30° and 35°C the shortest synnemata were observed. At 15°C synnemata of median length were recorded. At 40°C no synnemata could develop.

Longest synnemata can be seen at the pH range 8.6–9.0. Cultures exposed to short periods of illumination (one or 3 hours/day) were observed to favour the longest synnemata. No synnemata were detected in cultures exposed continuously to light.

Mannitol, glucose, lactose or sucrose as the sole source of carbon in the medium favoured synnemata of moderate length. Long synnemata were found to develop on galactose or starch. Maltose supports the development of the longest synnemata and it was noticed that the optimum concentration of maltose for maximum length of synnema was dependent on the temperature of incubation. Sporulation of *T. spiralis* could not be detected on acetate or citrate. Fumaric and succinic acids favoured short synnemata.

Longest synnemata were obtained on media with nitrate or glycine. Ammonium sulphate was inhibitory to the development of synnema, and on ammonium tartrate shortest synnemata were observed.

The addition of 1 M sodium chloride to the culture medium was suppressive to synnema development.

The antibiotic actidione in a concentration of 1 mg/ml of medium showed a retarding effect on the length of synnema.

In certain species of fungi, development of synnemata is apparently a necessary prerequisite to sporulation (LOUGHHEED, 1961). On the other hand, in other stilbaceous species, formation of synnemata is inconsistent and sporadic. Thus the significance of synnemata as a taxonomic criterion in the classification of the Hyphomycetes will not be clearly elucidated until the factors which control the development of these structures are better understood. Of the studies carried out so far in this line one can mention those of MANTEIFEL and SHAPOSHNIKOFF (1927). TABER and VINING (1959), TABER (1960; 1961), LOUGHHEED (1961), CARLILE *et al.*, (1961; 1962a, b), AL-HASSAN and FERGUS (1967), HARRIS and TABER (1970) and TRINCI and BANBURY (1967).

On cultivating *Trichurus spiralis* on agar media we have repeatedly observed that the dimensions of synnemata (coremia) vary considerably under different

cultural conditions (Fig. 1). It was thus thought that it might be worthy to study the effect of temperature, pH, light, carbon and nitrogen requirements, osmotic pressure and an antibiotic (actidione) on the frequency and length of such sporiferous structures and consequently on the degree of sporulation. The factors that effectively control spore formation are undoubtedly of significant importance when ap-

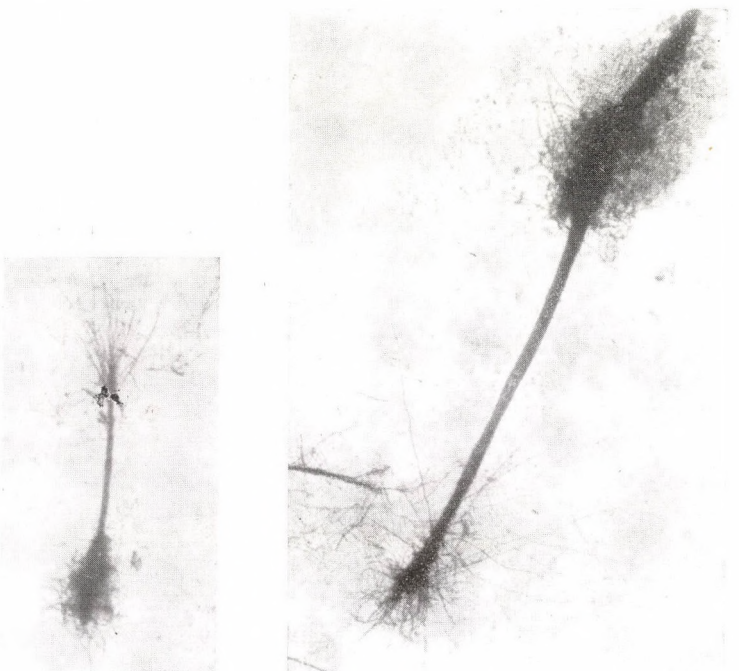

Fig. 1. Photomicrographs reproduced with the same magnification (×100 approx.) to represent two types of synnema of *Trichurus spiralis*. In spite of equal age, the synnema to the left is distinctly dwarf in comparison with the relatively giant one shown to the right. Either type is produced under conditions different from those favouring the development of the other

plied to restrict the spread of plant pathogenic fungi. *Trichurus terrophilus* and certain species of *Stysanus* were reported as causative agents of soft rots of wood (FINDLAY and SAVORY, 1954; LOHWAG, 1957; PRICE, 1957).

Material and Methods

The organism under investigation was isolated from an agricultural soil at Tanta, Egypt. Stock and plate cultures, unless otherwise specified, are maintained on glucose-nitrate agar (as g/l: glucose 10, $NaNO_3$ 2, KH_2PO_4 1.5, $MgSO_4$ $7H_2O$ 0.5, agar 15). The total length (covering stalk and head) of the synnema

in μ, as affected by the factors under investigation, was taken, unless otherwise stated, after a 3-week incubation period. In the majority of the experiments 75 synnemata were measured in each treatment, in a few other experiments, the length of 50 or 25 of these structures was considered. The results were statistically tested by carrying out an analysis of variance for each experiment to assess the degree of significance of variations in different treatments. It is the synnemata at the outer border of the colony that were measured in each treatment because they were more distinct and sufficiently isolated.

Results and Discussion

Temperature relations: By means of electrically heated incubators and constant temperature rooms, the effect of various temperature conditions (15, 20, 25, 30, 35 and 40°C) on the length of synnemata of *T. spiralis* was tested. Well developed long synnemata were observed at 20° and 25°C, while at 30° and 35°C the shortest synnemata were noticed. At 15° C synnemata of intermediate length were recorded, while no sporiferous structures could be detected at 40°C (Fig. 2). Maximum production of synnemata by *Stilbella thermophila* was observed within the range 27°–45°C (AL-HASSAN and FERGUS, 1967) and by *Ceratocystis ulmi* at about 25°C; at 30°C mycelial growth of the latter species free of synnemata was noticed (HARRIS and TABER, 1970).

pH-relations: On testing the effect of H-ion concentration on the length of synnemata, an organic nitrogen source (peptone, 3 g/l) was used instead of $NaNO_3$ and a phosphate buffer was added to the medium to keep the pH at almost its initial value.

Synnemata could develop on a relatively wide range of pH (3.9–9.0). A narrower range (5–8) was tested and reported to be favourable for synnemata production by *Stilbella thermophila* (AL-HASSAN and FERGUS, 1967). Measurements revealed longest synnemata of *T. spiralis* at pH range 8.6–9.0 Shorter synnemata were noticed at pH range 4.8–6.0. Still shorter ones were observed at pH 3.9, 7.4 and 7.9 (Fig. 3).

Effect of light: Inoculated media were exposed to zero, 1, 3, 7 or 24 hours illumination per day. The light used was of intensity 1000 foot candles/inch^2 and emitted by tubular fluorescent lamps. For the dark exposure periods, inoculated plates were placed in light-proof zinc boxes lined from inside with black paper and equipped each with two spirally twisted tubes one at each side of the box to allow for good aeration. The different treatments were put on one and the same glass shelf and at equal distances from the light source. Two fans were used, one at each side of the shelf, to dissipate excessive heat given off by the lamps. Temperature did not vary from one part of the shelf to the other and it was fairly constant at 23°C.

The total synnema length was found to increase with extension in the time of exposure to light up to 3 hours daily. A longer period of exposure (7 hours)

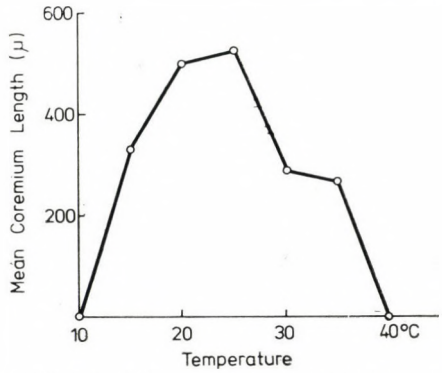

Fig. 2. Total length of synnemata of *T. spiralis* in 3-week-old cultures as affected by temperature

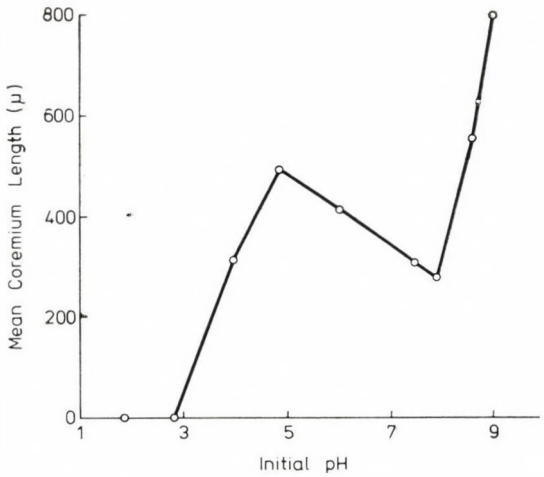

Fig. 3. Total length of synnemata of *T. spiralis* in 11-day-old cultures as affected by initial pH of the medium

favoured shorter synnemata. No synnemata could be seen in plates exposed continuously to light (Fig. 4).

Light is essential for initiation and continued growth of coremia of *Penicillium isariiforme* (CARLILE *et al.*, 1962a). However, for the development of structures in *Penicillium clavigerum* (CARLILE *et al.*, 1962b), *Isaria cretacea* strain A (TABER and VINING, 1959) and *Stilbella thermophila* (AL-HASSAN and FERGUS, 1967) light is not required, and for *Ceratocystis ulmi* (HARRIS and TABER, 1970) it was inhibitory.

Zonation, where rings of dense and sparse synnemata alternate was quite distinct in cultures exposed for 3 hours daily.

Carbon requirement: The effect of the carbon compound, mannitol, D(+) glucose, D(+) galactose, maltose, lactose, sucrose, starch, carboxymethylcellulose

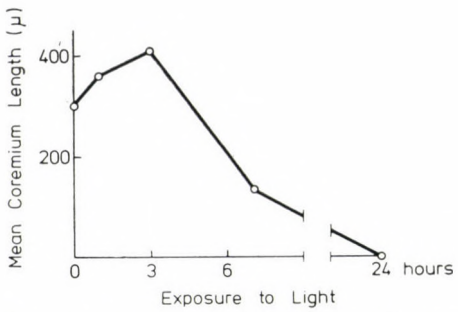

Fig. 4. Effect of the daily period of exposure to light on total synnema length in 2-week old cultures of *T. spiralis*

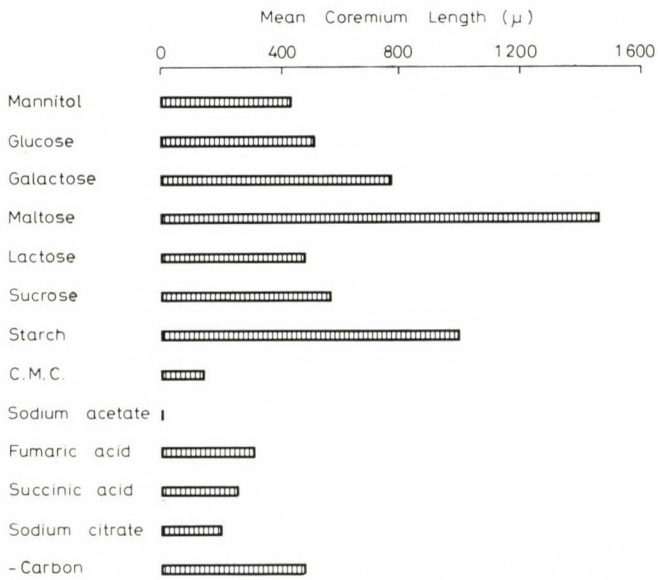

Fig. 5. Effect of the nature of carbon source on total synnema length in 3-week-old cultures of *T. spiralis*

(c.m.c.), sodium acetate, fumaric acid, succinic acid and sodium citrate, added individually in a concentration equivalent to 0.4% carbon to the basal medium lacking glucose, was tested on synnema length of *T. spiralis*. Starch and c.m.c. whose molecular weights are uncertain, were supplemented in amounts equal to that of glucose.

Synnemata were not observed on sodium acetate. Short synnemata were on c.m.c., fumaric acid, succinic acid and sodium citrate. Media lacking a carbon source or supplemented with mannitol, glucose, lactose or sucrose showed synnemata of moderate length. Long synnemata were favoured by galactose or starch. The longest ones were recorded on a maltose supplemented medium (Fig. 5).

Galactose, glucose, maltose lactose and starch permitted good production of synnemata by *Isaria cretacea* (TABER and VINING, 1959). Synnemata formation by this species was inhibited by mannitol, sucrose or acetate (TABER and VINING, 1959; TABER, 1961). AL-HASSAN and FERGUS (1967) recorded good production of synnemata by *Stilbella thermophila* on using glucose or starch, while with mannitol, galactose or sucrose the formation of synnemata was inhibited. A crude preparation of glucose is required for good production of synnemata by *Hirsutella gigantea* (LOUGHHEED, 1961). Lactose and starch supported good sporulation of *T. spiralis* var. *minuta* (MEHROTRA, 1964).

On testing the effect of different concentrations of maltose (0.25, 0.5, 1, 2, 5, 10, 15 and 20%) at the winter room temperature (15–25°C) on synnema length of *T. spiralis*, it was found that the longest synnema were on 10% maltose concentration and slightly shorter (but relatively long) ones were on 2, 5 and 15% of the sugar. Concentrations lower than 2 and higher then 15% favoured short synnemata (Fig. 6).

A second experiment was carried out for the same purpose, but with fixed incubation temperatures (7, 11, 20, 25, 30 and 35°C). The concentrations of maltose used were 0.5, 1, 2 or 5% in the same basal medium. Of the temperatures

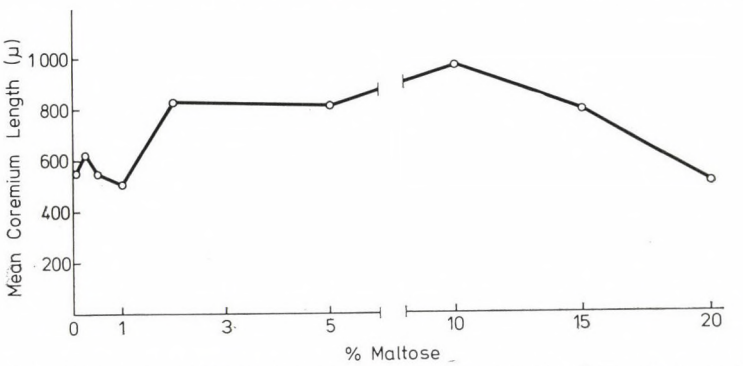

Fig. 6. Effect of maltose concentration on total synnema length of *T. spiralis* in 3-week-old cultures incubated at the winter room temperature (15–25°C)

used, 20°C was found to favour the longest synnemata on the lower sugar concentrations (0.5, 1 and 2%). With higher temperatures, significantly shorter synnemata were noticed. On 5% maltose, the longest synnemata were recorded at 20° and 25°C. Cultures exposed to 30°C and 35°C revealed short synnemata. At 7° and 11°C, the development of synnemata was completely suppressed (Fig. 7).

Comparison of the data obtained in these two experiments at the common temperature 20°C revealed concordant results (Figs 6 and 7).

Nitrogen requirement: The effect of the nature of nitrogen source on the length of synnema of *T. spiralis* was attempted using one at a time of the following nitrogen compounds: sodium nitrate, ammonium sulphate, ammonium tartarate, glycine, neopeptone, beef extract and casein hydrolysate in amounts equivalent

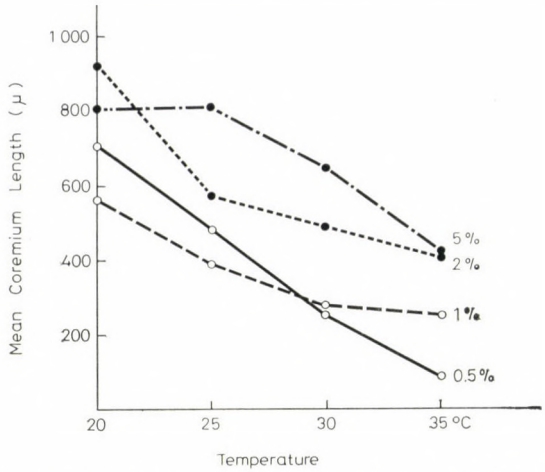

Fig. 7. Effect of maltose concentration on total synnema length of *T. spiralis* in 3-week-old cultures at different incubation temperatures

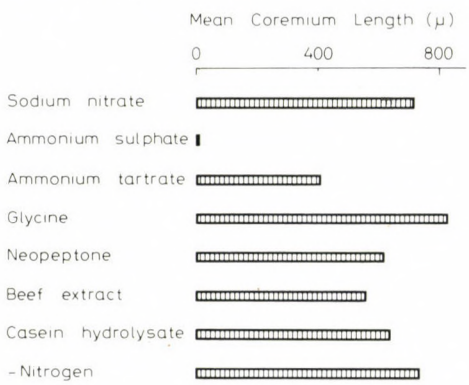

Fig. 8. Effect of the nature of nitrogen source on total synnema length in 26-day-old cultures of *T. spiralis*

to 2 g sodium nitrate per litre and added to the basal medium lacking a nitrogen source.

In 26-day-old cultures of *T. spiralis*, media with nitrate, glycine, or that lacking a nitrogen source supported longest synnemata. The shortest synnemata were detected in cultures supplemented with ammonium tartrate. On ammonium sulphate, no synnemata were seen, only chlamydospores. Synnemata with intermediate length were observed on the other sources of nitrogen used (Fig. 8). Thus the development of synnemata of *T. spiralis* appears not to require a specific nitrogen source, all the compounds tested (save ammonium sulphate) favoured spore formation. Similar results were obtained for *Graphium ulmi* (TABER, 1961).

Effect of osmotic pressure: To test the effect of various osmotic pressures on the length of synnemata of *T. spiralis*, different amounts of sodium chloride were

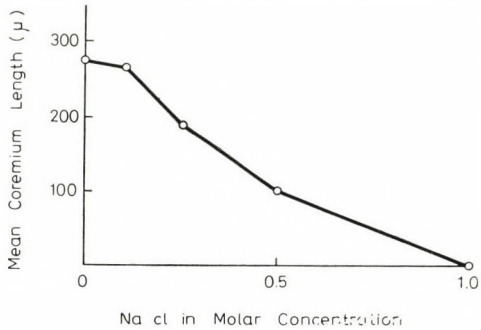

Fig. 9. Effect of sodium chloride molar concentration (M) in a solid medium on total synnema length in 3-week old cultures of *T. spiralis*

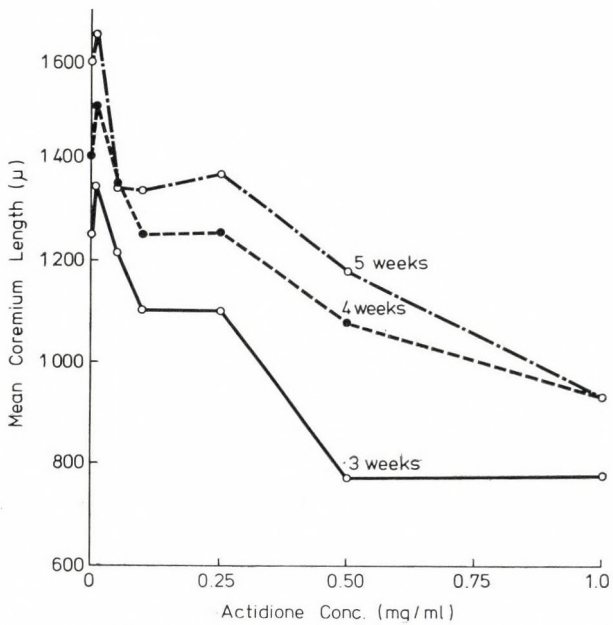

Fig. 10. Effect of actidione concentration on total synnema length of *T. spiralis*

added to agar basal media to give 0.00, 0.10, 0.25, 0.50, 1.00, 2.00 or 3.00 molar (M) final concentration of the salt.

Long synnemata were detected on the control medium (without sodium chloride) and on those furnished with 0.10 M of sodium chloride. Shorter synnemata were noticed on media with 0.25 or 0.50 M salt solution. On media with 1 M salt solution, synnemata were totally missing (Fig. 9). These results indicate that synnema formation by *T. spiralis* was depressed by sodium chloride. *Aspergillus niger* showed no conidia in a nutrient solution with 1% sodium chloride (MOLLIARD, 1918).

Effect of the antibiotic actidione: Actidione was applied in the final concentrations of 0.01, 0.05, 0.10, 0.25, 0.50 or 1.00 mg/ml of the basal solution. A higher concentration of the antibiotic could not be tested because of its restricted solubility. The basal medium without the chemical was used as a control.

Measurements of the synnema length in 3, 4, and 5-week-old cultures showed a slight stimulatory effect with the lowest actidione concentration (0.01 mg/ml). The higher the concentration of actidione used, the shorter would be the synnemata obtained (Fig. 10).

Because of an inhibitory action on plant pathogens, actidione was used in the control of diseases incited by these organisms (VAUGHN *et al.*, 1949; DE ZEEUW and VAUGHN, 1950; CATION, 1953; HILBORN, 1953; TODD, 1955; KENAGE and KIESLING, 1957; MOSS, 1957; WILSON and ARK, 1958; PRIDHAM, 1961). However, the antibiotic failed to control certain plant diseases (ZUCKERMAN, 1957).

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Review of the Mycoflora of Hungary

PART XI

Deuteromycetes: Moniliales

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The last two parts of this serial publication will present the Moniliales (Hyphomycetes) species of Hungary. Arrangement of this material differs basically from the previous parts, where the SACCARDO system of Sphaeropsidales and Melanconiales was applied. Conidium ontogeny proved to be a correct and dependable characteristic for the differentiation of suprageneric taxa (families) among Moniliales. For this reason a simple Moniliales system, based on these characters will be proposed, and outlined here.

The first modernistic Moniliales system, based on conidium ontogeny was created by HUGHES (1953). This system was improved by TUBAKI (1958; 1963). Groups, based on the mode of conidium development were raised to family rank by SUBRAMANIAN (1962). His family names were, however, extremely unfortunate. Names were not uniform: partly were formed from so-called "typical" generic names, and partly from conidium ontogeny types. Especially confusing names were Tuberculariaceae and Torulaceae. The former, combining the phialidic types in the SUBRAMANIAN system, is still used in the sense of SACCARDO for sporodochium forming fungi. On the other hand blastospore forming types were collected by SUBRAMANIAN in his family Torulaceae. Nevertheless it turned out, that Torula, the type genus of this family is characterized by the production of porospores, and not by blastospores.

In order to arrange the Hungarian Moniliales in a proper way, a new, simple system of these fungi had to be constructed. Family names were uniformly formed from conidium ontogeny types, according to BARRON (1968). To underline the imperfect character of these fungi, "-conidiaceae" endings were applied. Meristematic nature of the conidiophore, a character difficult to observe in the course of routine identification, was omitted from this system.

The following key serves both the definition, and the differentiation of the Moniliales families proposed here.

Key to families of Moniliales*

1 a Conidia are formed from pre-existing elements of the colony, they are not newly produced cells ("thallospores") 2

b Conidia are newly formed cells, blastospores in a wider sense ("conidia vera") 3

2 a Conidia are formed by the fragmentation of the conidiophore, or conidiogenous hypha, so they are actually arthrospores
1. family: *Arthroconidiaceae*

b Conidium is actually an apical cell, separated by a septum, and attached by a broad base. It is actually an aleuriospore, which can be defined as a holoblastic conidium as well. For this reason the first conidium formed on an annellophorum (see later) can be considered an aleurioconidium, too
2. family: *Aleurioconidiaceae*

3 a Both inner and outher walls of the conidiogenous cell are involved in the formation of the conidium (holoblastic conidia) 4

b None of the walls participate, or only the inner wall of the conidiogenous cell is involved in the formation of the conidium (enteroblastic conidia) . 7

4 a No change in length of the conidiophore during conidiogenesis 5

b Conidiophore length increases during conidiogenesis 6

5 a Conidia originate from one or from a few sites of the conidiogenous cell, usually in chains 3. family: *Blastoconidiaceae*

b Conidia originate simultaneously from the entire surface of the conidiogenous cell, usually solitary 4. family: *Botryoblastoconidiaceae*

6 a Conidiophore increases in length toward the main axis during conidiogenesis. Proliferation of the tip of the conidiogenous cell results annellation (annellophorum) 5. family: *Annelloconidiaceae*

b Conidiophore increases in length sympodially during conidiogenesis. As a result of this alternated sympodial development, sympodula type of conidiogenous cells are formed 6. family: *Sympoduloconidiaceae*

7 a Inner wall of the conidiogenous cell is involved in the formation of the conidium, which actually blows out together with the inner wall through a minute pore of the outher wall (porospore) 7. family: *Poroconidiaceae*

b None of the walls of the conidiophore participate in the formation of the conidia, which are produced from open phialides and are surrounded by newly developed cell walls 8. family: *Phialoconidiaceae*

Conidium ontogeny of a number of Hungarian Moniliales species is not yet known. In addition a few species have been described from Hungary on names, which are not legitimate anymore, the species in question, however, were never replaced into a valid genus. These species will be listed in an "Appendix", at the very end of this publication, and they will be arranged alphabetically.

* Formal desriptions of the proposed new families of Moniliales will be presented in the next, finishing part of this serial publication.

Ordo: *Moniliales*
Familia: *Arthroconidiaceae*
Genus: *Amblyosporium* FRES.
Amblyosporium botrytis FRES.
Syn.: Monilia albo-lueta SETCH.
MOESZ G. 1. (1459) as: Monilia albo-lueta SETCH.

Genus: *Coniosporium* LINK ex FR.

Coniosporium arundinis (CORDA) SACC.
MOESZ G. 1. (1635); MOESZ G. 13.
Coniosporium aterrimum (CORDA) SACC.
MOESZ G. 1. (1635); MOESZ G. 13.
Coniosporium bambusae (THÜM. et BOLLA) SACC.
MOESZ G. 1. (1635)
Coniosporium physciae (KALCHBR.) SACC.
HOLLÓS L. 1.
Coniosporium rhizophilum (PREUSS) SACC.
HOLLÓS L. 7.; MOESZ G. 1. (1635); MOESZ G. 11.
Coniosporium shiraianum (SYD.) BUBÁK
MOESZ G. 1. (1635); MOESZ G. 13.

Genus: *Coniothecium* CORDA

Coniothecium applanatum SACC.
HOLLÓS L. 1; HOLLÓS L. 7.
Coniothecium complanatum (NEES) SACC.
HOLLÓS L. 1.
Coniothecium epidermitis CORDA
HOLLÓS L. 1.
Coniothecium eryngii MOESZ
MOESZ G. 2.; MOESZ G. 13.
Coniothecium glumarum SACC.
MOESZ G. 1. (1735)
Coniothecium kabatii BRES.
MOESZ G. 1. (1735)
Coniothecium toruloides CORDA
HOLLÓS L. 1.

Genus: *Trichothecium* LINK ex FR.

Trichothecium candidum WALLR.
HOLLÓS L. 7.; MOESZ G. 1. (1598); MOESZ G. 13.
Trichothecium obovatum (BERK.) SACC.
HOLLÓS L. 1.
Trichothecium roseum LINK ex FR.
HOLLÓS L. 1.; HOLLÓS L. 7.; LEHOCZKY J. 3.; MOESZ G. 1. (1958); MOESZ G.

4.; MOESZ G. 11.; MOESZ G. 13.; NYERGESNÉ E. 1.; TÓTH S. 6.; TÓTH S. 7.; UBRIZSY G. 1.; VÖRÖS J. 4.

Familia: *Aleurioconidiaceae*

Genus: *Bactridium* KUNZE ex FR.

Bactridium flavum KUNZE
ZELLER L. 1.

Genus: *Clasterosporium* SCHW.

Clasterosporium scirpicolum (FUCKEL) SACC.
HOLLÓS L. 1.

Genus: *Epicoccum* LINK ex WALLR.

Epicoccum durieuanum MONT.
VÖRÖS J. 5.

Epicoccum granulatum PENZIG
HOLLÓS L. 7.

Epicoccum neglectum DESM.
HUSZ B. 1.; MOESZ G. 1. (1869); MOESZ G. 11.; MOESZ G. 13.; PETRÓCZY I. 1.

Epicoccum nigrum LINK
Syn.: Epicoccum effusum FUCKEL; Epicoccum herbarum CORDA; Epicoccum micropus CORDA; Epicoccum purpurascens EHRENB.
PETRÓCZY I. 1.; PODHRADSZKY J. 1.; TÓTH S. 9.; VASS A. 2.; as: Epicoccum effusum FUCKEL: MOESZ G. 1. (1869); as: Epicoccum micropus CORDA: HOLLÓS L. 1.; as: Epicoccum herbarum CORDA: BUBÁK F. 1.; MOESZ G. 13.; PETRÓCZY I. 1.; as: Epicoccum purpurascens EHRENB.: HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1869); MOESZ G. 7.; MOESZ G. 11.; MOESZ G. 13.; PETRÓCZY I. 1.; UBRIZSY G. 1.; VÖRÖS J. 3.

Epicoccum vulgare CORDA
HOLLÓS L. 1.

Genus: *Mycogone* LINK ex CHEV.

Mycogone cervina DITM.
MOESZ G. 1. (1606); MOESZ G. 4.; MOESZ G. 12.; MOESZ G. 13.

Mycogone pezizae (RICHON) SACC.
HOLLÓS L. 7.

Mycogone rosea LINK
MOESZ G. 1. (1606); MOESZ G. 13.; UBRIZSY G. 4.; VÖRÖS J. 12.

Genus: *Sepedonium* LINK ex FR.

Sepedonium chrysospermum (BULL.) FR.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1577); MOESZ G. 12.; MOESZ G. 13.

Sporidesmium foliculatum (CORDA) MASON et HUGHES

Syn.: Helminthosporium foliculatum CORDA
HOLLÓS L. 1. as: Helminthosporium foliculatum CORDA
Sporidesmium sicynum THÜM.
MOESZ G. 1. (1736); MOESZ G. 13.

Genus: *Sporotrichum* LINK ex FR.
Sporotrichum aranearum CAV.
MOESZ G. 1. (1564)
Sporotrichum aureum LINK
MOESZ G. 1. (1564)
Sporotrichum croceum KUNZE et SCHM.
MOESZ G. 1. (1564); MOESZ G. 24.
Sporotrichum sporulosum LINK
MOESZ G. 1. (1564); MOESZ G. 4.; MOESZ G. 11.

Genus: *Stephanoma* WALLR.

Stephanoma strigosum WALLR.
Syn.: Synthetospora electa MORGEN
MOESZ G. 1. (1579); MOESZ G. 13.; UBRIZSY G. 2.; MOESZ G. 1. (1625) as: Synthetospora electa MORGEN

Familia: *Blastoconidiaceae*

Genus: *Acremoniella* SACC.

Acremoniella atra (CORDA) SACC.
VASS A. 2.; VÖRÖS J. 3.; VÖRÖS J. 4.

Genus: *Arthrinium* KUNZE ex FR.

Arthrinium curvatum KUNZE
Syn.: Camptoum curvatum (KUNZE) LINK
VASS A. 2. as: Camptoum curvatum (KUNZE) LINK
Arthrinium phaeosporum (CORDA) ELLIS
Syn.: Melanconium sphaerospermum (PERS.) LINK; Papularia sphaerosperma (PERS.) HÖHNEL
as: Melanconium sphaerospermum (PERS.) LINK: HOLLÓS L. 1. as: Papularia sphaerosperma (PERS.) HÖHNEL: MOESZ G. 1.; MOESZ G. 13.
Arthrinium puccinioides (DC ex MÉRAT) KUNZE
Syn.: Goniosporium puccinioides (DC) LINK
as: Goniosporium puccinioides (DC) LINK: HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1655); MOESZ G. 11.
Arthrinium sporophleum KUNZE
MOESZ G. 1. (1654); MOESZ G. 11.; MOESZ G. 12.

Genus: *Aureobasidium* VIALA et BOYER

Aureobasidium pullulans (DE BARY) ARN.
Syn.: Dematium pullulans DE BARY
as: Dematium pullulans DE BARY: MOESZ G. 1. (1674); MOESZ G. 13.

Genus: *Bispora* CORDA

Bispora antennata (PERS. ex FR.) MOESZ
Syn.: Bispora monilioides CORDA; Torula antennata PERS.
as: Bispora monilioides CORDA: MOESZ G. 1. (1704); MOESZ G. 13. as: Torula antennata PERS.: HOLLÓS L. 1.

Genus: *Ceratosporium* SCHW.

Ceratosporium fuscescens SCHW.
Syn.: Ceratosporium strepsiceras (CES.) SACC.
HOLLÓS L. 7. as: Ceratosporium strepsiceras (CES.) SACC.

Genus: *Cladosporium* LINK ex FR.

Cladosporium aecidiicolum THÜM.
HOLLÓS L. 1.; MOESZ G. 1. (1709); MOESZ G. 2.; MOESZ G. 11.; MOESZ G. 13.; VASS A. 2.
Cladosporium elegans PENZIG
MOESZ G. 13.
Cladosporium bignoniae SCHW.
MOESZ G. 2.; MOESZ G. 13.
Cladosporium cucumerinum ELLIS et ARTH.
HÓDOSY S. 1.; UBRIZSY G. 1.
Cladosporium exoasci LINDAU
HOLLÓS L. 1.; HOLLÓS L. 7.
Cladosporium fuligineum BON.
MOESZ G. 12.; MOESZ G. 13.
Cladosporium fulvum COOKE
KASZONYI S. 1.
Cladosporium herbarum (PERS.) LINK
Syn.: Cladosporium epiphyllum (PERS.) MART.; Cladosporium fasciculatum CORDA; Cladosporium graminum CORDA; Cladosporium tomentosum CORDA
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1709); MOESZ G. 2.; MOESZ G. 11.; MOESZ G. 12.; MOESZ G. 13.; NYERGESNÉ E. 1.; UBRIZSY G. 1.; VASS A. 2. as: Cladosporium epiphyllum (PERS.) MART.: HOLLÓS L. 1.; MOESZ G. 1. (1709); MOESZ G. 2.; MOESZ G. 13. as: Cladosporium fasciculatum CORDA: MOESZ G. 1. (1709) as: Cladosporium graminum CORDA: HOLLÓS L. 7.; MOESZ G. 1. (1709); MOESZ G. 11.; MOESZ G. 13.; VASS A. 2. as: Cladosporium tomentosum CORDA: MOESZ G. 1. (1709)
Cladosporium herbarum (PERS.) LINK var. *nigricans* ROTH.
MOESZ G. 13.
Cladosporium laricis SACC.
MOESZ G. 1. (1709)
Cladosporium soldanellae JAAP.
MOESZ G. 1. (1709)

Cladosporium typharum DESM.
MOESZ G. 1. (1709); MOESZ G. 13.
Cladosporium vincae MOESZ
MOESZ G. 1. (1709); MOESZ G. 13.; MOESZ G. 15.

Genus: *Fusidium* LINK ex FR.

Fusidium aeruginosum LINK
Syn.: Cylindrium aeruginosum (LINK) LINDAU; Cylindrium flavo-virens (DITM.) BON.
as: Cylindrium aeruginosum (LINK) LINDAU: MOESZ G. 1. (1524); TÓTH S. 8.; VASS A. 2.; VÖRÖS J. 5. as: Cylindrium flavo-virens (DITM.) BON.: MOESZ G. 1. (1524); MOESZ G. 13.
Fusidium clandestinum CORDA
Syn.: Cylindrium clandestinum (CORDA) SACC.
MOESZ G. 1. (1524) as: Cylindrium clandestinum (CORDA) SACC.
Fusidium eburneum SCHROET.
MOESZ G. 1. (1523)

Genus: *Haplographium* BERK. et BR.

Haplographium bicolor GROVE
HOLLÓS L. 1.
Haplographium toruloides (FRES.) SACC.
Syn.: Cephalotrichum toruloides (FRES.) HÖHNEL
MOESZ G. 1. (1673); MOESZ G. 13. as: Cephalotrichum toruloides (FRES.) HÖHNEL

Genus: *Heterosporium* KLOTZSCH ex COOKE

Heterosporium avenae OUDEM.
MOESZ G. 1. (1727); MOESZ G. 13.; MOESZ G. 15.
Hterosporium echinulatum (BERK.) COOKE
KRENNER J. A. 1.; MOESZ G.1. (1727); MOESZ G. 13.; MOESZ G. 22.; UBRIZSY G. 3.
Heterosporium fraxini FRED. et WINGE.
MOESZ G. 1. (1727); MOESZ G. 13.
Heterosporium gracile SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; KRENNER J. A. 1.; MOESZ G. 1. (1727); MOESZ G. 11.; MOESZ G. 13.
Heterosporium robiniae KABÁT et BUBÁK
KRENNER J. A. 1.
Heterosporium variabile COOKE
KRENNER J. A. 1.; MOESZ G. 1. (1727); MOESZ G. 13.

Genus: *Monilia* BON.

Monilia aurea (PERS.) GMEL.

BUBÁK F. 1.; MOESZ G. 1. (1519); MOESZ G. 24.; TÓTH S. 8.; VASS A. 2.; ZELLER L. 1.

Monilia cinerea BON.

Syn.: Monilia laxa ADERH. et RUHL.

MOESZ G. 1. (1519); MOESZ G. 13.; HOLLÓS L. 1.; UBRIZSY G. 1.; VÖRÖS J. 4.; UBRIZSY G. 1. as: Monilia laxa ADERH. et RUHL.

Monilia fimicola COST. et MATR.

KRENNER J. A. 1.; MOESZ G. 1. (1519); MOESZ G. 13.

Monilia fructigena PERS.

HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1519); MOESZ G. 2.; MOESZ G. 13.; UBRIZSY G. 1.

Monilia linhartiana SACC.

MOESZ G. 1. (1519); UBRIZSY G. 1.

Monilia sitophila (MONT.) SACC.

VÖRÖS J. 3.

Genus: *Nigrospora* ZIMM.

Nigrospora oryzae (BERK. et BR.) PETCH

LELLEY I. 1.; PODHRADSZKY J. 1.

Genus: *Periconia* TODE ex SCHW.

Periconia atra CORDA

Syn.: Sporocybe atra (CORDA) FR.

as: Sporocybe atra (CORDA) FR.: HOLLÓS L. 1.; HOLLÓS L. 7

Periconia byssoidea PERS. ex SCHW.

Syn.: Periconia pycnospora FRES.; Sporocybe byssoidea (PERS.) FR.

HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1649) as: Periconia pycnospora FRES.: HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1649); MOESZ G. 11.; VASS A. 2.; VÖRÖS J. 3. as: Sporocybe byssoidea (PERS.) FR.: TÓTH S. 6.

Genus: *Polyscitalum* REISS

Polyscitalum saccardianum BRIZI

TÓTH S. 7.

Genus: *Zygosporium* MONT.

Zygosporium oscheoides MONT.

VASS A. 1.

Familia: *Botryoblastoconidiaceae*

Genus: *Botryosporium* CORDA

Botryosporium diffusum (ALB. et SCHW.) CORDA

VÖRÖS J. 3.

Botryosporium pyramidale (BON.) COST.

UBRIZSY G. 3.

Genus: *Botrytis* PERS. ex FR.

Botrytis allii MUNN.
VÖRÖS J. 4.

Botrytis cinerea PERS.
Syn.: Botrytis acinorum PERS.; Botrytis cinerea PERS. var. sclerotiophila (KLEB.) SACC.; Botrytis vulgaris FR.
HÓDOSY S. 1.; HOLLÓS L. 1.; MOESZ G. 1. (1567); MOESZ G. 4.; MOESZ G. 13.; NYERGESNÉ E. 1.; UBRIZSY G. 1.; UBRIZSY G. 2.; UBRIZSY G. 4.; VÖRÖS J. 1.; VÖRÖS J. 4. as: Botrytis acinorum PERS.: HOLLÓS L. 1.; MOESZ G. 1. (1567); MOESZ G. 13. as: Botrytis cinerea PERS. var. sclerotiophila (KLEB.) SACC.: HOLLÓS L. 1. as: Botrytis vulgaris FR.: HOLLÓS L. 1.

Botrytis cinerea PERS. f. *convallariae* KLEB.
HÓDOSY S. 1.

Botrytis densa DITMAR
Syn.: Botrytis tenella SACC.
as: Botrytis tenella SACC.: MOESZ G. 1. (1567); UBRIZSY G. 2.

Botrytis elliptica (BERK.) COOKE
VÖRÖS J. 1.; VÖRÖS J. 3.; VÖRÖS J. 7.

Botrytis epigaea LINK
HOLLÓS L. 1.; HOLLÓS L. 7.

Botrytis fabae SARD.
TÓTHNÉ Z. E. 1.

Botrytis infestans (HAZSL.) SACC.
UBRIZSY G. 2.

Botrytis paeoniae (OUDEM.) VAN BEYMA
HÓDOSY S. 1.; KASZONYI S. 1.

Botrytis reptans BON.
MOESZ G. 1. (1567)

Botrytis tulipae (LIB.) LIND
HUSZ B. 2.; VÖRÖS J. 4.; VÖRÖS J. 5.

Genus: *Gonatobotrys* CORDA

Gonatobotrys flava BON.
VÖRÖS J. 4.; VÖRÖS J. 5.

Genus: *Oedocephalum* PREUSS

Oedocephalum glomerulosum (BULL.) SACC.
MOESZ G. 1. (1528); MOESZ G. 13.; UBRIZSY G. 3.; VÖRÖS J. 4.; VÖRÖS J. 5.

Familia: *Annelloconidiaceae*

Genus: *Acrodictys* M. B. ELLIS

Acrodictys globulosa (TÓTH) M. B. ELLIS
Syn.: Monodictys globulosa TÓTH
TÓTH S. 3. as: Monodictys globulosa TÓTH

Genus: *Cephalotrichum* LINK

Cephalotrichum stemonitis (PERS.) LINK
Syn.: Stysanus stemonitis (PERS.) CORDA; Doratomyces stemonitis (PERS.) MORTON et SMITH
as: Stysanus stemonitis (PERS.) CORDA: MOESZ G. 1. (1789); TÓTH S. 6.; VÖRÖS J. 4.; VÖRÖS J. 5. as: Doratomyces stemonitis (PERS.) MORTON et SMITH: TÓTH S. 7.

Genus: *Deightoniella* HUGHES

Deightoniella arundinacea (CORDA) HUGHES
Syn.: Napicladium arundinaceum (CORDA) SACC.; Napicladium laxum BUBÁK
as: Napicladium arundinaceum (CORDA) SACC.: HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1725); MOESZ G. 11.; MOESZ G. 13.; VASS A. 1. as: Napicladium laxum BUBÁK: HOLLÓS L. 7.; MOESZ G. 1. (1725); MOESZ G. 13.

Genus: *Endophragmia* DUVERNOY et MAIRE

Endophragmia atra (BERK. et BR.) ELLIS
Syn.: Arthrobotryum atrum BERK. et BR.
VASS A. 2. as: Arthrobotryum atrum BERK. et BR.
Endophragmia atra (BERK. et BR.) ELLIS var. *majus* SACC.
Syn.: Arthrobotryum atrum BERK. et BR. var. majus SACC.
VASS A. 2. as: Arthrobotryum atrum BERK. et BR. var. majus SACC.
Endophragmia elliptica (BERK. et BR.) M. B. ELLIS
TÓTH S. 8.

Genus: *Graphium* CORDA

Graphium eumorphum SACC.
VÖRÖS J. 5.
Graphium pallescens (FUCKEL) MAGN.
HUSZ B. 1.
Graphium rigidum (PERS.) SACC.
MOESZ G. 1. (1780)
Graphium ulmi SCHWARZ.
MOESZ G. 13.; UBRIZSY G. 2.

Genus: *Mastigosporium* RIESS

Mastigosporium album RIESS
TÓTHNÉ Z. E. 1.

Genus: *Pollaccia* BALDACCI et CIF.

Pollaccia radiosa (LIB.) BALDACCI et CIF.
Syn.: Fusicladium radiosum (LIB.) LIND
VÖRÖS J. 1.; KRENNER J. A. 1. as: Fusicladium radiosum (LIB.) LIND

Genus: *Scopulariopsis* BAIN.

Scopulariopsis asperula (SACC.) HUGHES
Syn.: Torula asperula SACC.
as: Torula asperula SACC.: MOESZ G. 1. (1637); MOESZ G. 13.
Scopulariopsis brevicaulis (SACC.) BAIN.
Syn.: Penicillium brevicaule SACC.
MOESZ G. 13.; NYERGESNÉ E. 1.; VÖRÖS J. 2.; VÖRÖS J. 4.; KRENNER J. A. 1.
as: Penicillium brevicaule SACC.

Genus: *Septosporium* CORDA

Septosporium conjunctum PREUSS
HOLLÓS L. 1.

Genus: *Spilocaea* FR.

Snilocaea pomi FR.
Syn.: Fusicladium dendriticum (WALLR.) FUCKEL; Fusicladium dendriticum (WALLR.) FUCKEL var. sorbinum SACC.
as: Fusicladium dendriticum (WALLR.) FUCKEL: HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1706); MOESZ G. 11.; MOESZ G. 13.; UBRIZSY G. 1. as: Fusicladium dendriticum (WALLR.) FUCKEL var. sorbinum SACC.: MOESZ G. 13.

Genus: *Stigmina* SACC.

Stigmina carpophila (LÉV.) ELLIS
Syn.: Clasterosporium carpophilum (LÉV.) ADERH.: Clasterosporium amygdalearum (PASS.) SACC.
as: Clasterosporium carpophilum (LÉV.) ADERH.: HOLLÓS L. 7.; MOESZ G. 1. (1715); as: Clasterosporium amygdalearum (PASS.) SACC.: HOLLÓS L. 1.
Stigmina compacta (SACC.) ELLIS
Syn.: Steganosporium compactum SACC.; Thyrostroma compactum (SACC.) HÖHNEL
as: Steganosporium compactum SACC.: HOLLÓS L. 1. as: Thyrostroma compactum (SACC.) HÖHNEL: MOESZ G. 1.; MOESZ G. 13.
Stigmina glomerulosa (SACC.) HUGHES
Syn.: Exosporium glomerulosum (SACC.) HÖHNEL
HOLLÓS L. 7. as: Exosporium glomerulosum (SACC.) HÖHNEL
Stigmina palmivora (SACC.) HUGHES
Syn.: Exosporium palmivorum SACC.
as: Exosporium palmivorum SACC.: KRENNER J. A. 1.; MOESZ G. 1. (1887); MOESZ G. 13.

Familia: *Sympoduloconidiaceae*

Genus: *Arthrobotrys* CORDA

Arthrobotrys oligospora FRES.
TÓTH S. 6.; TÓTH S. 7.

Arthrobotrys superba CORDA
TÓTH S. 7.; TÓTH S. 9.; UBRIZSY G. 3.; VÖRÖS J. 4.

Genus: *Beauveria* VUILL.

Beauveria bassiana (BALS.) VUILL.
Syn.: Botrytis bassiana BALS.
MOESZ G. 13.; UBRIZSY G. 2.; VÖRÖS J. 3.; VÖRÖS J. 4. as: Botrytis bassiana BALS.: HOLLÓS L. 1.; MOESZ G. 1. (1567); MOESZ G. 7.

Genus: *Brachysporium* SACC.

Brachysporium coryneoideum (DE NOT.) SACC.
VASS A. 2.
Brachysporium gracile (WALLR.) SACC.
Syn.: Helminthosporium gracile WALLR.
as: Helminthosporium gracile WALLR.: MOESZ G. 1. (1723); MOESZ G. 7.

Genus: *Cercospora* FRES.

Cercospora acanthi PASS.
MOESZ G. 1. (1752); PÉNZES A. 1.
Cercospora aconiti PETRAK
PÉNZES A. 1.
Cercospora althaeina SACC.
HÓDOSY S. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 11.; PÉNZES A. 1.
Cercospora apii FRES.
PÉNZES A. 1.
Cercospora apii FRES. var. *carotae* PASS.
PÉNZES A. 1.
Cercospora apii FRES. var. *petroselini* SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; PÉNZES A. 1.; UBRIZSY G. 1.
Cercospora armoraciae SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 11.; MOESZ G. 13.; PÉNZES A. 1.
Cercospora bacilligera (BERK. et BR.) WOLLENW.
Syn.: Fusarium erubescens APP. et OV.
UBRIZSY G. 1. as: Fusarium erubescens APP. et OV.
Cercospora bellynckii (WESTEND.) SACC.
MOESZ G. 1. (1752); MOESZ G. 12.
Cercospora berteroae HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 17.; PÉNZES A. 1.
Cercospora beticola SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 12.; MOESZ G. 13.; PÉNZES A. 1.; UBRIZSY G. 1.; VASS A. 2.
Cercospora bizzozeriana SACC. et BERL.
HOLLÓS L. 1.; MOESZ G. 1. (1752); MOESZ G. 12.; MOESZ G. 13.; PÉNZES A. 1.

Cercospora boussingaultiae ROUM.
MOESZ G. 13.; PÉNZES A. 1.
Cercospora budapestiensis PÉNZES
MOESZ G. 13.; PÉNZES A. 1.; TÓTH S. 8.
Cercospora campi silii SPEG.
MOESZ G. 1. (1752); PÉNZES A. 1.
Cercospora carlinae SACC.
HOLLÓS L. 1.; MOESZ G. 1. (1752); MOESZ G. 13.; PÉNZES A. 1.
Cercospora carotae (PASS.) KAZN. et SIEM.
HÓDOSY S. 1.
Cercospora cerasella SACC.
BUBÁK F. 1.; MOESZ G. 1. (1752); MOESZ G. 12.; PÉNZES A. 1.
Cercospora chaerophylli HÖHNEL
HOLLÓS L. 7.; PÉNZES A. 1.
Cercospora chenopodiicola BRES.
PÉNZES A. 1.
Cercospora circumscissa SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 12.; PÉNZES A. 1.
Cercospora concors (CASP.) SACC.
PÉNZES A. 1.
Cercospora depazeoides (DESM.) SACC.
Syn.: Cercospora penicillata FRES.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 12.; MOESZ G. 13.; PÉNZES A. 1.; VÖRÖS J. 5.; HOLLÓS L. 1. as: Cercospora penicillata FRES.
Cercospora dubia (RIESS) WINTER
Syn.: Cercospora chenopodii FRES.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 4.; MOESZ G. 13.; MOESZ G. 26.; PÉNZES A. 1.; MOESZ G. 26. as: Cercospora chenopodii FRES.
Cercospora dulcamaraecola HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 3.; PÉNZES A. 1.
Cercospora elongata PECK
Syn.: Cercospora dipsaci HOLLÓS; Cercosporina elongata (PECK) SPEG.
HOLLÓS L. 1.; MOESZ G. 1. (1752); PÉNZES A. 1. as: Cercospora dipsaci HOLLÓS: HOLLÓS L. 2.; MOESZ G. 9. as: Cercosporina elongata (PECK) SPEG.: MOESZ G. 1.; MOESZ G. 7.; MOESZ G. 11.
Cercospora erythraeae HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 3.; PÉNZES A. 1.
Cercospora ferruginea FUCKEL
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 2.; MOESZ G. 4.; MOESZ G. 13.; PÉNZES A. 1.; TÓTH S. 8.
Cercospora fraxini (DC) SACC.

HOLLÓS L. 1.; HOLLÓS L. 7.; PÉNZES A. 1.

Cercospora galegae SACC.
MOESZ G. 1. (1752); MOESZ G. 4.; PÉNZES A. 1.

Cercospora gei (FUCKEL) BUBÁK
MOESZ G. 1. (1752)

Cercospora helvola SACC.
PÉNZES A. 1.

Cercospora kabatiana ALLESCH.
Syn.: Cercosporina kabatiana (ALLESCH.) MOESZ
as: Cercospora kabatiana (ALLESCH.) MOESZ: MOESZ G. 1.; MOESZ G. 11.

Cercospora ligustri ROUM.
VASS A. 2.

Cercospora loti HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 17.; PÉNZES A. 1.

Cercospora majanthemi FUCKEL
MOESZ G. 1. (1752)

Cercospora malvarum SACC.
HOLLÓS L. 1.; PÉNZES A. 1.

Cercospora medicaginis ELLIS et EVERH.
MOESZ G. 1. (1752); MOESZ G. 4.; PÉNZES A. 1.

Cercospora meliloti (LASCH.) OUDEM.
UBRIZSY G. 2.

Cercospora mercurialis PASS.
BUBÁK F. 1.; MOESZ G. 1. (1752); MOESZ G. 12.; MOESZ G. 13.; PÉNZES A. 1.

Cercospora microsora SACC.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1752); MOESZ G. 11.; MOESZ G. 12.; MOESZ G. 13.; MOESZ G. 20.; PÉNZES A. 1.

Cercospora myrthi ERIKS.
MOESZ G. 1. (1752)

Cercospora nasturtii PASS.
PÉNZES A. 1.

Cercospora nasturtii PASS. f. *sisymbrii* BAUML.
PÉNZES A. 1.

Cercospora nebulosa SACC.
PÉNZES A. 1.

Cercospora neriella SACC.
PÉNZES A. 1.

Cercospora nigellae HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 16.; MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora olivascens SACC.
HOLLÓS L. 1.; MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora olivascens SACC. var. *minor* SEREBR.
MOESZ G. 13.; PÉNZES A. 1.

Cercospora opuli (FUCKEL) HÖHNEL

HOLLÓS L. 7.; MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora paridis ERIKSS.
PÉNZES A. 1.

Cercospora periclymeni WINT.
MOESZ G. 1. (1752)

Cercospora plantaginis SACC.
HOLLÓS L. 7.; PÉNZES A. 1.

Cercospora polymorpha BUBÁK
MOESZ G. 1. (1752)

Cercospora radiata FUCKEL
HOLLÓS L. 1.; HOLLÓS L. 7.; KRENNER J. A. 1.; MOESZ G. 1. (1752); MOESZ G. 2.; MOESZ G. 12.; MOESZ G. 13.; PÉNZES A. 1.; UBRIZSY G. 1.; VÖRÖS J. 4.

Cercospora rautensis C. MASSAL.
MOESZ G. 1. (1752); MOESZ G. 11.

Cercospora resedae FUCKEL
HOLLÓS L. 1.; MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora rhamni FUCKEL
PÉNZES A. 1.

Cercospora rösleri (CATTAN.) SACC.
PÉNZES A. 1.

Cercospora rubi SACC.
HOLLÓS L. 7.

Cercospora sagittariae ELLIS et KELLERM.
MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora scandens SACC. et WINT.
PÉNZES A. 1.

Cercospora scandens SACC. et WINT. var. *macrospora* C. MASS.
Syn.: Cercospora tami HOLLÓS
HOLLÓS L. 7.; MOESZ G. 1. (1752) as: Cercospora tami HOLLÓS: HOLLÓS 2.; MOESZ G. 9.

Cercospora scandicearum MAGN.
HOLLÓS L. 1.; PÉNZES A. 1.

Cercospora smilacina SACC.
MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora taurica TRANZ.
HOLLÓS L. 1.; MOESZ G. 1. (1752); PÉNZES A. 1.

Cercospora thalictri THÜM.
MOESZ G. 1. (1752)

Cercospora traversiana SACC.
VÖRÖS J. 11.

Cercospora vexans MASSAL.
Syn.: Cercosporina vexans (MASSAL.) MOESZ
as: Cercosporina vexans (MASSAL.) MOESZ: MOESZ G. 1.; MOESZ G. 11.

Cercospora violae SACC.
BUBÁK F. 1.; HÓDOSY S. 1.; HOLLÓS L. 1.; MOESZ G. 1. (1752); MOESZ G. 2.; MOESZ G. 13.; PÉNZES A. 1.
Cercospora violae tricoloris BRIOSI et CAV.
PÉNZES A. 1.
Cercospora vitis (LÉV.) SACC.
Syn.: Cercospora viticola (CES.) SACC.
MOESZ G. 1. (1752); MOESZ G. 13.; PÉNZES A. 1.; HOLLÓS L. 1. as: Cercospora viticola (CES.) SACC.

Genus: *Cercosporella* SACC.
Cercosporella cana SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1624); MOESZ G. 4.; MOESZ G. 13.
Cercosporella inconspicua (WINT.) HÖHNEL
HOLLÓS L. 7.
Cercosporella narcissi BOUD.
Syn.: Cercosporella narcissi HOLLÓS
HOLLÓS L. 7. as: Cercosporella narcissi HOLLÓS: HOLLÓS L. 11.; MOESZ G. 10.
Cercosporella pastinacae KARST.
HOLLÓS L. 7.
Cercosporella podospermi HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 16.
Cercosporella primulae ALLESCH.
HUSZ B. 1.; MOESZ G. 1. (1624); MOESZ G. 12.
Cercosporella rapistri HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 5.
Cercosporella scirpi MOESZ
MOESZ G. 1. (1624); MOESZ G. 11.
Cercosporella verbasci HOLLÓS
HOLLÓS L. 7.; HOLLÓS L. 11.
Cercosporella virgaureae (THÜM.) ALLESCH.
MOESZ G. 1. (1624)

Genus: *Cordana* PREUSS em. SACC.
Cordana pauciseptata PREUSS
TÓTH S. 9.

Genus: *Dactylella* GROVE
Dactylella ellipsospora (PREUSS) GROVE
TÓTH S. 6.; TÓTH S. 7.

Genus: *Fusicladium* BON.
Fusicladium betulae ADERH.
HOLLÓS L. 7.

Fusicladium carpophilum OUDEM.
Syn.: Megacladosporium carpophilum (THÜM.) V. B.; Cladosporium carpophilum THÜM.
KASZONYI S. 1. as: Megacladosporium carpophilum (THÜM.) V. B.
Fusicladium crataegi ADERH.
HOLLÓS L. 1.
Fusicladium fraxini ADERH.
HOLLÓS L. 7.; MOESZ G. 1. (1706); MOESZ G. 13.
Fusicladium heterosporum HÖHNEL
Syn.: Didymaria epilobii HOLLÓS
MOESZ G. 1. (1706); MOESZ G. 11. as: Didymaria epilobii HOLLÓS: HOLLÓS L. 1.; HOLLÓS L. 15.
Fusicladium orbiculatum (DESM.) THÜM.
HOLLÓS L. 7.; MOESZ G. 1. (1706); MOESZ G. 13.
Fusicladium schnablianum ALLESCH.
MOESZ G. 1. (1706); MOESZ G. 12.
Fusicladium virescens BON.
Syn.: Fusicladium pirinum (LIB.) BON.
as: Fusicladium pirinum (LIB.) BON.: MOESZ G. 1. (1706); MOESZ G. 13.; MOESZ G. 26.; UBRIZSY G. 1.

Genus: *Hadrotrichum* FUCKEL

Hadrotrichum phragmitis FUCKEL
MOESZ G. 1. (1669)

Genus: *Helicosporium* NEES ex FR.

Helicosporium albidum GROVE
TÓTH S. 6.
Helicosporium lumbricoides SACC. em. MATR.
TÓTH S. 7.

Genus: *Isariopsis* FRES.

Isariopsis griseola SACC.
Syn.: Phaeoisariopsis griseola (SACC.) FERRARIS
MOESZ G. 1. (1800); UBRIZSY G. 1. as: Phaeoisariopsis griseola (SACC.) FERRARIS: MOESZ G. 1.; MOESZ G. 4.

Genus: *Monacrosporium* OUDEM.

Monacrosporium oxysporum SACC. et MARCH.
VÖRÖS J. 3.

Genus: *Passalora* FR. et MONT.

Passalora bacilligera MONT. et FR.
MOESZ G. 1. (1705)
Passalora depressa (BERK. et BR.) SACC.

Syn.: Fusicladium depressum (BERK. et BR.) SACC.; Megacladosporium depressum (BERK. et BR.) SACC.
MOESZ G. 1. (1705) as: Fusicladium depressum (BERK. et BR.) SACC: MOESZ G. 1. (1706); MOESZ G. 13.; VASS A. 2. as: Megacladosporium depressum (BERK. et BR.) SACC.: KASZONYI S. 1.; VÖRÖS J. 1.

Passalora graminis (FUCKEL) HÖHNEL
BÉKÉSI P. 1.

Passalora kirchneri (HEGYI) PETRAK

Syn.: Fusicladium depressum (BERK. et BR.) SACC. var. petroselini SACC.; Marssonina kirchneri HEGYI
HÓDOSY S. 1. as: Fusicladium depressum (BERK. et BR.) SACC. var. petroselini SACC.: KRENNER J. A. 1.; MOESZ G. 1. (1706); UBRIZSY G. 1.

Genus: *Pleiochaeta* (SACC.) HUGHES

Pleiochaeta setosa (KIRCHN.) HUGHES

Syn.: Ceratophorum setosum KIRCHN.
HÓDOSY S. 1. as: Ceratophorum setosum KIRCHN.: MOESZ G. 1. (1719); MOESZ G. 11.

Genus: *Polythrincium* KUNZE et SCHM. ex FR.

Polythrincium trifolii KUNZE et SCHM.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; MOESZ G. 1. (1708); MOESZ G. 4.; MOESZ G. 12.; MOESZ G. 13.

Genus: *Ramularia* SACC.

Ramularia aequivoca (CES.) SACC.
MOESZ G. 1. (1621)

Ramularia adenophorae MOESZ
MOESZ G. 28.

Ramularia agrestis SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)

Ramularia ajugae (NIESSL) SACC.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); UBRIZSY G. 3.

Ramularia alismatis FAUTREY
HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 12.; MOESZ G. 13.

Ramularia anchusae MASSAL.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)

Ramularia angelicae HÖHNEL
BUBÁK F. 1.; HOLLÓS L. 8.

Ramularia anthemidis HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; HOLLÓS L. 17.; MOESZ G. 1. (1621)

Ramularia aplospora SPEG.
Syn.: Ovoularia aplospora (SPEG.) MAGN.; Ovularia schroeteri (KÜHN.) SACC.; Ovularia haplospora (SPEG.) LINDAU
as: Ovularia haplospora (SPEG.) LINDAU: MOESZ G. 1. (1563); MOESZ G. 12.; ZELLER L. 1.
Ramularia archangelicae LINDR.
HUSZ B. 1.
Ramularia ari FAUTREY
UBRIZSY G. 1.
Ramularia armoraciae FUCKEL
MOESZ G. 1. (1621); UBRIZSY G. 1.
Ramularia aromatica (SACC.) HÖHNEL
MOESZ G. 1. (1621)
Ramularia arvensis SACC.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia atropae ALLESCH.
HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia bäumleriana MOESZ
MOESZ G. 1. (1621); MOESZ G. 13.; MOESZ G. 15.
Ramularia betae ROSTRUP
MOESZ G. 1. (1621); MOESZ G. 11.
Ramularia biflorae MAGN.
HUSZ B. 1.
Ramularia brunnea PECK
MOESZ G. 1. (1621); MOESZ G. 11.
Ramularia buniadis VESTERGR.
Syn.: Ramularia buniadis MOESZ
MOESZ G. 13. as: Ramularia buniadis MOESZ: MOESZ G. 1. (1621); MOESZ G. 15.
Ramularia calcea (DESM.) CES.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia calthae LINDR.
MOESZ G. 1. (1621)
Ramularia cardui KARST.
MOESZ G. 1. (1621)
Ramularia cardui KARST. var. *personatae* ALLESCH.
MOESZ G. 1. (1621)
Ramularia centaureae LINDR.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia centaureae atropurpureae BUBÁK
BUBÁK F. 1.; HOLLÓS L. 8.

Ramularia cerinthes HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 8.; HOLLÓS L. 15.
Ramularia cervina SPEG.
HOLLÓS L. 8.
Ramularia cirsii ALLESCH.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia coccinea (FUCKEL) WESTEND.
HOLLÓS L. 7.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia coleosporii SACC.
HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 12.
Ramularia conspicua SYD.
BUBÁK F. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia coronillae BRES.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; UBRIZSY G. 1.
Ramularia craccae LINDAU
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia cupulariae PASS.
Syn.: Ramularia inulae-britannicae ALLESCH.
HOLLÓS L. 7.; MOESZ G. 1. (1621) as: Ramularia inulae britannicae ALLESCH.: HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia cylindroides SACC.
BUBÁK F. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.; UBRIZSY G. 1.
Ramularia cynoglossi LINDR.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia cytisi HOLLÓS
HOLLÓS L. 2.; HOLLÓS L. 7.
Ramularia decipiens ELLIS et EVERH.
HOLLÓS L. 8.
Ramularia didyma UNGER
Syn.: Didymaria didyma (UNGER) SCHROET.
as: Didymaria didyma (UNGER) SCHROET.: MOESZ G. 1. (1604); MOESZ G. 11.
Ramularia didymarioides BRIOSI et SACC.
HOLLÓS L. 7.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia doronici (SACC.) THÜM.
BUBÁK F. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.
Ramularia dubia RIESS
MOESZ G. 1. (1621)
Ramularia echii HOLLÓS
HOLLÓS L. 2.; HOLLÓS L. 7.
Ramularia epilobii-parviflori LINDR.
MOESZ G. 1. (1621)
Ramularia epilobii-rosei LINDAU
MOESZ G. 1. (1621)

Ramularia equiseti MASSAL.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia filaris FRES.
HOLLÓS L. 8.
Ramularia filarszkyana MOESZ
MOESZ G. 16.
Ramularia galegae SACC.
HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 4.; MOESZ G. 11.; UBRIZSY G. 1.
Ramularia gei (ELIASS.) LINDR.
HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia geranii (WESTEND.) FUCKEL
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1721); MOESZ G. 11.; MOESZ G. 12.; TÓTH S. 8.
Ramularia geranii phaei (C. MASSAL.) MAGN.
BUBÁK F. 1.; HOLLÓS L. 8.
Ramularia hamburgensis LINDAU
BUBÁK F. 1.; HOLLÓS L. 8.
Ramularia harioti SACC.
Syn.: Ramularia brunellae ELLIS et EVERH.
MOESZ G. 1. (1621) as: Ramularia brunellae ELLIS et EVERH.: HOLLÓS L. 7.; MOESZ G. 1. (1621)
Ramularia hellebori FUCKEL
HOLLÓS L. 7.; VÖRÖS J. 5.
Ramularia heraclei (OUDEM.) SACC.
MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia jurineae HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; HOLLÓS L. 17.
Ramularia knautiae (MASSAL.) BUBÁK
HOLLÓS L. 7.
Ramularia krigeriana BRES.
MOESZ G. 1. (1621)
Ramularia lactea (DESM.) SACC.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.; MOESZ G. 13.
Ramularia lampsanae (DESM.) SACC.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia lathyri HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia leontodontis MOESZ
MOESZ G. 1. (1621); MOESZ G. 11.; MOESZ G. 13.; MOESZ G. 15.
Ramularia libanotidis BUBÁK
BUBÁK F. 1.; HOLLÓS L. 8.

Ramularia ligustrina MAUBL.
MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia lycopi HOLLÓS
HOLLÓS L. 1.; HOLLÓS L. 8.; HOLLÓS L. 17.
Ramularia lysimachiae THÜM.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.
Ramularia lysimachiarum LINDR.
MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia macrospora FR.
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; HUSZ B. 1.; MOESZ G. 1. (1621); MOESZ G. 2.; MOESZ G. 3.; MOESZ G. 7.; MOESZ G. 11.; MOESZ G. 13.
Ramularia macrospora FR. var. *asteris* TREL.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia macrospora FR. var. *campanulae trachelii* SACC.
HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia macularis (SCHROET.) SACC.
UBRIZSY G. 3.
Ramularia marrubii MASSAL.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.
Ramularia matronalis SACC.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia melampyrina MASSAL.
HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 4.
Ramularia menthicola SACC.
HOLLÓS L. 8.
Ramularia microspora THÜM.
BUBÁK F. 1.; HOLLÓS L. 8.
Ramularia monticola SPEG.
HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.
Ramularia nymphaeae BRES.
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia obovata FUCKEL
Syn.: Ovularia obovata (FUCKEL) SACC.
as: Ovularia obovata (FUCKEL) SACC.: HOLLÓS L. 7.; MOESZ G. 1. (1563)
Ramularia onobrychidis ALLESCH.
HOLLÓS L. 7.
Ramularia onopordi MASSAL.
HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)
Ramularia oreophila SACC.
HOLLÓS L. 8.; HUSZ B. 1.; MOESZ G. 1. (1621); MOESZ G. 12.
Ramularia parietariae PASS.

BUBÁK F. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.

Ramularia pastinacae BUBÁK

BUBÁK F. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.

Ramularia peucedani HOLLÓS

HOLLÓS L. 1.; HOLLÓS L. 8.; HOLLÓS L. 15.

Ramularia philadelphi SACC.

MOESZ G. 1. (1621)

Ramularia picridis FAUTREY et ROUM.

BUBÁK F. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)

Ramularia plantaginea SACC. et BERL.

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621)

Ramularia plantaginis ELLIS et MART.

MOESZ G. 1. (1621); VASS A. 2.

Ramularia phyteumatis SACC. et WINT.

HUSZ B. 1.; MOESZ G. 1. (1621)

Ramularia pratensis SACC.

HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.

Ramularia primulae THÜM.

MOESZ G. 1. (1621); MOESZ G. 13.; ZELLER L. 1.

Ramularia pulsatillae HOLLÓS

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.

Ramularia punctiformis (SCHLECHT.) HÖHNEL

Syn.: Cercospora epilobii SCHMIDT

MOESZ G. 1. (1621); PÉNZES A. 1.

Ramularia recognita MASSAL.

HOLLÓS L. 7.

Ramularia repentis OUDEM.

HOLLÓS L. 7.

Ramularia rosea (FUCKEL) SACC.

HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621)

Ramularia rubicunda BRES.

HUSZ B. 1.; MOESZ G. 1. (1621); MOESZ G. 12

Ramularia rumicis scutati ALLESCH.

HUSZ B. 1.

Ramularia sagittariae BRES.

MOESZ G. 1. (1621)

Ramularia salviae HOLLÓS

HOLLÓS L. 2.; HOLLÓS L. 7.

Ramularia sambucina SACC.

HÓDOSY S. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.; UBRIZSY G. 1.; VASS A. 2.

Ramularia saniculae LINHART

HOLLÓS L. 8.

Ramularia saxifragae SYD.

BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 13.

Ramularia scelerata COOKE

HOLLÓS L. 8.

Ramularia schulzeri BAUMLER

HOLLÓS L. 8.

Ramularia scrophulariae FAUTREY et ROUM.

MOESZ G. 1. (1621); MOESZ G. 13.

Ramularia senecionis (BERK. et BR.) SACC.

BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 8.

Ramularia sideritidis HOLLÓS

HOLLÓS L. 1.; HOLLÓS L. 8.; HOLLÓS L. 17.; MOESZ G. 1. (1621)

Ramularia silvestris SACC.

MOESZ G. 1. (1621)

Ramularia sonchi-oleracei FAUTREY

MOESZ G. 1. (1621)

Ramularia sorokinii SACC. et SYD.

Syn.: Ramularia leonuri SOROK.; Ramularia leonuri SACC. et PENZ.

as: Ramularia leonuri SOROK.: MOESZ G. 1. (1621); MOESZ G. 11. as: Ramularia leonuri SACC. et PENZ.: HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.

Ramularia stachydis (PASS.) MASSAL.

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.

Ramularia stachydis-germanicae MOESZ

MOESZ G. 1. (1621); MOESZ G. 21.

Ramularia succisae SACC.

HOLLÓS L. 1.; HOLLÓS L. 8.

Ramularia tanaceti LIND

HOLLÓS L. 7.; MOESZ G. 1. (1621); MOESZ G. 11.

Ramularia taraxaci KARST.

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.; MOESZ G. 13.

Ramularia thalictri HOLLÓS

HOLLÓS L. 2.; HOLLÓS L. 7.

Ramularia thesii SYD.

HOLLÓS L. 1.; HOLLÓS L. 6.; HOLLÓS L. 7.

Ramularia tricherae LINDR.

MOESZ G. 1. (1621); MOESZ G. 3.

Ramularia tulasnei SACC.

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 2.; MOESZ G. 11.; MOESZ G. 13.

Ramularia urticae CES.

HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; HUSZ B. 1.; MOESZ G. 1. (1621); MOESZ G. 12.; MOESZ G. 13.

Ramularia valerianae (SPEG.) SACC.

HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.

Ramularia variabilis FUCKEL
BUBÁK F. 1.; HOLLÓS L. 1.; HOLLÓS L. 7.; HOLLÓS L. 8.; MOESZ G. 1. (1621); MOESZ G. 11.; MOESZ G. 13.
Ramularia veronicae FAUTREY
HOLLÓS L. 1.; HOLLÓS L. 8.
Ramularia winteri THÜM.
MOESZ G. 1. (1621)

Genus: *Scolecotrichum* KUNZE ex FR.

Scolecotrichum clavariarum (DESM.) SACC.
MOESZ G. 1. (1707) as: Scolicotrichum clavariarum (DESM.) SACC.
Scolecotrichum graminis FUCKEL
as: Scolicotrichum graminis FUCKEL: HOLLÓS L. 1.; MOESZ G. 1. (1707); MOESZ G. 11.; MOESZ G. 12.; MOESZ G. 13.
Scolecotrichum graminis FUCKEL f. *avenae* ERIKSS.
UBRIZSY G. 1. as: Scolicotrichum graminis FUCKEL f. avenae ERIKSS.
Scolecotrichum fraxini PASS.
BUBÁK F. 1.

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Die Untersuchung der insekticiden Wirkung von Thiol-Reagentien

I. Thiocyanate, Isothiocyanate und potentielle Isothiocyanat-Bildner

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Der Verfasser hat die *in vitro* ausgeübte insekticide Wirkung einer Anzahl von Thiocyanaten und Isothiocyanaten sowie einer Reihe von Verbindungen, die sich *in vivo* leicht zu Isothiocyanaten umwandeln können, auf die Imagines und Larven des Kartoffelkäfers untersucht. Zum Vergleich hat er parallel die insekticide Wirkung einiger im Handel üblicher Stoffe unter identischen Versuchsumständen geprüft.

Dabei hat er die größte Mehrheit der untersuchten Thiocyanate und Isothiocyanate insekticid wirksam gefunden, während die überwiegende Mehrheit der potentiellen Isothiocyanatbildner sich bei den Untersuchungen als vollständig wirkungslos erwies.

Von den zum Vergleich untersuchten Insekticiden waren das Lindan, das Thiodan, und das Nikotin auf die Imagines und Larven des Kartoffelkäfers wirksam; ihre Wirkung wurde aber durch die des Neguvons und des Dichlorvos weit übertroffen, welch letztere sowohl im geschlossenen, wie auch im offenen System auch in einer 10–20mal niedrigeren Menge wirksam waren.

Von den untersuchten Verbindungen erwiesen sich das n-Heptyl-, n-Octyl-, n-Nonyl-, Benzyl-, 3- und 4-Chlor-phenyl-thiocyanat als die wirksamsten, deren insekticide Wirkung auf den Kartoffelkäfer nahe gleich groß war, wie die des Lindans, Thiodans, und des Nikotins; aber ihre Wirkung war viel niedriger als die vom Neguvon und Dichlorvos.

Der Verfasser bespricht die Zusammenhänge zwischen der chemischen Struktur und der insekticiden Wirkung der von ihm untersuchten Verbindungstypen, und beschäftigt sich mit den Hypothesen ihres Wirkungsmechanismus.

