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Gábor Ubrizsy, 1919–1973

Dr. GÁBOR UBRIZSY, member of the Hungarian Academy of Sciences, and editor in chief of Acta Phytopathologica passed away on May 25, 1973, at the age of 54, after a short illness. By his death Hungary has lost one of her most distinguished scientists of recent times.

During the course of his scientific career of three decades he covered a number of various fields, such as plant protection, phytopathology, mycology, weed research as well as environmental protection. Nevertheless his most remarkable achievement was the reorganization and expansion of plant protection research in Hungary, after the second World War. He has been the director of the Hungarian Research Institute for Plant Protection over a period of 20 years. Under his leadership a number of new research fields were initiated.

Besides publishing more than 300 scientific articles he is also the author of a number of important handbooks on practical plant protection, plant pathology, agricultural mycology, chemical weed control and a monograph on the smut fungi of the Carpathian Basin.

He was president of two Hungarian scientific societies and member of the presidential board of several Hungarian and international organizations, e.g. European Weed Research Council, Centre International des Antiparasitaires, etc.

His activities and results were highly appreciated by the Hungarian Government, too. He received the "Kossuth Award" in 1952, and was honoured by the golden degree of the "Order of Labour" in 1964. He has been a member of the Hungarian Academy of Sciences since 1965.

Dr. Ubrizsy was a man of many parts and his interests were broad. His unexpected early death means an inestimable loss for Hungarian plant protection research.

The Editorial Board

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Hypersensitivity and the Resistance of Potato Tuber Tissues to *Phytophthora infestans*

By

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The treatments of potato tuber slices either with chloramphenicol (50–800 ppm) or streptomycin (3–50 ppm) inhibited the growth of the infecting *Phytophthora infestans*. The inhibition was accompanied by hypersensitive-like reactions (tissue necrosis and browning). The increases both in the activity of polyphenoloxidase and peroxidase enzymes and in the amount of the phytoalexin rishitin referred to a similarity of this reaction with the natural hypersensitive reaction. As regards the cause-and-effect relationship between the inhibition of the growth of the fungus and the hypersensitive reaction, it was concluded that hypersensitivity associated with phytoalexin production is a consequence, rather than the cause of host resistance to infection.

In previous experiments we have shown that chloramphenicol, known as an antibacterial antibiotic inhibits the growth of *Phytophthora infestans in vivo* and *in vitro* (ÉRSEK et al., 1972). Furthermore, we experienced typical tissue necrosis on tuber slices treated with this antibiotic and infected with the fungus (KIRÁLY et al., 1972). Necroses were similar to those which developed in tuber tissues as a result of an incompatible host-pathogen interaction, at least macroscopically.

It is the purpose of the present investigations to gain a deeper insight into the following questions:

1. It would be possible to induce a hypersensitive-like phenomenon by applying antibiotics other than chloramphenicol?

2. Whether the natural hypersensitive necrosis is identical with tissue necrosis induced by the combined action of chloramphenicol and inoculation with the compatible pathogen?

3. What is the mechanism of the shift from a compatible host-pathogen interaction to an incompatible one under the influence of chloramphenicol or other antibiotics?

As regards the first question, streptomycin seems to be an antibiotic supposedly similar in action to that of chloramphenicol (MÜLLER et al., 1954; VÖRÖS, 1965).

Concerning the second question, microscopic investigations, measurements of activities of polyphenoloxidase and peroxidase enzymes as well as accumulation of the phytoalexin rishitin which all were regarded to be involved in the final

expression of the hypersensitive reaction (TOMIYAMA and STAHMANN, 1964; TOMIYAMA et al., 1968) contributed to a positive answer. In other words that the hypersensitive reaction induced by chloramphenicol and streptomycin in a compatible host–parasite combination is identical with the natural one.

Regarding the third question, the isolation and the role of an endo-toxin from both compatible and incompatible physiologic races of the fungus helped to understand why the antibiotics render the originally compatible host–pathogen interaction to an incompatible one.

Materials and Methods

Pathogen and plant materials

In the course of the present investigations tuber tissues of *Solanum tuberosum* L. cv. Gülbaba (*r*) and cv. Rotkelchen (R_1R_3) were inoculated with *Phytophthora infestans* (Mont.) de Bary. Race 1 of this fungus is incompatible to Rotkelchen, however, race 1.2.3.4 is compatible to the same cultivar. Gülbaba is compatible to both races of *Phytophthora infestans* used in these experiments.

About 8 mm thick tuber slices were immersed for 3 hrs in 0, 50, 100, 400 and 800 ppm water solution of chloramphenicol or 0, 3.125, 6.25, 12.5, 25 and 50 ppm solutions of streptomycin. One hour after this treatment the tubers were inoculated with 0.3 ml zoospore suspension (2×10^5 zoospores/1 ml distilled water). In the cases of treatments with 400 ppm chloramphenicol or 25 ppm streptomycin inoculation was carried out 24 hrs before treatments with the antibiotics. Tubers sections were made by a freezing microtome for microscopic studies 24 hrs after inoculation. Hyphae of the fungus were stained with a 0.05 per cent cotton blue in lactophenol.

Enzyme assays

Enzyme activities were determined in the 1.5 mm thick tuber slices 65 hrs after inoculation. Determination of the activity of polyphenoloxidase or peroxidase was carried out spectrophotometrically by a modified procedure of FEHRMANN and DIMOND (1967). 5 g samples were homogenized in 10 ml of 0.1 *M* phosphate buffer (pH 6.1) with quartz sand at 0°C, then filtered and centrifuged at 0°C with a Janetzki K23 type centrifuge at 5000 *g* for 20 min. The supernatant was filled up with the phosphate buffer to 20 ml. Catechol was used as a substrate for the polyphenoloxidase, and pyrogallol for peroxidase. In the case of peroxidase assays 0.1 ml of 0.2 *M* pyrogallol solution was added to a cuvette containing 1 ml of 0.1 *M* phosphate buffer (pH 6.1), 0.5 ml of 0.01 *M* peroxide solution and the enzyme extract with appropriate concentration, and distilled water to make a volume of 3 ml. The change in absorption at 430 nm was measured in a Unicam SP 800 spectrophoto-

meter. Enzyme activities were expressed as increase in absorption between 15 and 45 sec after the substrate was added in relation to the per cent of the uninfected and not treated control. As regards polyphenoloxidase, 0.2 ml of 0.2 *M* catechol served as substrate. The changes in absorption were measured at 400 nm.

Phytoalexin

The extraction of the phytoalexin rishitin was carried out by the method of SATO and TOMIYAMA (1969) 65 hrs after inoculation from 5 g of 1.0–1.5 mm thick tuber tissues. Rishitin was separated by thin-layer chromatography on plates coated with Silica Gel-G using ethyl ether as a solvent. The optical density of spots of rishitin was determined at 500 nm in a Unicam SP 800 spectrophotometer. Rishitin content of samples was expressed in μg/1 g fresh weight.

Inducer (endo-toxin) of necrosis and phytoalexin production

1 g mycelium of *Phytophthora infestans*, race 1 and 1.2.3.4 from a 2-week-old culture grown on a synthetic Henniger type medium was homogenized in 10 ml distilled water and then sonicated at 0°C with an MSE sonifier for 10 min at maximum intensity. Sonicates were centrifuged and applied to tuber surfaces of cultivar Gülbaba or Rotkelchen.

By another way, mycelium of the above-mentioned two races of the pathogen was placed on a watchglass and held for 24 hrs in a chloroform atmosphere under a glass jar. Killed cells of the mycelium released liquid on the watchglass. In addition, the mycelium was washed with distilled water (2 ml water to 1 g mycelium). The liquid was collected, filtered and applied to the surface of tuber slices of Gülbaba and Rotkelchen.

Results

In the course of experiments it was shown that the greater the concentration of chloramphenicol or streptomycin applied to tuber slices before inoculation, the lower the growth rate of the compatible race of the fungus. On the contrary, the brown discolouration (necrosis) of the tuber surface tissues is more prononunced with increasing concentrations of antibiotics (Table 1). Streptomycin, as compared to chloramphenicol, was more effective in inducing tissue necrosis and inhibition of fungal growth. As is shown in Fig. 1, 25 ppm streptomycin or 400 ppm chloramphenicol inhibited the fungus from further growth fully, and induced 2–3 mm deep small necrotic spots on the surface of tuber slices. Neither streptomycin and chloramphenicol nor the compatible race of the pathogen alone induced visible necrosis in tuber tissues. Tap water applied to tissues, as a check against antibiotics, did not influence the growth of *Phytophthora infestans*. Necrotization was on a lower rate in the case of 50 ppm streptomycin and 800 ppm chloramphe-

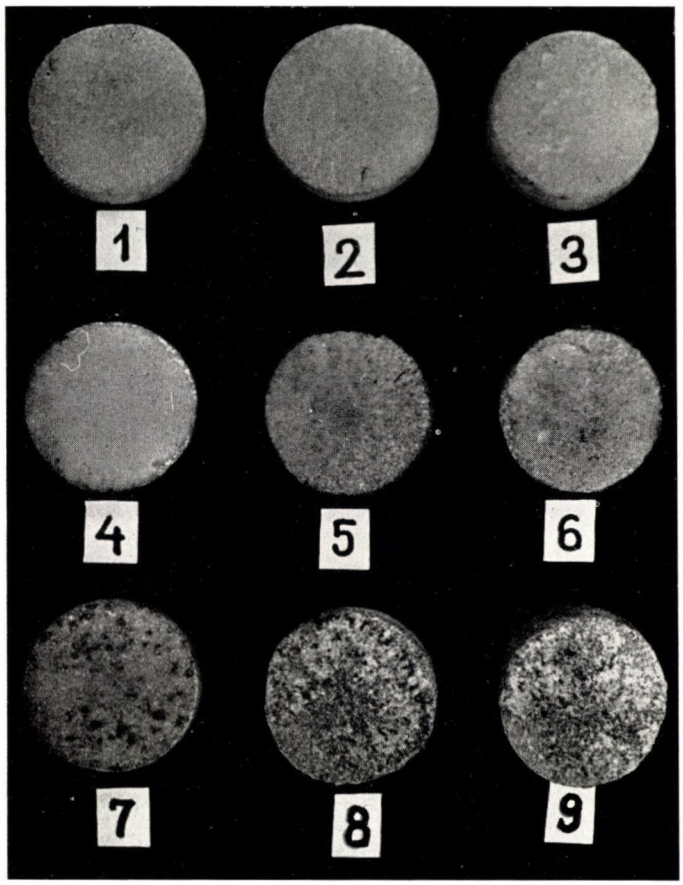

Fig. 1. Different treatments and inoculations on tuber slices of potato cultivar Gülbaba. 1: treated with tap water; 2: treated with streptomycin (25 ppm); 3: treated with chloramphenicol (400 ppm); 4: inoculated with race 1.2.3.4 of *P. infestans*; 5: inoculated with race 1.2.3.4, 24 h before treatment with streptomycin (25 ppm); 6: inoculated with race 1.2.3.4, 24 h before treatment with chloramphenicol (400 ppm); 7: treated with cell-free homogenate; 8. treated with streptomycin (25 ppm), 1 h before inoculation with race 1.2.3.4; 9: treated with chloramphenicol (400 ppm), 1 h before inoculation with race 1.2.3.4

nicol than with 25 ppm streptomycin and 400 ppm chloramphenicol although the fungus was seemingly inhibited from growth fully in all of the experiments (Table 1). When tuber slices were treated with the most effective concentrations of antibiotics (25 ppm streptomycin and 400 ppm chloramphenicol) 24 hrs *after* inoculation, surface growth of the fungus was fairly good and the extent of

Table 1

Growth of *Phytophthora infestans*, the degree of tissue necrosis, the activity of polyphenoloxidase (PPO) and peroxidase (PO) enzymes and the amount of rishitin in infected "Gülbaba" potato slices[a] treated with chloramphenicol or streptomycin

Concentration of antibiotics (ppm)	Growth of surface mycelium[b]	Degree of necrosis[c]	Activity[d] of		Rishitin content (μg/g fresh weight)
			PPO	PO	
0 (tap water)	100	0	166	125	trace
Chloramphenicol[e]					
50	80	+	200	135	41
100	40	++	255	150	62
200	20	++++	322	175	91
400	0	+++++	366	190	100
800	0	++++	344	185	83
Streptomycin[e]					
3.125	70	+	278	150	12
6.250	30	++	300	165	45
12.500	10	++++	344	185	86
25.000	0	+++++	366	195	115
50.000	0	++++	377	195	90

[a] Inoculation was carried out with race 1.2.3.4 of *Phytophthora infestans*, extractions of rishitin and enzymes were made 65 hours after inoculation.

[b] The growth rate of mycelium was expressed in percentage of control

[c] The degree of necrosis was estimated by visual observation. + weak necrosis, +++++ very intensive necrosis.

[d] Enzyme activity was expressed in percentage of noninoculated and tap water treated control (100%).

[e] Antibiotics were applied 1 hour before inoculation.

necrotization was weak (Table 2). The degree of necrosis was estimated on the third day after inoculation.

Microscopic investigations have shown that the penetration of the germination tubes of zoospores into tuber tissues was not inhibited by the application of antibiotics. It was seen under the microscope that 48 hrs after inoculation hyphae of *Phytophthora* was shrinken and apparently damaged in samples treated with streptomycin or chloramphenicol.

It is seen in Tables 1 and 2 that the activity of polyphenoloxidase and peroxidase enzymes is in a positive correlation with the degree of tissue necrosis. On the contrary, the lower the rate of fungus growth on the surface of the tuber slices, the higher the activity of the enzymes mentioned above. Enzyme activities were slightly augmented also in the non-infected slices which were treated with antibiotics. However, this increase in the activities was negligible even at the highest concentrations of antibiotics.

As regards rishitin extracted from 1.0–1.5 mm thick tuber slices it was also experienced that the greater the degree of necrosis in tuber tissues, the greater the phytoalexin (rishitin) content of samples. However, samples in which treatment with antibiotics were carried out 24 hrs after inoculation contained relatively high amount of rishitin in spite of the weak necrosis and the low rate of enzyme activities (Table 2). In uninoculated samples treated only with antibiotics no rishitin was detected.

Table 2

Effect of different treatments on the growth of *Phytophthora infestans*, the degree of tissue necrosis, the activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes and on the amount of rishitin in "Gülbaba" potato tuber slices[a]

Treatments and/or inoculations	Growth of surface mycelium[b]	Degree of necrosis[c]	Activity[d] of		Rishitin content (μg/g fresh weight)
			PPO	PO	
Inoculated (untreated)	100	0	166	125	trace
Treated with cell-free homogenate	0	+++++	356	190	123
Treated with chloramphenicol (400 ppm) 1 h before inoculation	0	+++++	366	190	100
Inoculated 24 h before treatment with chloramphenicol (400 ppm)	50	++	178	125	91
Treated with streptomycin (25 ppm) 1 h before inoculation	0	+++++	366	195	115
Inoculated 24 h before treatment with streptomycin (25 ppm)	50	++	170	120	86

[a] Inoculation was carried out with race 1.2.3.4 of *Phytophthora infestans*, extractions of rishitin and enzymes were made 65 hours after inoculation.

[b] The growth rate of mycelium was expressed in percentage of control.

[c] The degree of necrosis was estimated by visual observation. + weak necrosis, +++++ very intensive necrosis.

[d] Enzyme activity was expressed in percentage of noninoculated and tap water treated control (100%).

[e] Antibiotics were applied 1 hour before inoculation.

Both physiologic race 1 and 1.2.3.4 contained an extractable factor(s) which induced intensive necrosis of tissues on tuber slices of cultivars without regard to the compatible or incompatible nature of the original host-pathogen relation. In Table 3 the degrees of necrosis and the contents in rishitin as induced by endo-toxin(s) extracted from mycelium by sonication or by chloroform treatment are compared with values received with different host-pathogen interactions and with those induced by antibiotics. As is seen, the inhibited or damaged pathogen released a factor(s) which was in relation to rishitin production or to tissue necrosis.

Table 3

Hypersensitive reaction in potato-*Phytophthora infestans* and potato-toxin interactions

Host-pathogen/toxin interaction	Necrosis	Rishitin
Compatible[a]	0	0
Incompatible[b]	+	+
Compatible + treatment with streptomycin or chloramphenicol	+	+
Sonicated[c]		
Compatible	+	+
Incompatible	+	+
Chloroform-treated[d]		
Compatible	+	+
Incompatible	+	+

[a] Race 1 or race 1.2.3.4 of *P. infestans* on "Gülbaba" (r) or race 1.2.3.4 on "Rotkelchen" (R_1R_3) potato tuber slices.

[b] Race 1 on "Rotkelchen" potato tuber slices.

[c] Homogenates of mycelium of race 1 or 1.2.3.4 of *P. infestans* was sonicated. The cell-free liquids were applied to tuber slices of potato cultivar Gülbaba or Rotkelchen.

[d] Cell-free liquid released by race 1 or 1.2.3.4 as a result of treatment with chloroform was applied to tuber slices of potato cultivar Gülbaba or Rotkelchen.

Discussion

The hypersensitive reaction of the host, accompanied by necrosis and production of phytoalexin(s) can be induced in a compatible host-pathogen complex with chloramphenicol or with streptomycin. This latter antibiotic was more effective than chloramphenicol in inducing the hypersensitive reaction. Both antibiotics are similar in their action on *Phytophthora infestans:* only the growth of the mycelium is inhibited, neither zoospore release nor the germ tube formation of zoospores are influenced (ÉRSEK et al., 1972).

The natural hypersensitive reaction (incompatibility) in potato is characterized by the high level of the phytoalexin rishitin and the augmented activities of polyphenoloxidase and peroxidase, as compared to the compatible host–pathogen interaction. It was of interest to investigate these characteristics in the hypersensitive reaction induced by antibiotics in the originally compatible host–pathogen interaction too. The mechanism of the hypersensitive reaction of potato tuber tissues as characterized by the accumulation of a phytoalexin and by the increased activity of soluble oxidase seems to be identical both in the natural incompatibility and in the "induced incompatibility" in which latter case the common action of antibiotics and a compatible fungal infection results in a hypersensitive reaction.

One can suppose that in the antibiotically induced incompatibility some important metabolic alterations in tuber tissues render the compatible host to

become incompatible to the pathogen. However, experiments with another tuber-pathogenic fungus do not support this idea. *Rhizoctonia solani*, which is resistant to streptomycin or chloramphenicol grow well on tuber slices treated with both the antibiotics prior to inoculation. It would seem that the action of antibiotics is a direct influence on the growth of *Phytophthora infestans*. Treatments with antibiotics alone (without inoculation) never increased phytoalexin production and only slightly augmented enzyme activities.

We believe that the experiments with the fungal endo-toxin(s) give an explanation for the role of antibiotics in inducing hypersensitivity. Cell-free homogenate of the mycelium of *Phytophthora infestans* was able to induce the hypersensitive reaction in tuber slices whether the host was originally regarded as compatible (susceptible) or incompatible (resistant) to the fungus (cf. SAVELEVA and RUBIN, 1963; SATO et al., 1968; VARNS and KUĆ, 1971). We repeated this finding and speculated on the role of a factor(s) released by the fungus in incompatible host–pathogen relations. This factor(s) seems to be released from all the races of the pathogen if the fungus is damaged. In a resistant (incompatible) host the fungus is inhibited or somehow damaged as a consequence of the unknown mechanism of resistance. Subsequently, the factor(s) of hypersensitivity is released and this induces the characteristic symptoms of the hypersensitive reaction, like tissue necrosis, brown discolouration, activation of some oxidative enzymes and production of phytoalexin. One can suppose that the factor, which may be called endo-toxin, will not be released from the undamaged fungal pathogen in a compatible host. Consequently, metabolic alterations characteristic of the hypersensitive reaction will not be induced.

We have no direct evidence to offer as yet that an inhibitory mechanism is involved in damaging the fungus in the resistant host. When we simulated the injury to the mycelium by sonication or by treatment with chloroform on the one hand and treatments with antibiotics on the other, endo-toxin(s) was released causing the typical hypersensitive reaction. This explanation is in accordance with experiments on the action of antibiotics in different concentrations. The higher the concentration of streptomycin or chloramphenicol, the greater the amount of fungal mycelium damaged or killed in host tissue, and, consequently, the greater the amount of the endo-toxin released from the pathogen, the phytoalexin which accumulates in the tissue and the higher the activity of oxidases (Table 1).

The highest concentrations of chloramphenicol and streptomycin (800 and 50 ppm, respectively) inhibit the fungus from further growth very quickly. Thus, the relatively few mycelium releases only a few amount of endo-toxin which induce a slight necrosis in tissues and a slight increase in the activity of the soluble oxidases and in accumulation of rishitin. One can suppose that the degree of the hypersensitive reaction depends on the quantity of endo-toxin(s) released from the fungus.

It follows from the results outlined above that the concept of the hyper-

sensitive type resistance of plants must be revised (KIRÁLY et al., 1972). In the natural hypersensitive reaction neither the tissue necrosis nor phytoalexins are involved in the inhibition of the pathogen. It would seem that a *recognition phenomenon* of unknown mechanism is responsible in the resistant plant for the incompatibility between host and pathogen. Endo-toxin(s) is released as a consequence of damaging the fungus in the resistant host, and the hypersensitive reaction is only a consequence, not the cause, of resistance to *Phytophthora infestans*.

The phytoalexin rishitin do not seem to have a primary role in the resistance to *Phytophthora*. This can be deduced from experiments in which tuber slices were treated with antibiotics 24 hrs after inoculation with *Phytophthora*. A high amount of rishitin was produced in this case and still, the fungus produced abundant surface mycelium on tuber slices. The amount of rishitin was of the similar order as in a natural hypersensitive host–pathogen combination (VARNS et al., 1971). It is also important in this context that recently VARNS et al. (1971) detected a high level of phytoalexins in green sprout tissue of potato if it was in a compatible relation to *Phytophthora*, whereas in the incompatible (resistant) combinations tissue necrosis without detection of phytoalexins was observed. This finding with green sprouts is in contradiction with the phytoalexin theory of resistance.

On the basis of the above-mentioned evidences and other data, one can conclude that the hypersensitive tissue necrosis in potato is a consequence, rather than the cause, of resistance to infection of *Phytophthora infestans*.

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Phenol Localization in Rice Leaf Tissues

By

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Phenolic compounds were found to be localized in the bulliform cells and in the lipid droplets, distributed in the chlorophyll-containing parenchyma cells besides the already reported sites *viz.* the cell walls of vascular bundles, collenchyma and epidermal cells of rice leaves.

Introduction

Pathological disorders of rice leaves due to infection with fungi, bacteria and viruses cause brown discolouration of tissues at one stage or other. Browning of the plant tissues has been attributed to the oxidation of host phenols by phenol oxidases and polymerization of the resultant quinones to form melanin-like pigment (FARKAS and KIRÁLY, 1962). Various phenolic compounds, chlorogenic acid (SUZUKI et al., 1953), *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids (KUWATSUKA and OSHIMA, 1961), salicylic acid (ISHII et al., 1962), and protocatechuic, cinnamic, *o*-coumaric and caffein acids (VARGA, 1970), have been reported to be present in rice leaf tissues.

Presence of phenols in randomly occurring parenchyma cells of banana roots (MACE, 1963), species of *Eucalyptus* (WARDROP and CRONSHAW, 1962) and in various genera and species of the *Rosaceae* (POLITIS, 1957) has been shown. Within randomly scattered parenchyma cells of banana roots (BECKMAN and MUELLER, 1970) phenols localize in discrete, diffuse bodies or in larger globular bodies which occur singly or in aggregates arising from the dense peripheral cytoplasm. Chlorogenic acid was shown to be present in the cell membrane (walls) of vascular bundle, collenchyma and epidermal cells of rice leaves (SUZUKI et al., 1953). However, very little is known about the sites of synthesis and storage of phenols in rice leaves.

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Materials and Methods

Rice seedlings *(Oryza sativa* L.) of several cultivars, grown under greenhouse conditions were used.

Free hand sections of leaves obtained at various growth stages of the plant were stained either with 0.5 per cent aqueous methylene blue (MACE, 1963), 10 per cent aqueous nitrous acid (REEVE, 1951), both of which react strongly with *ortho*-dihydroxy phenols (MACE, 1963), ammonium solution or with a mixture of 10 per cent acetic acid-$NaNO_2$ (SUZUKI, 1965) and were examined under a light microscope.

Results and Discussion

The cell walls of vascular bundles, collenchyma and epidermal cells of rice leaves, the sites reported to contain chlorogenic acid by SUZUKI et al. (1953) were stained dark blue with methylene blue, red with nitrous acid, yellow with ammonium solution and with acetic acid-$NaNO_2$ reagent. Besides this, the bulliform cells were also stained readily with all the reagents tested (Fig. 1). One, two or all the cells in a bunch of bulliform cells gave positive reaction for the reagents, revealing the possible presence of phenolic compounds in them.

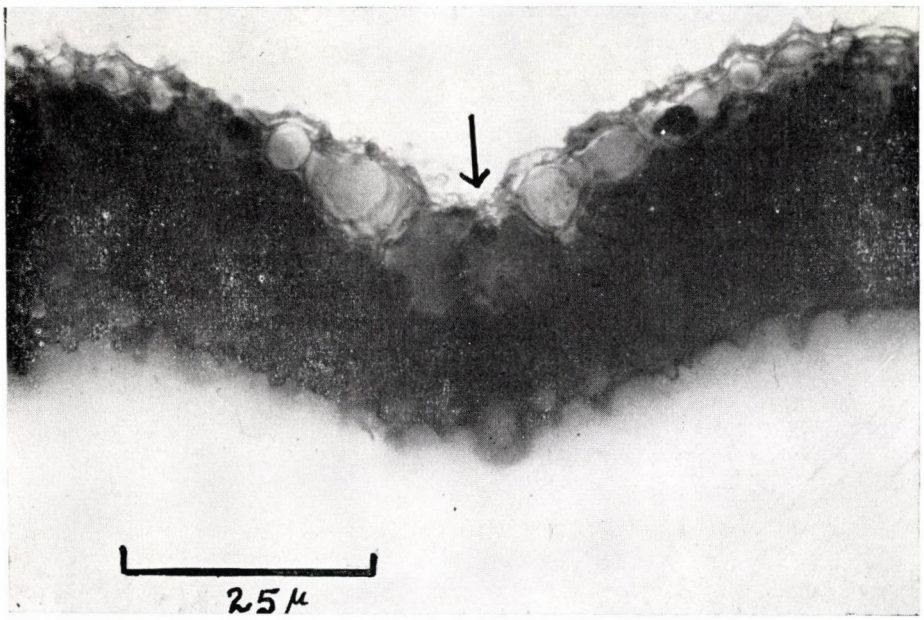

Fig. 1. Cross section of rice leaf showing bulliform cells stained with methylene blue (×450)

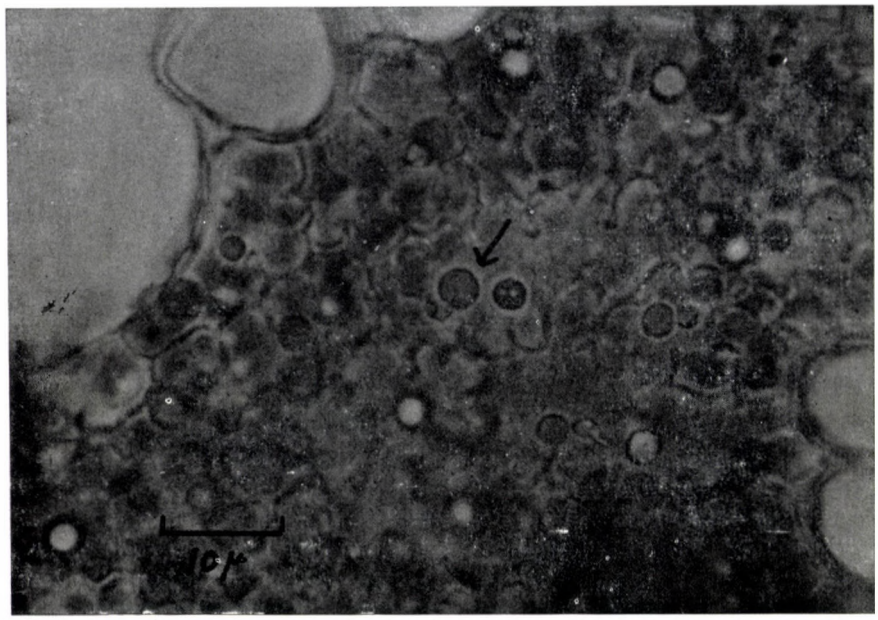

Fig. 2. Cross section of rice leaves showing the presence of lipid droplets distributed in the chlorophyll-containing parenchyma cells — unstained (×1000)

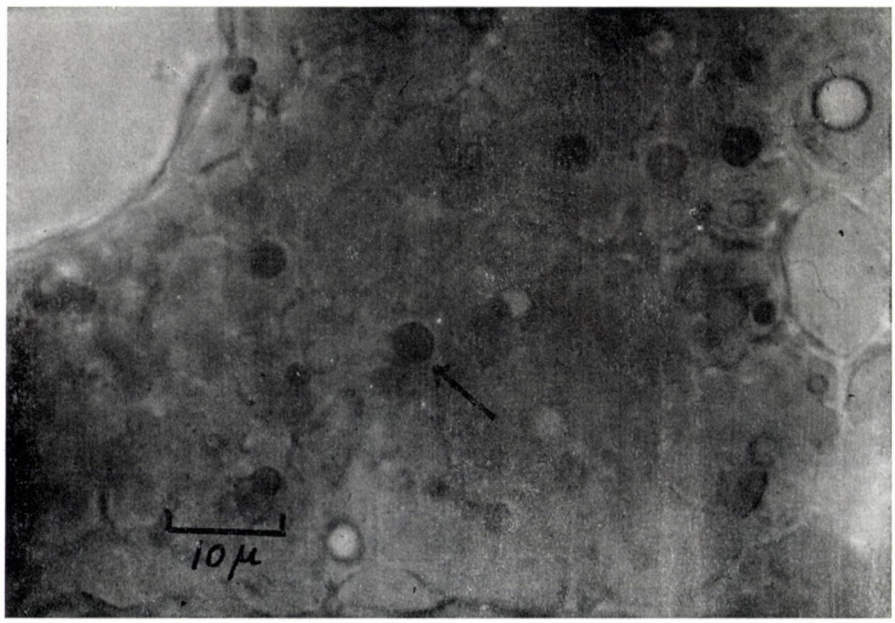

Fig. 3. Cross section of rice leaves showing the lipid droplets stained with methylene blue (×1000)

Methylene blue stained the lipid droplets, distributed in the chlorophyll-containing parenchyma cells (Fig. 2—4) in healthy tissues to blue, suggesting the localization of phenolic compounds in the lipid droplets. However, they were not stained by the other reagents, presumably due to their inability to penetrate the lipid globules. The lipid globules are discoloured brown before the visible discolouration of the tissues takes place during the infection with fungi, *Piricularia oryzae* Cav., *Helminthosporium oryzae* Breda de Haan, *Cercospora oryzae* Miyake, with bacterium *Xanthomonas translucens* f. sp. *oryzicola* (FANG et al.) Bradbury,

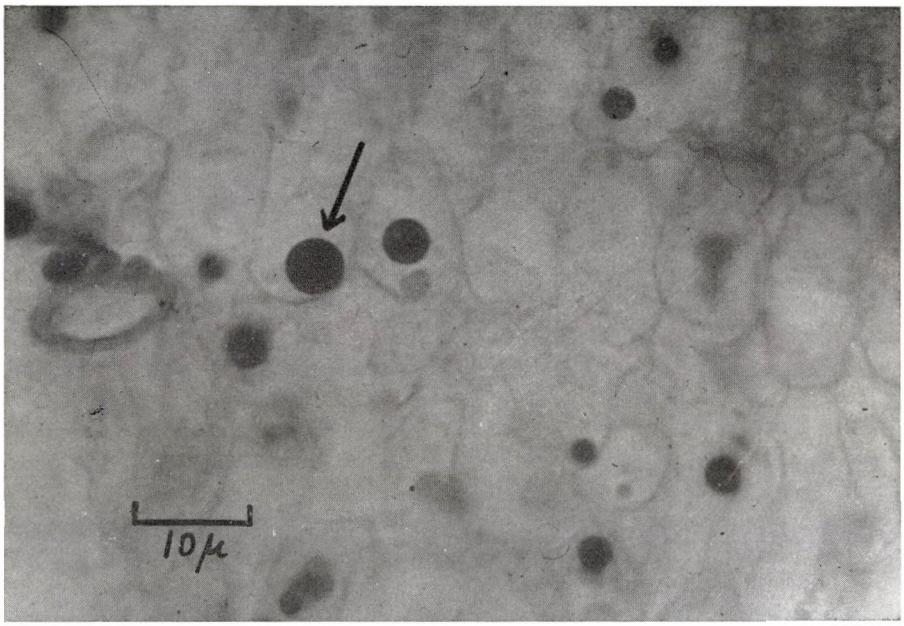

Fig. 4. Longitudinal section of rice leaves showing the lipid droplets stained with methylene blue (×1000)

and with tungro virus (SRIDHAR et al. 1972). In and around the diseased area the lipid droplets disappear (Fig. 5) and the brown pigment is dispersed with in the cells. The discolouration of the tissue is preceded by the dissolution of the discoloured lipid globules.

Bulliform cells are highly vacuolated cells, and they play a role in the hygroscopic opening and closing movements of mature leaves. The physiologic function of these cells other than that of water storage is poorly understood (ESAU, 1965). Phenolic compounds are frequently found stored in the vacuoles of the cells (ZIRKLE, 1932; PRIDHAM, 1965; LEDBETTER and PORTER, 1970). Phenols

are present at high concentrations in many plants but they are localized within specialized storage cells (POLITIS, 1957; WARDROP and CRONSHAW, 1962; MACE, 1963; BECKMAN and MUELLER, 1970) and thus spatially separated from their oxidases.

Phenolic compounds have been associated with defense mechanisms because of their general accumulation near the injured and infected tissues and that

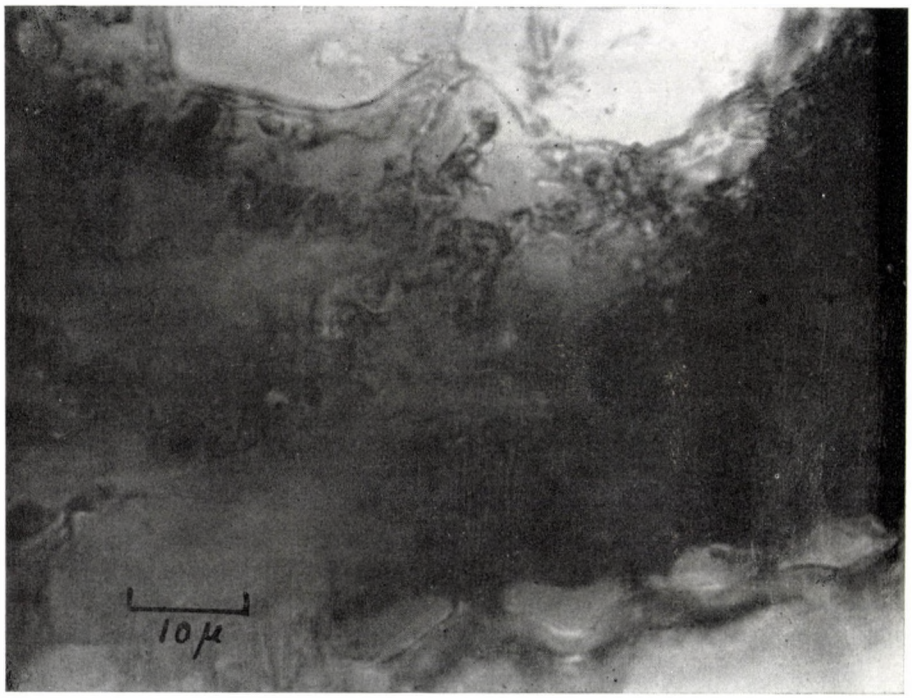

Fig. 5. Cross section through the blast diseased area showing the absence of lipid droplets due to their dissolution. (×1000)

phenols and their oxidation products are highly fungitoxic (FARKAS and KIRÁLY, 1962; CRUICKSHANK and PERRIN, 1964; RUBIN and ARTSIKHOVSKAYA, 1964; KUĆ, 1966). Activation of aromatic compounds is a characteristic feature of a plant under stress. Although the physiological role of phenolic compounds in plants is little understood, their involvement in many reactions is well documented (PRIDHAM, 1960; VAN FLEET, 1961). Further studies on their synthesis and release in response to pathogenic invasion will help to understand the host–parasite relationships.

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Conidial Germination in *Colletotrichum falcatum* — Study of physical factors and standardization of germination technique*

by

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The present investigations attempt to determine the optimum physical requirements of germinating conidia of ten isolates of *Colletotrichum falcatum* Went. These requirements include a direct contact of germinating conidia with water, 99—100% relative humidity, 18 hours' incubation at 26°C—27°C temperature, 12—16 days old cultures, about 6.0 pH, a spore concentration of 500,000 spores per cmm, sufficient supply of oxygen and carbon dioxide, and a certain amount of light. Besides, this work also suggests a technique for germinating conidia to obtain a relatively satisfactory germination in laboratory.

Introduction

Colletotrichum falcatum Went, causing the red rot of sugar-cane, is responsible for heavy losses to the crop in several sugar cane growing regions of the world. Considerable variations in cultural, physiological and pathogenic characteristics of the fungus have been demonstrated in India and elsewhere. As a result there have been encountered serious difficulties in complete understanding of the epidemiology of the disease. Since infection of healthy canes initiates with the germination of conidia in the nodal region; it was imperative to have a good understanding of the process of conidial germination, including the differentiation of germ tube and appressoria. The latter are small specialized cells formed before penetration of host tissue by the germ tube or the infection hypha (HASSELBRING, 1906; SIMMONDS, 1941; DICKINSON, 1960 and SUTTON, 1962, 1968). The importance of conidial germination and appressoria in the taxonomy of the genus *Colletotrichum* and in the establishment of infection by pathogenic species of the genus has been amply emphasized (SUTTON, 1968; MIEHLE and LUKEZIC, 1972).

VASUDEVA, CHONA and SRIVASTAVA (1953); CHONA and SRIVASTAVA (1954); and BAJAJ, GANJU and CHATRATH (1959) reported a low percentage of germination of densely granular conidia of the light type, highly sporing, virulent

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isolates as compared to a higher germination percentage of less granular conidia of the dark type, sparsely sporing, weakly pathogenic isolates. In 1962 VASUDEVA, BAJAJ and KHOSLA reported a higher germination percentage of the densely granular conidia of a light type, highly sporing, virulent isolate, No. *244. Such diverse reports necessitated detailed investigations on the germination of conidia of various isolates of *C. falcatum*. Studies conducted in this laboratory suggest that conidial germination in this fungus is influenced by several factors, including age of conidia, temperature of incubation, incubation period, light, concentration of conidial suspension, pH, oxygen, carbon dioxide, and last but not the least important is the technique of germination (SINGH, 1962). This paper presents the results obtained from the study of these factors and suggests a technique for the germination of conidia of *C. falcatum* under laboratory conditions.

Materials and Methods

Actively growing monosporic cultures of seven light type, highly sporing, virulent isolates (Nos* 244, 301, 304, 357, 382, 390 and strain "I") and three dark type, sparsely sporing, weakly pathogenic isolates (Nos* 7, 78 and 300) of *C. falcatum*, maintained on 2% oat-meal agar, were used in the present investigations. For all initial experiments, conidia harvested from 12–20 days old cultures were washed in distilled water through centrifugation (2800 rpm for 5 minutes) and made into suspensions of a concentration of 10–20 spores per microscopic field under the low power** in sterile glass-distilled water. The volume of the suspension drop was kept constant (0.03 ml – a normal size drop) in all experiments. Glass slides*** with germination drops were incubated at 25°C**** for about 16 hours in moist chambers, made from sterilized pairs of Petri plates.

The criterion for germination of a conidium was the emergence of a germ tube to a length exceeding its width. The data for each experiment was based on an average of about 3000 to 4000 spore counts/germ tube measurements.

Experimental Results

Germination on plain glass slides

Germination of unwashed conidia on dry glass slides and in conidial suspension. No germination of conidia was observed on dry glass slides both in the presence and in the absence of high humidity. There was also no germination of conidia in

* Indian Type Culture Collections, Indian Agricultural Research Institute, New Delhi, India.

** Low power means 10×10.

*** The slides were acid-cleaned, grease-free and dry.

**** Optimum temperature for the growth of the fungus.

suspension drops of distilled water in the absence of high humidity. However, conidia in suspension drops of distilled water did germinate in moist chambers with 100% relative humidity (Table 1). The data showed that conidia of all the ten isolates germinated in suspension drops under 100% relative humidity, thereby indicating an absolute requirement of direct contact with water as well as high humidity. The dark type isolates showed better germination than the light type ones. In the light type isolates, germination varied from an average of 19.0% in isolate No. 382 to an average of 31.2% in isolate No. 304. In the dark type isolates, the lowest average germination was 48.8% in isolate No. 78 and the highest was 50.6% in isolate No. 300. A similar trend was observed with the growth of the germ tube, which were longer (53.6 μ to 86.1 μ) in the dark type isolates than in the light type ones (42.2 μ to 78.0 μ long). The smallest germ tubes (42.2 μ) were observed in the strain "I".

Table 1

Germination of unwashed conidia of the ten *C. falcatum* isolates in suspension drops incubated at 25°C in moist chambers

Isolate No.	Average germination (%)	Average germ tube length (μ)
244	21.5	78.0
357	19.8	74.7
"I"	31.0	42.2
390	26.5	56.8
301	23.7	63.2
304	31.2	76.3
382	19.0	45.5
78	48.8	86.1
300	50.6	73.1
7	49.1	53.6

Germination of washed conidia in suspension

In the previous experiments, percentage of germination varied considerably between replicates and between repetitions of the experiment. This type of variation is known to be due to some external nutrients and mycelial fragments adhering to spores, which always come along, how-so-ever careful scrapping of the conidia is done. To exclude the effect of such nutrients and mycelial fragments, conidia were washed by centrifugation. Thick conidial suspensions in sterile distilled water were centrifuged at a speed of 2800 rpm for 5 minutes. The supernatant was then decanted of and the conidia were made into a fresh suspension (10–20 spores per microscopic field under low power of the microscope) in sterile distilled water, and germination studied at 25°C under 100% relative humidity after 16 hours of incubation.

The data (Table 2) showed that germination did take place but washing of the spores reduced the germination and the affect was more pronounced on the percentage of germination than on the length of the germ tube. The trend of germination was, however, the same as in the unwashed spores. The data obtained was fairly consistent and little variation was observed between replicates and repetitions of experiment, thus making washing of the spores necessary.

Appressorial formation was frequent. Most appressoria were terminal, rarely intercalary, broadly clavate, aseptate, smooth-walled and cinnamon buff or dark brown in color.

Table 2

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water

Isolate No.	Average germination (%)	Average germ tube length (μ)
244	13.2	73.3
357	13.3	66.8
"I"	23.4	42.2
390	17.4	55.2
301	16.0	60.1
304	21.4	71.5
382	8.1	40.6
78	41.0	87.7
300	43.5	79.6
7	41.9	48.7

Table 3

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water after one to three washings

Isolate No.	A		B				D	
	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)
244	17.4	71.5	13.6	55.2	3.9	45.5	22.4	81.2
357	16.2	65.0	12.9	58.5	6.9	45.5	21.4	61.7
"I"	28.7	39.0	23.1	32.5	13.1	22.7	33.1	42.2
390	19.1	52.0	15.1	45.5	2.3	13.0	21.9	52.0
301	23.1	61.7	15.6	52.0	6.0	42.2	29.3	61.7
304	30.1	65.0	24.3	61.7	6.2	32.5	35.7	74.7
382	11.5	45.5	8.0	42.2	2.8	26.0	18.4	48.7
78	40.6	91.0	35.3	81.2	12.6	48.7	46.9	97.5
300	45.9	65.0	42.0	61.7	25.1	35.7	51.4	81.2
7	39.6	52.0	32.7	52.0	16.4	39.0	46.3	55.2

The detrimental effect as a result of washing was further confirmed by giving three successive washings by centrifugations to the conidia – once (A), twice (B) and three times (C) at a speed of 2800 rpm for 5 minutes, each time. After each washing the supernatant was decanted off and the conidia were made into a new suspension in fresh distilled water. Finally each sample of the washed conidia was made into a suspension (10–20 spores per microscopic field) in fresh sterile glass-distilled water and germination studied at 25°C under 100% relative humidity after 16 hours of incubation. A control (D) was set up with unwashed conidia.

The results (Table 3) showed that conidia had germinated in all the four treatments (A, B, C and D) but repeated washings of the conidia induced a marked decrease in germination. After one washing there was a slight decrease in germination (Col. A). The affect was greater after two washings (Col. B) and still greater after three washings (Col. C). Germination in all cases was, however, normal with frequent appressorial formation in isolate Nos 78, 244, 300, 301, 304, 357, 390 and strain "I".

Germination of washed conidia in double distilled and tap water

Experiments with double distilled water showed that the percentage of germination as well as the length of germ tube decreased considerably in all the ten isolates. On the other hand, a stimulatory effect of tap water was observed (Table 4); the increase being both on the percentage of germination and the length of the germ tube. The type of germination in double distilled water and in tap water was, however, similar to that observed in single distilled water.

Table 4

Germination of washed conidia of the ten *C. falcatum* isolates in double distilled and tap water

Isolate No.	Double distilled water		Tap water	
	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)
244	4.8	65.0	22.4	91.0
357	3.3	41.1	21.2	74.7
"I"	14.4	39.0	38.7	48.7
390	9.5	56.3	28.8	60.0
301	7.0	60.6	28.8	78.5
304	11.7	76.1	36.5	86.1
382	1.9	41.1	17.5	50.3
78	25.4	84.5	56.8	99.1
300	23.3	69.3	62.4	71.5
7	19.3	49.8	52.0	53.6

Germination in hanging drops in cavity slides and in "Van Tieghem cells"

Experiments with the "hanging drop method" were conducted on cavity slides and in "Van Tieghem cells". In the former case, drops (0.03 ml) of the standard conidial suspensions (10–20 washed spores per microscopic field) of 12–20 days old cultures of the ten isolates were placed in the centre of acid-cleaned, dry coverslips. The coverslip was then inverted over a clean cavity of a cavity-slide, care being taken in not allowing the drop to spread while inverting the coverslip. These cavity slides, alongwith the inverted coverslips, were incubated at 25°C in high humidity for 16 hours.

The results (Table 5) showed that germination did take place, but the data varied considerably between germination drops. This indicated that the method

Table 5

Germination of washed conidia of the ten *C. falcatum* isolates in hanging drops in cavity slides

Isolate No.	Average germination (%)					Average germ tube length (μ)				
	R_1	R_2	R_3	R_4	R_5	R_1	R_2	R_3	R_4	R_5
244	4.5	1.6	17.7	24.3	3.2	72.5	65.0	78.0	52.0	66.8
357	11.0	2.8	10.4	5.6	0	56.8	54.1	56.3	49.8	—
"I"	17.6	13.1	8.6	7.8	2.8	28.1	39.0	30.3	39.0	34.6
390	11.4	17.6	3.7	16.2	9.4	54.1	56.8	45.5	56.8	39.0
301	19.2	6.4	24.6	3.5	14.4	63.2	39.0	56.3	42.2	50.6
304	20.8	4.9	11.2	7.4	6.7	69.3	33.2	44.6	52.0	34.8
382	2.7	1.4	5.6	8.7	0.3	23.0	41.1	34.6	28.4	—
78	44.1	21.7	12.2	9.7	19.3	47.6	65.0	56.0	53.4	45.5
300	22.2	31.2	4.5	11.6	7.8	53.6	65.0	52.0	47.6	58.5
7	34.1	21.9	14.6	33.0	9.2	49.8	30.3	41.1	30.8	54.1

(R_1 to R_5 are the repetitions of the experiment.)

is not reliable because the results obtained are not consistent. Also, the germination was reduced considerably in most drops and in most isolates, the effect was more pronounced on the percentage of germination than on the length of germ tube.

Similar erratic results were also obtained through germination in Van Tieghem cells. Germination was reduced still more, both in the percentage of germination and the length of the germ tube.

Study of physical factors

Physical factors influencing germination included temperature of incubation for germinating conidia, age of the culture, incubation period, pH, spore concentration, aeriation (oxygen and carbon dioxide), and light. The technique followed to study their effect has been described earlier in the germination of washed conidia in suspension on plain glass slides.

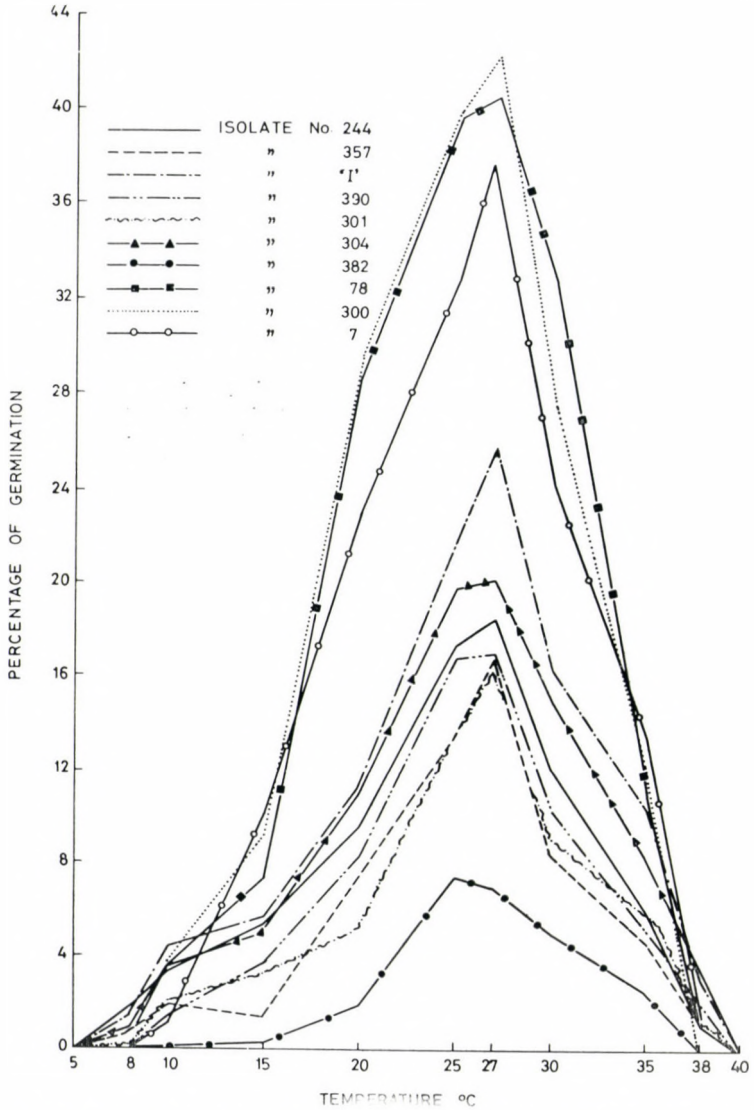

Fig. 1. Effect of temperature of incubation on the percentage of germination of spores in the ten isolates of *C. falcatum*

Temperature: The temperature requirements for germination of conidia of the ten isolates of *C. falcatum* was studied at temperatures ranging from 5°C to 41°C. It was found that the range of temperature required was wide and the three cardinals were as follows: Minimum, 7°C to 8°C; Optimum, 26°C to 27°C; Maximum, 38°C to 39°C. Optimum temperature for germination was between

26°C to 27°C; there was no germination at or below 5°C. Although germination in most isolates was initiated at 8°C, it was still poor at 10°C. With a rise in temperature from 10°C to 26°C, both the percentage of germination and the length of the germ tube showed a gradual increase, after which there was a sharp decline in germination. At or beyond 40°C there was no germination (Fig. 1). Response of the light and the dark type isolates was similar.

At the lower and the higher temperatures, germination was mostly unipolar with short germ tubes. However, germ tubes were well developed and often bipolar at the optimum temperature (27°C). Appressorium formation was abundant in most isolates (Nos 78, 244, 300, 301, 304, 357, 390 and strain "I") between 15°C to 26°C. There were not appressoria at 8°C or 10°C, very few at 12°C. There was a sharp decline in appressorium formation beyond 26°C and at 35°C there was not observed any appressorium in these isolates. The inhibitory effect on appressorium formation was more on the light type virulent isolates (Nos 244, 301, 304, 357) than on the dark type weakly pathogenic ones (Nos 7, 78, 300).

Age of culture: Optimum maturity of conidia of the ten isolates of *C. falcatum* was determined by setting up a germination test at 27°C using conidia of different ages, i.e. 6, 8, 12, 16, 20, 24, 28, 32 and 36 days old and the data obtained is presented in Fig. 2.

Conidia from 12–16 days old cultures showed the highest germination; however, conidia from 6 and 36 days old cultures showed the least germination. The percentage of germination as well as the length of the germ tube increased steadily with the increase in age from 6 to 16 days, beyond which the germination declined uptil the age of 36 days. The immature spores from 6 days or less old cultures either did not germinate or showed very poor germination; the same was true of the very old or the over mature spores (from 36 days or more old cultures). Isolate Nos 7, 304 and strain "I" showed maximum germination when their cultures were 12 days old. However, the other isolates showed maximum germination when their cultures were 16 days old. Conidia from 32 days old cultures of the dark type isolates (Nos 7, 78 and 300) did not show any germination at all. The light type isolates, however, retained their germination potential uptil the age of 36 days. Isolate No. 382 had the shortest viable phase, i.e. it initiated the germination as a 6 days old culture, reached a climax when it was 16 days old and showed no germination when the culture was 28 days old. Isolate No. 244 showed no germination when it was 32 days or more old.

The response in all the isolates was similar. Germination was normal, mostly unipolar, with frequent appressorial formation in most isolates when at optimum maturity (12–16 days old). Appressoria were absent in the younger cultures; the older cultures showed more appressorial formation than cultures of optimum maturity.

Incubation period: Different incubation periods tried for conidial germination were 6, 12, 18, 24, 36 and 48 hours. Conidial suspensions from 12 to 16 days old cultures of the ten isolates of *C. falcatum* were incubated at 27°C for these six

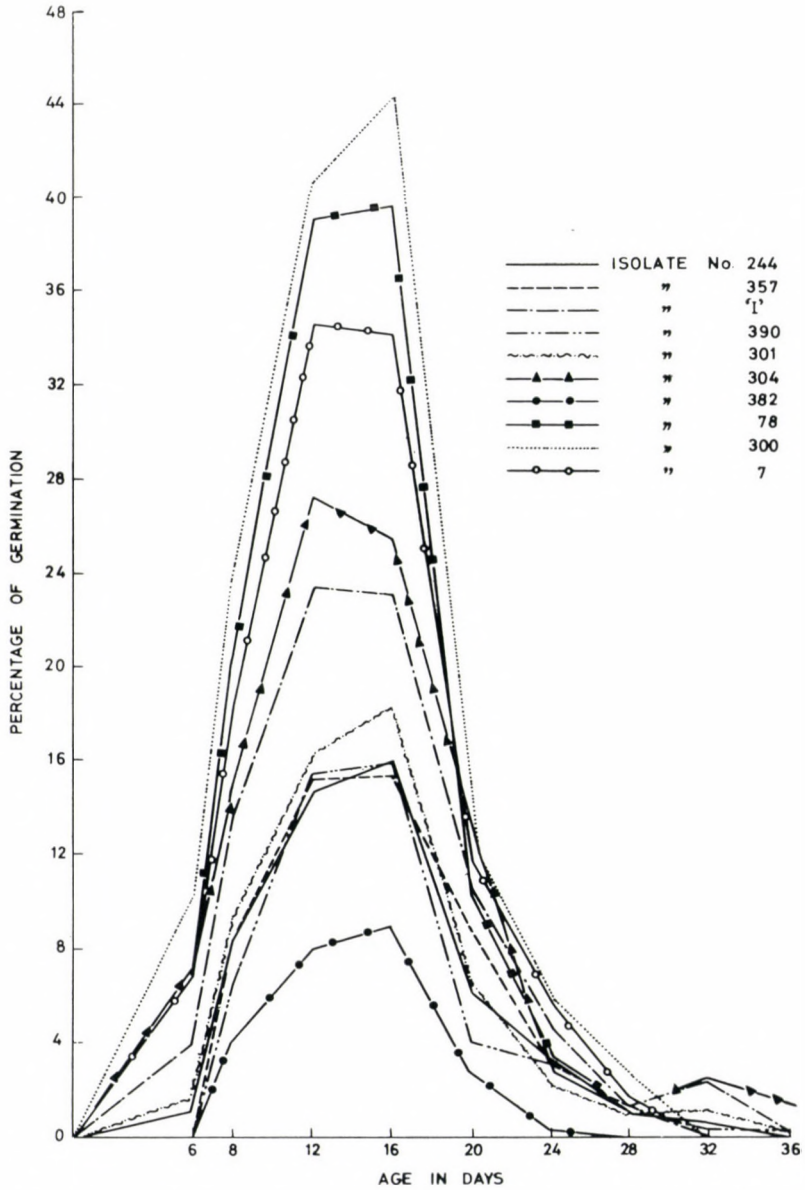

Fig. 2. Effect of age of culture on the germination of spores in the ten isolates of *C. falcatum*

incubation periods. Data obtained (Tables 6a and b) showed that the range of incubation period for germination of conidia was between 6 to 48 hours. Germination of most light type isolates initiated after 6 hours and that of the dark type isolates after 6 to 8 hours. Optimum germination of the light type isolates was obtained after 18 hours and that of the dark type ones after 24 hours. In the former case, there was also noticed a slight increase in the germination percentage from 18 hours to 24 hours, but there was no such increase after 24 hours in any of

Table 6(a)

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water at 27°C after different periods of incubation

Isolate No.	Average germination (%)					
	6 hours	12 hours	18 hours	24 hours	36 hours	48 hours
244	1.4	9.7	16.6	17.4	17.2	17.9
357	1.2	10.7	14.7	15.9	15.6	15.6
"I"	2.2	18.2	23.6	24.1	25.2	24.4
390	0.3	12.8	16.1	16.2	17.6	18.6
301	0.6	9.8	14.7	15.1	15.9	15.3
304	1.0	20.4	26.3	28.3	27.6	26.5
382	0	5.5	8.1	9.5	9.9	10.1
78	0	33.8	42.8	44.1	44.0	43.3
300	0	36.3	44.8	45.1	45.3	44.5
7	0	25.2	36.4	37.1	37.8	38.3

Isolate No.	Average germ tube length (μ)					
	6 hours	12 hours	18 hours	24 hours	36 hours	48 hours
244	19.5	52.0	71.5	117.0	Above 266.5	∞
357	35.7	55.7	61.7	178.7	Above 253.5	∞
"I"	16.2	26.0	39.0	120.2	214.5	∞
390	9.7	42.2	52.0	143.0	211.2	∞
301	13.0	61.7	78.0	214.5	∞	∞
304	26.0	61.7	84.5	242.0	∞	∞
382	0	32.5	48.7	81.2	97.5	∞
78	0	71.5	91.0	∞	∞	∞
300	0	65.0	81.2	∞	∞	∞
7	0	45.5	58.5	∞	∞	∞

these isolates, although the length of the germ tube continued to increase indefinitely. When the germ tube length reached 300 μ or more, the intertwined and could not be measured and has designated by the infinity sign (∞) in the tables.

Germination was normal, mostly unipolar with frequent appressorial formation after 12 hours' incubation in most isolates. No appressoria were formed before 8 hours' incubation. After 6 hours' incubation the germ tubes were just

protuberances and too small to be measured. However, after 36 hours' incubation, the germ tubes were more than 300 μ, very long and intertwined and could not be measured.

Spore concentration: During these investigations, it was often observed that highly concentrated spore suspensions gave poor or no germination as compared

Table 6(b)

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water at 27°C after different incubation periods

Isolate No.	Average germination (%)				
	12 hours	14 hours	16 hours	18 hours	20 hours
244	10.7	12.9	13.9	16.5	15.0
357	9.8	11.2	12.9	14.6	14.0
"I"	20.6	21.9	24.2	25.4	24.5
390	13.8	15.7	16.8	17.9	18.3
301	13.2	13.8	16.4	17.3	17.3
304	19.1	20.9	23.8	25.4	24.6
382	6.5	8.0	9.3	10.4	1.07
78	34.5	35.1	38.1	41.9	41.5
300	37.6	41.8	46.0	47.5	46.0
7	27.5	31.6	36.7	40.9	41.2

Isolate No.	Average tube length (μ)				
	12 hours	14 hours	16 hours	18 hours	20 hours
244	48.7	61.7	71.5	87.7	91.0
357	45.5	58.5	78.0	81.2	87.7
"I"	22.7	29.2	39.0	45.5	58.5
390	45.5	48.7	52.0	58.5	61.7
301	71.5	71.5	78.0	78.0	84.5
304	71.5	81.2	84.5	87.7	97.5
382	35.7	42.2	45.5	52.0	55.2
78	71.5	81.2	91.0	104.0	113.7
300	65.0	78.0	71.5	78.0	94.2
7	42.2	48.7	48.7	55.2	61.7

to the less concentrated suspensions. Thus standardization of conidial suspension to a concentration which would give optimum germination became imperative. Suspensions of washed conidia of the ten isolates of *C. falcatum* were prepared in sterile distilled water in seven different concentrations: A (120,000 spores/cmm), B (250,000 spores/cmm), C (500,000 spores/cmm), D (1,000,000 spores/cmm), E (2,000,000 spores/cmm), F (4,000,000 spores/cmm), G (8,000,000 spores/cmm) by

means of a haemocytometer (KOLMER and BOERNER, 1945) and the germination studied after 18 hours of incubation at 27°C.

The results (Table 7) showed that maximum germination occurred in column C with a spore concentration of about 500,000 spores/cmm (= 15 to 20 spores per microscopic field under the low power of microscope). However, till that optima reached there was a gradual increase in germination as seen in columns A to B and B to C of the Table. The effect was more pronounced on germination percentage than on the germ tube length. Further increase in the concentration of spores beyond the optima, i.e. from column C to D resulted in a sudden decrease

Table 7

Germination of washed conidia of the ten *C. falcatum* isolates in seven different concentrations of conidial suspension in distilled water

Isolate No.	Average germination (%)							Average germ tube length (μ)						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G
244	5.0	12.1	16.0	9.4	6.4	0	0	52.0	58.5	69.3	56.3	—	0	0
357	8.7	13.6	17.6	8.8	5.0	0	0	41.1	54.1	67.1	49.8	—	0	0
"I"	9.7	19.8	25.8	13.4	9.0	2.3	0	28.1	39.0	41.1	30.3	6.5	2.1	0
390	5.3	13.3	16.0	11.3	6.8	0	0	30.3	54.1	56.3	45.5	8.7	0	0
301	6.8	10.8	18.1	14.2	10.9	0.4	0	39.0	60.6	65.0	47.2	10.8	—	0
304	8.2	17.9	28.8	15.8	9.7	3.8	0	56.3	80.1	80.1	58.5	8.7	—	0
382	0.7	4.5	7.1	3.3	1.5	0	0	41.1	49.8	47.6	34.6	—	0	0
78	19.4	31.3	39.4	23.7	15.5	5.2	0	84.5	91.0	93.1	73.6	13.0	6.5	0
300	20.5	39.5	44.4	22.3	16.7	2.6	0	80.1	80.1	82.3	58.5	15.2	2.1	0
7	15.5	31.7	34.7	16.5	7.7	0	0	49.8	54.1	54.1	47.6	2.1	0	0

(— signifies germ tube length less than 6.5 μ)

in the percentage of germination as well as the length of the germ tube. In column E still a further decrease in germination was observed. In column F there was no germination in five of the ten isolates; the other five isolates showed only slight germination at times. In column G, there was no germination at all in any of the ten isolates.

The germination was normal, mostly unipolar in the columns B, C and D with abundant appressoria in most isolates. In columns E and F the appressoria were formed in only a few germinated conidia and the germ tubes were very short. In column A, almost every germinated conidium produced an appressorium.

Hydrogen-ion concentration: The effect of hydrogen-ion concentration on the germination of conidia was studied by using Sorensen's phosphate buffer mixture (CLARK, 1928), adjusted to pH levels, ranging from 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0. Conidial suspensions of the ten isolates were prepared in buffer solutions

at these pH levels and incubated at 27°C for 18 hours' incubation. A control was run alongwith in distilled water with pH of 6.1. The results obtained are presented in Fig. 3.

Like many fungi, pH requirements for the germination of conidia of *C. falcatum* were acidophilic, although the process of germination did extend into the

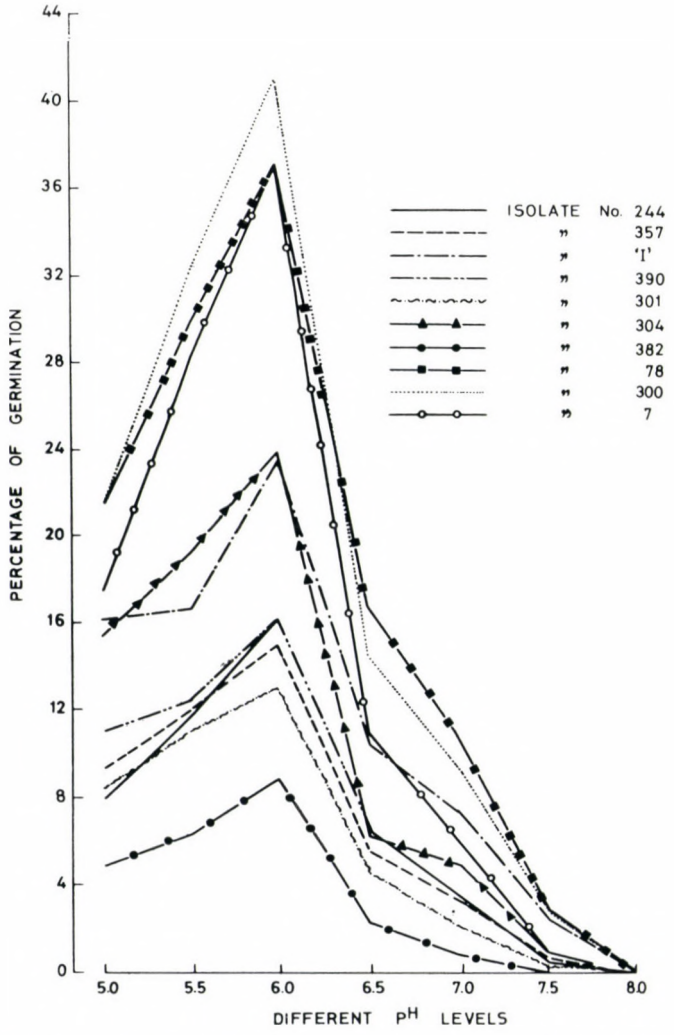

Fig. 3. Effect of different pH levels on the percentage of germination of spores in the ten isolates of *C. falcatum*

alkaline range of pH (7.5). Optimum pH for the percentage of germination and the length of the germ tube was between 5.5 to 6.5 (around 6.0). Germination started at a pH lower than 5.0 and increased gradually with the rise in pH from 5.0 to 6.0. At pH 6.5 it started declining and continued decreasing uptil a pH of 8.0 when there was not germination at all in any of the isolates. At pH 7.5, germination was very poor; isolate No. 382 did not show any germination at all at this pH. On the whole the pH range was narrow.

Table 8

Germination of washed conidia of the ten *C. falcatum* isolates in vacuum and in air devoid of oxygen

Isolate No.	Average germination (%)		Average germ tube length (μ)	
	In vacuum	In air devoid of oxygen	In vacuum	In air devoid of oxygen
244	0	1.5	—	—
357	0	0.7	—	6.5
"I"	0.1	0	—	8.7
390	0	3.8	8.7	10.8
301	0.4	1.5	10.8	10.8
304	0	0	0	0
382	0.3	3.3	6.5	10.8
78	1.0	1.5	2.1	9.7
300	1.4	0.6	—	13.0
7	0	1.2	0	8.7

(— signifies germ tube less than 6.5 μ)

Germination was normal, mostly unipolar; the appressorial formation was most abundant at pH 6.0 and 6.5, few appressoria were produced at the lower or the higher pH levels. Appressoria were almost absent at pH 7.5. The increase and decrease in the length of the germ tube was concordant with the rise and fall of the percentage of germination.

Oxygen: Investigations conducted in vacuum and in air devoid of oxygen* have shown that germination of conidia in distilled water was reduced considerably, in some cases to almost 0% (Table 8). This small amount of germination was perhaps due to the presence of the oxygen dissolved in distilled water used for making the spore suspension. To deplete the distilled water of this dissolved

* Freshly prepared "Berthelot's" solution containing equal volumes of pyrogallol solution (1 part of pyrogallol in 3 parts of water) and potassium hydroxide solution (1 part of potassium hydroxide in 3 parts of water), was used to remove oxygen from the air.

oxygen and nullify its influence on conidial germination the distilled water was boiled for one-half hour. The boiled distilled water was then cooled suddenly and divided into three parts (A, B, C). Part A was tested as such for germination. Fresh air was blown in for 10 minutes in part B by means of a pump and in part C, air was blown in for a longer time of about 30 minutes. Germination test for the conidia of the ten isolates was set up in the three parts (A, B and C) of the boiled distilled water; a control was also set up with unboiled, distilled water. This experiment was conducted in normal atmosphere.

Table 9

Germination of washed conidia of the ten *C. falcatum* isolates in boiled distilled water

Isolate No.	Average germination (%)				Average germ tube length (μ)			
	A	B	C	Control	A	B	C	Control
244	9.4	14.8	7.5	16.7	15.1	73.6	49.8	71.5
357	8.8	13.3	5.8	14.8	21.6	60.6	49.8	62.8
"I"	12.6	19.6	9.6	24.5	13.0	36.8	23.8	34.6
390	8.2	14.5	4.5	16.9	26.0	49.8	41.1	49.8
301	7.1	13.2	6.1	15.9	23.5	58.5	41.1	65.0
304	12.3	19.4	11.3	21.9	34.3	67.1	52.0	75.8
382	3.6	6.7	2.1	7.8	17.3	47.6	34.6	49.8
78	20.2	34.4	16.7	38.5	52.0	82.3	69.3	88.8
300	20.8	32.7	15.3	42.1	52.0	69.3	65.0	75.8
7	14.9	27.7	12.6	34.3	39.0	49.8	45.5	56.3

Germination percentage and the germ tube length was greatly reduced (3.6% to 20.8% and 13.0 μ to 52.0 μ respectively) in the water boiled for $^1/_2$ hour. (Table 9, Col. A); almost every germinated conidium produced an appressorium In Column B of the Table, the germination improved (6.7% to 34.4% and 36.8 μ to 82.3μ). However, in Column C, there was again a decline in the germination (2.1% to 16.7%); the germ tubes were also short (23.8 μ to 69.3 μ) and wavy. Appressoria were abundant, but of lighter color and tinner walls.

This experiment with boiled distilled water was also repeated in vacuum and in air devoid of oxygen; no germination was obtained at all in any isolate.

Carbon dioxide: Carbon dioxide has also been found necessary for conidial germination in *C. falcatum*. No germination was observed in atmosphere depleted of carbon dioxide. The latter was done by passing the air through 10% potassium hydroxide solution before supplying it to the germinating conidia. No detailed investigations were conducted on the effect of different concentrations of carbon dioxide on conidial germination and appressorium formation.

Light: Germination of conidia was studied in light and in complete dark at 27°C. In the former case, a 20 watts incandescent tungsten filament bulb (frosted)

was used. In the latter case thick black paper was wrapped round the Petri dishes, containing slides of conidial suspension drops for germination, during incubation.

Darkness was found to be detrimental and light stimulatory to germination (Table 10). The effect in either case was more pronounced on the length of the germ tube. The response of both the light and the dark type isolates was similar.

It has also been observed that strong light of 200 watts (frosted as well as unfrosted bulbs) is inhibitory to germination percentage and germ tube length of the conidia (Table 11).

Table 10

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water at 27°C in light and dark

Isolate No.	Average germination (%)		Average germ tube (μ)	
	In light	In dark	In light	In dark
244	16.4	10.4	214.5	45.5
357	15.3	12.8	190.6	39.0
"I"	32.8	26.9	134.0	23.8
390	15.6	13.7	173.1	32.5
301	17.9	14.7	182.0	43.3
304	24.6	21.4	179.8	36.8
382	6.9	6.9	147.3	21.6
78	41.8	37.2	149.5	54.0
300	41.0	37.0	153.6	43.3
7	37.1	34.8	106.1	38.6

Table 11

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water at 27°C in high and low light intensity

Isolate No.	Average germination (%)		Average germ tube length	
	In strong light	In low diffused light	In strong light	In low diffused light
244	11.2	15.2	34.6	117.0
357	11.5	16.2	43.3	112.6
"I"	18.6	22.9	14.0	88.8
390	9.7	15.2	28.1	179.8
301	10.7	16.6	43.3	175.5
304	17.3	26.8	49.8	173.3
382	6.6	8.4	36.8	149.5
78	38.4	41.2	49.8	138.6
300	35.4	43.3	49.8	117.0
7	30.9	37.0	30.3	106.1

Appressorium formation was similar in light and dark in most isolates. However, abundant appressoria were observed in strong light than in low and diffused light in most isolates.

Discussion on the factors and standardization of technique

Physiology of spore germination has been under investigation during the past 20 years and some excellent reviews on the subject have been published (GOTTLIEB, 1950; COCHRANE, 1958; ALLEN, 1965; SUSSMAN, 1965; SUSSMAN and HALVORSON, 1966; SKOROPAD, 1967).

In *C. falcatum* germination of conidia has been of great interest and investigations have revealed that it in influenced by several physical and nutritional factors (SINGH, 1962). Conidia show a positive tropism towards water and require a direct contact with it, besides a high humidity of 99–100% DORAN (1922), WALKER (1957), SCHNATHORST (1965) and BURNETT (1968) made similar observations in downy mildews. SUSSMAN and HALVORSON (1966) attributed this absolute requirement for water for the hydration of colloids in the spores, for several cases exist in which endogenous inhibitors must first be removed before germination can proceed. Under these conditions water might be required as a means through which the inhibitor can be dissipated. Further, it has been observed that the percentage of germination is lower in the light type isolates than in dark type isolates. There is indicated an inverse relationship between the germination percentage, some cultural characters and the virulence of an isolate. The light type highly sporing isolates which are also highly virulent and are with densely granular conidia exhibited a low percentage of germination, while the dark type sparsely sporing isolates which are weakly pathogenic and have hyaline spores, showed a comparatively higher percentage of germination. A similar relationship between germination and morphological characteristics of conidia and virulence of an isolate in *C. falcatum* has also been reported by VASUDEVA, CHONA and SRIVASTAVA (1953), and BAJAJ, GANJU and CHATRATH (1959).

Temperature is another important physical factor influencing germination; in certain cases it even determines the method of germination including appressoria formation (DORAN, 1922; TOGASHI, 1949; LILLY and BARNETT, 1951; COCHRANE, 1958; SUSSMAN, 1965). The three cardinals for the germination of conidia of *C. falcatum* were: minimum of 7°C to 8°C, optimum of 26°C to 27°C and maximum of 38°C to 39°C; thus the range of temperature requirement was wide. The unidirectional response of all the isolates indicated similar physiological requirements. It seems that most conidia had the capacity to germinate and the ability to differentiate into appressoria. However, the latter was lost at 35°C. Appressoria formation was more abundant at the lower temperatures than at the higher temperatures within the range of temperature required for germination. Similar observations were made by ISHIDA and AKAI (1969) in *Colletotrichum lagenarium*, RAHE and KUĆ (1970) in *C. lindemuthianum*, and MEHL and LUKEZIC (1972) in

C. trifolii. The results also showed that although the general temperature requirements for germination and appressoria formation were similar, optimum temperature range for conidial germination was wider than that for appressoria formation. The ability of conidia to form appressoria was more sensitive to temperature than their capacity to germinate. Besides, the inhibitory effect of temperature on appressorial formation was greater on the light type virulent isolates than on the dark type weakly pathogenic isolates of the fungus. These results apparently support VAN DER PLANK's hypothesis (1968).

The age of a culture influences germination and its technique through the period of maturity of the spores and the phase of optimum maturity varies between fungi and between spores. Young and old spores often show poor or no germination (GILBERT, 1929; BURGERT, 1934; LOWTHER, 1950). This may be due to immaturity in the former case and over-aging and senescence in the latter (GOTTLIEB, 1950). In *C. falcatum*, conidia from 12 to 16 days old cultures showed maximum germination while those from 6 and 36 days old cultures showed the least germination. Conidia of the dark type weakly pathogenic isolates showed germination for a short duration of time as compared to the light type highly virulent isolates, i.e. the light type virulent isolates started their germination earlier and retained their potential to germinate for a longer period. This may account for their greater infectivity period and better survival as compared to the dark type weakly pathogenic isolates (JONES, 1919; NOBLE, 1924; GUBA, 1925; KOHL, 1932; LILLY and BARNETT, 1951).

Optimum incubation period for the germination of conidia of most isolates of *C. falcatum* was found to be about 18 hours, beyond which only the length of the germ tube increased. However, the range of the incubation period was from 6 to 48 hours. VASUDEVA, BAJAJ and KHOSLA (1961) reported 16 hours at the optimum incubation period in one isolate of *C. falcatum*.

SUSSMAN and HALVORSON (1966) remarked that germination of spores is highly sensitive to pH effects and the optimum pH requirement for germination varies between fungi and between germinating media; at times germ tube elongation is more sensitive to pH than germ tube initiation (MATHRE and RAVENSCRAFT, 1966). The pH requirements for conidial germination in *C. falcatum*, like most fungi, were acidophilic, although germination did extend into the alkaline range. WEBB (1921) and KAUFMAN (1934) made similar observations in *Colletotrichum gossypii* and in certain basidiomycetes. The pH range for germination in *C. falcatum* varied from 5.0 to 8.0; the optimum pH level being around 6.0. The narrow pH range requirement indicated the importance of the factor in the germination process (LILLY and BARNETT, 1951).

Light does not seem to be an important factor in the germination of conidia of *C. falcatum*, although high intensity of light and complete darkness were inhibitory. Low and diffused light were stimulatory, more so for the growth of the germ tube.

The requirement for oxygen and carbon dioxide for germination of conidia

of *C. falcatum* was also found necessary, LILLY and BARNETT (1951), and SUSSMAN and HALVORSON (1966) remarked that since respiration is greatly accelerated during spore germination, an adequate supply of oxygen is a pre-requisite for germination. Germination of conidia in all isolates of *C. falcatum* was aerobic and it seems that oxygen consumption took place both from the atmosphere over the germination drop and from within the drop; in the latter case the oxygen is present in a dissolved state in the distilled water. Little or no germination was observed in vacuum or in atmosphere devoid of oxygen. Germination was also reduced considerably in suspensions made out of distilled water which had been boiled previously. Longer the boiling, more is the depletion of the dissolved oxygen and lesser is the germination. This observation was confirmed by an increase in germination in the boiled distilled water in which fresh air was blown in for some time. The requirement for oxygen was also indirectly indicated by a reduced germination in highly concentrated spore suspensions and good germination in diluted conidial suspensions. One of the reasons assumed for this was a lesser amount of available oxygen to a large number of germinating spores both from within the suspension drop and from the atmosphere outside the drop. Similar results have also been reported by SCHÜTT (1971), and PASS and GRIFFIN (1972) in *Scleroderris lagerbergii*, *Fusarium oxysporum*, *Lophodermium pinastri*, *Rhytisma acerinum* and *Aspergillus flavus*. SUSSMAN and HALVORSON (1966) remarked that competition for limited oxygen and nutrients, and release of some inhibitory substance by spores could explain for poor germination in highly concentrated spore suspensions.

It was also noted that spread of the germination drop into a thin film improved the germination considerably provided the film does not dry up. The latter was studied by an experiment where the germination drops were spread to lengths varying from 1 – 3 cm. An average of 5% – 54% increase in germination was observed in the drops spread to a length of 1.0 cm. The stimulation was mostly in the percentage of germination. In some replicates, the germination drops dried and hence no data could be obtained. In the drops spread to 2 – 3 cm length, no data could be obtained because in most slides the germination drops had dried completely or partially.

It has also been observed in some drops that submerged spores or overcrowded spores failed to germinate while spores on or near the surface of the drop germinated rapidly. This is assumed to be due to the availability of a greater amount of oxygen at or near the surface of the drop. Similar results were also obtained by DORAN (1922), EDGERTON (1958), and GOULD and SHAW (1969) in several other fungi.

Germination was better and fairly consistent in single distilled water than in double distilled or tap water; PASS and GRIFFIN (1972) have also reported little or no germination of washed conidia of *Aspergillus flavus* in double distilled water.

In view of the reduced germination and inconsistent results obtained in cavity slides and "Van Tieghen cells", plain glass slides seemed to be the most

satisfactory choice. At times, results obtained from plain glass slides were also inconsistent. In such cases, the suspension drops were of unequal size and shape which could be due to unequal volume of the drop; unequal spread of the drop either into an irregularly shaped drop or a thin film, the latter could also be due to unclean slides. The size of the germination drop can be kept constant by using the same volume (0.03 ml) of the conidial suspension in all experiments; the unequal spread of the drop can be avoided by using grease-free, acid-cleaned, dry plain glass slides, and by the use of a standardized technique.

To obtain a uniform size of the drop, an attempt was made to use ringed plain glass slides. Rings* or circles* of 1.0 cm, 1.5 cm, 2.0 cm and 2.5 cm diameter were marked on clean plain glass slides with the aid of several materials, including a glass marking pencil, vaseline, wax, collodion, canada balsam, euparol and 'cutex' nail polish (M/s Northam Warren Ltd., London). Equal volumes (0.03 ml) of the standardized conidial suspensions in distilled water were deposited and spread to cover each circle. No germination was obtained at all on slides ringed with wax, collodion, canada balsam and euparol. However, some germination was observed at times in a few isolates on slides ringed with glass marking pencil, but this device appeared crude because the markings by the pencil would peel off and float on the germination drops after a few hours, thus affecting the germination of conidia and giving erratic results. A highly stimulated germination was observed on slides ringed with vaseline and 'cutex' nail polish.

Maximum germination on slides ringed with 'cutex' was observed in rings 2.5 cm in diameter; however, on these slides the spore drops often receded or vanished because of partial or complete drying during 18 hours' incubation, resulting in little or no germination at all. Germination on 'cutex' nail polish-coated slides** also showed the presence of a similar stimulatory effect both in spread and unspread germination drops (Table 12).

From these investigations it appears that there is a definite stimulatory effect of the 'cutex' nail polish which evidently was not taken into account by VASUDEVA, BAJAJ and KHOSLA (1961) who recommended ringing of glass slides with this nail polish to study germination of conidia in *C. falcatum*. It appears that a high percentage of germination in isolate No. 244 as reported by VASUDEVA et al. (1961) is the result of stimulation provided by the nail polish as well as by the spread of the germination drop into a thin film. Strictly speaking a drop never remains a drop when it is spread, it becomes a thin film. The present investigations do agree with the findings of VASUDEVA et al. (1961) in that increased aeriation by spreading the drop or by direct aeriation of the distilled water stimulates germination.

* Ringing with 'cutex' nail polish, collodion, euparol, vaseline, wax and canada balsam was done with the help of a slide ringing table; rings of 'cutex', collodion, euparol and canada balsam were made 24 hours before use to allow enough time for drying. Once ringed slides can be conveniently used for 4—6 experiments.

** The 'cutex'-coated slides were dried for 24 hours before use.

Table 12

Germination of washed conidia of the ten *C. falcatum* isolates in distilled water on 'cutex' nail polish coated and ringed slides

Isolate No.	'Cutex'-coated slides		'Cutex'-ringed slides		Control	
	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)	Avg. germ. (%)	Avg. germ. tube length (μ)
244	34.7	76.3	25.0	84.5	14.2	66.6
357	25.8	63.3	22.0	76.3	11.0	65.0
"I"	42.3	43.8	41.4	50.3	22.2	35.7
390	26.3	53.6	33.9	56.3	16.1	50.3
301	26.8	63.3	23.2	73.3	12.4	61.7
304	34.2	72.5	35.9	79.6	21.5	66.8
382	17.4	40.6	16.3	48.7	7.6	45.5
78	56.3	84.5	51.4	94.2	36.7	84.5
300	67.2	71.5	58.5	75.0	39.1	61.7
7	51.5	48.7	53.6	56.8	34.6	50.3

The above discussion on the physical factors and standardization of technique indicates that after determining the optimum physical conditions of moisture, temperature, incubation period, age of culture, spore concentration, pH, etc., a certain technique has to be developed. Plain glass slides which are not ringed, but a grease-free, acid-cleaned and dry are by far the most satisfactory substrate with a constant volume and size of the drop of the standardized conidial suspension. This would give a fairly uniform spread and depth to the germination drop in all experiments, thus giving possibly the most consistent and reliable results under the laboratory conditions without any kind of stimulation being provided by ringing material or by unnatural spread of the drop.

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Effects of Cotoran (1,1-Dimethyl-3-α,α,α-Trifluoro-*m* Tolyl Urea) on the Growth of *Aspergillus flavus* in Soil and Liquid Culture

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The effect of the herbicide Cotoran (1,1-Dimethyl-3-α,α,α-trifluoro-*m*-tolyl urea), on growth of *Aspergillus flavus* was studied in soil and liquid cultures. In liquid cultures, growth of *A. flavus* was revealed by increased mycelial dry weight, relating to increased concentration of the herbicide. Total CO_2 production by the fungus in flasks of herbicide treated soil increased with time at concentrations of 1, 5, 10 and 20 μg per g of soil. Utilization of inorganic phosphorous was not significantly affected by the concentrations of the herbicide. pH values decreased with increasing concentration of herbicide.

Aspergillus flavus is a serious field and a storage pathogen of peanuts and cotton. The pod rot in peanuts and ball rot in cotton are produced by this fungus. *A. flavus* can survive in soil for years in the absence of host by producing spores.

Increased applications of herbicides to agricultural soils are a part of the rapid development of weed control in the past decade. Each year all over the world, especially in the United States, many millions of acres of crops are treated with herbicides. Extensive study has been made on the effects of herbicides on the physiological processes of higher plants whereas very little is known regarding their interactions with soil borne fungi. The effect of Cotoran (1,1-dimethyl-3-α,α,α-trifluoro-*m* tolyl urea) on the growth responses of *A. flavus* was investigated in soil and liquid culture.

Materials and Methods

Soil samples used throughout this investigation were collected from Prairie View Experiment Station Farm from a depth of six inches and two feet apart. Soil was air dried for 24 hours, screened, and thoroughly mixed. Technical grade cotoran was obtained from CIBA Agro-Chemical Company. A small quantity of herbicide was dissolved in 10 ml acetone and desired quantity was added to 200 g of soil and thoroughly mixed. Desired quantities from the above stock soil were further mixed with natural air-dried soil to provide concentrations of 1, 5, 10 and 20 ppm of herbicide plus a herbicide free check. The above soils were placed in

250 ml beakers covered with aluminium foil paper pierced with few holes. These were incubated at 25°C for 7 days, after which populations of fungi and bacteria were estimated by a modification of the syringe method of RODRIGUEZ-KABANA (1967). In another experiment 100 g (oven-dry basis) of soil were placed in each of six Erlenmeyer flasks with fitted (No. 6) rubber stoppers that had two holes in their short pieces of glass tubing were inserted with cotton lint loosely packed. The flasks thus prepared were autoclaved for one hour for three successive days. The inoculom was prepared by placing eight 4 mm mycelial discs from a young culture of *A. flavus* in a sterilized Monel semi-micro blender with 40 ml of sterile water and blended for 30 seconds. This suspension was then transferred from the blender into a flask containing 160 ml of sterile water. With a wide mouth pipette, two ml of this suspension were then aspetically added to each flask across the line, except the non-inoculated check. The flasks were incubated at 25°C for 48 hours.

At the end of incubation period, 10 ml of nutrient solution containing 200 ppm phosphorus as K_2HPO_4, 800 ppm of carbon as glucose, 120 ppm nitrogen as $NaNO_3$, plus the required concentrations of Cotoran from the stock solution. The soil moisture after application was 17% in each flask. The flasks were then connected to a vacuum assembly at random. Each concentration was triplicated and experiment was repeated.

The carbon dioxide production was determined at intervals of 3, 6 and 9 days. The CO_2 evolved was trapped and analyzed as described in RODRIGUEZ-KABANA et al. (1967). Phosphorus and pH were measured at the end of incubation period. Inorganic phosphorus was determined by the Vandaomolybdophosphoric yellow color method described by JACKSON (1958).

Effect of different concentrations of Cotoran on mycelial growth of *A. flavus* was also studied in modified Czapek's solution. A stock solution of Cotoran was prepared in acetone and desired quantities of this were added into 25 ml of Czapek's solution to provide 1, 5, 10 and 20 ppm concentrations of Cotoran plus a herbicide free check. This Czapek's solution was filtered sterilized. The solution in each flask was inoculated axenically with 4 mm mycelial disc from 72 hours old culture of *A. flavus*. The inoculated flasks were incubated at 25°C and fungal crop was harvested at the end of 9 days incubation period and the average dry weight of the mycelium was calculated.

Results

The fungal populations decreased with different concentrations of herbicide as compared to the control in all treatments (Table 1). The bacterial (+ actinomycetes) population revealed no appreciable differences in all the Cotoran treatments (Table 1).

The rate of CO_2 production by *A. flavus* during the 9-day incubation period in the soil experiment was highest at the first 3 days interval for all treatments, then

Table 1

Number of organisms per gram of soil (oven-dry equivalent) in natural sandy loam previously treated with Cotoran and incubated 7-days

Cotoran ppm	Fungi ($\times 10^4$)	Bacteria and Actinomycetes ($\times 10^5$)
0	4.4	1.0
1	3.3	1.1
5	4.3	1.0
10	4.0	1.0
20	3.5	1.0

continued at slower rates during the next 6 days. CO_2 production increased with increasing concentration of herbicide and was maximum in 20 ppm treatment (Table 2).

Table 2

Production of CO_2 by *A. flavus* in Cotoran-treated soil

Cotoran (ppm)	Meq. of C evolved/100 g of soil		
	3 days	6 days	9 days
Water check	7.13	10.38	11.6
Acetone check	7.5	10.8	11.8
1	4.38	7.63	8.5
5	4.69	8.5	9.0
10	6.88	10.13	11.3
20	8.00	11.75	13.0

The uptake of inorganic phosphorus was not affected at different concentrations as compared to control (Table 3). The pH values decreased with increasing concentration of herbicide (Table 4).

Table 3

Amount of phosphorus utilized in Cotoran-treated soil after growth of *A. flavus* for 9 days

Cotoran (ppm)	P/100 g of soil (ppm)
Water check	5000
Acetone check	4460
1	4400
5	4460
10	4680
20	4840

Table 4

pH of Cotoran-treated soil after growth of *A. flavus* for 9 days

Cotoran (ppm)	pH
Non-inoculated	6.3
Water check	5.20
Acetone check	5.25
1	5.20
5	5.15
10	4.94
20	4.83

In the liquid culture experiment the growth of *A. flavus*, as evidenced by mycelium produced, increased with increasing concentrations of herbicide (Table 5)

Table 5

Mycelial dry weight in Cotoran-treated liquid culture after growth of *A. flavus* for 9 days

Cotoran (ppm)	Mycelial dry weight (mg)
Water check	280
Acetone check	290
1	215
5	245
10	266
20	337

Discussion

Respiratory and reproductive life processes are most essential in the studies of herbicidal effects on soil borne pathogenic fungi. Total mycelial dry-weight of *A. flavus* was considerably increased with increased concentration of Cotoran and if abundance of mycelial dry weight is indicative of growth, then the degree of growth increase was directly related to increased herbicide concentration. If massive reproduction of mycelia is indicative of respiratory activities going on in fungus, then one can say that the production of CO_2 is related to the production of mycelium.

RODRIGUEZ-KABANA et al. (1970) studied the growth responses of *Sclerotium rolfsii* to the herbicide EPTC (ethyl-N,N-dipropylthio carbamate) in liquid culture and soil and reported that increase in titrable acidity with high concentration of

EPTC (25–100 g/m) is of particular significance with regard to mode of action against the pathogen. MAXWELL and BATEMAN (1968) studied the influence of carbon source and pH on oxalate accumulation in culture filtrate of *Sclerotium rolfsii* and reported that the acidity produced by this fungus under the conditions of the experiment was largely due to oxalic acid. However, the accumulation of acid, probably aspergillic acid, with increase in mycelial dry weight production could have resulted from the lack of blockage by Cotoran in the tricarboxylic acid cycle.

CHOPRA et al. (1970) studied the influence of prometryne (2,4-bis(isopropylamino)-6-meltylmercapto-s-triazine) in soil on growth related activities of *Fusarium oxysporum* f. *vasinfectum* and found that total CO_2 production was significantly reduced in treatments of 1, 5 and 10 ppm of prometryne and increased in 20 ppm treatment.

The investigation has revealed several significant points and suggests probable interactions of Cotoran with *A. flavus*. Field rate concentrations of the herbicide (1.25–3.33 lb/acre) would not be expected to enhance growth of this fungus, but very little is known about the residual nature of this herbicide in soil. If the herbicide accumulates in soil up to 20 ppm, it might considerably alter the fungistatic principle of natural soil.

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Cellulase Activity in Corn Stalk Tissues Naturally Infected with *Fusarium roseum* (Link) SN. et H.

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Hundred plants of inbred line(C5) were collected in October on the same day. Cellulase activity, pith decay and presence of rotting fungi in stalk nodal sections were determined. The occurrence and activity of cellulase were determined by the degree of hydrolysis of a soluble cellulose derivative CMC (cup-plate method) and by the degree of solubilization of insoluble cellulose. From the results of the present investigation it may be concluded that the cellulase activity of a given stalk tissue is related to pith decay and presence of rotting fungi. Infection by *Fusarium roseum* is far more common than that by other organisms. It was found that *F. roseum* is the main source of cellulase activity in the stalk tissues. We may assume that the cellulase in the nodal tissues disintegrate cell wall structure and weaken the stalk.

Very little has been reported concerning the relationship between rotting of corn stalks and details of enzymatic degradation of the stalk induced by microbial deterioration. The limited data available suggest that rotting of cellulosic components of stalk tissue is accomplished through action of cellulolytic enzymes, secreted by fungal pathogens (FOLEY, 1959; IKENBERRY and FOLEY, 1967; SZÉCSI, 1970). SIEGEL (1962) has reported on the chemical composition of the tissues of corn stalk in the following percentage distributions: cellulose-hemicellulose 79.9%, lignin 19.5%, other polysaccharides 8.3%, protein 1.9%, pectins 0.3%. On the basis of experimental data with artificially infected corn lines, it may be supposed — which are supported by the chemical composition outlined above — that the cellulase produced by the pathogenic fungi is primarily responsible for tissue decay (SZÉCSI, 1970). Among the various stalk rotting pathogens, *Fusarium roseum* var. *graminearum* (Schwabe) SN. et H. and *F. roseum* var. *culmorum* (Schwabe) SN. et H. proved to be the most important fungi responsible for root and stalk rot of corn in Hungary. These two Fusarium varieties are truly cellulolytic microorganisms (WOOD, 1969), because they are able to grow on native cellulose (unpublished data).

The objective of the present work was primarily to study the prevalence of cellulolytic enzymes in corn stalk tissues as related to the degree of their rotting and to the presence of fungi infecting the stalk.

Material and Method

Field procedures and sampling. For the investigation of the natural infection in the field, an inbred line from Martonvásár (C5) was planted in 50 × 80 cm rowes in an experimental nursery. Samples were taken in October, on the same day. Corn stalk nodal sections, including the first node above the uppermost brace roots were placed in refrigerator until time of preparation.

Extraction and precipitation of cellulase from corn stalk tissues. Each stalk sample was processed separately for recovery of cellulase present. The nodal section was first sliced into sections approximately five millimeters in thickness by using a sharp knife. A 5 g sample of the cut-up tissue was weighed and added to 20 ml of 0.1 *M* acetate buffer, pH 4.6 in a micro cup of "Biomix". It was essential to carry out the enzyme extraction process at a temperature as low as possible to avoid heat denaturation. Hence the "Biomix" was operated intermittently in an ice water bath for a 5 minutes period. The crude extract was filtered through a coarse grade sintered glass filter, into a flask kept in an ice water bath. This second extract was centrifuged for 30 minutes at 6000 rpm on 0°C. Precipitation of protein substances was achieved by addition of four volumes of acetone previously cooled to 0°C. Acetone was slowly introduced in a refrigerator, at 0°C. A magnetic stirrer was used to agitate the content of the bottle during this procedure. Following the complete addition of the four volumes of acetone, the bottle was capped and placed in the refrigerator for at least four hours to allow completion of precipitation.

The resulting flocculent precipitate was recovered by centrifugation at 6000 rpm for 30 minutes on 0°C. The supernatant was discharged and the precipitate re-dissolved in 10 ml of 0.1 *M* acetate buffer, pH 4.6. The remaining residue was removed by centrifugation at 6000 rpm for 30 minutes on 0°C. The resulting, clear supernatant was used for the determination of cellulase activity.

Cellulase activity of corn stalk samples. Determination of the degree of hydrolysis of the soluble cellulose derivative CMC with enzyme preparations. Cellulase activity was determined by means of the cup-plate method (DINGLE et al., 1953). The assay medium contained 1% CMC (Reanal), 2% agar (Difco) and 0.01% merthiolate (Reanal) in 0.1 *M* acetate buffer, pH 4.6. Wells (8 mm diameter) were made in the assay medium (5 mm thick), and filled with 0.2 ml of enzyme preparate. Plates were incubated at 37°C for 24 hrs, and then developed with 3% sugar of lead. As a result of enzyme activity, transparent zones develop on the opalescent plates. The plates were washed with distilled water and photographed. Enzyme activity was expressed as the diameter (mm) of the transparent zone per 37°C for 24 hrs.

Determination of the degree of solubilization of insoluble cellulose with enzyme preparations. The modified method of HALLIWELL (1961) was used for the determination of cellulase activity. In 20 ml test tube 3.5 ml of 0.1 *M* acetate buffer, pH 4.6 and 0.5 ml enzyme solution was added to 20 mg cellulose (Avicel-RC-581,

FMC Corporation American Viscose Division, Avicel Sales, Marcus Hook, Pa. USA). Test tubes with such mixtures were rotated in a thermostat for 6 hrs at 37°C. Then the enzyme was inactivated by the addition of sufficient 0.6 *N* sulphuric acid (about 0.5 ml), to lower the pH to 1.9. The same mixture treated with 0.5 ml 0.6 *N* sulphuric acid and kept in boiling water bath for 10 minutes were used as a control. After the incubation time the reaction mixtures were filtered on paper.

The "reducing" sugars in the filtrate were quantitatively determined with anthron-reagent (WHISTLER, 1963). 6 ml anthron-reagent was added to 3 ml filtrate (diluted 10×) slowly in a cold-water bath. After the addition of anthron-reagent, the mixture was heated in a boiling-water bath for 10 minutes, and transferred to a cold-water bath for 15 minutes. The absorbance of the mixtures were measured at 620 mμ by a Unicam SP 800 spectrofotometer. Absorbance data were calculated on the basis of a standard calibration curve prepared with glucose. The cellulase activity is given as: γ glucose/ml/ 6 h/37°C.

Detection of rotting fungi in corn stalk samples. The first mode of each plant was split longitudinally into four. One quarter of the first nodal section of each stalk was surface sterilized by immersion for 10 sec. into 0.5% sodium hypochlorite, followed by washing in water. The sections were placed on potato dextrose agar plates and incubated at room temperature. Fungal colonies on the stalk sections were transferred to potato dextrose agar tubes for identification. Fusarium-isolates were determined according to the system of MESSIAEN and CASSINI (1968).

Determination of pith decay (deterioration). The following pith decay scale was used as a criterion of stalk rot severity (IKENBERRY and FOLEY, 1967):

0 = no decay, no white (dead) cells in pith, tissue yellow green.
1 = white cells present, but no visible cell wall decay.
2 = pith tissue cavity present ($<^1/_4$ of cross sectional area).
3 = pith tissue cavity moderate (about $^1/_2$ of cross sectional area).
4 = pith tissue cavity about $^3/_4$ of the cross sectional area.
5 = stalk a cylinder of dry rind, only occasional pith tissue still visible.

Results and Discussion

The data obtained from these investigations are summarized in Table 1 and Table 2. In Fig. 1 longitudinal sections of corn stalks and their cellulase activity zone can be seen. On the basis of these data, it can be stated that the higher the cellulase activity of corn stalk the greater the degree of the pith decay. Cellulase activity of the stalk tissues were also related to the presence of rotting fungi. The data obtained by the two different cellulase determination methods were rather similar, but the cup-plate method is certainly more practical. Infection by *Fusarium roseum* is far more common than, that by other organisms. The cellulase found in corn stalk tissues is assumed to be of fungal origin (FOLEY, 1959; IKENBERRY and FOLEY, 1967), and its main source is the pathogenic fungus *F. roseum*.

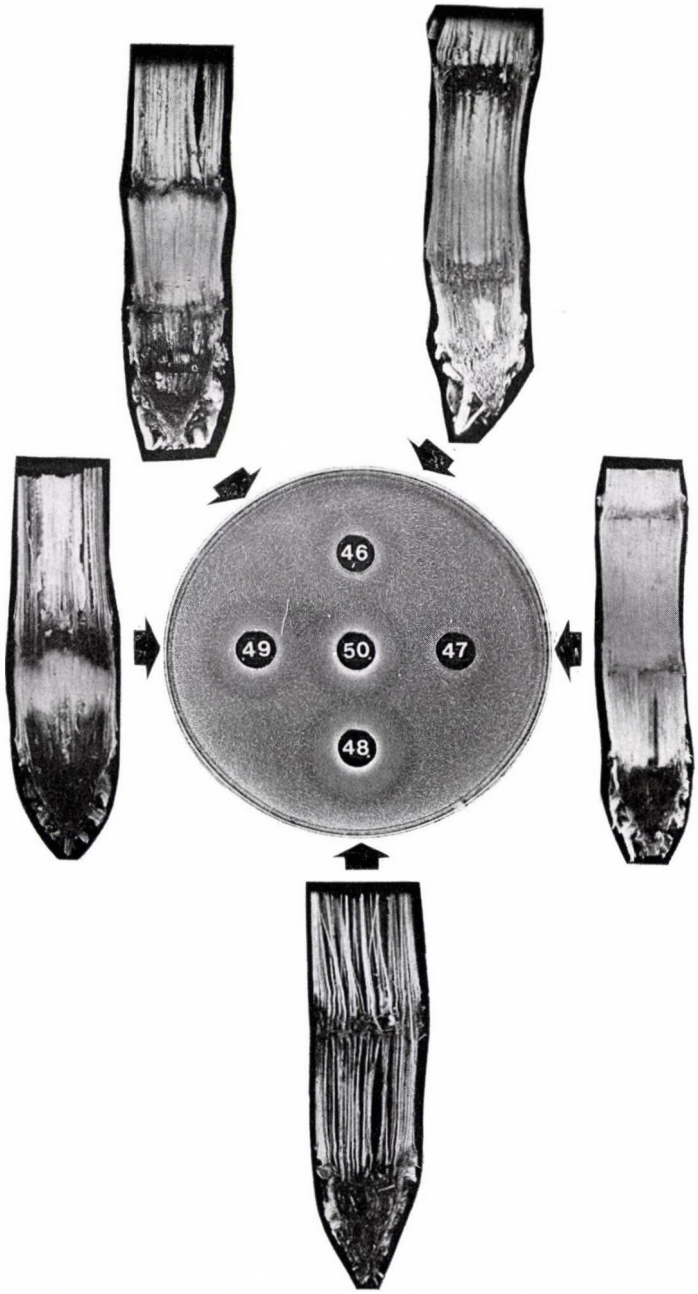

Fig. 1. Longitudinal sections of corn stalks and their cellulase activity zone

Table 1

Cellulase activity (degree of hydrolysis of soluble CMC) of corn stalk nodal sections related to pith decay and presence of pathogenic fungi

Plant no.	Degree of pith decay	Presence of fungi (+)r = F. roseum	Diameter of cellulase activity ring in mm	Plant no.	Degree of pith decay	Presence of fungi (+)r = F. roseum	Diameter of cellulase activity ring in mm
1	4	+r	21	51	4	+r	20
2	1	—	—	52	4	+r	21
3	5	+r	23	53	0	—	—
4	0	—	—	54	5	+r	22
5	4	+r	20	55	4	+r	20
6	1	—	—	56	4	+r	20
7	5	+r	24	57	0	—	—
8	0	—	—	58	4	+r	20
9	0	—	—	59	4	+	20
10	1	—	—	60	0	—	—
11	4	+r	21	61	1	—	—
12	3	+r	17	62	4	+r	19
13	3	+r	18	63	4	+r	21
14	0	—	—	64	3	+	18
15	1	—	—	65	4	+r	20
16	1	—	—	66	4	+r	21
17	0	—	—	67	4	+r	20
18	4	+r	21	68	0	—	—
19	1	—	—	69	2	+	14
20	3	+r	17	70	4	+r	19
21	4	+r	21	71	4	+r	21
22	0	—	—	72	3	+r	17
23	5	+r	23	73	3	+r	18
24	1	—	—	74	0	—	—
25	4	+r	21	75	0	—	—
26	1	—	—	76	0	—	—
27	1	—	—	77	1	—	—
28	0	—	—	78	4	+r	21
29	3	+	16	79	0	—	—
30	4	+	20	80	3	+r	17
31	0	—	—	81	4	+r	21
32	1	—	—	82	1	—	—
33	4	+r	20	83	5	+r	23
34	3	+r	17	84	1	—	—
35	1	—	—	85	4	+r	21
36	0	—	—	86	1	—	—
37	0	—	—	87	0	—	—
38	4	+r	20	88	1	—	—
39	4	+r	20	89	3	+r	16
40	2	+	12	90	5	+r	22
41	4	+r	21	91	0	—	—
42	0	—	—	92	0	—	—

Table 1 (cont.)

Plant no.	Degree of pith decay	Presence of fungi (+)r = F. roseum	Diameter of cellulase activity ring in mm	Plant no.	Degree of pith decay	Presence of fungi (+)r = F. roseum	Diameter of cellulase activity ring in mm
43	4	+r	19	93	4	+r	20
44	0	—	—	94	3	+r	17
45	4	+r	20	95	0	—	—
46	3	+r	17	96	1	—	—
47	1	—	—	97	1	—	—
48	5	+r	23	98	4	+r	21
49	4	+r	20	99	4	+r	20
50	4	+r	20	100	2	+r	15

Table 2

Cellulase activity (degree of solubilization of insoluble cellulose) of corn stalk nodal sections

Plant. no.	γ/ml/6h/37°C	Plant no.	γ/ml/6h/37°C
1	165	11	170
2	0	12	100
3	180	13	120
4	0	14	0
5	150	15	0
6	0	16	0
7	210	17	0
8	0	18	160
9	0	19	0
10	0	20	90

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Apoplexy of apricots

II. Cytosporal Die-back and the Simultaneous Infection of *Pseudomonas syringae* and *Cytospora cincta* on Apricots

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Cytospora cincta is an important pathogen in apricot orchards in Hungary. The symptoms of the cytosporal infection are the same as the symptom of bacterial canker and die-back caused by *Pseudomonas syringae*. Simultaneous inoculation with the bacterium and *Cytospora cincta* produced more extensive cankers than those caused by one of the pathogens alone. Freshly cut wounds were sensitive for a longer period to cytosporal infection than to bacterial inoculation. The knowledge of the annual-cycle of the cytosporal and bacterial diseases gives a chance for effective control against the apoplexy disease of apricots.

This work is a part of the study on the apoplexy disease (die-back, sudden wilt) of apricots. In an earlier work we reported on the role of *Pseudomonas syringae* on the development of cankers and die-back symptoms of apricot trees (KLEMENT, ROZSNYAY and VISNYOVSZKY, 1972). However, in some cases we were unsuccessful in the isolation of the bacterium from cankers and necrotized tissue. On these necrotized tissues and dead branches pycnidia of *Cytospora cincta* developed. On the other hand in many instances both of these microorganisms occurred together. In a number of cases when artificial inoculations were made with *Pseudomonas syringae* and the typical bacterial symptoms have developed, sometimes pycnidia of *Cytospora cincta* also appeared in the necrotized cortex after a considerable time. Therefore, several questions arise: what is the role of *Cytospora cincta* in the induction of apoplexy of apricots and what is the connection between the bacterial and cytosporal infection.