Es ist allgemein bekannt, daß eine Anzahl der Enzyme, welche die Stoffwechselprozesse aller Tier-, Pflanzen- und Mikroorganismenzellen katalisieren, zu den Thiolenzymen gehört, d. h. zum Kreis der Enzyme, deren Tätigkeit von der intakten Beschaffenheit einer oder mehrerer freier Thiol-Gruppen abhängt; wenn die Thiol-Gruppen der Enzyme solchen Typs mit irgendeiner Verbindung in irgendeiner Weise in chemische Reaktion treten, hört die Tätigkeit des betreffenden Enzyms in diesem Fall auf, was verschiedene Störungen des Stoffwechsels der Zelle, sogar eventuell die volle Lähmung, folglich das Aufhören der Zellenvermehrung, selbst eventuell auch die Abtötung der Zelle nach sich zieht. Die Verbindungen, die unter biologischen Umständen mit den Thiol-Gruppen der Thiolenzyme reagieren können, werden Thiol-Reagentien genannt. Die große Mehrheit der Thiol-Reagentien ist meistens imstande, eine antimikrobielle Wirkung von hohem Grad und oft auch die mit breitem Wirkungsspektrum auszuüben.

Es scheint die Annahme nahe zu liegen, daß eine Anzahl der Verbindungen im Kreise der Thiol-Reagentien vorzufinden wäre, die auch eine insekticide Wirkung kleineren oder größeren Grades auszuüben vermöchten.

Um diese Frage zu klären, haben wir die insekticide Wirkung zahlreicher Typen der Thiol-Reagentien auf den Kartoffelkäfer unter Laboratoriumsumständen untersucht, und die als wirksam gefundenen Stoffe wurden von uns grundsätzlich studiert. Es wurde bestimmt ihre niedrigste noch insekticid wirksame Menge, und es wurde untersucht, ob ihre insekticide Wirkung durch "Kontakt-Wirkung" oder "Gas-Wirkung" ausgeübt wird, ob sie auch außer Imagines auch für die Larven wirksam sind.

Ihre insekticide Wirkung wurde von uns mit der Wirkung der im Handel üblichen aktivsten insekticiden Mittel verglichen, ferner haben wir innerhalb der von uns untersuchten Grund-Typen der Thiol-Reagentien die Zusammenhänge gesucht, die zwischen der chemischen Struktur und der insekticiden Wirkung bestehen.

Untersuchungsmethode

Unsere Untersuchungen wurden an Kartoffelkäfern *(Leptinotarsa decemlineata)* durchgeführt, die von Kartoffelfeldern gesammelt wurden. Die von uns angewandten Kartoffelkäfer waren gut entwickelte Imagines mit einem individuellen Gewicht von 130 – 140 mg. Aus unseren Verbindungen wurden 0.2%-ige acetonische Lösungen verfertigt, deren 1.0, 0.5, 0.25 ml Mengen in Petri-Schalen mit einem Durchmesser von 9.2 cm eingemessen, und am Boden der Schalen ebenmäßig zerflößt wurden. Das Aceton verdünstete bei Zimmertemperatur in einigen Minuten, die untersuchten Verbindungen blieben am Boden der Petri-Schalen; sie bildeten im allgemeinen einen ziemlich gleichmäßigen Beschlag auf der Glasoberfläche. Dadurch betrug die Menge der untersuchten Verbindungen in den Petri-Schalen 2, 1 bzw. 0.5 mg, was mit Bezug auf 1 cm^2 30, 15 bzw. 7.5 μg, auf das Gebiet von einem Hektar umgerechnet (im Falle gleichmäßiger Berieselung) einer Wirkstoffsmenge 3.0, 1.5 bzw. 0.75 kg/Hektar entspricht.

Unmittelbar nach der Verdunstung des Lösungsmittels wurden 10 – 10 Kartoffelkäfer je Schale in die Petri-Schalen gelegt, die Petri-Schalen wurden mit ihren Deckeln zugedeckt, und die insekticide Wirkung (bzw. ihr Mangel) wurde nach einer Expositionszeit von 18 Stunden abgelesen. Die Versuche wurden bei 20 – 22°C Temperatur durchgeführt. Die Zahl der Parallelen betrug 2. Zur Kontrolle wurden sowohl verbindungsfreie "leere Kontrollen", wie auch sog. "aktive Verbindungskontrollen" zu jeder Serie angewandt, welche letzteren einen oder anderen in der Praxis gebräuchlichen insekticiden Stoff (z. B. Lindan, Nikotin, Neguvon usw.) enthielten. Diese Experimente bildeten die sogenannte "im geschlossenen System" durchgeführten Versuche.

Nach dem Beweis der verbindungsfreien "leeren Kontrollen" deckte die Luftmenge in den Petri-Schalen den Oxigenbedarf der je 10 Kartoffelkäfer für

18 Stunden reichlich; die Kontrollkäfer zeigten unveränderte Vitalität und Bewegungsfähigkeit nach der 18stündigen Expositionszeit.

Bei der Bewertung der Ergebnisse nach der Expositionszeit wurde darauf Rücksicht genommen, ob *alle* Käfer in den Petri-Schalen vernichtet bzw. völlig betäubt wurden, und sie nur auf die mechanische Berührung ihrer Fühler etwas Bewegungsreflex gaben, oder – wenn auch in mehr oder weniger krankem und hinfälligem Zustand – am Leben blieben, ob sie imstande waren, sich spontan in geringerem oder größerem Maße (eventuell nur ihre Gliedmaßen) zu bewegen.

Unsere Bewertungsmethode kann als ziemlich streng angesehen werden, weil nur "volle Ergebnisse" für maßgebend genommen wurden; Teilergebnisse wurden nicht berücksichtigt (weil das eine Anzahl von Irrtumsmöglichkeiten mit sich bringt, was sich auch durch eine Reihe der Rreproduktionen völlig nicht beseitigen läßt). Die erhaltenen Ergebnisse wurden von uns in allen Tabellen folgendermaßen bezeichnet:

\+ = 100% der Kartoffelkäfer wurde getötet, nicht einmal auf die Berührung ihrer Fühler zeigten sie ein Lebenszeichen. Da wurde die Verbindung als aktiv erachtet.

(+) = 100% der Kartoffelkäfer befand sich in einem vollständig betäubten Zustand, und sie zeigten nur auf Berührung ihrer Fühler einen gewissen Bewegungs-Reflex. In diesem Fall wurde die Verbindung als mittelmäßig wirksam bezeichnet.

– = Die Verbindung war wirkungslos. Die Mehrheit der Käfer blieb am Leben und war zur spontanen Bewegung fähig.

Wir möchten erwähnen, daß man durch diese Untersuchungsmethode nur das behaupten kann, ob eine Verbindung binnen relativ kurzer Einwirkungszeit eine insekticide Wirkung auf die Kartoffelkäfer ausüben könne. Die Verbindungen, welche ihre insekticide Wirkung nur nach relativ langer Einwirkungszeit ausüben (z. B. die chlorierten Kohlenwasserstoffe), erweisen sich bei der Anwendung dieser Untersuchungsmethode von vornherein als unwirksam. Unsere Untersuchungsmethode läßt nur darauf schließen, ob irgendwelche Verbindungen auf die Kartoffelkäfer unter *diesen Versuchsumständen* wirksam sind. Wir nehmen an, daß viele Verbindungen, die wir auf die Kartoffelkäfer als unwirksam gefunden haben, auf andere Insekten-Arten auch mit dieser Untersuchungsmethode, oder aber auch auf die Kartoffelkäfer selbst mit der Anwendung anderer Untersuchungsmethoden (z. B. im Falle längerer Einwirkungszeit, anderer Verbindungsmengen, usw.) als wirksam erwiesen hätten.

Diese Untersuchungsmethode haben wir darum gewählt, weil zwei Vertreter der Phosphatinsekticiden, nämlich die zum Vergleich untersuchten Neguvon und Dichlorvos, unter diesen Versuchsumständen sich als außerordentlich wirksam erwiesen haben, und sie bildeten deshalb den Beweis dafür, daß die durch uns angewandte Methode zur Auswahl solcher Verbindungen, die ihre insekticide Wirkung binnen kurzer Zeit ausüben können, geeignet ist. Die Verbindungen, die in einer Menge von 30 $\mu g/cm^2$ insekticide Wirkung von 100% hervorriefen,

wurden weiter ausführlicher, und zwar nicht nur im "geschlossenen System", sondern auch im "offenen System" untersucht. Diese Untersuchungsmethode lag den Umständen der *praktisen Anwendung* der insekticiden Stoffe wesentlich näher als die Umstände im "geschlossenen System". Die Untersuchung im "geschlossenen System" kann also als screening bezeichnet werden, mit dem man die Stoffe, die zur Durchführung weiterer Experimente geeignet zu sein schienen, wählen kann.

Die Untersuchungen im "offenen System" wurden wesentlich gleicherweise vorgenommen, wie die im "geschlossenen System". Ein Unterschied bestand nur darin, daß während die Petri-Schalen bei der letzteren mit Glasdeckel bedeckt waren, folglich ihr Luftraum von der äußeren Umgebung abgesondert (oder mindenstens die Möglichkeit der Verdunstung bedeutend vermindert) war, die Petri-Schalen bei den Untersuchungen im "offenen System" anstatt Glasdeckel mit perforierten Kunststoffplatten zugedeckt waren, wodurch der Luftraum der Petri-Schalen freien Ausgang zur Umgebung hatte und der eventuellen Verdunstung nichts im Wege stand, was die Verminderung der Menge am Boden der Petri-Schalen nach sich zieht, wie das bei der praktischen Anwendung der Insekticiden der Fall ist.

Unsere in beiden Systemen ausgeführten Untersuchungen waren zum Studium der *Kontaktwirkung* der Insekticide geeignet. Anhand dieser Untersuchungsmethode konnte sich die perorale Wirkung von vornherein nicht geltend machen, und infolgedessen fielen die Stoffe, die bloß *per os* insekticid wären (und auch jene, die ihre insekticide Wirkung nicht rasch auszuüben fähig sind), als wirkungslose Stoffe von vornherein aus.

Neben der Kontaktwirkung konnte man aber die simultane (eventuell ausschließliche) Geltung der eventuellen Gaswirkung in der Schaffung der insekticiden Wirkung nicht ausschließen. Auch zur Klärung dieser Frage wurden Versuche vorgenommen. Die Soffe, die sich anhand der Untersuchungen "im geschlossenen System" als insekticid in einer Menge von 30 $\mu g/cm^2$ erwiesen, wurden nicht nur "im offenen System", sondern *auch in einem solchen geschlossenen System* weiter untersucht, bei dem *eine perforierte Kunststoffplatte* zwischen die Verbindungen, welche sich am Boden der Petri-Schalen fanden, und die Untersuchungsobjekte (d. h. die Kartoffelkäfer) gelegt wurde, welche perforierte Kunststoffplatte die unmittelbare Berührung der Kartoffelkäfer mit den Verbindungen zwar verhinderte, aber die Wirkung der eventuellen Dämpfe der Verbindungen auf die Käfer nicht hemmte. Folglich wurde die Trennung der Kontakt- und der Gaswirkung ermöglicht.

Die Tabellen 3 und 4 enthalten unsere Untersuchungsergebnisse über die in der Praxis am meisten angewandten Insekticiden. Die Angaben dieser Tabellen sind besonders darum sehr wichtig, weil sie annährungsweise eine entsprechende Information darüber bieten, daß bei der Anwendung *identischer Untersuchungsmethode* und *desselben Testobjekts* wie sich die Wirkungsintensität unserer am wirksamsten gefundenen Verbindungen zu der Wirkungsgröße der in der Praxis bisher angewandten Insekticiden Stoffe verhält, und ob eine oder andere von uns

untersuchte Verbindung, wenn sie außer der *grundlegenden* insekticiden Wirkung auch noch einigen sonstigen Anforderungen (z. B. Mangel an Toxicität für Säugetiere und Mangel an Phytotoxizität, uzw.) entspräche, auf Grund des Wirkungsgrades geeignet wäre, als insekticides Mittel in der Praxis angewandt zu werden.

Die Verbindungen, die sich als wirksamste auf die Imagines der Kartoffelkäfers erwiesen, wurden auch in Hinsicht der larviciden Wirkung auf die Larven vom Entwicklungsstadium L_3 und L_4 des Kartoffelkäfers untersucht, um zu entscheiden, ob ein signifikanter Unterschied zwischen ihrer insekticiden und larviciden Wirkung bestehe. Diese Untersuchungen wurden im geschlossenen System vorgenommen. Die angewandte Methode war mit der vorher beschriebenen Methode gänzlich identisch, nur mit dem Unterschied, daß diesmal Larven als Testobjekte anstatt der Imagines angewandt wurden.

Ergebnisse

Die anhand der Untersuchung der insekticiden und larviciden Wirkung erhaltenen Ergebnisse wurden in den Tabellen 1–4 zusammengefaßt.

Tabelle 1

Die insekticide Wirkung von Thiocyanaten, Isothiocyanaten und potentiellen Isothiocyanat-*Bildnern*

Test-Organismus: Kartoffelkäfer (= Leptinotarsa decemlineata)
Zeichenerklärung: s. bei der Untersuchungsmethode

Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des "Gas-Testes"
	Verbindungsmengen µg/cm²			Verbindungsmengen µg/cm²			Verbindungsmengen µg/cm²
	30	15	7.5	30	15	7.5	30
Alkyl-thiocyanate R–S–CN							
R=							
n-C_6H_{13}–	+	+	–	+	(+)	–	(+)
n-C_7H_{15}–	+	+	(+)	+	(+)	(+)	(+)
n-C_8H_{17}–	+	+	+	+	+	(+)	(+)
n-C_9H_{19}–	+	+	(+)	+	(+)	(+)	(+)
n-$C_{10}H_{21}$–	+	(+)	(+)	(+)	(+)	–	(+)
n-$C_{12}H_{25}$–	+	–	–	(+)	–	–	–

Tabelle 1 (Fortsetzung)

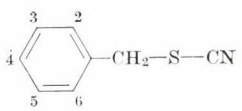
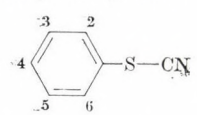
Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des "Gas-Testes"
	Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$
	30	15	7.5	30	15	7.5	30
Alkylen-di-(thiocyanate) NC−S−(CH_2)n−S−CN							
n=							
2	+	(+)	−	(+)	−	−	−
3	+	(+)	−	(+)	−	−	−
4	+	(+)	−	(+)	−	−	−
5	+	(+)	−	(+)	−	−	−
Alkyl-ester der Thiocyanoessigsäure NC−S−CH_2−COO−R							
R=							
CH_3−	+	−	−	−	−	−	(+)
C_2H_5−	+	−	−	(+)	−	−	(+)
n-C_3H_7−	+	−	−	(+)	−	−	(+)
n-C_4H_9−	+	−	−	+	−	−	(+)
n-C_5H_{11}−	(+)	−	−	(+)	−	−	−
n-C_6H_{13}−	(+)	−	−	(+)	−	−	−
n-C_9H_{19}−	(+)	−	−	(+)	−	−	−
n-$C_{10}H_{21}$−	(+)	−	−	(+)	−	−	−
n-$C_{12}H_{25}$−	(+)	−	−	(+)	−	−	−
Benzyl-thiocyanate 							
Substituenten des Benzol-Ringes:							
−	+	+	+	+	+	+	(+)
4-Cl	+	(+)	−	−	(+)	−	(+)
4-NO_2	+	−	−	(+)	−	−	−
2-NO_2, 4-Cl	+	−	−	(+)	−	−	(+)
Phenyl-thiocyanate 							
Substituenten des Benzol-Ringes:							
−	+	+	−	−	−	−	(+)
2-Cl	+	+	−	−	−	−	(+)
3-Cl	+	+	(+)	(+)	−	−	+
4-Cl	+	+	(+)	(+)	(+)	−	+

Tabelle 1 (Fortsetzung)

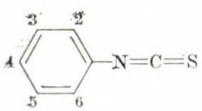
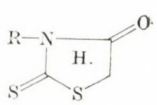
Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedinungen des "Gas-Testes"
	Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$			Verbindungs-mengen $\mu g/cm^2$
	30	15	7.5	30	15	7.5	30
Alkyl-isothiocyanate							
$R-N=C=S$							
R=							
$CH_2=CH-CH_2-$	+	(+)	−	−	−	−	+
n-C_4H_9−	+	−	−	−	−	−	−
cykl.C_6H_{11}−	(+)	−	−	−	−	−	−
Aralkyl-isothiocyanate							
$C_6H_5-(CH_2)n-N=C=S$							
n =							
1	+	+	−	−	−	−	+
2	(+)	−	−	−	−	−	(+)
Phenyl-isothiocyanate							
							
Substituenten des Benzol-Ringes:							
−	+	−	−	−	−	−	−
4-CH_3	+	(+)	−	+	−	−	−
3-Cl	+	(+)	−	−	−	−	−
4-Cl	+	(+)	−	−	−	−	−
Substituenten des Benzol-Ringes:							
4-Br	+	+	−	(+)	−	−	−
3,4-Cl_2	+	+	−	+	−	−	−
3-Cl, 4-Br	+	+	−	(+)	−	−	−
2,3-Benzo	+	−	−	(+)	−	−	−
N-Alkyl-rhodanine							
							
R=							
CH_3−	+	+	(+)	+	−	−	−
C_2H_5−	+	(+)	−	+	−	−	−
n-C_4H_9−	+	(+)	−	(+)	−	−	−

Tabelle 2

Unwirksam erwiesene Verbindungen

Die Verbindungen dieser Tabelle sind auf die Kartoffelkäfer in geschlossenen Petri-Schalen auch in der Menge von 30 $\mu g/cm^2$ ganz wirkungslos

Typ 1.

Ethyl-ester von α-Thiocyano-karbonsäuren

R = CH_3-, C_2H_5-, n-C_3H_7-, $-COO-C_2H_5$

Typ 2.

Alkylen-bis-(thiocyano-acetate)

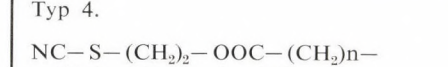
n = 2,4,5

Typ 3.

β-Thiocyano-ethyl-ester von Monokarbonsäuren

R = n-$C_5H_{11}-$, n-$C_7H_{15}-$, n-$C_8H_{17}-$, n-$C_{11}H_{23}-$, $CH_2=CH-(CH_2)_8-$, C_6H_5-, $C_6H_5-CH=CH-$

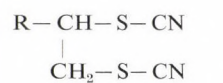
Typ 4.



Di-(β-thiocyano-ethyl-ester) von aliphatischen Dikarbonsäuren

n = 2,4,7,8

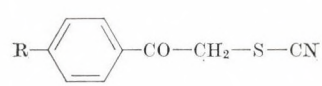
Typ 5.



Aliphatische Di(thiocyanate)

R = $-CH_2OH$, $-CH_2-OOC-C_8H_{17}$-n, $-(CH_2)_8-COOH$, $-(CH_2)_8-COO-CH_3$

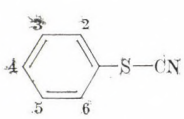
Typ 6.



Phenacyl-thiocyanate

R= H−, NO_2-

Typ 7.



Nitro-phenyl-thiocyanate

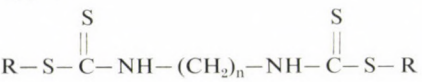
2-NO_2, 4-NO_2, 2-NO_2-4-Cl, 2,4-$(NO_2)_2$

Typ 8.

Ester von N-Alkyl-dithiocarbaminsäuren

R= CH_3-, C_2H_5-, cykl. $C_6H_{11}-$
R'= CH_3-, $CH_2=CH-CH_2-$, 4-Cl-$C_6H_4-CH_2-$

Typ 9.

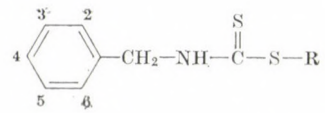


Ester von Alkylen-bis-dithiocarbaminsäuren

n = 2,6

R = CH_3-, C_2H_5-, $CH_2=CH-CH_2-$, 4-Cl$-C_6H_4-CH_2-$

Typ 10.

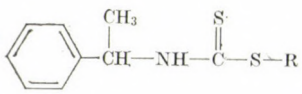


Ester von N-Benzyl-dithiocarbaminsäuren.

Substituenten des Benzol-Ringes:

–, 4-Cl, 3,4-$(O-CH_3)_2$,
R = CH_3-, C_2H_5-, $CH_2=CH-CH_2-$ $C_6H_5-CH_2-$, 4-Cl$-C_6H_4-CH_2-$

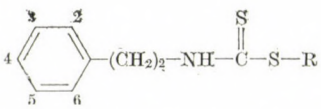
Typ 11.



Ester der N-(α-Phenyl-ethyl)-dithiocarbaminsäure

R = $CH_2=CH-CH_2-$, 4-Cl$-C_6H_4-$ $-CH_2-$,

Typ 12.

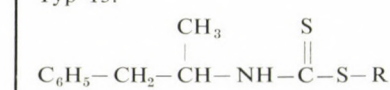


Ester von N-(β-phenyl-ethyl)-dithiocarbaminsäuren

Substituenten des Benzol-, ringes:

–, 3,4-$(O-CH_3)_2$, 3,4-$(O-C_2H_5)_2$
R = CH_3-, C_2H_5-, $CH_2=CH-CH_2-$, 4-Cl$-C_6H_4-CH_2-$

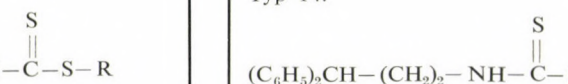
Typ 13.



Ester der N-(α-benzyl-ethyl)-dithiocarbaminsäure

R = CH_3-, C_2H_5-, $CH_2=CH-CH_2-$ 4-Cl$-C_6H_4-CH_2-$

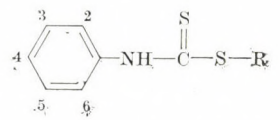
Typ 14.



Ester der N-(γ,γ-Diphenyl-propyl)-dithiocarbaminsäure

R = CH_3-, C_2H_5-, $CH_2=CH-CH_2-$ 4-Cl$-C_6H_4-CH_2-$

Typ 15.



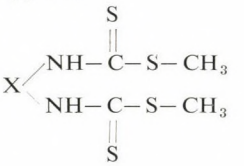
Ester von N-phenyl-dithiocarbaminsäuren

Substituenten des Benzol-Ringes:

—, 2-CH_3, 3-H_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 4-Br, 4-J, 2,5-Cl_2, 3,4-Cl_2, 3-Cl-4-Br, 3-Cl-4-J, 2-CH_3-4-Cl, 3-CH_3-4-Cl, 2-CH_3-4-Br, 3-CH_3-4-Br, 3-CH_3-4-J, 3-NO_2, 4-NO_2, 2-O—C_2H_5, 4-O—C_2H_5, 3-OH, 4-CO—CH_3, 2,3-Benzo

R= CH_3—, C_2H_5—, CH_2=CH—CH_2—, 4-Cl—C_6H_4—CH_2—

Typ 16.



Methyl-ester von Phenylen-bis-dithio-, carbaminsäuren

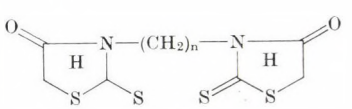
X=m-Phenylen, p-Phenylen

Typ 17.

Ester von N-Heterocyklyldithiocarbaminsäuren

R= Furfuryl-, 2-Pyridyl-, 2-Thiazolyl
R' = CH_3—, C_2H_5—, CH_2=CH—CH_2—

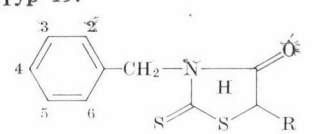
Typ 18.



Alkylen-bis-(rhodanine)

n = 2,6

Typ 19.

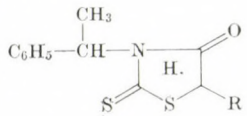


N-Benzyl-rhodanine

Substituenten des Benzol-Ringes:

—, 4-Cl, 3,4-(O—CH_3)$_2$
R= H—, CH_3—, C_2H_5—, n-C_3H_7—, n-C_4H_9—

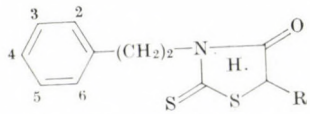
Typ 20.



N-(α-Phenyl-ethyl)-rhodanine

R= H–, CH_3–, C_2H_5–, n-C_3H_7–, n-C_4H_9–

Typ 21.

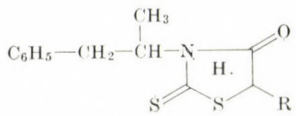


N-(β-Phenyl-ethyl)-rhodanine

Substituenten des Benzol-Ringes:

–, 3,4-(O–CH_3)$_2$, 3,4-(O–C_2H_5)$_2$
R= H–, CH_3–, C_2H_5–

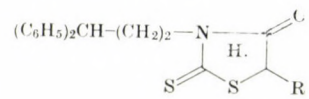
Typ 22.



N-(α-Benzyl-ethyl)-rhodanine

R= H–, CH_3–, C_2H_5–

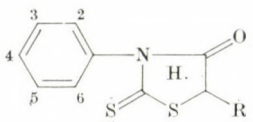
Typ 23.



N-(γ,γ-Diphenyl-propyl)-rhodanine

R= H–, CH_3–, C_2H_5–

Typ 24.

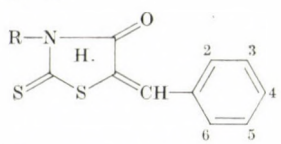


N- Phenyl-rhodanine

Substituenten des Benzol-Ringes:

–, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 4-Br, 4-J, 3,4-Cl_2, 3-Cl-4-Br, 3-Cl-4-J, 3-CH_3-4-Cl, 2-CH_3-4-Cl, 3-CH_3-4-Br, 3-CH_3-4-J, 2-O-C_2H_5, 4-O–C_2H_5, 4-O–C_2H_5, 3-OH, 4-OH, 2,3- Benzo
R= H–, CH_3–, C_2H_5–, n-C_3H_7–, n-C_4H_9–

Typ 25.

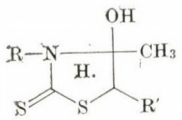


5-Benzal-rhodanine

Substituenten des Benzol Ringes:

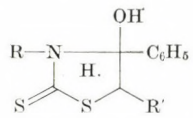
–, 4-Cl, 4-N(CH_3)$_2$, 4-OH
R= CH_3–, C_2H_5–, C_6H_5–CH_2–, C_6H_5–,

Typ 26.



4-Hydroxy-4-methyl-thiazolidin-2-thione

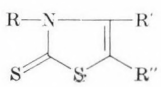
R'= $-COO.C_2H_5$, $-(CH_2)_2-COO-CH_3$
$C_6H_5-CH_2-$, C_6H_5-, $4\text{-}Cl-C_6H_4-$



4-Hydroxy-4-phenyl-thiazolidin-2-thione

R'= H–
R= CH_3-, C_2H_5-, cykl. $C_6H_{11}-$,

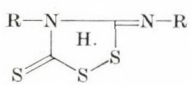
Typ 27.



Thiazolin-(2,3)-2-thione

R=$C_6H_5-CH_2-$, $3\text{-}Cl-C_6H_4-$, $4\text{-}Cl-C_6H_4-$
R'= CH_3-, C_6H_5-
R''= H–, $HO-(CH_2)_2-$

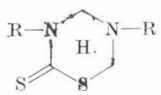
Typ 28.



1,2,4-Dithiazolidin-5-thione

R=$C_6H_5-CH_2-$, C_6H_5-, $4\text{-}Cl-C_6H_4-$

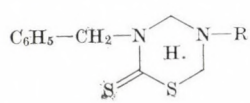
Ty 29.



3,5-Dialkyl- und 3,5-Di-(aralkyl)-tetrahydro-1,3,5-thiadiazin-2-thione

R= CH_3-, C_2H_5-, $n\text{-}C_4H_9-$, cykl. $C_6H_{11}-$, $C_6H_5-CH_2-$, $C_6H_5-(CH_2)_2-$

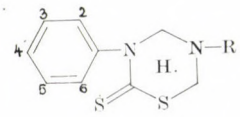
Typ 30.



3-Benzyl-5-alkyl-tetrahydro-1,3,5-thiadiazin-2-thione

R= CH_3-, C_2H_5-, $n\text{-}C_4H_9-$, cykl. $C_6H_{11}-$, $HO-(CH_2)_2-$

Typ 31.



3-Phenyl-5-alkyl-tetrahydro-1,3,5-thiadiazin-2-thione

Substituenten des Benzol-Ringes:

—, 3-CH_3, 4-CH_3, 3-Cl, 4-Cl, 3,4-Cl_2, 3-Cl-4-Br, 3-CH_3-4-Br
R = CH_3—, C_2H_5—, n-C_4H_9—, cykl. C_6H_{11}—, HO—$(CH_2)_2$—

Tabelle 3

Vergleichende Untersuchung der insekticiden Wirkung einiger im Handel gebräuchlicher insekticider Wirkstoffe

Zeichenerklärung: s. bei der Untersuchungsmethode

Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des "Gas-Testes"
	Verbindungsmengen µg/cm²			Verbindungsmengen µg/cm²			Verbindungsmengen µg/cm²
	30	15	7.5	30	15	7.5	30
Lindan	+	+	(+)	+	(+)	−	(+)
Thiodan	+	(+)	(+)	(+)	−	−	−
Nikotin	+	+	(+)	+	+	−	+
Neguvon (= Dipterex, Ditriphon)	+	+	+	+	+	+	+
Dichlorvos	+	+	+	+	+	+	+

Tabelle 4

Weitere Untersuchung der insekticiden Wirkung von Neguvon und Dichlorvos

Zeichenerklärung: s. bei der Untersuchungsmethode

Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des "Gas-Testes"
	Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$
	1.5	0.75	0.37	1.5	0.75	0.37	1.5
Neguvon	+	+	−	+	+	−	+
Dichlorvos	+	+	−	+	+	−	+

Bemerkung zu den Tabellen 2–4

Die larvicide Wirkung aller untersuchten Verbindungen auf die Larven des Kartoffelkäfers erwies sich als von gleicher Größenordnung wie die insekticide Wirkung derselben auf die Imagines. Die Verbindungen, deren untersuchte größte Menge (= 30 $\mu g/cm^2$) auch keine insekticide Wirkung auf die Imagines hatte, waren auch auf die Larven in dieser Menge vollständig wirkungslos.

Besprechung der Ergebnisse

Beim Überblick der in den Tabellen 1–4 zusammengefaßten Ergebnisse kann man Folgendes feststellen:

1. Alle von uns untersuchten Alkyl-thiocyanate, Alkylen-bis-(thiocyanate) und Benzyl-thiocyanate haben eine gleicherweise bedeutende insekticide Wirkung auf den Kartoffelkäfer und seine Larven. Das steht in Einklang mit den Ergebnissen anderer Verfasser, die von ihnen in früheren Arbeiten auf andere Test-Insekten erhalten wurden [1–3].

Das Phenyl-thiocyanat und seine halogen-substituierten Derivate üben im allgemeinen eine insekticide Wirkung gleichen Grades aus wie die Alkyl-thiocyanate; demgegenüber sind die Mononitro- und Dinitro-phenylthiocyanate völlig wirkungslos.

2. Die insekticide Wirkung der Alkyl-ester mit niedriger Kohlenstoffatomzahl der Thiocyanoessigsäure (Alkyl-Gruppen = von Methyl bis zum n-Butyl) ist mäßig, während die Ester, deren Alkyl-Gruppen höhere Kohlenstoffatomzahl besitzen (Alkyl-Gruppen = von n-Amyl bis zum n-Dodecyl), nur über eine ganz schwache insekticide Wirkung verfügen.

Die Alkyl-homologen des Thiocyanoessigsäure-ethyl-esters, d. h. die Ethylester der α-Thiocyano-Derivate der Propionsäure, der n-Buttersäure, der n-Valeriansäure, sowie auch das Thiocyanomalonsäure-diethyl-ester üben keinerlei insekticide Wirkung auf den Kartoffelkäfer und seine Larven aus. Gleicherweise sind die Alkylen-bis-(thiocyanoacetate), weiter die β-Thiocyano-ethyl-ester der aliphatischen und aromatischen Monokarbonsäuren, die Bis-(β-thiocyanoethylester) der aliphatischen Dikarbonsäuren sowie die Hydroxymethyl- und ω-Karboxyoctyl-Derivate des Ethylen-bis-(thiocyanats) und auch die Ester derselben ganz und gar unwirksam. Gänzlich wirkungslos sind das Phenacyl-thiocyanat und sein p-Nitro-Derivat.

3. Nach der Auffassung des Verfassers weicht der Wirkungsmechanismus der fungistatischen Thiocyanate vom Mechanismus ihrer insekticiden Wirkung mehr oder weniger ab. Durch die früheren Untersuchungen des Verfassers wurde nachgewiesen, daß die Benzyl- und Phenyl-thiocyanate, die eine bedeutende bakteriostatische und fungistatische Wirkung haben, ihre Wirkungen durch die Hemmung der Thiolenzyme ausüben, die grundlegend wichtige Rolle im Stoffwechsel der Mikroorganismen spielen [4, 5]. Sie bilden gemischte Disulfide mit den Thiol-Gruppen der Thiolenzyme, wobei Cyanhydrogen frei wird:

$$R-S-CN + HS\text{-Enzym} \rightarrow R-S-S\text{-Enzym} + HCN \ .$$

Man kann es dem Cyanhydrogen, daß sich während dieser Reaktion bildet, zuschreiben, daß die Thiocyanate für die tierischen Organismen (so auch für den Menschen) sehr giftig sind [5].

Die Benzyl- und Phenyl-thiocyanate, die anhand unserer Untersuchungen insekticide Wirkung aufweisen, üben ihre insekticide Wirkung – unserer Meinung nach – nur teilweise dadurch aus, daß sie gewisse Thiolenzyme der Kartoffelkäfer lähmen; in ihrer insekticiden Wirkung fällt eine entscheidende Rolle auch dem Cyanhydrogen zu, das während der Reaktion unter ihnen und den Thiolenzymen der Käfer zustande kommt, und das als ein bekannter Atmungsinhibitor wirkt, und als solcher allgemeine insekticide Wirkung besitzt.

Die Reaktionsfähigkeit der Alkyl-thiocyanate, der Alkylen-bis-(thiocyanate) und der Thiocyanoessigsäure-ester den Thiol-Gruppen gegenüber hat unter biologischen Umständen nur einen ganz niedrigen Grad, trotzdem sind sie als insekticid wirksam. Sie üben ihre insekticide Wirkung dementsprechend – unserer Meinung nach – nicht als Thiol-Reagentien aus, sondern dadurch, daß sie sich im Organismus der Käfer zu Thiol-Verbindungen und zum Cyanhydrogen reduzieren, und das während der Reduktion aus ihnen *in vivo* entstehende Cyanhydrogen den eigentlichen insekticiden Agenten spielt.

$$R-S-CN \xrightarrow{2DPNH \rightarrow 2DPN^{(+)}} R-SH + HCN$$

4. Die Aralkyl- und Aryl-isothiocyanate üben starke fungistatische, bakteriostatische, ascaricide und molluscicide Wirkung aus [4–6]. Einige von ihnen verfügen – nach dem Patentschrifttum – auch über eine nematocide und insekti-

cide Wirkung [7, 8]. Nach unseren Untersuchungen übt die Mehrheit derselben starke insekticide Wirkung sowohl auf die Imagines, wie auch auf die Larven des Kartoffelkäfers im geschlossenen System aus; im offenen System sind sie aber – wahrscheinlich wegen ihrer großen Flüchtigkeit – nur mäßig aktiv bzw. einige von ihnen sind ganz wirkungslos.

Es ist allgemein bekannt, daß die Isothiocyanate ihre antimikrobielle Wirkung durch die Lähmung der Thiolenzyme der Mikroorganismen ausüben [5]; und zwar dadurch, daß sie sich zu den Thiol-Gruppen der Enzyme auf Grund der folgenden chemischen Reaktion binden, wodurch dieselben inaktiviert werden:

$$R-N=C=S + HS\text{-Enzym} \rightarrow R-NH-\overset{\overset{S}{\|}}{C}-S\text{-Enzym} \quad .$$

Man kann annehmen, daß sie ihre insekticide Wirkung auf Grund eines gleichen Mechanismus wie ihre antimikrobielle Wirkung ausüben. Die Annahme wird dadurch glaubhaft gemacht, daß die insekticide Wirkung jener Isothiocyanate am intensivsten ist, deren antimikrobielle Wirkung auch einen sehr hohen Grad besitzt – und umgekehrt.

Es ist bekannt, daß alle Verbindungen, aus denen sich Isothiocyanate *in situ* bilden können, d. h. die sog. "potentiellen Isothiocyanatbildner" mehr oder weniger bedeutende antimikrobielle, ascaricide und molluscicide Wirkung auszuüben vermögen [4–6]. Die Intensität dieser Wirkungen – auf molarem Grunde – ist beinahe gleich groß wie die der Isothiocyanate, welche sich aus ihnen *in situ* bilden können [5, 6].

Auf diesem Grunde könnte man erwarten, daß die "potentiellen Isothiocyanatbildner" gleichweise und genau so insekticid wirksam seien, wie die Isothiocyanate selbst, mit denen sie in genetischer Knüpfung stehen. Es ist aber überraschend, daß sich nur die N-Methyl-, N-Ethyl- und N-(n-Butyl)-Derivate des Rhodanins von den von uns untersuchten potentiellen Isothiocyanatbildnern als insekticid erwiesen. Alle anderen isothiocyanat-bildenden Verbindungen (d. h. alle von uns untersuchten Vertreter der Verbindungstypen Nr. 8–31) erwiesen sich sowohl für die Imagines wie auch für die Larven des Kartoffelkäfers gleichweise als unwirksam, – trotz der Tatsache, daß die Aralkyl- und Aryl-isothiocyanate, die aus ihnen *in situ* frei werden können, eine starke insekticide Wirkung haben (s. Punkt 4!).

Ihre Unwirksamkeit läßt sich theoretisch auf viele Gründe zurückführen: unter den vielen Möglichkeiten finden wir die Erklärung naheliegendst, daß diese Verbindungen eine insekticide Wirkung aus dem Grunde nicht ausüben, weil ihre Entgiftung im Organismus der Kartoffelkäfer und ihrer Larven schneller vor sich geht, als die Bildung der eigentlichen aktiven Agentien der Isothiocyanate aus ihnen.

Die Wirksamkeit der N-Alkyl-rhodanine kann nur mit der Annahme erklärt werden, daß ihre Umwandlung zu Alkyl-isothiocyanaten im Organismus der Kartoffelkäfer und ihrer Larven – abweichend von den anderen potentiellen

Isothiocyanat-bildnern schneller vor sich geht als die Entgiftung dieser oder die der Alkyl-isothiocyanate, welche aus ihnen gebildet werden.

6. Von den zum Vergleich untersuchten insekticiden Mitteln erwiesen sich das Lindan, Thiodan, Nikotin, Neguvon und das Dichlorvos – sowohl im offenen, wie auch im geschlossenen System –, sogar mit Ausnahme des Thiodans auch unter den Umständen des "Gastestes" wirksam.

Die insekticide Wirkung des Lindans, des Thiodans und des Nikotins schien mit der der aktivsten Alkyl- und Aryl-thiocyanate nahe gleich groß zu sein, aber sie bleibt weit hinter der Wirkung von Neguvon und Dichlorvos zurück, die zum Kreis der Phosphat-insekticiden gehören. Die als Test-Organismen angewandten Kartoffelkäfer und ihre Larven erwiesen sich gegen Lindan und Thiodan empfindlich, aber die Wirkung dieser zwei Verbindungen kam langsam, erst nach verhältnismäßig langer Einwirkung (8–12 Stunden) zur Geltung. Das Nikotin, das Neguvon und das Dichlorvos sowie die aktivsten Thiocyanate, die Isothiocyanate und die N-Alkyl-rhodanine verfügten demgegenüber mit "knock down" Wirkung: nach einer Einwirkungszeit von 15–20 Minuten erfolgte die volle Lähmung der Bewegung der Test-Organismen, später, nach weiteren 40–60 Minuten waren sie alle getötet.

Die Möglichkeiten der praktischen Anwendbarkeit berücksichtigend, ist keine der von uns aktiv gefundenen Verbindungen mit den Phosphatinsekticiden konkurrenzfähig: demgegenüber scheinen das n-Heptyl-, n-Nonyl-, n-Decyl-, Benzyl-, das 3- und das 4-Chlor-phenyl-thiocyanat mit dem Lindan und Thiodan, weiterhin mit dem Nikotin – hinsichtlich der Wirkungsintensität – nahezu gleichwertig zu sein. Diese Thiocyanate sind zwar ziemlich toxische Verbindungen [5], aber ihre Toxicität ist nicht intensiver, als die des Nikotins, welches zur Vertilgung verschiedener Insekten auch heute noch im Gebrauch ist.

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Die Untersuchung der insekticiden Wirkung von Tiol-Reagentien

II. Verbindungen, welche die Thiol-Gruppen zu alkylieren, arylieren oder addieren vermögen

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Der Verfasser hat die *in vitro* ausgeübte insekticide Wirkung von 270 Verbindungen, deren Mehrzahl die Thiol-Gruppen zu alkylieren, arylieren oder addieren vermögen, auf die Imagines und Larven des Kartoffelkäfers untersucht. Zum Vergleich hat er parallel die insekticide Wirkung einiger Handelsinsekticiden unter identischen Versuchsumständen studiert.

Nur 19 der untersuchten Verbindungen haben sich als wirksam erwiesen; die überwiegende Mehrheit der Thiol-Reagentien erwies sich aber als ganz umwirksam. Von den als wirksam erwiesenen Verbindungen haben nur 6, nämlich α, β-Dibrompropionitril, β-Nitro-styrol-dibromid, 2,4-Dinitro-fluorbenzol, weiterhin das β-Nitrostyrol und dessen β-Methyl- und α-Brom-Derivate einige Handelsinsekticide (z. B. das Lindan, Thiodan, Nikotin) in Hinsicht der Wirkungsintensität annähert.

Die Versuche des Verfassers haben gezeigt, daß zwischen der Reaktivität einer Verbindung den Thiol-Gruppen gegenüber und deren insekticiden Wirkung kein enger Zusammenhang besteht.

Im Teil 1. dieser Mittellungsserie beschäftigten wir uns mit der insekticiden Wirkung der organischen Thiocyanaten, Isothiocyanaten und der potentiellen Isothiocyanat-Bildner [1]. Diese Verbindungen gehören — wie es bekannt ist — zu den Thiol-Reagentien, weil sie mit Thiol-Gruppen leicht in Reaktion zu treten imstande sind. Unsere Forschungen haben wir auch auf andere Klassen von Verbindungen ausgedehnt, nämlich auf solche, welche die Thiol-Gruppen leicht zu alkylieren, arylieren, oder addieren vermögen. Nach den Untersuchungen des Verfassers ist eine große Anzahl von solchen Verbindungen imstande, eine nennenswerte antimikrobielle und molluscicide Wirkung auszuüben [2, 3]. Aus der Fachliteratur ist bekannt, daß einige Vertreter dieses Typs von Verbindungen (z. B. die chlorierten Derivate des Nitromethans, Nitroethans, Nitropropans, Acetonitrils, Dimethylethers, weiterhin die Bromessigsäure-ester, das Acrylonitril, usw.) gegen gewisse Insektenarten eine bedeutende insekticide Wirkung ausüben [4—17]. Ihre insekticide Wirkung üben sie aller Wahrscheinlichkeit nach durch die Lähmung solcher Thiolenzyme aus, die in den Lebensprozessen der Insekten eine wichtige Rolle spielen. Wir nahmen an, daß auch viele andere Verbindungen außer den vorher genannten einigen Stoffen in der Reihe der Thiol-Reagentien vorzufinden wären, die eine insekticide Wirkung kleineren oder größeren Grades auszuüben vermöchten. Dies zu erklären, haben wir die auf den Kartoffelkäfer ausgeübte insekticide Wirkung solcher Verbindungen unter Laboratoriums-

umständen untersucht, die zur Alkylierung, Arylierung oder Addition von Thiol-Gruppen fähig sind, und die eventuellen Zusammenhänge zwischen ihrer insekticiden Wirkung und chemischen Struktur studiert.

Die bei unseren Untersuchungen angewandte Methode wurde im Teil 1 dieser Mitteilungsserie ausführlich beschrieben [1], daher wird von einer Wiederholung abgesehen.

Ergebnisse und Besprechung

Die im Laufe der Untersuchung der insekticiden Wirkung von Thiol-Reagentien gewonnenen Ergebnisse haben wir in den Tabellen 1 – 3 zusammengefaßt.

Tabelle 1

Die insekticide Wirkung der Verbindungen, welche Thiol-Gruppen zu alkylieren oder arylieren vermögen

Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des Gas-Testes
	Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$			Verbindungsmengen $\mu g/cm^2$
	30	15	7.5	30	15	7.5	30
α Brom-ketone							
ω-Brom-acetophenon	+	+	–	(+)	–	–	–
α-Brom-nitrile							
Monobrom-benzylmalonitril	+	–	–	–	–	–	–
Monobrom-(4-chlorbenzyl)-malonitril	+	–	–	–	–	–	–
α,β-Dibrom-propionitril	+	+	–	(+)	(+)	–	–
Nitro-styrol-dibromide							
β-Nitro-styrol-dibromid	+	+	(+)	(+)	(+)	–	–
β-Nitro-β-methyl-styrol-dibromid	+	(+)	–	(+)	–	–	–
Dinitroaryl-halogenide							
2,4-Dinitro-chlorbenzol	+	(+)	–	(+)	–	–	–
2,4-Dinitro-fluorbenzol	+	+	(+)	+	(+)	(+)	–
2,4-Dinitro-1-chlornaphtalin	+	(+)	–	(+)	–	–	–
Naphtochinone							
2-Brom-1,4-naphtochinon	+	(+)	–	(+)	–	–	–
2,3-Dichlor-1,4-naphtochinon	(+)	–	–	–	–	–	–
Zum Vergleich untersuchte Handelsinsekticide:							
Lindan	+	+	(+)	+	(+)	–	(+)
Thiodan	+	(+)	(+)	(+)	–	–	–
Nikotin	+	+	(+)	+	+	–	+

Tabelle 2

Die insekticide Wirkung der Verbindungen, welche Thiol-Gruppen zu addieren vermögen

Verbindungen	Insekticide Wirkung						
	In geschlossenen Petri-Schalen			In offenen Petri-Schalen			Unter den Bedingungen des Gas-Testes
	Verbindungsmengen µg/cm²			Verbindungsmengen µg/cm²			Verbindungsmengen ug/cm²
	30	15	7.5	30	15	7.5	30
β-Nitro styrole							
β-Nitro-styrol	+	+	−	+	(+)	−	(+)
α-Brom-β-nitro-styrol	+	+	−	+	−	−	−
β-Nitro-β-methyl-styrol	+	+	−	+	(+)	−	(+)
α-Brom-β-nitro-β-methyl-styrol	(+)	−	−	−	−	−	−
Brom-styrole							
α-Brom-styrol	(+)	−	−	−	−	−	−
α,β-Ungesättigte Nitrile							
Cinnamonitril	+	(+)	−	−	−	−	−
α-Brom-cinnamonitril	+	(+)	−	−	−	−	−
Naphtochinone							
2-Methyl-1,4-naphtochinon	(+)	−	−	−	−	−	−

Zeichenerklärung zu den Tabellen 1 und 2:

\+ = 100% der Kartoffelkäfer wurde getötet, nicht einmal auf die Berührung ihrer Fühler zeigten sie ein Lebenszeichen. Da wurde die Verbindung als aktiv erachtet.

(+) = 100% der Kartoffelkäfer befand sich in einem vollständig betäubten Zustand, und sie zeigten nur auf Berührung ihrer Fühler einen gewissen Bewegungsreflex. In diesem Fall wurde die Verbindung als mittelmäßig wirksam bezeichnet.

− = Die Verbindung war wirkungslos. Die Mehrheit der Käfer blieb am Leben und war zur spontanen Bewegung fähig.

Tabelle 3

Unwirksam erwiesene Verbindungen

Die Verbindungen dieser Tabelle sind auf die Kartoffelkäfer in geschlossenen Petri-Schalen auch in der Menge von 30 $\mu g/cm^2$ ganz wirkungslos

A) *Aus der Reihe der Verbindungen, welche zur Alkylierung oder Arylierung der Thiol-Gruppen imstande sind:*

Typ. 1.

$CH_2Cl-COO-R$

Monochloressigsäure-ester

R = CH_3-, C_2H_5-, n-C_3H_7-, n-$C_5H_{11}-$ n-$C_6H_{11}-$, n-$C_7H_{15}-$, n-$C_8H_{17}-$, n-$C_9H_{19}-$, n-$C_{10}H_{21}-$, n-$C_{12}H_{25}-$

Typ. 2.

$R-\underset{\displaystyle X}{\underset{|}{CH}}-COO-C_2H_5$

α-Halogen-carbonsäure-äthylester

X = Br−, J−
R = H−, CH_3-, C_2H_5-, n-C_3H_7-, $C_6H_5-CHBr-$, CH_2Br-, $CH_3-CHBr-$

Typ. 3.

$R-CHBr-CO-CH_3$

α-Brom-ketone

R = H−, $CH_3-COO-(CH_2)_2-$, C_2H_5-, $-COO\,.\,C_2H_5$

Typ 4.

$\begin{matrix} R \\ R' \end{matrix}\!\!\diagdown\!\!\diagup CBr-CN$

α-Brom-nitrile

R = $-CN$, H
R' = CH_3-, C_2H_5-, $-COO\,.\,CH_3$

Typ. 5.

$R-CBr_2-NO_2$

Brom-nitroalkane I.

R = Br−, CH_3-, C_2H_5-

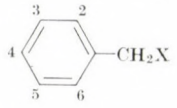
Typ. 6.

$R-\underset{\displaystyle CH_3}{\underset{|}{CBr}}-NO_2$

Brom-nitroalkane II.

R = CH_3-, C_2H_5-, n-C_3H_7-, n-$C_5H_{11}-$, C_6H_5-

Typ. 7.



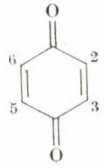
Benzyl-halogenide

X = Cl–, Br–, J–

Substituenten:

–, 4-Cl, 4-NO_2, 2-NO_2–4-Cl, 2,4-$(NO_2)_2$

Typ. 8.

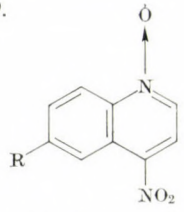


Halogensubstituierte Benzochinone

Substituenten:

2,6-Br_2; 2-CH_3-3,5,6-Cl_3; 2,3,5,6-Cl_4

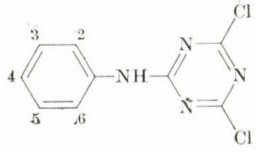
Typ. 9.



4-Nitro-chinolin-1-oxyde

R= H–, CH_3–

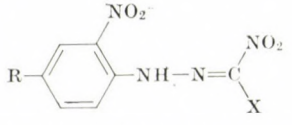
Typ. 10.



2-Arylamino-4,6-dichlor-1,3,5-triazine

Substituenten: –, 2-Cl, 3-Cl, 4-Cl, 2-CH_3, 3–CH_3, 4-CH_3, 3,4-Cl_2, 3-NO_2, 4-NO_2

Typ. 11.

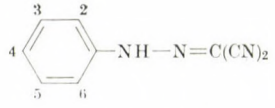


Nitroaryl-hydrazone von Nitroaldehyden

X= H–, CH_3–

R= H, Cl–, Br–, C_2H_5O–, CH_3–

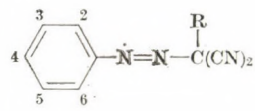
Typ. 12.



Mesoxalsäuredinitril-phenylhydrazone;

Substituenten: –, 2-Cl; 3-Cl-4-Cl; 4-Br; 4-J; 2,5-Cl_2; 3,4-Cl_2; 3-Cl–4-Br; 3-Cl-4-J; 2-CH_3; 3-CH_3; 4-CH_3; 2-CH_3-4-Br; 3-CH_3-4-Br; 3-CH_3-4-J; 2-O-C_2H_5; 4-O–C_2H_5; 2-NO_2; 3-NO_2; 4-NO_2; 2-Cl-4-NO_2; 3-Cl-4-NO_2; 2-NO_2-4-Cl; 4-CO–CH_3; 4-COOH; 4-COO–C_2H_5; 4-NH–CO–CH_3; 4-SO_2NH_2

Typ. 13.

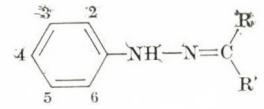


Arylazo-alkyl- bzw. aralkylmalonitrile

R = CH_3-, C_2H_5-, $C_6H_5-CH_2-$, 4-Cl-$C_6H_4-CH_2-$

Substituenten: –; 2-Cl; 3-Cl; 4-Cl; 2-CH_3; 3-CH_3; 4-CH_3;

Typ. 14.



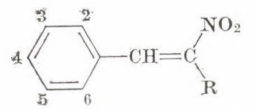
Arylazo-Derivate von aktive Methylen-Gruppe enthaltenden Verbindungen

a) R = –CN; R' = $-COO.CH_3$
b) R = CH_3-CO-, R' = $-COO-C_2H_5$
c) R = R' = CH_3-CO-
d) R = R' = $-COO.C_2H_5$
e) R = CH_3-CO- R' = C_6H_5-CO-
f) R = –CN, R' = $-CO-NH-C_6H_5$

Substituenten: –; 2-Cl; 3-Cl; 4-Cl; 2-CH_3; 3-CH_3; 4-CH_3; 2-NO_2; 3-NO_2; 4-NO_2; 4-O-C_2H_5; 4-$CO-CH_3$

B) *Aus der Reihe der Verbindungen, welche zur Addition von Thiol-Gruppen imstande sind:*

Typ. 15.



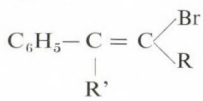
im aromatischen Kern substituierte β-Nitro-styrole

R = H–, CH_3-

Substituenten:

2-NO_2; 3-NO_2; 4-NO_2; 4-Cl; 4-O–CH_3

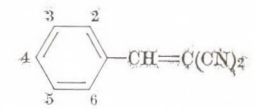
Typ. 16.



Brom-styrole

a) R = R' = H–
b) R = R' = Br–
c) R = Br, R' = H–

Typ. 17.

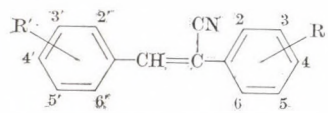


Benzal-malonitrile

Substituenten:

–; 4-Cl; 4-OH; 4-$N(CH_3)_2$

Typ. 18.



a) R = R' = H–
b) R = 4-Cl; R' = H–
c) R = 4-NO_2; R' = H–
d) R = 4-Cl; R' = 4'-Cl
e) R = 4-NO_2; R' = 4'-Cl

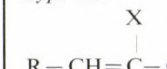
Typ. 19.

$CH_2=C-CN$

α,β-ungesättigte aliphatische Nitrile

X = H−, Br−

Typ. 20.

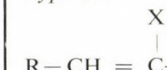


$R-CH=C-CHO$

α,β-ungesättigte aliphatische oder araliphatische Aldehyde

X= H−, Br−
R= H−, CH_3−, C_6H_5−

Typ. 21.



$R-CH = C-COO \cdot C_2H_5$

α,β-ungesättigte aliphatische oder araliphatische Carbonsäure-ester

X= H−, Br−
R=H−, CH_3−, C_6H_5−, −COO, $-C_2H_5$

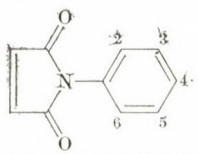
Typ. 22.

$C_6H_5-CH=CH-CO-R$

α,β-ungesättigte araliphatische Ketone

R= CH_3−; C_2H_5−; n-C_3H_7−; n-C_5H_{11}−; C_6H_5−; $C_6H_5-CH=CH-$

Typ. 23.



N-Aryl-maleinimide

Substituenten:

−; 3-Cl; 4-Cl; 3,4-Cl_2

Bemerkung zu den Tabellen 1−3

Die larvicide Wirkung aller untersuchten Verbindungen auf die Larven des Kartoffelkäfers erwies sich als von gleicher Größenordnung wie die insekticide Wirkung derselben auf die Imagines. Die Verbindungen, deren untersuchte größte Menge (= 30 $\mu g/cm^2$) auch keine insekticide Wirkung auf die Imagines hatte, waren auf die Larven in dieser Menge vollständig wirkungslos.

Die in den Tabellen 1−3 zusammengefaßten Ergebnisse gestatten die folgenden Feststellungen:

1. Es wurden insgesamt 270 Verbindungen, deren Mehrzahl mit Thiol-Gruppen zu reagieren imstande ist, in Hinsicht der insekticiden Wirkung untersucht. Nur 19 Verbindungen von ihnen üben eine insekticide Wirkung aus, dagegen er-

weisen sich 251 als ganz unwirksam. Von den Verbindungen, welche sich als wirksam erweisen, sind nur 6 (nämlich das α,β-Dibrompropionitril, β-Nitro-styrol-dibromid, 2,4-Dinitro-fluorbenzol, das β-Nitro-styrol und dessen β-Methyl- und β-Brom-Derivate), deren Wirkungsintensität die der zum Vergleich untersuchten einigen Handelsinsekticiden (d. h. die des Lindans, Thiodans bzw. Nikotins) annähern. Aber die Wirkungsintensität des zu den Phosphat-Insekticiden gehörenden Ditriphons und Dichlorvos wurde durch keiner von diesen 6 Verbindungen angenähert (die insekticide Wirkung der letzgenannten zwei Phosphat-Insekticiden S. im Teil 1 dieser Mitteilungsserie!). Demzufolge sind auch jene Verbindungen, die wir relativ als wirksamst gefunden haben, für praktische Zwecke ungeeignet.

2. Die Mehrzahl der als wirksam erwiesenen 19 Verbindungen ist in offenem System viel weniger aktiv als in geschlossenem System, ja einige von ihnen unter den letzteren Bedingungen untersucht erwiesen sich sogar als ganz unwirksam.

3. Die Verbindungen, die sich als insekticid wirksam erwiesen haben, sind gleicherweise auch larvicid wirksam; die Intensität ihrer Wirkungen auf die Imagines und auf die Larven der Kartoffelkäfer ist gleich.

4. Es ist überraschend, daß viele Thiol-Reagentien mit großer Reaktivität, die über eine bedeutende antimikrobielle und molluscicide Wirkung verfügen [2, 3], weder auf die Imagines, noch auf die Larven der Kartoffelkäfer eine insekticide bzw. larvicide Wirkung auszuüben fähig sind. Dies läßt darauf schließen, daß die Reaktivität einer Verbindung den Thiol-Gruppen gegenüber für sich selbst zum Zustandekommen der insekticiden Wirkung ungenügend ist; dazu sind auch andere Vorbedingungen (z. B. entsprechende Molekularstruktur und physikochemische Eigenschaften, usw.) unerläßlich.

5. Einige allgemein bekannte Thiol-Reagentien, die nach den Angaben der Fachliteratur gegenüber gewissen Insekten-Arten stark insekticid wirken (z. B. die halogensubstituierten Nitroalkane, die Brommessigsäure-ester, das Acrylonitril), haben sich in unseren Versuchen gegen die Kartoffelkäfer als total unwirksam erwiesen. Dies läßt darauf schließen, daß die insekticide Wirkung der Thiol-Reagenten nicht nur strukturspezifisch, sondern in Hinsicht der angewandten Test-Insekten auch stark Arten-spezifisch sei.

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Investigations into the Radioresistance of *Plodia interpunctella* (Hübner)*

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Of the various stages of metamorphosis of Indian-meal moth, the eggs were found most radiosensitive. Larvae did not emerge from eggs irradiated with 17.5 krad. Irradiation of larvae reduced feeding and inhibited pupation. Adults did not develop from irradiated larvae. When pupae were treated the percentage of emerging moths was reduced and the number of deformed moths increased. When pupae were treated with 35 krad or higher doses the eggs of the emerging moths were sterile and no progeny developed. The life span of irradiated moths was not reduced substantially, however the number of eggs decreased with increasing doses and the percentage of sterile eggs increased as well; at a dose level of 35 krad larvae did not emerge from the eggs. If the ground product contains only eggs or very young larvae 35 krad is sufficient to achieve disinfestation. If every stage of metamorphosis is present 70 krad seems more adequate.

Because of the problem of chemical residues existing in relation to fumigants used for insect disinfestation researchers have long been concerned with the radiation disinfestation of stored products and a great number of papers has been published on this subject (HASSETT and JENKINS, 1952; PROCTOR *et al.*, 1954; CORNWELL *et al.*, 1957; TÖRÖK *et al.*, 1959; TÖRÖK and FARKAS, 1960; SHCHEGOLEVA, 1963; FARKAS, 1966; TILTON *et al.*, 1966; QURESHI *et al.*, 1970; CALDERON and GONEN, 1971; GONEN and CALDERON 1971).

One of the most frequent and dangerous pest of stored foods, dried goods and seasonings is the polyphag Indian-meal moth, *Plodia interpunctella* (Hübner) *(Lepidoptera, Phycitidae)* (JÁVOR, 1969).

The aim of the present study was to establish the radioresistance of the various developmental stages of *Plodia interpunctella* infecting stored paprika products and the dose requirement of disinfestation.

Materials and Methods

A few adults of *Plodia interpunctella* (Hübner) obtained from the Entomology Department of the Research Institute for Plant Protection were used for reproduction. First ground noble sweet paprika was used as nutrition medium

* This paper was prepared during the tenure of a research contract with the International Atomic Energy Agency.

however it did not prove satisfactory for rapid mass-rearing. To speed up rearing the synthetic medium as suggested by MORRISON and CRAWFORD (1969) and modified by the authors was applied. The composition of the synthetic medium was as follows:

Ground corn	500 g
Ground wheat	550 g
Baker's yeast	250 g
Honey	300 g
Glycerol	250 g

The components were thoroughly mixed and NIPAGIN was added to arrest microbial spoilage (13 g NIPAGIN were dissolved in a little absolute alcohol and evenly distributed in 1 kg of the medium). The medium was kept in a refrigerator until used.

Jars of 1 litre capacity were filled to about one third with the medium and the just emerged moths were introduced. The jars were then covered with linen. Generally adults emerged within 3–4 days were placed in the same jar. After the death of moths the appearence of the first larvae was observed. Larvae grew rapidly on this medium and became larger than those reared on ground paprika. If necessary the medium was supplemented.

When the larvae reached the stage of pupation corrugated cardboard was placed in the jars. The larvae retreated into the openings of the cardboard for pupation. Thus were pupae obtained and the emergence of moths from the pupae was systematically observed.

When eggs were needed moths were placed in empty jars for oviposition and the eggs were collected carefully with a brush.

The cultures were kept in a room of controlled temperature at $26 \pm 3°C$ and 40% RH in natural light. Thus the cultures were illuminated during about 12 hours and were in the dark for the other 12 hours. According to data found in the literature such treatment stimulates oviposition (LUM and FLAHERTY, 1969; 1970).

Radiation treatment was carried out in a LMB-gamma-IM type ^{137}Cs radiation source of 3000 Ci activity at a dose rate of 280 krad/hour.

Every stage of development of the Indian-meal moth was treated with 17.5, 35 and 70 krad, respectively. Of every stage of development 3×20 individuals were exposed and after treatment the larvae were placed on the medium. The same treatment was given to the control samples except for irradiation.

The eggs were carefully collected with the help of a brush and transferred to a small beaker, 3×20 at every dose level. After treatment the hatching was observed.

3×20 larvae per dose level were placed in petri-dishes on synthetic food and pupation and development of moths was observed.

After irradiation pupae were left in the beaker and the development of moths was observed. The adults were then transferred into a larger beaker and their life span, oviposition and hatching of eggs was observed.

Since the collection of 3×20 adults of the same age was not possible, moths were radiation treated on several occasions using different numbers. After treatment the adults were placed in a jar and the jar was covered with a polyethylene pouch and oviposition and emergence were observed. Since the sexing of living adults encountered difficulties, the male : female ratio was established after their death.

Results

The eggs irradiated with 17.5, 35 and 70 krad, respectively, and the control samples were observed during 16 days after treatment. Data on their radiation sensitivity are summarized in Table 1.

Table 1

Susceptibility to radiation of the developmental stages of Indian-meal moth

Dose (krad)	Larvae from eggs[1], %	Pupae from older larvae[2], %	Adult from older larvae[2], %	Adult from pupa[3]		Adult[4]		
				total	deformed[5] %	life span	eggs	larva from egg
0	58.3	95.0	85.0	81.7	6.1	6 days	+++	+++
17.5	0.0	23.3	0.0	70.0	33.3	6 days	++	+
35.0	0.0	18.3	0.0	46.7	35.7	6 days	+	–
70.0	0.0	0.0	0.0	41.6	80.8	5.3 days	–	–

[1] eggs irradiated
[2] larvae irradiated
[3] pupa irradiated
[4] adult irradiated
[5] percentage of emerged moths
+++ many eggs or larvae
++ medium number of eggs or larvae
+ few eggs or larvae

While 35 to 75% of the eggs of the control samples hatched (58.3% in the average), not a single larva was found in the radiation treated samples, not even at the lowest level of treatment.

Larvae near the stage of pupation were radiation treated with doses given above. Data were summarized in Table 1.

Larvae in the control samples started pupation on the 2nd day. Radiation treated larvae were less motile than the control samples and formed pupae later or not at all.

On the fourth day after treatment about 30–60% of the larvae in the control samples started to enter the pupal stage, while the majority of the radiation treated larvae remained immobile, some of them on their back, others on their side. However they moved to the touch, thus they were not dead.

The first moths appeared on the 13th day after treatment in the control sample. Radiation treated larvae just started pupa formation at this time, formed only little web, did not crawl about and faeces was not observed indicating that feeding did occur.

On the 18th day after treatment the moths developing from untreated samples started oviposition while even larvae given the lowest dose were not finished with pupation, they prepared only the pupa cradle and withdrew into it.

On the 25th day after treatment some young larvae were observed in the control sample, while of the samples treated, only a few formed pupae, the majority remained in the initial stage of pupation or did not form pupae at all. Larvae treated with 70 krad were all killed. In the rest of the samples moths were not found even on the 80th day after treatment.

Data on radiation tolerance of pupae are also given in Table 1.

As seen from the table even exposure to 17.5 krad reduced moth development, from pupae. After 70 krad treatment only from 41.6% developed adults. While the percentage of adults decreased, the number of deformed moths increased. In the control sample 3 deformed moths (6.1%) were found. Of the moths developed from pupae treated with 70 krad 80% were deformed. During the mass rearing preceding this experiment not a single deformed moth was found.

The extent of deformation could be classified as follows:

(a) in the case of mild deformation the end of the wings is recurved and a few scales are missing (Fig. 1);

(b) the wings are recurved from the base, look broken, frequently the hind wings are similar (Fig. 2);

(c) both pairs of wings are completely recurved, colourless and look burnt. Hardly any scale or none is seen on the wings. Antennae and abdomen are also deformed (Fig. 3);

(d) the adult cannot emerge from the pupa (Fig. 4);

Fig. 1. Adult with slightly damaged wing emerged from pupa irradiated with 17.5 krad

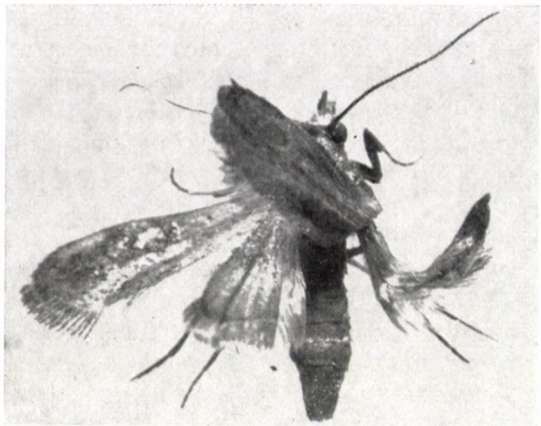

Fig. 2. Adult emerged from pupa treated with 35 krad

Fig. 3. Adult emerged from pupa treated with 70 krad

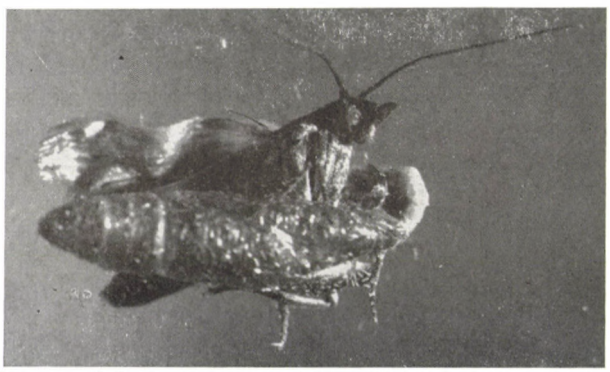

Fig. 4. Adult emerged from pupa treated with 70 krad. Emergence is not complete

Though some of the adults emerged from 17.5 krad irradiated pupae were extensively deformed and some of those treated with 70 krad showed only slight deformation, the extent of deformation increased with higher radiation dose.

Of the moths developed from irradiated pupae those seemingly sound could not fly properly, they hopped about only. The life span of the deformed moths was about 1 – 2 days.

The seemingly sound moths developed from pupae treated with 17.5 krad lived on the average 5 days and some of them produced eggs (about 30 – 50 eggs per female) and from these eggs emerged larvae.

The average life span of moths developing from pupae irradiated with 35 krad was 4 days and oviposition was not observed.

The seemingly sound moths, developed from pupae given 70 krad treatment, were on the 2nd day found laying on their backs and died on the fourth day after treatment. Oviposition was not observed.

Table 1 contains data on the life span of treated moths and observations as regards oviposition and emergence of larvae.

As seen, the life span was not reduced by treatment or slightly (70 krad treatment). Moths treated with 17.5 and 35 krad, respectively, layed eggs, even if in reduced number. Hatching however was observed only with eggs layed by 17.5 krad treated moths. (Neither the accurate number of eggs, nor the percentage hatching, only the occurrence or absence of the phenomenon was recorded.) No oviposition was observed with adults exposed to 70 krad.

The male : female ratio in adults treated was determined subsequently by investigation under the microscope. It was found to be nearly 1 : 1.

Discussion

Experiments carried out with *Plodia interpunctella* (Hübner) *(Lepidoptera, Phycitidae)* have shown the eggs to be the most susceptible stage of development to radiation treatment. Larvae did not hatch at the lowest dose given (17.5 krad) (Table 1).

Larvae were shown to be more radiation resistant. They did not die immediately upon irradiation even when 70 krad was applied. At lower doses feeding to a certain extent was observed and some web formation as well. Some of the radiation treated larvae formed pupae, but no moths developed (Table 1).

If pupae of Indian-meal moth are exposed to gamma radiation the possibility of adult emergence is reduced (Table 1). Moths developed from irradiated pupae, even if seemingly sound, can hardly fly or not at all, their life span is shorter and egg production is also lower. When pupae were treated with 17.5 krad the moths emerged laid eggs and even larvae hatched. The eggs produced by moths emerged from pupae irradiated with 35 krad were found sterile.

The life span of adults was not significantly reduced even by treatment with 70 krad. Oviposition decreases with increasing doses and the sterility of eggs

increases. From eggs of adults irradiated with 35 krad, larvae did not hatch (Table 1).

Though the primary object of our work was the determination of the radiotolerance of *Plodia interpunctella* living in ground paprika, because of the difficulties of rearing in paprika, the insect was reared on synthetic nutrient. In this case, to kill the eggs and active larvae, a radiation dose of 35 krad proved sufficient. If more developed larvae, pupae and adults were also present, treatment with 70 krad seemed more adequate. These results are in good agreement with data obtained for Indian-meal moth living in dried fruit (PAPADOPOULOU, 1963; TILTON *et al.*, 1966; COGBURN *et al.*, 1966; BROWER and TILTON, 1970). Presumably the viability of the *Plodia* population reared in ground paprika is different from that of insects reared on synthetic nutrient, thus heir radiosensitivity is also different. The elucidation of this question requires further investigations.

In experiments aimed at the reduction of cell count in ground paprika it was established that to achieve this 300–500 krad doses are required (FARKAS, 1971; FARKAS and BECZNER, 1971; FARKAS *et al.*, 1972). This dose is sufficient for the simultaneous disinfestation of ground paprika, too.

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Assay of Steroid Inhibitors for Insect Anti-hormone Activity

By

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Based on the similarities between the biosynthesis of cholesterol in human blood and of hormones regulating moulting in insects, several hypocholesterolaemic agents used in human medicine (I—X) were assayed for their influence on insect hormonal function. Further compounds, most of them known as fungicides acting by physicochemical adsorption to ergosterol (XI—XIII), were also tested as they can be regarded as potential ihnibitors of the steroid ecdysone hormones.

Cyclamin (XII), an antifungal saponin acting probably by adsorption to ergosterol, caused the greatest delay of pupation as well as the highest larval and pupal mortality in the test carried out on the flesh fly *(Sarcophaga bullata)*. In at least two of the three tests the compounds IX, VII, VIII, XI, II, IV, V and I (listed in a tentative order of activity) showed medium activity.

In an earlier paper (MATOLCSY *et al.*, 1973) we reported on the antifungal activity of hypocholesterolaemic agents used in human medicine for the inhibition of steroid biosynthesis. These studies were based on our assumption that inhibition of fungal steroid metabolism could result in selective action against fungi containing steroids as vital constituents of their protoplasmic membrane.

The present work and a series of subsequent works on this subject are aimed at exploring the possibilities of influencing the hormonal functions in insects by compounds acting as inhibitors of steroid metabolism.

Compared with the great volume of investigations carried out with natural insect hormones and their synthetic analogues, as potential third generation pesticides, relatively little effort has been devoted to developing compounds which could interfere with the biosynthesis and/or function of insect hormones regulating moulting and metamorphosis.

SVOBODA *et al.* (SVOBODA and ROBBINS, 1967; SVOBODA and ROBBINS, 1968; SVOBODA *et al.*, 1969) have shown that hypocholesterolaemic agents, such as 22,25-diazacholesterol and triparanol (1-[*p*-(2-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)-ethanol) inhibit Δ^{24}-sterol reductase in insects, thus affecting larval development regulated by the steroid ecdysone hormones. They expressed the idea that "continued research in these areas may lead to the development of safe and selective chemicals for insect control" (SVOBODA and ROBBINS, 1967).

Reports on further exploration of this aspect are missing however, from the

literature. In addition, inhibition of ecdysone biosynthesis represents only one of the potential means for interfering with the hormonal metabolism of insects. A comparative estimation of steroid metabolism in different organisms, as well as the abundant knowledge on the mechanism of action of hypocholesterolaemic agents used in human therapy may lead to conclusions serving as theoretical basis for new approaches to this problem.

Thus ecdysones could be potentially inhibited, besides blocking their biosynthesis, by compounds capable of physicochemical adsorption to these steroids, similarly to the antifungal action of the polyene macrolide antibiotics (LAMPEN *et al.*, 1962; GALE, 1963; LAMPEN, 1966; CHILD *et al.*, 1969; GOTTLIEB and SHAW, 1970) and of saponins (TSCHESCHE and WULFF, 1964; SCHLÖSSER, 1971) acting probably by adsorption to ergosterol present in the protoplasmic membrane.

The biosynthesis of juvenile hormones may serve as a further target for an interference by agents affecting steroid biosynthesis. This assumption is supported by the analogy between the early steps in human sterol biosynthesis starting from acetate and leading to farnesyl pyrophosphate (LANGDON and BLOCH, 1953; POPJÁK, 1954; CORNFORTH, 1954; RUDNEY, 1957; LYNEN *et al.*, 1958; BRODIE *et al.*, 1963; BLOCH, 1965), and the biosynthesis of insect juvenile hormones as proposed by KOYAMA *et al.* (KOYAMA *et al.*, 1972; OGURA *et al.*, 1972). It can be assumed that the formation of the hypothesized juvenile hormone precursors such as 3-ethyl-3-butenyl pyrophosphate, bis-homofarnesol and perhaps the yet unknown 3,5-dihydroxy-3-ethyl-valeric acid ("homomevalonic acid") could be inhibited by their synthetic methyl-, thio-, fluoro- and other analogues just as effectively as the formation of the corresponding intermediates in human cholesterol biosynthesis can be suppressed by their antimetabolite analogues (WRIGHT 1957; TAMURA *et al.*, 1958; STEWART and WOOLLEY, 1959; BERGMAN and COHEN, 1960; DAENIKER and DRUEY, 1960; TSCHESCHE and MACHLEIDT, 1960; KIRSCHNER *et al.*, 1961; SCHMIDT and JAHN, 1961; ROUSSEL-UCLAF, 1963; OGAWA *et al.*, 1965).

Based on these considerations, the present work comprehends a study of the developmental response of insects on treatment with compounds described in the literature partly as inhibitors of steroid biosynthesis (hypocholesterolaemic agents) used in human medicine for preventing atherosclerosis, partly as compounds, with one exception known as fungicides, capable of physicochemic l adsorption to steroids.

Materials and Methods

Compounds

Hypocholesterolaemic agents (potential inhibitors of ecdysone biosynthesis):

I. Butyl 2-phenyl-butyrate (COTTET *et al.*, 1953; THORP, 1962; WARING, 1962)
II. 2-(4-chlorophenyl)-valeric acid (COTTET *et al.*, 1953; THORP, 1962; WARING, 1962)
III. 2-(4-chlorophenyl)-valeronitrile (COTTET *et al.*, 1953; THORP, 1962; WARING, 1962)

IV. 2-(4-chlorophenoxy)-2-methylpropionic acid (WARING, 1962; THORP, 1963)
V. bis-(4-chlorophenoxy)-acetic acid (TIMMS *et al.*, 1968)
VI. 2-diethylaminoethyl bis-(4-chlorophenoxy)-acetate (TIMMS *et al.*, 1968)
VII. 1-cyano-1-phenylamino-cyclohexane (JONES *et al.*, 1961)
VIII. ethyl N-benzyl-N-benzyloxy-carbamate W-398[a] (BERGER *et al.*, 1963; DOUGLAS *et al.*, 1966; DOUGLAS, 1964)
IX. trans-1,4-bis-(2-chlorobenzylaminomethyl)-cyclohexane dihydrochloride AY-9944[b] (DVORNIK *et al.*, 1963; CHAPPEL *et al.*, 1964; KRAML *et al.*, 1964; HUMBER *et al.*, 1966)
X. nicotinic acid (ALTSCHUL, 1956)

Compounds (antifungal agents) known to act by adsorption to steroids (potential inhibitors of ecdysones by adsorption):

XI. nystatin (CHILD *et al.*, 1969; DEKKER, 1971)
XII. cyclamin[c] (TSCHESCHE and WULFF, 1964; SCHLÖSSER and GOTTLIEB, 1966; SCHLÖSSER, 1971)
XIII. digitonin (WINDAUS, 1907)

Some of the compounds were resynthesised according to methods described in the literature; others were obtained from commercial sources.

Assay

Last instar larvae of the flesh-fly, *Sarcophaga bullata* reared on pork liver at 23°C were used in our screening assays. The full-grown larvae were treated 24–36 hours before pupation (pupariation) after having left the food and the alimentary canal having been emptied. The insects were immobilized by being kept on the surface of thawing ice for 5–10 minutes.

The compounds were dissolved in ethanol–water (1 : 4) mixture. Though some of the chemicals proved to be poorly soluble in this solvent, the ratio of ethanol to water could not be raised because of its toxic effect on insects at higher concentrations. 5 μl of the solutions were injected into 24–30 larvae with a micrometer-driven tuberculin-syringe and No. 18 Record needle. The needle was thrust laterally into one of the last abdominal intersegments, the tip being moved forward to the thoracal body-cavity. After the treatment the larvae were put into Petri-dishes filled with saw-dust and kept at room temperature (20–24°C) and in total darkness.

The number of dead pupated insects was established twice a day. Solvent treated and untreated larvae were observed as controls. Each application was replicated 3 times.

[a] From Wallace Laboratories, Cranbury, N. J., U.S.A.
[b] From Ayerst Research Laboratories, Saint-Laurent, Quebec, Canada.
[c] From Professor G. WULFF, Institute of Organic Chemistry and Biochemistry, University of Bonn, Federal Republic of Germany.

Results and Discussion

The presumable anti-ecdyson effects of the compounds tested on *Sarcophaga* larvae were assessed on the retardation or total inhibition of pupation and was manifest in the prolongation of the mean period between the moment of treatment and pupation as well as in the mortality of larvae which had not been able to start metamorphosis. The stronger larval mortality became evident 24–48 hours after the treatment. On most of the treated insects even the primary symptoms of puparriation (rounding of body shape, sclerotization) were not detectable. In case of a real anti-hormone effect the inhibition of pupal–adult transformation could be assumed. Therefore the pupal mortality was also estimated.

Table 1

Influence of the tested compounds on timing of pupation, larval and pupal mortality of *Sarcophaga bullata*

Compound	Presumed mode of action[1]	Relative solubility in 1 : 4 EtOH – H_2O[2]	Dosage µg/larva	Delay of pupation[3]	Larval mortality %	Pupal mortality %
I	A	+++	2.5	1.5	6.7	—
II	A	++	0.5–1	2.2	4.0	16.7
III	A	+++	2.5	1.7	0.0	0.0
IV	A	++	0.5–1	2.0	0.0	9.9
V	A	+++	2.5	1.6	6.9	0.0
VI	A	+++	2.5	2.3	0.0	4.2
VII	A	+	0.5	2.4	6.7	21.4
VIII	A	+	0.5	2.3	6.7	10.7
IX	A	+	0.5	2.5	6.8	22.2
X	A	+++	2.5	1.0	3.3	—
XI	B	++	0.5–1	2.3	3.2	23.3
XII	B	++	0.5–1	2.9	40.0	77.8
XIII	B	+++	2.5	1.4	3.3	—
control (solvent)			0.0	1.0	0.0	0.0
control (untreated)			0.0	1.0	0.0	0.0

Key: (1) A: potential inhibition of ecdyson biosynthesis; B: potential blocking of ecdysone by adsorption;
(2) +++, ++ and + mean good, medium and low solubility in 1 : 4 ethanol–water mixture;
(3) The ratio of period necessary for the pupation of 50% of the treated larvae to the period necessary for pupation of 50% of the control larvae treated with the solvent only. The lengths of period were calculated by interpolation. Dead larvae were left out of consideration.

The solvent in itself caused neither delay in the development nor raised mortality. On the other hand, however, our experiences showed, that the success of screening-assays had been noticeably influenced by the exact timing of applications. The sensitivity of *Sarcophaga* larvae to chemicals with potential anti-ecdyson activity seemed to change rapidly with the age of insects presumably in correspondence with variations in the rate of ecdyson synthesis and release.

Results are summarized in Table 1. The relative solubilities of compounds in ethanol–water (1 : 4) mixture were also recorded. In case of compounds with low solubility the saturated (at room temperature) solutions of these compounds have been injected. In these cases the exact concentrations had not been determined. Therefore the effect of those compounds can be compared with which have similar solubilities.

In each three tests, the strongest inhibitory effect was exerted by cyclamin XII, an antifungal saponin present in Cyclamen species. In spite of the low concentration applied owing to its poor solubility in water–ethanol–mixture, this compound obviously impeded both pupation and pupal development.

The less soluble compounds IX, VII, VIII, the moderately soluble compounds XI, II, IV as well as compounds V and I having relatively good solubility (listed in a tentative order of activity) proved to be active in at least two of the three tests applied.

Considering the symptoms, the effective compounds tested on flesh-fly larvae seemed to influence the normal production or function of ecdysone. Nevertheless, other non-specific mechanisms of response could also be taken into account, which is the task of further investigations. In this field other studies are in progress.

*

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Effect of Climate on the Density and Distribution of some Mirid Pests of Lucerne (Heteroptera: Miridae)

By

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An attempt has been made to analyze the density of four heteropterous species (*Adelphocoris lineolatus*, *Lygus rugulipennis*, *Polymerus vulneratus*, *Lygus pratensis*) in the light of Selianinov's hydrothermic quotient calculated to characterize weather conditions. The analysis was based on data of the national plant protection light trap network and on the material of sweep samples taken in 1967 at five localities throughout the growing season. It has been found that *A. lineolatus* prefers balanced weather conditions and thrives well under cool and wet conditions, but its populations are adversly affected by warm and dry weather. *L. rugulipennis* prefers warm and dry climate, but owing to its wide ecological valency it also occurs in abundance in wet and cool areas. *P. vulneratus* shows preference to a moderately warm and dry climate, apparently it has a narrow ecological valency, and is scarce both in definitely warm and dry and in cool and wet regions. In contrast, *L. pratensis* does not thrive well under warm and dry conditions, it prefers a cool and wet climate. Due to the differences in the climatic demands of the investigated species their density and relative economic importance show marked differences parallel to weather changes as well as geographic and climatic shifts.

In Europe four species of Heteroptera (*Adelphocoris lineolatus* Goeze, *Lygus rugulipennis* Poppius, *Polymerus vulneratus* Panzer, *Lygus pratensis* Linnaeus) are generally listed as pests of lucerne grown for seed (Koppányi, 1959; Manninger, 1960; Josifov, 1962; Romankow, 1963; Obrtel, 1969; Benedek, Erdélyi and Jászai, 1970). Their proportion and density, however, was found to be widely different according to the findings of various authors. It was quite plausible to suppose that these differences were linked, primarily, with climatic effects.

Material and Method

In 1967 regular sweeps were made throughout the growing season at five localities in Hungary (Fig. 1). Samples were taken by 2×20 sweeps per plot at five different dates at Füzesgyarmat and Gyoma, at three dates at Kevermes, at six dates at Székkutas and at five dates at Júliamajor in 1–5 chosen fields at each locality. The investigated fields were divided into two plots. For obtaining stands of different phenological stages at each field one of these plots was cut

2–3 weeks later than the other. Sweeps were taken in both plots of the fields at each occasion, so the density of the investigated bugs was only slightly affected by migration linked with the phenological stage of stands. The investigated species gave a total of 424, 847, 434, 334 and 21 specimens for the five localities listed above. The mean density of adults related to 20 sweeps and the percentual ratio of the four species were calculated for each locality.

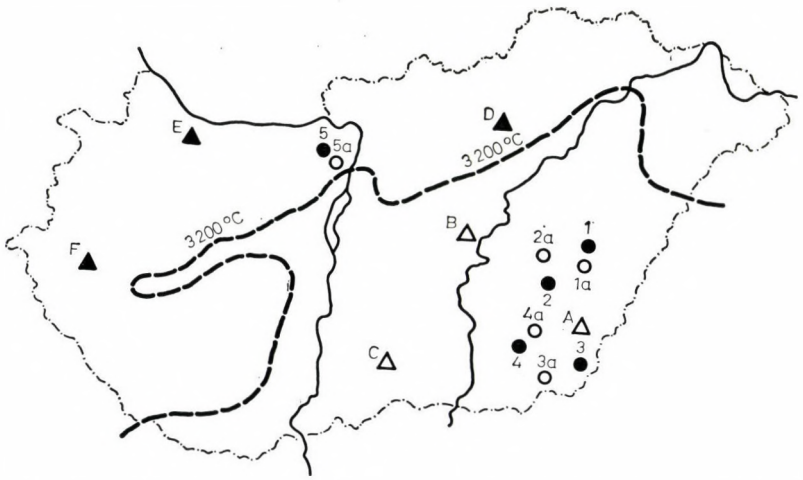

Fig. 1. Distribution of investigated sites and meteorological stations selected for this st udy The broken line indicates the isotherm of the 3200°C cumulative degree days for the growing season. Areas southwards and northwards of the isotherm are called as "warm summer" and "cool summer" territories, respectively. Sites of sweep surveys: 1 = Füzesgyarmat, 2 = Gyoma, 3 = Kevermes, 4 = Székkutas, 5 = Júliamajor. Meteorological stations utilized for sweep samples: 1a = Szeghalom, 2a = Túrkeve, 3a = Mezőhegyes, 4a = Orosháza, 5a = Budapest (Csillagda). Meteorological stations utilized for light trap catches: A = Békéscsaba, B = Szolnok, C = Kiskunhalas, D = Eger, E = Győr, F = Zalaegerszeg

All specimens of the four species taken in the 20 traps of the national plant protection light trap network were identified between 1964 and 1966, their number gave a total of 54,377 specimens. In evaluating the data we followed a method elaborated by REICHART and SZŐCS (1961) currently used to process light trap data in Hungary. Hungary was divided into two regions one above and one below 3200°C cumulative degree days for the growing season. These regions were denoted as "warm summer" and "cool summer" territories, respectively. Each territory was covered by 10 light traps. In the analysis the total annual catch of 10 light traps of the respective region was used for each species.

SOUTHWOOD (1960) pointed out that the flight activity of *A. lineolatus* is mostly nocturnal while that of *L. rugulipennis* is mostly diurnal. Thus, according to our opinion, the number of specimens caught by light traps does not reflect a true proportion of the species. In this respect only the sweep samples reveal relevant data.

SELIANINOV's (cit. WALTER, 1955) hydrothermic quotient (HTQ) was used for characterizing weather and climatic conditions. This quotient is valid for the months of the growing season with an average temperature higher than 10°C. To calculate the quotient the monthly total of precipitation in mm was devided by the threefold of the monthly average temperature in °C. Under the temperate belt, the weather may be considered balanced if the quotient gives the value of 1; if it is less or more, the season is recorded "warm and dry" and "cool and wet".

Against the data of sweeps, the HTQ values covering the period between April and August were set, since the last sweep was done in August. With the light trap data, the HTQ values for the April–September period were taken. In the case of sweep samples, the data of the meteorological stations in close vicinity of the investigated sites were used to calculate HTQ values. These stations in the order of investigation sites were as follows: Szeghalom, Túrkeve, Mezőhegyes, Orosháza, Budapest (Csillagda). Meteorological data from three characteristic stations of both the warm summer and cool summer regions were related to the light trap data. They were Békéscsaba, Szolnok and Kiskunhalas for warm summer, and Eger, Győr and Zalaegerszeg for cool summer territories (Fig. 1). Data of sweep samples and light traps together with relevant HTQ values were subjected to regression analysis.

Results

1. Effect of climate on density and light trap catch

Adelphocoris lineolatus. The regression line calculated from the data of sweep samples shows that the density increase follows an exponential curve from warm and dry region towards balanced conditions (Fig. 2a). Simultaneously,

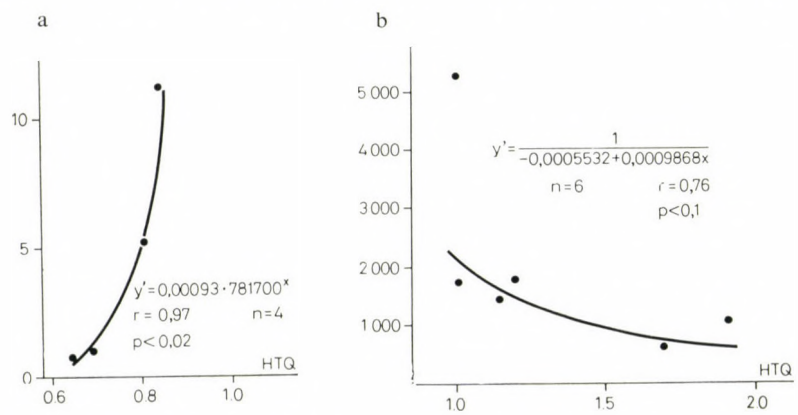

Fig. 2. Effect of weather conditions in the growing season on the density of *Adelphocoris lineolatus* in lucerne fields (a) and on the size of its light trap catches (b). Scale: density per 20 sweeps (a) and total number of specimens taken in 10 light traps (b)

the equation based on the light trap data presents a step by step decrease in the catch from balanced conditions towards the cool and wet region (Fig. 2b). The single sweep sample of this region (Júliamajor: 1.1 adult per 20 sweeps) was not taken into account because it should belong to the descending portion of the density curve as suggested by the light trap data.

Lygus rugulipennis. Its number gradually decreases from the warm and dry region, through balanced conditions, towards the cool and wet region (Figs 3a, b). However, its quantity remains considerable in the latter region, too.

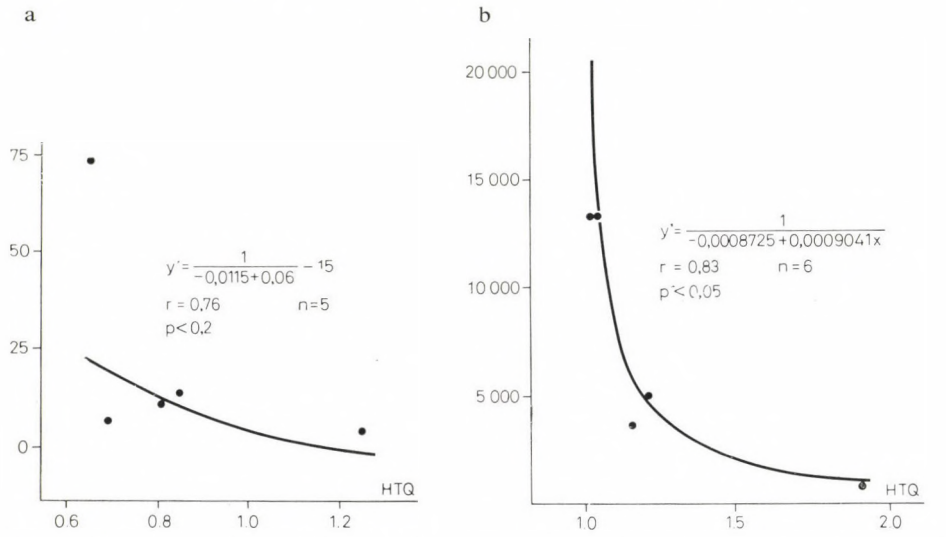

Fig. 3. Effect of weather conditions in the growing season on the density of *Lygus rugulipennis* in lucerne fields (a) and on the size of its light trap catches (b). Explanations as in Fig. 2

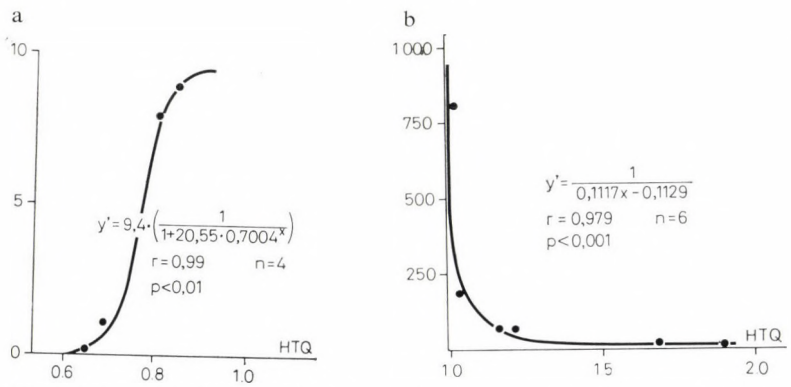

Fig. 4. Effect of weather conditions in the growing season on the density of *Polymerus vulneratus* in lucerne fields (a) and on the size of its light trap catches (b). Explanations as in Fig. 2

Polymerus vulneratus. Approaching balanced conditions from warm and dry zone density initially shows an exponential increase then it merges into a sigmoid curve (Fig. 4a). It is striking how suddenly the catch curve falls moving from balanced conditions towards cool and wet zone (Fig. 4b). This may explain why no specimen of the species was found in the Júliamajor sweep samples (the weather here was definitely cool and wet; HTQ = 1.25).

Lygus pratensis. This species was scarcely present in the material of our sweep samples. Only a few specimens were caught in light traps, and the number showed an irregular variation to HTQ values. For this reason no regression curve can be plotted.

2. *Size of light trap catches in warm summer and cool summer territories*

Only one species, *L. pratensis*, was found in larger quantities in the cool summer territories. *P. vulneratus*, on the other hand, derived up to 90% from the warm summer territories. More than 77% of the trapped *L. rugulipennis* material together with 69% of that of *A. lineolatus* was captured in warm summer regions. The proportion of adults trapped in cool and warm summer territories differed significantly ($p < 0.05$) in all the four species (Fig. 5).

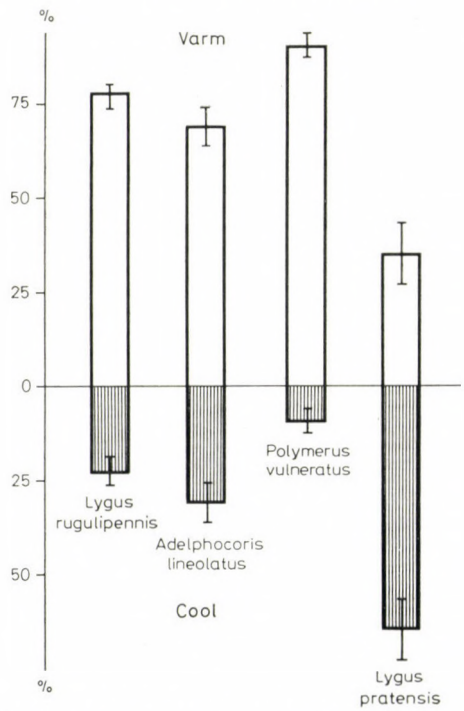

Fig. 5. Percentual distribution of the annual total catches of 20 light traps in the "warm summer" and "cool summer" regions in Hungary

3. Effect of climate on the proportion of species in the sweep samples

Figure 6 demonstrates that the proportion of *L. rugulipennis* was overwhelming at the warmest and dryest locality of the investigated ones (HTQ = 0.65). Approaching balanced conditions, then the cool and wet weather regions its proportion gradually decreased. The proportion of *A. lineolatus* followed an opposite trend, its relative density was the highest in the cool and wet and the lowest in the warm and dry territories. The proportion of *P. vulneratus* showed a peculiar pattern, i.e. it was scarce both in definitely warm and dry, and cool and wet territories but attained a considerable proportion under slightly warm and dry weather conditions (HTQ = 0.81 – 0.85). The ratio of *L. pratensis* was around zero, except in Júliamajor. The latter locality, however, was in the cool and wet territories. Here, *L. pratensis* achieved a marked density.

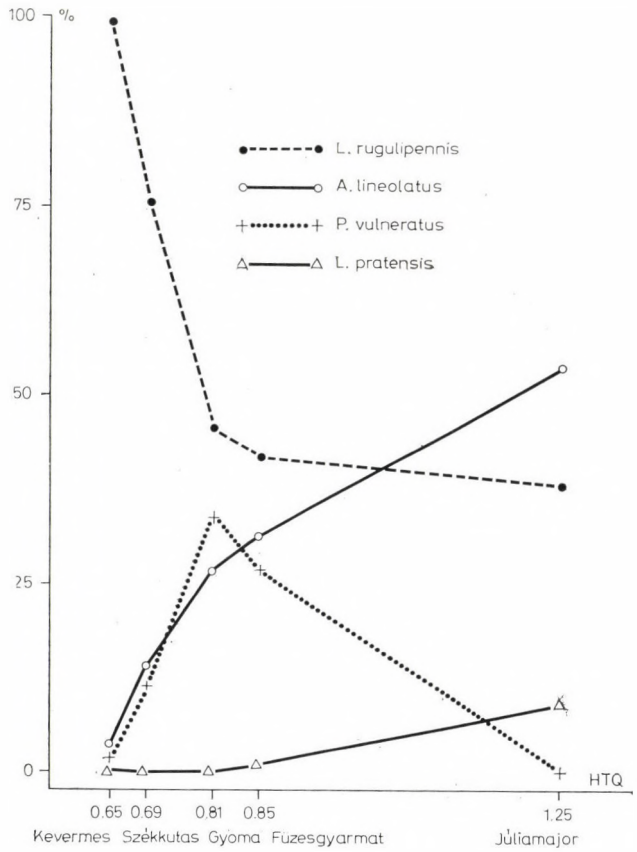

Fig. 6. Effect of weather conditions in the growing season on the percentual relative density of four heteropterous pests of lucerne

Discussion and Conclusions

The results strongly suggest that balanced climatic or weather conditions are most favourable for *A. lineolatus* though its ecological valency appears to be rather wide. It occurred in a small density even in the extreme warm and dry regions and was found in a considerable number in cool and wet regions. This indicates that it rather endures cool and wet than warm and dry conditions. This finding is in accordance with MANNINGER's (1951) observation. He stated that neither cool and rainy nor dry and warm seasons are favoured by the species. PUCHKOV (1966) reported that in the Soviet Union, forest steppe provides the best conditions for this species. This belt has a balanced climate (HTQ = 1 or so) so PUCHKOV's experiences also confirm our establishment.

L. rugulipennis was extremely abundant at the locality of definitely warm and dry weather conditions (HTQ = 0.65) and it occurred in a high density also in regions of balanced as well as of cool and wet weather (HTQ = 1.91). No doubt, its ecological valency is extremely wide, still the definitely warm and dry conditions are most favourable for it. In warm and dry regions its ecological demands seem to be the very opposite of those of the former species. Therefore, it is plausible to say that the densities of these two species shift in opposite directions as a result of weather changes and climatic differences. Such shifts were reported by PUCHKOV (1966) in 1947, 1951 and 1953 with warm and dry summer, when the *A. lineolatus* populations of the second generation fell to the tenth of the first one and the number of *L. rugulipennis* simultaneously increased.

We may conclude that moderately or slightly warm and dry conditions are optimal for *P. vulneratus*, the ecological valency of which appears to be narrow, since it hardly occurred in regions of definitely warm and dry (HTQ = 0.65) and cool and wet conditions (HTQ = 1.91). PUCHKOV (1966) stated that in the forest steppe belt of the European territories of the Soviet Union it was greatly abundant in 1930–1931 and also in 1951–1953. It was established that during the growing season in these years, the weather was moderately warm and dry. This is in a good agreement with our statement.

L. pratensis was scarcely present in our sweep samples, therefore, its ecological demands may be approached mainly on the basis of our light traps. Since in the cool summer regions it has been taken in a significantly larger number it may be concluded that, in contrast to the former species, cool and wet weather conditions are favourable for the species. It was most abundant in 1966 of a definitely cool and wet summer in the cool summer regions (HTQ = 1.7). However, a considerably smaller number of its adults was taken in 1964 and 1965 when weather conditions in summer were much less and slightly more cool and wet, respectively (HTQ = 1.2 and 1.9, resp.). This indicates that even moderately cool and wet conditions (HTQ = 1.2) are unfavourable for this species, and apparently extremely cold and rainy summer is also disadvantageous.

Accordingly, it seems clearly evident that there are considerable differences in the ecological demands of the investigated species. No doubt, this is of a pri-

mary influence on their geographical distribution and population density. This may explain away the great differences in their density and ratio reported by various authors.

As a consequence of its wide ecological valency, *L. rugulipennis* is the most widely distributed of the four species. It spreads all over the Palaearctic region from the tundras to the deserts of North Africa. It was shown to occur in all countries of North Africa (PUCHKOV, 1966), i.e. even in the hottest and dryest eastern ones with a rainfall of next to nothing in the April–September period (e.g. Alexandria: 3 mm, Idris: 26 mm). It is common also in Finland (LINNAVUORI, 1951) where the climate is definitely cool and wet (HTQ is 1.6 and total rainfall is 335 mm in Helsinki in the April–September period). Because of its frequent occurrence, it is considered as pest in its whole area including Finland, Sweden and countries at the southern border of its distribution. It often attains a strikingly high density. For example, we have found 192 individuals per 20 sweeps and PUCHKOV (1966) has recorded as high densities as 600–800 individuals per 100 sweeps.

A. lineolatus is a widely spread species but its distribution area does not expand exactly as far as the southern and northern limits of the area of *L. rugulipennis*. The northern limit of its distribution lies southwards of some five latitude degree than that of *L. rugulipennis*. It is well known from Europe and Palaearctic Asia, and was introduced also into North America. North Africa is the most southern region of its distribution where it spreads over the less dry western countries with, for example, a 125 and 148 mm rainfall in the April–September period for Tunis and Algiers, respectively, but it is missing from the extremely dry eastern territories (STICHEL, 1955–1962). Since, it is common almost all over its distribution area it is widely known as a pest of fodder legumes.

The distribution of *P. vulneratus* covers an area bordered by Marocco, Sweden and West Siberia (STICHEL, 1955–1962; PUCHKOV, 1966). Owing to its narrow ecological valency it must be a rather scarce species in most regions of its distribution. This is confirmed by ROMANKOW's (1963) report spread over several years; among thousands of *A. lineolatus* and *L. rugulipennis* only a few specimens of *P. vulneratus* have been found in Poland. In Hungary it seems to be abundant in certain years in the central and southern regions, the greatest part of which is of a moderately warm and dry climate, but the species is scarce in the cool and wet western and northern regions of the country.

Taking the ecological demands of *L. pratensis* into consideration it can be concluded that its geographical distribution is much less expanded than was generally thought to be. It has been recorded all over the Palaearctic region, however, the areas favourable for it must be restricted to the northern cool and wet regions. This contradiction may be explained by the fact that most records on its distribution had been published before the well-known taxonomic confusions relating to its species-group were solved. In all probability the density of *L. pratensis* nowhere achieves that of *L. rugulipennis* even in areas favourable for it. For example, in our sweep samples its ratio to *L. rugulipennis* was 1 : 250 and

in ROMANKOW's (1963) the ratio was 1 : 16. LINNAVUORI (1951) established *L. rugulipennis* to be much more common than *L. pratensis* even in Finland.

No doubt, the density, the proportion and, consequently, the economic importance of the investigated species are greatly different under diverse climatic conditions. Owing to its ecological demands *L. rugulipennis* must be the most abundant, hence, economically most important species in the southern countries of definitely warm and dry climate. In moderately warm and dry conditions *L. rugulipennis* retains its importance but two additional species, *A. lineolatus* and *P. vulneratus*, attain a high density. In countries with balanced climate, the density of *A. lineolatus* greatly increases and exceeds that of *L. rugulipennis*. In moderately cool and wet territories the density of *A. lineolatus* decreases but remains higher than that of *L. rugulipennis*. This is the reason why in Central Europe and in adjacent countries *A. lineolatus* is the most devastating pest of the heteropterous species in lucerne fields. Here, at the same time, the occurrence of *P. vulneratus* is scarcely noticeable and *L. pratensis* turns up in small numbers only. Under extreme cool and wet conditions, i.e. in northern territories, the density of the investigated species suddenly decreases, except *L. rugulipennis*. Although, its density is also lower than in more southern regions, as a consequence of its wide ecological valency, it maintains well even under extremely cool and wet climate unfavourable for the other species.

Similar shifts occur when weather conditions change. In territories of definitely warm and dry climate, seasons with unusually high rainfall make the density of *A. lineolatus* significant besides that of the highly abundant *L. rugulipennis*. In regions with a balanced or moderately cool and wet climate dry seasons cause an increase in the population density of *L. rugulipennis* and a decrease in that of *A. lineolatus*. Additionally, a mass appearance of *P. vulneratus* may also occur. In cool and wet territories, seasons with relatively low rainfall, increase the relative importance of *L. rugulipennis* parallel with the decrease of proportion of *A. lineolatus*. In extremely cool and wet seasons the density of *A. lineolatus* suddenly decreases and a simultaneous but, presumably, less abrupt decline occurs in the population density of *L. rugulipennis*.

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Book review

VISHWA NATH PATHAK: Essentials of Plant Pathology. Prakash Publishers, Jaipur, 1972. Pp. I—XV. and 1—448., with 89 figures

This volume will be welcome by both teachers and students of plant pathology, all over the world. Structure and content of the book can be considered as an outline of any university course in phytopathology.

The first chapter summarizes the definition, classification and importance of plant diseases. After a short historical background the main characteristics of plant pathogens (taxonomy, physiology, variation, pathogenicity) are discussed in four chapters. The following part of the book (five chapters) deals with the development of the plant diseases, including the responses of the host plant, too. Basic elements of plant disease control are given in chapter 12. Chapter 13, a detailed description of methods in plant pathology seems to be the most valuable and useful part of the volume.

Material about specified plant pathology is presented in a rather unusual order: descriptions of fungal diseases are followed by bacterial, virus, nematode, phanerogamic and finally non-parasitic diseases. The last two chapters separately deal with postharvest and mycoplasma diseases respectively, which procedure can also be debated.

A glossary of the mos' important terms and a chart for the identification of the more frequent plant pathogens are certainly useful supplements of the book.

J. VÖRÖS

ANNOUNCEMENT

On the occasion of the 150th anniversary of the founding of the Hungarian Academy of Sciences, an International Symposium on

Current Topics in Plant Pathology

will be held in Budapest, Hungary, June 24–27, 1975. The Symposium will be under the auspices of the Research Institute for Plant Protection in Budapest and is sponsored by the Hungarian Academy of Sciences.

Participants are invited to present papers on the following topics:

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All correspondence relating to the Symposium should be addressed to:

Dr. Z. KIRÁLY, Chairman Organizing Committee for the Symposium on Current Topics in Plant Pathology
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von *T. Zsolnai*

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Jonathan Spot Induced by Ultraviolet Light

By

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Experiments were conducted to demonstrate the spot-inducing effect of ultraviolet light in Jonathan spot of mature apples (*Malus sylvestris* Miller). Ultraviolet light irradiation either from the sun under orchard conditions or artificially induced by UV germicidal lamp causes Jonathan spot. In the spotted apple peel tissues anthocyanins (mainly cyanidine-3-galactoside) were oxidized or destroyed. Simultaneously with UV light irradiation, there were increases in the activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase.

In the last decade considerable quality reduction in stored Jonathan apples is due to formation of brown spots in the peel tissue. Both the commercial value and export potential are reduced in apples showing these symptoms. The causes of spot formation are not clear and opinions are conflicting in the literature.

PENTZER (1925) observed that the pH of spotted tissue was higher than that of adjacent normal tissue and suggested that the spot symptoms originated from the anthocyanin color conversion from red to blue. The spotted tissue was characterized by a marked accumulation of cations, mainly K^+, Ca^{++} and Mg^{++}. Perhaps there is a relationship between pH changes, binding of the organic acids by cations and peel tissue neutralization.

The relationship between cation accumulation and organic synthesis and the development of Jonathan spot on apple fruit was demonstrated by RICHMOND *et al.* (1964). They found that the epidermal tissues of Jonathan apples affected with Jonathan spot contained more than twice as much K^+ and four times as much Mg^{++} and Ca^{++} as adjacent normal tissues. The total acidity of spotted tissue was twice that of normal tissue while the pH was 0.7 unit higher. They also found that the citric acid content of spotted tissue was considerably higher than that found in normal tissue.

In spite of these data, several investigators have traced the Jonathan spot back to calcium deficiency and have offered calcium-fertilizer spray application as a control measure for Jonathan spot (BEYERS, 1963,, SCHACHRESTANY, 1964., SCHUMACHER and FANKHAUSER, 1966., PAIS and PETHŐ, 1971). Apparently these recommendations were connected with the decreased calcium fertility levels of orchards. On the other hand, JERMY (1962) measured the temperatures of apple trees on different sides of the crown and the sun-tanned side and the shaded side

of the apple fruit. He found a 12 °C difference between the two sides in the sunny heat of September. According to observations, by orchardists if the weather is sunny and hot during the ripening period, apples have increased spotting under storage.

HADWIGER and SCHWOCHAU (1971) demonstrated that pea pods irradiated with ultraviolet light showed increased phenylalanine ammonia lyase (PAL) activity. In our preliminary experiments we established that the PAL acitivity was higher in spotted peel tissue than in the normal tissue (BALÁZS and TÓTH, 1973). However we did not observe any biochemical differences between the Jonathan spot and Lenticel spot as described by TRECCANI (1960), in spite of the fact that the two types of peel spots are different from a histological point of view.

On the basis of these former observations, we believed that the ultraviolet light of the sun causes increased enzyme activity in the irradiated peel tissue and destroys the anthocyanins. This study demonstrated that the Jonathan spot was induced by ultraviolet light and that UV light promoted through increased enzyme activity, the oxidation of anthocyanin and caused the Jonathan spot.

Materials and Methods

Plant material

Mature Jonathan apples (*Malus sylvestris*. Miller) were harvested and maintained in storage at approximately +4 °C for six months to obtain fruit with varying degrees of spot development.

Artificial Jonathan spot induction

Jonathan apples were placed under a germicidal lamp (Germicid F type, Tungsram) with an energy output between 185 – 578 nm. The major energy output is at 253.7 nm.

The apples were irradiated 40 cm from the lamp surface. The controls were the unirradiated sides of the apples. The time of UV irradiation was dependent upon the colour of the peel, and the amount of light received by the plant during its growing period. After UV treatments (for 1, 2, 3, 4 and 5 h) the apples were stored at 4 °C for six months. The apples were observed at weekly intervals during the storage period.

Isolation and purification of anthocyanins

The spotted tissue (both from naturally induced Jonathan spot and artificially induced Jonathan spot) and the adjacent normal red peel tissue were carefully scraped from the pulp. Samples of spotted tissue and normal peel tissue were dried in a desiccator above P_2O_5 for 3 days in the dark under vacuum. 1 – 1 g

dried peel samples were extracted with 25 ml $HCl-CH_3OH$ (1 : 99 v/v) for 24 h at 4 °C. The peel samples were washed two times with 5 ml $HCl-CH_3OH$ (1 : 99 v/v) and the extracts were collected and filtered through G 2 glass filters. The filtered extracts were concentrated in a vacuum desiccator above anhydrous NaOH for 12 h. The final volume of the extracts was approximately 5 ml. The extracts were filtered again through Whatman No. 1 paper and were washed with 20 ml distilled water. These aqueous extracts were passed through a column containing 7 ml of DOWEX 50 W-X 4 (H^+ 400 mesh) ion exchange resin (RODNEY and LUH, 1965).

Table 1

Enzyme activities in apple peel tissue

Tissue	Activity of		
	PO	PPO	PAL
Jonathan spot (natural)	151±8	128±6	135±5
UV germicidal lamp-induced Jonathan spot	158±6	127±5	136±5

Enzyme activity was expressed in percentage of adjacent red peel tissue control (100%). The enzyme measurement was repeated five times. The enzyme activity was calculated both for fresh weight and 10 μg protein. The time of UV irradiation was 4 hours as optimum time. Measurements were made three months after UV treatment

The column was washed with 10 ml distilled water and 5 ml acetone three times. The anthocyanins were eluted from the resin with 50 ml $HCl-CH_3OH$ (5 : 95 v/v) mixture. The eluted anthocyanins were concentrated to 10 ml under vacuum at 35 °C, and the samples were concentrated further in a vacuum desiccator above anhydrous NaOH to the desired degree. This purified anthocyanin (mainly, cyanidine-3-galactoside) was measured spectrophotometrically (UNICAM SP 800) between 350–550 nm and compared to spectral data of known quantities of pure cyanidine-3-galactoside (HARBORNE, 1967).

Enzyme assays

Peroxidase (PO) and polyphenol oxidase (PPO) extractions and assays. For examining PO and PPO activities, the samples were taken from the normal red and spotted peel tissues that were carefully scraped from pulp. Samples were homogenized (5–5 g fresh weight) in 50 ml of 0.1 *M* phosphate buffer pH 6.5, with quartz sand in a pestle and mortar at 4 °C. They were filtered through four layers of cheesecloth and centrifuged at 0 °C with a Janetzky K 23 type centrifuge at 5000 *g* for 30 min. The supernatant was used for the determination of the activity of PO and PPO, using the modified method of FEHRMANN and DIMOND (1967). Catechol (0.2 *M*) was used as a substrate for PPO and pyro-

gallol (0.2 *M*) for PO. Increases in absorbance at 400 nm and at 430 nm were recorded in a UNICAM SP 800 spectrophotometer, respectively.

Phenylalanine ammonia lyase (PAL) extraction and assay: 5—5 g fresh weight spotted and normal red peel tissues were carefully scraped from pulp and homogenized at 4 °C in a pestle and mortar, with 0.1 *M* borate buffer (25 ml) pH 8.8 and quartz sand. After centrifugation at 5000 g at 0 °C for 20 min in a Janetzky K 23 type centrifuge, the supernatant was centrifuged further at 20,000 *g* for 30 min (MOM G 120 Ultracentrifuge), and this supernatant was used for measuring the PAL activity. An appropriate amount (0.1 ml) of the enzyme solution, 200 mmoles of borate buffer pH 8.8 plus 60 mmoles of l-phenylalanine and distilled water for a total volume of 3 ml were mixed in a 1 cm path cuvette. After a 1-h incubation period increase in absorbance at 290 nm against controls without phenylalanine was recorded with a UNICAM SP 800 spectrophotometer (BELLINI and HILLMAN, 1971). The protein content was determined by the method of LOWRY *et al.* (1951).

Results and Discussion

The irradiation of Jonathan apples with UV light promoted the Jonathan spot development under storage. We found that the irradiated sides of Jonathan apples were affected with Jonathan spot to a large extent, while the non-irradiated sides were not spotted. The number and degree of spot development were dependent upon the time of UV irradiation and the red colour intensity of peel tissue. We also found that the artificially induced Jonathan spot appeared at the same time as the natural Jonathan spot. We demonstrated that in the Jonathan spot anthocyanin was oxidized or destroyed (Fig. 1). This caused the visible symptoms

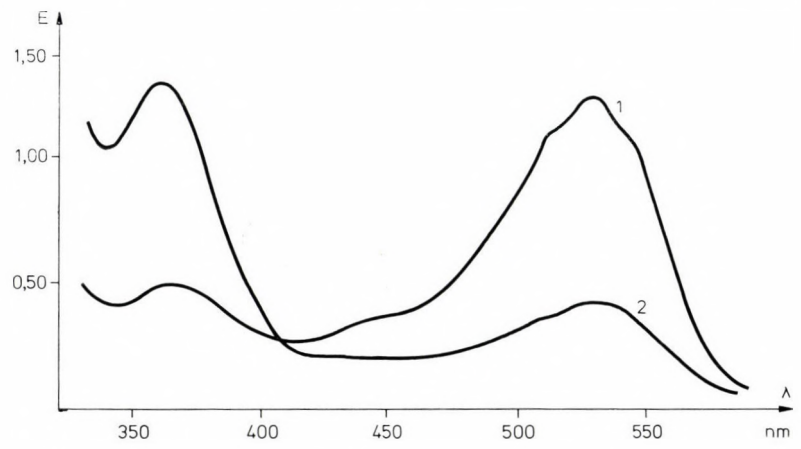

Fig. 1. Ultraviolet spectra of cyanidin-3-galactoside purified from apple peel tissue. 1. UV spectra of anthocyanin purified from normal red Jonathan apple peel; 2. UV spectra of anthocyanin purified from Jonathan spot tissue. Solvents: HCl—CH_3OH (1 : 99 v/v)

on the apple peel tissue. It is noteworthy that if we did not use ion exchange resin in the purification we were unable to demonstrate any differences between the anthocyanin content of spotted and adjacent normal tissue. Mainly in the spotted tissue we found large quantities of phenolic compounds that altered our control. No differences were observed between the anthocyanins of artificially induced and natural sports.

Our experiments proved that the peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities were higher in spotted tissue than in normal tissue. In respect to these enzymes, we could not detect any differences between the natural and artificially induced Jonathan spot (Table 1). The observed UV-induced increase in PAL activity relates well to other literature (HADWIGER and SCHWOCHAU, 1971).

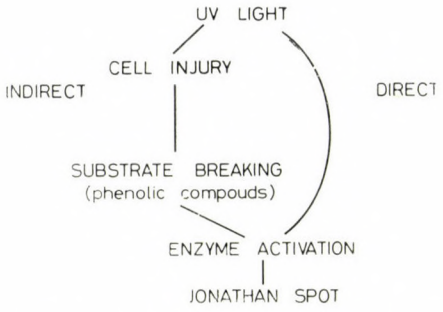

Fig. 2. Two theoretical pathways of the UV light-induced Jonathan spot formation

We proposed two theoretical pathways for the UV light-induced Jonathan spot formation: through direct enzyme activation and indirect enzyme activation (Fig. 2). Our experiment demonstrated that UV light from the sun is involved with Jonathan spot on the apple peel tissue. We were also able to induce Jonathan spot with a germicidal lamp. In the Jonathan spot anthocyanin was destroyed or oxidized. The peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activities were higher in spotted than in normal tissue. These observations correlated well with the ecological effects of high temperatures and concentrated sunshine during the ripening period in September. Enzyme activity is inhibited under low storage temperatures (0–4 °C); therefore the spots appear only in the second or third months of storage.

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The authors thank Prof. Einar W. PALM (University of Missouri) for help in the preparation of this paper.

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Phenylalanine Ammonia-Lyase and Polyphenoloxidase Activities Correlated with Necrogenesis in Cauliflower Mosaic Virus Infection

By

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Biochemical assays of phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) activities were carried out on leaves of Chinese cabbage systemically infected with cauliflower mosaic virus (CaMV). Light microscopic observations of the lesions after specific lignin staining as well as autoradiographic studies after phenylalanine-^{3}H administration were also carried out. As compared with controls, a slight — though significant — increase of the two enzymatic activities was found in the infected tissues. This increase is probably correlated to the few and tiny necrotic lesions present in this host–virus combination. A moderate lignification was present in the outer zone of the necrotic lesions, but no phenylalanine incorporation was seen in the living infected cells. The role of PAL and PPO in the development of localized resistance is discussed.

The formation of virus-induced local lesions in hypersensitive hosts implies a necrogenic process in which many phenolic compounds are involved (FARKAS *et al.*, 1960; FARKAS and KIRÁLY, 1962; TANGUY, 1971; FRITIG *et al.*, 1972). Parallel to the development of such necrotic lesions there is an increased activity of phenylalanine ammonia-lyase (PAL) (FARKAS and SZIRMAI, 1969; SIMONS and ROSS, 1971 *b;* PAYNOT *et al.*, 1971, 1973; FRITIG *et al.*, 1973), an enzyme involved in the metabolism of phenolic compounds, some of which enter the pathway of lignin synthesis (NEISH, 1960; CONN, 1964; PRIDHAM, 1965). It is known that in higher plants lignin is deposited around a lesion whenever the latter undergoes necrosis (LIPETZ, 1970; WU, 1973), and recently FRITIG *et al.* (1973) demonstrated that the increased PAL activity found in hypersensitive hosts is related to necrogenesis and not to the amount of virus responsible for infection.

Another enzyme involved in necrogenesis is polyphenoloxidase (PPO), which also increases parallel to the development of local lesions. However, its role in this process is not clear (FARKAS *et al.*, 1960; FARKAS and KIRÁLY, 1962; BOZARTH and ROSS, 1964; VAN KAMMEN and BROUWER, 1964; PARISH *et al.*, 1965; JOCKUSH, 1966; CABANNE *et al.*, 1968, 1971; SIMONS and ROSS, 1971 *a*; VAN LOON and GEELEN, 1971).

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The aim of the present study was to see how PAL and PPO activities behave when necrotic symptoms are present but inconspicuous, and to investigate if a deposition of lignin could be demonstrated not only in the zone delimiting the necrotic lesions but also in the walls of the living cells surrounding it, in view of the fact that an increased PAL activity was found also at the level of such cells (FRITIG *et al.*, 1973). We carried out our studies on leaves of *Brassica chinensis* L. systemically infected with cauliflower mosaic virus (CaMV), because in this host–virus combination a few tiny necrotic lesions develop, and because CaMV induces marked morphological alterations in the walls of the host cells (CONTI *et al.*, 1972), suggesting an altered cell wall metabolism and/or composition.

Our experiments included biochemical assays of PAL and PPO activities, light microscopic observations of the virus-induced lesions by specifically staining lignin, and autoradiographic studies of phenylalanine-^{3}H incorporation in the infected but living cells surrounding the necrotic lesions, since phenylalanine is a precursor of lignin (NEISH, 1960; YOUNG *et al.*, 1966; RUBERY and NORTHCOTE, 1968; WOODING, 1968).

Materials and Methods

Host plant inoculation

All the experiments were carried out on young plants of *Brassica chinensis* L., grown in an insect-screened greenhouse where the temperature averaged 20–25 °C and the relative humidity 70–90%. The plants were inoculated in the early stages of growth with the sap extracted from CaMV-systemically infected leaves. Inoculation was made by the usual mechanical transmission, using carborundum powder (600 mesh) as an abrasive. The sap was diluted with 0.01 *M* phosphate buffer, pH 7.2. Control plants were rubbed with buffer alone.

Samples were taken from either healthy or systemically infected leaves, 15 days after inoculation, when the plants showed very clear systemic symptoms (mosaic, circular yellow or chlorotic spotlets, short necrotic lines and very small necrotic lesions). For the experiments of electron microscopic autoradiography, samples were taken from a narrow zone around the necrotic lesions. These were carefully avoided because they might have hampered the absorption of the tracer.

Measurement of PAL activity

The analysis of PAL activity was made on acetone powder preparations, using 20 g of leaf blade for each sample. Two grams of acetone powder were suspended in cold 0.1 *M* Tris-HCl buffer (pH 8.5) containing 5×10^{-3} *M* 2-mercaptoethanol. The mixture was occasionally stirred for 60 min; the extract was squeezed through cheese cloth and centrifuged at 20,000 *g* for 10 min. The supernatant was treated with ammonium sulphate up to 50% saturation to pre-

cipitate the soluble proteins. The precipitate was sedimented by centrifugation at 21,000 *g* for 10 – 15 min and the pellet was dissolved in Tris-HCl buffer. All these operations were carried out at 4 °C.

PAL activity was assayed in this partially purified preparation by measuring the amount of trans-cinnamic acid produced, as described by O'NEAL and KELLER (1970).

Measurement of PPO activity

PPO activity was measured in the same leaf tissues as used for the measurement of PAL activity. Samples made from 1 g of leaf blades were homogenized in a mortar with quartz sand, using 5 ml of Mc Ilvaine buffer (pH 7.0) containing 0.175 *M* disodium phosphate and 0.016 *M* citric acid. The homogenate was filtered through glass wool and centrifuged at 8000 *g* for 1 hour. The supernatant was dialyzed for 20 hours against the same buffer. All the operations were performed at 4 °C. PPO activity was measured in the dialyzed sap by the rapid spectrophotometric method described by VAN KAMMEN and BROUWER (1964).

Light microscopy

Large portions of the leaf blade were stained with basic fuchsin following the method of FUCHS (1963). This method is specific to lignin, which stains purple. Micrographs were taken with a Leitz Ortholux microscope.

Electron microscopic autoradiography

Strips of the leaf blade, 3 × 5 mm, were incubated for 30 and 60 min respectively on sterilized water containing 2000 μC/ml phenylalanine-^{3}H (L-3-phenylalanine-^{3}H [G], specific activity 1.0 Ci/mmole) at room temperature, and then washed in a medium containing unlabelled phenylalanine (250 μg/ml) for 60 min.

The strips were then fixed in phosphate buffered 3% glutaraldehyde, pH 6.9, for 2 hrs, washed in buffer overnight, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite. Ultrathin sections were mounted on gold grids, double stained with uranyl acetate and lead citrate, coated with a thin carbon layer, and then covered with Ilford L4 emulsion (CARO and VAN TUBERGEN, 1962). After a 3-week exposure in the dark at 4°C the autoradiographs were developed with Kodak Microdol X and examined in a Siemens Elmiskope 1A.

Results

PAL and PPO activities

The healthy leaves exhibited very low PAL and PPO activities. A significant, though slight, increase of these activities was observed in the infected leaves, as shown in Table 1.

Table 1

Phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) activities in healthy and CaMV-infected leaves. Means of 3 experiments ± s.e.m.

		Healthy leaves	Infected leaves	t	P
PAL[1]	m	4.20	5.80	11.299	<0.01
	± s.e.m.	0.114	0.077		
PPO[2]	m	0.44	0.95	3.850	<0.05
	± s.e.m.	0.001	0.126		

[1] PAL activity is expressed as μg of transcinnamic acid produced per gram of leaf tissue (fresh weight) per hour

[2] PPO activity $= \frac{\Delta A/sec}{mg\ dry\ weight/ml} \times 10^5$. The reaction mixture contained 0.6 ml chlorogenic acid at a concentration of 0.1 mg/ml, 0.8 ml enzyme extract, and 1.6 ml buffer

Light microscopy

In the leaf areas showing only mosaic and chlorotic or yellow spotlets no staining specific to lignin was found, and such areas appeared as optically empty zones (Fig. 1, arrows). On the contrary, the necrotic lesions appeared delimited by a stained ring (Fig. 2, arrows).

Electron microscopic autoradiography

After 30 min incubation, the label uptake was rather scanty, while after 60 min it reached a fairly high level. Both in the control and the virus-infected leaves some label was found in all the cell components but it was particularly concentrated in the tracheid walls, as expected (Fig. 3). On the contrary, the walls of the parenchyma cells were not labelled, not even at the level of the outgrowths nduced by the virus (Fig. 4, arrow).

Discussion

The low PAL and PPO activities found in healthy leaves of *B. chinensis* are probably due to the nature of the plant, since parallel assays carried out on healthy or TMV infected leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris* gave results comparable to those given by other authors (FARKAS *et al.*, 1960; FARKAS and SZIRMAI, 1969).

The slight, though significant increase of PAL activity found in CaMV infected samples can probably be related not only to necrogenesis, but also to the moderate lignification process taking place at the level of the tiny necrotic

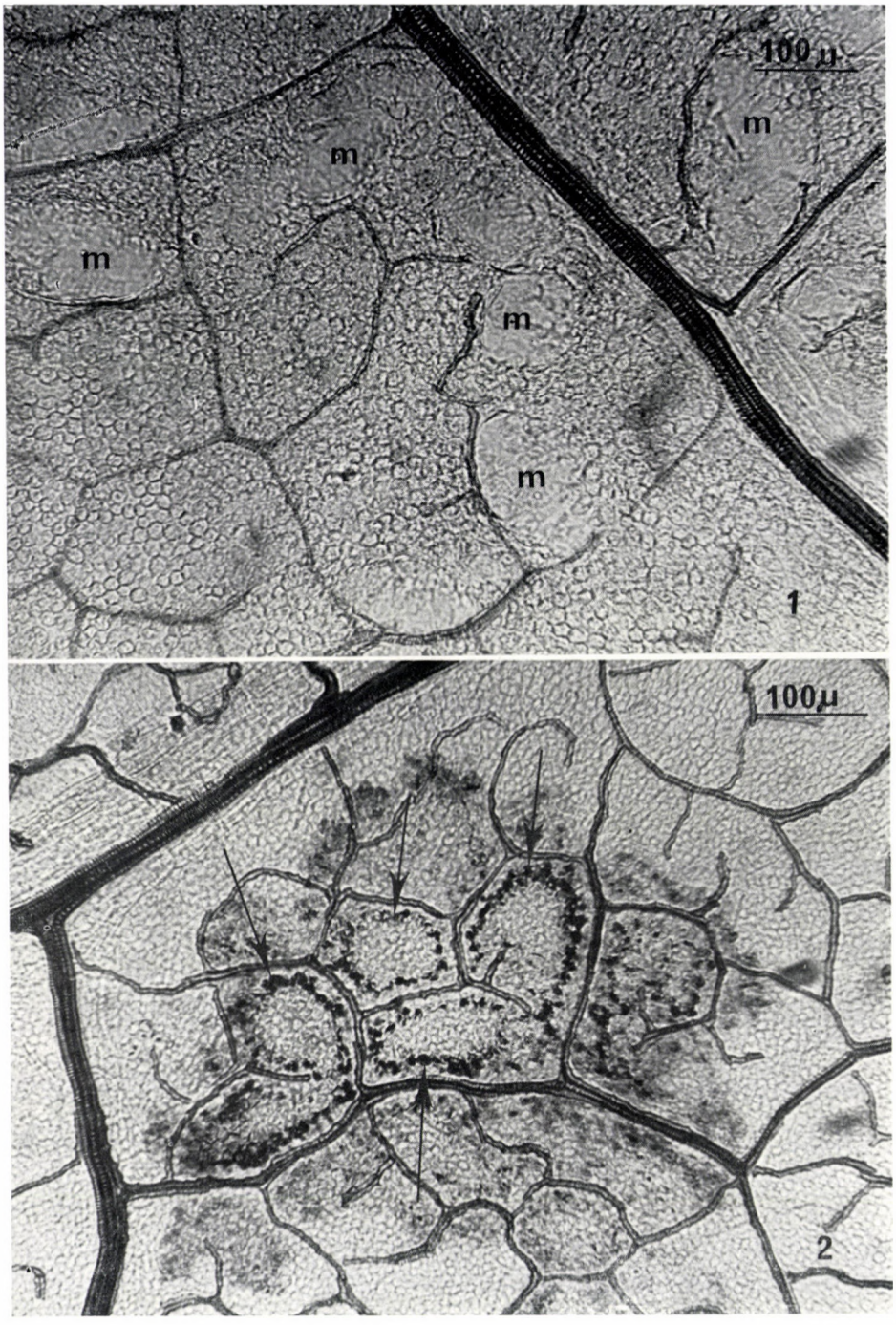

Fig. 1. Light micrograph of a CaMV-infected leaf after fuchsin staining. Area with mosaic symptoms. The lesions (m) appear as optically empty zones without a neatly defined outline
Fig. 2. Light micrograph of a CaMV-infected leaf after fuchsin staining. Area with necrotic lesions. The single lesions appear outlined by a dark ring (arrows) and a diffuse halo is seen in the adjacent necrotic region

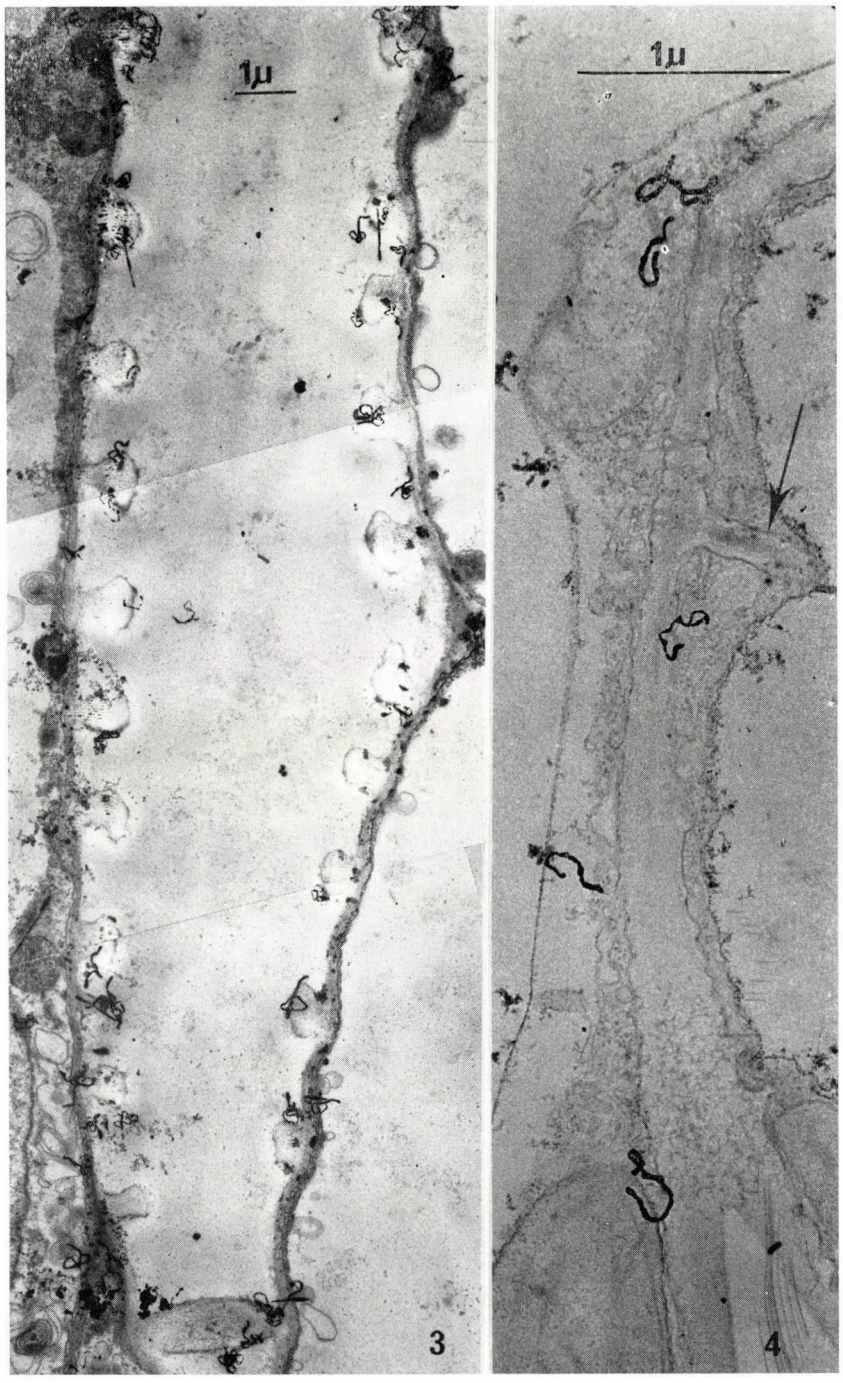

Fig. 3. Electron autoradiograph of a tracheid in a control leaf after phenylalanine-^{3}H administration. The silver grains are localized over the cell-wall thickenings

Fig. 4. Electron autoradiograph of two parenchyma cells in a CaMV-infected leaf. Area confining with a necrotic region. No silver grains are visible over the cell-walls. Also the cell-wall digitation (arrow) is unmarked

lesions induced by CaMV in systemically infected leaves, as shown by our light microscopic observations. In fact, as already pointed out, PAL catalyzes the biosynthesis of a wide variety of phenolic compounds and is therefore involved in lignin synthesis (NEISH, 1960; CONN, 1964; PRIDHAM, 1965). However, our autoradiographic experiments did not reveal any incorporation of phenylalanine-^{3}H in the walls of the living cells surrounding the necrotic lesions, not even at the level of their numerous outgrowths. This suggests that the deposition of lignin takes place only where the necrogenic process is going on, and it seems likely that the augmented PAL activity, as found by FRITIG *et al.* (1973) in the area surrounding the necrotic lesions, is to be related to its increased synthesis in this area.

If PAL is involved in necrogenesis, it can also be said to be involved in the development of local resistance to infection, since the necrotic process which takes place in hypersensitive hosts creates a barrier which blocks a further spreading of the virus and provides the plant with a localized mechanism of defense (SOLYMOSY *et al.*, 1959; FARKAS *et al.*, 1960; KOSUGE, 1969; LOEBENSTEIN, 1972). It might be postulated that also in systemically infected tissues a limited number of cells can develop a hypersensitive reaction.

Also PPO activity increases parallel to the development of necrotic lesions in virus-infected tissues (FARKAS *et al.*, 1960; SOLYMOSY and FARKAS, 1963; VAN KAMMEN and BROUWER, 1964; CABANNE *et al.*, 1971; SIMONS and ROSS, 1971 *a*; VAN LOON and GEELEN, 1971), and this can explain the faint increase of its activity found in CaMV infected leaves, where necrosis is scanty. But while the results obtained by some authors indicate that PPO plays an important role in the resistance phenomenon (FARKAS and KIRÁLY, 1962; PARISH *et al.*, 1965), other results do not support the idea that the increased PPO activity is responsible for necrosis and localization of infection (BOZARTH and ROSS, 1964; VAN KAMMEN and BROUWER, 1964; JOCKUSH, 1966; CABANNE *et al.*, 1968 and 1971; SIMONS and ROSS, 1971 *a*; VAN LOON and GEELEN, 1971). The fact that we have found an increased PPO activity in tissues infected long before and showing stabilized symptoms seems to confirm the view that the enhanced PPO activity is a consequence and not a cause of the death of the cells.

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Effect of Systemic Infection by TMV on Cytokinin Level of Tobacco Leaves and Stems

By

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Cytokinin extracts from *Nicotiana tabacum* L. cv. Samsun were prepared one month after systemic infection with tobacco mosaic virus U 1 strain. Cytokinin activity of the extracts from leaves and stems was determined on the basis of soybean callus bioassay.

The same active compounds, chromatographically similar to zeatin and zeatin riboside, are present in both healthy and diseased leaves and stems. However the cytokinins in infected plants exhibited higher cell division activity in the tissue culture bioassay. The virus-infected leaves contained two additional components with cytokinin activity which were not present in traceable quantities in the extracts from healthy leaves. It is believed that the increase in cytokinins may result in stabilizing the host–parasite relationship, and in helping the structures carrying out the virus biosynthesis.

Virus multiplication proceeds by directing the host cells to manufacture virus nucleic acid and virus protein. The stimulation of RNA and protein synthesis is one of the known biochemical effects of cytokinins (BURDETT and WAREING, 1966; ZWAR, 1973; MOTHES *et al.*, 1961). The effect of cytokinins on plant-virus multiplication has been studied (DAFT, 1963; KIRÁLY and SZIRMAI, 1964; SELMAN, 1964; KIRÁLY *et al.*, 1968). However, until recently, the changes of cytokinin level in virus infected plants have not been investigated. There are several phenomena caused by virus infections which can be associated with increases in cytokinins. For instance, increased RNA content, tumor production and accelerated metabolic processes suggest higher cytokinin activity in some virus-host systems. In the present study the cytokinin level was examined in leaves and stems of systemically infected tobacco plants.

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Materials and Methods

Plant material

Tobacco plants (*Nicotiana tabacum* L. cv. Samsun) grown under normal greenhouse conditions were inoculated with tobacco mosaic virus (TMV U1 strain) in the 6–8 leaf stage. The stems and leaves of both control and infected plants were harvested one month after inoculation.

Cytokinin extraction and determination

Thirty grams of leaves and stems, respectively, were homogenized with 60 ml phosphate buffer pH 6.5 and extracted with 200 ml of 96% ethanol for 12 h at 4 °C. The extracts were filtered and centrifuged at 6000 *g* for 15 minutes (Janetzki K 23), and then the supernatants were concentrated to the aqueous phase under vacuum at 35 °C. Samples were centrifuged at 6000 *g* for 30 minutes. The supernatants were adjusted to pH 9.0 with 1 *N* NaOH and extracted with three equal volumes of petroleum ether (b.p. 40–70 °C) in separatory funnels. After removing the petroleum ether, the aqueous phases were adjusted to pH 2.5 with 1 *N* HCl and shaken with 3 equal volumes of ethyl acetate. The aqueous fractions were concentrated to 25 ml, and then passed through columns containing Dowex 50 W–X 8 H^+ (100–200 mesh) cation exchange resin. The columns were washed with bidistilled water, and then the active materials were eluted with 4 *N* NH_4OH. After removing the ammonia, the water residue was adjusted to pH 7.8 and shaken with 3 equal volumes of water-saturated *n*-butanol, and the active materials went into the *n*-butanol phase. The *n*-butanol fractions were dried under vacuum and washed five times with bidistilled water and evaporated. The residues were dissolved in 96% ethanol, and were applied as a streak on Whatman No. 1 chromatography paper. The chromatograms were developed in a solvent: *t*-butanol–ammonia–water (3 : 1 : 1; v/v). The migration of active compounds in stem and leaf extracts was compared with that of known cytokinins. The standards, zeatin and zeatin riboside, were detected at the R_F range given as ultraviolet absorbing bands.

After drying, the chromatograms of extracts from stems and leaves were divided into 5 and 10 equal strips, respectively. Cytokinin activity was tested on the basis of soybean callus bioassay.

Twenty ml of tissue culture basal medium, supplemented with portions of chromatograms corresponding to specific R_F regions, was added to each 50 ml Erlenmeyer flask. The pH was adjusted to 5.8 with 1 *N* NaOH, and the medium was autoclaved at 0.8 atm. for 45 minutes. Three and five pieces, respectively, of soybean callus tissue were placed in each flask. Cultures were maintained at 27 °C for 28 days and then weighed.

For extraction and purification of cytokinins we used modified procedures of KIRÁLY *et al.*, MILLER and van STADEN *et al.* (KIRÁLY *et al.*, 1967; MILLER,

1967; van STADEN *et al.*, 1972). The determination of cytokinin activity was carried out according to the method of MILLER (1965). The experiments were repeated with similar results in five cases. The results of one representative experiment are shown in Table 1 and Fig. 1.

Table 1

Growth of soybean callus on mediums containing extracts from stems of healthy and infected plants

	Callus yield (mg/flask)				
R_F	0.0–0.2	0.2–0.4	0.4–0.6	0.6–0.8	0.8–1.0
Healthy	145	123	136	115	338
Infected	135	140	127	171	459

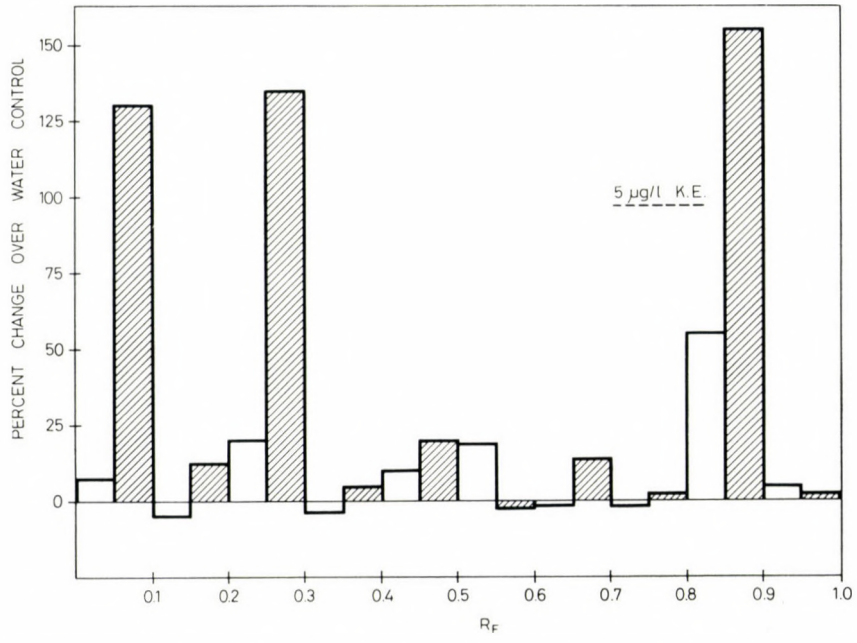

Fig. 1. The paper chromatographic separation of cytokinin-extracts from healthy (□) and infected (▨) leaves. The chromatograms were developed in *tercier*-butanol–ammonia–water (3 : 1 : 1; v/v/v). The extracts were obtained from 50 g fresh weight of leaves 1000/1000 ml medium. (K. E. = Kinetin equivalent)

Results and Discussion

Considering the growth inhibition of tobacco plants induced by virus infection, it seemed appropriate to compare the cytokinin levels of virus-infected and healthy stems. Cytokinin activities of extracts prepared from identical fresh weights of stem of healthy and infected plants were determined on the basis of growth of soybean callus (Table 1.). Neither extract had great activity at the low R_F regions. Most of the cytokinin activity was found at the R_F 0.8 – 1.0 from both the healthy and infected stem extracts. The extracts from infected stems exhibited higher activity. The standards, namely zeatin and zeatin riboside were detected at R_F 0.81 – 0.86 and at R_F 0.89 – 0.95 respectively.

As illustrated in Figure 1, the peak of cell division activity of extracts from healthy leaves was at R_F 0.8 – 0.9. At this R_F region, cytokinin activity also was found in the extracts of virus-infected leaves. However, it was higher than that of the extracts from healthy leaves. Simultaneously, two additional peaks of growth-promoting activity were present in the extracts of infected leaves, at R_F 0.0 – 0.1 and R_F 0.2 – 0.3 respectively. These peaks could not be found in the cytokinin extracts from healthy leaves. The results obtained show that the total cell division activity in extracts from virus-infected plants was much greater than that in extracts prepared from healthy plants.

There are numerous references of increased cytokinin level in diseased plants infected by different plant pathogens (KIRÁLY *et al.*, 1967; THIMANN and SACHS, 1966; DEKHUIJZEN and OVEREEM, 1971; SZIRÁKI *et al.*, 1974). The increase in cytokinins has been suggested to account for the altered transport and accumulation of materials, the deposition of starch, and the formation of green islands around infection sites of numerous obligate parasites (cf., DEKHUIJZEN and STAPLES, 1968).

Plant viruses, which are obligate parasites, also cause increased metabolic activity in systemically infected plants. DOKE and HIRAI studied the radioautograms of the leaves of systemically TMV-infected hosts which were exposed to $^{14}CO_2$ in light. They found lesions having localized and enhanced radioactivity. The virus multiplication and $^{14}CO_2$ fixation were more intense in the leaf areas of high radioactivity than in leaf areas of low radioactivity (DOKE and HIRAI, 1970).

Our data suggest that the increase in cytokinins may serve to enhance protein and RNA synthesis, as well as other metabolic processes of systemically infected tobacco plants. The increase in the cytoplasmic mass and increased numbers of mitochondria, ribosomes and membranes of the cells of infection sites may be associated with enhanced cytokinin activity. A further investigation is needed to determine the origin and mechanism of increased cytokinin levels in virus-infected tissues.

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Uptake of ^{14}C-TMV by Tobacco Leaves. Effect of Poly-L-Ornithine and Metabolic Inhibitors

By

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The uptake of ^{14}C—TMV by tobacco leaves was studied. In accordance with the literature, poly-L-ornithine was shown to stimulate TMV adsorption and cause lower infectivity of the preparation. This was oberved in experiments concerning systemic and local lesion host plants. The stimulation of TMV adsorption depends on virus–polycation relationship in the inoculum; it also depends on how poly-L-ornithine is used, i.e. whether it is incubated with the virus prior to inoculation, or prespread onto the leaves, which are then washed off to be inoculated with a virus suspension.

Decreased infectivity of the virus treated with poly-L-ornithine is observed irrespective of whether stimulation of uptake occurs or not. Apparently, two poly-L-ornithine effects are to be distinguished: action on virus and on leaf cells.

Inhibitors of energy metabolism — sodium azide (0.65 mg/ml), sodium fluoride (0.84 mg/ml) and 2.4-dinitrophenol (0.02 mg/ml) — cause a decrease in virus retention by leaves; however, inhibitors of protein sythesis — puromycin (0.1 mg/ml), cycloheximide (0.01 mg/ml) and chloramphenical (0.1 mg/ml) — do not reduce retention. The last two substances cause strong stimulation of TMV retention at high virus concentrations in the inoculum.

Treatment of leaves with poly-L-ornithine strongly reduces dependence on the action of the inhibitors.