Cytosporal infection of apricots was investigated by many research workers, however, the results were obscure and confused. Namely, DEFAGO (1935), SCHMIDLE (1961), KOVÁCS (1970) and STANOVA (1968, 1970) obtained positive results on apricot trees inoculated with *Cytospora (Valsa) spp.* however, others (CHABROLIN, 1927; JOESSEL and BORDAS, 1931; RIEUF, 1950; and PLOCK, 1960; MAZZUCHI, 1966) were unable to get definite symptoms with these fungi. For this reason a general opinion developed in many apricot growing countries that *Cytospora* is only a secondary organism on the weakened grown apricot trees.

To study the role of *Cytospora cincta* in the pathogenesis of apoplexy disease of apricots artificial inoculations were carried out with different strains of *Cyto-*

spora cincta as well as with *Pseudomonas syringae* singly and simultaneously in the course of one year. The results of these experiments clarified the etiology and the annual cycle of the disease induced by *Cytospora cincta* and the relation to the bacterium.

Methods

Isolation of fungi. In the course of summer, 1970, 12 *Cytospora* strains were isolated from different parts of Hungary. Eight strains from apricots, 2 strains from peaches and 2 from cherry trees were isolated. These strains were obtained from the part of necrotized phloem and browned xylem. The surface of the infected branch was desinfected by 96% alcohol and it was flamed for a moment. The suspension of pycnospores or a piece of necrotized tissue was put onto potato-dextrose-agar medium. After 3–5 days incubation period the white mycelium of *Cytospora* grew out at 25°C. Subcultures were obtained from these cultures on slant agar medium.

Fig. 1. The inoculated site covered with a wet cotton and aluminium foil

Artificial inoculation with *Cytospora cincta* was carried out on 4 and 10-year-old apricot trees. In both experiments 3–3 apricot trees were inoculated per month. On every tree 5–5 inoculations were made. Cortex of tranks and main branches were cut in with a knife and small pieces of the mycelial cultures of *Cytospora cincta* were put into the wounded cortex or onto pruning surface of branches. The inoculated sites were covered with a wet cotton and after this with aluminium foil (Fig. 1). The cotton and the foil were removed after two weeks. Six-day-old cultures were used for inoculation (STANOVA, 1968).

Simultaneous inoculation with Pseudomonas syringae and Cytospora cincta. In both experiments 3 trees were inoculated with the bacterium 3 trees with *Cytospora cincta* and 3 apricots with the bacterium and fungi simultaneously per month. In the case of the simultaneous infection at first the suspension of bacterium was brushed onto the same wounded surface before the fungal inoculation. When the bacterium was used alone as a control treatment, the inoculated wounds were also covered with wet cotton and the aluminium foil.

The artificial inoculations were carried out every month from December 1970 to November 1971. The results were evaluated after a one-year-cycle but the infected trees were observed during a two years period. The extent of damage of phloem tissues was determined from the diameter of the developed cankers and that of the xylem from the browning of the xylem tissues.

Symptoms

Symptoms of the cytosporal infection are the same as the symptoms of bacterial canker and die-back caused by *Pseudomonas syringae* which has been characterized in another paper (KLEMENT, ROZSNYAY and VISNYOVSZKY, 1972). The characteristic symptoms of apoplexy are the development of cankers and die-back of branches or the whole trees. In the case of die-back the foliage of the infected branches the whole tree wilts quickly in the course of about a few days.

Both causal organisms infect phloem and xylem, however, only the necrotized phloem and cambium result in die-back and sudden wilt of branches or whole tree. If damage of phloem and cambium girdled the branch or the trank, some of the branches or the whole tree above the girdled part die in spring, summer or early autumn. In the case of the cytosporal infection die-back symptom usually appears during summer and early autumn while bacterial die-back develops mostly in spring and early summer.

When the desctruction of cambium does not engirdle the branches and stem, the newly growing tissues tend to overgrow the necrotized phloem and so cankers develop. The form of cankers caused by the two pathogens are indistinguishable. Cankers usually develop at the pruning points. Infected areas are slightly sunken and darker brown in colour than the surrounding healthy bark. The freshly destroyed browned phloem is soft and wet, gum-soaked and has a characteristic sour smell. The colour of cortical tissues of the cankered area varies from bright orange to brown. Later the necrotized phloem dries out and black pycnidia of *Cytospora cincta* develop under the bark. Since the cytosporal canker grows from one year to the other on the margin of the old canker the new infected zone appears during the sensitive period of tree. The browning of tissues is often followed by gum exudation.

The damage of xylem tissues appears in the form of brown-black stripes which extend by more than 5–30 cm necrosis of phloem. These brown strips in the xylem are also very similar to those caused by *Pseudomonas syringae.*

Results

The causal organism of the cytosporal infection

Twelve *Cytospora* strains were isolated from apricots, peaches and cherry trees. The cultures originated from apricots and peaches produced greyish-white mycelium while the strains isolated from cherry trees grow in a form of oil-green mycelium on potato-dextrose-agar medium. Pycnidia developed in 4—5 week old cultures. On the basis of the inner structure of the pycnidia the fungi isolated from apricots and peaches were determined as *Cytospora cincta* Sacc.

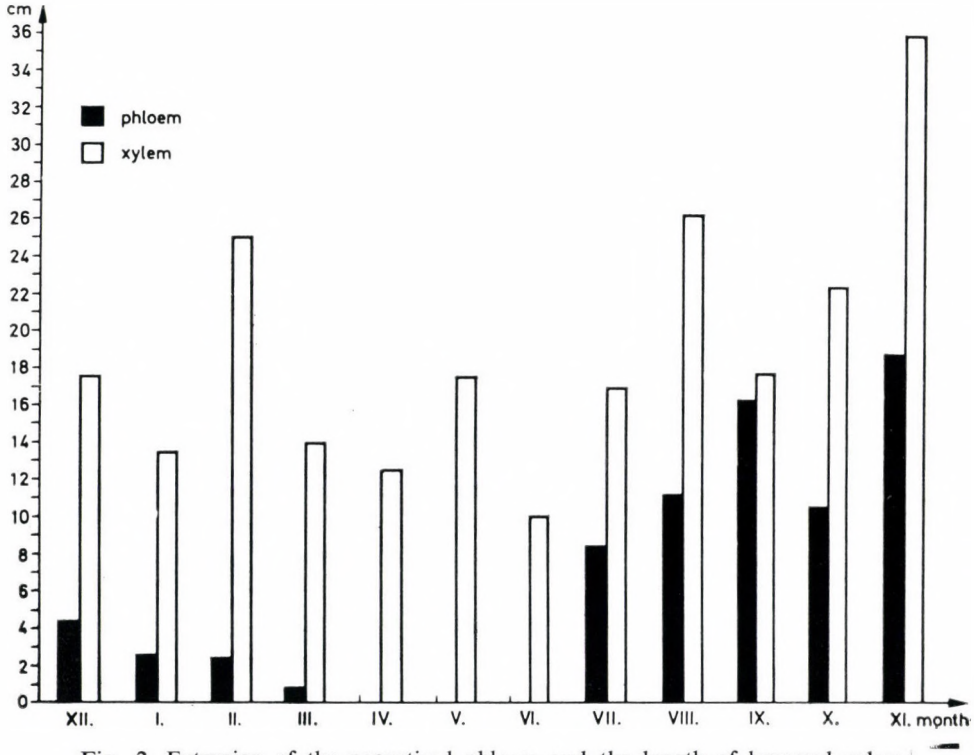

Fig. 2. Extension of the necrotized phloem and the length of browned xylem induced by *Cytospora cincta*

The virulence of the various strains on apricot was different but there was no correlation between their virulences and their host origin. Since the strain No. 6 isolated from apricot proved to be the most virulent, this strain was used for further inoculations.

Artificial inoculations with Cytospora cincta

All artificial inoculation with the mycelium culture or the suspension of pycnospores through the surface of pruning or wounding sites were successful. These experiments were carried out every month so as to establish the development of the disease in the course of one year. These results are summarised in Fig. 2.

It was proved that *Cytospora cincta* was able to infect apricots all the year round. The xylem was sensitive every month, however, phloem and cambium were necrotized only from July till the beginning of vegetation. The wounds which were inoculated in spring and early summer were overgrown by the new

Fig. 3. The results of the artificial inoculation with *Cytospora cincta* inoculated on the 14th September, 1971

callus. It was concluded that the most sensitive period, when the fungus is very active in the phloem tissue, is September, October and November, but the infections during winter period too are also able to produce cankers and die-back (Fig. 3, 4).

After the inoculation the mycelium grows continuously in the tissues, however, cankers or die-back will be visible in the next summer. The pycnidia under the necrotized bark develop during 3–5 months after the inoculation.

Simultaneous infection with Pseudomonas syringae and Cytospora cincta

The artificial inoculations were conducted from December, 1970 to November, 1971, in every month 1. with *Pseudomonas syringae*, 2. with *Cytospora cincta*, 3. with *Pseudomonas syringae* and *Cytospora cincta* simultaneously. This experiment was evaluated on December 9, 1971 (Table 1) and later on July 18, 1972 (Table 2).

The results in Table 1 are not completely suitable for evaluation because

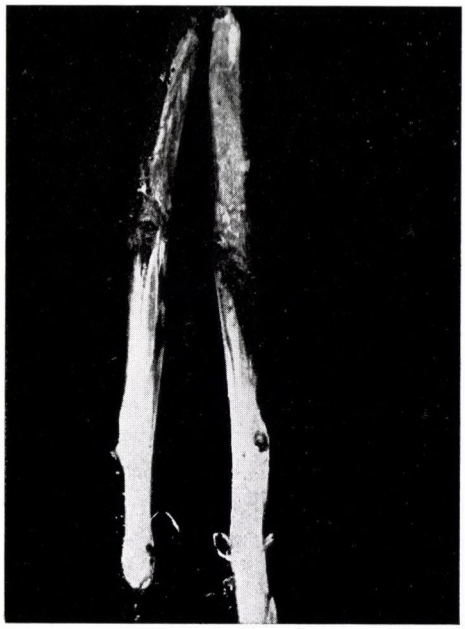

Fig. 4. The effect of *Cytospora cincta* necrotizing phloem and xylem on one year old branches

Table 4

Different pathogens which cause die-back symptoms.

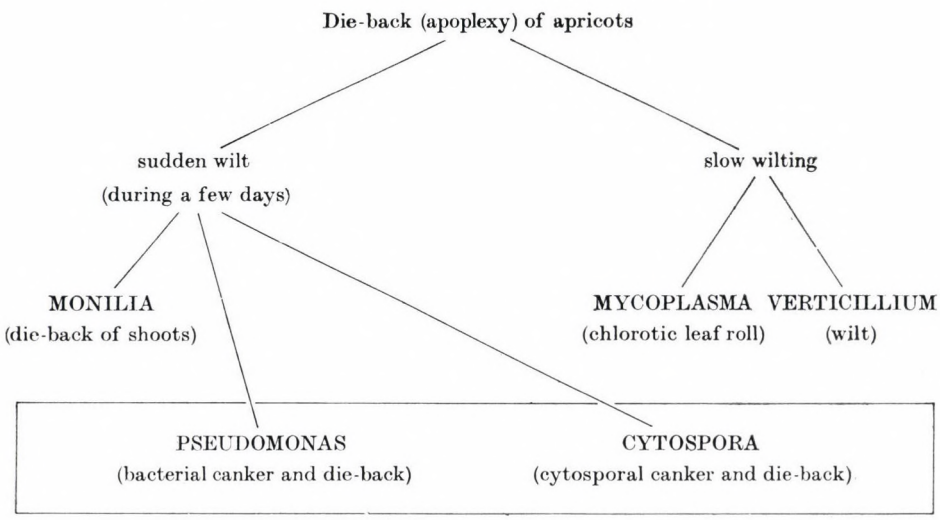

Table 1

Extension of cankers (necrotized phloem) in cm induced by *Cytospora cincta* and *Pseudomonas syringae* and by the *two pathogens* simultaneously. Beginning of the experiment: December 1970. The data of the first evaluation: December 9th, 1971

The date of inoculation	*Cytospora cincta*	*Pseudomonas syringae*	*Cytospora cincta* + *Pseudomonas syringae*
December 8	2.86	3.61	13.9
January 13	1.50	4.40	15.55
February 11	2.4	9.51	17.01
March 10	0.2	0.45	0.5
April 9	0.00	0.41	0.41
May 12	0.27	0.00	0.51
June 3	0.73	0.43	3.37
July 13	4.42	3.2	3.65
August 11	2.9	2.46	2.71
September 1	2.7	2.58	3.6
October 7	2.23	2.32	2.4
November 5	Cannot be estimated at this time		

Table 2

Extention of cankers (necrotized phloem) in cm induced by *Cytospora* and *Pseudomonas syringae* and by the *two pathogens* simultaneously. Beginning of the experiment: December 1970. The data of the second evaluation: July 18th, 1972

The date of inoculation	*Cytospora cincta*	*Pseudomonas syringae*	*Cytospora cincta* + *Pseudomonas syringae*
December 8	4.2	4.4	14.9
January 13	2.5	2.1	13.8
February 11	2.4	8.6	16.7
March 10	0.7	8.4	3.4
April 9	0	0	0
May 12	0	0	0
June 3	0	0	2.5
July 13	8.2	0	2.5
August 11	11.2	0	9.4
September 1	16.2	0	13.9
October 7	10.5	0	10.4
November 5	18.7	2.5	9.2

there was not enough time for the development of cankers. However, the comparison of the data in Table 1 and Table 2 gives us some information on the extension of the disease.

The symptoms induced by the bacterium and *Cytospora cincta* were indistinguishable. Both causal organisms infected phloem and xylem, however, only the

Table 3

Correlation between the age of the pruning wounds and the extension of cankers and length of browned xylem in cm induced by *Cytospora cincta*

The age of wounds	Experiment I (September 6th 1971)			Experiment II (September 24th 1971)		
	In phloem	In xylem	Development of pycnidia	In phloem	In xylem	Development of pycnidia
0 h	9.8	12.2	+++	6.0	6.5	—
1 h	12.6	13.8	+++	5.0	5.7	—
3 h	8.7	13.2	++	6.2	6.3	—
6 h	7.0	11.5	+	2.0	2.0	—
24 h	9.8	10.0	+++	11.0	11.0	—
48 h	8.0	10.7	+++	1.2	2.5	—
5 days	11.0	12.5	+++	3.0	3.0	—
8 days	3.3	4.3	+	2.0	3.0	—
10 days	4.0	4.5	+	0	0	—
16 days	2.0	4.5	--	0	0	—
24 days	0	0	—	0	0	—
29 days	6.0	14.0	++	0	0	—
40 days	0	0	—	0	0	—

The extent of production of pycnidia: + weak; ++ medium +++ strong; — no pycnidia.

diseased and necrotized phloem and necrotized cambium caused die-back symptom on the both cases.

It was proved that *Pseudomonas syringae* is able to infect xylem all the year round but phloem and cambium were infected only from leaf-fall to the beginning of vegetation. Therefore, the infections during the vegetation period do not cause cankers and die-back. Die-back symptom usually developed in spring or early summer after the winter inoculations.

As it was shown above, *Cytospora cincta* was also able to infect apricot trees, however cankers and die-back developed if the inoculations were done from July to March. The wounds which were inoculated in spring and early summer were overgrown by the new callus.

When *Pseudomonas syringae* and *Cytospora cincta* were used as inocula simultaneously the symptoms were stronger than those of the cankers which were caused by only one of the pathogens (Fig. 5). However, this synergistic effect appears only if the inoculation was carried out in the dormant stage of the trees. There are no synergistic effects if the inoculation was made during the vegetation period. The size of cankers was two-three times larger than those which were induced by the bacterium and five-six times larger than cankers caused by *Cytospora cincta.*

Pseudomonas syringae could be re-isolated from freshly necrotized tissues only from December to the middle of June. On the contrary, the re-isolation of

Fig. 5. One year old canker inoculated simultaneously with *Pseudomonas syringae* and *Cytospora cincta*

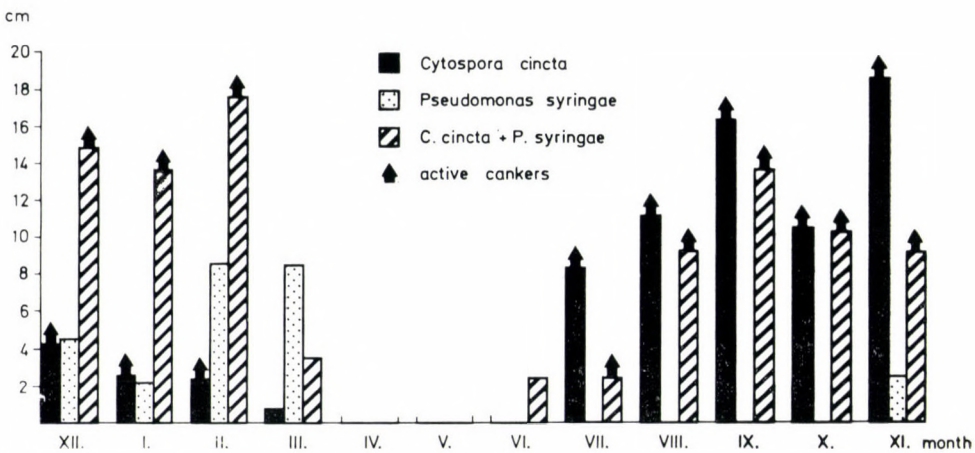

Fig. 6. Effect of the inoculation with *Cytospora cincta*, *Pseudomonas syringae* and with *both pathogens simultaneously*, in the phloem and the reactivation of cankers in the following year

Cytospora cincta was successful all over the year. As we have seen after a three-year-period of artificial inoculations the spread of *Pseudomonas syringae* in tissues is rapid but does not extend from one year to the other. Therefore, the bacterial apoplexy seems to be an acute disease. On the other hand, *Cytospora cincta* grows slower than the bacterium but it causes a chronic disease and so cankers are developed and reactivated year by year (Fig. 6).

Correlation between the age of wound and its infectivity

Both, *Pseudomonas syringae* and *Cytospora cincta* infect trees through natural wounds but more frequently at pruning points. Therefore, an important epidemiological question arises: How long does a freshly cut wound serve as an open door for infection?

In an earlier experiment it was proved that only fresh wounds could be infected by *Pseudomonas syringae*. If wounds were older than 5 hours the inoculation with the bacterium was unsuccessful.

The experiments with *Cytospora cincta* is demonstrated in the Table 3. It was interesting to see, that wounds were sensitive for a much longer period to cytosporal infection than to bacterial infection. For instance, in a few experiments wounds were sensitive to *Cytospora cincta* also on the 29th day after wounding, generally only 8–10 days after pruning.

Discussion

The presence of *Pseudomonas syringae* and *Cytospora cincta* in apricots have been demonstrated by many research workers, however, they have not emphasized the significance of these pathogens or the two pathogens together in the pathogenesis of the apoplexy of apricots. Furthermore, the importance of these pathogens was not realized sufficiently in the apricot producing countries.

The definition of the apoplexy of apricots should be clarified too. So far all kinds of apricot damage are called apoplexy. This leads to misunderstanding. In the human pathology the apoplexy is reserved only for a symptom which appears suddenly. In future it would be advisable to reserve the term apoplexy of apricots to those diseases which are infectious and result in a sudden death of trees (Table 4). Other symptoms caused by organisms, like *Verticillium* and mycoplasma, can be characterized by slow destruction of trees (e.g. yellowing, early leaf fall, distortion), should be distinguished from apoplexy. This would clarify and prevent misunderstanding on this problem.

In Hungary *Pseudomonas syringae* and *Cytospora cincta* or the two pathogens together are responsible for the development of apoplexy of apricots. The *Verticillium spp.* occur only rarely in Hungary and the symptoms caused by these fungi differ externally from those of typical apoplexy (BEREND, 1958).

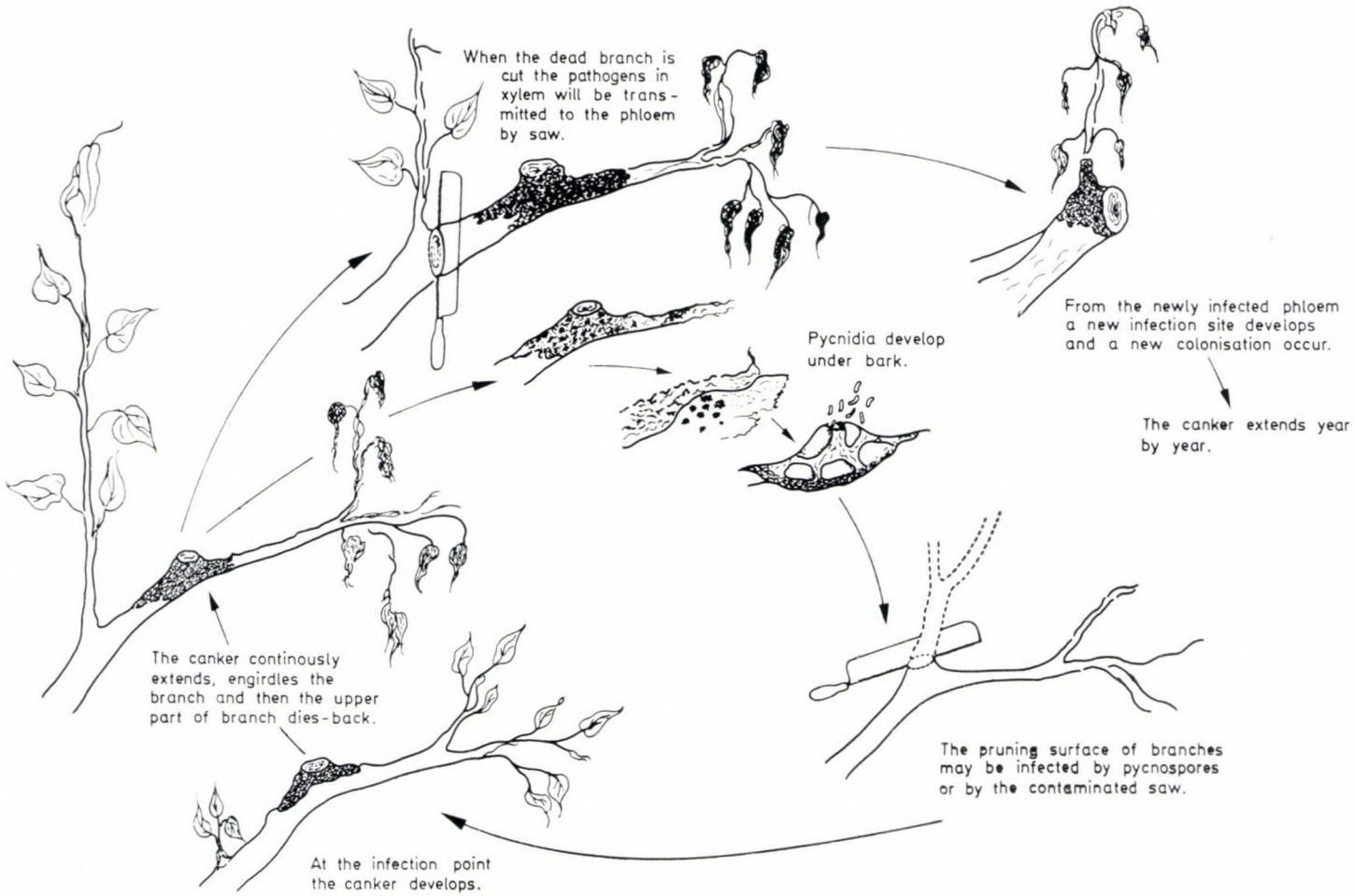

Fig. 7. Annual cycle of cytosporal apoplexy of apricots

*Monilia spp.*which cause wilt and die-back of young shoots appear only in early spring and only rarely, *Chlorotic leaf roll* of apricots which is induced by a mycoplasma (MORVAN, 1968) has not been determined in this country.

Our investigations have clearly shown that *Cytospora cincta* is a true pathogen on apricots and not only a secondary parasite on the weakened trees. Our experiments were conducted on very well growing trees and the artificial inoculations with this fungus were successful in each case if they were made in the sensitive stage of the tree.

These results call attention to the importance of the combined infection of apricots. In orchards there are many possibilities for the combined infection with *Pseudomonas syringae* and *Cytospora cincta.*

The identity of symptoms produced by these pathogens caused many misunderstandings in the past as regards apoplexy of apricots. The results of the study outlined above dissolved the problem.

The knowledge of the annual-cycle of the disease (Fig. 7) gives a chance for the effective control against the apoplexy disease of apricots. Since both pathogens infect through wounds, the time of pruning must be changed. Namely, it seems necessary to work out a new pruning system. At present, pruning of apricots is usually carried out during the dormant stage of trees. However, at this time apricot trees are in the most sensitive physiological condition against *Pseudomonas syringae* and *Cytospora cincta.* Therefore, it is not advisible to prune apricots during the winter period. In the new system pruning of the trees should be made from the start of vegetation till the middle of June. During this period trees are resistant to both of the pathogens (Fig. 6). In this time both pathogens infect only the xylem but not phloem, and the infection is localised by the new callus produced. If pruning is carried out in the spring, all other control measures would seem to be unnecessary.

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Inhibition de la réaction hypersensible par des extraits de feuilles

Par

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Extracts from healthy leaves of tobacco and cabbage preinfiltrated into the leaves of tobacco can inhibit the hypersensitive reaction induced by a plant pathogenic bacterium (*Pseudomonas phaseolicola*). Leaves were ground by hand in a precooled mortar with buffer and precooled sand. The grinding was continued for 4 min. The resulting slurry was squeezed through two layers of cheese-cloth and centrifuged for 1 min at 500 g to sediment whole cells, debris and sand. The supernatant was collected and centrifuged for 10 min at 6000 g. The sediment consisted mainly of chloroplasts, and the supernatant contained mitochondria, soluble fraction, and some broken chloroplasts. The chloroplast fraction was suspended in water and infiltrated into tobacco leaves. The inhibition of the hypersensitive reaction was detected 6—8 hours after the infiltration of the extracts. The supernatant was fractionated by $(NH_4)_2SO_4$, and different fractions was also infiltrated into leaves. The chloroplast fraction gave the best protective effect. Other fractions were less inhibitory depending on the concentration of $(NH_4)_2SO_4$.

La plupart des bactéries phytopathogènes injectées à un hôte hétérologue y induisent une réaction hypersensible. Ceci se traduit par l'apparition d'un flétrissement localisé (collapse) qui ne se manifeste que pour autant que la concentration des germes injectés ait dépassée 2×10^6 germes/ml (KLEMENT, 1967). La réaction peut être inhibée par différents traitements: infiltration d'ion calcium (COOK et STALL, 1970), température élevée (SÜLE et KLEMENT, 1971), cytokinines (NOVACKY, 1972), prétraitement par des bactéries ou des extraits de bactéries dont l'infiltration préalable développe un état de prémunité. KLEMENT (1967) a ainsi obtenu une inhibition de la réaction avec des bactéries saprophytes et LOZANO et SEQUEIRA (1970) avec des bactéries tuées.

D'après nos expériences (LE NORMAND *et al.*, 1972) induire une prémunité chez un hôte hétérologue est une propriété commune à tous les types de bactéries phytopathogènes. La dose prémunisante est généralement de 2×10^4 germes/ml cependant, elle peut atteindre 10^8 germes/ml pour des bactéries qui ne déclenchent

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pas la réaction hypersensible, c'est le cas par example pour *Corynebacterium fascians* et *Pseudomonas marginalis*. SEQUEIRA *et al.* (1972) ont pu isoler à partir d'extraits bactériens des protéines responsables de cet effet. Ils ont également montré que l'injection de protiénes d'une autre origine (caséine, trypsine, ribonucléase . . .) était incapable de déclencher ce même état. On pouvait donc être amené à conclure que la prémunité est un phénomène déclenché par l'injection de protéines bactériennes spécifiques. Le but de cette étude est de montrer qu'il n'en est rien et que l'on peut obtenir le même résultat avec des extraits de feuilles.

Matériel et Méthodes

1. Plantes et bactéries. Des tabacs *(Nicotiana tabacum var. Xanthi)* sont utilisés au stade 6–8 feuilles. Ils sont placés dans une chambre climatisée à 25°C et 70% d'humidité et sous un éclairement continu de 7000 lux. La bactérie choisie est *Pseudomonas phaseolicola* (Burk) Dowson, souche 88 de notre collection. Après 24 heures de culture sur milieu de King B, les germes sont prélevés, lavés et suspendus en eau distillée. La suspension est ajustée à 5×10^6 bactérie/ml.

2. Extraction. On prélève les trois feuilles médianes de chaque plante puis on retire les nervures centrales de chacune d'elles. Les feuilles sont alors rassemblées puis placées au réfrigérateur durant trente minutes, elles sont ensuite pesées et broyées au mortier avec du sable de Fontainebleau et en présence d'acide ascorbique (0.5% du poids frais). Le broyat est alors passé sur étamine et le liquide récolté est centrifugé 1 minute à 500 g afin de sédimenter sable et gros débris. Le surnageant est alors centrifugé à 6000 g durant 10 minutes. Le culot est repris dans 5 fois son volume d'eau distillée: fraction E_1. La fraction E_1 et son surnageant sont injectés directement dans les feuilles. On a également préparé des extraits protéiques; dans ce cas le broyage de feuilles a été réalisé dans un faible volume de tampon phosphate 0.02 M pH 6.5 additionné de chlorhydrate de cystéine (0.1% du poids frais). Ce dernier est en effet le seul corps efficace en présence de quantités importantes d'acide chlorogénique et de phénolases comme c'est le cas chez tabac (ANDERSON, 1968; KHANNA *et al.* 1968), on procède ensuite comme précédemment. Le dernier surnageant est ensuite précipité par de l'acéton pure ou de l'alcool éthylique porté à la température de −25°C ou encore par une solution saturée de sulfate d'ammonium. Dans ce cas on a désigné la fraction précipitée entre:

0 et 25 % de saturation par F_1
25 et 35 % de saturation par F_2
35 et 55 % de saturation par F_3
55 et 80 % de saturation par F_4

Les précipités ont dans tous les cas été repris dans 5 fois son volume d'eau distillée et dialysés contre de l'eau courante jusqu'à élimination de l'ammoniaque.

L'ensemble des opérations décrites a été effectué à +2°C.

3. Technique. Les differents extraits ont été infiltrés dans des espaces internervaires de feuilles de tabac à l'aide de la technique décrite par KLEMENT (1963). Les infiltrations ont toujours été réalisées sur des feuilles de même âge en position médiane, les plantes sont ensuite placées en éclairement continu durant 6–8 heures. La suspension bactérienne d'épreuve est alors injectée dans les mêmes espaces internervaires. On a réservé un espace témoin par feuille (qui n'a donc reçu que l'injection bactérienne), et par ailleurs on a éprouvé la suspension sur des plantes témoins n'ayant reçu aucune injection préalable de façon à déceler un éventuel effet systémique.

Résultats

La fraction E_1 comprenant essentiellement les chloroplastes et les débris de chloroplastes inhibe totalement la réaction hypersensible. Le surnageant récupéré après la sédimentation des éléments ayant servi à la préparation de E_1, présente le même effet inhibiteur, cependant conservé à la température ambiante (28°C) il brunit très vite et perd simultanément son activité inhibitrice.

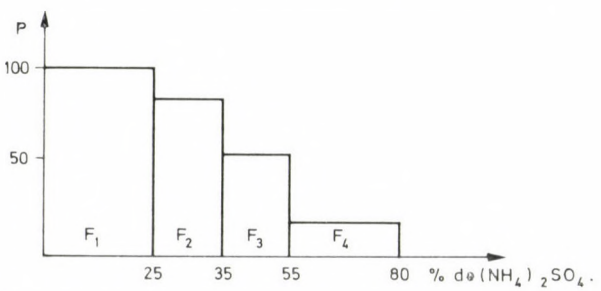

Fig. 1. Effet de protection (P) en pour cent obtenu par les différentes fractions de l'extrait de plante

Les extraits obtenus après dissolution des précipitations alcooliques ou acétoniques ont également un effet inhibiteur mais bien inférieur à celui obtenu avec E_1. Quant aux extraits obtenus après précipitation fractionnées au sulfate d'ammonium, ils s'avèrent d'autant moins inhibiteurs que le relargage a été obtenu avec une concentration plus grande.

La figure 1 montre que la fraction F_1 (–25%) qui présente encore une couleur verte due à la présence d'éléments chloroplastiques, inhibe fortement la réaction hypersensible, la fraction F_2 (25–35%) inhibe encore notablement; par contre la fraction F_3 (35–55%) n'inhibe plus qu'à 50%, quant à la fraction F_4 (55–80%) son effet est discutable. La plupart des feuilles injectées avec cette dernière fraction présentent le symptôme de collapse, cependant quelques feuilles ne flétrissent qu'à moitié.

Nous avons pu noter, en outre, un effet systémique qui s'observait sur les espaces internervaires voisins et cela dans le cas des trois extraits: E_1, F_1, F_2. Cet effet ne se manifeste pas dans le cas de F_3 et F_4.

Afin d'apprécier l'effet de la lumière sur cette inhibition, nous avons parallèlement maintenu une série de plantes à l'obscurité et nous les avons traitées de la même façon que précédemment. Cependant 5 heures après l'injection bactérienne ces plantes sont retirées de l'obscurité et mises sous éclairage faible (2000 lux). Dans ce cas on constate que l'effet inhibiteur se manifeste aussi mais son intensité est plus faible que dans les séries précédentes. Nous avons enfin examiné la thermosensibilité de cette propriété de la façon suivante. Tous les extraits ont été chauffés 1 à 2 minutes à 80°C, il apparaît des précipités que l'on maintient en suspension et on injecte le tout comme précédemment. On constate que cette opération ne fait pas perdre l'effet inhibiteur des fractions E_1 et F_1 alors que les fractions F_2, F_3, F_4 perdent graduellement le leur.

Par ailleurs, cet effet ainsi mis en évidence ne semble pas être spécifique, nous l'avons obtenu également en injectant des extraits de choux (préparés comme précédemment) dans des feuilles de tabac.

Discussion

Les résultats montrent que l'on peut prémunir contre la réaction hypersensible non seulement avec des bactéries ou des extraits bactériens mais encore par des extraits de feuilles. Le fait que toutes les fractions aient présenté un effet inhibiteur plus ou moins marqué donne à penser que l'aptitude à prémunir n'est pas due à un seul type de substance.

Il semble aussi que, bien que la lumière favorise la prémunition, elle ne constitue cependant pas un facteur essentiel. Enfin, puisque ces fractions riches en chloroplastes ou en fragments de chloroplastes présentent l'effet maximal, on peut penser que cette propriété leur est liée.

Sommaire

Les extraits de feuilles saines possèdent des propriétés inhibitrices sur la réaction hypersensible. La fraction comprenant essentiellement les chloroplastes inhibe totalement la réaction hypersensible.

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Problem of Variation in *Xanthomonas malvacearum*

By

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Less virulent or weakly pathogenic isolates of races A, B, C and D of *Xanthomonas malvacearum* did not show specific relationship of L-serine inhibition of bacterial growth to host specificity or virulence. Race D is suggested to have been derived from Race A.

Introduction

I had earlier suggested the recognition of 4 races in *Xanthomonas malvacearum* (E. F. Sm) Dowson, and biotypes within each (NAYUDU, 1964). The races A, B, C and D are distinguished based on their restricted pathogenicity to the 4 species of cotton, *Gossypium hirsutum L, G. barbadense L, G. arboreum L,* and *G. herbaceum L.* Hirsutum and barbadense are allotetraploids (n: 26), and arboreums and herbaceums are diploids (n: 13). Race A is pathogenic to all 4 species, race B is non-pathogenic to *G. herbaceum*, race C is non-pathogenic to *G. arboreum*, and race D is non-pathogenic to both herbaceums and arboreums. Biotypes are recognized on the basis of variation in pathogenicity to varieties within a host species. I had also suggested derivation of races B and C from race A or D by two different mutations.

$$A \rightleftharpoons B \rightleftharpoons D$$
$$A \rightleftharpoons C \rightleftharpoons D$$

Early in 1968, most of the "5-year-old" cultures, particularly of races A, B and C, were lost due to a mistake. In November 1968, isolations were made from infected material of *G. arboreum* var. Adonicum and *G. herbaceum* var. Westerns-1, sent by the senior cotton Assistant, Adoni, Andhra Pradesh. Sixteen arboreum-isolates and 6 herbaceum-isolates were tested against the 4 species of cotton. Results are presented in Table 1 for some of the isolates.

To elucidate the possible amino acid nutritional relationship to variation in virulence, L-glutamic acid and L-serine relationships were studied. Methods were described earlier (NAYUDU, 1970). Table 2 presents the data.

Results and Discussion

None of the 22 isolates gave highly virulent reaction on any host, and most isolates were weakly pathogenic in contrast to isolates I worked with earlier. Isolates 066, 068 and 071 fall under race A; isolates 060, 065 and 074 under race B; isolate 053 under race C; and isolates 054 and 059 under race D. Thus arboreum-isolates fall under races A, B and D, while herbaceum-isolates fall under races C and D.

I have no evidence so far of any isolate from hirsutum or barbadense infecting the other two host species, and all these isolates are of D race only. Nor has

Table 1

Pathogenicity of isolates of *X. malvacearum* to 4 species of cotton

	053*	054	059	060	065	066	068	071	074
Laxmi*** (Hirsutum)	2**	2	1	2	2	1	2	2	2
Adonicum (Arboreum)	0	0	0	2	1	3	3	1	1
Westerns-1(Herbaceum)	2	0	0	0	0	2	3	2	0
Sea Island (Barbadense)	2	2	1	1	1	1	1	1	1

* 053, 054: Isolates from *G. herbaceum* var. Westerns-1. 059 to 074: Isolates from *G. arboreum* var. Adonicum.

** 0: no lesion; 1: lesions 10 or less per leaf, brown, minute and dry from the beginning, no yellowing; 2: lesions 100 or less per leaf, minute, brown and dry from the beginning, slight yellowing in the vicinity of lesions; 3: lesions 100 or more per leaf, water soaked and/or brown from the beginning, 1 mm, leaf yellowing; 4: lesions 100 or more per leaf, remain water soaked and continually expand up to one-and-half months time, 1—3 mm, turn brown and coalesce with time, leaf yellowing, drying, shedding, vein infection.

*** Standard differentials in use since 1963.

Table 2

Growth of different isolates of *X. malvacearum* as affected by L-serine*

Isolate**	Serine: μg N/ml		
	0	50	13
011	0.155	0	0.249
053	0.269	0	0.227
054	0.155	0.01	0.017
059	0.269	0	0.064
060	0.119	0.005	0.109
068	0.172	0.01	0.095
071	0.155	0	0.092
074	0.120	0	0.020

* Medium: K_2HPO_4: 0.12%; KH_2PO_4: 0.08%; $MgSO_4 \cdot 7H_2O$: 0.02%; Sucrose: 1%; L-glutamic acid: 200 μg N/ml.

** Isolate 011: from a hirsutum. Isolates 053, 054: from herbaceum. Isolates 059 to 074: from arboreum.

any D-type isolate over the 6-year period in culture changed to B or C type. Original A, B and C race-cultures remained stable during 1963–1968. Highly virulent race D culture has not so far been obtained from naturally infected arboreums or herbaceums.

The present recording of races A, B and D from arboreum, and C and D from herbaceum, even though of low pathogenicity, suggest derivation of race D from race A. HUNTER *et al.* (1968) recognize 15 races in U.S.A. using 7 hirsutum differentials. This probably satisfies the local needs where hirsutum and barbadense only are grown. Where four different species are grown even in adjacent fields as in India the problem is more complex.

In earlier work both a less virulent and a highly virulent isolate, derived from a hirsutum variety, were reported to be inhibited by even 13 μg N L-serine per ml but the less virulent isolate was inhibited less than the other (NAYUDU, 1970). The inhibitory effect was attributed to the amino group of serine probably involving polymerization. Using L-glutamic acid-1-^{14}C, it has been demonstrated that it is accumulated to a very high concentration in the presence of 50 μg N/ml of serine (NAYUDU, 1972). It was suggested that either bacterial cell permeability was altered allowing glutamic acid accumulation to toxic proportions, or the glutamic acid–serine polymer was not hydrolysed in the cells leading to their starvation.

Isolate 011 and other isolates from hirsutum and barbadense varieties in the present study were totally inhibited by 50 μg N serine per ml, but stimulated by 13 μg N serine per ml. Among the less virulent isolates derived from arboreum and herbaceum varieties, races A (068, 071), B (074) and D (054 and 059) are greatly inhibited by 13 μg N serine per ml, while the B type (060) and C type (053) are inhibited very little.

These results demonstrate the basic phenomenon of inhibition of bacterial growth by L-serine. At this stage, they cannot be related to host specificity of the races or virulence of the pathogen. This inhibitory effect of serine is already reported for *X. vesicatoria* (NAYUDU and WALKER, 1961), and is also noted in *X. Citri* (unpublished). Therefore, other phenomena will have to be looked for to explain host specificity of a pathogen and its virulence.

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Growth Inhibition of Virus-infected Plants: Alterations of Peroxidase Enzymes in Compatible and Incompatible Host-Parasite Relations

By

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The rate of growth inhibition of inoculated tobacco cultivars, *Nicotiana tabacum* L. cv. Samsun and cv. Xanthi-*nc* was measured one month after systemic infection with tobacco mosaic virus (TMV), U_1 strain and cucumber mosaic virus (CMV) white strain, respectively. A positive correlation was determined between the virus-induced host stunting and the enhanced activity of peroxidase enzymes of the host stem. The systemic infection did not induce synthesis of new peroxidase isozymes. The growth inhibition of the TMV-infected hypersensitive cultivar (Xanthi-nc) was not remarkable. Similarly, no significant change was experienced in this case in the peroxidase activities, except the soluble fraction of the inoculated leaves. New peroxidase isozymes appeared in the soluble and cell-wall bound fraction of the inoculated leaves, but there was no change in the isozyme spectra of the stem fractions. Permanent effect of the local virus infection on the peroxidase activity was detected only in the soluble fraction of the young, non infected leaves, which may be in relation to the systemic acquired resistance. No change of activities was registered in the peroxidase fractions of the stem, according to the nearly normal growth of the host. The dwarfing effect of systemic virus infection was reversed by treatment with gibberellic acid. Since gibberellin treatment may reduce indolaecetic acid oxidase activity in the plant cell-wall fraction one can suppose that the virus-induced dwarfing effect could be reversed by this way.

The pathogen increases without significant limitation in virus infected susceptible host plants inducing a systemic type of infection (compatibility). The systemic infection may be symptomless or visible symptoms may be found such as mosaic, chlorosis, leaf and flower abnormalities. One of the most characteristic symptoms is the inhibition of growth of diseased plants. This growth inhibition can be expressed by reduction of leaf size and the shortening of internodia. In most cases dwarfing induced by virus infection is the cause of yield loss having significant economic importance (Cf. HORVÁTH, 1971). In spite of this the metabolism of growth inhibition induced by virus infection has not been clarified from a pathophysiological point of view.

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Resistant host plant reacts to the infection of pathogen by a hypersensitive reaction and localizes it in the site of infection (incompatibility). In this host-parasite relationship (resistant plant) growth inhibition can not be expected or if so to a limited extent.

The explanation of the significant difference in the symptoms of the two host-parasite relationships may be connected to different metabolic changes in the host plant. The different metabolism of local and systemic virus infections has been reported (FARKAS, KIRÁLY and SOLYMOSY, 1960; FARKAS and SOLYMOSY, 1965; GOODMAN, KIRÁLY and ZAITLIN, 1967). The intention was primarily centered on the cause of the resistance. Particularly, the increased activity of peroxidase was involved in the formation in active zones surrounding of local lesions (ROSS, 1961a and b).

The indoleacetic acid oxidase enzyme – indirectly regulating growth – was found to be identical with peroxidase (RAY, 1958). Determining its localization in the cells (HACKETT and RAGLAND, 1962; RIDGE and OSBORNE, 1971; SÁGI, 1970, 1971) provided the opportunity for the investigation of this enzyme not only in the soluble, but in the cell-wall bound fraction too.

The peroxidase activity of virus infected plants has been measured in many cases. However, former investigations did not extend to the separation of certain peroxidase fractions, on the other hand the obtained results were not related to the abnormal growth due to virus infection.

In the present study the activity of peroxidase was examined in the leaf and stem tissues. Our results indicated, that after the systemic virus infection the activity of peroxidase enzyme increases permanently both in stem and leaf in all of the fractions, without the formation of new isozymes. On the contrary in resistant host plants, in the course of the hypersensitive reaction, marked activity can be detected both in the soluble and the cell-wall bound fraction of infected leaves. This, however, is accompanied by the appearance of new isozymes. New isozymes do not occur in the bound and soluble fractions of the stem. The rise of peroxidase activity in stems is only temporary.

Material and Method

Host-parasite relations. For investigating of induced growth inhibition by virus infection tobacco (*Nicotiana tabacum* L. cv. Samsun and cv. Xanthi-*nc*) seems to be a suitable material. Tobacco plants, grown under normal greenhouse conditions, were used for virus inoculation in the 6–8 leaf stages.

Tobacco mosaic virus (TMV, U_1 strain) and cucumber mosaic virus (CMV, white strain) were used for inoculations. One month after virus infection remarkable growth inhibition was detectable in the compatible host-parasite relation. A hypersensitive tobacco (Xanthi-*nc*) was applied as local host plant for TMV infection. Estimation of growth inhibition of diseased plants and investigation on

the activity of peroxidase enzyme were carried out on systemically infected plants one month after inoculation.

In the case of local infections peroxidase activity was measured 5–6 days after inoculation i.e. at the very time of symptom development. For investigating the permanent effect of local virus infection on peroxidase of this enzyme was measured one month after inoculation in the non-infected leaves and stems. The measurements were carried out in each season.

Peroxidase. For examining of peroxidase activities the samples were taken from upper third of plants. Ten grams of fresh weight of leaf and stem samples were ground in equal volume of cold distilled water in mortar and then were homogenized in a Waring blendor. After centrifugation of aqueous extract (1500 rev/min, for 10 min) the supernatant was separated. The sediment was suspended again in distilled water, ground in a mortar and centrifuged again. The supernatant fractions were collected in 8 replications. The 8–10. fractions having no more peroxidase activity were discarded. The collected supernatants were centrifuged in a preparative ultracentrifuge (Janetzki VAC 60 type, 105.000 g, for 1 h) and the supernatant was used for measuring the soluble peroxidase activity.

The cell-wall bound peroxidase activity was measured in the residue of distilled water fractionation, with a phosphate buffer (0.15 *M*, pH 7.2) containing 0.3 *M* NaCl. The activities were measured according to the method of BELOSERSKI and PROSKURJAKOV (1956). For determining the protein content the method of LOWRY *et al.* (1951) was used. An aliquot of the extracts was used for polyacrylamide gel electrophoresis. The dialized enzyme extracts were concentrated in 50% polyethylenglycol (Carbowax 20.000). Amounts equivalent to 100–200 μg protein were taken on gel (Cyanogum 41.5%) and were run in 0.1 *M* TRIS-EDTA buffer (30 min 2.5 mA and 90 min 5 mA per tube). The gels were soaked in 0.2 *M* Na-acetate buffer (pH 5) and the peroxidase activities were determined by incubation in solutions of benzidine and hydrogen peroxide.

Results and Discussion

Growth inhibition induced by virus infection

Systemic mosaic symptoms of TMV and CMV appeared 12–15 days after inoculation. Growth inhibition could be observed from the second week following inoculation. The number of leaves developed on the infected plants was similar as on the control, however, the internodia became shorter. The growth inhibition induced by virus infections are summarized in Table 1.

The resistant host plants react to virus infection by developing local necrosis (incompatibility). Local necroses can be seen from the second day following infection. At the high inoculum concentration used by us 50% of the leaf surface

Table 1

Growth and leaf weight of healthy and virus infected plants*

Host-parasite relation	Stem length (cm)	Leaf weight (g)
TMV–Samsun (compatibility)	8.6	61.5
Control Samsun	19.0	89.0
CMV–Xanthi (compatibility)	9.5	50.0
Control Xanthi	16.3	101.5
TMV–Xanthi (incompatibility)	16.5	88.5
Control Xanthi	17.9	99.6

* Measures one month after inoculation
TMV = tobacco mosaic virus; CMV = cucumber mosaic virus

necrotized. One can see in Table 1 that the growth of resistant Xanthi plants were not significantly influenced by TMV-infection.

Changes in peroxidase activity

Investigations so far have shown that the oxidative metabolism of systemically infected leaves slightly increases with the appearance of symptoms. In certain cases it is in correlation with virus multiplication (LOEBENSTEIN and LINSEY, 1961; NOVACKY and HAMPTON, 1967; WOOD and BARBARA, 1971; STAHMANN and DEMOREST, 1972). According to our measurements (Table 2) peroxidase activity

Table 2

Peroxidase activities in susceptible host–parasite relation

Plant part	Peroxidase fraction	Peroxidase activity*	
		TMV–Samsun	CMV–Xanthi
Leaf	soluble	1.30	0.99
	bound	1.49	1.60
Stem	soluble	2.69	1.80
	bound	2.07	1.32

* Specific activity rates presented per 10 μg protein
TMV = tobacco mosaic virus; CMV = cucumber mosaic virus

showed a slight rise in the leaves, both in the cytoplasmic and the cell-wall fractions. Only the activities in the cytoplasmic fraction make a comparison with date of literature possible. The so-called "soluble fraction" does not contain ribosomal or mitochondrial peroxidase. In this way our results are in accordance with other

data, although in our experiments the values are generally lower. This is especially the case in Xanthi-TMV relationship.

The peroxidase enzyme activities measured in the stem, similarly showed an increasing tendency, which proves the permanent efficiency of a general stress effect connected with infection. Activities measured in soluble and bound fraction of the infected plants rise by about twofold as compared to the control. The increased peroxidase activity measured in the stem could have a remarkable role in the oxidation on indoleacetic acid responsible for cell elongation.

In the case of resistant plants the necrogenic reaction connected with virus infection activates a series of enzymes in infected leaves both at the site of necroses and in the zones surrounding the lesions (SOLYMOSY and FARKAS, 1963). The peroxidase activities show a maximum at the very time of symptom development (VAN LOON and GELEEN, 1971).

Peroxidase activities established 5–7 days after inoculation in the inoculated leaves are shown in Table 3. The data obtained at time of symptom appearance

Table 3

Peroxidase activities in incompatible host–parasite relation

Plant part	Peroxidase fraction	Peroxidase activity*	
		TMV-Xanthi**	TMV-Xanthi***
Leaf	soluble	3.60	1.29
	bound	1.29	0.98
Stem	soluble	0.95	0.72
	bound	1.44	1.02

* Specific activity rates presented per 10 μg protein
** Measurement 6 days after inoculation
*** Measurement 30 days after inoculation in the newly developed parts

demonstrate deviations according to the enzyme source. The marked increase in the activity of the soluble fraction of inoculated leaves is well-known from the literature. Much more important is the fact that the peroxidase activity of other leaf or stem fractions shows only a slight change.

Our data are in accordance with symptom observations. After inoculation a stress effect is going on in the resistant plants and if the inoculation is accompanied by necrosis the host may lose one or more leaves. Thus, disturbances in growth are only temporary in the host, therefore plants can grow normally. According to SIMONS and ROSS (1970, 1971) peroxidase activity in the upper non-infected leaves of locally infected host plant remains at a high level three weeks following inoculation. In the newly developed healthy leaves the peroxidase activity is in

correlation with the systemic acquired resistance. As regards enzymes other than peroxidase such lasting effect could not be observed.

This results are apparently in contradiction with the quite normal development of infected plants. If the peroxidase activities are measured in the cell-wall bound and soluble fractions both in the leaves and the stems, this contradiction disappears. One month after infection peroxidase activities are smaller than the values obtained during the lesion development. The activities in bound fraction or the stem fractions are similar to the control. An increase in the enzyme activity occurs only the enzyme fraction having no importance from the point of view of stem elongation.

Peroxidase isozymes

Not only the activities of peroxidase enzymes show changes as a result of virus infection, but the number of components are also altered. According to the data so far, the enzyme composition in systemic host-parasite relations does not change, but in the case of local lesion hosts new isoenzymes appear after infection (LOEBENSTEIN and LINSEY, 1961; FARKAS and STAHMANN, 1966; VAN LOON and GEELEN, 1971; STAHMANN and DEMOREST, 1972). In contrast to these observations NOVACKY and HAMPTON (1967; 1968) and CHANT and BATES (1970) found that in the local lesion hosts the "new" peroxidase isoenzymes are only the already existing isoenzymes activated during the process of senescence. FARKAS and STAHMANN (1966) and STAHMANN and DEMOREST (1972) also found new isoenzymes related to senescence, but among these was one new isozyme which occurred only in virus infected plants. The development of the new isozyme can be prevented by protein inhibitors.

It must be stressed that the distribution of peroxidase isozymes in virus-infected plants was investigated in the above-mentioned cases according to a quite different experimental design. So there is no possibility to compare the data of the literature with our results except one field e.g. the cytoplasmic fraction (SOLYMOSY *et al.*, 1967).

As was expected, no new isoenzymes appear in the cytoplasmic fraction of the leaf of systemically infected tobacco (Fig. 1). Only the different discolouration of certain isozymes refers to the enhanced peroxidase activity of infected plants. Similar results were obtained in the other fractions, too. Results of figures are only representative ones. Measurements were repeated several times, during the four seasons, but in the systemic infection of TMV and CMV we have never found new isoenzymes, although there were some differences in the number of isoenzymes. MCCOWN *et al.* (1969) also reported on similar alterations in the isoenzyme spectra in relation to the seasons. Certain isozymes of systemically infected plants showed markedly high activity which is in accordance with the spectrophotometrical investigations.

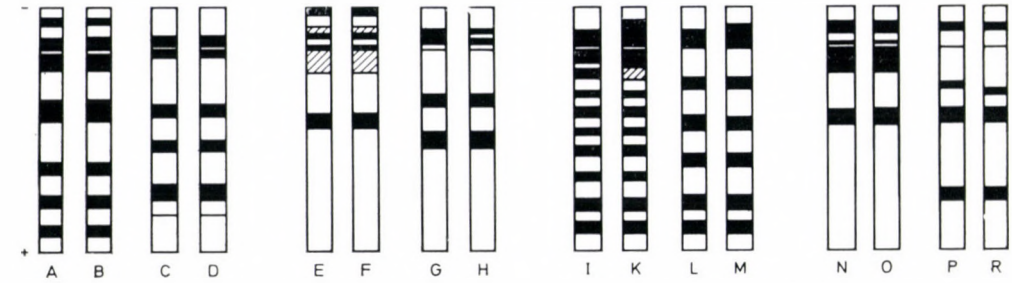

Fig. 1. Peroxidase isozyme spectrum in compatible host-parasite relation, in the leaf and stem fractions of systemically infected tobaccos
Leaf soluble: A TMV – Samsun; B C – Samsun; C CMV – Xanthi; D C – Xanthi. Leaf bound: E TMV – Samsun; F C – Samsun; G CMV – Xanthi; H C – Xanthi. Stem soluble: I TMV – Samsun; K C – Samsun; L CMV – Xanthi; M C – Xanthi. Stem bound: N TMV – Samsun; O C – Samsun; P CMV – Xanthi; R C – Xanthi. TMV = tobacco mosaic virus; CMV = cucumber mosaic virus; C = control

Fig. 2. Peroxidase isoenzymes in incompatible host-parasite relation, in the stem and leaf fraction of local infected tobaccos
Leaf soluble: A C – Xanthi; B TMV – Xanthi; Leaf bound: C C – Xanthi; D TMV – Xanthi. Stem soluble: E C–Xanthi; F TMV – Xanthi. Stem bound: G C – Xanthi; H TMV – Xanthi

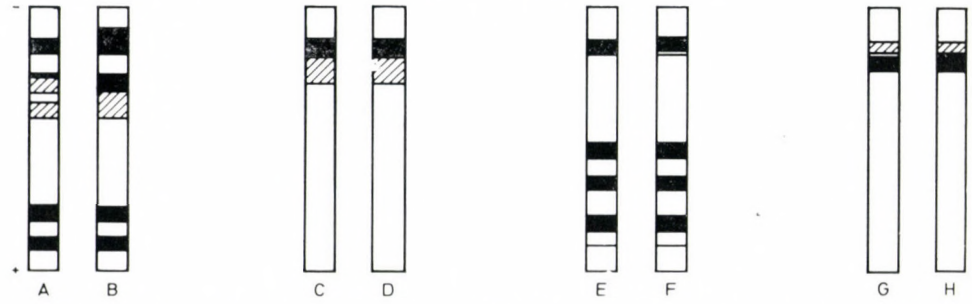

Fig. 3 Peroxidase isoenzymes in incompatible host-parasite relation 5 and 30 days after inoculation
Leaf soluble: A Inoculated leaf; B Newly developed leaf. Leaf bound: C Inoculated leaf; D Newly developed leaf. Stem soluble: E Five days after inoculation; F Thirty days after inoculation. Stem bound: G Five days after inoculation; H Thirty days after inoculation

In the resistant plants new isoenzymes appear as a consequence of local virus infection, not only in the cytoplasmic but also in the cell-wall bound fraction, indicating significant change in the enzyme system of the cell-wall fraction. Two new isozymes appear in the soluble and one in the cell-wall bound fraction (Fig. 2).

Especially important is the fact that the new isozymes induced by TMV are partly cationic peroxidases. We should like to note that one cationic isozyme of horse-radish peroxidase is more active in ethylene production than the anionic ones (YANG, 1968). The rise of peroxidase activity in hypersensitive tobaccos may be related to the enhanced ethylene production of infected plants (GÁBORJÁNYI, BALÁZS and KIRÁLY, 1971; NAKAGAKI and HIRAI, 1971).

We have not found new peroxidase isozymes in stem fractions, which mean that in this case the virus infection has only local effect on peroxidases. After a one-month-infection isoenzyme changes were not observed in the stem of resistant host plant compared with the control, whereas isozymes of soluble and bound peroxidase showed a higher activity than the control (Fig. 3). This higher activity is connected with the increased activity of peroxidase measured spectrophotometrically. Permanent effect of virus infection, therefore, is detectable only in the newly developed leaves.

Reversion of induced dwarfing

The dwarfing effect induced by systemic virus infection may be due to peroxidase enzymes (primarily to the indoleacetic acid peroxidase). One can suppose that by treatment with hormones, the stunting caused by the virus can be reversed. For this purpose we treated tobacco plants systemically infected with cucumber mosaic virus with indole-3-acetic acid, 3-indolyl-acetamide, 5-hydroxy-indole-3-yl-acetic acid, gibberellic acid (GA_3), benzyladenine four times, weekly. The inhibition of growth caused by virus infection could be reversed by gibberellic acid (Table 4).

Table 4

Reverse of dwarfing effect of systemic virus infection on Xanthi tobaccos*

Treatment	Stem length (cm)	Leaf weight (g)
Indol-3-acetic acid (10 ppm)	17.2	61.3
3-Indolyl-acetamide (10 ppm)	14.7	57.6
5-Hydroxy-indole-3-yl-acetic acid (10 ppm)	14.2	59.6
Gibberellic acid (GA_3) (10 ppm)	20.4	72.0
Benzyladenine (30 ppm)	12.9	41.5
Non-infected control	21.8	85.7
Infected control	14.4	59.2

* Measurement a month after inoculation with cucumber mosaic virus (Averages from 20-20 plants)

The indoleacetic acid increases the weight of plants only in a small extent and was not effective enough in reversing of dwarfing. The other hormons proved to be ineffective. The exogenous indoleacetic acid has a slight effect because one part of it becomes bound (cf.: ANDREA E and GOOD, 1955), on the other hand, as a substrate of indoleacetic acid peroxidase induces the formation of this enzyme (GALSTON and DALBERG, 1954).

The results achieved with gibberellic acid are in accordance with earlier observation of KURAISHI and MUIR (1962). Gibberellin treatment increased the endogenous auxin level of plants. In addition, SÁGI (1972) detected a lower activity of indoleacetic acid peroxidase in the cell-wall fraction of gibberellin treated plants. In this way the stunting effect induced by a virus infection can be controlled by gibberellic acid. MARAMOROSCH (1957) and ORLOB and ARNY (1961) similarly reversed dwarfing in plants infected by virus or mycoplasma. Our experimental results may refer to the reversing mechanism of virus-induced dwarfing effect.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

VIII. Purification, Properties and Serology of *Erysimum* Latent Virus

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Erysimum latent virus (ELV) was sufficiently purified by the butanol-chloroform method and by two cycles of differential centrifugations. After density gradient centrifugation, two virus-specific bands were found, top and bottom components (TC and BC). Only the latter proved to be infective. Sedimentation coefficient (S_{c20}) of BC was determined as 113.4. BC possesses UV absorption properties of a nucleoprotein. The extinction quotient at wave lengths of 280 and 260 nm ($Q_{E280:260}$) was 0.54 to 0.56 and the E_{max} : E_{min} ratio fluctuated between 1.3 and 1.5. UV absorption of TC indicated the presence of a protein with additional substances strongly absorbing in UV range between 280 and 250 nm. The TC complex could not be separated by repeated density gradient centrifugations. Phenol-extracted RNA of ELV has maximum and minimum UV absorption at 258 and 230 nm, respectively, and a $Q_{E280:260}$ between 0.47 and 0.48. In dilution tests, RNA had an infectivity by about 14 per cent in comparison to intact virus. An ELV antiserum had a titre of 1 : 1024 against the homologous virus in OUCHTERLONY test. No serological differences could be detected between TC and BC preparations. The antiserum neither reacted with healthy plant sap nor with radish mosaic, turnip crinkle, turnip rosette, turnip yellow mosaic, and cauliflower mosaic viruses. Electron microscopical investigations showed that BC consisted of intact virus particles whereas TC represented empty protein shells. The diameter of intact ELV particles proved to be 21 nm in negative staining preparations and 30 nm in shadow casting preparations. In comparison to other crucifer viruses, ELV seems to be most similar to turnip yellow mosaic virus. However, the investigations supported the idea that ELV is a fifth distinct entity within the group of beetle-transmissible crucifer viruses.

Erysimum latent virus (ELV) was described as a previously unknown crucifer virus (SHUKLA and SCHMELZER, 1972). Its host range is mainly confined to species of *Cruciferae*. ELV has a dilution end point at 1 : 500,000, a thermal inactivation point between 76° and 78°C and is stable in crude sap at room temperature up to 21 days. It is easily transmissible by mechanical inoculation, but transmission tests with *Myzus persicae* Sulz. as well as through seeds failed. These properties are similar to those of other crucifer viruses, known to be transmitted by chewing insects. However, no close relationship could be demonstrated between

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ELV and the earlier found beetle-transmitted crucifer viruses in serological and cross-protection tests (SHUKLA and SCHMELZER, 1972). The present investigations were conducted to find a method of purifying ELV, to characterize its physical properties and its morphology and to develop an antiserum for further studies on its relationships.

Materials and Methods

Culture and assay of ELV. The virus was propagated for purification in *Sinapis alba* L. or in *Brassica chinensis* L. because it was shown that ELV is rather highly concentrated in these species (SHUKLA and SCHMELZER, 1972) and they grew readily in the greenhouse under the normal conditions maintained in the tests. Methods of preparing inoculum and performing inoculations were the same as described earlier (SHUKLA and SCHMELZER, 1970a). Infectivity assays were done on *Sinapis alba* and/or *Brassica napus* var. *napobrassica*. The latter is a good local lesion host of ELV.

Purification. Systemically and locally infected leaves, together with their petioles, were harvested two weeks after inoculation and homogenized in a Waring blendor in 1 : 2 (weight per volume) 0.05 or 0.067 *M* phosphate buffer, pH 7.0. This buffer was used throughout the experiments. The resulting pulp was pressed through Dederon cloth and the sap was subjected to one of the following three procedures for abolishing host constituents: ether/carbontetrachloride (WETTER, 1960), butanol (TOMLINSON *et al.*, 1959) and butanol-chloroform (STEERE, 1956). The latter method was eventually found best as the partially purified virus preparations were highly infective and relatively clear when examined under electron microscope in comparison to the other methods. Therefore, this procedure was followed in subsequent trials:

To the squeezed sap, a mixture of equal volumes of n-butanol and chloroform twice in volume of sap was added slowly. The liquid was constantly stirred for 20 minutes. The resulting emulsion was separated by low-speed centrifugation (Janetzki GDR, K 23, 2000 g for 15 minutes). The clear yellow supernatant aqueous phase was subjected to two cycles of differential centrifugation (high-speed-Janetzki GDR VAC 60, 80,000 g, 120 minutes and 110,000 g, 60 minutes for first and second cycles, respectively; low-spead-Janetzki GDR K24, 10,000 g, 10 minutes, common to both cycles). The final pellets were resuspended in 1 to 1.5 ml phosphate buffer per 100 gram of starting material. All operations, except centrifugations, were carried out at room temperature. Most of the work was done in cooler months. Infectivity tests were performed after each step of purification.

Density gradient centrifugation. By the aid of preparative density gradient centrifugations in sucrose (BRAKKE, 1953), the partially purified virus preparations were fractioned. All density gradient centrifugations were done in Spinco L-2 50 using the rotors SW 25.2, 25.1 (preparative run) and SW 39 (analytical run).

a) *Preparative density gradient centrifugation.* The gradients were prepared by layering 50, 40, 30, 20 and 10 per cent (w : v) sucrose solutions in phosphate buffer into the tubes. The tubes of rotors SW 25.2 and SW 25.1 were filled with 13, 10, 10, 10, 12 ml and 5, 7, 7, 7 ml respectively. In the latter case the most concentrated solution was omitted. The gradients stood for 24 to 36 hours at 4°C before being used. On the large gradients 3 ml and on the little 1 to 2 ml of partially purified virus preparations were layered. The rotors were centrifuged at 24,000 rpm for 3 hours at 5°C.

The opalescent bands, seen after centrifugation, were sucked by puncturing the tubes with a hypodermic syringe. The extract was diluted 2 to 3 times of its volume in phosphate buffer and centrifuged at 110,000 g for 90 minutes. The virus sediment was dissolved in a small quantity of buffer and finally centrifuged at 10,000 g for 10 minutes.

In some experiments we fractionated the whole sucrose gradients. For this the bottom of the tubes was punctured with a syringe and a 60 per cent sucrose solution was pumped slowly into the tube in order to press the gradient column through a capillar into the flow cell of the photometer Uvicord II (LKB-Producter, Stockholm, Sweden), where the UV-absorption was measured at 254 nm and recorded.

b) *Density gradient centrifugation in acrylamide.* To determine purity and homogenity of the virus preparations, fixing and staining of proteins in polymerized sucrose gradients were done by the method of JOLLY *et al.* (1967). We used sucrose gradients of 5 to 20 per cent in 7.5 per cent acrylamide, pH 7.0. After a centrifugation at 128,000 g for 90 minutes in SW 39 rotor, the tube content was photo-polymerized and subsequently fixed and stained with 0.07 per cent amido black solution, prepared in 7 per cent acetic acid, for 45 minutes. The excess dye was washed off with 7 per cent acetic acid.

Analytical ultracentrifugation. Sedimentation constants were determined in the analytical ultracentrifuge U 50 L (Phywe AG Göttingen, FRG) using schlieren optics. The data were calculated by the graphical (MARKHAM, 1960) and integral (ELIAS, 1961) methods.

Isolation of RNA. ELV RNA was isolated by treating the purified virus (bottom component) with phenol in the presence of bentonite and sodium dodecyl sulphate in the manner described by BOCKSTAHLER and KAESBERG (1965). Infectivity of virus and RNA, obtained from the same preparation, was compared in four concentrations on *Brassica napus* var. *napobrassica* immediately after isolation. Dilutions were prepared with phosphate buffer. The test plants were selected for uniformity and only the second and third leaves of each plant were inoculated. Six plants per treatment were used. Local lesions were counted 5 days after infection.

UV absorption. All ultraviolet absorption determinations of the virus components and RNA were done with an universal spectrophotometer of VEB Carl Zeiss Jena, GDR.

Preparation of antiserum. Antiserum to ELV was prepared by injecting a rabbit three times intravenously at 3 day intervals with 2 ml partially purified virus preparations. Two days after the last injection, blood was taken and the antiserum was tested against ELV and other viruses by the agar-gel double diffusion method as described by SHUKLA and SCHMELZER (1970b). The antiserum was stored in a refrigerator at about $-5°C$.

Electron microscopy. Samples of purified virus preparations were examined under an Elmi D (VEB Carl Zeiss Jena) electron microscope. For shadow mounts, a droplet of the virus solution was placed onto collodion-coated grids and then shadowed with a mixture of Platinum and Iridium (4 : 1) at an angle of 35°. Negative staining preparations were made by mixing equal parts of a 4 per cent aqueous solution of sodium tungstate (pH 5.0) and virus suspension.

Results

Purification and properties

ELV preparations, obtained after two cycles of differential centrifugations, were bluish-opalescent and contained high concentrations of virus. The partially purified virus, when subjected to density gradient centrifugation, was found to consist of several components. Besides a diffuse light scattering zone just below the meniscus, indicating unspecific impurities, two clearly defined opalescent bands were visible in the middle range of the gradients (Fig. 1 below). They are hereafter referred to as top (TC) and bottom (BC) components, respectively. Fractionation of the gradients showed that TC absorbed considerably less light at 254 nm than BC (Fig. 1, above). Only samples taken from the highly opalescent BC proved to be infective. After repeated concentration of the both united components, the preparations did not contain any impurity which could be stained by amido black in polymerized gradients but only the two clearly separated bands TC and BC (Fig. 2).

Preparations from healthy plants, made in the same manner as from diseased ones, did not show any opalescent band, but only the diffuse light scattering zone below the meniscus of the gradients.

The purified virus preparations proved not to contain impurities of high molecularity also in analytical centrifugations. BC sedimented as a homogeneous gradient (Fig. 3). The slight distortion of the gradients in the direction of sedimentation indicated a certain amount of aggregated material. The sedimentation coefficient (S_{c20}) was found to be 113.4. The S-value of TC could not be determined due to weak concentration.

BC possessed UV absorption properties of a nucleoprotein with a maximum and minimum of 258–259 and 239, respectively (Fig. 4). The quotient of extinction at 280 and 260 nm ($Q_{E280:E260}$) gave values between 0.54 and 0.56. The $E_{max} : E_{min}$ ratio fluctuated between 1.3 and 1.5.

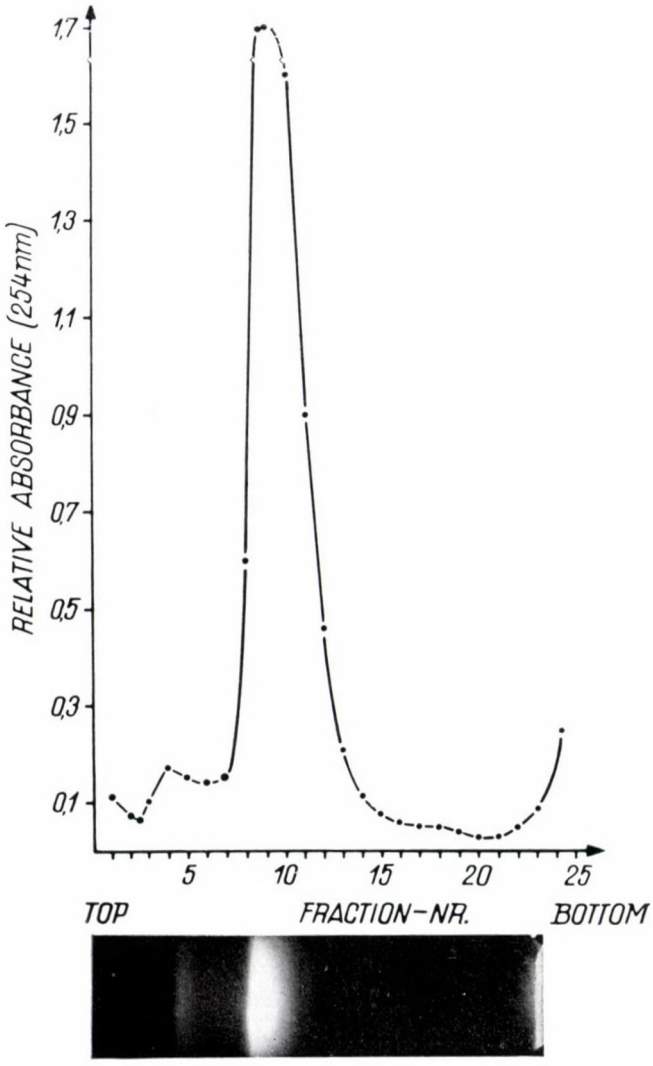

Fig. 1. The two components of ELV after density gradient centrifugation. Above: relative UV absorbance of the different fractions at 254 nm, below: opalescence of the two components of ELV in a sugar gradient tube. The positions of strongly UV absorbing fractions and opalescent bands in the tube are in good accordance

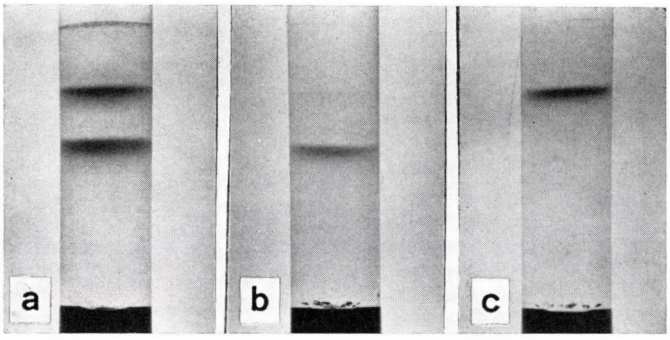

Fig. 2. ELV density gradients in acrylamide, stained with amido black. a: both components together, b—c: separation of the two bands in bottom (b) and top (c) components after fractionation. The third band in a is due to unspecific colour adsorbing substances at the meniscus

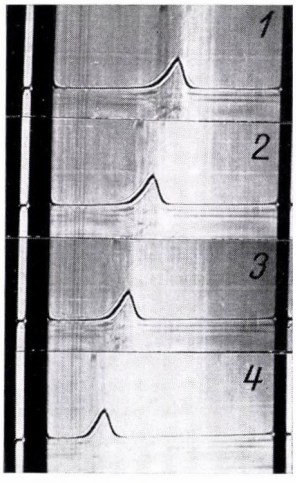

Fig. 3. Sedimentation pattern of the infective bottom component of ELV in analytical centrifugation. Photos taken at intervals of 4 minutes, centrifugation at 27,000 rpm

UV absorption measurements of TC showed neither a typical protein nor a nucleoprotein spectrum. The trend of the absorption curve suggested for a protein with adherent substances which strongly absorb in UV range between 280 and 250 nm. A repeated purification of TC by density gradient centrifugation did not lead to any change in its UV absorption properties. In polymerized gradients only one component could be detected. Because of these results, it was thought that BC consists of intact ELV virus particles whereas TC represents mostly hollow protein shells of that virus.

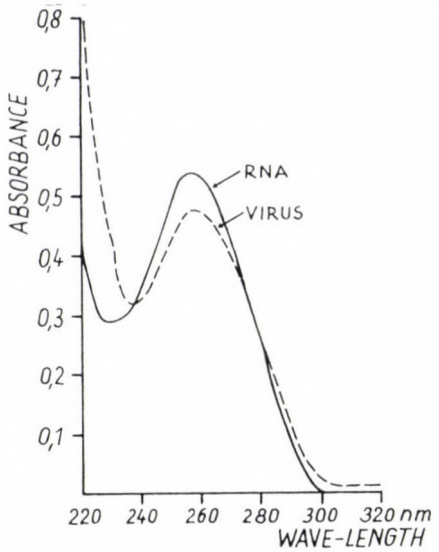

Fig. 4. UV absorption curves of the bottom component (infective virus) and of RNA preparation of ELV

Virus RNA and its infectivity

The phenol-extracted ELV RNA possessed following UV absorption characteristics: maximum and minimum at 258 and 230 nm, respectively (Fig. 4) and an extinction quotient ($Q_{E220:260}$) between 0.47 and 0.48. The percentage RNA content of the virus was not investigated. Infectivity tests proved that ELV RNA is infectious by about 14 per cent in comparison to the intact virus. Table 1 shows the obtained data at different dilutions.

Table 1

Infectivity comparison of intact ELV and its RNA on *Brassica napus* var. *napobrassica*

Concentration	Intact virus	RNA	Per cent infectivity of RNA
Undiluted	457.0*	65.1	14.2
1 : 10	262.2	34.5	13.7
1 : 100	164.8	24.8	15.0
1 : 1000	71.6	10.0	12.5

* Average number of local lesions per leaf, calculated from 12 leaves per treatment

Serology

An antiserum, produced by injection of partially purified ELV, reacted positively with crude sap of *Brassica chinensis*, *B. juncea* (L.) Czern. at Coss., *B. pekinensis* (Lour.) Rupr., and *Sinapis alba* systemically infected by this virus. A maximum titre of 1 : 1024 was obtained in OUCHTERLONY tests. Always only one virus specific line occurred. No precipitation line of any type could be seen when the antiserum was tested against crude sap from healthy plants of the mentioned species. Purified virus preparations were also tested. TC and BC reacted at maximum antiserum dilutions of 1 : 128 and 1 : 512, respectively. No spur formation was observed when the two components were tested side by side (Fig. 5), thus

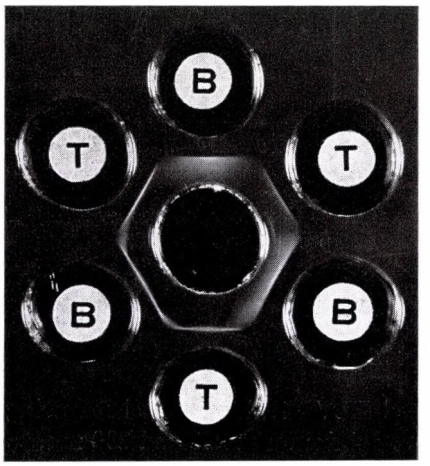

Fig. 5. OUCHTERLONY tests with ELV antiserum (middle well) and top (T) and bottom (B) components of this virus. There is no spur formation

showing that the antigenic active parts of both are identical. The antiserum dilution used for this test was 1 : 16. Crude sap of *Brassica napus* var. *napobrassica* and *B. pekinensis* infected with radish mosaic, turnip crinkle, turnip rosette, and turnip yellow mosaic viruses did not give any reaction with ELV antiserum. The antiserum showed no reaction also with crude sap from cauliflower leaves infected with cauliflower mosaic virus.

Morphology of the virus particles

Purified TC and BC of ELV, obtained after density gradient centrifugation, were examined separately under electron microscope. Virus particles in the shadowed BC and negatively stained TC preparations were found to be uniform and polyhedral in shape (Fig. 6a and 7b). In the negatively stained BC pre-

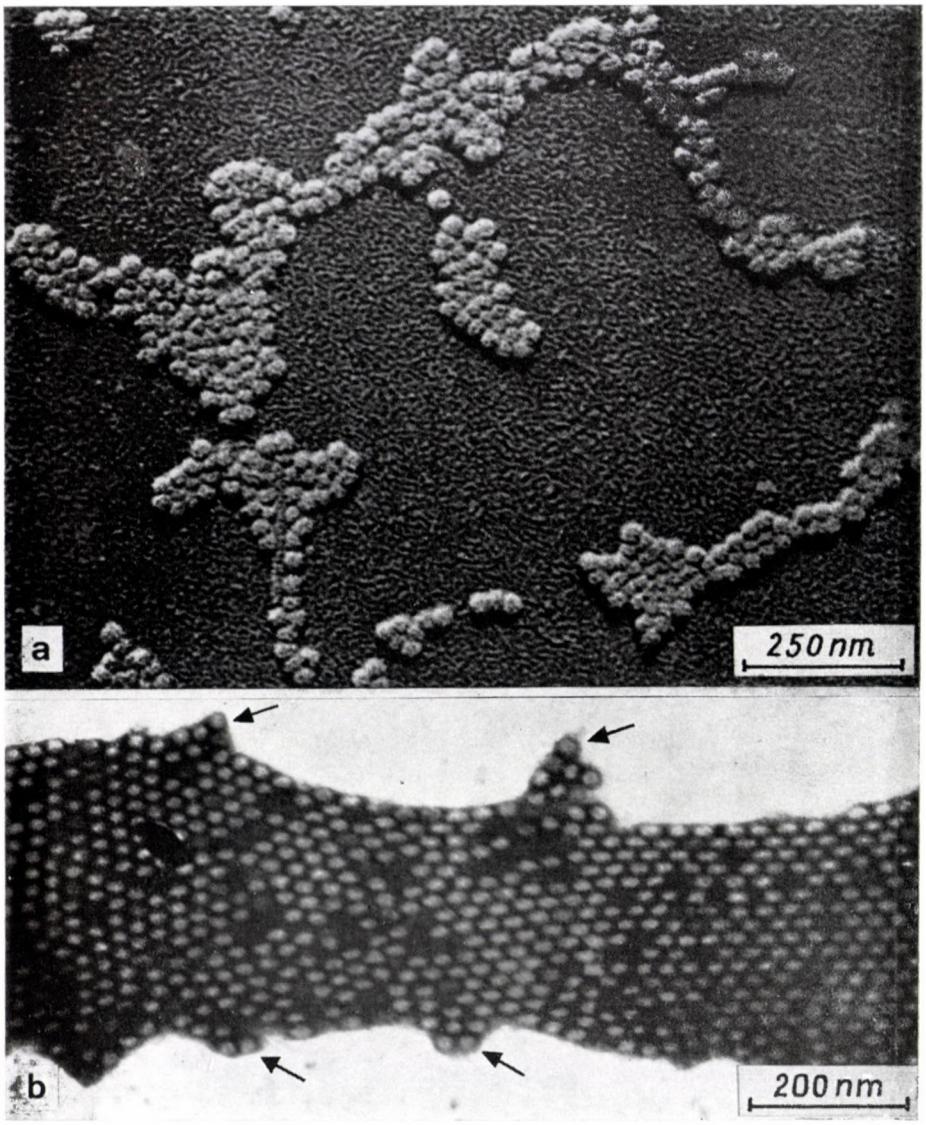

Fig. 6. Electron microscopy of bottom component of ELV (intact virus). a: metal-shadowed preparation, b: negative stain preparation. Arrows indicate not stretched particles

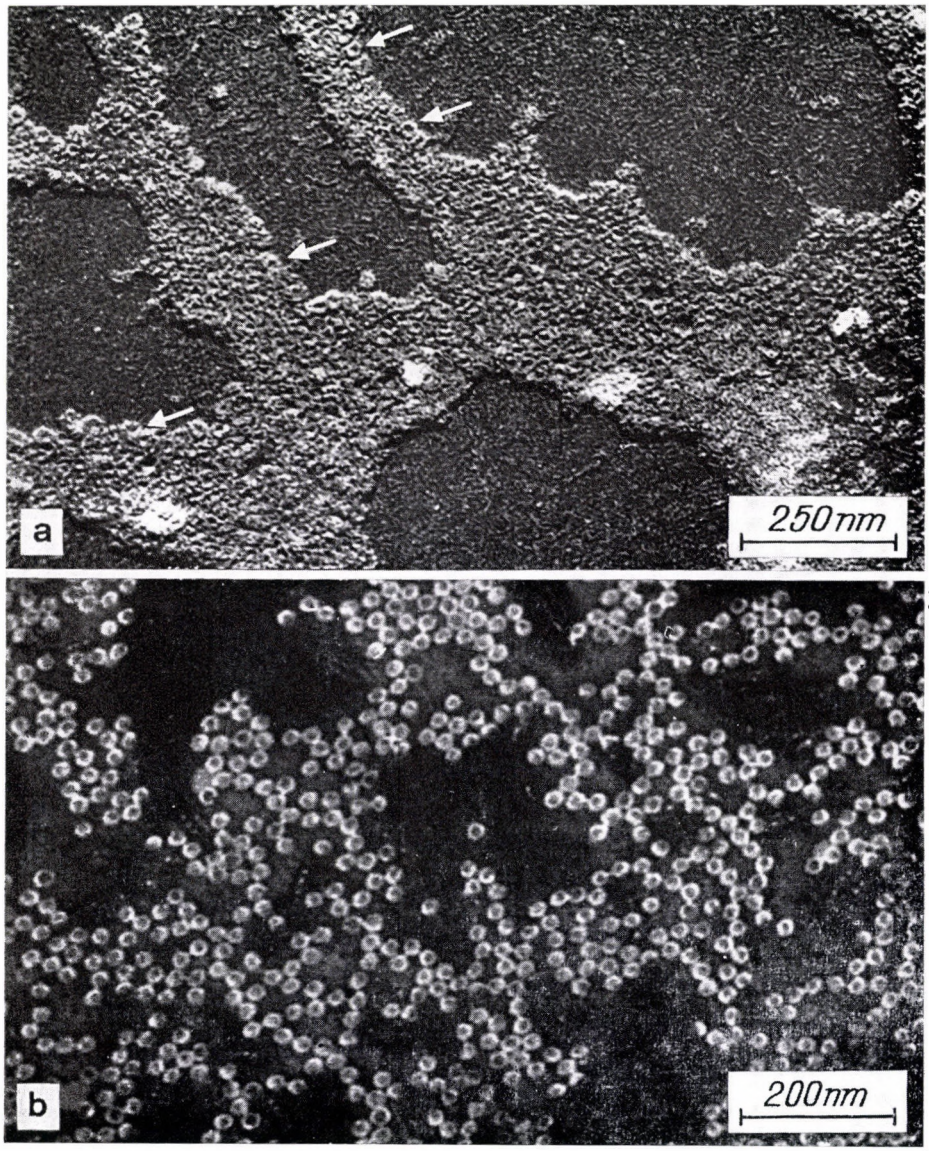

Fig. 7. Electron microscopy of top component of ELV (hollow protein shells). a: metal-shadowed preparation, b: negative stain preparation. Arrows indicate hollow particles in the shadowed preparation

paration, the particles are mostly to be seen stretched and closely packed together (Fig. 6b). However, this is believed to be an artifact. Particles in the shadowed preparation from TC are seen strongly aggregated and appear to have lost their structural integrity. Some of them, especially those lying on the border of the aggregated mass, show a hollow centre as in the negatively stained preparation of TC (Fig. 7a). The average diameter of virus particles in the shadowed preparation was 30 nm and in the negatively stained preparation 21 nm. Although it was rather difficult to measure the exact diameter of the particles in the shadow mounts of TC, apparently there was no difference in size between particle of TC and BC.

Discussion

The evidence presented in this paper indicates that ELV occur in considerably high concentration in its hosts. Butanol-chloroform procedure is obviously very suited for purifying such a stable virus by removing most of the impurities due to host plants. This seems to be the reason for lack of host antibodies in the antiserum. The same result was obtained by other workers when this method was used (DIAS and HARRISON, 1963; TAYLOR and HEWITT, 1964). As photometric scanning and analytical ultracentrifugation showed, highly purified preparations of ELV can be obtained by density gradient centrifugation.

ELV has been found to be a two component virus having top (TC) and bottom (BC) components. This result is substantiated by the absence of opalescent zones in healthy preparations and by serology as well as by electron microscopical investigations of both components. The non-infectious nature of TC and its hollow appearance in the negatively stained preparations suggests that the particles of this component consist of only protein shells without RNA, a characteristic known for several other viruses, especially for turnip yellow mosaic virus (KAPER and STEERE, 1959). Also for this virus it was described that negative stain penetrates into the space enclosed by the capsid which is occupied by RNA in normal virus particles (FINCH and KLUG, 1967). There was no difference in antigenic properties of TC and BC of ELV. A relatively low antiserum titre obtained with the former may be due to its lower concentration.

About 14 per cent infectivity of RNA in comparison to the intact ELV appears to be rather high. The remarkable constant results in comparing four different concentrations of both indicated that *Brassica napus* var. *napobrassica* may be a sensitive and reliable local lesion host of ELV and its RNA. However, this should be confirmed in further investigations.

The average diameter of virus particles in the negatively stained preparations has been found to be 21 nm as against 30 nm in shadow mounts. This result is not surprising because in general negatively stained preparations give lower values for virus diameters (HARRISON and NIXON, 1960; SCHMIDT, 1967).

SHUKLA and SCHMELZER (1972) did not find serological relationships between

ELV and antisera against radish mosaic, turnip crinkle, turnip rosette, and turnip yellow mosaic viruses. The negative results found in serological tests performed vice versa in the present investigations further confirm this fact. HOLLINGS and STONE (1969) investigated properties and serology of the 4 above mentioned beetle-transmissible viruses of crucifers and did not find serological relationships between them. These authors described turnip crinkle and turnip rosette viruses as containing only one component in density gradient centrifugation whereas radish mosaic and turnip yellow mosaic viruses proved to consist of two components. Turnip yellow mosaic virus is distinguished from radish mosaic virus because the former has a non-infective TC. In this respect, ELV appears to be more related to turnip yellow mosaic virus than to any other of the beetle-transmissible viruses. The sedimentation coefficients of both the viruses also does not differ much (ELV: 113.4 S, TYMV: 119 S). The results of measurements of virus particle diameter depend on several circumstances, thus different values for turnip yellow mosaic virus are reported (26 nm – COSENTINO *et al.*, 1956, 28–30 nm – HOLLINGS and STONE, 1969). ELV may also have similarities in diameter with this virus and they perhaps belong to the same subgroup. In spite of their similarities, ELV and turnip yellow mosaic virus are serologically distinct and differ in many other properties. On the basis of the results presented in this paper, it is concluded that ELV is a fifth entity within the group of beetle-transmissible crucifer viruses.

Acknowledgements

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Studies on Viruses and Virus Diseases of Cruciferous Plants

IX. *Brassica napus* var. *napobrassica*, a Sensitive and Reliable Local Lesion Host for *Erysimum* Latent Virus

By

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The cultivar 'Gelbe' of *Brassica napus* var. *napobrassica* showed between individual plants and between leaves in the same position only very little differences in numbers of necrotic local lesions induced by *Erysimum* latent virus. In order to compare different solutions of this virus within one trial, only equal batches of young plants having the same age, size, structure, and leaf numbers are needed in order to obtain reliable results. In contrast to numerous other combinations of viruses and test plants, the combination investigated here yielded most local lesions on the upper and largest lesions on the lowest leaves.

Since HOLMES (1929) showed that the number of local lesions produced by tobacco mosaic virus on *Nicotiana glutinosa* L. depends on the virus content of the inoculum, this assay method has been used in many virus-host combinations for quantitative virus studies. BAWDEN (1950) and later on MATTHEWS (1970) concluded from literature that 10 to 20% difference in virus content or activity between solutions may be detected in comparisons done in the same test if a suitable local lesion host is used. However, local lesion counts are only reliable when factors affecting local lesion numbers are considered carefully. Some of them are uniformity in size, age, and susceptibility of plants, numbers, positions, and side of leaves, and uniform method of inoculation. Especially difficult is to work with plants giving only chlorotic local lesions, because these lesions may vary from clear-cut spots to faint yellow areas which can be counted only arbitrarily. In these and in other cases where no local reaction at all is visible, differences in starch content between infected and non-infected areas were utilized for showing lesions by the starch-iodine reaction (HOLMES, 1931). This method, however, did not come into general use. Therefore with many viruses no suitable local lesion hosts are known for quantitative determinations. For instance this is also true with turnip yellow mosaic virus, which was studied very intensively and gave many fundamental informations on various aspects of plant virology, although DIENER and JENIFER (1964) found that in nitrogen-deficient Chinese cabbage (*Brassica pekinensis* (Lour. Rupr.) well-defined purple local lesions may be induced by this virus.

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While working with *Erysimum* latent virus (ELV), SHUKLA and SCHMELZER (1972) found *Brassica napus* var. *napobrassica* (L.) Rchb. to be a suitable local lesion host. It produced small necrotic local lesions constantly and systemic mottling very seldom. In further trials SHUKLA *et al.* (1972) compared the infectivity of intact ELV and its ribonucleic acid at different concentrations using the mentioned species as a bioassay host and obtained rather uniform results. In the present investigation we have examined the exactness of local lesion trials on *Brassica napus* var. *napobrassica* taking into account the influence of individual plants, position and side of leaves, virus content of inocula and dependency of local lesion size on leaf position. ELV has some similarities to turnip yellow mosaic virus in being fairly stable, occurring under natural conditions in crucifers only, being not transmissible by aphids and reaching high concentrations in its hosts. Perhaps the advantage in having a reliable local lesion host makes ELV more adapted for fundamental investigations than turnip yellow mosaic virus.

Material and Methods

ELV infected *Brassica pekinensis* provided the source of inoculum throughout the experiments. Dilutions were prepared by grinding the infected leaves (w/v) in 0.067 *M* phosphate buffer pH 7.0. Plants of *Brassica napus* var. *napobrassica* cv. 'Gelbe' were raised first in a large pot and then planted singly in pots of 9 cm size, filled with mixtures consisting of soil, sand, and peat. Inoculations were made with glass spatulas on leaves dusted with carborundum carefully and the plants were rinsed with water immediately after inoculation. Young plants as similar in size as possible and of the same age were selected for each experiment. They were always standing under normal greenhouse conditions where the temperatures fluctuated in the range 17 to 25°C. Local lesions were counted 5 days after inoculation with the aid of an Atherman lamp put under opalescent glass.

In trials, aimed to find out variations in the number of local lesions due to leaf side and leaf position, the two sides of the 3rd, 4th and 5th leaves of 6 plants were inoculated with a 1 : 500 preparation of ELV. Before inoculation, the inoculum was divided in two parts and used separately on the same leaves after the half-leaf method. For determining the variations in the number of local lesions due to virus content of the inoculum, leaf position and plants, the first 4 true leaves of 10 plants were inoculated with each of the 3 dilutions, namely 1 : 100, 1 : 500 and 1 : 1000 of ELV. In order to find out number of lesions per square cm leaf area, the leaves from the 4 different positions of the 10 plants inoculated with 1 : 1000 diluted inoculum were collected separately, the leaf areas were drawn on paper, cut out and weighed with an analytical balance. From the weight of a square cm of paper the square cm of leaf area could be calculated. The length and breadth of the local lesions were measured on the 1st, 2nd, 3rd and 4th leaves inoculated with 1 : 1000 diluted inoculum of ELV with the aid of a light microscope using an ocular micrometer. 25 lesions were measured from each position

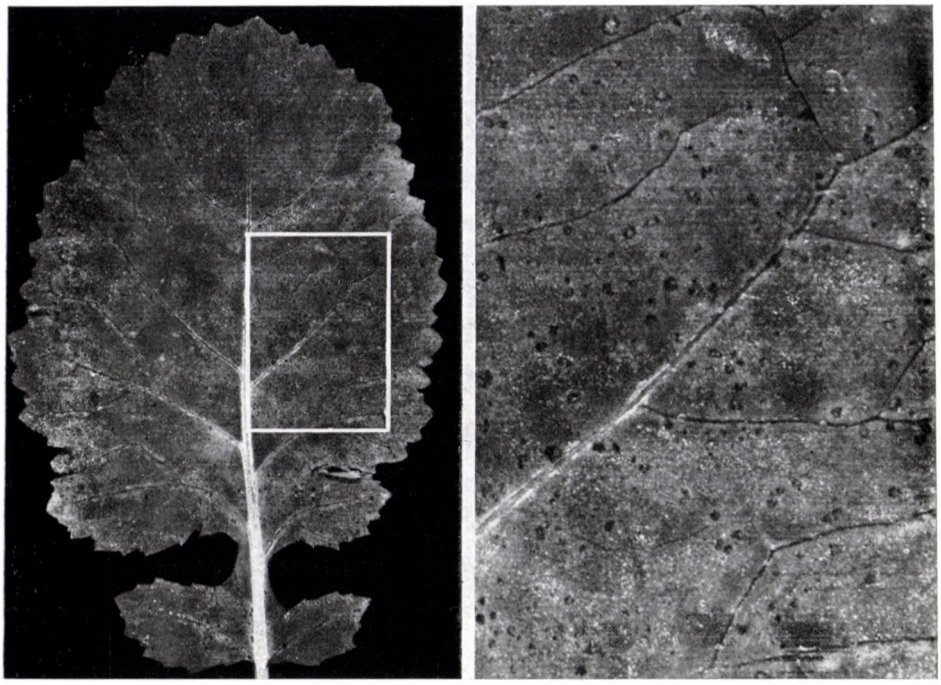

Fig. 1. A young leaf of *Brassica napus* var. *napobrassica* cv. 'Gelbe' showing small local lesions after inoculation with *Erysimum* latent virus. Left: whole leaf, right: leaf area enlarged 3.6 times in comparison to left

of leaves. By assuming that a local lesion has the shape of an ellipse, the local lesion area was calculated after the formula (length × breadth × π)/4. All local lesion data were analyzed using standard statistical techniques.

Results

The investigation on the influences of leaf side, leaf position, and of individual plants on local lesion numbers shows at the first glance that there was no important influence of the leaf side, since the average difference between left and right leaf halves was about 3 to 4 per cent (Table 1). Also the influence of individual plants on local lesion number was remarkably low. The only important factor of variation proved to be the leaf position. The analysis of variance secured these findings.

After this we inoculated plants with a virus solution which was diluted in different steps. For this trial, batches of 10 plants each were separately rubbed with the virus suspensions so that the variation of individual plants could have

great importance for the results. In this case, too, the differences between individual plants and between leaves of the same position from various plants proved to be very low (Table 2). The variation between plants and between leaves in the same position decreased with increasing virus concentration. Again, however, the leaf position was very important. Between the dilutions of 1 : 100 and 1 : 500 the fall in number of lesions was proportionally smaller than the dilution, but between 1 : 500 and 1 : 1000 change of concentration produced an almost equivalent change in lesion number.

In contrast to findings with many other virus-host combinations where always less lesions were found on younger than on older leaves, ELV induced

Table 1

Influence of leaf positions, leaf sides, and individual plants on the result of local lesion tests with *Erysimum* latent virus on *Brassica napus* var. *napobrassica* (virus dilution 1 : 500)

Plant number	Leaf position						Total number of local lesions per plant
	III		IV		V		
	left	right	left	right	left	right	
1	57*	62	88	93	112	128	540
2	62	55	87	81	109	122	516
3	56	68	75	80	129	137	545
4	54	46	77	89	135	126	527
5	69	67	88	96	132	139	591
6	58	56	83	87	138	137	559
Total number of local lesions	356	354	498	526	755	789	9443

* Number of local lesions per half leaf
—— maximum } values in comparison of results with different plants
.... minimum }

Table of variance	Variance	Degrees of freedom	F-values	F-values for P = 1%
Total	33,412.96	35		
Plants	546.29	5	0.89	3.86
Positions	29,683.62	2	121.28	5.57
Sides	62.18	1	0.51	7.77
Positions × sides	61.49	2	0.25	5.57
Error	3,059.38	25		

Table 2

Influence of inoculum dilutions, leaf positions and individual plants on the results of local lesion tests with *Erysimum* latent virus on *Brassica napus* var. *napobrassica*

Plant number	Virus dilution 1 : 100					Virus dilution 1 : 500					Virus dilution 1 : 1000				
	leaf position				total number of local lesions per plant	leaf position				total number of local lesions per plant	leaf position				total number of local lesions per plant
	I	II	III	IV		I	II	III	IV		I	II	III	IV	
1	69*	143	272	446	930	48*	69	82	314	513	11*	32	90	162	295
2	62	129	289	392	872	42	92	126	274	624	19	36	74	149	278
3	72	121	242	413	848	47	83	162	275	567	21	39	71	159	290
4	97	178	286	378	939	45	97	128	265	535	27	56	98	188	369
5	75	159	298	426	958	39	81	171	287	578	25	37	96	165	323
6	56	198	232	487	973	52	79	147	279	557	28	38	82	126	274
7	93	135	301	456	985	56	74	212	281	623	22	35	68	156	281
8	83	146	267	474	970	51	82	166	292	591	14	26	62	113	215
9	67	149	297	484	997	37	98	171	313	619	23	34	76	147	280
10	85	126	283	477	971	31	88	143	289	551	17	58	88	143	106
Average number of local lesions	75.9	148.4	276.7	443.3	944.3	44.8	84.3	159.8	286.9	575.8	20.7	39.1	80.5	150.8	291.1

* Number of local lesion per half leaf

—— maximum } values in comparison of results with different plants

. . . . .minimum }

Table of variance	Variance	Degrees of freedom	F-values	F-values for P = 1%
Total	1,808,670.38	119		
Plants	2,804.80	9	0.66	2.59
Positions	1,060,400.21	3	751.27	3.98
Dilutions	536,233.61	2	569.87	4.82
Positions × dilutions	162,653.26	6	57.62	2.99
Error	46,578.50	99		

most local lesions on the youngest leaves of *Brassica napus* var. *napobrassica* cv. 'Gelbe'. Comparing the local lesion numbers on leaves of different positions we found 3.14, 3.67, 4.20 and 9.92 lesions per square centimeter for the first (oldest) till to the fourth (youngest expanded) leaf at the virus dilution of 1 : 1000. Because the average areas of a local lesion for the leaves were 0.475, 0.309, 0.092 and 0.025 mm^2, the following percentages of leaf areas of the oldest till to the youngest leaves were necrotized: 1.49, 1.13, 0.39 and 0.25. The values are statistically significant at the 0.1% level. The youngest leaves had the least necrotized areas due to the fact that their larger numbers of lesions did not compensate the larger necrotized spots of older leaves with fewer lesions.

Discussion

Due to the fact that *Brassica napus* var. *napobrassica* belongs to the cross-pollinated plants which in general are not genetically uniform, the little variation in local lesion numbers between individual plants induced by the same dilution of ELV is astonishing. After our experience, *Nicotiana glutinosa*, the classic local lesion host for TMV, shows much more variation in this respect. Also other trials, represented in the next publications of this series, showed the high reliability of the test system ELV – *Brassica napus* var. *napobrassica*. During our investigations we found that the mentioned plant species reacts with a few necrotic local lesions after infections with turnip crinkle, and occasionally with turnip rosette viruses whereas turnip yellow mosaic virus induces only sometimes chlorotic local lesions and radish mosaic as well as cauliflower mosaic viruses do not cause local reactions at all. Therefore this plant is also a useful differential host for crucifer viruses with particles of isometric shape. However, we do not know whether all cultivars of *Brassica napus* var. *napobrassica* are so well adapted for quantitative and qualitative trials with ELV as 'Gelbe'. Using this cultivar, only equal batches of young plants having uniform age, size, structure, and leaf numbers are needed within one trial in order to obtain reproducible results. To arrange the treatments on leaves in the manner of a Latin square or some similar designs are not essential. Also the half-leaf method for comparisons with a standard solution seems to be superfluous. However, care should be taken while counting the local lesions on the youngest leaves in order not to overlook the very small lesions.

In contrast to numerous combinations of viruses and test plants in which most local lesions appear on leaves inserted in the middle or in the lower part of a stem or rosette, ELV infected *Brassica napus* var. *napobrassica* cv. 'Gelbe' form the highest number of lesions on the youngest expanded and the largest lesions on the lowest leaves. The reasons for this are unknown, but it should be investigated if the differences in number and size of lesions depends on substances formed before or after the virus infections. Perhaps these differences can be influenced by

external conditions. Comparing treatments including virus-host combinations which normally show most local lesions on middle or lower and largest lesions on upper leaves may be useful for clearing up the mechanisms involved in these phenomena.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

X. Effects of Temperature on Symptom Expression and Concentration of *Erysimum* Latent Virus in *Brassica Chinensis* and *B. Juncea*

By

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Plants of two *Brassica* species were infected with *Erysimum* latent virus and kept under uniform conditions at 18°, 22°, 25°, and 32°C. Symptom development and virus content, measured by local lesion numbers on *Brassica napus* var. *napobrassica*, showed that 22° was the best temperature followed by 25° and 18°C, whereas at 32°C no symptoms could be observed and only very little virus accumulation was demonstrated. *Brassica chinensis* reached higher virus concentration than *B. juncea*. With the exception of 32°C, where the youngest inoculated leaves contained comparatively most virus, the following rule was observed. The younger the unfolded leaf, the higher the virus content. There were only two exceptions. In contrast to *Brassica chinensis*, *B. juncea* contained only little virus in the top leaves 7 days after inoculation. 21 days after inoculation the virus content of the uppermost leaf in both species was lower than in the preceeding leaf. The virus content of all tested leaves was highest after 14 days, whereas after 21 days it decreased nearly to the same level as at 7 days after inoculation. In general, virus concentration and symptom expression were in good conformity. After the results of this paper, *Erysimum* latent virus seems to be adapted to the climate of the moderate zone.

Among various environmental conditions that influence symptom development and accumulation of viruses in infected plants, temperature is considered to be one of the most important factors. In the past, effects of this factor on a number of viruses have been investigated and now it is well established that plant viruses vary considerably in their temperature requirements. A particular temperature best suited for one virus may be disadvantageous for others. In general, plant viruses fall into two groups with respect to temperature effect on multiplication, those like tobacco mosaic virus, potato viruses X and Y and tomato spotted wilt virus which can multiply at temperatures up to 36°C and those like tobacco necrosis and tomato bushy stunt viruses which do not multiply at 36°C (MATTHEWS, 1970). The subject has critically been reviewed by KASSANIS (1957) and more recently by WOLFFGANG (1970).

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In the present work, we report the effects of temperature on symptom development and concentration of *Erysimum* latent virus (ELV) which was described recently and obviously belongs to the group of beetle-transmissible crucifer viruses (SHUKLA and SCHMELZER, 1972a; SHUKLA *et al.*, 1972). The fact that the activity of ELV in plant sap can be measured very exactly (SHUKLA and SCHMELZER, 1972b), facilitated our task considerably.

Material and Methods

ELV infects a large number of crucifers in which distinct symptoms are induced, but for the present investigation *Brassica chinensis* L. and *B. juncea* (L.) Czern. et Coss. were selected because they were found to contain high virus concentrations and could readily be grown under normal greenhouse conditions (SHUKLA and SCHMELZER, 1972a). The plants were raised first in large pots and then transferred in pair into small pot of 9 cm size containing equal amounts of a steam-sterilized soil mixture. Before the beginning of the temperature treatments, the plants were maintained under normal greenhouse conditions. Almost uniform plants were selected and inoculated with the virus when they had developed 4 true leaves. All 4 leaves were rubbed with sap of freshly infected *Brassica chinensis*. Methods of preparing and applying inoculum were the same as described earlier (SHUKLA and SCHMELZER, 1970). Just after inoculation, 30 plants of each species were put into each of the 4 chambers of the "climate house" of our institute (WACHE and WOLFFGANG, 1966). In these chambers 18°, 22°, 25° and 32°C (±1°) were constantly maintained. Except the temperatures, other conditions in the chambers were the same, i.e. always 70% (±5%) relative humidity, continuous light of mixed wave lengths produced by combining fluorescent tubes "white" and "Lumoflor" (1 : 1), distance of lamps from the pallets 120 cm, and light intensity about 1200 lux. The latter was measured with the Luxmeter LMI (VEB Carl Zeiss Jena, GDR) which gave only approximate values because of the different spectral sensitivity of the photo elements. The plants in the chambers were inspected twice a day and the symptoms were recorded. At weekly intervals, beginning 7 days after inoculation, 4 samples from 10 plants of each species per chamber were taken three times and assayed for virus activity. Because of the fact that during the trial new top leaves were formed and older inoculated leaves died, it was not possible to investigate always the same leaves at the three different times of testing. Details of the samples in the three harvestings are given below (Table 1).