Intensive research by COCKING (1966, 1970) and TAKEBE (OTSUKI *et al.*, 1972) gave rise to new concepts on the pinocytotic mechanism of the uptake of viral particles by protoplasts. The results of electronmicroscopic observations by the above authors convincingly show that pinocytotic vesicles, containing viral particles, are formed in the cytoplasm of inoculated protoplasts, processes similar to those involved in the uncoating of viral RNA take place. However, rapid deproteinization of TMV in the vesicles indicates to high hydrolytic activity in these formations, and this may impede realization of the genetic information of virus RNA. This circumstance, as well as the fact that increased pinocytotic uptake of TMV is not accompanied by a rise in the number of affected protoplasts (HIBI and YORA, 1972) have both led to the assumption that protoplast infection is caused by a small number of TMV particles penetrating the protoplast while bypassing the pinocytotic vesicles (BURGESS *et al.*, 1973).

To elucidate the role of pinocytosis in the infection, further research is essential. However, even if the virus really penetrates the protoplasts as stated above, the process, nevertheless, may not take part in the virus uptake by plant

leaf cells. In other words, viruses possibly penetrate into protoplasts and leaf cells in different ways. This possibility may be inferred from the works of SHAW (1972), who showed tobacco leaves to possess very low sensitivity to the action of poly-L-ornithine. In these experiments, the poly-L-ornithine concentration essential to stimulate uptake was 1000 times higher than in the experiments of TAKEBE and OTSUKI (1969).

This discrepancy could have partly resulted from procedural differences, since TAKEBE and OTSUKI preincubated the virus with poly-L-ornithine to use the mixture for subsequent inoculation, while SHAW first treated the leaves with polycation and then inoculated them with a TMV suspension. Such different uses of poly-L-ornithine may have basic significance in explaining its effect, since preincubation possibly causes in the virus charge and degree of aggregation, whereas pretreatment of leaves with poly-L-ornithine changes the state of the cell membranes.

We have made an attempt to establish whether the degree of leaf sensitivity to poly-L-ornithine is really lower than that of protoplasts. At the same time, we tried to ascertain the significance of the use of poly-L-ornithine for virus penetration and infectivity in leaves. The present paper gives a detailed description of the effect of the polycation on ^{14}C-TMV absorption with different inculation procedures and shows the dependence of the process on certain metabolic inhibitors.

Materials and Methods

Plants. Plants of *Nicotiana tabacum* L. cultivar Samsun, and *Nicotiana glutinosa* (both 5–6 weeks old) grown in the greenhouse were inoculated with labelled TMV.

Labelled virus. ^{14}C-TMV, specific radioactivity 5–100 Ci/mg, was prepared by a technique used previously (ZHURAVLEV, MINSKAYA and SHUMILOVA, 1974).

Method of using poly-L-ornithine. Two methods were applied in these experiments.

The first method of TAKEBE and OTSUKI (1969) involves preincubation of the virus and the polycation in 0.1 *M* phosphate buffer, pH 7.0, for 10 min and subsequent inoculation of leaves (0.1 ml/leaf). Leaves then were washed with tapwater.

In the second method (SHAW, 1972), the leaves were first rubbed with poly-L-ornithine by the aid of a glass spatula, then quickly washed with water and instantly inoculated with the virus in a phosphate buffer. The inoculum was washed off after 10 min.

Assay of TMV infectivity. In experiments with local lesion hosts, virus infectivity was assayed by the number of local lesions formed on inoculated leaves. In tests with *N. tabacum* a systemic host, discs were punched out from inoculated leaves at definite time intervals, homogenized in an 0.1 *M* phosphate buffer, and then the homogenate was centrifuged at 8000 *g* in the cold. The suitably diluted

supernatants were used for inoculating leaves of *N. glutinosa*. Each sample was applied onto at least twenty half leaves.

Determination of TMV retention. A cork borer was used to punch out discs (twelve pieces in each sample) from leaves inoculated with the labelled virus. The discs were then homogenized in an 0,05 *M* phosphate buffer, pH 7.0, and centrifuged at 8000 *g* for 15 min. The supernatants were dried on aluminium planchets to determine radiactivity by the aid of a thin-end-window tube T-25-BFL.

Effect of inhibitors. The discs from tobacco leaves were kept for 4 h in inhibitor solutions, then quickly blotted dry and inoculated with labelled TMV. The inoculum was washed off with tap-water prior to homogenizing the discs; the homogenate was centrifuged as described above to subsequently determine the radioactivity of the supernatant.

Results

TMV uptake with different inoculation procedures

We repeated SHAW's experiments and obtained similar results. Pretreatment of leaves with poly-L-ornithine stimulated TMV attachment at polycation concentrations higher than 1mg/ml (Table 1). Under the conditions when polycation was applied as in the experiments of TAKEBE *et al.* (TAKEBE and OTSUKI, 1969; TAKEBE *et al.*, 1971), the stimulating effect of poly-L-ornithine on TMV attachment by *N. glutinosa* leaves was also observed (Table 1). Increased virus- or poly-

Table 1

Effect of pretreatment of *N. glutinosa* leaves with poly-L-ornithine on ^{14}C-TMV attachment and appearance of local lesions

Poly-L-ornithine mg/ml	^{14}C-TMV attachment, cps 10^{-2}	Number of lesions per leaf
0	28	25
1	101	21
5	119	16
7.5	110	19

L-ornithine concentrations led to greater retention. Poly-L-ornithine in concentrations above 100 μg/ml caused a decrease in retention. At concentration from 0.01 to 0.1 mg/ml, a stimulation of TMV retention was observed in the inoculum, irrespective of virus concentration.

An approximately continuous increase in virus retention takes place with 0.2 to 2.0 mg/ml TMV content in the inoculum.

Infectivity of retained TMV

SHAW (1972) reported, without any numerical data, that an increase in TMV attachment after treatment of leaves with poly-L-ornithine is not accompanied by an increased number of local lesions on the inoculated leaves of local lesion host.

The same results were obtained by us in experiments with *N. glutinosa* (Table 1). Decreased infectivity was also observed after inoculating the leaves of *N. tabacum*, a systemic host (Table 2), with poly-L-ornithine though, in this case, increased TMV attachment was likewise noted.

Table 2

Infectivity of juice of tobacco leaf inoculated with TMV after treatment with poly-L-ornithine (number of local lesions per half leaf)

Leaf treatment	Days after inoculation		
	1	2	3
Poly-L-ornithine	13	65	89
Water	20	96	113

Table 3

Attachment of ^{14}C-TMV to and appearance of local lesions mixture of ^{14}C-TMV and poly-L-ornithine

Host plant	TMV concentration, μg/ml	Poly-L-ornithine, μg/ml	^{14}C-TMV attachment, cps 10^{-2}	Number of leasions per half leaf
Nicotiana glutinosa	1	0	47	3.7
	1	1	74	1.0
	1	10	120	1.6
	1	100	96	1.3
	1	1000	91	0.4
	3	0	55	18.0
	3	1	61	5.0
	3	10	72	3.7
	3	100	7	5.5
	3	1000	52	4.5
	20	0	—	72.0
	20	10	—	23.0
	20	100	—	10.0
	20	1000	—	8.0
	200	0	—	107.0
	200	10	—	48.0
	200	100	—	46.0
	200	1000	—	59.0
Nicotiana tabacum	20	0	76	—
	20	10	122	—
	20	100	92	—
	20	1000	65	—
	220	0	52	—
	220	10	111	—
	220	100	68	—
	220	1000	58	—

In experiments with *N. glutinosa* TMV infectivity also decreased when the leaves were inoculated by the procedure used by TAKEBE (with a mixture of the virus and poly-L-ornithine). An intensive virus retention by the leaves was observed with poly-L-ornithine concentrations 10 to 100 μg/ml, whereas maximum infectivity was noted in the absence of poly-L-ornithine (Table 3). Infectivity is sometimes very strongly suppressed at high poly-L-ornithine and low TMV concentrations.

Effects of inhibitors

The effect of six metabolic inhibitors was studied. Only three of them, namely sodium azide, sodium fluoride and 2,4-dinitrophenol, obviously inhibit TMV attachment. The inhibiting effect is more pronounced at high virus concentrations in the inoculum (Table 4). Puromycin does not have any remarkable effect on virus retention, whereas chloramphenicol and cycloheximide cause intensive stimutation of retention at high virus concentrations in the inoculum.

Table 4

Effects of inhibitors on ^{14}C-TMV retention by discs from *N. tabacum* leaves

Version	Inhibitor concentration mg/ml	^{14}C-TMV retention, μg/sample				
		TMV concentrations in inoculum, μg/ml				
		20	120	270	520	1020
Control	—	1.00	4.50	11.00	25.00	57.00
Sodium fluoride	0.84	1.00	2.40	8.00	16.00	39.00
Control	—	0.20	1.00	2.40	3.80	10.00
Sodium azide	0.65	0.10	0.40	0.70	1.30	2.50
Control	—	0.15	0.75	0.60	1.90	5.00
2,4-dinitrophenol	0.092	0.10	0,50	1.20	2.60	6.00
Control	—	0.10	0.40	1.00	1.00	4.00
2,4-dinitrophenol	0.92	0.05	0.15	0.30	0.75	2.30
Control	—	0.40	2.10	4.90	9.00	18.50
Cycloheximide	0.002	0.40	2.00	4.80	9.00	35.00
Control	—	0.20	1.00	2.40	4.60	10.00
Cycloheximide	0.01	1.00	2.90	2.90	6.50	17.50
Control	—	0.10	0.40	1.20	1.80	3.50
Puromycin	0.10	0.10	0.50	0.75	1.60	3.25
Control	—	0.20	0.50	1.50	3.00	6.50
Chloramphenicol	0.10	0.40	1.80	0.40	3.30	8.50

The above-mentioned effect of the inhibitors on TMV uptake is also observed on leaves of *N. glutinosa* (Table 5). (In these experiments, the inoculum concentration was 200 μg TMV/ml).

Table 5

Effects of inhibitors on ^{14}C-TMV retention by discs from *N. glutinosa* leaves

Inhibitor	Inhibitor concentration, mg/ml	^{14}C-TMV retention, cps $.10^{-2}$
Control	—	54
Sodium fluoride	0.84	55
Cycloheximide	0.01	57
Chloramphenicol	0.10	56
Sodium azide	0.65	29
2,4-Dinitrophenol	0.92	27

The ability of inhibitors of energy metabolism to arrest TMV uptake by tobacco leaves sharply decreases after treatment with poly-L-ornithine. 2,4-Dinitrophenol (0.9 mg/ml) TMV decreases attachment in leaves by 36%. However, after a previous treatment of leaves with poly-L-ornithine, 2,4-dinitrophenol decreased the attachment by only 6% (Table 6).

Table 6

Effect of 2,4-dinitrophenol on virus retention by discs from *N. tabacum* leaves depending on pretreatment with poly-L-ornithine

Versions		^{14}C-TMV retention, cps $.10^{-2}$	Inhibition, %
2,4-Dinitrophenol, mg/ml	Poly-L-ornithine, mg/ml		
—	—	66	—
—	1	127	—
0.92	—	42	36
0.92	1	119	6

Discussion

Besides poly-L-ornithine, other polycations can also lower TMV infectivity (STAHMANN *et al.*, 1951; STAHMANN and GOTHOSKAR, 1958). One of the possible causes for decrease in infectivity could be the formation of virus–polycation complexes; another reason could be virus adsorption on physiologically inactive structure of leaves treated with poly-L-ornithine. One can not exclude the possibility of a direct effect of polycations on TMV structure.

Our data confirm the assumption that, at least in the case of poly-L-ornithine, the polycation affects either the virus itself or the leaf cells. The effect on the virus causes decrease in infectivity, though, under certain conditions, one can observe

increased virus uptake. This is apparent at poly-L-ornithine concentration from 10 to 1000 μg/ml, whereas direct treatment of leaves with poly-L-ornithine proves to be effective only at concentrations above 1000 μg/ml. Hence, the second reason for decreased TMV infectivity (virus adsorption on physiologically inactive structures) appears to be less probable, since decrease in infectivity begins with poly-L-ornithine concentrations lower than those at which stimulation of virus attachment begins.

Although in our experiments (in accordance with TAKEBE's scheme) the stimulating effect of poly-L-ornithine starts to display itself at lower concentrations than in the experiments performed by SHAW's scheme, those concentrations are, nevertheless, one order higher than in the experiments on protoplasts. Hence, the assumption that the protoplast plasmalemma and the cell plasmalemma in the leaf possess different sensitivities to poly-L-ornithine, still remains valid.

Virus absorption of the leaf is apparently a physiological process, since it is inhibited by substances arresting cell energy metabolism and is stimulated by antimetabolites of protein synthesis. Virus absorption is evidently not equal to attachment, which latter is stimulated by poly-L-ornithine, since after the polycation treatment of leaves the sensitivity of virus retention to inhibitors sharply decreases. On the basis of this fact it is hard to assume that the virus penetrates the leaf in the absence of poly-L-ornithine by pinocytosis. This latter process does not depend on metabolic inhibitors, as was shown for several subjects of animal origin (RYSER, 1968). Virus retention also hardly depends on these inhibitors in the case of protoplasts if the latter are inoculated by a mixture of TMV and poly-L-ornithine (manuscript to be published). One can assume that only leaf treatment with poly-L-ornithine stimulates pinocytotic uptake, whereas with the conventional mechanical inoculation (without poly-L-ornithine) virus penetration proceeds along different pathways. One of these, could be TMV penetration into the plant conductive elements.

When the leaf is subjected to mechanical inoculation, the virus chiefly contacts with epidermal cells, whereas protoplasts originate from mesophyll cells. This circumstance alone may be the reason for different sensitivities of these systems to poly-L-ornithine. The effect of metabolic inhibitors on TMV uptake by leaves in the absence of poly-L-ornithine can be considered as indicative of the process being closer to phagocytosis, or to one kind of pinocytosis related to formation of rather large vesicles. Such processes are known to be sensitive to the effect of inhibitors in animal systems (COHN and PARKS, 1967; CHAMPTAN-ANDERSEN, 1967). It would seem that this conclusion is in accordance with the fact that the epidermis, among other functions, also fulfils a protective function, which may involve elements of phagocytesis. However, more detailed studies are necessary for final conclusions.

The increase of TMV absorption under the effect of the inhibitors of protein synthesis is also an interesting phenomenon. Presumably, stimulation takes place because the inhibitors impede the synthesis of certains proteins that regulate membrane transport. However, the inhibitor concentration that we used are high

enough to suppress all sensitive syntheses. Similar concentrations of cycloheximide in the experiments of TAKEBE *et al.* (1968) strongly suppressed TMV reproduction in cells isolated from tobacco leaves.

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Isolierung des Kirschenblattroll-Virus (*cherry leaf roll virus*) aus *Sambucus nigra* L. in Ungarn

Von

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In Hungary the same virus could be isolated from elder (*Sambucus nigra* L.) showing on the leaves either yellow net or chlorotic ringspot. On test plants the virus induced typical symptoms of a *NEPO* virus and the properties *in vitro* supported this diagnosis. By serological tests the virus was identified as belonging to the red elder ringspot serotype of cherry leaf roll virus. This is the first proof of that virus on *Sambucus* in Hungary. *Ammi visnaga* (L.) Lam., *Aptenia cordifolia* (L.) Schwantes, *Browallia americana* L., *B. roezli* Nichols, *Commelina communis* L., *C. graminifolia* H. B. K., *C. tuberosa* L., *Erodium cicutarium* (L.) L'Herit. ex Ait., *E. malacoides* Willd., *E. moschatum* (L.) L'Herit ex Ait., and *Obione sibirica* (L.) Fisch. are new detected experimental hosts of cherry leaf roll virus.

Einleitung

Virusverdächtige Erkrankungen und Virusinfektionen wurden an verschiedenen *Sambucus*-Arten in Nordamerika und Europa festgestellt. Vor etwa fünfzig Jahren stellte MARTIN (1925) in Amerika eine Mosaikkrankheit an Traubenholunder (*Sambucus racemosa* L.) fest, und fünf Jahre später wurde in Europa eine Ringfleckenkrankheit am Schwarzen Holunder (*S. nigra* L.) beobachtet (BLATTNÝ, 1930). In beiden Fällen erfolgte keine hinreichende Charakterisierung der Viren, die möglicherweise die Erkrankungen auslösten. Unseres Wissens wurden seit Anfang der fünfziger Jahre folgende elf Viren von *Sambucus*-Arten isoliert: Tabakringflecken-Virus (*tobacco ringspot virus* — WILKINSON, 1952; UYEMOTO und Mitarb., 1971), Gurkenmosaik-Virus (*cucumber mosaic virus* — SMITH, 1952—54; UYEMOTO und Mitarb., 1971), Arabismosaik-Virus (*arabis mosaic virus* — CADMAN, 1960; HARRISON und WINSLOW, 1971), Latentes Erdbeerringflecken-Virus (*strawberry latent ringspot virus* — LISTER, 1964), Kirschenblattroll-Virus (*cherry leaf roll virus* — SCHMELZER, 1965, 1966; ŠTEFANAC, 1969; MAMULA und MILIČIĆ, 1974), Tomatenschwarzring-Virus (*tomato black ring virus* — SCHMELZER, 1965, 1966), Tabakmosaik-Virus (*tobacco mosaic virus* — MUELLER, 1967), Tomatenringflecken-Virus (*tomato ringspot virus* — UYEMOTO, 1970; JONES, 1972), Tabaknekrose-Virus (*tobacco necrosis virus* — UYEMOTO und Mitarb., 1971), Latentes Holunder-Virus (*elderberry latent virus* — JONES,

1972) und ein noch nicht näher charakterisiertes Virus mit fadenförmigen Partikeln von etwa 650 nm Länge, das in Großbritannien den vorläufigen Namen Holunder-Virus A (*elderberry virus A*) erhielt (UYEMOTO und Mitarb., 1971; JONES, 1972). Virusverdächtige Krankheiten des Holunders ohne exakte Ermittlung der Ursachen wurden in weiteren Arbeiten erwähnt (MALLACH, 1957; BRČÁK, 1962; KRISTENSEN, 1964; HÄRDTL, 1967). In Nordamerika soll außerdem die von mykoplasmaähnlichen Organismen bewirkte PIERCEsche Krankheit symptomlos an *Sambucus* auftreten können (FREITAG, 1951).

In Ungarn haben zuerst SCHMELZER und Mitarb. (1969) virusverdächtige Symptome an *Sambucus nigra* beobachtet, jedoch keine erfolgreichen Virusisolierungsversuche vorgenommen. Auf Grund der Häufigkeit von Virusinfektionen an *Sambucus nigra* in vielen Ländern erschien es wünschenswert, auch in Ungarn entsprechende weitere Untersuchungen durchzuführen. Die vorliegenden Ergebnisse stellen jedoch nur einen Anfang dar.

Fundort, Symptome und Isolierungsversuche

Objekt unserer Untersuchungen waren zwei im Stadtpark von Keszthely wachsende Sträucher von *Sambucus nigra*. Der eine davon zeigte ein typisches Gelbnetz (*YN*) an den Blättern, der andere ein chlorotisches Ringmuster (*CR*, Abb. 1, A und B). In den Sommermonaten 1972 und 1973 erfolgten mit ihrem Blattmaterial mechanische Beimpfungen auf Testpflanzensortimente, die aus *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Nicotiana megalosiphon*, *N. tabacum* und anderen Arten bestanden. Von beiden Sträuchern gelang es mehrfach, Virusisolierungen zu erhalten, die auf das Vorliegen von *NEPO*-Viren hindeutende Krankheitserscheinungen an den genannten Testpflanzen verursachten.

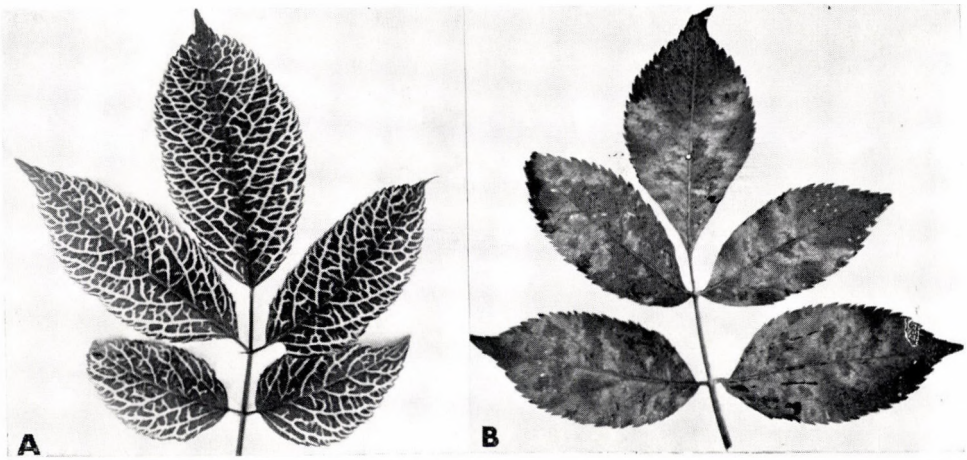

Abb. 1. Symptome der vom Kirschenblattroll-Virus infizierten Pflanzen von *Sambucus nigra*. A: Gelbnetz, B: chlorotische Ringe

Tabelle 1

Wirtspflanzen und Symptome des Isolats aus gelbnetzkranker *Sambucus nigra* L.

Wirtspflanzen	Symptome (I = Symptome der abgeriebenen Blätter, II = Folgesymptome)
AIZOACEAE	
Aptenia cordifolia (L.) Schwantes	I: graue nekrotische, sich ausdehnende Flecke II: keine Symptome
Tetragonia tetragonoides (Pall.) O. Ktze.	I: chlorotische, später nekrotische Ringe II: gelegentlich Spitzennekrose
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	I: chlorotische Flecke und Ringe (Abb. 2A) II: chlorotische und nekrotische Flecke, später Nekrose der Triebspitze; gelegentlich Erholung (Abb. 2B)
C. murale L.	I: nekrotische Flecke und Ringe, Blattabfall II: Nekrose, Blattdeformation, oftmals Absterben der Triebspitze
C. quinoa Willd.	I: chlorotische, später nekrotische Flecke, Blattabfall II: Nekrose, oftmals Absterben der Triebspitze
Obione sibirica (L.) Fisch.	I: chlorotische Flecke, Blattabfall II: chlorotische Flecke, Blattverkleinerung, Wachstumhemmung
Spinacia oleracea L.	I: schnelles Absterben II: starke Chlorose, Blattverkleinerung, später Absterben der Pflanzen
COMMELINACEAE	
Commelina communis L.	I: keine Symptome II: streifige gelbe Scheckung
C. graminifolia H.B.K.	I: keine Symptome II: streifige gelbe Scheckung
C. tuberosa L.	I: keine Symptome II: streifige gelbe Scheckung
COMPOSITAE	
Zinnia elegans Jacq.	I: keine Symptome II: chlorotische Scheckung, später Erholung
CUCURBITACEAE	
Cucumis sativus L.	I: chlorotische Flecke oder Ringe II: chlorotische Ringe und Eichenblattmuster, Erholung ist möglich (Abb. 2C)
GERANIACEAE	
Erodium cicutarium (L.) L'Herit. ex Ait.	I: keine Symptome II: sehr starkes Mosaik und Perlmuster
E. malacoides Willd.	I: keine Symptome II: chlorotische Flecke
E. moschatum (L.) L'Herit. ex Ait.	I: keine Symptome II: sehr starkes Mosaik und Perlmuster (Abb. 3A)

Wirtspflanzen	Symptome (I = Symptome der abgeriebenen Blätter, II = Folgesymptome)
LEGUMINOSAE	
Phaseolus vulgaris L. cv. 'Pinto' und 'Red Kidney'	I: braune nekrotische Flecke mit chlorotischem Hof; die Lokalläsionen treten manchmal nach dem Erscheinen der systemischen Symptome auf II: chlorotische Flecke oder Ringe, Blattdeformationen und später Nekrose der Triebspitze (Abb. 3B)
MALVACEAE	
Lavatera trimestris L.	I: chlorotische Flecke oder Ringe II: chlorotisches Eichenblattmuster an älteren Blättern
SOLANACEAE	
Browallia americana L.	I: keine Symptome II: graue nekrotische Flecke (Abb. 3E)
B. roezli Nichols	I: keine Symptome II: graue, oft ringförmige Flecke
Nicotiana megalosiphon Heurck. et Muell.	I: chlorotische bis nekrotische Flecke und Ringe II: Adernaufhellung, nekrotisches Muster, Erholung
N. tabacum L. cv. 'Bel 61—10', 'Samsun' und 'Xanthi-nc'	I: nekrotische oder chlorotische Ringe, später meist von Ringen umgehen (Abb. 3C und D) II: nekrotische oder chlorotische Ringe, Bogen- und Eichenblattmuster, Blattdeformationen, Erholung
UMBELLIFERAE	
Ammi majus L.	I: chlorotische Flecke II: chlorotisches bis gelbliches Muster, Erholung
A. visnaga L. (Lam.)	I: chlorotische Flecke II: chlorotisches bis gelbliches Muster, Erholung

Eine Reihe von Reaktionen ließen vermuten, daß beide Isolierungen weitgehend identisch waren, nur an *Chenopodium quinoa* bewirkte das Isolat aus dem gelbnetzkranken Strauch stärkere, an *Nicotiana tabacum*, Zuchtstamm 'Bel 61—10', dagegen schwächere Symptome als das Isolat aus dem ringfleckenkranken Strauch.

Wirtskreis und Eigenschaften in vitro

Zum weiteren Wirtspflanzenkreisuntersuchungen und zur Ermittlung der Eigenschaften *in vitro* wurde ausschließlich das Gelbnetz-Isolat (*YN*) verwendet. An 23 Pflanzenarten, die 10 Familien angehören, wurden die in Tabelle 1 verzeichneten Krankheitserscheinungen hervorgerufen. Sie sind zum Teil auf den Abb. 2 und 3 wiedergegeben. Die folgenden 19 Pflanzenarten blieben ohne erkennbare Reaktionen: *Amaranthaceae: Amaranthus caudatus* L., *Gomphrena globosa* L.; *Caryophyllaceae: Saponaria officinalis* L.; *Cucurbitaceae: Bryonia alba* L., *B. dioica* Jacq., *Citrullus lanatus* (Thunb.) Mansf., *Cucumis myriocarpus* Naud., *Cucurbita pepo* L.; *Leguminosae: Cassia tora* L., *Vigna sinensis* Savi ex

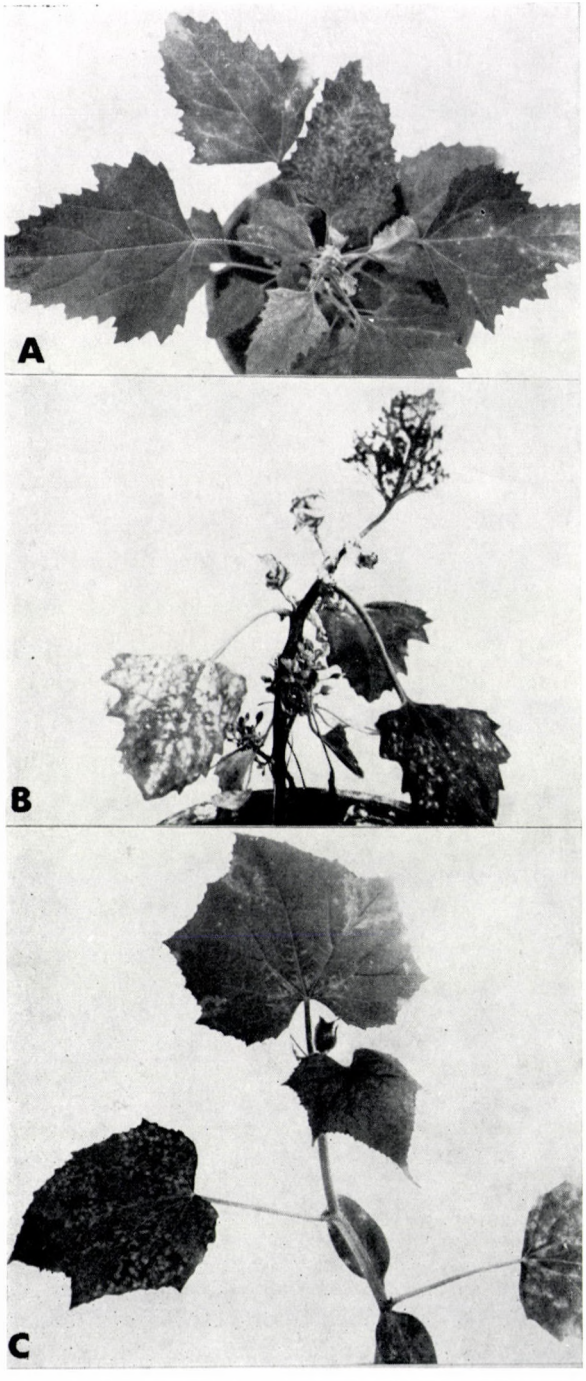

Abb. 2. Symptome des Kirschenblattroll-Virusstammes *YN* an Testpflanzen. A und B: *Chenopodium amaranticolor*, A: lokale, B: systemische Krankheitserscheinungen; C: *Cucumis sativus*, systemische Krankheitserscheinungen

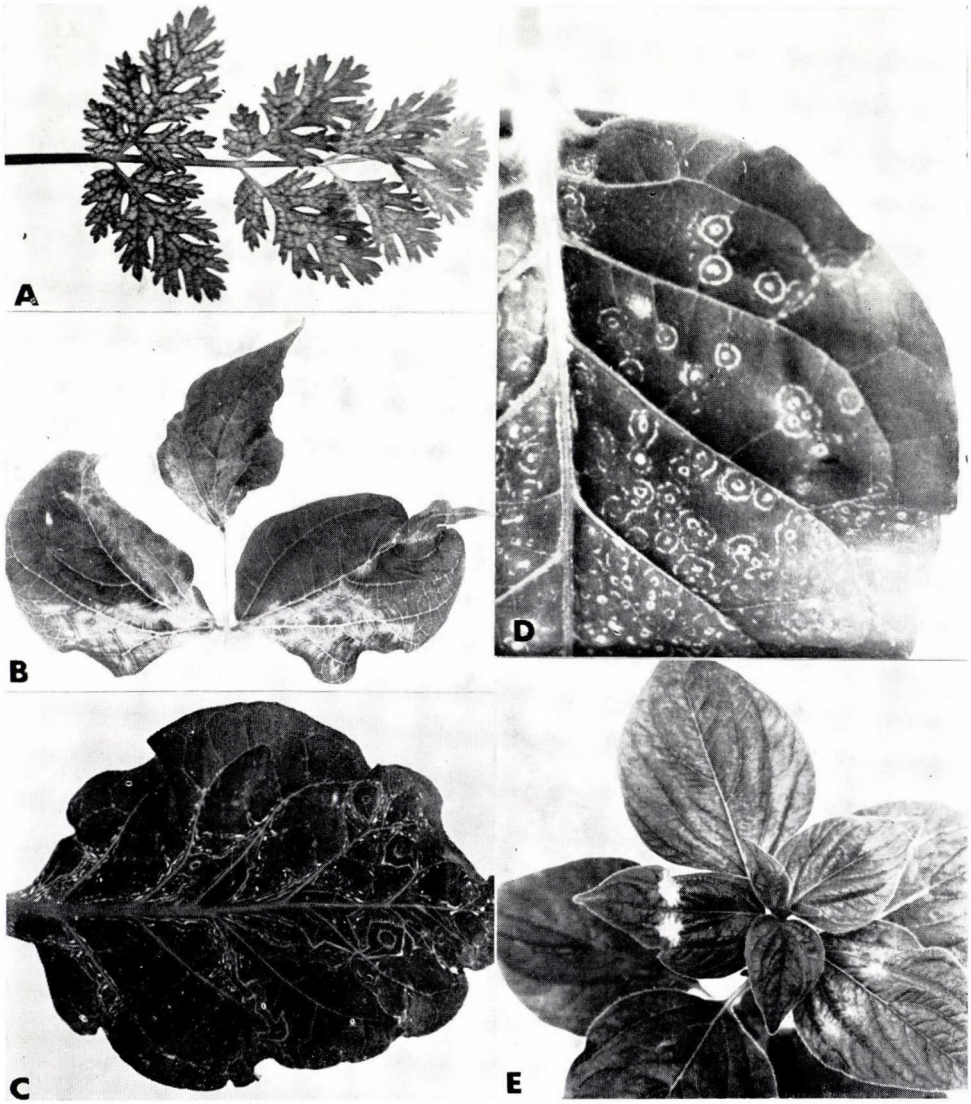

Abb. 3. Symptome des Kirschenblattroll-Virusstammes *YN* an Testpflanzen. A: *Erodium moschatum;* B: *Phaseolus vulgaris* cv. 'Red Kidney'; C—D: *Nicotiana tabacum,* C: cv. 'Xanthi-nc', D: cv. 'Samsun'; E: *Browallia demissa;* A, B, C und E: Folgesymptome, D: abgeriebenes Blatt

Hassk.; *Scrophulariaceae: Paulownia tomentosa* (Thunb.) Steud.; *Solanaceae: Capsicum annuum* L., *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *Petunia hybrida* hort. ex Vilm., *Petunia parviflora* Juss., *Solanum rostratum* Dun.; *Vitaceae: Vitis vinifera* L.

Für die Feststellung der Eigenschaften des Virus *in vitro* dienten mechanisch infizierte *Nicotiana tabacum* 'Xanthi-nc' als Virusquellen und junge *Chenopodium amaranticolor* als Testpflanzen. Weitere methodische Einzelheiten wurden bereits an anderer Stelle veröffentlicht (HORVÁTH, 1969). Die Ergebnisse verzeichnet Tabelle 2.

Tabelle 2

Eigenschaften *in vitro* des Isolates aus gelbnetzkranker *Sambucus nigra* L.*

Versuchsdatum	TIP	VEP	Biv	BTBT
23. 8. 1973	58	2×10^{-3}	6	über 90 Tage
12. 11. 1973	56	10^{-3}	9	über 170 Tage
4. 2. 1974	58	10^{-3}	9	über 230 Tage
4. 3. 1974	56	10^{-3}	9	über 300 Tage**

* Abkürzungen: TIP, thermaler Inaktivierungspunkt in °C; VEP, Verdünnungsendpunkt; B*iv*, Beständigkeit *in vitro* in Tagen; BTNT, Beständigkeit nach Trocknung bei niedrigen Temperaturen (+3 bis 5 °C) über Calciumchlorid

** Nur eine von zehn *Chenopodium amaranticolor* zeigte Symptome nach Infektion mit Blattmaterial, das 300 Tage bei niedrigen Temperaturen über Calciumchlorid getrocknet worden war

Serologische Untersuchungen

Alle Untersuchungen, sowohl am Isolat aus gelbnetzkrankem als auch aus ringfleckenkrankem Schwarzen Holunder, gaben keinen Hinweis auf ein etwaiges Vorliegen von Mischinfektionen. Weitere Aufschlüsse über die Reinheit der Isolate und ihre Identität wurden von serologischen Untersuchungen erwartet. Als Antigenquellen dienten 8–20 Tage zuvor infizierte *Chenopodium quinoa*. Die Antiseren hatten sich in zahlreichen früheren Untersuchungen bewährt. Methodisch wurde nach dem Agargel-Doppeldiffusionstest vorgegangen (SCHMELZER, 1970). Die Ergebnisse sind der Tabelle 3 zu entnehmen. Danach gehören beide Isolate zum Kirschenblattroll-Virus und zwar zum Traubenholunderringflecken-Serotyp (SCHMELZER, 1972b). Die ebenfalls am Schwarzen Holunder nachgewiesenen *NEPO*-Viren Arabismosaik-, Tomatenschwarzring- und Latentes Erdbeerringflecken-Virus hatten zu den untersuchten Isolaten keine Beziehungen.

Diskussion

Die Isolierung des Kirschenblattroll-Virus (KBRV) aus *Sambucus nigra* stellt einen Erstnachweis dieser Viruswirtkombination in Ungarn dar. Das KBRV wurde in diesem Land zuerst von wildwachsendem *Ligustrum vulgare* L. in einem Waldgebiet am Plattensee isoliert (SCHMELZER, 1972b). In Ungarn scheint ein Spontanbefall von Gehölzen durch das KBRV insbesondere in Wäldern vorzu-

kommen, da es inzwischen bei zahlreichen Sträuchern von *Sambucus nigra* in den Budaer Bergen zu ermitteln war.

Tabelle 3

Serologische Reaktionen der Isolate aus *Sambucus nigra* L.

Antiserum / Isolat	Arabismosaik-Virus "FII" (8000)	Tomatenschwarzring-Virus		Latentes Erdbeerringflecken-Virus "RoT 5" (4096)	Kirschenblattroll-Virus	
		Kartoffelbukett-Serotyp "RoU" (512)	Rübenringflecken-Serotyp "HoG" (1027)		Kirschenblattroll-Serotyp "Pt" (256)	Traubenholunderringflecken-Serotyp "BeRi" (256)
Gelbnetz (*YN*)	0	0	0	0	64	128
Ringflecken (*CR*)	0	0	0	0	32	128

Zeichenerklärung: Die Abkürzungen in Anführungsstrichen geben jeweils den Virusstamm an, gegen den das Antiserum hergestellt wurde. Die Zahlen in Klammern verzeichnen den reziproken Wert des Serumtiters mit dem homologen Virusstamm. Die übrigen Zahlen stellen die reziproken Werte der Serumtiter mit den geprüften Isolaten dar.

Unseres Wissens wurde das KBRV außer in *Sambucus nigra*, *S. racemosa*, *S. canadensis*, *S. ebulus* und *Ligustrum vulgare* in Form mehrerer Stämme bzw. Serotypen bisher in folgenden Pflanzen gefunden: *Ulmus americana* L. (VARNEY und MOORE, 1952; FULTON und FULTON, 1970), *Prunus avium* L. (CROPLEY, 1961), *P. cerasus* L. (KEGLER, 1963, 1968), *Rheum rhaponticum* L. (TOMLINSON und WALKEY, 1967), *Rubus* sp. (CROPLEY und TOMLINSON, 1971), *Betula pendula* Roth, *Ptelea trifoliata* L. (SCHMELZER, 1972a, 1972b), *Rumex obtusifolius* L. (WALKEY und Mitarb., 1973) und *Cornus florida* L. (WATERWORTH und LAWSON, 1973). Übereinstimmend waren beide in der vorliegenden Arbeit näher untersuchten *Sambucus nigra* vom Traubenholunderringflecken-Serotyp befallen. Das ist um so bemerkenswerter, als zwei gelbnetzkranke *Sambucus nigra* aus verschiedenen Gegenden der DDR unterschiedliche KBRV-Serotypen enthielten (SCHMELZER, 1972b). Die an zahlreichen Pflanzenarten verursachten Reaktionen stimmten weitgehend mit denjenigen überein, die der typische Vertreter des Traubenholunderringflecken-Serotyp ausgelöst hatte (SCHMELZER, 1966). Die vorliegenden Untersuchungen stellten folgende Pflanzenarten als bisher unbekannte experimentelle Wirte des KBRV heraus: *Ammi visnaga*, *Aptenia cordifolia*, *Browallia americana*, *B. roezli*, *Commelina communis*, *C. graminifolia*, *C. tuberosa*, *Erodium cicutarium*, *E. malacoides*, *E. moschatum* und *Obione sibirica*. Besonders bemerkenswert ist, daß es nach der erstmaligen Feststellung einer Monokotyle (*Commelina coelestris* Willd., vgl. SCHMELZER, 1966) gelang, noch weitere Wirte aus der gleichen Gattung zu ermitteln. Die hier geschilderten Experimente lassen den Zusammenhang zwischen der Symptomausprägung an *Sambucus nigra* und dem isolierten Virus offen. Vom Gelbnetz des Schwarzen Holunders ist bekannt, daß es außer vom

KBVR sowohl vom Arabismosaik- als auch von verschiedenen Serotypen des Tomatenschwarzring-Virus verursacht werden kann (SCHMELZER, 1966). Inwieweit das KBRV eine Ringfleckigkeit am Schwarzen Holunder zu bewirken vermag, müßte noch untersucht werden. Die ganz ähnliche Ringfleckigkeit am Traubenholunder, die durch das KBRV verursacht wird, deutet allerdings auf diese Möglichkeit hin. Die Klärung der Frage, warum der eine Strauch von *Sambucus nigra* mit Gelbnetz, der andere aber mit Ringfleckung auf eine Infektion mit dem gleichen Serotyp des KBRV reagiert, wäre dann von Interesse.

Zusammenfassung

Vom Schwarzen Holunder (*Sambucus nigra* L.) mit Gelbnetz- oder chlorotischen Ringfleckensymptomen an den Blättern konnte in Ungarn das gleiche Virus isoliert werden. Es verursachte an Testpflanzen typische Symptome eines *NEPO*-Virus und die Eigenschaften *in vitro* unterstützten diese Diagnose. Mittels serologischer Untersuchungen wurde das Virus als zugehörig zum Traubenholunderringflecken-Serotyp des Kirschenblattroll-Virus identifiziert. Das ist der erste Nachweis dieses Virus an *Sambucus* in Ungarn. *Ammi visnaga* (L.) Lam., *Aptenia cordifolia* (L.) Schwantes, *Browallia americana* L., *B. roezli* Nichols, *Commelina communis* L., *C. graminifolia* H.B.K., *C. tuberosa* L., *Erodium cicutarium* (L.) L'Herit. ex Ait., *E. malacoides* Willd., *E. moschatum* (L.) L'Herit. ex Ait. und *Obione sibirica* (L.) Fisch. sind neu festgestellte experimentelle Wirte des Kirschenblattroll-Virus.

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Studies on Viruses and Virus Diseases of Cruciferous Plants XV. NEPO Viruses in Ornamental and Wild Species

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Including the results of previous papers (SHUKLA and SCHMELZER, 1970a, 1970b), 43 species of ornamental and wild crucifers belonging to 14 genera proved to be naturally infected by tomato black ring virus. It seemed to induce no symptoms in these plants. In all 58 isolates of the virus were obtained from 9 botanical gardens and plantations in the GDR. From these isolates, 25 and 33 were classified as beet ringspot and potato bouquet serotypes, respectively. At some locations only one serotype was found or nearly so, but at two places the isolates belonged to the serotypes in a ratio of about 1 : 1. However, there was no indication for mixed infections with both serotypes in an individual plant. Samples of 4 species, coming from 2 to 4 places, were infected with only one serotype each, whereas for 4 other species the serotype varied with the locations. Arabis mosaic virus was demonstrated only in 4 species from two genera of the crucifers. Also the infection with this virus seemed to induce no symptoms. *Iberis saxatilis* proved to be infected with raspberry ringspot virus at 3 places. No other NEPO viruses were detected during the investigations described here. All the demonstrated natural combinations between viruses and ornamental and wild crucifers were not found before.

It is astonishing that the knowledge of NEPO viruses in Europe was very limited for a long time. Now it is obvious that they are rather frequent, especially in perennial plants. Nevertheless, the real distribution of NEPO viruses in different regions and plant families is not explored until now. Intensive studies can add a large amount of previously unknown natural hosts. In two earlier papers we described the detection of some new hosts of NEPO viruses among ornamenta and wild crucifers (SHUKLA and SCHMELZER, 1970a, 1970b). During the continuation of these studies we found very often plants infected with tomato black ring virus (TBRV) but only sometimes with arabis mosaic virus (ArMV) and only one time with raspberry ringspot virus (RRSV). A summarizing report on our results is given here.

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Materials and Methods

Botanical gardens and plantations in different regions of the GDR were the places where samples of crucifer species with and without symptoms were collected and tested after the methods already described in earlier papers (SHUKLA and SCHMELZER, 1970a, 1970b).

Results

Tomato black ring virus

In general the following test plant reactions were obtained:

Chenopodium amaranticolor Coste et Reyn. I chlorotic to necrotic spots; II mottling, curling, and necrotic spots or tip necrosis
C. murale L. I necrotic spots; II tip necrosis
C. quinoa Willd. I chlorotic to necrotic spots; II mottling, curling, and necrotic spots or tip necrosis
Cucumis sativus L. II chlorotic spots, mottling, stunting, quick recovery
Nicotiana megalosiphon Heurck et Muell. I necrotic spots and rings; II necrosis and mottling, quick recovery
N. tabacum L. cv. 'Samsun' I necrotic rings; II necrotic to chlorotic ring and oak-leaf pattern, recovery
N. tabacum, cv. 'Bel 61 – 10' I necrotic spots and rings; II chlorotic to necrotic pattern, curling, mottling, and recovery
Petunia hybrida hort. ex Vilm. I necrotic spots; II necrosis and mottling, recovery
I = inoculated leaves, II = top leaves

These symptoms are characteristic for TBRV. The identity of all the isolates was confirmed by serological investigations. Systemically infected *Chenopodium quinoa* plants were used as virus sources and their saps were tested with antisera against ArMV, RRV, strawberry latent ringspot virus and against both serotypes of TBRV. The antisera against strains "Ca N" and "Ro U" represented beet ringspot and potato bouquet serotypes, respectively (SCHMELZER, 1970). The isolates reacted only with the antisera of TBRV. Merely one isolate reacted additionally with the ArMV antiserum, thus indicating that it consisted of a mixture of TBRV and ArMV. Although all TBRV isolates reacted with the antisera against both serotypes, each showed a special affinity to one of them. The isolates still reacting with higher dilutions of "Ca N" antiserum were considered as members of beet ringspot serotype (BRS), whereas the isolates reacting more markedly with "Ro U" than with "Ca N" antisera were classified as belonging to potato bouquet serotype (PBS). Species names, places of collection, and the serotype obtained are recorded in Table 1.

Table 1

Natural hosts of tomato black ring virus among ornamental and wild crucifers

Plant species	Places of collection	Serotype	
		BRS	PBS
Alyssum cuneifolium Ten.	Greifswald		+
A. idaeum Boiss. et Heldr.	Greifswald	+	
A. ovirense A. Kern	Berlin		+
A. saxatile L.	Rostock	+	
Arabis alpina L.	Eberswalde	+	
A. androsacea Fenzl	Halle	+	
A. x arendsii Wehrh.	Greifswald		+
A. caucasica Willd.	Berlin		+
	Eberswalde	+	
	Gatersleben*	+	
	Greifswald	+	
	Potsdam*		+
A. corymbiflora Vest	Rostock	+	
A. ferdinandi-coburgi Kell. et Suenderm.	Greifswald		+
A. jacquinii Beck	Eberswalde	+	
A. scopoliana Boiss.	Rostock		+
A. sicula Huet.	Rostock		+
Aubrieta columnae Guss.	Berlin	+	
A. croatica Schott, Nym. et Kotschy	Rostock		+
A. deltoides (L.) DC.	Jena	+	
	Potsdam*		+
A. deltoides var. *graeca* Regel	Potsdam*	+	
A. intermedia Heldr. et Orph.	Berlin		+
A. cultorum Bergm. cv. Leichtlinii	Berlin		+
Barbarea iberica (W.) DC.	Berlin		+
B. intermedia Bor.	Berlin		+
B. stricta Fries	Berlin		+
B. vulgaris R. Br.	Berlin		+
	Greifswald		+
Cardamine pratensis L.	Berlin		+
Cheiranthus cheiri L.	Berlin		+
	Rostock	+	
Cochlearia officinalis L.	Berlin		+
Draba bryoides DC. var. *imbricata* C. A. Mey.	Greifswald		+
D. carinthiaca Hoppe	Greifswald	+	
D. hispanica Boiss.	Greifswald	+	
D. lasiocarpa Rochel	Rostock		+
D. mollissima Stev.	Potsdam*		+
D. sibirica (Pall.) Thell.	Eberswalde	+	
	Greifswald	+	
D. siliquosa M. B.	Greifswald	+	
Hesperis matronalis L.	Potsdam*		+
	Rostock	+	

Plant species	Places of collection	Serotype	
		BRS	PBS
Hutchinsia alpina (L.) R. Br.	Dresden	+	
	Eberswalde	+	
	Greifswald	+	
	Rostock	+	
H. auerswaldii Willk.	Berlin		+
Iberis gibraltarica L.	Rostock	+	
I. jordanii Boiss.	Rostock		+
I. pruitii Tineo	Rostock		+
I. sempervirens L.	Berlin		+
	Potsdam*		+
	Rostock		+
Lunaria annua L.	Berlin		+
Schivereckia bornmuelleri Prantl	Rostock		+
S. doerfleri (Wettst.) Bornm.	Greifswald	+	
Thlaspi montanum L.	Berlin		+

* Already mentioned in a previous paper (SHUKLA and SCHMELZER, 1970b)

Including the findings of the previous paper (SHUKLA and SCHMELZER, 1970b), the presence of TBRV could be demonstrated in 43 species of ornamental and wild crucifers. The material came from nine botanical gardens and plantations. From the visited places only the botanical garden in Leipzig never contained this virus. Obviously, all species mentioned in Table 1 are new natural hosts of TBRV. With the exception of *Cochlearia officinalis*, the investigated species did not show any symptom when found infected in their habitats. However, the mentioned species contained also cucumber mosaic virus. Apparently TBRV induces only latent infections in crucifers. Back transmissions of the virus (isolate from Gatersleben) onto young seedlings of *Arabis caucasica* (SHUKLA and SCHMELZER, 1970b) as well as artificial infections of some cruciferous species done by earlier authors yielded only latent infections and support this presumption.

The two serotypes of TBRV were found to be rather equally distributed among the investigated samples, since 25 and 33 isolates belonged to BRS and PBS, respectively. There was no evidence for the existence of additional TBRV serotypes. Both serotypes could be isolated from plants of the same habitat (Berlin, Greifswald, Potsdam, and Rostock). This result was explained in our previous paper by the fact that already infected mother plants were brought to the nursery at Potsdam. However, crucifers in botanical gardens are mostly grown from seeds. TBRV is known to be rather well seed transmissible (LISTER and MURANT, 1967). Therefore it is possible that the more frequent occurrence of PBS than that of BRS in Berlin (15 : 1) and Potsdam (5 : 1) is due to the fact that the first mentioned serotype is naturally present there and the latter is introduced from other places by plant material or seeds. The demonstration of only BRS in all the five isolates from Eberswalde confirms the findings of HARRISON

(1964) that presumably due to the different ecological requirements of the two nematode species responsible for transmission only one is present within a natural virus outbreak. On the other hand, the isolation of PBS and BRS in nearly the same amount at Greifswald (5 : 8) and Rostock (8 : 6) may be caused by a mixed population of *Longidorus attenuatus* and *L. elongatus*. Also other factors may be involved, for example unknown vector species. At any rate it is of interest that in no case a mixed infection with both serotypes was detected within one plant. Specimens of *Barbarea vulgaris*, *Draba sibirica*, *Hutchinsia alpina*, and *Iberis sempervirens* were collected at 2 to 4 places and contained uniformly either PBS or BRS. However, samples of *Arabis caucasica*, *Aubrieta deltoides*, *Cheiranthus cheiri*, and *Hesperis matronalis* demonstrated that the same cruciferous species can be infected singly by both serotypes at different locations. Specimens of *Cheiranthus cheiri* and *Cochlearia officinalis* both collected twice in different seasons, namely May and September 1971, from the botanical garden in Berlin-Baumschulenweg yielded always only PBS. This indicated that the plants were indeed infected only by one serotype and that there was no transitory suppression of the other one within the same plant individuum.

Arabis mosaic virus

From the used test plants *Chenopodium amaranticolor*, *C. murale*, and *C. quinoa* reacted against ArMV in a similar manner as against TBRV. The reactions of the other symptom showing plant species were as follows:

Cucumis sativus I chlorotic to necrotic spots; II mottling and necrotic spots, mostly stunting
Nicotiana megalosiphon I chlorotic to necrotic spots and rings; II mottling, weak necrosis, recovery
N. tabacum, cv. 'Samsun' I chlorotic to necrotic spots and rings; II chlorotic to necrotic ring and line pattern, mottling, recovery
N. tabacum, cv. 'Bel 61 – 10' I necrotic spots and rings; II necrotic spots, rings and mosaic, recovery
I = inoculated leaves, II = top leaves

The symptoms are in accordance with earlier findings. With the exception of *Cucumis sativus*, however, none of these plants gives the possibility to differentiate between ArMV and TBRV. For these reasons the identity of the ArMV isolates had to be checked by serology. From the antisera against NEPO viruses only that against the specific virus reacted. The one exception, in which a mixed infection of ArMV and TBRV was found, is *Arabis androsacea* from Halle. *Arabis caucasica* from Aschersleben, already mentioned earlier (SHUKLA and SCHMELZER, 1970a), *Draba bryoides* from Jena, and *D. carinthiaca* from Leipzig are the only findings of ArMV in ornamental and wild crucifers during our studies. The virus seemed to be present in these hosts without inducing symptoms.

Raspberry ringspot virus

We did not succeed in detecting more hosts of RRV. The only known natural host *Iberis saxatilis* L., which had been found earlier at Gatersleben and Potsdam (SHUKLA and SCHMELZER, 1970a), proved to be also infected by RRSV in the botanical garden Berlin-Baumschulenweg. The new isolate seemed to be different from a Scottish strain as already shown in the mentioned paper for the earlier investigated isolates.

Discussion

In the beginning of our investigations we did not expect that the ornamental and wild crucifers in botanical gardens and in some other plantations of the GDR would be so often infected by TBRV. Apparently, the virus has rather good chances to invade the plants at some of the places, but there is also no doubt that many crucifers are very susceptible to it. Our results suggest that the investigated plant group may be an important source of TBRV under natural conditions. ArMV had been detected for the first time in a crucifer species and thus it was named after it (SMITH and MARKHAM, 1944). It seems, however, that the affinity of crucifers to this virus is comparatively low. During the trials described here only two genera have been found to be infected by it. The very frequent infestation of horseradish by ArMV (HICKMAN and VARMA, 1968; SHUKLA and SCHMELZER, 1972) is apparently an exception. We found that 6 cruciferous species out of 39, belonging to 22 genera, were susceptible to artificial inoculations with RRSV (SHUKLA and SCHMELZER, 1970a). This indicated that some crucifers may also be infected by the virus in nature, but it was detected only in *Iberis saxatilis*. The reason for the result can be the rare presence of RRSV in the GDR. This suggestion is supported by the fact that the virus was found there only once in a fruit crop (RICHTER *et al.*, 1966) whereas it was shown to be present rather frequently in cherries, raspberries or red currants at certain places of Britain, the Netherlands, FRG, and Switzerland (BLUMER and GEERING, 1950; CADMAN, 1956; KUNZE, 1953; VAN DER MEER, 1960, 1965; MULDER, 1951; PFAELTZER, 1959). Crucifers can be spontaneous hosts also of other NEPO viruses. In our trials, however, we did not succeed in demonstrating any other than the three viruses already mentioned here. Tobacco ringspot and tomato ringspot viruses are apparently very seldom in Europe. During sap inoculation tests of the junior author (SCHMELZER, 1966, 1969), strawberry latent ringspot and cherry leafroll viruses could be artificially transmitted to crucifers only to one species each. Therefore, crucifers may not be very adapted as hosts of both viruses. These facts suggest that the four last mentioned NEPO viruses scarcely occur in crucifers at least in the GDR.

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Studies on Viruses and Virus Diseases of Cruciferous Plants XVI. Purification and Serology of a GDR Isolate of Radish Mosaic Virus and Its Comparison with an American and a Yugoslavian Isolate

By

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One GDR isolate (DT4) of radish mosaic virus (RMV) was partially purified by butanol-chloroform method and by two cycles of differential centrifugation. Isometric virus particles, observed under electron microscope in metal shadowed preparation, measured 24 nm in aggregated material and 32 nm when scattered. An antiserum of this isolate had a titre of 1 : 8192 with the homologous virus in OUCHTERLONY test and did not react with healthy plant sap. Comparisons between an American (type strain), a Yugoslavian (HZ) and the mentioned GDR isolate of RMV, based on test plant reactions, showed considerable differences and it was concluded that they should be three distinct strains. However, in serological tests the isolates DT4 and HZ could not be differentiated whereas the type strain was distinct. JS 30, another GDR isolate of RMV, reacted in the same manner as DT4 and HZ. Obviously the GDR isolates are closely related to the "European strain" of RMV described by ŠTEFANAC and MAMULA (1971).

CAMPBELL (1964) succeeded in purifying radish mosaic virus (RMV) and reported polyhedral particles of about 30 nm diameter. Particle diameter of about 25 nm in purified preparations was found with a Yugoslavian isolate of this virus (ŠTEFANAC and MAMULA, 1971). In serological and biological tests, a Japanese isolate of RMV obtained from radish and an American isolate from turnip proved to be identical (CAMPBELL and TOCHIHARA, 1969), whereas the latter, when compared with a Yugoslavian isolate, obtained from turnip, was found rather closely related but not identical in test plant reactions and spur tests (ŠTEFANAC and MAMULA, 1971).

We reported for the first time about the occurrence of RMV in the GDR on three ornamental and wild cruciferous species, namely *Erysimum crepidifolium* Rchb., *E. hieraciifolium* Jusl. and *E. silvestre* (Cr.) Scop. which were previously unrecorded hosts of this virus (SHUKLA and SCHMELZER, 1973). The present paper

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deals with purification and serology of one of the GDR isolates of RMV and its comparison with an American and a Yugoslavian isolate on the basis of test plant reactions and serological properties.

Materials and Methods

The GDR isolate of RMV (DT4), used in the present studies, was isolated from *Erysimum silvestre* (SHUKLA and SCHMELZER, 1973). The American (type strain) and the Yugoslavian (HZ) isolates and their antisera were obtained from Dr. HOLLINGS (England) and Dr. ŠTEFANAC (Yugoslavia), respectively. The methods of preparing inoculum, inoculation and serology were the same as described in our earlier papers (SHUKLA and SCHMELZER, 1970a; 1970b). The test plants used in the comparison trials were mostly those already reported by previous workers as symptom-producing hosts of RMV. The three isolates were propagated in *Sinapis alba* L. and the test plants were inoculated with them on the same day. Susceptibility of the test plants was considered on the basis of symptoms produced on them. No attempt was made to show if the symptomless species were infected latently. The isolate DT4 was purified using butanol-chloroform for sap clarification and two cycles of differential centrifugation and an antiserum was prepared against it by immunizing a rabbit intravenously and intramuscularly as described in case of *Erysimum* latent virus (SHUKLA *et al.*, 1973). For purification purpose, the isolate was propagated both in *Brassica perviridis* Bailey and in *Sinapis alba*.

Results

The partially purified preparations of DT4 isolate of RMV were found rather clean when seen under electron microscope. The particles were isometric and had an average diameter of 24 nm in aggregated material but they measured 32 nm when present separately in metal shadowed preparations (Fig. 1). The antiserum prepared against this isolate had a serum titre of 1 : 8192 with the homologous virus in agar-gel double diffusion test and did not react with healthy plant sap.

Considerable differences were observed when the American, the Yugoslavian and the GDR isolates were compared basing on the reaction of 27 test plant species (Table 1). Some of the more important differences worth special mentioning are as follows: (1) cauliflower and cabbage showing pronounced symptoms by the infection of type strain remained symptomless by the other two isolates; (2) turnip, which was damaged heavily by HZ isolate and displayed somewhat less severe symptoms by DT4, showed no symptoms by type strain; (3) the type strain induced severe systemic necrosis and DT4 showed pronounced mottling and severe chlorotic rings on *Nicotiana clevelandii* but the species was symptomless when tested with HZ; (4) severe symptoms were induced by HZ and a little

less severe by DT4 on swede but the type strain produced only mild symptoms on this host; (5) *Chenopodium foliosum* reacted only locally by the infection of HZ and DT4 isolates, whereas the type strain produced systemic chlorosis and severe necrosis additionally. Therefore, on the basis of test plant rections, the three isolates can be considered distinct strains, but in general HZ and DT4 appear to be more similar to each other than to the type strain.

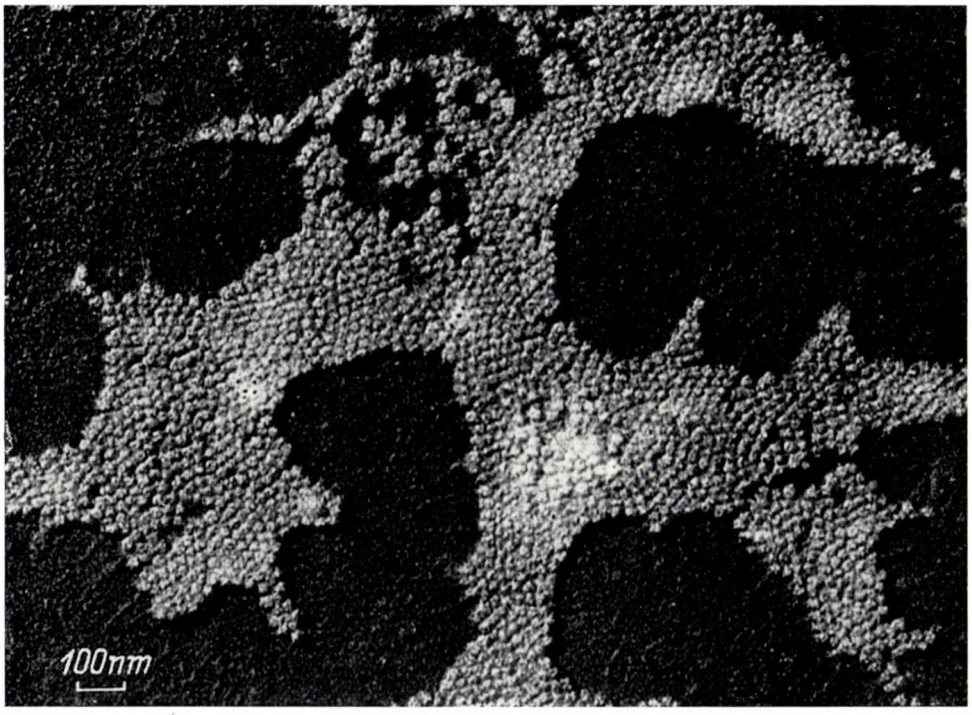

Fig. 1. Virus particles of radish mosaic virus in partially purified, metal-shadowed preparation. Magnification about 1 : 60 000

In serological investigations, when the three isolates were tested in spur tests in all possible combinations using symptom-showing *Sinapis alba* as source in each case, DT4 and HZ reacted identically and the type strain proved to be distinct. DT4 and HZ formed spurs against the type strain when the antisera of the first two mentioned isolates were used and vice-versa. No difference was observed in serological reactions of DT4 and HZ isolates when they were tested side by side with both of their antisera. JS 30, the other GDR isolate of RMV obtained from *Erysimum hieraciifolium* (SHUKLA and SCHMELZER, 1973), also reacted in the same manner as DT4 and HZ. A serum titre of 1 : 1024 was obtained with DT4 and JS 30 with type strain antiserum.

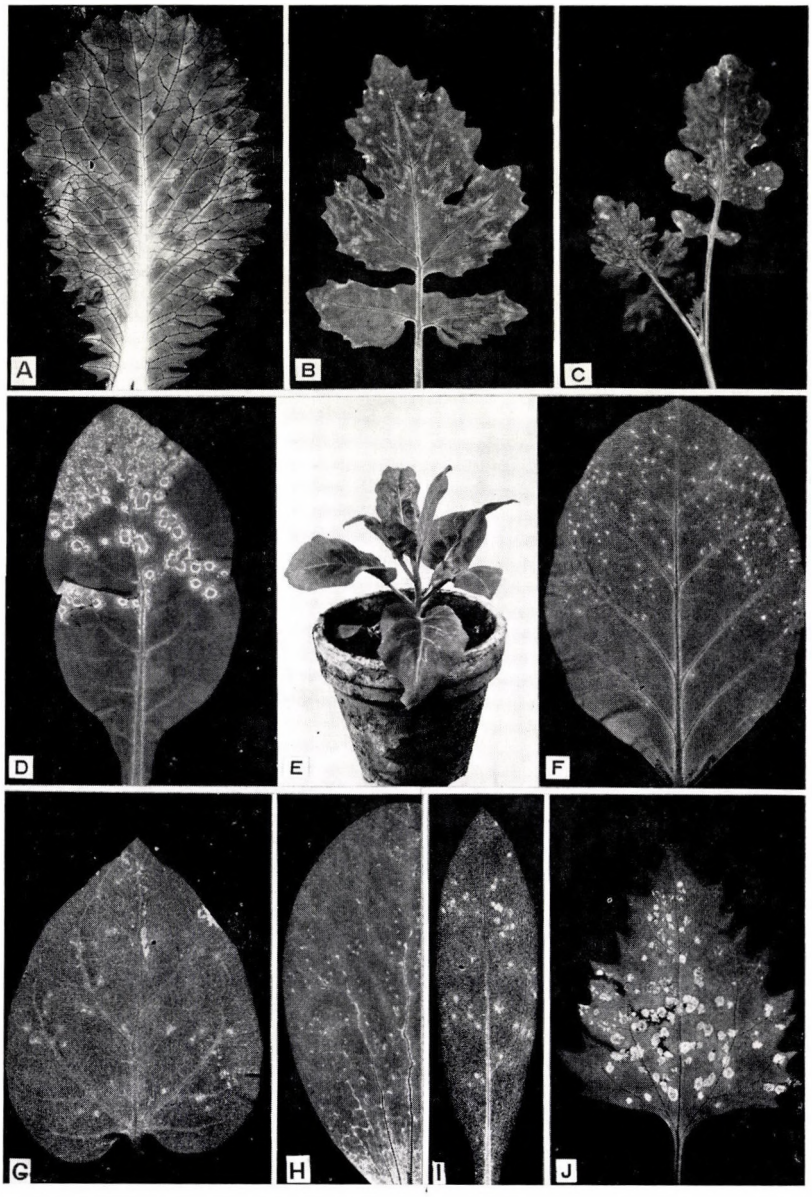

Fig. 2. Symptoms of radish mosaic virus (isolate DT4) on different test plants. A: *Brassica pekinensis*; B—C: *Sinapis alba*; D—E: *Nicotiana megalosiphon*; F: *N. tabacum* cv. 'Samsun'; G: *N. glutinosa*; H: *Cucurbita pepo*; I: *Gomphrena globosa*; J: *Chenopodium murale*. D, F—J: Local infection; A, C, E: systemic infection

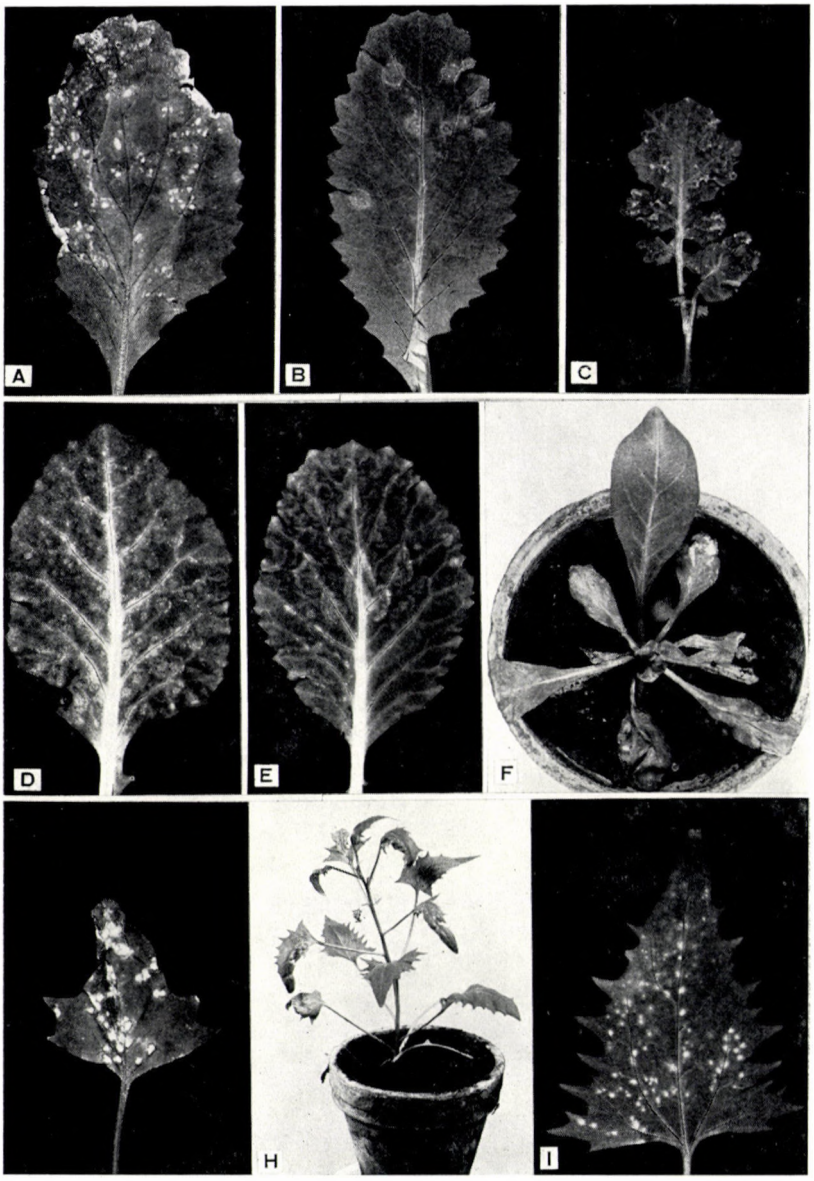

Fig 3. Symptoms of radish mosaic virus (type strain and HZ isolate) on different test plants. A—B: *Brassica pekinensis*; C: *Sinapis alba*; D—E: *Brassica oleracea* var. *botrytis*; F: *Nicotiana clevelandii*; G: *Chenopodium quinoa*; H—I: *C. foliosum*. A, D, G, I: local infection; B, C, E, F, H: systemic infection, A—H: infection by type strain; I: infection by HZ isolate

Table 1

Reactions of an American, a Yugoslavian and a GDR isolate of radish mosaic virus on various test plants

Plant species	Isolates and their reactions		
	Type strain	*H Z*	*DT 4*
Crucifers			
Brassica juncea (L.) Czern. et Coss.	I no; II M	I no; II M	I no; II **M**
B. napus var. *napobrassica* (L.) Rchb.	I no; II (cF)	I **nL**; II **M**, N	I **nL**; II M
B. nigra (L.) Koch	I nL; II M, C, N	no	no
B. oleracea var. *botrytis* (L.) Alef.	I cR, cF; II **cR** (Fig. 3, D–E)	no	no
B. oleracea var. *capitata* (L.) Alef.	I cR; II cR	no	no
B. pekinensis (Lour.) Rupr.	I **nL**; II cR, nR (Fig. 3, A–B)	I (nL); II (M)	I (nL); II (M) (Fig. 2, A)
B. rapa var. *rapa* (L.) Thell.	no	I cL, nL; II **M**, **N**	I no; II **M**, C
Capsella bursa-pastoris (L.) Med.	I no; II (M)	I no; II **M**, *C*	I **nL**; II **M**, **C**
Raphanus sativus L.	no	no	no
Sinapis alba L.	I no; II M, **C**, N (Fig. 3, C)	I no; II M, cF, P	I no; II M, cF, P (Fig. 2, B–C)
S. arvensis L.	I **nL**; II **M**, C	I no; II cR, **M**, N	I cR, nR; II **M**, **N**
Non-crucifers			
Beta vulgaris L.	no	no	no
Chenopodium amaranticolor Coste et Reyn.	no	no	no
C. foliosum Aschers.	I cL, nL; II **Ch**, **N** (Fig. 3, H)	I **cL**; nL; II no (Fig. 3, I)	I cL, nL; II no
C. murale L.	I (nL); II no	I nL; II no	I **nL**; II no (Fig. 2, J)
C. quinoa Willd.	I **nL**; II no (Fig. 3, G)	I (nL); II no	I (nL); II no
Cucumis sativus L.	no	no	no
Cucurbita pepo L.	no	no	I nL; II no (Fig. 2, H)
Datura stramonium L.	no	no	no
Gomphrena globosa L.	I **nL**; II no	I nL; II cF, M	I nL; II cF, M (Fig. 2, I)

Plant species	Isolates and their reactions		
	Type strain	*H Z*	*DT 4*
Nicotiana clevelandii Gray	I cL, nL; II **N** (Fig. 3, F)	no	I cL, nL; II **M**, **cR**
N. glutinosa L.	I nL; II no	no	I (nL); II cF (Fig. 2, G)
N. megalosiphon Heurck et Muell.	I nL; II **M**, cR, nR	I **nR**; II **M**, **cR**, nR	I **nR**; II M, **cR**, nR (Fig. 2, D–E)
N. tabacum L. cv. 'Bel 61–10'	I nR; II no	I (nR); II no	I (nR); II no
N. tabacum cv. 'Samsun'	I nL; II no	I nL, II no	I nL; II no (Fig. 2, F)
Petunia hybrida Vilm.	no	no	no
Spinacia oleracea L.	I no; II **M**, N	I no; II (M)	I no; II M

no Symptoms not observed
I symptoms of inoculated leaves
II symptoms of tip leaves
cF chlorotic flecks
C crinkling
cL Chlorctic local lesions
nL necrctic local lesions
M mottling
cR chlorctic rings
nR necrotic rings
N necrosis
P pattern
Ch Chlorosis
( ) weak symptoms
— fat letters severe symptoms

Discussion

RMV has been sufficiently purified using butanol (CAMPBELL, 1964; HOLLINGS and STONE, 1969) and chloroform (ŠTEFANAC and MAMULA, 1971) for sap clarification and two cycles of differential centrifugation. In the present work, clean preparations of the virus were obtained by butanol-chloroform method. Although we did not compare the mentioned other two methods, perhaps our method is at least equally suited for the purification of RMV. It was found best for purifying *Erysimum* latent virus (SHUKLA *et al.*, 1973) and gave high virus yield in the case of turnip yellow mosaic virus (unpublished). CAMPBELL (1964) and ŠTEFANAC and MAMULA (1971) reported particle diameter of RMV as 30 and 25 nm, respectively. In our studies it was found 24 nm in aggregated material and 32 nm when the particles were lying separately. This is not surprising because the results of measurement of virus particle diameter depend on several factors, for example also on their more or less close packing.

The test plant reactions obtained in the present investigation by the type strain and HZ isolate are almost in accordance with the earlier descriptions of the two isolates (CAMPBELL, 1964; ŠTEFANAC and MAMULA, 1971). None of the three isolates induced symptoms on *Beta vulgaris*, *Datura stramonium*, *Raphanus sativus*,

Chenopodium amaranticolor, *Cucumis sativus*, and *Petunia hybrida*. The first three mentioned species are known to display symptoms by the infection of type strain, the following two by type strain as well as by HZ isolate and the last mentioned species has never been infected by any one of them (CAMPBELL, 1964; CAMPBELL and TOCHIHARA, 1969; ŠTEFANAC and MAMULA, 1971). *Nicotiana glutinosa*, shown by CAMPBELL (1964) not to be susceptible to type strain, produced severe necrotic lesions in the present trial. Similarly *Brassica pekinensis*, found to react only locally by type strain (CAMPBELL, 1964) and *Chenopodium foliosum* reported to show local as well as systemic symptoms by HZ isolate (ŠTEFANAC and MAMULA, 1971), reacted in our investigations by systemic and local symptoms, respectively, with the corresponding isolates. However, these discrepancies can be attributed to the differences in varietal response and in the effect of different environment on host plant susceptibility. Similar conclusions were also drawn by CAMPBELL and TOCHIHARA (1969) while comparing the Japanese and the type strain of RMV.