In this way mostly 10, sometimes 20 leaves, were combined for giving one inoculum. By the aid of cleaned and sterilized pressing tongs and Dederon cloth, sap was extracted and diluted to 1 : 100 in 1/15 *M* phosphat buffer (pH 7.0). The different inocula were assayed for virus activity on *Brassica napus* var. *napobrassica* cv. 'Gelbe' which has been found to be a very sensitive and reliable local lesion host of ELV (SHUKLA and SCHMELZER, 1972b). Plants of this species were raised

Table 1

Details of the samples taken at different intervals from ELV infected leaves of *Brassica chinensis* and *B. juncea*

Harvesting time after inoculation in days	Number of sample			
	I	II	III	IV
7	1 + 2*	3	4	5
14	3	4	5 + 6	7
21	4	5 + 6	7	8

* Position of leaves, no. 1 is the oldest leaf, 1—4 inoculated leaves, 5—8 top leaves

at weekly intervals in order to use them at the same age for testing all the three harvestings. They were planted singly in pots of 9 cm diameter and were maintained always under normal greenhouse conditions. Only the first two fully expanded true leaves dusted with carborundum were rubbed with the inocula using clean, sterilized glass spatula. 20 leaves of 10 plants were allotted to each inoculum. The local lesion plants were selected for uniformity. Lesions were counted 5 days after inoculation.

Results

Symptom development

The two plant species exposed to different temperatures developed the following symptoms:

Brassica chinensis — The first symptoms were chlorotic local lesions appearing 3, 4 and 5 days after inoculation on the plants exposed to 25°, 22° and 18°C, respectively. At the first two mentioned temperatures the third as well as the fourth inoculated leaves produced local lesions whereas at the last mentioned temperature they were observed only on the 4th leaf. Apparently there was no difference in size of local lesion at 22° and 25° on comparable leaves, but at 18°C they were smaller. Systemic symptoms as vein clearing appeared after 6 days at 25° and 22° but at 18°C these symptoms were seen only after 8 days. Plants at 25° and 22° produced leaf mottling after 9 days, but at 18°C after 12 days. Mottling on the 8th leaf was milder in comparison to that of the preceding leaf at all the three above mentioned temperatures. Although local lesions were first seen at 25° and the development of other symptoms was simultaneous at 22° and 25°C, more severe disease symptoms were noticed at 22° followed by 25° and 18°C. All plants at 32°C remained symptomless on rubbed and top leaves throughout the experiments.

Brassica juncea — Almost similar types of symptoms, as described in case of *Brassica chinensis*, were also noticed on *B. juncea*, but in this species they were

expressed rather slowly. Chlorotic local lesions appeared only on the 4th inoculated leaves after 7 days at 25° and 22° and after 8 days at 18°C. Vein clearing was visible after 10 days at 25° and 22° and after 12 days at 18°C. At 25° and 22°, plants started to develop leaf mottling after 12 days, at 18°C after 14 days. Most of the local lesions became necrotic 10, 11 and 13 days after inoculation at 22°, 25° and 18°C, respectively. The 8th leaf produced only mild mottling compared to the preceding leaf. The most severe symptoms also in this species was found at 22° followed by 25° and 18°C. Again at 32°C all the plants did not show any symptom.

Virus concentration

Effects of different temperatures on ELV concentration in *Brassica chinensis* and *B. juncea* 7, 14 and 21 days after infection are shown in Figs 1 and 2. The values are based on local lesion counts.

Brassica chinensis – Looking to the curves obtained at different temperatures in Fig. 1, 22°C gave the highest values in all the three harvestings, followed by 25° and 18°C, whereas 32°C allowed only a minimum virus accumulation. At 18°, 22° and 25°C the virus contents 7 and 14 days after infection showed always the same tendency. The oldest leaves contained the less virus, the youngest the most and the values of the other leaves fit well forming rather straight lines. These six lines are nearly parallel. All the values for the harvestings after 14 days are higher in comparison to those after 7 days for the same treatments tested. In contrast to this, at 32°C the virus content was highest in the fourth, the youngest inoculated leaf, and was lower in top leaves. Also at this temperature higher values were obtained 14 days after inoculation than after 7 days. 21 days after inoculation at 18°, 22° and 25°, the values for the youngest inoculated and for the oldest top leaves dropped to almost the same level which were obtained 7 days after inoculation. With the exception of 18°C, where there was no change, the virus content of the 7th leaf decreased to the same extent as with the other leaves in comparison to the harvesting after 14 days. Nevertheless the 7th leaf had the highest virus content than all the other leaves tested after 21 days because the youngest top leaf reached only values not very much different from those of the oldest systemically infected leaves. At 32°C, all leaves had rather uniform very low virus contents.

Brassica juncea – In comparison to *Brassica chinensis*, the virus content in leaves of *B. juncea* was always lower in all the treatments. However, also in this species the maximum virus concentration was achieved at 22°, followed by 25°, 18° and 32°C (Fig. 2). With some exceptions, the trend of the curves was the same as it was found in case of *Brassica chinensis*. The main difference is that 7 days after inoculation the top leaves had the lowest virus content instead of the highest

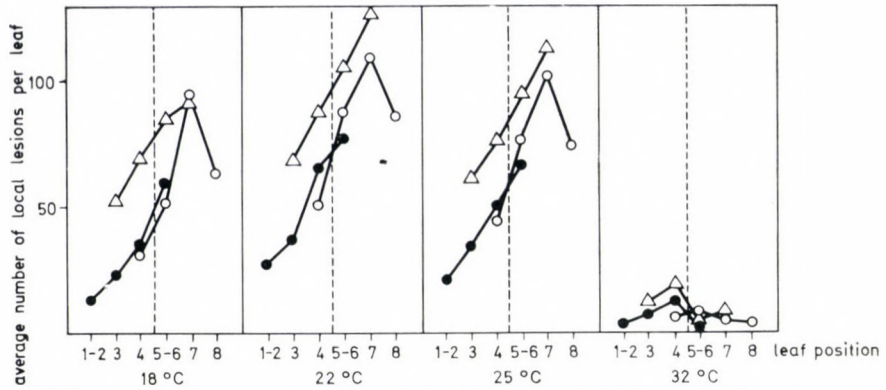

Fig. 1. Effects of temperature on concentration of *Erysimum* latent virus in *Brassica chinensis* The values are based on average numbers of local lesions on *Brassica napus* var. *napobrassica* cv. 'Gelbe'. ●——● after 7 days, +——+ after 14 days, ○——○ after 21 days

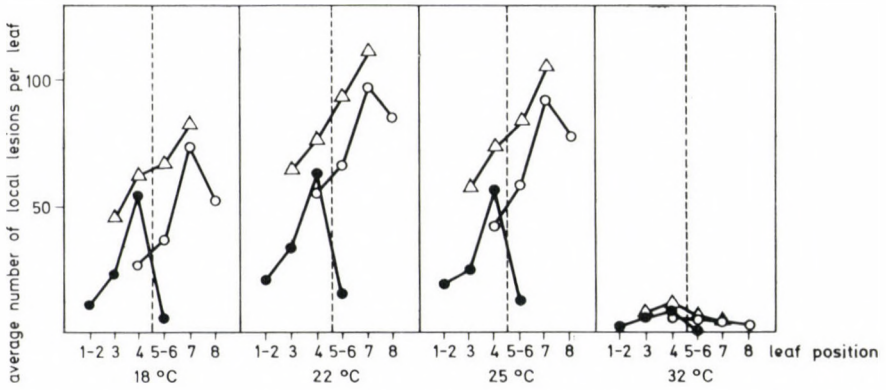

Fig. 2. Effects of temperatures on concentration of *Erysimum* latent virus in *Brassica juncea.* The values are based on average numbers of local lesions on *Brassica napus* var. *napobrassica* cv. 'Gelbe' ●——● after 7 days, +——+ after 14 days, ○——○ after 21 days

as in case of *Brassica chinensis*. At 18°C even 14 days after inoculation the combined 5th and 6th leaves gave a value only little better than the 4th leaf, indicating that there may be areas not occupied by the virus. Also 21 days after inoculation the youngest leaf gave lower values than the 7th. However, the tendency of decreasing virus concentration was less pronounced in comparison to *Brassica chinensis* and the virus content of the 8th leaf was always considerably higher than that of the 5th plus the 6th leaves. The curves obtained with saps from plants treated at 32°C showed again that this temperature is very unfavourable for ELV.

Discussion

All the findings of the present and of the preceding papers (SHUKLA and SCHMELZER, 1972b) indicate that our local lesion countings on *Brassica napus* var. *napobrassica* cv. 'Gelbe' reflect not only activity but also concentration of ELV. The results of the temperature treatments from both the plant species investigated confirmed each other. Leaves of *Brassica chinensis* contained always more virus than those of *B. juncea*. The studies demonstrated that 22°C was the best suited temperature for symptom expression and concentration of ELV in comparison to the other applied temperatures. If the real optimum is not coinciding with 22°C, it seems to be more probable between 22° and 25° than between 18° and 22°, since 25° proved to be better than 18°C. In the range of 18° to 25°C symptom expression and concentration of ELV in both species were in good conformity. Such results have also been found in case of tobacco mosaic virus and potato virus X in tobacco in the range 16° to 28°C (BANCROFT and POUND, 1954; POUND and HELMS, 1955). At 32°C, however, ELV accumulated although very slowly, but symptoms could not be observed. At this temperature, the virus concentration in leaves developing after inoculation was remarkably low. At the other temperatures, the following rule was valid: the younger the unfolded leaf, the higher the virus concentration. There were only two exceptions. 7 days after inoculation top leaves of *Brassica juncea* had low virus content. This indicates that in early stages of infection ELV moved slowly into upper parts of this species. This conclusion is supported by the observations on symptom development, as in *Brassica juncea* the first systemic symptom appeared only after 10 days. The other exception was found 21 days after inoculation where the uppermost of the tested leaves contained less virus than the preceding leaf. About 14 days after inoculation seems to be the optimum for ELV concentration. Therefore at this time plants should be harvested for virus purification. Due to some unknown mechanisms and/or substances acting 7 days later, the virus content decreased considerably in those leaves which were already developed at optimum time. In the newly formed leaf discolouration due to virus and virus concentration both did not reach to the same extent as in the preceding leaf. It is of interest to note that the uppermost leaf of *Brassica juncea* showed a comparable smaller decrease in virus concentration than *B. chinensis* 21 days after inoculation. This has some similarities with the retarded multiplication of ELV in newly formed top leaves of *Brassica juncea* in early stages of infection. Similar dropping of virus content some weeks after inoculation as in case of ELV have been reported with other viruses, too (FROST and HARRISON, 1967; LEBEURIER and HIRTH, 1966).

After our results, ELV belongs to the group of viruses best adapted to the climate of the moderate zone. Its optimum temperature for multiplication is higher than for the viruses from cooler regions, i.e. parsnip yellow fleck, Andean potato latent, and potato mop-top viruses (ABU SALIH *et al.*, 1967; GIBBS and HARRISON, 1969; HARRISON and JONES, 1971). ELV do not reach high concen-

trations in plant at temperatures often prevailing in tropical and subtropical countries. It should be investigated whether ELV and similar viruses may easily develop mutants that can multiply well at higher temperatures. Another interesting problem arises from the question if plants infected by ELV may easily be cured by heat treatments.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

XI. Effects of Light on Symptom Expression and Concentration of *Erysimum* Latent Virus in *Brassica chinensis*

By

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Plants of *Brassica chinensis* in 4 leaf-stage were infected with *Erysimum* latent virus and kept in climate chambers at 22°C and 70 per cent air humidity. The plants received light intensities of 75, 300, 1200 lx continuously or 1200 lx for 16 hours per day. Symptom development of the test plants and measurements of local lesion numbers on *Brassica napus* var. *napobrassica* proved that continuous light of 1200 lx was best for plant growth, virus symptoms, and virus contents, followed by 16 hours of 1200, 300, and 75 lx. The plants of the chamber with the last mentioned light intensity died before the end of the trial. 14 days after inoculation comparatively highest virus content was found in all leaves and treatments, whereas after 21 days it decreased almost to the same lavel as it existed 7 days after inoculation. As in case of temperature treatments, also in the present trials the virus concentration was relatively highest in the youngest unfolded leaf and decreased with leaf age. Exceptions were found only in some top leaf samples. 7 days after inoculation no and less virus was found in them at 75 and 300 lx, respectively. Also after 14 days at 75 lx and with both treatments of 1200 lx after 21 days top leaves showed somewhat decreased virus contents.

The role played by intensity and duration of light in influencing concentration and subsequently symptom development of viruses in plants is rather well known. In general, under artificial conditions, high light intensities and long days favour virus multiplication (CHEO and POUND, 1952; POUND and BANCROFT, 1956; BANCROFT, 1958; POUND and GARCES-OREJUELA, 1959). However, recent research on the subject have demonstrated that the requirements of light intensity and duration for plant viruses depend mostly on virus-host combination. OPEL (1970) published almost a complete review on this problem.

The aim of the work presented here was to investigate effects of different light intensities on symptom development and concentration of *Erysimum* latent virus (ELV) which has also been examined concerning its temperature requirements and seems to be very suitable for quantitative virus determinations (SHUKLA and SCHMELZER, 1972a; 1972b).

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Material and Methods

Results of temperature experiments showed that the symptom development in *Brassica chinensis* was quicker and more pronounced than in *B. juncea* and the former contained higher virus concentration. Therefore, in the present investigation only *Brassica chinensis* was used. Raising, selection, and treatment of the plants were done in the same manner as described earlier (SHUKLA and SCHMELZER, 1972b). After inoculation, 30 plants were put into each of the 4 chambers of the "climate house" (WACHE and WOLFFGANG, 1966). In these chambers different light intensities were maintained, namely 75, 300 and 1200 lx continuously and 1200 lx for 16 hours per day. All the other conditions in the chambers were constant: temperature 22°C (±1°), relative air humidity 70% (±5%), and a mixed quality of light produced by combining fluorescent tubes "white" and "Lumoflor" (1 : 1). The light intensity was measured with the luxmeter LMI (VEB Carl Zeiss Jena, GDR) which gave only approximate values because of the different spectral sensitivity of the photoelements for different light qualities. While working on effects of temperature on ELV, the above mentioned temperature was found best both for symptom expression and virus concentration in *Brassica chinensis* (SHUKLA and SCHMELZER, 1972b).

Symptom expression was inspected twice a day. In order to determine virus concentration, 4 samples from 10 plants per chamber were harvested at weekly intervals starting 7 days after inoculation. Due to death of lower leaves and formation of new leaves during the 4 weeks needed for the experiments, a uniform criterion of sampling could not be maintained between different dates of harvesting. Also it was not possible to have uniform samples from all the 4 chambers at one date because the plants kept at lower light intensities grew very slowly and produced fewer leaves. The plants of the chamber with 75 lx died before the end of the trial. Details of the samples in the three harvestings are given below (Table 1).

Table 1

Details of the samples taken at different intervals from ELV infected leaves of *Brassica chinensis*

Harvesting time after inoculation in days	Treatment at 1200 lx number of sample				Treatment at 300 lx number of sample				Treatment at 75 lx number of sample			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
7	1 + 2*	3	4	5	1 + 2	3	4	5	1 + 2	3	4	5**
14	3	4	5 + 6	7	3	4	5	6	3	4	5	—
21	4	5 + 6	7	8	4	5	6	—	plants dead			

* Position of leaves, no. 1 is the oldest leaf, 1—4 inoculated leaves, 5—8 top leaves

** Due to bad development, this leaf sample was grinded in a mortar 1 : 100 (w/v) with phosphate buffer, instead of being pressed and the juice diluted afterwards

10 or 20 leaves were united in one sample. Each sample was treated and then tested on *Brassica napus* var. *napobrassica* cv. 'Gelbe' in the same manner as described earlier (SHUKLA and SCHMELZER, 1972b). 20 leaves of 10 plants of this cultivar were inoculated with the juice of one sample each. Local lesions were counted 5 days after inoculation and the average value was recorded.

Results

Symptom development

First symptoms, consisting of chlorotic local lesions, appeared on the 3rd as well as on the 4th inoculated leaves 4 and 3 days after inoculation in plants standing at 1200 lx (16 hrs day) and 1200 lx (continuous) light intensities. Plants at 75 and 300 lx produced this symptom only on the 4th inoculated leaf after 12

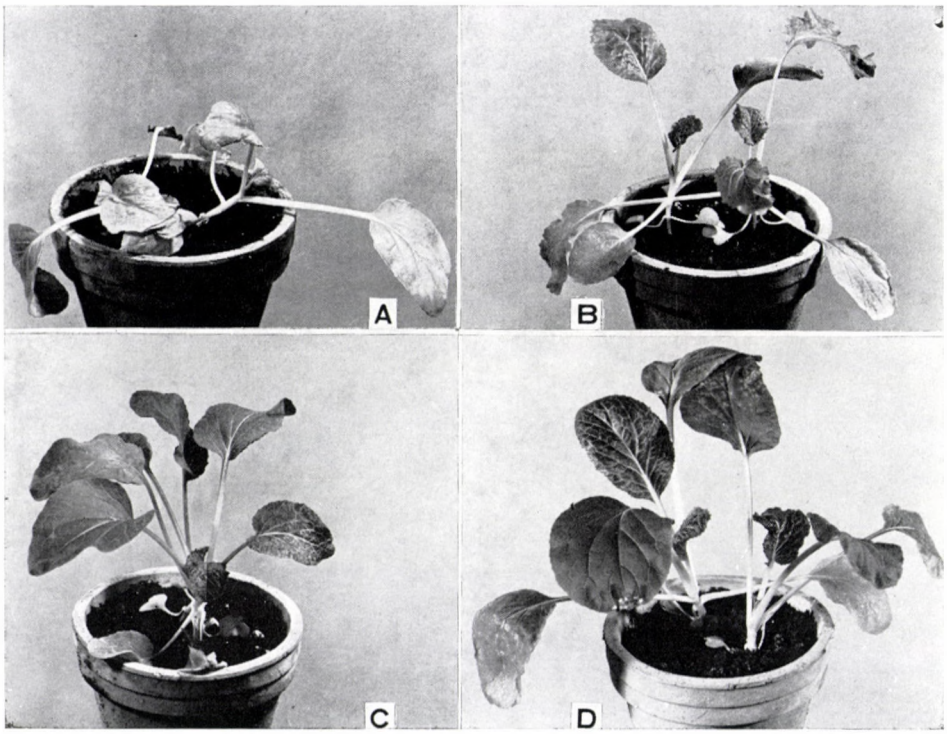

Fig. 1. Plants of *Brassica chinensis* 11 days after infection by *Erysimum* latent virus subjected to a constant temperature of 22°C, 70 per cent relative air humidity, and different light intensities. A: continuous 75 lx, B: continuous 300 lx, C: 16 hours per day 1200 lx, D: continuous 1200 lx

and 6 days. Vein clearing on top leaves was noticed after 10, 7 and 5 days and mottling after 14, 9 and 7 days at 300, 1200 (16 hrs day) and 1200 lx (continuous), respectively. Some of the chlorotic local lesions changed to necrotic after 8 and 7 days at the last two mentioned light intensities whereas at 75 and 300 lx no necrotic lesions were observed. None of the plants getting 75 lx showed vein clearing and mottling and they died before the end of the trial. In general, vigour of the plants was greatly affected by lower light intensities. Especially at 75 lx, but also at 300 lx, the leaves began to show chlorosis after beginning of the trial thus indicating that these light intensities are unfavourable for plant growth (Fig. 1). Severity of disease symptoms and plant vigour was found best at continuous 1200 lx followed by 16 hrs per day 1200 lx.

Virus concentration

Results of virus concentration determinations in leaves of *Brassica chinensis*, subjected to different light intensities and harvested 7, 14 and 21 days after infection, are shown in Fig. 2.

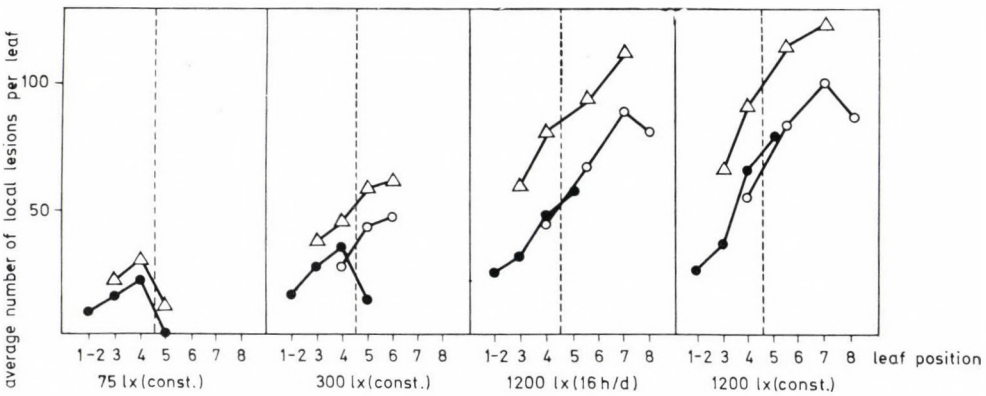

Fig. 2. Effects of light on concentration of *Erysimum* latent virus in *Brassica chinensis*. The values are based on average numbers of local lesions on *Brassica napus* var. *napobrassica* cv. 'Gelbe'. ●——● after 7 days, +——+ after 14 days, ○——○ after 21 days

The curves obtained from different light intensities showed that the plants subjected to continuous 1200 lx developed highest virus concentrations throughout the trial followed by 16 hrs per day 1200, 300 and 75 lx. From Fig. 2 it can be seen that there was no detectable virus at the first harvesting in the 5th (youngest) leaf of the plants standing at 75 lx. At 300 lx the virus multiplied in this leaf but the concentration was lower than in the inoculated leaves. On the other hand, at 16 hrs 1200 lx and continuous 1200 lx the 5th leaf contained maximum amount of

virus and the trend was in an ascending order, i.e. the lower leaves had lower virus concentration.

14 days after inoculation the virus concentration in plants increased at all light intensities in comparison to the first harvesting. Plants at 75 lx stopped forming new leaves. However, in their 5th leaves the virus did multiply but the concentration was lower than in the directly inoculated leaves. In the other treatments the virus concentration curves were rather straight. The younger the tested leaf, the more virus was found.

At the end of the third week determination of virus content in plants kept at 75 lx was not possible because none of them survived. At the other light intensities the virus concentration decreased in comparison to the values obtained 14 days after inoculation. With both treatments at 1200 lx the curves appeared to be elongations of those after 7 days. Only the 8th leaf had less virus content than the foregoing. At 300 lx the 6th (youngest) leaf possessed the relatively highest virus concentration.

Discussion

The results on the effects of light intensity on ELV supported the findings of the earlier paper (SHUKLA and SCHMELZER, 1972b). The harvestings 14 days after inoculation yielded more virus than after 7 days. Also the decrease in virus content of the 8th leaf in comparison to the 7th leaf 21 days after inoculation fitted well. Obviously, in the present trials the light intensity did not transgress the optimum and therefore virus content increased with increasing intensity and duration of light. The same was true with symptom expression and growth of the plants. Apparently the effects of light on ELV in our trials was indirect. The light increased photosynthesis first and by this virus multiplication. From Figs 1 and 2 it can be concluded that low light intensities reduce considerably plant vigour and virus multiplication. At the lowest light intensity the virus was not detectable in newly formed leaves 7 days after inoculation. Also 14 days after inoculation the virus content was rather low in comparison to plants getting more amounts of light. This indicates slow multiplication and/or movement of ELV under bad light conditions. Eventually the plants died. At light intensities of 1200 lx the plants grew well and showed pronounced virus symptoms. The results of the present and the foregoing papers suggest that young *Brassica chinensis* submitted to continuous light of 1200 lx in climate chambers at 22°C yield very high ELV concentrations 14 days after infection. This may be of interest for obtaining starting material in virus purification trials.

Acknowledgements

We are grateful to Dr. H. OPEL for his assistance in performing these investigations, to Mr. W. KILIAN for the drawings, 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

XII. A Complex Disease of Fodder Rape

By

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In late summer and harvest of 1971, a field of fodder rape (*Brassica napus* var. *napus* f. *annua*) was observed near Aschersleben in which 95 per cent of the plants were stunted and had only small leaves showing pronounced vein clearing, vein banding, yellow-green mosaic, crinkling, and deformation. 14 leaf samples, taken at different times, were found to be all infected simultaneously by cabbage black ring (CBRV), cucumber mosaic (CMV), and nasturtium ringspot (NRSV) viruses. Two other samples, taken rather late, proved to be additionally infected by cauliflower mosaic virus. In back transmission tests with the first three mentioned viruses, singly and in different combinations, it was found that CBRV was responsible for crinkling. This virus and sometimes CMV were singly able to induce mosaic symptoms on rape. CBRV + CMV caused severe damage and even death of young plants. Obviously, NRSV did not have marked influence on the severity of the complex disease. In single infection it was symptomless. NRSV and also the complex of three and even four viruses were never described before on rape.

A virus disease of 'Liho' rape, a cultivar of *Brassica napus* var. *napus* f. *annua* (Schübl. et Mart.) Thell., was observed in a field near Aschersleben in August 1971. The crop was cultivated for being used as fodder, but the growth was so retarded that harvesting could not be done at the intended time. A preliminary survey showed that most of the plants were severely infected by a virus disease. This led us to identify the viruses involved and to investigate the aetiology of the disease. The results are presented in this paper.

Material and Methods

The field was surveyed several times in the season, symptoms were noticed and per cent incidence of the disease was recorded. Leaves from the symptom-showing plants were brought to the laboratory and examined for the viruses. In all, 16 leaf samples from various plants, collected in the field at different places and times, were investigated. Test plants, methods of isolation, insect transmission, and serology were the same as described earlier (SHUKLA and SCHMELZER, 1970). In order to reproduce the field symptoms in greenhouse, young seedlings of 'Liho'

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rape were inoculated with the isolated viruses singly and in all possible combinations. Back transmission tests from these plants to indicators were performed one month later.

Results

Symptoms in the field

The common symptoms consisted of pronounced vein clearing, vein banding, faint to severe yellow mosaic, crinkling and deformation of leaves. Leaves and stems were strongly reduced in size giving a stunted appearance of the infected plants (Figs 1, 2, 3). From some distance, the diseased plants looked yellowish in comparison to the dark green symptomless plants standing nearby. At the end of the season, more than 95 per cent of the whole crop showed distinct virus symptoms.

Isolation and identification of the viruses

The sap from 14 samples, when rubbed onto usual test plants, induced chlorotic flecks and rings on top leaves of *Brassica oleracea* var. *botrytis*; chlorotic to necrotic local lesions and tip necrosis on *Chenopodium quinoa*; systemic mottling on *Cucumis sativus*; local necrotic spots and rings, followed by some necrosis, chlorotic rings and mottling on top leaves of *Nicotiana megalosiphon*; brown necrotic spots, concentric rings on inoculated leaves and mottling, rings, and oak-leaf pattern on top leaves of *N. tabacum* cv. 'Samsun'; and systemic vein clearing turning to mosaic on *Sinapis alba*.

The test plant reactions suggested the presence of more than one virus in the isolates. Most of the symptoms indicated the involvement of cabbage black ring virus (CBRV). Some, for instance, mottling on *Cucumis sativus*, were typical for cucumber mosaic virus (CMV). Tip necrosis in *Chenopodium quinoa*, rings and oak-leaf pattern on *Nicotiana tabacum* were reactions known to be caused by most of the NEPO and some aphid transmissible viruses. Thus it was supposed that at least three different viruses were involved. These could be obtained separately without difficulties by using top leaves of some infected test plants. Cauliflower yielded CBRV, *Cucumis sativus* CMV, and *Chenopodium quinoa* the entity belonging to NEPO or other viruses.

The presence of CBRV in the isolates was confirmed by insect transmission tests and by electron microscopical examinations. The virus could successfully be transmitted by *Myzus persicae* Sulz. from cauliflower to cauliflower. Flexuous virus particles of a length of about 750 nm were seen in metal-shadowed dip preparations from symptom-showing cauliflower leaves.

To demonstrate the presence of CMV, serological investigations were performed. Crude sap of infected *Nicotiana tabacum*, when tested with an antiserum

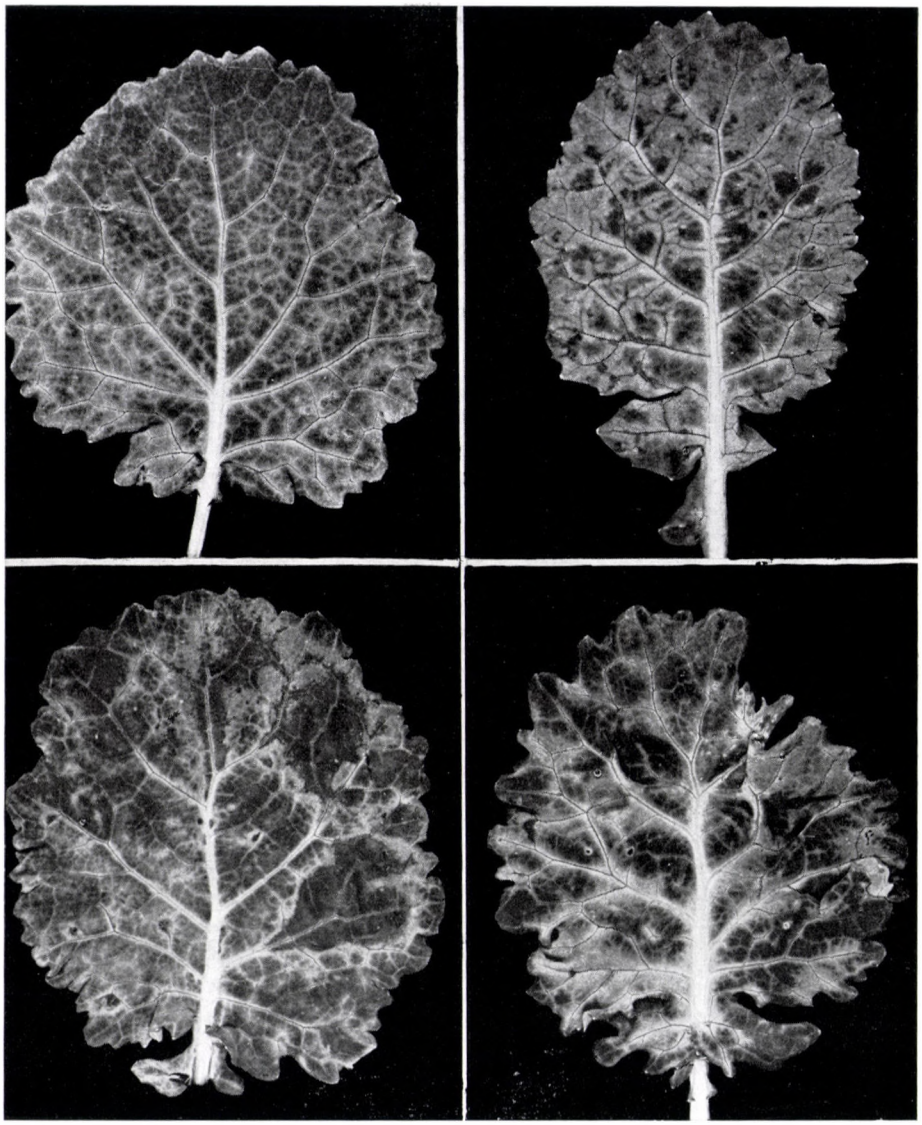

Fig. 1. Leaves of spontaneously infected 'Liho' rape

against a common strain of the virus, gave positive reactions in agar-gel double diffusion trials.

For the identification of the unknown virus (or viruses), systemically infected leaves of *Chenopodium quinoa* were tested with antisera against alfalfa mosaic, arabis mosaic, nasturtium ringspot (NRSV), raspberry ringspot, strawberry latent ringspot, and the two serotyps of tomato black ring viruses in OUCHTERLONY tests. All the 14 isolates gave positive reactions only with NRSV antiserum. The maximum serum dilution successfully tested was 1 : 256. Thus it was demon-

Fig. 2. Tops of spontaneously infected 'Liho' rape

strated that the third virus involved in the rape infection was NRSV. In further trials, one of the rape isolates of NRSV was compared with isolates obtained from *Tropaeolum majus* L. (SCHMELZER, 1970), *Sinapis alba* L. (SHUKLA and SCHMELZER 1972a), and *Eryngium campestre* L. (WOLF, 1972) in serological tests by placing them alternately in peripheral holes and NRSV antiserum prepared against the mentioned *Tropaeolum* isolate in the central hole. All the four isolates reacted well without forming any spur. This indicated that the isolates, although obtained from different plant species, may be identical in serological respect.

Fig. 3. Details of the spontaneously infected 'Liho' rape field at the end of October

The remaining two leaf samples of 'Liho' rape, besides inducing the test plant reactions mentioned above, caused vein clearing, slight veinal necrosis and stunting on cauliflower. These symptoms are known to be produced by cauliflower mosaic virus (CIMV). Therefore CBRV and CIMV seemed to be present in the inoculated cauliflower plants simultaneously. In order to separate CIMV from CBRV, the fact was used that the latter virus has a lower thermal inactivation temperature. Two ml sap from cauliflower leaves infected with each of the two isolates were filled into small glass tubes and heated in an ultrathermostate at 68°C for 10 minutes, cooled with tap water and rubbed immediately onto virusfree

cauliflower seedlings. After 15 days, typical symptoms of ClMV appeared. As it was found in electron microscopical examinations, metal-shadowed dip preparations from such symptom-showing cauliflower leaves did not contain elongated virus particles.

None of the isolates reacted with antisera against 4 beetle-transmissible crucifer viruses, namely radish mosaic, turnip crinkle, turnip rosette, and turnip yellow mosaic viruses. Attempts to prove, if the newly described *Erysimum* latent virus (SHUKLA and SCHMELZER, 1972b; SHUKLA et al., 1972a) was present in the isolates, also failed.

Reproduction of the original disease symptoms in greenhouse

Leaves from a spontaneously infected rape plant with pronounced disease symptoms, supposed to contain all the 4 viruses isolated, were brought from the field and served as a source in an aphid transmission test. After 3 hours of fasting, *Myzus persicae* were allowed to feed on the leaves for 15 minutes and then 10 aphids were transferred to each of 10 young virusfree seedlings of 'Liho' rape, raised in the greenhouse. 16 hours later the insects were killed by an insecticide. Only 6 of the seedlings showed vein clearing and mottling (Figs 4A, B). In back transmission tests from these plants only CBRV was recovered. The seedlings without symptoms yielded no virus.

In a further series of trials mechanical inoculations were done. CBRV, CMV, and NRSV were used singly and in all possible combinations. 5 plants were allotted to each of the 7 treatments. ClMV was not included in the trials because its occasional presence was demonstrated only late in the season when this experiment was already started. The inocula contained (1) CBRV, (2) CMV, (3) NRSV, (4) CBRV + CMV, (5) CBRV + NRSV, (6) CMV + NRSV and (7) CBRV + CMV + NRSV. All the plants inoculated with CBRV singly or in combinations showed vein clearing and/or green mosaic with certain differences in severity of the disease symptoms connected with crinkling (Figs 5A, D, E and G). Type and severity of symptoms were almost the same in treatments (1) and (5) (Figs 5A and E). Combinations of CBRV and CMV severely affected the plants, induced pronounced stunting and even killed the seedlings (Figs 5D and G). There was no difference in double (4) and triple (7) infections. Only 3 of 5 plants inoculated with CMV developed at first a faint vein clearing and later on a distinct white-green mosaic (Figs 5B; 4C and D). The presence of CMV in symptom-showing plants could be demonstrated in serological investigations whereas from the symptomless seedlings CMV was recovered only with test plants. None of the plants inoculated with NRSV alone or with CMV + NRSV showed any symptom (Figs 5C and F). However, the viruses were transmitted from them to differential hosts.

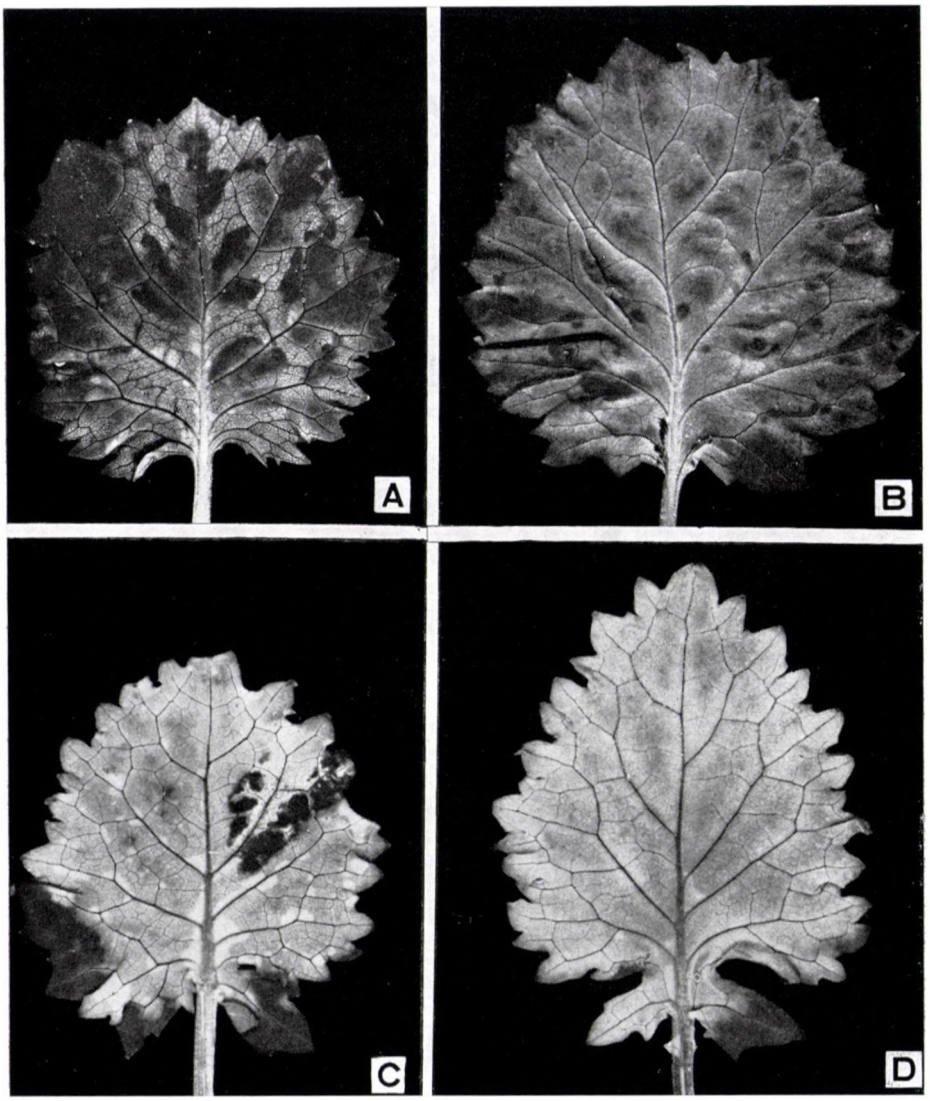

Fig. 4. Leaves of artificially infected 'Liho' rape. A and B: aphid transmission from the natural complex yielding only cabbage black ring virus; C and D: mechanical transmission of the cucumber mosaic virus component singly

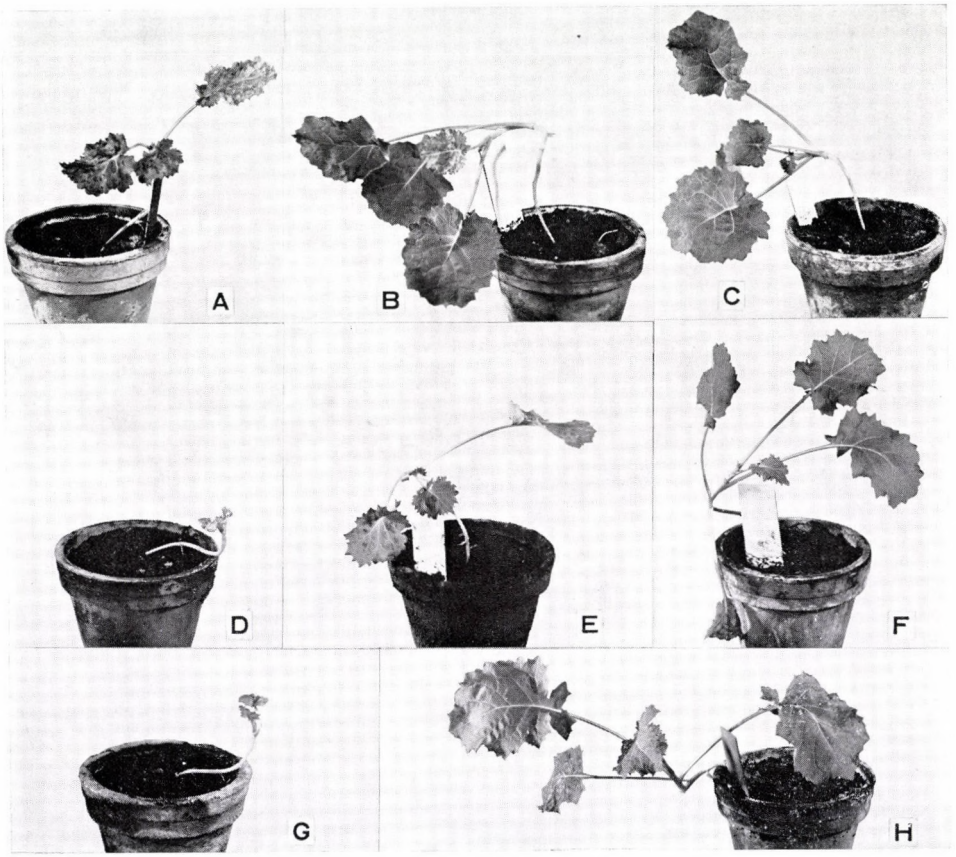

Fig. 5. Mechanically infected 'Liho' rape showing the effect of the viruses involved in spontaneous infections. A – C: single infections, D – G: mixed infections, H: healthy control; A: CBRV, B: CMV, C: NRSV, D: CBRV + CMV, E: CBRV + NRSV, F: CMV + NRSV, G: CBRV + CMV + NRSV

Discussion

As far as we know, virus diseases of rape were reported for the first time from New Zealand (CHAMBERLAIN, 1936) and Central Europe (KAUFMANN, 1936) simultaneously. In both cases, strains of CBRV seemed to be the cause. Strains of CBRV appeared to be present in rape also in China (LING and YANG, 1940; SHEN and PU, 1965). Although several workers described the susceptibility of rape to ClMV (TOMPKINS, 1937; CALDWELL and PRENTICE, 1942; MAMULA and MILIČIĆ, 1968), obviously only one report was published on natural infections (FRY, 1952). Until now, CMV was found on field plants of rape in Belgium (SEMAL, 1958) and

Hungary (HORVÁTH, 1969). The last mentioned author claimed that the virus caused severe diseases of rape in certain parts of his country. Thus all the viruses which we isolated from rape were found already earlier on this crop. The only exception is NRSV. It was never isolated before from members of the genus *Brassica*. After our knowledge, however, nobody analysed and identified until now virus complexes in rape consisting of 3 or even 4 viruses. The reason for this may be unsufficient methods for detection and differentiation of viruses in this crop. Judging from disease descriptions, we can not exclude that such mixed infections did exist in Central Europe at least since more than 35 years.

In case of the heavily infected field which was investigated by us, circumstances extraordinarily favoured virus infestation. In July and perhaps also in August 1971, large numbers of winged aphids were active in different regions of the GDR, but especially near Aschersleben. The fodder rape field was situated close to gardens and fields where overwintered plants and perennials of different kinds, including crucifers, were growing. So a severe loss resulted. Attempts to measure the damage exactly failed because almost all the rape plants seemed to be infected. Those without symptoms were weak in growth, obviously due to bad water supply. A field of 'Liho' rape at more than 10 kilometers distance from Aschersleben lying far away from gardens and other infection sources contained only few mosaic symptom showing plants and grew well.

We do not have secured facts in order to explain, how the mixed infections took place, but it seems unlikely that single aphids coming from outside transmitted 3 or even 4 viruses in complex. In theory, all the viruses could be transmitted simultaneously by single members of different aphid species. For example, *Brevicoryne brassicae* and *Myzus persicae* are known to be vectors of all the four viruses which had infected rape. But there was no indication for the very existence of corresponding source plants harbouring all the viruses at the same time. The one aphid transmission test, performed by us with obviously mixed infected leaves as source, even does not support the idea that from rape to rape plant several viruses were transmitted in a single feeding act. With some security it can be said that ClMV was only seldom and infected the plants rather late in the season. This may be the consequence of few virus sources at least in the beginning of aphid flights. ClMV was shown to be relatively rare in the GDR from 1969 to 1971 (SHUKLA and SCHMELZER, 1972a). Perhaps all the viruses arrived singly to the rape plants, however, their sequence is unknown.

The trials to demonstrate the importance of the various viruses for the origin of the symptoms and the damage of naturally infected 'Liho' rape indicated that CBRV is the main factor. This virus is also known to induce severe symptoms and damage a number of other cruciferous crop plants in Europe (BROADBENT, 1957; SHUKLA and SCHMELZER, 1972c; SHUKLA *et al.*, 1972b).

Occurrence of CMV in several crucifers is reported in literature. The virus is known to multiply in them generally without producing symptoms. Our earlier and present results clearly indicate that CMV is often associated with other viruses

in crucifers. In accordance with the statements of HORVÁTH (1969) we found in experimental transmissions that rape can react with pronounced symptoms after CMV infections. Sometimes, however, latent infections may occur. In such cases virus concentration is reduced. Obviously the virus intensifies symptom expression in mixture with CBRV.

Although all the 16 naturally infected plants tested contained NRSV, it apparently did not increase the severity of the field symptoms since greenhouse plants remained unaffected. On the other hand, in combination with CMV it seemed to suppress symptom expression of the latter. The reason for this is not known. Symptomless infections by NRSV were also obtained in case of *Sinapis alba* L., the only other cruciferous plant demonstrated before to be a natural host of NRSV (SHUKLA and SCHMELZER, 1972a). An attempt to infect 3 other crucifers in the mentioned paper gave only latent infections. 'Liho' rape appears to be very susceptible to NRSV, whereas 55 cultivars of brassica and radish crops were inoculated in greenhouse without any success. KARL *et al.* (1972) showed that 21 of 22 aphid species transmitted the virus more or less frequently. Therefore the chances of the virus to be transmitted to rape are high in years with strong aphid flights.

Acknowledgements

Our thanks are due to Dr. J. RICHTER for providing the CMV antiserum, to Dr. H. B. SCHMIDT for electron microscopical examinations, to Dr. C. LEHMANN (Gatersleben) for seeds of 'Liho' rape, 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

XIII. Cabbage Black Ring, Nasturtium Ringspot and Alfalfa Mosaic Viruses in Ornamental and Wild Species

By

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During 1970 and 1971, investigations were performed concerning the natural infection of viruses within ornamental and wild crucifers growing in eleven botanical gardens and plantations located in different regions of the GDR. With the exception of two, cabbage black ring virus was found at all the places. The 45 species, which proved to be spontaneous hosts of this virus, belong to 29 genera. Thirty of the species are previously unknown natural hosts. Only one half of the species displayed disease symptoms. Nasturtium ringspot virus was detected at three locations and in 8 species belonging to 7 genera. All the species were never shown before to be naturally infected by the virus. It seemed that only latent infections occurred. Alfalfa mosaic virus was isolated from *Lunaria annua* and *Malcolmia bicolor* growing in two botanical gardens. The latter species is a new spontaneous host. Both species seemed to be infected latently.

The history of virus diseases in ornamental crucifers goes back to 1783 when a report from an anonymous author was published in the first issue of the first German garden journal "Journal für die Gartenkunst" on pages 58 to 59. Here the beauty and the possibility of artificial induction of different colours in the same flower of stock (*Matthiola incana* R. Br.) was described. Now it is known that this phenomenon is caused by cabbage black ring virus (CBRV), but the virus nature of flower breaking in stock was established more than 150 years later by TOMPKINS (1934) in the USA. Soon thereafter similar diseases also of other ornamental crucifers were recognized in England and CBRV, too, was found to be the cause of them (SMITH, 1935; 1947). However, *Matthiola incana* remained so far the most investigated species and the occurrence of CBRV on it has been stated in numerous countries (NOBLE *et al.*, 1934; OLIVEIRA and BORGES, 1944; JEFFERSON and EADS, 1951; JOHNSON and BARNHART, 1956; MISCHKE, 1957; USCHDRAWEIT and VALENTIN, 1957; SCHWARZ, 1959; BHARGAVA and JOSHI, 1960; MILIČIĆ, 1962; SAN JUAN and POUND, 1963; SERGEEVA, 1964).

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Besides *Cruciferae*, CBRV is known to infect plant of several other families in nature. USCHDRAWEIT and VALENTIN (1957, 1959) isolated it from 30 ornamental and wild plant species belonging to 25 genera and 11 families. The virus has often been reported from various parts of the world occurring spontaneously with pronounced disease symptoms on different ornamental and wild crucifers. As far as we know, the number of stated natural CBRV hosts of this kind in crucifers has now risen to almost 40 (TOMPKINS, 1934, 1939; SMITH, 1935, 1947; MILIČIĆ, 1956, 1962; USCHDRAWEIT and VALENTIN, 1957; MILIČIĆ *et al.*, 1958; LOVISOLO and BENETTI, 1959; SCHWARZ, 1959; ARNOLD and BALD, 1960; BHARGAVA and JOSHI, 1960; LOVISOLO, 1960; BRČÁK and POLÁK, 1963; ŠTEFANAC-UDBINAC *et al.*, 1963; SERGEEVA, 1964; POLÁK, 1965; SHUKLA and SCHMELZER, 1970a; FELDMAN and GRACIA, 1972). The fact that most of the natural CBRV hosts among ornamental and wild crucifers have been reported from only one country shows that there are considerable gaps in our knowledge concerning this question.

Although some ornamental and wild crucifers have been demonstrated in the past to be experimental hosts of nasturtium ringspot virus (NRSV) (SCHMELZER 1960; SHUKLA and SCHMELZER, 1972), its natural occurrence in members of the family *Cruciferae* is reported only in mustard and rape (SHUKLA and SCHMELZER, 1972; 1973).

Alfalfa mosaic virus (AMV) is known to possess the widest host range after cucumber mosaic virus among aphid transmissible plant viruses but its spontaneous occurrence in crucifers was recognized only recently. SCHMELZER *et al.* (1973) demonstrated for the first time two ornamental crucifers, namely *Iberis umbellata* L. and *Lunaria annua* L., as natural hosts of AMV.

While working on viruses in ornamental and wild crucifers during the vegetation periods of 1970 and 1971, we isolated and identified CBRV, NRSV and AMV from a large number of species. The results are presented in this paper.

Material and Methods

Plant material for isolation of viruses was collected from different botanical gardens and plantations in the GDR. Symptomless as well as symptom-showing young leaves of ornamental and wild crucifers, sown or planted mostly for botanical purposes, served as inocula. Methods of isolation, raising of test plants, and performing of serological investigations were the same as described in earlier papers (SHUKLA and SCHMELZER, 1970a, 1970b).

Results

Cabbage black ring virus

The test plant reactions induced by the CBRV isolates in our trials were as follows:

Brassica oleracea L. var. *botrytis*	II chlorotic flecks, chlorotic to necrotic rings and mottling
Chenopodium amaranticolor Coste et Reyn.	I necrotic lesions II not infected
C. murale L.	I necrotic lesions II not infected
C. quinoa Willd.	I chlorotic to necrotic lesions II not infected
Nicotiana megalosiphon Heurck et Muell.	I necrotic spots and rings II mottling, crinkling, and leaf distortion
N. tabacum L. cvv. 'Bel 61 – 10' and 'Samsun'	I brown necrotic lesions II not infected
Petunia hybrida hort. ex Vilm.	I black necrotic lesions II vein clearing, mottling, crinkling and stunting
Sinapis alba L.	II mosaic and crinkling

I and II represent rubbed leaves and systemically infected leaves, respectively.

In insect transmission tests, performed only with few isolates, the virus could be transmitted successfully by *Myzus persicae* Sulz. from cauliflower to cauliflower in the non-persistent manner. Using the dip method, virus particles of a length of about 750 nm were seen under electron microscope in preparations from cauliflower leaves showing pronounced symptoms.

In our investigations the virus was isolated from 45 different plant species collected in 9 botanical gardens or plantations. Only the samples from Greifswald and Potsdam did not yield this virus. Table 1 records names of the species, places of their collection, and the symptoms observed on them.

The occurrence of CBRV in 15 plant species mentioned in Table 1 has already been described in literature. However, the other 30 species are being reported here for the first time as natural hosts of the virus, although a number of them had been found susceptible to CBRV in mechanical inoculation tests. CBRV infections at 2 to 4 locations were shown for 13 of the species.

Some ornamental and wild crucifers, from which seeds were available, were tested in greenhouse for their reactions against CBRV. *Bunias erucago* showed

Table 1

Natural hosts of CBRV among ornamental and wild crucifers

Plant species	Places of collection	Symptoms observed in nature
**Alliaria officinalis* Andrz.	Berlin, Dresden**, Halle	mosaic
Alyssoides utriculatum (L.) Med.	Jena**	mosaic, stunting
Alyssum altaicum C. A. Mey.	Dresden	no
**A. argenteum* Vitm.	Berlin, Dresden**, Jena	mosaic
A. idaeum Boiss. et Heldr.	Leipzig**	mosaic, chlorotic rings
**A. saxatile* L.	Eberswalde, Jena**	mosaic (fig. 1, G)
Arabis androsacea Fenzl.	Halle	no
A. caucasica Willd.	Eberswalde	no
A. jacquinii Beck	Halle**, Jena	mosaic, chlorotic rings
A. ludoviciana C. A. Mey.	Dresden**	mosaic
A. muralis Bert.	Jena**	mosaic
A. procurrens Waldst. et Kit.	Dresden, Jena, Leipzig	no
A. vochinensis Spreng.	Dresden	no
Aubrieta olympica Boiss.	Eberswalde	no
Barbarea vulgaris R. Br.	Berlin	no
Berteroa incana DC.	Dresden,** Halle, Jena Leipzig	mosaic
Biscutella lyrata L.	Halle**	mosaic
Brassicella erucastrum (L.) O. E. Schulz	Leipzig	no
Cakile maritima Scop.	Leipzig	no
Capsella grandiflora (Bory et Chaub.) Boiss.	Halle**	vein clearing, mosaic
**Cheiranthus cheiri* L.	Berlin, Leipzig	no
**Cochlearia officinalis* L.	Halle**	mosaic
**Diplotaxis muralis* (L.) DC.	Leipzig	no
D. tenifolia DC.	Halle, Jena, Leipzig	no
Draba fladnizensis Wulf.	Dresden**	chlorotic rings (fig. 1, D)
D. hispanica Boiss.	Jena	no
**Eruca sativa* Mill.	Leipzig, Rostock**	mosaic, chlorotic rings, tip necrosis, stunting
Erysimum crepidifolium Rchb.	Dresden**	chlorotic flecks and mosaic
E. hieraciifolium Jusl.	Jena	no
E. odoratum Ehrh.	Leipzig	no
**Hesperis matronalis* L.	Halle**, Jena**, Rostock	mosaic, stunting (fig. 1, J)
H. steveniana DC.	Dresden**	mosaic
**H. tristis* L.	Jena	no
Iberis amara L.	Halle	no
**I. umbellata* L.	Leipzig	no
Lobularia maritima (L.) Desv.	Jena, Leipzig	no
**Lunaria annua* L.	Berlin, Halle**, Rostock	mosaic, stunting (figs 1, H—I)
Malcolmia bicolor Boiss. et Heldr.	Leipzig	no

(Table 1, continued)

Plant species	Places of collection	Symptoms observed in nature
**Matthiola incana* R. Br.	Halle**, Rostock**, Quedlinburg**	mosaic, stunting, flower breaking
**Moricandia arvensis* (L.) DC.	Leipzig	no
**Raphanus raphanistrum* L.	Leipzig**	mosaic
Schivereckia podolica Andrz. et Bess	Leipzig	no
**Sinapis arvensis* L.	Gatersleben**	mosaic, crinkling (fig. 1, A)
Thlaspi rotundifolium (L.) Gaud.	Leipzig	no
Vesicaria utriculata Lam. et DC.	Halle**	mosaic, stunting

* Plant species already described as spontaneous hosts of CBRV in literature
** Only at the indicated location the concerning species showed symptoms

chlorotic to necrotic lesions, systemic vein clearing and mottling (Figs 1,B – C); *Lunaria annua* displayed black necrotic local lesions, systemic vein clearing and mottling (Fig. 1,H – I); *Malcolmia bicolor* and *M. flexuosa* produced systemic mottling and stunting. The systemic symptoms on *Lunaria annua* were similar to those found in nature, whereas *Malcolmia bicolor* showed pronounced symptoms after artificial inoculation but seemed to be latently infected in the garden. *Bunias erucago* and *Malcolmia flexuosa* are new experimental hosts. The former can also be used as test plant for CBRV as it produces defined symptoms within 3 to 4 days after inoculation.

Nasturtium ringspot virus

The test plant reactions induced by NRSV in our trials were as follows:

Chenopodium amaranticolor,	I chlorotic to necrotic lesions, death of leaves
C. murale, and *C. quinoa*	II chlorosis, wilting, tip necrosis
Nicotiana megalosiphon	I necrotic spots and rings
	II necrosis, necrotic to chlorotic pattern, dwarfing, recovery
N. tabacum cvv.	I necrotic rings and spots
'Bel 61 – 10' and 'Samsun'	II chlorotic to necrotic ring and oak-leaf pattern, mottling, recovery
Petunia hybrida	II chlorotic spots, rings and mosaic, no recovery

I and II represent rubbed leaves and systemically infected leaves, respectively.

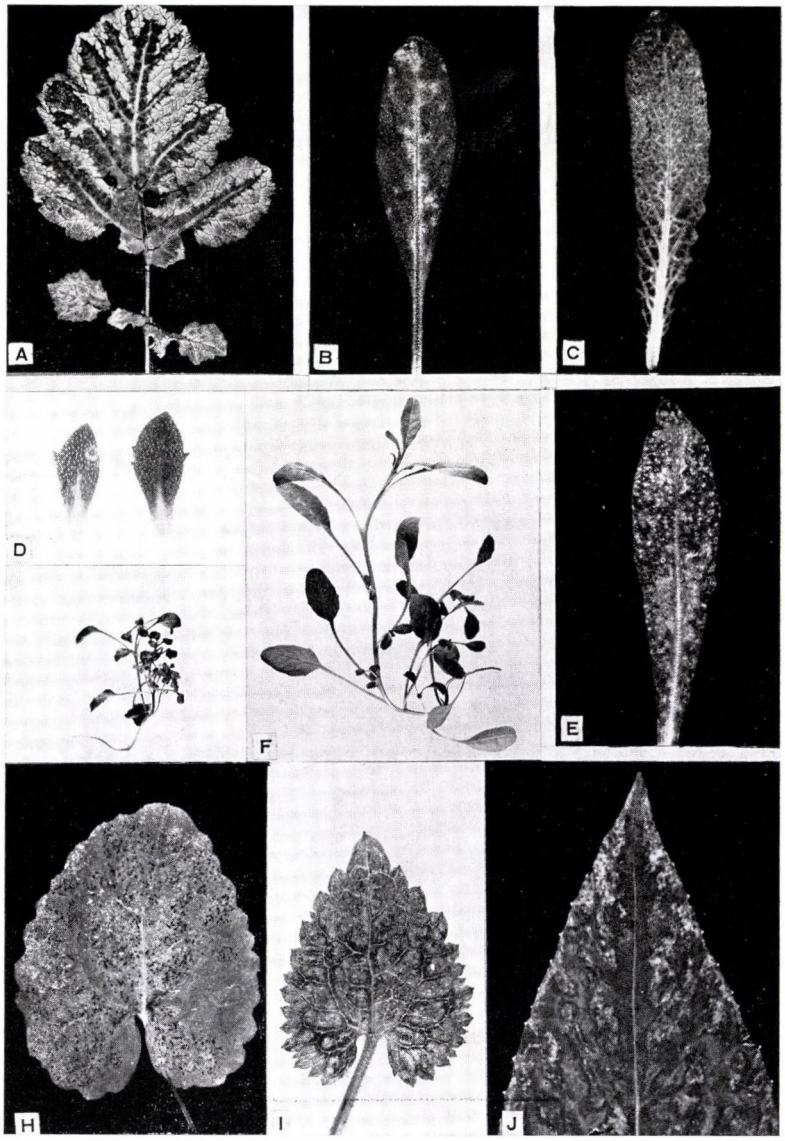

Fig. 1. Symptoms of cabbage black ring virus on ornamental and wild crucifers. A: *Sinapis arvensis*; B–C: *Bunias erucago*; D: *Draba fladnizensis* (on the left diseased, on the right healthy); E–F: *Malcolmia bicolor*; G: *Alyssum saxatile*; H–I: *Lunaria annua*; J: *Hesperis matronalis*; A, D, G, T, J: spontaneous infections; B, C, E, H: artificial infections; F: control; B, H: local infections; A, C, D, G, E, G, I, J: systemic infections

The test plant reactions of NRSV were in accordance with the earlier descriptions (SCHMELZER, 1960, 1970). The identity of all obtained isolates was confirmed by serological investigations. When tested with antisera against arabis mosaic, AMV, NRSV, raspberry ringspot, strawberry latent ringspot viruses and against the two serotypes of tomato black ring virus, using systemically infected leaves of *Chenopodium quinoa* as source, the isolates always reacted only with NRSV antiserum. The latter was prepared against an isolate of the virus from *Tropaeolum majus* L. (SCHMELZER, 1970) and gave a maximum serum titer of 1 : 256. There was no important difference between the various NRSV isolates. In Table 2 are given the data for the NRSV infected species found among ornamental and wild crucifers in three botanical gardens.

Table 2

Natural hosts of NRSV among ornamental and wild crucifers

Plant species	Places of collection	Symptoms observed in nature
Alliaria officinalis	Berlin	no
Biscutella lyrata	Halle	mosaic
Cakile maritima	Leipzig	no
Erysimum perovskianum Fisch. et Mey.	Halle	no
Iberis amara	Halle, Leipzig	no
I. umbellata	Leipzig	no
Lunaria annua	Halle	mosaic, stunting
Malcolmia bicolor	Leipzig	no

Out of the 8 species mentioned in Table 2 are, symptoms were observed only on 2 of them and these contained also CBRV simultaneously which may be responsible for symptom production. All species in Table 2 previously unknown natural hosts of NRSV.

Alfalfa mosaic virus

In the present investigation AMV could be detected only in *Lunaria annua* and *Malcolmia bicolor* collected from the botanical gardens in Halle and Leipzig, respectively. The latter species is a new natural host of the virus. In both cases AMV occurred in mixed infections with three other viruses. Therefore, on the basis of test plant reactions in the isolation experiments no conclusion could be drawn about the presence of AMV in the isolates, since *Chenopodium amaranticolor* and *C. quinoa* reacted with systemic mottling, curling and necrosis of top leaves which is typical for AMV, NRSV and a number of NEPO viruses. Serological investigations were the main criterion for identification. Positive reactions

were obtained with AMV antiserum with both the isolates. No attempts were made to separate the AMV components from the complex infections. It seems probable that both plant species were infected by AMV only latently. SCHMELZER *et al.* (1973) also did not observe AMV symptoms on spontaneously infected ornamental crucifers.

Discussion

As a result of the present paper, the knowledge on the natural host range of cabbage black ring virus within the *Cruciferae* has considerably been increased. Most of the CBRV hosts mentioned in Table 1 are overwintering or even perennial plants which may serve as important virus sources when they grow in larger numbers in or adjacent to gardens and fields. Although the situation in this respect is even worse with other viruses in ornamental and wild crucifers, the presence of CBRV cannot always be recognized by disease symptoms. Only one half of the species in Table 1 showed symptoms and when they did it, symptoms were not seen at all locations at which the plants were found to be infected. This fact underlines the danger of the virus sources.

Also among the new natural crucifer hosts of NRSV there are some which may act as virus reservoirs. Now the overwintering of NRSV under natural conditions and its sudden appearance in annual plants, problems which were rather obscure until recently, may be better understood. Besides *Scrophulariaceae* (SCHUMANN, 1963), *Umbelliferae* (SCHMELZER and WOLF, 1969; WOLF, 1970; WOLF and SCHMELZER, 1972), and probably some woody plants, *Cruciferae* may have some importance in this respect.

Possibly, ornamental and wild crucifers do not play any practical role as sources of AMV because other plants, especially lucerne, are much more often infected by this virus. Nevertheless, the fact is of interest that among the already known 150 natural AMV host species (SCHMELZER *et al.*, 1973), there are also some crucifers.

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We are grateful to Dr. J. SCHULTZE—MOTEL, Gatersleben, for help in nomenclature of *Cruciferae*. Dr. H. B. SCHMIDT and Dipl.-Landw. K. EISBEIN performed the electron microscopical investigations. Miss H. C. NORDMANN and Miss U. BRUNNE made the photographs.

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Studies on Viruses and Virus Diseases of Cruciferous Plants

XIV. Cucumber Mosaic Virus in Ornamental and Wild Species

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During two years of investigation 80 species of ornamental and will crucifers, belonging to 38 genera, were shown to be infected by cucumber mosaic virus (CMV). With the exception of 8 species they are previously unknown natural hosts of the virus. The material was collected in 9 botanical gardens or nurseries distributed over a large part of the GDR. Nineteen of the species proved to be infected at 2 to 5 different locations. Most of the investigated species were perennials. From these results it can be concluded that long living ornamental and wild cruciferous plants are potential sources of CMV. Most of the crucifers, in which the virus was detected, did not show symptoms. Eight cruciferous species became infected without displaying symptoms after artificial inoculation with a normal strain of CMV. Therefore it seems that the virus is mostly latent in ornamental and wild crucifer species and only special strains may induce pronounced reactions on a majority of them.

Soon after the discovery of cucumber mosaic virus (CMV) by DOOLITTLE (1916), its importance in causing severe diseases in *Cucurbitaceae* and members of other families was recognized by workers in many countries. DOOLITTLE and WALKER (1926) were also the first who suggested the eradication of wild host plants which may serve as bridge hosts or reservoirs for CMV. It is now known to have the widest host range within the plant viruses infecting species of almost all families of dicotyledonous and monocotyledonous groups. While working on viruses in ornamental and wild perennial plants, USCHDRAWEIT and VALENTIN (1956, 1959) found CMV more often than any other virus. They detected it in 129 plant species belonging to 30 families and concluded that two thirds of the diseased plants were infected by CMV alone.

Also ornamental and wild crucifers are known to be infected by CMV in nature. Until now 15 different species of them have been stated as natural CMV hosts (SMITH, 1946; POUND and WALKER, 1948; LIHNELL, 1951; ANONYM, 1954; USCHDRAWEIT and VALENTIN, 1956; HEROLD and BREMER, 1958; SCHWARZ, 1959; NITZANY, 1960; BRČÁK and POLÁK, 1963; SHUKLA and SCHMELZER, 1970a). Additionally, almost an equal number of ornamental and wild crucifers are known

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to be susceptible to the virus in artificial inoculations (POUND and WALKER, 1948; SMITH, 1952; SCHWARZ, 1959; KLINKOWSKI and co-workers, 1968).

During an investigation on viruses in ornamental and wild crucifers in the vegetation periods of 1970 and 1971, we isolated and identified CMV from a large number of plant species. The results are presented in this paper.

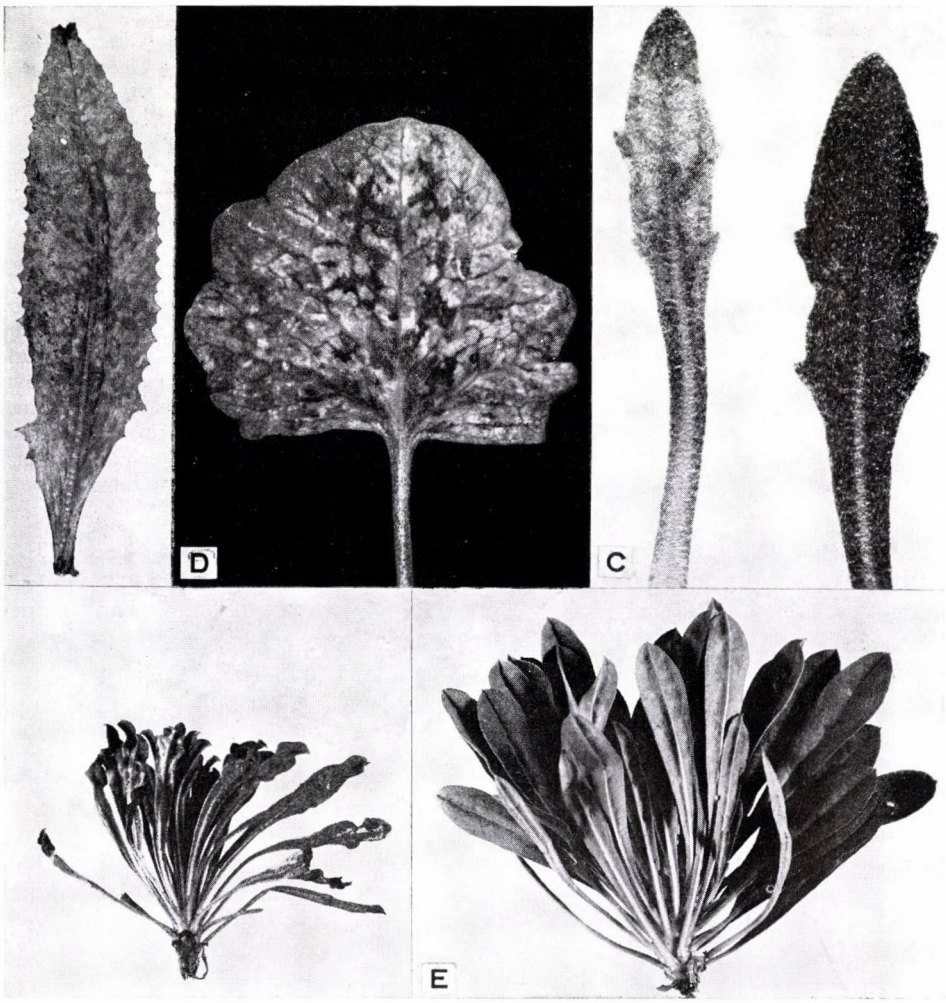

Fig. 1. Symptoms of ornamental and wild crucifers, spontaneously infected by cucumber mosaic virus and possibly induced by it. A: *Bunias orientalis*; B: *Cochlearia officinalis*; C: *Fibigia clypeata* (on the left diseased, on the right healthy); D—E: *Alyssoides utriculatum*, D: diseased, E: healthy

Material and Methods

Symptomless as well as symptom-showing young leaves from ornamental and wild crucifer species were collected in the botanical gardens of Berlin (Baumschulenweg), Dresden, Eberswalde, Greifswald, Halle, Jena, Leipzig, Rostock and in a nursery near Potsdam for virus isolation. Test plants, methods of isolation, cross-protection and serology were done in the same manner as described in earlier papers (SHUKLA and SCHMELZER, 1970a; 1970b).

Results

The obtained CMV isolates induced the following test plant reactions in our trials:

Chenopodium amaranticolor Coste et Reyn. Ia, small necrotic local lesions; IIb) not infected
C. murale L. I necrotic local lesions; II not infected
C. quinoa Willd. I chlorotic to necrotic local lesions; II not infected
Cucumis sativus L. II mottling, mostly stunting
Nicotiana megalosiphon Heurck et Muell. I necrotic local lesions, sometimes rings; II necrosis, mottling, gradual recovery
N. tabacum L. cv. 'Bel 61 – 10' I chlorotic to slight necrotic spots; II mottling
N. tabacum L. cv. 'Samsun' I slight necrotic concentric rings and patterns; II mottling
Petunia hybrida hort. ex Vilm. I sometimes chlorotic spots; II vein clearing and mottling
Sinapis alba L. II occasionally mottling

a) inoculated leaves
b) top leaves

The reactions were in accordance with those earlier described for CMV (SCHMELZER, 1962/63; SHUKLA and SCHMELZER, 1970a). The identity of the isolates was confirmed either by cross-protection tests or by serology. As already described by SCHMELZER (1962/63), *Nicotiana megalosiphon* was found to be the best plant species for cross-protection tests also in the present investigation and was used invariably. Plants recovered after infection with the isolates in question were reinoculated with a common strain of CMV, named "GM I", as challenge virus and vice-versa. All tested isolates of CMV from crucifers gave positive reactions. In serological tests, using crude sap of systemically infected leaves of *Nicotiana tabacum* cv. 'Samsun' as source for virus antigen, the isolates reacted positively with the antiserum prepared against the "GM I" strain of the virus. At least the major part of the isolates seemed to belong to the common form of CMV which occurs most frequently in the GDR. Table 1 records names of the plant species, places of collection and the symptoms observed on them in nature.

Table 1

Natural hosts of cucumber mosaic virus among ornamental and wild crucifers

Plant species	Places of collection	Observed symptoms
Aethionema grandiflorum Boiss. et Hohen.	Leipzig, Potsdam	no
A. pulchellum Boiss. et Huet.	Eberswalde	no
**Alliaria officinalis* Andrz.	Berlin, Halle	no
Alyssoides utriculatum (L.) Med.	Jena**, Leipzig**	mottling and stunting (Figs 1, D—E)
Alyssum bornmuelleri Hausskn.	Dresden**	mosaic
**A. saxatile* L.	Potsdam	no
A. spinosum L.	Halle, Leipzig	no
Arabis alpestris Rchb.	Rostock	no
A. blepharophylla Hook. et Arn.	Jena	no
A. caerulea All.	Leipzig	no
A. caucasica Willd.	Halle, Leipzig	no
A. jacquinii Beck	Eberswalde	no
A. muralis Bert.	Jena**	mosaic
A. scopoliana Boiss.	Dresden	no
A. stelleri DC.	Leipzig	no
A. sudetica Tausch.	Jena	no
A. vochinensis Spreng.	Dresden	no
**Aubrieta deltoides* (L.) DC. var. *graeca* Regel	Berlin	no
A. erubescens Griseb.	Dresden, Greifswald	no
A. hesperidiflora G. Don	Dresden, Eberswalde	no
A. intermedia Heldr. et Orph.	Jena	no
A. italica Boiss.	Dresden	no
A. olympica Boiss.	Eberswalde	no
A. pinardi Boiss.	Dresden	no
A. suendermanii Host	Dresden	no
Barbarea iberica (W.) DC.	Jena	no
B. intermedia Bor.	Berlin	no
B. vulgaris R. Br.	Berlin, Greifswald, Halle	no
Berteroa incana DC.	Dresden**	mosaic
Biscutella lyrata L.	Halle**	mosaic
B. raphanifolia Poir.	Jena	no
Brassicella erucastrum (L.) O. E. Schulz	Leipzig**	mosaic
Bunias erucago L.	Halle**	mosaic
**B. orientalis* L.	Dresden**, Jena, Leipzig**, Rostock, Greifswald	vein clearing, chlorotic spots and mosaic (Fig. 1, A)
Cakile maritima Scop.	Leipzig	no
Campe barbarea F. W. Wight	Leipzig	no
Capsella grandiflora (Bory et Chaub.) Boiss.	Halle**	mosaic
Cardamine pratensis L.	Berlin	no
Cheiranthus cheiri L.	Berlin	no

continuation of table 1

Plant species	Places of collection	Observed symptoms
Cochlearia glastifolia L.	Jena, Leipzig	no
**C. officinalis* L.	Berlin**, Dresden, Halle**	mosaic (Fig. 1, B)
Crambe maritima L.	Eberswalde, Halle	no
Draba aizoides L.	Dresden, Jena	no
D. daurica DC.	Dresden**	mosaic
D. hispida Willd.	Jena	no
D. hoppeana Rchb.	Halle	no
D. loiseleurii Boiss.	Rostock	no
D. longirostra Schott, Nym. et Kotschy	Halle	no
D. sibirica (Pall.) Thell.	Jena	no
Eruca sativa Mill.	Leipzig, Jena	no
Erysimum helveticum (Jacq.) DC.	Dresden, Eberswalde	no
E. hieracüfolium Jusl.	Jena	no
E. ochroleucum DC.	Leipzig	no
E. odoratum Ehrh.	Leipzig	no
E. perovskianum Fisch. et Mey.	Halle, Jena**	mosaic
E. pumilum Gaud.	Berlin	no
Fibigia clypeata (L.) Med.	Dresden**, Jena	mosaic (Fig. 1, C)
**Hesperis matronalis* L.	Berlin, Eberswalde**, Halle**, Jena**, Rostock	mosaic, stunting
H. steveniana DC.	Dresden**	mosaic
H. tristis L.	Jena	no
Hutchinsia alpina (L.) R. Br.	Eberswalde	no
H. brevicaulis Hoppe	Halle	no
**Iberis amara* L.	Halle	no
I. umbellata L.	Leipzig	no
Isatis tinctoria L.	Leipzig	no
Lepidium stalatum Lag. et Rodr.	Dresden	no
Lobularia maritima (L.) Desv.	Jena, Leipzig	no
Malcolmia bicolor Boiss. et Heldr.	Leipzig	no
Matthiola vallesiaca Jaccard	Leipzig	no
Peltaria alliacea Jacq.	Halle	no
**Raphanus raphanistrum* L.	Leipzig**	mosaic
Rorippa silvestris Bess	Potsdam	no
Schivereckia podolica Andrz. et Bess	Dresden**	mosaic
Schizopetalon walkeri Sim.	Rostock	no
Sisymbrium loeselii L.	Leipzig**	mosaic
S. officinale (L.) Scop.	Halle	no
Thlaspi arvense L.	Halle**	mosaic
T. bellidifolium Griseb.	Leipzig	no
T. montanum L.	Berlin	no
Vesicaria utriculata Lam. et DC.	Halle**, Greifswald**	mosaic, stunting

* Species already described in literature as natural host of CMV
** Locations where the plants showed disease symptoms

From Table 1 it is to be seen that 80 species of ornamental and wild crucifers, belonging to 38 genera, proved to be natural hosts of CMV. Eight of them have already been reported as spontaneous hosts of the virus by previous workers whereas 72 other species are being reported for the first time. CMV infections at 2 to 5 different locations were shown for 20 of the species.

Only a quarter of the cruciferous species in Table 1 showed disease symptoms. In order to have more informations on the symptomatology of CMV in crucifers the followings 8 species were inoculated mechanically in greenhouse: *Bunias erucago* L., *Camelina sativa* (L.) Crantz, *Conringia orientalis* (L.) Dumort., *Lepidium sativum* L., *Malcolmia bicolor* Boiss. et Heldr., *M. flexuosa* Sibth. et Sm., *M. littorea* R. Br., and *Raphanus caudatus* L. None of them showed any symptom even one month after inoculation. However, the virus could be recovered from them in back transmission tests to indicator hosts. *Bunias erucago* and *Malcolmia bicolor* were also found spontaneously infected by CMV in the present investigation. The other 6 species infected mechanically are new experimental hosts.

Discussion

During our investigation on cruciferous plants we found CMV as the most frequent virus in ornamental and wild species. It was the only virus whose presence was proved in all the botanical gardens and plantations investigated thoroughly by us. Rather often the same species was detected as a host of CMV at more than one location indicating its usual presence in them. Most of the CMV infected crucifers are perennials. From these facts it can be concluded that long living ornamental and wild cruciferous plants are potential sources of CMV under natural conditions.

A remarkable fact is that most of the species were found latently infected by CMV. In a majority of the symptom-showing species cabbage black ring (CBRV) and sometimes other viruses were additionally present. Therefore, it is difficult to say which virus induced the symptoms unless it is demonstrated experimentally. The ability of CBRV to induce pronounced disease symptoms in ornamental and wild crucifers is well-known (SHUKLA and SCHMELZER, 1973). The artificial inoculations described here did not sustain the idea that CMV causes always well visible damage in this plant group. Only little attention has been paid to this problem in literature. USCHDRAWEIT and VALENTIN (1956), who intensively worked on virus infestations in perennial ornamentals, did not record the symptoms induced by CMV in nature. SCHWARZ (1959) could transmit the virus to only 4 out of 11 ornamental and wild crucifers tested. Obviously, these species were also infected latently as the author did not mention symptoms produced on them. POUND and WALKER (1948) infected successfully all the 15 cruciferous species used in their trials including 10 ornamental and wild species. All species except one reacted with symptoms after mechanical inoculations with two isolates obtained from naturally infected *Hesperis matronalis*. It may be, however, that

they worked with special strains capable to induce more or less severe damage in crucifers. The same may have been happened in case of *Bunias orientalis* in Sweden (LIHNELL, 1951). Our earlier (SHUKLA and SCHMELZER, 1970a) and the present findings suggest that common strains of CMV mostly occur in latent form in ornamental and wild crucifers.

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Serologische Bestimmung des Trespenmosaikvirus in Extrakten aus Gerstenblättern in absoluten Gewichtseinheiten

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A modification of the method of SCHULTZE and SCHWICK (1959) for quantitative serological determinations is described. This method enables the determination of brome mosaic virus in clarified extracts from infected barley plants in absolute weight units.

Serologische Konzentrationsbestimmungen von phytopathogenen Viren in Saft bzw. Extrakten aus infizierten Pflanzen werden fast ausschließlich durch Austitern des Saftes vorgenommen. Die Nachteile dieses Verfahrens liegen in seiner Ungenauigkeit begründet und in der Tatsache, daß es keine absoluten Werte liefert. Im medizinischen Bereich wurden in den letzten Jahren eine Reihe von relativ einfachen Verfahren ausgearbeitet — insbesondere zur Bestimmung von Plasmaproteinen —, die diese Nachteile nicht aufweisen (siehe BECKER u. a., 1968; FRIEMEL u. a., 1969). Bei den meisten der hierfür verwendbaren Verfahren läuft die Immunpräzipitation in festem Medium ab. Es ist jedoch auch möglich, in flüssigem Medium zu arbeiten und den Antikörper-Überschußbereich von Präzipitationskurven, die bei der Reaktion von Antigen-Verdünnungsreihen mit einer konstanten Antiserum-Menge erhalten werden (HEIDELBERGER und KENDALL, 1935), zur Konzentrationsbestimmung des Antigens zu verwenden. Die auf diese Weise erhaltenen Präzipitate werden üblicherweise abzentrifugiert, gewaschen und ihr Proteingehalt auf chemischem Wege ermittelt. Einfacher und weniger zeitaufwendig ist es jedoch, die Präzipitatmengen über Trübungsmessungen zu bestimmen. SCHULTZE und SCHWICK (1959) entwickelten eine Methode, bei der Antigen und Antiserum verdünnt miteinander zur Reaktion gebracht werden und die Extinktion bei 450 nm als Maß für die Trübung verwendet wird.

Wir prüften, ob sich diese Methode auch zur Konzentrationsbestimmung phytopathogener Viren in Extrakten aus infizierten Pflanzen eignet. Die Versuche wurden mit dem Trespenmosaikvirus (TrMV) in Gerste durchgeführt.

Material und Methoden

Die Vermehrung des Trespenmosaikvirus (Stamm Lo) erfolgte in Gerste, Sorte "Xenia". Für die Versuche wurde ein Gramm Blattmaterial mit 5 ml 0,1 m Phosphatpuffer, pH 5,0 homogenisiert, das Homogenat durch ein Dederontuch gepreßt und anschließend 5 min bei 60°C erhitzt. Nach Abzentrifugation der groben Bestandteile (15 min bei 5000 U/min) bleibt eine vollkommen klare Lösung zurück, in der auch nach einstündigem Erhitzen bei 37°C keine Trübung auftrat. Vorversuche mit gereinigtem TrMV hatten ergeben, daß bei dieser kurzfristigen Temperaturbehandlung das Virus vollständig im Überstand verbleibt. Die Versuche wurden wie folgt in Reagenzgläsern (100 × 10 mm) angesetzt:

Versuchsansatz: 1,8 ml verdünntes Antiserum + 0,2 ml Extrakt aus infizierten Pflanzen + 0,2 ml Puffer

Kontrollansatz: 1,8 ml verdünntes Antiserum + 0,2 ml Extrakt aus gesunden Pflanzen + 0,2 ml Puffer

Standardansatz und Eichkurven: 1,8 ml verdünntes Antiserum + 0,2 ml Extrakt aus gesunden Pflanzen + 0,2 ml gereinigtes Virus in Puffer.

Zur Verdünnung der Reaktionspartner dienten 0,1 m Phosphatpuffer pH 5 (Pflanzenextrakte, Virus) bzw. 0,15 m NaCl (Antiserum). Die Antiseren wurden vor Gebrauch 30 min bei 56°C erhitzt. Für die Standardansätze und die Aufstellung der Eichkurven wurde gereinigtes TrMV (Herstellung nach PROLL und SCHMIDT, 1964, jedoch mit Fraktionierung über Sephadex G-200 anstelle von Dialyse als letzten Reinigunsschritt) verwendet, dessen Konzentration spektrophotometrisch (PROLL, 1967) ermittelt wurde.

Die Reaktionsgemische wurden 30 min bei 37°C in einem Ultrathermostaten inkubiert, in kaltem Wasser abgekühlt und anschließend in Küvetten überführt. Die Bestimmung des Trübungsgrades erfolgte durch Messung der Extinktion bei 450 nm im Spektralkolorimeter SPEKOL (VEB Carl Zeiss Jena, DDR). Für die Messung wurde der Extinktionsmeßansatz EK mit 0,5 cm-Küvetten (Füllvolumen: 1,8 ml) verwendet. Die Versuchsansätze (in wechselnder Anzahl pro Versuch) und die Standardansätze (zwei bis drei pro Versuch) wurden jeweils gegen den Kontrollansatz (Kontrolle = 0) gemessen. Jeder Ansatz wurde in zweifacher Wiederholung durchgeführt.

Ergebnisse

Aufstellen von Eichkurven

In Vorversuchen wurde zunächst die optimale Serumverdünnungsstufe ermittelt. Da die Eichkurve nur für die jeweils verwendete Antiserum-Charge benutzt werden kann, wurde i. a. ein Mischserum hergestellt, um eine möglichst große Menge zur Verfügung zu haben. Die Verdünnung erfolgte soweit, daß mit 100 μg Virus ein Wert von 75 – 100 für E_{450} ($\times$ 1000) erreicht wurde. Nach dem Verdünnen

wurde dem Antiserum 0,02% Natriumazid zugefügt. Die Aufbewahrung erfolgte bei 4°C.

Für die Aufstellung der Eichkurven wurden jeweils 6–8 verschieden stark verdünnte TrMV-Lösungen mit einer Konzentration von 5–100 µg (= 25–500 µg/ml) verwendet (Abb. 1). Wichtig ist, daß für die Konzentrationsbestimmungen nur der Antikörper-Überschußbereich verwendbar ist. Wir ermittelten diesen Bereich durch Testung der Überstände mit Hilfe von TrMV bzw. TrMV-Antiserum,

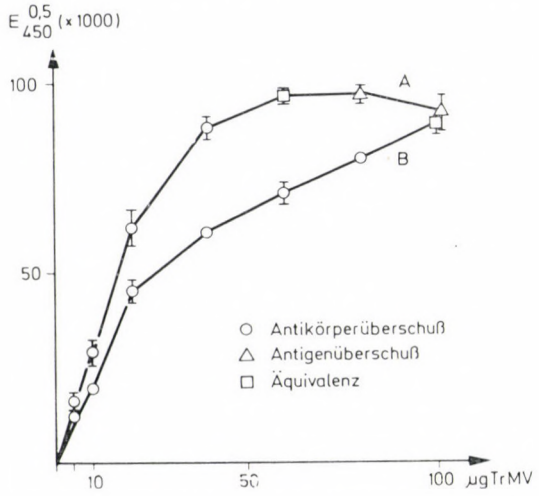

Abb. 1. Eichkurve zur serologischen Konzentrationsbestimmung des TrMV

nachdem zuvor die Antigen-Antikörper-Präzipitate, die sich nach 24-stündigem Stehen bei 4°C gebildet hatten, abzentrifugiert worden waren. Kurve B zeichnet sich gegenüber Kurve A durch einen größeren Meßbereich aus. Dies ist insofern ein Vorteil, als die Gefahr geringer ist, bei Bestimmungen an unbekanntem Material den Meßbereich zu verfehlen. Allgemein sollten stets zwei verschiedene Verdünnungen der zu testenden Probe geprüft werden, da nur so gesichert werden kann, daß der eigentliche Meßwert auf dem aufsteigenden Teil der Präzipitationskurve liegt.

Genauigkeit der Methode

Die errechneten Standardabweichungen wurden in die beiden Präzipitationskurven eingetragen (Abb. 1). Prozentual beliefen sie sich im mittleren und oberen Teil der Kurven (bei Viruskonzentrationen von 20–100 µg) auf ±2 bis 8%. Im unteren Teil der Kurve können die Werte auf ±12% ansteigen.

Um zu überprüfen, inwieweit die Methode richtige Werte liefert, wurden Zusatzversuche durchgeführt, bei denen eine bekannte Menge an gereinigtem

TrMV zu Ansätzen gegeben wurden, die mit Extrakt aus gesunden bzw. kranken Pflanzen hergestellt worden waren (1,8 ml Antiserum + 0,2 ml Extrakt + 0,2 ml gereinigtes TrMV). Parallel dazu wurden Versuche mit Zusatz von 0,2 ml Puffer anstelle von Virus durchgeführt, um die Virusmenge im vorgelegten Extrakt zu ermitteln. Tabelle 1 enthält als Beispiel Ergebnisse, die mit einer Antiserum-Charge erhalten wurden.

Tabelle 1

Verhältnis von zugegebenem zu wiedergefundenem TrMV in einem Gerstenextrakt-haltigen Antigen—Antikörper-Reaktionsgemisch

µg Virus zugegeben/vorgelegt	Insgesamt		% der zugegebenen Virusmenge (wiedergefunden)
	berechnet	gefunden	
10/0	10	10	100,0
10/5	15	16	110,0
10/43	53	53	100,0
10/85	95	95	100,0
20/11	31	32	105,0
20/20	40	40	100,0
20/46	66	64	90
40/0	40	44	110,0
40/5	45	54	122,5
40/43	83	85	105,0

Die mittlere Abweichung vom tatsächlichen Wert (100%) beträgt ±8,66%. Somit bestätigen diese Versuche die Brauchbarkeit des beschriebenen Verfahrens.

Anwendung der Methode

Wir verfolgten den Verlauf der Virusvermehrung im 1. Folgeblatt von Gerste vom 5. Tage nach der Infektion der Primärblätter mit TrMV an. Zu jeder Probenahme wurden 6—10 Blätter abgeerntet und aufgearbeitet (siehe Material und Methoden). Die Ergebnisse von 2 Versuchen sind in Abb. 2 wiedergegeben.

Es zeigt sich, daß der Verlauf der Virusvermehrung gut zu erfassen ist. Die Versuche fanden in einem normalen Gewächshaus in den Monaten März (Versuch A) bzw. Mai (Versuch B) statt. Aus den unterschiedlichen Umweltbedingungen resultieren die deutlichen Differenzen in der Vermehrungsgeschwindigkeit. Bei Versuch A war das Maximum der Viruskonzentration erst nach 20 Tagen erreicht, außerdem lag die TrMV-Konzentration um ca. 20% unter dem Wert, der in Versuch B erhalten wurde. Im letztgenannten Versuch wurde das Maximum bereits nach 12 Tagen erreicht. Nach Erreichen des Maximums deutet sich eine

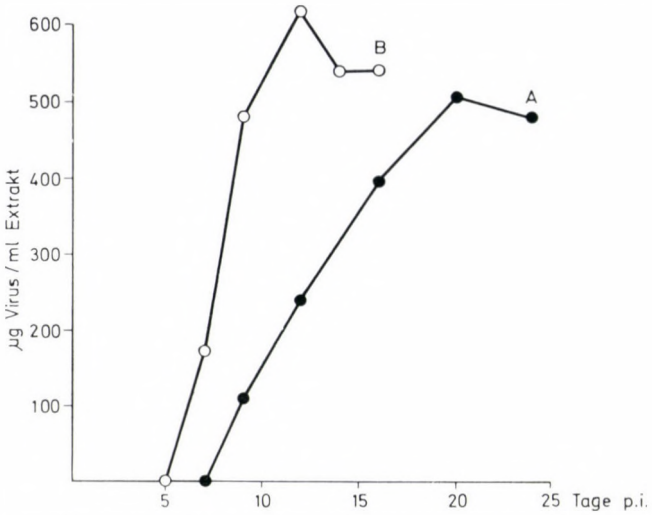

Abb. 2. Konzentration des TrMV in Gerste (Sekundärblatt) in Abhängigkeit vom Infektionszeitpunkt. Die Infektion erfolgte am Primärblatt

geringe Abnahme der Viruskonzentration an. Einen ähnlichen Verlauf der TrMV-Vermehrung in Sekundärblättern von Gerste wie in Versuch B fand PROLL (1967) mit Hilfe einer spektrophotometrischen Bestimmungsmethode.

Diskussion der Ergebnisse

Serologische Methoden zur Konzentrationsbestimmung haben aufgrund ihrer hohen Spezifität gegenüber anderen Verfahren den Vorteil, daß fremde Proteine bzw. Viren den Nachweis nicht stören. Es besteht daher die Möglichkeit, die Bestimmungen in ungereinigten Extrakten vorzunehmen. In der vorliegenden Arbeit wurde der Nachweis geführt, daß eine serologische Bestimmung der absoluten Viruskonzentration in Pflanzenextrakten prinzipiell möglich ist. Bei der von uns verwendeten Methode ist allerdings die Klärung der Extrakte unbedingt notwendig. Im Falle der TrMV-infizierten Gerstenpflanzen ist dies durch Verwendung eines schwach saueren Extraktionspuffers und eine nachfolgende Temperaturbehandlung (5 min bei 60 °C) relativ einfach zu erreichen.

Zwei Möglichkeiten deuten sich an, um auch in ungeklärten bzw. grob geklärten Extrakten serologische Konzentrationsbestimmungen dieser Art durchführen zu können:

1. Anwendung des klassischen Verfahrens der Präzipitatbestimmung (Zentrifugation und mehrmalige Waschung des Sedimentes sowie Bestimmung des Proteingehaltes). Auf diese Weise ermittelten z. B. KLEINKOPF u. a. (1970) die

Konzentration von Fraktion-1-Protein in Homogenaten von Gerstenblättern. Allerdings dürfte in rohen Extrakten die Gefahr gegeben sein, daß das Präzipitat Begleitsubstanzen enthält, die das Ergebnis verfälschen.

2. Anwendung eines Gelpräzipitations-Verfahrens. Besonders häufig wird in letzter Zeit die Radial-Immunodiffusion nach MANCINI u. a. (1965) verwendet. Diese Methode ist auch zum qualitativen Nachweis pflanzenpathogener Viren geeignet (SHEPARD und SECOR, 1969). Für quantitative Bestimmungen auf diesem Gebiet wurde sie unseres Wissens bisher noch nicht herangezogen. Ihre Eignung in dieser Hinsicht sollte in nächster Zeit überprüft werden. – Was die Fehlerbreite der Methode angeht, so ist diese wesentlich geringer als bei der herkömmlichen Bestimmung des Antigentiters. Bei letzterer kann sie dann, wenn Versuche innerhalb einer Serie an verschiedenen Tagen durchgeführt werden, einen Faktor von ± 2 (Verdünnungsstufen) betragen, das sind (je nach dem Bezugssystem) $100-200\%$. Unsere Methode arbeitet demgegenüber mit einem Fehler, der im Durchschnitt 10% nicht übersteigt. Demzufolge kann beispielsweise der Verlauf der Virusvermehrung auch besser erfaßt werden als durch Titerbestimmungen.

Zusammenfassung

Es wird eine Modifikation der Methode von SCHULTZE und SCHWICK (1959) beschrieben, mit deren Hilfe serologische Konzentrationsbestimmungen am Trespenmosaikvirus (TrMV) durchgeführt wurden. Die Methode ermöglicht die Bestimmung des TrMV in geklärten Extrakten aus infizierten Gerstenblättern in absoluten Gewichtseinheiten.

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Paulownia fargesii Franch. (Family: *Scrophulariaceae*) As a New Host Plant for Several Plant Viruses[1]

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Paulownia fargesii Franch. (Family: *Scrophulariaceae*) was mechanically inoculated with crude sap from the following plant leaves infected with thirteen plant viruses listed below: *Brassica rapa* L. var. *rapa* (radish mosaic virus, R/✳ : ✳/✳ : S/S : S/Cl; turnip yellow mosaic virus, R/1 : 1.9/37 : S/S : S/Cl), *Nicotiana glutinosa* L. (potato aucuba mosaic virus, ✳/✳ : ✳/✳ : E/E : S/Ap; tobacco rattle virus, R/1 : 2.3/5 : E/E : S/Ne), *Nicotiana tabacum* L. cv. *Samsun* (alfalfa mosaic virus, R/1: 1.3/18 : U/U : S/Ap; cucumber mosaic virus, R/1 : 1/18 : S/S : S/Ap; potato virus X, R/1 : ✳/6 : E/E : S/[Fu]); potato virus Y, ✳/✳ : ✳/✳ : E/E : S/Ap; tobacco mosaic virus, R/1 : 2/5 : E/E : S/✳), *Nicotiana tabacum* L. cv. *Xanthi-nc* (tobacco ring spot virus, R/1 : 1.8/42 : S/S : S/Ne), *Phaseolus vulgaris* L. cv. *Red Kidney* (bean [common] mosaic virus, ✳/✳ : ✳/✳ : E/E : S/Ap) and *Solanum tuberosum* L. cv. *Bintje* (potato virus M, ✳/✳ : ✳/✳ : E/E : S/Ap) as well as *Solanum tuberosum* L. cv. *Fortuna* (potato virus S, ✳/✳ : ✳/✳ : E/E : S/Ap).

Only local lesions were obtained on the inoculated leaves of *Paulownia* plants after infection with potato aucuba mosaic virus, potato virus X, tobacco mosaic virus and tobacco rattle virus. Lesions were dark brown, necrotic and in certain cases green in the yellowing leaves. These four viruses could be readily recovered exclusively from *Paulownia* leaves that were initially inoculated. Local yellowing symptoms occurred on the inoculated *Paulownia* leaves after infection with alfalfa mosaic virus and potato virus Y. These viruses could not be recovered from the inoculated leaves of *Paulownia* as it was indicated by back transfers to *Phaseolus vulgaris* L. as well as to *Nicotiana tabacum* L. cv. *Samsun*.

Paulownia fargesii Franch. proved to be highly susceptible to various strains of cucumber mosaic virus and to tobacco ring spot virus. Local symptoms (chlorotic spots, leaf dropping) developed eight days after inoculation with cucumber mosaic virus. As a consequence of cucumber mosaic virus infection, by the 12th and 15th days, systemic vein clearing, vein banding, mosaic and severe growth reduction appeared on the newly developed, non-inoculated leaves. *Paulownia fargesii* Franch. reacted with necrotic local lesions, four days after inoculation, with tobacco ring spot virus. At high temperatures (about 30°C) systemic symptoms, leaf dropping and top necrosis developed. These last two viruses were readily recovered in high titer from the inoculated and non-inoculated or subsequently developed leaves of *Paulownia fargesii* Franch.

No symptoms developed on *Paulownia* plants inoculated individually with crude saps containing bean (common) mosaic virus, potato virus M, potato virus S,

[1]In memoriam Prof. Dr. G. UBRIZSY († 25. May 1973)

radish mosaic virus as well as turnip yellow mosaic virus. These viruses could not be recovered from the inoculated and non-inoculated leaves of *Paulownia* plants.

On the basis of these studies *Paulownia fargesii* Franch. as a new indicator plant can be used for the detection and separation of certain plant viruses.

As far as we known the family *Scrophulariaceae* includes about seventy susceptible species to two mycoplasmas (aster yellows mycoplasma, ⁎/⁎ : ⁎/⁎ : ⁎/⁎ : S,I/Au; KUNKEL 1926, FRAZIER and SEVERIN 1945, SEVERIN and FREITAG 1945, as well as Vaccinium [cranberry] false-blossom mycoplasma, ⁎/⁎ : ⁎/⁎ : ⁎/⁎ : S/Au; KUNKEL 1945) and about forty plant viruses. Out of the virophilic members of the family *Scrophulariaceae* about twenty species are susceptible to the polyphagous tobacco mosaic virus (R/1 : 2/5 : E/E : S/⁎), seventeen to alfalfa mosaic virus (R/1 : 1.3/18 : U/U : S/Ap), fourteen to cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap), nine species to tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne) and tobacco necrosis virus (R/⁎ : ⁎/⁎ : S/S : S/Fu) as well as six plant species to tobacco etch virus (⁎/⁎ : ⁎/⁎ : E/E : S/Ap). The more important publications on this subject (cf. PRICE 1940, HOLMES 1946, HOLLINGS 1955, SCHMELZER 1957, SCHUMANN 1963a, b, SCHMELZER 1963a, b, c, DE ZEEUW 1965, HOLLINGS and STONE 1965, SCHMELZER 1966, THORNBERRY 1966, HULL 1969, BECZNER 1973) indicate that e.g. the *Antirrhinum*, *Digitalis*, *Linaria*, *Mimulus*, *Namesia*, *Penstemon*, *Torenia*, *Verbascum* and *Veronica* species are especially important in virus work. The following species proved to be the most useful: *Namesia strumosa* Benth., *Veronica longifolia* L., *Mimulus luteus* L., *Verbascum phoenicum* L., *Zaluzianskya villosa* F. W. Schmidt and more recently *Antirrhinum majus* L. and *Torenia fournieri* Lind. These two later plants are susceptible to many viruses. HOLLINGS and STONE (1955) reported, that *Torenia fournieri* Lind. is very suitable for obtaining purified preparations of several plant viruses.

Recently it was possible to isolate the polyphagous cucumber mosaic virus too, from spontaneously diseased member of the family *Scrophulariaceae: Paulownia tomentosa* (Thunb.) Steud. (syn.: *Paulownia imperialis* S. et Z.) in Germany and Hungary (cf. SCHMELZER 1969, HORVÁTH 1973).

Several isolates or strains of alfalfa mosaic virus, tomato black ring virus (⁎/⁎ : ⁎/⁎ : S/S : S/Ne), tobacco rattle virus (R/1 : 2.3/5 : E/E : S/Ne), tomato spotted wilt virus (R/⁎ : ⁎/⁎ : S/S : S/Th), and potato virus X (R/1 : ⁎/6 : E/E : S/[Fu]), caused necrotic local lesions, nasturtium ring spot virus (⁎/⁎ : ⁎/⁎ : ⁎/⁎ : S/Ap) induced symptomless local or systemic infections, arabis mosaic virus (R/1 : ⁎/41 : S/S : S/Ne) and cucumber mosaic virus showed systemic symptoms on *Paulownia tomentosa* (Thunb.) Steud. after artificial inoculations (cf. SCHMELZER 1969).

Paulownia fargesii Franch. (Family: *Scrophulariaceae*) is not listed in recent host range compilations for several plant viruses, therefore we investigated the reactions of this plant species to several plant viruses.

Material and Methods

Young *Paulownia fargesii* Franch. plants were mechanically inoculated in 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 destilled water after inoculation.

The following viruses were used: alfalfa mosaic virus (strain K2, BECZNER 1972), bean (common) mosaic virus (✳/✳ : ✳/✳ : E/E : S/Ap, BCMV-F23 a newly isolated strain in Hungary), four strain of cucumber mosaic virus (cf. 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 (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, strain HZ, kindly supplied from DR. Đ MAMULA, Zagreb, and RMV-H7 a newly isolated strain in Hungary), strain U1 of tobacco mosaic virus (SIEGEL and WILDMAN 1954), tobacco rattle virus (kindly supplied from DR. H. A. VAN HOOF, Wageningen), tobacco ring spot virus (kindly supplied from DR. J. W. DEMSKI, Experiment) and turnip yellow mosaic virus (R/1 : 1.9/37 : S/S : S/Cl, strain Y65, kindly supplied from DR. Đ. MAMULA, Zagreb, and TYMV-H4 a newly isolated strain in Hungary). The pure viruses were kept in *Brassica rapa* L. var. *rapa* (radish mosaic virus, turnip yellow mosaic virus), *Nicotiana glutinosa* L. (potato aucuba mosaic virus, tobacco rattle virus), *Nicotiana tabacum* L. cv. *Samsun* (alfalfa mosaic virus, cucumber mosaic virus, potato virus Y, potato virus X, tobacco mosaic virus), *Nicotiana tabacum* L. cv. *Xanthi-nc* (tobacco ring spot virus), *Phaseolus vulgaris* L. cv. *Red Kidney* (bean [common] mosaic virus) and *Solanum tuberosum* L. cv. *Fortuna* (potato virus S) as well as *Solanum tuberosum* L. cv. *Bintje* (potato virus M).

Symptoms on the inoculated *Paulownia fargesii* Franch. plants were regularly registered. In addition potato virus M and potato virus S infected plants were serologically investigated too. Serological investigations were carried out with plants about two or three weeks after inoculation. For serological tests the saps from the inoculated and non-inoculated leaves were clarified at approximately 6000 *rpm* for 15 minutes. Inoculated leaves of *Paulownia fargesii* Franch. were washed with tap water before any serological investigations. 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 reisolated from inoculated and non-inoculated leaves of *Paulownia* and tested on the following plant species: *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* (alfalfa mosaic virus, potato virus Y), *Nicotiana tabacum* L. cv. *Xanthi-nc* (tobacco

mosaic virus, tobacco ring spot virus), *Phaseolus vulgaris* L. (alfalfa mosaic virus), *Phaseolus vulgaris* L. cv. *Red Kidney* (bean [common] mosaic virus) and *Tetragonia expansa* Murr. (syn.: *Tetragonia tetragonoides* [Pall.] O. Ktze., cucumber mosaic virus). Inoculated *Paulownia* leaves were washed with tap water before the reisolation of viruses. Before the reisolation of the investigated viruses, the expressed saps from the inoculated and non-inoculated leaves of infected *Paulownia* plants were diluted 1 : 1 with tap water.

Results

Table 1 illustrates the viruses tested and the corresponding reactions (symptoms) of *Paulownia fargesii* Franch. to each of them. The results of the reisolation of the examined viruses as well as the results of the serological tests are also indicated. In the case of tobacco ring spot virus the first visible symptoms appeared four days after inoculation on *Paulownia fargesii* Franch. in the form of necrotic local lesions on the inoculated leaves (Fig. 1A). At high temperatures

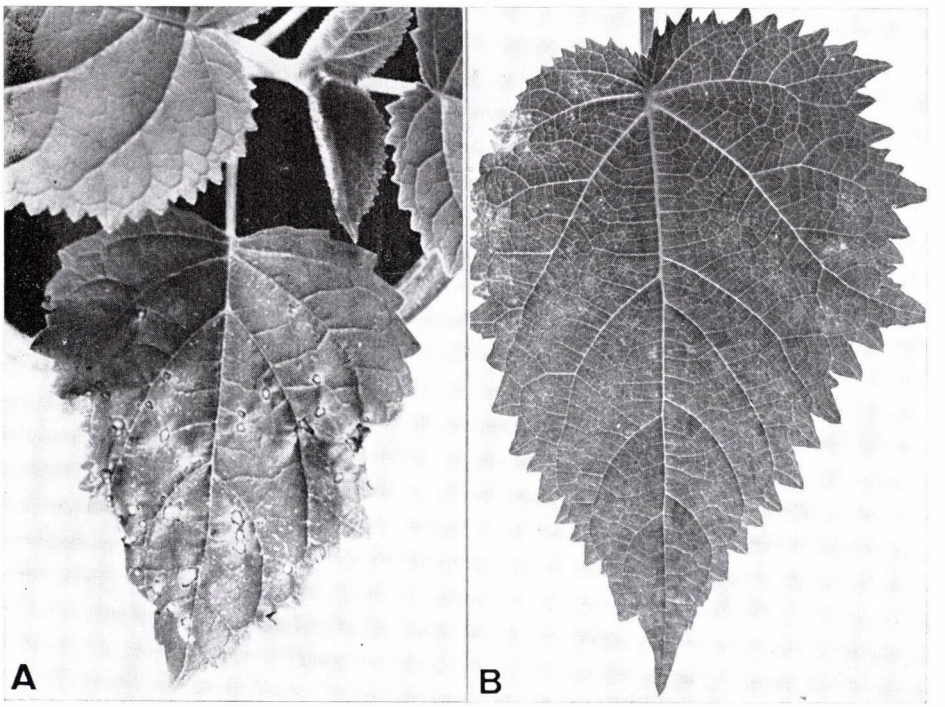

Fig. 1. Symptoms on *Paulownia fargesii* Franch. after mechanical inoculation with two different plant viruses. A: tobacco ring spot virus induced necrotic local lesions, B: cucumber mosaic virus (strain W) induced systemic mosaic symptoms

Table 1

Reaction of *Paulownia fargesii* Franch. to some plant viruses after mechanical inoculation

Viruses	Symptoms*	Results of the reisolation or serological test of the investigated viruses**
Alfalfa mosaic virus	IL: yellowing	IL: negative
	NIL: no symptoms	NIL: negative
Bean (common) mosaic virus	IL: no symptoms	IL: negative
	NIL: no symptoms	NIL: negative
Cucumber mosaic virus	IL: chlorotic spots, leaf dropping (at high temperatures necrotic lesions)	IL: positive NIL: positive
	NIL: vein clearing, vein banding, mosaic symptoms, severe growth reduction (at high temperatures necrotic lesions and leaf deformation, leaf dropping, top necrosis)	
Potato aucuba mosaic virus	IL: yellowing with green islands	IL: positive
	NIL: no symptoms	NIL: negative
Potato virus M	IL: no symptoms	IL: negative
	NIL: no symptoms	NIL: negative
Potato virus S	IL: no symptoms	IL: negative
	NIL: no symptoms	NIL: negative
Potato virus X	IL: necrotic lesions	IL: positive
	NIL: no symptoms	NIL: negative
Potato virus Y	IL: yellowing	IL: negative
	NIL: no symptoms	NIL: negative
Radish mosaic virus	IL: no symptoms	IL: negative
	NIL: no symptoms	NIL: negative
Tobacco mosaic virus	IL: yellowing with green islands	IL: positive
	NIL: no symptoms	NIL: negative
Tobacco rattle virus	IL: necrotic lesions	IL: positive
	NIL: no symptoms	NIL: negative
Tobacco ring spot virus	IL: necrotic lesions 4 days after inoculation (at high temperatures leaf dropping)	IL: positive NIL: positive
	NIL: at high temperatures top necrosis and leaf dropping	
Turnip yellow mosaic virus	IL: no symptoms	IL: negative
	NIL: no symptoms	NIL: negative

* IL: inoculated leaves, NIL: non-inoculated or subsequently developed leaves of *Paulownia fargesii* Franch.

** Potato virus M and potato virus S were not reisolated from the inoculated *Paulownia* plants, but were serological tested in the inoculated (IL) and non-inoculated (NIL) leaves of *Paulownia fargesii* Franch.

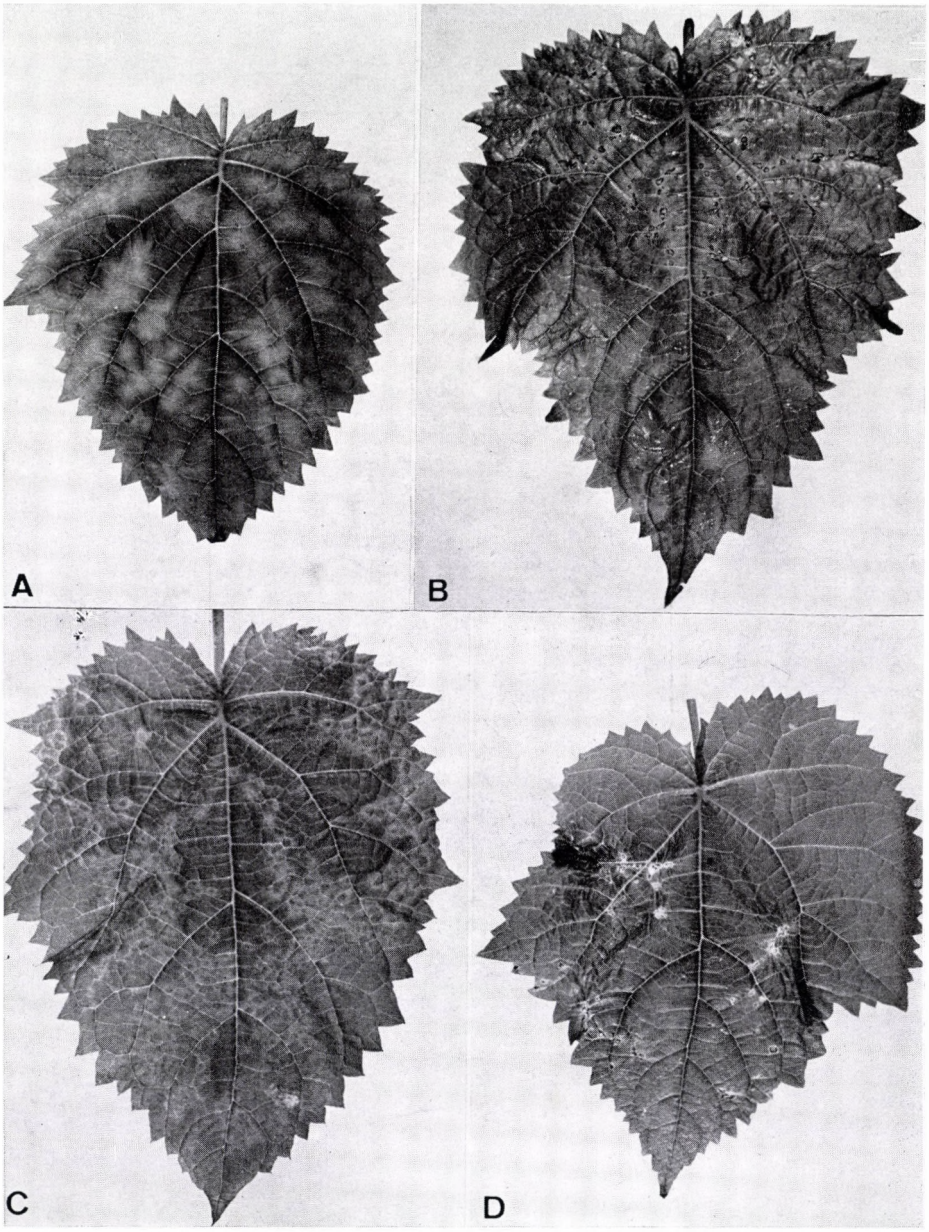

Fig. 2. Symptoms induced by two cucumber mosaic virus strains on *Paulownia* leaves. A and B: strain R of cucumber mosaic virus, C and D: strain T of cucumber mosaic virus. A and C: systemic symptoms on the non-inoculated leaves at normal temperatures, B and D: necrotic symptoms and leaf deformation at high (about 30°C) temperatures

(about 30°C) infected plants showed top necrosis and leaf dropping. The virus was readily recovered in high titer from the inoculated and non-inoculated leaves of the infected *Paulownia* plants and transferred to *Nicotiana tabacum* L. cv. *Xanthi-nc* tobacco. Eight days after inoculation cucumber mosaic virus showed chlorotic spots, leaf dropping on the inoculated leaves, followed by severe vein banding, vein clearing, and mosaic symptoms on the non-inoculated, young or subsequently developed ones (Fig. 1B, Fig. 2A and C). When infection was severe, *Paulownia* plants were strongly stunted. At about 30°C the infected *Paulownia* plants showed necrotic local lesions, necrotic systemic symptoms, and severe

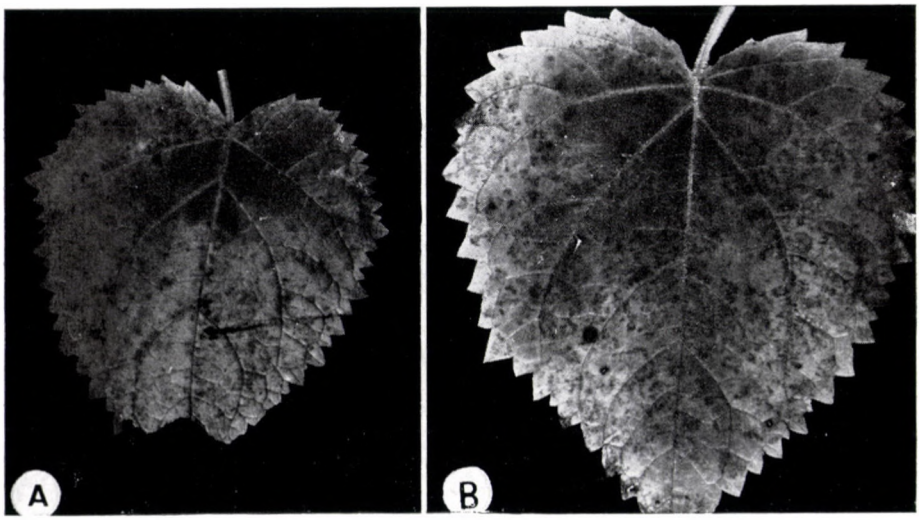

Fig. 3. Leaves of *Paulownia fargesii* Franch. inoculated with alfalfa mosaic virus (A) and tobacco rattle virus (B)

leaf deformations (Fig. 2B and D). After inoculation with alfalfa mosaic virus (Fig. 3A), potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus and tobacco rattle virus (Fig. 3B) only local symptoms developed (yellowing or necrotic local lesions as well as green lesions on the yellowing leaves) on *Paulownia fargesii* Franch. Alfalfa mosaic virus and potato virus Y could not be recovered from the inoculated leaves.

No symptoms were observed on *Paulownia fargesii* Franch. plants inoculated individually with crude saps containing bean (common) mosaic virus, potato virus M, potato virus S, radish mosaic virus and turnip yellow mosaic virus. The last two viruses were not recovered neither from the inoculated, nor from the non-inoculated or subsequently developed leaves of *Paulownia* plants.

Thus, *Paulownia fargesii* Franch. appears to be a suitable new host or indicator plant for the following six plant viruses out of the thirteen investigated

ones: cucumber mosaic virus, potato aucuba mosaic virus, potato virus X, tobacco mosaic virus, tobacco rattle virus, and tobacco ring spot virus. *Paulownia fargesii* Franch. was immune to alfalfa mosaic virus, bean (common) mosaic virus, potato virus M, potato virus S, potato virus Y, radish mosaic virus and turnip yellow mosaic virus.

Discussion

As can be seen from Table 1 six plant viruses, showing systemic and/or local symptoms are newly recorded from *Paulownia fargesii* Franch. This study newly demonstrated, that the temperature has an effect on the production of systemic or localized symptoms in a virus-host-system. *Paulownia fargesii* Franch. can be used as a very good local lesion host for tobacco ring spot virus particularly at low temperature. It has been found that the reaction of *Paulownia fargesii* Franch. to the different viruses varies greatly. Therefore it can be used as a suitable indicator and/or screening host for certain plant viruses.

On the basis of these experimental data it is presumed that *Paulownia fargesii* Franch. similar to *Paulownia imperialis* S. et Z. (syn.: *Paulownia tomentosa* [Thunb.] Steud.) (see SCHMELZER 1969, HORVÁTH 1973) plays an important role as a virus reservoir.

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Natural Occurrence of Turnip Yellow Mosaic Virus in Hungary

By

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Turnip yellow mosaic virus (TYMV, R/1 : 1.9/34 : S/S : S/Cl) was isolated from the field turnip plants (*Brassica rapa* L. var. *rapa*) showing pronounced vein clearing and vein yellowing, outstanding variegation and mosaic with yellow, sometimes nearly white areas on leaves. This is apparently the first report about the presence of TYMV in Hungary. Identification was based on symptoms, serological reactions and light microscope studies of cytoplasmic inclusion bodies.

Crambe abyssinica Hochst. ex R. E. Frees (*Cruciferae*) was demonstrated as a new host plant for several isolates of TYMV.

The Hungarian isolate (TYMV-H2) was not serologically identical with the Yugoslav strain of TYMV (TYMV-Y65), but they were closely related. It was concluded, that TYMV-H2 could not be differentiated from TYMV-Y65 by means of immunoelectrophoresis.

The process of alteration of chloroplast in plants infected with TYMV was slower in subepidermal than in epidermal cells.

Turnip yellow mosaic virus (TYMV, R/1 : 1.9/34 : S/S : S/Cl) was first recorded in the United Kingdom (MARKHAM and SMITH 1946, 1949) and later in six other countries in Europe. The virus was found only recently in Hungary, near the Southern border of the country, Borsfa.

This paper is a further contribution to the knowledge of the natural spread of viruses on turnip plants in Hungary. The first contribution was published by MAMULA *et al.* (1972). The present study deals predominantly with the identification of TYMV from naturally infected turnip plants.

Materials and Methods

Young leaves of ten spontaneously infected turnip plants (*Brassica rapa* L. var. *rapa*) were separately collected in Borsfa during October, 1972, and brought to the Laboratory of the Research Institute for Plant Protection at Keszthely, Hungary, in plastic bags. Inocula were prepared by grinding the tissue in mortars by applying phosphate buffer (0.15 *M*, pH 7.0) in a ratio of about 1 : 1. Inoculations were made with glas rod using carborundum as abrasive to young turnip plants and to seven *Crambe* species (*C. abyssinica* Hochst. ex R. E. Frees, *C.*

armena N. Busch, *C. cordifolia* Stev., *C. hispanica* L., *C. maritima* L., *C. orientalis* L., *C. tatarica* Sebeók) as well as to *Ocimum canum* Sims. Inoculated leaves were rinsed with tap water after mechanical inoculation. Ten isolates from the naturally infected turnip plants were designated by the symbols from H1 to H6 and from H9 to H12.

Serological reactions were performed by means of agar gel double diffusion test using the procedure described previously (VAN REGENMORTEL 1966, 1967; WETTER and LUISONI 1969; cf. MATTHEWS 1970a) and intragel absorption test (0.9% Difco-Bacto agar) according to VAN REGENMORTEL (1966, 1967). As precipitin lines in the latter test were not strong enough for photographing, the lines were strengthened by the modified method of WETTER (1967). This author strengthened the lines by fixing the preparations directly in diluted acetic acid. Our modification consisted in putting the preparation in an acetic acid atmosphere. This atmosphere was made by about 0.5 *M* Na-acetate buffer or acetic acid which were placed at the bottom of the box. The immunoelectrophoretic tests were carried out according to the procedure described by HIRSCHFELD (1960). Buffered Difco-Noble agar (0.9%) was used in immunoelectrophoresis. Veronal buffer in agar and electrode vessels had pH 8.6 and ionic strength 0.1. The electrophoretical analysis was carried out at room temperature without cooling, under a potential of 7–8 V/cm for 3.5 hours. The antiserum against TYMV-Y65 was prepared by MAMULA (1968).

Tissue sections for light microscopic investigations were taken on midrib from the lower leaf surface and immersed in tap water. Both epidermal and subepidermal cells were investigated, the sections being observed in the first case from unhurted, and in the second from hurted side. Only living cells were taken into account in the investigation. To make sections more transparent for taking photographs, we infiltrated them with tap water by means of a water suction-pump. Both infected and healthy plants were investigated microscopically.

Results

Reactions on test plants, the serological results and results in relation to the inclusion bodies confirmed the presence of TYMV in H1, H2, H3, H4, H5 and H6 as well as in H12 isolates. Isolate H9 contained only radish mosaic virus (RMV, (R/* : */* : S/S : S/Cl), while isolates H10 and H11 were probably identical with the cauliflower mosaic virus (CaMV, D/2 : 4.5/16 : S/S : S/Ap) and/or turnip mosaic virus (TuMV, */* : */* : E/E : S/Ap). However, more investigations are in course to tell more about these results.

A pronounced vein clearing and yellowing, conspicuous variegation and mosaic with yellow, more rarely with almost white areas were observed on leaves of turnip plants infected with TYMV in Borsfa from which the materials were collected for virus isolation (Fig. 1A, 2A and 3A). The incidence of the disease

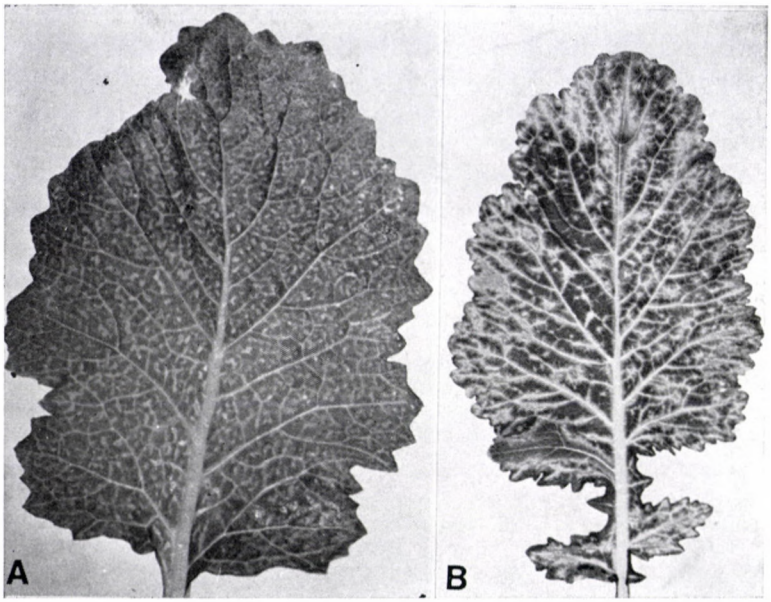

Fig. 1. *Brassica rapa* L. var. *rapa* leaves systemically infected with TYMV (TYMV-H2). A: naturally infected, B: experimental infection

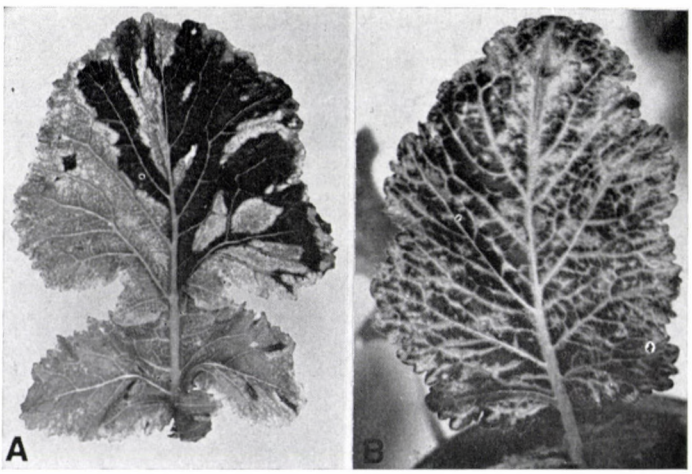

Fig. 2. *Brassica rapa* L. var. *rapa* leaves systemically infected with TYMV (TYMV-H3). A: naturally infected, B: experimental infection

varied from 20 to 30 per cent. We have found, that white variety of turnip in the field was more susceptible to the virus than the red variety. Turnip as a test plant showed the same type of symptoms after mechanical inoculation as the naturally infected field plants. However, symptoms on the artificially inoculated plants were weaker than those on the field specimens (Fig. 1B, 2B and 3B). From investigated *Crambe* species only *Crambe abyssinica* reacted with symptoms. The isolates of the virus first induced bright yellow mosaic symptoms on non-inoculated newly

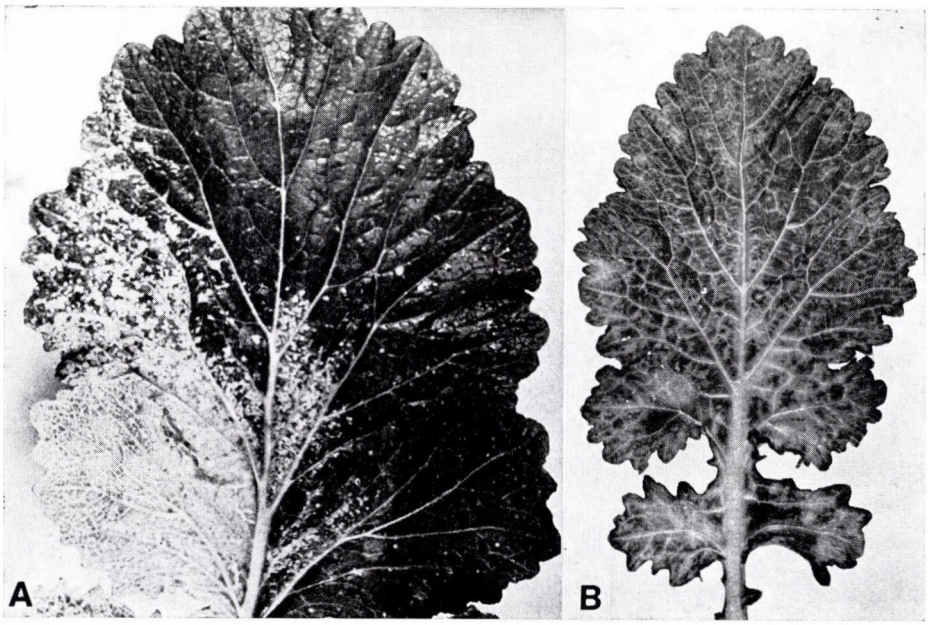

Fig. 3. *Brassica rapa* L. var. *rapa* leaves systemically infected with TYMV (TYMV-H4). A: naturally infected, B: experimental infection

developed leaves and on axillary shoots of this species. It was demonstrated that the inoculated symptomless leaves of *Crambe abyssinica* also contained TYMV, while the inoculated and non-inoculated leaves of *Crambe armena*, *C. cordifolia*, *C. hispanica*, *C. maritima*, *C. orientalis* and *C. tatarica* did not. The other investigated species, *Ocimum canum* did not react to isolates H1 to H6 and H12 of TYMV. In serological experiments isolates H1 to H6 and isolate H12 produced very strong precipitin bands with the antiserum TYMV-Y65 in agar gel double diffusion tests. These were almost indistinguishable from the bands of the homologous isolate TYMV-Y65.

More experiments were made to establish serological relationship between TYMV-Y65 and H2 isolate. In experiments carried out by means of agar gel

double diffusion method, isolate H2 could not often be distinguished from TYMV-Y65 because spur formation occurred only in a few tests (Fig. 4A). However, intragel absorption tests showed that isolate H2 can be always fairly distinctly differentiated from TYMV-Y65 when antiserum was used against TYMV-Y65 (Fig. 4B). Namely, TYMV-Y65 antiserum, which had been absorbed with isolate

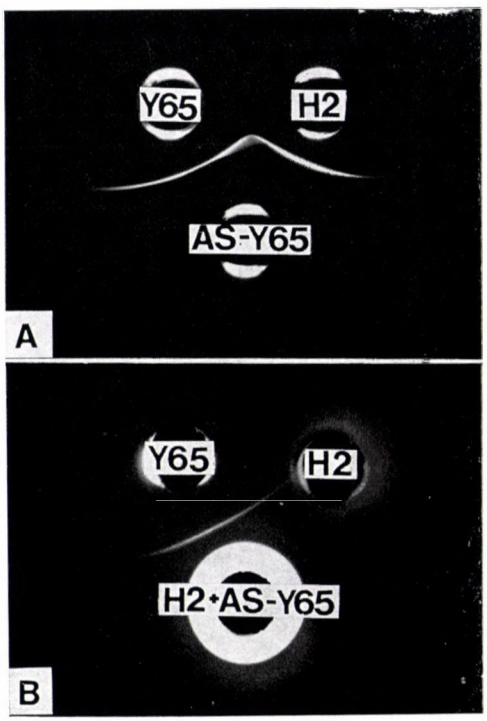

Fig. 4. Results of agar gel double diffusion test (A) and intragel absorption test (B) experiments. Y65: Yugoslav strain of TYMV, AS-Y65: Antiserum to Y65 strain, H2: Hungarian isolate of TYMV. The precipitin line in (B) is strengthened through the action of acetic acid

H2, always reacted with the homologous virus. Consequently, isolate H2 is not serologically identical with TYMV-Y65, but they are closely related.

Immunoelectrophoretic experiments revealed that isolate H2 could not be distinguished from TYMV-Y65, i.e. isolate H2 migrated in immunoelectrophoresis at a rate which was identical with the one of TYMV-Y65 isolate (Fig. 5A). When the mixture containing isolate H2 and TYMV-Y65 was immunoelectrophoretically analysed, a separation of the isolates did not occur (Fig. 5B). Therefore, it was concluded that isolate H2 could not be differentiated from TYMV-Y65 by means of immunoelectrophoresis.

In light microscope investigations of leaf tissue we have found conspicuous alterations in chloroplasts of plants infected with the Hungarian virus isolates. Formation of vacuoles in chloroplasts, followed by clumping and degeneration of plastids to form irregular alveolar inclusion bodies was very distinct in epidermal cells (Fig. 6). Appearance of vacuoles themselves was even better visible in chloroplasts of subepidermal cells, where the plastids are greater than in epi-

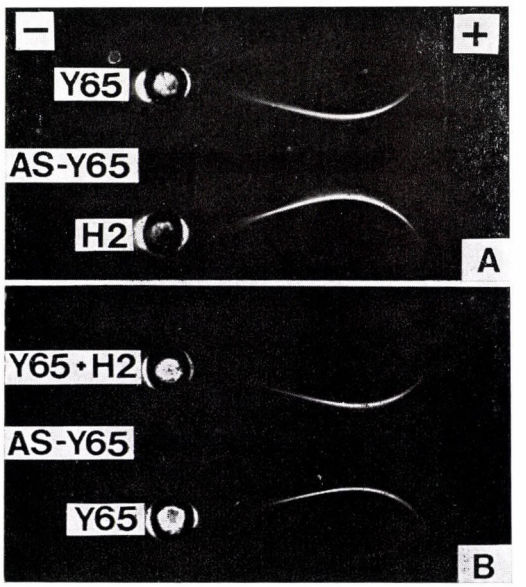

Fig. 5. Results of immunoelectrophoretic experiments with isolates Y65 and H2 of TYMV separately (A) and in mixture (B). Y65: Yugoslav strain of TYMV, AS-Y65: Antiserum to Y65 strain, H2: Hungarian isolate of TYMV

dermis, but it was followed in these cells only by partial clumping of such altered plastids. In a single chloroplast we could notice one bigger or two or a few smaller vacuoles (cf. Fig. 6). Further complete clumping and degeneration of the chloroplasts in subepidermal cells could be seen quite rarely in comparison with chloroplasts in epidermis. Also, we noticed that the whole process of chloroplast alterations ran more readily in epidermis than in cells of subepidermal layers. So we could see in the same section and at the same time already formed alveolar bodies in epidermal cells and only partially vacuolated and clumped chloroplasts in subepidermal cells (see Fig. 6). The changes in chloroplasts we have found in field as well as in artificially inoculated turnip plants. In cells of healthy plants no chloroplast abnormalities were detected.

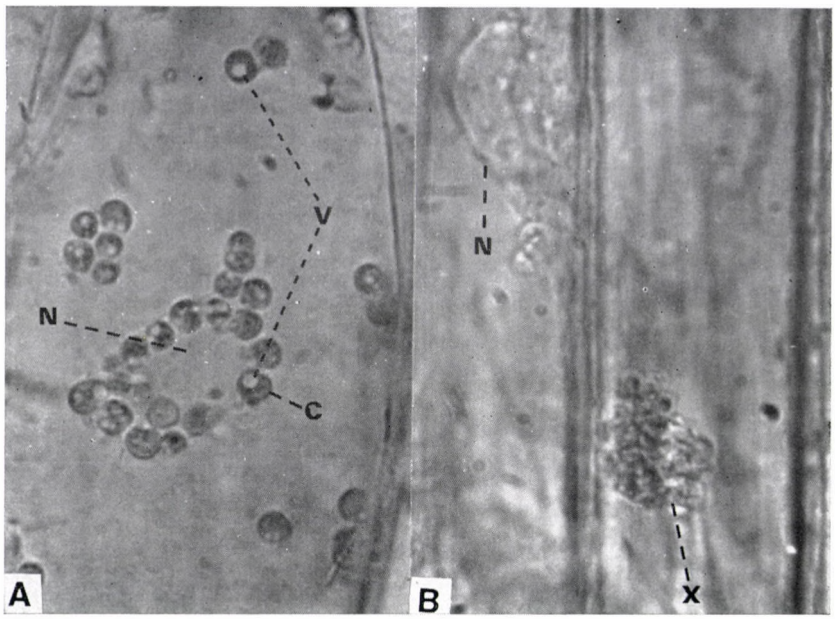

Fig. 6. Light micrograph of a thin section of turnip plant (*Brassica rapa* L. var. *rapa*) infected with isolate H2 of TYMV. C: chloroplast, N: nucleus, V: vacuole, X: clumped chloroplasts in form of X-body. A: Subepidermal cell on lower leaf surface, midrib region; chloroplasts are vacuolated and partially clumped. B: Epidermal cell on lower leaf surface plastid are degenerated and clumped in form of X-body. Magnification (A and B): 1500×

Discussion

Natural occurrence of TYMV has been established in several European countries till now. A few years ago the virus was recorded in Yugoslavia and Austria (MAMULA and MILIČIĆ 1971). Recently it was also found in the German Democratic Republic (SHUKLA and SCHMELZER 1973). Our present finding of TYMV in Hungary is a new contribution to the knowledge about the geographical distribution of this virus in the Middle Europe. The finding of TYMV in Hungary was supported by several experiments among which the most important were serological and cytological studies.

Our serological tests have shown repeatedly that the intragel absorption test is more sensitive than the agar gel double diffusion test (*Ouchterlony* method). This fact was earlier observed by WETTER and LUISONI (1969) in experiments with tobacco mosaic virus (TMV, R/1 : 2/5 : E/E : S/∗) mutants. We find interesting to mention the advantage of the application of acetate in serological reactions in agar when precipitin bands are faintly expressed. The application of acetate (cf. WETTER 1967) proved to be very appropriate in our intragel absorption tests.

With respect to cytological changes caused in the plants infected by isolate H2, it is to be pointed out that the changes observed completely agreed with the descriptions of this phenomenon reported by other workers. The vacuolisation, clumping and degeneration of chloroplasts was found by RUBIO (1956). Later this phenomenon has also been investigated by some other workers (cf. CHALCROFT and MATTHEWS 1966, 1967a, b; MILIČIĆ and ŠTEFANAC 1967; MILIČIĆ *et al.* 1969; MATTHEWS 1970b). Studies of the chloroplast abnormalities contributed to a more ready identification of the isolates. It is of interest also that the process of chloroplast alteration was slower in subepidermal than in epidermal cells (cf. MILIČIĆ and ŠTEFANAC 1967).

In addition to its susceptibility to some other viruses, and its properties as a screening plant for some viruses (HORVÁTH 1969, 1972; HORVÁTH *et al.* 1973), it can be pointed out that *Crambe abyssinica* was established as a new host plant for TYMV.

Acknowledgements

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Mathematical Method for the Determination of Sterile Insect Population Competitiveness

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A mathematical method has been developed to determine competitiveness of sterilized adults when both sterile males and females are released into a normal population. The correctness of the mathematical model has been proved by laboratory experiments carried out with the bean weevil. A dose of 10 krad gamma radiation did not affect the competitiveness of the sterile population.

Competitive ability of sterilized males as compared to normal males is one of the key points in developing the sterile male technique. It is, therefore, of vital importance to use appropriate methods for the exact determination of it. FRIED (1971) developed a method for calculating competitiveness in laboratory trials with experimental populations not containing sterile females. However, since in mass release programs there is generally no possibility of sexing the sterilized populations, the evaluation of competitiveness has to be determined by trials using both sterilized sexes. In this case the situation is more complicated since the average number and the viability of the eggs laid by sterilized females differs from that of the eggs laid by normal females, the attractiveness of sterilized females may deviate from that of normal ones, etc. Thus in such cases the term of "male competitiveness" cannot be used: the ratio of non-viable eggs found in such experiments, as compared to the estimated ratio, expresses the "competitiveness of the sterilized population".

To our knowledge the mathematical aspects of such experimental situations have not been investigated so far. In this paper experiments carried out on the bean weevil, *Acanthoscelides obtectus* Say (*Coleoptera, Bruchidae*) are used as a model.

Materials and Methods

Adults produced in a laboratory mass rearing (SZENTESI, 1972) were used for the experiment. (The mass rearing had been started 3 years prior to the experiment and the population had been refreshed each year by mixing it with strains collected in nature). For getting virgin adults Jermy's hatching device was used in which the infested beans are stuck by melted paraffin drops on the inside

of a glass cylinder, the whole inner surface of the latter is covered by talcum powder so that the hatching adults slip into a funnel put under the cylinder. The funnel stands in a jar cooled by ice cubes where the adults are immobilized before having the opportunity to mate.

The adults were irradiated in a ^{60}Co-source with a dose of 10 krad (dose rate 373 rad/min).

Competition experiments. The experiments were carried out in 2 litre glass jars (18 cm in diameter). About 30 dry beans were put on the bottom of each jar in one layer and a corrugated paper cylinder standing on the beans provided a bigger surface for the distribution of the adults. The jars were closed by linen cloth and were kept at 23°C and about 50 to 60% relative humidity.

Using different ratios on sterile and normal males altogether 200 adults were put in each jar. Each variant was repeated 5 times and the whole series of experiments has been carried out twice so that each result is the average of 10 replicates. Table 1 shows the ratios used.

Prior to the experiment both the normal and the sterilized sexes were kept separately so that copulation could take place only in the mixed population.

Twenty days after the beginning of the experiments the number of viable and non-viable eggs was determined. By that time all adults died and all viable eggs hatched.

Experiments for determination of egg production. Pairs of the four possible combinations, i.e. $N♂/N♀$, $S♂/N♀$, $N♂/S♀$ and $S♂/S♀$, were kept for 20 days at 23°C and 50 to 60% relative air-humidity in 10 ml glass vials containing 5 dry beans. Five pairs were put in each vial. At the end of the experiments the number and the percentage of viability of the eggs were determined.

Calculation of competitiveness. For this purpose first of all the ratio of non-viable eggs being expected (S_c) in the experiment is to be calculated. If an experimental population consists of N_m normal males, N_f normal females, S_m sterile males, and S_f sterile females, the probability of the four possible copulae is

$$(N_m \times N_f) : (S_m \times N_f) : (N_m \times S_f) : (S_m \times S_f) \qquad (I)$$

E.g., in a population consisting of

$$N_m : N_f : S_m : S_f = 1 : 1 : 9 : 0$$

the probability of the four copulae is 1 : 9 : 0 : 0 i.e., only the two first type of matings occur and if the sterilizing method used causes 100 per cent sterility in the males, *10 per cent* of the matings will result in fertile eggs.

However, if the composition of the experimental population is 1 : 1 : 9 : 9, then all four types of mating are possible, and the probability of them calculated by formula (I) will be: 1 : 9 : 9 : 81, i.e., in the case of 100 per cent sterility of

Table 1

Results of competition experiments

Exp. No.	No. of replicates	No. of adults				Ratio	No. of eggs (mean)		Percentage of non-viable eggs		Competitiveness C_p
		N♂	N♀	S♂	S♀	N♂ : N♀ : S♂ : S♀	total	non-viable	found ($S_x \times 100$) (mean and stand. dev.)	calculated ($S_c \times 100$)	
1.	2.	3.				4.	5.	6.	7.	8.	9.
1	10	10	10	90	90	1 : 1 : 9 : 9	2011.3	1983.6	98.4 ± 1.7	98.2	1.12
2	10	20	20	80	80	1 : 1 : 4 : 4	2014.7	1913.1	94.4 ± 3.5	94.9	0.91
3	10	30	30	70	70	1 : 1 : 2.3 : 2.3	2072.4	1833.0	89.2 ± 2.7	90.1	0.91
4	10	40	40	60	60	1 : 1 : 1.5 : 1.5	2099.1	1901.6	85.8 ± 4.2	83.9	1.13
5	10	50	50	50	50	1 : 1 : 1 : 1	2003.4	1447.3	71.8 ± 6.5	76.3	0.83
6	10	60	60	40	40	1 : 1 : 0.6 : 0.6	2053.8	1386.4	67.2 ± 2.9	67.1	1.00
7	10	100	100	—	—	1 : 1	2302.4	415.2	17.4 ± 3.9		

Average: $C_p = 0.98$

Table 2

Results of egg-production experiments

Pairs	No. of replicates	Mean no. of eggs/♀	Mean non-viable eggs (%)	Correction factors	
				non-viability	No. of eggs
N♂/N♀	15	49.7 ± 15.1	15.6	$k = 0.16$	—
S♂/N♀	5	50.1 ± 10.2	98.4	$x = 0.98$	$a = 1.0$
N♂/S♀	5	26.3 ± 8.2	90.5	$y = 0.91$	$b = 0.5$
S♂/S♀	5	22.6 ± 2.3	100	$z = 1.00$	$c = 0.4$

both sexes, and if the number of eggs laid by normal females equals to the number of eggs laid by the sterilized females, only *1 per cent* of eggs will be fertile.

For an exact calculation of the expected ratio of non-viable eggs (S_c), the following factors are to be taken into consideration: (1) the ratio of non-viable eggs laid by normal females mated with normal males, (2) the ratio of non-viable eggs resulting from S♂/N♀, N♂/S♀ and S♂/S♀ pairs, respectively, (3) the difference between the mean number of eggs resulting from S♂/N♀, N♂/S♀ and S♂/S♀ pairs, respectively, and (4) the mean number of eggs resulting from N♂/N♀ pairs.

The following formula contains all these correction factors:

$$S = \frac{k(N_m \times N_f) + ax(S_m \times N_f) + by(N_m \times S_f) + cz(S_m \times S_f)}{(N_m \times N_f) + a(S_m \times N_f) + b(N_m \times S_f) + c(S_m \times S_f)} \qquad \text{(II)}$$

If the sex ratio is one in both the sterile and the normal populations, i.e., if

$$N_m = N_f = N \text{ and } S_m = S_f = S$$

then

$$S_c = \frac{kN^2 + (ax + by) \times (SN) + czS^2}{N^2 + (a + b) \times (SN) + cs^2} \qquad \text{(III)}$$

where

$$k = \frac{\text{mean No of non-viable eggs resulting from N♂/N♀ pairs}}{\text{mean No of eggs resulting from N♂/N♀ pairs}}$$

$$x = \frac{\text{mean No of non-viable eggs resulting from S♂/N♀ pairs}}{\text{mean No of eggs resulting from S♂/N♀ pairs}}$$

$$y = \frac{\text{mean No of non-viable eggs resulting from N♂/S♀ pairs}}{\text{mean No of eggs resulting from N♂/S♀ pairs}}$$

$$z = \frac{\text{mean No of non-viable eggs resulting from S♂/S♀ pairs}}{\text{mean No of eggs resulting from S♂/S♀ pairs}}$$

$$a = \frac{\text{mean No of eggs resulting from S♂/N♀ pairs}}{\text{mean No of eggs resulting from N♂/N♀ pairs}}$$

$$b = \frac{\text{mean No of eggs resulting from N♂/S♀ pairs}}{\text{mean No of eggs resulting from N♂/N♀ pairs}}$$

$$c = \frac{\text{mean No of eggs resulting from S♂/S♀ pairs}}{\text{mean No of eggs resulting from N♂/N♀ pairs}}$$

Competitiveness of sterile population (C_p) can be regarded as the degree of its sexual activity as compared with the activity of normal populations as unity. Competitiveness can be calculated in the following way:

Considering the correction factor k as zero, and all the others as 1, the calculated non-viability ratio would be:

$$S_c = \frac{SN + NS + SS}{NN + SN + NS + SS} = \frac{(S + N)^2 - N^2}{(S + N)^2}$$

and the calculated *viability* ratio:

$$1 - S_c = \frac{N^2}{(S + N)^2}$$

In order to get the experimental value of the viability ratio, the number of sterile adults is to be multiplied by C_p:

$$1 - S_x = \frac{N^2}{(C_p S + N)^2}$$

Dividing the first equation with the second:

$$\frac{1 - S_c}{1 - S_x} = \frac{(C_p S + N)^2}{(S + N)^2}$$

From this:

$$C_p = \frac{\sqrt{\frac{1 - S_c}{1 - S_x}}\,(S + N) - N}{S} \qquad \text{(IV)}$$

The reciprocal value of C_p gives the factor by which the number of sterile adults must be increased in order to get the effect on reduction of egg-viability that fully competitive sterile adults would have given.

Results and Discussion

The data summarized in Table 2 show the differences in viability and number of eggs laid by pairs of different combinations. It can be concluded that the sterilizing dose used in the experiments caused nearly full sterility in both males and females, and that the number of eggs laid by the sterilized females was significantly smaller than with normal ones. The correction factors calculated from the results by the formulae given above are also included in Table 2. The difference between factor *b* and *c* is not significant. Thus it can be concluded that the sterile females produced roughly 50% less eggs in these experiments.

The results of the competition experiments shown in Table 1 partly contradict the data of Table 2, since the number of eggs does not decrease significantly when the proportion of the sterile females increases (from Exp. 6 to Exp. 1). Thus, it has to be supposed that in the competition experiments, due to some unknown factors, e.g., crowding effect, possibility of females mating with more males, etc., the number of eggs laid by sterilized females did not differ significantly from that laid by normal ones. Therefore, in the calculation of competitiveness 1.0 has been used for the correction factors *b* and *c* instead of 0.5.

The difference in the mean number of eggs laid by one female, as represented in Table 1 and 2, respectively, is probably due to the differences in the degree of crowding or to some other unknown factors.

Taking into consideration the sterile/normal ratios given in column 4 of Table 1, the expected percentage of non-viable eggs has been calculated by the formula (III). The results are shown in column 8 of the same table.

The sterile population competitiveness calculated by the formula (IV) does not differ significantly from 1.0. Thus the sterile population can be regarded as equally competitive to the normal one. This agrees with the data of former experiments showing no reduction or even a slight increase of the competitiveness in sterilized bean weevil males (JERMY and NAGY, 1969) as well as with the findings of WIENDL (1971) who has shown that the lowest doses causing full sterility increased the longevity of the weevil *Zabrotes subfasciatus* (Boh.).

The good accordance of the experimental and the calculated values clearly indicate that the sterilized population plays really that role in population reduction as it has been assumed in our calculations. Thus, e.g., a sterile/normal *population* ratio of 9 : 1 results in roughly 1% of viable eggs instead of 10% resulting from a 9 : 1 sterile/normal *male* ratio. It can be assumed that the sterilized bean weevil females are equally "competitive" to the normal ones.

It has to be mentioned that ANDREEV and co-workers (1969) found a considerable *decrease* of the impact of sterile adults on the dynamic of the normal population when both sterile males and females were used, which contradicts both the theoretical assumptions, and our experimental findings. Since the authors cited did not publish details of their experiments, the cause of this inconsistency cannot be made clear. However, such an effect can result, e.g., if mating of the sterile adults among themselves, and the same among the normal adults, can take place before the experiment starts. Since the bean weevil can mate just after emerging from the beans, we carefully prevented early mating (see the methods above).

The fact, that the average number of eggs produced by females in the competition experiments did not show the differences which should have appeared regarding the correction factors *b* and *c*, indicates that in competition experiments also the average egg-production must be examined and compared to the values found in egg-production experiments where one type of mating can take place only.

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Reproductive Activity of Codling Moth (*Laspeyresia pomonella* L. *Lepidopt.; Tortr.*) Exposed to Short Photophase during Preimaginal State

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Codling moth populations having developed under field conditions have a very low average fecundity (17–23) after their diapause, in contrast to the 1st generation (76–83 eggs). The causes for the low fecundity after diapause are; (1) only 40–50% of the adults mate, (2) only about 24% of the females lay eggs, (3) the number of eggs laid per one fertilized female is lower after diapause (71) than in summer (105).

Females having flown in August and September mated approximately as frequently (71%) as those of the first generation (71–81%), but only 43% laid eggs. The mean fecundity of the fertilized females, however, was as low as after diapause (77). This phenomenon is all the more interesting as the females flying in August and September are heavier than those of the other generations. Thus the positive correlation between weight and fecundity can be considered valid only for females swarming during the same period.

It has been shown in laboratory experiments that the alteration in the reproductive activity of adults is due to the short photophase during egg, larval and pupal stages. Under field conditions the diapause itself seems to intensify the mal effect of the short photophase on the reproductive activity. Thus, short day conditions must be considered not only as a factor inducing diapause, but as a factor controlling the number of individuals in a given orchard as well. This statement is probably valid for other lepidopterous species with similar life history.

Codling moths overwinter in their cocoons as last larval instars; the adults appear after pupation in spring. There may be more than one generation annually, depending upon the geographic regions and climate (SHEL'DESHOVA, 1962, 1965; BALACHOWSKY, 1966); in Hungary maximally two generation develop (SZELÉNYI, TERÉNYI and VIKTORIN, 1953). Diapause is induced by shortening daylength in the second half of the summer (DICKSON, 1949; RUSS, 1966; JERMY, 1967, WILDBOLZ and RIGGENBACH, 1969; SÁRINGER, 1974). In the case of Hungarian populations diapause is induced by a photophase shorter than 16–17 hours at 23°C (JERMY, 1967).

A large number of data concerning fecundity of the codling moth has been

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published (reviewed by BUTT, 1970, 1971, 1972). These data show differences according to the locations, conditions and generations.

In the present paper the causes for the differences in fecundities in various generations, observed under natural conditions, are discussed (Table 7).

Materials and Methods

Different populations of codling moth

(1) Field populations of codling moth were collected in different regions of Hungary in 1970, 1971 and 1972 by the specialists* of stations for Plant Protection (Fig. 1).

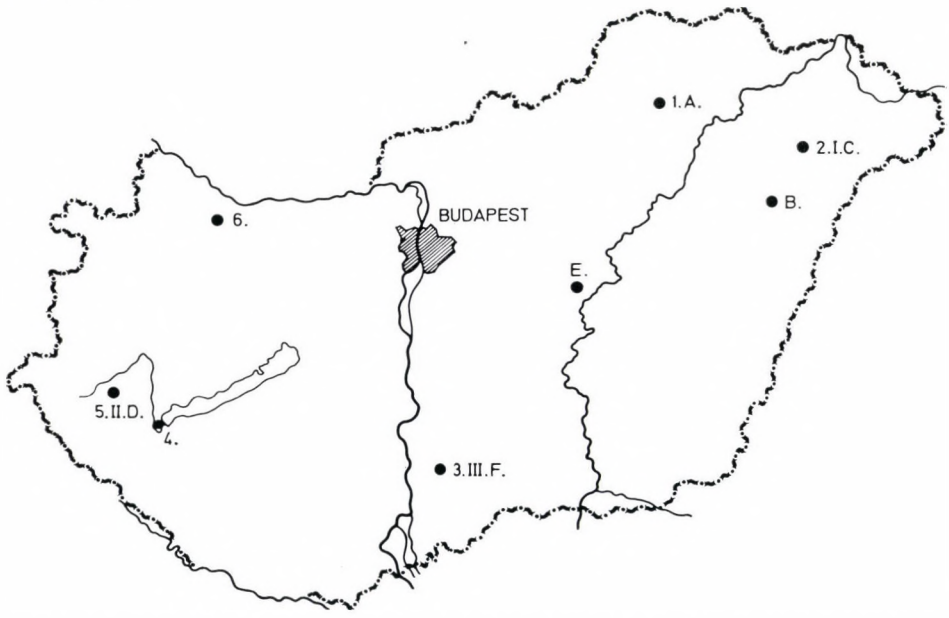

Fig. 1. Regions of Hungary where codling moth larvae were sent from (Data in Tables 1,2,3,4,5)

From the end of July until the end of September, at intervals of about 14 days, corrugated paper belts were fastened on the trunks of apple trees, to collect the larvae entering diapause. During winter, the belts were kept in the open, in cages; after pupation in spring, adults were observed in the laboratory.

In June and July, 1972 infested apples were also obtained from the same regions of the country where the diapausing material was collected. Thus we had

* We wish to thank the Laboratories of the Stations for Plant Protection (Budapest, Győr-Kismegyer, Kállósemjén, Kenderes, Mikepércs, Miskolc, Tass, Velence, Zalaegerszeg) for having collected the codling moths.

an opportunity to compare the ones after diapause with adults of the summer generation(s) having developed under field conditions as well.

(2) The Yakima population was maintained in the laboratory according to the rearing principles of the laboratory in Yakima (Washington, USA). This was reared in our laboratory on green apples at 23°C. Before pupating, larvae spun cocoons in corrugated paper; they were then placed into hygrostates where the adults emerged. Recognition of virgin females was carried out as described earlier (DESEŐ, 1971). This population is characterized by the great mating activity of males as well as the great receptivity of females.

The emerging adults were weighed, then kept by pairs or by groups of ten ($5♀+5♂$) in glass vessels with airing covers. The different populations, furthermore males and females reared at different photophases were kept under arrangement as indicated below. All adults were fed with 5–10% honey-water, which was changed every second day, and a green apple was placed into each jar to give the olfactory stimuli possibly necessary for egglaying.

Treatments. In order to study the effect of photophase during ontogenesis, eggs on green apples were exposed to and reared until adult emergence at 8/16, 14/10, 16/8 and 17/7 hours L/D photoperiod.

In other experiments 1 μg of Farnesymethyl-ether (FME) in acetone solution was dropped on the abdomen of the chilled male or female, according to the method described earlier (DESEŐ, 1972).

The eggs laid were counted; in certain observations dead females were dissected. In these cases ripe eggs were counted and mating was verified by the presence of spermatophores.

Results

The results of the year 1971 are shown in Table 1. It appears from this Table that, apart from the place of origin, there are differences in the average fecundities

Table 1

Differences in the number of eggs laid of females of the overwintered and the 1st generations (1970)

1970 Originating places of codling moths	Generation after diapause			1st generation	
	No. of ♀♀	% of ♀♀ with spermatophor	Mean fecundity (No. of eggs laid per 1 ♀)	No. of ♀♀	Mean fecundity
1. Gyöngyös	13	32	18	—	—
2. Kállósemjén	15	74	14	10	93
3. Kiskőrös	18	55	37	—	—
4. Keszthely	21	43	27	10	62
5. Pacsa	17	46	18	10	72

Table 2

Occurrence of mating and egglaying in the native codling

Populations	No mating, no egglaying			No mating, egglaying			
	No. ♀♀	Weight (mg) mean	No. of matured eggs (mean)	No. ♀♀	Weight (mg) mean	No. of matured eggs (mean)	No. of eggs laid
I.	11	31.4	86	6	35.4	111	16
II.	7	28.7	75	4	33.0	112	32
III.	4	32.8	82	4	37.5	117	25

of the overwintered and the summer generations: mean fecundities after diapause are very low. Two questions arise in connection with this:

(1) Does a loss of weight occur during overwintering which would account for the smaller number of eggs after the diapause? This question is justified as in our earlier studies on laboratory populations a positive correlation was found between the weight and fecundity of females (DESEŐ, 1971).

(2) Does every female begin to lay eggs after mating? When comparing the rates of mated females to the numbers of eggs laid per female no correlation can be shown.

1. Correlation between the weight and fecundity of adults during the season

The first task was to establish whether the overwintering affects the following vitellogenesis? Females of populations obtained from different regions of the country had always many ripe eggs, proving that the maturation of eggs did take place. In each population a positive correlation of the weight of the female and the number of ripe eggs was found, though the correlation was not always close (Fig. 2). For the Yakima population (continuously reared in the laboratory) shown on Fig. 2, the slope of the regression line is more steeper than for the natural population after their diapause. This difference draws attention to the fact that similar variances may occur even within the same population during the season.

In Table 2 a comparison of the weights of females after diapause (1971) to oviposition and average number of ripe eggs within the females is presented. It is apparent that with increasing weight the readiness to lay eggs rises; however, as it was expected, the weight is not correlated to the readiness to mate.

In 1972 not only adults after diapause and those of the first generation, but also those flying in August and September were weighed. (Among the latter population individuals belonging to the 2nd generation may already be present.) The data summed up in Table 3 show that during the season the mean weight of males increases by 2.5 mg and those of females by 1.3 mg. However, the difference in the mean weight of females after diapause and those of the late-summer

moth populations after diapause (1♀ + 1♂ in one jar) (1971)

Populations	Mating, no egglaying			Mating, egglaying			
	No. ♀♀	Weight (mg) mean	No. of matured eggs (mean)	No. ♀♀	Weight (mg) mean	No. of matured eggs (mean)	No. of eggs laid
I.	2	31.5	104	1	37.5	166	150
II.	—	—	—	1	39.4	310	190
III.	—	—	—	—	—	—	—

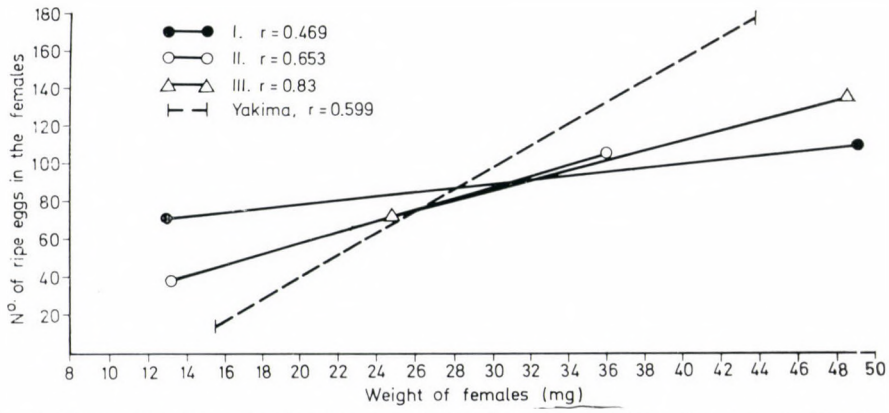

Fig. 2. Regression lines showing the correlation between weight and numbers of matured eggs in the females without egglaying

population is 4.2 mg, in case of males only 2.7 mg. Although the mean weights are different for each population, a tendency for an increase in weight is apparent in every case as the season progresses.

The extent of fecundity of females is shown in Table 4. Females of the first generation (preimaginal development in June) are the most fertile ones; the late-summer adults and those after diapause are less fecund. Thus, no correlation can be shown between the fecundity and weights in this case.

2. Alterations in the reproductive activity of females during the season

It was shown already by the data obtained in 1970 (Table 1) that after diapause a considerable percentage of females did not mate. About 90% of the over-wintered population of 1971, kept by pairs did not mate, and only half of those having mated laid eggs. In all probability one of the causes for this low reproductive

Table 3

Mean weight (mg) of females and males of the codling moth generations during one season, collected in the same regions of Hungary

Regions	Females			Males		
	Generations			Generations		
	overwintered	I	I–II*	over-wintered	I	I–II*
A.	26.6± 2.7**	28.7± 2.4	34.3	20.7± 1.0	20.1±0.2	20.15± 1.6
B.	28.0± 1.5	30.5± 0.8	32.2± 1.7	19.0± 1.2	21.3± 0.2	24.1
C.	28.0± 1.5	31.5± 1.9	—	20.7± 1.2	20.3± 1.0	—
D.	29.1± 1.1	29.8	—	19.4± 1.0	19.9± 1.5	—
E.	—	33.7± 1.2	—	—	19.3± 1.1	—
F.	—	—	29.4± 2.5	—	—	21.6± 2.5
G.	—	—	32.7± 2.1	—	—	24.8± 2.7
Average	27.9	30.8	32.1	19.9	20.1	22.6
No. of adults	108	130	73	105	126	73

* Emerged in August and September from infested apples collected at the end of July
** Mean ± S.E. (Mean without S.E. means that the amount of females was less than 10)

activity was that the adults did not have the possibility to choose their mate (see "B"). For this reason, in 1972, 5♀ and 5♂ were kept together in each jar.

Data in Table 5 show that the females of the 1st generation (which had developed in June when days were lengthening), mate very readily. Similarly, a great proportion of the females developed during shortening days in July and August mated. After the diapause, however, only half of the animals mated.

It is also shown in Table 5 that mating does not mean that oviposition will begin at the same time. Compared to the 1st generation, in autumn about half and after diapause only about one-third of the females laid fertile eggs. Further studies were focused on the causes for this decreased reproductive activity.

(A) *Treatments with Farnesylmethyl-ether*

In certain species corpora allata affect the receptivity of females (ENGELMANN, 1960 a, b; BARTH, 1961, 1962) and in others the activity of males (see reviewed in WAJC and PENER, 1969). Thus it was obvious to make an attempt at increasing the mating activity of adults by a juvenile hormone analogue.

Ten females treated with FME after diapause and kept with untreated males, and ten males treated with FME kept with untreated females did not show an

Table 4

Reproductive activity of the codling moth during the season of 1972 (5♀ + 5♂ in one jar)
1. Mean fecundities (numbers of eggs laid per one female) of the populations

Regions	No. of eggs per female			No. of eggs per mated female			No. of eggs per fertilde (mated and oviposited) female		
	Generations			Generations			Generations		
	overwintered	I	I–II*	over-wintered	I	I–II*	over-wintered	I	I–II*
A.	24.8 ± 8.2**	84.7 ± 6.1	43.9 ± 11.9	53.3 ± 13.5	104.7 ± 6.9	55.5 ± 11.9	71.1 ± 9.9	104.7 ± 6.9	77.8 ± 11.8
B.	20.8 ± 14.5	84.6 ± 7.4	37.1 ± 10.0	44.8 ± 16.3	99.7 ± 5.4	51.9 ± 15.0	65.4 ± 8.1	99.7 ± 5.4	88.1 ± 11.8
C.	13.0 ± 4.9	86.4 ± 11.9	—	30.0 ± 26.3	106.4 ± 12.4	—	70.1 ± 23.6	115.5 ± 10.7	—
D.	8.6	—	—	26.0	—	—	78.0	—	—
E.	—	74.5 ± 13.1	—	—	89.0 ± 7.3	—	—	101.3 ± 3.9	—
F.	—	—	38.7 ± 12.3	—	—	57.4 ± 19.4	—	—	69.7 ± 14.1
G.	—	—	26.8	—	—	42.1	—	—	73.7
Average	16.8	83.3	36.6	38.5	99.9	51.7	71.1	105.3	77.3

* Emerged in August and September from infested apples collected at the end of July
** Mean ± S.E. (Mean without S.E. means that the amount of females was less than 10)

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Table 5

Reproductive activity of the codling moth during the season of 1972 (5♀ + 5♂ in one jar)
2. Percentages of females mated and fertilized

Regions	Mated ♀♀ (%)			Fertiie (mated and oviposited) ♀♀ (%)			Females without fertile eggs (%)		
	Generations			Generations			Generations		
	overwintered	I	I–II*	over-wintered	I	I–II*	over-wintered	I	I–II*
A.	46.5	82.0	86.6	34.8	82.0	46.6	65.2	18.0	53.4
B.	46.0	86.6	70.8	31.6	86.6	41.6	68.4	13.4	58.4
C.	37.5	80.0	—	18.8	76.0	—	81.2	24.0	—
D.	33.4	—	—	11.1	—	—	88.9	—	—
E.	—	77.3	—	—	68.2	—	—	31.8	—
F.	—	—	65.2	—	—	47.8	—	—	52.2
G.	—	—	63.6	—	—	36.4	—	—	63.6
Average	40.85	81.4	71.5	24.1	78.2	43.2	75.9	21.8	56.8

* Emerged in August and September from apples collected at the end of July

increased mating activity. None of the couples mated when males as well as females were treated with FME after the diapause. Thus this juvenile hormone analogue proved to be ineffective on the mating behaviour of the codling moth.

(B) *Mating of adults belonging to different populations and generations*

Our further investigations started with the supposition that during diapause irreversible changes take place in the moths.

Of native populations 10 females after diapause were kept with males of the continuously reared Yakima population. Only one pair mated; the female laid eggs. On repeating the experiment, no better result was obtained even when there were two males with one female. The result was similar if continuously reared Yakima females were kept with native males after their diapause in the arrangement described above.

When native females after the diapause were mated with native males of the 1st generation, having developed under natural conditions, again only one couple mated and fertile eggs were laid.

As no difference could be established in favour of the males of the 1st generation of native populations, in our further experiments native adults after diapause were kept together with the Yakima population. If more males and females were kept together in one jar, the number of matings increased as compared to those kept as single pairs. Native males after diapause kept with Yakima females and native females after diapause kept with Yakima males mated and also laid eggs in 42.7 per cent of the females.

(C) *Reproductive activity of adults developed at different photophases*

Since adults flying in late summer mate normale (see Table 5), but only half of the mated females begin to lay eggs, attention was focused on the effect of the environmental conditions changing during the season. During June, July and August the monthly average temperatures are insignificantly different (a deviation of 2°C); therefore we studied the effect of the photophase which changes in the course of summer.

Portions of the continuously reared laboratory population were exposed to short (8/16, 14/10 L/D) and long photophases (16/8 L/D), while the check were kept at 17/7 L/D photoperiod. (The diapause inducing marginale photophase in this population was 14.30 hours at 23°C (SÁRINGER, 1974).) In this experiment the rearing temperature was 29.±1°C, under which conditions (DICKSON, 1949) only 10% of the the population reared at 8/16 L/D entered diapause. Larvae developed in small, young, green apples. Thus the reproductive activity of these populations could be studied under the sole influence of daylength (Table 6).

Nearly 100% of the females in the check and of those developed under LD conditions (16h) mated and laid eggs (Table 6). Part of the adults developing from

Table 6

The effect of the photophase during the ontogenesis on the reproductive behaviour of the codling moth developed without diapause (29 ± 1°C)

Photophase (h) during larval development	No. of pairs	Mating and egg-laying (%)	Mating but no egglaying (%)	No mating (%)
17 h ♀ + 17 h ♂ (check)	50	96	0	4
16 h ♀ + 16 h ♂	50	96	0	4
14 h ♀ + 14 h ♂	18	72.3	27.7	0
8 h ♀ + 16 h ♂ 16 h ♀ + 8 h ♂	24	62.5	16.5	21
8 h ♀ + 8 h ♂	25	55	12	33

the population exposed to 14 hours SD did not lay eggs after mating, and a large proportion of those illuminated for 8 hours did not mate at all. If one of the partners had been reared at short and the other at long photophases the resulting percentages were between the two extremes.

Discussion and Conclusion

The results of our investigations, conducted during three years show that the mean fecundities of the overwintered codling moth populations are considerably lower (17–23 eggs) than those of the generation swarming in summer (76–83). The average number of eggs laid by the codling moth population swarming late in summer is also smaller (36.6) than the value for the summer generation. It is shown in the Tables that the low average fecundity results from three factors: (1) The mating frequency is reduced after diapause; it is only about the half of the summer value. (2) While in summer females only occasionally fail to oviposit after mating, in autumn half and after diapause only about one third of the mated females lay eggs. (3) The number of eggs per fertilized female is also different: in summer it is considerably higher (105) than after the diapause (71) or late in summer (77).

The latter phenomenon is all the more remarkable as just the females swarming near the end of summer weigh the most. Consequently, the positive correlation between the weight of the females and the number of eggs laid by them (Deseő, 1971) is valid only for females flying during the same period. It is as yet impossible to tell what changes take place in the insect resulting in so great a deviation from the expected values.

The data appear to show that it is first of all the short photophases during the eggs, larval and pupal stages and not diapause itself that negatively influences the oviposition in a mated female. Apparently if the photophase is shortening during the ontogenesis, mating is very often not followed by oviposition.

Mating frequency itself seems to become reduced after diapause; a considerable part of the overwintered population (50%, (57–90%) 60%) did not mate in any of the three years of our observations.

Under laboratory conditions the photophase of 14 hours during the ontogenesis (marginale photophase for diapause inducing was 14^{30} hours) seemed already to influence the beginning of oviposition after mating. An extremely short photophase (8 hours) affected apparently not only the triggering of oviposition, but had an unfavorable effect both on the receptivity of females and mating activity of males. Since diapause did not occur, this phenomenon must be independent from the "preparation" for diapause; therefore we consider it as a by-effect of the short (-ening) photophase (DESEŐ, 1973). An other important point of view is that in this laboratory-experiment young, green apples were used. Thus the probability of the unfavourable effect of the ripe apples on the reproductive activity of the adults was excluded.

HARCOURT and CASS (1966) describe the effect of short photophase in the larval stage on mean fecundity of *Plutella maculipennis* Curt. These authors assumed that the shortening of daylength accounts for the decrease in fecundities of the consecutive summer generations. They argue that changes in the metabolism of insect preparing for diapause may be responsible for the observed phenomenon.

LUM and FLAHERTY (1969, 1970) also observed an effect of the photophase during larval stage in *Plodia interpunctella* Hbn. Males reared from larvae in continuous light caused a decreased fecundity of females bred under natural conditions, for their sperm was inadequate to fertilize all eggs.

The difference in the behaviour of adults after their diapause and that of the summer generations has been already noticed in the case of plum fruit moth (*Grapholitha funebrana* Tr.) (DESEŐ and SÁRINGER, 1970). After overwintering there were spermatophores in 66.7% of the females, but only 34% laid eggs. The laboratory experiment with larval populations reared at photophases of different lengths also revealed the unfavorable effect of the shortening daylength on the number of ovipositing females.

These observations carried out in laboratory and under field conditions suggest that a short or shortening photophase experienced by the egg, larval and pupal stages may exert a similar effect (DESEŐ, 1973) on the reproductive activity of codling moth populations living at different locations as well as that of other species. From this point of view only species overwintering as pupae or prepupae in diapause induced by short photophase have to be taken into consideration.

In the literature on plant protection, the number of eggs per generation is reported for a few species only; these are summed up in Table 7. Even these few

Table 7

Fecundity of different generations of some lepidopterous species hibernating as prepupae or pupae in diapause, induced by short photophase

Species	Diapausing form	Fecundity of 1♀ or mean fecundity of the population		Author	Region
		overwintered	Summer		
Laspeyresia pomonella L.	prepupa	75	100	Radetzky, 1913	Turkestan (USSR)
		94	173, 100	Newcomer and Whitecomb, 1924	Yakima (USA)
		64	83	Hall, 1929	Canada
		17 (max 79)	22 (max 106)	Selkregg and	Delaware
		21 (max 99)	25 (max 209)	Siegler, 1928	(USA)
		61	103	Vleeuwen, 1929	Georgie (USA)
Hyphantria cunea Drury	pupa	566	799	Gere and Reichart, 1957	Budapest (Hungary)
Grapholitha funebrana Tr.	prepupa	20–85	100–200	Bobirnac, 1958	Roumania
		45	80	Arakeljan, 1962	Caucasus (USSR)
		8	18–59	Deseő, 1967	Budapest (Hungary)
Lobesia botrana Den. et Schiff.	pupa	77	140, 91	Reichart, 1968	Balaton (Hungary)
Cochylis ambiguella Hb.	pupa	50	73	Reichart, 1969	Balaton (Hungary)

data show that codling moth populations living in distant geographical regions have a reduced mean fecundity in the spring. We consider this phenomenon to be due to the changes in the reproductive activity of the overwintered generation, brought about in all probability by the short photophase preceding diapause.

Additional factors related in some way to the diapause syndrome may influence fecundity as well. In the plum fruit moth (DESEŐ and SÁRINGER, 1970), fewer of the adults that resulted from diapausing larvae of the 2nd generation laid fertile eggs (27%) than those originating from the similarly diapausing 3rd generation (41%). On the ground of the experiments described above, the adults of the 2nd generation could be expected to lay fertile eggs in a higher proportion, for their preimaginal development occurred in a period when days were longer than those during the development of the 3rd generation. For this reason the duration of the diapause may be supposed to act as another factor to decrease the reproductive activity of the adults of the overwintering population.

It would need further investigations to clear up the question whether short-day conditions disturb the endogen rhythm of the animal in a sensitive period and, wether it is a genetic feature of a part of the population that shortening illumination during preadult state has no influence on the reproductive activity of the adults.

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Influence of Photoperiod and Temperature on the Food Consumption of the Alfalfa Beetle, *Subcoccinella vigintiquatuorpunctata* L. (*Coleoptera: Coccinellidae*)

By

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Data on the food consumption of the alfalfa beetle, *Subcoccinella vigintiquatuorpunctata* L. (Col., Coccinellidae) were recorded. These data were gained from our experimental breedings at three controlled temperatures: 18 ± 1 °C, 23 ± 0.8°C, and 28 ± 0.5°C and five photoperiods: 17/7, 16/8, 15/9, 14/10 and 13/11 hours of light/dark per day.

In general, the high temperature activates the beetles more than the low one. So the food consumption increases with the increase of temperature. One beetle consumed from emergence until the onset of diapause 379.4 mg green leaves at 18°C, while this amount increases at 28°C up to 603.2 mg.

The same effect have been observed at one photoperiod and different temperatures.

The influence of photoperiod or day-length is more different. It is of interest to know that the food consumed by 5 beetles is very high at long day (17 hrs light per day), low at intermediate day (15 hrs light per day); the food uptake begins to increase again under short day (13 hrs light per day) condition. These interesting observations can be explained by the diapause in the adults of this species.

The relation between food consumption and adult age was also investigated. All the experimental results are statistically analyzed.

Diapause or the rest period is one of the enviromental adaptations manifested in insect species and ascribed mainly to the influence of photoperiod (WAY and HOPKINS, 1950; LEES, 1955; DE WILDE, 1955; SÁRINGER, 1966).

Other behavioural phenomena of insect species beside diapause such as locomotion, mating, length of different developmental stages (HODEK, 1958; ALI, 1970) and oviposition (ANDERWARTHA and BIRCH, 1954; DE WILDE, and BONGA, 1958) are not only attributed among others to the influence of duration of light, but also to its intensity.

Daily rhythms of feeding activity have been studied on leaf feeding caterpillars (EDWARD, 1964, 1965), also the effects of long-day and short-day conditions on the food consumption of *Tanymecus dilaticollis* Gyll., larvae of *Colaphellus sophiae* Schall. and *Athalia glabricollis* Thomson, were investigated (SÁRINGER, 1954, 1960, 1961).

The alfalfa beetle, *Subcoccinella vigintiquatuorpunctata* L. (Col., Coccinellidae) is one of the dangerous pests of alfalfa (*Medicago sativa* L.) and causes

damage not only on alfalfa, but also on about 70 plant species with preference to alfalfa and clover, *Trifolium* spp. (CSEHI, 1961; SZELÉNYI, 1944; TANASIJEVIC, 1958).

The interaction of temperature and photoperiod is too complex to be demonstrated, therefore, there are only few data in the literature concerning their effects on insect behaviour.

The present investigation deals with the influence of photoperiod and temperature on the food uptake of the adults of *S. vigintiquatuorpunctata* L. The food consumption was evaluated at three different temperatures and five photoperiods. Only the food consumption during the experimental time was determined in these experiments, not the changes in adult body weight.

Day-length, temperature and food consumption give rise to several questions about their relationships. Within the scope of this investigation, the following questions are of importance:

- Are there great differences in the food consumption at different temperatures at the same duration of light?
- Whether the amount of food consumption under long-day condition equals that of short day at the same temperature?
- After how many days reach the adults their peak of feeding activity?

Material and Methods

Adults of alfalfa beetle required for the experiments were obtained from laboratory mass rearing after 12 hours of their emergence. 5 newly emerged beetles were placed on each hygrostate culture, containing of a glass dish half filled with water and covered with linen (fastened by the aid of a rubber band) with filter paper and petri-dish cover.

The alfalfa plants (*Medicago sativa* L) serving as food were obtained from the laboratory garden. Young leaves of the topical part of plant were used usually as food.

The food consumed was assessed according to the method described by WALDBAUER (1964). This method can be summarized as follows: the leaves administered to the beetles are cut into two symmetrical portions along the mid-rib. One half is weighed and given to the beetles, the other half is weighed fresh and then dried in an oven at 105 °C for 24 hours and then dried to a constant weight to determine the dry weight percentage.

Before the remnants of food had dried, excrements adhering to the leaves were washed off carefully with a brush dipped into distilled water. Plant leaves were changed every 24 hrs, and the amount of food consumption during 24 hours was equal to the weight of food introduced, minus the weight of the left over food.

Rearing were made in replicates and kept at three constant temperature rooms: 18 ± 1°C, 23 ± 0.8°C, 28 ± 0.5°C, each of which is accomodated with five

photoperiods: 17/7, 16/8, 15/9, 14/10, and 13/11 hours of light/dark per day and illuminated with about 1500 lux light intensity.

The statistical analyses of the experimental results were carried out according to SNEDECOR, 1956 and KASEM&HINDY, 1964 systems.

Results and Discussion

Data obtained from the food consumption experiments lead to the following results:

influence of temperature on food consumption

Results on effects of temperature given in Table 1, indicate that the amount of food consumption by one beetle increases by the increase of temperature. The highest amount of green leaves consumed (744.6 mg) was observed at 28 °C, while the least one (268.1 mg) was at 18°C at the same light exposure period. These results can be ascribed to the increase activity due to the increase of temperature.

At one and the same light exposure period, there are noticeable differences

Table 1

Food consumption of *S. vigintiquatuorpunctata* L. kept at different temperatures and light conditions

Temperature °C	Photo-period hrs/day	No. of beetles	Feeding during 34 days			
			Total weight of food (g)	Total weight of food consumption (g)	Weight of food consumption/ beetle (g)	% green leaf consumption
18 ± 1	17	5	3.5062	1.8967	0.3794	54.0
	16	5	3.4266	1.6700	0.3340	48.7
	15	5	3.6926	1.3371	0.2676	36.2
	14	5	3.4403	1.4165	0.2833	41.2
	13	5	2.3910	1.3405	0.2681	56.0
23 ± 0.8	17	5	4.6003	2.6291	0.5258	57.1
	16	5	4.6285	2.4487	0.4898	53.0
	15	5	3.8430	1.4271	0.2854	37.1
	14	5	4.4645	2.5367	0.5074	56.8
	13	5	4.2010	2.6053	0.5211	62.0
28 ± 0.5	17	5	5.3267	3.8221	0.7644	71.4
	16	5	5.3465	3.4267	0.6853	64.0
	15	5	4.9653	3.0161	0.6032	60.7
	14	5	5.3567	3.3132	0.6627	61.9
	13	5	5.7695	3.7236	0.7446	64.5

in the amount of green leaf consumption. Statistical analysis proved that under long-day conditions (17 hrs light per day) there are no significant differences in the food consumption of beetles at different temperatures (Table 2 and Fig. 1 — A).

Table 2

Influence of different temperatures on food consumption under long-day condition

Factors tested	Analysis of variance		Calculated	Tabular
	Sources	Average of food consumption (M.S.)	F	$F_{0.05}$
28 ± 0.5°C 23 ± 0.8°C 18 ± 1.0°C	Between Trs. Within Trs.	0.3823 3.4714	0.110	3.68

* Not significant. Trs. = Treatments

On the other hand, highly significant differences were found between the values of food consumption at both of intermediate-day (15 hrs light per day) and short-day (13 hrs light per day) conditions as shown in table 3.5 and Fig. 1 — B and C.

By comparing the amounts of food uptake at different temperatures and at intermediate-day conditions and by using the least significant differences test "L.S.D." (Table 4), the following conclusions are derived.

(a) There are no significant differences between the food consumption at 28°C or at 23°C.

(b) The food consumed by beetles at 23°C or at 28°C surpasses significantly the amount consumed at 18°C.

Although there were considerable differences in the food consumption at short-day conditions, the L.S.D. test showed that there were no significant differences in the food uptake at 28°C and 23°C or between 23°C and 18°C, and the only significant differences were observed between 28°C and 18°C (Table 6).

Table 3

Influence of different temperatures on food consumption under intermediate-day conditions

Factors tested	Analysis of variance		Calculated	Tabular F	
	Sources	Average of food consumption (M.S.)	F	$F_{0.05}$	$F_{0.01}$
28°C 23°C 18°C	Between Trs. Within Trs.	50.7535 1.7620	28.71**	3.68	6.26

** Highly significant

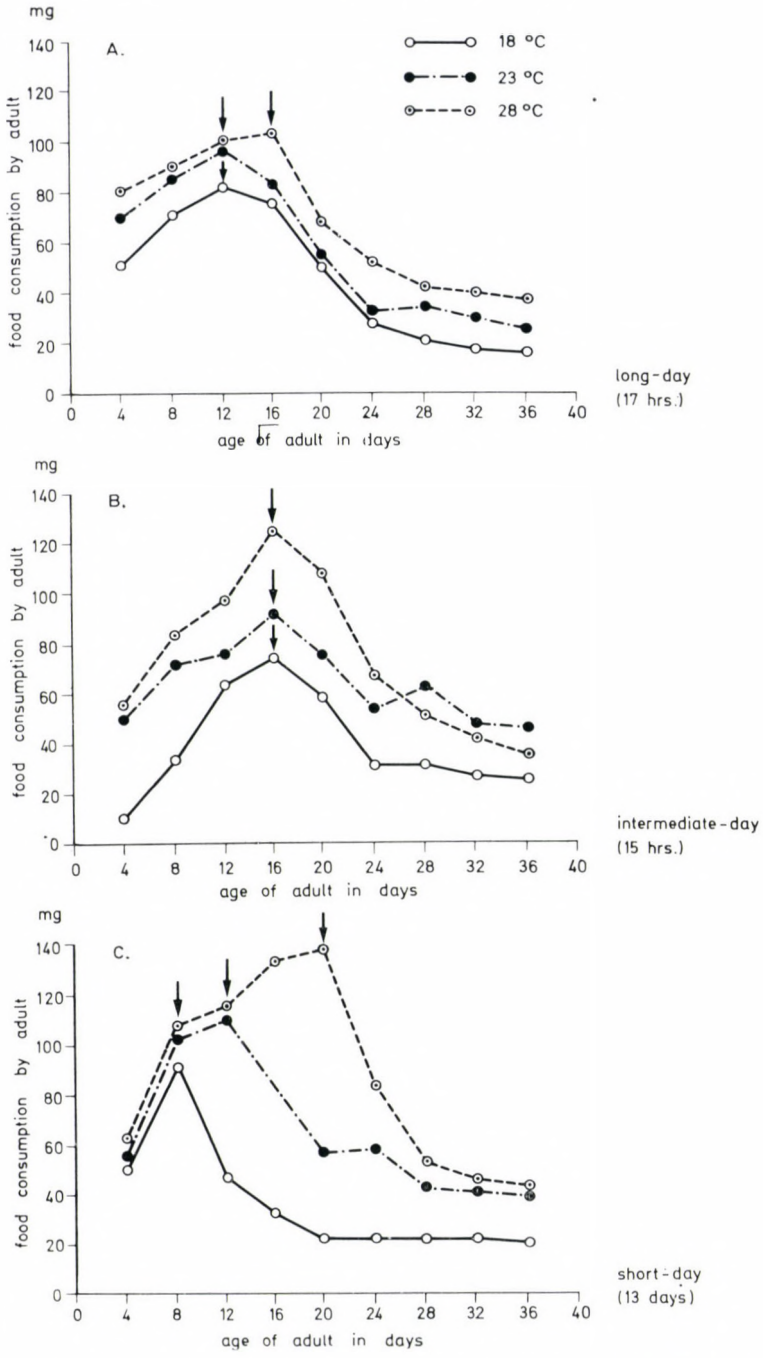

Fig. 1. Effect of different temperatures: 18°, 23° and 28°C on the food consumption by Alfalfa beetle adults, *S. vigintiquatuorpunctata* L. A- Long-day conditions (17/7 hrs light/dark per day); B- Intermediate-day (15/9 hrs light/dark per day); C- Short-day conditions (13/11 hrs light/dark per day)

Table 4

Comparison between the food consumption at different temperatures and under intermediate-day conditions

Treatments	Average of food consumption x			L.S.D.$_{0.05}$
	$\bar{x}_1$	—	$\bar{x}_2$	
(a) −28°C: a−b	408.6	—	413.4 = −4.8	
(b) −23°C: a−c	408.6	—	222.8 = 185.8*	162.95
(c) −18°C: b−c	413.4	—	222.8 = 190.6*	

* Significant

Table 5

Influence of different temperatures on food consumption under short-day conditions

Factors tested	Analysis of variance		Calculated	Tabular F	
	Sources	Average of food consumption (M.S.)	F	$F_{0.05}$	$F_{0.01}$
28°C 23°C 18°C	Between Trs. Within Trs.	15.0371 1.8070	8.32**	3.68	6.26

** Highly significant

Table 6

Comparison between the food consumption at different temperatures and under short-day conditions

Treatments	Average of food consumption $\bar{x}$			L.S.D.$_{0.05}$
	$\bar{x}_1$	—	$\bar{x}_2$	
(a) −28°C: a−b	438.1	—	386 = 52.1	
(b) −23°C: a−c	438.1	—	223.9 = 214.2*	208.7
(c) −18°C: b−c	386.0	—	223.9 = 162.1	

* Significant

Influence of photoperiod on food consumption

According to the data got in this investigation or the same aspect with the larvae of *Colaphellus sophiae* Schall. (Col., *Chrysomelidae*) by SÁRINGER, 1960, it is expected for the present species that the beetles reared under long-day conditions would consume more green leaves than those kept under short-day condi-

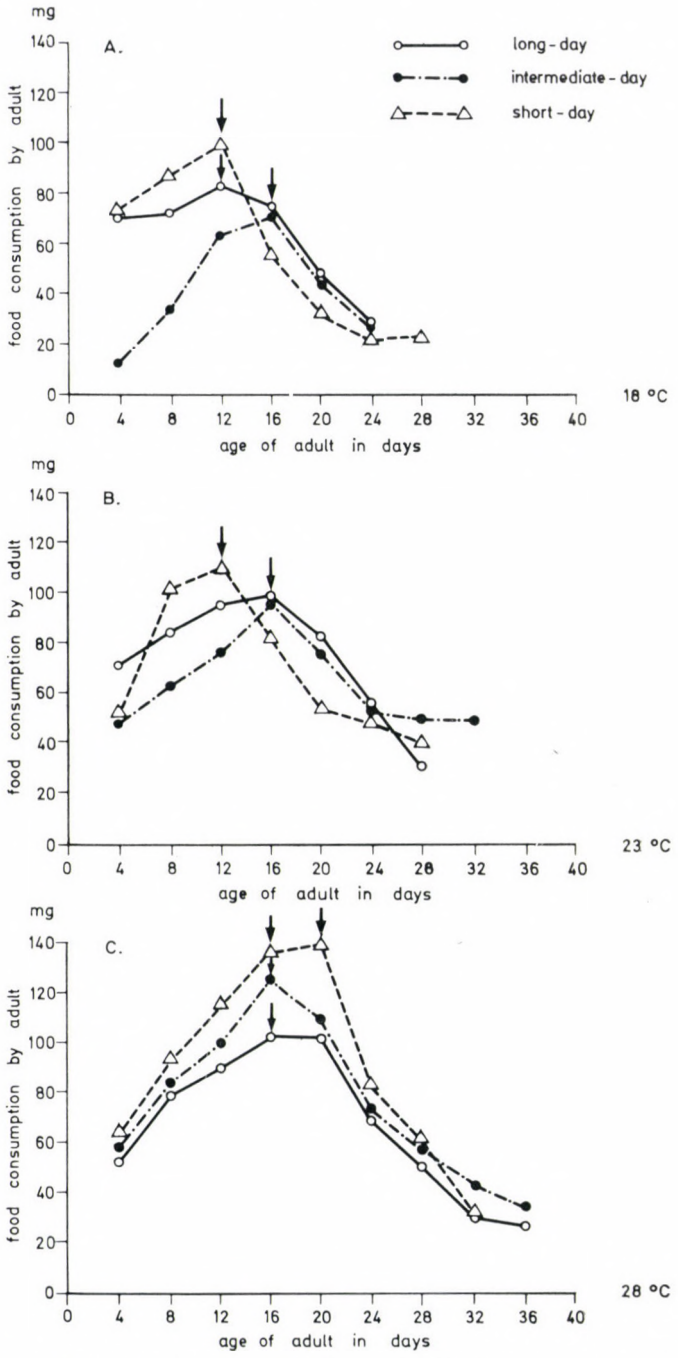

Fig. 2. Influence of day-length (Photoperiod) on the food consumption by Alfalfa beetle adults, *S. vigintiquatuorpunctata* L. A — 18°C; B — 23°C; C — 28°C

tions; the present results showed, however, that the green matter consumed fluctuated due to the fluctuation of day-length or the duration of light. The percentages of food consumption (Table 1) were usually high at long-day conditions, decreased at intermediate-day, then began to increase again to reach their maximum at short-day conditions.

The great amount of food consumed by the beetles at short day and at 18°C, 23°C and 28°C conditions can be regarded as related to the diapause phenomenon induced in the adults of this species. The author (ALI, 1970) found that beetles of *Subcoccinella vigintiquatuorpunctata* L. went into diapause when they had been exposed to short photoperiod of 14/10 light/dark per day; also several studies about the imaginal diapause of Coccinellids (HODEK, 1962) correspond with this observation. Imaginal diapause is usually accompanied with the tendency to store food reserves in form of lipids, glycogen and protein granules to enable themselves to survive through the hibernating or aestivating period (WAY, 1962). This fact also explain the high feeding activity at short-day conditions. (Fig. 2 – A, B and C).

Although the percentages of food consumption both at low or high temperatures (Table 1) show some differences under different duration of light, the statistical analyses of the results showed that these were not significant. In other words, the amount of food consumed by the alfalfa beetle, *S. vigintiquatuorpunctata* L. at various light conditions and at the same temperature presents no statistically significant differences.

As regards the relations between the food consumption and the age of adults, it was found that the adults reach their highest peak of feeding activity at an age ranging from 14 to 26 days depending on the day length and temperature conditions.

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Combustion Products of Carbon Disulphide for Killing Mercury Light Trap Catches

By

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The authors give the description of a modified light trap. The insects captured by the mercury vapour light trap are collected during the night in a large cage. The fragile *Lepidoptera* climb up the sides and separating walls of the cage and rest there, whereas the majority of the large *Coleoptera* (e.g. *Carabidae*) remains on the bottom. The damage caused by the latter in the susceptible lepidopterous insects can be thus prevented. The catch is killed in the following morning by burning carbon disulphide in the air space of the cage.

This light trap – called also the "Bečej" type – has been operating in Bečej (Yugoslavia) since 1969 successfully, yielding continually well preserved insect material.

For studying the seasonal activity of agricultural insect pests and for the continuous survey of their population dynamics an efficient light trap, collecting a large amount of insects was needed in the region of the Bečej Agricultural and Industrial Combine (PIK). As is well-known, many insects are attracted by the mercury-vapour lamps, so we began to work with a 250 Watt mercury light trap. The handling of the large insect material caused, however, some problems. As the determination and evaluation of the catches was carried out mostly during the winter following the given vegetation period, it became an outmost necessity to collect and preserve the catches in a condition as good as possible.

So emerged the idea to place a spacious collecting cage under the funnel of the light trap, instead of the traditional killing jar. The larger space enables the moths and other fragile insects to separate themselves from the hard, active beetles (e.g. *Carabidae*) also caught in high numbers, thus the injury of the former can be prevented.

The prototype of this light trap has been operating in Yugoslavia since 1969, yielding an extremely large insect material in a well preserved state, reducing thus the problems of determination and evaluation. During the vegetation period of 1969 the light trap captured about 95.000, in 1970 about 77.000 lepidopterous specimens, not mentioning other insect groups.

In Yugoslavia and in Hungary this light trap is known as the "Bečej" type; this modified trap was mentioned the first time by ČAMPRAG (1971), who presented also its photograph. The results achieved during the first two years by using this

type, have been summarized in two separate papers (MÉSZÁROS, VOJNITS and VARGA, 1971; VOJNITS, MÉSZÁROS and VARGA, 1971).

Based on this type, further light traps have been constructed and operated in Yugoslavia since 1971 in Prilep (Macedonia) and since 1972 in Osijek (Croatia).

Description and handling of the light trap

The 250 Watt, 220 Volt mercury vapour light bulb (type IPR WTF) of the trap is set 2 m above the ground; the trap – mounted on a metal stand – is protected against the sweeping-in of rain by a circular roof, fastened above the bulb (diameter: 50 cm). The light bulb itself is placed into the centre of three radial metal wings, closing in 120 degrees. Under the bulb is attached the funnel of the trap

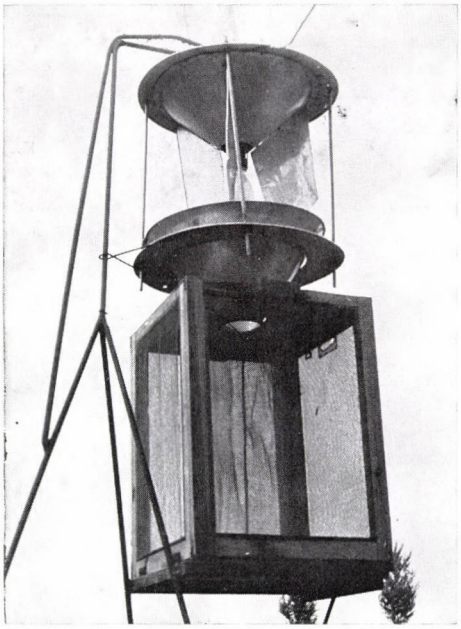

Fig. 1. Light trap type "Bečej"

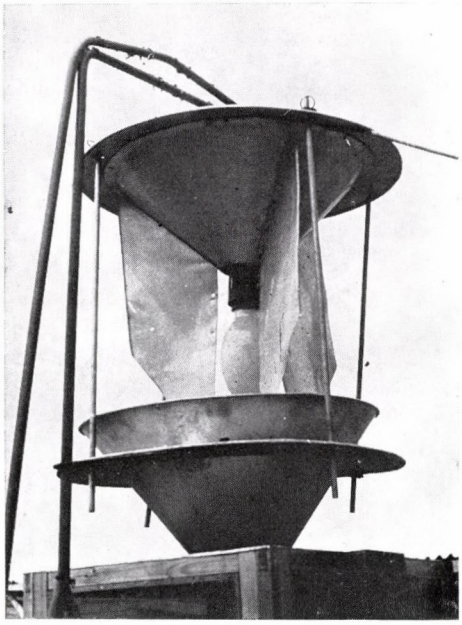

Fig. 2. The mercury vapour lamp with the radial wings

(diameter: 50 cm, height: 30 cm) and through the latter get the insects – attracted by the light – into the collecting cage. The collecting cage consists of a wooden frame (50 × 50 × 70 cm), covered by plastic screen. The inner space of the cage is subdivided into four parts with two perpendicular, vertical screen walls, which end 5 cm above the bottom; the bottom of the cage is a perforated metal tray (with holes of 2–3 mm in diameter).

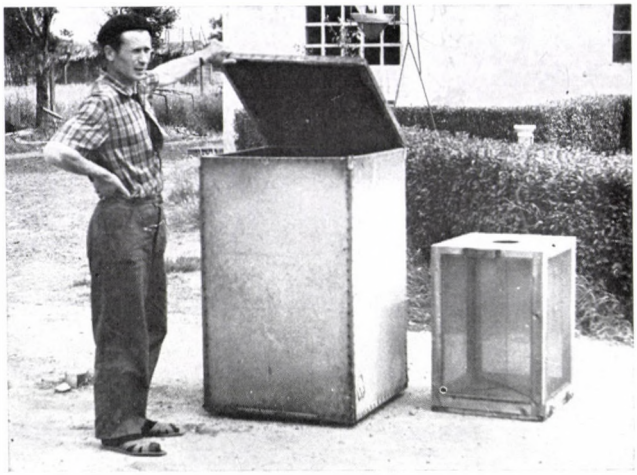

Fig. 3. The collecting cage and the killing box

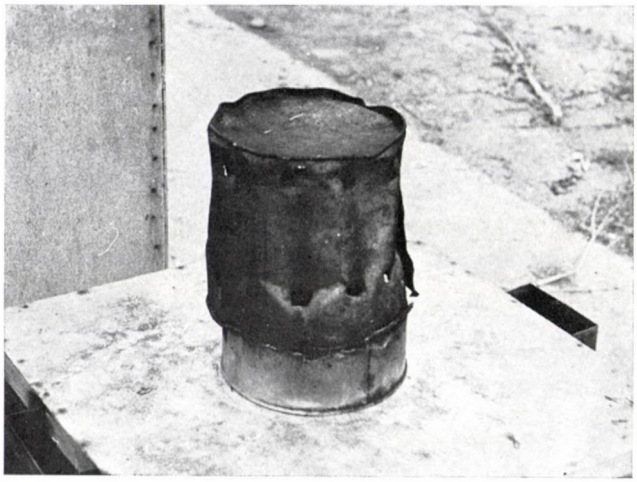

Fig. 4. Metal tray and cylinder used for burning the carbon disulphide

The majority of the *Lepidoptera* and other fragile insects captured, seeks less disturbed resting place and separates itself from the other insects, by climbing the side and separating walls of the cage; they are found there at the morning inspection. Other insects with hard integument (like *Carabid* beetles caught sometimes in high numbers) remain in the communicating bottom space and cause no injury to the soft-bodied other insects.

The lamp of the trap is put out at sunrise by the attendant; the cage is then taken off the stand and placed into a killing box made from plywood panels ($70 \times 70 \times 120$ cm). On the top of the collecting cage 20–30 ml carbon disulphide is poured into a metal tray, lighted and covered by a perforated metal cylinder. Finally, the killing box is covered by a non-airtight cover. The combustion gases, generated by the burning carbon disulphide, kill the insect catch very fast. It is advisable, however, to keep the box covered for an other 3–4 hours to kill also the most resistant beetles. As the carbon disulphide is very dangerous explosive material, care has to be taken at handling and storing.

The light trap operates every night during the vegetation period, even in unfavourable weather. The insect material is well preserved, ready for instant evaluation or, properly dried and stored, for an ensuing examination.

Acknowledgements

The authors are very much indebted for their contribution to Dr. B. Todorovski (Tobacco Institute, Prilep) and to Ing. I. Šilješ (IPK "Osijek", Osijek) who continue the work with the modified light trap described above.

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Damage Caused by *Haplodiplosis equestris* Wagn. in Hungary and the Analysis of Its Extent

By

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In 1966 and 1971 damages caused by *Haplodiplosis equestris Wagn.* were observed in Hungary.

The subject of this paper is the analysis of the material collected during damages in 1971. On the ground of the evaluation it was shown that in the case of the winter wheat sample examined (variety Bezostaja 1) stem length decreased by 15.49 cm, the number of grains per ear by 6.36 pc, the weight of grains per ear by 0.3673 grammes and the weight of a thousand grains by 10.8 grammes in average as compared to undamaged plants.

A significant appearance of *Haplodiplosis equestris* in Hungary was first observed in 1966. At that time the pest damaged several thousand kilohectares of cereal crops (CSÖRGŐ, PATAKI, TÓTH 1967).

In 1971, in the Southern region of Hungary (Csongrád county), after a period more rainy than usual, *H. equestris* appeared and caused damages again.

Materials and Methods

Wheat samples (Bezostaja 1 variety) collected at different points of the damaged corn field and ripened wheat samples were examined.

In the course of evaluation, spear length (cm), the number of grains per ear (pc), and the weight of grains per ear (grammes) were measured, furthermore the number of damages per shoot (pc) and the places of damage on the individual joints (pc) counted.

305 shoots were analysed altogether, from among which broken plants, those damaged by other pests and not allied were omitted.

After the analysis the proportion of all damaged and undamaged plants, furthermore within the plants damaged the values of the indices according to the extent of damage were mathematically evaluated.

Results

From the 305 plants examined 245 proved to be suitable for further analysis. 60.41 % of the shoots were damaged.

The formation of the indices discussed above as compared to control, i.e. undamaged plants was found as follows (Table 1):

Table 1

Damage caused by *Haplodiplosis equestris* Wagn. on winter wheat (Bezostaja 1 variety) on the basis of the comparison of undamaged and damaged plants

	Stem length (cm)	Number of grains per ear (pc)	Weight of grains per ear (grammes)	Weight of thousand grains (g)	n
Undamaged	92.23	26.10	0.8812	32.8	97
Damaged	76.74	19.74	0.5139	22.0	148
Difference of the two mean values	15.49	6.36	0.3673	10.8	
Sz. D. 0.1	4.68	3.67	0.17	4.61	
Extent of damage in %	16.8	24.4	41.7	32.9	

1. The stem is shorter by 15.49 cm.
2. In the average of ears, the number of grains decreased by 6.36 pc.
3. The average weight of grains per ear decreased by 0.3673 grammes.
4. The weight of a thousand grains lessened by 10.8 grammes.

Plants were usually damaged on the top internodium, the one under the ear (68.7 %); the 1st and 2nd internodia, altogether, were already less damaged (25.9 %); further towards the soil damages became more and more insignificant or ceased (Table 2).

Table 2

Damage caused by *Haplodiplosis equestris* Wagn. on the individual joints

Variations	Number of shoots	
	pc	%
Damage on the 1st (top) joint only	127	68.7
Damage on 1st and 2nd joints	48	25.9
Damage on 2nd joint only	9	4.9
Damage on 1st, 3rd and 4th joints	1	0.5

Damages were analysed in detail on the increasing extent of damage (Table 3).

Table 3

Damage caused by *Haplodiplosis equestris* Wagn. on winter wheat (Bezostaja 1 variety), as a function of the extent of damage

Extent of damage per plant	Spear length (cm)	Decrease of spear length (cm)	Number of grains per ear (pc)	Weight of grains per ear (grammes)	Weight of a thousand grains (grammes)	Number of shoots analysed	
						n	%
Undamaged	92.23	0	26.10	0.8812	33.8	97	39.6
1 damage	83.39	9	24.29	0.8535	35.1	28	11.4
2—3 damages	74.16	18	18.64	0.4735	25.4	25	10.2
4—5 damages	76.89	15	19.04	0.4263	22.4	27	11.0
6—9 damages	75.84	16	18.54	0.4369	23.6	37	15.1
10 damages or more	73.74	18	18.58	0.4077	21.9	31	12.7

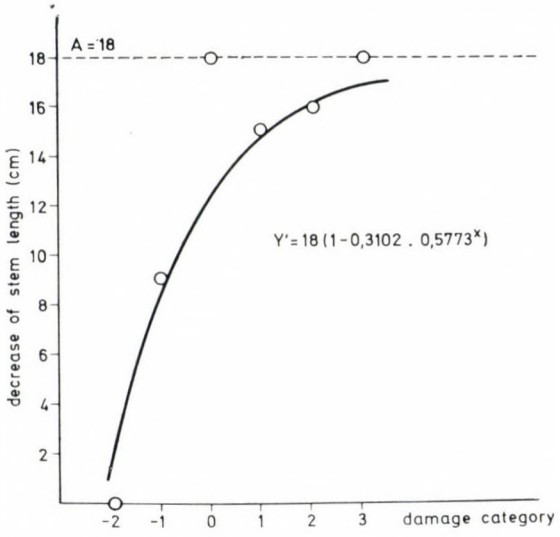

Fig. 1. As a result of the damage caused by *Haplodiplosis equestris*, the decrease of stem length takes the course of a saturation line

The decrease of stem length is described, by a saturation line (Fig. 1), pointing to the fact that owing to damages spear length approaches a well-defined value (in this case 18 cm).

The decrease of the number of grains per ear (Fig. 2), the weight of grains per ear (Fig. 3) and the weight of a thousand grains (Fig. 4) in the function of the extent of damage are described by hyperbolic curves. According to all of the three lines, the number and weight of grains and the weight of a thousand grains approach "O", a value which may occur in practice too.

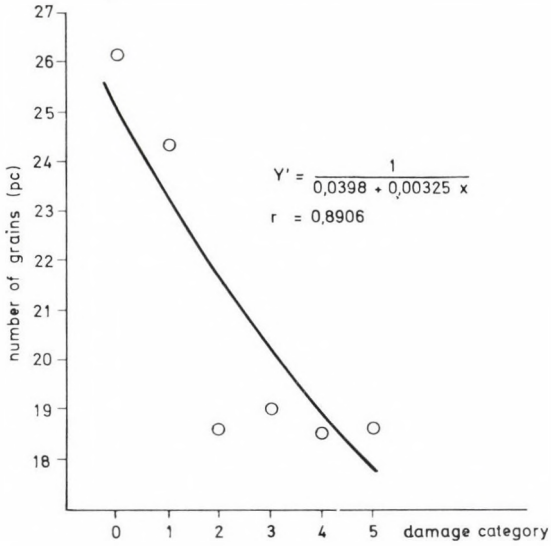

Fig. 2. Decrease of the number of grains per ear (pc) occurring as a consequence of damages of different extents caused by *Haplodiplosis equestris*

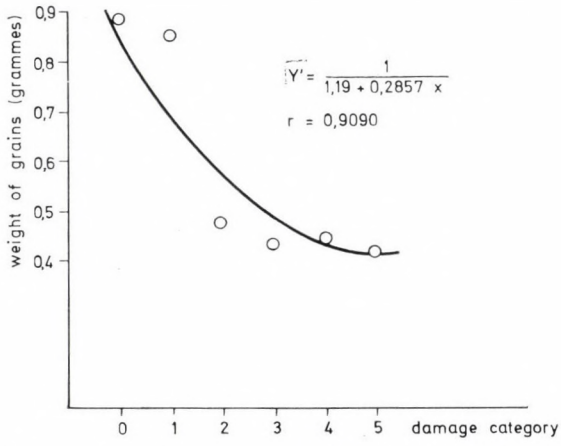

Fig. 3. Decrease of the weight of grains per ear (grammes) occurring as a consequence of damages of different extents caused by *Haplodiplosis equestris*

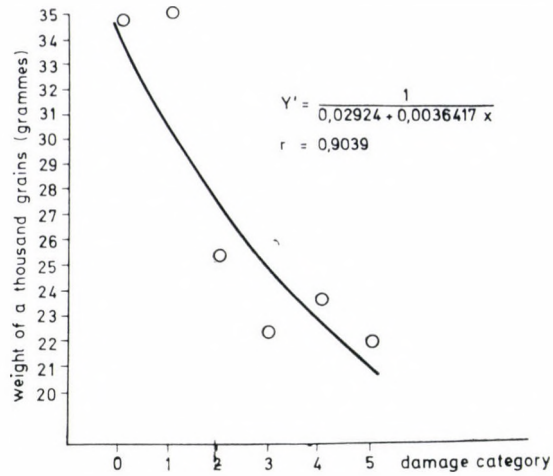

Fig. 4. Decrease of the weight of a thousand grains (grammes) occurring as a consequence of damages of different extents caused by *Haplodiplosis equestris*

According to the data in Table 3 the considerable decrease of the individual indices can be observed until one damage per 2—3 plants only, further on the extent of the decrease is much more insignificant.

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Observations on the Autumn Mass Flight of *Frankliniella intonsa* Trybom (*Thysanoptera, Thripidae*)

By

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Publications dealing with the mass flight of Thysanoptera present only few or no data on *Frankliniella intonsa* Trybom. By working up the materials collected by a suction trap operated for two vegetation periods (1965, 1967) in Keszthely, Hungary, data were obtained on the seasonal flight activity of the species. While in April-May only a moderate activity was noticed, the number of captured exemplars increased from June in both years. In the second half of August and in September a mass flight was observed under weather conditions similar to the ones characterized in details by Lewis, 1964 (anticycline). The flight was directed very likely towards the hibernation sites.

The basic handbooks and references dealing with the flight habits of Thysanoptera (KÖRTING 1930; LEWIS 1964, 1965; JOHNSON, 1969) report only few or no data on *Frankliniella intonsa*. A material collected by a suction trap which was operated in Keszthely (South-West Hungary) gave a good opportunity to obtain data on the seasonal activity of this species, which occurs on many crops as pest.

Material and Methods

The suction trap was run in Keszthely, in an orchard of the Laboratory of Research Institute for Plant Protection. It collected the samples through a tube (12 cm in diameter) from a height of 6 m. In earlier studies of JOHNSON (1969) this height proved to be suitable for collecting flying *Thysanoptera*. The air was taken in by the sucking action of a powerful ventilator (capacity 1000 m^3 per hour), which produced a vacuum in a chamber of $100 \times 60 \times 60$ cm. The insects sucked in with the air stream were swept in the vacuum chamber into a glass jar fastened to the lower end of a plastic screen funnel, which was in turn, attached to the end of the sucking tube. This collecting jar contained a mixture of alcohol and glycerine for preserving the insect material. The suction trap operated in 1965 from the begin of June until 20 September and in 1967 from 11 April until 10 October. In these periods the trap was run continuously, with the collecting jar changed every day between 7 and 9 a.m.

The trap was constructed under the guidance of Dr. T. JERMY on the model of JOHNSON's suction trap. The meteorological data were furnished by the Hungarian Institute of Meteorology and by the Laboratory of the Research Institute for Plant Protection.

Results

According to our data the flight of *F. intonsa* has begun in 1967 at the begin of April, as shown by the presence of sporadic individuals until the end of May. In June the suction trap collected a higher number of individuals in both years. In July the number of trapped *F. intonsa* exemplars fluctuated, with a marked rise towards the end of the month. Until the end of August the number of flying individuals further increased and in both years the catches attained their maximum in the first week of September. Their number proved to be high for the rest of September and in 1967 the number of *F. intonsa* dropped to a minimum in the samples at the begin of October.

From the begin of August until the second half of September the mass flight developed always on days when the weather displayed signs of an anticyclone, the maximum of air temperature reaching or exceeding 20°C (with an average of 16°C

Table 1

Frankliniellia intonsa Trybom individuals collected by using suction trap. Catch numbers added up per ten day's periods (Keszthely 1965, 1967)

Date		Year	
		1965	1967
April	11—20.	—	3
	21—30.	2	—
May	1—10.	—	—
	11—20.	—	—
	21—31.	—	4
June	1—10.	—	11
	11—20.	8	3
	21—30.	50	48
July	1—10.	11	29
	11—20.	66	19
	21—31.	82	48
August	1—10.	27	40
	11—20.	355	19
	21—31.	236	81
September	1—10.	1195	96
	11—20.	982	58
	21—30.	—	78
October	1—10.	—	6

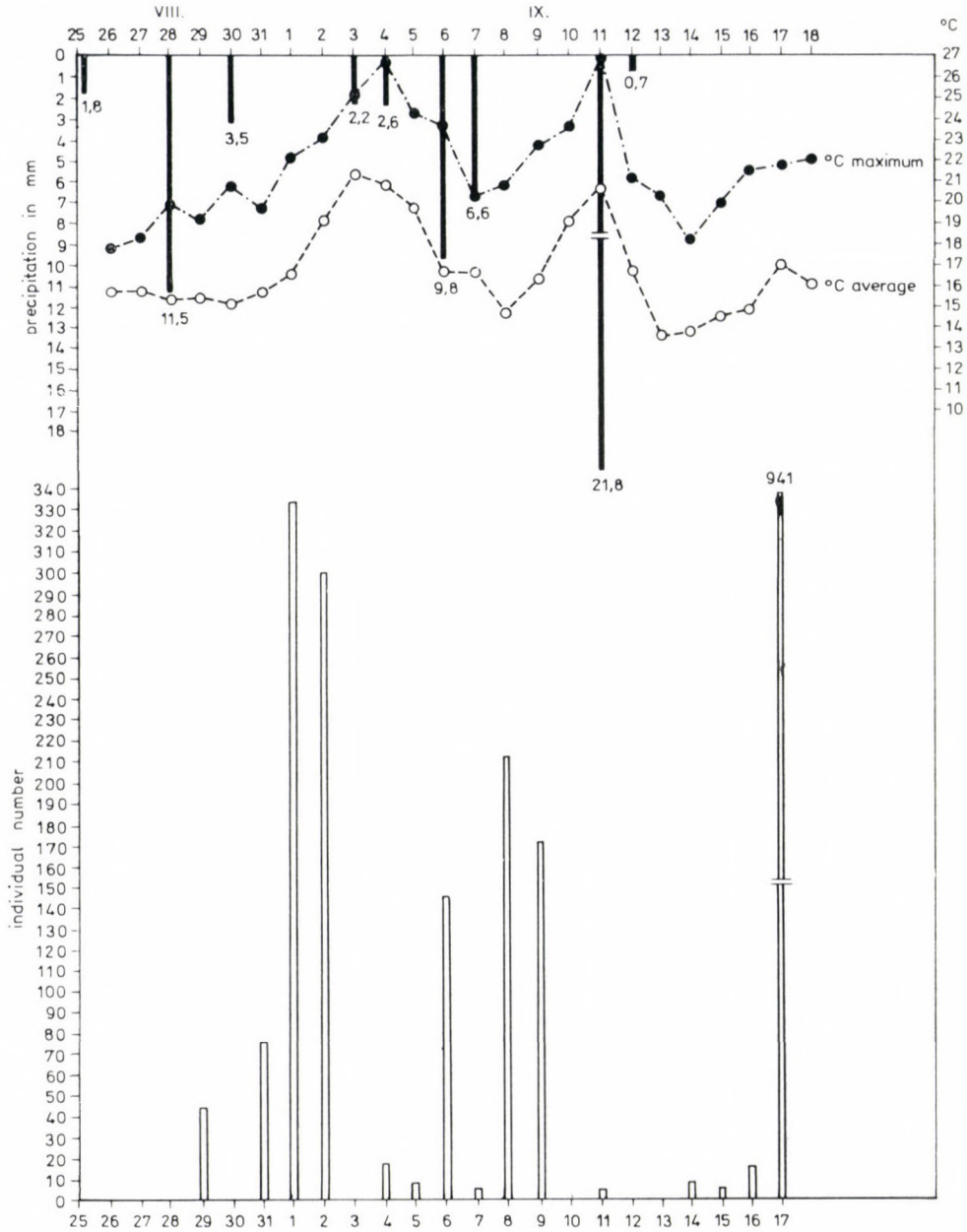

Fig. 1. Data on flight activity of *Frankliniella intonsa* Trybom (Keszthely), Number of individuals caught in August and September 1965

and a minimum above 10°C). Even small amounts of rain (2–2.6 mm) disturbed noticeably the mass flight, although sporadic flights could be even then observed. In our observations the mass flight of *F. intonsa* occurred under weather conditions corresponding to the ones described by LEWIS (1964) as optimal for the flight of many Thysanopteran species as well (Fig. 1, Table 1).