During comparison of the isolates of RMV originating in USA, Yugoslavia and in the GDR on the basis of test plant reactions, varying degrees of differences were noticed and it was concluded that the three investigated isolates are distinct but in serological tests the GDR and the Yugoslavian isolates could not be differentiated and the American one proved to be distinguishable. On the other hand, the Japanese and the American isolates have also been reported to possess the same antigenic properties. It is of interest to see that the isolates of RMV found in the GDR and Yugoslavia on one hand and those in USA and Japan on the other are serologically more closely related. This speaks for connections between serological relationship and geographical distribution, although Japan and USA are separated from each other by the Pacific.

The various isolates of RMV obtained and investigated in Yugoslavia did not differ in their antigenic properties (ŠTEFANAC and MAMULA, 1971). The same may be true for the GDR isolates as two of them tested here proved to be identical. ŠTEFANAC and MAMULA (1971) joined their RMV isolates under the name "European strain". In spite of certain differences in test plant reactions, our isolates of the virus are closely related or may belong to the "European strain" because of similar antigenic properties.

Acknowledgements

We are highly grateful to Dr. M. HOLLINGS, Littlehampton (England) and Dr. Z. ŠTEFANAC, Zagreb (Yugoslavia) for kindly providing antisera and specimens of radish mosaic virus. Our thanks are also due to Dr. H. B. SCHMIDT for electron microscopy and to Miss H. C. NORDMANN and Miss U. BRUNNE for the photographs.

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Studies on Viruses and Virus Diseases of Cruciferous Plants XVII. Serologically Distinct Strains of Turnip Yellow Mosaic Virus Occurring in the GDR

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Four distinct strains of turnip yellow mosaic virus (TYMV) were recognized among eight GDR isolates investigated on the basis of test plant reactions and spur forming capability. The strains consisted of the following isolates: 1. JS 1, JS 12 (strong); 2. DT 22, ED 3 (moderate); 3. HS 28 (moderate); 4. JS 22, HS 21, RK 18 (weak). The results showed that serologically differing forms of TYMV differ also in their test plant reactions. More than one strain of the virus could be isolated from samples collected at one place. In another case, a single plant species, collected from two different places, proved to contain two distinct strains of TYMV. The type strain from Cambridge and a Scottish isolate of the virus, used for comparison purpose, were found to have the same antigenic properties and formed spurs against all the eight GDR isolates. The only antiserum, used throughout the present work, was one against the type strain.

The existence of turnip yellow mosaic virus (TYMV) strains in laboratory isolates and field infections is already known from literature. MATTHEWS and RALPH (1966) found very occasionally, following inoculation of Chinese cabbage leaves with the Cambridge-type strain, the production of a necrotic local lesion, which when subcultured gave a pure necrotic strain of the virus. MATTHEWS (1970) obtained three distinct isolates from different coloured blocks of tissue in a Chinese cabbage leaf infected with the Cambridge stock culture of TYMV, indicating the presence of different strains in the same leaf.

BROADBENT and HEATHCOTE (1958) described three strains of TYMV, namely E, B and N, isolated from different localities in Great Britain. E was the type strain from Cambridge. They noticed small symptom differences induced by the isolates in certain cruciferous hosts, however, no plant was found that was susceptible to one strain and not to the others. Serological tests indicated that B and E were closely related, whereas N was serologically distinguishable. In cross-protection tests, the authors found that Chinese cabbage and turnip infected with E or N could still become infected with B as detected by serological tests.

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SYMONS *et al.* (1963), while working with six isolates of TYMV obtained from various sources in England, FRG and Denmark, recognized two distinct groups on the basis of amino acid composition of coat protein, base analysis of RNA and oligonucleotide frequencies.

During the investigation of TYMV isolates, obtained in the GDR from various ornamental and wild cruciferous species (SHUKLA and SCHMELZER, 1973), differences between the isolates were observed in biological and serological tests which are described here.

Materials and Methods

The eight GDR isolates of TYMV, used in the present investigation, are listed in table 1 (SHUKLA and SCHMELZER, 1973).

Table 1

List of the GDR isolates of turnip yellow mosaic virus compared in the present investigation

Isolate	Original host	Place of collection (botanical gardens)
HS 28	*Arabis jacquinii* Beck	Halle
JS 1	*A. jacquinii* Beck	Jena
JS 22	*A. japonica* A. Gray	Jena
DT 22	*A. serpyllifolia* Vill.	Dresden
JS 12	*Biscutella laevigata* L.	Jena
RK 18	*Draba athoa* (Griseb.) Boiss.	Rostock
ED 3	*D. fladnizensis* Wulf	Eberswalde
HS 21	*Schivereckia bornmuelleri* Prantl	Halle

For preparation of inoculum, inoculation and serology only those methods were followed which have already been described in our previous papers (SHUKLA and SCHMELZER, 1970a; 1970b). The test plants were selected for their seed availability and for the fact that they are all known as symptom-producing hosts of TYMV from literature (BROADBENT and HEATHCOTE, 1958). The symptoms produced by different isolates on them were recorded. No attempt was made to demonstrate, if the symptomless species were infected latently. The two foreign isolates of TYMV, used for comparison purpose in the present studies, were the type strain from Cambridge and a Scottish isolate. The type strain and its antiserum were obtained from Dr. HOLLINGS (England). The Scottish isolate is being maintained in our greenhouse for several years (MAMULA, 1969). The type strain antiserum had a titre of 1 : 16384 with the homologous virus in tube test (HOLLING's personal communication) and was used throughout the present investigation. However, in our Ouchterlony tests the titre was lower. All the ten TYMV isolates were propagated in *Sinapis alba* L.

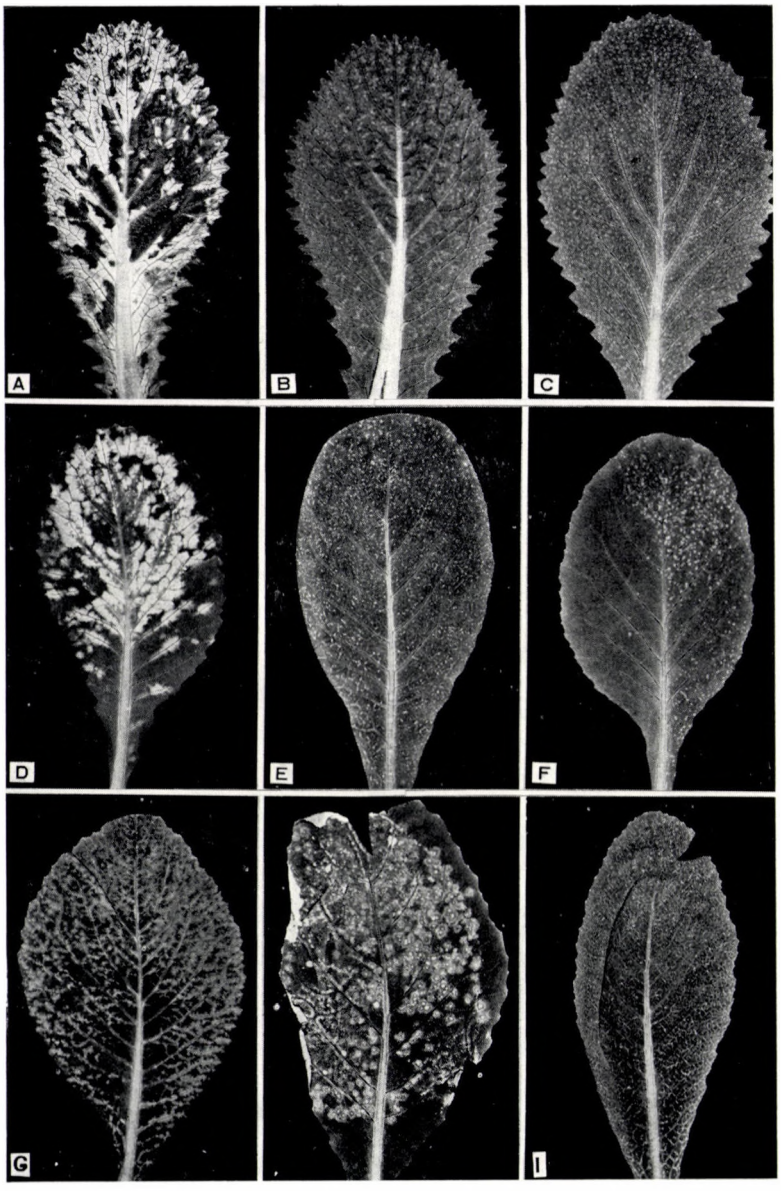

Fig. 1. Symptoms of different GDR isolates of turnip yellow mosaic virus on two *Brassica* spp. A—C: *Brassica pekinensis*; D—I: *B. perviridis*. H: local infection; A—G, I: systemic infections. A, D: isolate JS 1; G: JS 12; B, E: ED 3; C: DT 22; F: JS 22; H—I: HS 28

Table 2

Test plant reactions and antiserum titre of

Test plants and antiserum titre	Isolates and their reactions		
	RK 18	HS 21	JS 22
Brassica napus var. *napobrassica* (L.) Rchb.	no	no	no
B. nigra (L.) Koch	no	no	no
B. oleracea var. *botrytis* (L.) Alef.	no	no	no
B. pekinensis (Lour.) Rupr.	I no; II (M)	I cL; II (Vc), (M)	I nL; II (cF)
B. perviridis Bailey	I no; II (Vc) M, Vn	I cL, nL; II (Vc), (M)	I cL; II (Vc), (M), cF (Fig. 1, F)
B. rapa var. *rapa* (L.) Thell.	no	I no; II Vc, M	I no; II cF, (M)
Reseda odorata L.	no	I cL; II no (Fig. 2, E)	no
Sinapsis alba L.	I no; II Vc, M	I no; II Vc, M	I no; II Vc, M
S. arvensis L.	I no; II (Vc), M, Vn (Fig. 3, D)	I no; II (Vc), M	I no; II Vc, M
Antiserum titre	1 : 1024	1 : 2048	1 : 2048

no symptoms not observed
I symptoms of inoculated leaves
II symptoms of tip leaves
cF chlorotic flecks
cR chloritic rings
nR necrotic rings
Vc vein clearing
Vn veinal necrosis

Results

When the eight GDR isolates of TYMV, mentioned in table 1, were compared, differences were found in their test plant reactions and serological properties (Table 2). From table 2 it can be seen that the individual isolates differed considerably in their reactions on various test plants. However, taking into consideration the degree of severity in symptom, a tentative grouping of the isolates could be made. The isolates JS 1 and JS 12 produced the most severe reactions on all the hosts tested except *Brassica nigra* and the type and degree of symptom severity were the same in both the cases. Although the reactions of DT 22 and ED 3 were different on some hosts, they showed similarity in their symptom pro-

eight GDR isolates of turnip yellow mosaic virus

Isolates and their reactions				
HS 28	ED 3	DT 22	JS 1	JS 12
I no; II (cF) (Fig. 2, D)	I nL; II cF	no	I cL; II **Vc, M** (Fig. 2, A—B)	I cL; II **Vc; M**
I no; II **M**	no	I cR; II Vc, M	no	no
no	I no; II (Vc), cF	no	I no; II Vc, cF (Fig. 2, C)	I no; II Vc, CF
I cL, **nL**; II M, cF	I no; II (Vc) M, cF (Fig. 1, B)	I cL; II (Vc), M, cF (Fig. 1, C)	I cL; II **Vc, M** (Fig. 1, A)	I cL; II **Vc, M**
I cR, nR; II Vc, M, Vn (Fig. 1, H—I)	I cL; II (Vc), M, **Vn** (Fig. 1, E)	I cL; II (Vc), M	I cL; II **Vc, M** (Fig. 1, D)	I cL; II **Vc,** M (Fig. 1, G)
I no; II Vc, M	I no; II cF, (M)	I no; II Vc, M	I cL; II **Vc, M**	I cL; II **Vc, M**
I nL; II no (Fig. 2, F)	I cL; II no	I cL; II no	I nL; II no	I nL; II no
I no; II **Vc,** M	I no; II (Vc) M, C	I no; II **Vc,** M	I no; II **Vc,** M	I no; II **Vc, M**
I cR, nR; II **Vc, M** (Fig. 3, A–B)	I no; II (Vc), M	I cL; II (Vc), M	I cL; II **Vc, M** (Fig. 3, C)	I nL; II **Vc, M**
1 : 1024	1 : 1024	1 : 2048	1 : 1024	1 : 1024

C crinkling
cL chlorotic local lesions
nL necrotic local lesions
M mottlings

( ) weak symptoms
— fat letters severe symptoms

duction on other hosts and hence they may belong to one group. HS 28 was the isolate also with moderate symptoms but the type of symptoms induced by this isolate was very different in comparison with the other isolates. HS 28 produced defined chlorotic to necrotic local rings and systemic veinal necrosis on *Brassica perviridis* and *Sinapis arvensis* and severe necrotic local lesions on *Brassica pekinensis*. JS 22, BK 18 and HS 21 generally induced the weakest test plant reactions and caused symptoms on least number of test plants with almost the same degree of severity and type. Thus, on the basis of these results the eight isolates can be classified under four groups: 1. JS 1, JS 12 (strong); 2. DT 22, ED 3 (moderate); 3. HS 28 (moderate); 4. JS 22, RK 18, HS 21 (weak). The second and third groups were considered as moderate but kept separately because of differences in the type of symptoms induced by them. It is of interest to note that the isolates JS 1 and

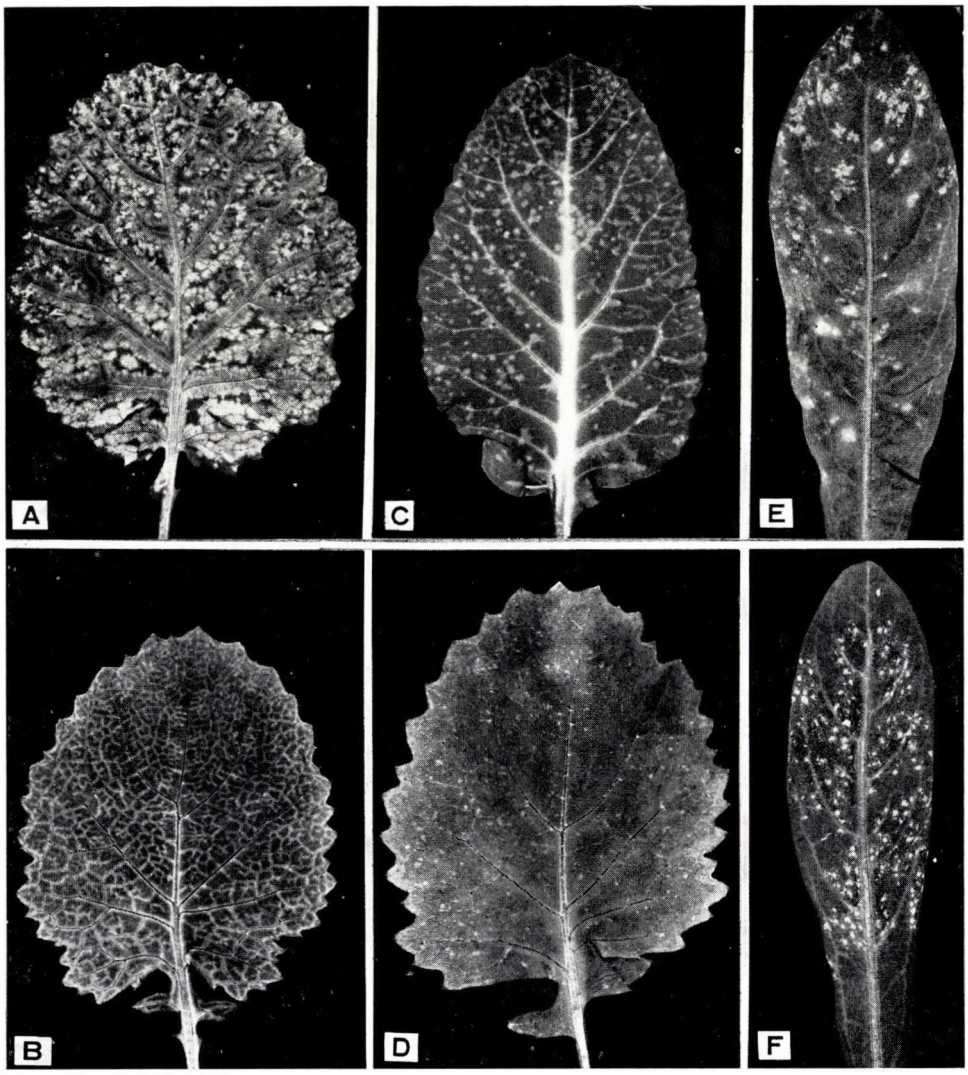

Fig. 2. Symptoms of different GDR isolates of turnip yellow mosaic virus on different test plants. A, B, D: *Brassica napus* var. *napobrassica*; C: *B. oleracea* var. *botrytis*; E—F: *Reseda odorata*. E—F: local infections; A—D: systemic infections. A—B: isolate JS 1; D, F: HS 28; E: HS 21

HS 28 were obtained from the same plant species (*Arabis jacquinii*), but collected at two different places, showed different test plant reactions and hence were kept in two separate groups.

Looking to the serum titre in table 2, it is to be found that the isolates did not differ very much from each other. However, the fact of special interest here

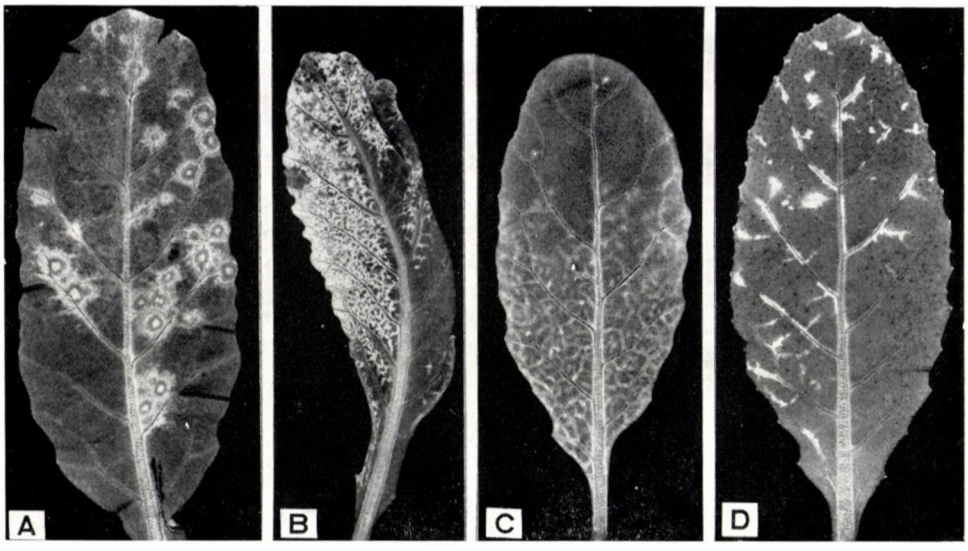

Fig. 3. Symptoms on different GDR isolates of turnip yellow mosaic virus on *Sinapis arvensis*. A: local infection; B—D: systemic infections. A—B: isolate HS 28; C: JS 1; D: RK 18

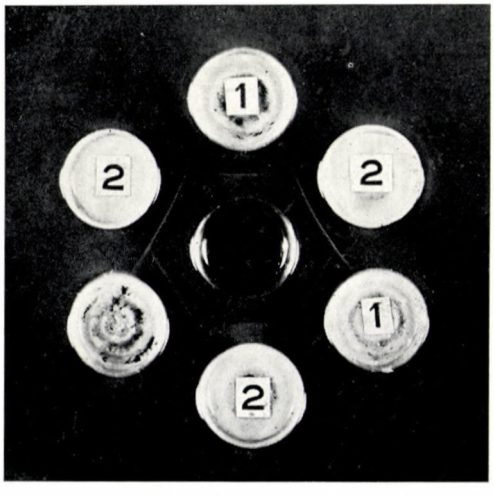

Fig. 4: Spur test with turnip yellow mosaic virus. The central well was filled with the type strain antiserum and the wells 1 and 2 contained JS 1 and type strain, respectively. Spur formation by type strain

is that the strong symptom producing isolates (JS 1, JS 12) gave lower titres than the weak isolates (JS 22, HS 21). The antiserum titre obtained with the type strain and the Scottish isolate of TYMV was found to be 1 : 4096 in our investigation.

To find out antigenic differences, the eight mentioned GDR isolates and the type and the Scottish isolates of TYMV were compared in spur tests in all 45 possible combinations using the type strain antiserum. The results are presented in table 3. From the table it can be seen that the ten isolates formed five different groups and thus they belong to five different strains of TYMV. The type strain and the Scottish isolate behaved identical and formed spurs against all the eight GDR isolates tested (Fig. 4). Among the GDR isolates, existence of four different strains could be demonstrated. On the basis of decreasing spur forming potency the strains can be put in the following sequence. (1) JS 1, JS 12; (2) DT 22, ED3; (3) HS 28; (4) JS 22, HS 21, RK 18.

Table 3

Serological reactions of 10 different turnip yellow mosaic virus isolates in all possible combinations in spur test

Isolates and their origin	German Democratic Republic								Great Britain	
	RK 18	HS 21	JS 22	HS 28	ED 3	DT 22	JS 1	JS 12	Scottish isolates	type strain
German Democratic Republic										
RK 18										
HS 21	−									
JS 22	−	−								
HS 28	+	+	+							
ED 3	+	+	+	+						
DT 22	+	+	+	+	−					
JS 1	+	+	+	+	+	+				
JS 12	+	+	+	+	+	+	−			
Great Britain										
Scottish isolate	+	+	+	+	+	+	+	+		
type strain	+	+	+	+	+	+	+	+	−	

− = no spur formation
+ = spur formation; the spur forming isolates are indicated on the left side of the table.

Discussion

The results of test plant reaction as well as the serological findings clearly demonstrated that different strains of TYMV exist in the GDR. Possibly the six other GDR isolates, reported by us (SHUKLA and SCHMELZER, 1973) and not included here, would have yielded some more strains of TYMV. The four groups of the eight GDR isolates formed tentatively on the basis of test plant reactions coincided well with their antigenic properties, indicating that serologically differing forms of TYMV differ also in their test plant reactions. The example of JS1, JS 12 and JS 22 obtained from Jena and of HS 21 and HS 28 from Halle showed that two or more strains of this virus may exist at one place. *Arabis jacquinii*, collected from Jena and Halle, proved to harbour two distinct strains separately. This indicated that one species can be infected by various strains of TYMV in nature.

The strongest GDR strain (JS 1, JS 12) appeared to be very close to the English one in spur tests. Although the latter was not compared with the former on the basis of test plant reaction simultaneously, the type and severity of symptoms induced by the latter on Chinese cabbage, swede and turnip were approximately the same as compared to the former. None of the GDR isolates produced systemic symptoms on *Reseda odorata*, whereas the English strain induced chlorotic local lesions and vein clearing on this host.

As indicated by MATTHEWS (1970), for viruses that multiply to high concentrations in plants, there is ample opportunity for spontaneous mutation to occur. Therefore, it is not surprising that TYMV was recognized in four distinct strains among the eight GDR isolates in the present investigation. Although this virus has been investigated very intensively by many authors, a review of the available literature on strains (BROADBENT and HEATHCOTE, 1958; SYMONS *et al.*, 1963; MATTHEWS and RALPH, 1966; MATTHEWS, 1970) reveals that TYMV has not been well studied concerning its strains occurring in nature. This may be due to the mostly low frequency of the virus in crucifers.

Acknowledgements

We are grateful to Dr. M. HOLLINGS, Littlehampton (England) for kindly sending specimen and antiserum of the Cambridge-type strain of turnip yellow mosaic virus and to Miss H. C. NORDMANN and Miss U. BRUNNE for the photographs.

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The Effect of Post-Inoculation Temperature on the Number of Local Lesions and Symptoms Expression Induced by Systemic and Necrotic Strains of Alfalfa Mosaic Virus on French Beans (*Phaseolus vulgaris* L.)*

By

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In experiments at temperatures maintained exactly at 15 °, 22 °, and 30 °C and with a localized necrotic and two systemic non-necrotic strain of alfalfa mosaic virus (AMV) on *Phaseolus vulgaris*, the following results were obtained in sap transmission tests on French beans: the largest numbers of local lesions appeared at a constant temperature of 22 °C. In general, at 30 °C more local lesions appeared than at 15 °C. Symptoms expression of the non-necrotic systemic strain on the top leaves was most marked at 22 °C. The necrotic strain induced systemic necrosis at 15 °C and was restricted to inoculated leaves at the higher temperatures. When the plants were subjected to changing temperatures, the necrotic strain produced fewer local lesions, when 15 °C prevailed. However, two days at normal or higher temperatures in the beginning were sufficient to induce comparatively large local lesion numbers. At a constant temperature of 15 °C the necrotic local lesions had a rather small diameter, but after 9 days at 15 °C, only one day at 30 °C was sufficient to induce very large local lesions. The enlargement of the local lesions was considerably less after shorter times at 15 °C followed by longer periods at higher temperatures. A constant temperature of 30 °C caused somewhat bigger lesions than at 22 °C. Local lesions induced by the non-necrotic strains at changing temperatures often had the shape of rings. Obviously 15 °C and 30 °C broke down the resistance against local migration of the necrotic strain. The extent of this phenomenon depended on the sequence and the duration of the temperatures. Systemic symptoms by the non-necrotic strains were not visible during an observation time of ten days if the plants were kept for at least 9 days at 30 °C. The necrotic strain developed a few necrotic spots on top leaves when the plants were kept for only one day p.i. at higher temperatures and later on at 15 °C. In this case, already two days p.i. at higher temperatures prevented the appearance of systemic symptoms. However, 8 days at 15 °C followed by 2 days of higher temperature also induced systemic infections. It is supposed that low temperatures in greenhouses during spring time enable local necrotic AMV strains to induce systemic necrotic reactions of French beans. So it does not seem to be justified to believe that systemic necrotic reactions in *Phaseolus vulgaris* after AMV inoculations are always due to special strains.

The effect of temperature on multiplication and/or symptom expression of several mechanically transmissible plant viruses has been studied by many

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authors (for instance by BEST, 1936; POUND, 1949; CHEO and POUND, 1952; KASSANIS, 1952; 1957; HARRISON, 1956; HITCHBORN, 1956; LI and SCHMELZER, 1964; HELMS, 1965; BRČAK *et al.* 1966; FROST and HARRISON, 1967; GÁBORJÁNYI and EL HAMMADY, 1969; MUSIL and LEŠKOVA, 1969; HARRISON and JONES, 1971; SHIMOMURA, 1971; SHIMOMURA and OHASHI, 1971; SHUKLA and SCHMELZER, 1973). Recently, summarizing surveys about this subject were given by HORVÁTH (1969, 1972) and WOLFFGANG (1970).

Alfalfa mosaic virus (AMV) was studied in a number of papers in which different hosts were used in order to establish the effect of temperature on the development of symptoms (WEIMER, 1931; DIACHUN and HANSON, 1957) and on susceptibility to the virus (PANZER, 1958; BODNÁR and KVIČALA, 1968; DESJARDINS, 1969; CRILL *et al.*, 1970).

The French bean is a main test plant for AMV. The type strain of AMV produces brown necrotic local lesions on inoculated bean leaves. This reaction helps to identify AMV and makes it possible to determine infection rates of lucerne stands (BURKE, 1963; FROSHEISER, 1964; BECZNER and MANNINGER, 1973). The bean test is also the basis of the screening method suggested by CRILL *et al.* (1970) for recognizing lucerne plants resistant to AMV. Additionally, the local lesions on primary French bean leaves are well adapted for determination of infectivity of AMV in crude saps and in preparations.

Recently in Europe the number of articles has increased in which AMV strains are described producing mostly chlorotic or necrotic local lesions on inoculated French bean leaves and later on becoming systemic (ŠUTIĆ, 1959; ZSCHAU, 1964; VERHOYEN, 1964; SCHMELZER *et al.*, 1972; BECZNER, 1972). However, the reaction of beans to AMV strains seems also to depend on experimental conditions. The aim of the present paper was to investigate comparatively the effect of temperature on reaction of beans against the two types of AMV strains, a problem which does not seem, to have been studied so far.

Materials and Methods

Phaseolus vulgaris L. cv. "Fullcrop" and "Pinto" were used as test plants at the age of 10 to 14 days, just when the primary leaves were fully expanded. They were inoculated mechanically. Inocula were prepared by grinding leaves of *Nicotiana tabacum*, breeding line "Bel 61 – 10", together with 0.067 *M* phosphate buffer pH 7.0 in sterile mortars, 7 to 10 days after infection. Inoculations were carried out with glass spatulas under normal greenhouse conditions. Carborundum was used as an abrasive. Inoculated leaves were rinsed with tap water immediately after rubbing. "Pinto" was inoculated only in preliminary experiments. The main test were performed with "Fullcrop".

The necrotic strain AM-K_2 and the systemic strains AM-N_1 and AM-14 of AMV were used for the trials (BECZNER and SCHMELZER, 1972).

After inoculation the bean plants were divided into three groups and put into climate chambers at 15 °C, 22 °C, and 30 °C. One, two, four or eight days after inoculation several plants of each group were transferred to the other two chambers for determining the effect of changing temperature in post-inoculation period.

The climate chambers were the same as described by WACHE and WOLFFGANG (1966). The temperature was always constant. The chambers were lighted by fluorescent tubes giving 1200 lux for 16 hours per day. Prominence, development and type of symptoms were observed daily. After 10 days, at the end of the experiment, the number of local lesions was counted and the average was calculated for one primary leaf. In one treatment 16 primary leaves were examined in addition, the size of the local lesions was determined.

Results

Number of local lesions and symptom expression at constant temperatures

The number of visible local lesions depended on temperature. As it is shown in Table 1, the necrotic AMV strain AM-K_2 in three trials as well as the systemic strains AM-N_1 and AM-14 induced the largest numbers of local lesions at 22 °C. With only one exception (AM-14) 30 °C was more favourable for local lesions number than 15 °C. At this temperature the expression of local lesions was delayed.

Table 1

Effect of constant temperatures on local lesion numbers of AMV strains

Strain	Number of experiment	Local lesions					
		at 15 °C		at 22 °C		at 30 °C	
		number	per cent	number	per cent	number	per cent
AM-K_2 (necrot.)	I.	12.6	9.3	135.8	100	79.2	58.3
	II.	30.1	6.0	~500	100	320.9	64.1
	III.	4.2	13.7	30.6	100	27.7	90.8
AM-N_1 (system.)		198.9	49.7	~400	100	213.8	53.5
AM-14 (system.)		130.2	32.6	~400	100	105.5	26.4

Table 2 shows that the local lesions induced by the systemic AMV strains were always chlorotic whereas plants inoculated with necrotic AMV formed necrotic local lesions. However, there were some differences in spreading of the local lesions according to various constant temperatures. At 22 °C the systemic strains induced systemic mottling. At 15 °C the bean plants developed no further leaves and therefore no symptoms caused by systemic spread of the virus strains could be recognized. At 30 °C the plants developed symptomless top leaves which

nevertheless contained the virus. Of special interest is the fact in all three trials the plants inoculated with the necrotic strain and kept at 15 °C developed stem necrosis. At the other temperatures this strain induced no systemic reactions.

Table 2

Effect of constant temperatures on symptom expression of AMV strains

	AM-14 and AM-N_1		AM-K_2	
	local	systemic	local	systemic
15 °C	chlorotic spots	plants do not develop	some small necrotic local lesions, large light green areas	stem necrosis and necrotic spots
22 °C	chlorotic spots and rings	chlorotic pattern	pronounced necrotic local lesions	no symptoms
30 °C	chlorotic spots and chlorotic pattern, especially along the veins	no symptoms	diffuse brown necrotic local lesions with pronounced centre	no symptoms

Number and size of local lesions at changing temperatures

Very often it was rather difficult to count the non-necrotic local lesions induced by the systemic AMV strains. Mostly they were only diffuse without defined borders and often they were confluent. For these reasons the numbers and diameters of local lesion induced by these strains were not recorded at changing temperatures. The results with the necrotic strain AM-K_2 are summarized in Table 3. In confirmation of the results at constant temperature the largest numbers of local lesions were formed at 22 °C, whereas the least numbers of lesions appeared at 15 °C. Generally, a temperature of 30 °C was not unfavourable for lesion numbers. One or two days first at 15 °C diminished the local lesion numbers only slightly. Four days were not detrimental if afterwards the plants were subjected to 22 °C. However, when the temperature was raised to 30 °C, the local lesion numbers were considerably lower. Two days at 22 °C or 30 °C followed by 15 °C were sufficient to induce rather high local lesion numbers, but only one day at the higher temperatures was insufficient.

The following results were obtained in respect of local lesions diameter. At a constant temperature of 15 °C only very small lesions were formed. Nevertheless, at this temperature the virus migrates, because even after only one day at 30 °C large necrotic spots appeared on the inoculated leaves. Even four days of low temperature immediately after infection were sufficient to induce a considerable enlargement of the lesions, when 30 °C or 22 °C followed. A constant temperature of 30 °C caused somewhat larger lesions than 22 °C. Alterations of temperature not especially had intermediate effects.

Table 3

Effect of different temperatures treatments on number and size of local lesions of AM-K_2 strain

Treatment (in days)		Local lesions		Treatment (in days)		Local lesions	
		percentage*	diameter (mm)			percentage*	diameter (mm)
15 °C	*22 °C*			*15 °C*	*3 °C*		
10	0	14	0.5	10	0	14	0.5
				9	1	27	7–8
4	6	79	3–4	4	6	34	3–4
2	8	65	1–1.5	2	8	74	1–1.5
1	9	66	1–1.5	1	9	105	1–1.5
22 °C	*15 °C*			*22 °C*	*30 °C*		
10	0	100	1.5–2	10	0	100	1.5–2
4	6	63	1.5–2	4	6	72	2–2.5
2	8	62	0.5–1	2	8	95	1.5–2
1	9	10	0.5–1	1	9	63	1 5–2
30 °C	*15 °C*			*30 °C*	*22 °C*		
10	0	90	2–2.5	10	0	90	2–2.5
4	6	95	1–1.5	4	6	91	2–2.5
2	8	82	1–1.5	2	8	93	1.5–2
1	9	7	0.5–1	1	9	101	1.5–2

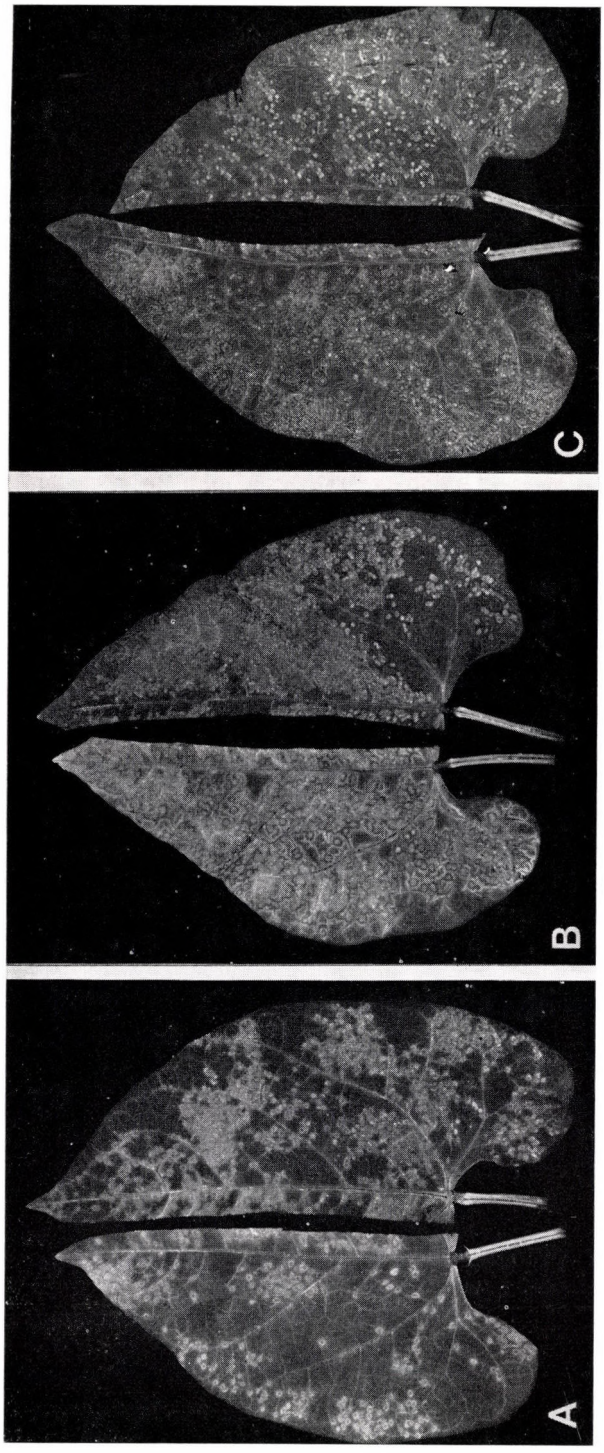
* An average local lesion number of 31 per leaf at a constant temperature of 22 °C was the basis for the calculation of the percentages

Symptom expression at changing temperatures

In Table 4 the symptoms of French beans inoculated with systemic and necrotic strains of AMV and subjected to different temperatures and temperature sequences are recorded.

Very often with the systemic strains but only seldom with the necrotic strain the local lesions had the shape of rings. More often halos showed that the virus could migrate from the original infected cells to surrounding tissue. Obviously, both the temperatures of 15 °C and 30 °C broke down the resistance to local migration of the necrotic AMV strain. The degree of this phenomenon depended on the sequence and the duration of the temperatures. 15 °C had more effect, than 30 °C. In case of the non-necrotic systemic AMV strains, constant high or low temperature followed by high temperature induced spread of symptoms on the inoculated leaves.

In nearly all cases the systemic strains induced systemic infections but only differences in leaf colour and no necrosis. The one exception was a treatment of two days at 22 °C, followed by 15 °C after which mild diffuse necrosis was shown. Systemic symptoms were not visible when the plants developed no top leaves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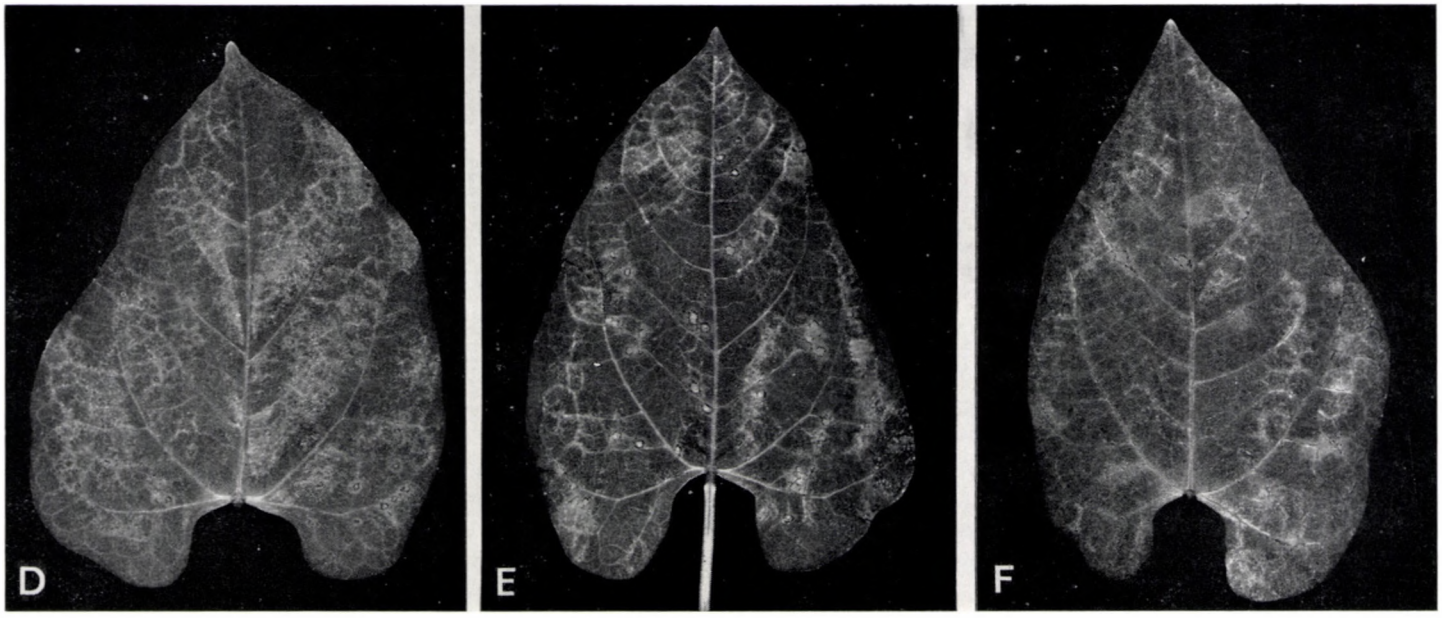

Fig. 1. Symptom expression of alfalfa mosaic virus strains on inoculated leaves of *Phaseolus vulgaris*, cv. "Full crop" ten days after inoculation, maintained at different temperatures. A–C: Systemic strain AM-N_1, D–F: necrotic strain AM-K_2. A: left: 1 d 22 °C + + 9 d 15 °C, right: 10 d 22 °C; B: left: 4 d 15 °C + 6 d 30 °C, right: 4 d 30 °C + 6 d 15 °C, C: left: 1 d 15 °C + 9d 30 °C, right: 10 d 30 °C; D: 2 d 15 °C + 8 d 22 °C; E: 4 d 15 °C + 6 d 22 °C; F: 4 d 15 °C + 6 d 30 °C

Table 4

Effect of different temperature treatments on symptom expression of AMV strains[1]

Treatment (in days)		AM-14 and AM-N1		AM-K_2	
		local	systemic	local	systemic
15°	*22°*				
10	0	chlorotic spots	plants do not develop	small necrotic spots, large light green areas	stem necrosis and necrotic spots
9	1			large necrotic spots	stem and top necrosis, death of the plants
4	6	pronounced chlorotic spots	vein clearing	brown necrotic rings (Fig. 1, E)	no symptoms
2	8	chlorotic spots	yellow spots	brown necrotic spots with light green halo (Fig. 1, D)	no symptoms
1	9	chlorotic spots and rings	vein clearing, chlorotic spots	pronounced brown necrotic spots	no symptoms
15°	*30°*				
4	6	chlorotic spots with halo (Fig. 1, B left)	plants do not develop	brown necrotic rings (Fig. 1, F)	no symptoms
2	8	chlorotic spots surrounded by light green rings	a few chlorotic spots	necrotic spots with diffuse necrotic halo	no symptoms
1	9	chlorotic spots surrounded by chlorotic rings (Fig. 1, C left)	no symptoms	necrotic spots with diffuse necrotic halo	no symptoms
22°	*15°*				
10	0	chlorotic spots and rings (Fig. 1, A right)	chlorotic patterns	pronounced brown necrotic spots	no symptoms
4	6	pronounced chlorotic rings	strong chlorotic pattern along the veins	necrotic spots with small necrotic halo	no symptoms
2	8	chlorotic spots, mild diffuse necrosis	little chlorotic leaves	necrotic spots, surrounded by diffuse necrosis	no symptoms
1	9	chlorotic spots and rings (Fig. 1, A left)	vein clearing	a few necrotic spots, chlorotic areas	some necrotic spots

Table 4 (continued)

Treatment (in days)		AM-14 and AM-N1		AM-K_2	
		local	systemic	local	systemic
22°	*30°*				
4	6	pronounced chlorotic spots and rings	chlorotic pattern	pronounced necrotic spots with small necrotic halo	no symptoms
2	8	chlorotic rings	chlorotic spots	necrotic spots surrounded by diffuse necrosis	no symptoms
1	9	chlorotic rings and irregular chlorotic pattern	no symptoms	pronounced necrotic spots	no symptoms
30°	*15°*				
10	0	chlorotic spots and chlorotic patterns especially along the veins (Fig. 1, C right)	no symptoms	diffuse necrotic spots without pronounced centre	no symptoms
4	6	chlorotic spots and rings (Fig. 1, B right)	mild vein clearing	diffuse asteroid necrotic spots	no symptoms
2	8	chlorotic spots surrounded by dark green rings	vein clearing and chlorosis	pronounced necrotic spots surrounded by diffuse necrosis	no symptoms
30°	*15°*				
1	9	chlorotic spots, later remaining green when the leaf becomes yellow	vein clearing	a few necrotic spots	necrotic spots
30°	*22°*				
4	6	chlorotic spots, surrounded by light green rings	vein clearing and chlorotic spots	solid brown necrotic spots	no symptoms
2	8	chlorotic spots and rings	chlorotic spots	necrotic spots	no symptoms
1	9	pronounced chlorotic spots and rings	vein clearing and chlorotic spots	pronounced necrotic spots	no symptoms

[1] In all the cases symptoms were described ten days after inoculation

during the time of observation due to low temperature or when the plants grew for at least 9 days at 30 °C. The necrotic strain produced necrotic systemic symptoms at a constant temperature of 15 °C, and a few necrotic spots on top leaves when only one day at 22 °C or 30 °C after infection the plants grew for nine days at 15 °C. Already two days after infection at the higher temperatures prevented the appearance of systemic symptoms. In a further trial, not recorded in Table 4, 8 days at 15 °C followed by 2 days at 22 °C induced chlorotic spots with necrotic cent on the top leaves (Fig. 2).

Fig. 2. Symptom expression of the necrotic alfalfa mosaic virus strain AM-K_2 ten days after inoculation. The plant was maintained 8 d at 15 °C followed by 2 d at 22 °C. Note the symptoms of the top leaf

Discussion

As already mentioned, the effect of temperature on symptom expression of AMV was studied earlier only with strains which induce necrotic local lesions on French beans. The papers described effects of short periods before or after infection (PANZER, 1958; BODNÁR and KVIČALA, 1968). According to BODNÁR

and KVIČALA (1958) and CRILL *et al.* (1970) high temperatures in the post inoculation period decreased local lesion numbers. This is in agreement with our results.

However, as far as we could see, no earlier paper has dealt with the effect of exactly maintained low temperature after infection of French beans. Therefore it is of special interest that we found low temperature to be the cause of systemic spread of a typical necrotic and localized AMV strain in *Phaseolus vulgaris*. Earlier results obtained by one of us and by other authors may be explained by this observation. It was stated that an AMV isolate from *Viburnum opulus* L. was able to induce necrosis of top leaves and even death of *Phaseolus vulgaris* plants. This reaction was only obtained in trials during spring and not in summer (SCHMELZER, 1962/63; BURKE, 1963), working in USA, Washington state, found that lucerne samples taken in fall, winter or spring very often induced systemic necrosis in *Phaseolus vulgaris* whereas those taken in August induced local infections and only exceptional systemic necrosis. In the trial with the *Viburnum* isolate the systemic necrotic reaction occurred after lower temperatures than normal in the greenhouse. Unfortunately, nothing is said about greenhouse temperature in Washington, but it may be that due to the rather cold climate in this region there were also rather low temperatures. We cannot claim that no AMV strains are able to induce systemic necrosis in bean at higher temperatures. Some isolates may induce a large number of local lesions on inoculated leaves of *Phaseolus vulgaris* and this fact seems to favour systemic infections. The cause of large local lesion numbers is obviously a high virus concentration which may be a special property of such isolates. On the other hand, it seems advisable not to believe that all systemic necrotic reactions in French beans are due to strains clearly distinguished from other ones. This question should be investigated more in detail, because until now the systemic reaction of French beans – chlorotic as well as necrotic – was believed to be a reliable distinctive mark for AMV strains (BANCROFT *et al.*, 1960). Without any doubt, however, the AMV strains inducing systemic chlorotic mottle only on *Phaseolus vulgaris* are quite different from the necrotic strains.

The systemic necrosis at low temperatures by otherwise local necrotic strains may also occur in *Vigna sinensis*. This is indicated by results in the trials earlier mentioned with the *Viburnum* isolate of AMV.

In the virus–host combination of AMV and French bean the variance in diameter of necrotic local lesions due to post-inoculation temperature is not so obvious as in case of TMV and *Nicotiana* species (KASSANIS, 1952; LI and SCHMELZER, 1964; BRČÁK *et al.*, 1968; GÁBORJÁNYI and EL HAMMADY, 1969). SHIMOMURA (1971) reported that in TMV-infected *Nicotiana glutinosa* there is a virus limiting factor which localizes the virus at lower temperatures. Higher temperatures inactivate this factor reversibly. If such factor is also involved in the combination of French bean and necrotic AMV strains, it seems to be more inactivated at lower than at higher temperatures. In this connection it may be mentioned that KASSANIS (1952) as well as LI and SCHMELZER (1964) found no pronounced activating effect of high temperatures on the spread of necrosis due to normal TMV strains of *Phaseolus vulgaris*. The non-necrotic AMV strains, however, induced

more extended local symptoms at higher temperatures. On the other hand, these strains spread to the top leaves obviously always when temperatures allowed new leaves to develop. Duration of 30 °C for 9 or 10 days did not seem to prevent systemic infection but symptom expression of systemic AMV strains on top leaves. This may be the same phenomenon as with lucerne plants under field conditions. After infection with various AMV strains they can show yellow spotting in spring time when temperatures are still relatively low but not at higher temperatures in summer time.

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Identification and Serological Properties of Tomato Mosaic Virus Isolated in Hungary

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Identification of tomato mosaic virus (ToMV) in tomato plants (*Lycopersicon esculentum* cv. Moneymaker) is reported from Hungary. The virus ToMV-H was identified on the basis of test plants reaction, cell inclusions, serological properties and physical properties *in vitro*. It caused only local lesions on various test plants (e.g. *Datura stramonium*, *Nicotiana glutinosa*, *N. sylvestris*), while certain plants, such as *Chenopodium amaranticolor*, *Ch. quinoa* and several species of *Browallia* reacted with local and systemic symptoms.

Twenty six plant species (*Aptenia cordifolia*, *Browallia cordata*, *B. demissa*, *B. grandiflora*, *B. roezli*, *B. viscosa*, *Cucumis myriocarpus*, *Erodium ciconium*, *E. cicutarium*, *E. gruinum*, *E. moschatum*, *Gomphrena decumbens*, *Ocimum basilicum*, *O. canum*, *Pentstemon alpinus*, *P. attenuatus*, *P. calycosus*, *P. cardinalis*, *P. hirsutus*, *P. laevigatus*, *P. murrayanus*, *P. ovatus*, *P. whippleanus*, *Roripa islandica*, *R. sylvestris* and *Tetragonia echinata*) were demonstrated as new experimental hosts of ToMV-H.

The virus induced hexagonal virus crystals in cells, which are characteristic of tobacco mosaic and tomato mosaic viruses. ToMV-H proved serologically identical with the type "*Dahlemense*" isolate of ToMV. The Hungarian isolate of ToMV had a thermal inactivation point between 92 and 94 °C, dilution end-point between 2×10^{-6} and 10^{-6}, and retained its infectivity *in vitro* after a period of two months. In desiccated tobacco leaf tissue it remained highly infectious after two years storage.

Introduction

Tomato plants are often affected by viruses, either in field or in greenhouse. The list of viruses isolated from this species hitherto is fairly long and includes well-known and wide-spread viruses (cf. SOLYMOSY, 1959; THORNBERRY, 1966; HORVÁTH, 1968; KLINKOWSKI, 1968; SCHMELZER and WOLF, 1971; SMITH, 1972). Isolates associated with tobacco mosaic virus (TMV, R/1 : 2/5 : E/E : S/*) have been repeatedly found in tomato plants. One of them was the so-called tomato mosaic virus (ToMV, "*Dahlemense*" isolate) described for the first time by MELCHERS *et al.* (1940). For a fairly long period TMV-like viruses from tomato have been considered as strains of TMV, but none of them was confirmed to be a separated virus other than TMV. However, KNIGHT (1963) and WITTMANN (1965) established differences in chemical composition between common TMV and the

"*Dahlemense*" isolate of TMV. Afterwards WANG and KNIGHT (1967) and WETTER (1968) found distinct serological differences between common TMV and tomato isolates associated with TMV. On the basis of those data the mentioned authors concluded that some TMV-related isolates from tomato represent an individual virus named tomato mosaic virus (ToMV), belonging to the same group of viruses as common TMV, that is to tobamovirus group (cf. HARRISON *et al.*, 1971). Recently, MOSCH *et al.* (1973) found the same buoyant density and the same *S* value between 18 TMV-isolates from tomato. The common strain ("Vulgare" strain) of TMV differed from the others only in buoyant density.

Tomato mosaic virus, sometimes still named tomato strain of TMV, has been isolated from tomato plants by more authors (cf. VERONA and TREGGI, 1957; NAGAICH, 1958; GIGANTE, 1958; MILLER and THORNBERRY, 1958; BROADBENT, 1962; BROADBENT and FLETCHER, 1966; WANG and KNIGHT, 1967; RAST, 1967; JURETIĆ, 1970; LISA and LOVISOLO, 1973). These isolates originated from distant regions in the world.

Materials and Methods

The virus isolate ToMV-H (earlier designated by TMV-T) from tomato plants (*Lycopersicon esculentum* cv. Moneymaker) which is the subject of this paper, was isolated in Hungary in 1972 (cf. HORVÁTH and BECZNER, 1973). Tomato plants were collected in the areas where tomato cultivation was developed. Infected plants exhibited mild mosaic and fern leaf symptoms, as well as moderate stunting.

For comparative purposes three tobamoviruses, i.e. common TMV, Holmes' ribgrass mosaic virus (RMV) – both originating from Yugoslavia (JURETIĆ *et al.*, 1969) – and the "*Dahlemense*" (type) isolate of ToMV (ToMV-D, cf. MELCHERS *et al.*, 1940) were involved in these investigations, particularly in serological experiments.

ToMV-H was studied by means of test plants, investigations of cytoplasmic inclusion bodies in light microscope and serologically, as well as with respect to its physical properties.

Mechanical inoculation was carried out with diluted infectious sap, the leaves being previously dusted with carborundum (500 mesh). After inoculation test plants were rinsed with water.

Light microscope observations were performed on living cells in several plants, but especially on systemically infected leaves of *Lycopersicon esculentum*, *Nicotiana tabacum* cv. White Burley and *Chenopodium amaranticolor* plants. In the first two plants quoted, cca 0.5 mm wide and some millimeters long area on the margin of leaf blade was cut and marginal hair-cells were examined. In *Chenopodium amaranticolor*, lower epidermis strips taken from midrib region of the leaves were observed. Better transparence of the preparations in the latter

case was achieved by replacing gas content from the intercellular spaces with tap water, by means of water suction pump.

The viruses ToMV-H, ToMV-D, common TMV and RMV were purified according to the procedure of GOODING and HEBERT (1967) with small changes after WETTER and JURETIĆ (not published) concerning the clarification step. This included the presence of 0.2% DIECA, 0.2% Na_2SO_3 and 0.05 *M* EDTA in 0.01 *M* pH 7.5 phosphate buffer and the use of 1 : 1 (v/v) chloroform-(n) butanol mixture. Such a mixture was added to strained homogenate in the ratio of 1 : 4 (v/v). Yield and purity of viruses was checked spectrophotometrically and serologically. Antisera against three tobamoviruses, i.e. ToMV-D, common TMV and RMV were applied to serological experiments. These antisera have been prepared earlier (JURETIĆ *et al.*, 1969; JURETIĆ, 1971). Determination of homologous and heterologous antisera titres was performed through microprecipitin drop method. Immunodiffusion experiments were set in 0.9% Difco Noble agar in saline containing 0.025% NaN_3. Straight gel-diffusion tests (spur test) were performed after VAN REGENMORTEL (1967) and intragel absorption tests after WETTER and LUISONI (1969). Serological reactions in agar were observed during a period of three days after being set.

Physical properties (thermal inactivation point, dilution end-point, ageing *in vitro*, storage in leaves dehydrated over $CaCl_2$) were performed after HORVÁTH (1966), except that *Nicotiana glutinosa* and *N. tabacum* cv. Xanthi-nc were used as test plants.

Results

Investigations on test plants

The Hungarian virus isolate from tomato plants (ToMV-H) was transmitted through sap inoculation to 42 herbaceous plants belonging to 11 families. These plants are listed in Table 1, together with symptoms that appeared on their leaves (Figs 1 and 2). Generally, the reactions of test plants corresponded to those provoked by some tobamoviruses. In this respect particularly interesting was the reaction of *Datura stramonium*, and *Nicotiana glutinosa* which both repeatedly exhibited symptoms on inoculated leaves only. However, the lack of systemic symptoms in *Nicotiana sylvestris* indicated that ToMV-H differed from common TMV. Reaction of some valuable differential test plants following inoculation with ToMV-H and several common tobamoviruses is shown in Table 2.

As visible in Table 2, ToMV-H and ToMV-D caused completely similar symptoms on all the differential test plants quoted. They both produced symptoms partially similar to those of TMV only in *Lycopersicon esculentum*. ToMV-H and ToMV-D differed symptomatologically from RMV on *Chenopodium quinoa* and *Lycopersicon esculentum* (see Table 2). The following plants were found

Table 1

Reaction of test plants following inoculation with the Hungarian isolate of tomato mosaic virus (ToMV-H) from Hungary

Inoculated plants	Symptoms*
AIZOACEAE	
Aptenia cordifolia (L.) Schwantes**	IL: Large grey necrotic lesions NIL: No symptoms, immune
Tetragonia echinata Ait.**	IL: Necrotic lesions NIL: No symptoms
Tetragonia tetragonoides (Pall.) O. Ktze	IL: Necrotic lesions NIL: No symptoms
AMARANTHACEAE	
Gomphrena decumbens Jacq.**	IL: Chlorotic and later necrotic spots NIL: Chlorotic and brown necrotic lesions
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	IL: Numerous, 1—2 mm large chlorotic lesions three or more days a. i., usually with simple or ringlike necrotic centre (sometimes minute) immediatelly or later; during spring till autumn brown-red ring of living cells on the periphery of lesions (Fig. 1B) NIL: Chlorotic (in older stages of infection mainly) and necrotic spots, often close to leaf veins, a week or more a. i.; leaf malformations and stunting (Fig. 1A)
Chenopodium murale L.	IL: Numerous necrotic lesions in 3—4 days a. i.; lesions size 1—2 mm NIL: Chlorotic and necrotic spots 1—2 mm large, 10 days a. i.; leaf malformations, stunting, sometimes mosaic (Fig. 1C)
Chenopodium quinoa Willd.	IL: A great number of chlorotic lesions cca 4 days a. i.; some lesions later convert to necrotic lesions NIL: Two weeks after inoculation or somewhat earlier rare chlorotic spots up to 1 cm large, seldom with normally green centre; sometimes sporadic chlorotic bands along bigger veins (Fig. 1D)
*CRUCIFERAE****	
Roripa islandica (Oeder) Borb.**	IL: Susceptible without symptoms NIL: Susceptible without symptoms
Roripa sylvestris (L.) Besser**	IL: Susceptible without symptoms NIL: Susceptible without symptoms
CUCURBITACEAE	
Cucumis myriocarpus Naud.**	IL: Chlorotic, later necrotic lesions 3—4 days a. i. NIL: No symptoms, immune
GERANIACEAE	
Erodium ciconium (L.) L'Hérit ex Ait.**	IL: Susceptible without symptoms NIL: No symptoms, immune
Erodium cicutarium (L.) L'Hérit ex Ait.**	IL: Susceptible without symptoms NIL: No symptoms, immune
Erodium gruinum (L.) L'Hérit ex Ait.**	IL: Susceptible without symptoms NIL: No symptoms, immune

Table 1 (continued)

Inoculated plants	Symptoms*
Erodium moschatum (L.) L'Hérit ex Ait.**	IL: Susceptible without symptoms NIL: No symptoms, immune
LABIATAE	
Ocimum basilicum L.**	IL: 2—4 mm large necrotic lesions violet-brown coloured, 10 days a. i.; some lesions are of the zonal type (Fig. 2C) NIL: No symptoms, immune
Ocimum canum Sims**	IL: Necrotic local lesions violet-brown coloured NIL: No symptoms, immune
LEGUMINOSAE	
Phaseolus vulgaris L. cv. Pinto	IL: No symptoms, immune NIL: No symptoms, immune
Phaseolus vulgaris L. cv. Red Kidney	IL: No symptoms, immune NIL: No symptoms, immune
PLANTAGINACEAE	
Plantago major L.	IL: 2 mm large chlorotic lesions in a week a. i. NIL: About 2 mm large chlorotic spots
SCROPHULARIACEAE	
Pentstemon alpinus Torr.**	IL: Irregular severe chlorotic, later necrotic lesions NIL: No symptoms, immune
Pentstemon attenuatus Dougl.**	IL: Chlorotic and brown necrotic lesions NIL: No symptoms, immune
Pentstemon calycosus Small.**	IL: Weak chlorotic and necrotic lesions NIL: No symptoms, immune
Pentstemon cardinalis Woot. et Standl.**	IL: Severe chlorotic and necrotic lesions NIL: No symptoms, immune
Pentstemon hirsutus (L.) Willd.**	IL: Severe chlorotic and later necrotic lesions NIL: No symptoms, immune
Pentstemon laevigatus Ait.**	IL: Weak chlorotic and necrotic lesions NIL: No symptoms, immune
Pentstemon murrayanus Hook.**	IL: Weak and small chlorotic and necrotic lesions NIL: No symptoms, immune
Pentstemon ovatus Dougl.**	IL: Weak and small chlorotic and necrotic lesions NIL: No symptoms, immune
Pentstemon whippleanus A. Gray**	IL: Weak and small chlorotic and necrotic lesions NIL: No symptoms, immune
SOLANACEAE	
Browallia cordata G. Don.**	IL: Necrotic lesions, premature leaf dropping NIL: Mosaic, leaf deformations, necrotic lesions, premature leaf dropping; symptoms of the same type on axillary shoots
Browallia demissa L.**	IL: Necrotic lesions, premature leaf dropping NIL: Mosaic, leaf deformations, necrotic spots, premature leaf dropping; symptoms of the same type on axillary shoots
Browallia grandiflora R. Grah.**	IL: Necrotic lesions, premature leaf dropping NIL: Mosaic, leaf deformations, necrotic spots, premature leaf dropping; symptoms of the same type on axillary shoots

Table 1 (continued)

Inoculated plants	Symptoms*
Browallia roezli Nichols.**	IL: Necrotic lesions, premature leaf dropping NIL: Mosaic, leaf deformations, necrotic spots, premature leaf dropping; symptoms of the same type on axillary shoots (Fig. 1E)
Browallia viscosa H. B. et K.**	IL: Necrotic lesions, premature leaf dropping NIL: Mosaic, leaf deformations, necrotic spots, premature leaf dropping; symptoms of the same type on axillary shoots
Datura stramonium L.	IL: 1–2 mm large necrotic lesions three or more days a. i. NIL: No symptoms, immune
Lycopersicon esculentum Mill.	IL: No symptoms, latent susceptible; some varieties (e.g., Ruttgers, Pigmeo, Pécs Gyöngye, Humbert König, Essex Wonder, Carrick, Carro Red, Beste von Allen) reacted 2 weeks a. i. with brown necrotic lesions (cf. HORVÁTH and BECZNER 1973, pp. 227) NIL: Mild mosaic in 2 weeks a. i., growth reduction
Nicandra physaloides (L.) Gaertn.	IL: No symptoms, latent susceptibility NIL: 2 weeks a. i. necrosis along leaf veins surrounded by larger chlorotic areas
Nicotiana glutinosa L.	IL: Numerous 1–3 mm large necrotic lesions 2–3 days a. i. NIL: No symptoms, immune
Nicotiana sylvestris Speg. et. Comes	IL: 3–4 days a. i. dark brown necrotic lesions up to 5 mm in size when full developed; later several concentric rings (layers) can be seen in some lesions (Fig. 2B) NIL: No symptoms, immune
Nicotiana tabacum L. cv. Turkish (syn.: *N. tabacum* L. cv. Samsun)	IL: 3–4 days a. i. brown necrotic lesions 1–2 mm in diameter; lesions do not appear regularly. In the experiments of HORVÁTH and BECZNER (1973) Samsun tobacco reacted only with systemic symptoms NIL: Interveinal blister-like deformations, sometimes with green islands scattered on leaf blade; severe mottling and mosaic (Fig. 2D)
Nicotiana tabacum L. cv. White Burley	IL: 3–4 days a. i. brown necrotic lesions of 1–2 mm diameter (Fig. 2A) NIL: Interveinal blister-like deformations (somewhat milder than in Samsun tobacco); mottling and mosaic, sometimes vein banding
Nicotiana tabacum L. cv. Xanthi-nc	IL: Expressive brown necrotic lesions 2–3 days a. i. NIL: No symptoms, immune
Physalis alkekengi L.	IL: Necrotic lesions occasionally NIL: Mosaic, leaves blistered

* IL: Inoculated leaves, NIL: Non inoculated or subsequently developed leaves

** New test plants of ToMV

*** A tobamovirus (ribgrass mosaic virus) was recorded spontaneously infecting a single species of the genus *Roripa* (*Roripa amphibia* [L.] Bess), a member of the family *Cruciferae* (JURETIĆ *et al.* 1973)

as new experimental hosts for ToMV: *Aptenia cordifolia*, *Browallia cordata*, *B. demissa*, *B. grandiflora*, *B. roezli*, *B. viscosa*, *Cucumis myriocarpus*, *Erodium ciconium*, *E. cicutarium*, *E. gruinum*, *E. moschatum*, *Gomphrena decumbens*, *Ocimum*

Table 2

Reaction of some differential test plants for tobamoviruses following inoculation with ToMV-H, ToMV-D, TMV and RMV*

Test plants	Viruses**			
	ToMV-H	ToMV-D	TMV	RMV
Chenopodium amaranticolor Coste et Reyn.	LS	LS	L	LS
Chenopodium quinoa Willd.	LS	LS	L	L
Lycopersicon esculentum Mill.	S	S	S	L
Nicotiana sylvestris Speg. et Comes	L	L	LS	L
Plantago major L.	LS	LS	L	LS

* L: Symptoms on inoculated leaves; S: systemic symptoms; LS: Local and systemic symptoms

** ToMV-H: Hungarian isolate of tomato mosaic virus from tomato; ToMV-D: "*Dahlemense*" isolate of tomato mosaic virus; TMV: Common tobacco mosaic virus; RMV: Strain of Holmes' ribgrass mosaic virus from Yugoslavia

basilicum, *O. canum*, *Pentstemon alpinus*, *P. attenuatus*, *P. calycosus*, *P. cardinalis*, *P. hirsutus*, *P. laevigatus*, *P. murrayanus*, *P. ovatus*, *P. whippleanus*, *Roripa islandica*, *R. sylvestris* as well as *Tetragonia echinata*.

Inclusion bodies

Cytological observations were performed on plants infected with ToMV-H in order to establish type of cytoplasmic inclusion bodies, if present. In *Lycopersicon esculentum* and *Nicotiana tabacum* cv. White Burley leaf hair-cells well developed crystalline inclusions in form of more or less regularly shaped hexagonal prisms were found (Fig. 3A). In a single hair-cell one to several, sometimes more than 10 such crystals could be seen. *Chenopodium amaranticolor* leaf epidermis cells also contained virus crystals of the same type which were somewhat less frequently of regular hexagonal shape (Fig. 3B and C) than in *Lycopersicon esculentum* and *Nicotiana tabacum*. In average, the crystals were present in greater number but were of smaller size in *Chenopodium amaranticolor* than in the two mentioned solanaceous species. In the three above-quoted species the crystals were often clustered inside a cell in one larger aggregate (Fig. 3B and C).

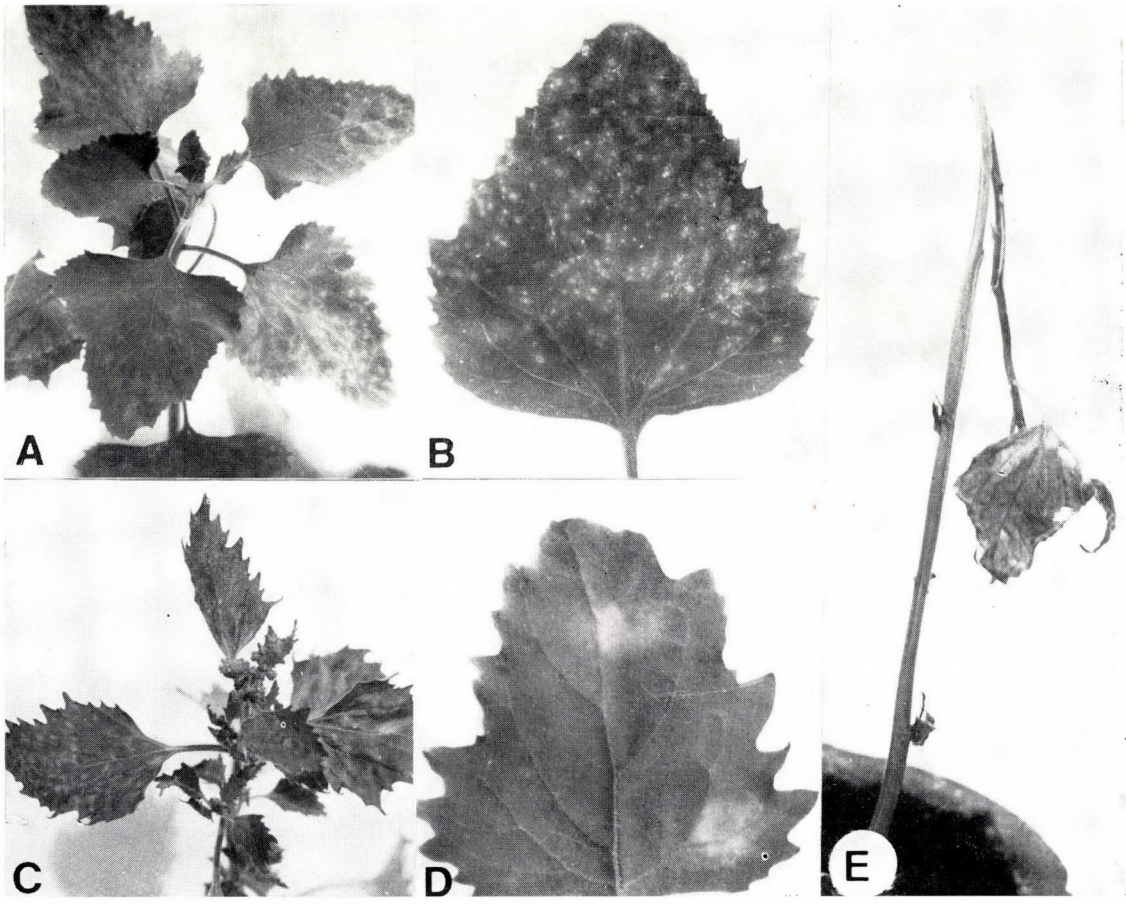
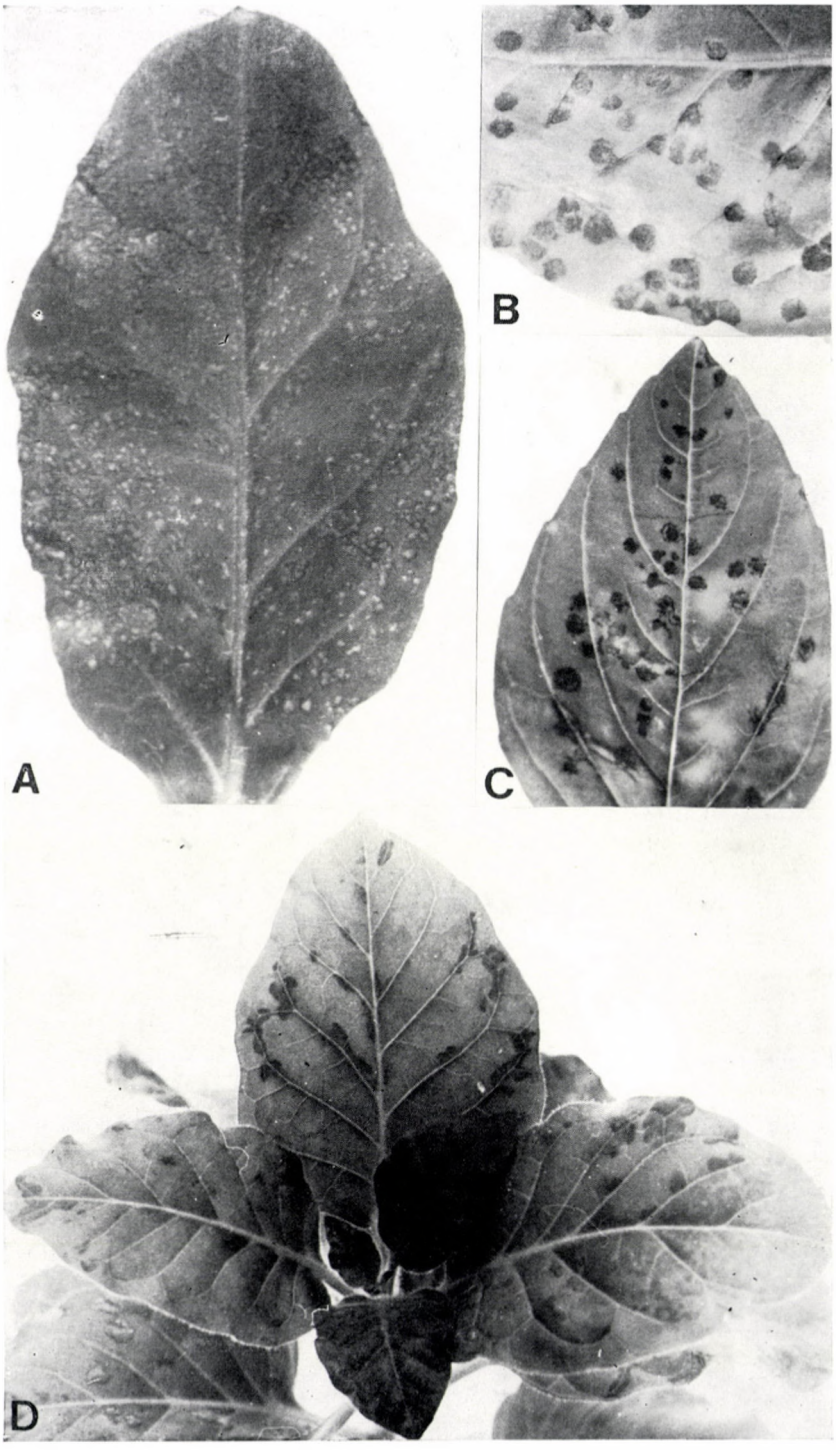


Fig. 1. Reactions of various test plants to the infection with Hungarian isolate of tomato mosaic virus (ToMV-H) from tomato. A: Systemic chlorotic spots on leaves of *Chenopodium amaranticolor* Coste et Reyn.; B: chlorotic-necrotic lesions on inoculated leaf of *Chenopodium amaranticolor*; C: *Chenopodium murale* L. with systemic chlorotic and necrotic spots and leaf deformations, also exhibiting reduction in leaf size; D: chlorotic spots on the subsequently developed leaf of *Chenopodium quinoa* Willd., E.: systemically infected *Browallia roezli* Nichols. plant

Fig. 2. Reactions of various test plants to the infection with ToMV-H. A: necrotic local lesions on inoculated leaf of *Nicotiana tabacum* L. cv. White Burley; B: necrotic lesions on inoculated leaf of *Nicotiana sylvestris* Speg et Comes; C: *Ocimum basilicum* L., inoculated leaf with dark brown-violet coloured lesions; some lesions are of zonal type; D: systemic symptoms on *Nicotiana tabacum* L. cv. Samsun leaves →


A
B
C
D

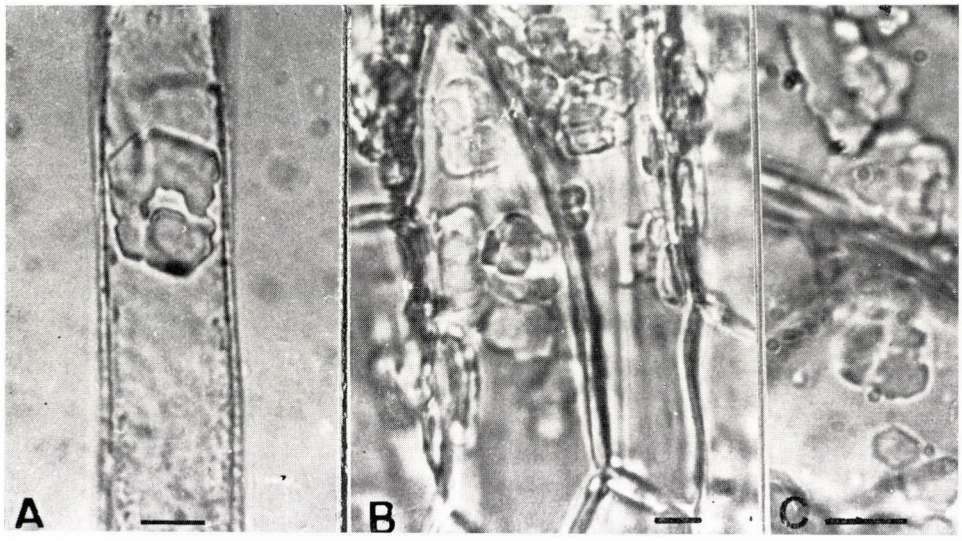


Fig. 3. Crystalline inclusion bodies of ToMV-H in systemically infected leaves of artificially infected plants. A: *Lycopersicon esculentum* Mill., leaf hair-cell containing hexagonal prism in stage of disaggregation; B: and C: *Chenopodium amaranticolor* Coste et Reyn. leaf lower epidermis cells (midrib region) containing numerous inclusions (partially aggregated); some inclusions are of the hexagonal shape. A, B, and C: Bar represents 5 μm

Purification

Partially purified preparations of ToMV-H, ToMV-D, common TMV and RMV gave a weak serological precipitation of normal plant proteins, while the virus precipitation followed relatively high dilutions of the preparations. When assayed spectrophotometrically the preparations showed value of 1.2 for the ratio $E_{260/280}$.