This section of the seasonal activity of *F. intonsa* i.e. the autumn flight was not established so far in spite of the fact that the species is quite common and appears regularly as pest on cultivated plants. In systematic collections carried out by suction traps in South England (LEWIS, 1964, 1965) only the spring flight of overwintered individuals was noticed, but no reference was given to the autumn mass movements. Our data – completed by other observations – may give an explanation to the phenomenon reported by SCHLIEPHAKE (1961). This author has found in Germany in systematically sampled alfalfa stands a marked – and unexplained – decrease in the individual density of *F. intonsa* populations towards the end of summer.

The autumn mass flight of *F. intonsa* observed in Keszthely was very likely in connection with the migration towards the hibernation sites.

Acknowledgement

I gratefully acknowledge the kindness of Dr. T. JERMY for putting the valuable material collected by the suction trap at my disposal.

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Effect of Different Degrees of Antennectomy on the Mating Behaviour of Bean Weevil, *Acanthoscelides obtectus* Say *(Col.; Bruchidae)*, Males

By

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The role of the antennae and palpi of bean weevil males in the mating behaviour was investigated. The antennae were amputated in different degrees while the palpi were entirely cut off. The sexual activity of the males towards females, manifesting itself in various behavioural patterns, decreased in all cases, but did not disappear completely. The amputation of the three or four terminal segments of the antenna considerably (10—30%) decreases the sexual activity. Consequently the 3rd and 4th segments and one or two further ones must play a prominent role in influencing the mating activity of bean weevil males. On further amputation the activity level remains constant or shows a tendency to decline. The activity of normal females towards antennectomized males increased remarkably.

The antennae and palpi of the bean weevil play an important role in the recognition of other individuals of the species and of food. From among the several kinds of receptors generally situated on the antennae of insects, the olfactory and tactile stimuli of the mentioned origin excite different sorts of chemo- and mechano-receptors; visual stimuli are secondary (SNODGRASS, 1935; DETHIER, 1953; SLIFER, 1967). NAKAMURA (1969) reported that antennectomized males of *Callosobruchus chinensis* L. were unable to find the females not even if they were kept in narrow tubes with females, from which he concluded that the females are recognized by olfactory stimuli. The experiments carried out with species of different insect genera revealed the arising or the lack of some kind of physiological, behavioural responses in the case of the injury or amputation of the antenna (SYRJÄMÄKI, 1962; BENZ, 1970; LEVINSON—BAR ILAN, 1970; MYERS—WALTER, 1970; POUZAT, 1970; SÁRINGER, 1970). The reactions can be brought about by means of cutting off some segments or the whole of the antenna, or else by inhibiting the individual chemoreceptors. The investigations to be discussed in this paper were based upon the observation of the effect of antenna, palpus maxillaris and palpus labialis amputations on the behaviour of the male and the response of the female.

Materials and Methods

Non-mated young males and virgin females were obtained from the laboratory mass rearing of a Hungarian bean weevil strain by means of JERMY's hatching equipment ((SZENTESI, 1972).

The antennae and palpi of the males were amputated two days after hatching. The joint between two antennal segments and the bases of the palpi were cut with fine scissors. Prior to the treatment the males were refrigerated until reaching stiffness on a refrigerating plate. The temperature of the refrigerating plate was constant during one treatment (cca. 1–3 min), while in the course of a whole series of treatments it varied between 0.5 and 4°C. (Bean weevils are completely stiff up to 5°C). For one day after the treatment the animals were kept at 28°C, then, one by one, they were put into 10 cm^3 empty glass vials each with a virgin female. The observations were carried out at 28°C. During 30 min of observation time the manifestations of the mating activity of the male and female and the occurrence of mating were recorded. (Under normal conditions mating does occur within 30 min between 3-day-old virgin males and females.) In each experiment the behaviour of 20 amputated males was observed twice in each case. Investigations were carried out during the period of highest activity of the weevils in the laboratory, i.e. immediately before noon and early in the afternoon.

The following factors that could decrease to some extent the activity of treated males as compared to the non-treated ones, affected all treated males in the same way: refrigeration during treatment, traumatic character of the treatment (loss of haemolymph, etc.), activity level of the female.

The behaviour of females was also influenced by several factors, such as the degree of the initiative ability of the males and the suitability of the two individuals to each other (occasionally very different dimensions).

For the sake of lucidity, the treatments and the variants of observations are summed up in Table 2. Three forms of male activity following each other were recorded; (1) courtship activity, (2) activities of attempting mating, and (3) mating activity (see further explanation, below). The three forms of activity were treated separately, and the number of specimens having reacted was indicated as a percentage of the number of all the specimens. The data obtained were evaluated by mathematical methods. The line describing the distribution of the data observed was drawn by means of orthogonal polynoms and partly by fitting to them a regression line. The decrease of the males' activity is approximated by several regression lines; in accordance with the qualitative difference between the different parts of the antenna however, these are not uniaxial ones, and in some particular cases they cannot even be fit because of the considerable dispersion of the data observed. In the cases where no fitting could be achieved only the original points are indicated. The equations of the lines, the values of the correlation coefficients and the result of the statistical essay are presented. The number of antennal segments cut off is indicated on the abscissa.

Additionally the morphological examination of the antennae was accomplished. For this purpose the antennae were boiled in a 10% KOH solution for 1 day until they became suitable for morphological observations.

Results and Discussion

The description of the antenna

The antennae of bean weevils are dorso-laterally flattened, and consist of 11 segments: 5 terminal and 6 basic ones. These two groups differ from each other; within one group, however, the individual segments are approximately of similar shape and size. The palpus maxillaris contains 3 segments, and the palpus labialis 2 ones (Fig. 1).

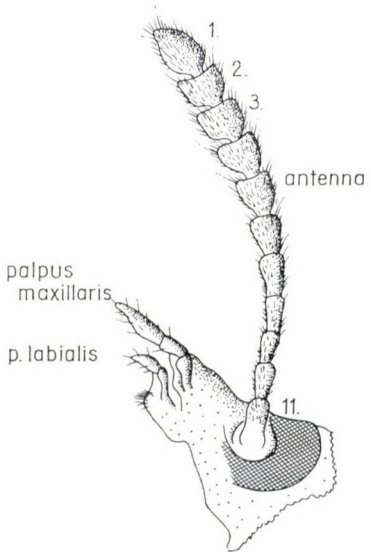


Fig. 1. The antenna and palpi of the bean weevil (rough sketch of the left side)

On the antennal segments and on the palpi hairs of different lengths and small pegs can be observed. In the case of this species no data are known about their function, however it seems probable that they play a role in tactile perceptiva and chemoreception. On each segment there are very short, medium sized and long hairs. Long sensory hairs are located on the upper third of the segments, arranged like a wreath, and on the top of the last segment. The longest hairs are situated on the inner side of the antennae (Fig. 1).

Measurements concerning the size of the antennae and palpi were also carried out (Table 1). The distribution of hairs of different sizes – considering

Table 1

Dimensions of the antennae and palpi of *Acanthoscelides obtectus* Say

	mm	pc.
Length of antennae	1.0–1.5	
Length of antennal segments	0.11–0.13	
Length of terminal segment	0.17	
Breadth of antennal segments	0.07–0.13	
Length of hairs	0.02–0.06	
Dimensions and number of hairs pro segment		
– short hairs	0.02	10–100
– medium sized hairs	0.03	10– 50
– long hairs	0.06	1– 10
Length of palpus maxillaris	0.3 - 0.5	
Length of palpus labialis	0.17	

the increasing surface of the individual segments towards the end of the antenna – is not uniform. As to their number on a given surface of the antennal segment there are only estimations. In Fig. 2 points of rise of hairs and small pegs are shown.

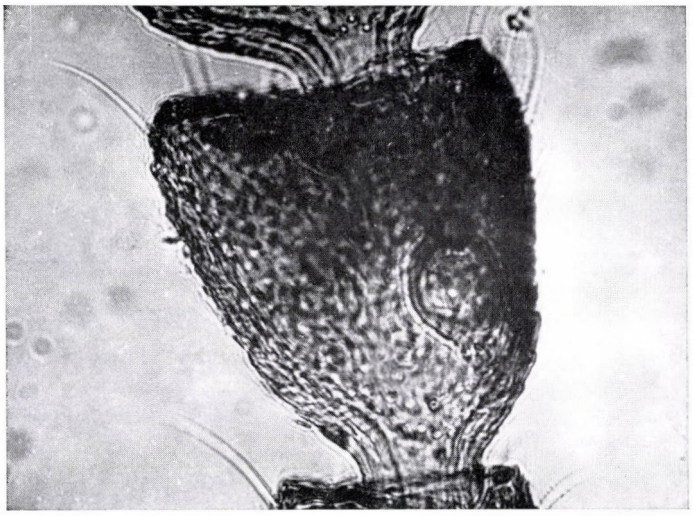


Fig. 2. Segment of the dorso-laterally flattened antenna of a bean weevil, from above. Laterally and on the upper part of the segment long hairs are shown (× 500)

In order to compare more easily the differences between the behaviour of treated and non-treated animals, the behaviour of normal adults was also investigated.

Mating behaviour of normal males and females

In the sexes' finding and recognizing each other, several, somewhat contradictory, factors are supposed to act.

a) By means of extraction with n-hexane, HOPE et al. (1967) obtained a substance continually produced by males. These authors suggest that, on the one hand, this substance encourages the hatching of females (males hatch earlier), on the other hand, it may act as a sex-attractant;

Table 2

Different extent of amputation, performed on males of *Acanthoscelides obtectus* Say, and the behavioural patterns investigated. (For detailed description of the individual behavioural patterns see the text)

	I. Antennectomy and palpectomy	II. Antennectomy (intact palpi)
a)	*Courtship behaviour* of amputated males towards normal females	
b)	Behaviour of intact females towards amputated males	
c)	"*Attempt to mate*" behavioural pattern of amputated males towards intact females	
d)	*Mating behavioural pattern* of amputated males towards intact females	

b) LABEYRIE (1970) considers important the inducing effect of the host plant, influencing first of all, the egglaying female. On the other hand, if a virgin female finds a bean plant, after having got to a state of excitation it begins to produce sexual pheromone, thus alluring the male from a short distance. Prior to this, the male is attracted by the plant.

c) In granaries, large quantities of dried beans arrest the adults. In case of a great number of individuals, the sexes may find each other by chance too, as a result of constant movement. In agreement with the observations of LABEYRIE (1970) we also found that visual stimuli were effective only from a very short distance, about 5—10 mm within which the male or female automatically starts to follow the animal moving in front of it. It is likely that both sexes produce a pheromone effective within a short distance or even as a contact stimulus, and the gathering, stimulating effect of the host plant may be of at least the same importance in finding the other sex.

The mating behaviour of a normal male and female finished by mating can be divided into several phases:

a) the phase of *courtship* manifesting itself in recognizing the other sex (mainly by touching with the antennae), in turning around each other and in

following the other sex closely, even along a distance of 10–15 cm. This phase changes into,

b) *the phase of trying to mate*, when the excited male tries to climb on the back of the female and to mate with it. Often 2–3 attempts are unsuccessful and the courtship behaviour returns, as in the case of an unsuccessful mating the female shakes off the male. It can be proved by measuring the length of time of the pseudocopulation that no real mating occurs. After a successful attempt, during the phase of

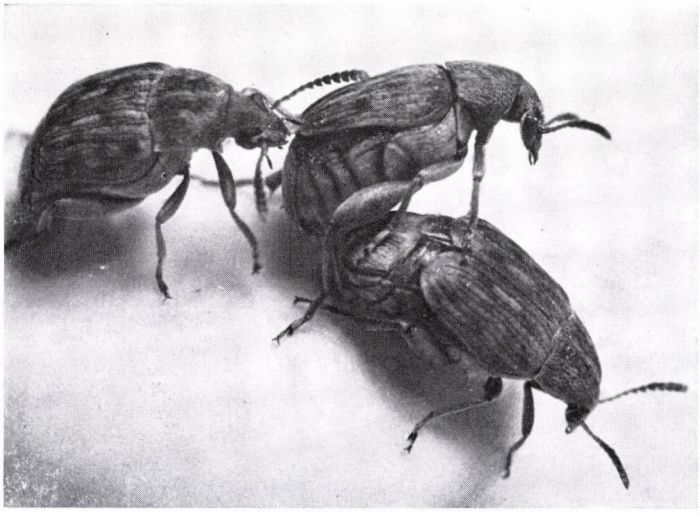


Fig. 3. Mating bean weevils. Another female touches the male with its antennae and palpi

c) *mating* both the male and female are calm, no essential change can be observed. The male often touches the wing-sheats of the female with the antennae.

On progressive amputation, the antennectomized males are able to accomplish some phases only, which gives reason for the division of the process, otherwise homogeneous.

Thus the courtship and mating behaviour of two normal adults in each other's vicinity can be started by the touching of the antennae by the pheromone which is perceptible on contact, and by the recognition of the behaviour of the animal touched. It can often be observed with recently mated females that they scare away the attempting males by strong sweeping movements of the hind legs. In case of a great population density mating is often disturbed by another individual, e.g. by a female (Fig. 3) allured by the copulating male.

Mating behaviour of amputated males

We had previously supposed that the palpi also played some role in recognizing the other sex and in courtship. Therefore the importance of their function was also investigated in the two series of experiments accomplished. In all the experiments, females were intact.

Series I

Males: antennae amputated to different degrees, palpi amputated.

Control males: intact antennae, amputated palpi.

a) Amputation of the antennae to different degrees decreases the courtship activity of males (Fig. 4).

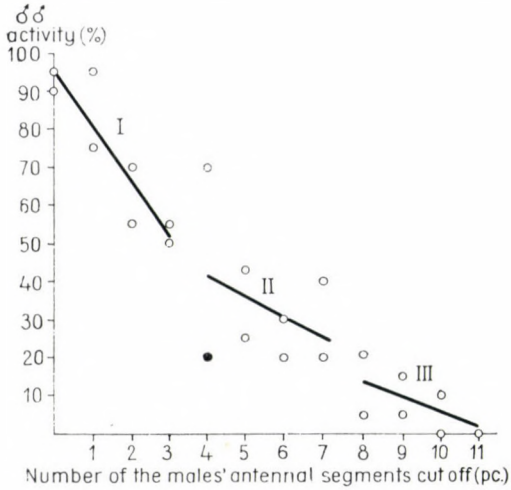


Fig. 4. Courtship activity of males as a function of the degree of antennectomy (palpi amputated). (1 point = average of 20 repetitions)

$Y_I = 94.48 - 14.24\ X;$ $\quad r_I = -0.9807$
$Y_{II} = 73.84 - 5.34\ X;$ $\quad r_{II} = -0.8140$
$Y_{III} = 42.20 - 3.64\ X;$ $\quad r_{III} = -0.9945$

The points obtained show linear correlation, however, the line can be divided into several parts. In case of cutting off the terminal antennal segments (part I) the activity decreases considerably, then from the third terminal segment on the decrease becomes slower (part II). The final part (III) is again uniform. The correlation of the decrease in activity to the number of the antennal segments cut off is close, which is demonstrated – beside the correlation coefficients (-0.9807, -0.8140 and -0.9945 respectively) – by the fact that part I and part III are significant on a level of 5% and 10%, respectively. Amputation until the 3rd to

4th antennal segments, causes a considerable decrease in activity. Thus, it is not only the declining character of the courtship activity that can be observed, but it can also be concluded that at a certain number of antennal segments the initiative ability suddenly decreases. A decrease in activity of the same character but of a much smaller extent results on cutting off the 8th segment. In case of the total amputation of the antennae and palpi of the males, their activity may disappear completely.

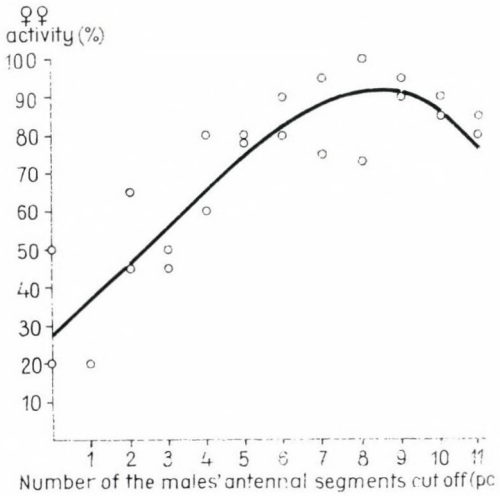


Fig. 5. Mating activity of females as a function of the degree of antennectomy on males (palpi amputated). (1 point = average of 20 repetitions) r = 0.9536

b) As to the behaviour of the females towards males described above, it is just the opposite process that takes place. As the males' activity decreases because of shorter and shorter antennae, that of the females increases and reaches a maximum (Fig. 5). The answer to the question as to why that maximum is not more prominent can be sought among the various factors having an effect on females (see above). The course of the experimental points obtained is shown by the line fitted to them by means of orthogonal polynoms, which is described the most precisely by a cubic equation. The correlation coefficient of the line is 0.9536, indicating a very close correlation. The linear part of the line is highly significant on a level of 0.1 % and the quadratic part is significant on a level of 1 %. The third part is not significant which means that the activity of the females does not decrease when the males' antennae consist of only 1 – 2 segments. The relationship resulting from the point of intersection of the line and the ordinate, is also noteworthy, indicating that a "basis" activity of a considerable level must be present on the females' side, even when the antennae of the males are intact. The behaviour of

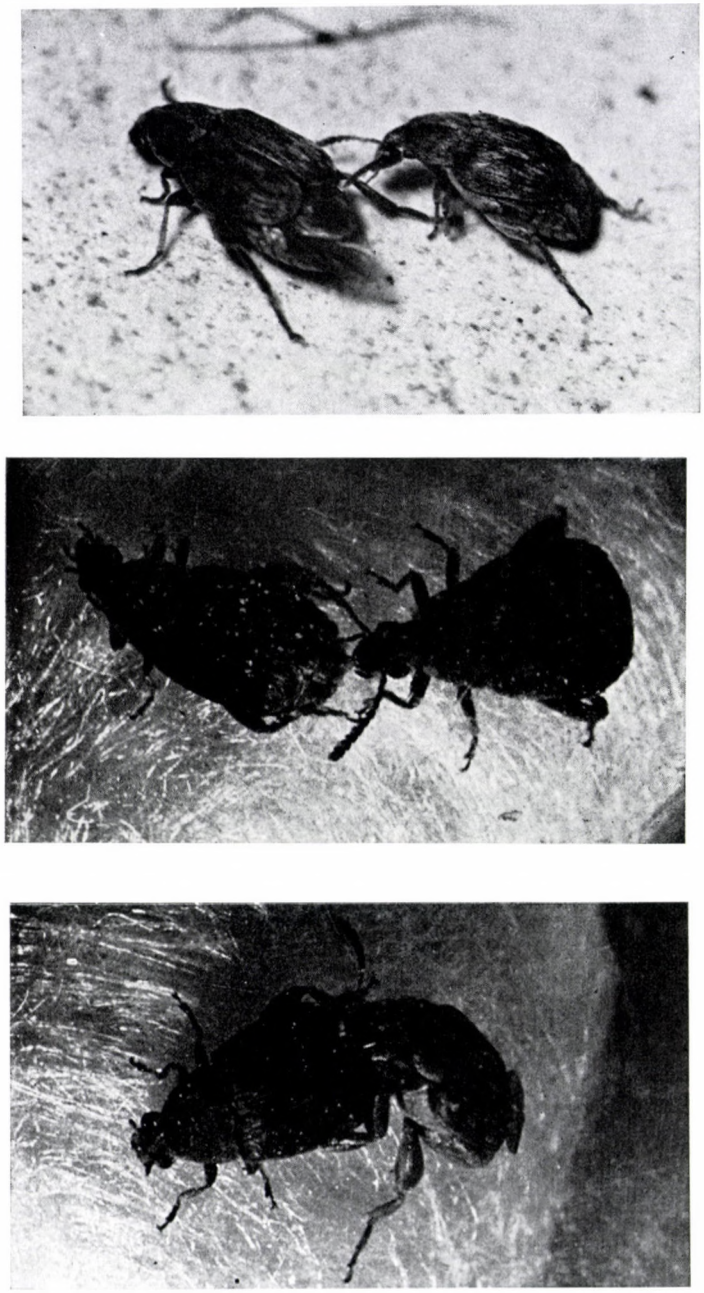


Fig. 6. a, b, c. Behaviour of normal female and antennectomized male bean weevils

the females towards antennectomized males shows certain elements of the corresponding behaviour of normal males: they touch the males, feel them with their antennae and even climb on the back of the males. These observations are illustrated by Figs 6a, b and c.

c) In the course of the activation of the males, during the phase preceding mating the male attempts to mate but that is generally unsuccessful. The activity of the males is characterized by the "attempt to mate" too, and the level of this kind of activity is also changed by amputation (Fig. 7). The correlation of the points are approximated by 3 regression lines. These can be characterized in the

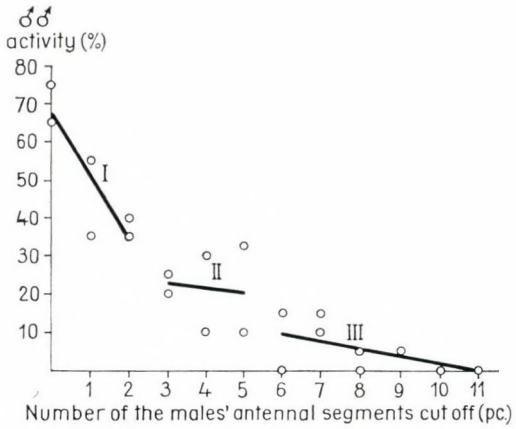


Fig. 7. Changes in activity for "attempt to mate" at different degrees of antennectomy (palpi amputated) (1 point = average of 20 repetitions)

$Y_I = 89.1 - 15.0\ X;$ $r_I = -0.9966$
$Y_{II} = 41.6 - 3.5\ X;$ $r_{II} = -0.8652$
$Y_{III} = 27.41 - 2.49\ X;$ $r_{III} = -0.8619$

same way as Fig. 4. The correlation coefficients of the three parts are -0.9966, -0.8652, and -0.8619, respectively. The first and third parts are significant at a level of 10% and 5%, respectively. Sudden changes take place when cutting off the 3rd and the 6th segments.

d) On the female's initiative, antennectomized males are able to mate too, depending upon the number of antennal segments left (Fig. 8). This fact indicates that on this level other sense-organs may serve as tactile receptors, perhaps chemo- and tactile receptors scattered in the genital region are to transmit stimuli, for though on cutting off some antennal segments mating occurs considerably rarer, it disappears only in case of total antennectomy. The correlation describing this phenomenon indicates a decrease of a very great rate and extent in mating activity (on cutting off the 1st and 2nd segments, a decrease of 40%, on removing one more

segment a decrease of 10%); after further segments it is less pronounced. On cutting off the 3rd to 5th segments no significant change occurs, while further on mating activity gradually disappears. All this is reflected by the correlation coefficients expressing the closeness of the correlations: phase I: −0.9787, II: −0.6000, III: −0.7991. The validity of phases I and II is small, no significant correlation can be revealed, while phase III is significant on a level of 5%.

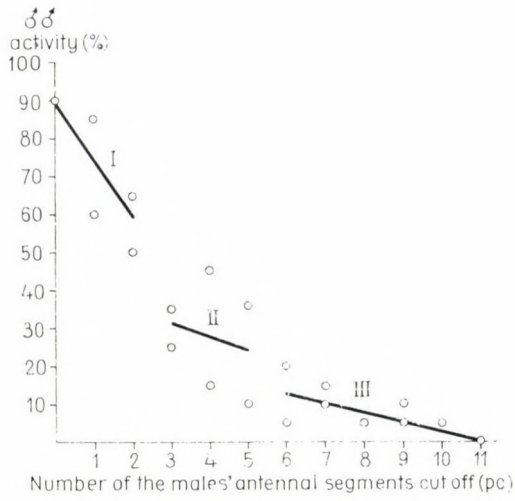


Fig. 8. Changes in mating activity of males at different degrees of antennectomy and total palpectomy. (1 point = average of 20 repetitions)

$Y_I = 67.05 - 16.25\ X$; $r_I = -0.9787$
$Y_{II} = 24.7 - 0.75\ X$; $r_{II} = -0.6000$
$Y_{III} = 22.09 - 2.06\ X$; $r_{III} = -0.7991$

Series II

Males: antennectomy of different degrees, intact palpi.

Control males: intact antennae and palpi. Looking at the distribution of the data observed (Figs 9, 10, 11 and 12), it is obvious that with all three behavioural pattern the correlation is not so consistent as in the previous series. The first part of the decrease in the males' activity is generally unambiguous (a regression line can be fitted to it), but sometimes it is not. Thus, it is obvious that if the males' palpi are left intact, the activity value characterizing the different behavioural stages is conserved on a higher level. On the other hand, a rather high percentage of the activity is still left after cutting off several antennal segments, and it does not cease on even total antennectomy.

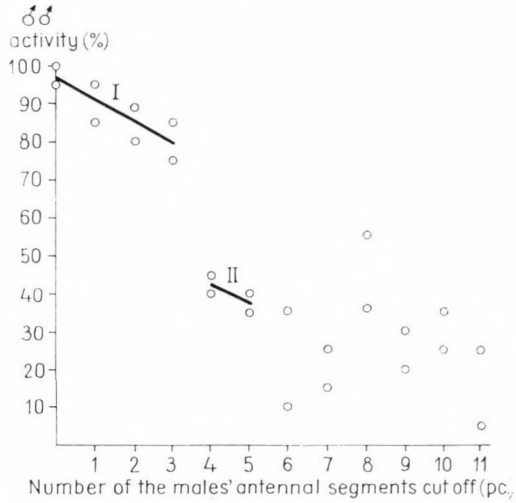


Fig. 9. Courtship activity of males as a function of the degree of antennectomy (intact palpi). (1 point = average of 20 repetitions)

$$Y_1 = 96.73 - 5.74\ X; \qquad r_1 = -0.9930$$

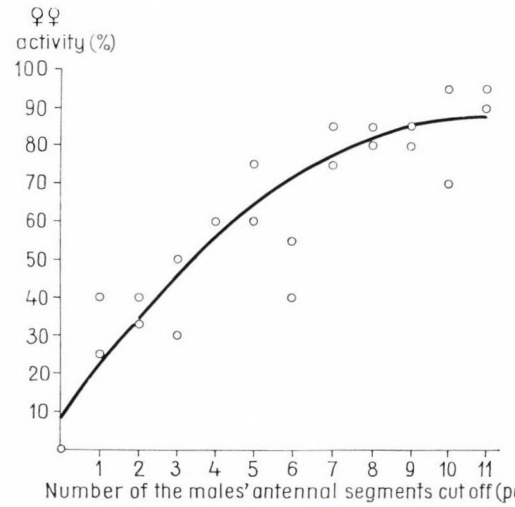
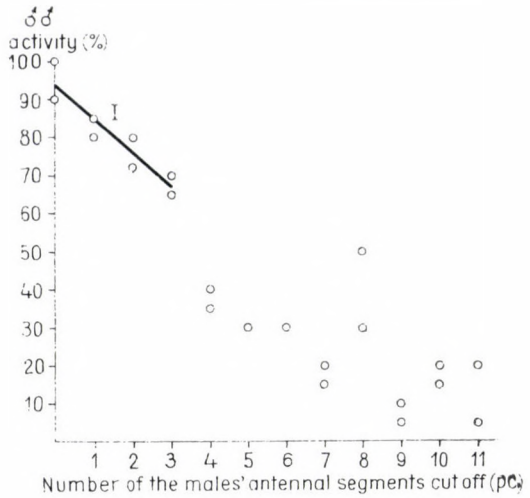


Fig. 10. Changes of sexual activity of females towards males antennectomized to different degrees (palpi intact). (1 point = average of 20 repetitions) r = 0.9805

a) On antennectomy of different degrees the ability of the males to initiate mating decreases (Fig. 9). The decrease is not considerable; in part I it is 25%. Part II begins after cutting off the 4th segment and here the decrease is 30%. The



Fig, 11. Changes in activity for "attempt to mate" at different degrees of antennectomy (intact palpi). (1 point = average of 20 repetitions)

$Y_I = 93.59 - 8.88\ X; \qquad r_I = -0.9866$

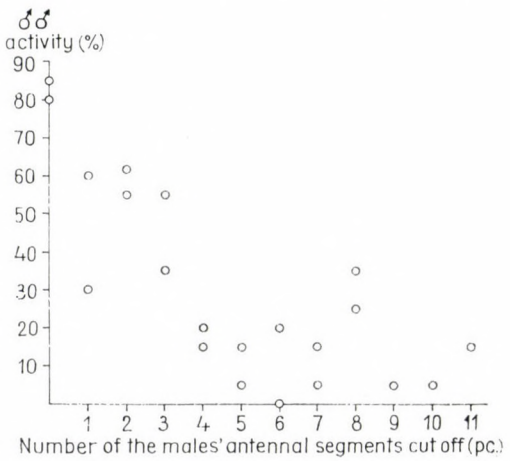


Fig. 12. Changes of mating activity of males at different degree of antennectomy (intact palpi). (1 point = average of 20 repetitions)

points of the supposed part III show a considerable deviation, but they do not reach the abscissa (indicating that the activity is not reduced to zero). The remaining activity may be attributed to various factors: to the *antennal segments left*, or to the intact palpi, or perhaps to the cumulative effect of both, for the latter can take over the function of the missing antennal receptors to a certain extent. The correlation coefficient of part I is -0.9930, and is significant at a level of 1%.

b) The females' activity increases uniformly, in relation to the extent of the males' antennectomy (Fig. 10). The data obtained can be described with a quadratic line the linear part of which is highly significant at a level of 0.1%; its quadratic part is significant at a level of 1%. The correlation coefficient is 0.9805, indicating a very close correlation between of the two factors, i.e. the cutting off one segment of the males' antennae, and the courtship activity of the females. The line and the ordinate intersect under 10%, showing higher activity of males with intact palpi and consequently lower "basis" activity of the females.

c) The males' activity aiming at "attempts to mate" tends to decrease too (Fig. 11). It is only the first part of the points that can be evaluated; it has a close correlation coefficient (−0.9866) but a lower level of significance (5%). The points of the further parts deviate very much. The activity decreases until reaching a minimum, but it does not disappear.

d) On progressive amputation mating activity decreases (Fig. 12), but — deviation being considerable — it is not worth applying any method of fitting. Activity still persisting after total amputation refers to the role of the palpi. All these facts prove that antennectomy has an effect on the mating activity of males, but the tendency to decrease is moderated by the presence of the palpi.

Investigating the importance of the function of the palpi further variations of amputation were tried too, i.e. amputation of one of the antennae and the palpi, or cutting off only the palpus maxillaris or the palpus labialis. The results obtained in this way are in good agreement with those discussed above.

Conclusions

1. The courtship activity of bean weevil males decreases gradually, according to the extent of antennectomy. The presence or absence of the palpi does not change this fact *essentially*, however after palpectomy, the general level of activity decreases and the data obtained show greater variation. Thus, the antennal segments left, and the palpi can take over the function of the whole antennae to a certain extent, at least regarding sexual activity.

2. The decreasing sexual activity of antennectomized males results in the increased activity of normal females, so mating generally takes place. There is a very close correlation between the decrease in the number of the males' antennal segments and the increase of the females' activity.

3. It can be supposed that in the case of bean weevils normal individuals of the two sexes are perfectly equivalent as concerns bringing about mating, but this is concealed by the greater initiative ability or "basis activity" of the males and it is only when the antennae of the male are damaged that the female takes over this role. The supposed phenomenon is of importance only in individual cases or in populations where the sex ratio is shifted in favour of the females, for then also males with damaged antennae may mate. With a great number of

individuals where the male : female ratio is normal or is shifted in favour of the males, males with damaged antennae are ousted from reproduction as they are not active enough, and are oppressed by unhurt males.

4. Even the amputation of the terminal segments of the antennae is of great importance as regards the males' mating activity. A uniform decrease in activity of a considerable extent (30–40%) takes place until the amputation of 3 to 4 terminal segments. The 3rd and 4th segments are of a special importance; in this region removal of one segment causes a decrease in activity of 10–30%. From this fact we conclude that on the terminal segments a great number of uniformly distributed chemoreceptors is situated which participate to the same extent in maintaining the male's activity. On the 4th segment, however, their number may suddenly diminish (and be the same until the 6th–7th segments), since from this region on, amputation brings about a decrease in activity of an equal extent. A greater decrease occurs again on cutting off the 7th to 8th segments. Consequently, the antennal segments are not equivalent from the point of view of the function of recognizing the other sex, for the number of receptors alters periodically.

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Synergie de quelques mélanges herbicides et l'interprétation physiologique et biochimique de ce phénomène

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Herbicides containing several active components are used in a wide sphere, but knowledge on the joint action of the components is rather scanty. The author carried out exact experiments with the patented herbicide mixtures *Synpran-111* (DPA + phenoxy ester) and *Buvinol* (atrazine + phenoxy ethanol), developed under his co-operation, to clear the measure and the nature of the synergism. Physiological and biochemical parameters, evaluated on the basis of preliminary assumptions have been checked by means of the biometrical model developed by PLACKETT and HEWLETT, (1952—1963) for the investigation of the synergism of insecticides. The comparison of the estimated and measured values made possible the physiological and biochemical interpretation of the interactions between the active components. In the case of *Buvinol*, the differences between the reactions of the one year old monocotyledon *(Echinochloa crus-galli)*, the one year old dicotyledon *(Sinapis alba)* and the perennial dicotyledon *(Convolvulus arvensis)* indicator plants could also be rationally explained, under consideration of the characteristics of mitosis, nutrient flow and metabolic mechanism.

L'utilisation des pesticides contenants plusieurs substances actives se propage dans les rayons de plus en plus étendus. Cela se comprend, parce que l'élargissement du spectre d'action et quelquefois d'autres avantages de l'action contribuent aux avantages technologique évidents. Dans les groupes des insecticides et des fungicides des mélanges au point de vue de la biochimie et de la physiologie synergistes «véritables» sont connus. Ce qui donne une plus grande difficulté, c'est l'étude de l'action jointe des mélanges de herbicides appartenant aux substances actives de plusieurs types, parce que dans ce domain de la protection des plantes la lutte s'effectue habituellement simultanément contre plusieurs espèces des mauvaises herbes, alors on peut bien déterminer l'élargissement du spectre d'action, mais la vérification de la synergie véritable est bien compliquée.

Il y a plusieurs années que dans le laboratoire biologique de l'*Usines Chimiques de Budapest (Budapesti Vegyiművek)* nous continuons des études fondamentales concernant l'action jointe des mélanges herbicides (BÁNKI, 1969a, b et 1971), dont le principe nous faisons connaître par deux produits qui présentent une synergie véritable, d'une part et dont la synergie nous pouvons expliquer, d'autre part. Notons que tous les deux produits sont protégés dans plusieurs pays

par brevets et ils sont fabriqués par l'*Usines Chimiques de Budapest* dans les tonnages de plusieurs centaines depuis plusieurs années, alors nos essais sont vérifiés par l'utilisation de grande échelle au point de vue du pratique.

Modèle 1. Synpran-111. Une herbicide supersélective de la culture de riz

Il est bien connu que la protection chimique des cultures de riz peut être considérée comme résolue postémergent par DPA (propanil) contre les espèces de l'*Echinochloa*, mais pas contre des *Scirpes maritimes (Bolboschoenus maritimus)*, particulièrement résistants et polluants et des autres espèces de mauvaises herbes vivaces de marais (*Alisma* spp., *Cyperus* spp., *Schoenoplectus* spp., *Typha*

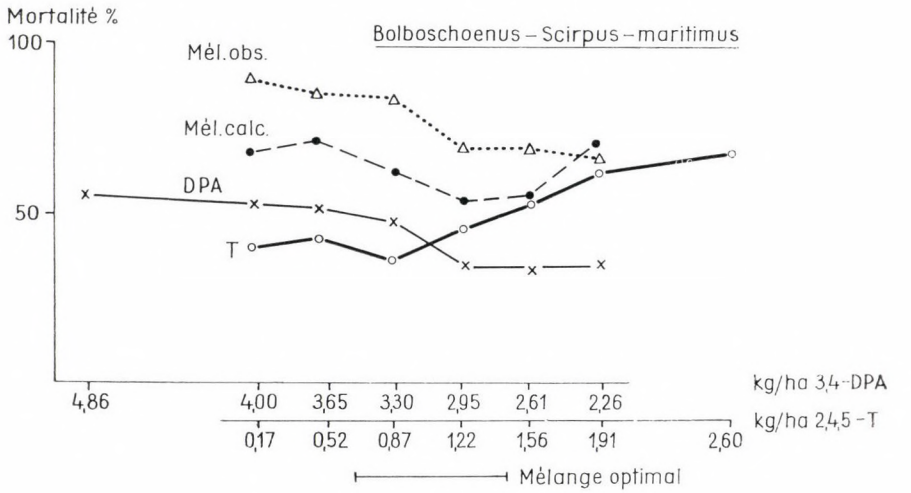

Fig. 1

spp.). Pour l'extirpation de celles-ci avec le repoussement simultané des espèces d'*Echinochloa* est très appropriée le *Synpran-111*, un produit DPA contenant une substance synergiste de type éphnoxy éthanol. La synergie se présent (BIHARI, SZILVÁSSY et VÁSÁRHELYI, 1967 et 1970) dans un rayon très large de la proportion des components du mélange (Fig. 1) mais dans notre pratique la zone moyenne s'est réalisée au point de vue de l'économie et de la sécurité d'opération. Enfin, le Synpran-111 dans sa forme finale contient quatre parties de DPA et une partie de l'esther isoamilde 2,4,5-T.

La vérification décisive de la synergie a été effectuée par un essai de microparcelle sur un champ de riz contaminé par des pieds de plusieurs centaines de *Scirpe maritime*, par traitement. Les components actifs ont participé dans un

rayon large avec 8 – 8 doses, le Synpran-111 avec 4 doses (Fig. 2). Après la calculation répandue dans la pratique

$$\frac{(100 - a) \cdot b}{100}$$

qui suppose un effet entièrement indépendant et dont on ne peut considérer qu'une approximation grossière – le taux de la synergie soit 1,3 dans la moyenne des quatre doses du mélange.

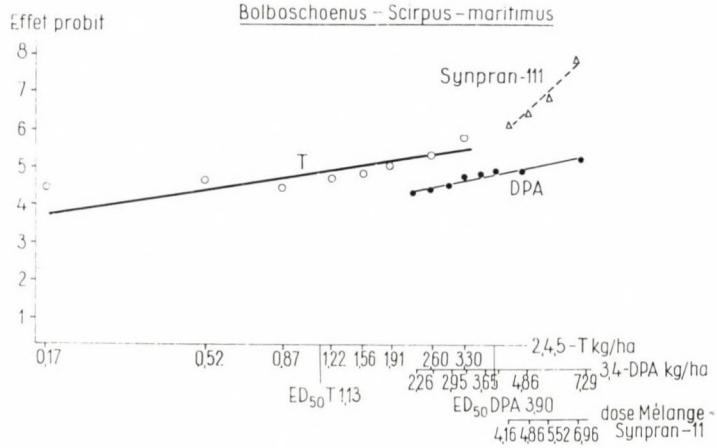

Fig. 2

Nous avons analysé les données avec l'utilisation du modèle biométrique de HEWLETT et PLACKETT mis au point pour insecticides et très important au point de vue biologique et biométrique. De leurs formules nous avons calculé avec leur équation générale (1963) présentée dans la Fig. 3. Après l'hypothèse des auteurs, si le calcul ou l'estimation des paramètres biologiques étant présents dans la formule est adéquate, «l'effet de mélange présumé» calculé par leur procédé doit consonner avec «l'effet de mélange mesuré» (voir en détail (BÁNKI, 1971; PLACKETT et HEWLETT, 1952, 1963a et 1963b). *La différence entre les effets présumés et mesurés a été éliminée* selon la fonction du modèle après plusieurs itérations quand «*le coefficient de pénétration*» de DPA a été $\eta_D = 0{,}2 - 0{,}3$ et celui du component phénoxy $\eta_{PH} = 0{,}1$ et la mesure de «*la similitude de l'effet*» a été $\lambda = 0{,}8 - 0{,}9$.

Ces données semblent être à l'opposé des caractéristiques physiologiques et biochimiques des deux substances actives. On considère le DPA comme un herbicide «contact» qui empêche présomptivement la chaîne de transport des

La formule employée (Plackett-Hewlett, 1963)

$$h^{\frac{\mathfrak{Z}_1-\mathfrak{Z}}{\lambda\theta_1}} + h^{\frac{\mathfrak{Z}_2-\mathfrak{Z}}{\lambda\theta_2}} = 1$$

h = base du logarithme
$\mathfrak{Z}$ = réponse probit
λ = paramètre du degré de la similitude de l'effet
$0<\lambda<1$

$\theta = \frac{b}{\eta}$ b = tangente de la dose-réponse ligne
η = „coefficient de la pénétration"

Fig. 3

électrons dans les cytochromes et par conséquent la réaction de Hill (AUDUS, 1964). Le component phenoxy lypophil utilisé par nous pénètre dans la plante relativement facilement et il agit dans son métabolisme par intensification nocive du mythose. Sur sa base on pourrait attendre a) un coefficient de la pénétration de DPA proche à 1 ; b) un λ proche à 0 à cause des méchanismes d'action essentiellement différents des substances actives. L'explication de la déviation est la suivante :

ad a) Le DPA n'est «un herbicide contact» qu'apparemment, qui est en effet une classification simplifée. En effet il peut arriver par plusieurs voies sur le lieu d'action. Soit, il peut pénétrer à travers de la cuticule et de l'épiderme cirées de la plante étudiée jusqu'à ce qu'il puisse toucher les chloroplasts incorporés dans le tissu du parenchyme. Soit, — en partie — par une voie plus directe, c'est à dire il arrive sur le même lieu à travers des trachées, hydatodes ou des fils de plasme. Par conséquent le changement quantitatif de la substance active survient, donc la diminution de η_D est bien motivée.

ad b) L'esther de 2,4,5-T, étant présent dans une quantité moindre, est mobile, qui peut hydroliser aisément et engage vite la mitose nocive et intensifie fort indirectement la dissimilation. Par contre le DPA qui se meut lentement et agit par l'empêchement de la réaction de HILL, diminue l'assimilation soutenant et relativement lentement. Alors au point de vue *de la totalité de l'organisme vivant de Scirpe maritime* on ne peut pas considérer les deux méchanismes d'action comme entièrement différents, parce que la *dissimilation élevée et l'assimilation diminuée* causent ensemble le *dépérissement de la plante*, donc la valeur de λ obtenue est réelle. Ainsi *on peut expliquer la «synergie» forte, mesurée par expériences dans notre série d'essais par l'aide du modèle et on peut vérifier sa validité.*

Modèle 2. Buvinol. Une herbicide sélective, utilisable dans un grand nombre de cultures, avec un spectre très large

C'est une produit contenant une partie d'Atrazine et une partie de 2,4,5-T-phénoxy éthanol (BIHARI, CSAPÓ et KISS, 1967). Nous avons conseillé son utilisation pendant des années uniquement comme un traitement préémergent parce que après

les approximations faits avec les modèles empiriques il semblait que l'action de «l'empêchement de la germination» du component de phénoxy éthanol et celle de l'empêchement de la réaction de HILL de l'Atrazine absorbé à travers des racines se présentent indépendantes, quasi en succession, quoique la synergie déterminée par le dépérissement des mauvaises herbes existe (BIHARI, CSAPÓ et KISS, 1967; BIHARI et RADVÁNY, 1970). Dans nos expériences récentes se donnent des résultats surprenants: dans le cas de l'utilisation postémergente les espèces de mauvaises herbes présentent par rapport de leur stade de croissance une synergie encore plus forte. Pour sa mesure et nature nous pouvons donner le résumé suivant.

Le mécanisme d'action des components et leurs mélanges:

a) Le 2,4,5-T-phénoxy éthanol d'un caractère des précurseurs se transforme en présence de NAD, déshydrogénase d'alcool et oxydase d'aldéhyde – sans compter les réactions secondaires – en deux échelles en 2,4,5-T et se produit 2 $NADH_2$ (BIHARI et al., 1970). D'abord il intensifie avantageusement le transport mitochondrial des électrons et la formation de l'ATP et le 2,4,5-T commence à élever l'action intensifiant la mitose. Puis les procès oxydatifs se transposent en partie dans le système directement oxydatif qui ne produit pas d'énergie à cause du chargement de la chaîne de transport des électrons. Le résultat par rapport de l'effet: une dissimilation élevée et une mitose aphysiologique.

b) Atrazine, comme c'est connu (AUDUS, 1964), empêche le transport des électrons des cytochromes et ATP ne se forme pas, l'assimilation s'arrête, le $NADH_2$ physiologique s'accumule. La plante jaunit, se fane et dépérit.

c) Avec le *Buvinol* sur la base des précédents, le $NADH_2$ physiologique et se formant par l'intoxication s'accumule (comme effet: addition). La division cellulaire s'intensifie: chez dicotylédones le tortillement des feuilles et de la tige et la prolifération des tissus; chez les monocotylédones l'élongation de la partie de la tige au-dessous du nodus. Le fonctionnement de la chaîne de transport des électrons, et la formation de l'ATP s'arrêtent totalement. Le seul qui fonctionne encore, c'est le système directement oxydatif. (Comme effet ensemble: c'est la synergie.)

Evidemment le niveau de NAD physiologique des différentes espèces de mauvaises herbes (élevé dans les grains germinants et dans les feuilles des dicotylédones comme c'est bien connu) et des stades de croissance, le pH des sèves des cellules (par exemple l'oxydase d'aldéhyde ne fonctionne que dans un domaine faiblement alcalin) la mesure des procès mitotiques physiologiques et la direction du courant des nutrients *varient bien ce procès complexe biochimique et physiologique très compliqué.*

Par conséquent il existe une différence fondamentale entre la sensibilité des mauvaises herbes monocotylédones annuelles, des dicotylédones annuelles et en général celle des mauvaises herbes pérennes.

Ainsi les mauvaises herbes *dicotylédones annuelles* sont sensibles contre le *Buvinol* dès la germination dans n'importe quelle phénophase, parce que la division secondaire des cellules est en cours durant toute l'augmentation de la plante,

donc le produit est actif également dans les traitements préémergents et postémergents. Par exemple, dans les essais effectués avec les plantes à étudier *Sinapis alba* nous avons déterminé dans un rayon large de doses une synergie empirique d'une moyenne 1,3 dans le traitement préémergent et une synergie de grade 1,4 dans le stade feuillé avec un traitement postémergent.

Des plantes *monocotylédones annuelles* peuvent réagir très différemment dans les différentes phénophases. Chez celles-ci il n'y a pas de la division secondaire des cellules, à cause de cela elles sont sensibles à l'effet du component hormonique seulement pendant la période de la division cellulaire primaire. Nous avons effectué des expériences très exactes avec un pulvérisateur logarithmique afin de constater la sensibilité de l'*Echinochloa crus-galli* (Tabl. 1).

Table 1

Les données du Buvinol ED_{84} (probit 6) dans les phénophases différentes de l'*Echinochloa crus-galli*

L'état de racine	Nombre des feuilles	Type du traitement	Dose de Buvinol kg/ha
Direct à la germination	— —	Préémergent	6—10
Racine de germe primaire	1—2	Postémergent	4— 8
Relais de racine (apparence de la racine de couronne, le début du tallage)	2—3	Postémergent	1— 2
Le développement de la racine de couronne	4—6	Postémergent	4—10
	plus que 7	Postémergent	10—20

On voit des données que pour développer l'effet correspondant de la valeur ED_{84} (probit 6) dans la période du relais de racine, une quantité de *Buvinol* 4—10-fois moins était nécessaire, que dans les phases résistantes. Nous avons déterminé *une synergie empirique d'une moyenne de 3,5-fois* dans une expérience de modèle très précise dans cette période (Fig. 4), que nous pouvons interprété par le fait, que le *Echinochloa* présente dans cette phase choisie non seulement un métabolisme très fort, mais la «transformation» de celui-ci est aussi en train, ainsi la totalité de l'organisme est touchée (comme nous l'avons dit sur le *Synpran-111)* par une action très forte, «une action nocive additionnée». C'est très important surtout dans la production par exemple du maïs, où avec une seule dose de 5 kg/ha environ, bien réglée à temps, on peut obtenir une extirpation complète sans le risque de post-effet de triazine.

Des *plantes vivaces* nous présentons nos expériences effectuées sur le *Convolvulus arvensis* lequel, comme c'est bien connu on ne peut pas extirper aisément. Le *Buvinol* utilisé comme préémergent ne développe son effet à 100 % que dans

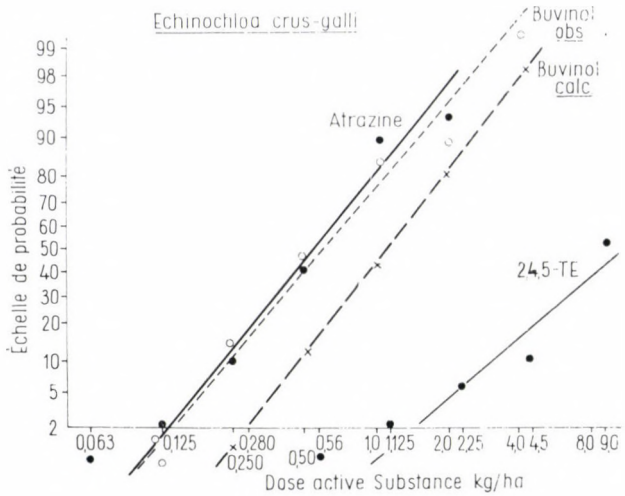

Fig. 4

une dose relativement élevée (10 – 12 kg/ha). La mesure de la synergie est dans ce cas aussi environ 1,4. La mauvaise herbe est plus sensible par ordre de grandeur si l'activité des tissus étant en division cellulaire ne s'arrête pas encore, mais le courant des nutrients commence déjà à se diriger vers les tissus des réserves souterraines. C'est l'état qu'on peut trouver dans le *Convolvulus* avant l'exanthèse et au début de mûrissement des grains. La translocation basipetalaire transporte alors très vite les métabolites activées de 2,4,5-T-phénoxy éthanol. Le component d'Atrazine arrive relativement facilement sur le lieu d'action à cause de la superficie d'assimilation très grande. Ainsi l'action complexe biochimique toxique du *Buvinol* déjà mentionnée commence à dominer presque tout de suite dans la totalité de la plante. Après nos mesures précises effectuées dans ce stade la dose de substance LD_{100} est:

2,4,5-T-phénoxy éthanol seul	3,0
Atrazine seul	5,0
Buvinol	2,5 kg/ha

Le grade de la synergie empirique est alors 3,0.

Les feuilles des *plantes ligneuses* (p. e. les fruitiers) sont les plus sensibles dans la période de la croissance active des feuilles, même avant la cutinisation. Au contraire par la suite, ou avant la période de végétation, même les drupacés et les cultures à baies très sensibles supportent très bien le *Buvinol.*

Notons que nous avons controlé les données des essais de modèle effectués avec *Buvinol* à l'aide du modèle biométrique de HEWLETT et PLACKETT. Les paramètres calculés sont les suivants:

Echinochloa crus-galli	$\eta_{\text{Atr.}} = 0{,}9$	$\eta_{\text{T.Et.}} = 0{,}1$	$\lambda = 0{,}6$
Sinapis alba	$\eta_{\text{Atr.}} = 0{,}8$	$\eta_{\text{T.Et.}} = 0{,}1$	$\lambda = 0{,}2$

Une différence plus importante ne se manifeste que chez le paramètre λ. Cet écart est dûment justifié par la différence mentionnée du méchanisme de la division cellulaire des plantes monocotylédones et dicotylédones annuelles.

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Synthesis and Antifungal Properties of Dithiocarboxylic Acid Derivatives

III. Dimethylaminomethyl-dithiocarboxylates and ß-dithiocarbamates

By

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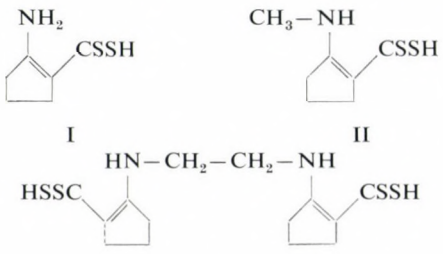
Dimethylaminomethylesters of 2-amino-1-cyclopentene-1-dithiocarboxylic acids and dialkyldithiocarbamic acids were tested comparatively with their parent acids or salts, respectively, and their methylesters for antifungal and polyphenol oxidase inhibitor activity. While methylesters of these acids possess a sharply decreased activity their dimethylaminomethylesters reveal biological properties similar to the parent acids or their salts, respectively.

In our previous studies (MATOLCSY et al., 1970; 1971) we demonstrated that 2-amino-1-cyclopentene-1-dithiocarboxylic acid (I) described by TAKESHIMA et al. (1969) as well as its N-methyl-derivative prepared by us (BORDÁS et al., 1971) (II) exert a marked antifungal action. In the literature listed above compound I is mentioned as 2-imino-cyclopentane-dithiocarboxylic acid; using infrared and nuclear magnetic resonance spectroscopy data we have proved however (BORDÁS et al., 1971) that both compounds possess the enamine form represented by formulae I and II, respectively.

Methyl esters of these acids were shown by us to be poorly active (MATOLCSY et al., 1971). This is consistent with the finding of KLÖPPING and VAN DER KERK (1951) that alkyl esters of dithiocarbamic acids are inactive and a metabolic splitting is necessary to restore the active dithiocarbamate ion.

In the present study the dimethylaminomethylesters of I, II and of ethylenebis-[2,2'-amino-1,1'-cyclopentene-1,1'-dithiocarboxylic acid] (III) described in our previous paper (BORDÁS et al., 1971) were tested for antifungal action.



I II

III

The parent acids I, II and III as well as their methyl esters were simultaneously tested in order to investigate the contribution to activity of the dialkylaminomethylester function.

It seemed us indicated to extend these studies also on the dithiocarbamates. Although a great number of dithiocarbamic acid derivatives were studied by BARRAT and HORSFALL (1947), KLÖPPING (1951), PLUIJGERS (1959), TORGESON et al. (1960), RICH and HORSFALL (1961), CARTER et al. (1964) and others, the antifungal properties of their dialkylaminomethylesters were studied sporadically (BOEHME and OTTO, 1967). However, compounds of this type were thoroughly investigated by SCHOENBERGER and ADAM (1964; 1965) and by NIEGER (1966) as potential aminoalkylating agents to be used in cancer therapy. In this case the dithiocarbamate moiety serves as a carrier for the biologically active dialkylaminomethyl function.

Experimental

Antifungal activity of the compounds was measured on six fungi, by using two different methods. The spore germination and growth inhibition method (BÁNKI et al., 1966) was used to test activity against *Alternaria tenuis* and *Botrytis allii; Fusarium moniliforme*, *Rhizoctonia* sp., *Aspergillus niger* and *Helminthosporium sativum* served as test fungi in the widely applied agar plate paper disc method.

Polyphenol oxidase activity of the compounds was parallely tested by means of the dihydroxyphenylalanine test described by KAARS SIJPESTEIJN and PLUIJGERS (1962).

Results and Discussion

Results of the spore germination, growth inhibition and polyphenol oxidase inhibition tests are presented on Table 1, those of the agar plate tests on Table 2.

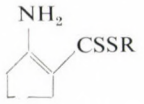
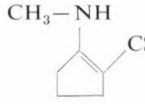
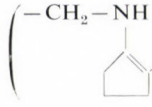
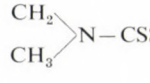
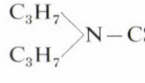
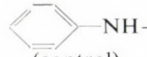
The general conclusion to be drawn is that dimethylaminomethylesters both of dithiocarboxylic and of dithiocarbamic acids resemble in their antifungal properties to the parent acids or their salts, respectively and greatly differ from their alkyl esters possessing a sharply decreased antifungal activity. This finding might be explained by the fact that the methylene group located between S and N atoms is activated by their $-I$ effects, thus faciliating the attack by OH^- ions and finally the formation of dithiocarboxylate anion.

Polyphenol oxidase inhibition shows a rough parallelism with antifungal activity, demonstrating that the dimethylaminomethylesters resemble to their parent acids also in this respect.

The dimethylaminomethylesters both of the acids II and III show, in contrast to their parent acids, also a selective antisporulation activity against *Hel-*

Table 1

Antifungal and polyphenol oxidase inhibitory action of the compounds studied. The data of antifungal tests represent the minimal concentration in % required for a 50% inhibition of germination and growth, respectively. Polyphenol oxidase inhibitory activity is expressed by the minimal concentration in p. p. m. necessary for complete inhibition

Compound	R	*Alternaria tenuis* inhibition of		*Botrytis allii* inhibition of		Polyphenol oxidase inhibition
		germination	growth	germination	growth	
	H	0.006	0.002	0.006	0.002	10
	$-CH_3$	0.01	0.008	0.03	0.01	50
	$-CH_2N(CH_3)_2$	0.005	0.002	0.005	0.006	5
	H	0.09	0.006	0.003	0.001	100
	$-CH_3$	0.1	0.06	0.1	0.02	>1000
	$-CH_2N(CH_3)_2$	0.002	0.001	0.006	0.006	200
	H	0.06	0.03	0.05	0.03	>1000
	$-CH_3$	>1	>1	>1	>1	>1000
	$-CH_2N(CH_3)_2$	0.005	0.003	0.001	0.003	>1000
	Na	0.0004	0.0002	0.00005	0.00001	10
	$-CH_3$	0.01	0.01	0.05	0.01	>1000
	$-CH_2N(CH_3)_2$	0.0007	0.0001	0.000007	0.000002	50
	Na	0.06	0.05	0.04	0.08	>1000
	$-CH_3$	0.1	0.1	0.1	0.3	>1000
	$-CH_2N(CH_3)_2$	0.09	0.05	0.009	0.003	>1000
 (control)		—	—	—	—	2

minthosporium sativum. In this respect the dimethylaminomethylesters exceed their parent acids, it seems probable therefore that also the aminoalkylating capacity of these esters, partly investigated by SCHOENBERGER and ADAM (1964; 1965) and by NIEGER (1966), contributes to biological action.

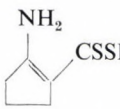
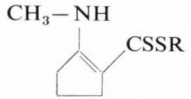
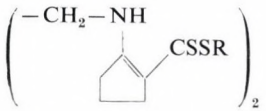
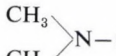
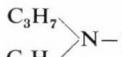
This finding deserves further attention as sporulation is a specific property of fungi, so it appears to be a hopeful target for the selective control of fungal diseases of agricultural crops (HORSFALL and LUKENS, 1968).

Thanks are due to Mrs E. FORRAI and Miss P. BERENCSI for valuable technical assistance.

Table 2

Antifungal activity of the compounds studied assessed in the agar plate-paper disc tests using three different concentrations for each compounds

Numbers: diameter of the zone of inhibition in mm; numbers in parentheses: width of the annulus of selective antisporulation activity in mm surrounding the zone of complete inhibition;

Compound	R	*Fusarium moniliforme*			*Rhizoctonia* sp.			*Aspergillus niger*			*Helminthosporium sativum*		
		1	0.5	0.25	1	0.5 %	0.25	1	0.5	0.25	1	0.5	0.25
	H (I)	29	23	+	23	20	+	23	+	0	24	21	+
	$-CH_3$	0	0	0	0	0	0	0	0	0	+	+	0
	$-CH_2N(CH_3)_2$	28	24	22	22	20	21	37	32	27	45	38	38
	H (II)	+	+	0	+	+	+	+	+	+	+	+	+
	$-CH_3$	0	0	0	0	0	0	0	0	0	+	+	0
	$-CH_2N(CH_3)_2$	0	0	0	0	0	0	0	0	0	+ (43)	+ (38)	+ (35)
	H (III)	0	0	0	0	0	0	0	0	0	+	+	+
	$-CH_3$	0	0	0	0	0	0	0	0	0	0	0	0
	$-CH_2N(CH_3)_2$	0	0	0	0	0	0	0	0	0	+ (44)	+ (43)	+ (40)
	Na	21	19	19	28	25	24	29	26	24	45	44	42
	$-CH_3$	+	+	+	0	0	0	0	0	0	+	+	0
	$-CH_2N(CH_3)_2$	23	19	22	25	24	20	20	23	23	39 (6)	43 (7)	41 (5)
	Na	+	+	+	+	+	0	+	+	+	+	0	0
	$-CH_3$	0	0	0	0	0	0	0	0	0	0	0	0
	$-CH_2N(CH_3)_2$	+	0	0	+	0	0	+	+	+	+	+	+

+ inhibition on the treated paper disc only;
0 no inhibition.

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Defence Reaction Induced by a Primary Inoculation with Barley Mildew on Wheat Seedlings

Short communication

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Induced defence reaction seems to be a rather widespread phenomenon among plants. In such cases a primary inoculation of the host with an avirulent (or incompatible) pathogen can decrease or inhibit completely the disease development caused by a subsequent inoculation with a virulent (or compatible) pathogen.

This phenomenon is best known in the case of virus diseases ("cross-protection") but data are also published from the fields of bacterial and fungal diseases. In the case of certain fungal diseases, immunity of a given host variety has been succesfully induced against the pathogen with its attenuated strain and/or incompatible varietal non-pathogenic race. However, only a very limited amount of data are available concerning defence reaction induced by species non-pathogenic fungi (cf. KIRÁLY, 1968; MATTA, 1971).

This paper reports on the phenomenon, that a primary inoculation of wheat plants with *Erysiphe graminis* DC. f. sp. *hordei* March., a species non-pathogenic fungus to wheat, induces defence reaction to its specific pathogenic powdery mildew, *E. graminis* DC. f. sp. *tritici* March.

Seedlings of wheat (*Triticum aestivum* L. 'Bezostaya 1') were grown under ordinary greenhouse conditions. The primary leaves were inoculated with conidia of the powdery mildew fungus, *E. graminis* f. sp. *hordei* 6—7 days after planting (2—3 days after emergence) by brushing them gently with heavily mildewed plants. A number of seedlings preinoculated with barley mildew were reinoculated with *E. graminis* f. sp. *tritici* after 48 hours, and at the same time an other group of plants was inoculated only with wheat mildew.

On the third day after inoculation with barley mildew diffuse chlorosis was observed on the leaves. As a consequence of the reinoculation with wheat mildew the leaf-tops began to turn yellow and finally they withered. Conidia of barley mildew germinated usually with 2—3 germ tubes on the wheat leaves, like *in vitro* on cellophane. Although the formation of appressoria occurred, haustoria did not develop even 48 hours after inoculation. This observation is in correspondence with ELLINGBOE's data (ELLINGBOE, 1968, 1972). At the same time conidia of wheat

mildew germinated generally with a single germ tube and produced haustoria in every case.

On the wheat leaves "immunized" with barley mildew only some rudimentary colonies of wheat mildew could be observed on the fourth day after reinoculation with wheat mildew. On the contrary, a great number of well-developed mildew colonies were observed on the leaves inoculated only with wheat mildew.

The counting of colonies on the fifth day after reinoculation has shown that the number of colonies on the "immunized" wheat leaves was thoroughly reduced as compared to the "non-immunized" control. The preinoculation of wheat with barley mildew reduced the number of the colonies of the wheat-pathogenic fungus by 81 per cent. At the same time, growth rate of the colonies was also inhibited to a great extent.

A similar inhibition of rust development, as well as simultaneous yellowing of leaf tops were observed, when wheat seedlings "immunized"with barley mildew were reinoculated with *Puccinia graminis* Pers. f. sp. *tritici* Erikss. et Henn. (race 11) two days after "immunization". Potted plants were kept in a moist chamber (100% relative humidity at 27°C) for 12 hours following rust inoculation. The rate of rust infection was evaluated after one week. Rust development, on the basis of the number of pustules, was inhibited by 90 per cent on the "immunized" leaves.

On the new parts of the "immunized" primary leaves, grown after the first inoculation, no significant inhibition occurred. Number as well as maturity of both mildew and rust pustules were not influenced on the newly developed leaf parts. This phenomenon clearly indicates, that the action (inhibition) induced by the primary inoculation is exclusively local.

In summary, it can be concluded, that preinoculation of wheat with a species non-pathogenic fungus reduces the development of species-pathogens, such as wheat mildew and rust. This reducing effect is not specific, but it seems to be a general phenomenon, which develops locally, only in the preinoculated leaf parts, and does not spread toward the new parts of the primary leaves.

These phenomena, described here briefly draw a number of important questions such as: What is the actual cause of the inhibition? What changes does the preinoculation induce, and where is the site of action? What is the inducer causing the changes? How do the the changes act on the development of the pathogen?

Further studies are in progress in order to answer these questions, and a better understanding of defence reaction.

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Book Review

K. MARAMOROSCH and H. KOPROWSKI (editors): Methods in Virology. Academic Press. New York and London, Vol. I, 1967. XVI + 640 p., Vol. II, 1967. XVI + 682 p., Vol. III, 1967. XIV + 677 p., Vol. IV, 1968. XVI + 764 p., Vol. V, 1971. IX + 530 p.

The five volumes of the "Methods in Virology" published so far contain the most important biological, physical, physico-chemical techniques and experimental statistical methods currently used in the study of viruses and mycoplasmas. The volumes, destined for the use of virologists and students working in the fields of human, animal, plant, insect and bacterial viruses, deal on 3293 pages, in 81 chapters with the methods and techniques which became indispensable in an up-to-date research and teaching. About 20 chapters contain informations on the research of plant viruses; at the end of each chapter comprehensive lists of references are given, which provide further sources of information for the reader. The five volumes of "Methods in Virology" contain nearly eight thousand references and about seven hundred figures. It has been an invaluable merit of the Editors to have selected hundred specialists of international fame for writing the different chapters. As a results a handbook was produced which enables virologists, graduate students and prospective students not only to appreciate the diversity of the methods currently being used in the different fields of virology, but also to develop a sense to evaluate the possible advantages and disadvantages of those. The individual volumes contain the following chapters, each written in a diversified individual style, according to the subjects treated and to the personalities of their authors:

Vol. I: *1.* Natural ecology (H. N. Johnson), *2.* Virus hosts and genetic studies (A. G. Dickinson and J. M. K. MacKay), *3.* Methods for the study of mosquitoes as virus hosts and vectors (R. W. Chamberlain and W. D. Sudia), *4.* Methods of studying and mites as virus hosts and vectors (C. B. Philip), *5.* Methods of studying plants as virus hosts (L. Bos), *6.* Laboratory methods of virus transmission in multicellular organisms (D. Blaškovič and B. Styk), *7.* Mechanical transmission of plant viruses (C. E. Yarwood and R. W. Fulton), *8.* Plant virus transmission by insects (K. G. Swenson), *9.* Nematode transmission (D. J. Raski and W. B. Hewitt), *10.* Methods for experimenting with mite transmission of plant viruses (J. T. Slykhuis), *11.* Fungus transmission of plant viruses (D. S. Teakle), *12.* Plant viruses: transmission by dodder (C. W. Bennett), *13.* Graft transmission of plant viruses (L. Bos), *14.* Insect pathogenic viruses (K. M. Smith), *15.* Bacteriophage techniques (A. Eisenstark), *16.* Animal tissue culture (J. S. Porterfield), *17.* Plant tissue culture (B. Kassanis), *18.* Invertebrate tissue culture (C. Vago).

Vol. II: *1.* The ultracentrifuge (R. Markham), *2.* Equilibrium ultracentrifugation (H. M. Mazzone), *3.* Density-gradient centrifugation (M. K. Brakke), *4.* Miscellaneous problems in virus purification (M. K. Brakke), *5.* New centrifugal methods for virus isolation (N. G. Anderson and G. B. Cline), *6.* Chromatography and membrane separation (L. Philipson), *7.* Water organic solvent phase systems (L. Philipson), *8.* Virus concentration by ultrafiltration

(K. Strohmaier), *9.* Diffusion (R. Markham), *10.* Two-phase separation of viruses (Per-Åke. Albertsson), *11.* Purification of virus by adsorption on cells and elution (F. E. Wassermann), *12.* Molecular sieve methods (G. K. Ackers and R. L. Steere), *13.* Filtration techniques (V. P. Perry and M. M. Vincent), *14.* Electrophoresis of viruses (A. Polson and B. Russell), *15.* Labeling of viruses with isotopes (C. Henry), *16.* Separation of viruses into components (R. K. Ralph and P. L. Berquist), *17.* Methods of degrading nucleic acids and separating the components (T. H. Lin and R. F. Maes), *18.* Assay of infectivity of nucleic acids (S. Sarkar).

Vol. III: *1.* Analysis of protein constituents of viruses (H. Fraenkel-Conrat and R. R. Rueckert), *2.* Analysis of lipid components of viruses (D. Kritchevsky and I. L.Shapiro), *3.* RNA virus RNA polymerase: detection, purification, and properties (J. T. August and L. Eoyang), *4.* Immunological techniques for animal viruses (J. Casals), *5.* Serological techniques (plant viruses) (R. E. F. Matthews), *9.* The plaque assay of animal viruses (P. D. Cooper), *7.* Transformation assays (M. G. P. Stoker and I. A. Macpherson), *8.* Methods for selecting RNA bacteriophage (M. Watanabe and J. T. August), *9.* Structural studies of viruses (J. T. Finch and K. C. Holmes), *10.* Microscopic techniques (R. S. Spendlove), *11.* Electron microscopy of isolated virus particles and their components (R. W. Horne), *12.* The application of thin sectioning (C. Morgan and H. M. Rose), *13.* Autoradiographic methods for electron microscopy (N. Granboulan).

Vol. IV. *1.* Techniques for the study of interferons in animal virus-cell systems (R. R. Wagner, A. H. Levy and T. J. Smith), *2.* Methods for the study of viral inhibitors (F. E. Wassermann), *3.* Methods of inactivation by ultraviolet radiation (A. Kleczkowsky), *4.* Inactivation of viruses by ionizing radiation and by heat (W. Ginoza), *5.* Methods for testing antiviral agents (F. Link), *6.* Techniques for studying defective bacteriophages (A. M. Campbell), *7.* Methods for the study of defective viruses (H. Hanafusa), *8.* Cell cultures and pure animal virus in quantity (H. L. Bachrach and S. S. Breese, Jr.), *9.* Methods in human virus vaccine preparation (L. Potash), *10.* Methods for containment of animal pathogens at the Plum Island Animal Disease Laboratory (J. J. Callis and G. E. Cottral), *11.* Methods of storage and preservation of animal viruses (T. G. Ward), 12. Methods of preservation and storage of plant viruses (H. H. McKinney and G. Silber), *13.* The optical diffractometer (R. Markham), *14.* Contamination of cell cultures by mycoplasma (PPLO) (A. Brown and J. E. Officer), *15.* Methods for the study of colicine and colicinogeny (H. Ozeki), *16.* Methods of virus classification (C. H. Andrewes), *17.* Experimental design and statistical methods of assay (A. Kleczkowski).

Vol. V: *1.* Fusion of cells for virus studies and production of cell hybrids (J. F. Watkins) *2.* New approaches to ultracentrifugation (A. Polson), *3.* Small circular viral DNA: preparation and analysis (J. S. Pagano and C. A. Hutchinson, III), *4.* Polyacrylamide gel electrophoresis of viral RNA (M. Adesnik), *5.* Polycrylamide gel electrophoresis of viral proteins (J. V. Maizel, Jr.), *6.* DNA—RNA and DNA—DNA hybridization in virus research (H. J. Raskas and M. Green), *7.* Techniques of RNA—DNA hybridization in solution for the study of viral transcription (K. Bøvre, H. A. Lozeron and W. Szybalski), *8.* Methods for the study of mRNA synthesis in bacteriophage-infected *E. coli* (J. S. Salser), *9.* Reactions of viruses in agar gel (A. L. Barron), *10.* Methods for the study of virus-antibody complexes (B. Mandel), *11.* Techniques of ferritin-tagged antibodies (S. S. Breese, Jr. and K. C. Hsu), *12.* The immunoperoxidase technique: localization of viral antigens in cells (E. Kurstak), *13.* Leaf-dip serology (E. M. Ball), *14.* Procedure to increase virus yield from infected plants (C. E. Yarwood), *15.* Freeze-etching technique for the study of virus ultrastructure (H. Bauer and E. L. Medzon).

The volumes of "Methods in Virology" have been received in the international scientific world with an enthusiasm which they merited: this worthy appreciation may be attributed with good reasons not only to their pioneering character but also to the high standard of their content.

J. HORVÁTH

Review of treatises of agricultural interest published in the series of *World Meteorological Organisation Technical Notes*

From the angle of agriculture — to be more exact, from that of plant protection — the following WMO booklets are considered to command interest: No. 96, 97, 99, 101, 118, 119 and 122. No. 96: "*Air Pollutants, Meteorology, and Plant Injury*" treats briefly the problems of air pollution, chemical reactions in polluted air, chemistry of pollutants. The author touches on the effect of emissions caused by extensive use of chemicals in industry and agriculture. A description of meteorological conditions of air pollution is given in length together with ways of determination of its extent. A chapter of special interest summarizes the physiological effect of air pollution on plants.

No. 97: "*Practical Soil Moisture Problems in Agriculture*" deals with questions of water-logging with special regard to irrigation.

No. 99: "*Meteorological Factors Affecting the Epidemiology of Wheat Rusts*" is the most important study as far as plant protection is concerned. This note beside describing in general terms the most important rusts of cereals comprises the distribution of them (black rust, brown and yellow rust) according to Continents, their symptoms and effects. The periodical character of their appearance has also been pointed out. A separate chapter is devoted to the epidemiology of wheat rusts and to the significance of weather conditions linked with spore transmission. A possibility of forecast has been discussed together with relevant approaches in different countries. In the end a survey of those meteorological conditions, methods, and prognostical experiences are presented which could provide a sound bases for the estimation of contributory factors of rust epidemics.

No. 101: "*Meteorology and Grain Storage*". This booklet gives an account of the problems of grain storage. It provides evidence to prove that 50 per cent of the stored grains fall victim to adverse environmental conditions, to pests and fungi. The moisture content of the grain, temperature and humidity of store as well as the associated organisms are mainly responsible for the success of storage. A detailed description of handling of grains is given, also methods of drying and fumigation has been dealt with.

No. 118: "*Protection of Plants Against Adverse Weathers*" discusses possible out-door measures against extreem weather conditions. A special attention is given to the climatical conditions in glass and plastic houses, to irrigation, heating, airing and mulching. The other part of the study discusses the climatical problems of out-door growing: sunshine and radiation, precipitation, wind and other meteorological factors, including humidity, wind borne vectors and indirect pests.

No. 119: "*The Application of Micrometeorology to Agricultural Problems*" presents findings on bio-and microclimatological phenomena occurring in the ecosphere of cultivated plants. Problemes of radiation, atmospheric motion, irrigation and the carbondioxid content of the air has been discussed in the first place; in the second part of the book a closer look is taken at problems encountered already in practice, with special bias to spreading of pests, diseases, to air and soil pollution, hurricanes, frosts and forest fires. A special emphasis is laid on questions related with plant physiology. The third part of the note outlines the prospects of micrometeorology then it ends with a list of micrometeorological research organisations in the world.

No. 122: "*Some Environmental Problems of Livestock Housing*". In the concentrated husbandry the housing of livestock show an ever increasing importance; as the prolonged environment of the livestock, it assumes a special significance from the angle of meteorological factors. Conditions that effect health — temperature, germs, contaminations, air-borne pollutants — has been analyzed together with the direct environment of livestock yard.

In the end a detailed survey is presented of those implements which monitor microclimatical conditions — temperature, air circulation, humidity — in the interior of the buildings. Studies of WMO reviewed above are of special agricultural interest, provide useful hints and view points for both the practical agriculturalist and research worker. G. UBRIZSY

Harry J. HUDSON: Fungal Saprophytism. Studies in Biology, No. 32. Edward Arnold Ltd, London, 1972. Pp. 1—68.

The richly illustrated booklet deals with the life processes, ecological conditions of saprophytic micro- and macrofungi; these are presented in a very clear, up-to-date form. In the introduction definitions are given on the saprophytism and parasitism respectively, as life forms or types of nutrition; then the roles of main nutrients (carbon, nitrogen, sodium, vitamin sources) are discussed. A special chapter is dedicated to the biochemical characterization of cellulose degradation and wood decay, with special interest to sources of cellulases, decay of living and dead wood, dry rot and blue stain of soft wood. The ecological significance of saprophytic fungi lies above all in the decomposition and mineralization of leaf litter and other debris in woodlands; the special chapter summarizes also the results of experimental ecological work on woodland fungi. Here also the pyrophilous fungi (the ones occurring on sites of forest fires), the micro-fungi of leaf litter, the species participating in the decomposition of pine needles and the chitinolytic fungi are presented. Special chapters contain the descriptions of the coprophilous, osmophilous and aquatic fungi with special regard to their ecological conditions, mode of nutrition and their role in the environmental contamination. In the chapter dealing with thermophilic fungi the mushroom cultivation and the cultivation of other edible *Basidiomycetes* is described, besides other interesting species occurring in birds's nests, grain storage silos and in tropical lands.

The last chapter deals with the industrial use of saprophytes, as many species produce antibiotics, steroids, acids, vitamins. Finally, fungi are described as food source, utilized not only by man but also by fungus growing insects. The booklet gives an excellent and variegated introduction to mycology for university students and any biologist interested in fungi.

G. UBRIZSY

George Baker CUMMINS: The Rust Fungi of Cereals, Grasses and Bamboos. Springer Verlag, Berlin, New York, 1971. Pp. 1—570 with 364 figures

The monograph gives a remarkable and up-to-date summary of the rust fungi parasitizing on different members of *Gramineae*. The construction of the volume is very appropriate as the reader finds already on the pages 1—40 keys to the species by genera of hosts, followed on pages 41—525 by the descriptions and characteristics of the different rust species; there also, within the rust genera further keys are given. The author took by all means the efforts to give a clear review of species which had been described under many synonyms. It was not easy, however, to agree in some cases with the author, who interpreted the concept of collective species or aggregates in a too wide sense, as in the case of *Puccinia recondita* Rob. ex Desm., where not less than 52 synonimes were united. Even the author called the example mentioned a "species complex" and ranged there all the rust fungi which form aecidia on *Balsaminaceae*, *Boraginaceae*, *Hydrophyllaceae* and *Ranunculaceae*, parasitizing on countless species of 37 different genera. Fusions of such extent are hardly justified, even if positive results of inoculation experiments are available. In some other cases similarly exaggerated fusions can be witnessed (e.g. *Puccinia hordei* Otth., *Puccinia poarum* Niels., *Uromyces setariae-italicae* Yosh. etc.), which are supported neither by the scientific research, neither by our present taxonomic knowledge.

The monograph may be considered as extremely useful for all who want to study the rust fungi of wild growing and cultivated *Gramineae*, from point of view of plant pathology or from other reasons. The detailed, clear figures help substantially in the identification of the species

G. UBRIZSY

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Begründet 1930 von Prof. Dr. E. SCHAFFNIT

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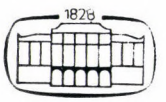

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Systemic and Chemotherapeutic Fungicidal Activity–Chemical Structure Relationship of Some 4-Methyl-5-thiazolecarboxylic Acid Derivatives. — Laboratory Screening Tests

By

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Institute of Organic Chemistry and Technology, Technical University (Politechnika), Warszawa, Poland

One hundred and thirtyseven compounds have been synthesized including 108 new (so far undescribed) ones which are derivatives of 4-methyl-5-thiazolecarboxylic acid. They were subjected to screening tests in order to obtain a better knowledge of their chemical structure–activity relationships. As bioindicators in the laboratory tests *Alternaria tenuis*, *Phytophthora infestans*, *Rhizoctonia solani*, *Tilletia caries* and *Venturia inaequalis* were used.

According to the results of these tests a great number of the compounds were selected for further glasshouse and field tests.

The first information about the systemic activity of 4-methyl-5-thiazolecarboxylic acid derivatives was given in the paper by NOVAKOVA (1968) and it concerned the compounds designated by the code number F-849 and G-696, introduced and investigated by the company UniRoyal Chemical. The former compound, 2-amino-4-methyl-5-carboxy-anilidothiazole was tested according to BERG (1970) under the name Seedvax, but in field conditions it proved to be insufficiently active (1971).

Later SNEL et al. (1970, 1971) and MATHRE (1971) compared the activity of the above-mentioned anilides with carboxine and oxycarboxine derivatives, claiming that the former have a weaker action than oxathiine derivatives. Seedvax and G-696 that is 2,4-dimethyl-5-carboxyanilidothiazole and three analogues of the former were investigated in more detail by HARDISON (1971) who tested their chemotherapeutic activity against smut and rust pathogens of Kentucky bluegrass (*Poa pratensis*). The author evaluated them as highly active but too phytotoxic.

Our investigations on 4-methyl-5-thiazolecarboxylic acid derivatives were undertaken at the end of 1968. The studies were started because of the facility of preparation of these substances and of developing the procedure to a production scale inclusively. The purpose in view was a systematic study of the influence of the chemical structure on the biological activity of this group of compounds.

Therefore, under laboratory conditions, the following compounds were synthesized and their activity was evaluated:

(i) – 4-methyl-5-thiazolecarboxylic free acid and esters with various substituents in position 2,

(ii) – 4-methyl-5-carboxyanilidothiazole; the influence of substituents in position 2 on the biological activity was elucidated,

(iii) – the carboxyanilide rest; the influence of the kind and positions of the substituents in the benzene ring was investigated, mainly in the case of new Seedvax and G-696 analogues and some compounds described in the quoted patent literature (British, S. Afric., and U.S. pats).

Material and Methods

The tested compounds with the structure corresponding to the general formula (A) were prepared according to the method described in WILEY's (1951) or SPRAGUE's (1959) monographs or else they were synthesized as described in the papers of ABDEL-LATEEF et al. (1971, 1972). The physical properties and yield of type (A) compounds are listed in Table 1.

All the compounds designated with bold type are new substances, while those listed in ordinary type are known and described in the literature. It should be stressed that elemental analysis data were in excellent accord with those calculated for compounds (1)–(137). The IR spectra also confirmed the structure of all the synthesized compounds of type (A).

Free carboxylic acids of type (A) were tested as aqueous solutions of their sodium salts, whereas esters and anilides were assayed in the form of emulsions with Tween 80 as tenside, prepared by the method described by JONES et al. (1951).

2-Amino-4-methyl-5-carboxyanilidothiazole (94) that is F-849 or Seedvax and 2,4-dimethyl-5-carboxyanilidothiazole (110) that is G-696 and their mixtures with Cu-oxine were tested in the present work under the respective code symbols ALF and ALG, ALF-59-16 and ALG-59-16 (details in the second part of this paper).

For comparative purposes also commercial preparations used as systemic fungicides were used such as:

Benlate – DuPont's commercial 40 WP containing benomyl as active ingredient,

Vitavax – technical product of UniRoyal Chemical, 75 per cent carboxine as wettable powder (WP).

In the assay of the effectiveness of type (A) compounds by the BLUMER–KUNDERT method (1950) 4-day-old spores of *Alternaria tenuis* Ness. and 16-day-old spores of *Venturia inaequalis* (Cooke) Aderh. were used. The latter were cultivated on one-year-old apple tree shoots of the Boskoop variety. The substance tested in the form of solution of suitable concentration was sprayed onto slides

by means of a Potter's metal bell-jar and after drying kept in a humid chamber. The spore suspension (ca. 3200 in 1 cm^3) was placed on the slides with a micropipette in the amount of 0.05 cm^3. After incubation at 20–22°C or 18–20°C the number of germinating spores was counted. The effectiveness was calculated as per cent of the control.

In the case of stinking smut of wheat (*Tilletia caries* (Bjerk.) Wint.) teliospores were used for the tests. The experiments were run similarly as the previously described ones but slides covered with 2 per cent collodion (modification of KOTHERBY's method). The 0.05 cm^3 drops of the spore suspensions micropipetted onto the slides contained about 20,000 spores in 1 cm^3. The slides were incubated at 9–11°C at 100 per cent moisture for 8 days.

In the case of *Rhizoctonia solani* Kühn the method of VINCENT (1947) described by BORECKI et al. (1965) was applied. It consists of inhibition of mycelium growth on sterile potato-glucose-agar (PGA) culture plates, by admixture of suitable concentrations of the tested fungicide. Radial growth of the fungus was compared with that of the control between the 5th and 20th day at 20–22°C.

Laboratory investigations of compound (94) and (110) that is ALF and ALG, and of their mixtures with Cu-oxine (ALF-95-16 and ALG-59-16) were performed and they were compared with Vitavax (UniRoyal Chemical) as seed dressing. The method of SETHOFER (1946/1947) was used with a PGA medium. As bioindicators spores of *Fusarium lini* Bolley and bean seeds naturally infected with *Collectotrichum lindemuthianum* Bri. and Cav. were used. Flax seeds (variety Wiera) deprived of germination power or viable bean seeds (variety Saxa) were infected with spores and incubated at 20–22°C. The degree of seed infection was calculated after 14–15 days.

Laboratory screening tests for systemic and chemotherapeutic activity were performed by the newly developed method. Tomato leaves of the Kondine Red or Potentat variety, obtained from plants grown under fungus-free conditions in a greenhouse, were cut and inoculated on both sides with 7-day-old spores of *Phytophthora infestans*. In the tests for systemic action, the inoculated leaves were kept in a humid chamber for 5 hr. Then the ungerminated spores were washed off with sterile distilled water and the leaf stalks were clipped to leave three apical leaflets on each leaf. Two leaflets were treated with the test solution so as to leave the apical leaflet free of the tested compounds (diagram – ).

A second lot of leaves was removed from the incubation chamber 22 hr after infection with fungal spores and was treated in an analogous manner. The extension of the incubation period permitted the evaluation of the chemotherapeutic activity of the tested substances.

In a similar manner new series of tomato leaves three apical leaflets were prepared, and only the apical leaflet was treated with the test solution of the compound tested (diagram – ).

Results and Discussion

From among the results obtained in biological activity tests of all the compounds synthesized (1)–(137) only some were selected for listing in Tables 2 and 3 or for graphic presentation in Diagram 1 and 2 (Figs 1 and 2). Selection was based on equal biological activity – within the limits of experimental error – with the standard substances Vitavax or Benlate. The results of *in vitro* tests of activity towards *T. caries*, *A. tenuis* and *V. inaequalis* spores are shown in Table 2.

It is noteworthy that the products prepared for field application, from the synthesized preparation ALF (94) and ALG (110), in the form of 75 per cent WP exhibited a higher biological activity than the pure anilides in respect to the spores of *T. caries*. From among the compounds listed in Table 2 the activity of the standard preparations towards this phytopathogen is exceeded by the ethyl ester of 2-(4′-chlorophenyl-amino)- (20), 2-phenylthio-4-methyl-5-thiazolecarboxylic acids (27), and by the anilide of the latter (80).

A high fungitoxicity with respect to the spores of *A. tenuis* and *V. inaequalis* is exhibited by compound (55) that is 2-(2′,3'-dimethylphenoxy)methyl-4-methyl-5-carboxyanilidothiazole, but it shows no activity towards *T. caries*. From the point of view of control of *V. inaequalis* exclusively, 2-methoxyanilide (98) and 2-bromoanilide of 2-amino-4-methyl-5-thiazolecarboxylic acid (105) deserve attention. These compounds have a higher activity than the standard Benlate.

Particularly interesting results were obtained with the compounds (1)–(137) in tests for their ability to inhibit growth of *Rhizoctonia solani* mycelium. It was found as regards compounds (13, 16, 20, 44, 45, 76, 78, 81, 86, 88, 90, 91, 101, 109, 114, 119, 120, 123, 131, 136, ALF-75 and ALF-59-16) that in concentrations from 0.2 to 0.0062 per cent they inhibited mycelium growth completely for 20 days. Similar qualities as regards this pathogen of crop plants are noted in Vitavax when used in amounts equivalent to the active ingredient.

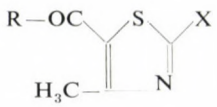
As regards other compounds (2, 21, 41, 46, 53, 60, 81, 86, 110, 135, ALG-75 and ALG-59-16) growth of mycelium was noted at concentrations of 0.025, 0.0125 and 0.0062 per cent, and in dependence on time it ran the course shown in the diagrams (Fig. 1 and 2). The height of the bars in the diagrams denotes the diameter of the fungus colony (mm) for the successive concentrations of 0.025, 0.0125 and 0.0062 per cent, respectively, of the substance investigated on the days of measurement.

In the case of 2-ethyl-4-methyl-5-carboxyanilidothiazole (46), the first growth of mycelium was observed at a 0.025 per cent concentration as late as after 7 days of incubation (Fig. 1) whereas in the case of 2-piperydylthiocarbamoyl-4-methyl-5-carboxyanilidothiazole (83) this effect was observed at the same concentration on the 6th day of incubation (Fig. 2).

On the basis of the laboratory studies it may be stated that 4-methyl-5-thiazolecarboxylic acid derivatives are systemic fungicides with a high activity against *R. solani*.

Table 1

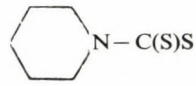
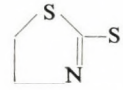
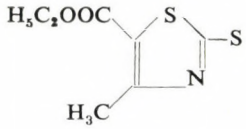
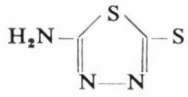
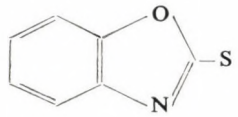
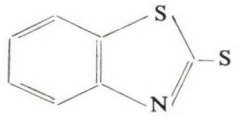
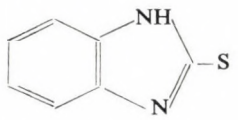
Properties of synthesized and laboratory tested 4-methyl-5-thiazolecarboxylic acid derivatives of the general formula (A):



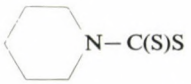
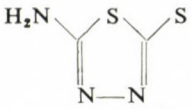
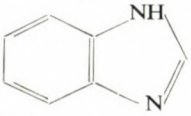
(A)

Comp. No.	X	R	M.p.s' (°C)	Yield (%)
1	2	3	4	5
1	H	OH	211–212	55.7
2	H	OC_2H_5	27–28	43.0
3	CH_3	OH	231–233	61.0
4	CH_3	OC_2H_5	51–52	75.0
5	$C_6H_5OCH_2$	OH	219–220	83.1
6	$C_6H_5OCH_2$	OC_2H_5	63.5–65.5	60.5
7	$C_6H_5OCH(CH_3)$	OH	162–163	86.0
8	$C_6H_5OCH(CH_3)$	OC_2H_5	112.5–114.5	59.8
9	2-$CH_3C_6H_4OCH_2$	OH	153–155	80.2
10	2-$CH_3C_6H_4OCH_2$	OC_2H_5	102–104	58.4
11	(2)-$C_{10}H_7OCH_2$	OH	216–217	81.3
12	(2)-$C_{10}H_7OCH_2$	OC_2H_5	114.5–115.5	60.7
13	C_6H_5O	OC_2H_5	59.5–60.5	78.0
14	3-CH_3-4-ClC_6H_3O	OC_2H_5	56.5–57.5	76.0
15	SH	OH	209–211	77.5
16	SH	OC_2H_5	153–154	75.0
17	NH_2	OH	168–169	91.7
18	NH_2	OC_2H_5	177–178	96.0
19	C_6H_4NH	OC_2H_5	142–143	89.7
20	4-ClC_6H_4NH	OC_2H_5	150–151	89.3
21	Cl	OC_2H_5	50–51	50.0
22	Br	OH	163–164	73.2
			(180–182)	73.2
23	Br	OC_2H_5	70–71	72.0
24	I	OC_2H_5	86–87	52.5
25	CH_3S	OC_2H_5	33–34	76.0
26	n-C_4H_9S	OC_2H_5	oil	82.0
27	C_6H_5S	OC_2H_5	66–67	83.0
28	4-$HOOCCH_2OC_6H_4S$	OH	209–211	59.0
29	4-$CH_3OOCCH_2OC_6H_4S$	OC_2H_5	87–88	84.0
30	4-$HOOCCH_2OC_6H_4S$	OC_2H_5	157.5–159.5	81.0
31	4-$CH_3OOCCH_2OC_6H_4S$	OH	192–193	48.0
32	$(CH_3)_2NC(S)S$	OC_2H_5	141–143	75.0
33	$(C_2H_5)_2NC(S)S$	OC_2H_5	81–82	77.6

(Table 1 continued)

Comp. No.	X	R	M.p.s' (°C)	Yield (%)
1	2	3	4	5
34		OC_2H_5	87–89	72.0
35		OC_2H_5	152–154	15.0
36		OC_2H_5	96–97	83.0
37		OC_2H_5	157–158	77.8
38		OC_2H_5	85–86	76.0
39		OC_2H_5	87.5–89.5	84.7
40		OC_2H_5	103.5–105.5	68.0
41	$C_6H_5SO_2$	OC_2H_5	111.5–113.5	80.0
42	4-$CH_3C_6H_4SO_2$	OC_2H_5	133–134.5	78.2
43	4-$CH_3CONHC_6H_4SO_2$	OC_2H_5	164.5–165.5	72.0
44	4-$ClC_6H_4SO_2$	OC_2H_5	129–130.5	76.0
45	H	NHC_6H_5	157–159	68.2
46	C_2H_5		153.5–155.5	67.0
47	$C_6H_5CH_2$		124.5–126	62.5
48	$(C_6H_5)_2CH$		190–191	78.2
49	$C_6H_5OCH_2$		156–158	62.0
50	2-$CH_3C_6H_4OCH_2$		179.5–180.5	61.2
51	3-$CH_3C_6H_4OCH_2$		162–163	73.2
52	4-$CH_3C_6H_4OCH_2$		182–183	65.6
53	2-$C_2H_5C_6H_4OCH_2$		178–179	82.4
54	4-t-$C_4H_9C_6H_4OCH_2$		132–132.5	69.5

(Table 1 continued)

Comp. No.	X	R	M.p.s' (°C)	Yield (%)
1	2	3	4	5
55	2,3-$(CH_3)_2C_6H_3OCH_2$		190–192	58.5
56	2,4-$(CH_3)_2C_6H_3OCH_2$		195–196	76.5
57	2,6-$(CH_3)_2C_6H_3OCH_2$		147.5–148.5	68.4
58	2-CH_3-4-$ClC_6H_3OCH_2$		201–201.5	69.0
59	3-CH_3-4-$ClC_6H_3OCH_2$		164.5–165.5	78.4
60	2-C_2H_5-4-$ClC_6H_3OCH_2$		189–191	64.5
61	4-$CH_3OC_6H_4OCH_2$		156–157	74.4
62	4-FC_6OCH_2		158.5–159.5	71.5
63	2-$ClC_6H_4OCH_2$		162–163	70.2
64	3-$ClC_6H_4OCH_2$		159–160	70.0
65	4-$ClC_6H_4OCH_2$		173–173.5	72.1
66	2,4-$Cl_2C_6H_3OCH_2$		202–202.5	66.0
67	2,5-$Cl_2C_6H_3OCH_2$		176–177	67.5
68	2,4,5-$Cl_3C_6H_2OCH_2$	NHC_5H_6	173.5–174.5	64.4
69	2-$BrC_6H_4OCH_2$		179–180	72.6
70	4-$BrC_6H_4OCH_2$		183–184.5	70.8
71	4-Br-2,5-$Cl_2C_6H_2OCH_2$		156.5–158.5	68.4
72	2,4-$Cl_2C_6H_3OCH(CH_3)$		151–152	69.7
73	(2)-$C_{10}H_7OCH_2$		193–193.5	51.3
74	C_6H_5O-$CH_2CH_2CH_2$		131.5–132.5	62.4
75	C_6H_5		170–171	73.1
76	2,6-$Cl_2C_6H_3$		167.5–168.5	59.2
77	C_6H_5O		202–204	87.2
78	3-CH_3-4-ClC_6H_3O		146.5–147.5	85.5
79	n-C_4H_9S		106.5–107.5	83.3
80	C_6H_5S		114.5–116.5	78.0
81	$(CH_2)NC(S)S$		188–189	60.0
82	$(C_2H_5)_2NC(S)S$		154–155.5	63.4
83			184.5–186	62.2
84			181–182	76.2
85			191.5–192.5	82.4
86	$C_6H_5SO_2$		206–208	65.4
87	4-$CH_3C_6H_4SO_2$		188–189	71.3
88	4-$ClC_6H_4SO_2$		214–216	65.0
89	4-$CH_3CONHC_6H_4SO_2$		233–235	67.2
90	Cl		104.5–105.5	52.5
91	Br		131–132	65.3

(Table 1 continued)

Comp. No.	X	R	M.p.s' (°C)	Yield (%)
1	2	3	4	5
92	SH		264 – 265	72.5
93	SH	NHC_6H_4CL-2	233 – 234	85.0
94	NH_2	C_6H_5NH	219 – 222	91.6
95		2-$CH_3C_6H_4NH$	228 – 230	81.0
96		3-$CH_3C_6H_4NH$	196 – 197	75.9
97		4-$CH_3C_6H_4NH$	244 – 245	47.3
98		2-$CH_3OC_6H_4NH$	251 – 252	51.5
99		3-$CH_3OC_6H_4NH$	186 – 187	84.5
100		4-$CH_3OC_6H_4NH$	226 – 227	48.3
101		2-ClC_6H_4NH	264 – 265	95.0
102		3-ClC_6H_4NH	211 – 212	84.4
103		4-ClC_6H_4NH	275 – 277	93.5
104		3,4-$Cl_2C_6H_3NH$	245 – 246	93.5
105		2-BrC_6H_4NH	263 – 265	80.2
106		4-BrC_6H_4NH	276 – 278	96.2
107		2-CH_3O-4-ClC_6H_3NH	263 – 265	84.0
108		4-CH_3O-3-IC_6H_3NH	275 – 276	66.0
109	CH_3	cyclohexyl-NH	135.5 – 136.5	47.0
110		C_6H_5NH	146	73.3
111		2-$CH_3C_6H_4NH$	123.5 – 124.5	62.0
112		3-$CH_3C_6H_4NH$	83.5 – 84.5	51.2
113		4-$CH_3C_6H_4NH$	115.5 – 116.5	53.0
114		2-$C_2H_5C_6H_4NH$	108.5 – 110.5	48.0
115		2,5-$(CH_3)_2C_6H_3NH$	136.5 – 138.5	52.2
116		2-$C_6H_5C_6H_4NH$	138 – 140	66.7
117		4-CH_3-2-ClC_6H_3NH	105.5 – 106.5	72.0
118		2-CH_3-4,6-$Cl_2C_6H_2NH$	188 – 190	52.1
119		2-$CH_3OC_6H_4NH$	97 – 99	63.7
120		3-$CH_3OC_6H_4NH$	104 – 105	55.4
121	CH_3	4-$CH_3OC_6H_4NH$	126.5 – 127.5	63.2
122		2-CH_3O-4-ClC_6H_3NH	136.5 – 138	58.0
123		2-CH_3O-5-$NO_2C_6H_3NH$	172 – 173	47.0
124		4-CH_3O-3-IC_6H_3NH	179 – 181	61.3
125		4-FC_6H_4NH	158 – 160	66.7
126		4-F-2-ClC_6H_3NH	134.5 – 136.5	67.2
127		2,4-$F_2C_6H_3NH$	178 – 180	68.0
128		2-ClC_6H_4NH	112 – 113	69.0
129		2,5-$Cl_2C_6H_3NH$	129.5 – 131.5	65.7
130		2,6-$Cl_2C_6H_3NH$	188 – 190	71.5
131		2,5-Cl_2-4-$NO_2C_6H_2NH$	150.5 – 151.5	61.0
132		2,4,5-$Cl_3C_6H_2NH$	152.5 – 153.5	56.2
133		2,4,6-$Cl_3C_6H_2NH$	207 – 208	65.0
134		4-BrC_6H_4NH	146 – 148	49.0
135		3,4-$Br_2C_6H_3NH$	168 – 170	68.2
136		4-IC_6H_4NH	169 – 171	47.2
137		(2)-$C_{10}H_7NH$	143.5 – 144.5	43.5

Table 2

Antifungal activity of some compounds of (A) type

Compound tested	Inhibition of spore germination, in % of the control								
	Tilletia caries			*Alternaria tenuis*			*Venturia inaequalis*		
	Chemical concentration in %								
	0.2	0.1	0.05	0.2	0.1	0.05	0.2	0.1	0.05
1	2	3	4	5	6	7	8	9	10
Vitavax	100.0	80.5	20.1	64.0	0.0	0.0	100.0	88.5	74.5
Benlate	—	—	—	0.0	0.0	0.0	90.0	75.0	43.0
ALF-75	100.0	51.3	24.0	—	—	—	—	—	—
ALF-59-16	100.0	80.5	35.7	—	—	—	—	—	—
ALG-75	100.0	93.0	48.7	—	—	—	—	—	—
ALG-59-16	100.0	72.7	40.9	—	—	—	—	—	—
18	0.0	0.0	0.0	100.0	56.0	0.0	0.0	0.0	0.0
20	100.0	80.5	18.2	0.0	0.0	0.0	0.0	0.0	0.0
27	100.0	80.5	33.1	0.0	0.0	0.0	0.0	0.0	0.0
46	100.0	67.5	18.1	0.0	0.0	0.0	0.0	0.0	0.0
52	100.0	72.1	17.1	0.0	0.0	0.0	0.0	0.0	0.0
55	0.0	0.0	0.0	100.0	84.5	31.2	100.0	75.3	27.2
76	100.0	65.6	15.6	0.0	0.0	0.0	100.0	45.0	0.0
80	100.0	74.7	20.1	0.0	0.0	0.0	100.0	71.5	0.0
87	0.0	0.0	0.0	100.0	52.5	0.0	100.0	63.0	0.0
94 (ALF)	100.0	62.1	28.4	—	—	—	—	—	—
98	—	—	—	0.0	0.0	0.0	100.0	100.0	100.0
105	—	—	—	0.0	0.0	0.0	100.0	93.1	74.3
110 (ALG)	100.0	74.7	10.4	0.0	0.0	0.0	92.0	84.0	56.8
112	0.0	0.0	0.0	0.0	0.0	0.0	100.0	87.0	46.5
113	100.0	75.9	17.4	0.0	0.0	0.0	0.0	0.0	0.0
121	0.0	0.0	0.0	0.0	0.0	0.0	98.5	83.0	54.5
131	92.2	79.5	20.0	0.0	0.0	0.0	87.5	73.5	20.5
136	93.5	67.4	10.3	0.0	0.0	0.0	88.0	63.5	8.5

In laboratory tests of the activity against *Fusarium lini* only compound (110) that is 2,4-dimethyl-5-carboxyanilidothiazole (ALG) was used and compared with Vitavax. Both fungicides have to be used in high concentration for obtaining a 95 per cent inhibition of pathogen development. Moreover, the results show no significant differences between compound (110) and Vitavax in 0.4, 0.3 and 0.2 per cent concentrations. However, Vitavax was more active than ALG in 0.1 per cent concentration.

Bean seeds (variety Saxa), naturally infested with *Colletotrichum lindemuthianum* causing anthracnose, were tested with ALF (94) and ALG (110) preparation for their fungitoxicity, and the germination power of the seed treated

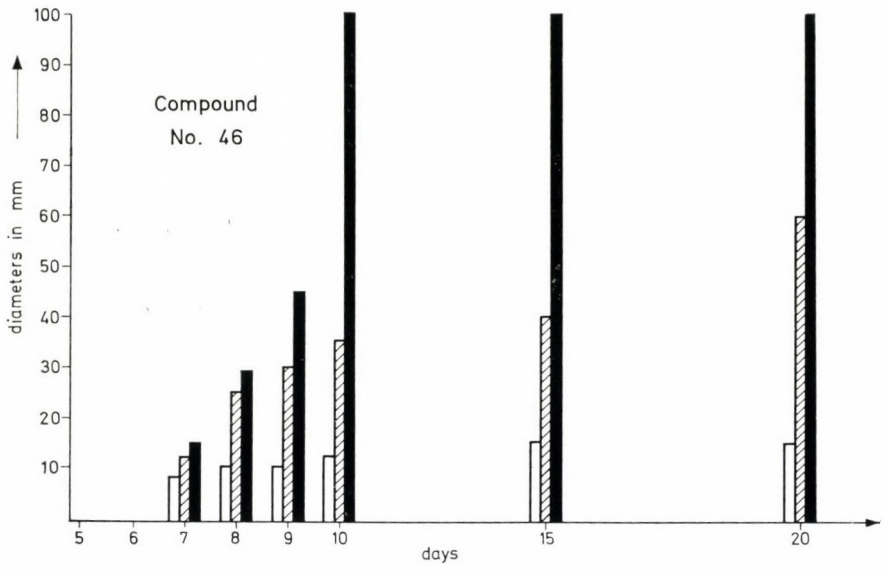

Compound
No. 46
diameters in mm
100
90
80
70
60
50
40
30
20
10
5
6
7
8
9
10
15
20
days

Fig. 1

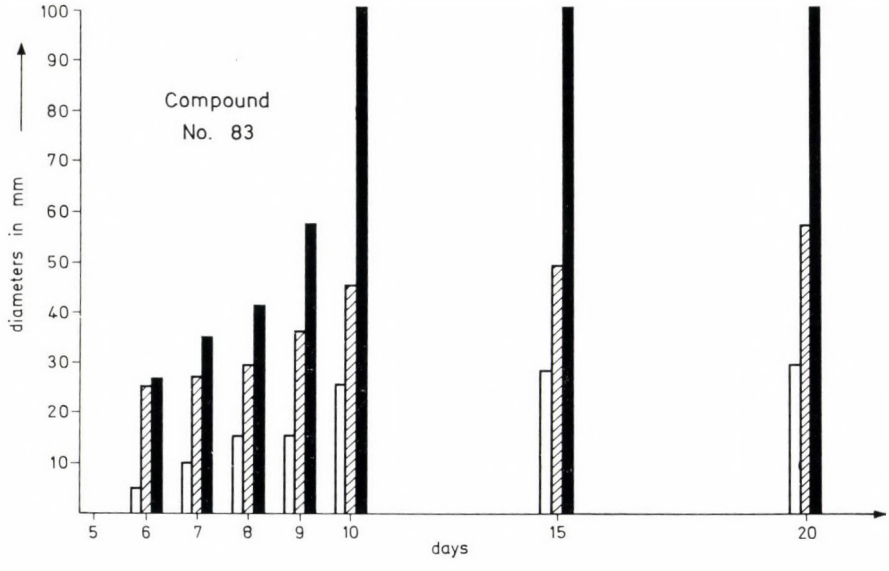

Compound
No. 83
diameters in mm
100
90
80
70
60
50
40
30
20
10
5
6
7
8
9
10
15
20
days

Fig. 2

with this dressing was established. For these tests products for field experiment in form of 75 WP (ALF-75 and ALG-75) and their mixtures with 16 per cent Cu-oxine (ALF-59-16 and ALG-59-16) as well as Vitavax were used. The results are shown in Table 3.

The level of infestation was estimated 15 days later. According to the results in this Table, it may be concluded that the ALF-59-16 and ALG-59-16 mixtures are more active or equal to Vitavax as fungicides an d so is ALG-75 against *C. lindemuthianum*.