Serology

First ToMV-H was tested with antiserum against common TMV, using microprecipitin drop method. The reaction was positive, followed by occurrence of very expressive precipitation which showed that the virus belonged to tobamoviruses. However, ToMV-H reacted with this serum to a serum titre which was several steps of serum twofold dilutions lower than the homologous one (Table 3). Therefore a further experiment was undertaken in which ToMV-H was compared with ToMV-D and RMV using antisera of the two latter viruses. As shown in Table 3, ToMV-H reacted with ToMV-D-antiserum to the same antiserum dilution as did the homologous virus. The reaction between ToMV-H and RMV-antiserum was, on the contrary, very weak, as compared with the homologous

Table 3

Results of determinig homologous and heterologous titres of antisera to TMV, ToMV-D and RMV*

Antiserum to	Viruses**			
	TMV	RMV	ToMV-D	ToMV-H
TMV	1/512	(−)***	(−)	1/32
RMV	(−)	1/256	(−)	1/4
ToMV-D	(−)	(−)	1/256	1/256

* Determined in microprecipitin drop test

** TMV: Common tobacco mosaic virus; RMV: A strain of Holmes' ribgrass mosaic virus from Yugoslavia; ToMV-D: "*Dahlemense*" (type) isolate of tomato mosaic virus; ToMV-H: Hungarian isolate of tomato mosaic virus from tomato

*** Not tested (−)

reaction (see Table 3), so that the difference between RMV-antiserum homologous and heterologous titre was comparatively larger than in TMV-antiserum. All these results indicated close serological similarity of ToMV-H and ToMV-D.

Some additional experiments were carried out afterwards by means of straight gel-diffusion method. In these experiments ToMV-H and TMV reacted with TMV-antiserum and spur formation occurred. A similar reaction was obtained when ToMV-D and TMV were compared by means of the same antiserum (Fig. 4A). Analogous result was achieved when ToMV-D-antiserum was employed in place of the TMV one (Fig. 4B). However, when ToMV-D were compared using ToMV-D-antiserum only the coalescence of the precipitation lines without any spur formation appeared, i.e. the reaction of identity was obtained (Fig. 4C). The reaction of ToMV-H with the antiserum to RMV in agar-gel was too weak to compare these two viruses by means of gel diffusion methods.

The results of straight gel diffusion experiments were checked through intragel absorption experiments. Antisera to TMV and ToMV-D were absorbed in separate experiments with ToMV-H. Using such absorbed antisera, homologous reaction occurred only with TMV but not with ToMV-D (Fig. 4D and E). This confirmed the results of the foregoing serological experiments. Thus, ToMV-H was found to be serologically identical or very closely related to ToMV-D, but conspicuously different from TMV and RMV (Fig. 4F).

Physical properties

Thermal inactivation point of ToMV-H ranged between 92 °C and 94 °C. The dilution end-point was in the range from 2×10^{-6} to 10^{-6}, the longevity *in vitro* was greater than two months. When kept in dried Samsun tobacco leaf tissue at 3 − 5 °C, ToMV-H remained highly infectious after a period of two years.

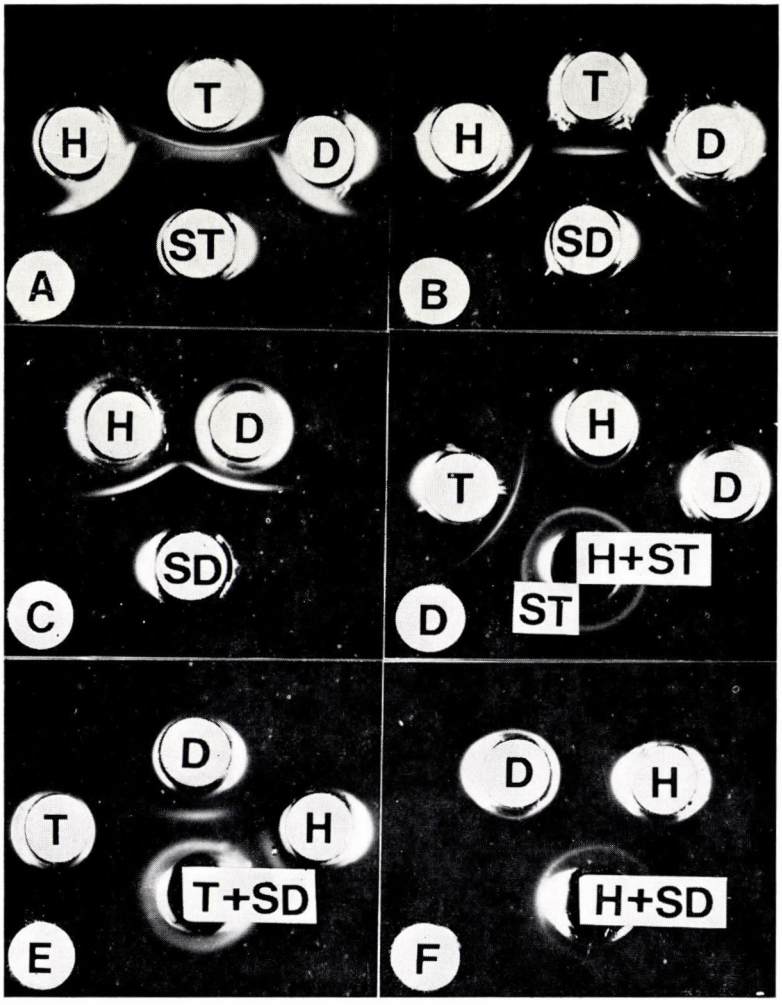


Fig. 4. Serological agar gel double diffusion reactions of ToMV-H (H), ToMV-D (D) and common TMV (T) with antisera to common TMV (ST) and ToMV-D (SD). A, B and C: Straight gel diffusion experiments (spur test); D, E and F: intragel absorption experiments. A: Comparison of H with T and D by means of antiserum ST; precipitation band of T formed spur with the ones of H and D; B: analogous experiment as in A but with antiserum SD; in this case precipitation lines of D and H formed spur with line of T; C: comparison of H with D using antiserum SD; precipitation lines of D and H coalesced without forming spur; D: serum ST previously absorbed with H (H + ST) still reacted with the homologous virus T; D did not react, the same as H did not; E: similar as in D but with serum SD which was absorbed with T (T + SD); homologous virus and H reacted quite similar with residual antibodies which could not be absorbed by T; F: D did not react with its homologous serum previously absorbed through H (H + SD), which showed the identity of D and H viruses

Discussion

Our investigations on ToMV-H isolate supported the conclusion that this virus was the isolate of ToMV but not common TMV in several aspects. This became obvious from serological experiments in particular. Moreover, serological experiments revealed serological identity of ToMV-H and ToMV-D, at least through the use of ToMV-D antiserum. This fact is in agreement with the results obtained by WANG and KNIGHT (1967), who found that 13 isolates of ToMV from tomato plants originating from very distant regions were serologically identical with ToMV-D and mutually. JURETIĆ (1971) also established serological identity of one ToMV isolate from Yugoslavia and ToMV-D. The results of the just mentioned authors together with the results of the present paper indicate insignificant or no serological variability in ToMV isolates from tomato plants. Similar is in isolates from some other plants (cf. FACCIOLI and PAUL, 1967), including woody hosts (OPEL *et al.*, 1969). Conversely, isolates of some other tobamoviruses, as for instance RMV exhibit quite distinct serological differences between each other (JURETIĆ *et al.*, 1969; JURETIĆ and WETTER, 1973; JURETIĆ, 1974).

It seems that the predominantly spread tobamovirus in tomato crops is ToMV. This fact is particularly supported by the investigations of BROADBENT (1962), WANG and KNIGHT (1967), LISA and LOVISOLO (1973), HORVÁTH and BECZNER (1973), as well as BECZNER and HORVÁTH (1974). It is obvious that the members of tobamovirus group are adapted to a more or less restricted range of plant species they affect (cf. WITTMANN, 1965). For instance, Sammons' opuntia virus (SAMMONS and CHESSIN, 1961) and cucumber green mottle mosaic virus (USCHDRAWEIT, 1955) were found in nature affecting only species from family *Cactaceae* and *Cucurbitaceae* respectively, while apparently the only tobamovirus repeatedly detected in cruciferous plants so far is RMV (reviewed by JURETIĆ *et al.*, 1973).

Beyond tomato, several other plant species have been found infected spontaneously with ToMV, as for instance: *Capsicum annuum* (MILLER and THORNBERRY, 1958; CONTI *et al.*, 1973), *Asparagus officinalis*, *Cucurbita pepo* and *Vicia faba* (FACCIOLI and PAUL, 1967) and *Nicotiana tabacum* (KOMURO and IWAKI, 1968). It is interesting that even woody plants, i.e. apple and pear trees were found in nature attacked with ToMV (cf. GILMER and WILKS; OPEL *et al.*, 1969).

The pepper tobamovirus investigated by FELDMAN and OREMIANER (1972) could also belong to ToMV. That virus differed greatly from common TMV in serological experiments performed by these authors, and provoked cytoplasmic hexagonlike crystals, i.e. inclusions associated with TMV and ToMV (cf. MILIČIĆ and JURETIĆ, 1971). Moreover, it caused merely symptoms on inoculated leaves of *Nicotiana sylvestris*, while common TMV, in addition, infects this species systemically. However, that virus and the tomato strain of TMV, also investigate by FELDMAN and OREMIANER (1972), did not infect *Chenopodium amaranticolor* and *Ch. quinoa* systemically. In the present investigations we demonstrated sys-

temic infection caused through ToMV-H on both *Chenopodium* species (cf. MELCHERS *et al.*, 1940; JURETIĆ, 1971), by back inoculations to "White Burley" tobacco, and in addition on *Chenopodium amaranticolor* cytologically. ToMV-H also caused systemic infection in *Chenopodium murale*, but in our experiments that plant revealed systemic infection after inoculation with common TMV as well.

The Pinto bean variety (*Phaseolus vulgaris*) is commonly used in the U.S.A. as a local lesion test plant to common TMV (cf. PIACITELLI and SANTILLI, 1961). It is interesting to mention that various bean varieties (e.g. Pinto, Red Kidney) did not react to ToMV-H and several other TMV-like tomato isolates from Hungary, but we obtained good reaction on these bean varieties with the U_1 strain of TMV (HORVÁTH, 1972). Consequently ToMV-H and the just mentioned tomato isolates from Hungary differed from common TMV also with respect to the reaction of these bean varieties.

In the literature available (KLINKOWSKI, 1968; SCHMELZER and WOLF, 1971; SMITH, 1972) we could not find evidence on transmitting tobamoviruses to anyone of 26 plant species (see Table 1, marked with two asterisks), to which ToMV-H was successfully transmitted in the course of the presented investigations. We also did not find in the corresponding literature data that inclusion bodies of ToMV were observed and found in systemically infected leaves of *Chenopodium amaranticolor*.

It was shown in our experiments that the method after GOODING and HEBERT (1967) was not only good enough in purification of common TMV, but also with ToMV and RMV.

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Phenols in Relation to Pathogenesis Induced by Avirulent and Virulent Strains of *Erwinia amylovora*

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Both avirulent (AV) and virulent (V) strains of *Erwinia amylovora* when infiltrated into apple leaf tissues at 10^8 cells/ml multiplied logarithmically for a period of 24 h, after an initial 5 to 6 h period of decline. Thereafter, they multiplied at a reduced rate. The total phenols in the leachates of the same leaf tissues during the initial period did not alter between the control and V and AV strains. After the initial period, a rising trend of phenolics leaking into the leachate of either the AV or V strain-infiltrated leaves was observed which reached a peak at approximately 24 h. The symptoms of browning appeared at about 12 h.

Chromatographic data of the leaf leachate at 24 h revealed that phloretin was uniformly present in the control as well as AV and V strain-infiltrated leaves. Most of the components of the infected leaves were chalcones and aurones. No significant difference in the phenolic constituents could be discovered between the leaves infiltrated with AV and V strain. The three major aglycones were characterized as: a) phloretin, b) quercetin, and c) pedospicatin.

In recent years the rate and intensity of a hypersensitive reaction (HR) has been repeatedly demonstrated to vary with the inoculum concentration and virulence of living phytopathogenic bacteria (BURKOWICZ and GOODMAN, 1969; GOODMAN and BURKOWICZ, 1970; KLEMENT *et al.*, 1974; KLEMENT and GOODMAN, 1967; KLEMENT and LOVREKOVICH, 1961). However, the nature of the physiological and/or morphological host factors responsible for this defense reaction have not been conclusively determined. Numerous reports of phenolic accumulation during the process of pathogenesis or HR exist (CONDON *et al.*, 1963; FARKAS and KIRÁLY, 1962; MATTA *et al.*, 1967, 1969; PERRIN and CRUICKSHANK, 1965; TOMIYAMA *et al.*, 1955) which suggest that probably these compounds play a role in the HR. Quinones produced by oxidation of phenols may be indirectly toxic to many plant pathogens by causing the HR and, subsequently, host cell death. Studies were therefore undertaken (i) to determine the relationship between

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bacterial multiplication and the level of total phenol in Jonathan apple leaves inoculated with avirulent and virulent strains of *Erwinia amylovora;* and (ii) to characterize the important components of phenols that are released during pathogenesis and/or HR.

Materials and Methods

Lyophilized cultures of bacteria were used in all inoculation experiments following methods by BURKOWICZ and GOODMAN (1969). Inocula concentrations were calibrated in a Spectronic 20 colorimeter at a wave length of 525 nm and usually contained 10^8 living bacterial cells/ml of either avirulent (AV) or virulent (V) strains of *Erwinia amylovora* (Burrill) Winslow *et al.*

Year-old greenhouse-grown apple trees (*Malus pumila* Mill. 'Jonathan') were used in all experiments. Leaves for inoculation were collected usually within 3 to 4 weeks after bud break. Only the 4th and 5th almost fully expanded leaves, counting from the apex of a shoot, were selected for inoculation. The selected leaves were detached from the shoots, washed for 0.5 h in running tap water, rinsed in sterile distilled water, and blotted on sterile paper towels. They were subsequently immersed in bacterial suspensions and infiltrated under vacuum for about 1 h. Fully infiltrated leaves were sorted out from those partially infiltrated and rinsed in sterile distilled water to remove as many of the bacteria adhering to the surface as possible. The leaves were blotted and allowed to equilibrate with the atmosphere (1 to 3 h) till the water-soaked appearance of the leaves largely disappeared. They were then placed in moist chambers constructed of either 2 tops or 2 bottoms of sterile petri plates lined with a water-soaked filter paper and containing a micro dish filled with 2 ml of sterile water. The moist chambers were sealed with scotch tape and incubated at 28 °C for different periods of time. Leaves infiltrated with either sterile distilled water or sterile phosphate buffer served as controls.

Population trends in inoculated leaves were followed at intervals over a period of 72 h. The sampling was started immediately after inoculation. Two disks 2.0 cm in diameter were cut from each of 2 leaves with a No. 14 cork borer. Triplicate samples of 4 disks were immediately homogenized in 1 ml of 0.05 *M* sterile phosphate buffer (pH 7.0) with a mortar and pestle. A series of 10-fold dilution of 0.1 ml of each sample homogenate in phosphate buffer (pH 7.0) were made. The bacterial population was determined by the serial plate dilution procedure (in triplicate) on nutrient yeast dextrose agar (5) plates. Bacterial colonies were counted after 48 h incubation at 28 °C and are presented as numbers of bacteria per cm^2 of leaf area.

For estimation of total phenols, 8 leaf disks 2.0 cm in diameter, 2 from each of 4 infiltrated leaves, served as a sample and each sample was run in duplicate. Care was taken to put an equal number of disks from basal and terminal portions of the leaves in each sample. These were shaken in 25 ml of distilled water

in 250 ml Erlenmeyer flasks for 20 min at a speed of 150 strokes per min. The leaf leachates were passed through millipore filters (0.22 μ) to remove the bacteria. The leachates were then evaporated to dryness in a rotary evaporator at a temperature not exceeding 40 °C. The residues were taken in 5-ml quantities of 80% aqueous methanol (v/v) and centrifuged at 10,000 r.p.m. for 15 min in an angle head Servall centrifuge to remove the protein precipitates. The supernatants were decanted and filtered again through millipore filters (0.22 μ) and stored at 2 to 4 °C for subsequent estimation of total phenols. Total phenols were determined according to SPIES (1955) using Folin-Ciocalteu Phenol Reagent (2N) (Fisher Scientific Co.). In this procedure, 10 ml of 14% aqueous sodium carbonate solution was added to 5 ml of the methanolic solution of the extract which was then mixed with 3 ml of diluted phenol reagent (phenol reagent-water, 1 : 2 v/v) while agitating. The precipitate formed was removed by centrifugation at 5000 r.p.m. for 15 min. One h after mixing of the phenol reagent, the absorbance was determined in 1-mc cells at 650 nm in a Beckman DB-G spectrophotometer. The results are expressed as μg equivalent of chlorogenic acid per cm^2 of leaf area.

Characterization of phenolic compounds. For determining the specific phenolic compounds released in the leachate following bacterial infiltration with either the AV or the V strains, 24-g samples of whole infiltrated apple leaves were shaken for 20 min in 12 500-ml Erlenmeyer flasks each containing 2 g of leaves in 100 ml of sterile distilled water on a mechanical shaker at 150 strokes per min. The leachates were decanted and combined at the end of 20 min, after which the extracts were prepared as follows:

The combined leachate was filtered through Whatman No. 1 filter paper in a Buchner funnel and centrifuged for 30 min at 10,000 r.p.m. in cold (0 °C) in an angle head Servall centrifuge. The supernatant was decanted and the pellet was resuspended with a little water and recentrifuged. The supernatant was decanted and combined with the earlier supernatant and filtered through a millipore filter (0.22 μ) to get rid of the bacteria. The filtrate was then put in a boiling water-bath for 5 min to preclude enzymatic hydrolysis of glycosides. It was then evaporated in a rotary evaporator below 40 °C and the residue was taken up in 30 ml of aqueous 80% methanol and centrifuged at 10,000 r.p.m. for 20 min to remove the protein precipitates. The supernatant was decanted and filtered through millipore filter (0.22 μ) and stored at 2 to 5°C for subsequent chromatographic analysis.

Thin layer chromatography was used for the separation of phenolic compounds. Precoated cellulose glass plates (layer thickness 0.1 mm) supplied by Brinkman Instruments Inc., Westbury, N. Y. 11590 were used. The extracts and the marker solutions were applied with a Hamilton microsyringe, and the plates were developed in the following solvent systems: *n*-butanol–acetic acid–water (4 : 1 : 5 by volume) top layer, acetic acid (2% and 6%, v/v), and Forestal (acetic acid–conc. HCl–water, 30 : 3 : 10, by volume). For detection and identification purposes, ultraviolet fluorescence, ammonia vapour and chromogenic sprays such as 5% ethanolic aluminium chloride, 5% aqueous sodium carbonate, a fresh mixture of equal volumes of 1% aqueous ferric chloride and 1% aqueous ferri-

cyanide, a fresh mixture of equal volumes of tetrazotized benzidine and sodium nitrite (RANDERATH, 1968), ethanolic magnesium-HCl (SHINODA, 1928), and ammoniacal silver nitrate (ROUX and MAIHS, 1960) were used. R_f values were determined by marking the point of maximum concentration of each band under the ultraviolet light. The numbering of the phenolic bands began with the fastest moving phenolic compound. For spectral studies, bands were scraped with a razor blade and removed from the thin layer plates by reduced pressure. Their components were eluted from the cellulose with 95% ethanol. The ultraviolet absorption spectra of the eluates were determined with a Beckman DB-G spectrophotometer.

Results

Population trend. The rates of growth of the virulent (E-9) and avirulent (E-8) strains of *Erwinia amylovora* are depicted in Fig. 1. It is apparent that both grew similarly and well in apple leaf tissue. A very high recovery of bacteria could be obtained immediately after infiltration. On a leaf area basis, this was found to be 6×10^6 and 8×10^6 cells/cm^2 for V- and AV-strains respectively. Subsequently

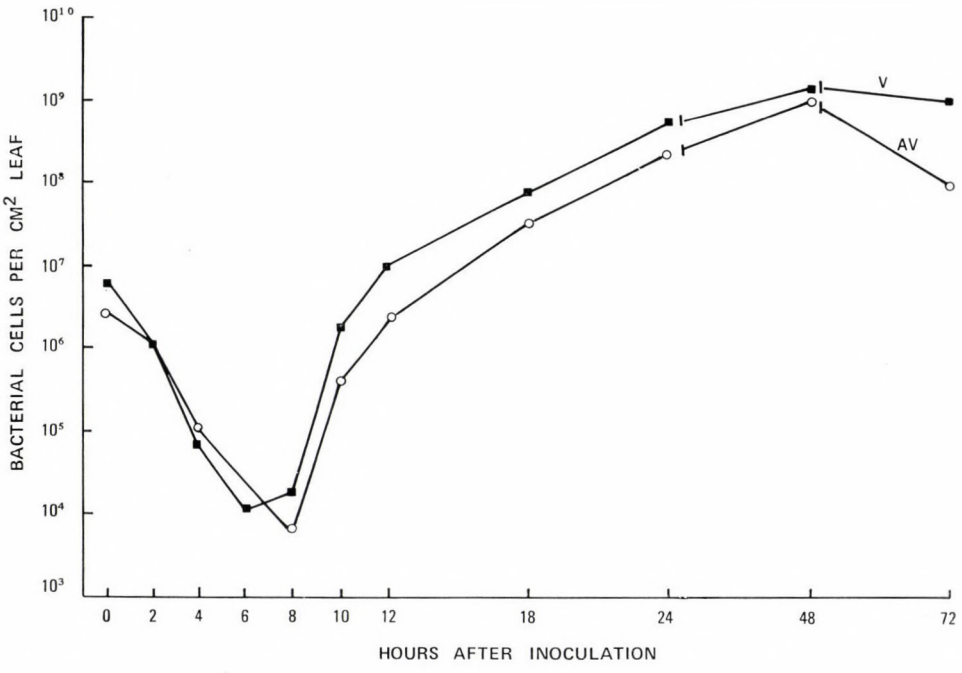

Fig. 1. The rate of growth of avirulent (AV) and virulent (V) strains of *Erwinia amylovora* in 4-week-old Jonathan apple leaf tissue inoculated with 10^8 cells/ml

the population of both strains decreased for 6 to 8 h to 10^4 cells/ml and then rose exponentially for 16 to 18 h, when a stationary phase was reached which lasted for 24 h. The population maxima were reached about 48 h after inoculation, when they started to decline. Death of the AV strain occurred more rapidly than that of the V strain.

Quantitative changes in phenols as a function of time in inoculated leaves. Hypersensitive reactions are produced in apple leaves infiltrated with both V- and AV-strains of *Erwinia amylovora*. Light brown colored materials appear around 12 h after infiltration over the entire leaf lamina. These become darker in color with time, and intensively dark brown color of the leaf is seen at 24 h after infiltration. A more distinct reaction is produced with AV-strain than with V-strain. Phenols appeared in inoculated apple leaves but not in uninoculated controls (Fig. 2). Approximately a four-fold increase in total phenols occurred in inoculated leaves as compared with controls at 24 h after inoculation (Fig. 2).

Partial characterization of phenols. Chromatographic data indicate that the same major phenols appear in both V- and AV-strain inoculated leaves (Fig. 3). However, phloridzin was detected in high concentration in inoculated leaves as well as in uninoculated controls (Band No. 1). Other major compounds (Band

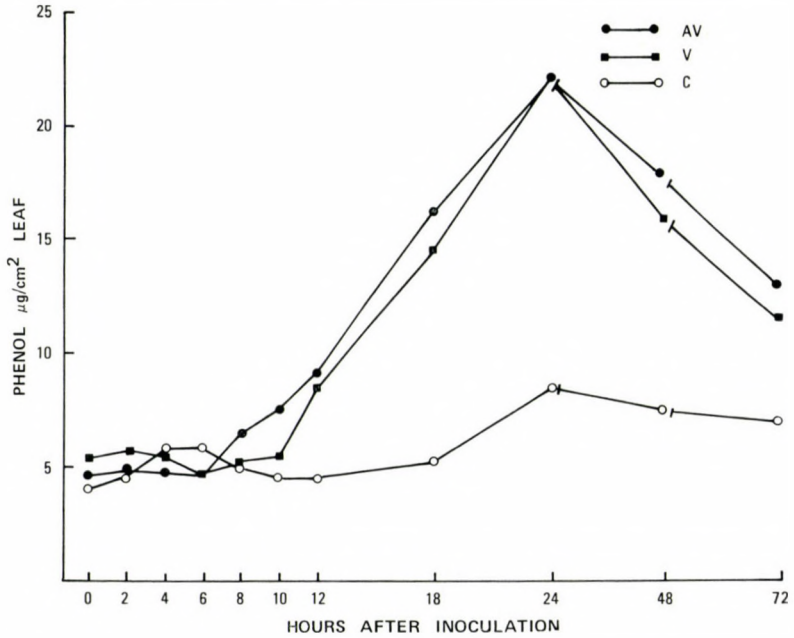

Fig. 2. The rate of appearance of total phenols as chlorogenic acid equivalents in 4-week-old Jonathan apple leaf leachate. Apple leaves had been inoculated with 10^8 cells/ml of avirulent (AV) and virulent (V) strains of *Erwinia amylovora* and controls (C) with 0.05 *M* phosphate buffer

Nos 4, 5, and 7) that appeared after inoculation were not detected in extracts of uninoculated controls. Separation of the extract using *n*-butanol–acetic acid–water (4 : 1 : 5) and 2.2 % acetic acid in a 2-dimensional chromatogram (ascending), followed by spraying with tetrazotized benzidine, ammoniacal silver nitrate and ultraviolet light reactions with and without ammonia suggest that most of the compounds are chalcones and aurones and some with *o*-dihydroxylation or vicinal trihydroxilation. Altogether 20 phenolics could be detected.

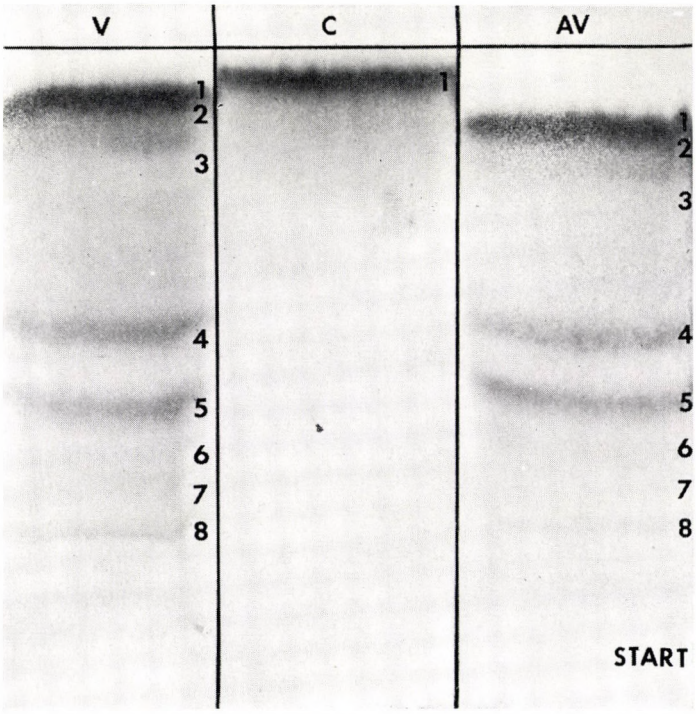

Fig. 3. One-dimensional chromatogram of phenolics in 4-week-old Jonathan apple leaf leachate. Apple leaves had been inoculated with 10^8 cells/ml of virulent (V) and avirulent (AV) strains of *Erwinia amylovora* and controls (C) with water. Identity of bands: Band Nos 1. dihydrochalcone; 2. unidentified; 3. chalcone and/or aurone; 4. chalcone and/or aurone; 5. chalcone and/or aurone; 6. chalcone and/or aurone; 7. isoflavone; 8. isoflavone

Since many of these phenolics were glycosides, leachates were hydrolyzed for identification of aglycones. In this procedure 4 ml of the leachate extract were evaporated to dryness in a rotary evaporator and taken up in 4 ml of 1 *N* HCl and hydrolyzed in a sealed tube by boiling in a water-bath at 100 °C for 3 h. After hydrolysis, the ether fraction contained the major phenolics. The spectral properties and chromatographic data of the 3 major aglycones are presented in Table 1. The absorption spectra are presented in Figs 4, 5, and 6. The tentative iden-

tification of the band Nos 1 and 6 (Table 1) are based on comparison with authentic phloretin and quercetin whereas the band No. 3 in the same Table was identified as podospicatin by comparing the R_f values and ultraviolet absorption spectrum to published values (BRIGGS and CAIN, 1959). This compound has not been previously reported from apple.

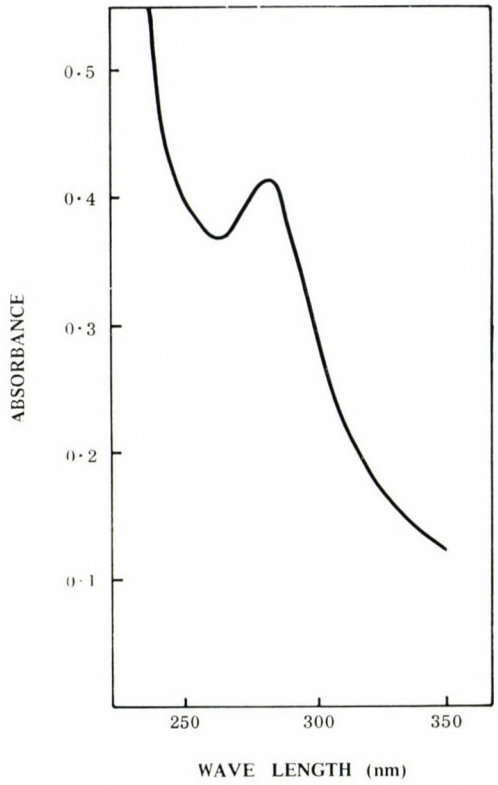

Fig. 4. Absorption spectrum of the aglycone Band No. 1 (see Table 1). Solvent 95% ethanol

Table 1

Spectral maxima and R_f value of major aglycones

Aglycone Band No.	Rf in				In EtOH		Tentative identification
	BAW	2% HOAc	6% HOAc	Forestal	max (nm)	Mg-HCl	
1	0.93	0.00	0.07	0.98	282, —	—	Phloretin
3	0.85	0.40	0.55 0.70[a]	0.75	260, 295	—	Podospicatin
6	0.65	0.00	0.00	0.36	256, 376	+	Quercetin

[a] R_f in 30 % HOAc

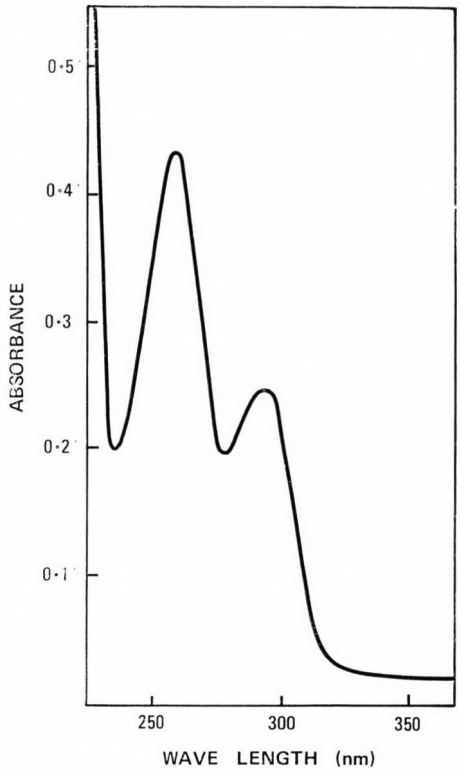

Fig. 5. Absorption spectrum of the aglycone Band No. 3 (see Table 1). Solvent 95% ethanol

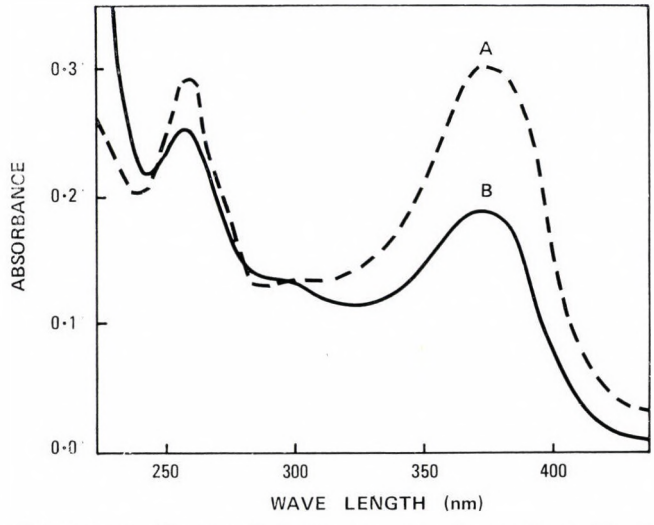

Fig. 6. Absorption spectra of quercetin (curve A) and aglycone Band No. 6 (curve B) (see Table 1). Solvent 95% ethanol

Discussion

In vivo, multiplication of the virulent and avirulent strains of *Erwinia amylovora* was typical of different phytopathogenic bacteria in various host plants. In these studies, it was the incompatible combination (virulent strains in resistant host or avirulent strain in susceptible host) that revealed an earlier and more intense symptom expression.

In the present investigation, determination of bacterial population at 2 h intervals during the initial 12 h after infiltration presented only a slightly different pattern from that noted in the literature. There was an initial decline in the bacterial population for the first 6 to 8 h and thereafter the rate of multiplication went up exponentially for a period of 18 h. This initial decline in population may be due to several factors which have not been fully investigated. However, experimental evidence of a decline of surface-borne bacteria have been obtained in our laboratory (ADDY, 1970) which might be at least partly responsible for the decline.

The population trend showed some reduction in the rate of multiplication after 24 h but the total cell numbers per unit leaf area continued to increase (Fig. 1). The browning symptom on the leaf lamina was first visible about 12 h after infiltration. Symptom expression in the case of the avirulent strain occurred only one-half h earlier than with the virulent strain. But with the lapse of another 6 to 8 h the difference in the intensity of tissue darkening between the 2 strains was discernibly more intense with the avirulent strain.

The symptom expression is closely linked with significant appearance of phenol (Fig. 2). The leakage of phenol is also closely associated with permeability changes (ADDY, 1970). Since phenolic substances occur in many plant species in almost all parts of the plant, either as free phenolic acids or as polymerized products such as tannins, lignins, etc. (BONNER, 1957), a possibility exists, that phenolic substances in the middle lamella and other components of the wall may be released by the degradative effect of the bacteria on the host cell wall. Subsequently these polyphenolic substances may be oxidized to quinones which could in turn inhibit enzymes affecting wall metabolism, denature structural protein in the wall *per se* or denature membrane protein. There is, however, no concrete evidence to date indicating that *Erwinia amylovora* possesses the enzymes necessary to release or degrade the polyphenolic compounds of either the middle lamella or the primary cell wall.

The other possibility is that phenolic substances like tannins may be present in individual cells or in special containers termed tannin sacs which are a common ingredient of vacuoles (ESAU, 1963), or they may occur in the cytoplasm proper in the form of small droplets which eventually fuse. BECKMAN and MUELLER (1970) presented evidence of localized concentrations of phenols, stored in discrete bodies within randomly scattered parenchyma cells in banana roots. Globular bodies of phenolic substances were often present as aggregates and extended well into the vacuoles. Bacteria may in some way alter the solubility of phenols in the cell proper, permitting easy leakage through as yet undamaged membranes.

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Altered Levels of Indoleacetic Acid and Cytokinin in Geranium Stems Infected with *Corynebacterium fascians*

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Tumor tissues of leafy galls of geranium (*Pelargonium zonale* cv. Irene) infected with *Corynebacterium fascians* contain decreased amount of indole-3-acetic acid (IAA) and exhibit increased cytokinin activity in the tissue culture bioassay compared with healthy stem tissues. Three active compounds, chromatographycally similar to zeatin, zeatinriboside and $N^6(\Delta^2$-isopentenyl)-adenine are present both in healthy and infected stems. Tumor tissues of leafy galls contain an additional cytokinin which is not present in traceable quantities in the extracts from healthy stems. The total cytokinin activity in extracts from leafy gall tissues was much greater than that in extracts prepared from healthy stem tissues.

The characteristic symptoms of the disease caused by *Corynebacterium fascians* are the shortening of internodes, leafy gall formation and high water content of the affected tissues. There are several reports which explain the growth abnormalities relative to hormonal imbalances (THIMANN and SACHS, 1966; KIRÁLY *et al.*, 1967; VAN ANDEL and FUCHS, 1972; SZIRÁKI *et al.*, 1975). AMES (1974) has shown that in the young seedlings of the tumor-prone amphiploid *Nicotiana suaveolens* X *Nicotiana langsdorffii* the IAA concentration decreased over 9 days. HAMILTON *et al.* (1974) established that no free IAA was present in tumor tissue of *Parthenocissus tricuspidata* and they separated several IAA metabolites by thin-layer chromatography. Two papers reported that *Corynebacterium fascians* can break down IAA (LACEY. 1948; KEMP and STEENSON, 1971). This phenomenon, namely decreasing of IAA level in the plant, may have a trigger effect in tumor formation (*cf.* AMES, 1974).

On the other hand, THIMANN and SACHS (1966) demonstrated that the diseased tissues of peas and the cultures of the infecting *Corynebacterium facians* contained chloroform-soluble cytokinins. Cytokinin measurements were based on retention of chlorophyll and on senescing of oat leaves. SEQUEIRA (1973) in his review expresses some doubt as to the nature of the presumed cytokinins because these above-mentioned bioassays are not sensitive and specific. KLÄMBT *et al.* (1966) isolated the cytokinins from cultures of *Corynebacterium fascians* and they were identified as N^6-(Δ^2-isopentenyl)-adenine, nicotinamide and 6-methylaminopurine by HELGESON and LEONARD (1966). These studies have attempted to interpret alterations in normal growth and development as the result of changes in cytokinin or in auxin levels of the infected plants.

In this paper we try to demonstrate that the extract of infected germanium stems shows altered cytokinin activity and altered level of IAA as compared to the extract of healthy stems. These hormonal changes may play an important role in leafy gall formation and development.

Materials and Methods

Plant material: Geranium (*Pelargonium zonale* (L.) Ait. cv. Irene) stems naturally infected by *Corynebacterium fasciens* (Tilford) Dowson, and uninfected healthy stems were harvested in greenhouse from several stocks. Infected stems had characteristic leafy tumors.

Cytokinin extraction and bioassay: Thirty grams of stems, healthy and in fected respectively, were washed and homogenized with 60 ml phosphate buffer pH 6.5 and extracted with 200 ml of 96% ethanol for 12 h at 4 °C. The extracts were filtered and centrifuged at 6000 *g* for 15 minutes and then the superantants were concentrated to the aqueous phase under vacuum at 35 °C. For extraction and purification of cytokinins the procedures of KIRÁLY *et al.* (1967), MILLER (1967) and VAN STADEN *et al.* (1972) were followed as we described previously (SZIRÁKI and GÁBORJÁNYI, 1974; SZIRÁKI *et al.*, 1975). Further purification of the cytokinins was carried out by paper chromatography on Whatman No. 1 paper. The solvent systems were: *t*-butanol : conc. NH_4OH : water (3 : 1 : 1, v/v/v) and 0.03 *M* borate buffer, pH 8.4. The samples to be chromatographed were dissolved in 90% ethanol and streaked onto chromatography papers. After drying, the chromatograms were divided into 10 equal stripes and tested for cytokinin activity in the soybean callus bioassay using the method of MILLER (1965) modified by KRASNUK *et al.* (1971). The migration of active compounds in stem extracts was compared with known cytokinins. The standards, namely zeatin (Z), zeatinriboside (ZR) and N^6-(Δ^2-isopentenyl)-adenine (2iP) were spotted as markers.

Twenty ml of tissue culture basal medium supplemented with agar and portions of chromatograms corresponding to specific R_F regions was added to each 50 ml Erlenmayer flask. After adjusting the pH to 5.8 with NaOH, the medium was sterilized by heating twice, at 100 °C for 45 min. A one-day-interval was allowed between heat treatments. Five callus explants were transferred to each flask. Cultures were maintained at 27 °C for 28 days and then weighed.

The experiments were repeated with similar results in five cases. Data of Tables show the results of one representative experiment. All the data are expressed on a dry weight basis, because the water content of infected stem is always higher than that of healthy stems. The dry material content of healthy stems was 19.2%, while that of the infected ones only 9.8%.

Extraction and assay of indole compounds: Healthy and infected stems were rinsed in tap water and blotted dry. Thirty grams fresh weight samples were extracted with 300 ml of 60% ethanol in a Waring Blendor for 10 min. As regards the extraction of indole compounds, we slightly modified the procedure of WIESE and

DE VAY (1970), (*cf.* SZIRÁKI *et al.*, 1975). For identification of the separated indole compounds authentic samples were spotted as markers. The markers were as follows: indole acetonitrile, indole propionic acid, indole-3-acetic acid, indole acetamide, 5-hidroxy-indole-3-yl acetic acid and tryptophane. The separated indole compounds on developed chromatograms were located and characterized by applying the SALKOWSKI's reagent and measured spectrophotometrically. The R_F regions from chromatograms corresponding to indole-3-acetic acid were eluted with ethanol and the adsorbance of eluates were measured at 280 nm using a UNICAM SP 800 spectrophotometer. The spectrophotometric measurements were carried out according to FLETCHER and ZALIK (1963). The experiments were repeated with similar results in five cases. Data are from one representative experiment, and expressed on a dry-weight-basis.

Results and Discussion

It was shown that geranium stems infected by *Corynebacterium fascians* contained lower level of indoleacetic acid than the healthy ones (Table 1). Other

Table 1

Spectrophotometric estimation of contents of indole-3-acetic acid in healthy and in *Corynebacterium fascians*-infected germanium stems on a dry weight basis*

Compound	µg/kg dry weight	
	healthy	infected
Indole-3-acetic acid	23±4	11±2

* Compounds were located by spraying a portion of the chromatographic paper (Whatman No. 1) with the SALKOWSKI's reagent. The corresponding R_F regions from unsprayed portion of the paper were eluted with ethanol and the absorbance of the eluate at 280 nm was measured spectrophotometrically and compared to known quantities of authentic indole-3-acetic acid.

authentic samples (mentioned in the Material and Methods) were also applied but we could not detected any of them. Only the IAA was located and characterized from plant extracts by applying the SALKOWSKI's reagent. The IAA content was estimated spectrophotometrically. The R_F regions from chromatograms corresponding to IAA were eluted with ethanol and the absorbance of eluates was measured at 280 nm and compared to known quantities of authentic IAA. Decreased level of IAA in the affected tissues corresponds well with that shown by KEFFORD (1959) in the frenching disease of tobacco. Very recently AMES (1974) has shown that in a tumor-prone amphiploid hybrid tobacco the concentration of IAA decreased. This was confirmed by HAMILTON *et al.* (1974) in an other tumor

Table 2

Soybean callus bioassay of chromatograms of purified extracts of geranium stems, healthy and infected by *Corynebacterium fascians**

	Callus yield (mg/flask)									
	R_F 0.0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
Healthy	166	326	390	184	144	353	391	211	234	403
Infected	977	263	421	396	255	163	318	411	180	357
Control (without extract)	220									

* Purified extracts were chromatographed on Whatman No. 1 paper in *t*-butanol–conc. NH_4OH–water (3 : 1 : 1, v/v/v). Cultures were grown on basal medium supplemented with portions of chromatograms corresponding to R_F regions. Each flask contained 5 callus explants. The experiments were repeated with similar results in five cases. Data of Table show the results of one representative experiment. The extracts were obtained from 10 g dry weight of stem/1000 ml medium.

Table 3

Soybean callus bioassay of rechromatographed extracts of chromatograms of geranium stems, healthy and infected by *Corynebacterium fascians**

	Callus yield (mg/flask)									
	R_F 0.0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0 5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1.0
Healthy	247	261	241	264	295	235	274	260	257	235
Infected	260	303	237	252	360	236	236	320	247	225
Control (without extract)	235									

* Samples were chromatographed on Whatman No. 1 paper in borate buffer (0.03 *M*, pH 8.4). Cultures were grown on basal medium supplemented with portions of chromatographed extracts in *t*-butanol–conc. NH_4OH–water (3 : 1 : 1, v/v/v) corresponding to 0.0–0.1; 0.2–0.3 and 0.6–0.8 R_F regions. The R_F regions from chromatograms were eluted with ethanol. Each flask contained 5 callus explants.

tissue. One can suppose that the decrease of IAA stems from the ability of *Corynebacterium fascians* to break down indoleacetic acid.

We found in this study that infected geranium stems contained increased level of cytokinin as compared to the healthy ones. The chromatographed extracts were tested in the soybean bioassay. The chromatograms were divided ten R_F regions and the individual regions were incorporated into the medium for bioassaying cytokinin activities. After a 28-day incubation period the fresh callus yields were weighed (Table 2). It is seen from the Table 2 that purified extracts represented a mixture of active materials. The extract purified from healthy stems shows high cytokinin activity at R_F 0.1–0.3; 0.5–0.7 and 0.9–1.0 regions. The extract purified from infected stem tissues exerts high cell division activity at 0.0–0.1; 0.2–0.4; 0.6–0.8 and 0.9–1.0 R_F regions. It is seen from these results that the extract of healthy plants contains active materials at three R_F regions and the extract of infected plants at four R_F regions. There is no active material in traceable quantities in the extracts of healthy stems at R_F 0.0–0.1. The peak migration of the active material was at R_F 0.9–1.0 and R_F 0.0–0.1 in healthy and infected stems, respectively. It is seen also from the data of Table 2 that the total cytokinin activity from infected tissues is higher than that of the healthy ones. The standards namely zeatin, zeatinriboside and isopentenyladenine were detected at R_F 0.81–0.86; 0.89–0.95 and 0.91–0.96, respectively. We suppose that the high cytokinin activity at R_F 0.9–1.0 both with healthy and infected stems originated from the isopentenyladenine content of plant material.

For characterization of the factors responsible for stimulating callus proliferation at the R_F regions 0.5–0.7 and 0.6–0.8 of healthy and infected samples respectively, and at the lower R_F regions, a second chromatographic separation was employed. The active materials from the given regions were eluted and rechromatographed in borate buffer (0.03 *M*, pH 8.4). It is seen from the Table 3 that both the extracts from healthy and infected tissues contain active material at 0.4–0.5 R_F region. It could be concluded that both extracts contain zeatin at these R_F regions, since the authentic zeatin was detected at R_F 0.48–0.53. The extracts of infected stems show high activity at R_F 0.1–0.2 and at 0.7–0.8 too. At these R_F regions the healthy stems show low, if any, activities. It is probable that the cell division activity located at R_F 0.7–0.8 is due to zeatinriboside (R_F 0.81–0.87).

One can conclude from these data that the infection of geranium by *Corynebacterium fascians* causes increasing cytokinin and decreasing auxin levels. This altered auxin–cytokinin rate could be the cause of development of leafy gall in geranium stems.

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Hypersensitive Reaction of Rust-Infected Wheat in Compatible Host–Parasite Relationships

By

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In a study of compatible wheat-stem rust relationships the pathogen was selectively destroyed by heat treatment (50°C, 20 min) or fungicides (Blasticidin-S or nickel nitrate). As a result of the appropriate treatments, necrotic hypersensitive reactions occurred in the originally compatible wheat leaves. Besides the necrotic symptoms, a marked and rapid increase in peroxidase enzyme activity was observed, suggesting a similarity in this artificially induced reaction with the natural hypersensitive reaction. These results, supported by previous findings with other host–parasite relationships, again suggest that hypersensitive necrosis associated with augmented peroxidase activity are the consequence rather than the cause of the host resistance to infection.

The most widespread "defence reaction" against pathogens of plants is the hypersensitive reaction. Characteristic of the reaction are browning of tissues and necrosis. Pathogens become localized in the necrotic tissues and are unable to extend further and sooner or later die. Considering that the phenomenon was first observed in obligate parasites, it may be supposed that the necrosis forming with the hypersensitive reaction localizes the pathogen since obligate parasites can develop only in living tissues. Experiments in the areas of physiology and biochemistry of plant disease resistance almost exclusively deal with the hypersensitive reaction. Among the important areas of research in these fields, in respect to effects of infection are the sudden rise in respiration intensity, phenol metabolism changes and the remarkable increased activities of peroxidase, polyphenol oxidase and ascorbic acid oxidase (FARKAS and KIRÁLY, 1958; DALY *et al.*, 1961; FARKAS and KIRÁLY, 1962). Since these changes are more prominent in hypersensitive (resistant) host plants as compared with susceptible plants they may be associated with plant resistance. Many publications have appeared on this problem, but hypersensitive necrosis and the metabolic changes involved this phenomenon and plant resistance is still a debated question.

In spite of the extensive body of data, several fundamental questions still remain: What are the cause and effect relationships between these metabolic changes in the hypersensitive reaction and plant resistance? Are these changes only the consequences of the incompatibility between the host and its attacking pathogen? Or quite the contrary, is the hypersensitive reaction (with augmented

enzyme activities, accumulation of phenols and/or phytoalexins) the cause of plant resistance, by which the pathogen is damaged and/or destroyed?

The following investigations were carried out to examine these cause and effect relationships.

The pathogen in the wheat–stem rust relationship was destroyed by heat treatment or fungicides in the plant. These treatments substituted the action of a "defence reaction" that occurs under natural conditions in connection with incompatibility. Experiments were designed to determine whether the conditions could be reproduced that take place under natural incompatible relationships.

Materials and Methods

The wheat cultivars, Little Club (*Triticum compactum* L.) and Reliance (*Triticum aestivum* L.) were used in the experiments. Races 11 and 21 of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. et E. Henn. were used as inocula. The Little Club cultivar is susceptible to both rust races; the Reliance cultivar is susceptible to race 11. The plants were grown under normal greenhouse conditions and were inoculated at the one-leaf stage 8 days after sowing. The inoculum consisted of a tap water suspension of uredospores collected on the day of inoculation.

Inoculation

Before inoculation the plant leaves were washed with tap water. Both sides of the leaves were brushed with a spore suspension and the plants were placed in a moist chamber for 24 hours.

Heat treatment

Separate plant samples were heat-treated at different time periods, every second day. The heat treatments were started two days before and were continued up to 10 days after inoculation. The infected plants were dipped into 50°C water for 20 sec. Then the rust fungus present in the tissues died selectively, but the host plant at this temperature obviously was not damaged (YARWOOD, 1963).

Treatment with fungicides

Plants were treated with a 500 ppm solution of $Ni(NO_3)_2$ or a 50 ppm solution of Blasticidin-S. The same time intervals were used as with the heat-treated plants. Both nickel ion and Blasticidin-S inhibit fungus infection. Thus prevent the rust fungus from spreading without damaging the plant. The treatments were carried out on two subsequent days, twice daily. The appropriate concentration of the above fungicides was brushed or sprayed on the leaves.

Peroxidase enzyme assays

Samples for determining the enzyme activities were taken at inoculation and 2, 4, 6, 7, 8 and 9 days after the inoculation.

The samples were the following:

1. Uninoculated and untreated control plants.
2. Inoculated and untreated plants.
3. Uninoculated and treated plants.
4. Inoculated and treated plants.

The plants were sprayed twice daily with a 50 ppm solution of Blasticidin-S on the 4th and 5th days of infection. Only the first leaves of the plants were examined. The method of FEHRMANN and DIMOND (1967) for determinig enzyme activity was used with slight modification. Two gram samples were macerated in 6 ml 0.2 *M* acetate buffer (pH 4.6) at 4 °C with quartz sand in a mortar and pestle. The samples were filtered through 4 layers of cheesecloth. The filtrate was centrifuged at 0°C with a Janetzki K 23 centrifuge at 5000 *g* for 40 min. For measuring enzyme activity, pyrogallol was used as a substrate. The enzyme extracts occurred in the optimal concentration (0.2 ml) in the final volume of 3 ml reaction mixture. Measurement was made at 430 nm in Unicam SP 800 spectrophotometer. Enzyme activities were expressed as the per cent increase in absorption between 15 and 45 sec after the enzyme extract was added, in relation to the untreated and uninoculated control.

Ultrasonic treatment

One hundred milligram of uredospores of the stem rust races (21, 11, 41 and 331) were floated on the surface of tap water in Petri dishes of 40 cm diameter. The freshly collected spores on the surface of the water completely germinated in 24 hours. These were collected and homogenized with 2 ml 0.15 *M* phosphate buffer (pH 6.5) and quartz sand in a precooled mortar and pestle. After 5 min homogenization, the mixture was exposed to ultrasonic treatment. A MSE sonifier was used at 0 °C for 10 min at maximum intensity. The homogenate was centrifuged at 0 °C with a Janetzki K 23 centrifuge at 5000 *g* for 20 min. After centrifugation the supernatant was injected into the primary and secondary leaves of the different wheat cultivars of the STAKMAN differential sortiment using the KLEMENT technique (1963).

Results

Through the use of heat treatments and fungicides on the infected plants, practically all resistant relationships between wheat and stem rust can be obtained from every susceptible host–parasite relationship (e.g. Little Club–race 11 or 21 and Reliance–race 11). The reaction type depended on the time in which the fungus was destroyed by the treatment. If heat treatment was made before

inoculation, the originally susceptible relationship remained unchanged (3–4 type reaction).

However, if the treatment was made after inoculation, but before the appearance of the symptoms, the relationship remained " immune" (0 type reaction) e.g. no visible symptoms occurred on the leaves. When the treatment was made at the chlorotic stage (4–6 days after inoculation) hypersensitive necrotic spots occurred on the leaves (0; and type 1 reaction). If the treatment was made at the

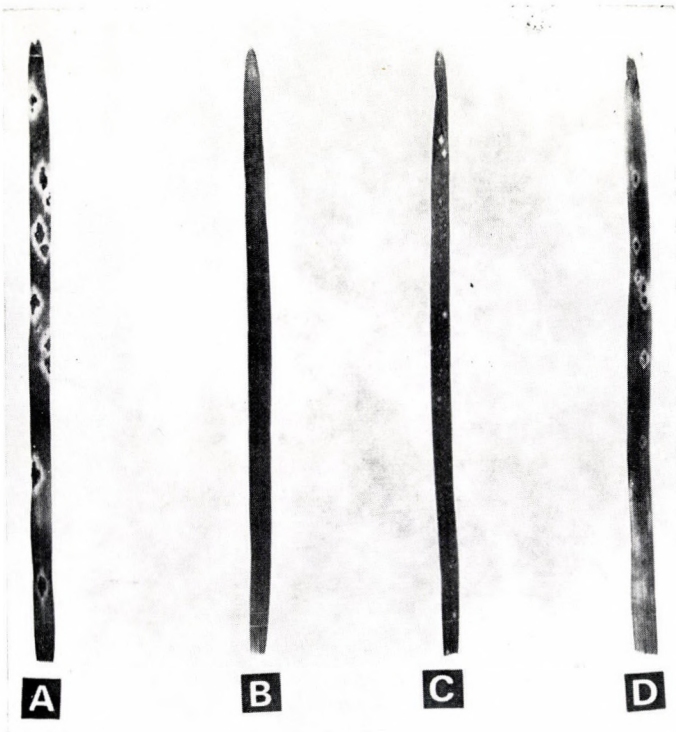

Fig. 1. Wheat leaves (cv. Little Club) inoculated with *P. graminis tritici* race 11 and treated by heat (50 °C, 20 min) at various time periods; 2 days before (A), 2 (B), 5 (C) and 6 days after inoculation (D)

beginning of the sporulation phase (6–8 days after inoculation) necrotic rings formed around the small spore colonies. The effect of treatment destroyed the spore colonies as indicated by the blackening of the spores (Fig. 1).

The results obtained with fungicides were identical with heat treatments with one exception. When the fungicide treatment was made before inoculation this pretreatment prevented the infection.

Peroxidase activity

The peroxidase activities of the different samples were closely correlated with the visible symptoms. The appearance of necrosis in the case of the originally susceptible relation was accompanied by a marked increase in peroxidase activity (Fig. 2). The treatment in chlorotic stage (4th and 5th day after inoculation) was selected among the treatments because we obtained the most characteristic

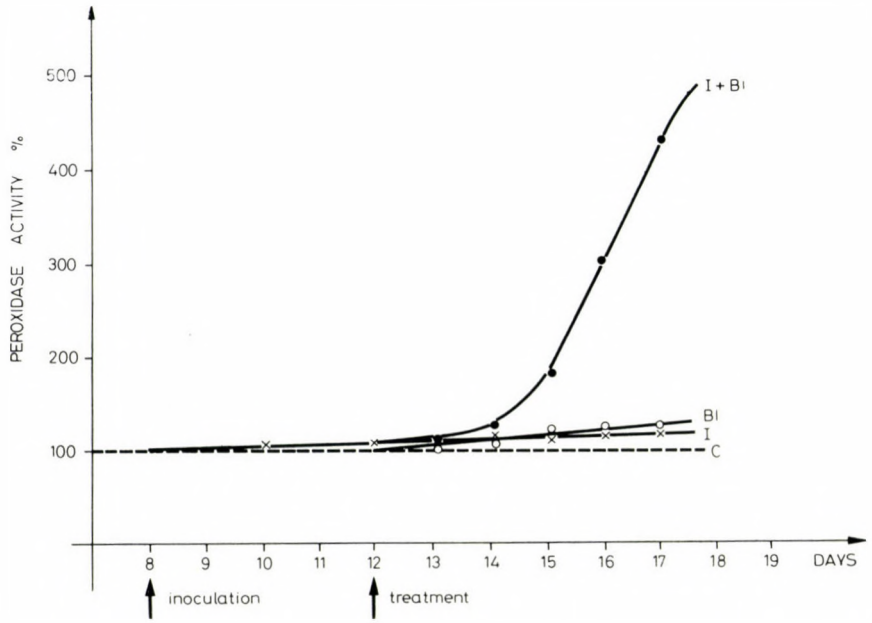

Fig. 2. Peroxidase activity of Little Club wheat leaves inoculated with *P. graminis tritici* race 11 and/or treated with Blasticidin-S (50 ppm) expressed as percentage of control
C = control (uninoculated, untreated)
I = inoculated with *P. graminis tritici*
Bl = treated with Blasticidin-S
I + Bl = inoculated and treated leaves
Each value is the average of five replications

hypersensitive picture at that stage. Peroxidase activities were the highest at that stage whereas if the treatment was made in an earlier stage, the increase of activity was much less.

The extent of necrosis and the peroxidase activity of the leaves were in close correlation as shown in Fig. 3. It was demonstrated that when more dilute spore suspension was applied (causing 15–20 necrotic flecks/leaf) the rise in the enzyme activity was about 70–80% while in the case of a more concentrated spore suspension (80–100 necrotic flecks/leaf) this rise was 325–351%.

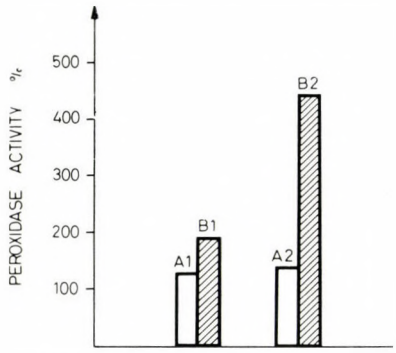

Fig. 3. Peroxidase activity of Little Club wheat leaves inoculated with different uredospore concentrations of *P. graminis tritici* race 11 and treated with Blasticidin-S (50 ppm) in the percentage of uninoculated and untreated control
A_1 = inoculated (15—20 flecks/leaf)
B_1 = inoculated and treated (15—20 flecks/leaf)
A_2 = inoculated (80—100 flecks/leaf)
B_2 = inoculated and treated (80—100 flecks/leaf)
Each value is the average of five replications

Ultrasonic treatment

Ultrasonic treatments were carried out to determine whether the toxic material released from the destroyed fungus material would cause necrosis and the hypersensitive reaction. Since the rust fungus is an obligate parasite, rust mycelium would be difficult to obtain from artificial media. Instead a large mass of germinated spores were exposed to ultrasonic treatment. The results of leaf injection with the homogenate, were negative. Only in the case of the largest concentration did a faint chlorosis appear on the leaf surface.

The endotoxin hypothesis could not be supported by the results of the experiment. The chlorotic spots appeared after injection on the cultivars also, which gave no hypersensitive reaction with living fungi (Little Club, Reliance). Chlorotic spots did not appear in cultivars producing the hypersensitive reaction with living fungi (Vernal, Khapli).

Discussion

Similar results as with the wheat–stem rust relationship have been obtained with other host-parasite relationships. Streptomycin or chloramphenicol treatments in *Phytophthora infestans*–potato have induced the necrotic reaction and the death of the fungus. With nickel nitrate or heat treatments in the *Uromyces fabae*–broad bean and *Uromyces phaseoli*–bean, with heat treatment in the *Erysiphe graminis*–wheat host–parasite relationships, it was possible also to induce the hypersensitive necrotic reaction with the destruction of the pathogen (KIRÁLY *et al.*, 1972). The results of these investigations indicate that, at least in certain

obligate parasites, the hypersensitive reaction is the consequence of the destruction of the pathogen. Besides macroscopic observations, we established through biochemical investigations in the *Phytophthora infestans*–potato relationship that there were similarities in the natural and artificially induced hypersensitive reactions (ÉRSEK *et al.*, 1973).

The peroxidase enzyme activity was examined in the wheat–stem rust relationship. Increased enzyme activity has been a characteristic of most natural hypersensitive reactions. Since certain authorities have considered peroxidase to have special role in the resistance of wheat to stem rust, it was desirable to study the activity of this enzyme in an artificially created hypersensitive reaction. According to MACKO *et al.* (1968) the peroxidase enzymes have a direct inhibitory effect on the development of the stem rust fungus.

The results of our investigations indicated that the increase in peroxidase enzyme activity was a consequence of tissue necrosis. Significant increase in the enzyme activity was obtained only when there was tissue necrosis in the wheat leaves.

In previous studies with *Phytophthora infestans* extracts of fungus mycelium were prepared which, when applied to potato slices, induced the hypersensitive reaction. However, in similar experiments in the rust–wheat relationship such efforts to determine endotoxinlike compounds were not successful. There may be two reasons for this differences:

1. Germinated spores were used instead of fungus mycelium.
2. There are certain differences in the mechanism of the development of necrosis in the course of the hypersensitive reaction.

There is no need to explain the first reason which may be the cause of the unsuccess. As for the second presumable reason, in this case also as in the *Phytophthora infestans*–potato relationship, the destruction of the pathogen is the cause of necrosis. However, there is no evidence that an endotoxin released from the fungus mycelium causes the necrosis. In compatible relationship the pathogen changes the host metabolism to favour its own development. However, if the pathogen is destroyed, the "high obligate" relationship is cut. The host cell cannot return to its original metabolism and becomes necrotic. In the case of incompatibility the pathogen is unable to completely influence the host cell in its own favour. The susceptible relationship cannot be established and the pathogen and host cell sooner or later die.

We believe that in both cases the effect is a mutual relationship between the pathogen and host cell. In cases of incompatibility the injury of the pathogen and of the host cell cannot be separated. Perhaps in certain cases the visible injury occurs earlier in the host cell but the fungus inhibition can also be detected by its slower development (MACLEAN *et al.*, 1974).

The results of the present experiment suggest some answers to the question of the cause and effect relationships. The hypersensitive-necrotic reaction and increased peroxidase enzyme activity associated with it are the consequences and not the causes of plant resistance.

These observations are in close correlation with the data which have appeared in the literature in recent years and which could not be explained by the earlier concepts. BROWN *et al.* (1966) were unable to show any correlation between the rust colony size and the size of the necrosis formed around it. DALY and co-workers, using wheat cultivars which change their resistance to stem rust under different temperatures did not find any correlation between resistance and phenol content, either before or after infection (SEEVERS and DALY, 1970; DALY *et al.*, 1971). Their investigations also include peroxidase isoenzymes. However, their results were negative; they could not demonstrate a correlation between resistance and peroxidase activity (DALY *et al.*, 1971).

From the experiments in the present study, supported by the data of other authors, we believe that the necrosis and changes in peroxidase activity in the course of the hypersensitive reaction are not the causes of resistance but the results of the incompatibility between wheat and the stem rust fungus.

Acknowledgement

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Apoplexy of Apricots

IV. Studies on the Toxin Production of *Cytospora (Valsa) cincta* Sacc.

By

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A toxic substance was obtained from three isolates of the fungus, *Cytospora (Valsa) cincta* Sacc. When absorbed by attached young apricot shoots the toxin caused leaf collapse, gum production and necrotic wounds.

Optimum toxin production was achieved when fungus mycelium was cultivated on cellophane disks placed on potato dextrose agar for 15 days and subsequently extracted by floating the disks on distilled water for 72 hours.

Experimental results indicated that the toxic substance was an exotoxin of high molecular weight and highly heat stable. A protein component was present, but was not the toxic factor. There was also no evidence to indicate that a lipid component was responsible for the toxicity. The carbohydrate component present was believed to be an important part of the toxic agent.

Further studies are needed to characterize the toxic compound. The exotoxin may have an important role in apricot tissue necrosis and canker development in the apricot die-back disease.

Cytospora (Valsa) cincta Sacc. is a significant pathogen of apricot and peach trees. The fungus plays an important role in the apricot die-back and canker and the early death of peach trees. (ROZSNYAY and KLEMENT, 1973; STANOVA, 1968).

The pathogen has a relatively wide host range, it is able to infect apricot, peach, cherry, plum, sour-cherry and other *Prunus* species (SORAUER, 1928; VIENNOT-BOURGIN, 1949; HELTON and MOISEY, 1955).

The fungus infects the trees through wounds. At the site of fungus penetration the cortical tissues of apricot trees become necrotic. As a result of tissue collapse well defined sunken areas develop that gradually expand into large cankers. Usually gum exudates are present on the cankered branches. The infection process takes place within a relatively short time in the case of young (2—3 year-old) branches. It can continue for several years in the older branches. The extension of necrosis is more rapid longitudinally than laterally. Moreover, the infection spreads more rapidly in the xylem than in the phloem. Longitudinal and sectorial browning and necrosis develop in the xylem.

Isolation of the fungus was successful in every case from the brown-colored phloem. However, in most cases it was unsuccessful from brown-colored xylem tissues 8—10 cm from the inoculation site. The inability to isolate the fungus from the discolored xylem, coupled with the wilting symptoms of the infected

shoots before they are completely girdled by necrosis, suggest the role some toxic material produced during the infection process. (Fig. 1)

A number of toxins produced by micro-organism are known to cause plant disease symptoms. The term "toxin" used in a broader sense, involves any substance produced by a given pathogen that contributes to its pathogenicity in the development of a disease. Accordingly, certain enzymes transferred to plant tissues by pathogens can also be called toxins.

Fig. 1. Wilting symptoms on the young apricot tree infected by *Cytospora cincta*

CAKADZE (1959) detected a toxin-like material from *Cytospora leucostoma*. A similarity was shown between *C. leucostoma* and *C. cincta* in respect to their tissue injury and symptom expression (TASNÁDY and LEHOCZKI, 1966). On the basis of these disease symptoms and the above-mentioned literature we tried to demonstrate some toxic material produced by *Cytospora cincta*.

Materials and Methods

Culturing of fungus

The initial inoculum was obtained from the mycelia of the C_1, C_2, and C_6 strains of *C. cincta* isolated from diseased apricot trees and cultured on potato dextrose agar. For the production of the toxin, the method of BICKEL (1961)

was applied with some modifications. The surfaces of the potato dextrose agar were covered with sterilized cellophane disks. The mycelium of the fungus, as an inoculum, was placed on the surface of the cellophane and incubated in a thermostate at 23 – 25 °C. Cultures of *Rhizoctonia solani* and *Alternaria crassa* were used as controls. Potato dextrose agar plates covered with non-inoculated cellophane disks served as absolute controls. After the cellophane disks were overgrown by the fungus, they were removed and floated on 10 ml of sterilized distilled water in Petri dishes for 24, 48 or 72 hours. This liquid was passed through a Seitz-filter (G6) in order to obtain a culture filtrate free from conidia and mycelia.

Determination of phytotoxicity

For determining the toxicity of the culture filtrates, the following bio-assays were used:

a) The culture filtrate was injected into intervenial areas of the primary leaves of Pinto bean (*Phaseolus vulgaris* L. cv. 'Pinto') and the leaves of tobacco plants (*Nicotiana tabacum* L. cv. Xanthi-n.c.) at the 6-leaf stage using KLEMENT's (1963) method. Both the degree and the type of tissue necrosis were examined 24 – 28 hours after the injection.

Fig. 2. Fungus filtrate take up into young apricot shoots. Close to the site of treatment 2 withered leaves can be seen

b) Rootles seedlings of young tomato and bean plants and excised shoot of young apricots 20–25 cm in length, were placed in the culture filtrate.

c) Using the method of DE VAY *et al.* (1968), cotton threads 4–5 cm in length were threaded through young apricot shoots and branches leaving the ends of the thread hanging down into tubes containing culture filtrate (Fig. 2). In order to reduce evaporation the tubes were covered with aluminium foil.

Control materials used in each bio-assay were the filtrates of *Rhizoctonia solani* and *Alternaria crassa* without dilution. The absolute control material used was the distilled water on which non-inoculated cellophane disks was floated.

Optimal period for toxin production

In order to determine the optimum period required for toxin production, the following experiments were carried out:

C. cincta was cultivated on the cellophane disks over P.D.A. for 5, 10 and 15 days. Then the cellophane disks from each series of the different ages were floated on distilled water for 24, 48 and 72 hours, respectively. These fluids were passed through Seitz-filters. A dilution series of 1 : 2, 1 : 4, 1 : 8 was made up of the filtrates using tap water. The diluted samples were injected into the primary leaves of 2-week-old bean plants. The development of necrosis was evaluated after 48 hours.

Source of the toxin

In order to determine whether the toxin was endogenous or exogenous, cell-free homogenates were made. A 2 gr sample of mycelium obtained from the shaken culture was washed thoroughly in distilled water. The mycelium suspension was centrifuged at 4 °C in a "Janetzki K 23" centrifuge at 5000 *g* for 20 minutes. The supernatant was injected into the primary leaves of two-week-old bean plants. After 48 hours the development of necrosis was evaluated.

Characterization of toxin-like material

The fluids obtained after flotation were passed through Seitz-filters. These filtrates were used as starting materials for further steps in characterization of the toxin. The products from all of the procedures below were assessed by injections into the primary leaves of bean plants. The results were evaluated 48 hours after injection.

1. *Evaporation*: 50 ml of filtrate was evaporated to dryness at 100 °C in vacuum. The residue was dissolved in 10 ml of distilled water. Dilutions of 1 : 2, 1 : 4, 1 : 8 were made prior to injection into bean leaves.

2. *Dialysis*: 2 ml of filtrate was dialyzed against distilled water, tap water, and 0.15 *M* phosphate buffer (pH 6.4), respectively, at 4 °C for 24 hours.

3. *Chromatography*: 20 ml of filtrate was dialyzed against polyethyleneglycol (Type 20 000) at 4 °C overnight. The material, concentrated to a volume of 4 ml, was eluted through a Sephadex G-25 column (2 × 25 cm) with distilled water. The fractions of the eluate (4 ml each) were subjected to various bioassays and the UV absorption of eluent fractions was determined at 280 nm in a Unicam SP 800 spepctrophotometer.

4. *Precipitation with acetone*: 10 ml of filtrate was precipitated with 20 ml acetone at −20 °C with frequent stirring. The mixture was centrifuged at 5000 *g* for 20 minutes. Subsequently the acetone was evaporated from the supernatant. The residue was dissolved in 5 ml of 0.15 *M* phosphate buffer (pH 6.4).

5. *Precipitation with ammonium sulfate*: Finely powdered ammonium sulfate was added to 10 ml of the filtrate to 90% saturation with frequent stirring at 4°C. The mixture was centrifuged at 5000 *g* for 20 minutes. The supernatant was dialyzed against 0.15 *M* phosphate buffer (pH 6.4) at 4 °C overnight. The residue was dissolved in 5 ml of 0.15 *M* phosphate buffer (pH 6.4) and dialyzed overnight.

6. *Heat treatment*: 2 ml samples of the filtrate were boiled in a water bath for 5, 10, 30 and 60 minutes, respectively. After boiling, the solutions were brought up to 2 ml with distilled water.

7. *Extraction with ether*: 10 ml of filtrate was extracted three times with the same volume of ethyl ether, and the ether phases were combined. Ether was evaporated at room temperature and the residue dissolved in 4 ml of 0.1% tap water solution of Tween 40 (palmitylsorbitan polyethylenglicolether). From the water phase the ether was evaporated. Both the material dissolved in Tween 40 and the water phase were injected into bean leaves.