Table 3

Antifungal activity against anthracnose *(C. lindemuthianum)* of bean and effect on germination of seeds dressed with preparation of compound (94) and (110)

Preparation	Prep. dose g/100 kg seeds	Infested seeds (%)	Fungitoxicity (%)	Seeds germinating power, %
Control	–	100	0.0	93.3
Vitavax	200	11.2	88.8	96.0
ALF-75	200	17.5	82.5	96.0
ALF-59-16	200	6.5	93.5	95.2
ALG-75	200	13.1	86.9	86.5
ALG-59-16	200	4.1	95.9	98.6

The systemic and chemotherapeutic action of the group of thiazole derivatives investigated has been unequivocally proved for compounds (1)–(137) by tests on the three apical leaflets of tomato leaf inoculated with *Phytophthora infestans*. All these leaflets were kept during the test in moist chambers at 18°C. The two systems of covering with the active substance, shown in the diagrams allowed simultaneously to reveal the preferred way of translocation of the active substance in the case of the given compound. The most interesting results are presented in Table 4.

Most noteworthy among the results shown in this Table is the systemic action of the 2-amino- (18) and 2-phenylthio-4-methyl-5-thiazolecarboxylic acid ethyl esters (27) and the activity of the anilides (84), (94) and (110). The latter two are identical with the UniRoyal products tested under the name of Seedvax and the code number G-696.

It results from the laboratory investigations of 137 various 4-methyl-5-thiazolecarboxylic acid derivatives that systemic fungitoxicity of compounds of this structure differs widely as regards the range of activity and depends on the substituents modifying not only the function of the carboxyl group, but occupying also position 2 in the heterocyclic ring. On the other hand, fungitoxicity is not

Table 4

Systemic and chemotherapeutic activity of some (A) type compounds by the method of tomato apical leaflets inoculated with tomato blight *(Phytophthora infestans)*

Comp. No.	Concent. of tested comp., %	Effectiveness after Abbot's formula, %			
		System.	Chemoth.	System.	Chemoth.
1	2	3	4	5	6
18	0.2	95.0	95.0	90.0	90.0
	0.1	50.0	70.0	50.0	70.0
	0.05	25.0	35.0	0.0	20.0
27	0.2	97.5	100.0	95.0	97.5
	0.1	95.0	95.0	85.0	95.0
	0.05	40.0	45.0	25.0	37.0
45	0.2	70.0	85.0	70.0	95.0
	0.1	35.0	55.0	60.0	56.0
	0.05	0.0	0.0	0.0	0.0
54	0.2	40.0	90.0	90.0	90.0
	0.1	10.0	56.5	37.6	55.0
	0.05	0.0	0.0	0.0	0.0
55	0.2	50.0	84.0	88.0	85.0
	0.1	0.0	35.0	75.0	36.0
	0.05	0.0	0.0	12.0	0.0
67	0.2	95.0	95.0	70.0	90.0
	0.1	26.0	30.0	31.0	30.0
	0.05	0.0	0.0	0.0	0.0
69	0.2	90.0	90.0	90.0	90.0
	0.1	31.5	42.3	30.0	42.0
	0.05	0.0	0.0	0.0	0.0
77	0.2	90.0	95.0	90.0	95.0
	0.1	36.3	40.5	39.0	43.5
	0.05	0.0	0.0	0.0	0.0

Table 4. (cont.)

Comp. No.	Concent. of tested comp., %	Effectiveness after Abbot's formula, %			
		System.	Chemoth.	System.	Chemoth.
1	2	3	4	5	6
78	0.2	0.0	100.0	95.0	100.0
	0.1	0.0	36.5	41.3	36.0
	0.05	0.0	0.0	0.0	0.0
84	0.2	95.0	98.0	97.5	100.0
	0.1	56.5	60.0	61.3	61.5
	0.05	15.1	23.6	19.5	25.0
85	0.2	90.0	95.0	90.0	95.0
	0.1	31.5	42.5	42.0	42.0
	0.05	0.0	0.0	0.0	0.5
90	0.2	90.0	92.5	90.0	95.0
	0.1	65.0	70.0	63.0	71.5
	0.05	0.0	0.0	0.0	0.0
94 (ALF)	0.2	93.7	95.0	95.0	92.5
	0.1	72.5	75.0	70.0	90.0
	0.05	17.5	25.0	50.0	50.0
98	0.2	95.0	35.0	95.0	50.0
	0.1	90.0	0.0	0.0	0.0
	0.05	25.0	0.0	0.0	0.0
108	0.2	97.3	100.0	95.0	0.0
	0.1	55.0	0.0	50.0	0.0
	0.05	0.2	0.0	0.0	0.0
110 (ALG)	0.2	90.0	100.0	97.5	100.0
	0.1	50.0	80.0	50.0	95.0
	0.05	0.0	80.0	17.0	85.0
135	0.2	65.0	89.0	65.0	90.0
	0.1	0.0	69.0	24.0	60.0
	0.05	0.0	0.0	0.0	0.0

conditioned exclusively by the presence of the carboxyanilido group forming the crotonic acid anilide rest as the structural element (ABDEL-LATEEF et al.,1971). This earlier conclusion of ours, based on observation of the structure of other presently synthesized or investigated systemic fungicides should be considered as a first approximation. The high fungitoxicity of ethyl esters and anilides with an ethyl, 2,6-dichlorophenyl, phenylthio, aryloxymethyl and arylsulphonic groups in position 2 indicates that systemic fungitoxicity is a function of the 4-methyl-5-thiazolecarboxylic acid molecule as a whole. Adequate substituents in position 2 and rests modifying the function of the carboxyl group play here the role of activators determining the spectrum and level of the systemic fungitoxicity of the compounds under study.

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Comparison of the Systemic Fungicidal Activity of 2-Amino-4-methyl-5-carboxyanilidothiazole (ALF) and 2,4-Dimethyl-5-carboxyanilidothiazole (ALG) in Greenhouse and Field Tests

By

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The effectiveness of ALF (Seedvax) and ALG (G-696) for pathogen control in crop and ornamental plants were investigated in greenhouse and field conditions. Both preparations were also investigated in the form of mixtures with Cu-oxine as seed dressings. In most greenhouse and field tests ALF and ALG were compared with various systemic and protective fungicides recommended for plant protection, e.g. Vitavax, Plantvax, Benlate, captan, Zaprawa nasienna T etc.

Unfortunately, there was no possibility to check all the most active compounds selected in the laboratory tests and described in previous papers (ABDEL-LATEEF, 1971; ABDEL-LATEEF et al., 1973).

Only two compounds: ALF synthesized by us (UniRoyal's Seedvax) and ALG (G-696 of the same firm) were used in greenhouse and field tests. Both compounds are interesting for two important reasons: they are easy to synthesize and show low toxicity to warm-blooded animals. According to the data of NOVAKOVA (1968), the acute oral DL_{50} is 1410 or 5620 mg/kg for rats.

Material and Methods

The authors' isolates of *Fusarium lini* and *Tilletia caries* as well as *Colletotrichum lindemuthianum* and other naturally occurring phytopathogens *Graphiola phenicis*, *Exosporum palmivorum*, *Ustilago nuda*, *Botrytis* sp., *Verticillium* sp. and *Septoria gladioli* were used in this study.

The following synthesized compounds and chemicals used for comparison were tested:

1. ALF – technical grade 2-amino-4-methyl-5-carboxyanilidothiazole,
2. ALF-75 – own preparation WP (wettable powder) containing 75% ALF, 5% tenside and 20% carrier,

3. ALF-59-16 – WP containing 59% ALF, 16% Cu-oxine, 5% tenside and 20% carrier,
4. ALG–technical grade 2,4-dimethyl-5-carboxyanilidothiazole,
5. ALG-75 – own preparation WP containing 75% ALG, 5% tenside and 20% carrier,
6. ALG-59-16 – WP containing 59% ALG, 16% Cu-oxine, 5% tenside and 20% carrier,
7. Benlate – DuPont's commercial preparation contains 50% benomyl used as WP,
8. benomyl – methyl ester of 1-(butylcarbamoyl)-2-benzimidazolinocarbamic acid,
9. Vitavax-7-WP – preparation synthesized by the authors containing 75% carboxine, 5% tenside and 20% carrier,
10. Vitavax – UniRoyal's technical product containing 75% carboxine,
11. Quinolate Vitavax V-4-X – commercial preparation of La Quinoleine, containing 59% carboxine and 16% Cu-oxine,
12. carboxine – 2,3-dihydro-5-carboxyanilido-5-methyl-1,4-oxathiine (authors' synthesis also),
13. Plantvax – UniRoyal's technical product, 75 WP containing oxycarboxine,
14. oxycarboxine – S,S-dioxy-2,3-dihydro-5-carboxyanilido-5-methyl-1,4-oxathiine,
15. Daconil – preparation containing chlorothalonil (2,4,5,6-tetrachloro-isophthalonitrile) as active ingredient,
16. captan – N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide; in preparations: Kaptan Azot, Kaptan NA or Kaptan 80,
17. maneb – manganese ethylene-1,2-bisdithiocarbamate as 80% WP; in preparations: Maneb Azot, Luxan and Manzate 80 DuPont,
18. Cynkotox Azot – preparation contains 65% zineb,
19. Zaprawa nasienna T – Azot preparation with about 50% thiuram as active ingredient,
20. Panogen 0.8 – Casco technical product contains methyl mercury cyanoguanidine as active ingredient equivalent to 0.8% mercury.

Laboratory studies

Wheat seeds *(Triticum aestivum)* of a quickly growing variety were infested with *Tilletia caries* (Bjerk) Wint. spores (5 g/kg of seed) harvested on the field according to HARNAK's method (1965). Slurry seed dressing was performed in glass containers by shaking with fungicides for 10 min.

Infected and dressed wheat seeds were sown in boxes previously filled with soil prepared according to HARNACK. The boxes were kept at 8–15°C until the appearance of the first leaf and then transferred to 8–20°C. In these conditions

wheat was allowed to grow until it reached dough ripeness. For calculating the degree of infestation with *T. caries*, all the ears, both healthy and infected ones, were taken. The effectiveness was calculated as per cent in respect to the control according to ABBOT's formula.

Fungitoxicity of some preparations was tested on bean seeds (var. Saxa) naturally infected with anthracnose *(C. lindemutianum)* in greenhouse on garden soil in Mitscherlich pots. The effects of chemical seed dressing, seedling development and level of infestation were evaluated by the method of ZALEWSKI (1952). The thiazole compounds were tested at one concentration and compared with Vitavax, Zaprawa nasienna T and Kaptan 80.

A similar test was carried out with naturally infested yellow lupin seeds (var. Zółty Popularny IHAR) in garden soil for damping off and brown foot rot (*Fusarium* sp. and *Pythium* sp.), respectively. The number of germs, infestation level and the effect of chemicals on plant development (weight and length) were determined 21 days after sowing. Only ALG was tested for biological activity as dressing of flax seeds infected with *Fusarium lini*. The results were compared with the activity of Vitavax, Zaprawa nasienna T and Panogen 0.8 (a Casco product).

Greenhouse experiments on gerbera *(Gerbera jamessoni)* were performed on plantations of annual plants naturally infested by *Verticillium* sp. Chemicals were sprayed with a hand sprayer (1.5 dm^3) type Pomasa. The sprayings were repeated four times (at 14-day intervals). The entire surface of the plant was covered uniformly with the spray. Plant infestation was analysed during the test period with elimination of diseased plants.

Analogous investigations were performed on 2- and 3-year-old date-palm seedlings naturally infested by russet maculation *(Exosporium palmivorum)* and leaf spot *(Graphiola phenicis)*. In the latter case the palm leaves were heavily infected, more than 25 sori were located in the apical section of the leaves. Therefore, for carboxine and ALG sprayings emulsions were used with Tween 80 as tenside. The treatment was applied at the 4th stage of well developed leaves at 14-day intervals. The first treatment was applied at the moment of appearance of single spots on the leaves. Infestation level on the leaf surface was estimated three weeks after the last spraying.

Field studies

All field studies were performed according to the random-blocks method, unless specified otherwise, in three replicates (BARBACKI, 1953), and seeds were tested according to DORYWALSKI et al. (1964). The experimental preparations from ALF and ALG and the commercial products for comparison were tested against loose smut *(Ustilago nuda)* on barley. Barley seeds were also subjected to anaerobic and hot water treatment. The formulations were tested by the Plant Breeding and Seed Production Association in SHR Sobótka as seed dressing on spring

barley (var. Alsa). The field was divided into 50 m^2 experimental plots. Barley seeds originated from barley naturally infected with loose smut. The preparations were used in doses of 300 g/100 kg seeds. The chemical dressing was applied in a seed pickling machine.

The field test on beans (var. Saxa) was carried out in loamy-sandy soil on a farm field divided into 10 m^2 plots. Bean seeds were dressed one day before sowing, and sown with a hand seeder (100 kg/ha). The effect of the chemicals on germination and development of bean plants (weight and length) was investigated. Observations on seed germination were recorded after two weeks. The infestation level and effect of chemicals on weight and length of the plants were analysed two months later than the first observations.

Investigations on plants naturally infested with anthracnose were performed by an analogous method on yellow lupin (var. Zółty Popularny IHAR) seeds. The lupin was sown in the amount of 160 kg/ha with a hand seeder on 5 m^2 plots.

Fungitoxicity of the ALG-75 preparation against *Fusarium lini* was tested on 6 m^2 plots. Seeds of long-fibre flax (var. Wiera) were infected with an aqueous suspension of *F. lini* spores (250,000 in 1 cm^3). After drying, the seeds were dressed with the tested preparations and sown by hand in rows so as to use the same amount of seeds (140 kg/ha) on each plot. The activity of the thiazole derivative was compared with Vitavax and Zaprawa nasienna T. Infection was analysed in detail and the plants were measured when the flax reached a length of about 10 cm.

Field tests on gladioli (var. Picardo) were performed on a commercial plantation in Zywiec. Plants, 30 on each plot, were naturally infected with core rot (*Botrytis* sp.) and hard rot (*Septoria gladioli*). Plants were treated when they were 15–20 cm high and showed no disease symptoms. Spraying was done at 10–14-day intervals. Infestation was assayed (20 plant from each plot) on 14 July, 1970, and the per cent of leaf infestation was calculated on 24 Sept., 1970. A six-grade score was applied in the analysis: 0 – free of fungus, 1 – 5%, 2 – 10%, 3 – 10–30%, 4 – 30–50% and 5 – above 50% infection of leaf surface. The per cent of corm infestation was calculated after their harvest and drying on 5 Dec., 1970 for *Botrytis* sp., *Septoria gladioli* and *Fusarium* sp. jointly.

Results and Discussion

On the basis of the greenhouse tests, the thiazole compounds ALF and ALG may be considered as excellent fungitoxicants against *Tilletia caries* on wheat plants (Table 1).

The results in this table show that ALG-75 is as toxic as Vitavax and more active than ALF-75. The data of greenhouse tests also indicate that Plantvax is completely inefficient against smut of wheat.

Tests of anthracnose control on bean seeds (var. Saxa) (Table 2) show that the thiazole preparations equal Vitavax in activity. The data, however, reveal

Table 1

Fungicidal activity of some compounds on wheat infected with stinking smut *(T. caries)* in greenhouse test by Harnak's method

Preparation	Concentration of preparation %	Infested wheat ears %	Mean length of one plant cm	Fungitoxicity %
Control	—	63.5	48.5	—
ALF-75	0.2	9.3	51.5	85.4
	0.15	15.0	50.3	76.4
	0.1	19.1	49.1	64.9
	0.05	21.3	51.2	66.5
ALG-75	0.2	0.0	51.0	100.0
	0.15	6.0	49.0	90.6
	0.1	6.3	55.1	90.1
	0.05	15.1	49.3	76.2
Vitavax	0.2	0.0	50.1	100.0
	0.15	0.9	53.2	98.6
	0.1	6.8	54.1	89.3
	0.05	10.3	53.2	83.8
Plantvax	0.2	52.0	47.0	18.1
	0.15	58.0	48.3	8.7
	0.1	61.0	51.0	3.9
	0.05	65.0	47.2	0.0

the unfavourable influence of Cu-oxine, particularly in the case of ALG-59-16 as admixture to thiazole preparations. Cu-oxine lowers the fungitoxicity of the basic compounds towards *C. lindemuthianum*.

All the preparations tested had no toxic effect on seed germination, length and weight of the plant root system and above-ground parts when tested after DORYWALSKI et al. (1964).

In greenhouse tests of the selected dressings, listed in Table 3, against damping off, brown foot rot and ear blight on yellow lupin, only 50–60 per cent effective fungicidal activity was noted. This low activity may be due to the heavy infestation with pathogens of the soil under experiment. The tested compounds, however, stimulated somewhat the development of lupin plants (Table 3), except the ALG-75, ALG-59-16 and ALF-75 preparations.

Table 2

Fungicidal activity of some preparations on bean seeds infected with anthracnose *(C. lindemuthianum)* (greenhouse test)

Preparation	Dose of preparation g/100 kg seeds	Infested seeds %	Fungitoxicity %
Control	—	57.1	—
Vitavax	200	21.0	63.4
ALF-75	200	22.8	60.2
ALF-59-16	200	24.5	57.2
ALG-75	200	20.3	64.6
ALG-59-16	200	37.9	33.0
Zaprawa nasienna T	200	31.5	45.0
Zaprawa nasienna T	300	25.0	56.0
Kaptan 80	200	23.6	58.8

Table 3

Phytotoxic effects of the fungicidal active preparations on germination and development of yellow lupin

Preparation	Dose of preparation g/100 kg seeds	Seedlings %	Length of one plant cm	Weight of one plant (g)
Control	—	29.0	10.1	1.1
Vitavax	200	66.0	13.9	1.3
Vitavax V-4-X	200	71.0	11.9	1.5
ALF-75	200	58.0	10.4	1.4
ALF-59-16	200	43.0	13.2	0.8
ALG-75	200	43.0	15.4	0.9
ALG-59-16	200	46.0	14.4	0.7
Zaprawa nasienna T	200	68.0	10.4	1.3
Zaprawa nasienna T	300	81.0	11.5	1.5
Kaptan 80	200	89.0	13.4	1.2

The latter caused a weaker growth of the leaf blades as manifested in the weight of one plant.

The use of ALG in comparative studies on *Fusarium lini* control (Table 4) showed that this preparation is equivalent with Vitavax in a 0.4 and 0.3 per cent concentration; in a 0.2 per cent concentration the activity of ALG was somewhat lower.

Table 4

Comparison of ALG with other fungicides on flax infected with *Fusarium lini*

Preparation	Concentration of the preparation %	Per cent of infested plants after days,						Fungitoxicity in %, after 40 days
		6	12	18	24	30	40	
Control	—	81.0	89.0	90.0	90.0	90.0	90.0	—
Vitavax	0.4	0.0	1.5	2.0	2.0	2.0	2.0	97.8
	0.3	10.0	10.0	11.0	11.0	11.0	11.0	84.4
	0.2	22.0	22.0	23.0	23.0	23.0	23.0	74.4
ALG-75	0.4	3.2	3.5	4.0	4.0	4.0	4.0	95.6
	0.3	12.3	12.3	13.0	13.0	13.0	13.0	85.6
	0.2	31.5	32.0	25.0	35.0	35.0	35.0	61.1
Zaprawa nasienna T	0.4	15.3	16.0	18.0	25.0	27.0	29.0	67.8
Zaprawa nasienna T	0.3	28.2	29.0	38.0	41.0	42.0	49.0	45.6
Panogen 0.8	0.4	0.0	3.2	4.1	5.3	6.9	9.3	89.7
(Casco)	0.3	5.4	6.3	9.1	11.4	19.0	25.0	72.2
	0.2	11.3	12.0	12.4	21.0	29.0	48.0	46.1

In greenhouse tests, however, ALG was more active than Zaprawa nasienna T and Panogen 0.8 (Casco). The latter case is particularly interesting in view of the general tendency to elimination of mercury dressings in plant protection.

Furthermore, the mixtures of ALF and ALG with Cu-oxine were satisfactorily fungitoxic against *Verticillium* sp. causing gerbera wilt (Table 5). Both preparations, however, were less active than Vitavax V-4-X. Preparations ALF-59-16 and ALG-59-16 were less active than Vitavax-75WP (synthesized by the authors) and Vitavax UniRoyal in the case of date-palms infested with *E. palmivorum* (Table 6).

In greenhouse tests of *G. phenicis* control on date-palms, ALG and Vitavax-75WP were used in the form of diluted emulsions to obtain full coverage of foliage. Both have given excellent results (ALG was better) as regards fungitoxicity, without any noticeable side-effects in the plants. On the contrary, the growth and colour of the foliage greatly improved. The results of fungitoxicity tests are shown in Table 7.

Field tests show noteworthy fungicidal activity of ALF and ALG. In most cases these thiazole derivatives were either as fungitoxic as Vitavax or more.

Table 5

Spray control of gerbera wilt (*Verticillium* sp.) in greenhouse

Preparation	Concentration of the preparation %	Diseased plants %	Fungitoxicity %
Control	—	67.45	—
Vitavax	0.15	14.3	78.8
Vitavax V-4-X	0.15	4.9	92.7
ALF-59-16	0.1	14.0	78.8
ALG-59-16	0.1	12.0	82.2
Maneb Luxan	0.2	68.3	0.0
Benlate	0.2	12.3	81.8
	0.15	28.8	57.3
	0.1	41.6	38.2

Table 6

Control of russet maculation of palm *(E. palmivorum)* on date-palm in the greenhouse

Preparation	Concentration of the preparation %	Surface infested leaves %	Fungitoxicity %
Control	—	69.5	—
Vitavax	0.2	5.0	92.8
Vitavax-75WP	0.15	4.3	93.8
Benlate	0.15	11.9	82.9
Plantvax	0.2	10.6	84.7
ALF-59-16	0.15	23.2	66.7
ALG-59-16	0.15	19.8	71.4
Maneb Luxan	0.2	18.5	73.4
Maneb Azot	0.2	19.3	72.3
Kaptan Azot	0.25	19.7	71.7
Kaptan NA	0.25	28.9	58.5

Table 7

Fungicidal activity of ALG on date-palm infected with *Graphiola phenicis*

Preparation	Conc. %	Spraying every 14 days,		Spraying every 30 days,	
		infected leaves (%)	fungitoxicity (%)	infected leaves (%)	fungitoxicity (%)
Control	—	100.0	—	100.0	—
ALG	0.3	5.1	94.9	4.9	95.0
	0.2	6.5	93.5	7.4	92.6
	0.1	34.5	65.5	15.1	84.9
	0.05	83.0	17.0	—	—
Vitavax-75WP	0.3	4.1	95.9	3.5	96.5
	0.2	5.1	94.9	9.2	90.8
	0.1	41.0	59.0	17.1	82.9
	0.05	79.5	20.5	—	—
Manzate 80	0.3	11.5	88.5	17.0	83.0
	0.2	31.0	69.0	41.5	58.5

Comparison of the fungitoxicity towards loose smut on barley (Table 8) shows that ALG-75 and ALG-59-16 were as active as Vitavax UniRoyal. However, ALF and ALG-59-16 were not only less fungitoxic, but they were also phytotoxic to barley. The results obtained with Vitavax-75WP are difficult to explain logically, but, in contrast to thiazole preparations, Vitavax did not reduce the energy and power of seeds' germination neither did it lower the seed yield. Examination of this phenomenon in detail revealed that with the time of storage of dressed seeds, the phytotoxicity of the thiazole dressing subsides without affecting the fungitoxicity.

The results of field tests of anthracnose control on beans and of damping off on yellow lupins are listed in Tables 9 and 10. They show the high fungitoxicity of both the mixed preparations ALF-59-16 and ALG-59-16. In the case of bean, however, the thiazole preparations reduced the germination power of the seeds and sometimes also the length of the plants.

In the case of lupin, a reduction in the crop of the treated plants was noticed as compared with the control. Reduction of crop may be due to the delayed maturity. It is an important point that snow had fallen (September, 1970) at the time of this experiment and caused damage to the hulls.

In experiments on plots with flax infected with *F. lini*, the activity of ALG

Table 8

Comparison of the fungitoxicity of ALF and ALG preparations with other fungicides and seed treatments against loose smut *(Ustilago nuda)* of barley

Preparation or method of treatment	Amount of infected plants amount / %	Energy of germination	Germinating power	Seed crop kg/ha
Control	35 / 100	85	100	54.3
Hot water treatment	1 / 2.8	77	90	51.4
Anaerobic treatment	0.5 / 1.4	75	90	50.4
Vitavax	0 / 0	87	97	53.6
Vitavax-75WP	2.5 / 7.1	85	100	53.1
ALF-75	0 / 0	60	85	46.7
ALF-59-16	2.0 / 5.6	65	85	43.6
ALG-75	0 / 0	81	97	53.3
ALG-59-16	0 / 0	80	92	53.6

in 0.4 and 0.3 per cent concentrations was higher than that of Zaprawa nasienna T, but equal to that of standard Vitavax. A delay in flax germination was observed as compared to the control, pointing to an unfavourable influence of the dressing on the energy and germination power of the dressed seeds.

The control of *Botrytis* sp. and *Septoria gladioli* on commercial plantations of gladioli (var. Picardo) was achieved by spraying of the leaves with the preparations listed in Table 11.

Table 9

Control of anthracnose *(C. lindemuthianum)* on bean in field test

Preparation	Dose g/100 kg seeds	Germs (%) in relation to control	Weight of one plant g	Mean length for one plant cm	Infested plants %	Fungi-toxicity %
Control	—	100.0	2.1	16.4	86.6	—
Vitavax	200	131.1	2.7	15.4	14.0	83.8
Vitavax V-4-X	200	135.2	3.1	17.1	15.0	82.2
ALF-75	200	194.2	2.5	18.7	11.8	86.4
ALF-59-16	200	65.3	2.2	15.9	3.5	95.9
ALG-75	200	79.2	1.8	15.4	14.3	83.5
ALG-59-16	200	65.3	2.2	15.9	3.5	95.9
Zaprawa nasienna T	200	135.2	3.1	17.1	23.0	73.4
Zaprawa nasienna T	300	193.4	3.2	18.0	14.0	83.8
Kaptan 80	200	142.8	2.9	16.5	28.0	67.7
Minimum significant difference, t = 0.95					11.9	

Table 10

Control of root rots on yellow lupin

Preparation	Dose g/100 kg seeds	Weight of one plant g	Mean length for one plant cm	Infested plants %	Fungi-toxicity %	Yield in cwt per ha
Control	—	4.5	17.5	48.0	—	12.1
Vitavax	200	5.9	17.2	6.0	87.5	9.9
Vitavax V-4-X	200	4.9	17.1	5.5	88.5	10.6
Vitavax-75WP	200	4.9	16.1	5.6	88.3	10.8
ALF-75	200	5.0	14.2	5.6	88.3	5.6
ALF-59-16	200	3.9	14.5	2.6	94.6	4.6
ALG-75	200	3.3	16.2	5.3	88.9	7.4
ALG-59-16	200	3.9	14.5	2.6	94.6	4.6
Zaprawa nasienna T	200	4.8	16.9	13.7	71.5	6.0
Zaprawa nasienna T	300	5.4	17.9	4.4	90.8	11.1
Kaptan 80	200	5.2	17.5	6.5	86.5	8.3

Table 11

Control of core rot (*Botrytis* sp.) and hard rot (*Septoria gladioli*) on gladiolus (var. Picardo)

Preparation	Concentration %	*Botrytis* sp.		*S. gladioli*		Infested bulbs %	Fungitoxicity %
		Surface infested leaves %	Fungitoxicity %	Surface infested leaves %	Fungitoxicity %		
Control	—	26.1	—	84.3	—	63.2	—
Vitavax	0.2	13.0	50.2	24.5	70.8	15.7	75.3
Vitavax-75WP	0.2	8.1	69.9	7.7	90.8	12.5	80.2
Vitavax V-4-X	0.2	2.8	89.5	5.3	93.7	4.7	92.6
ALF-59-16	0.2	3.5	86.6	6.2	92.7	7.6	87.9
ALG-59-16	0.2	3.4	86.9	7.5	91.1	9.1	85.6
Cynkotox Azot	0.3	17.3	33.9	24.7	70.7	20.5	67.6
Benlate	0.1	5.2	79.9	5.5	98.5	2.2	96.5
	0.2	2.5	90.6	3.0	96.5	1.7	97.3

The results obtained indicate that all the tested compounds exhibit a satisfactory activity. No phytotoxic effects were observed and the mixed preparations ALF-59-16 and ALG-59-16 were only slightly less fungitoxic than Vitavax V-4-X against pathogens infesting gladiolus corms.

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Systemic Activity of Benomyl in Urd Bean (*Phaseolus mungo* L.)

By

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Uptake of benomyl by germinating seeds and its subsequent translocation to roots, stems and leaves of urd bean (*Phaseolus mungo* L.) was studied by bioassay method using *Rhizoctonia solani* as the test organism. The fungitoxicant, when used as a seed treatment, first accumulated in a short time in the seed coat and then moved into the cotyledons. Later on the chemical was found in the roots, stems and leaves, maximum accumulation being in roots followed by stems and leaves.

Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazole carbonate, when used as a seed or/and soil treatment is effective against *Verticillium albo-atrum* (BIEHN, 1969, 1970), *Fusarium oxysporum* f. *lycopersici* (BIEHN and DIMOND, 1969, 1970), *Rhizoctonia solani* (JHOOTY and BEHAR, 1960), *Thielaviopsis basicola* (HARPER, 1968), *Plasmodiophora brassicae* (JACOBSEN and WILLIAMS, 1970), *Sphaerotheca* spp. (SCHROEDER and PROVVIDENTI, 1968), *Podosphaera leucotricha* (GILPATRICK, 1969), *Erysiphe* spp. (HAMMETT, 1968; NETZER and DISHON, 1970; JOHNSTON, 1970), *Botrytis cinerea* (LENZ, PAULOS and BALD, 1971) and *Cercosporella herpotrichoides* (WITCHALLS and CLOSE, 1971) in various crop plants.

In our experiments, seed treatment with benomyl controlled pre- and post-emergence root rot caused by *Rhizoctonia solani*, and reduced the intensity of powdery mildew *(Erysiphe polygoni)* and leaf spots *(Cercospora* spp., *Macrophomina phaseoli)* in urd bean (unpublished data). Evidently, the fungitoxicant was absorbed by the seeds and moved into the system of the plant and acted against these pathogens. This paper deals with relative quantitative uptake of the fungitoxicant by germinating seeds, its movement from outside to inside and subsequent translocation in different parts of the seedlings of urd bean *(Phaseolus mungo* L.*)* in relation to time and concentration.

Materials and Methods

An isolate of *Rhizoctonia solani* isolated from roots of urd bean was maintained on potato dextrose agar and used throughout the study. Benomyl (50% WP) was supplied by Agromore Limited, Bangalore.

The bioassay methods used in the present study were modifications of the techniques employed by ALLAM, SCHILLING and SINCLAIR (1969) and AL-BELDAWI and PINCKARD (1970). For studying the uptake of fungitoxicant by germinating seeds, urd bean seeds (variety Pb No. 1) treated with different concentrations of benomyl were germinated on sterile filter papers in the Petri dishes (9 cm diam.) containing moist sterile sand. The Petri dishes were placed at 28°C and seeds allowed to germinate. The samples were withdrawn at desired intervals for analysis. An aliquot of 5 g of germinating seeds were taken, rinsed thoroughly with sterile water to remove the adhering chemical and sand and dried between the layers of sterile filter papers. In order to determine the region of seed in which fungitoxicant was absorbed, seed coats and cotyledons of the treated seeds were separated. These samples were then ground in pestle and mortar in sterile distilled water and the volume made to 30 ml.

For detecting the systemic activity of the fungitoxicant in different parts of the seedlings, seeds were treated with benomyl at the rate of 1.25 and 2.50 mg/g seed. These were then sown in enamelled iron trays (37 × 30 cm) containing sterilized sand. Seedlings were uprooted on the 13th day of sowing. The leaves, stems and roots of these seedlings were separated, weighed and analysed.

Each prepared sample was mixed with an equal volume of double strength Czapek's nutrient agar medium. For prevention of bacterial contamination one drop of Dicrysticin (Strepto-penicillin) was added to each flask containing the media and tissue extracts. Petri dishes were poured of each sample media and each Petri dish was then seeded with 5 mm diameter disc of *R. solani* and incubated at 28°C. Radial growth of the fungus was measured and data recorded. A standard growth curve was first made by recording the radial growth of *R. solani* with known concentrations of benomyl. This was used to determine the quantitative amounts of the fungitoxicant in various tissues of urd bean. Suitable controls were maintained in all cases.

Results

The fungitoxicant was absorbed by the seeds during germination (Figure 1). With the lapse of time, there was increased accumulation of the fungitoxicant in the seeds. Maximum uptake took place during the early stages of germination, i.e. during the first 12 hours and then with the further lapse of time its accumulation was slow. Seeds treated with higher concentrations of benomyl absorbed more fungitoxicant than the seeds treated with lower concentrations. Thus the maximum uptake took place when the seeds were treated at the rate of 5 mg/g seed.

Seed coats and cotyledons analysed after 12 and 24 hours of germination showed that the accumulation of the chemical in the seed coats was at least double or more as compared to the amount accumulated in cotyledons (Figure 2). After

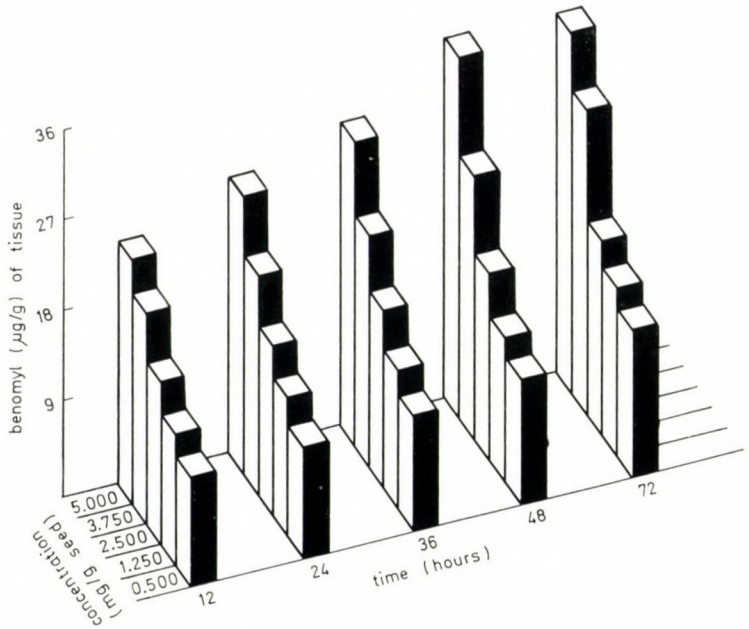

Fig. 1. Uptake of benomyl by seeds of *Phaseolus mungo* during germination

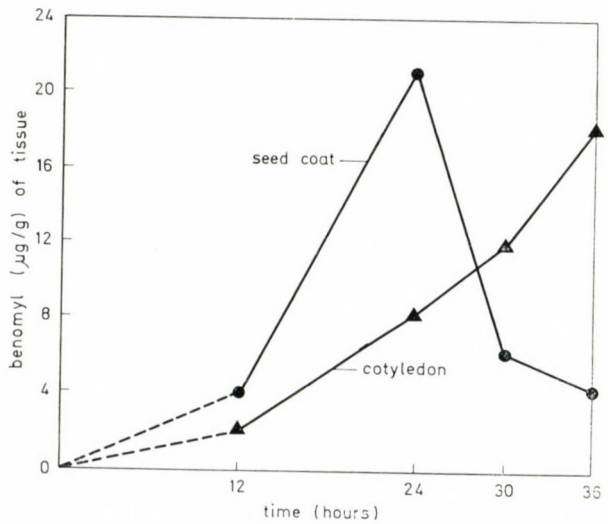

Fig. 2. Uptake of benomyl by seed coat and cotyledons of *Phaseolus mungo* during germination

30 hours, however, the amount of chemical decreased in the seed coats and increased in the cotyledons, thus indicating a flow of the chemical from seed coat to cotyledons.

The fungitoxicant was translocated to different parts of the seedlings (Table 1). Seedlings raised from seeds treated with benomyl at the rate of 2.50 mg/g seed accumulated more fungitoxicant than seeds treated at the rate of 1.25 mg/g. The amount of fungitoxicant accumulated was highest in roots followed by stems and leaves.

Table 1

Translocation of benomyl in roots, stems and leaves of urd bean

Tissue bioassayed	Rate of application of benomyl (mg/g seed)	% *R. solani* inhibition	Amount of benomyl and/or MBC (ug/g fresh weight)
Roots	1.25	58.33	8.69
	2.50	62.77	9.53
Stems	1.25	15.00	2.28
	2.50	23.88	3.30
Leaves	1.25	11.66	1.90
	2.50	20.55	2.74

Discussion

Several workers have reported translocation of benomyl and methyl 2-benzimidazole carbamate (MBC) in different plant parts of dicotyledons (SIM, MEE and ERWIN, 1969; PETERSON and EDGINGTON, 1970, 1971; GRAY and SINCLAIR, 1970; THAPLIYAL and SINCLAIR, 1971; NICHOLSON et al., 1972) and in monocotyledons (MEYER, NICHOLSON and SINCLAIR, 1971; SABET, SAMARA and ABDEL-RAHIM, 1972). These workers used bioassay or bioautography methods to detect the systemic activity of the chemicals. Most of these workers applied benomyl either to the soil wherein plants were grown or the roots of seedlings were fed in a nutrient solution containing the chemical. In the present study, however, urd bean plants were raised from the fungicide treated seeds and the movement of benomyl was determined in various parts of the seedlings by bioassay method using *R. solani* as the test organism. It was noted that the chemical first accumulated in short time in the seed coat and then moved into the cotyledons. Later on the chemical was found in the roots, stems and leaves, maximum accumulation being in roots followed by stems and leaves.

THAPLIYAL and SINCLAIR (1971) detected benomyl in roots, cotyledons and leaves of soyabean seedlings raised from the seeds treated with labelled benomyl.

However, 'no' benomyl/MBC was detected in hypocotyl tissues. They reported the poor stand of the seedlings because of lack of accumulation of the fungitoxicant in hypocotyl (THAPLIYAL and SINCLAIR, 1970, 1971). It may be true that the fungitoxicant may not accumulate in the hypocotyl but it should have been detected at a given time because it is through seed or hypocotyl that the further movement of this chemical takes place to the cotyledons or the leaves. Our studies, however, indicated that the seedlings of urd bean raised from benomyl treated seeds got excellent protection against *R. solani*. This resulted in good stand of the seedlings of urd bean, thus achieving the objective of protection against the attack of *R. solani*.

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Studies on the Plant Growth-regulating Activity of Some Xanthene Derivatives

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The growth regulating activity of some xanthene derivatives has been observed. On the basis of their morphological effects it was tried to relate these compounds to one of the categories of the known growth regulators. However, it was concluded that they do not belong either to auxins or to morphactins. Measuring the fluorescence quenching of some xanthene derivatives we assumed, that these compounds exert their biological activity by disturbing the processes of energy transfer.

Introduction

The close structural similarity of the xanthene and fluorene derivatives initiated our work to compare their plant growth-regulating activity. The only available data in the literature on this subject have been reported by BUCHENAUER and GROSSMAN (1970) and BUCHENAUER (1971). Previously other authors working with xanthene derivatives have not observed their growth-regulating activity (HEACOCK et al., 1958).

Our aim was to study the morphological effects and identify the type of the observed growth-regulating activity of these compounds.

Materials and Methods

The xanthene derivatives used in our experiments have been prepared by the Chinoin Chemical and Pharmaceutical Works Ltd. The fluorene derivatives are the products of the Merck Company.

In the paper we use the following abbreviations:

Xanthydrol (XH)
Xanthene-9-carboxylic acid (XA)
4-(9-xanthyl)-antipyrine (XP)
Xanthene-9-carboxylic acid p-chlorophenyl ester (XPhe)
5-(9-xanthyl)-salicylic acid (XS)
4-(6-hydroxy-pirydazine)-9-xanthyl ester (XHP)

α-(N-)9'-xanthyl(-carboxamido)-α-phenylbutyronitril (PheN)
9-Hydroxy-fluorene-9-carboxylic acid butyl ester (Fl)
Fluorenol-carboxylic acid butyl ester (FBu)
Fluorenol-carboxylic acid methyl ester (FMe)

The plant growth-regulating activity of the xanthene derivatives has been studied in the laboratory on cucumber and oat seedlings and in the greenhouse on tomato plants in pots. The type of effect was identified by combined treatments.

The fluorescence measurements were carried out with Hilger spectrophotometer in the region under 365 nm as exciting radiation.

Laboratory tests. The physiological effects of the xanthene derivatives were studied in morphology tests on seedlings (SÜDI et al., 1960, 1962) using 2,4-dichloro-phenoxyacetic acid (2,4-D) and Fl as reference substances. Cucumber and oat seeds were germinated on filter-paper disks (11 cm in diameter) previously impregnated with the acetone solutions of the tested compounds and after the evaporation of the acetone each moistened with 12 ml of distilled water. A series of different concentrations of compounds tested in this experiment was applied. The highest level was roughly (mg/Petri dish) equivalent to the active material contained in 12 ml of 1000 ppm aqueous solution. By diluting this solution we obtained dilutions of 100, 10 and 1 ppm. Actually, the concentration calculated from the highest dose of compounds was higher than the concentration of the saturated solution since the fine dispersion of the solid phase of the active material on the filter paper disks caused a permanent supply of the compound tested for the water solution. Therefore, the concentrations over the limit of water solubility have only biological but not physical meaning.

The morphological effects observed on the 6-day-old seedlings were grouped in three categories: Non-specific toxic (or growth-inhibitory) effect, stimulating or other specific effects.

In the seedling morphology test cucumber as representative of *Dicotyledonae* (with seed germination very sensitive to the effects of growth-regulators) and oat seedlings representing the *Monocotyledonae* were used as experimental material. In the greenhouse test compounds selected in the seedling morphology test were applied to the leaf of potted tomato in the 5—6 leaf stage in acetone solutions. Plant cultivars were as follows: *Cucumis sativus* cv. Marketer; *Lycopersicon esculentum* cv. Kecskeméti 362.

Results

Seedling morphology test. XH, XA and XPheN were the only compounds which exerted a stimulatory activity (swelling of shoots or root tissues) on cucumber seedlings when the overall morphological effect was observed. No stimulation has been observed on oat seedlings. XH in a 100 ppm concentration induced

hypocotyl swellings next to the root (Fig. 1). As is seen in Fig. 2 swelling of primary root tips on most seedlings occurred as a result of treatment with XA at 100 and 10 ppm concentrations. On cucumber seedlings previously germinated on distilled water for two days a "spindle lie" swelling of hypocotyl was induced by 1000 ppm of XA (Fig. 4). XPheN was similar in its effect to XH but in a concentration of 1000 ppm.

As to the growth inhibitory effects, the reduction of the length of seedling shoots, the primary and secondary roots was observed in the case of XH, XA as

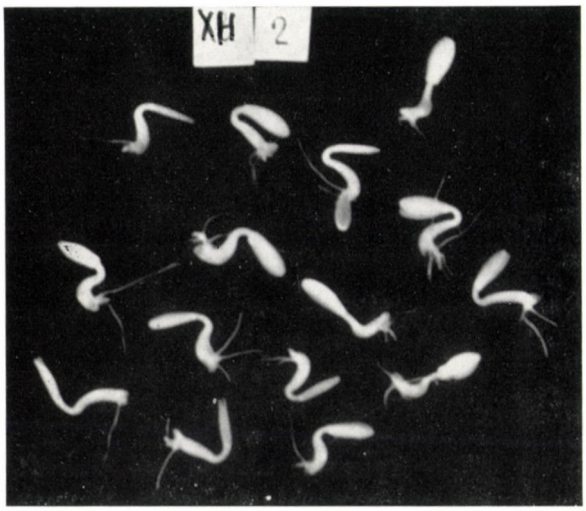

Fig. 1. The morphological reaction of cucumber seedlings following the treatment with XH (100 ppm)

well as Fl. The occurrence of the inhibitory effect and its relative extent were different with different compounds tested. XH in a concentration of 100 ppm significantly reduced the length of the hypocotyl, the primary and secondary roots (Fig. 1). Primary roots were more sensitive than the secondary roots. XH in a concentration of 1000 ppm almost completely suppressed the germination of seeds (Fig. 6). The morphological effect of XA was rather similar to that of Fl (Fig. 3), but more pronounced. Both compounds induced growth inhibition of secondary roots. Secondary root formation was fully inhibited by 10 ppm of XA. The growth inhibition of primary roots as induced by 100 ppm of XA was more pronounced than the inhibition evoked by 100 ppm of Fl. The curvature of the inhibited primary roots, as a specific toxic symptom, was constantly observed following treatments with 100 ppm of XA (Fig. 2). These two compounds were similar in disturbing geotropism of 3-day-old seedlings of cucumber which resulted in upward-growing of roots and in shoot growth directed downward.

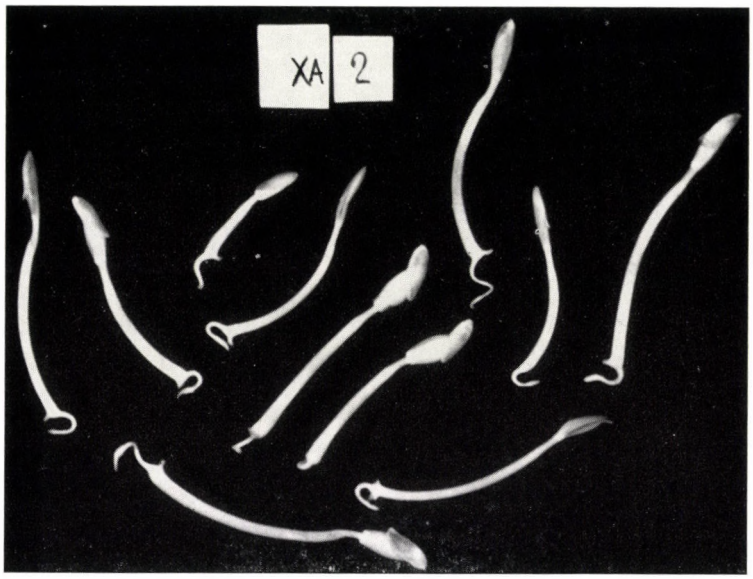

Fig. 2. The morphological reaction of cucumber seedlings following the treatment with XA (100 ppm)

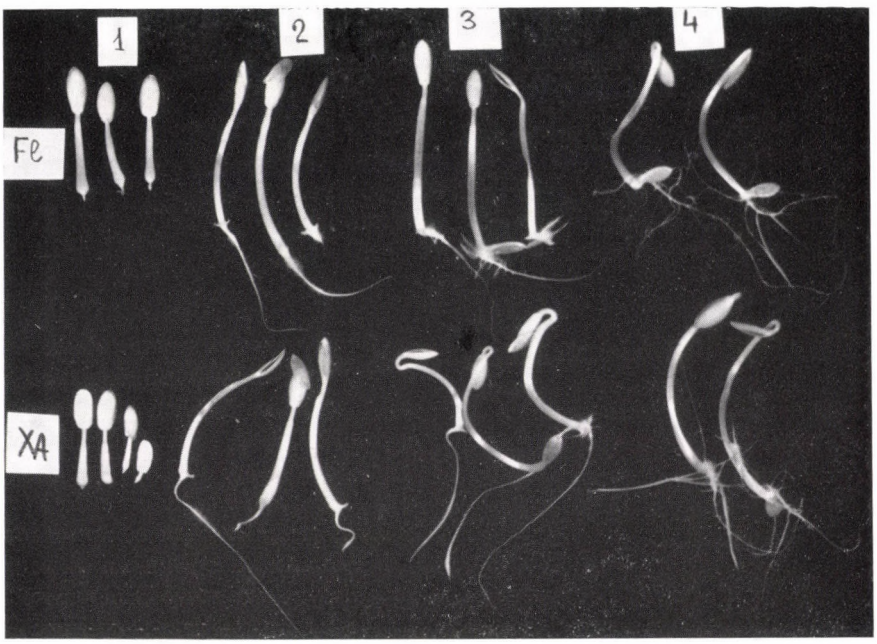

Fig. 3. The morphological reaction of cucumber seedlings following treatments with different concentrations of Fl or XA. (1) 1000 ppm, (2) 100 ppm, (3) 10 ppm, (4) 1 ppm

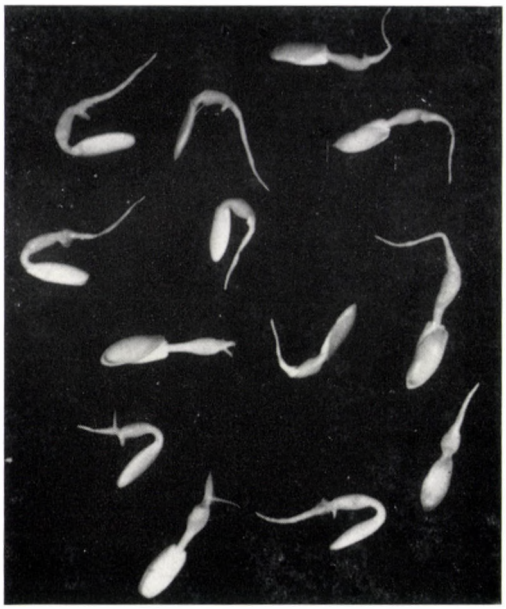

Fig. 4. Cucumber seedlings, previously germinated on distilled water, after 2-day-treatment with XA (1000 ppm)

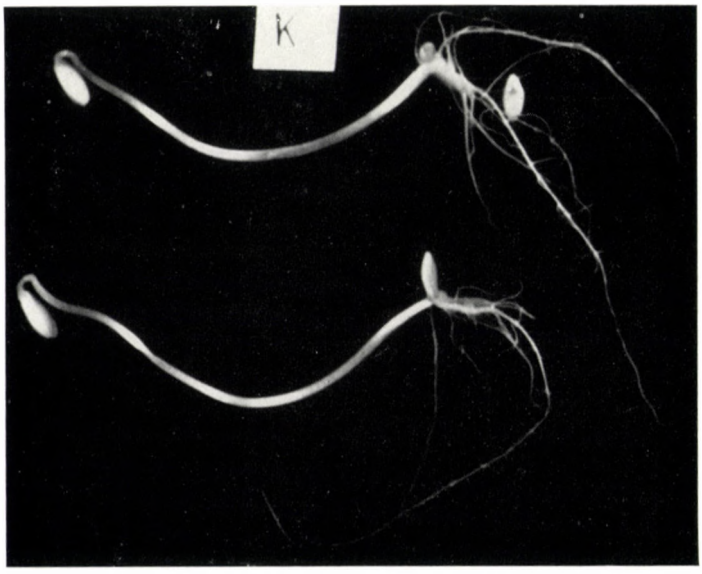

Fig. 5. Control (non-treated) cucumber seedlings

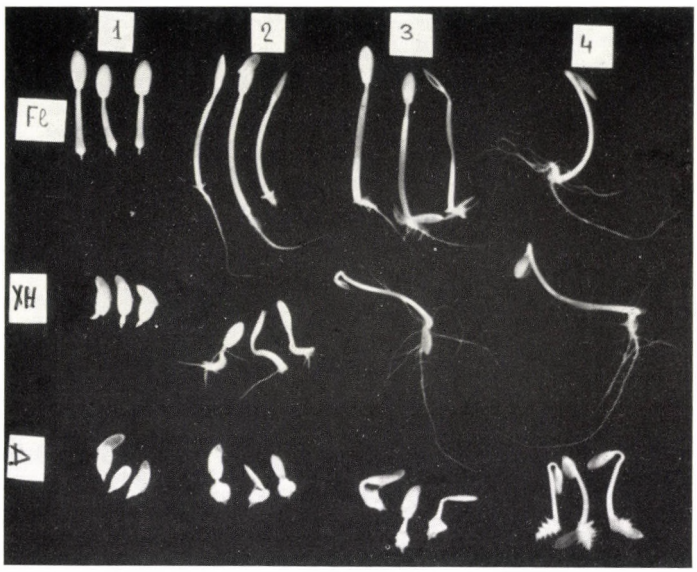

Fig. 6. The morphological reaction of cucumber seedlings following treatments with different concentrations of Fl, XH and D: (1) 1000 ppm, (2) 100 ppm, (3) 10 ppm, (4) 1 ppm

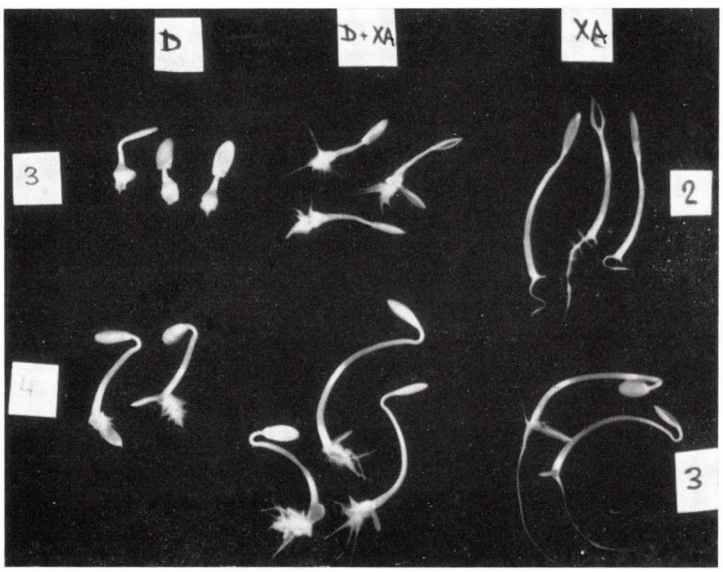

Fig. 7. Cucumber seedlings after single treatments with D3 and D4 or XA2 and XA3 and after combined treatments (D3 + XA2) or (D4 + XA3). Concentrations symbols: (2) 100 ppm, (3) 10 ppm, (4) 1 ppm

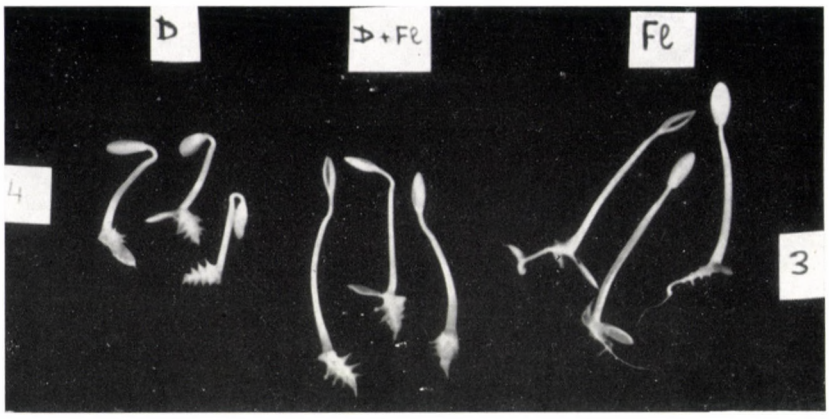

Fig. 8. Cucumber seedlings after single treatments with D4 or Fl3 and combined treatment (D4 + Fl3). Concentrations symbols: (3) 10 ppm, (4) 1 ppm

Treatment with 1000 ppm of XPheN resulted not only in the swelling of hypocotyl tissues but also in a pronounced inhibition of the growth of hypocotyl, primary and secondary roots. Primary roots were more sensitive to the inhibitory effect than the secondary ones.

Non-specific toxic effects were observed following the treatments with 1000 ppm of XS and XHP. In these cases growth of the primary as well as secondary roots was inhibited to a lesser extent. On the other hand, there was a stimulation effect upon root hair growth. All the other compounds tested had no effect concerning the growth of root hairs.

As regards the growth-regulating activity of XH and XA on oat seedlings swelling was never observed and a slight inhibition of root growth occurred when these compounds were applied in 1000 ppm.

To get a deeper insight into the mechanism of the hypocotyl and root tip swellings caused by treatments with XH and XA on cucumber seedlings a very active anti-auxin (3,5-dichlorophenoxyacetic acid) was applied in different experiments. Results obtained with the combined action of XH, XA and 3,5-D have shown that neither tissue swellings nor other effects on the seedlings were antagonized by 3,5-D. Consequently, an auxin type of action was not involved.

Greenhouse experiments. When XH, XA and Fl were applied to the leaves of potted tomatoes in 0.01, 0.1 or 1 per cent acetone solutions there was not observed any similarity in the effects of xanthene derivatives as compared to those of fluorene compounds. XH and XA in a 0.1 per cent acetone solution induced an increased growth of tomato plants. XH in a 0.03 per cent aqueous suspension as well as XA in a 0.3 per cent concentration caused an increased tomato yield in small plot open field experiments. XH or XA in combination wih 2,5-D (1 : 1) in experiments with potted plants diminished some of the growth-regulating effects

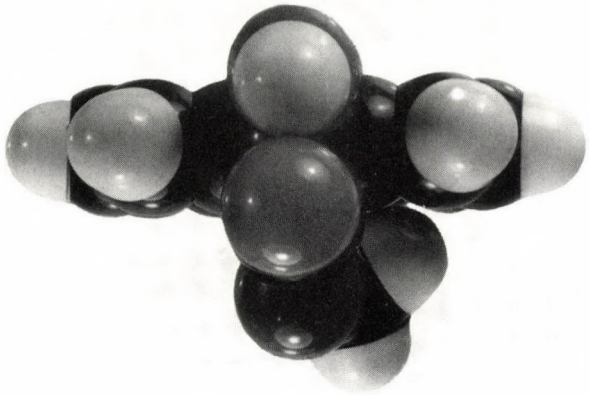

Fig. 9. Structure model of FMe molecule

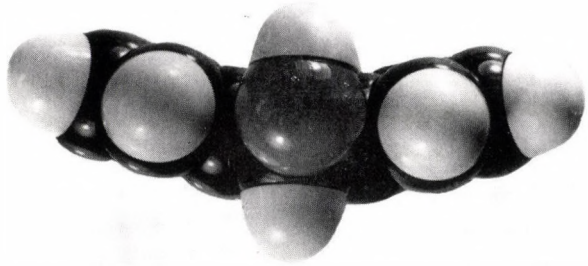

Fig. 10. Structure model of XH molecule

of 2,4-D like leaf epinasty, and stem curvature. We supposed antagonistic effects between 2,4-D and xanthene-derivatives. Consequently, 2,4-D and XH, XA or Fl was combined (1 : 10) and applied to the seedlings of cucumber in the seedling morphology test. As is shown in Fig. 7, XH was not antagonistic to 2,4-D, however, XA in 10 or 100 ppm concentrations was able to antagonize some of the effects of 2,4-D if the latter compound was applied in the concentrations of 1 or 10 ppm. Fl in 10 ppm also antagonized the inhibition of hypocotyl growth as induced by 1 ppm 2,4-D (Fig. 8).

The quenching of fluorescence was measured in alcoholic solutions. Table 1 shows the extent of fluorescence caused by the compounds tested in this study.

Table 1

The influence on the fluorescence of eosin of some xanthene derivatives

Compounds and concentrations	Extent of fluorescence in per cent
Eosin (10^{-5} *M*)	100.0
Xanthydrol (10^{-3} *M*)	2.3
Xanthene-9-carboxylic acid (10^{-3} *M*)	1.2
9-Hydroxy-fluorene-9-carboxylic acid (10^{-3} *M*)	3.0
E + XH (10^{-5} *M* + 10^{-3} *M*)	82.0
E + XA (10^{-5} *M* + 10^{-3} *M*)	50.0
E + FL (10^{-5} *M* + 10^{-3} *M*)	44.0

Discussion

From the results of the foregoing experiments it is concluded that compounds derived from XH or XA exhibited only little if any growth-regulating activity. Therefore, the effects of the basic compounds (XH and XA) will be discussed here.

If one compares the molecular structure of XH and FMe on structure models (Figs 9, 10), the difference in the steric position of the benzene rings is conspicuous. In the case of FMe molecules benzene rings are in the same plane, however, they form an angle with each other in the XH molecules. In the latter case centres of the two benzene rings are close to each other just as in the fluorenol derivatives. In spite of these structural similarities, xanthene compounds do not show the morphactin-specific morphogenetic activity on tomato plants, which is characteristic for fluorenol derivatives. However, XH and XA exhibited a pronounced growth-regulating activity when the over-all morphological effects of these compounds were investigated. This was experienced when XH and XA were applied alone or in combination with 2,4-D. In the first case there was a tissue swelling effect, and in the latter case XA turned to be antagonistic to 2,4-D.

The effect on tissue swelling of XH and XA cannot be considered as a specific auxin-type action, since an auxin-antagonist (3,5-D) was ineffective in inhibiting swelling of tissues. As regards the antagonism of XA to 2,4-D, one cannot suppose that XA acts like a competitive antagonist because the structure of the two compounds is quite different. It may however be antagonistic by influencing processes of energy transfer.

As to the auxin-antagonistic effect of Fl there is no comment in the literature on it (SCHNEIDER, 1970). It seems that the effect of Fl in combination with 2,4-D depends on the environmental conditions.

In fluorescence measurements 2,4-D was similar to the xanthene derivatives

as well as to Fl as regards the quenching of the fluorescence of eosin. One can suppose that there is an overlap in the ability of these compounds in the transfer of energy.

The difference in effects of XH and Fl on plants cannot be explained by the lack of $-COOH$ in the molecules of XH, because the latter compound has a series of additional effects as compared to those of Fl and the lack of an active group cannot induce new effects. A more convincing proof of this statement would have been the comparison of two perfectly analogous compounds: 9-hydroxy-fluorene-9-carboxylic acid and 9-hydroxy-xanthene-9-carboxylic acid. The latter compound turned to be very labile and, therefore, unsuitable for experimental purposes.

On the basis of the morphological effects of xanthene derivatives we tried to relate these compounds to several known categories of growth regulators. However, it was concluded from our experiments that they do not belong either to auxins or to morphactins at least not all of them and not in every test (e.g. XA and Fl were similar in action in the seedling morphology test but exerted different effects in greenhouse experiments on fully developed plants).

*

The author is greatly indebted to Dr. G. Josepovits for his help and useful discusssion of the subject and also to Chinoin Chemical and Pharmaceutical Works Ltd. for providing xanthene compounds used in this study.

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Viruskrankheiten der Möhre (*Daucus carota* L.)

Von

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From carrot plants (*Daucus carota* L.) with spotting, mottling, mosaic, line and ring patterns, malformations, and dwarfing, seven different viruses could be isolated and identified as alfalfa mosaic (LMV), arabis mosaic (AMV) carrot mottle (MSV), celery mosaic (SMV), cucumber mosaic (GMV), nasturtium ringspot (TRMV), and tobacco rattle (TMaV) viruses. The aphid-transmissible viruses are commonly occurring on carrots in the GDR. The nematode-transmissible AMV and TMaV were found only in the Spreewald region. Alfalfa mosaic, arabis mosaic, nasturtium ringspot, and tobacco rattle viruses are described for the first time on carrot plants. Especially remarkable is the first demonstration of the natural occurrence of tobacco rattle virus within the plant family of *Umbelliferae*. Each year in the harvest the carrot crops in the GDR are almost completely infected by different viruses. The losses due to these viruses reach generally more than 50 per cent. After epidemiological observations the losses can be reduced by cultivation methods and hygiene measures.

Die Möhre besitzt von allen Umbelliferen die größte wirtschaftliche Bedeutung in der DDR. So werden dort etwa 5000 ha Möhren als Gemüse und über 7000 ha für Futterzwecke angebaut (ANONYM 1970). In den eigenen Untersuchungen über Viruskrankheiten an Umbelliferen nahm deshalb die Möhre eine vorherrschende Stellung ein.

Viruskrankheiten an Möhren wurden relativ spät nachgewiesen. Erstmals gelang es SEVERIN und FREITAG (1938), an chlorotisch gescheckten Möhrenpflanzen das Selleriemosaik-Virus (SMV – celery mosaic virus) festzustellen. STUBBS and GRIEVE (1944) sowie STUBBS (1948, 1952) beschrieben eine neue Viruskrankheit – die Scheckige Verzwergung der Möhre (carrot motley dwarf) – für die eine Gelbgrünscheckung der Blätter und eine Verzwergung der gesamten Pflanze charakteristisch sind. Das ursächliche Virus erhielt neuerdings die Bezeichnung Möhrenscheckungs-Virus (MSV – carrot mottle virus). Eine Gelbscheckung der Möhre durch das Gurkenmosaik-Virus (GMV – cucumber mosaic virus) wurde von ROLAND (1961) beobachtet. TUITE (1960) wies das Tabakringflecken-Virus (TRV – tobacco ringspot virus) in Möhren nach. Möhren mit Rötungserscheinungen der Blätter enthalten nach den Untersuchungen von WATSON, SERJEANT und LENNON (1964) das Möhrenrotblättrigkeits-Virus (MRV –

carrot red leaf virus), das häufig mit dem MSV vergesellschaftet ist und mit diesem den Komplex der Scheckigen Verzwergung der Möhre bildet. Außerdem soll das MRV für die persistente Blattlausübertragung des MSV erforderlich sein. Chlorotische Flecke, die später ein mehr oder weniger deutliches Mosaik bilden, sowie Deformierungen und Mißbildungen, werden durch das Möhrenmosaik-Virus (carrot mosaic virus) hervorgerufen (CHOD 1965 a und b, 1966). Eine schwach hellgrüne bis gelbliche Scheckung, unterschiedliche Kräuselung des Laubes sowie geschlitzte bis fadenförmige Endfiedern der befallenen Möhrenpflanzen beschrieben SCHMELZER und WOLF (1969; vgl. auch Abb. 1). Sie isolierten von diesen Pflanzen sowohl das *Tropaeolum*-Ringmosaik-Virus (TRMV – nasturtium ringspot virus) als auch das SMV und diskutierten eine mögliche Identität dieser Mischinfektion mit dem von CHOD (1965 a und b, 1966) beschriebenen Möhrenmosaik-Virus. RUSH und GOODING (1970) erhielten von natürlich infizierten Möhrenpflanzen das Tomatenringflecken-Virus (ToRV – tomato ringspot virus).

Auch Mykoplasmen sind die Ursache von weit verbreiteten Krankheitserscheinungen an Möhren. Sie wurden nach früherer Kenntnis als Infektionen mit dem Asternvergilbungs-Virus beschrieben. In der DDR und der BRD wurde die Asternvergilbung bisher nicht an der Möhre nachgewiesen. Wahrscheinlich steht dies in engem Zusammenhang mit der geringen Verbreitung der als Vektoren fungierenden Zikaden in diesen Ländern. Als Fehldeutung kann an dieser Stelle der Nachweis einer Asternvergilbungsinfektion der Möhre durch LEH (1970) hinzugefügt werden. Es gelang diesem Autor bei seiner Untersuchung über den Nährstoffgehalt virusinfizierter Möhren in Berlin-Dahlem (Westberlin) von den untersuchten Möhren ein Virus zu übertragen. Er schloß jedoch trotz der wahrscheinlich mechanischen Übertragung fälschlich auf Grund der Ähnlichkeit mit einem von KOCHMAN und STACHYRA (1957) in der VR Polen beschriebenen Symptombild auf das Vorliegen der Asternvergilbung.

Material und Methoden

Methodisch wurde in ähnlicher Weise, wie bereits beschrieben, vorgegangen (WOLF, 1970). Die in der vorliegenden Arbeit ermittelten Wirtspflanzenreaktionen sowie die Eigenschaften der von Möhren isolierten Viren glichen den in der angeführten Arbeit erwähnten und werden deshalb nicht noch einmal geschildert. Die Identifizierung der Virusisolierungen erfolgte außer an Hand der Wirtspflanzenreaktionen beim GMV durch Prämunitätsversuche, beim Arabismosaik-Virus (AMV – arabis mosaic virus), Luzernemosaik-Virus (LMV – alfalfa mosaic virus), Tabakmauche-Virus (TMaV – tobacco rattle virus) und beim TRMV durch den serologischen Agargel-Doppeldiffusionstest, sowie beim SMV durch den elektronenmikroskopischen Nachweis der fadenförmigen Viruspartikeln. Die homologen Titer der verwendeten Antiseren sind in Tab. 1 dargestellt.

Tabelle 1

In den Untersuchungen verwendete Antiseren und ihre homologen Titer

Antigen	Bezeichnung der Virusstämme bzw. Antiseren	Blutentnahme am	Homologer Titer
AMV	F II	13. 04. 1967	1/512
		17. 04. 1967	1/8000
		14. 06. 1967	1/8000
		26. 03. 1968	1/1024
LMV	LM I	14. 12. 1966	1/256
		10. 03. 1967	1/128
TMaV	Ra I	09. 08. 1968	1/128
	Ra II	20. 03. 1970	1/128
TRMV	TRM	22. 06. 1967	1/256

Inwieweit etwaige Virusinfektionshemmstoffe der Möhre Virusübertragungen hindern, wurde mit Blattpreßsaft gesunder Möhren geprüft, der virusinfizierten Testpflanzenextrakten im Verhältnis 1 : 1 zugesetzt worden war. Als Vergleich dienten Mischungen gleicher Konzentration mit Blattpreßsaft gesunder Tabakpflanzen. Weitere methodische Einzelheiten werden an entsprechender Stelle beschrieben.

Ergebnisse und Besprechung

I. Isolierungsversuche von virusverdächtigen Möhren

Von den seit 1967 in Möhrenbeständen der DDR beobachteten virusverdächtigen Symptomen waren schwache bis starke Blattscheckungen besonders im Herbst überall sehr häufig zu finden. Sie wurden meist von Wuchsbeeinträchtigungen, sowie von Deformierungen oder Rötungen der Blätter begleitet (Abb. 1, 2, 3, 4).

Von gescheckten Pflanzen ließen sich außer dem GMV nur unter Schwierigkeiten das LMV, MSV und TRMV isolieren. In Möhren mit mehr oder weniger deformierten Blättern, die gelegentlich ein schwaches Mosaik zeigten, konnte das SMV allein oder als Komponente von Mischinfektionen nachgewiesen werden (vgl. auch Abb. 1). Mögliche Mischinfektionen mit schwer isolierbaren Viren (z. B. MSV) sind mit den derzeitigen Mitteln und Methoden nur schlecht zu erfassen.

Abb. 1. Symptome an Möhrenblättern, die das TRMV und das SMV in Mischinfektion enthielten

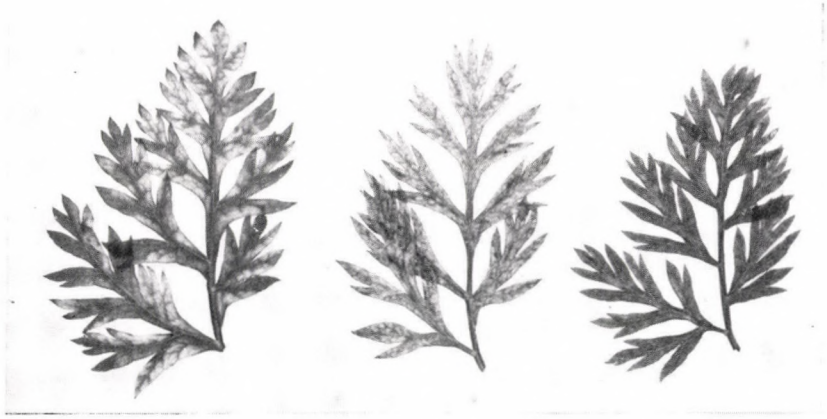

Abb. 2. Offensichtlich virusbedingte Scheckungen an Möhrenfiederblättern (rechts sehr schwache Scheckung)

Im Sommer 1970 wurden in Burg/Spreewald[1] Möhren mit starker gelber bis weißer Scheckung gefunden. Aus ihnen war ohne Schwierigkeiten das TMaV zu gewinnen. Zu einem späteren Zeitpunkt konnte von Pflanzen mit chlorotischen Flecken und Ringen auf den Blättern (Abb. 5) ebenfalls das TMaV isoliert werden.

[1] Wir möchten auch an dieser Stelle dem Pflanzenschutzmeister Herrn Paul SCHULZ in Burg/Spreewald für seine Unterstützung bei der Auffindung viruskranker Pflanzen herzlich danken.

Abb. 3. Virusbedingte Scheckungen und schwache Deformierungen an Möhrenblättern

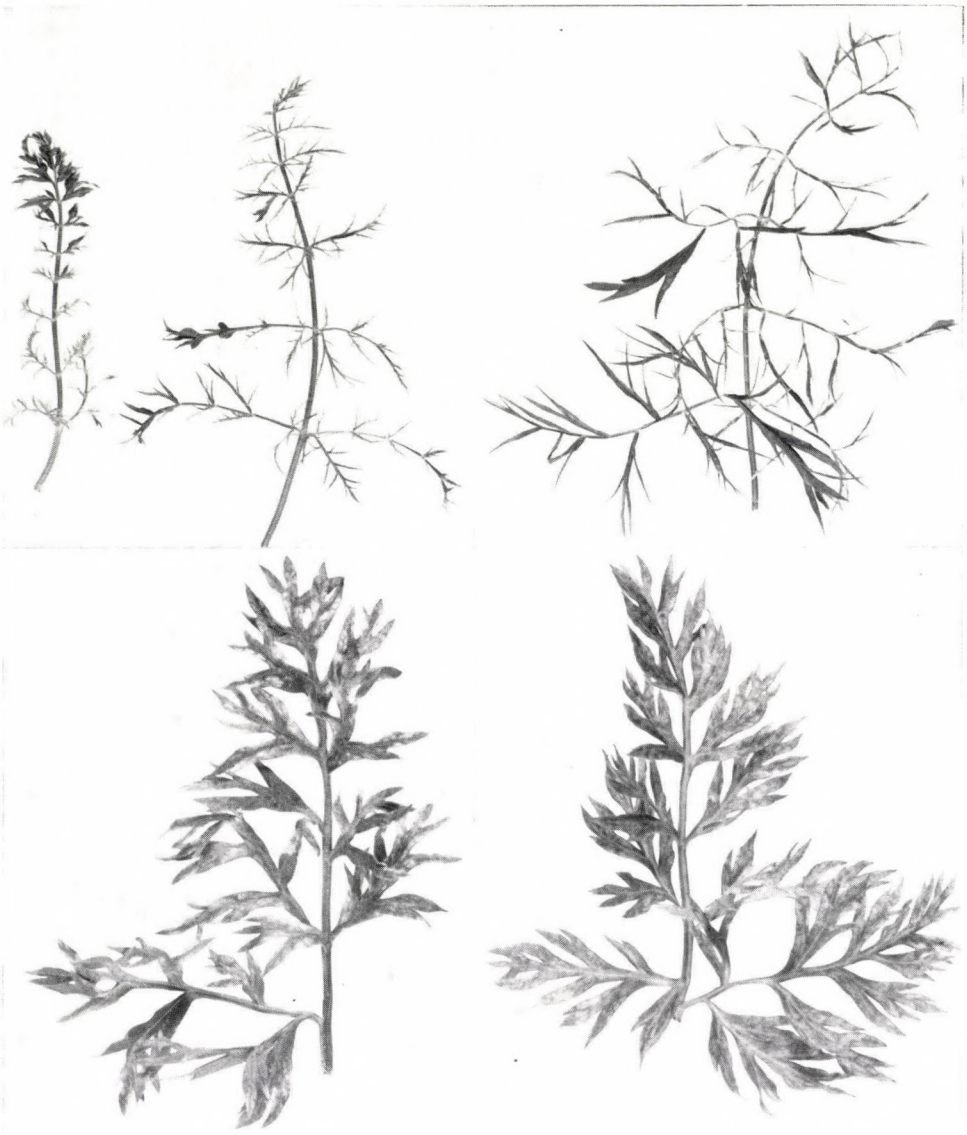

Abb. 4. Virusbedingte starke Deformierungen (oben) sowie Scheckungen und Deformierungen (unten) an Möhrenblättern

Von verzwergten und schwach deformierten Möhrenblättern mit hellgrünem bis gelbem Mosaik wurde das AMV erhalten. Aus Möhren mit schwach deformierten Blättern und deutlichen Adernaufhellungen (Abb. 6) ließ sich kein Virus übertragen. Eine auf Grund der spontanen Versuchung des betreffenden Standortes mit virustragenden Nematoden vermutete Infektion durch das Tomatenschwarzring-Virus (ToSRV – tomato black ring virus) konnte bisher in mehr als sechs Isolierungsversuchen von im Gewächshaus ausgepflanzten virusverdächtigen Möhren nicht bestätigt werden. Inwieweit hierbei eine zu niedrige Konzentration

Abb. 5. Symptome an einem Möhrenblatt, von dem das TMaV isoliert wurde

des Virus als Ursache vorlag, bleibt noch zu prüfen. Die vergleichsweise leichte Isolierbarkeit des TMaV und das AMV aus Möhre sowie des ToSRV aus verschiedenen anderen Umbelliferenarten in eigenen Versuchen unterstützen diese Möglichkeit nicht. Außerdem konnten von virusverdächtigen Möhren nicht identifizierte Viren gewonnen werden, die hier keine weitere Erwähnung finden sollen.

Rückübertragung auf Möhre und Reisolierung von künstlich infizierten Pflanzen gelangen nur mit dem SMV, dem GMV und dem TRMV. Dabei wurden wesentlich schwächere Symptome als bei Spontanbefall beobachtet. Das TRMV war nur mit Hilfe von *Myzus persicae* (Sulz.) auf Möhre übertragbar.

Von allen geprüften kultivierten Umbelliferen erwies sich die Möhre als diejenige, von der Viren am schlechtesten isoliert werden konnten. Dies dürfte der entscheidende Grund dafür sein, daß sie bisher in virologischer Hinsicht ver-

nachlässigt worden war. Von Samenträgern gelingen Virusübertragungen gewöhnlich etwas besser als von einjährigen Möhrenpflanzen.

Die Prüfung der Möhre auf Infektionshemmstoffe ergab bei der Virus-Wirt-Kombination SMV + *Chenopodium quinoa* Willd. 24,4 Läsionen je Blatt bei Möhrenpreßsaftzusatz und 18.8 bei den Tabakpreßsaftkontrollen. Bei der Virus-Wirt-Kombination GMV + *Nicotiana tabacum* L., Sorte 'Samsun', wurden als entsprechende Werte 12.2 und 2.2 Läsionen je Blatthälfte ermittelt. Aus beiden Versuchen ergab sich kein Hinweis auf das Vorhandensein von Virushemmstoffen in Möhren. Es erscheint sogar als möglich, daß der Möhrenpreßsaft Verringerungen der Infektiosität verhindert, die durch Virusaggregation zustandekommen.

Abb. 6. Vermutlich virusbedingte Adernaufhellung an Möhrenblättern

Da das eingangs erwähnte persistente MRV nicht mechanisch übertragbar ist, wurde es in der vorliegenden Untersuchung nicht erfaßt. In einem Übertragungsversuch (KARL und WOLF unveröffentlicht) mit *Cavariella aegopodii* Scop. konnten jedoch im Gewächshaus Blattrötesymptome an der Möhre reproduziert werden.

II. Identifizierung der erhaltenen Virusisolate

An dieser Stelle sollen lediglich die beiden neu an Möhren identifizierten Viren, das AMV und das TMaV, eingehender besprochen werden. Für die übrigen Viren (SMV, GMV, LMV, MSV und TRMV) trifft das an gleicher Stelle über die

Viruskrankheiten der Petersilie ausgeführte zu (WOLF, 1970). Außer Testpflanzenreaktionen waren der elektronenoptische Nachweis 750 nm langer Viruspartikeln beim SMV, Prämunitätsreaktionen beim GMV und beim MSV sowie serologische Reaktionen im Agargeltest beim LMV und beim TRMV von ausschlaggebender Bedeutung für die Identifizierung. Besonders zu erwähnen ist der ebenso wie bei der Petersilie in einigen Fällen möglich gewesene serologische Direktnachweis des LMV in Möhren.

Ermittlungen an Hand von Literaturangaben weisen nach, daß viröse und virusverdächtige Erscheinungen auf fast allen Kontinenten vorkommen. In folgenden Ländern wurden bisher Viren festgestellt (mit einem ? versehene Angaben bedeuten nicht identifizierte bzw. vermutete Identität):

Australien: MSV (STUBBS und GRIEVE, 1944); Belgien: GMV (ROLAND, 1961); BRD: MSV? (HEINZE, 1968); Bulgarien: GMV (KOVACHEVSKY, 1965); ČSSR: SMV? (CHOD, 1965); Dänemark: GMV und MSV (KRISTENSEN, 1964); DDR: GMV und SMV (WOLF, 1969); TRMV (SCHMELZER und WOLF, 1969); England: MSV (WATSON, 1960); Frankreich: SMV? (MARROU und LECLANT, 1966); JAPAN: MSV (KOMURO und YAMASHITA, 1956), GMV und SMV (IWAKI und KOMURO, 1970); Neuseeland: MSV (SMITH, GIESEN und ALLEN, 1960); Niederlande: MSV (VAN BAKEL und DE KRAKER, 1961); Polen: GMV? und SMV? (KOCHMAN und STACHYRA, 1957); Schottland: MSV (MURANT, GOOLD, ROBERTS und CATHRO, 1969); USA: SMV (SEVERIN und FREITAG, 1938), MSV (STUBBS, 1956), TRV (TUITE, 1960), ToRV (RUSH and GOODING, 1970).

1. Arabismosaik-Virus (AMV – arabis mosaic virus)

Im Jahre 1961 stellten HARRISON und WINSLOW das AMV erstmals innerhalb der Familie der *Umbelliferae* und Bleichsellerie in England fest. Eine Bestätigung dieses Befundes ohne Erwähnung des Erstnachweises erfolgte ebenfalls in England durch WALKEY und MITCHELL (1969).

Das AMV ließ sich in den eigenen Versuchen sehr leicht von den damit befallenen Möhren isolieren. Folgende Reaktionen wurden auf den AMV-beimpften Testpflanzen beobachtet (I bedeutet lokale, II systemische Infektion):

Apium graveolens L. var. *rapaceum* (Mill.) Gaud.	II symptomloser Befall;
Chenopodium amaranticolor Coste et Reyn.	I nekrotische Flecke; II chlorotische Scheckung, Deformierung, Spitzennekrose;
C. murale L.	I nekrotische Flecke; II Deformierung, Spitzennekrose;
C. quinoa	I chlorotische, zum Teil nekrotische Flecke und Ringe; II Scheckung, Deformierung, Spitzennekrose, Absterben;
Cucumis sativus L.	I chlorotisch-nekrotische Flecke oder Ringe;

	II Mosaik, nekrotische Flecke, Absterben der Triebspitze;
Nicotiana megalosiphon Heurck et Muell.	I nekrotische Flecke und Ringe;
	II Adernaufhellung, -mosaik, Erholung;
N. tabacum Zuchtstamm 'Bel 61—10'	I starke nekrotische Flecke und Ringe,
	II weißlich-nekrotische Ringe und Muster, Mosaik;
Petunia hybrida Vilm. Sorte 'Schneeglocke'	I violett-braune nekrotische Flecke und Ringe;
	II weißlich-gelbes Mosaik, Deformierung;
Phaseolus vulgaris L. Sorte 'Orella'	I braune nekrotische, zum Teil in den Adern verlaufende Flecke;
	II chlorotische Scheckung, Triebspitzennekrose;
Tetragonia tetragonioides (Pall.) O. Kuntze	II Mosaik, gelbe chlorotische Flecke und Ringe.

Die geprüften 10 Arten aus den Familien *Aizoaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Leguminosae*, *Solanaceae* und *Umbelliferae* stimmen in ihren Reaktionen gut mit den von SCHMELZER (1962/63) sowie von WALKEY und MITCHELL (1969) beobachteten überein.

Das AMV konnte in den infizierten Testpflanzen serologisch im Agargeltest sehr leicht nachgewiesen werden (Titer höher als 1/1024). Das AMV wurde an der Möhre bisher nur im Spreewald gefunden.

2. Tabakmauche-Virus (TMaV — tobacco rattle virus)

Das TMaV wurde bisher an vielen spontan befallenen Pflanzen, aber noch nicht an Umbelliferen nachgewiesen. Die eigenen Isolierungen von Möhre sind der erste Nachweis dieses geographisch weit verbreiteten Virus an Umbelliferen.

In der DDR wurde das TMaV von KIEWNICK (1957) an Tabak und im gleichen Jahr an Kartoffel (SCHMELZER, unveröffentlicht), von SCHMELZER (1966, 1970a, b) an *Gerbera jamesonii* Bolus, *Helianthus annuus* L., *Chionanthus virginicus* L., *Ribes sanguineum* Pursh und *Hydrangea arborescens* L. gefunden.

Die mechanische Übertragung des TMaV von den befallenen Möhren auf Testpflanzen gelang sehr leicht. Die infizierten Testpflanzen zeigten folgende Symptome:

Ammi majus L.	I chlorotische, zum Teil nekrotische Flecke;
Apium graveolens var. *rapaceum*	I chlorotische, zum Teil nekrotische Flecke;
Blackstonia perfoliata (L.) Huds.	I nekrotische Flecke,
	II ohne Symptome;
Celosia argentea L.	I nekrotische Flecke;
Chenopodium amaranticolor	I nekrotische Flecke;
C. murale	I nekrotische Flecke, Punkte, Ringe;
C. quinoa	I viele kleine nekrotische Flecke;
Coriandrum sativum L.	II ohne Symptome;
Datura stramonium L.	I ohne Symptome;

Daucus carota	nicht anfällig
Dorotheanthus bellidiformis (Burm. f.) N. E. Br.	II chlorotische Scheckung;
Nicotiana megalosiphon	I nekrotische Flecke, Ringe und Muster, II Mosaik;
N. tabacum, Zuchtstamm 'Bel 61—10'	I starke nekrotische Flecke, Ringe, Linien und Muster;
Ocimum basilicum L.	I große schwarze nekrotische Flecke;
Petunia hybrida	II Mosaik, chlorotisch-nekrotische Linien und Muster;
Phaseolus vulgaris Sorte 'Orella'	I kleine braune nekrotische Flecke;
Pisum sativum L.	I wenige nekrotische Flecke;
Tetragonia tetragonioides	I große nekrotische Flecke, II ohne Symptome;
Vicia faba L.	I schwarzviolette nekrotische Flecke;
Vigna sinensis (L.) Savi ex Hassk. ssp. *sinensis*	I kleine braune nekrotische Flecke.

Die geprüften 19 Arten aus den Familien der *Aizoaceae*, *Amaranthaceae*, *Chenopodiaceae*, *Gentianaceae*, *Leguminosae*, *Solanaceae* und *Umbelliferae* zeigten ähnliche Symptome wie die bei SCHMELZER (1957, 1970b) beschriebenen.

SCHMELZER (1957), der die ausgedehnteste Prüfung des TMaV-Wirtspflanzenkreises durchführte, gelang es ebenfalls nicht, die Möhre mechanisch mit diesem Virus zu infizieren. Wie schon ausgeführt und auch von anderen Untersuchern festgestellt wurde, ist die Möhre sehr schwer mechanisch zu infizieren. Deshalb lassen negative Befunde über die Virusanfälligkeit von Möhren in künstlichen Infektionsversuchen keine Schlüsse auf den natürlichen Virusbefall zu. Bei einigen blattlausübertragbaren Viren scheint die Übertragung durch Aphiden die erfolgreichste Methode zu sein. Inwieweit beim TMaV analog die Möglichkeit einer Übertragung durch die als Vektoren fungierenden Nematoden (*Trichodorus* spp.) besteht, bleibt noch zu prüfen. Das Virus ließ sich in den infizierten Testpflanzen relativ leicht mit TMaV-Antiserum im Agargeltest nachweisen (Titer 1/128).

Da das TMaV ebenso wie das AMV an der Möhre nur im Spreewald gefunden wurde, scheint die Gefahr für den gesamten Möhrenanbau auf Grund des Übertragungsmodus gering zu sein. Die bonitierten Spreewälder Möhrenflächen (etwa 3 ha in verstreutliegenden Parzellen) zeigten einen bis zu zehnprozentigen Befall mit nematodenübertragbaren Viren. Beide genannten Viren wurden nicht in Mischinfektionen miteinander oder mit aphidenübertragbaren Viren an den untersuchten Möhren festgestellt.

III. Zur Schadwirkung und Epidemiologie von Viruskrankheiten der Möhre

Die alljährliche Virusdurchseuchung der Möhrenbestände, die im Herbst meist hundertprozentig befallen sind, ließ eine starke negative Ertragsbeeinflussung vermuten. Im Jahre 1969 wurde dazu eine orientierende Untersuchung durch-

geführt, die gleichzeitig epidemiologische Schlußfolgerungen ermöglichte. An einen Möhrenschlag der Sorte 'Lange Rote Stumpfe' grenzte unmittelbar im Osten ein Samenträgerbestand und im Westen ein später gedrillter Schlag der gleichen Sorte. Bonitiert wurden Deformierungen, Scheckungen, starke Scheckungen, Röte sowie Scheckungen plus Deformierungen an jeweils 4 × 20 Pflanzen je Reihe. In Richtung zur Infektionsquelle – dem Möhrensamenträgerbestand – wurden die Reihen in engerem Abstand bonitiert (Samenträgerbestand, Reihe Nr. 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 24, . . . usf.).

Die Bonitierung am 25. 7. 1969 ergab eine stetige Abnahme der Anzahl virusinfizierter Pflanzen mit zunehmender Entfernung vom Samenträgerbestand. Zum Erntezeitpunkt, am 16. 9. 1969, wurde im gesamten Bestand der im Früh-

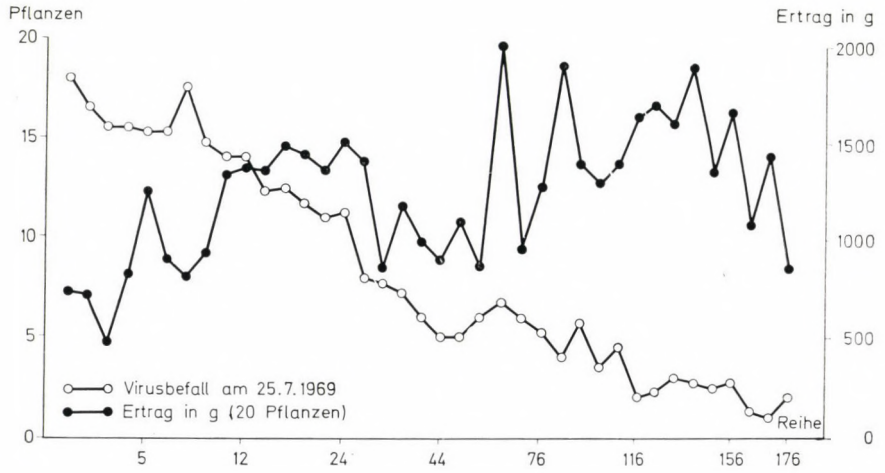

Abb. 7. Der Zusammenhang zwischen Abstand von der Infektionsquelle (Samenträgerbestand — linker Bildrand), Infektionsrate und Ertragsminderung in einem Konsummöhrenbestand (der Virusbefall zum Erntezeitpunkt, am 16. 9. 1969, betrug nahezu 100% und wurde aus Gründen der Übersichtlichkeit nicht eingezeichnet)

jahr des gleichen Jahres ausgesäten Möhren ein beinahe vollständiger Virusbefal (97.4%) festgestellt. Der Gesamtertrag an Wurzelmasse (Möhren ohne Laub) von 20 Möhrenpflanzen je Reihe zeigte eine gegenläufige Tendenz im Vergleich zum Virusbefall am 25. 7. 1969 (Abb. 7). Daraus wird ersichtlich, daß früher Virusbefall zu den stärksten, sehr später dagegen nur zu geringen negativen Ertragsbeeinflussung führt. Insgesamt erfolgten 760 Einzelwägungen. Die in Abb. 7 dargestellte Bonitierung des Virusbefalls gibt den Mittelwert aller vier Abschnitte wieder. Die Erträge wurden dagegen nur an dem Abschnitt, der auf das Vorgewende folgte, ermittelt. Bezogen auf die oben angeführten Symptome wurden im Durchschnitt je Pflanze signifikante Ertragsdepressionen ($\alpha = 0.1\%$) von

etwa 46 bis 65% festgestellt (Abb. 8). Dabei ist zusätzlich zu berücksichtigen, daß diese Werte den Durchschnitt aller Bonitierungen des gleichen Symptomtyps darstellen und durch die miterfaßten Spätinfektionen die Unterschiede zu den symptomlosen Pflanzen verringert werden. Die Zunahme der einzelnen Symptome von der ersten Bonitur bis zum Erntetermin gibt Tabelle 2 wieder.

Wie aus Tabelle 2 hervorgeht, war eine besonders große Zunahme der stark scheckigen sowie der scheckigen und deformierten Pflanzen bis zur Ernte zu verzeichnen. Wie bereits oben ausgeführt, trat besonders bei diesen beiden Symptomtypen eine Abschwächung der Ertragsunterschiede ein.

Ähnlich wie in England (WATSON und SERJEANT, 1964) dürfte auch bei uns die festgestellte starke Schadwirkung von Viruskrankheiten bei Möhren den Ein-

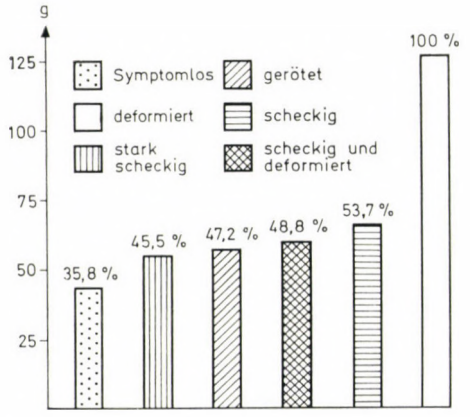

Abb. 8. Der Einfluß des Symptomtyps auf den durchschnittlichen Wurzelertrag je Pflanze bei Möhren

Tabelle 2

Zunahme der Virussymptome bis zur Ernte in einem Möhrenfeld

Bonitur am	Virussymptome					Symptomlos	Insgesamt
	deformiert	stark schekkig	gerötet	scheckig	scheckig und deformiert		
25. 7. 1969	37[1]	18	23	194	16	472	760
16. 9. 1969	94	116	42	345	143	20	760
Zunahme	154%	544%	83%	73%	794%	—	—

[1] Anzahl der bonitierten Möhrenpflanzen.

satz von Insektiziden zumindest zur Ausschaltung der persistenten Viren – MSV und MRV – sowie zur Einschränkung der nichtpersistenten aphidenübertragbaren Viren erforderlich machen. Dabei muß aber erwogen werden, daß die Möhre als eines der bedeutendsten diätetischen Säuglings- und Kleinkindernahrungsmittel strengen hygienischen Vorschriften hinsichtlich Insektizidrückständen unterliegt. In Zukunft sollten Möhrenflächen vertraglich für die Konservenindustrie gebunden werden, die für den genannten Zweck unbehandelt und sogar mit nicht inkrustiertem Saatgut bestellt sein müssen. Nur für alle übrigen Möhren anbauenden Betriebe kann ein Insektizideinsatz unter Beachtung der vorgeschriebenen Karenzzeiten ertragssteigernd wirksam werden. Im Möhrensamenbau, der unter anderem durch Viruskrankheiten starke Verluste erleidet, ist mit Ausnahme der Blütezeit der Möhren eine ständige Insektizidanwendung ohne Bedenken möglich (WOLF, KARL und ARNDT, 1969).

Unter Berücksichtigung der Epidemiologie von Möhrenviruskrankheiten ist bei der Anlage von Möhrenkulturen auf eine genügende Entfernung von möglichen Infektionsquellen zu achten. Im Möhrensamenbau ist von der häufig zu beobachtenden Praxis des benachbarten Anbaus von Stecklings- bzw. überwinterten Möhren und Neuansaaten abzugehen, um diese Gefährdungsmöglichkeit auszuschließen (vgl. auch Abb. 7). Wie aus Tabelle 2 ersichtlich ist, nimmt der Virusbefall besonders stark im Verlaufe des Spätsommers zu. Zur Infektionseinschränkung ist deshalb ein frühzeitiger Anbau von Möhren durchzuführen. So zeigten zum Beispiel in Frühbeeten gezogene Möhren meist keine Virusinfektionen. Inwieweit eine Möglichkeit zur Einschränkung von Virusinfektionen im Möhrensamenbau durch Hygienemaßnahmen im ersten Anbaujahr besteht, ist noch zu untersuchen. Analog zur Kartoffel ist außerdem eine Verlagerung des Möhrenanbaus zur Stecklingsgewinnung in sogenannte Gesundheitslagen in Erwägung zu ziehen. Ausschließlich anbauhygienische Maßnahmen zur Virusinfektionseinschränkung kommen bei den bereits erwähnten Möhrenkulturen für die Säuglingsernährung in Betracht: Vermeidung der Nachbarschaft möglicher Infektionsquellen, wie Gärten und gärtnerische Kulturen, Umbelliferen-Samenträgerkulturen, mehrjährige Luzerneschläge, ausgedehnte Wiesen- und Weideflächen; frühzeitiger Anbau möglichst in der freien Feldflur, Niederhalten benachbarter Graben- und Ödlandfloren, die häufig einen sehr starken Umbelliferenbesatz aufweisen.

Die Bekämpfung der als Vektoren fungierenden Nematoden beim AMV und TMaV auf den versuchten Flächen würde eine weitere Ausbreitung verhindern. Gegenwärtig sind chemische Mittel dazu aus Kostengründen nicht anwendbar. Eine entsprechend gestaltete Fruchtfolge könnte als anbauhygienische Maßnahme jedoch zur Einschränkung dieser Virusinfektionen beitragen.

Die alljährlich starke Verseuchung der Möhrenflächen in der DDR mit Viruskrankheiten verursacht bei vorsichtiger Schätzung eines Ertragsverlustes von 10% in der DDR allein im Möhrengemüsebau eine finanzielle Einbuße von mehr als drei Millionen Mark. Im Futtermöhrenanbau und im Möhrensamenbau

dürften die virusbedingten Verluste fast ebenso groß sein, so daß die Verluste insgesamt noch bedeutend höher zu veranschlagen sind (etwa fünf Millionen Mark). Ertragsverluste dieser Größenordnung weisen die zwingende Notwendigkeit von Virusbekämpfungsmaßnahmen im Möhrenanbau nach.

*

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Zusammenfassung

Von Möhren (*Daucus carota* L.), deren Blätter Fleckungen, Scheckungen, Mosaik, Linien- und Ringmuster, Deformierungen und Zwergwuchs aufwiesen, konnten 7 verschiedene Viren isoliert und als Selleriemosaik- (SMV), Gurkenmosaik- (GMV), Luzernemosaik- (LMV), Arabismosaik- (AMV), Tabakmauche- (TMaV), Möhrenscheckungs- (MSV) und *Tropaeolum*-Ringmosaik-Virus (TRMV) identifiziert werden. Am verbreitetsten sind davon die blattlausübertragbaren Viren. Das AMV und das TMaV wurden bisher nur im Spreewald nachgewiesen. Das LMV, das AMV, das TMaV und das TRMV wurden erstmals an der Möhre festgestellt. Völlig neu ist der Befund, daß das TMaV spontan an Umbelliferen vorkommt. Der Virusbefall erreicht an Möhren alljährlich im Herbst nahezu 100%. Virusinfizierte Möhren zeigen durchschnittlich einen mehr als 50% betragenden Ertragsausfall. Unter Berücksichtigung epidemiologischer Erkenntnisse läßt sich das Schadausmaß durch entsprechende Kultur- und Hygienemaßnahmen einschränken.

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Untersuchungen über eine Virose der Wildgurke (*Echinocystis lobata* [Michx.] Torr. et Gray)

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A mosaic disease of wild cucumber (*Echinocystis lobata* [Michx.] Torr. et Gray) occurs in several parts of Hungary. From mosaic diseased plants virus isolates (designated by *E*) readily could be transmitted mechanically and with *Myzus persicae* Sulz. as well as with seeds of infected plants. The *E*-isolate reacted positively to antisera in agar-gel double diffusion test of an isolate of cucumber mosaic virus (CMV, R/1 : 1/18 : S/S : S/Ap). For the *E*-isolate of CMV (CMV-E) the following physical properties were established: thermal inactivation point, 68—70°C; dilution end point, $10^{-4}-2\times10^{-5}$; longevity *in vitro* 19—22 days; storage over $CaCl_2$ in a desiccator at about 4—5°C, over 405 days. Five new host plants (*Geranium dissectum* Jusl., *Geranium sibiricum* L., *Ocimum canum* Sims, *Paulownia fargesii* Franch. and *Tetragonia echinata* Ait.) of CMV-E could be detected. Cross protection was observed between *E*-isolate and *W*-strain of CMV when tested in *Nicotiana tabacum* L. cv. *Samsun*. Interference was demonstrated between CMV-E and U_1-strain of tobacco mosaic virus (TMV, R/1 : 2/5 : E/E : S/*) in *Nicotiana tabacum* L. cv. *Xanthi-nc* tobacco. The percentage protection varied between 24 and 53 per cent. The highest protection was observed in the six-leaf stage.

I. Einleitung

A) In der Literatur an Wildgurken beschriebene Virosen

Virusverdächtige Symptome an Wildgurken (*Echinocystis lobata* [Michx.] Torr. et Gray, syn.: *Micrampelis lobata* Greene) wie Mosaikerscheinungen wurden nach Angaben von DOOLITTLE und GILBERT (1918) bereits seit mehr als fünf Jahrzehnten in den Vereinigten Staaten von Amerika beobachtet, und als Krankheitserreger wurde das Gurkenmosaik-Virus (*cucumber mosaic virus*, R/1 : 1/18 : : S/S : S/Ap) festgestellt. Dieses Virus kommt an Wildgurken-Pflanzen sehr verbreitet vor, und sein Vorkommen ist auch in Europa bekannt (VALENTA 1960). Nach MCCLANAHAN (1964) spielt *Echinocystis lobata* (Michx.) Torr. et Gray als Reservoir bei der Übertragung des Gurkenmosaik-Virus mit *Myzus persicae* Sulz. auf *Cucumis sativus* L. und *Asclepias syriaca* L. eine sehr große Rolle. Es wurde auch bestätigt, daß das Virus mit Samen kranker Wildgurken-Pflanzen übertragen wird (DOOLITTLE und WALKER 1925). Diese Angaben betrachten wir als drit-

ten Hinweis für die Virusübertragung durch Samen neben der Übertragung des gewöhnlichen Bohnenmosaik-Virus (*bean [common] mosaic virus*, ✳/✳ : ✳/✳ : : E/E : S/Ap) mit krankem *Phaseolus vulgaris* L. — Samen (REDDICK und STEWART 1919) und dem Grünscheckungsmosaik-Virus der Gurke (*cucumber green mottle mosaic virus*, R/✳ : ✳/✳ : E/E : S/✳, DOOLITTLE und GILBERT 1919).

Zu Beginn der zwanziger Jahre wies DOOLITTLE (1921) in den Vereinigten Staaten von Amerika in erkrankten Wildgurken das Grünscheckungsmosaik-Virus nach, und stellte auch fest, daß dieses Virus mit Samen von kranken *Echinocystis*-Pflanzen übertragen wird. Vier Jahre später hat FERNOW (1925) ein neues Virus, das sog. Wildgurkenmosaik-Virus (*cucumber [wild] mosaic virus*, R/1 : 2.4/35 : : S/S : S/Cl) aus *Echinocystis lobata* (Michx.) Torr. et Gray isoliert. Wie neuere Untersuchungen erwiesen, ist dieses Virus serologisch verwandt mit dem Wasserrübengelbmosaik-Virus (*turnip yellow mosaic virus*, R/1 : 1.9/37 : S/S : S/Cl; vgl. MACLEOD und MARKHAM 1963) und dem Gelbmosaik-Virus des Kakaos (*cacao yellow mosaic virus*, R/1 : ✳/38 : S/S : S/✳; vgl. BRUNT et al. 1965).

In der zweiten Hälfte der fünfziger Jahre wurde aus Wildgurken-Pflanzen das Kürbismosaik-Virus (*squash mosaic virus*, R/1 : 2.4/35 : S/S : S/Cl) isoliert (LINDBERG, HALL und WALKER 1956). Ein Jahr später konnte ihre Wirtseignung für die sog. "*Fulton's viruses*" wie Weidenblättrigkeit der Pflaume (*Fulton's virus B*, syn.: *prune dwarf virus*, ✳/✳ : ✳/✳ : S/S : S/✳), Ringfleckigkeit des Pfirsichs (*Fulton's virus A*, syn.: *Fulton's virus G*, *Fulton's cherry virus E*, *Prunus necrotic ring spot virus*, ✳/✳ : ✳/✳ : S/S : S/✳) festgestellt werden (FULTON 1957). Die aufgeführten Viren rufen an dieser Art systemische Symptome hervor.

B) Vorkommen einer Virose bei Wildgurke in Ungarn

Der zweite Autor dieser Arbeit beobachtete im Jahre 1959 in der Nähe von Szombathely, längs des Perint-Flußes Krankheitserscheinungen an Wildgurken, für die in der ungarischen Literatur zur damaligen Zeit keine Hinweise zu finden waren. Es bestand eine markante Ausbreitungstendenz in Nord-Südrichtung. Damals wurde die Virusnatur dieser Krankheit durch mechanischen Übertragung auf *Capsicum annuum* L., *Cucumis sativus* L., *Echinocystis lobata* (Michx.) Torr. et Gray, *Nicotiana glutinosa* L. und *Nicotiana tabacum* L. cv. *Samsun* bestätigt. Auf Grund der Symptombildung der Testpflanzen wurde angenommen, daß das Virus an Wildgurken mit dem Gurkenmosaik-Virus identisch ist. Die rasche Verbreitung der Wildgurkenvirose zwischen 1959 und 1969 war damit erklärbar, daß Wildgurkenpflanzen in unmittelbarer Nähe von Infektionsquellen, z. B. Melonenfelder — die über 80% mit dem Gurkenmosaik-Virus verseucht waren — wuchsen. In der Nähe von Szombathely wurden häufig mehrere Jahre nacheinander Melonen und in zahlreichen Gärtnereien verschiedene zur Familie der *Cucurbitaceen* gehörende Pflanzenarten angebaut. Durch die wechselseitige Infektion ausgehend von Melonen und anderen *Cucurbitaceen*, sowie Melonen- und Wildgurken-

Pflanzen könnte der Verseuchungsgrad letzterer im Laufe der Zeit ansteigen. Nach dem Jahre 1969 wurden die Gärtnereibetriebe einschließlich Melonenanbau aus städtebaulichen Gründen aufgegeben und infolgedessen ließ sich im Jahre 1972 eine Verminderung des Virusbesatzes bei Wildgurken feststellen.

Der erste Autor dieser Arbeit beobachtete erst im Jahre 1969 in der Umgebung zwischen Keszthely und Hévíz (längs des Hévízkanals) sowie bei Szedres (längs des Nádor- oder Sárvízkanals) die Krankheitserscheinungen an Wildgurke. In Anbetracht dessen, daß diese Pflanzenkrankheit in Ungarn nicht bearbeitet wurde, untersuchten wir ihre Ätiologie und stellten die Eigenschaften des Krankheitserregers fest.

II. Das Krankheitsbild in Wildgurke

Die betroffenen *Echinocystis lobata* (Michx.) Torr. et Gray-Pflanzen zeigten in den Sommermonaten mosaikartige Blattaufhellungen, meist gleichmäßig über die Blattspreite verteilt (Abb. 1). In anderen Fällen beobachteten wir starke Blattdeformationen, die von einer Adernbänderung und starker Adernaufhellung begleitet waren (Abb. 2). Die kranken Pflanzen zeigten starke Wuchsdepressionen. Außer den Blattsymptomen konnten wir eine Verminderung des Triebdurchmessers, sowie ausgeprägte Samenverkleinerungen und -deformationen feststellen. Es wurde auch beobachtet, daß aus kranken Pflanzen stammende Samen in

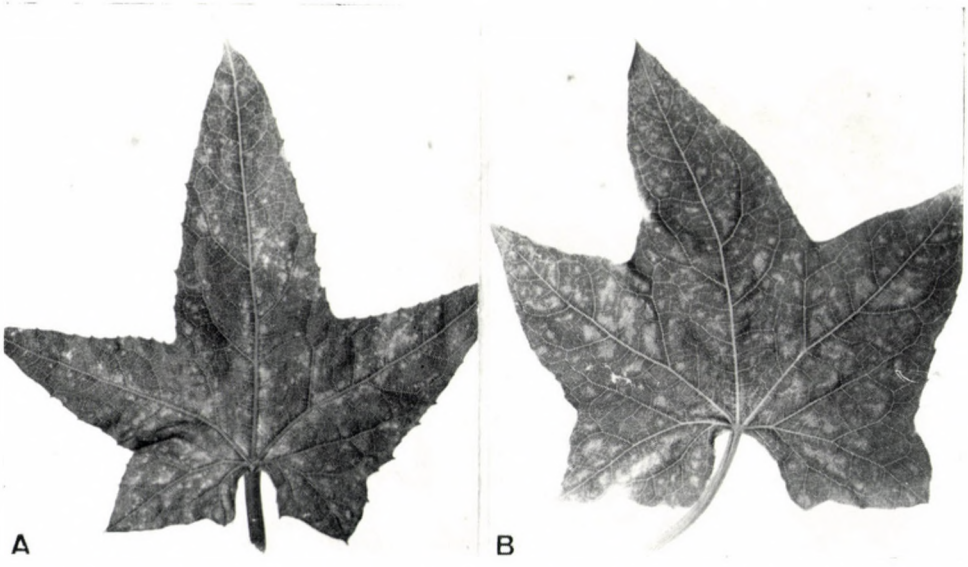

Abb. 1. Krankheitserscheinungen an *Echinocystis lobata* (Michx.) Torr. et Gray. A: Blatt einer spontan infizierten Freilandpflanze, B: Mit dem *E*-Isolat künstlich infiziertes Folgeblatt

vielen Fällen deformierte kleinblättrige Sämlinge und Zwillingsprosse hervorbringen (Abb. 3). Es wurde auf ähnliche Erscheinungen bei Kulturgurken seit mehr als drei Jahrzehnten hingewiesen (SZIRMAI 1941).

Abb. 2. Adernbänderung, Adernaufhellung und Blattdeformationen an einem *Echinocystis lobata* (Michx.) Torr. et Gray-Blatt

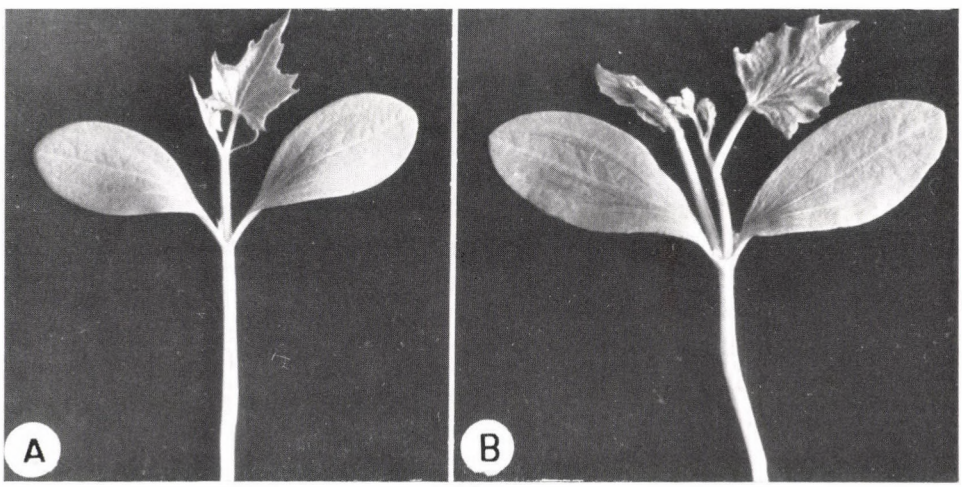

Abb. 3. Junge *Echinocystis lobata* (Michx.) Torr. et Gray-Pflanzen. A: Gesunde Pflanzen, B: Kranke Pfianzen

III. Experimentelle Ergebnisse

A) Isolierung eines Virus

Aus mehreren kranken Wildgurkenpflanzen wurden unter Zusatz von neutralem *M*/15 Phosphatpuffer im Verhältniss 1 : 2 Blattpreßsäfte hergestellt und auf verschiedene Testpflanzen wie *Chenopodium amaranticolor* Coste et Reyn., *Cucumis sativus* L., *Nicotiana glutinosa* L., *Nicotiana tabacum* L. cv. *Samsun* und *Tetragonia tetragonoides* (Pall.) O. Ktze. (syn.: *Tetragonia expansa* Murr.) verimpft. Die Versuche wurden mit zwei Isolierungen mehrfach wiederholt. Das Isolat *E* stammt aus der Umgebung von Hévíz und die Abimpfung *EN* aus Szedres. Aus jungen erkrankten Wildgurken-Pflanzen konnten wir aber nur selten ein Virus isolieren, was vermutlich auf eine zu geringe Viruskonzentration zurückzuführen ist. Das Isolat *E* ist näher untersucht worden.

B) Charakterisierung und Identifizierung des E-Isolates

1. Wirtspflanzenkreis

Unter Verwendung infektiöser Preßsäfte aus *Nicotiana tabacum* L. cv. *Samsun* und *Nicotiana glutinosa* L. wurden jeweils fünf bis zehn Testpflanzen verschiedener Arten abgerieben. Die Preßsäfte wurden im Verhältnis 1 : 1 mit destilliertem Wasser verdünnt. Vor der Inokulation haben wir die Testpflanzen mit Karborundpuder (500 *mesh)* bestäubt. Die beimpften Blätter wurden nach der Inokulation mit Leitungswasser abgespült. Die Bonitierung der Symptome erfolgte fortlaufend, die Rückteste wurden aber nicht in jedem Fall vorgenommen. Sie unterbleiben vor allem bei Pflanzenarten, die gut erkennbare Symptome zeigten. Die Rückteste erfolgten auf *Chenopodium amaranticolor* Coste et Reyn., und *Nicotiana tabacum* L. cv. *Samsun* sowie auf *Nicotiana glutinosa* L. Die Ergebnisse der Wirtspflanzenuntersuchungen sind in Tabelle 1 aufgeführt. Es wurden insgesamt 42 Arten, die zehn Familien angehören, geprüft. Davon stellten wir 29 sichtbar erkrankte und zwei symptomlose anfällige, sowie elf resistente Pflanzenarten fest. Als neue, in der Pflanzenvirologie noch nicht berücksichtigte virusanfällige Arten wurden *Geranium sibiricum* L., *Ocimum canum* Sims., *Paulownia fargesii* Franch. sowie *Tetragonia echinata* Ait. nachgewiesen (Abb. 4A, B und C).

2. Eigenschaften in vitro

Die Eigenschaften *in vitro* wurden nach den üblichen Methoden ermittelt (vgl. HORVÁTH 1969a). Beim Isolat *E* dienten als Virusquelle *Nicotiana tabacum* L. cv. *Samsun*-Pflanzen und *Tetragonia tetragonoides* (Pall.) O. Ktze. und *Tetragonia echinata* Ait. als Testpflanze.

Tabelle 1

Wirtspflanzen des *E*-Isolates

AIZOACEAE	
Tetragonia echinata Ait.*	AB: chlorotische rundliche Flecke FB: chlorotische rundliche Flecke
Tetragonia tetragonoides (Pall.) O. Ktze.	AB: chlorotische Läsionen, Lo FB: symptomlos
AMARANTHACEAE	
Amaranthus caudatus L.	AB: nekrotische Läsionen, Lo FB: symptomlos
Amaranthus tricolor L.	AB: nekrotische Läsionen, Lo FB: symptomlos
Gomphrena globosa L.	AB: schwache nekrotische Flecke FB: dunkelgrün Scheckung und Formveränderungen der Blätter (Abb. 4D)
CHENOPODIACEAE	
Chenopodium amaranticolor Coste et Reyn.	AB: chlorotische und nekrotische Läsionen FB: symptomlos
Chenopodium murale L.	AB: nekrotische Flecke, Lo FB: symptomlos
Chenopodium quinoa Willd.	AB: chlorotische und nekrotische Flecke, Lo FB: symptomlos
Spinacia oleracea L.	AB: symptomlos FB: gelbliche Aufhellung der Interkostalfelder, starke Wachstumhemmung, vorzeitiges Absterben der Pflanzen
COMPOSITAE	
Zinnia elegans Jacq.	AB: symptomlos FB: Adernaufhellung, gelbe Scheckung
CUCURBITACEAE	
Bryonia alba L.	AB: symptomlos FB: Adernaufhellung, schwache Mosaikscheckung, Wachstumhemmung
Bryonia dioica Jacq.	AB: symptomlos FB: Adernaufhellung, schwache Mosaikscheckung, Wachstumhemmung
Cucurbita pepo L.	AB: symptomlos FB: Adernaufhellung, Mosaikscheckung
Cucumis sativus L.	AB: chlorotische Flecke FB: Adernaufhellung, starke Mosaikscheckung (Abb. 5)
Echinocystis lobata (Michx.) Torr. et Gray**	AB: symptomlos FB: starke Adernaufhellung, Adernbänderung und Mosaik

Tabelle 1 (Fortsetzung)

GERANIACEAE	
Geranium cristatum Stev.	AB: symptomlos FB: symptomlos, RN
Geranium columbianum L.	AB: symptomlos FB: symptomlos, RN
Geranium dissectum Jusl.*	AB: symptomlos FB: symptomlos, RP
Geranium lucidum L.	AB: symptomlos FB: symptomlos, RN
Geranium molle L.	AB: symptomlos FB: symptomlos, RN
Geranium pratense L.	AB: symptomlos FB: symptomlos, RN
Geranium pusillum Burm.	AB: symptomlos FB: symptomlos, RN
Geranium pyraenicum Burm.	AB: symptomlos FB: symptomlos, RN
Geranium robertianum L.	AB: symptomlos FB: symptomlos, RN
Geranium rotundifolium L.	AB: symptomlos FB: symptomlos, RN
Geranium sibiricum L.*	AB: symptomlos FB: symptomlos, RP
LABIATAE	
Ocimum basilicum L.	AB: symptomlos FB: Adernaufhellung, starke Mosaikscheckung
Ocimum canum Sims*	AB: symptomlos FB: Adernaufhellung, Adernbänderung, starke Mosaikscheckung, Deformationen, Wachstumhemmung
LEGUMINOSAE	
Cassia tora L.	AB: symptomlos FB: symptomlos, RN
Phaseolus lunatus L.	AB: nekrotische Läsionen FB: Adernaufhellung, starke Mosaikscheckung und nekrotische Flecke, sowie Wachstumhemmung (Abb. 6)
Phaseolus vulgaris L. cv. *Red Kidney*	AB: symptomlos FB: symptomlos, RN
Vicia faba L.	AB: nekrotische Läsionen, Lo FB: symptomlos
SCROPHULLARIACEAE	
Paulownia fargesii Franch.*	AB: chlorotische Flecke FB: Adernaufhellung, Adernbänderung, starke Mosaikscheckung, Wachstumhemmung

Tabelle 1 (Fortsetzung)

SOLANACEAE	
Capsicum annuum L.	AB: schwache nekrotische Flecke, in einigen Fällen Blattabfall FB: Adernaufhellung, Mosaikscheckung
Datura stramonium L.	AB: symptomlos FB: Mosaikscheckung
Lycium halimifolium Mill.	AB: braune nekrotische Lokalläsionen, Absterben der Blätter, Lo FB: symptomlos
Lycopersicon esculentum Mill.	AB: symptomlos FB: schwache Mosaiksymptome
Nicotiana glutinosa L.	AB: symptomlos FB: dunkelgrüne Scheckung, Blattdeformationen, Blattverschmalerung und Wuchsstauchung; eventuell Erholung (Abb. 4E)
Nicotiana megalosiphon Heurck et Muell.	AB: symptomlos FB: chlorotische Flecke, unscharf abgegrenzte nekrotische Flecke; Blattverkleinerung und eventuell Erholung
Nicotiana tabacum L. cv. *Samsun*	AB: symptomlos FB: Mosaikscheckung, Erholung
Nicotiana tabacum L. cv. *Xanthi-nc*	AB: symptomlos FB: Mosaikscheckung, Erholung
Solanum demissum Lindl. *Hybride-A6*	In Petrischalen punktförmige, schwarze Läsionen

Abkürzungen: AB: abgeriebene Blätter, FB: Folgeblätter, Lo: Infektion bleibt lokal, RN: Reisolierung war nicht möglich, RP: Reisolierung war möglich. Die mit * gekennzeichneten Pflanzenarten sind neue Wirte für das Gurkenmosaik-Virus. Die mit ** gekennzeichneten *Echinocystis lobata* (Michx.) Torr. et Gray-Pflanzen konnten wir nur in Primärblattstadium erfolgreich inokulieren.

Die Ergebnisse sind in Tabelle 2 zusammengefaßt. Der thermale Inaktivierungspunkt lag zwischen 68–70 °C, und der Verdünnungsendpunkt zwischen 10^{-4} bis 2×10^{-5}. Die Beständigkeit *in vitro* belief sich auf 19–22 Tage. Das *E*-Isolat blieb nach Trocknung und Aufbewahrung infizierter Wildgurkenpflanzen und auch in *Samsun*-Tabakpflanzenblättern bei niedrigen Temperaturen zwischen 3 bis 5 °C länger als 405 Tage infektiös.

3. Blattlausübertragbarkeit

In den Experimenten prüften wir die Blattlausübertragbarkeit des *E*-Isolates mit *Myzus persicae* Sulz. Als Infektionsquelle dienten spontan erkrankte *Echinocystis lobata* (Michx.) Torr. et Gray-Pflanzen und künstlich infizierte *Samsun*-Tabakpflanzen. Die Aphiden entstammten virusfreien Zuchten an *Brassica rapa*

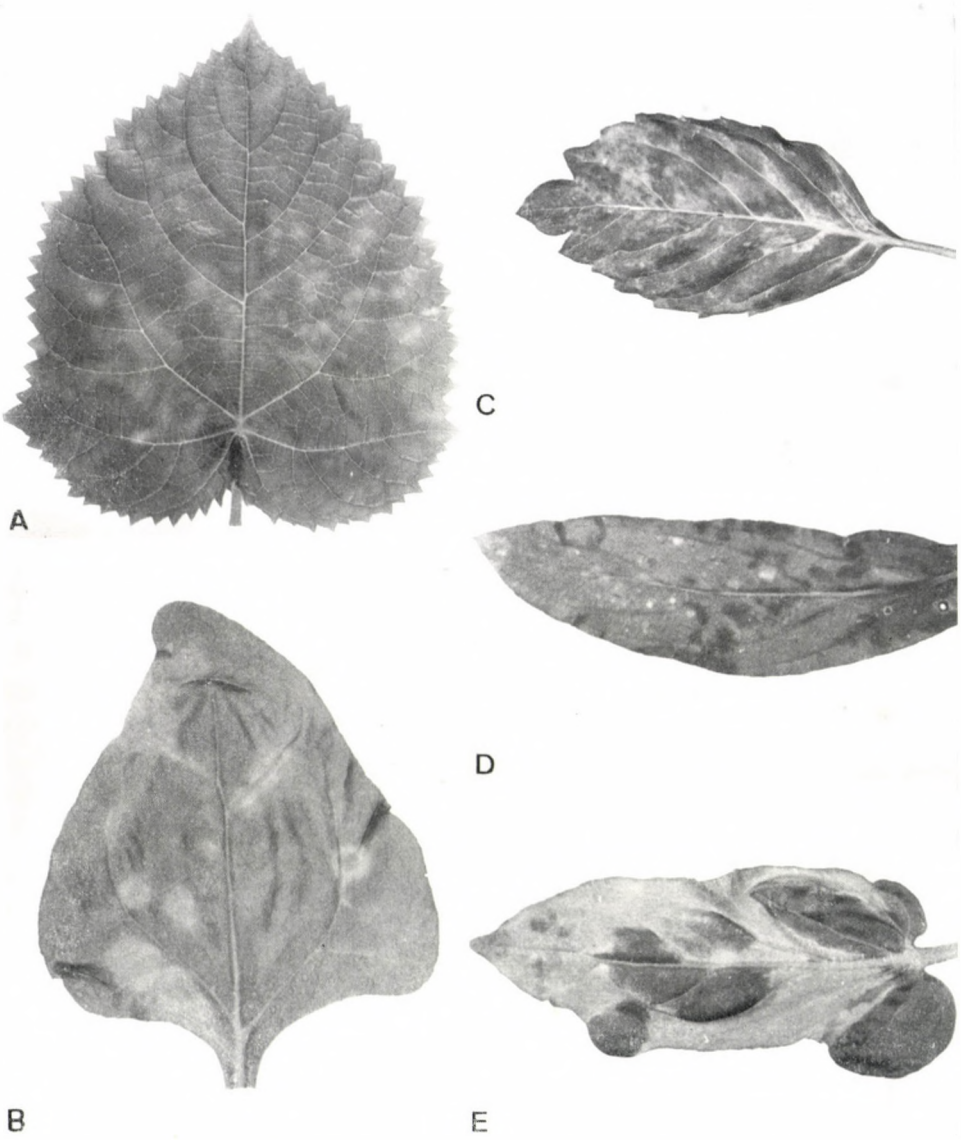

Abb. 4. Symptome des *E*-Isolates des Gurkenmosaik-Virus. A: *Paulownia fargesii* Franch., B: *Tetragonia echinata* Ait., C: *Ocimum canum* Sims., D: *Gomphrena globosa* L. und E: *Nicotiana glutinosa* L. A—C: Neue Wirte des Gurkenmosaik-Virus

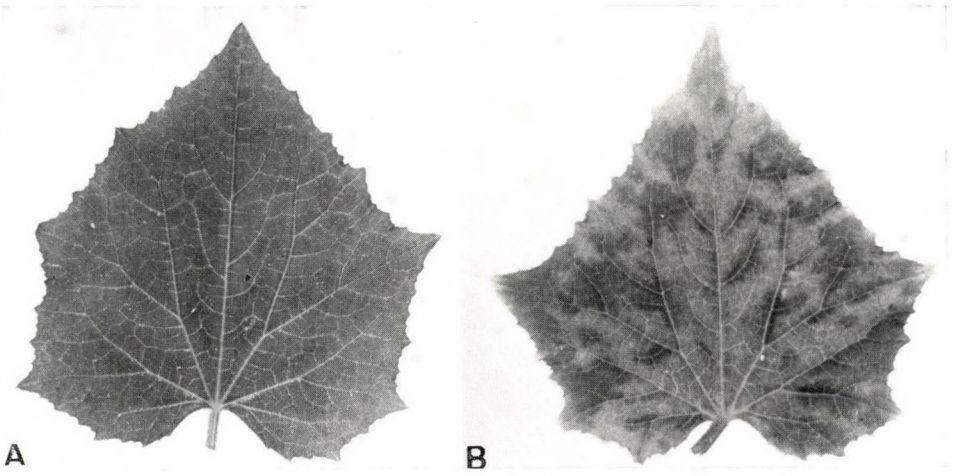

Abb. 5. Symptome des *E*-Isolates des Gurkenmosaik-Virus an *Cucumis sativus* L. A: Kontrollblatt, B: Infiziertes Blatt

Tabelle 2

Eigenschaften des *E*-Isolates *in vitro**

Versuch	TIP	VEP	BIV	BTNT
1	70	10^{-4}	19	über 243
2	70	2×10^{-5}	22	über 365
3	68	2×10^{-5}	20	über 405

* TIP: thermaler Inaktivierungspunkt in °C, VEP: Verdünnungsendpunkt, BIV: Beständigkeit *in vitro* in Tagen, BTNT: Beständigkeit nach Trocknung bei niedrigen Temperaturen (+ 3 bis 5 °C) über Calciumchlorid in Tagen.

L. var. *rapa*-Pflanzen. Nach einer Hungerzeit von drei Stunden, einer Virus-Aufnahmezeit von 8–10 Minuten und einer -Abgabezeit von 24 Stunden wurden junge *Samsun*-Tabakpflanzen im 2–3. Blattstadium infiziert. Die einzelnen, mit 10–15 Blattläusen besogenen Testpflanzen wurden unter Glasglocken bei künstlicher Belichtung (300 W, Höhe der Lichtquelle 30 cm) gehalten. Nach Beendigung der Testzeit wurden die Blattläuse mit Phosdrin abgetötet.

In getrennten Versuchen erkrankten 20 von 30 Pflanzen (bei Wildgurkenpflanzen als Infektionsquelle) und 24 von 30 Pflanzen (bei *Nicotiana tabacum* L. cv. *Samsun* als Infektionsquelle). Wie die Ergebnisse zeigen, konnte *Myzus persicae* Sulz. das *E*-Isolat übertragen. Es war den nichtpersistenten *(stylet-borne)* Viren zuzuordnen.

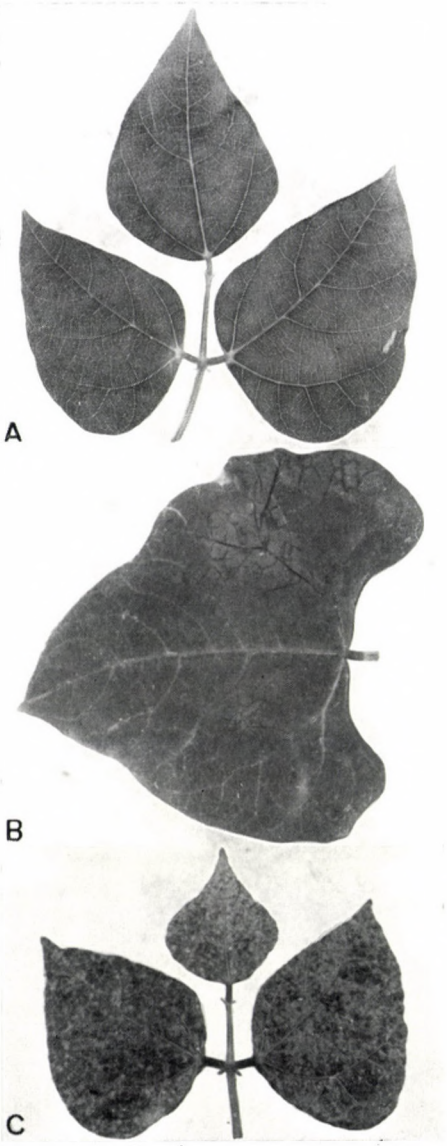

Abb. 6. Symptome des *E*-Isolates des Gurkenmosaik-Virus an *Phaseolus lunatus* L. A: Kontrollblatt, B: Lokal infiziertes Blatt, C: Systemisch infiziertes Blatt

4. Samenübertragung

Es wurde auch geprüft, ob aus spontan infizierten *Echinocystis lobata* (Michx.) Torr. et Gray-Pflanzen stammender Samen das Virus an die Jungpflanzen weitergibt. Aus spontan infizierten und gesunden Wildgurken-Pflanzen wurde Samen gewonnen und unter Gewächshausbedingungen in den Wintermonaten zu jeweils 100 Korn ausgelegt. Die daraus hervorgegangenen Pflanzen wurden bonitiert und im Alter von 60 Tagen auf *Nicotiana tabacum* L. cv. *Samsun* rückgetestet.

Wir stellten fest, daß von 100 kranken Samen bis sechs Wochen nach der Aussaat nur insgesamt 7, und von 100 gesunden Samen nur 9 keimten. Die aus krankem Samen hervorgegangenen Pflanzen zeigten nur zum Teil Krankheitssymptome. Von 7 Pflanzen blieben 4 gesund, die anderen erkrankten typisch. Aus solchen Pflanzen konnten wir das Virus auf *Nicotiana tabacum* L. cv. *Samsun* übertragen. Aus gesundem Samen entstandene Pflanzen zeigten keine Symptome und enthielten kein Virus. Infolge der schlechten Keimung konnte die prozentuale Samenübertragung des *E*-Isolates nicht exakt festgestellt werden. Es erscheint aber eindeutig, daß das *E*-Isolat durch Wildgurkensamen übertragen werden kann. Die schlechte Keimung hängt wahrscheinlich mit einer längeren Ruheperiode der Samen zusammen. Diese Vermutung unterstützten Untersuchungen, die wir 6 Monate nach der Ernte unter optimalen Bedingungen in Petrischalen durchführten. In diesen Versuchen haben wir aus 50 Samen nur 4 Pflanzen erzielt.

5. Samengewicht

Es ließen sich große Unterschiede zwischen dem Gewicht virusinfizierter und gesunder Wildgurkensamen feststellen. Es wurde gefunden, daß das Tausendkorngewicht der virusinfizierten Wildgurkensamen 125 g und das der gesunden Samen 268 g beträgt. Aus den Untersuchungen geht hervor, daß das Virus einen großen Einfluß auf das Samengewicht ausübt.

6. Serologie

Mit freundlicher Unterstützung von Herrn D. Z. Maat (Institut für Pflanzenkrankheiten, Wageningen, Niederlande) wurden im Jahre 1971 serologische Untersuchungen mit einem Gurkenmosaik-Virus-Antiserum durchgeführt, da auf Grund unserer Untersuchungen die Annahme berechtigt war, daß das *E*-Isolat wahrscheinlich mit dem Gurkenmosaik-Virus identisch ist. Bei den serologischen Untersuchungen reagierte das *E*-Isolat mit dem genannten Antiserum im Agargel-Doppeldiffusionstest deutlich positiv. In eigenen Untersuchungen prüften wir außer dem *E*-Isolat das sog. *EN*-Isolat aus *Echinocystis lobata* (Michx.) Torr. et Gray, das aus Szedres, und zwar aus dem Gebiet des Nádor- bzw. Sárvizkanal stammte und ein *R*-Isolat aus Raps (*Brassica rapa* L., vgl. Horváth 1969a).

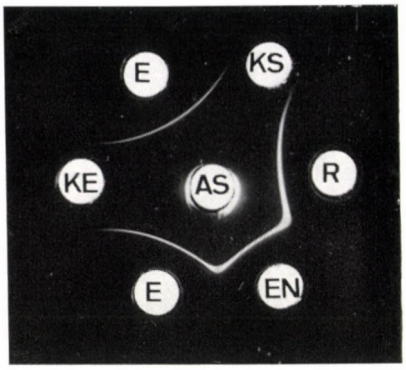

Abb. 7. Agargel-Doppeldiffusionstest der verschiedenen Isolate des Gurkenmosaik-Virus. Zeichenerklärungen: AS: Antiserum gegen Gurkenmosaik-Virus, E: *E*-Isolat des Gurkenmosaik-Virus, EN: *EN*-Isolat des Gurkenmosaik-Virus, KE: Kontrolle (gesunder *Echinocystis lobata* [Michx.] Torr. et Gray Preßsaft), KS: Kontrolle (gesunder *Nicotiana tabacum* L. cv. *Samsun* Preßsaft), R: *R*-Isolat des Gurkenmosaik-Virus

Im Agargel-Doppeldiffusionstest stellten wir fest, daß außer dem *E* und *EN* auch das *R*-Virusisolat deutlich positiv mit Gurkenmosaik-Virus-Antiserum reagierte (Abb. 7). Wir konnten auch feststellen, daß die drei Gurkenmosaik-Virus Isolate serologisch identisch sind.

7. Prämunitätsversuche zwischen dem W-Stamm und dem E-Isolat des Gurkenmosaik-Virus

Es wurden Prämunitätsversuche mit dem *E*-Isolat durchgeführt, um auch hierdurch die Identität mit dem Gurkenmosaik-Virus unter Beweis zu stellen. Wir infizierten junge *Nicotiana tabacum* L. cv. *Samsun*-Tabakpflanzen mit dem *E*-Isolat und rieben die Kontrollpflanzen mit destilliertem Wasser ab. In einem anderen Versuch infizierten wir getrennt junge *Samsun*-Pflanzen mit dem *W*-Stamm des Gurkenmosaik-Virus (SCHMELZER 1962, SKIEBE und SCHMELZER 1967). Die Kontrollpflanzen wurden ebenfalls mit destilliertem Wasser abgerieben. In einem dritten Versuch wurden die inokulierten Pflanzen nach Ausbildung der systemischen Symptome mit dem *W*-Stamm bzw. mit dem *E*-Isolat überimpft. Die Kontrollpflanzen wurden wiederum mit destilliertem Wasser behandelt.

Die Infektion mit dem *E*-Isolat führte zu schwachen Mosaikscheckungen, während der *W*-Stamm starke Vergilbungen aufwies. Wenn die Erstinfektion mit dem *E*-Isolat vorgenommen wurde, war eine deutliche Schutzwirkung gegen den *W*-Stamm festzustellen. Diese Ergebnisse der Prämunitätsversuche bestätigten ebenfalls die Identität des *E*-Isolates mit dem Gurkenmosaik-Virus.

8. Interferenz zwischen dem E-Isolat des Gurkenmosaik-Virus und dem U_1-Stamm des Tabakmosaik-Virus

Es wurden 20 *Nicotiana tabacum* L. cv. *Xanthi-nc*-Tabakpflanzen mit dem *E*-Isolat des Gurkenmosaik-Virus und die gleichen Blätter 21 Tage später mit dem U_1-Stamm des Tabakmosaik-Virus (TMV, R/1 : 2/5 : E/E : S/✳) beimpft (SIEGEL und WILDMAN 1954). Weitere 20 Pflanzen wurden nur mit dem Tabakmosaik-Virus (U_1-Stamm) inokuliert. Für beide Gruppen dienten gleichaltrige Pflanzen, es wurden stets das 4., 5. und 6.-Blattpaar (von unten nach oben) je Pflanze abgerieben und danach mit Wasser abgespült. Für die Beurteilung der Hemmwirkung der systemischen Infektion mit dem *E*-Isolat des Gurkenmosaik-Virus auf das Tabakmosaik-Virus wurde die Anzahl nekrotischer Lokalläsionen mit der

Tabelle 3

Hemmung der Anfälligkeit von *Nicotiana tabacum* L. cv. *Xanthi-nc* gegenüber dem Tabakmosaik-Virus unter dem Einfluß einer systemischen Infektion mit dem *E*-Isolat

Blattsequenz	Anzahl der Lokalläsionen auf Blattscheiben, die nur mit dem Tabakmosaik-Virus infiziert wurden*	Anzahl der Lokalläsionen an Blattscheiben die mit dem *E*-Isolat und dann mit dem Tabakmosaik-Virus infiziert wurden*	Relative Hemmung der Blätter der Anfälligkeit verschiedener Sequenzen bei *Xanthi-nc*-Tabakpflanzen
4**	720	547	24
5	360	216	40
6***	240	112	53

* Die Anzahl der Lokalläsionen wurde mit insgesamt 120 Blattscheiben (226 cm^2) Blattsequenz festgestellt.
** Ältestes Blatt.
*** Jüngstes Blatt.

nicht mischinfizierter Pflanzen verglichen. Die Untersuchungen wurden 4. und 5. Tage nach der Infektion mit dem Tabakmosaik-Virus durchgeführt. Insgesamt wurden 60 Blätter je Versuch in der Weise ausgewertet, daß wir aus jedem Blatt sechs Blattscheiben, die jeweils eine Größe von 2.26 cm^2 aufwiesen, ausgestanzt haben. Wir stellten dann die Anzahl der Lokalläsionen der einzelnen Blattscheiben fest.

Wie die Tabelle 3 ausweist, konnte die Gurkenmosaik-Virusinfektion eine Zweitinfektion beimpfter Tabakpflanzen Sorte *Xanthi-nc* durch den U_1-Stamm des Tabakmosaik-Virus hemmen. Es wurde festgestellt, daß die Anzahl der Lokal-

läsionen an den *Xanthi-nc*-Tabakblättern, die nur mit dem Tabakmosaik-Virus inokuliert wurden, in der Blattfolge von unten nach oben abnimmt. Eine Vorinfektion der Pflanzen mit dem Gurkenmosaik-Virus verminderte die Zahl der durch Tabakmosaik-Virus hervorgerufenen Lokalläsionen um 24–53%. Es ist interessant, daß die Hemmung der mit Gurkenmosaik-Virus infizierten *Xanthi-nc*-Tabakpflanzen bei den untersuchten alternden Blättern mit 24% die niedrigste war. Die jüngeren Blätter bildeten eine geringere Anzahl der Lokalläsionen und wiesen meist eine stärkere Hemmung der Tabakmosaik-Virusinfektion auf.

IV. Diskussion

Im Rahmen experimenteller Wirtspflanzenkreisuntersuchungen wurden erstmalig die spontane Virusinfektion sowie die Möglichkeit der künstlichen Infektion der in Ungarn sehr verbreiteten Wildgurke (*Echinocystis lobata* [Michx.] Torr. et Gray) nachgewiesen. Schon vor Jahren machten wir darauf aufmerksam, daß die Wildgurke als Virusreservoir dienen kann (vgl. HORVÁTH und POCSAI 1972a). Die durchgeführten Untersuchungen bestätigten diese Vorstellungen. Die Ergebnisse mit *Echinocystis*-Pflanzen weisen darauf hin, daß unter den Wildpflanzen-Infektionsquellen das Gurkenmosaik-Virus vorkommen, von denen ausgehend Infektionen von Kulturpflanzen durch Blattläuse möglich sind. Auf Grund unserer Beobachtungen dürfte *Echinocystis* vor allem in Feldern, die mehrere Jahre nacheinander eine Gurkenmosaik-Virus anfällige Kultur tragen, eine sehr gefährliche Infektionsquelle darstellen. Untersuchungen im Ausland (DOOLITTLE und GILBERT 1919, DOOLITTLE und WALKER 1925) sowie eigene Befunde erwiesen, daß das Gurkenmosaik-Virus mit den Samen von Wildgurken-Pflanzen übertragen wird. Die Verbreitung infizierten Samens stellt eine wesentliche Verseuchungsgefahr dar. Im Hinblick darauf, daß längs des Perint-Flusses (bei Szombathely), des Hévízkanals (bei Keszthely und Hévíz) sowie des Nádor- oder Sárvízkanals (bei Szedres), wo die Wildgurken-Pflanzen sehr verbreitet sind, sich zahlreiche Gärtnereien befinden, erschien es als verständlich, weshalb Paprika- und Gurkenfelder bei Szedres und Melonenfelder bei Szombathely sehr stark durch das Gurkenmosaik-Virus verseucht waren. Analog den Angaben aus der Literatur, wonach bei Gurkenmosaik-Virus-Befall anderer Pflanzen, z. B. *Cucumis sativus* L. und *Capsicum annuum* L. (SZIRMAI 1941) eine allgemeine Beeinträchtigung des Wachstums zu verzeichnen ist, zeigten die kranken *Echinocystis*-Pflanzen eine starke Verminderung der Triebdicke und die deformierten und kleinblättrigen Sämlinge bildeten Zwillingssprosse. Diese Symptome könnte man vielleicht zur Virus-Frühdiagnose bei infizierten Pflanzen verwenden. Wir haben bei Wildgurken auch eine starke Samenverkleinerung und -deformation, sowie bedeutungsvolle Samengewichtsverluste, ähnlich wie bei infizierten Rapssamen beobachtet (vgl. HORVÁTH 1969a) festgestellt.

Die Untersuchungen auf den Gebiet der Serologie und Prämunität zeigten eindeutig, daß das *E*-Isolat zum Gurkenmosaik-Virus gehört, und daß die untersuchten ungarischen Gurkenmosaik-Virus Isolate aus *Brassica rapa* L. (*R*-Isolat, HORVÁTH 1969a), und aus *Echinocystis lobata* (Michx.) Torr. et Gray (*E*- und *EN*-Isolat) serologisch identisch sind. Die durchgeführten Versuche ergaben weiterhin, daß eine systemische Infektion mit dem *E*-Isolat des Gurkenmosaik-Virus in *Nicotiana tabacum* L. cv *Xanthi-nc* die Anfälligkeit dieser Tabakpflanze für das Tabakmosaik-Virus um 24–53% herabsetzte. Ähnliche Ergebnisse wurden in den Arbeiten von MCKINNEY (1941), GARCES-OREJUELA und POUND (1957), THOMSON (1958), BRČÁK (1962), NITZANY und SELA (1962), HORVÁTH (1969b) festgestellt. Wir möchten darauf hinweisen, daß die relative Hemmung der Tabakmosaik-Virus-Anfälligkeit mit Gurkenmosaik-Virus infizierten Tabaks von der Blattsequenz und vom Alter der Blätter abhängt. Wir stellten fest, daß unter Einfluß der Gurkenmosaik-Virus-Infektion z. B. die alternden Blätter die größte Anfälligkeit gegenüber dem Tabakmosaik-Virus aufweisen. Damit wurden frühere Untersuchungen (POZSÁR und KIRÁLY 1965, KÖHLER 1966, KIRÁLY et al. 1968, HORVÁTH 1969b, HORVÁTH und POCSAI 1972b) nochmals bestätigt.

Zusammenfassung

Unsere Untersuchungen über den Wirtspflanzenkreis eines aus *Echinocystis lobata* (Michx.) Torr. et Gray isolierten Virus, sowie dessen Eigenschaften *in vitro*, die Blattlausübertragbarkeit, die Samenübertragung, die positive serologische Reaktion, Prämunitäts- und Interferenzversuche erwiesen, daß das erhaltene Isolat mit dem Gurkenmosaik-Virus identisch ist. Das Krankheitsbild konnte durch Rückübertragung des Gurkenmosaik-Virus auf die genannte Pflanzenart reproduziert werden.

*

Für die freundliche Durchführung der serologischen Untersuchungen mit dem *E*-Isolat und für die Überlassung von Gurkenmosaik-Virus-Antiserum sei auch an dieser Stelle Herrn D. Z. MAAT (Institut für Pflanzenkrankheiten, Wageningen, Niederlande) verbindlichst gedankt. Unser besonderer Dank gilt Herrn Dr. H. E. SCHMIDT (Institut für Phytopathologie, Aschersleben, DDR) für die sprachliche Verbesserung unseres Manuskriptes. Für die technische Mitarbeit danken wir Frl. A. TŐKÉS, Frl. K. MOLNÁR und Frl. M. BOLLÁN.

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Studies on the Effect of Herbicides on Virus Multiplication

I. Effects of Trifluralin to Alfalfa Mosaic and Tobacco Mosaic Viruses on *Phaseolus vulgaris* L. cv. Pinto

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Trifluralin herbicide *(a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine)* incorporated into the soil (forest soil : peat : sand, 3 : 2 : 1, v/v/v) remarkably inhibited the multiplication of alfalfa mosaic virus (R/1 : 1.3/18 : U/U: S/Ap) and tobacco mosaic virus (R/1 : 2/5 : E/E : S/∗) in the intact primary leaves of bean (*Phaseolus vulgaris* L. cv. Pinto). In the first series of experiments, when the virus inoculation followed the herbicide incorporation after 17 days, trifluralin reduced the number of local lesions of alfalfa mosaic virus by 75.6 per cent and the local lesions of tobacco mosaic virus by 64.6 per cent. In the second series of experiments the virus inoculation followed the incorporation after 66 days and trifluralin reduced the multiplication of alfalfa mosaic virus by 57.9 per cent and to a lesser extent the multiplication of tobacco mosaic virus by 25.0 per cent. The mechanism of inhibition of virus multiplication, caused by trifluralin, is not known as yet.

Introduction

Research work on the interaction of herbicides and plant viruses started only a few years ago. There are only a few papers dealing with this topic. The interactions between triazine herbicides (cf. ULRYCHOVÁ and BLATTNÝ, 1961; MILLIKAN et al., 1966; SCHUSTER, 1969; COLE et al., 1968, 1969; MACKENZIE et al., 1970), substituted ureas (e.g. monolinuron, linuron, see by SCHUSTER, 1972), phenoxyacetic acids (e.g. 2,4-D, JANKOWSKI cit. SCHUSTER, 1972) and MH (maleic hydrazide, see by SCHUSTER, 1972) and maize mosaic virus and maize dwarf mosaic virus (strains of the sugarcane mosaic virus, ∗/∗ : ∗/∗ : ∗/∗ : S/Au), 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/∗) have mainly been examined.

In our first paper we report on the results of experiments obtained in detecting the effect of trifluralin (soil applied herbicide) on polyphagous alfalfa mosaic virus (R/1 : 1.3/18 : U/U : S/Ap) and tobacco mosaic virus (R/1 : 2/5 : E/E : S/∗). This herbicide has been used for chemical weed control in cultivated plants including *Cruciferae*, *Leguminosae* and *Solanaceae*.

Material and Methods

In these experiments we examined the effect of trifluralin herbicide (Fig. 1). Prepared soil (forest soil: peat : sand, 3 : 2 : 1, v/v/v) has been treated with 3.5 l/ha commercial form of trifluralin herbicide – generally applied dose in practice. Because of the considerable loss of trifluraline due to photochemical decomposition or volatilisation, the herbicide has homogeneously been incorporated into the soil. After the 8th and 56th days following the incorporation, plastic pots with 10 cm diameter each, were filled with herbicide-treated and control soil. Two seeds of bean (*Phaseolus vulgaris* L. cv. Pinto) were sown in each pot. As is well known, Pinto bean is a good test plant of alfalfa mosaic and tobacco mosaic viruses (HORVÁTH,

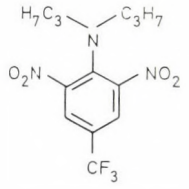

Fig. 1. Trifluralin (*a,a,a,-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine*)

1972; HORVÁTH and BECZNER, 1972). On the 9 – 10th days after planting, intact primary leaves of bean were inoculated with the local strain of alfalfa mosaic virus (AMV-L, cf. BECZNER, 1973) and with U_1 strain of tobacco mosaic virus (TMV-U_1, cf. SIEGEL and WILDMAN, 1954). The virus inoculum obtained from tobacco (*Nicotiana tabacum* L. cv. Samsun) was diluted 1 : 1 with distilled water. Leaves were previously dusted with carborundum (500 mesh), and, after inoculation, were rinsed with distilled water. During the first series of experiments 21 days after incorporation the number of local lesions was established on 108 bean leaves, inoculated with alfalfa mosaic virus and on 116 bean leaves in the case of tobacco mosaic virus. In the second series of experiments 70 days after herbicide incorporation, 64 bean leaves inoculated with alfalfa mosaic virus and 160 leaves inoculated with tobacco mosaic virus were examined to count the local lesions. In the case of control, during both series of experiments, the virus symptoms were obtained on the basis of similar number of leaves.

Results and Conclusions

The results of the experiments are shown in the Table 1. According to the figures, there is a considerable decrease in the number of local lesions, particularly in the first series of experiments. In the second series of experiments the reduction

Table 1

Effects of trifluralin on the production of local lesions produced by alfalfa mosaic and tobacco mosaic viruses on *Phaseolus vulgaris* L. cv. Pinto leaves*

Experiment series	Inhibition (in per cent) in relation to the control bean leaves	
	Alfalfa mosaic virus (AMV-L)	Tobacco mosaic virus (TMV-U_1)
I.	75.6	64.6
II.	57.9	25.0

* In the first series of experiments 8 days after incorporation of trifluralin into soil, bean seeds were planted; on 17th day primary leaves were inoculated and on the 21st day after incorporation the number of local lesions was counted. In the second series of experiments, plantation took place on 56th day, inoculation on 66th day and counting of local lesions was carried out on 70th day after herbicide incorporation.

of multiplication of viruses is probably due to a degradation and inactivation of herbicide (through 66 days from incorporation of herbicide to inoculation), which resulted in a decrease of lesser extent in virus inhibition. In both cases, inhibition of multiplication of alfalfa mosaic virus proved to be more pronounced than in the case of tobacco mosaic virus. It is especially noteworthy that in spite of the fact that the considerable part of trifluralin herbicide had been degraded, there was a reduction of nearly 60 per cent in alfalfa mosaic virus multiplication. As is known, alfalfa mosaic virus infects beans in field conditions (cf. VERHOYEN, 1966; reviewed by SCHMELZER et al., 1972), therefore, trifluralin, used for chemical weed control in beans, may be important not only as a herbicide but probably as a viricide too. The mechanism of inhibition of virus multiplication caused by trifluralin is not known as yet.

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Polyethylene Glycol as a Means for Improving Titers of Antisera Against Plant Viruses

Short Communication

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Addition of eight per cent polyethylene glycol to weak or inactive antisera from rabbits against plant viruses followed by low-speed centrifugation yielded precipitates which had considerably higher titers than it could be expected from the concentrating effect after resuspension in saline solutions.

After the first publication about the possibility to precipitate and to concentrate plant viruses by polyethylene glycol (HEBERT, 1963), a large number of research workers used this method with good results for many plant viruses. In own experiments we found it very useful for improving the antigen quality in agar-gel double-diffusion tests. However, as far as we know there exists no publication on the effect of the mentioned chemical for concentrating antibodies against plant viruses. Already in our first experiments it became obvious that the chemical had no deleterious effect on the antibody activity of rabbit antisera against plant viruses.

In several series of experiments we used the following method: Eight per cent (wt./vol.) polyethylene glycol, mol. wt. 6000, was added to an antiserum and the mixture was stirred for some minutes in order to solve the chemical. Afterwards the mixture was centrifuged at 3000 *g* for 10 minutes. The resulting pellet was resuspended in 0.85 per cent saline solution. By this way we concentrated the antisera ten to fifty-fold. The titers of untreated and treated lots of antisera in agar-gel double-diffusion tests are given in Table 1.

Surprisingly we obtained considerably higher titers than it could be expected from the concentration of the antiserum after treatment. The reasons for this need further investigation, but it seems probable that components of the antisera responsible for blocking of the precipitin reactions are left in the supernatant. Already MATTHEWS (1957) claimed the existence of such components. The supernatant fluids of our treated antisera gave no or only slight serological reactions in agar-gel double-diffusion tests.

It is obvious that the described method is able to improve considerably the titers of weak antisera against plant viruses. The effect was not restricted to antisera against certain virus types. The investigated isometric viruses belong

Table 1

Titers of antisera against plant viruses untreated and precipitated with eight per cent polyethylene glycol, respectively

Antiserum against	Titer in agar-gel double-diffusion test		Antibodies concentrated after precipitation
	untreated	precipitated	
Alfalfa mosaic virus	1/8	1/8000	12-fold
Broad bean wilt virus (= nasturtium ringspot virus)	1/4	1/128	10-fold
Celery mosaic virus*	0	1/1024	50-fold
Strawberry latent ringspot virus	1/128	1/4096	10-fold
Tomato ringspot virus	0	1/16	10-fold

* The virus was disintegrated by treatment with 0.1 methylene-diamine tetraacetic acid di Na-salt dihydrate before test.

both to aphid and nematode transmissible groups. Not only well diffusing viruses gave good results, like isometric viruses and the bacilliform alfalfa mosaic virus, but also the thread-like celery mosaic virus which needs disintegration for the tests (WOLF and SCHMELZER 1972).

Zusammenfassung

Zusatz von 8% Polyäthylenglykol zu schwachen oder inaktiven Kaninchenantiseren gegen Pflanzenviren sowie nachfolgende niedertourige Zentrifugation führte zu Präzipitaten, die nach Auflösen in physiologischer Kochsalzlösung zu einer wesentlich höheren Titersteigerung führten als auf Grund der erfolgten Konzentrierung zu erwarten war.

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Ocimum canum Sims as a New Indicator Plant for the Strains of Alfalfa Mosaic Virus

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Since the discovery of the polyphagous alfalfa mosaic virus (AMV, R/1 : : 1.3/18 : U/U : S/Ap) it has been reported as an important pathogen on many crops and wild plants (reviewed by SILBER and HEGGESTAD, 1965; THORNBERRY, 1966; HULL, 1969; SCHMELZER, 1969; CRILL et al., 1970; SCHMELZER et al., 1972; BECZNER, 1973). As far as we know, there are fifteen plant species in the family of *Labiatae* which are susceptible to AMV. One of them is *Ocimum basilicum* L. which is susceptible to several plant viruses (cf. LOVISOLO, 1966; HORVÁTH, 1974). But this plant is especially important as a differential indicator or test plant for AMV (LOVISOLO, 1960; 1961). The reaction of an other *Ocimum* species, *Ocimum canum* Sims to the infection of AMV is not entirely known.

We investigated the reaction of *Ocimum canum* Sims against two strains (AMV-L and AMV-S, cf. BECZNER, 1972) of AMV. Inocula were prepared from *Nicotiana tabacum* L. cv. *Samsun* plants 7 to 10 days after inoculation. The other details of the experiment were already described in a previous paper (cf. BECZNER and SCHMELZER, 1972).

Ocimum canum Sims was systemically susceptible to both strains of AMV. It was found that the investigated plant reacted with severe symptoms to AMV-S. Strong yellow mosaic developed after seven or eight days of incubation (Fig. 1A). No growth reduction was observed. The yellow mosaic symptoms appeared on the leaves of the axillary shoots, too (Fig. 1B and C). *Ocimum canum* Sims seems to be a suitable indicator or screening plant for differentiation of AMV from other plant viruses. According to HORVÁTH (1974) the following six viruses can not infect *Ocimum canum* Sims: bean (common) mosaic virus (∗/∗ : ∗/∗ : : E/E : S/Ap), potato virus M (∗/∗ : ∗/∗ : E/E : S/Ap), potato virus S (∗/∗ : : ∗/∗ : E/E : S/Ap), potato virus Y (∗/∗ : ∗/∗ : 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). It should be mentioned that *Ocimum canum* Sims was found to be host plant for the following viruses in addition to AMV: tomato black ring virus (∗/∗ : ∗/∗ : S/S : S/Ne, cf. SCHMELZER, 1963), cherry leaf roll virus (∗/∗ : : ∗/∗ : S/S : S/∗, cf. SCHMELZER, 1966), and poplar mosaic virus (∗/∗ : ∗/∗ : : E/E : S/∗, cf. SCHMELZER, 1966) as well as tobacco rattle virus (R/1 : 2.3/5 :

: E/E : S/Ne), 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/∗) and tobacco ring spot virus (R/1 : 1.8/42 : S/S : S/Ne, cf. HORVÁTH, 1974).

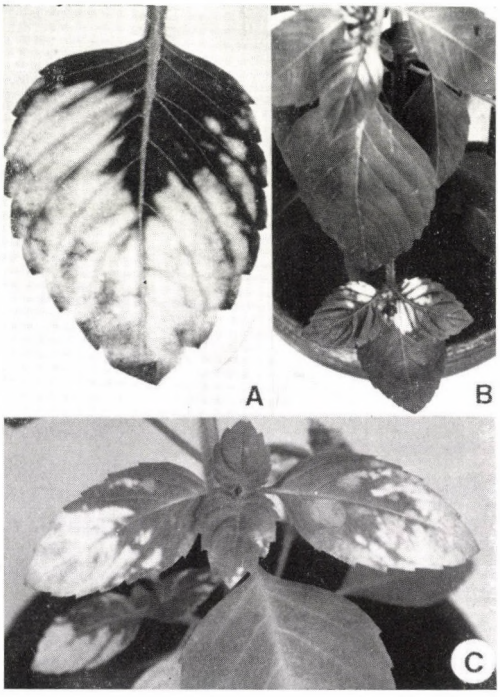

Fig. 1. Symptoms of alfalfa mosaic virus strains on *Ocimum canum* Sims. A and B: Local strain of alfalfa mosaic virus (AMV-L). A: Systemically infected leaf with the local strain of alfalfa mosaic virus. B and C: Symptoms on the axillary shoots. C: Systemic strain of alfalfa mosaic virus (AMV-S)

In spite of the susceptibility of *Ocimum canum* Sims to the above-mentioned viruses this plant is one of the best indicator plant for AMV because of the strong yellow mosaic symptoms which develop on the *Ocimum* after a short incubation period. Other viruses never cause symptoms similar to those induced by AMV.

*

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Fluorescent Antibody Staining of *Fusarium culmorum*

By

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A strain-specific antiserum has been prepared against the mature hyphae of *Fusarium culmorum* (W. G. Smith) Sacc. Both the direct and indirect methods of the fluorescent antibody technique resulted in a specific yellow-green immunofluorescence, however, indirect staining was always more intense. There was a significant difference in the intensity of fluorescence between the mycelia of the homologous *F. culmorum* strain and those of the heterologous *F. graminearum* and *F. culmorum* strains. All the other *Fusarium* species tested showed no detectable fluorescence. The heterologous strains of *F. culmorum* and *F. graminearum* could not be distinguished. No significant difference was found between the "culmorum" strains from different host plants. The serum specific for *F. culmorum* and *F. graminearum* is suitable to separate these species from other fungi. Morphological examination and culturing processes are not necessary because mycelia sufficient for fluorescent antibody staining usually grow out from infected plant material on to a microscope slide in 48—72 hours.

Fluorescent antibody staining, when an antigen is incubated with antibody labelled with a fluorescent dye, and the reaction is examined by a fluorescence microscope is wide-spread in the detection of bacteria and viruses. However, very few results have only been reported in the field of plant pathogenic fungi. SCHMIDT and BANKOLE (1962, 1965) detected *Aspergillus flavus* in soil, KUMAR and PATTON (1964) gave a short account of the work with *Polyporus tomentosus*. BENO and ALLEN (1964) used the direct method for the identification of *Puccinia sorghi* urediospores. BURREL et al. (1966) found the technique to be useful in differentiating three *Phytophthora* species. AMOS and BURREL (1967) also used it for taxonomical purpose in the case of the genus *Ceratocystis*. PREECE and COOPER (1969) prepared antiserum for *Botrytis cinerea*. CHOO and HOLLAND (1970) detected the hyphae of *Ophiobolus graminis* in the rhizosphere of wheat and oat plants, while WARNOCK (1971) succeeded in assaying *Penicillium cyclopium* mycelium in the palea and the lemma of barley grains by the means of fluorescent antibody staining.

In the present investigation our attempt was to apply the method for the rapid detection of the hyphae of *Fusarium culmorum* (W. G. Smith) Sacc. The fluorescent antibody technique has not been applied till now for the diagnosis of *Fusarium* species, but MARCHANT and SMITH (1968) determined serological

differences between the various parts of the colony of *F. culmorum* using this method. Serological techniques other than immunofluorescent labeling were used in experiments with *Fusarium* species in a few cases. TEMPEL (1959), as well as BUXTON, CULBRETH and ESPOSITO (1961) established serological differences among the formae speciales of *Fusarium oxysporum*, while SPAAR and VESPER (1970) found serological relationships among several *Fusarium* species.

Material and Method

Isolation of fungi. Fusarium culmorum (strains C1, C2, C3, C4, C6), *F. graminearum* (G1, G2, G3) and *F. moniliforme* strains isolated from maize were obtained from the culture collection of Dr. Á. SZÉCSI (Research Institute for Plant Protection, Budapest). Further strains of *F. oxysporum*, *F. sporotrichioides* and *F. solani* (from alfalfa), *F. culmorum* (strains W1, W2 and W3), *F. acuminatum*, *F. tricinctum*, *F. equiseti*, *F. sambucinum* and *F. sulphureum* (from wheat) were isolated by usual methods and were identified according to the BOOTH system (1971).

Immunization. 8-day-old culture of *Fusarium culmorum*, strain C6 grown on Czapek's liquid medium with 1% yeast extract (Difco) at 25°C was three times washed with physiological saline. Rabbits were injected four times a week with an initial amount of 0.1 ml antigen administered intramuscularly. The procedure lasted for 4 weeks and the final dose was 5.0 ml antigen suspension. A week's rest was followed by a hyperimmunization for 2 weeks. Seven days after the last injection the animals were bled from the carotid artery.

Immunodiffusion. Cultures were grown as described above and 0.1 g dry weight of mycelium was suspended in 5 ml physiological saline. The suspension was treated with ultrasonic desintegration for 15 min. Tests were carried out in the media described by GOODING (1966) with the antiserum in the inner well and the antigens in the outer wells. Results were recorded after 4 days' incubation in a moist chamber at 25°C.

Preparation of fluorescein isothiocyanate (FITC)-labeled antibody. The method of DEDMON, HOLMES and DEINHARDT (1965) was used to prepare FITC conjugated gamma-globulin. The globulin was precipitated with half-saturated ammonium sulfate solution and sedimented by centrifugation at 1800 *g* for 30 minutes at 4°C. After decanting the supernatant the precipitated globulins were dissolved in distilled water to approximate the original serum volume. Three precipitations were made and the last dissolution was followed by a dialysis overnight against 0.15 *M* NaCl to remove residual sulfate. The presence of sulfate was tested by adding 2% barium sulfate solution to the dialyzing medium. The protein concentration was determined by the biuret method described by GORNALL, BARDAWILL and DAVID (1949). Extinctions were read on a Unicam SP 800 A spectrophotometer at 555 nm, and the protein concentrations were calculated

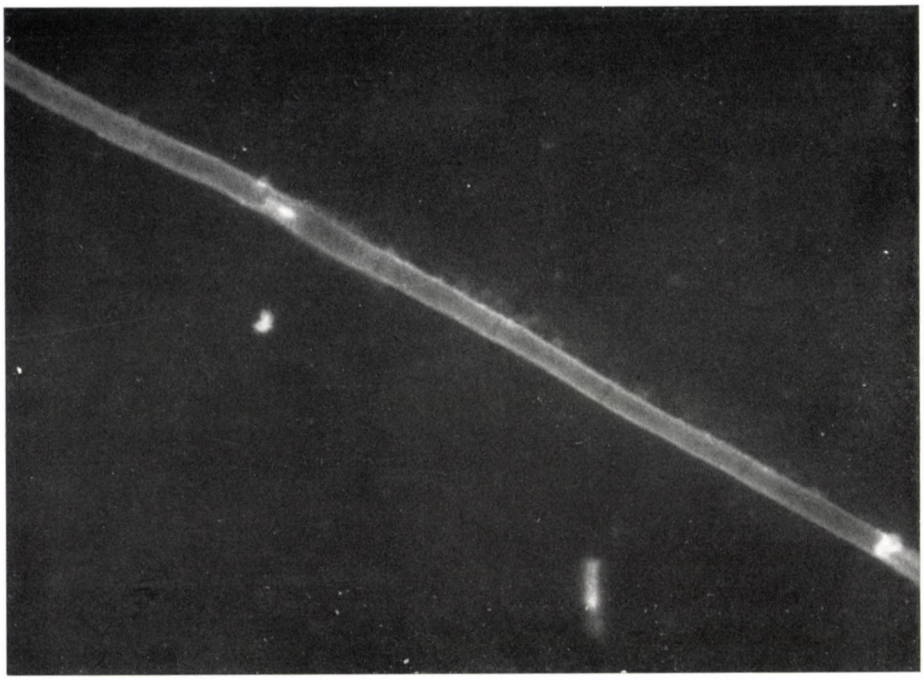

Fig. 1. Indirect staining of *Fusarium culmorum* C6 with its homologous antiserum diluted 1 : 8. ×2000

on the basis of a standard curve prepared with a standard solution of crystalline bovine albumin. Powdered FITC (SPOFA, Praha) was dissolved in 0.025 *M* carbonate-bicarbonate buffer, pH 9.0 containing 0.15 *M* NaCl at a rate of 0.1 mg/ml. The protein concentration was adjusted to 1% by adding 0.15 *M* NaCl and the solution was placed in a dialyzing bag. Labeling was carried out through the dialyzing membrane. The ratio of the carbonate-bicarbonate buffer containing FITC and the protein solution was 10 : 1. The conjugation process was allowed to proceed for 18 hours while the dialyzing buffer was stirred. Uncoupled dye was removed by gel filtration through G-25 Sephadex (Pharmacia Fine Chemicals AB, Uppsala, Sweden) equilibrated and eluted with phosphate-buffered saline (0.01 *M* phosphate, 0.15 *M* NaCl, pH 7.4). The diameter to height ratio of the column was 1 : 10, with the sample load 25% of the bed volume. Collecting elute was ceased when the last material eluted from the dark yellow band. All the conjugation, dialyzing, and gel-filtration procedures were carried out in a dark room at 4°C.

Preparation of slide cultures. A procedure similar to the method of PREECE (1969) was applied. A small amount of Czapek's agar with 1% yeast extract was dropped on a sterile microscope slide and covered with a coverglass. Fungi grown

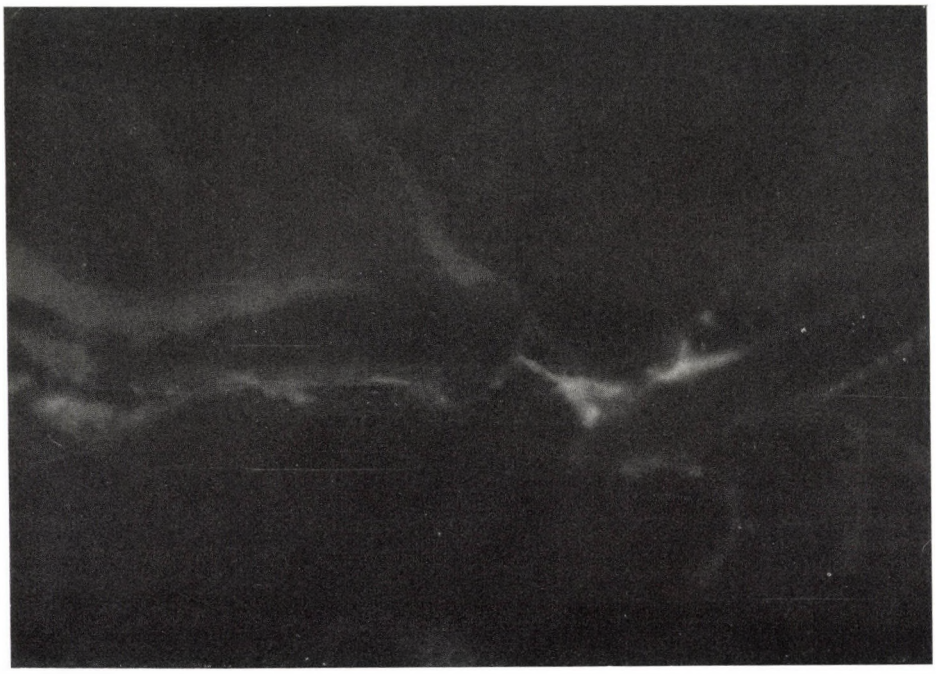

Fig. 2. Heterologous fluorescence. White areas represent nonserologically bound antiserum. The reaction grade is "—" in this case. ×2000

on potato-dextrose agar were inoculated under the coverglass, and after an incubation for 48–72 hours in a moist chamber at 25°C the specimen was ready for staining. A similar scheme was used to demonstrate the fungus from plant material, except that the medium was water agar with 1 % glucose. Small pieces of the inner tissues of artificially inoculated four-week-old maize plants were placed under the glass in this case.

Direct and indirect fluorescent antibody staining. Mycelia located on the slide were dried at room temperature for 5 min and fixed in aceton for 20 min. Antigen was allowed to be stained with the conjugate for 40 min at 25°C in a moist chamber and washed free of unreacted serum with phosphate-buffered saline for 10 min followed by rinsing in distilled water. After drying, the specimen was mounted in phosphate-buffered glycerol. Indirect staining was carried out by applicating FITC-labeled anti-rabbit-globulin from sheep serum (Institut Pasteur).

Specificity testing. Unlabeled antibodies were incubated with their homologous antigen to block staining with the labeled antibodies (KUMAR and PATTON, 1964). Heterologous antigens were also examined.

Microscopy and photomicrography. Preparations were observed under bright-field illumination transmitted from a HBO 50 light source. Two 2 mm BG 12

exciter filters, a trough containing 3 per cent copper sulfate, a ×16 apochromatic objective and a ×10 ocular fitted with suppression filter OG 1 were used. Photographs were taken with an Exacta Varex IIa camera on Fortepan (27 DIN) film using an exposure time of 3.5 min.

Results and Discussion

The titre of serum determined by the gel diffusion method was at least 1 : 16 in the case of the strain used for immunization (C6) and 1 : 4 in the case of other "culmorum" and "graminearum" strains. One precipitation line was detected on all occasions. *Fusarium moniliforme*, *F. acuminatum*, *F. oxysporum*, *F. solani*, *F. tricinctum*, *F. sporotrichioides*, *F. equiseti*, *F. sambucinum* and *F. sulphureum* gave no detectable reaction even if undiluted serum was used. These examinations were necessary in order to determine if the serum had any antibody nature.

Using the fluorescent antibody technique both the direct and the indirect methods resulted in specific yellow-green immunofluorescence at a maximum dilution of 1 : 16. Dilutions of 1 : 4 and 1 : 8 proved to be the most suitable ones for the differentiation between C6 and other "culmorum" and "graminearum" strains (Table 1). There is a significant difference in the intensity of fluorescence

Table 1

The fluorescence of the mycelia of different "culmorum" and "graminearum" strains observed under bright-field illumination using two BG 12 exciter filters, a ×16 apochromatic objective and a ×10 ocular fitted with suppression filter OG 1

Mycelia	Intensity of fluorescence			
	Direct staining		Indirect staining	
	1 : 4	1 : 8	1 : 4	1 : 8
F. culmorum C6	++	++	+++	+++
F. culmorum C1	+	±	+	+
F. culmorum C2	+	−	+	±
F. culmorum C3	+	−	+	−
F. culmorum C4	+	±	+	+
F. culmorum W1	+	−	+	+
F. culmorum W2	+	−	+	−
F. culmorum W3	+	±	+	+
F. graminearum G1	+	±	+	+
F. graminearum G2	+	±	+	+
F. graminearum G3	+	−	+	−

− indicates autofluorescence; ± indicates visibility; +++ indicates bright fluorescence.

between the mature hyphae of the homologous antigen and those of the heterologous ones (C1, C2, C3, C4, W1, W2, W3, G1, G2, G3). When indirect staining was applied with undiluted serum or sera diluted 1 : 1 and 1 : 2, C6 and heterologous "culmorum" and "graminearum" strains could not be distinguished. Although differences between the C, W and G mycelia and the other *Fusarium* species were easily detected, a very weak fluorescence of these species existed with the undiluted serum. There were differences in the degree of intensity between direct and indirect staining of the same dilutions. Indirect staining was always more intense.

Hence, the serum prepared against the mature hyphae of strain C6 of *F. culmorum* is a strain-specific one, and the heterologous "culmorum" and "graminearum" strains are also distinguishable from all the other *Fusarium* species tested, but they cannot be distinguished from one another. On the basis of gel diffusion experiments SPAAR and VESPER (1970) found that the two species were closely related serologically.

No significant difference was found between strains C1, C2, C3, C4 and W1, W2, W3 by this technique. This method, however, is not a sufficient tool for separating cultures from different host plants.

Removing cross-reacting antibodies to prepare a species-specific conjugate cross-absorption was not attempted. When the technique of the inhibition of specific staining by unlabeled antibody (KUMAR and PATTON, 1964) was used to judge the specificity of serum, no difference was found between the degrees of blocking the specific staining, either strains C or G were examined.

The strain-specific serum will be useful to carry out certain model experiments with *Fusarium culmorum* C6, to examine host–parasite relations, and to examine mycelial development following artificial inoculation. The serum specific for *F. culmorum* and *F. graminearum* is suitable to separate these species from other fungi without morphological examination and culturing process because mycelia sufficient for fluorescent antibody staining usually grow out from infected plant material on to the slide in 48 – 72 hours.

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The authors wish to thank Dr. Á. SZÉCSI for his helpful discussion about this work.

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The Occurrence of Bunt Fungi in Wheat Inoculated by *Tilletia caries* and *T. foetida*

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Inoculations with separate and mixed spores of *T. foetida* and *T. caries* on 16 wheat varieties were carried out prior to sowing. The aim was to clarify: what kind of bunt species appear in the ears? 20,794 grains were subjected to mycological investigations. Species of bunts and their proportions were defined. We have identified *T. foetida*, *T. caries* and the hybrid of these: *T. intermedia*. Separation within ears established: grains were infected by one of the bunts only. However, the spores of the bunts mixed in single grains also appeared. No conclusive evidence supports an antagonism between *T. caries* and *T. foetida*.

This paper is a summary of a work extending over some years to investigate the relation of the bunt fungi with each other and with their host. The basis of our investigations was a series of trials and a country-wide collection initiated by Dr. J. PODHRADSZKY, whose data and material — after his sudden death — were placed at the author's disposal.

At the beginning of investigations *Tilletia foetida* was considered by PODHRADSZKY as a dominating pathogen. On regions bordering with Austria and Czechoslovakia *Tilletia caries* was also fairly known, but it went down rather for a curiosum than for a menace to be counted with. PODHRADSZKY indicated in 1950 that only a fragment portion (0.24 per cent) of the total *Tilletia* infections was caused by *Tilletia caries*, in spots where this pathogen did occur at all. It was in 1953 that PODHRADSZKY for first time found heterozygote hybrid *Tilletia intermedia* in a kernel of wheat in which *T. caries* and *T. foetida* also occurred. In the later years on the gradual spread of *T. caries*, PODHRADSZKY launched a resistance trial with new and old varieties. The main issue was to clarify the degree of resistance of these varieties to bunt especially *T. foetida*, then widely spread over the country.

At the beginning, the seeds of the trial were inoculated only with *T. foetida*. Later, *T. caries* with lattice spores was collected in such a quantity as to enable PODHRADSZKY to include it into his trials. In the last etape of his work, when the spread of *T. caries* took larger proportions, PODHRADSZKY started a series of provocative trials using *T. foetida* and *T. caries* separately as well as in mixture. The aim was to find out what bunt species develop on the treated plots and whether a suppressing effect of *T. caries* on *T. foetida* could be demonstrated.

PODHRADSZKY's idea was to form a research group for treating his material collected in 18 years. Because of his sudden death the plan could not be realized.

Recently, the author was commissioned to complete the mycological investigation of the material, which was stored in a herbarium.

Material and Method

In two sites (Keszthely and Mosonmagyaróvár) at harvest time ears were sampled. A batch of ear samples generally contained 200–250 ears. Unfortunately, as the microscopical study went on, it turned out that they derived from different plants with unknown identity. Therefore, we cannot decidedly say that ears infected only with *T. caries* or only with *T. foetida*, in fact, were grown in the same plant or not.

Data recorded with the samples included: wheat variety, site, plot, time of harvest, bunt applied for inoculation. The preparation for microscopic study was done as follows: spores were obtained from 3 kernels of each ear. The sampled

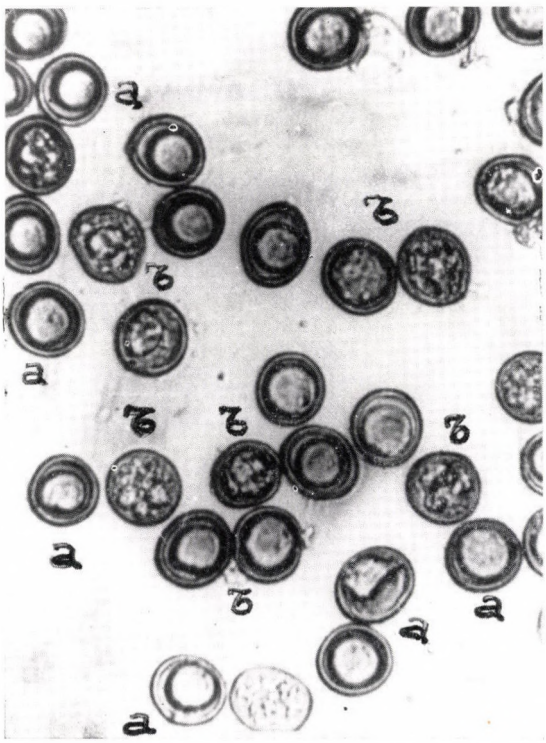

Fig. 1. Spores of *Tilletia foetida: a)* normal spores; *b)* spores similar to *Tilletia caries*

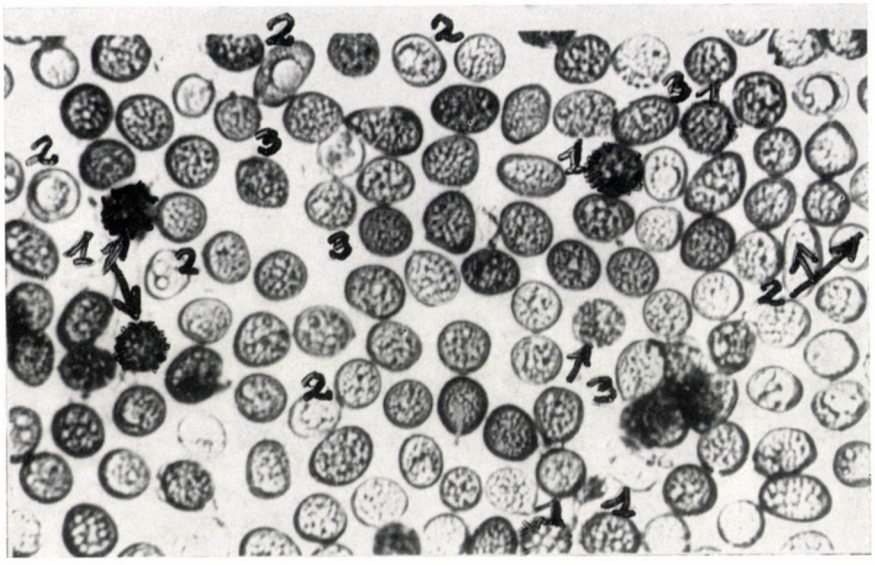

Fig. 2. *Tilletia caries* (1), *T. foetida* (2) and *T. intermedia* (3) spores from the same grain

grains – amounting to about 21,0000 – were handled by the same person. We must have applied a comparatively simple, fast method which also excluded the hazard of spore residues remaining on the slide. That is the reason why we do not applied cover-slip during microscopic investigations. Each ear tested was laid on the slide by its stalk. Three grains per ear were thrusted through with a needle-like object. The needle, by tunelling the grains, delivered a scarcely visible amount of spore onto the slide. This was done with 3 grains – with 2 cm distances between them – in succession.

The method to obtain spores by grounding or pinching the grains, frequently published in the literature, was too slow and constituted a grave risk of mixing the spores.

By tunelling the grains we obtained 400–600 spores each time, thus on the average 1500 spores appeared in the field of sight of the slides. This amount of spores formed 3 well separated groups, which, by the adjustment of the objective table, could be scrutinized without difficulty. Spores with their fatty consistency adhere well onto the slide, but they do not wet readily with tap or distilled water. A solution of 0.25 per cent Ultra washing powder wetted very well, and resulted in a layer of spores, thus adjustment of the microscope was but a very few times necessary. Such slides could be easily washed even after they got dry. A thorough wetting was all the more necessary, because – due to the refractive power of the air – old spores cannot be investigated: only small black spots with hazy contours appear under the microscope. When larger magnification is used (40×)

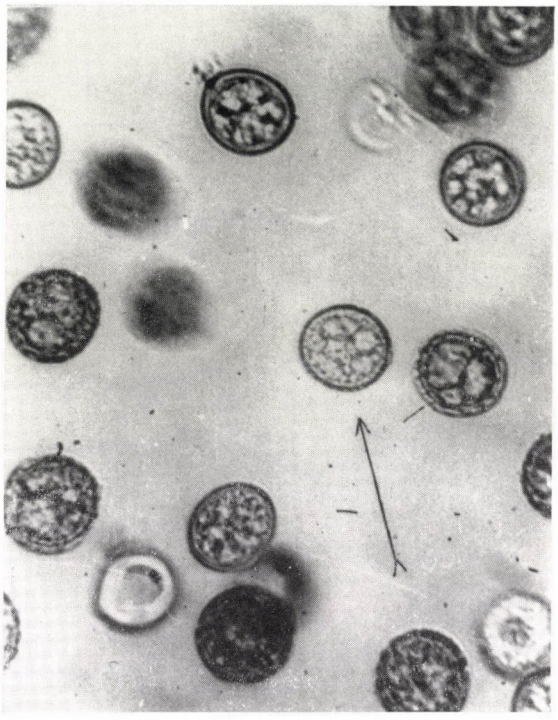

Fig. 3. *Tilletia intermedia* (arrowed) among *T. caries* and *T. foetida*

the distance between slide and objective diminishes to such an extent that the latter can merge into the water. This snag is avoidable if the drop of water applied is of modest size. If the drop appears to be too large, we have to wait a minute or so till the preparation dries a little.

The identification of *T. intermedia* spores is – owing to their placement and the refraction – rather uncertain: a larger magnification is necessary to separate the outer hyaline reticulate from the darker inner part. On stirring the water drop with a slice of blotting paper, the spores of *T. foetida* would only slide, while those of *T. intermedia* roll in the same way as the spores of *T. caries*. When this happens the reticulate of *T. intermedia* with its less projective character becomes quite visible.

Results

The smut balls, symptoms of bunt, are not distorted kernels but special formations, developed instead of grains, and they contain of about 6–8 million spores. The fish-like stink of the bunt spores results from trimethylamine but

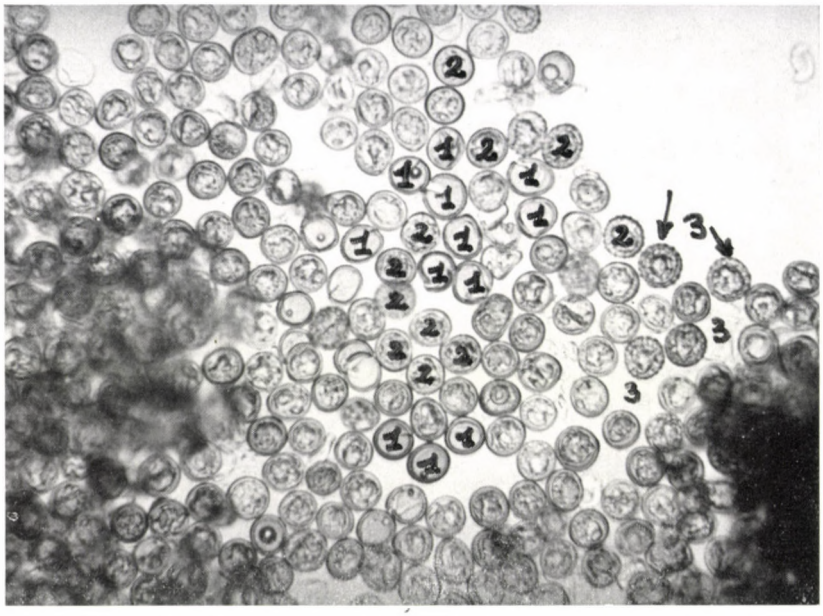

Fig. 4. *T. foetida* (1), *T. intermedia* (2) and *T. caries* (3) Mixed occurrence of spores within the same grain

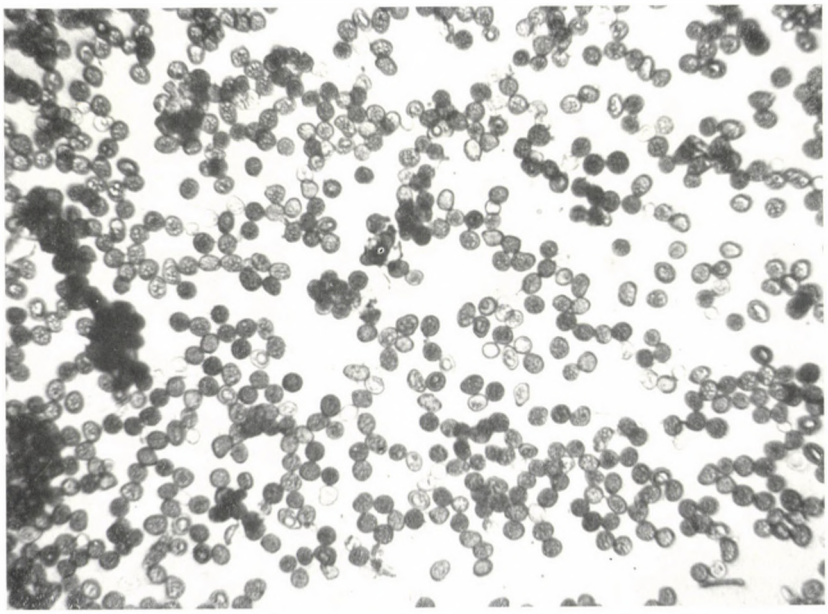

Fig. 5. Simultaneous appearance of *T. foetida* (1), *T. intermedia* (2) and *T. caries* (3) in case of inoculation by *T. caries*

Table 1

Occurrence of *Tilletia* species in kernels of wheat varieties inoculated with *Tilletia foetida* or *T. caries*

Wheat varieties	Inoculation of seeds with		Number of grains	Occurrence of T. species found in the grains			
	T. caries	T. foetida		T. caries	T. foetida	T. intermedia	Mixed appearance within the same grain
Bánkuti 1201	T. caries	—	975	966	→	→	3 = T. caries + T. foetida 6 = T. caries + T. foetida + T. intermedia
Bánkuti 1201	—	T. foetida	240	→	183	→	57 = T. caries + T. foetida + T. intermedia
Buzogány	—	T. foetida	393		393		
Buzogány	T. caries	T. foetida	1068	1029	39		
Fertődi 481	—	T. foetida	840		786	8	27 = T. foetida + T. intermedia 19 = T. caries + T. foetida + T. intermedia
Fertődi 293	T. caries	—	840	838	→	→	2 = T. caries + T. foetida + T. intermedia
Fertődi 293	—	T. foetida	960		936	→	24 = T. foetida + T. intermedia
Fertődi 293	T. caries	T. foetida	1878	689	60	→	1090 = T. caries + T. foetida 39 = T. caries + T. foetida + T. intermedia
Kompolti 169	T. caries	T. foetida	696	552	144		
Kompolti 169	T. caries	—	1260	1227	→	→	39 = T. caries + T. foetida + T. intermedia

Kompolti 169	—	T. foetida	1195		195		
Karcagi 388	T. caries	T. foetida	1197	334	782	→	81 = T. caries + T. foetida + T. intermedia
Karcagi 388	T. caries	—	810	810			
Karcagi 344	—	T. foetida	660		638	→	22 = T. foetida + T. intermedia
Nagykunsági 22	—	T. foetida	600		600		
Nagykunsági 34	—	T. foetida	120	35	76	→	9 = T. caries + T. foetida + T. intermedia
Bezostaja 1	T. caries	—	708	708			
Bezostaja 1	—	T. foetida	270		210	→	60 = T. foetida + T. intermedia
Miranowskaja 808	T. caries	—	2059	2003	27	→	12 = T. caries + T. foetida 17 = T. caries + T. foetida + T. intermedia
Miranowskaja 808	—	T. foetida	276		276		
Skorospelka	—	T. foetida	633		633		
Skorospelka	T. caries	T. foetida	693	60	633		
Veselopodjanskaja	T. caries	—	1254	1207	→	→	38 = T. caries + T. foetida 9 = T. caries + T. foetida + T. intermedia
Libellula	T. caries	—	477	477	→	→	
San Pastore	T. caries	—	510	506			4 = T. caries + T. foetida + T. intermedia
Variety not identified	T. caries	T. foetida	1200	400	800		

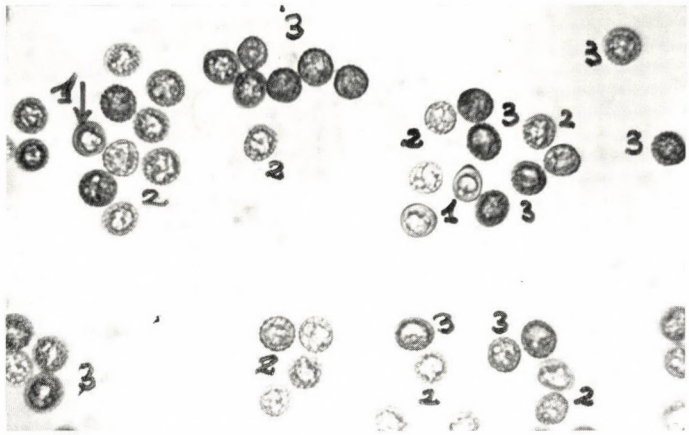

Fig. 6. *Tilletia foetida* (1), *T. intermedia* (2) and *T. caries* (3) occurring together in the same grain. Inoculation was carried out with mixed spores of *T. caries* and *T. foetida*

perfectly odourless spores *(Tilletia inodor)* were also found in Somogy by OLGYAY (1949).

OLGYAY, GIMESI and FRENYÓ (1949) found the trimethylamine to inhibit germination of the *Tilletia* spores and to lose this capacity after getting into the soil. The spores can germinate direct after maturity, but even 3–4 months later their germinating capacity and germination energy still remains poor.

The life span of spores is estimated to 11–15 years, but BEREND (1953, 1956) found much older spores capable for germination as a consequence of treatment with ultraviolet- and X-ray.Table 1 gives result of microscopical study.

In the ears of Fertődi 293 *T. foetida* occurred only in the lower grains. In those ears where *T. caries* and *T. intermedia* also occurred, the infection spread higher up, sometimes as far as the tip of the ears. In this wheat variety we found among the average sized spores (diameter: 15–17 micron) giant ones, too (34–36 micron in diameter).

In the wheat Veselopodjanskaja the very opposite was the case: the infection of the tip of the ear was frequently found with a healthy lower part. Smaller than average-sized, almost black *T. caries* spores (diameter: 9–10 micron) cropped up, too.

Skorospelka inoculated with a mixture of spores *(T. caries* and *T. foetida)* showed *T. foetida* spores which were similar to those of *T. caries*. Though the spores of *T. foetida* were smooth, their colours varied between vague gray and brown. Instead of long, irregular shape, so characteristic of the spores, round, thick-walled spores with inner granules occurred. Their average size ranged between 10–12 micron.

In some kernels all the three bunt species *(T. caries, T. foetida, T. intermedia)* were found. Fertődi 293 presented an unusual form of mixed infection: in grains

placed side by side or alternately in front of each other, *T. caries* or *T. foetida* was found, but within a single kernel never more than one of the bunts occurred only. In these ears no *T. intermedia* was demonstrated.

Conclusions

The size of ears does not affect the formation of different spores. Is there any difference between the proportions of the mixed spore formation within grains in case of inoculation carried out with *T. caries* or with *T. foetida* or with the mixture of both? No, there is not. The mixed spore formation within the same grain is comparatively infrequent. This stands for *T. intermedia*, too. Only in a single case (in Fertődi 481) occurred a mixed infection of about 6 per cent. Here the inoculation was done with *T. foetida*. Within a single grain a mixture of *T. foetida*, *T. caries* and *T. intermedia* spores appeared.

Although the inoculation was done with a mixture of spores (*T. caries* and *T. foetida*), very often only one or the other spore appeared. There were instances when the colour and shape of *T. foetida* spores was not the same as usual. Is it possible to find *T. foetida* spores in wheat inoculated with *T. caries* or *vice versa?* Yes, but to a small extent, accompanied mostly with *T. intermedia*. *T. caries* does not exercise a suppressive effect on *T. foetida*.

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Investigations on the Effect of *Orobanche cumana* on Sunflower

By

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Over the years from 1958 to 1970 trials were conducted in county Szabolcs-Szatmár to assess damages to sunflower caused by *Orobanche cumana*. We have chosen to probe "Kisvárdai" which, being a late-maturing variety, is also very sensitive to this parasite. *Orobanche cumana* causes heavy losses especially when it shoots in the first half of the sunflower's growing time, invading the main roots of the plant. With the parasitic shoots sprouting in the second half of vegetation, the sunflower survives since only its diminutive roots suffer. A sunflower stand infected by *Orobanche cumana* may yield by 30 per cent less seed than an intact one; also it shows a considerable loss — up to 15 per cent — in the oil content.

In the sunflower fields of Hungary *Orobanche ramosa* L. a native sort and *Orobanche cumana* Wallr. of Chinese origin are widely distributed. *Orobanche cumana*, causing the heavier losses, occurs all over the country in the association of sunflower, tobacco, tomato and also in that of a biennial weed *Carduus nutans*.

The individual plants of *Orobanche* can be found bunched together subsisting on the roots of sunflowers; their numbers range between 3 to 200. *Orobanche* affects sunflower by forming different morphological abnormities as a consequence of the toll of nutrients and water taken on the plant.

Often a small number of parasites kill the host, on other occassions, however, it may survive with hundreds of *Orobanche* shoots sponging on it. Data of workers and our own observations suggest to regard the time and not the mass of parasitic sprouting as decisive in the extent of damage caused to the host; the mode of attachment of parasites on the roots is also important.

Material and Method

The effect of *Orobanche cumana*, a phanorogamous parasite, on sunflower was investigated in county Szabolcs-Szatmár from 1958 to 1970. Our choice has fallen on this county because it forms the very region where the first *Orobanche cumana* occurrence in the country had ever been registered, and the well-known

long maturing *Orobanche*-sensitive "Kisvárdai", together with some similar regional sorts, has been grown.

Over the years of our trial the parasite turned up in each season causing losses of varying degree, dependent on given meteorological conditions.

The localities chosen for the experiments were the following: Kisvárda, Nyírderzs, Gégény.

Time of sprouting of *Orobanche* relative to the flowering time of the sunflower attacked was recorded, and control plants in correspondent situation and number were chosen. When shoots of *Orobanche* were about to dry away, we measured the height of the sunflower, the circumference of its stem together with the diameter of its head. At the same time the number of *Orobanche cumana* shoots found on the infected plant was also taken.

The mode of attachment of *Orobanche* plants to the roots of sunflowers was studied by the washing out technique. After harvesting seed yield and oil content were recorded.

Results

In county Szabolcs-Szatmár the sprouting of *Orobanche* began in second half of July (Figure 1).

At this time variety "Kisvárdai" does not flower yet. *Orobanche* sprouts developing prior to flowering of sunflowers form characteristic bunches near and round the base, but no bunching occurs when sprouts follow the flowering time of the host. Figure 1 illustrates this, too.

Orobanche sprouts take 8–10 days to reach full height. At this time *Orobanche* begins to flower. This, in our case, coincided with the flowering time of the host plant.

It was at the appearance of the parasitic sprouts when our observations on the injurious effects of *Orobanche* with relevant examinations actually started.

Mention was already made that sprouts of *Orobanche* on variety "Kisvárdai" can develop before the host flowers. In this case a delay of 7–9 days in the flowering time of the host plant may occur. It was rather unexpected to see that sunflowers infected before their flowering time died within a fortnight, developing no yield at all.

Tabulated effect of *Orobanche* according to its time of sprouting can be seen in Table 1. These data verify our claim: whenever *Orobanche* sprouts before the host flowers, a significant damage ensues. This stands for the majority of cases, even in the presence of only a few *Orobanche* individuals; while post flowering infection, severe it may be, does not always kill the host, in fact, does not prevent it from yielding. Workers explain this with disturbances in water and nutrient supply. According to our observations, however, early dying of sunflowers – even under optimal soil conditions – occurs, thus calling in question the explanation referred above.

Fig. 1. Shoots of *Orobanche cumana* around the stem of sunflower

Table 1

Damages caused by *Orobanche cumana* to sunflower before and after flowering of the host

Number of plants checked	Time of appearance of *Orobanche* shoots relative to flowering of host	Counted mean of *Orobanche* shoots pro host	Circumference of stem	Height of stem	Diameter of head
			of host		
10	no infection	—	3.85	247.55	29.30
10	before	33.65	2.28	135.40	no head
10	after	123.17	2.89	213.90	16.50

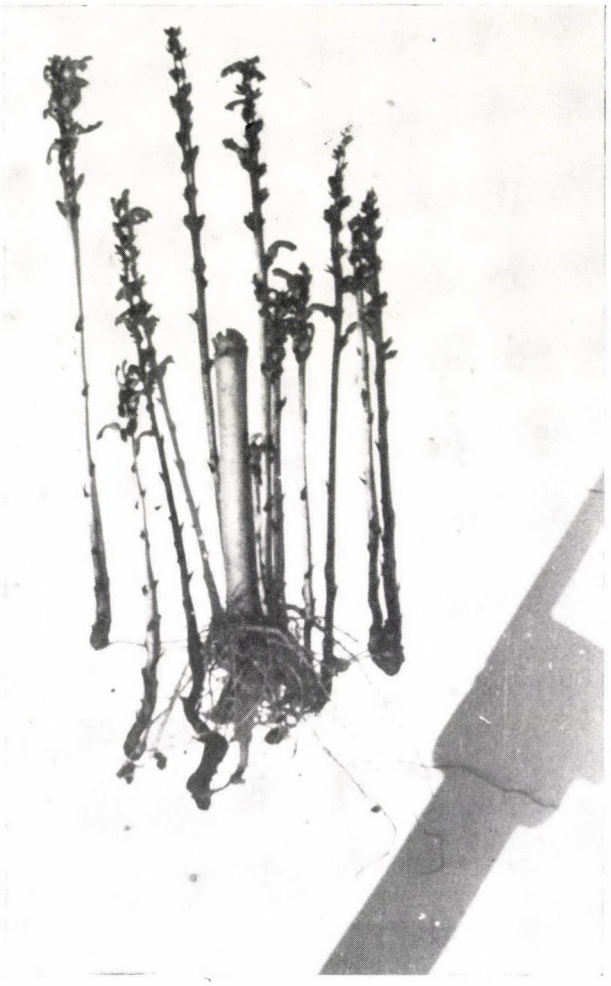

Fig. 2. Mode of attachment of *Orobanche* shoots on secondary roots of sunflower

Having taken a closer look on the roots of dying sunflowers infected with *Orobanche* prior to flowering, we learned that the parasitic shoots are subsisting – without exception – on the primary roots of the host plant; on the other hand, host plants with post flowering infection show only their diminutive roots being sponged by *Orobanche* shoots (Figure 2).

From these observations we have got the following conclusion: whenever the shoots of *Orobanche* develop in first half of vegetation – that is in a period when the primary roots of sunflower are not lignified yet – the host plant either

Table 2

Effect of *Orobanche cumana* on quantity and quality of yield of sunflower

Number of plants estimated	Healthy plants repetition I.	II.	III.	IV.	Mean
Counted number of *Orobanche* shoots on 25 sunflower plants	—	—	—	—	
Mean of counted number of *Orobanche* shoots pro sunflower plant	—	—	—	—	
Yield of 25 sunflower plants in g	1250	1310	1276	1250	1271.50
Average yield relative to control	—	—	—	—	100.00
Per cent of oil content	31.08	30.65	31.70	30.88	31.07

Number of plants estimated	Infected plants repetition I.	II.	III.	IV.	Mean
Counted number of *Orobanche* shoots on 25 sunflower plants	1689	1511	1705	1487	1598
Mean of counted number of *Orobanche* shoots pro sunflower plant	67.56	60.44	68.20	54.48	63.92
Yield of 25 sunflower plants in g	828	861	793	807	822.25
Average yield relative to control	—	—	—	—	64.66
Per cent of oil content	18.99	20.07	19.25	19.50	19.45

dies or brings but scanty yield. Infections taking place in the second half of vegetation cause less significant damages.

In the course of our experiment we have estimated the extent of qualitative and quantitative reduction of yield caused by *Orobanche*. Results are tabulated in Table 2.

These data do show that *Orobanche* infections cause serious damages both in quality and quantity of the yield. We must add, however, that our data concern exclusively the Hungarian variety "Kisvárdai".

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Developing Concepts of Plant Resistance to Infections

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Plant incompatibility versus plant resistance, pathogenicity and virulence, the specialization of hosts and pathogens, the intraspecific subdivision of hosts and pathogens, immunity, and the recognition of foreignness are treated in this paper from a terminological point of view.

Plant incompatibility versus plant resistance

When a pathogenic organism is able to cause disease on some individuals within a plant *species* but not on some other members of the host, in this case the latter group can be designated as "resistant". This determination points out the behaviour of the *host species* as a whole. Most of the plant species do not have the ability to serve as hosts for a given pathogen. In this case, not a single member of the species has the ability to enter in a host-pathogen relationship with the otherwise pathogenic microorganism. The plant species may not be regarded as resistant, however simply a "non-host". The microorganism can be designated as "*species-nonpathogenic*": it is not pathogenic to any plant individual within that particular species.

When some individuals, types or cultivars of a plant species are susceptible to a given pathogen, only in this case can we define the not diseased or slightly damaged types of the *same species* as "resistant" ones. A cultivated host species consists of different resistant and susceptible cultivars. The pathogenic microorganism is indeed "*cultivar-pathogenic*" to susceptible types of host species and "*cultivar-nonpathogenic*" to resistant plants. Pathogens are never pathogenic to the plant species as a whole.

From the foregoing it clearly follows that the relationship between the host plant and the pathogenic microorganism is one which can be characterized by interdependency. Susceptible host cultivars are diseased only by cultivar-pathogenic types, and resistant ones by cultivar-nonpathogens. The concept of susceptibility or pathogenicity in the host-pathogen combination depends on the other partner. How is it then possible to use such terms as susceptibility and resistance on the one hand, and pathogenicity or non-pathogenicity on the other, if the host-

pathogen relation is regarded as a complex? It would seem that these principles do not have their values in themselves. They are interdependent. They have been created from an anthropocentrical point of view. It is more exact to designate a certain host-pathogen complex as a *compatible* or *incompatible* one. In an incompatible host-pathogen combination the host cultivar is a resistant one and the infecting microorganism is a cultivar-nonpathogenic type. Nonhost plants (species) and species-nonpathogens may also be regarded as incompatible partners to each other. There are good grounds for the hypothesis according to which a primitive form of immunity (not-self recognition) plays a primary role in the incompatibility of plants of non-host species as well as in resistant cultivars of a host species (DeVay, Schnathorst and Foda, 1967; Burnet, 1971; Király, Barna and Érsek, 1972). It remains, however, to be seen that the physiological mechanism of incompatibility in both cases is indeed similar or identical.

In spite of the above-mentioned evidences it is inevitable to use such terms as resistant, susceptible, pathogenic or non-pathogenic, at least from a practical (agricultural) point of view. A few examples will show the issue better. The host-pathogen combination of the wheat cultivar Vernal-physiologic race 21 of stem rust *(Puccinia graminis* f. sp. *tritici)* is an incompatible one, in other words, Vernal is a resistant host producing necrotic spots. The same wheat cultivar, however, is susceptible to another race of stem rust, race 40, which produces abundant spores in the pustules. Vernal may be considered in the first case as a resistant, and in the latter case as a susceptible wheat cultivar, depending its character on the race with which it creates a complex. Strictly speaking, this host-pathogen combination may be compatible or incompatible and, in fact, the host plant as such is neither susceptible nor resistant. Similarly, races of the rust fungus are neither pathogenic nor nonpathogenic in themselves. Still, if we consider that race 21 is the most important form of the pathogen on a large area (in most European countries), Vernal can be regarded as a resistant cultivar on this wide area, at least from a practical (agricultural) point of view. The mass-attack of stem rust races other than 21 on this area is improbable at present.

If we evaluate the behaviour of a series of cultivars to a single race of the pathogen, the resistant or susceptible character of the host cultivar seems to be a practical (real) concept. For example in the following combinations:

race 21 – Marquis (compatible)
race 21 – Arnautka (compatible)
race 21 – Reliance (incompatible)
race 21 – Vernal (incompatible)

Marquis and Arnautka are really susceptible cultivars and Reliance or Vernal are practically resistant.

Another example shows the resistance in relation to the preformed resistance, in other words, in relation to resistance factor(s) which exists in the plant prior

to infection. TURNER (1961) was able to demonstrate that a toxic glucoside exists in the root of oats which is resistant to *Ophiobolus graminis*, the causal agent of take-all disease of cereals. This compound inhibits both infection and growth of the pathogen. *Ophiobolus graminis* consists of several variants (varieties) to which the host is resistant, except one, *O. graminis* var. *avenae*. This latter, as was shown by TURNER, degrades the toxic glucoside in roots by a glucosidase enzyme. In this case the host is resistant to a series of variants of the pathogen but susceptible to var. *avenae*. In the agricultural practice the concept of resistance is a necessary requirement because it seems possible to increase the content of the toxic glucoside in the host by breeding, increasing thereby resistance of the plant to the take-all fungus. One can suppose, however, that var. *avenae* will adjust itself to the host by selecting out a physiologic race with high glucosidase activity. But for all that plant breeders may evaluate the high content in glucoside as a sign of resistance at least from a practical point of view.

Compatible but relatively resistant hosts

From these examples it is seen that resistance of the host plant is relevant for only certain types (biotypes, races, variants) of the pathogen, and, therefore, it is always relative. In fact, host plants are resistant if the non-diseased group of plants (for example a cultivar) represents a genetically pure line and the cultivar-nonpathogen as a partner consists of a single pure line biotype. Host cultivars and pathogenic biotypes (or races) are *nearly pure lines*. Nearly but not perfectly. This creates in the practice further complications.

Plant pathologists and breeders are faced mostly with cultivars and races. Resistance of the host and pathogenicity of the pathogen is determined on the basis of compatibility or incompatibility. However, these are qualitative terms. Compatibility or incompatibility do not refer, as a rule, to the degree of resistance of the host or to the degree of pathogenicity of the pathogen. It became evident from practical observations that some cereal cultivars although they were regarded as compatible to a certain physiologic race of stripe rust *(Puccinia striiformis)*, produced spores in quite different rate. Some cultivars had "slow-rusting" ability as compared to others, and, therefore, were regarded as more resistant ones, JOHNSON (1972) for example has shown that wheat cultivars which are in the same reaction class or in other words they are all regarded to be compatible (susceptible) to the attack of a given stripe rust race, may produce different amounts of uredospores in the field. This is very important from the point of view of the *degree* of resistance of that particular cultivars.

JOHNSON compared the cumulative weights of uredospores of two isolates of a single physiologic race of stripe rust on two wheat cultivars. Cultivar Joss Cambier was more resistant than cultivar H46 to one of the two isolates (WYR 69/10), in spite of their similarity in producing susceptible reaction classes (both are designated as compatible on the basis of infection types). If we take another

isolate of the same race, namely WYR 71/2, the situation is reversed: H46 being the slow-rusting cultivar (more resistant) and Joss Cambier is more susceptible. These two cultivars exhibit different degree of resistance although they are similarly compatible on the basis of the generally applied method for assessment of compatibility. A more quantitative procedure, however, the assessment of the spore production, permits to designate the degree of susceptibility (or the degree of resistance).

This strange situation was experienced because the physiologic races of rusts are nearly but not perfectly pure from a genetical point of view. Race 104 E137 of *Puccinia striiformis* contains at least two biotypes (isolates) which sporulate differently on Joss Cambier and H46. Isolate WYR 69/10 preferred cultivar H46 and isolate WYR 71/2 preferred Joss Cambier.

Pathogenic (compatible) and relatively aggressive isolates: The concept of virulence

Considering this phenomenon in relation to the pathogen, it is clear that the above-mentioned cultivar-pathogenic race of stripe rust has at least two differently *virulent* isolates. One is more aggressive on cultivar H46, the other on Joss Cambier. *Virulence* of the pathogen refers to the intensity of infection, to growth rate in the host and to the rate of sporulation. One of the most important tasks of pathophysiology will be in the future to throw some light on the biochemical or physiological basis of the factors of virulence of plant pathogenic agents. The evaluation of resistance of host plants must be carried out not only in relation to pathogenicity (compatibility) of the disease agent, however, occasionally also in relation to the virulence of the pathogen.

Summarizing, the pathogenic or non-pathogenic character of the infecting agent refers to a compatible or incompatible host-pathogen relationship, respectively. The term virulent or non-virulent can be used to characterize a pathogenic microorganism in compatible host-parasite relationships quantitatively. In the case of fungus diseases the virulence of the fungal pathogen is expressed in most instances in the rate of the sporulation.

Specialization of host and pathogen

Plant resistance is very finely specialized and it seems to be much more specific than resistance of human types or races to infectious microorganisms. Differences between human varieties (or races) to infectious diseases have been only scarcely demonstrated. One example is the weak resistance of negroes to small pox. In most cases, however, human races are similarly susceptible or resistant to infectious agents. In the plant kingdom, however, plant species consist of a series of different biotypes. This complexity of species, first of all crop and ornamental plant species, has been enormously increased by plant breeding, creating thereby innumerable new biotypes or cultivars with very different degree of

susceptibility to disease. Resistance or susceptibility of these biotypes is correlated with non-pathogenicity or pathogenicity of infectious microorganisms. The gene-for-gene theory convincingly supports this idea.

The gene-for-gene theory

This concept was introduced by FLOR (1955) as a result of studying inheritance of pathogenicity in the flax rust fungus *(Melampsora lini)*. He has found that on cultivars of flax that have one gene for resistance to the cultivar-nonpathogenic parent race of rust, F_2 cultures of the fungus segregated into monofactorial ratios. Furthermore, on cultivars of flax having 2, 3 or 4 genes for resistance to the cultivar-nonpathogenic parent race of the fungus, the F_2 rust cultures segregated into bi-, tri- or tetrafactorial ratios, respectively. FLOR suggested that for each gene that conditions a host response to infection there is a corresponding gene in the pathogen that conditions pathogenicity.

The gene-for-gene concept is suitable for the explanation of the co-evolution of host-pathogen systems. The natural or artificial selection for resistance in the host leads, as a rule, to selection for pathogenicity in the population of the pathogen. Unopposed selection for resistance in the host would lead to elimination of the pathogen. This, in fact, never occurs. On the other hand, unopposed selection for pathogenicity in the infectious microorganism would lead to elimination of the host. However, in this case too, the selection for pathogenicity, which produces new cultivar-pathogenic races of the microorganism, leads to selection for resistance in the host (PERSON, SAMBORSKI and ROHRINGER, 1962; FLOR, 1971). Thus the host-pathogen interaction on a population level is controlled by the selective pressure exerted by one on the other.

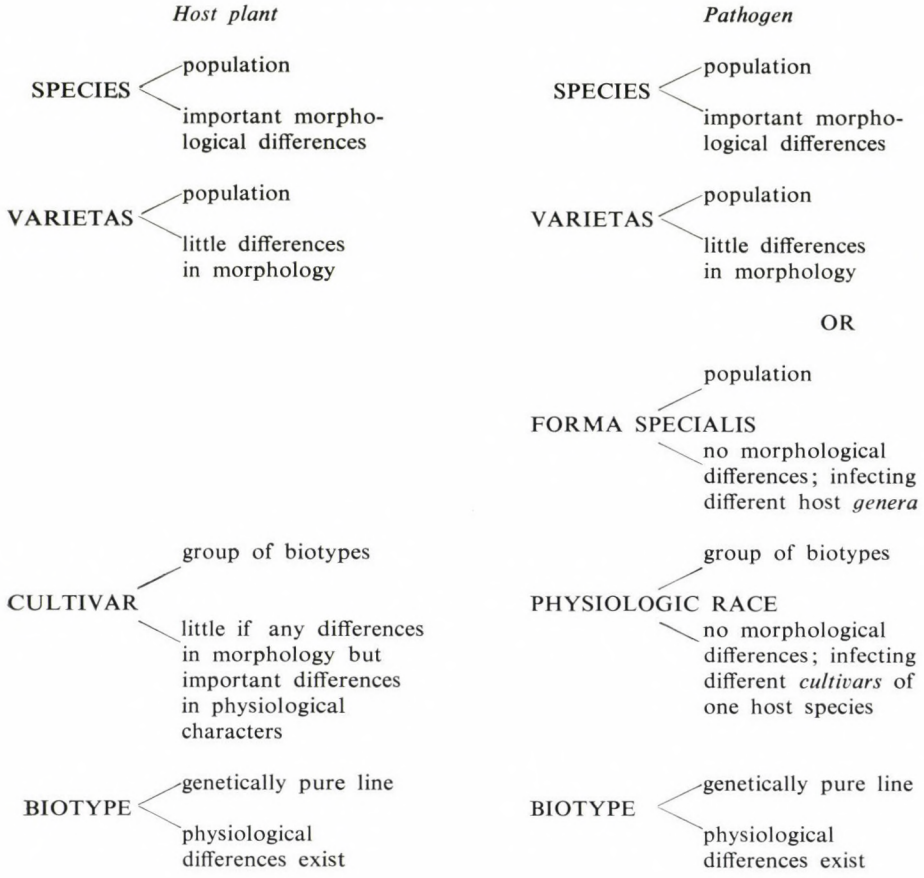
Biotypes, physiologic races, cultivars etc. (Intraspecific subdivision of host and pathogen)

It is clear from the foregoing that host biotypes are confronted by pathogen biotypes. In the everyday practice pathogenic microorganisms are regarded as cultivar-pathogenic types, which means that they are able to cause disease only in certain biotypes (cultivars) of the host. On the other hand, the same biotype which is cultivar-pathogenic to certain host biotypes (cultivars) may be cultivar-nonpathogenic to other cultivars of the host.