8. *Extraction for lipid components from complex*: The extraction of potentially toxic lipids was carried out with a mixture of chloroform and methanol (c. f. WILLIAMS and CHASE, 1968). After extraction the residue was taken up in a 0.1% tap water solution of Tween 40.

9. *Hydrolysis in HCl*: 5 ml of filtrate was boiled together with 5 ml of 1 *N* HCl for 20 min and allowed to stand overnight. The material was neutralized with 1 *N* NaOH and dialyzed against 0.15 *M* phosphate buffer (pH 6.4) for 12 hours.

10. *α-naphtol test for carbohydrates*: 0.1 ml α-naphtol (1% w/v in 96% ethanol) was added to 1 ml of the filtrate. Then 1 ml conc. H_2SO_4 was surface layered. The results were evaluated after 16 hours.

Results

Twenty four hours after the injection with the filtrates of *Cytospora cincta* red-brown edged necroses were beginning to form and were fully developed on the bean leaves by the following day. The injected interveinal areas of the leaf became very necrotic and then withered within 6 days (Fig. 3). Neither the filtrates

Fig. 3. Necrotic intervenial area of bean leaf 3 days after the injection of the fungus filtrate

of *Rhizoctonia* and *Alternaria* nor those of the absolute controls caused any visible necrosis in the injected bean leaves. None of the filtrates caused necrosis in tobacco leaves. After 4–5 days only chlorotic spots appeared.

The filtrates of the three strains of *C. cincta* caused wilting on the apricot shoots and on rootless bean and tomato seedlings. In every case the symptoms appeared 24–36 hours after placing the shoots and plants in the filtrates. No wilting symptoms appeared in the controls.

The filtrates of three strains of *C. cincta* conducted through the intact apricot shoots with the cotton thread wicks caused collapse after 48 to 72 hours. These symptoms usually appeared on the apex or edge of the leaves (Fig. 4), after which the leaves withered, curled and finally dropped off (Fig. 2).

Fig. 4. Tissue collapse on the apex of apricot leaf after take up the *Cytospora* filtrate

The leaf collapse did not appear on the whole shoots since the filtrate take up was limited. By the third day, around the cotton thread inserted through the shoot, a minor wound developed that later became dry. Around the site of treatment in the young branches, the phloem was necrotic while the xylem showed vertical black striped discoloration. Furtheı more, gum production and sunken areas formed around the wounds. The control filtrates (including both the absolute and fungal controls) caused callus formation that later overgrew the wound. The *Cytospora* filtrates inhibited callus formation in every case. From the site of the symptoms caused by *Cytospora* filtrates the fungus could be not isolated.

Optimum cultivation and flotation time of C. cincta for the toxin production

The experimental results summarized in Table 1 indicate that the most effective phytotoxicity was produced by the 15-day-old culture floated for 72 hours. Using longer periods than 15 days and 72 hours the cultures became old and little quantities of a viscous material were produced.

Table 1

The toxicity of *Cytospora*-filtrate in respect to cultivation and flotation period of the fungus
+: positive reaction, −: negative reaction in bean leaf

Age of fungus culture	Time of flotation	Dilutions			
		1×	2×	4×	8×
5-day-old	24 h	−	−	−	−
	48 h	−	−	−	−
	72 h	−	−	−	−
10-day-old	24 h	−	−	−	−
	48 h	+	+	−	−
	72 h	+	+	+	+−
15-day-old	24 h	−	−	−	−
	48 h	+	+	+	+−
	72 h	+	+	+	+

The homogenate of mycelium injected into bean leaves caused no visible symptoms. The toxic material is produced by the living fungus. It is not a decomposition product of the mycelium, and is therefore an exotoxin.

Characterization of the toxin-like material

1. *Evaporation*: The evaporation to dryness at 100 °C reduced phytotoxicity of the filtrate. The residue taken up in 10 ml distilled water produced positive reaction but its dilutions caused no symptoms.

2. *Dialysis*: After dialysis the filtrate produced positive reaction with unchanged intensity in every bioassay.

3. *Chromatography*: Toxicity and UV absorption at 280 nm was found in the fractions No. 7−12 of the column chromatography eluate. In the course of preliminary calibration with albumin of the column the same fractions contained a material of high molecular weight. The results of chromatography corresponding with those of dialysis, clearly indicated that the molecular weight of the toxic material was more than 5000.

4. *Precipitation with acetone*: After precipitation with acetone, the supernatant showed a weak reaction. However the residue dissolved in buffer was intensively effective in bio-assays.

5. *Precipitation with ammonium sulfate*: Only the precipitate dissolved in buffer proved to be toxic. The supernatant had no effect.

6. *Heat treatment*: Each sample boiled for 5, 10, 30 and 60 minutes, respectively, was toxic without any decrease in intensity.

7. *Extraction with ether*: The combined ether phases contained no toxic material while the water phase remained toxic despite the three extractions with ether.

8. *Extraction for lipids*: Since the toxic material might be a lipid, extraction for lipid was carried out. Toxic lipids usually occur in the form of complexes. After the procedure, there was no toxic effect in the lipid containing factors.

9. *Hydrolysis in HCl*: In the course of hydrolysis the material lost the toxic character.

10. *α-naphthol test*: The toxic *Cytospora*-filtrate exhibited positive reaction with α-naphthol (violet discoloration) while the nontoxic control filtrates showed no discoloration.

Discussion

Experimental results with C_1, C_2 and C_6 strains of *C. cincta* indicated that the fungus produces a phytotoxic substance. This toxic substance caused interveinal necrosis when injected in Pinto bean leaves. Young apricot shoots and young bean and tomato plants with excised roots placed in a culture filtrate of the fungus showed severe wilting symptoms. When the culture filtrate was introduced into attached apricot shoots by way of cotton thread wicks, leaf collapse or formation of gummy wounds in the shoots occurred, followed by necrosis of the stem tissues and canker development.

Optimum toxin production was obtained by cultivation for 15 days and flotation for 72 hours.

The fact that the toxin is a product of the living fungus and not a decomposition product of the macerated mycelium suggests that an exotoxin is involved.

Furthermore, the results indicated that the exotoxin obtained was a high molecular weight complex compound more than 5000.

The absorption maximum at 280 nm and the precipitation with acetone and ammonium sulfate suggested the presence of a protein component. The phytotoxic substance was highly heat stable. Therefore, it is unlikely that the protein component was responsible for the toxicity.

There was no experimental evidence to indicate that a lipid-fraction was involved in the toxic effects.

Since the toxic substance gave positive reaction with α-naphtol reagent it is believed that a carbohydrate component is present, and that it is an important part of the toxic agent. Further studies are necessary to determine the exact characterization of the toxic compound.

The exotoxin may have an important role in the necrosis of apricot tree tissues and the development of cankers in the apricot die-back disease, and therefore merits continual investigation.

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Studies on the Biology and Ecology of Onion Downy Mildew (*Peronospora destructor* (Berk.) Fries) in Hungary

I. Overwintering of the Pathogen in Onion Bulbs

By

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On the basis of histological and symptomatological studies carried out during the years, 1971—1973, the following conclusion could be drawn: *Peronospora destructor* mycelia overwintering in onion bulbs are the main sources of the primary infection in Hungary.

Even on heavily infected onion fields grown for seed, the highest percentage of plants with systemic symptoms remains under 1 per cent. The infection rate depends on the length of the vegetation period as well as on the climatic conditions of the previous year.

The determination of primary infection sources is very important for the understanding of the development of a given disease. In this work it was aimed to study the mode of the overwintering of the onion downy mildew fungus under Hungarian climatic conditions.

MURPHY (1921), MURPHY and MCKAY (1926) and KATTERFELD (1926) demonstrated that *P. destructor* are able to grow into onion bulbs as mycelia and these bulbs are the primary sources of downy mildew infections in the next spring. This mode of overwintering was also stated by other authors (YARWOOD, 1943; VAN DOORN, 1959; RONDOMANSKI, 1964). Data of these authors mentioned above, however, differ greatly in regard to percentage of infection rate. The two extremes are represented by MURPHY and MCKAY (1926) in Ireland with 100 per cent, and by YARWOOD (1943) in California with 0.004 per cent. Recently, RONDOMANSKI (1967) affirmed that under Polish conditions the percentage of systemic infection depends upon the development of the disease and on the length of the growing season of the previous year.

Material and Methods

To determine the occurrence of downy mildew mycelia, bulbs of heavily infected onion plants were collected from fields as well as artificial inoculations were carried out by means of YARWOOD's (1943) method.

The inner scales of the collected and artificially inoculated bulbs were fixed in WILLER's (1970) solution for 24 hours. After being washed with 70 per cent

ethanol, sections at a thickness of about 20 μ were made either by hand or by a freezing microtome. The sections were placed on microscopic slides, and were stained with 0.05 per cent cotton blue in lactophenol for 5–10 minutes. Mycelia were readily recognized by their dark blue appearance (Fig. 1). Numerous field investigations were carried out during the spring in 1971, 1972, and 1973, in order to observe and count the number of onions showing primary disease symptoms.

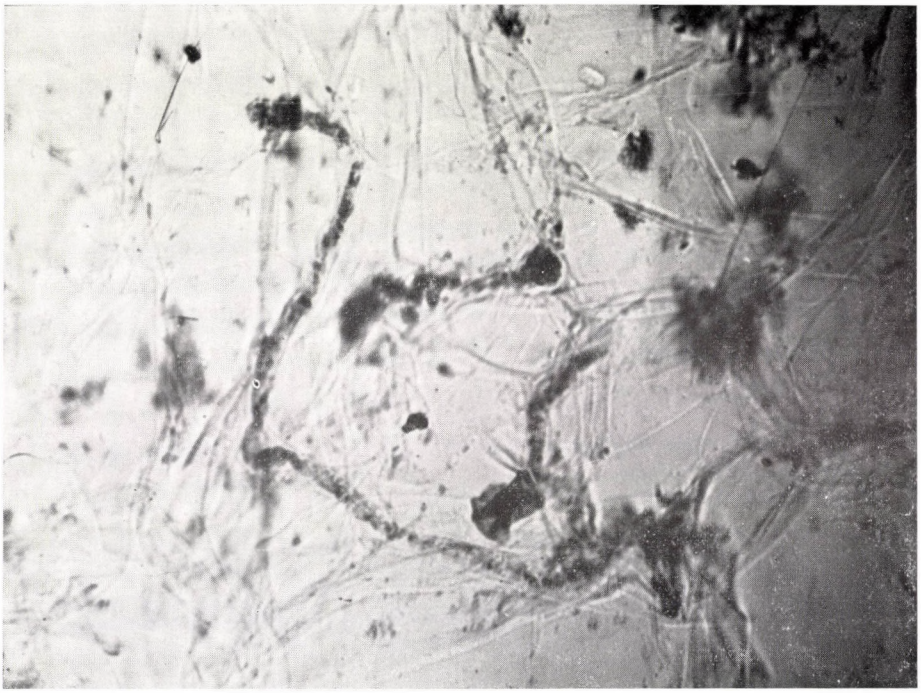

Fig. 1. *Peronospora destructor* hyphae inside the fleshy scale of an onion bulb (cross-section) 100×

Results

Among the bulbs of onion plants heavily infected in the previous year, the fungus mycelia occurred only in a single bulb. On the other hand, all the artificially infected bulbs contained rests of conidia, germ tubes and hyphae branching irregularly (Fig. 1). Further studies showed that mycelia are present not only in the fleshy scales, but also in the stem as well as in the rudimentary central shoots. Histological examinations of the sprouting leaves proved that the pathogen grows with its host plant simultaneously. On the basis of these results the following conclusion could be drawn: the pathogen overwinters as mycelium inside the onion bulbs causing primary infections in the field after sprouting.

About half of the onion bulbs artificially inoculated with the pathogen resulted in plants having primary symptoms of the disease, as it was indicated by bent and light green leaves (Fig. 2). Onion bulbs in the field, however, rarely became naturally infected, as it was found during the three-year period. Onion plants grown from sets proved to be healthy, whereas those grown for seed showed primary infections usually in 0.1 – 0.5 per cent. The highest percentage of systemic infection (0.75 per cent) was observed in an experimental field, where onion bulbs obtained from heavily infected plots were replanted.

Fig. 2. Onion grown for seed systemically infected with *Peronospora destructor*

Discussion

Both symptomatological and histological studies proved that mycelia overwintering in onion bulbs are the main sources of primary infection of *P. destructor* in Hungary. KATTERFELD (1926) described a hypha-form from bulbs which differs morphologically from those found in other parts of the invaded onion plant. In contrast with KATTERFELD's (1926) statement, at least under Hungarian conditions, mycelia of various types were found both in bulbs and other parts of the infected onion plants. Though, the occurrence of mycelia in the diseased plants

can be readily recognized by histological examinations, this method appears to be unsuitable for practical use because of the scarce occurrence of systemically infected bulbs.

The observation of the onion plants showing primary symptoms in the field seems to be more dependable.

During the three years, even in the case of heavy epidemic, the highest percentage of systemic infection reached only 0.75 per cent. VAN DOORN (1959), JOVIČEVIĆ (1964) and RONDOMANSKI (1964) obtained similar results. On the other hand, some authors reported much higher infection rates. Both KATTERFELD (1926) in the Soviet Union and JAMALAINEN (1952) in Finland found systemic infection in a rate of 80—100 per cent. This contradiction can be explained by the differences in the climatic conditions of various countries. In the northern lands, for example in Ireland, the vegetation period is much longer than in countries with a long and hot summer. The former conditions certainly promote the growing of the fungus mycelia into the bulb. Climatic conditions can be markedly different even within a single country resulting in different infection rates as it was observed by RONDOMANSKI (1967) in Poland.

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Studies on the Biology and Ecology of Onion Downy Mildew (*Peronospora destructor* (Berk.) Fries) in Hungary

II. Factors Influencing Sporulation and Conidium Germination

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Previous light effect and at least 95 per cent relative humidity proved to be essential for the conidium formation of the onion downy mildew fungus, *Peronospora destructor*. Optimum temperature for sporulation varies between 11—13 °C. Conidia exposed to direct sunlight loose viability in a few hours, on the other hand, those kept in the shade germinate even after 24 hours.

Germination occurs only in free water, at a range of temperature between 6 and 27 °C, with an optimum of 10—12 °C. The first germ tubes develop in 2—4 hours under favourable conditions.

YARWOOD (1937) described, that sporulation of several downy mildew fungi occurred only in the dark after the host plant had been exposed to a light effect.

According to TALIEVA (1966) sugar content of plant infected with *P. destructor* seems to be much higher before, than after sporulation.

RONDOMANSKI (1967) stated, that both low and high temperature delayed sporulation and caused certain morphological changes.

Conidia attached to the conidiophores keep viable much longer than those detached and lying on the surface of the host plant (YARWOOD, 1943; RONDOMANSKI, 1967). COOK (1932) found that lake water was more favourable for conidium germination than distilled water. On the other hand, RONDOMANSKI (1964) achieved the best result, when 0.01 *M* glucose solution in twice distilled water was applied. A considerable number of data is available concerning the effect of temperature on conidium germination. All of these indicate the optimum between 10 and 13 °C.

Materials and Methods

Onion leaves and seedstalks collected from infected plants were placed into the humid chamber in various hours of the day, from the morning hours till the late afternoon. During this period (16—24 hours) the temperature varied between 10 and 20 °C. Fresh conidia formed in the night were examined either directly on microscopic slides, or by means of germination tests. In the latter case

conidia were separated from the plant surface, and were transferred to slides by shaking or wiping them with a brush. Most frequently, however, conidia were washed off with distilled water. Germination tests were made on dry slides, on slides covered with small droplets of dew, or in drops of conidium suspension placed on the slide. The density of suspension was checked by means of a haemacytometer, and was adjusted to $2 \times 10^4 - 6 \times 10^5$ conidium/ml.

In all cases the microscopic slides were placed into Petri dishes with wet filter paper and were kept in various temperatures from 6 to 27 °C for 2, 4, 6, 24 and 48 hours. After these intervals the slides were removed and evaluated by counting at least 300 conidia per every treatment.

Results

Sporulation occurred only on those infected plants which were taken into the humid chamber in the afternoon. Sporulation was promoted by wiping the plant surface with cotton. On the other hand, sporulation during the previous day totally blocked the recurrence of conidia.

Sporulation often took place on onion leaves and seedstalks without any previous symptoms.

Making a large number of germination tests, it was found that germ tubes developed either in condensed water, or in drops of conidium suspension put on the slides.

Germination seems to be inhibited in distilled water, produced by a distilling apparatus made by copper. Best results were obtained in twice distilled water containing 0.01 *M* glucose. Germination tests made on various temperatures showed, that the highest number of germ tubes developed at 10–12 °C (Table 1).

Table 1

Conidium germination of *Peronospora destructor* on various temperatures

Temperature °C	Germination %
6	27.7
11	34.4
12	37.1
21	21.3
27	1.6

The viability of conidia depends on their age. Conidia placed every two hours in a humid chamber germinated equally well up to the sixth hour, however, those placed there a longer time after their harvest lost viability (Table 2). The

minimum length of time necessary for conidium germination proved to be 2–4 hours, and after 24 hours further germ tube development could scarcely be observed.

Table 2

Effect of age of *Peronospora destructor* conidia on the germination

Length of time after harvest; hour	Germination %
0	67.6
2	51.0
4	4.7*
6	67.0
8	12.0
24	4.3

* Due to unknown failure

Discussion

In agreement with YARWOOD's (1943) and VAN DOORN's (1959) results it was found, that the onion downy mildew fungus requires previous light effect and at least 95 per cent relative humidity for conidium formation.

Sporulation often occurred without previous lesion formation on the infected leaves and seedstalks. Such a phenomenon has been rarely mentioned in the literature (COOK, 1932; RONDOMANSKI, 1967).

Sporulation prevents further production of conidiophores and conidia during the next 24 hours, which is due to the lack of nutrients in the host tissue.

Observations carried out in Poland revealed, that both low and high temperatures induce the formation of irregular conidia (RONDOMANSKI, 1967). Such abnormal forms have been repeatedly observed by the author.

Conidia, when detached, were more sensitive to direct sunlight and high temperature than those remaining on the conidiophores. In the latter case the rate of germination started to decrease after six hours, nevertheless some new germ tubes were observed even after 24 hours.

YARWOOD (1943), VAN DOORN (1959), RONDOMANSKI (1967) and others indicated, that *P. destructor* conidia germinate only in free water. Author's results completely confirm this observation. Similarly to RONDOMANSKI (1967) the best germination was found in twice distilled water containing 0.01 *M* glucose. There is a common opinion in the literature, that optimum temperature for germination varies between 10 and 13 °C. A great number of tests carried out by the author gave similar results.

There is no difference in dynamics of conidium germination found in the

literature and observed by the author. In agreement with the opinion expressed by RONDOMANSKI (1967), the germination of the conidia begins in 2–4 hours under favourable conditions.

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Black Dead-arm Disease of Grapevine Caused by *Botryosphaeria stevensii* Infection

By

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The infection of *Botryosphaeria stevensii* Shoem. (st. con. *Sphaeropsis malorum* Berk.) that caused partial and total grapevine destruction resembling dead-arm disease is at present sporadic in the Hungarian grape-growing regions.

The pathogenic fungus did not infect the green parts of the vines nor the clusters. Phloem and the xylem tissues of the infected woody parts were black discoloured. Therefore the name "black dead-arm disease" seems to be appropriate to distinguish from the disease caused by *Phomopsis viticola* Sacc. ("dead-arm disase"). The pathogenic fungus sometimes associated with the infection of *Phomopsis viticola* Sacc. and with the serious xylem disordering induced by the rugose wood (legno riccio) virus disease. The rootstock tissues of infected vines (Teleki Kober 5BB and 5C) became diseased too, in a few cases.

Research on the etiology of vine destructions like dead-arm disease, primarily proved the occurrence of the pathogenic fungus *Phomopsis viticola* Sacc. in Hungarian grape-growing districts (LEHOCZKY, 1972) but at the same time gave also some evidences that other pathogenic fungus can cause similar symptoms characteristic of the dead-arm disease. In the period 1970—73 we found in several Hungarian grape-growing districts (Tokaj, Mátra, Lake-Balaton and Great Plain grape-growing districts) some vines which showed dead-arm diseaselike symptoms caused by *Botryosphaeria stevensii* Shoem. (= *Physalospora mutila* (Fries) N. E. Stevens; st. con.: *Sphaeropsis malorum* Berk. = *Diplodia mutila* (Fries) Mont.) The isolation of the fungus from the bark and xylem tissues of the diseased parts was equally successful.

Literary data on the occurrence of the fungus on grapevines

Only few data can be found in the literature on the occurrence of *Sphaeropsis malorum* Berk. on grapevines. STEVENS (1933) found a difference between *Sphaeropsis malorum* Berk. and *Sphaeropsis malorum* Peck and he considered them, with the exception of the apple, as polyphage saprophytes on the grapes.

VERWOERD and DIPPENAAR (1930) reported the first proof of the fact that *Sphaeropsis malorum* Berk. may become pathogenic on the grapes because it caused serious berry and cluster rot on the *Vitis vinifera* cultivars in South Africa (White and Red Hanepoot). The berries became infected in a stage close to rip-

ening, then discoloured to dark brown, shrivelled and mummified. Sometimes it caused complete destruction on clusters. The yield loss was 25–30%. Symptoms on other vine parts, were not reported.

Recently CHAMBERLAIN *et al.* (1964) in Canada isolated *Sph. malorum* Berk. from the woody parts of cv. Concord vines (*Vitis labrusca* L.). They found that this fungus together with *Sph. malorum* Peck associated with the dead-arm disease initiated by *Phomopsis viticola* Sacc. infection. The occurrence of the other species *Sph. malorum* Peck was rather frequent and sometimes it occurred even independently. That is why they supposed that it has a role in certain phases of the development of dead-arm disease.

The pathogen

For the identification of the pathogenic fungus some papers (STEVENS, 1933; SHOEMAKER, 1964) were taken into consideration to see distinctly for the taxonomic confusion between *Sph. malorum* Berk. and *Sph. malorum* Peck. The pathogen found and isolated in Hungary, was identical with *Sph. malorum* Berk. (= *Botryosphaeria stevensii* Shoem.).

The hypha septate, initially hyaline later oil brown coloured. The pycnidia develop singly or sometimes in groups in the dead cortex tissue are 130–195 μ in size, have ostioles and 33–195 μ long beaks (Fig. 1), their walls dark brown and rather thick. Conidia hyaline, one-celled, cylindric with thick, smooth glassy wall, broadly rounded ends and with guttules in the plasma (Fig. 2). The size of conidia developed on dead woody parts as natural substrate was 24.0–27.3 × × 10.1–13.0 μ. Length/width quotient 2.2. Conidia after discharge remain hyaline for more than 15 days under humid conditions and give a white mass around the ostioles. More rarely they form short tendrils and slowly discolour to slightly brown. Two-celled brown coloured conidia rarely occur.

The perithecium of the pathogen so far has not been found in Hungary on grapevine.

Infection and Symptoms

So far symptoms on green parts and on clusters have not been found from infection of *Sph. malorum* Berk. in Hungary. Whereas the fungus was isolated repeatedly from the woody parts of many-year-old vines (Fig. 3) showing the dead-arm diseaselike symptoms.

The main season for infection of woody parts and the infection courts have not been clarified. It may be supposed that the main season for infection is depending on the weather conditions in spring when the pycnidia ripen in mass about May and June. Probably the pathogen invades through the wounds of woody parts, pruning wounds, stubs (Fig. 4) or through the residue of woody tendrils

Fig. 1. Pycnidia on the decayed bark tissue. Magn. ×100

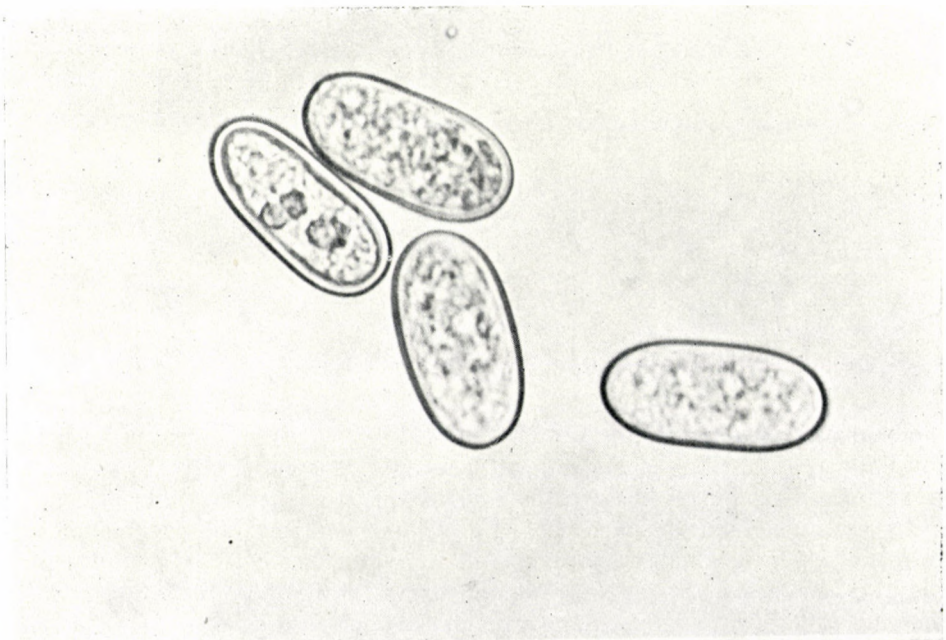

Fig. 2. Conidia. Magn. ×1200

Fig. 3. Symptoms resembling "dead-arm disease" [cv. Furmint/T. K. 5C]

and forms a wide and rather deep wound (Fig. 5). After penetration depending on the age of the infected tissue the disease remains latent covered by rhytidome for some years until the diseased part of the vine dies.

On certain parts of many-year-old but well nourished vines, sometimes on whole branches, the buds do not burst and these later totally die (Fig. 3). The bark tissue of the diseased parts and trunks collapses at the site of infection and discolours to dark brown, black and pycnidia develop on the surface (Fig. 4). The fungus sometimes invades the xylem deeply and the tissue decayed wide-spreading-

Fig. 4. Died young trunk with stubs and pycnidia on the surface

Fig. 5. Deep and enlarged wound around a woody residue of tendril was the site of fungal invasion. Around wound the xylem tissue black coloured under the peeled rhytidome and bark tissue

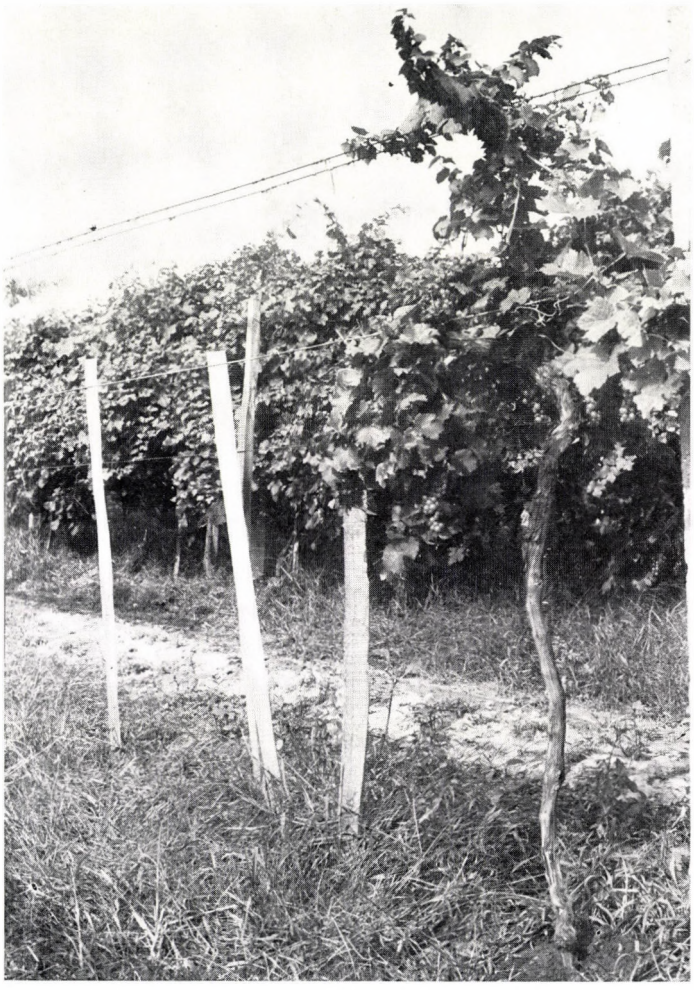

Fig. 6. 11-year-old weakly growing declined vine [cv. Chasselas/T. K. 5BB]

ly then becomes black coloured (Fig. 5). The black discolouration may possibly originate from the colour of the fungus mycelium. The spreading of the fungus in the xylem can happen downwards as well as upwards in a narrow straight stripe.

In some cases the fungus can associate with other diseases and in this way accelerates the partial or total death of the vine. Similarly to that CHAMBERLAIN *et al.* (1964) observed, we also found *Sph. malorum* Berk. pycnidia which developed on the surface of the dead-arm diseased parts, together with the pycnidia

Fig. 7. On the same vine, shows Fig. 6, serious symptoms of rugose wood virus disease on the lower part of the trunk [cv. Chasselas/T. K. 5BB]

Fig. 8. On the same vine, shows Fig. 6, association of *B. stevensii* infection with the symptoms of rugose wood [cv. Chasselas T. K. 5BB]

Fig. 9. On the same vine, shows Fig. 6, independently infection of *Phomopsis viticola* Sacc. on the trunk above the site where *B. stevensii* associated with rugose wood [cv. Chasselas T. K. 5BB]

of *Phomopsis viticola* Sacc. It may also associate with the rugose wood (legno riccio) virus disease. On the lower part of the trunk of an 11-year-old declined vine (Fig. 6), we found serious symptoms of rugose wood (Fig. 7) to that to be associated the infection of *Sph. malorum* Berk. which caused black colouration in the outer part of xylem (Fig. 8). Additional, on the same vine another infection developed at a height of 40 cm around an older stub caused by *Phomopsis viticola* Sacc. (Fig. 9). It is expected that these together cause the total vine destruction in a short time.

It is interesting to note that we have found infection of *Sph. malorum* Berk. on the rootstocks (Teleki Kober 5BB and 5C.) of diseased vines in a few cases (Tokaj, Eger).

The pathogenic fungus is able to infect not only the mature vines but it may cause necrosis of nurseried young grafts (LEHOCZKY, 1974).

Discussion

The disease of the vascular tissue system caused by the infection of *B. stevensii* that leads to partial or total vine death, has a great economic significance. Fortunately, its occurrence in Hungarian grape-growing districts so far is only sporadic, therefore the loss may still be tolerated economically. At present the knowledge of pathological fact is more important, that another pathogenic fungus apart from *Phomopsis viticola* is also able to cause partial or total dead-arm destruction, accompanied by symptoms resembling dead-arm disease. According to the Hungarian observations there is a difference between the infection of *Phomopsis viticola* and *B. stevensii*, namely the last one, does not infect the green parts of the vine and the clusters, but regarding vine destruction, the symptoms are identical. The black discolouration in the phloem and xylem tissues as a consequence of *B. stevensii* infection, may be useful for distinguishing, because it is lacking in the case that initiated by *Phomopsis viticola*. Therefore the proposed popular name "black dead-arm disease", may be suitable to differentiate diagnostically between the two diseases.

There is a contrast between that VERWOERD and DIPPENAAR (1930) found and the Hungarian experiences, namely that berry infection has not occurred in Hungary, while from South Africa reported nothing about vine destruction. Two reasons could explain it: 1. different biotypes, of the pathogenic fungus 2. differences in sensibility among cultivars.

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Necrosis of Nurseried Grapevine Grafts of *Botryosphaeria stevensii* Infection

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The infection of *Botryosphaeria stevensii* Shoem. (st. con. *Sphaeropsis malorum* Berk.) caused necrosis on 26.5% of the nurseried and classified Traminer grafts in the course of winter storing. The phloem and xylem tissues of the scions died and black discoloured and pycnidia developed on their surfaces. The investigations did not provide proofs about when the grafts were infected and where the infection ccurt was.

It is quite exceptional, when among nurseried grapevine grafts after classification and careful winter storing diseased ones can be found in a high percentage. It is a well-known fact that *Botrytis cinerea* Pers. and according to a newer observation *Phomopsis viticola* Sacc. can in the course of forcing, or outdoors during the nursery period cause graft necrosis (SIMON *et al.*, 1969). These diseased grafts, however, can be screened by classification carried out in the autumn after the grafts were being digged up in the nursery. That is why the occurrence of *Botryosphaeria stevensii* Shoem. (st. con. *Sphaeropsis malorum* Berk.) infection has been surprising on the nurseried grafts after classification in the course of winter storing.

Casuistic Description

In one of the Hungarian grape-growing districts (near Eger) grape grafts were stored in the cellar in sand during the winter of 1971/72 for a new plantation. In spring, at the beginning of April when the grafts were selected, it became obvious that of the 10,000 pieces of Traminer grafts on the rootstock Teleki Kober 5BB, 2655 were infected (26.5%) (Fig. 1). Among grafts of other cultivars stored at the same time (Müller-Thurgau, Red Veltliner, Kékfrankos) there were no diseased ones or occurred only in a much smaller percentage.

The origin of the disease is unknown but it can be supposed that the grafts were infected in the field in nursery and the symptoms appeared after a long incubation in the course of winter storing.

On the scion of the diseased grafts, pycnidia developed visibly in spring (Fig. 2) and on some ones hyaline conidium mass accumulated around the ostioles. The conidia were one-celled and their size $26.7-28.4 \times 11.5-13.3\ \mu$. After

discharge they remained hyaline for a considerable time. On the scions of the smaller part of infected grafts pycnidia did not develop, although the phloem and xylem tissues around the unit black discoloured (Fig. 3). On 74.0% of the diseased grafts, pycnidia developed well. The infection did not spread from the scion into the rootstock tissues, herein only a slight physiological browning occurred below the unit (Fig. 3).

Fig. 1. Diseased Traminer grafts on the rootstock Teleki Kober 5BB

Fig. 2. Pycnidia on the surface of the scion (cv. Traminer/T. K. 5BB)

The investigations did not give evidences of when the grafts became infected and where the infection court was, furthermore, whether on the scion the upper cutting wound has had a role in the infection or not.

The pathogenic fungus may also infect mature vines. The symptoms resembling dead-arm disease, causing partial or total vine destructions (LEHOCZKY, 1974).

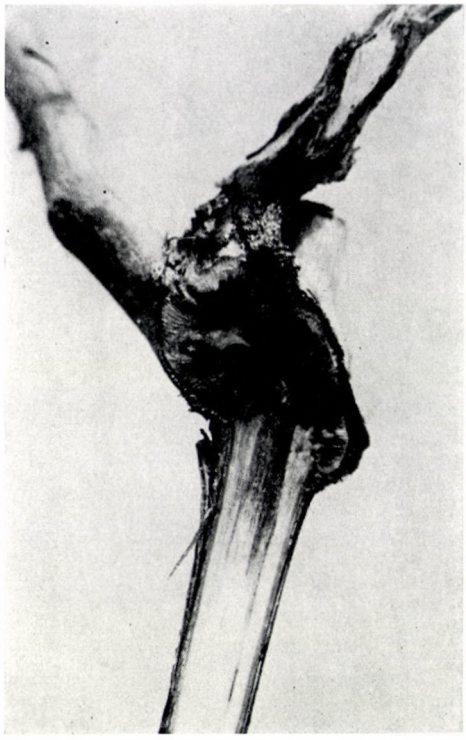

Fig. 3. Longitudinal section of the diseased graft: necrosed and black-coloure dbark and xylem tissues of scion and below the unit physiologically brown coloured rootstock tissue

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Review of the Mycoflora of Hungary

Part XII. *Deuteromycetes: Moniliales* and *Myceliales*

By

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The finishing part of this serial publication covers the rest of the Hungarian Moniliales species and the *Myceliales (Mycelia sterilia)*. Descriptions and a key of the new *Moniliales* families, based on conidium ontogeny were presented in the previous part (Part XI). This paper is actually a list of species belonging to the following families: *Poroconidiaceae*, *Phialoconidiaceae*. Conidium ontogeny of a number of species is not yet known. Others were described under illegitimate names. These species are listed alphabetically at the end of the *Moniliales*. *Myceliales* species are arranged also in an alphabetical order.

In an appendix, formal descriptions of the new, proposed families of *Moniliales* are given.

Familia: *Poroconidiaceae*
Genus: *Alternaria* Nees et Wallr.

Alternaria brassicae (Berk.) Bolle
Ubrizsy G. 1.
as: *Alternaria brassicae* (Berk.) Sacc.: Moesz G. 1. (1749); Hollós L. 7.

Alternaria crassa (Sacc.) Rands.
Moesz G. 13.; Ubrizsy G. 1.

Alternaria dauci (Kühn.) Groves et Skolko f. sp. *solani* (Ell. et Mart.) Neerg.
Syn: *Macrosporium solani* Ell. et Mart.; *Alternaria solani* Ell. et Mart.
as: *Macrosporium solani* Ell. et Mart.: Moesz G. 1. (1643)
as: *Alternaria solani* Ell. et Mart.: Moesz G. 1. (1749); Moesz G. 11.; Moesz G. 13.; Ubrizsy G. 1.

Alternaria dianthi Stevens et Hall.
Moesz G. 1. (1749); Moesz G. 13.; Moesz G. 22.; Vass A. 2.

Alternaria dianthicola Neerg.
Hódosy S. 1.

Alternaria grossulariae Jacz.
Hódosy S. 1.

Alternaria nucis Moesz
Moesz G. 2.; Moesz G. 13.

Alternaria tabacina (Ellis et Everh.) Gulyás
Ubrizsy G. 1.
Alternaria tenuis Nees
Syn: *Macrosporium caudatum* Cooke et Ellis
Hódosy S. 1.; Hollós L. 7.; Moesz G. 1. (1749); Moesz G. 11.; Moesz G. 13.; Nyergesné E. 1.; Tóth S. 7.; Ubrizsy G. 1.; Vass A. 1.; Vass A. 2.
Alternaria tomato (Cooke) Brinkm.
Ubrizsy G. 1.
Alternaria violae Gall. et Dors.
Ubrizsy G. 1.
Alternaria zinniae Pape
Hódosy S. 1.

Genus: *Corynespora* Güssow
Corynespora melonis (Cooke) Lindau
Syn: *Cercospora melonis* Cooke
Pénzes A. 1.

Genus: *Dendryphion* Wallr.
Dendryphion comosum Wallr.
Moesz G. 1. (1733); Moesz G. 13.
Dendryphion penicillatum (Corda) Fr.
Hollós L. 1.
Dendryphion toruloides (Fres.) Sacc.
Hollós L. 7.

Genus: *Diplococcium* Grove
Diplococcium strictum Sacc.
Vass A. 2.

Genus: *Helminthosporium* Link
Helminthosporium anthyllidis Baudys
Tóthné Z. E. 1.
Helminthosporium apiculatum Corda
Tóth S. 1.
Helminthosporium atrovirens (Harz.) Mason et Hughes
Syn: *Helminthosporium solani* Dur. et Mont.; *Spondylocladium atrovirens* Harz.
Hollós L. 1. as: *Spondylocladium atrovirens* Harz.
Helminthosporium betulinum Corda
Hollós L. 1.
Helminthosporium cylindrosporum Sacc.
Moesz G. 1. (1723); Moesz G. 13.
Helminthosporium cynodontis Marignoni
Moesz G. 1. (1723); Moesz G. 11.
Helminthosporium fusiforme Corda
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1723)

Helminthosporium gramineum Rabenh.
Moesz G. 1. (1723); Moesz G. 13.; Ubrizsy G. 1.
Helminthosporium microsorum D. Sacc.
Moesz G. 1. (1723)
Helminthosporium rhopaloides Fres.
Hollós L. 1.; Moesz G. 1. (1723)
Helminthosporium sativum Pam, King et Bakke
Vörös J. 4.
Helminthosporium scolicoides Corda
Moesz G. 1. (1723)
Helminthosporium siccans Drechs.
Vörös J. 1.
Helminthosporium teres Sacc.
Ubrizsy G. 1.
Helminthosporium tiliae (Link) Fr.
Syn: *Exosporium tiliae* Link
Moesz G. 1. (1723); Hollós L. 7.
as: *Exosporium tiliae* Link: Hollós L. 1.
Helminthosporium turcicum Pass.
Podhradszky J. 1.; Vörös J. 3.; Vörös J. 7.
Helminthosporium velutinum Link ex Fr.
Syn: *Helminthosporium macrocarpum* Grev.
as: *Helminthosporium macrocarpum* Grev.: Hollós L. 1.; Hollós L. 7.

Genus: *Spadicoides* Hughes

Spadicoides obovatum (Cooke et Ellis) Hughes
Syn: *Acrothecium obovatum* Cooke et Ellis
as: Acrothecium obovatum Cooke et Ellis: Moesz G. 1. (1732); Moesz G.13.

Genus: *Stemphylium* Wallr.

Stemphylium alternariae (Cooke) Sacc.
Moesz G. 1. (1742); Moesz G. 13.
Stemphylium amoenum Oudem.
Moesz G. 1. (1742)
Stemphylium botryosum Wallr.
Syn: *Macrosporium commune* Rabenh.; *Macrosporium parasiticum* Thüm.
Hollós L. 1.; Vass A. 2.; Vörös J. 3.
as: *Macrosporium parasiticum* Thüm.: Moesz G. 1. (1743)
Stemphylium juniperum Karst. var. *microsporum* Dom.
Hollós L. 7.
Stemphylium macrosporoideum (Berk. et Br.) Sacc.
Hollós L. 1.
Stemphylium radicinum (M., Dr. et E.) Neerg.
Lehoczky J. 2.

Stemphylium ramulosum Sacc.
Syn: *Macrosporium ramulosum* Sacc.
Moesz G. 1. (1743) as: *Macrosporium ramulosum* Sacc.
Stemphylium sarciniforme (Cav.) Wiltshire
Syn: *Macrosporium sarciniforme* Cav.
Ubrizsy G. 1. as: *Macrosporium sarciniforme* Cav.

Genus: *Torula* Pers. ex Fr.

Torula cistina Thüm.
Moesz G. 1. (1637)
Torula herbarum (Pers.) Link
Syn: *Torula abbreviata* Corda; *Torula expansa* (Kunze) Pers.; *Torula herbarum* (Pers.) Link var. *affinis* Sacc.
Hollós L. 1.; Moesz G. 1. (1637); Moesz G. 4.; Tóth S. 7.
as: *Torula abbreviata* Corda: Moesz G. 1. (1637); Moesz G. 2.; Moesz G. 13.
as: *Torula expansa* (Kunze) Pers.: Hollós L. 1.
as: *Torula herbarum* (Pers.) Link var. *affinis* Sacc.: Moesz G. 1. (1637)
Torula monilioides Corda
Moesz G. 1. (1637); Moesz G. 12.
Torula palmigena Bubák
Bubák F. 1.; Moesz G. 13.
Torula pulveracea Corda
Moesz G. 1. (1637)
Torula viticola Allesch.
Hollós L. 7.

Familia: *Phialoconidiaceae*
Genus: *Acremonium* Link ex Fr.

Acremonium strictum Gams
Syn: *Cephalosporium acremonium* Corda
as: *Cephalosporium acremonium* Corda: Husz B. 1.; Moesz G. 1. (1534); Moesz G. 6.; Moesz G. 11.; Moesz G. 13.; Vass A. 2.; Vörös J. 4.

Genus: *Acrocylindrium* Bon.

Acrocylindrium granulosum Bon.
Hollós L. 1.

Genus: *Acrostalagmus* Corda

Acrostalagmus aphidum Oudem.
Moesz G. 1. (1894)
Acrostalagmus cinnabarinus Corda
Moesz G. 1. (1591); Moesz G. 13.; Vörös J. 1.; Vörös J. 3.

Genus: *Aspergillus* Mich. ex Fr.

Aspergillus archiflavipes Blochwitz
Vörös J. 2.

Aspergillus awamori Nakazawa
Nyergesné E. 1.; Vörös J. 2.
Aspergillus caespitosus Raper et Thom
Nyergesné E. 1.; Vörös J. 2.
Aspergillus candidus Link
Syn: *Sterigmatocystis szurákiana* Moesz
as: *Sterigmatocystis szurákiana* Moesz: Moesz G. 1. (1546); Moesz G. 6.; Moesz G. 13.
Aspergillus chevalieri (Mangin) Thom et Church
Nyergesné E. 1.
Aspergillus clavatus Desm.
Vörös J. 2.
Aspergillus effusus Tiraboschi
Nyergesné E. 1.
Aspergillus elegans Gasperini
Vörös J. 2.
Aspergillus flavipes (Bain. et Sart.) Thom et Church
Nyergesné E. 1.; Vörös J. 2.
Aspergillus flavus Link
Nyergesné E. 1.; Vörös J. 2.
Aspergillus foetidus (Naka.) Thom et Raper
Nyergesné E. 1.; Vörös J. 2.
Aspergillus fumigatus Fres.
Nyergesné E. 1.; Vörös J. 2.
Aspergillus humicola Chaudhuri et Sachar
Nyergesné E. 1.; Vörös J. 2.
Aspergillus japonicus Saito
Nyergesné E. 1.
Aspergillus luchuensis Inui
Vörös J. 2.
Aspergillus mangini (Mangin) Thom et Raper
Vörös J. 2.
Aspergillus nidulans Eidam
Nyergesné E. 1.; Moesz G. 13.; Vörös J. 2.
Aspergillus niger Van Tieghem
Syn: *Sterigmatocystis nigra* Van Tieghem
Kászonyi S. 1.; Nyergesné E. 1.; Vörös J. 2.
as: *Sterigmatocystis nigra* Van Tieghem: Moesz G. 1. (1546); Moesz G. 13.; Hollós L. 1.
Aspergillus niveus Blochwitz
Vörös J. 2.
Aspergillus ochraceus Wilhelm
Vörös J. 2.

Aspergillus oryzae (Ahlb.) Cohn
Nyergesné E. 1.; Vörös J. 2.
Aspergillus panamensis Raper et Thom
Nyergesné E. 1.; Vörös J. 2.
Aspergillus petrakii Vörös
Vörös J. 6.
Aspergillus phoenicis (Corda) Thom
Nyergesné E. 1.; Vörös J. 2.
Aspergillus proliferans G. Smith
Nyergesné E. 1.
Aspergillus pseudoglaucus Blochwitz
Nyergesné E. 1.
Aspergillus repens De Bary
Vörös J. 2.
Aspergillus restrictus Smith
Nyergesné E. 1.
Aspergillus ruber (Konig, Spick. et Brem) Thom et Church
Nyergesné E. 1.; Vörös J. 2.
Aspergillus sclerotiorum Huber
Nyergesné E. 1.; Vörös J. 2.
Aspergillus sydowi (Bain. et Sart.) Thom et Church
Vörös J. 2.
Aspergillus terreus Thom
Vörös J. 2.
Aspergillus umbrosus Bain. et Sart.
Nyergesné E. 1.; Vörös J. 2.
Aspergillus unguis (Emile-Weil et Gaudin) Thom et Raper
Nyergesné E. 1.
Aspergillus ustus (Bain.) Thom et Church
Nyergesné E. 1.; Vörös J. 2.
Aspergillus versicolor (Vuill.) Tiraboschi
Nyergesné E. 1.; Vörös J. 2.
Aspergillus wentii Wehmer
Nyergesné E. 1.; Vörös J. 2.

Genus: *Ciliciopodium* Corda emend. Sacc.

Ciliciopodium sanguineum Corda
Moesz G. 1. (1764)

Genus: *Cladobotryum* Nees

Cladobotryum dendroides (Bull. per Mérat) Gams et Hoozemans
Syn: *Dactylium dendroides* (Bull.) Fr.
as: *Dactylium dendroides* (Bull.) Fr.: Moesz G. 1. (1616); Moesz G. 12.; Ubrizsy G. 4.

Cladobotryum varium Nees per Duby
Syn: *Diplocladium minus* Bon.
as: *Diplocladium minus* Bon.: Moesz G. 1. (1602); Ubrizsy G. 4.
Cladobotryum verticillatum (Link per S. F. Gray) Hughes
Syn: *Verticillium agaricinum* (Link) Corda; *Verticillium lactarii* Peck
as: *Verticillium agaricinum* (Link) Corda: Moesz G. 1. (1582); Moesz G. 13.
as: *Verticillium lactarii* Peck: Moesz G. 1. (1582)

Genus: *Cylindrocarpon* Wollenw.

Cylindrocarpon album (Sacc.) Wollenw.
Syn: *Fusarium album* Sacc.
as: *Fusarium album* Sacc.: Hollós L. 1.; Moesz G. 1. (1861)
Cylindrocarpon candidum (Link) Wollenw.
Syn: *Fusidium candidum* Link
as: *Fusidium candidum* Link: Hollós L. 1.
Cylindrocarpon destructans (Zins.) Scholten
Syn: *Moeszia cylindroides* Bubák
as: *Moeszia cylindroides* Bubák: Moesz G. 1.; Moesz G. 13.
Cylindrocarpon obtusisporum (Cooke et Harkn.) Wollenw. var. *medium* Jörg.
Syn: *Fusarium lineare* Moesz
as: *Fusarium lineare* Moesz: Moesz G. 1. (1861); Moesz G. 6.; Moesz G. 13.

Genus: *Fusarium* Link ex Fr.*

Fusarium aquaeductum Lagh.
Syn: *Fusarium sphaeriaeforme* Sacc.
Moesz G. 1. (1861) and Moesz G. 13. as: *Fusarium sphaeriaeforme* Sacc.
Fusarium avenaceum (Corda ex Fr.) Sacc.
Syn: *Fusarium aecidii tussilaginis* Allesch.; *Fusarium corallinum* Sacc.; *Fusarium graminum* Corda; *Fusarium herbarum* (Corda) Fr.; *Fusarium viticolum* Thüm.; *Fusarium zeae* (Westend.) Sacc.
Ubrizsy G. 1.
as: *Fusarium aecidii tussilaginis* Allesch.: Moesz G. 1. (1861)
as: *Fusarium corallinum* Corda: Moesz G. 1. (1861); Moesz G. 4.
as: *Fusarium graminum* Corda: Moesz G. 1. (1861)
as: *Fusarium herbarum* (Corda) Fr.: Ubrizsy G. 1.

* The following Fusaria described from Hungary are not listed here due to their uncertain taxonomic position:
Fusarium discoidum Fautrey
Moesz G. 1. (1861)
Fusarium exobasidii Moesz
Moesz G. 1. (1861)
Fusarium rosae (Preuss) Sacc.
Moesz G. 1. (1861)

as: *Fusarium viticolum* Thüm.: Moesz G. 1. (1861); Moesz G. 13.
as: *Fusarium zeae* (West.) Sacc.: Moesz G. 1. (1861)

Fusarium culmorum (W. G. Smith) Sacc.
Krenner J. A. 1.; Moesz G. 13.

Fusarium graminearum Schwabe

Syn: *Fusarium roseum* Link var. *maydis* Sacc.
Hollós L. 1. as: *Fusarium roseum* Link var. *maydis* Sacc.

Fusarium heterosporum Nees ex Fr.

Syn: *Fusarium heterosporum* Nees ex Fr. var. *lolii* (W. G. Smith) Wollenw.
Moesz G. 1. (1861); Moesz G. 13.
as: *Fusarium heterosporum* Nees ex Fr. var. *lolii* (W. G. Smith) Wollenw.): Moesz G. 1. (1861); Ubrizsy G. 1.

Fusarium lateritium Nees emend. Snyder et Hansen

Syn: *Fusarium episphaericum* (Cooke et Ellis) Sacc.; *Fusarium pseudacaciae* Rapaics; *Fusarium putaminum* (Thüm.) Sacc.; *Fusarium pyrochorum* (Desm.) Sacc.; *Fusarium roseum* Link var. *buxi* Sacc.; *Fusarium roseum* Link var. *dulcamarae* Sacc.; *Fusarium salicis* Fuckel
Hollós L. 1.; Hollós L. 7.; Ubrizsy G. 1.; Vörös J. 1.
as: *Fusarium episphaericum* (Cooke et Ellis) Sacc.: Hollós L. 7.
as: *Fusarium pseudacaciae* Rapaics: Ubrizsy G. 1.
as: *Fusarium putaminum* (Thüm.) Sacc.: Hollós L. 1.
as: *Fusarium pyrochorum* (Desm.) Sacc.: Hollós L. 1.; Hollós L. 7.
as: *Fusarium roseum* Link var. *buxi* Sacc.: Hollós L. 7.
as: *Fusarium roseum* Link var. *dulcamarae* Sacc.: Hollós L. 1.
as: *Fusarium salicis* Fuckel: Moesz G. 1. (1861); Moesz G. 13.

Fusarium lateritium Nees emend. Snyder et Hansen f. sp. *mori* (Desm.) Mauro et Sato

Syn: *Fusarium urticearum* (Corda) Sacc.
Moesz G. 1. (1861) as: *Fusarium urticearum* (Corda) Sacc.

Fusarium moniliforme Sheldon

Syn: *Fusarium moniliforme* Sheldon var. *minus* Wollenw.
Kaszonyi S. 1. as: *Fusarium moniliforme* Sheldon var, *minus* Wollenw.

Fusarium nivale (Fr.) Ces.
Ubrizsy G. 2.

Fusarium oxysporum Schlecht.

Syn: *Fusarium aurantiacum* (Link) Sacc.
Hollós L. 1.
as: *Fusarium aurantiacum* (Link) Sacc.: Hollós L. 7.

Fusarium oxysporum Schlecht. f. sp. *callistephi* (Beach) Snyder et Hansen
Kaszonyi S. 1.

Fusarium oxysporum Schlecht. f. sp. *conglutinans* (Wollenw.) Snyder et Hansen
Kaszonyi S. 1.

Fusarium oxysporum Schlecht. f. sp. *lycopersici* (Sacc.) Snyder et Hansen

Syn: *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* Sacc.; *Fusarium bulbigenum* Cooke et Mass. f. *lycopersici* (Brushi) Wollenw. et Reinking
as: *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* Sacc.: Hollós L. 1.; Ubrizsy G. 1.
as: *Fusarium bulbigenum* Cooke et Mass. f. *lycopersici* (Brushi) Wollenw. et Reinking: Ubrizsy G. 1.

Fusarium oxysporum Schlecht. f. sp. *niveum* (E. F. Smith) Snyder et Hansen
Syn: *Fusarium bulbigenum* Cooke et Mass. var. *niveum* (E. F. Smith) Wollenw. et Reinking
Kaszonyi S. 1.; Kaszonyi S. 2.
as: *Fusarium bulbigenum* Cooke et Mass. var. *niveum* (E. F. Smith) Wollenw. et Reinking: Ubrizsy G. 1.

Fusarium oxysporum Schlecht. f. sp. *pini* (Hartig) Snyder et Hansen
Syn: *Fusarium oxysporum* Schlecht. var. *aurantiacum* (Link) Wollenw.; *Fusarium oxysporum* Schlecht. f. *aurantiacum* Corda
as: *Fusarium oxysporum* Schlecht. *var. aurantiacum* (Link) Wollenw.: Moesz G. 13. as: *Fusarilium oxysporum* Schlecht. f. *aurianticum* Corda: Hollós L. 1.; Krenner J. A. 1.

Fusarium oxysporum Schlecht. f. sp. *tracheiphilum* (E. F. Smith) Snyder et Hansen
Syn: *Fusarium tracheiphilum* (E. F. Smith) Wollenw.
Moesz G. 1. as: *Fusarium tracheiphilum* (E. F. Smith) Wollenw.

Fusarium oxysporum Schlecht. f. sp. *vasinfectum* (Atk.) Snyder et Hansen
Syn: *Fusarium vasinfectum* Atk.
Ubrizsy G. 1. as: *Fusarium vasinfectum* Atk.

Fusarium roseum Link emend. Snyder et Hansen*
Syn: *Fusarium roseum* Link
as: *Fusarium roseum* Link: Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1861); Moesz G. 13.; Moesz G. 22.; Vörös J. 5.

Fusarium sambucinum Fuckel
Syn: *Fusarium sarcochorum* (Desm.) Sacc.; *Fusarium sclerodermatis* Oudem.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1861); Moesz G. 2.; Moesz G. 13.
as: *Fusarium sarcochorum* (Desm.) Sacc.: Hollós L. 7.
as: *Fusarium sclerodermatis* Oudem.: Hollós L. 1.; Moesz G. 1. (1861)

Fusarium solani (Mart.) Sacc.
Ubrizsy G. 1.

Fusarium sphaeriae Fuckel
Hollós L. 1.; Moesz G. 1. (1861)

Genus: *Gliocladium* Corda

Gliocladium roseum (Link) Bain.
Vörös J. 2.; Vörös J. 4.; Vörös J. 5.

* According to Booth (1971) this species is not valid. It was maintained here only for traditional reasons.

Genus: *Gonytrichum* Nees et Nees

Gonytrichum caesium Nees et Nees
Hollós L. 1.; Tóth S. 9.
Gonytrichum macrocladum (Sacc.) Hughes
Syn: *Mesobotrys macroclada* (Sacc.) Sacc.
Tóth S. 7.

Genus: *Hyalostachybotrys* Srinivasan

Hyalostachybotrys bisbyi Srinivasan
Ubrizsy G. 3.

Genus: *Menispora* Pers. ex Chev.

Menispora caesia Preuss
Hollós L. 1.

Genus: *Metarrhizium* Sorok.

Metarrhizium anisopliae (Metschn.) Sorok.
Moesz G. 1.; Moesz G. 13.

Genus: *Myrothecium* Tode ex Fr.

Myrothecium inundatum Tode
Moesz G. 1. (1877)
Myrothecium gramineum Lib.
Hollós L. 7.
Myrothecium roridum Tode
Hollós L. 1.; Hollós L. 7.; Vörös J. 5.
Myrothecium verrucaria (Alb. et Schw.) Ditm.
Hollós L. 1.; Hollós L. 7.

Genus: *Paecilomyces* Bain.

Paecilomyces elegans (Corda) Mason et Hughes
Syn: *Spicaria elegans* (Corda) Harz
Tóth S. 7.; Tóth S. 9.
as: *Spicaria elegans* (Corda) Harz; Ubrizsy G. 3.
Paecilomyces farinosus (Dicks. ex Fr.) Brown et Smith
Syn: *Isaria farinosa* Dicks. ex Fr.
Hollós L. 1.: as: *Isaria farinosa* Dicks. ex Fr.
Paecilomyces fimetarius (Moesz) Brown et Smith
Syn: *Spicaria fimetaria* Moesz
as: *Spicaria fimetaria* Moesz: Moesz G. 1. (1593); Moesz G. 6.
Paecilomyces marquandii (Massee) Hughes
Syn: *Spicaria violacea* Abbott
Vörös J. 4. as: *Spicaria violacea* Abbott

Paecilomyces varioti Bain.
Nyergesné E. 1.

Genus: *Penicillium* Link ex Fr.

Penicillium asperum (Shear) Raper et Thom
Nyergesné E. 1.
Penicillium aurantio-violaceum Biourge
Nyergesné E. 1.
Penicillium brevi-compactum Dierckx
Nyergesné E. 1.; Vörös J. 2.
Penicillium capsulatum Raper et Fennell
Nyergesné E. 1.
Penicillium chrysogenum Thom
Nyergesné E. 1.
Penicillium claviforme Bain.
Nyergesné E. 1.
Penicillium crustaceum Fr.
Hollós L. 1.; Moesz G. 12.; Moesz G. 13.
Penicillium cyclopium Westling
Nyergesné E. 1.
Penicillium expansum Link
Syn: *Coremium glaucum* Fr.
Vörös J. 2.
as: *Coremium glaucum* Fr.: Hollós L. 1.
Penicillium frequentans Westling
Nyergesné E. 1.; Vörös J. 2.
Penicillium glaucum Link
Hollós L. 1.
Penicillium granulatum Bain.
Nyergesné E. 1.
Penicillium herquei Bain.
Nyergesné E. 1.
Penicillium implicatum Biourge
Nyergesné E. 1.
Penicillium italicum Wehmer
Hollós L. 1.; Nyergesné E. 1.
Penicillium javanicum Van Beyma
Nyergesné E. 1.
Penicillium nigricans (Bain.) Thom
Nyergesné E. 1.
Penicillium oxalicum Currie et Thom
Nyergesné E. 1.
Penicillium purpurogenum Fleroff-Stoll
Nyergesné E. 1.

Penicillium purpurescens (Sopp) Raper et Thom
Nyergesné E. 1.
Penicillium putterillii Thom
Nyergesné E. 1.
Penicillium roqueforti Thom
Nyergesné E. 1.
Penicillium roseo-purpureum Dierckx
Nyergesné E. 1.
Penicillium rotundum Raper et Fennell
Nyergesné E. 1.
Penicillium stoloniferum Thom
Nyergesné E. 1.
Penicillium striatum Raper et Fennell
Nyergesné E. 1.
Penicillium waksmani Zaleski
Nyergesné E. 1.

Genus: *Sesquicillium* W. Gams

Sesquicillium buxi (Schmidt in Link) W. Gams
Syn: *Verticillium buxi* (Link) Lindau; *Fusidium buxi* Link
as: *Verticillium buxi* (Link) Auersw. et Fleisch.: Hollós L. 1.
as: *Fusidium buxi* Link: Hollós L. 1.
Sesquicillium candelabrum (Bon.) W. Gams
Syn: *Verticillium candelabrum* Bon.
Moesz G. 1. (1582) sa: *Verticillium candelabrum* Bon.

Genus: *Stachybotrys* Corda

Stachybotrys alternans Bon.
Tóth S. 7.; Tóth S. 9.
Stachybotrys chartarum (Ehrenb.) Hughes
Syn: *Stachybotrys atra* Corda
as: *Stachybotrys atra* Corda: Vörös J. 3.; Vörös J. 5.
Stachybotrys lobulata Berk.
Moesz G. 1. (1644); Moesz G. 13.; Tóth S. 9.; Vörös J. 3.

Genus: *Stilbum* Tode ex Mérat

Stilbum erythrocephala Ditm.
Syn: *Stilbella erythrocephala* (Ditm.) Lindau
as: *Stilbella erythrocephala* (Ditm.) Lindau: Moesz G. 1. (1760); Moesz G. 26.; Hollós L. 7.

Genus: *Thielaviopsis* Went

Thielaviopsis basicola (Berk. et Br.) Ferraris
Vörös J. 4.; Vörös J. 5.

Genus: *Trichoderma* Pers. ex Fr.

Trichoderma koeningi Oudem.
Vörös J. 3.
Trichoderma lignorum (Tode) Harz
Moesz G. 13.

Genus: *Tubercularia* Tode ex Fr.

Tubercularia atra Pass.
Moesz G. 1. (1814)
Tubercularia berberidis Thüm.
Hollós L. 7.
Tubercularia confluens Pers.
Hollós L. 1.; Moesz G. 1. (1814); Moesz G. 7.; Moesz G. 13.
Tubercularia granulata Pers.
Hollós L. 7.
Tubercularia kmetiana Baumler
Moesz G. 1. (1814)
Tubercularia nigricans (Bull.) Link.
Hollós L. 1.; Moesz G. 1. (1814)
Tubercularia minor Link
Hollós L. 1.
Tubercularia pezizoides Schum.
Moesz G. 13.
Tuberdularia sarmentorum Fr.
Hollós L. 1.; Hollós L. 7.
Tubercularia sphaeroidea Cooke et Harkn.
Hollós L. 1.
Tubercularia vulgaris Tode
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1814); Moesz G. 2.; Moesz G. 12.; Moesz G. 13.; Vörös J. 1.
Tubercularia vulgaris Tode var. *betulae* Wallr.
Hollós L. 1.

Genus: *Verticillium* Nees ex Wallr.

Verticillium albo-atrum Reinke et Berth.
Krenner J. A. 1.; Ubrizsy G. 1.
Verticillium candidulum Sacc.
Moesz G. 1. (1582); Moesz G. 13.
Verticillium glaucum Bonord.
Moesz G. 1. (1582); Moesz G. 13.
Verticillium lateritium Berk.
Hollós L. 1.; Moesz G. 1. (1582); Moesz G. 13.
Verticillium niveostratosum Lindau
Moesz G. 1. (1582)

Verticillium rexianum Sacc.
Moesz G. 1. (1582); Moesz G. 13.
Verticillium terrestre (Link) Sacc.
Moesz G. 1. (1582); Moesz G. 12.

Genus: *Volutella* Tode ex Fr.

Volutella buxi (Corda) Berk.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1844); Moesz G. 11.
Volutella buxi (Corda) Berk. var. *rusci* Kickx
Moesz G. 1. (1844); Vass A. 2.
Volutella ciliata (Alb. et Schw.) Fr.
Moesz G. 1. (1844); Moesz G. 13.
Volutella gilva (Pers.) Sacc.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1844); Moesz G. 11.; Moesz G. 13.
Volutella gilva (Pers.) Sacc. var. *rosea* Sacc.
Moesz G. 1. (1844); Moesz G. 13.
Volutella therryana Sacc.
Vass A. 2.

Genera with uncertain conidium ontogeny

Genus: *Arthrosporium* Sacc.

Arthrosporium albicans (Sacc.) Sacc.
Syn: *Arthrobotryum albicans* Sacc.
Hollós L. 1.

Genus: *Bostrichonema* Linder

Bostrichonema alpestre Cesati
Moesz G. 1. (1605); Moesz G. 7.; Husz B. 1.

Genus: *Chaetostroma* Corda

Chaetostroma atrum Sacc.
Hollós L. 1.; Hollós L. 7.

Genus: *Cylindrocolla* Bon.

Cylindrocolla urticae (Pers.) Bon.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1836); Moesz G. 2.; Moesz G. 13.; Ubrizsy G. 1.; Ubrizsy G. 3.; Vörös J. 4.; Vörös J. 5.

Genus: *Didymaria* Corda

Didymaria linariae Pass.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1604); Moesz G. 11.; Moesz G. 13.

Genus: *Didymopsis* Sacc. et March.

Didymopsis helvellae (Corda) Sacc. et March.
Hollós L. 1.; Moesz G. 1. (1597); Moesz G. 12.

Genus: *Discocolla* Prill. et Delacr.

Discocolla pirina Prill. et Delacr.
Moesz G. 1. (1858); Moesz G. 11.

Genus: *Fusicladiella* Höhnel

Fusicladiella melenae (Fuckel) Hughes
Syn: *Fusicladium aronici* Sacc.
as: *Fusicladium aronici* Sacc.: Bubák F. 1.; Husz B. 1.; Moesz G. 1. (1706); Moesz G. 12.; Moesz G. 13.

Genus: *Fusoma* Corda

Fusoma veratri Allesch.
Husz B. 1.; Moesz G. 1. (1609)

Genus: *Gibellula* Cav.

Gibellula pulchra (Sacc.) Cav.
Hollós L. 1.; Moesz G. 1. (1774)

Genus: *Illosporium* Mart. ex Fr.

Illosporium carneum Fr.
Moesz G. 1. (1825); Moesz G. 12.

Genus: *Isaria* Pers. ex Fr.

Isaria arachnophila Ditm.
Hollós L. 1.
Isaria eleutheratorum Nees
Hollós L. 7.; Moesz G. 24.
Isaria lecaniicola Jaap.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1771)
Isatia umbrina Pers.
Syn: *Trichosporum umbrinum* (Pers.) Fr.
Bubák F. 1.; Moesz G. 1. (1771); Moesz G. 13.

Genus: *Kabatiella* Bubák

Kabatiella caulivora (Kirchn.) Karak.
Syn: *Gloeosporium caulivorum* Kirchn.
Ubrizsy G. 1. as: *Gloeosporium caulivorum* Kirchn.
Kabatiella microsticta Bubák
Moesz G. 1.; Moesz G. 13.
Kabatiella tubercularioidea (Sacc.) Moesz
Syn: *Gloeosporium tubercularioides* Sacc.
Moesz G. 5.

Genus: *Mucrosporium* Preuss

Mucrosporium sphaerocephalum (Berk.) Lind.
Vörös J. 4.

Genus: *Ovularia* Sacc.

Ovularia asperifolii Sacc.
Bubák F. 1.; Hollós L. 7.; Moesz G. 1. (1563)
Ovularia asperifolii Sacc. var. *symphyti tuberosi* Allesch.
Moesz G. 1. (1563)
Ovularia bistortae (Fuckel) Sacc.
Husz B. 1.
Ovularia decipiens Sacc.
Hollós L. 7.; Moesz G. 1. (1563)
Ovularia duplex Sacc.
Hollós L. 1.; Hollós L. 7.
Ovularia farinosa (Bon.) Sacc.
Hollós L. 7.; Moesz G. 1. (1563)
Ovularia gnaphalii Syd.
Hollós L. 1.
Ovularia obliqua (Cooke) Oudem.
Hollós L. 1.; Hollós L. 7.; Moesz G. 13.; Vass A. 2.
Ovularia rigidula Delacr.
Hollós L. 7.
Ovularia rubi Bubák
Bubák F. 1.
Ovularia schroeteri (Kühm) Sacc.
Husz B. 1.; Moesz G. 1. (1563)
Ovularia stellariae (Rabenh.) Sacc.
Bubák F. 1.; Moesz G. 1. (1563)
Ovularia tuberculiniformis Höhnel
Moesz G. 1. (1563); Moesz G. 4.; Moesz G. 11.; Moesz G. 13.
Ovularia veronicae (Fuckel) Sacc.
Moesz G. 1. (1563)
Ovularia villiana Magn.
Moesz G. 1. (1563); Moesz G. 13.

Genus: *Periola* Fr.

Periola tomentosa Fr.
Vörös J. 1.

Genus: *Sarcopodium* Ehrenb. ex Wallr.

Sarcopodium circinatum Ehrenb.
Syn: *Sarcopodium roseum* (Corda) Fr.
as: *Sarcopodium roseum* (Corda) Fr.: Moesz G. 1. (1679); Moesz G. 11.
Sarcopodium fuscum (Corda) Sacc.
Vass A. 2.

Genus: *Septocylindrium* Bon. ex Sacc.

Septocylindrium morchellae Oudem.
Hollós L. 1.; Hollós L. 7.
Septocylindrium virens Sacc.
Moesz G. 1. (1623)

Genus: *Sphacelia* Lév.

Sphacelia typhina (Pers.) Sacc. var. *aeruginosa* Sacc.
Moesz G. 1. (1821); Moesz G. 13.

Genus: *Titaea* Sacc.

Titaea ornitomorpha Trott. emend. Moesz
Moesz G. 1. (1632); Moesz G. 11.

Genus: *Trichaegum* Corda

Trichaegum rhizospermum Corda
Hollós L. 1.; Hollós L. 7.
Hollós L. 1.; Hollós L. 7.

Genus: *Trinacrium* Riess

Trinacrium subtile Riess
Hollós L. 1.

Genus: *Tuberculina* Sacc.

Tuberculina persicina (Ditm.) Sacc.
Syn: *Tubercularia persicina* Ditm.
Bubák F. 1.; Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1809); Moesz G. 2.; Moesz G. 4.; Moesz G. 11.; Moesz G. 12.; Moesz G. 13.
as: *Tubercularia persicina* Ditm.: Moesz G. 1. (1814)
Tuberculina vinosa (Sacc.) Sacc.
Moesz G. 1. (1809); Moesz G. 13.

Invalid genera

Genus: *Acrotheca* Fuckel

Acrotheca caulinum Sacc.
Tóth S. 6.

Genus: *Cercosporina* Speg.

Cercosporina scrophulariae Moesz
Moesz G. 1.; Moesz G. 11.

Genus: *Cylindrium* Bon.

Cylindrium elongatum Bon.
Moesz G. 1. (1524); Moesz G. 13.

Genus: *Fumago* Pers.

Fumago vagans Pers.
Hollós L. 1.; Moesz G. 1. (1750); Moesz G. 7.; Moesz G. 12

Genus: *Gyroceras* Corda

Gyroceras plantaginis (Corda) Sacc.
Moesz G. 1. (1640); Moesz G. 11.

Genus: *Helicotrichum* Nees et Nees

Helicotrichum obscurum (Corda) Sacc.
Hollós L. 1.

Genus: *Hormiscium* Kunze ex Wallr.

Hormiscium altum Ehrenb.
Moesz G. 1. (1639); Moesz G. 12.
Hormiscium antiquum (Corda) Sacc.
Moesz G. 1. (1639); Moesz G. 13.
Hormiscium laxum Wallr.
Hollós L. 1.
Hormiscium pinophilum (Nees) Lindau
Moesz G. 12.
Hormiscium stilbosporum (Corda) Sacc.
Moesz G. 13.; Vörös J. 1.

Genus: *Hymenula* Fr.

Hymenula antherici Hollós
Hollós L. 2.; Hollós L. 7.
Hymenula artemisiae Hollós
Hollós L. 2.; Hollós L. 7.
Hymenula pellicula (Desm.) Sacc.
Moesz G. 1. (1812)

Genus: *Hyphoderma* Fr.

Hyphoderma roseum (Hoffm.) Fr.
Moesz G. 1. (1558); Moesz G. 13.

Genus: *Macrosporium* Fr.

Macrosporium avenae Oudem.
Ubrizsy G. 1.
Macrosporium cladosporioides Desm.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1743); Moesz G. 13.
Macrosporium concinnum Berk. et Br.
Hollós L. 1.

Macrosporium cookei Sacc.
Moesz G. 1. (1743)
Macrosporium saponariae Peck
Hollós L. 1.
Macrosporium savulescui Tóth
Tóth S. 5.
Macrosporium sparganii Lindau
Moesz G. 1. (1743); Moesz G. 11.
Macrosporium trichellum Arc. et Sacc.
Moesz G. 1. (1743); Moesz G. 13.
Macrosporium uredinis Ellis et Barth.
Vass A. 1.
Macrosporium uvarum Thüm.
Ubrizsy G. 1.

Genus: *Monosporium* Bon.

Monosporium spinosum Bon.
Moesz G. 1. (1565); Moesz G. 12.

Genus: *Oidium* Link ex Fr.

Oidium aceris Rabenh.
Hollós L. 1.; Hollós L. 7.
Oidium balsamii Mont.
Moesz G. 1. (1520)
Oidium chrysanthemi Rabenh.
Hódosy S. 1.
Oidium crataegi Grogniard
Moesz G. 1. (1520); Hollós L. 7.
Oidium cyparissiae Syd.
Moesz G. 1. (1520); Moesz G. 13.
Oidium erysiphoides Fr.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1520); Moesz G. 2.; Moesz G. 7.; Moesz G. 11.; Moesz G. 12.; Moesz G. 13.
Oidium euphorbiae Thüm.
Moesz G. 1. (1520)
Oidium evonymi japonicae (Arc.) Sacc.
Krenner J. A. 1.; Moesz G. 1. (1520); Moesz G. 13.
Oidium farinosum Cooke
Moesz G. 1. (1520)
Oidium leucoconium Desm.
Hollós L. 7.
Oidium lini Skoric.
Ubrizsy G. 1.

Oidium monilioides (Nees) Link
Hollós L. 1.; Hollós L. 7.; Moesz G. 2.
Oidium quercinum Thüm.
Hollós L. 1.; Hollós L. 7.; Moesz G. 1. (1520); Moesz G. 3.; Moesz G. 7.; Moesz G. 11.
Oidium tuckeri Berk.
Hollós L. 1.; Hollós L. 7.

Genus: *Oospora* Wallr.

Oospora cuniculina Massal.
Tóth S. 7.
Oospora scabies Thaxt.
Ubrizsy G. 1.

Genus: *Pachybasium* Sacc.

Pachybasium pyramidale (Bon.) Sacc.
Tóth S. 8.

Genus: *Papularia* Fr.

Papularia polydera Moesz
Moesz G. 16.

Genus: *Pirobasidium* Höhnel

Pirobasidium sarcoides (Dicks.) Höhnel
Moesz G. 1.; Moesz G. 13.

Genus: *Riessia* Fr.

Riessia semiophora Fres.
Moesz G. 1. (1806)

Genus: *Stysanus* Corda

Stysanus cybosporus D. Sacc.
Vörös J. 1.

Genus: *Trichofusarium* Bubák

Trichofusarium candidum Wallr.
Hollós L. 7.

Ordo: *Myceliales* (Mycelia sterilia)
Genus: *Ozonium* Link ex Fr.

Ozonium auricomum Link
Moesz G. 1. (1913); Moesz G. 12.; Moesz G. 13.; Moesz G. 24.
Ozonium stuposum Pers.
Moesz G. 24.

Genus: *Racodium* auct. non Pers.

Racodium cellare Pers. ex Wallr.
Moesz G. 1. (1914); Moesz G. 13.; Moesz G. 24.; Nyergesné E. 1.
Racodium nigrum (Link) Schum.
Moesz G. 1. (1914); Moesz G. 24.

Genus: *Rhizoctonia* Dc. ex Fr.

Rhizoctonia crocorum Fr.
Syn: *Rhizoctonia violacea* Tul.; *Rhizoctonia crocorum* (Pers.) Dc.
as: *Rhizoctonia violacea* Tul.: Moesz G. 1. (1907)
as: *Rhizoctonia crocorum* (Pers.) Dc.: Ubrizsy G. 2.
Rhizoctonia solani Kühn
Syn: *Moniliopsis aderholdi* Ruhl.
Ubrizsy G. 2.; Vörös J. 4.
as: *Moniliopsis aderholdi* Ruhl.: Vörös J. 4.

Genus: *Rhizomorpha* Roth ex Fr.

Rhizomorpha subcorticalis Pers.
Moesz G. 1. (1910); Moesz G. 12.

Genus: *Sclerotium* Tode ex Fr.

Sclerotium cepivorum Berk.
Ubrizsy G. 1.
Sclerotium durum Pers.
Moesz G. 1. (1904); Moesz G. 13.
Sclerotium fulvum Fr.
Moesz G. 1. (1904); Moesz G. 12.
Sclerotium liliorum Schw.
Moesz G. 13.
Sclerotium rhizoides Auersw.
Moesz G. 1. (1904)
Sclerotium semen Tode
Moesz G. 1. (1904); Moesz G. 2.; Moesz G. 13.
Sclerotium sphagni (Therry) Moesz
Moesz G. 13.; Moesz G. 16.
Sclerotium stercorarium Dc.
Moesz G. 1. (1904); Moesz G. 7.; Moesz G. 13.

Appendix

Description of the proposed new families of *Moniliales*

1. *Arthroconidiaceae* nov. fam.

Conidia (thallosporae) per phragmentationes hypharum orta (arthroconidia) et catenata.

Conidia are formed from pre-existing elements of the colony, they are not newly produced cells (thallospores). Conidia are produced by the fragmentation of conidiogenous hyphae (arthrospores) in chains. (Figs 1, 2)

2. *Aleurioconidiaceae* nov. fam.

Conidia (thallosporae) difficiliter separabilia, per inflationem cellularum terminalium orta, singulariter formata, cum basibus truncatis (aleurioconidia).

Conidia (thallospores) are inflated apical cells, with a broad base. They are formed singly and separate with difficulty. These aleurioconidia can be defined as holoblastic conidia as well. (Fig. 3)

3. *Blastoconidiaceae* nov. fam.

Murus (externus et internus) utriusque fit particeps formationis conidiorum. Conidia catenata sunt singularia seu nonnulla. Conidiophora per conidiogenesim non extenduntur.

Conidia are newly formed cells. Both inner and outer walls of the conidiogenous cell are involved in the formation of the conidia (blastospores). Conidia in chains, originate from one or a few sites of the conidiogenous cell. No change in length of the conidiophore during conidiogenesis. (Fig. 4)

4. *Botryoblastoconidiaceae* nov. fam.

Blastoconidiaceae et Botryoblastoconidiaceae congruunt inter se excepto quod hic conidia sunt singularia et in tota superficie conidiophorum orta.

The main characteristics of both Blastoconidiaceae and Botryoblastoconidiaceae are the same, except that here conidia are formed singly and simultaneously on the entire surface of the conidiogenous cell. (Figs 5, 6)

5. *Annelloconidiaceae* nov. fam.

Durante conidiogenesi conidiophora recte, cum annellationibus extenduntur.

Conidiophore increase in length toward the main axis during conidiogenesis. Proliferation of the tip of the conidiogenous cell results annellation (annellophorum). (Fig. 7)

6. *Sympoduloconidiaceae* nov. fam.

Durante conidiogenesi conidiophora tortuose extenduntur.

Conidiophore increases in length sympodially during conidiogenesis. As a result of this, alternated sympodial development, sympodula type of conidiogenous cells are formed. (Fig. 8)

7. *Poroconidiaceae* nov. fam.

Murus internus solus fit particeps formationis conidiorum. Conidia per poros orta.

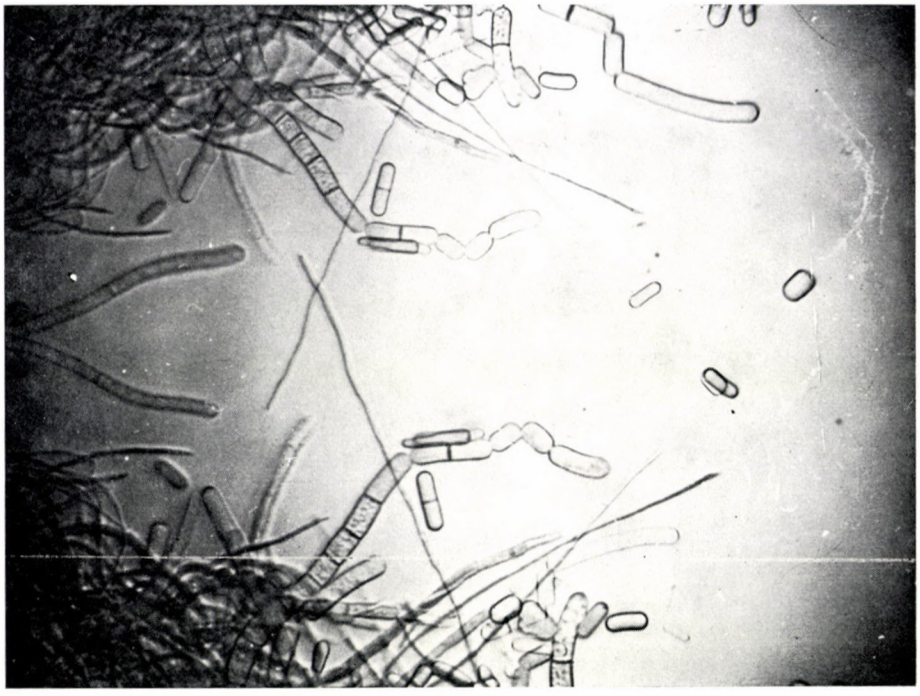

Fig. 1. *Arthroconidiaceae: Geotrichum candidum*

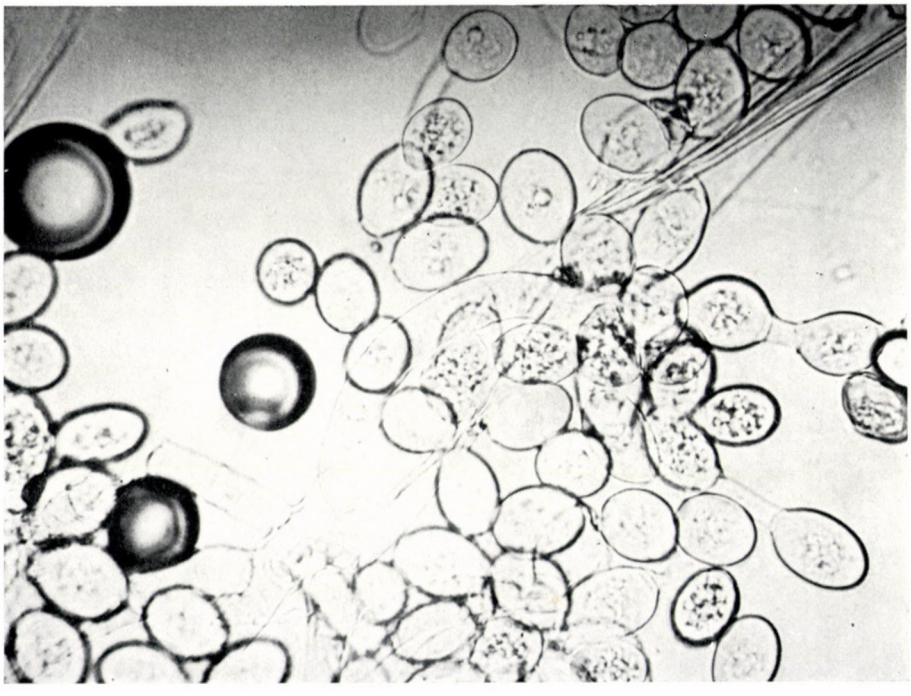

Fig. 2. *Arthroconidiaceae: Amblyosporium botrytis*

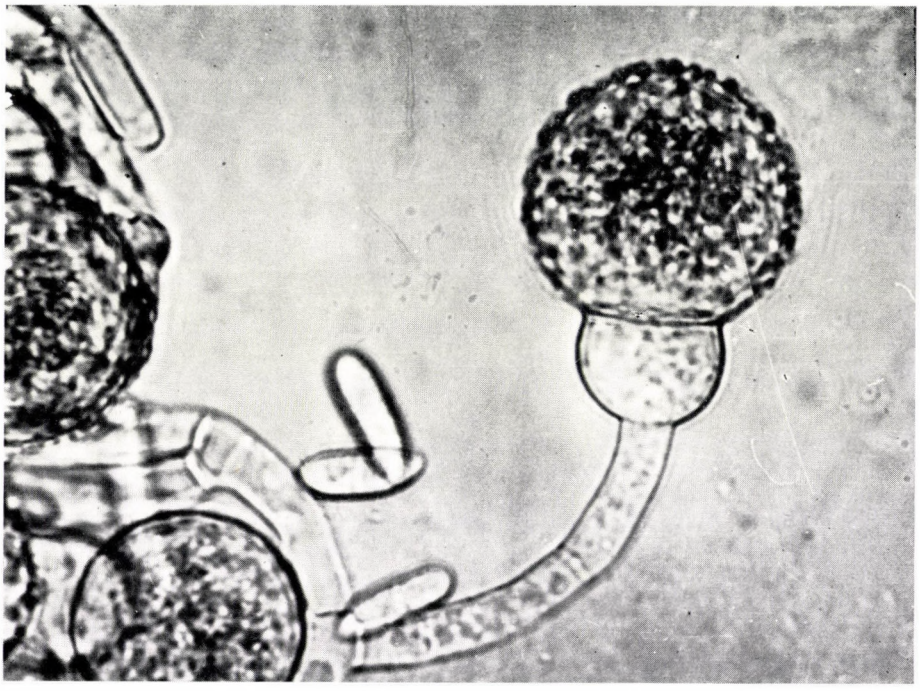

Fig. 3. *Aleurionidiaceae: Mycogone rosea*

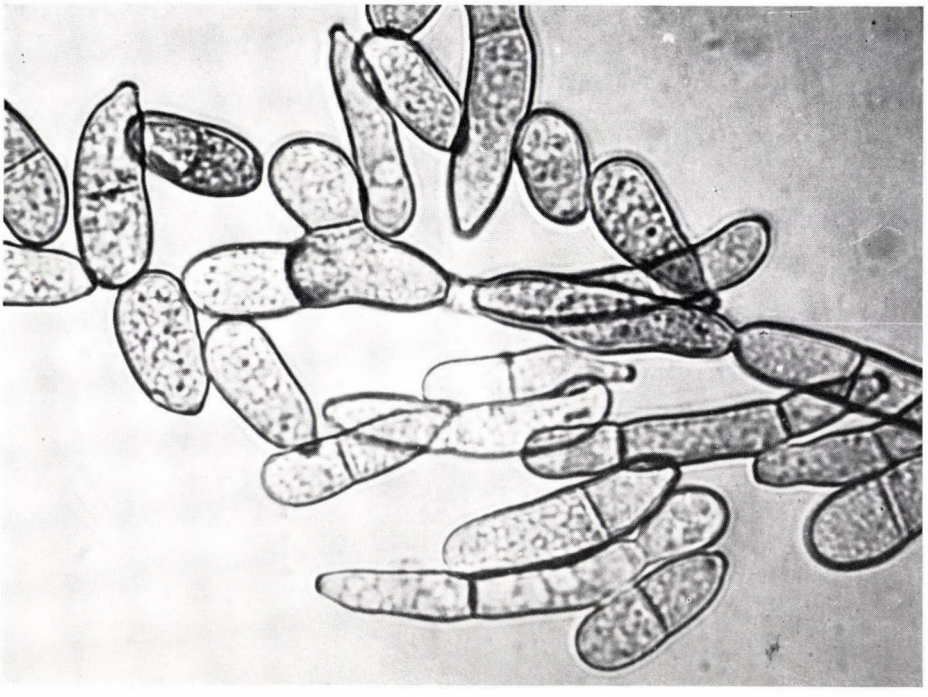

Fig. 4. *Blastoconidiaceae: Cladosporium fulvum*

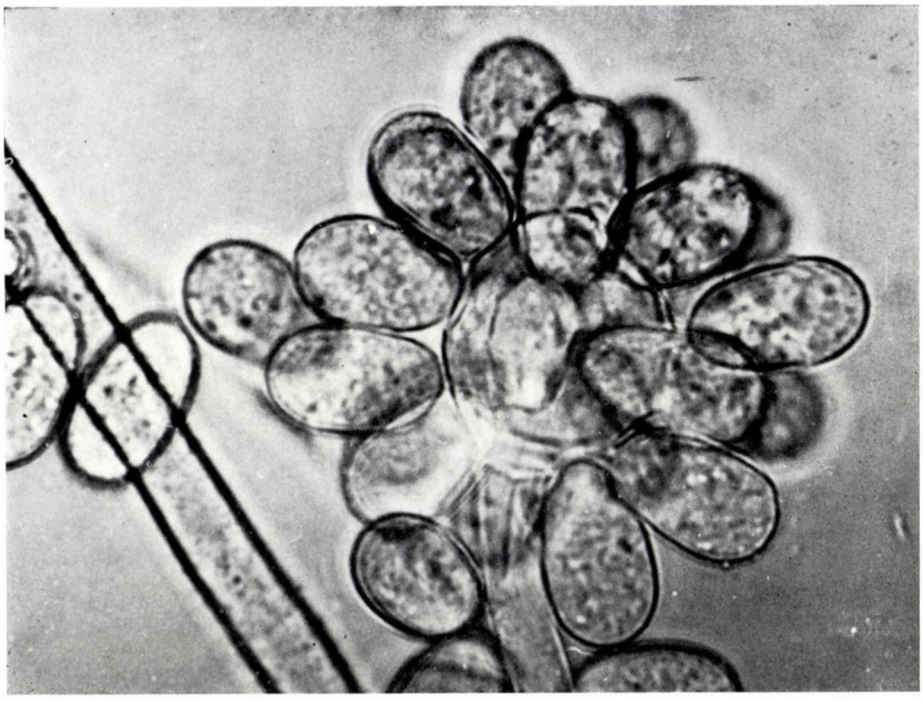

Fig. 5. *Botryoblastoconidiaceae: Oedocephalum glomerulosum*

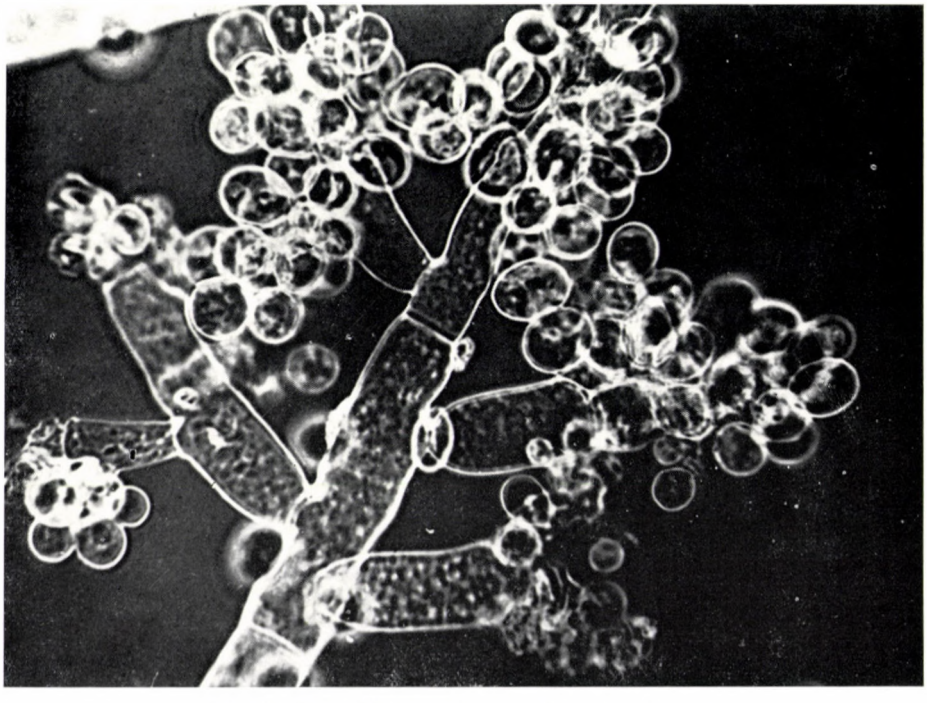

Fig. 6. *Botryoblastoconidiaceae: Botrytis cinerea*

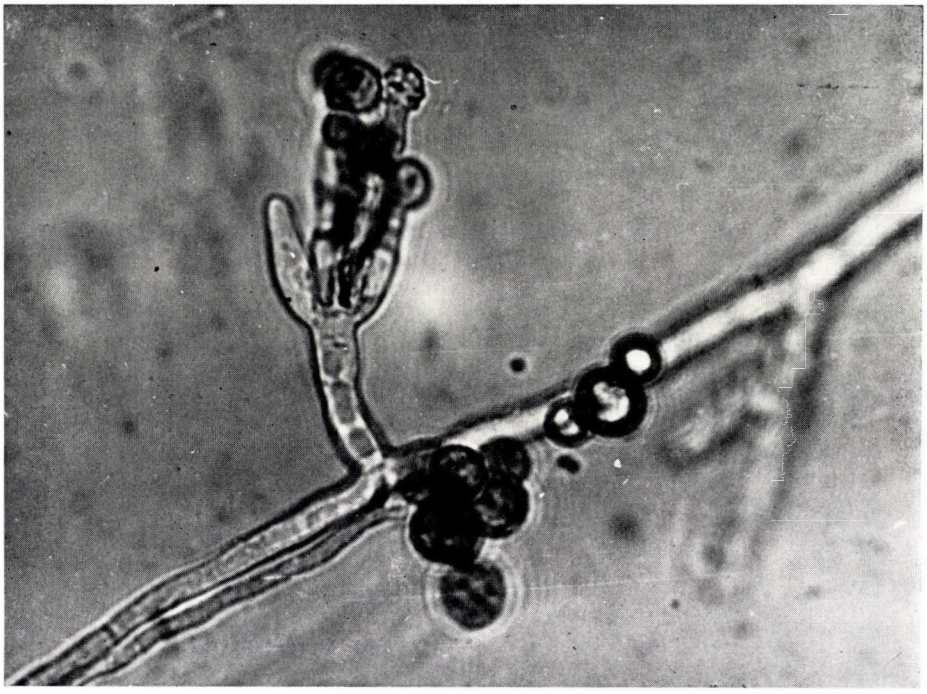

Fig. 7. *Annelloconidiaceae: Scopulariopsis brevicaulis*

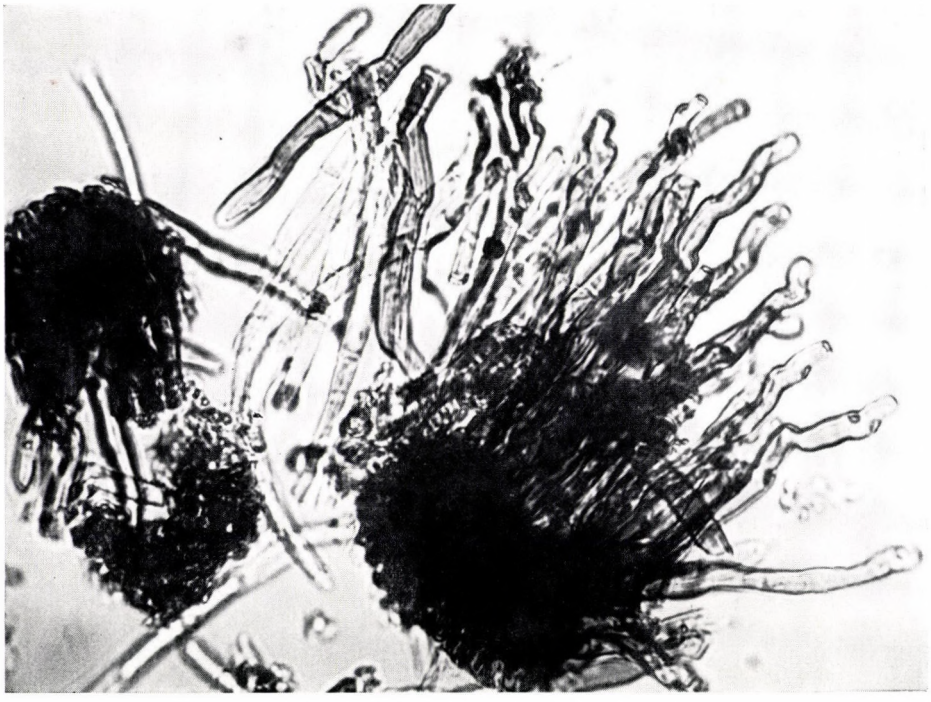

Fig. 8. *Sympoduloconidiaceae: Cercospora traversiana*

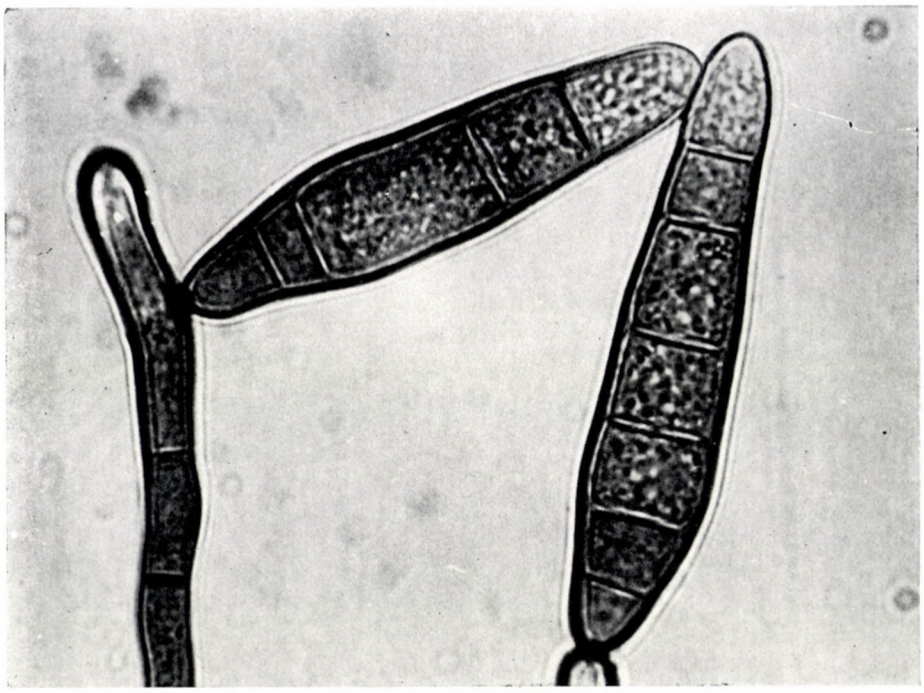

Fig. 9. *Poroconidiaceae: Helminthosporium turcicum*

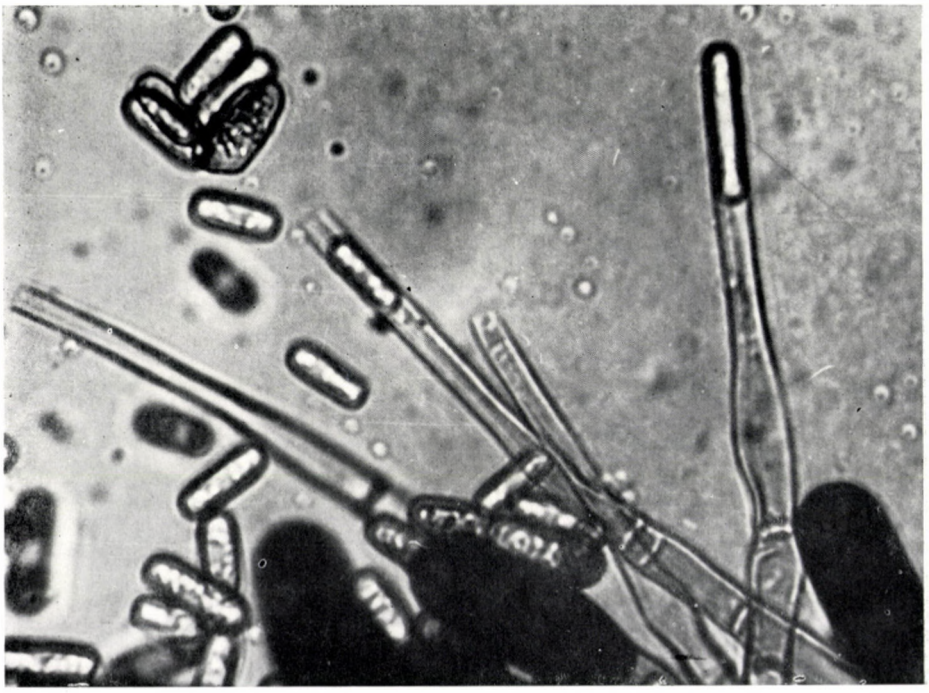

Fig. 10. *Phialoconidiaceae: Thielaviopsis basicola*

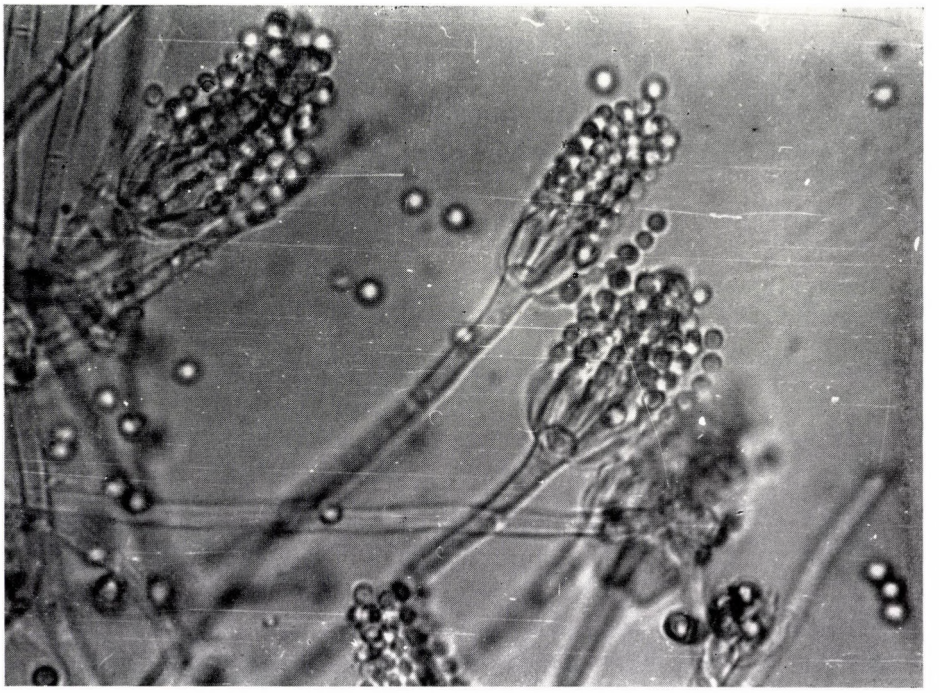

Fig. 11. *Phialoconidiaceae: Penicillium frequentans*

Only the inner wall of the conidiogenous cell is involved in the formation of the conidia, which actually blow out together with the inner wall of the conidiophore, through a minute pore of the outer wall (porospore). (Fig. 9)

8. *Phialoconidiaceae* nov. fam.

Nullus murorum conidiophororum fit particeps formationis conidiorum. Conidia per cellulas apertas orta.

None of the walls of the conidiogenous cell participate in the formation of the conidia, which are produced from open phialides, and are surrounded by newly developed cell walls. (Figs. 10, 11)

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The Role of Extreme Temperature Fluctuations in the Population Dynamics of Overwintering Eggs of *Panonychus ulmi* KOCH

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The extreme fluctuations of winter temperature can at times cause a mortality rate of 100% in the overwintering populations. This fact is to be appreciated when compiling data for prognosis of plant protection or estimating role of abiotic factors in population dynamics.

The rate of mortality was more than once reduced by repeated coolings and warming up, which can be well explained by the repetition of stimulated and interrupted dormancy.

Temperature applied in period of reactivation did not effect the begin of spring hatching, a fact that excludes applicability of the method of totalled temperature.

Climate of Hungary can be characterized, among others, by a marked fluctuation of winter temperature. January readings have already been +18 °C maximum and −33 °C minimum as well, while in March +25 °C and −20 °C were the corresponding figures. (Fortnight prognosis of Meteorological Institute, 1971). This fluctuation bridging over ±25 °C constitute a serious trial bordering with destruction for the different forms of living organisms. Almost every monographic work dealing with insect pests refers to the fact that winter fluctuation of temperature results in marked reduction of individual density.

There are several comprehensive works available on the effect of low temperatures (LOZINA-LOZINSKII, 1972; SALT, 1961; USATINSKAYA, 1957). These workers all point out that the frost tolerances of healthy insect populations are capable of coping with the lowest temperatures of given environment; in the temperate zone it is only the fluctuation of temperatures that takes heavy toll of insect populations. The question of temperature was highlighted by some other workers (BODENHEIMER 1958; DANILEVSKII, 1961; GORYSIN, 1966; JAKHONTOV, 1969; KOZHANCHIKOV, 1961; SCHWERDTFEGER, 1963) etc. but they hardly touched on the topics dealt with in present paper or on the problem of fluctuating temperatures. Works on insect ecology do not present data either on questions of this field, though related data are of paramount importance for in Hungary comprehending problems in population dynamics or composing prognosis for plant protection. In winters of 1971, 1972, and 1973 we sought answers to the following questions: to what extent do warmings up of different degree, their duration and frequency excert an influence upon the death of the overwintering form and on start of swarming.

Materials and Methods

From an apple orchard at Bakonyszentlászló, branches of trees carrying heavy infestation of *P. ulmi* eggs were collected and kept in outdoor insectariums. Pieces of infested twigs were glued onto the bottom of Petri dishes and surrounded with rings of vaseline to facilitate counting of swarming individuals. Petri dishes were kept in outdoor insectariums. Stimulated warmings up were effected by placing the dishes into climate chamber with illumination and set temperature together with 80% air humidity. Duration of illumination was 17 hours. Low temperatures were insured by placing dishes in question on cooling shelves with a temperature of -17 °C ± 2 °C.

Hatchings were studied under cytoplast microscope, and larvae stuck in the vaseline stripes were removed. Mortality rate of eggs was established by counting unhatched ones following the hatching. The reason why $+25$ °C was chosen as upper limit is that this very temperature caused mostly 100% mortality (KOZÁR and SÁNTHA, 1970) and constitutes about the maximum record in Hungary over the period of February and March.

The investigations were carried out in 1–3 repetition. Amount of eggs ranged between 20–945. Total amount of eggs scrutinized have approached 20.000.

Results and Discussion

I.

The effect of extreme warming up on egg mortality is shown in Table 1. A single warming up of 6 day duration doubled mortality rate on the average. Repetition of warming failed to increase mortality, in fact, certain decrease was

Table 1

Effect of extreme winter warmings up ($+15$ °C) on mortality of eggs of *P. ulmi* in 1971–73

Duration of warming up	Year	Mortality of eggs (%)	
		single warming up	repeated warmings up (+ 6 day)
6 days	1971	10.12	44.10
	1972*	60.60	51.87
	1973**	60.00	52.94
12 days	1971	7.18	64.70
	1972*	36.60	34.49
	1973*	54.83	40.92
Control	1971	14.38	
	1972*	49.54	
	1973**	24.07	

* Not significant
** LSD = 27.65

experienced. Warming up of 12 day duration caused certain increase of mortality, too, though it never reached the rate scored by 6 day warming. Data obtained differ from year to year which can be well explained by assuming diverse physiological conditions.

Mortality of eggs effected by extreme temperatures showed an increasing tendency from January till April in 1971.

A 6 day warming up to 25 °C caused a 50% mortality of eggs in 1971. This warming up with a longer duration resulted in egg hatching and terminated in a complete destruction of population when cooling followed.

II.

Coolings of 6 and 12 day duration caused a significant mortality, especially in 1972. Repetition of cooling reduced mortality of eggs. Any increase of duration of cooling caused reduced mortality in 1972, while in 1973, an increased mortality was observed (Table 2).

Table 2

Role of drops of temperatures in winter in mortality of *P. ulmi* eggs (-17 ± 2 °C)*

Duration of drop of temperature	Year	Mortality of eggs %			
		Single drop of temperature		Repeated drops of temperature	
		January	February	January	February
6 days	1972	93.27	69.59	59.61	28.03
	1973	27.43	27.16	53.80	36.59
12 days	1972	75.65	44.02	50.78	50.66
	1973	32.56	35.75	32.54	41.92
Control	1972	29.54			
	1973	24.07			

* No LSD between data (CV = 65.6%) though certain trends are present

III.

In the other series of experiments, the joint effect of warming up and cooling was studied. (Table 3). Data indicate no significant increase in mortality in January either by single or repeated warmings and coolings. By increasing the first warming up to 12 days, repeated warmings and coolings increased mortality. In February even a single 6 day warming doubled mortality. Doubled repetition of warmings and coolings pushed mortality rate up to 94%. When the first warming up was extended to 12 days, an increased mortality resulted, and a single warming up has caused an 80% mortality. In every variation the repeated treatments increased mortality almost up to 100%.

Table 3

Effect of warming up (+15 °C) and coolings (−17±2 °C) on the mortality of *P. ulmi* eggs

Frequency of treatments	Mortality of eggs %			
	January		February	
	6 days	12 days	6 days	12 days
Single warming up	40.54	30.76	57.50	80.00
Warming and cooling	35.97	29.13	57.14	99.24
Warming, cooling and warming again	17.48	52.58	77.61	100.00
2 warmings up and 1 cooling	39.90	56.63	94.44	100.00
Control	24.07			

Discussion

It has been proved that great fluctuation of temperature under experimental conditions can cause in given cases a mortality rate of about 100% in *P. ulmi* eggs. The adverse effect of temperature can be enhanced or tempered by humidity or other factors, joint effect of which is yet to be clarified.

Extreme fluctuations of temperature has an increased significance in case of insects with a temperature controlled reactivisation. Population dynamics remain relatively unaffected by great temperature fluctuations in species whose reactivisation is controlled by photoperiod.

Several variations of warmings and coolings showed a marked decrease in mortality when single shock only was applied. Supposedly this is an instance of repeatedly effected dormancy when, owing to lack of reactivisation, a single shock kills the experimental object, while more than one shocks ensure reactivization and hatching follows with insignificant mortality. This group of questions constitutes a part of problems of dormancy and merits further attention.

From the aspect of forecasting for plant protection, it has to be pointed out that extra increase of temperature in the period of reactivisation does not influence begin of hatching. Our results have also disclosed that the method of totalled temperature is not applicable with this pest, since during warmings up two, even three-fold mean temperature sum values were reached before the begin of hatching.

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Effect of Substances with Juvenile Hormone Activity on the Imaginal Diapause of Alfalfa Ladybird, *Subcoccinella 24-punctata* L. (*Coleoptera: Coccinellidae*) I. Termination of Diapause

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Adults of alfalfa ladybird, *Subcoccinella 24-punctata* L. kept at short photoperiod do not mate or oviposit but enter diapause about 20 days after emergence. Diapause can be terminated by treating adults with juvenilizing compounds, R-20 458, ZR-512, SJ-53-Fchl, and Cecropia juvenile hormone in dosages of 2, 8, 10, 14, 50, and 72 μg which induce rapid reactivation, mating and oviposition.

Juvenile hormone analogues can successfully terminate diapause up to 73% of insects treated. The effect of juvenoids is much more evident under short-day conditions than at long photoperiod. So, the high percentages in adult reactivation are due to effect of juvenoids and not to the combined effect of juvenoids and the photoperiod, as it had been proven under long-day conditions.

Among the compounds tested R-20 458 is the most effective, and the suitable doses are between 24 and 50 μg. The highest percentages of reactivated adults have been registered 12—15 days after treatment. Applying higher dosages, oviposition increases but the egg-hatchability is not significantly influenced.

The alfalfa ladybird, *Subcoccinella 24-punctata* L. (*Coleoptera: Coccinellidae*) is one of the most serious pests of alfalfa (*Medicago sativa* L.) in the south-western part of Hungary. The species develops in 2—3 generations a year depending on the environmental conditions (DESEŐ in MANNINGER, 1960; CSEHI, 1964). Under natural circumstances, adults of the last generation — appearing in September-October — hibernate and remain in diapause till the next spring.

The imaginal diapause of the alfalfa ladybird is induced by photoperiod and temperature (ALI, 1971). At the same time, the influence of genetical factors is also evident as it was also found by HODEK and CERKASOV (1961) in *Coccinella septempunctata* populations.

Termination of imaginal diapause in insect pests by chemical treatments e.g. with the use of juvenoids would be of great practical importance. This reactivation would force the insects to feed and reproduce under unfavourable ecological conditions thus causing them to commit an 'ecological suicide'. A few publications showed some approach to this new selective method of pest control. So, treatment of diapausing adults of the alfalfa weevil, *Hypera postica* with

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10,11-epoxy-farnesenic acid methyl ester could terminate diapause and induced normal feeding and oviposition in adults (BOWERS and BLICKENSTAFF, 1966). Laboratory tests were successfully performed also on diapausing females of *Draeculacephalus crassicornis* which were topically treated with synthetic Cecropia JH (KAMM and SWENSON, 1972). Similar results were also reported on the cereal leaf beetle (CONNIN *et al.*, 1967), the Colorado beetle (DEWILDE, 1969; SCHOONEVELD, 1973; DE WILDE and LUTKE SCHIPHOLT, 1974), and the cereal bug (BUROV *et al.*, 1972; KONTEV *et al.*, 1974).

The aim of the present work was to test the effect of some compounds with juvenile hormone activity on the termination of diapause in adults of the alfalfa ladybird and to investigate the behaviour and reproduction of treated beetles under long- and short-day conditions.

Materials and Methods

Adults of the alfalfa ladybird, which had been in diapause for 2–3 weeks, were narcotized by dipping them under water for 10–15 minutes. Then they were treated topically on the ventral surfaces of the abdomen with 2 μl of acetone solutions of Cecropia juvenile hormone, methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate as well as of a few juvenoids: 1,4-ethyl-phenoxy-6,7-epoxy-3,7-dimethyl-2-octene (R-20458, Stauffer Chem. Co.), ethyl-3,7-11trimethyl-2,4-dodecadienoate (ZR-512, Zoecon Corp.), and ethyl-11-chloro-3,7,11-trimethyl-2-dodecanoate (ŠJ-53-FChl, Institute of Organic Chemistry, CSAV) in dosages ranging from 2 to 72 μg per specimen. Control groups were either treated with 2 μl of acetone or left untreated. Treatments were replicated 3 times, in each with 20 beetles (10 females, 10 males).

Treated and control groups were reared at 23 °C at long (17/7 hr L/D) and short photoperiods (13/11 h L/D). The number of reactivated beetles, number of eggs laid and hatched as well as the number of dead adults was recorded – according to different experiments – for 30 or 21 days.

Results

Termination of diapause at long photoperiod

Regarding the proportion of adults remaining in diapause for a period of 30 days, the application of 10 and 50 μg of the juvenoid, R-20458 could significantly ($P < 0.01$) reduce diapause to 27.1 and 20.1%, respectively (Table 1); 39,7% of adults remained in diapause in the control groups. The behaviour of adults received 2 μg of R-20458 did not differ greatly from that of control animals. Treatments with 10 and 50 μg of the juvenile hormone analogue ZR-512 were

Table 1

Termination of imaginal diapause in alfalfa ladybird by juvenile hormone analogues at two different photoperiods (Temp.: 23 °C)

Juvenoid	Dose in μg	Long-day conditions (17/7 h L/D)				
		Diapausing adults %	Number of eggs per female	Egghatch-ability %	Mortality %	
					♀♀	♂♂
Control	0	39.7±4.8	90	53.4	10	13
R-20 458	2	27.1±2.8	176	53.4	15	15
	10	27.1±2.8	248	53.4	15	15
	50	20.1±2.1	284	57.1	30	35
ZR-512	2	34.5±1.6	96	53.2	15	26
	10	27.0±2.6	121	56.4	15	20
	50	22.6±1.5	258	61.0	10	20

Juvenoid	Dose in μg	Short-day conditions (13/11 h L/D)				
		Diapausing adults %	Number of eggs per female	Egghatch-ability %	Mortality %	
					♀♀	♂♂
Control	0	82.3±3.6	0	—	10	10
R-20 458	2	43.0±4.8	0	—	20	20
	10	43.0±3.8	0	—	20	10
	50	29.8±3.1	30	80	30	30
ZR-512	2	48.0±3.0	0	—	10	10
	10	39.6±4.1	0	—	10	10
	50	27.2±2.6	0	—	15	17

also more effective for breaking diapause than those with 2 μg of the same juvenoid. No significant differences were found between the percentages of adults remaining in diapause in the groups which had received 2 μg and those received 10 μg of ZR-512, while the differences were quite evident between the behaviour of adults treated with 2 μg and 50 μg of the juvenoid ($P < 0.01$).

Adults in which diapause was successfully terminated by different doses of R-20458 began to lay eggs 8–12 days after treatment. Beetles received 2 and 10 μg of R-20458 oviposited first on the 8th day, while those received 50 μg started the egg-laying 4 days alter. Nevertheless, these females deposited the highest number of eggs. (Fig. 1-A) The number of eggs increased by the higher doses applied (Table 1). Treatments with the same doses but of ZR-512 resulted in similar effects on oviposition (Fig. 1-B; Table 1). Females treated with this JH analogue laid eggs first on the 6th day. Control insects started egg-laying on the

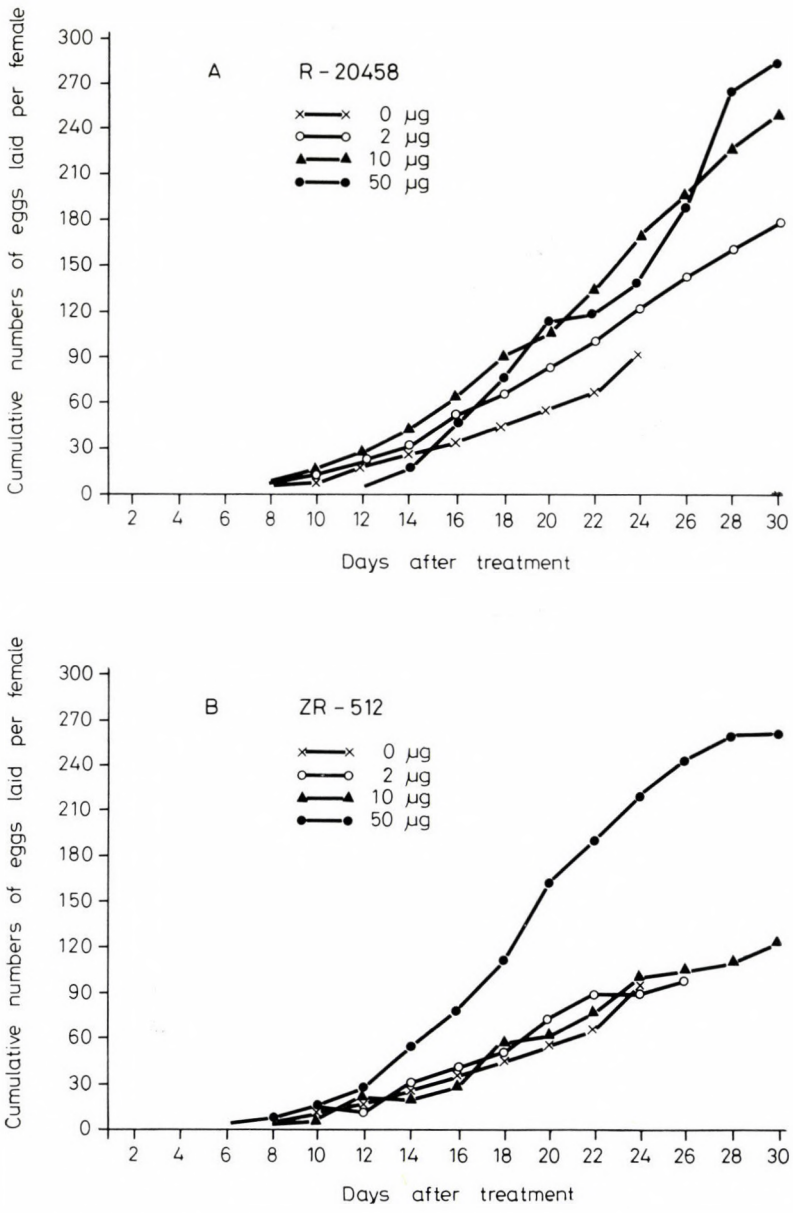

Fig. 1. Cumulative numbers of eggs laid by females of alfalfa ladybird treated with juvenile hormone analogues (Temp.: 23°C; photoperiod: 17/7 h L/D)

8th day of the experiment but deposited less eggs than females treated with juvenoids. Moreover, untreated females stopped oviposition 6 days earlier than treated ones.

No significant differences were found between the percentages of viable eggs laid by females treated with different doses of juvenoids in spite of the slight increase in the hatchability of eggs deposited after treatments with 50 μg of JH analogues (Table 1).

The mortality of treated beetles was slightly higher than that of controls, especially after applying 50 μg of R-20458 (Table 1).

Termination of diapause at short photoperiod

The effect of juvenoids on imaginal diapause of Subcoccinella was much evident when the experiment was performed under short-day conditions (Table 1).

Untreated beetles showed slight activity for 8—10 days, thereafter nearly all of them started to hide again.

Diapausing adults which received 2 μg of one of the two juvenoids could terminate diapause in about 50% of the population treated. The effectivity of juvenile hormone analogues increased by the higher dosages applied. Treatments with 50 μg of ZR-512 and R-20458 reduced the percentage of diapausing insects to 27 and 30%, respectively. Though ZR-512 appeared to be slightly but not significantly more effective in reactivation than R-20458, it failed to stimulate the ovaries. Females received ZR-512 did not lay eggs even when high doses were applied. On the other hand, 50 μg of R-20458 induced oviposition and the viability of eggs was also high.

The mortality of adults increased when higher dosages of juvenoids were applied. Treatments with R-20458 were accompanied by higher mortality than that recorded for ZR-512.

The results of experiments described above were of great encouragement to study the effect of other juvenilizing compounds and to compare the efficiencies of treatments with different substances.

Table 2

Reproductive activity of alfalfa ladybird females treated with juvenilizing compounds (Temp.: 23 °C; photoperiod: 13/11 h L/D)

Compound	Number of eggs per female			Egg-hatchability %		
	Dose in μg: 8	24	72	8	24	72
R-20458	14	60	171	78	80	73
Cecropia JH	15	34	0	87	78	—
ŠJ-53-FChl	10	0	0	50	—	—
ZR-512	0	5	25	—	100	88

Table 3

Percentages of adults in diapause in 3 days intervals after treatment with different juvenilizing compounds (Temp.: 23 °C; photoperiod: 13/11 h L/D)

Compound:	R-20 458				Cecropia JH			
Doses in μg:	8	24	72	Mean	8	24	72	Mean
Experimental period in days								Diapausing
3	35.0	13.8	23.0	23.9	28.8	49.0	29.8	35.8
6	23.9	13.3	28.7	21.9	22.5	14.7	19.5	18.9
9	18.6	11.9	35.6	22.0	17.0	12.5	27.0	18.8
12	12.0	13.9	35.0	20.3	11.4	19.6	29.8	16.9
15	25.9	13.3	29.6	22.9	19.4	29.4	41.1	29.9
18	32.9	20.8	20.6	24.7	32.0	23.3	44.5	33.2
21	32.5	24.3	16.3	24.3	40.0	31.4	31.1	34.1
Average:	25.8	15.9	26.9	22.8	24.4	24.2	31.8	26.8

Control insects fed for 3–5 days but did not lay eggs, then all the partially reactivated beetles went into diapause again. On the other hand, adults treated with juvenilizing compounds showed considerable activity for a longer period, moreover, some of them mated and laid eggs (Table 2). The changes in behaviour and reproduction depended on the compounds and the dosages used.

The percentages of diapausing adults which were recorded in 3-day intervals are summarized in Table 3. In comparison to control groups, treated insects exhibited high activity even within a short period after treatment. This ratio still increased later. After the first 6 days, the percentages of beetles remained in diapause in control groups ranged from 70 to 75% with a standard deviation of 17.3%. However, it has to be noticed that this percentage increased up to 100% by the 12th day after treatment.

To make a comparison between the efficiency of juvenilizing substances in terminating imaginal diapause, the least significant difference test and the analysis of variance were used. The average percentages of diapausing beetles (remained in or reentred diapause) indicate that (without taking into consideration the doses used) R-20458 proved to be the most effective but the action of Cecropia JH was not significantly different (Table 3). Nevertheless, ZR-512 and ŠJ-53-FChl can be also accepted as good juvenoids for breaking diapause if compared with the control.

The lowest percentages of diapausing beetles in treated groups were registered 12 (9–15) days after treatment. Thereafter the ratio of insects re-entred diapause increased gradually (Table 3).

Regarding the doses applied, it was found that the application of 24 μg could terminate diapause in the alfalfa ladybird with the greatest efficiency (Table 3). In comparison to control groups, lower or higher dosages could also consider-

ŠJ-53-FChl				ZR-512				Control
8	24	72	Mean	8	24	72	Mean	0
adults %								
49.9	54.5	45.2	49.8	41.5	49.3	48.9	45.5	70.0
34.8	33.2	28.5	32.1	38.4	36.9	37.4	37.5	75.0
11.6	18.9	11.0	13.8	23.7	20.7	26.7	23.7	89.3
15.5	20.4	13.5	13.1	15.5	17.5	30.0	21.0	100.0
29.8	35.6	26.4	30.6	11.8	22.5	21.2	15.1	100.0
31.9	41.0	29.1	34.0	41.0	30.5	40.0	37.1	100.0
52.3	18.8	42.2	37.7	47.4	43.2	44.8	45.1	100.0
30.8	31.7	27.9	30.1	32.8	28.5	35.5	32.8	

L.S.D. 5% of effect of the juvenilizing compounds = 5.1
L.S.D. 5% of effect of the doses applied = 4.8
L.S.D. 5% of effect of the experimental periods = 6.7

ably reactivate diapausing adults. A relatively good relationship between doses applied and the percentages of diapausing beetles existed only after applying ŠJ-53-FChl. In case of the three other compounds 72 μg resulted in an increase of percentages of diapausing adults, and it also caused higher mortality.

Discussion

Diapause in adults of alfalfa ladybird, *Subcoccinella 24-punctata* could be terminated by treatments of diapausing adults with Cecropia juvenile hormone or juvenile hormone analogues. Juvenilizing compounds could stimulate ovaries and promote egg-laying, in addition to breaking of diapause, especially under long-day conditions. These results are in good agreement with the data obtained by BOWERS and BLICKENSTAFF (1966), DE WILDE (1969), SCHOONEVELD (1973), DE WILDE and LUTKE SCHIPHOLT, 1974 and CONNIN and HOOPINGARNER (1971) on adults of other *Coleoptera: Hypera postica*, and *Leptinotarsa decemlineata*, and *Oulema melanopus*, respectively.

Applying synthetic juvenile hormone or juvenoids, a definite dose-response relationship could be observed on alfalfa ladybird adults like in the cases of the above-mentioned species. 100 μg of the synthetic Cecropia juvenile hormone produced the greatest number of eggs in the case of alfalfa weevil, and 10–50μg doses gave only partial reactivation (BOWERS and BLICKENSTAFF, 1966). In *Subcoccinella 24-punctata*, doses ranging between 10 and 50 μg of R-20458 or ZR-512

promoted reproduction to the highest degree, and 72 μg of R-20458 was stimulating on fecundity at short photoperiod as well.

Most of the investigations published so far on the application of juvenilizing substances for breaking imaginal diapause have been carried out under long-day conditions. We assume, therefore, that these results concerning the reactivation of adults can be attributed more conveniently to the combined or simultaneous effect of JH treatment and photoperiod than to the action of the juvenilizing substance alone. Our results obtained in experiments which were performed at two different photoperiods may support this hypothesis.

In a definite percentage of the population, the alfalfa ladybird adults, like other coccinellids in Central Europe (HODEK and CERKASOV, 1961) enter diapause even under long-day conditions and remain in this state for a certain period. Treating them with juvenilizing compounds can significantly reduce the percentage of diapause. We supposed that the diapause-breaking capacity of some chemicals with JH activity could be more expressed at short photoperiods when the positive influence of long-day conditions was precluded. Actually, by comparing the data regarding the behaviour of control and treated insects (see Table 1), we can state that juvenoids terminate diapause in the adults of *Subcoccinella 24-punctata* at short photoperiod to a greater extent than under long-day conditions. Nevertheless, the percentages of diapausing adults remained higher in treated groups under short-day conditions than at long-photoperiods. This shows that exogenous JH supply can not replace in every respect the influence of long photoperiods on termination of imaginal diapause.

The reproductive activity of *Subcoccinella* adults was stimulated by JH treatments at both photoperiods. Beetles proved to be much 'resistent' in this respect under short-day conditions. These data are in good accordance with the results of SCHOONEVELD (1973) on Leptinotarsa decemlineata who emphasized the importance of the photoperiodic control of brain activity (neurosecretion) in reproductive processes.

We can suppose that in the maintenance of imaginal diapause in alfaifa ladybird, in addition to the strong effects of genetic factors, and the influence of temperature, photoperiodism plays the decisive role. The latter in turn, does not exert its influence only through the control of corpus allatum activity or 'tuning in' a definite JH titer but possibly also via nervous and/or neurosecretory processes. This fact may render the effectiveness of treatments with juvenoids for complete termination of diapause in adults very questionable. On the other hand, it seems reasonable that even an imperfect (*i.e.* temporary, reversible) breaking of imaginal diapause in a large proportion of the population, which was also observed in our experiments, may make an end of a mass-appearance of the insect pest under appropriate ecological conditions.

The necessity of applying relatively (in relation to the body size) high dosages of juvenoids for termination of imaginal diapause in the alfalfa ladybird brings us to the conclusion that, for a possible field use in the future, more active and supposedly more stable compounds will be required. The other difficulty

arises from the simple fact that diapausing beetles exhibit a severe hiding behaviour which enables them to escape from the direct contact with the chemicals sprayed on the plant and soil surfaces. Such technical difficulties, which led to the failure of effective control with juvenoids in the field was encountered i.e. in the case of *Eurygaster integriceps* (KONTEV *et al.*, 1974). The inhibition of diapause incidence with juvenile hormone analogues seems, therefore, more perspectivic.

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Effect of Substances with Juvenile Hormone Activity on the Imaginal Diapause of Alfalfa Ladybird, *Subcoccinella 24-punctata* L. (*Coleoptera: Coccinnellidae*) II. Inhibition of Diapause Incidence

By

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Topical applications of juvenoids, R-20458 and Zr-512 to active adults of alfalfa ladybird, *Subcoccinella 24-punctata* L. prevented a high percentage of population from entering diapause. Experiments were performed under long-day and short-day conditions.

At long photoperiod, treatments with 12 and 36 μg of R-20 458 inhibited the incidence of diapause in 78% and 88% of adult population, respectively. At the same time, treated females laid significantly more eggs than control insects.

Under short-day conditions, 96,4% of control beetles entered diapause within 30 days and females did not lay eggs. Among adults received 4 μg of the juvenile hormone analogues the percentage of diapausing insects was reduced to 50%. Doses of 12 and 36 μg per specimen prevented diapause in about 70% of the population. R-20 458 proved to be more efficient than ZR-512, stimulating also the egg-laying of treated females.

Termination of imaginal diapause in some insect species can be successfully elicited by chemicals showing juvenile hormone activity. Synthetic Cecropia juvenile hormone and some juvenoids proved to be especially effective as reported on alfalfa weevil (BOWERS and BLICKENSTAFF, 1966), cereal leaf beetle (CONNIN *et al.*, 1967), a leafhopper (KAMM and SWENSON, 1972), Colorado beetle (DEWILDE, 1969; SCHOONEVELD, 1973, DE WILDE, and LUPKE SCHIPHOLT, 1974) and cereal bug (BUROV *et al.*, 1972; KONTEV *et al.*, 1974). As it was described in our previous paper (ALI *et al.*, 1974), the diapause in adults of the alfalfa ladybird, *Subcoccinella 24-punctata* L. could be also effectively terminated by applying to the diapausing beetles some of the most active juvenilizing substances. At the same time, we are not aware of any literary data concerning another possible control method interfering chemically with imaginal diapause, i.e. the inhibition of diapause incidence with the use of juvenile hormone analogues.

Most adults, which enter diapause in a certain period of their development, exhibit a hiding behaviour and rest thereafter in concealment; these insects can be chemically treated in the field only with difficulties and with low efficiency. On the

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other hand, spraying the field still before the incidence of diapause, the active insects can much easier get in contact with a juvenoid. Under appropriate circumstances, this advantage may render such a selective control quite effective and economic.

In present investigations, our aim was to study the possibility to inhibit the diapause in the alfalfa ladybird with application of two juvenoids to active beetles and with subsequent rearing them under long-day or short-day conditions.

Materials and Methods

Similar method as described in our previous paper (ALI *et al.*, 1974) was used for treating adults of *Subcoccinella* with juvenile hormone analogues. 3–5 days old active beetles were topically treated with 4, 12, and 30 μg of two juvenoids: 1,4-ethylphenoxy-6,7-epoxy-3,7-dimethyl-2-octene (R-20458, Stauffer Chem. Co.), and ethyl-3,7,11-trimethyl-2,4-dodecadienoate (ZR-512, Zoecon Corp.). Control groups received only 2 μl of acetone or were left without any treatment. 3 replications were used, in each with 20 beetles (10 females, 10 males). The insects were reared then at 23 °C and under long-day (17/7 hr L/D) or short-day (13/11 hr L/D) conditions for 30 days.

Results

Inhibition of diapause incidence at long photoperiod

In this experiment only R-20458 was tested (Table 1). The applications of 12 and 30 μg of juvenoid prevented 78 and 88 % of adults population from entering diapause, respectively. Adults which had received only 4 μg did not behave differently than control insects. In control groups 40% of beetles went into diapause.

Treated and control females started egg-laying 9–15 days after treatments, however, treated insects laid more eggs than control ones. Adults received 12 or 36 μg of R-20458 produced significantly more eggs than control females or those treated with 4 μg of the juvenoid. ($P < 0.01$) No great differences were observed in the egg-hatchability of treated and untreated groups.

The mortality was higher with the increase of doses used. Treatments with 36 μg of the juvenile hormone analogues caused two times higher adult mortality than which was registered in control insects or in those received 4 μg of the same compound.

Inhibition of diapause incidence at short photoperiod

The effects of juvenile hormone analogues were much more evident when the experiment was performed under short-day conditions. In this case each of the juvenoids, R-20458 and ZR-512 was applied.

Table 1

Inhibition of diapause incidence in adults of *Subcoccinella 24-punctata* L. by using juvenile hormone analogues at two different photoperiods (Temp.: 23 °C)

Juvenoid	Dose in µg	Long-day conditions (17/7 h L/D)				
		Diapausing adults %	Number of eggs per female	Egghatchability %	Mortality % ♀♀	Mortality % ♂♂
Control	0	40.1± 5.3	45	84	10	15
R-20, 458	4	35.4± 3.3	62	84	15	10
	12	22.4± 3.4	114	70	20	30
	36	12.9± 0.5	137	79	25	30
ZR-512	4					
	12					
	36					

Juvenoid	Dose in µg	Short-day conditions (13/11 h L/D)				
		Diapausing adults %	Number of eggs per female	Egghatchability %	Mortality % ♀♀	Mortality % ♂♂
Control	0	96.4± 1.3	0	—	15	10
R-20, 458	4	50.6± 2.2	0	—	10	10
	12	37.2± 6.4	15	71	20	25
	36	37.2± 6.4	28	90	20	20
ZR-512	4	50.6± 1.9	0	—	17	17
	12	24.9± 3.2	0	—	17	30
	36	30.6± 7.2	0	—	16	23

As data given in Table 1 show, the applications of juvenoids successfully inhibited the incidence of diapause in adults of the alfalfa ladybird even when small dosages were used. Active beetles received 4 µg of the compounds entered diapause in about 50 % of the population. Insects treated with 36 µg of R-20458 and ZR-512 went into the resting stage in 36 and 31 %, respectively. In control groups diapause appeared in 96.4 %. So, concerning the ratio of population entering diapause a definite dose-response relationship was encountered.

Though ZR-512 could reduce the percentage of diapause in higher dosages slightly better than R-20458, only latter compound proved effective in stimulating reproduction. Untreated females and females received 4 µg of R-20458 as well as those treated with different doses of ZR-512 did not lay eggs at all. Adults received 36 µg of R-20458 laid twice as many eggs than females received 12 µg of the same juvenoid.

The hatchability of eggs laid by JH treated beetles was entirely normal. The mortalities of adults in treated groups were hardly higher than in controls.

Discussion

According to our data published earlier (ALI *et al.*, 1974), the diapause in adults of the alfalfa ladybird, *Subcoccinella 24-punctata* can be successfully terminated by applying substances with juvenile hormone activity. The present results show that the inhibition of diapause incidence in the same species could be also achieved. Such treatments are effective either at long or short photoperiods. Applying 36 μg of the juvenoid R-20458 only about 13% of the adult population enter diapause under long-day conditions. Taking into consideration the natural ecological conditions in the habitat of the species at the beginning of autumn (September-October), the results obtained under short-day conditions are still more remarkable. These show that treatments with juvenoids can efficiently reduce the ratio of insects which enter diapause, moreover, reproductive activity will be also stimulated.

By spraying chemicals with juvenile hormone activity in alfalfa fields infested by Subcoccinella in the period of late summer or autumn when the day-length determines otherwise the diapause of adults, a large proportion of the population would be forced to feed and reproduce again. Within a few weeks thereafter, this prolonged activity, owing to the lack of suitable food and the inadequate weather conditions would cause the death of beetles. In comparison to the possible termination of imaginal diapause in insect pest, with the use of juvenoids – often suggested in the literature – the inhibition of diapause incidence in the same species seems more perspectivic, since this in case not hiding but active insects would be treated.

Furthermore, for a future field use, more active and possibly more stable juvenoids are required as the effective doses of present compounds seem to be relatively high in relation to the body-size of beetles.

Acknowledgement

The authors are very indebted to Mrs. Enayate Salama for her valuable help in tending the insect material. Thanks are also due to the chemists of the Institute of Organic Chemistry, CSAV, Prague, Czechoslovakia for providing us with JH active compounds. For the critical reading of the manuscript we wish to express our sincere thanks to Dr. KATALIN DESEŐ and L. SZALAY-MARZSÓ.

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Mycoplasma- or Chlamydiaelike Bodies in Grape, Affected by Marbour

(*Short Communication*)

By

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VUITTENEZ (1966) described a grape vine mosaic, widespread in South-East of France, the symptoms of which differed from those of court-noué, he termed this disease "marbour". Afterwards, this disease was found in other countries, namely in Ukraina and Moldavia (SHTERENBERG *et al.*, 1973; MARINESKU, 1973). The marbour manifested itself latently on many *Vitis vinifera* and stock varieties. It is only when cuttings from infected vines are grafted on Rupestris du Lot stock, that severe symptoms appear. The clearing of veins of 3—4th order and of a small adjacent leaf area appears on the leaves of the stock, they roll upwards in a spoonlike manner, and the vigour of vines decreases. The absence of endocellular cordons in Rupestris du Lot and Coberr 5BB shoots, as well as the impossibility of mechanical transmission on herbaceous test-plants and the absence of serologic reaction with court-noué antiserum (VUITTENEZ, 1970) distinguish marbour from court-noué:

The disease symptoms, mentioned above, together with the failure of mechanical transmission on herbaceous test-plants, the possibility of diagnosing it only by grafting, led us to presume that marbour is induced by mycoplasma- or chlamidiae-like organisms.

DOI *et al.*, (1967) were first to report mycoplasma-like bodies in ultrathin section of yellow infected plants. Many reports of similar structures in plants infected by yellow type diseases and in cells of leafhopper-vectors have appeared since then (BELLI *et al.*, 1972; DARPOUX, 1971; BOWYER and ATHERTON, 1970; HIRUMI and MARAMOROSCH, 1969). Mycoplasmalike bodies were found also in grape vine tissue infected by flavescence dorée (GIANNOTTI *et al.*, 1969). GOHEEN *et al.* (1973) have shown that Pierce disease of grape vine is induced by rikettsia-like organisms. The recovery of infected plants was achieved by thermotherapy, but they remained sensitive to reinfection by leafhoppers.

Materials and Methods

The veins of 3—4th order were cut from the leaves of Rupestris du Lot infected by marbour, in a drop of fixing solution. The material was fixed at room temperature for 2 hours in 6.5% glutaraldehide solution and then for 2 hours

in 1% osmium tetroxide solution with phosphate buffer, at +4 °C (MILLONIG, 1961). After dehydration in increasing concentration of etanol the samples were put in metacrilate or epon-812. Sections, made with ultratome LKB-8800, were dyed in lead citrate (REYNOLDS, 1963) and investigated under the electron microscope.

Results and Discussion

The bodies that should rather be considered, due to their morphology, as micro-organisms of PLT-group than mycoplasma-like ones, had been found in the sieve cells of the plants infected by marbour. These bodies were mostly oval or spheroid and had a distinct triple-layered unit membrane. Their approximate dimension was 150–180 nm (Fig. 1). An electrondense material – a nucleoid, was observed inside the micro-organism. But we also met bodies without this

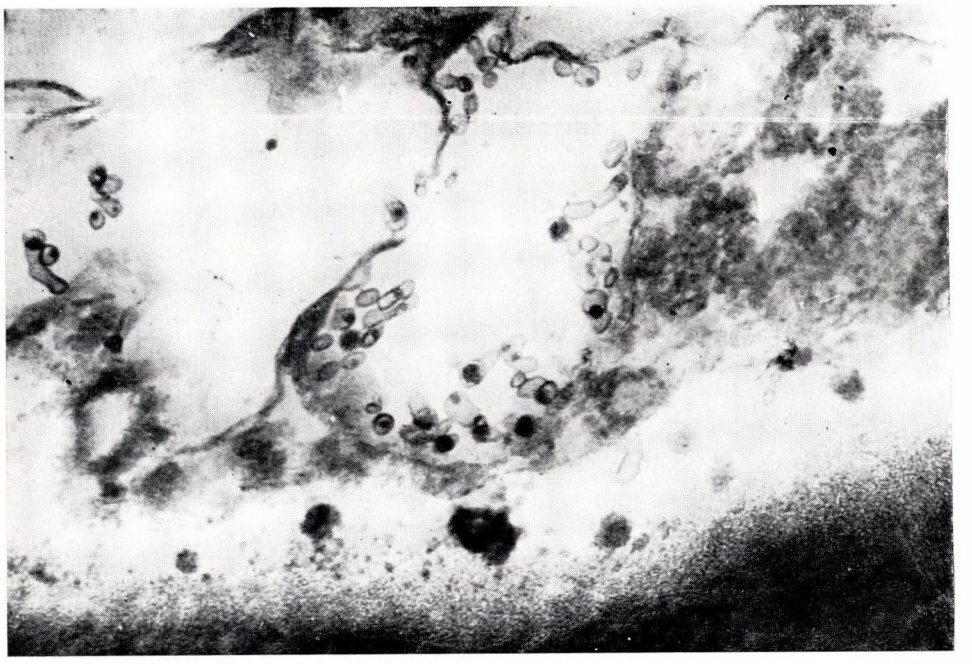

Fig. 1. Chlamydiaelike bodies in the phloem cell of the vein of grape leaf (×30, 000)

electrondense centre. Chlamydiaelike bodies were surrounded by common membrane, that most probably had been derived from plasmalemma of host cell. Not all the sieve cells in the vascular bundle investigated contained chlamydiae-like bodies. In one part of the vein chlamydiae-like bodies were abundant, in others they were absent. The cells, adjacent to the infected ones, appeared anatomically normal.

The number of chlamydiae-like bodies in phloem cells, observed on early stages of infection, was small, but at the later stages their number increased considerably. According to some workers (LIN and LEE, 1967/68), the number of mycoplasma-like bodies in the phloem of plants infected by yellow-type diseases is in correlation with the infection stage and intensity of disease symptoms.

The antibiotics, that have proved to be effective against diseases induced by mycoplasma in mammals and birds (HAYFLICK and CHANOCK, 1965; NAVNHAM and CHU, 1965), clearly inhibit the symptoms of yellow-like diseases in plants (DAVIS and WHITCOMB, 1969; GRANADOS, 1969; ISHIE *et al.*, 1967).

Our experiments have shown, that 0.25% solution of chlortetracycline produces inhibitory effect on grape vine marbour. The disease symptoms disappeared after months of treatment of vine roots on the effect of above solution. They did not appear the following year either. The disease might have passed into a latent stage.

On the basis of the presence of chlamydiae-like bodies in the tissues of diseased plants or their absence in analogous tissues of healthy plants and also the inhibitory effect of tetracycline antibiotics on cane, we believe with a considerable degree of probability that, chlamydiae-like bodies are the agent of grape vine marbour.

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Book Review

L. B. MacHattie and F. Schnelle: An Introduction to Agrotopolimatology. Technical Note, No. 133. World Meteorological Organization, Geneva, 1974. Pp. 1—129.

An understanding of the local climate of a field or forest, as influenced by the local topography, is very important in agriculture for such purposes as the estimation of productivity and accurate planning. In this new booklet of WMO, one can find data of the mechanism how topography influences climatic elements. Moreover, the booklet offers a wide-ranging overview of the more important topoclimatological studies that have been published during the last fifty years.

In Introduction the aims and uses of topoclimatology with examples are discussed.

Chapter 2 deals with the elements of topoclimatology, considering the interaction of the various individual elements, too. Chapter 3 draws attention to factors to be considered when planning and carrying out agrotopoclimatological surveys, then examples of surveys are given in Chapter 4. Last but not least, the bibliography is an extensive collection of the literature on agrotopoclimatology taken from a wide variety of meteorological, geographical and agricultural journals.

This booklet, just as the previous ones of WMO, is very important and provides useful hints for both agricultural experts and research workers.

T. Érsek

Lozina-Lozinskij, L. K. (1972): Ocherki po kryobiologii. (Adaptation of living organisms and cells to low and ultra low temperatures.) Leningrad. Nauka. 288. p.

Author reports his results in kryobiology, a new branch of biology. Kryobiology is a study into the effects of deep (0—80 °C) and ultra deep freeze on the living organisms.

Ten parts of the book deal with these questions. The first three chapters form a treatment of organisms in and near the poles and in high mountains; they give a study of physiological adoptation systems together with reactions of unicellular organisms to low temperatures.

The forth chapter gives an analysis of the phenomenon linked with cooling, freezing of water and biological liquids.

The fifth chapter is a study of frost resistance of vertebrates, insects, fermentive and tumorous cells.

The sixth and seventh chapters cover the question of resistance of vertebrates to deep-freeze.

In the eighth chapter an account is given of damages caused by cooling, freezing und of the resistance to them.

The ninth chapter is devoted to the anabiosis following ultra deep freeze.

The last chapter considers the cosmobiological aspects of cryobiology.

The questions touched on, beside having theoretical significance, are of importance from practical aspects, too. Owing to evaporation of water and precipitation of proteins, life is destroyed beyond a certain level of high temperature; whereas low temperatures render life to carry on existing. In case of larvae of Nematodes survival was observed after an exposure to −196 °C. Diapausing larvae of Ostrinia nubilalis survived −79 °C; Pieris brassicae, P. rapae and Saturnia pyri survived for a couple of days after being kept in a temperature of −196 °C. Deep freezing cannot be however survived without special gradual cooling and warming techniques.

For the practical application of anabiosis, artificial insemination offers a good example. Now it is proved that the sperms of bulls can be kept for years at −78 °C without loss of vigour.

Based on facts listed in the book, prolonged existence of organisms under cosmic conditions, in state of anaboisis is not excluded.

Author piled up data existing in a relatively moderate number in the most diverse fields of biology with a good sense of proportion and completed them with his own results. The only draw-back is in his experiments the small number of individuals.

The book is a useful tool for workers of entomology and for all those who seek the most general relationships of biology.

F. KOZÁR

M. UJVÁROSI: Weeds (Gyomnövények). Mezőgazdasági Kiadó, Budapest, 1973, pp. 833. Figs 810, Ft. 125

The author, a renowned botanist, has been working intensively for 3 decades on the weed flora of Hungary. His oeuvre comprises already many valuable papers on this important discipline, among them three books.

With this work he has succeeded in writing a textbook which is an answer to any professional expectation. This book is not only useful for research workers and for plant protection engineers working in practice but its style and structure makes it possible also to use it as a school manual.

In this volume on nearly 800 pages the descriptions of 805, weeds are given which are completed by the excellent drawings of Vera Csapodi, herself also author of many fine works on plants. The drawings — comprising details on seeds and seedlings — illustrate the plants with the correct precision of a scientist, an achievement which may be regarded in this genre more than art.

By utilizing the data of a vast literature on weeds, the author uses the experiences of his long research. Following a chapter on general plant morphology, the weed species are presented in a systematic order; the most important species are emphasized both by longer descriptions and different lettering.

The scientific names are mentioned according to the most recent nomenclature, together with their synonyms, making the identification of earlier data possible.

The plant descriptions follow a rigorous, yet clear logical sequence, comprising form, size, characteristic colours, shape of the fruits. In places where these play a role in the determination, the characteristics of seeds are also described. From the descriptions and illustrations even those plant protection specialists are able to determine a plant, whose field of work is far from botany.

The descriptions also include data on the life form of the given weed species, on its occurrence in different crop stands, on its ecological requirements and distribution. In some cases other properties like the presence of toxins or active materials used in human medicine are mentioned, providing information on the species from medical or veterinary points of view. The methods of weed control receive only general conclusions in this book, as practical weed control will be treated in a different volume.

T. HALÁSZ

Content

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