Table 1 gives an outline of the intraspecific position of biotypes, physiologic races, cultivars, variants etc. of both host plant and pathogen. Because different terms are used today for the intraspecific categories I have tried to apply terms on a common ground in cases of both host and its pathogen in Table 1. It is seen that biotypes and physiologic races of the pathogen as well as biotypes and cultivars of the host plant are regarded as more or less pure lines or groups of lines which exhibit rather uniformity from a genetical point of view. Certainly, they are not

Table 1

Comparison of intraspecific subdivision of host and pathogen



regarded as populations. Plant cultivars being relatively pure can be properly designated as *resistant* plants in combination with biotypes or physiologic races of pathogens which are also pure from a genetical point of view. On the contrary, plant species or variants always represent populations consisting of different biotypes. The dominating biotypes in the host species are changing under the influence of the environmental conditions. Naturally, disease resistance of the host *species* is not permanent, however, it is continuously changing, which is expressed in alterations in the biotype composition of the host. In Table 2 incompatible and compatible host-pathogen relations and intraspecific subdivision of host and pathogen are exemplified on powdery mildew disease of barley.

Table 2

Host-pathogen relations and intraspecific subdivision of host and pathogen as exemplified on powdery mildew of barley

INCOMPATIBLE COMBINATION	HOST	COMPATIBLE COMBINATION
Species	Species	Species
Erysiphe cichoracearum (Species-nonpathogenic)	*Hordeum vulgare*	*Erysiphe graminis* (Species-pathogenic)
Forma specialis	Variant	Forma specialis
Erysiphe graminis f. sp. *tritici* (Species-nonpathogenic)*	*Hordeum vulgare* var. *distichon*	*Erysiphe graminis* f. sp. *hordei* (Species-pathogenic)*
Physiologic race	Cultivar	Physiologic race
Erysiphe graminis f. sp. *hordei* physiologic race 1 (Cultivar-nonpathogenic)	*Hordeum vulgare* var. *distichon* cultivar Weihenstephan CP 127422	*Erysiphe graminis* f. sp. *hordei* physiologic race 8 (Cultivar-pathogenic) → Virulent (abundantly sporulating); Non-virulent (sporulation is relatively slow)

* Variant-nonpathogenic or variant-pathogenic microorganisms are not known as yet.

Resistance and immunity

Plant pathological literature commonly uses the term "resistance", however, occasionally (mostly in Slavic languages) "immunity" is applied as an equivalent expression. Unfortunately enough, a generally accepted nomenclature was not created and has not been distributed. This is because the basic events of plant resistance and immunity were not discovered and basic principles were not pronounced on a biochemical or even biological level. Resistance and susceptibility are two extreme possibilities but several steps are also existing between these two. Different types of resistance and susceptibility and the intermediates are defined in the literature mostly on the basis of the infection types of STAKMAN and co-

workers (1944, 1962), based on size of pustules of rust spores and the condition of the surrounding wheat tissues. This scale was proposed originally only for distinguishing rust races and for practical evaluation of one of the different forms of cereal resistance to stem rust (*Puccinia* spp.). The highest grade of resistance in this scale is called "immune" type of reaction. No rust pustules develop and no other symptoms are seen on the host. This, however, does not mean that the host is, in fact, in an immune state, in other words, that host condition has been changed in consequence of an immune reaction.

This term is untenable in this form both from pathological and immunological point of view. One can not use this term simply designating a high degree of resistance which is entirely without symptoms. Strictly speaking, the state of immunity means that the host is physiologically altered as a result of a primary infection (inducer), and, therefore, it will not be diseased by a subsequent (challenge) infection of the same pathogen. It is an acquired state, an acquired resistance to subsequent infections. This phenomenon is well known among higher animals. Newly discovered phenomena in plant pathology (cf. KIRÁLY, 1968) clearly show that the state of immunity caused by an inducer infection also exists among higher plants and the immune plants are resistant to challenge infections. As to the mechanism of the immune reaction, it remains to be seen the nature of the biochemical alterations in host physiology.

It follows from the foregoing that the term "immune" cannot be applied to designate a very high degree of plant resistance (without symptoms), even if this used to be a common practice in the past in plant pathological literature and in the every-day wording of plant breeders.

According to GAUMANN (1946) two forms of resistance exist: 1. *Preformed* resistance or axeny (inhospitableness, incompetence, natural resistance) and 2. the *defence* reaction.

Preformed resistance is present before infection, prior to the plant becoming a host. This characteristic of the plant exists before attack by the pathogen, i.e. it is a preformed resistance. It is based on morphological or chemical barriers. For example, if the plant contains high concentrations of materials that are toxic to the pathogens or if the tissues do not contain compounds that are essential to the pathogens, we are dealing with preformed resistance.

Defence reaction, on the other hand, was regarded as a postinfectional characteristic which begins to operate only after infection. It would be an active, a real "reaction", an induced capacity.

Recognition of foreignness

However, it turned out that "defence" against pathogenic microorganisms is only an anthropomorph aspect of the more general phenomenon of "recognition of foreignness" by the organisms whether they are plants or animals (cf. BURNET, 1971). In spite of the fact that pathogenic microorganisms are inhibited from

further growth or even killed in resistant plant tissues, one can regard this type of incompatibility (resistance) as being related to the phenomena of "self" and "not-self" recognition, rather than defence to pathogens. The plant is able to recognize "not-self" macromolecules (proteins) of infecting microorganisms by a primitive immunity phenomenon. As a result of the recogition of foreignness a positive rejection occurs, and in most cases necrotic material develops between the host and the infecting ("foreign") agent. The necrosis of the host tissue is the most characteristic sign of the hypersensitive reaction. However, we have shown (KIRÁLY, BARNA and ÉRSEK, 1972) that hypersensitive necrosis connected with the production of phytoalexins is only a consequence, not the cause, of resistance to different fungi. The unknown mechanism of the primary step in host resistance is certainly connected with the recognition of the foreign "not-self". It may be connected with the lack of common antigenic proteins in host and pathogens (DOUBLY et al., 1960; WIMALAJEEWA and DEVAY, 1971). According to the "common antigen theory" serologically similar proteins (and other antigens) occur in the compatible host-pathogen relationship, but common antigens are lacking in the incompatible host-parasite relations. Host incompatibility (resistance) may be dependent upon unsuccessful molecular mimicry by the infecting angent. The recognition of the foreign infecting microorganism which does not share serologically similar proteins with the higher plant would be the basic phenomenon of incompatibility (resistance). There is a growing tendency to regard recognition as the basic phenomenon of immunity. It must be stressed that recognition of foreignness is not analogous to the adaptive immunity of vertebrates, however, it is probable a primitive step in the evolution of the immune systems of higher organisms (BURNET, 1971). In summary, plant resistance phenomena can be divided into three groups as follows: 1. *Preformed resistance.* 2. *Recognition of foreignness* (incompatibility, primitive immunity, lack of common antigens between host and pathogen). 3. *Acquired immunity* (a primary infection induces resistance to a challenge infection).

A strange host-pathogen combination develops if the host is susceptible to infection but not to the disease. In other words it is resistant to disease though susceptible to infection. This state of the plant is called *tolerance* (cf. SCHAFER, 1971).

With regards to terminology, it is necessary to deal here with two additional terms which are used by plant pathologists and plant breeders interested in practical questions of disease resistance. These terms are: "*vertical*" and "*horizontal*" resistance. When a cultivar is more resistant to some biotypes or physiologic races of a pathogen than to others the resistance is called vertical. On the other hand, when host resistance is equally spread against all biotypes or races it is called horizontal (VAN DER PLANK, 1968). Usually, but not necessarily, vertical resistance (race-specific resistance) is monogenic and easily overcome by new physiologic races. Horizontal (not race-specific) resistance is polygenic in character and is safer than the former one. These terms are practical in nature. It is

very difficult to find a physiological explanation for them. In addition, vertical resistance probably never occurs unaccompanied by horizontal resistance, so the plant cultivars do not have either pure vertical or pure horizontal resistance. Since the aim of this paper is to approach plant disease resistance from a biological, not epidemiological point of view, the terms vertical and horizontal resistance will not be treated in detail. Similarly, expressions like seedling resistance and field resistance (adult plant resistance) will not be considered because these have nothing to do with the mechanism of plant resistance to microorganisms.

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Theoretical Concepts of Disease Resistance

By

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Several concepts in disease resistance are examined. The terms "immunity" and "immune" which are currently used in plant pathology do not convey the exact meanings as they literally should. Resistance carries more meaning than immunity and is recommended to be used.

Two types of resistance have been recognized: general resistance and specific resistance. General resistance is the resistance of plants against several organisms. Specific resistance is the resistance of plants against specific parasites.

Resistance against parasites is not due to structural barriers such as thick epidermis, lack of leaf hairs, thick cuticle, sugar content, osmotic pressure, pH and rH and other features. Chemical toxicants such as prohibitins, phytoalexins and other post-infectionally formed inhibitory substances appear to be important in defense reactions.

Prohibitins have been classified into general and specific prohibitins. General prohibitins are present in several plant species and sometimes in different families of different orders, conferring general protection against several parasitic microorganisms. Specific prohibitins are present in specific species and their occurrence may be confined to specific tissues and may be active against a few parasites.

Some of the recent results on phytoalexins have been re-examined. Because there are certain structural relationships between the various phytoalexins produced by closely related plant species, they are classified into general and specific phytoalexins.

All these views have been integrated with general resistance and specific resistance. General resistance includes the participation of general prohibitins and general phytoalexins while specific resistance is displayed through specific prohibitins and specific phytoalexins.

The theory on specificity is re-examined and is supported with data. Applying the principles of the theory and considering the fact that nutritional requirements of plant parasites do not differ, a classification of plant parasites is proposed. Accordingly, they are considered as monophagous, oligophagous and polyphagous parasites depending upon the number of species of plants they infect. Monophagous parasites infect only one species of plants, oligophagous parasites infect several species of plants of one family and polyphagous parasites infect plants of several families.

It can be claimed that since the publication of *Principles of Plant Infection* (GÄUMANN, 1950) there have been no major conceptual advances in disease resistance. What has happened in the last 20 years is the revival of some of the older

concepts that have remained obscure. Since the beginning of the sixties, the revival of phytoalexin theory (CRUICKSHANK, 1963; MÜLLER, 1969), phenol theory (FARKAS and KIRÁLY, 1962; HERRMANN, 1962; KUC, 1963; MAHADEVAN, 1966) and prohibitin concept (MAHADEVAN, 1970) has given fresh impetus to workers in disease resistance. Concomitant to these is the availability of coherent information concerning the chemical make-up of plant species and in particular, their secondary constituents (KARRER, 1958; HEGNAUER, 1962, 1963, 1964, 1966, 1969). Apart from their usefulness as taxonomic markers, these display antimicrobial activity and naturally their importance is being emphasized in disease resistance (ERDTMAN, 1949; STOESSL, 1970). On the whole, the number of publications on disease resistance in the last few years has been exponentially expanding. Consequently several new interpretations on resistance have been presented by a few reviewers to accommodate anomalous results (WOOD, 1967; METLITSKIJ and OZERETSKOVSKAYA, 1968; FAWCETT and SPENCER, 1969). Some of these interpretations are not only confusing but at times challenge the very validity of the fundamental postulates of theories on resistance. Moreover, some of the discarded concepts, e.g. structural barriers, have been revived (WOOD, 1967; AGRIOS, 1969) apparently based on meager experimental evidences. Naturally a critical appraisal of the literature – old and new – and presentation of newer theoretical interpretation to the various findings, wherever necessary, become indispensable. These have been attempted here.

Immunity and immune. Frequent references to immunity and resistance are common in literature. The meanings of immunity and resistance require a critical evaluation. In a broad sense, these two terms are complementary but strictly from a scientific view, immunity and resistance are two different things. VAVILOV (1950) believed "By the term immunity we mean the lack of susceptibility of organisms to diseases". More explicitly RUBIN and ARTSIKHOVSKAYA (1963) defined immunity as "The ability of a living organism to resist infection when brought into direct contact with the causative agent of a particular disease . . . We consider that it would be more correct to understand immunity as nonsusceptibility of the organism to disease, no matter whether any interrelations between the plant and parasite are established on contact, and no matter what are the causes of the resistance". KUC (1966) put it somewhat differently but with the same implication "Various degrees of resistance are possible, with immunity being the ultimate in resistance. Immunity is absolute, therefore an immune plant would not be attacked by an infectious agent". A similar view was upheld by WOOD (1967) among others.

"Immunity" was borrowed from the medical literature where it, however, means anything concerned with resistance. CAMERON (1956) aptly pointed out that immunity is an indefinite term, is most frequently used as a generalized word and it may be natural, acquired, absolute or partial. WELSH (1961) a plant pathologist, after critically evaluating the meaning and implication of immunity in plant pathology explicitly states "Use of the term 'immunity' is fraught with comparable risk". Moreover, virtually every plant is infected in the sense that it

carries microorganisms, even "germ free" tissues may carry viruses. Therefore continued usage of "immunity" to indicate "absolute resistance" is certainly misleading in plant pathology.

Another equally erroneous word is "immune". Literally it means "exempt" or "free" (Oxford English Dictionary, 1959), and presumably free from infection or disease. In fact as early as 1907, MARRYAT believed in the "immune" nature of plants with this reserved meaning in mind. Furthermore, the immune state is due to the presence of specific substances, "antibodies". Virtually nothing is known about the existence of antibody in plants. Therefore this term ought to be avoided in the literature.

When one carefully examines the voluminous literature on host-parasite interaction, one is clearly struck by the banal fact that the capacity to resist parasites is not uniformly distributed in a plant; it varies from one tissue to another, from one organ to another, and from one parasite to another. Consider that leaf-spotters seldom infect roots and root-invaders scarcely colonize leaves!

Moreover, the literature tells us that resistance is not a stable character of plants; it is highly conditioned by environment, among others. As an example, consider the classic observation of WATERHOUSE (1929). Marquis wheat varied from complete susceptibility to forms *Puccinia graminis tritici* 46 and 55 during summer, to moderate resistance in winter. Normally Marquis is moderately resistant to form 46 but to 55, it is highly susceptible. If, however, the environmental conditions change its reaction to form 55 from susceptibility to moderate resistance, the line of distinction between the two forms largely disappears. Evidences of this nature are abound in the literature (REED, 1935; GÄUMANN, 1950). To these, one may recall VAVILOV's notation that the thing which is inherited is not definite degree of "immunity" or susceptibility, but a norm of reaction under different conditions (VAVILOV, 1950). Naturally, therefore, one cannot remain without casting doubts on the validity of the grades of resistance proposed by several workers for various host plants, e.g. the grades of rust resistance for wheat varieties proposed by STAKMAN and LEVINE (1922).

Admittedly the relationship between resistance and susceptibility is a subtle one and these terms are in fact complementary. Susceptible means least resistance, and therefore the word resistance qualifies for the relative expression of a character. In disease resistance a relative term such as resistance serves its purpose as long as one qualifies it with proper adjectives, "extreme", "high", "moderate", "partial" and "least". Least resistance is a state of susceptibility and therefore this word becomes redundant in plant pathological literature; KEYWORTH (1955) among others, strongly agrees with this view.

Types of resistance. There have been attempts to differentiate the resistance of plants. TOMIYAMA (1963) considered two types of resistance: "major gene resistance" and "field resistance". In the former, resistance is controlled by specific genes. Varieties having this qualitative type of resistance are usually highly resistant to specific races of parasites but are susceptible to others. Field resistance

is quantitative, i.e. varieties having field resistance may differ in the level of resistance and generally the degree of resistance is easily effected by environmental factors.

VAN DER PLANK (1968) reinterpreted this view and presented "vertical resistance" and "horizontal resistance" concepts. Vertical resistance or differential resistance is the "differentially interacting resistance or susceptibility of a host". A vertically resistant plant has greater resistance relatively to some races of the parasite than to others. Horizontal resistance or uniform resistance is the resistance spread evenly against all races of a parasite.

Vertical resistance and major gene resistance signify the same thing. Similarly field resistance and horizontal resistance are functionally ambiguous. However, when one considers the various manifestations displayed by plants in response to infection by microorganisms, certain general defense reactions are activated in almost all the plants. And in the cells that are in direct proximity with the infectious agent, besides the general mechanisms, some specific reactions are activated primarily to combat the infecting parasite. It is here that varieties differ in their intensities of response. Therefore, two kinds of resistance response displayed by infected plants are recognized: general mechanisms of resistance and specific mechanisms of resistance.

General resistance. This is the resistance of plants against several organisms including parasitic microorganisms. This resistance is spread throughout the plant. Because of general resistance, most plants are resistant to most microorganisms.

Specific resistance. This is the resistance of plants active against specific parasites. This resistance is presumably due to the activity of a few genes. A plant may have several specific resistances to combat specific parasites. Because most parasites have tissue preferences, this resistance is apparently confined to special tissues.

Both general resistance and specific resistance are characteristic of all plants regardless of susceptible and resistant varieties. The difference is the velocity with which the varieties mount their defense; resistant plants do so faster presumably due to their "acceleration genes" (MÜLLER and BÖRGER, 1939) than the susceptible, in which these genes are functionally recessive, hence they respond slowly. In the absence of vigorous defense, the parasite will obviously continue its onslaught.

It is interesting to note that VAVILOV (1950) formulated generic resistance (= "generic immunity") and specific resistance (= "specific immunity") to distinguish the resistance displayed by genera and species of plants. This approach is based on the evolutionary principles if one recalls PLUNKETT's (1944) remarks "While some of the specificity of organisms is individual, the greater part of it is group specificity common to a group of individuals". To a large extent, general resistance and specific resistance are complementary to generic resistance and specific resistance.

Structural barriers and resistance. Where there is a revival of opinion that certain special structures in plants confer resistance (WOOD, 1967; AGRIOS, 1969), earlier progressive plant pathologists always preferred to discount their importance. As early as 1902, WARD recognized: "The capacity for infection, or for resistance to infection, is independent of the anatomical structure of the leaf, and must depend on some other internal factor or factors in the plant". More explicitly WARD (1905) stated: "Nothing to do with anatomy, but depends entirely on physiological reactions of the protoplasm of the fungus and the cells of the host".

From plant breeders' view, ORTON (1908) summed up the situation. "Structural differences do not seem to play much part in enabling plants to resist the true parasite. Satisfactory demonstration of cases where resistance to highly adapted parasites is due to thickened epidermis, development of hairs etc. are lacking. It is hard to understand why a thick cell wall should protect from infection a leaf which has many thousand openings as breathing pores through which a fungus might enter." In fact there is no difference between the velocity of penetration of the cell walls of resistant and susceptible cells by a parasite; the difference in response is found only in the attitude of host cells against the parasite after penetration (WOOLMAN, 1930; MEYER, 1939; TOMIYAMA, 1963). Rightly GÄUMANN (1950) pointed out that "The plant is susceptible to infection but is not habitable".

Yet it will remain a great mystery in disease resistance why these pioneering views were overlooked by some of the reviewers (RUBIN and ARTSIKHOVSKAYA, 1963) who emphasized the significance of morphological and anatomical features in disease resistance.

Similarly these reviewers content that cuticle offers a strong passive barrier against the penetration of parasitic microorganisms. MARTIN (1966, 1967) who extensively investigated the chemistry of cuticle and its relationship to resistance was skeptical about cuticle in preventing the penetration of parasites. Aptly MARTIN (1967) concluded: "The cuticles of many plants are extremely fragile and if anything, contribute to susceptibility by permitting the outward diffusion of nutrients rather than to the resistance. The heaviest cuticle that we have so far found, that of an ornamental plant, is readily penetrated by a powdery mildew."

Other tissue barriers. Features such as osmotic pressure, sugar content, and acidity of cell sap as factors of resistance could be excluded from the defense strategy of plants. In support of this, ORTON (1908) postulated: "The evidence indicates that the resistance is due to a specific protective reaction of the host cell against the parasite. So in plants the evidence leads us to believe that more is involved than the acidity of the cell sap or the chemotactic effect of sugars or other food substances. The first group is the most important, relating as it does to diseases due to the most highly developed parasites, such as the rusts, mildews and other injurious fungi."

More recently, RADTKE (1969) after critically investigating the problem concluded that neither osmotic pressure nor sugars in the resistant potato cultivar contribute to the resistance against *Fusarium caeruleum.* The growth of plant

parasitic fungi and bacteria is not much affected by pH and rH over a wide range (KERN, 1956). Naturally they can be precluded as resistance factors.

Nutrients offered by a prospective host plant have been considered as an important aspect of the resistance mechanisms. MÜLLER and BÖRGER (1939) conclusively ruled out the nutrient aspect as the resistance factor in potato against *Phytophthora infestans*. Moreover, nutritional requirements of mutants of *Venturia inaequalis* and *Colletotrichum coccodes* did not limit their pathogenicity (WILLIAMS and BOONE, 1963; LOPRIENO et al., 1964). Furthermore all plants contain nutrient substances even though these may differ in concentration and quality. Yet most plants seem to be remarkably free from diseases. Moreover, the nutrient hypothesis says nothing about the plants that are not infected by parasites.

Therefore the best strategy of plants against parasites is to inhibit them. This inhibition is achieved by preformed inhibitory substances—"prohibitins" and post-infectionally formed inhibitory substances—"phytoalexins". Other chemically conditioned barriers such as "wound healing" and "physical barricades" develop in response to infection (NAEF-ROTH, 1948; NOLL, 1949). These also contain a spectrum of substances toxic to microorganisms (BOPP, 1959; MCKEE, 1961; METLITSKIJ and OZERETSKOVSKAYA, 1968). Clearly the principal gambit of plants is the chemical armory.

Prohibitins and prevention of infection. Extensive occurrence of prohibitins in plants was reviewed by SKINNER (1955) and MAHADEVAN (1970) and their importance in disease resistance was emphasized by FAWCETT and SPENCER (1969), MAHADEVAN (1970) and STOESSL (1970). A few model cases that have remained virtually obscure in the literature will be considered here.

A classic observation on the participation of prohibitins in preventing the development of parasites was made by RUDLOFF (1935). Extract prepared from *Pyrus communis* leaves at 1 : 250 dilution inhibited the conidial germination of *Venturia inaequalis*, the apple-scab parasite but caused no effect on *V. pirina*, the pear-scab fungus. The extract contained arbutin (I) and hydroquinone (II) (Fig. 1). In extremely low concentrations, these were highly toxic to the conidial germination and mycelial growth of *V. inaequalis*. Understandably RUDLOFF proposed that *V. inaequalis* does not parasitize pear because of the presence of potential toxic substances in the leaf. It is pathogenic to apple because apple does not contain such toxic substances and *V. inaequalis* does not appear to be sensitive to whatever toxic substances apple contains. In fact KIRKHAM (1957) observed that phloridzin (III), a phenolic glucoside present in apple, strongly stimulated the conidial germination of *V. inaequalis*.

Similarly VON GUTTENBERG and STRUTZ (1952) found that *Zea mays* extract inhibited the germination of bunt spores of *Ustilago avenae* and *U. hordei* but had no effect on *U. zeae*. Likewise an extract from *Avena sativa* inhibited the germination of *U. zeae* and *U. hordei* but was nontoxic to *U. avenae*, pathogenic to oats!

Recently SCHÖNBECK (1967) presented the most convincing evidence on the

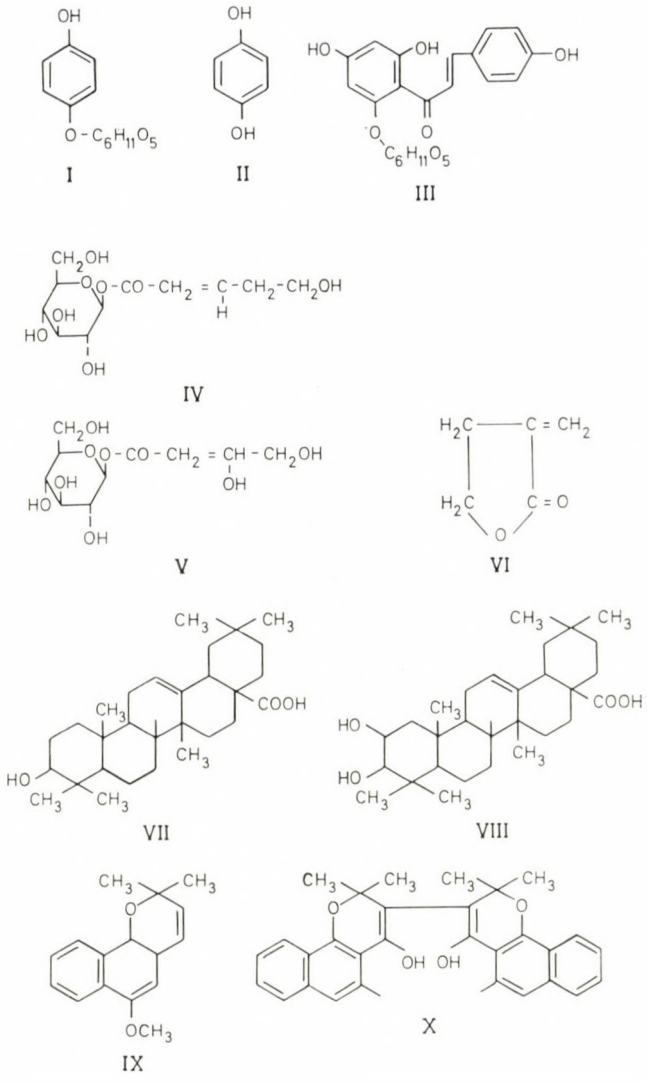


Fig. 1

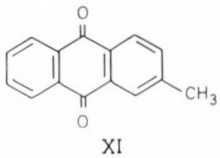
participation of prohibitins in *Tulipa gesneriana*. *Botrytis tulipae* specifically infects tulips; it penetrates the pistil not only through the stigma but also from the petals. Other fungi such as *B. cinerea*, *Phytophthora cactorum*, and *Pythium debaryanum* which are pathogenic to a large number of cultivated plants do not penetrate pistils and not even through the wounded pistils. Coinciding to this observation is the fact that the various parts of flowers, in particular pistil and stigma

contain high concentrations of inhibitory substances (Fig. 3). SCHÖNBECK believed that the actual concentration of the toxic substance must be more than several times necessary to cause inhibition of *B. cinerea* and *P. debaryanum*. The inhibitor from tulip was toxic to several fungi except to *B. tulipae*. But when the inhibitors were removed by leaching, these fungi promptly infected the tissues. The inhibitors are tuliposides A and B (TSCHESCHE et al., 1968) (IV,V) and possibly α-methyl-butyrolactone (VI) (TSCHESCHE et al., 1969). This classic study of SCHÖNBECK is conclusive in that prohibitins insure protection against the infection of parasitic microorganisms.

There are several other interesting evidences presented by tree pathologists. Oak (*Quercus* sp.) is not attacked by *Polyporous betulinus* because of its high tannin content (LUTZ, 1929). Similarly the heartwood of *Abies balsamea* is highly resistant to the growth of *Cytospora* sp., *Corticium polygonium*, *Rhizopus nigricans*, *Cladosporium herbarum* and *Penicillium* sp. (ETHERIDGE, 1962). But both *Quercus* sp. and *A. balsamea* wood upon heat treatment became susceptible to the fungi since the heat treatment removed the inhibitory substances. According to KLINGSTROM (1969) when the wood of *Pinus sylvestris* was leached in water, it became susceptible to *Melanospora pinitorqua* because leaching removed the toxic substances from the wood.

More conclusive are the findings by RUDMAN and his group in Australia. DA COSTA and RUDMAN (1958) found that healthy and ether extracted heartwood of *Eucalyptus microcorys* was remarkably resistant to decay caused by *Trametes lilacinogliva*, *Coniophora cerebella*, *Coriolus versicolor* and *Fomes durus*. But methanol extracted sawdusts were highly susceptible to the fungi. Methanol obviously removed the toxic substances from the heartwood. In fact when the methanol extract was added to the sawdust of susceptible *E. regnans*, it conferred resistance to the test fungi. Similarly RUDMAN (1963) extracted the heartwood of *E. marginatus* in 0.1 *N* NaOH. This fraction was highly toxic to *Coniophora olivaceae*. Not surprisingly, the extracted heartwood lost its resistance to the fungus.

One is most impressed by the finding of SANDERMANN and FUNKE (1970). Some of the temples of the classic Maya period in Mexico contain door lintels and carvings made from the wood of *Zapote faigan*, *Z. blanco*, *Z. rojo* and *Z. morado*. These have resisted parasitic fungi for more than 1200 years. This resistance is due to the sapogenins, hydroxy and dihydroxyoleanolic acids(VII, VIII). But what is surprising is the fact that the inhabitants of the Maya period without knowing the chemical basis of disease resistance fully exploited it! The same generality holds good for the temples built by the ancient Hindus who made the door lintels, carvings, palanquins and chariots from teak wood *(Tectona grandis)* among others. According to SANDERMANN and DIETRICH (1959) the heartwood of *T. grandis* is enriched with lapa chonon (IX), tectol (X), tectoquinone (XI) and dehydrotectol (XII). These are highly toxic even in extremely low concentrations to wood rotting fungi such as *Coniophora olivaceae*, *Lentinus lepideus*, *Lenzites trabea* and *Poria monticola* (RUDMAN, 1961, 1963).



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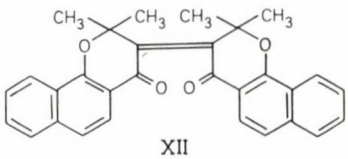

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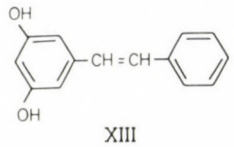

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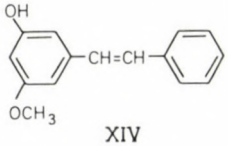

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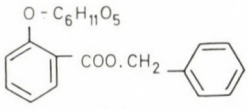

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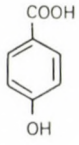

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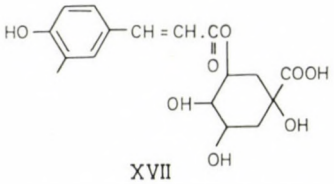

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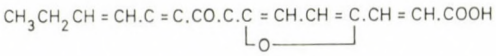

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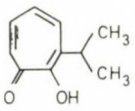

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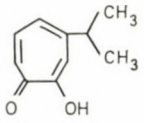

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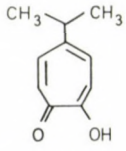
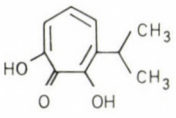


XXI



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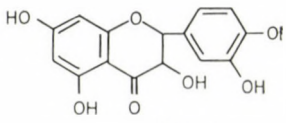


XXIII

Fig. 2

Furthermore it is relevant to recall that several plant parasites are considered to be wound parasites because wounding the tissues enables them to infect the tissues. Paradoxically wounding effectively removes the barriers that contain substances toxic to the infectious agents, at least this is what has been reported for banana (WARDLAW et al., 1939; MULVANA et al., 1969), orange (EL-TOBSHY and SINCLAIR, 1964) and tulip (SCHÖNBECK, 1967; BERGMAN and BEIJERSBERGEN, 1968).

Considering the results of other workers and these, one is persuaded to believe that prohibitins confer protection against the infection of parasites and a decrease in concentration will be directly reflected on the expression of resistance. This conclusion has formed part of the theory on specificity (MAHADEVAN, 1969).

Ancilliary to the prohibitin concept is an idea presented by RENNERFELT in 1945. RENNERFELT examined the decay resistance of several trees and concluded that decay resistance is intimately associated with the presence of certain toxic substances in the trees. For example, *Pinus* spp. contain pinosylvin (XIII) and pinosylvinmonomethyl ether (XIV). Both are extremely toxic to decay fungi such as *Coniophora puteana*, *Lentinus lepideus* and *Merulius lacrymans*, but pinosylvinmonomethyl ether was more toxic than pinosylvin was. *Pinus* spp. however, differ in their prohibitin content; all the species contain pinosylvin while pinosylvinmonomethyl ether is present in a few species. Consequently RENNERFELT proposed that pinosylvin acts as a *general toxicant* against fungal parasites while pinosylvinmonomethyl ether, being extremely toxic and being present in specific tissues acts as a *specific toxicant*. This unorthodox idea, like the prohibitin concept, did not capture the imagination of disease resistance workers.

But when one considers the chemical make-up of plant families (KARRER, 1958; HEGNAUER, 1962, 1963, 1964, 1966, 1969), RENNERFELT's idea adds a new dimension to the prohibitin concept. For instance, the extensive literature on prohibitins can be reconciled with the general and specific toxicants concept. Accordingly we recognize two kinds of prohibitins: general and specific prohibitins.

General prohibitins. These are present in several plant species and sometimes, in families of different orders (Table 1). Most of them are toxic to microorganisms and there is no specificity of toxic action since tannins, catechol, pyrogallol etc. inhibit bacteria, fungi and viruses. Therefore their presence in plant tissues may be associated with general protection from microorganisms.

Specific prohibitins. In contrast to general prohibitins, specific prohibitins seem to be present in a few species only and less commonly being present in some specific tissues. For example, xanthatin is highly concentrated in the walls of burs of *Xanthium pennsylvanicum* (LITTLE et al., 1950). In *Populus trichocarpa*, trichocarpin (XV), the anti-*Dothichiza populea* factor is present in large amounts in leaves and buds (BUTIN, 1960). In fact the literature is sprinkled with such factual data (Table 2). Specific prohibitins may be active against a few specialized parasites.

True, the lists of prohibitins are impressive, but there is an extreme paucity of accurate quantitative data on the distribution of general and specific prohibitins

Table 1

General prohibitins of plants (compiled from ABRAHAM, 1949; KING, 1953; DUQUENOIS, 1955; SKINNER, 1955; THAPLIYAL and NENE, 1967)

Anthocyanidin	Pinosylvin
	Pinosylvinmonomethyl ether
Caffeic acid	Protocatechuic acid
Catechins	Pyrogallol
Catechol	Quercetin
Carvarcrol	pQuinone
Chlorogenic acid	Hydroquinone
p-Coumaric acid	Hydrothymoquinone
Coumarin	Thymoquinone
Cyanidin	Salicylic acid
Ellagic acid	Scopoletin
Eugenol	Scopolin
Ferulic acid	Syringic acid
Gallic acid	Tannic acid
Guaiacol	Tannin
Kaempferol	Thujaplicins
Lapachonon	Thymol
Leucocyanidin	Umbelliferone
Oleuoresin	Vanillin
Phloroglucinol	
α-Pinene	

in different plant species and in different tissues and on their antimicrobial activity. Therefore it is not presently possible to make well-founded generalizations concerning the discrete contribution made by prohibitins to the overall defense strategy of plants.

Phytoalexins. Since the appearance of CRUICKSHANK's review "Phytoalexins" in 1963, the subject has been attracting many workers in different countries and since then, the number of publications on phytoalexins has been growing almost in an exponential fashion. Naturally with new information, newer approaches to phytoalexin have been made. One of the newest interpretations is a provocative classification of phytoalexins presented by FAWCETT and SPENCER (1969). They classified phytoalexins into true-, pseudo-, and false-phytoalexins. A true phytoalexin is produced only "in the living host in response to the invading pathogen" and pseudo-phytoalexin is "produced not only in response to contact with pathogens but also by a chemical or physical treatment of the host". Examples of pseudo-phytoalexin, according to FAWCETT and SPENCER, are pisatin and orchinol. False phytoalexins "can be produced in the living host in response to the invading pathogen but they originate from host-borne precursors which may also be degraded to the fungitoxic compounds in the absence of the living host". An example of this group is the production of 4-hydroxybenzoic acid (XVI) by *Sclero-*

tinia fructigena from chlorogenic acid (XVII) in apple fruit or from chlorogenic acid added to the fungus in culture medium (FAWCETT and SPENCER, 1968).

There are several grave questions concerning the validity of this classification because of the recent understanding on some of the so-called phytoalexins. FAWCETT and SPENCER proposed this classification presumably based on CRUICKSHANK's paper. He considered ipomeamarone, isocoumarin and trifolirhizin as phytoalexins besides pisatin and orchinol. There are creditable arguments presented among others by CRUICKSHANK to consider pisatin and orchinol as phytoalexins. But the role of ipomeamarone, isocoumarin and trifolirhizin in disease resistance is doubted by many workers.

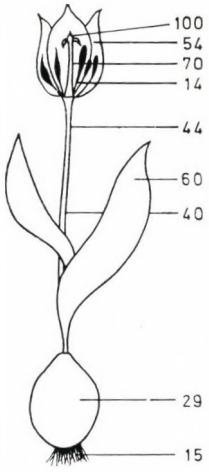


Fig. 3. Relative inhibitor content of different parts of tulip (from SCHÖNBECK, 1967)

First, when one considers the various publications concerning the formation of ipomeamarone and isocoumarin in *Ipomoea batatas* and in *Daucas carota*, respectively, the following conclusions are inescapable: 1. There is no hypersensitive reaction involved either in sweet potato or in carrot, 2. These substances are synthesized by tissues in response to mechanical injury, chemical toxicants and environmental stress, particularly freezing. 3. These are more injurious to the cells that produce them than to parasites such as *Ceratocystis fimbriata*. 4. The participation of ipomeamarone in the resistance of *I. batatas* against parasitic infection was repeatedly questioned by URITANI himself (HYODO et al., 1968; 1969) and WEBER and STAHMANN (1966) and isocoumarin in *D. carota* by CHALUTZ et al. (1969) and STOESSL (1969). URITANI and his associates who examined the production of ipomeamarone by sweet potato in response to inoculation with several races of *C. fimbriata* found no relationship between ipomeamarone production

and the spread of the pathogen in the tissues. Consequently they concluded: "Furanoterpenoides including ipomeamarone may not be associated with a defense action of the host" (HYODO et al., 1969).

Similar discrepancies have come into light concerning the role of isocoumarin in the resistance of carrot root. CHALUTZ et al. (1969) measured the toxicity of isocoumarin on several isolates of *C. fimbriata* and found no correlation between pathogenicity and concentration required to inhibit the isolates. Rightly they concluded: "The role of MMHD* in the resistance of carrot roots to *C. fimbriata* should be re-examined. The fact that this fungus is inhibited in culture by MMHD in concentrations lower than those found in the host may or may not be relevant to substantiation of the role attributed to MMHD in resistance, particularly in view of the present results." Another formidable objection to the consideration of isocoumarin as a phytoalexin is that it is synthesized by *C. fimbriata* in culture medium (STOESSL, 1969) and possibly in the infected tissues. Cautiously STOESSL put it: "One must conclude that a critical experimental re-examination is mandatory before the dihydroisocoumarin can be accepted as a carrot phytoalexin."

Secondly, consideration of trifolirhizin as phytoalexin is not warranted because the information is disappointingly meager. True, it is present in *Trifolium pratense* (BREDENBERG and HIETALA, 1961) but nothing is known about its antimicrobial activity. Moreover virtually nothing is known about its synthesis by the infected clover plants. Therefore one wonders why in the first place trifolirhizin was considered as a phytoalexin! Actually it should have been considered as a prohibitin, and in this paper, it is considered as a prohibitin (Table 2).

Clearly therefore the lines of distinction drawn by FAWCETT and SPENCER (1969) for true- and pseudo-phytoalexins disappear. The false phytoalexin concept proposed by these workers is intriguing but a major criticism that can be levied against it is that cleavage of a substance such as chlorogenic acid can be brought about by enzymes of the plant and in some instances by the invading parasite. Several other evidences of this nature are abound in the literature, e.g. arbutin is readily broken down to hydroquinone and glucose by β-glucosidase of *Pyrus communis* (SIEBS, 1964) as well as by the β-glucosidases of parasitic microorganisms. Obviously one wonders whether the appearance of new compounds can be considered as "false phytoalexins"!

In this connection, however, it is worthwhile to examine a recent finding of HIGGINS and MILLAR (1970). *Stemphylium loti* weakly pathogenic to alfalfa, induces the production of phytoalexin in the plants but the amount of the phytoalexin in the infection droplets was small. The pathogen rapidly degraded the phytoalexin and the degradation product was highly toxic to the germ-tube growth of the conidia. Similarly *Colletotrichum phomoides* not pathogenic to alfalfa, also degraded the phytoalexin but converted it into efficient toxicant. In these instances,

* Isocoumarin

Table 2

Occurrence of specific prohibitins (constructed from references quoted in Table 1)

Plant	Prohibitin
Acacia georgina	Fluoroacetate
Agropyron repens	Agropyrene
Allium spp.	Allicin,
	S-Methylcysteine sulphoxide
	S-Propylcysteine sulphoxide
Anacardium occidentale	Anacardic acid
Anemone pulsatilla	Protoanemonin-anemonin
Artemisia capillaris	Capillin
Avena sativa	Avenacin
Brassica spp.	Sinigrin
Callitris spp.	Callitrol
Capsicum spp.	Capsicin, Capsaicin
Cheiranthus cheiri	Cheirolin
Chlorophora excelsa	Chlorophorin
Citrus reticulata	Tangeritin
	5,4-Dihydroxy,6,7,8,3'-tetramethoxy-flavone
	5,4-dihydroxy 6,7,8-trimethoxyflavone
	Nobiletin
Colchicum autumnale	Nobiletin
Crepis taraxacifolia	Crepin
Cupressus spp.	Nootkatin
Curcuma tinctoria	Curcumin
Diachapetalum cymosum	Fluoroacetate
Ferula spp.	Galbanic acid
Fraxinus excelsior	Tyrosol
Gossypium spp.	Gossypol
Ginkgo biloba	α-Hexenal
Hordeum vulgare	Hordatines, p-Coumaroylagmatine
Humulus lupulus	Lupulun, Humulum
Impatiens balsamina	2-Methoxy-1,4-naphthoquinone
Juglone regia	Juglone, Hydrojuglone
Knophofia uvaria	1,8-Dihydoxy, 3-anthraquinone carboxylic acid
Lawsonia inermis	Lawson
Libocedrus decurrens	Libocedrol
Linum spp.	Linamarin-HCN
Medicago sativa	Coumestrol
Melilotus spp.	Dicoumarol
Pisum sativum	α-amino-γ-butyrylactone
Plumbago spp.	Plumbagin
Plumeria multiflora	Plumericin
Podophyllum peltatum	Podophyllin
Poterium sanguisorba	Tormentol
Populus trichocarpa	Trichocarpin, Benzylgentisate
P. tacamahacca	a,a-Bisabolal

Table 2 (cont.)

Pterocarpus spp.	Prunetin, Pterocarpin, Homopterocarpin, Pterostilbene
Pyrus communis	Arbutin, Hydroquinone
P. malus	Phloridzin, Phloretin, Phloretic acid
Ranunculus spp.	Anemonin, Protoanemonin
Robinia pseudoacacia	Robinetin
Sanguinarine canadensis	Sanguinarin
Secale cereale	2,3-Benzoxolinone
Solanum spp.	Tomatine, Solanine
Tectona grandis	Dihydrotectol, Tectoquinone, Tectol
Toxylon pomiferum	2,3',4,5'-tetrahydroxystilbene
Trifolium pratense	Biochanin-A, Trifolirhizin
Triticum spp.	6-Methoxybenzoxolinone Purothionin
Vicia faba	Wyerone
Xanthium spp.	Xanthatin
Zapota spp.	Sapogenin A_1, A_2
Zea mays	6-Methosxybenzoxolinone
Zelkova serrata	Keyakinin, Keyokinol

the phytoalexin has been made effective by the fungi and this process is analogous to "lethal synthesis" of PETERS (1963). Whether the effective form of phytoalexin should be considered as phytoalexin or be called by some other name depends upon future research in this line.

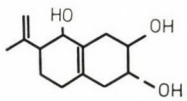
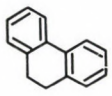
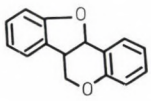
Concluding this section, one must remember that the mere presence of a substance in an infected plant does not guarantee its participation in defense reactions and its consideration as a phytoalexin. Because let us not forget that MÜLLER and BÖRGER (1939) carefully pointed out that the role of phytoalexin should lie on its function as a defensive compound. This evidently seems to have been overlooked by some of the enthusiastic workers.

General and specific phytoalexins. Of course, there are certain structural relationships between the phytoalexins produced by closely related species of plants (Table 3). Apparently plant species belonging to a family have common mechanisms by which they synthesize defense factors resulting in the appearance of similar substances. For example, hircinol and orchinol are produced by orchidaceous plants; phaseollin, pisatin, viciatin and the soybean-phytoalexin produced by leguminous plants, and rishitin by solanaceous plants. Therefore such common phytoalexins are considered as *general phytoalexins.* Functionally to ward off the common less specialized parasites, plants produce general phytoalexins in response to the initial infection stimulus.

In contrast to general phytoalexins, to combat specific parasites, plant species synthesize *specific phytoalexins.* Wyerone acid(XVIII) produced by *Vicia faba*

Table 3

Structural relationship among the various phytoalexins produced by plants

Plant	Phytoalexin	Common structure	Reference
Glycine max *Phaseolus vulgaris*	Phaseollin		KLARMAN and SANFORD (1968) CRUICKSHANK and PERRIN (1963)
Pisum sativum	Pisatin		Ibid (1960)
Vicia faba	Viciatin		CRUICKSHANK (1963)
Orchis militaris *Orchis* spp	Orchinol		BOLLER et al. (1957)
Loroglossum hircinum	Hircinol		URECH et al. (1963)
Lycopersicon esculentum	Rishitin		SATO et al. (1968)
Solanum tuberosum	Rishitin		TOMIYAMA et al. (1968)

in response to inoculation with *Botrytis cinerea* and *B. fabae* appears to be the only example of specific phytoalexin (LETCHER et al., 1970).

This approach to disease resistance is essentially a corollary to the ideas advanced by GÄUMANN (1950; 1964) that resistance to spread is distributed throughout the tissues as well as localized in certain special tissues.

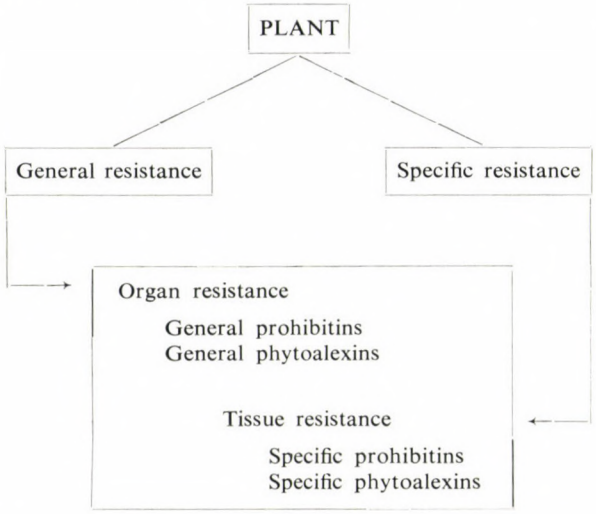
Organ resistance and tissue resistance. When orchid bulbs are infected by parasites, they do not react uniformly (GÄUMANN and HOHL, 1960); in the infected zone, the defense reactions are highest and in the adjoining cells, less pronounced. To explain this phenomenon, these workers proposed that the overall resistance response of the bulbs to parasites be called *organ resistance* ("Organimmunität") and the resistance of the infected tissues, *tissue resistance* ("Gewebeimmunität"). Evidences of phytoalexin theory and general activation of defense mechanism (TOMIYAMA et al., 1967) are clearly compatible with the postulates of GÄUMANN and HOHL.

Both organ resistance and tissue resistance can be reconciled with the general resistance and specific resistance. Organ resistance is general resistance and tissue resistance is specific resistance. Applying the prohibitin and phytoalexin concepts to general and specific resistances, it follows that general resistance is mediated by general prohibitins and general phytoalexins while specific resistance, through specific prohibitins and specific phytoalexins (Table 4).

Specificity of plant parasites. The basic principles discussed in the previous sections can be extended to explain the specificity of plant parasites and their

Table 4

A summary of different kinds of resistance displayed by plants



tissue preferences. In fact, I did postulate a theory (MAHADEVAN, 1969). The theory states: "Inhibitory substances, both preformed or post-infectionally formed in the plants, govern the specificity of pathogenic microorganisms; when the inhibitors are removed or their activity is overcome, the organisms become pathogenic to that particular plant."

Any theory on host-parasite interaction should obey the gene to gene relationship between host and parasite formulated by OORT (1944) and confirmed by FLOR (1956) and PERSON et al. (1962). Indeed this theory does obey the gene to gene relationship. For gene expression is by means of biochemical activity and there is a specificity of interaction between chemical stimuli and genetic elements (JAAOB et al., 1965). Since enzyme synthesis is governed by the inducer (JACOB and MONOD, 1961) particularly true for the synthesis of catabolic enzymes, an extension of this theory to host-parasite interaction implies that the host substrate "directs" the invading organism to synthesize an enzyme that will cleave the substrate which is also the "inducer". Assuming the inducer is an inhibitory substance, any enzyme produced by the parasite will necessarily cleave the inhibitor. If such a mechanism were to operate *in vivo*, it would consequently remove the chemical barrier in advance of infection. Microorganisms that do not produce the detoxifying enzymes will naturally succumb to the inhibitors and evidently will be eliminated at the portal of entry.

Several lines of evidence are compatible with the postulates of the theory. In Table 5, the relationship between the sensitivity of parasites to prohibitins of plants and their pathogenicity is summarized. These evidences indicate that micro-

Table 5

Relationship between the sensitivity of plant parasites to prohibitins and pathogenicity

Plant	Prohibitin	Microorganism	Sensitivity	Pathogenicity	Reference
Abies balsamea	Unidentified	*Cladosporium herbarum*	+	−	ETHERIDGE (1962)
		Corticium polygonium	+	−	
		Cytospora sp.	+	−	
		Penicillium sp.	+	−	
		Rhizopus nigricans	+	−	
		Stereum sanguinolantum	−	+	
Acer spp.	Acetone extract	*Ceratocystis ulmi*	+	−	MAY et al. (1958)
Avena sativa	Avenacin	*Ophiobolus graminis*	+	−	TURNER (1961)
		O. graminis avenae	+	+	
Brassica oleracea	Mustard oil	*Fusarium oxysporum* f. spp.	+	−	DAVIS (1963)
		F. oxysporum f. *conglutinans*	−	+	
Lycopersicon esculentum	Tomatine	*Septoria lycopersici*	−	+	ARNESON and DURBIN (1967)
		Many fungi	+	−	Ibid (1968)
Oryza sativa	Flavonoids	*Pyricularia oryzae*	−	+	WAKIMOTO et al. (1960)
		Helminthosporium oryzae	−	+	
Pinus contorta latifolia	Pinosylvin	*Peniophora pseudopini*	−	+	LOMAN (1970)
	Pinosylvinmono-methyl ether	*Tympanis hypopodia*	−	+	
		Coryne sarcoides	−	+	
	Pinocembrin	*Stereum sanquinolentum*	−	+	
	Pinobanksin	*Fomes pini*	−	+	
		Coniophora puteana	−	+	

Pinus spp.	Acetone extract	*Ceratocystis ulmi*	+	−	MAY et al. (1958)
Pyrus communis	Arbutin-Hydro-quinone	*Venturia inaqualis*	+	−	RUDLOFF (1935)
		V. pirina	−	+	
Quercus spp.	Acetone extract	*Ceratocystis ulmi*	+	−	MAY et al. (1958)
Solanum tuberosum	Solanine	*Fusarium caeruleum*	−	+	MCKEE (1959, 1961)
		F. solanii	+	−	METLITSKIJ and MUKHIN (1964)
		Helminthosporium carvonum	+	−	ALLEN and KUC (1968)
Tulipa gesneriana	Tuliposides	*Botrytis tulipae*	−	+	SCHÖNBECK (1967)
		B. cinerea	+	−	
		Phytophthora cactorum	+	−	
		Pythium debaryanum	+	−	
Ulmus americana	Acetone extract	*Ceratocystis ulmi*	−	+	MAY et al. (1958)
U. carpinifolia	Ibid	Ibid	−	+	
U. pumila	Ibid	Ibid	−	+	

organisms specifically pathogenic to host plants are hardly sensitive to their prohibitins. This is evidently due to their ability to degrade the prohibitins during infection.

Apart from the detoxification of prohibitins, pathogenic microorganisms also detoxify post-infectionally formed inhibitory substances phytoalexins, and those that destroy them are pathogenic while those that do not, are not pathogenic to the plants. This relationship is strikingly illustrated in Table 6.

Direct evidences like these are disappointingly few. But there is an extensive amount of literature on the detoxification of naturally occurring phenolic substances by phytopathogenic bacteria and fungi (BAVENDAMM, 1929; FÄHRAEUS, 1952; RICH and HORSFALL, 1954; LYR, 1958a,. b, 1961, 1962a, b; FLAIG and HAIDER, 1961; NORD and SCHUBERT, 1961; WESTLAKE et al., 1961; ROSCH, 1966; ARMAND-FRAYSSE and LEBRETON, 1969; JAYASANKAR et al., 1969; TROJANOWSKI et al., 1970). Bacteria such as *Pseudomonas* spp., and fungi such as *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Collybia velutipes*, *Endothia parasitica*, *Fistulina hepatica*, *Lenzites saepiaria*, *Penicillium* spp., *Polyporous* spp., *Poria* spp., *Pullularia pullulans* and *Rhizoctonia* spp. are pathogenic to many plant species because of their unique capacity to produce enzymes that detoxify a variety of toxic substances, primarily aromatic substances present in plants.

All these findings strongly reinforce the postulates of the theory on specificity and certainly give credence to the theory. Consequently one has to consider, in the light of the theory, that the pathogenicity of microorganisms is intimately associated with their capacity to degrade the toxic substances present in the plants. Since plant species differ considerably in their prohibitin content, and in the systems associated with the formation of phytoalexins, pathogenicity of microorganisms will necessarily differ from one plant species to another. Moreover, the commonly held belief that virulent and avirulent parasites differ notably in their cell-wall dissolving enzymes and toxin producing capacities now requires a fresh thinking in the light of the theoretical ideas presented here. There is every reason to believe that virulence of parasites is intimately associated with their capacity to detoxify and/or tolerate the toxic substances of plants. This is an area of study which requires investigation.

Monophagy, oligophagy and polyphagy. The important object of all parasites is to colonize tissues that contain food materials which support growth and reproduction. To achieve it, parasites have not only developed offensive weapons such as detoxifying and cell-wall dissolving enzymes and phytotoxins but a discrete preference to colonize certain plant species. Consider the fact that *Puccinia buxi* specifically parasitizes *Buxus sempervirens*, while *Urocystis occulta*, on *Secale cereale*. There is an extreme group of microorganisms such as tobacco mosaic virus, ringspot virus, *Rhizoctonia solani*, *Verticillium albo-atrum*, *Pythium debaryanum*, *Phymatotrichum omnivorum*, *Agrobacterium tumefaciens* and *Erwinia carotovora* that has a wide host range (GÄUMANN, 1950; WHEELER, 1968). Between these two extremes is an intermediate group comprising *Endoconidiophora fagacearum*,

Xanthomonas campestris, *Pseudomonas solanacearum* etc., that infects several species of plants belonging to one family and sometimes, plant species of closely related families.

Despite the recognition of such a phenomenon, the underlying mechanism has been to date virtually not understood other than several speculative proposals which in no way satisfactorily explain it.

But a profitable understanding of this relationship can be acquired from a study of host preference of insects, which also display in many respects a parallel relationship. For a long time, the selective host preference of insects, like microbial parasites was attributed to the exact recipe of nutrients held by plants that sustain growth and multiplication of the insects. FRAENKEL (1959) who critically examined this problem concluded that the food specificity of insects is based solely on the presence or absence of secondary substances such as phenolic glycosides, saponins, tannins, alkaloids, terpenes and essential oils, etc., in plants which serve as repellents to insects. Fraenkel emphasized that the basic composition "the primary substances" such as carbohydrates, proteins and lipids of all plant species is very much alike but the secondary substances differ considerably from one plant species to another and even within tissues, both in quality and quantity. Chemotaxonomists overwhelmingly substantiate this view (KARRER, 1958; HEGNAUER, 1962, 1963, 1964, 1966, 1969).

With this preamble consider the data on the nutritional requirements of pathogenic bacteria and fungi (viruses being not known). Their basic food requirements do not differ appreciably from one microorganism to another (COCHRANE, 1958; TANDON, 1961). These include the major nutrients such as carbohydrates, nitrogen, phosphorus and growth factors-vitamins and trace elements. Furthermore, most plant parasites are not fastidious in their nutrient requirements. Most plants contain them, although differences in concentration and in quality may exist. Therefore, theoretically microorganisms can procure these from any plant, but they seem to be selective and some of them, e.g. rusts appear to be super-selective. Naturally the reason for the selective preference of plant pathogens, like insects, must be intimately associated with their sensitivity to secondary substances.

Indeed, a critical apprisal of the literature on the sensitivity of microorganisms to toxic substances of plants reveals that they differ considerably in their sensitivity (SKINNER, 1955; PERRIN and CRUICKSHANK, 1969; FAWCETT and SPENCER, 1969; MAHADEVAN ,1970 and earlier part of this paper). Therefore their presence in tissues will obviously influence the development of microorganisms; more of these substances and of different quality will inhibit the development of some microorganisms but exerting little effect on others. Since in tissues, the distribution of secondary substances differs markedly, their resistance to microorganisms will consequently vary. As an example, consider the resistance displayed by the heartwood of *Thuja plicata*. The heartwood is enriched with a, b and v-thujaplicins(XIX, XX, XXI) and thujaplicinol(XXII) which are toxic to wood rotting

Table 6

Sensitivity of plant parasites to phytoalexins and their capacity to cause disease

Plant	Phytoalexin	Parasite	Sensitivity	Pathogenicity	Reference
Medicago sativa	Unidentified	*Helminthosporium turcicum*	+	−	HIGGINS and MILLAR (1969, 1970)
		Stemphylium loti	+	−	
		Colletotrichum phomoides	+	−	
		S. botryosum	−	+	
Orchis militaris	Orchinol	*Rhizoctonia repens*	−	+	GÄUMANN and KERN (1959a, b)
		Many fungi	+	−	
Phaseolus vulgaris	Phaseollin	*C. lindemuthianum*	−	+	CRUICKSHANK and PERRIN (1963)
		Monilia fructicola	+	−	
		R. solani	−	+	PIERRE and BATEMAN (1967)
		Fusarium solani	−	+	
Pisum sativum	Pisatin	*M. fructicola*	−	+	CRUICKSHANK (1965)
		Many fungi and bacteria	+	−	
		Ascochyta pisi	−	+	UEHARA (1964)
		F. oxysporum	−	+	
		F. solani	−	+	NONAKA (1967)
		F. oxysporum f. *pisi* race 1	−	+	DE-WIT ELSHOVE (1969)

		F. solani f. *pisi*	−	+	
		Mycosphaerella pinodes	−	+	
		A. pisi	−	+	
		F. solani	−	+	
		A. pisi (nonpathogenic strain)	+	−	
		Glomerella cingulata	+	−	
		Botrytis fabae	+	−	
		C. lindemuthianum	+	−	
		Cladosporium cucumerinum	+	−	
		Aspergillus fumigatus	+	−	
Ulmus hollandica	Monsonone E, Monsonone F	*Ceratocystis ulmi*	−	+	Overeem and Elgersma (1970)
		Botrytis allii	+	−	
		Penicillium italicum	+	−	
		Cladosporium cucumerinum	+	−	
		Aspergillus niger	+	−	
Vicia faba	Wyerone acid	*B. fabae*	−	+	Deverall and Vessey (1969)
		B. cinerea			

fungi. But the adjoining sapwood cells are remarkably free of these substances. Naturally microorganisms easily infect these cells as do wood rotting fungi (MACLEAN and GARDNER, 1956). Similar relationships can be established for other host-parasite systems.

Clearly therefore the host preference of microorganisms and of insects is based on the same principle. Entomologists classify the insects based on their host preference into monophagous, oligophagous and polyphagous. There is no reason why such a classification cannot be extended to plant pathology?

Most rusts, mildews and viruses are so specialized that they usually infect only one plant species. Therefore they should be considered as monophagous parasites. Several fungi such as *Pythium*, *Fusarium*, *Cercospora*, *Alternaria* and *Rhizoctonia* and viruses such as tobacco mosaic virus and cucumber mosaic virus feed on several species of plants of different families; these are the polyphagous parasites. Between these two extremes are the oligophagous parasites such as *Xanthomonas campestris*, *Ceratocystis* spp., *Helminthosporium* spp., etc.

This approach to a classification of plant pathogens is analogous to the proposal advanced by VAVILOV (1950) and GÄUMANN (1950). NEERGARD and NEWHALL (1951), MOLOT and SIMONE (1965) and BEYRIES and MESSIAEN (1969) have recognized the polyphagous nature of fungi in the sense presented here.

Theoretically monophagous parasites are extremely sensitive to most secondary substances of plants except to those present in the plants that they infect. In contrast, polyphagous parasites must be practically insensitive to most of the secondary substances. (It is an outstanding coincidence that tobacco mosaic virus is extremely resistant to high concentrations of several alkaloids such as aconitine, morphine, saponin digitalin (FUKUSHI, 1930) and to several organic toxicants, notably phenols (STANLEY, 1935). Oligophagous parasites represent an intermediate group in their sensitivity.

Certainly this mechanism associated with the selective host preference is radically different from the conventionally accepted nutritional theories. Even if there is some doubt concerning the validity of this mechanism, it can be dispelled if one realizes that more than 80 years ago, the German botanist STAHL (1888) presented such a radical view. He postulated that it is only the secondary chemical substances ("Exkrete") of plants that protect them against the infection of snails. Whereas STAHL's postulate was rigorously tested and verified in plants-animals including insects interaction (FRAENKEL, 1959) this requires verification in plant pathology. However, it is heartening to read the authoritative claim "Regarding the specificity, there is something common between the big buds and the small ones, the microorganisms" (KÜHN and LÖW, 1955), who showed that steroid alkaloids—demissine and tomatine— in solanaceous plants inhibit the development of *Leptenotarsa decemlineata* as well as *Saccharomyces cerevisiae*. Hopefully plant pathologists will extend this observation.

Target site. Plant parasites are not only selective in their preference to colonize plants but a preference to infect special tissues rather than the whole plant

per se. Such a tissue evidently represents the target for the pathogen. MACLEAN and GARDNER (1956) called the tissue that is colonized by wood rotting fungi "archery target". These targets or sapwood cells in *Thuja plicata* are relatively free of heartwood prohibitins such as α-, β- and γ-thujaplicins and thujaplicinol so that infection by wood rotting fungi is not prevented.

KENNEDY and WILSON (1956) presented almost an identical view. The wood of *Pseudotsuga menziesii* shows ring-like patterns of heartwood and sapwood cells. The heartwood contains high concentrations of taxifolin (XXIII), toxic to wood rotting fungi whereas the sapwood cells are deficient of the prohibitin. Consequently these cells offer little resistance to *Fomes annosus* and *Lentinus lepideus*. These cells, KENNEDY and WILSON believed, represent the target of attack for the wood rotting fungi. I propose the expression "target site" for the tissue that is infected by a parasitic microorganism. Figure 4 illustrates the target site concept.

In fact the target site concept explains the tissue preferences of plant parasites when one considers that wilt parasites prefer to colonize vascular vessels and mildews and rusts, leafy tissues. Since quantitative data on the prohibitin content of target tissues and their resistance to parasites are limiting, it is presently not possible to reconcile this concept with field observations.

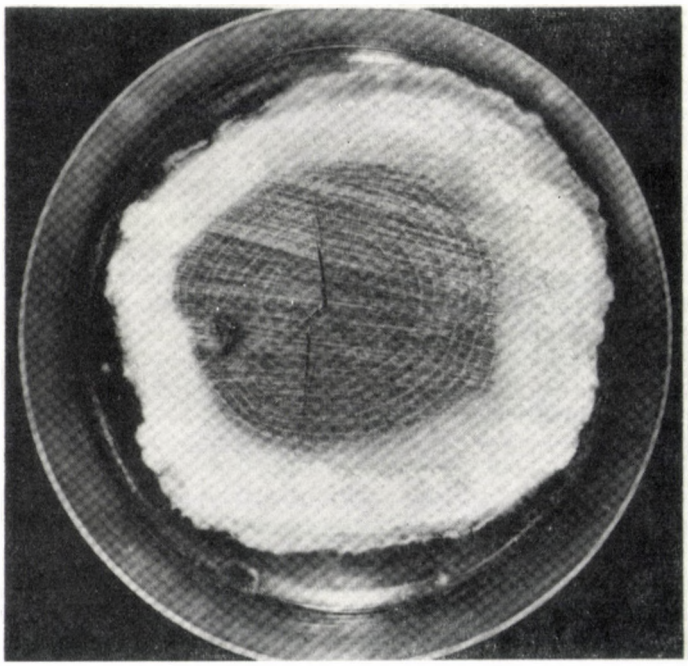


Fig. 4. *Ceratocystis fagacearum* growing on the sapwood (= target site) of *Quercus rubra*. Note the inner heartwood is free from fungal growth (from BILBRUCK, 1959)

Concluding remarks. The theoretical concepts discussed in this paper have been mostly derived from the data that had been accumulated in the literature. These concepts are certainly different from the traditionally held ones.

Theoretical principles can be extended to interpret the intricacies of practical problems confronting the plant pathologists and their dependents, notably plant breeders. For example, the most effective approach to breeding resistant varieties must involve the manipulation of the genes that are associated with the syntheses of prohibitins and phytoalexins. But either breeding for resistant varieties against polyphagous parasites or searching for highly resistant breeding stocks against them is bound to yield little success as predicted by VAVILOV (1950). This prediction has withstood all these years when one realizes that decades of effort to breed varieties resistant against polyphagous parasites has so far yielded a minuscule of success. In contrast, breeders have been successfully bringing out new varieties in almost every season, resistant against monophagous parasites, e.g. wheat varieties resistant against rust. This practical success is a corollary to the theoretical prediction of VAVILOV (1950).

Indeed there is a constant demand for new varieties that will withstand new races of pathogens because monophagous parasites tend to mutate more frequently than polyphagous parasites do. There is a large dossier on the physiological races of rusts and smuts. In contrast, only a few races of *Phymatotrichum omnivorum*, *Rhizoctonia solani* and TMV, among the polyphagous parasites exist. These observations can be reconciled with an ecological theory where it is axiomatic that increased species diversity leads to increased stability (MACARTHUR, 1955; PIMENTEL, 1966). This can be reformulated in plant pathology as "increased host-specificity leads to increased mutability of the parasite". This is reasonably true when one considers that chances of survival for the monophagous parasites are always threatened in the event of a new host variety, they have to constantly mutate for virulence to overcome specific factors of resistance in the host. But for polyphagous parasites, presumably there is little need to mutate in order to survive on new varieties since survival as a rule is not threatened in the absence of one particular host.

Much is now being said about soil-borne pathogens and their control. Actually their control should not be too difficult as long as one approaches it with a proper theoretical understanding. For instance, addition of crop residues or crop rotation can be formulated based upon their toxic action against soil-borne inocula, as SCHWINN (1965) did. In these days of ecological awareness and increased accusation of fungicides and antibiotics in disease control, substances of plant origin because they are selectively toxic and are easily degraded by soil microorganisms into nontoxic components, offer the greatest hope to chemotherapists.

I am grateful to Prof. Dr. F. SCHÖNBECK, University of Bonn, Germany, for permission to use Figure 1.

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Microbial Colonization of Aerial Parts of Plants — A Review

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The surfaces of different plant organs represent a colourful array of microbes (comprising both parasitic and non-parasitic microorganisms) unique to them. This fact has led various investigators to explore the problems related to the biological activities going on in the respective ecological niches (LAST, 1955; TYNER and BARBARA, 1964; LAST and DEIGHTON, 1965; CALDWELL, 1963; HANCOCK, 1966; SINHA, 1965, 1971; RUINEN, 1956, 1961, 1971; DICKINSON, 1965, 1967; KERLING, 1958, 1964; SMITH and WIERINGA, 1963; LEBEN et al., 1968; GREGORY, 1971; WEBSTER, 1956, 1957; HUDSON and WEBSTER, 1958; PUGH, 1958; PUGH and WILLIAMS, 1968; LAI and BRUEHL, 1966; FRASER, 1971; BHURAT and SEN, 1970; HUDSON, 1962, 1968; HYDE and WILLIAMS, 1953; LEBEN, 1965, 1969, 1971; SHARMA and MUKERJI, 1972; BURGESS and GRIFFIN, 1967; YADAV, 1960; WAKSAM, 1922, 1931; VOZNYAKOVSKAYA and KHUDYAKOV, 1960; SAITO, 1956, 1957 and BUTLER, 1953a, b).

The recent trend in the study of microbial colonization has been mainly to understand the problems such as: the relationship between the plant surface microflora and the environmental factors affecting it; the effect of foliar application of a fungicide or a substrate or any chemical factor e.g. growth hormones and inhibitors, on the biology of plant surface microbes; the interactions among the surface colonizers themselves, and in response to the host-activity; pollution as a factor interacting with the surface colonizers; the role of plant organ which can have a carry-over-effect on the spread of a particular disease; leaching as a process in relation to plant surface colonization and mechanism of succession on a plant surface initially devoid of any medium. The studies of this nature provide answers to the questions regarding: (1) the role of plant surface microbes in biological control of certain epidemic diseases, (2) their role as active decomposers of different plant organs in relation to soil fertility, (3) the evaluation of the role of a pathogen in the absence of visible symptoms, (4) the means to enhance the activity of plant surface nitrogen-fixers, and (5) the establishment of the cause of certain allergies common to mankind.

Methodology

The surfaces of different plant organs are distinct both in the nature of their substrates as well as in microflora harboured by them (DALBRO, 1956; GOOD and TUKEY, 1966; MITCHELL, 1967 and LEBEN et al., 1970). But, it is difficult to select out a particular technique for performing quantitative and qualitative studies on the natural plant surface microbes, because the immediate vicinity of the aerial plant parts – the micro environment, is rendered unstable by the continually changing macroenvironmental factors (temperature, light, windspeed, rain and pollution), to which it is directly exposed, and, the indeterminate growth of the plant parts themselves.

DICKINSON (1971) has reviewed the significance of different cultural techniques employed in recent years. He explains that although dilution plate technique is useful for collecting data on sporing and unicellular forms, it suffers from several disadvantages. For instance, this technique assumes the possibility of some unavoidable events such as: 1. the number of viable propagules on the sample is equal to the number of colonies developing in the culture plates, 2. development of one colony is independent of any other colony developing in the same plate, and 3. all the surface microbes have an identical growth response to a medium employed for the purpose. However, he (1971) suggested several ways to increase the efficiency of this technique and overcome these difficulties.

DICKINSON (1971) has also cited the shortcomings of the use of: culturing of the surface washed explants (DICKINSON, 1967); leaf impression technique (POTTER, 1910); maceration technique (LEBEN, 1970; 1971); direct examination of plant surfaces (BEECH and DAVENPORT, 1971; JANES, 1962 and ISAAC, 1960) and indirect examination of plant surfaces (MCCOY and DIMOCK, 1971). DAVENPORT (1970) has reported that leaf impression technique, although provides data for microbial distribution on leaf surface, it is insufficient to provide data for all the detachable propagules.

DICKINSON (1967) has reported that the moist chamber technique, devised by KEYWORTH (1951), is advantageous in one respect that it provides data about the microorganisms which take active part in plant decomposition. We have observed that in addition to this, the technique is good for isolation of myxomycetes from the decaying plant organs (SHARMA and MUKERJI, 1972). The application of plastic bags to provide a continuous high humidity *in situ* has been found useful in promoting the growth of *Helminthosporium* on *Secale*-leaves (FOKKEMA, 1971). In addition to these, some selective methods to study special groups of fungi, have also been employed. For instance, spore-fall method has been useful in isolating the members of the *Sporobolomycetaceae* (LAST, 1955) and several species of *Cladosporium* (DICKINSON, 1971). Several zoosporic fungi can be isolated by baiting techniques where the choice of bait determines the isolation of fungus (SPARROW, 1960). Nitrogen-fixing bacteria of *Pseudotsuga* needles have been isolated by employing ASHBY's combined nitrogen-free medium (JONES, 1970) and

cellulolytic fungi of *Lactuca* leaves have been isolated on a cellulose agar medium (DICKINSON, 1971). Certain selective media have been employed by TSAO (1970) to isolate several pathogenic fungi.

In short, each of the techniques discussed above is satisfactory for one aspect but, at the same time, extremely insufficient for some other aspects. Thus, to obtain reliably significant data for all aspects of a problem, a suitable combination of several techniques should be employed and analysed statistically (DICKINSON, 1971).

The colonisers

According to LAST and WARREN (1972), the principle leaf surface population (phylloplane) comprises the bacteria and yeast-like fungi including members of the *Cryptococcaceae* (asporogenous), *Sporobolomycetaceae* (ballistospore producers), and of the genera *Aureobasidium* and *Candida*. However, they have mentioned that some hyphomycetes and ascomycetes, algae including members of the *Chlorophyceae* and *Cyanophyceae*, and lichens, also occur uncommonly on foliage in wet tropics. *Actinomycetes*, although abundant in soil, have rarely been recorded on leaves. During our studies on the foliage of *Sesamum* and *Gossypium* (unpublished), the leaves at seedling stage were found to be highly populated by actinomycetes which were gradually replaced by bacteria, yeasts and filamentous fungi, and, they disappeared completely when the plants attained the age of 45–60 days.

HUDSON (1968) has grouped the saprophytic plant surface colonizers into two categories. The first category comprises the ubiquitous microbes, grouped under the term – common primary saprophytes e.g. *Cladosporium* spp., *Alternaria* spp., *Aureobasidium pullulans*, *Epicoccum nigrum* and *Botrytis cinerea*. These fungi, also designated as "field fungi" with few exceptions, are more commonly associated with freshly decaying green parts of plants. The second category comprises microorganisms having restricted host range, the so-called restricted primary saprophytes e.g. *Readeriella mirabilis* and *Piggotia stellata* are restricted to *Eucalyptus* (MACAULEY and THROWER, 1966); *Ascochytula obiones* to *Halimione portulacoides* (DICKINSON, 1965); *Leptosphaeria* spp. to members of *Gramineae; Fusicoccum bacillare* and *Sclerophoma pithyophila* are common on pine needles and some other conifer leaves; and *Hyalodendron* only on *Quercus* leaves (TUBAKI and YOKOYAMA, 1971).

RUINEN (1956), from her studies on certain epiphytes, has concluded that phylloplane of different plants is characterized by specific microorganisms, especially the bacteria and yeasts; the first colonizers being bacteria, followed by actinomycetes, fungi, lichens and arthropods in succession. The food for the growth and reproduction of these colonizers is supplied by leaf exudates and the nitrogen fixing bacteria of the phylloplane itself. Prior to this, LAST (1955) had reported

that phylloplane of cereals was characterized by the presence of *Sporobolomyces, Bullera, Cladosporium* and *Tilletiopsis.*

MENNA (1959; 1971) reported the occurrence of certain yeasts *(Sporobolomyces, Rhodotorula, Cryptococcus* and *Candida curvata), Aureobasidium pullulans, Cephalosporium, Cladosporium herbarum, Fusarium* spp., some pycnidial forms, *Myrothecium* spp. (cause of myrothecitoxicosis) and *Pithomyces chartarum* (cause of facial eczema in animals) on the leaves of some pasture plants. SINHA (1971) isolated certain leaf surface saprophytes which were exclusively associated with four solanaceous members. Saprophytic fungi have also been reported from leaves of *Secale cereale* and *Beta vulgaris* (KERLING, 1958; 1964); *Saccharum officinarum* (HUDSON, 1962); *Nothofagus truncata* (RUSCOE, 1971); *Carex paniculata* (PUGH, 1958); *Halimione portulacoides* and *Pisum sativum* (DICKINSON, 1965; 1967); *Fagus sylvatica* (HOGG and HUDSON, 1966); *Oryza sativa* (MISHRA and SRIVASTAVA, 1971); some pteridophytes (KAMAL and SINGH, 1970); *Nicotiana* (WELTY and LUCAS, 1968); *Gossypium hirsutum* (SHARMA and MUKERJI, 1972); *Paspalum dilatatum, Salix babylonica* and *Eucalyptus stellulata* (LAMB and BROWN, 1970), and decaying petioles of *Pteridium aquilinum* (FRANKLAND, 1966).

The most prominent fungal species on the leaf surfaces of *Beta vulgaris* have been reported to be *Aureobasidium pullulans* and *Cladosporium cladosporiodes* whereas *Alternaria chartarum, Botrytis cinerea, Epicoccum nigrum* and *Phoma* spp. being isolated less frequently (STOTT, 1971). We (1972) have found the following dominant species on cotton leaves: *Alternaria* spp., *Fusarium* spp., *Fusidium viride, Cladosporium herbarum, Stachybotrys, Memnoniella* and *Candida albicans.* On green leaves, *Chaetomium* spp., but on dried and decaying leaves certain myxomycetes *(Physarum vernum, P. cinereum* and *Didymium saturnus)* were predominant.

KLINCARE et al. (1971) have reported that the predominant epiphytic bacteria of lupin, lucerne, rye, barley, oats, cabbage and sugar-beet belong to the genera *Pseudomonas, Bacterium, Mycobacterium, Chromobacterium, Micrococcus, Sarcina* and *Bacillus.* Both Gram positive and Gram negative organisms were reported from apple leaves (HISLOP and COX, 1969). Microfungi have been isolated from various other sources also, for instance, from beech cupules (CARRE, 1964); decaying logs and branches (CHESTER, 1950); oak leaf litter (HERING, 1967); conifer litter (BRANDESBERG, 1969 and WARD, 1952) decaying stems of *Agropyron repens* (HUDSON and WEBSTER, 1958); leaf litter of *Pinus sylvestris* (KENDRICK and BURGES, 1962 and KENDRICK, 1963); beech litter (SAITO, 1960; 1965; 1966 and CALDWELL, 1963); wilted jute plants (WADUD and AHMED, 1962), decaying stems of *Urtica dioica* (YADAW and MADELIN, 1968); decaying stems of *Triticum vulgare* L. and *Andropogon sorghum* L. (LAL and YADAV, 1964); wheat straw compost (CHANG, 1967); decaying cocks-foot culms (WEBSTER and DIX, 1960), and decaying wood (MERRIL and FRENCH, 1966; 1964 and VAARTAJA, 1968).

Both pathogenic and saprophytic bacteria (including nitrogen-fixers), yeasts and fungi have been isolated from the vegetative buds of tropical woody plants,

and some annuals (LEBEN et al., 1970; LEBEN, 1969, 1971 and DAVENPORT, 1970). Some plant pathogenic fungi have been reported to have their source of inoculum in the buds e.g. *Tilletia caries* (LEBEN, 1971) and *Venturia inaequalis* (PREECE, 1963). LEBEN (1971) has suggested that the primary site for the growth of non-pathogenic and pathogenic bacteria is the active bud. According to him, these bacteria are distributed onto the maturing leaves as they unfold which then act as the sites for their multiplication.

Several pathogenic and saprophytic fungi have been isolated from the onion crop in the field and during storage (EL-HELALY et al., 1962). SINHA (1965), during his studies on *Eruca sativa*, *Sesamum indicum* and *Linum usitatissimum*, reported several saprophytic fungi, some bacteria and actinomycetes on the surfaces of flowers, fruits and stored seeds. On the other hand, MONTEGUT (1967) has reported a parasitic complex which attacks the upper parts of cotton plants during decomposition.

The Ecological Niche

The plant surface-niche comprises the substrate (exudates from the host plant, chemical compounds resulting due to the biological activity of nitrogen fixers and the components which are the results of atmospheric pollution) and the microorganic entities which come to lie on the plant surfaces in due course. The latter consists of the fungal spores, pollen grains, spores of other plants, bacterial cells, yeasts, actinomycetes and insects. These entities get deposited on the surfaces in four principle ways: wind-borne route, in rain drops, rain splash droplets and by biological activities. The equilibrium of plant surface niche is controlled by various factors including the influence of host on the colonizers and vice-versa.

The structure of the epidermis and especially, that of the cuticle, is the first thing to interfere with the plant surface colonizers (MARTIN, 1964). The cuticle is known to interact with the entry of insecticides, fungicides or any other chemical sprayed for the specific purpose (MARTIN and JUNIPER, 1970). Electron microscopic studies have shown that cuticular surface exhibits a characteristic morphology due to polymerization and crystallization of some waxy compounds exuded from the epidermal cells (HALLAM and JUNIPER, 1971). The cuticle morphology may interact indirectly with the plant surface biological entities by influencing the balance of cuticular to stomatal transpiration, the movement of solutes in surface moisture, the degree of wettability, and the contact angle of the water droplets with the plant surface. The major chemical components of the cuticle are lipids, wax and cutin. Cutin is the chief structural component, and is a polymer comprising fatty and hydroxy fatty acids with wax embedded within the membrane and exuded over its surface (HOLLOWAY, 1971). Cellulose, pectin and some polyphenolic compounds comprise other structural components of the cuticle (BAKER and MARTIN, 1967; HOLLOWAY and BAKER, 1970 and BAKER, 1971).

Of the cuticular waxes, some are known to be stimulatory while others are inhibitory to the surface colonizers. HEATHER (1967) has reported that glaucous coating of *Eucalyptus bicostata* affects the deposition of conidia of *Phaeoseptoria eucalypti*. ROBINSON (1967) has found that spores of *Botrytis cinerea*, if deposited on the cuticle, get fully established even if exposed to only a few hours of dew at night which shows the significance of spore-wettability. Several microbes colonize the plant surface by virtue of their lipolytic activity. This has been demonstrated by RUINEN (1966), who detected the secretion of lipases by *Cryptococcus laurentii* and *Rhodotorula glutinis* when they were cultured on the stripped epidermis of *Aloe* and cuticle fragments of *Sanseviera*.

Raspberry plants are usually sensitive to spur blight *(Didymella aplanata)*, grey mould *(Botrytis cinerea)*, and cane spot *(Elsinoe veneta)*. Symptoms of spur blight are confined to nodes but those of the latter two, to the internodes. The seedlings with hairy nodes, spine free and wax free or non-pigmented canes are resistant to spur blight but susceptible to cane spot. JENNINGS (1962) has suggested that hairy nodes promote water shedding and thus prevent occurrence of moist conditions at nodes which is unfavourable for spur blight. On the other hand, since waxy coatings are fungistatic, wax-free canes are vulnerable to cane spot and grey mould.

The cuticular trichomes seem to be vulnerable sites for the entry of several bacteria and viruses. It has been suggested that the ectodesmata on the plant surfaces may provide zones of weakness in the epidermal cell walls through which viruses may seek entrance (MARTIN and JUNIPER, 1970). Bacteria are known to penetrate the epidermal cells through natural openings also. For instance, fire-blight pathogen *(Erwinia)* which is disseminated by bees and wasps, when sprayed on the blossoms, was found to cause infection at nectaries, stigmatic surfaces and undehisced anthers (HILDEBRAND and MACDANIELS, 1935). HILDEBRAND (1942) showed that a normal abrasion of healthy leaves against infected leaves was adequate to create openings in cuticle sufficient for bacteria to enter. Insect colonization was also affected by the waxiness or nonwaxiness of the plant surfaces. The resistance of several species of *Nicotiana* to the aphid, *Myzus persicae*, had been attributed to the secretion of alkaloids (nicotine) by the trichomes.

The substrate of the plant surfaces comprises macro- and micro-elements, large amounts of organic substances including free sugars, pectic substances, sugar alcohols, amino acids, oranic acids, gibberellins, vitamins and phenolic substances (MITCHELL, 1968). The rate and extent of plant surface leaching varies with the plant species and the action of the aqueous solutions such as rain, dew, mist and fog (TUKEY, jr., 1971). Earlier also, it had been reported that high light intensities, high temperatures, windspeed, dew, mist and fog could enhance leaching to a great extent (TUKEY et al., 1958; MITCHELL, 1968; MECKLENBURG et al., 1966; BHAN et al., 1959; LINSKENS, 1952; PHILLIS and MASON, 1942 and MORGAN and TUKEY, 1964). In addition, the young plant parts are prone to a low degree of leaching as compared to older and mature parts (TUKEY et al., 1958). MITCHELL

(1968) reported that leaching of carbohydrates from *Chrysanthemum* and *Poinsettia* increased as flower buds were initiated, reached a peak at flowering and then decreased during senescence.

It appears that the inorganic nutrients are leached from the translocation stream within the plant part, into the leaching solution on the plant surface involving exchange reactions and diffusion through the areas devoid of cuticle (TUKEY and TUKEY, 1962). LANSBERG (1935) reported that upper leaf surfaces exhibit 70 per cent greater nutrient loss than the lower surface indicating that stomata are not the primary avenues for the loss. Loss of nutrient by guttation from hydathodes and trichomes, and secretion from nectaries can also be included in the process of leaching.

A high degree of leaching reduces crop yields in strawberry and raspberry (TUKEY et al., 1958). On the other hand, leaching is known to be beneficial in controlling the accumulation of lethal concentrations of salt in the leaves of *Ricinus communis* growing in a high salt root medium (ARENS, 1950). It supports the general observation that house plants get improved in quality when their leaves are cleaned with moist cloth. BLAKEMAN and FRASER (1971) have found that microbes can also add to the pool of leaf surface nutrients. They demonstrated that spores of *Botrytis cinerea* and *Mycosphaerella ligulicola* leak amino acids and carbohydrates, a process exhibited more efficiently by the former than the latter.

The atmospheric pollutants, for instance, soot, smoke, dust, carbon monoxide, sulphur dioxide, sulphuric acid droplets, hydrogen sulphide, fluorine and fluorides, chlorine, bromine, iodine, certain metals, pesticides, herbicides, fungicides, fertilizers, photochemical smog, ozone, peroxyacetyl nitrate, oxides of nitrogen and aldehydes influence the plant surfaces to a great extent. Some oxidant pollutants are known to kill some bacteria in vitro, for example, *Serratia*, *Escheritia coli* and *Photobacterium phosphoreum* are very susceptible to such pollutants. HEAGLE (1970) reported that on oats, uredospore production by *Puccinia coronata* could be inhibited by 10–20 ppm ozone. *Diplocarpon rosae* and *Hysterium pulicare* disappeared from the areas where SO_2 concentrations were high (SAUNDERS, 1966). The use of SO_2 has also been suggested to control *Botrytis cinerea* (COUEY and UOTA, 1961) and *Alternaria* spp. (COUEY, 1965). The latter are extremely sensitive to SO_2 at higher relative humidities (95–100 per cent). In addition, toxicity of SO_2 is increased by low pH values at the plant surface. On the other hand, SO_2 can be reduced to less toxic derivatives under controlled conditions of temperature, pH and SO_2 concentration both in solution on the surface and in the atmosphere (GILBERT, 1968 and SAUNDERS, 1971).

SUTTON (1953) has suggested that microclimate of a leaf comprises a thin layer of air influenced by the leaf surface. Leaf influences this layer by bringing about variations in the levels of ambient temperature, moisture, radiation and windspeed. The thickness of the layer varies depending upon the leaf size, leaf thickness and leaf morphology and the variations in environmental factors. This

has been supported by BURRAGE (1971) who found that the variations in the ambient leaf temperature, humidity, radiation, energy-exchanges and windspeed were very important as regards the survival and penetration of microorganisms on the leaf surface. The incidence of black rust of wheat depends upon the pattern of dew distribution which is determined by the wettability of leaf surface (BURRAGE, 1969).

WEBSTER (1956, 1957) and HUDSON and WEBSTER (1958) showed that the stem base fungi (at higher water content) could fruit on upper internodes (at fluctuating water content) of the cut-down stems. They cited that consistently higher water content of the lower internodes was unfavourable for the sporulation of the colonizing fungi. *In vitro*, WEBSTER (1957) also showed that in comparison to fungi from upper internodes, the stem base fungi required a higher humidity for germination and germ tube growth. In addition, WEBSTER and DIX (1960) demonstrated that primary colonizers inhabiting the upper internodes (regions of fluctuating relative humidities) grew faster than the secondary colonizers confined to lower internodes only.

BURGES (1958) has suggested that decaying pine needles present an improved habitat for fungal colonization because of their high water holding capacity. We (1972) found that in rainy season highest fungal population inhabited the dried intact leaves of *Gossypium*. It was suggested that higher humidity during rainy season caused delay in the falling off of the senescent leaves. The decomposition of these leaves progressed while they were still intact on the plant causing sporulation of the fungal colonizers. In moist chamber studies, higher moisture conditions promoted the predominance of myxomycetes on the senescent leaves, but limited supplies of water promoted the growth of filamentous fungi as well.

ROBINSON (1967) demonstrated that spores of *Plasmopara viticola* infect the mature vine leaves at 80 per cent humidity but at 70 per cent humidity they infect only the young leaves. This shows the significance of the amount of moisture in fungal colonization. MANNERS (1971) found that spore production in *Erysiphe graminis* and *Puccinia striiformis* was greatly influenced by environmental factors such as temperature, relative humidity and the incidence of light. BRUEHL and LAI (1968) have shown that *Cephalosporium gramineum* survives well at the highest moisture levels but relative humidities (RH) of 90 per cent and 86 per cent are deleterious. *C. gramineum* competes well at RH 100 per cent but at RH 90 per cent or 86 per cent; *Penicillium* spp. act as vigorous competitors and can even replace it completely. GRIFFIN (1963) found that penicillia were among the most dominant fungi colonizing the bait at RH values below 90 per cent (indicating thereby the role of atmospheric moisture content). On the other hand, BRUEHL and LAI (1968) showed that on three soils at RH 90 per cent and 86 per cent, the same *Penicillium* spp. dominated the wheat straw (showing the importance of substrate in the fungal colonization).

High moisture conditions beneath the sand surface seem to favour high frequency of *Chaetomium* spp. on the buried parts of *Salsola kali* (PUGH and WIL-

LIAMS, 1968). In contrast to this, LAST (1955) found that a higher incidence of *Sporobolomyces* on wheat on upper leaf blades was due to higher humidity at that level. WEBSTER and DIX (1960), on the other hand, suggested that the case of *Sporobolomyces* could be correlated with higher nutrient status of the upper leaf blades.

DIEM (1971) studied the effect of low humidity on the survival of germinated spores of *Alternaria tenuis*, *Stemphylium botryosum*, *Helminthosporium* sp., *Cladosporium herbarum*, *C. cladosporioides*, *Colletotrichum graminicola*, *Aspergillus* spp. and *Penicillium* spp. Intermediate humidity (76 per cent) was found to be deleterious for *S. botryosum*, *A. tenuis* and *Cladosporium* spp. The spores of *Colletotrichum graminicola* and *Helminthosporium sativum* were killed within four hours at 73 per cent. The hyaline spores were sensitive to anhydrous desiccation due to calcium chloride. This was suggested to be the cause of scarcity of *Aspergilliacea* in the phylloplane and prevalence of dematiaceous members under conditions of desiccation.

Minimal, maximal and optimal growth of various fungi is found to be limiting at different pH values (CANTRELL and DOWLER, 1971; YADAV, 1968 and MUKERJI, 1966). BRUEHL and LAI (1968) reported that the best growth of *Cephalosporium gramineum* in infested wheat straw occurred in the pH range 3.9 – 5.5. Certain nutrients and light have also been reported to influence growth of *Ceratocystis ulmi* (HARRIS and TABER, 1970) and the rate of wood-decay (DUNCAN, 1967).

The fluctuations in the plant surface temperatures are also very important in controlling microbial colonization. The temperature beyond 60°C, is usually the upper limit for fungal growth (COONEY and EMERSON, 1964; LANGE et al., 1958 and CHANG and HUDSON, 1967). But still thermophilic fungi have been isolated from self heating hay and mushroom compost (FERGUS, 1964), and wheat straw compost (CHANG and HUDSON, 1967). It has been reported that primary colonizers disappear once the thermophilic and thermotolerant fungi make their appearance (GREGORY and LACEY, 1963). We (1972) found that moderate temperatures of October favoured the appearance of a higher fungal population on cotton leaves, but very low temperatures of January and December resulted in a decline in the total fungal population. Some fungi showed significant correlation with temperature. For instance, *Candida* and *Phoma* were never recorded during the high temperature days of June, however, they were infrequent when extreme low temperatures of December and January prevailed. *Aspergillus niger* was also sensitive to low temperatures of winter months. In addition, temperature variations are also known to bring about changes in growth pattern of *Pythium* and *Rhizopus stolonifer* (SINGH, 1964 and PIERSON, 1966).

In general, it is not possible to characterize the factors determining sequence of microbial colonization on plant surfaces. However, RUINEN (1961) has suggested that yeast colonization might depend on the primary activities of bacteria during which essential nutrients are released. On the other hand, the progression

from bacteria to yeasts may reflect changes in nutrients released by the ageing host (TUKEY, 1971). LAST (1955) also reported that numbers of *Sporobolomyces* colonies isolated from cereals increased with increasing leaf age.

Substrate Characteristics in Relation to Microbial Colonization

The earliest plant surface colonizers are aided by an absence of competition but after the arrival of secondary colonizers, competition begins among them due to the creation of a relatively hostile environment. HERING (1965) reported that in ash, birch, hazel and oak, up to one year after leaf-fall, a characteristic flora of pyrenomycetes and deuteromycetes existed on leaves, but after their decay for one year, species of *Penicillium* and *Trichoderma* dominated. DICKINSON (1965, 1967) showed that young leaves were colonized by a very restricted mycoflora as compared to the moribund and senescing leaves. Our studies (1972) on *Gossypium* also indicated the colonization of green leaves by a fewer species as compared to that on dried intact, dried fallen and dried decaying leaves. KAMAL and SINGH (1970) found that the members of sphaeropsidales, and certain genera viz. *Fusarium*, *Alternaria* and *Curvularia* whose spores are usually present on relatively decomposed leaves of several pteridophytes became active when the leaves came in contact with soil. On the other hand, PUGH and BUCKLEY (1971) showed that a percentage of spores of *Cladosporium herbarum* and *Epicoccum nigrum* consistently germinated on green leaves, but their subsequent rates of growth and sporulation were low as compared to those on moribund leaves. This observation, therefore, implies that these fungi are active colonizers of green leaves.

WEBSTER and DIX (1960) reported that during the months of May, June and July, upper leaf-sheath tissues were found to be capable of supporting more rapid growth of the primary fungi than the lower internode tissues. In later months, the situation became reversed and the lower internode tissues supported more rapid growth of the fungi. The leaf blades, however, bore the same sporulating fungi on the upper as well as lower internodes. ARCHBOLD and MUKERJEE (1942) reported similar observations in barley at maturity, wherein the variation in the carbohydrate content had been suggested to control the differential fungal distribution. Similar studies have been made on *Heracleum sphondylium* (YADAV, 1966), *Dactylis glomerata* and *Agropyron repens* (WEBSTER, 1956; 1957; HUDSON and WEBSTER, 1958 and HUDSON, 1962).

RUINEN (1970) reported that mature and senescent stages of leaf as well as the leaf sheath and lamina were harboured by a distinct mycoflora. This has been supported by DICKINSON and MORGAN-JONES (1966) who reported the occurrence of vegetative state of *Ascochytula* on the green functional leaves, and its sporulating state on moribund leaves. Maximum occurrence of *Ascochytula obiones* was found in May and September on all types of leaves in all the three *Halimione*

communities, they investigated. They further demonstrated that during the months of January and March, *Ascochytula* disappeared but *Fusarium* spp. became the dominant colonizers of the moribund leaves. The presence of similar dominant pycnidial fungi in all the three communities has been considered a good evidence for the host determined nature of colonizers.

Sometimes, the response to ageing tissues described by PUGH and BUCKLEY (1971), may be confused with effects of changing season. When age and season were separated in an analysis of the occurrence of *Sporobolomyces* on the differing leaves of winter-sown wheat, as expected, fewer colonies were isolated from leaves that unfurled during winter than from those that unfurled during the spring and summer. MENNA (1959) detected a similar seasonal trend with *Rhodotorula* and *Sporobolomyces* isolated from New Zealand pasture grasses, but the total number of colonies was more or less maintained by a compensating increase in the population of *Cryptococcus laurentii* and *Torulopsis ingeniosa*.

A few more host specific microbes can be mentioned here. DICKINSON (1967) reported *Ascochytula pinodes* to be a fungus 'unique' on the senescent *Pisum* leaves. Similarly, HUDSON and WEBSTER (1958) found *Pullularia pullulans* to be an important primary colonizer of *Agropyron repens*. HOGG (1966) and PUGH and WILLIAMS (1968) demonstrated that the fungi which were frequent on the aerial plant parts, were found to disappear from the buried parts. On the other hand, YADAV and MADELIN (1968) during their studies on the ecology of microfungi on decaying stems of *Urtica dioica*, divided the sporulating fungi into three categories: *(i)* the fungi which were more or less uniformly distributed e.g. *Cladosporium*, *Sphaerospermum*, *Dendryphium comosum* and *Periconia cookei*, *(ii)* the fungi which were more frequent on upper segments e.g. *Alternaria tenuis* and *Cladosporium herbarum*, and *(iii)* the fungi which were more frequent on the lower segments e.g. *Leptosphaeria acuta*, *L. doliolum*, *Pleurophragmium simplex* and *Torula herbarum*.

LAST (1955), LAST and DEIGHTON (1965), DICKINSON (1967), FRIEND (1965) and PUGH and BUCKLEY (1971) have demonstrated that most of the fungi on the leaf surfaces are restricted to the veins and veinlets. PUGH and BUCKLEY (1971) suggested the following reasons for such a distribution: 1. Presence of channels over the veins where water droplets carrying the spores may flow. 2. High rate of exudation of nutrients along the veins. 3. Vein extension cells along the veins may cause enrichment of exudates in these regions. 4. Aphid punctures over the veins may increase exudation and provide avenues for the entry of fungi into the tissues. 5. Fungi may be present in the vascular tissues and may reach the surface along the veins and veinlets.

TUBAKI and YOKOYAMA (1971) studied the mycoflora on sterilized leaves placed in the natural litter and placed the isolated fungi in four groups. First group comprised those which were represented by detachable propagules and germinated in the available moisture e.g. the members of *Mucorales*. The second group comprised those forms which grew actively and sporulated on the leaf

surface during the entire period of decay e.g. *Penicillium* spp., *Calcarisporium* and *Trichoderma*. The third group was represented by those fungi which were found during the early decay process but had a tendency to disappear later e.g. the species of *Ceratocystis*, *Subulispora* and *Sympodiella*. The fourth group comprised those fungi which were rare in the early periods of decay but common during the latter half of the decay period e.g. *Toxotrichum*, *Calcorisporium*, *Chalara*, *Codinaea*, *Crinula*, *Dactylaria*, *Oidiodendron*, *Thysanophora* and *Verticillium*. *Subulispora* and *Pestalotia* were common only on sterilized green leaves during very early stages of decay, *Calcarisporium* on newly dead leaves while *Oidiodendron* grew actively only on the dead leaves.

JENSEN (1971) reported that principal bacterial colonizers of above ground parts of beech plants were the fluorescent pseudomonas but non-fluorescent bacteria also occurred in large numbers on both living and dead plant material. Phylloplane bacteria were described to have simpler growth requirements than the soil or litter bacteria. LANGE and LEBEN (1971) have reported on the infection of cucumber leaves by *Pseudomonas lachrymans* while the leaves were within the bud. These leaves have been reported to provide a continuous source of inoculum for disease spread after their unfolding.

RUINEN (1971) has demonstrated that grass sheath is a very important niche for the nitrogen fixing bacteria *(Spirillum* and *Beijerinckia)*. The reason has been suggested to be the presence of optimal conditions for the catchment of water and leaching of carbohydrates and nutrients in higher concentrations in the microclimate of the leaf-sheaths. JONES (1970), using radioactive nitrogen (N^{15}), not only detected nitrogen fixing microbes superficially colonizing the leaves of *Pseudotsuga* but also proved that these microbes could fix nitrogen in this ecological niche in field conditions. MENNA (1971) reported the occurrence of *Pithomyces chartarum* and *Metarrhizium anisopliae* from the surfaces of green leaves. In nature, the former is a saprophyte and the latter is an insect pathogen. It had been suggested that this behaviour was due to the contamination of green leaf surfaces by some organic substrates and the insects parasitizing the host plant, respectively.

Interactions on the Plant Surfaces

There are evidences to indicate that the plant surface microbes maintain a dynamic equlibrium. The balancing mechanism operates not only between pathogens and non-pathogens but also within them. The plant surface saprophytes may interfere with the pathogens in two ways: by inhibiting the infection or by modifying the course of disease after infection. CROSSE (1971) observed several saprophytes co-existing on leaf surfaces which included pseudomonads, coliforms and the *Erwinia*-like organisms. When the isolates from the latter group were co-inoculated with *Pseudomonas morsprunorum* into the leaf scars, they reduced the incidence of infection caused by *P. morsprunorum* and the size of the resulting can-

kers. This inhibitory action could be destroyed if the *Erwinia*-like organisms were heated before inoculating them along with the *P. morsprunorum* cells. This indicated that the biologically active systems were responsible for the inhibition.

LAST (1955) showed that in the beginning colonies of *Sporobolomyces* could be isolated from the entire surface of wheat leaves but suddenly they were found restricted to the margins, the remainder of the lamina being colonized by *Tilletiopsis*. A similar instance was reported by PUGH and BUCKLEY (1971) wherein they noticed that on sycamore leaves, *Aureobasidium pullulans* grew actively until populations of *Cladosporium* and *Epicoccum* were not high. In due course, cells of *A. pullulans* were thickened and became pigmented (resting stages) which, however, could be induced by culturing *A. pullulans* close to the colonies of *Cladosporium* and *Epicoccum*.

BRUEHL and LAI (1966) and MACER (1961) showed that primary colonization of wheat straw by certain fungi resulted in the prevention of invasion by other fungi. COOK (1970) found that colonization of wheat straw by *Fusarium roseum* went on declining as the period of exposure of the straw to weathering prior to burial increased. The saprophytic colonization by *F. roseum* increased if the weathered straw was autoclaved prior to burial. It was suggested that the establishment of other saprophytic fungi in the straw prior to burial was the reason for this effect.

WEBSTER and DIX (1960) suggested that competition with other fungi in damper conditions, for instance, on the lower internodes, was the reason for non-sporulation of the primary colonizers on the leaf blades of lower internodes. Thus, the fungi having high competitive ability, sporulated first and constituted the dominant fungi. Our studies (1972) also indicated that non-sporulation of myxomycetous members in the presence of higher moisture conditions might have been due to competition between the myxomycetous members and other colonizing fungi.

MÜLLER and BÖRGER (1940) were the first to invoke the idea that post-infectionally produced fungitoxic compounds were responsible for inducing resistance of potato tubers following inoculation with *Phytophthora infestans*. They named such inhibitory compounds as "phytoalexins" and defined a phytoalexin as "an antibiotic which is produced as a result of the interaction between two metabolic systems, the host and the parasite, and which inhibits the growth of microorganisms pathogenic to plants".

BLAKEMAN (1971) has described that the phytoalexins can operate in three different situations:

1. Pathogens and saprophytes can induce phytoalexin but the pathogens are able to tolerate its effects e.g. *pisatin* is known to be broken down by pathogens.

2. Pathogens are unable to induce phytoalexins in their hosts but they are sensitive to phytoalexin induced by non-pathogens or saprophytes. In alfalfa, *Helminthosporium turcicum* (saprophyte) and *Stemphylium loti* (weak pathogen), are known to induce phytoalexin formation in larger and smaller amounts, re-

spectively. But in the presence of alfalfa pathogen, *S. botryosum*, the phytoalexin could not be detected probably due to its breakdown by the pathogen. Fungitoxic concentrations of phytoalexin, *in vitro*, were, however, effective against all the three fungi.

3. Pathogens are able to induce phytoalexins only in resistant varieties of the host e.g. *Helminthosporium turcicum* causes phytoalexin formation only in the resistant varieties of corn whereas *Phytophthora megasperma* does so only in the resistant varieties of soybean. Application of the phytoalexins on lesions of a susceptible variety results in the protection of plant against infection.

Phytoalexin production has been reported in the case of several pathogenic and saprophytic fungi, for instance, *Sclerotinia fructicola* (CRUICKSHANK and PERRIN, 1960), *Ascochyta pisi* (UEHARA, 1958; 1959), *Septoria pisi*, *Fusarium solani* var. *martii* f. *pisi*, *Botrytis alli*, *Penicillium* spp., *Erysiphe* sp. and *Uromyces fabae; Aspergillus nidulans*, *Rhizopus nigricans* and *Neurospora crassa* (CRUICKSHANK and PERRIN, 1961; 1965). Fungitoxicity and chemical nature of several phytoalexins viz. orchinol, pisatin, phaseollin, wyerone acid, rishitin, ipomeamarone, an isocoumarin and safynol, have been determined. It appears that differential sensitivity in terms of the ability of pathogenic fungi to degrade phytoalexin is responsible for the incidence of a disease (BAILEY, 1971).

Mechanism of phytoalexin production is still a matter of dispute. CRUICKSHANK (1963; 1968) has suggested that phytoalexin formation is induced by metabolites of the invading fungus. He isolated a metabolite, monilicolin A from *in vitro* culture of *Sclerotinia fructicola*. This compound has been proved to be an active phytoalexin inducer even at very low concentrations. WOOD (1967) suggested that fungal metabolite enters into the cell through cuticle and induces the underlying cells to produce the inhibitory substances before they are penetrated by the infection hyphae. This inhibitor later may move onto the plant surface and kill the invader in early stages of infection.

BAILEY (1969) suggested that the active growth of phylloplane microflora when the leaves senesce, is due to a decreased phytoalexin production. In this connection, KLARMAN and GERDEMANN (1963) have shown that when the phytoalexin produced by soybeans resistant to *Phytophthora sojae*, was leached away, the fungus caused extensive water-soaked lesions on the host.

BAILEY (1971) has given an account of the role of phaseollin in restricting hyphal growth in the hypersensitive reaction on *Phaseolus vulgaris* as a result of infection by *Collectotrichum lindemuthianum*. The fungus was very sensitive to phaseollin which was detected in tissues containing necrotic cells. Thus, hypersensitive reaction, here, was associated with phaseollin production which in turn resulted in the cessation of growth of *C. lindemuthianum*.

HEUVEL (1971) demonstrated that the presence of *Alternaria tenuissima* (saprophyte) on the bean leaves did not allow the infection by the pathogenic species *A. zinniae*. He has produced evidence that this inhibition was due to some inhibitory substances produced by *A. tenuissima*. MCBRIDE (1971) showed that

development of *Meria laricis* (a pathogen) and *Cladosporium gerbarum* (a saprophyte) was inhibited by bacteria and yeasts isolated from the phylloplane of larch. In addition, he found that inhibitory factors from *Pseudomonas* sp. and *Sporobolomyces roseus* varied depending upon the nutrient level in the media. LEBEN and DAFT (1965) and MCBRIDE (1969) demonstrated that foliar spray of nutrient suspensions containing the spores of microflora could reduce disease incidence to a great extent.

JOHNSTON and HUFFMAN (1958) showed that the incidence of *Puccinia triticina* on a susceptible wheat variety could be reduced by pre-inoculation of the leaf with uredospores of *Puccinia coronata* (crown rust of oats). On the other hand, MOSEMAN and GREELEY (1964) have demonstrated that the presence of *Puccinia graminis tritici* on wheat predisposes the plant to infection by *Erysiphe graminis hordei* (parasite on barley). BIER (1966) reported that development of *Melampsora occidentalis* on living needles of *Pinus trichocarpa* could be inhibited by artificial introduction of *Trichoderma* and *Epicoccum*. RIBERAU-GAYON et al. (1955) showed that the growth of *Botrytis cinerea* beneath the skin of the grape resulted in the reduction of sugar content of the grapes and its antibiotic effect replaced the common fermenting yeast, *Saccharomyces* by the species of *Torulopsis*. Studies on the artificial inoculation of dry conidia of *Botrytis cinerea* onto the detached clover leaflets have indicated an inhibition of development of *Erysiphe polygoni* by as much as 80 per cent (BARNES, 1971). TVEIT and WOOD (1955) reported that *Fusarium* blight in oat seedlings could be controlled by antagonistic species of *Chaetomium*.

Field studies by MORTON and PETERSON (1960) and WIBE and MORTON (1962) indicate that early infections of leaf blades by *Helminthosporium sativum* inhibit the latter development of *Septoria passerinii* in leaf sheaths. BIER and ROWAT (1962) have demonstrated that the presence of saprophytes on bark of poplar and willow is inhibitory to canker caused by *Hypoxylon pruinatum*. The host cuttings could be rendered susceptible to this fungus by surface sterilization. SINHA (1965) found that *Alternaria* blight of chillies could be controlled by the presence of a bacterium. When the bacterial population was high (from March to June), the disease incidence was low but disease incidence increased as there was decline in the frequency of bacterium (from July onwards). BAMBERG (1931) isolated a bacterium from corn which when mixed with smut spores of *Ustilago zeae*, reduced the infection rate of corn and inhibited the germination of chlamydospores. VOZNYAKOVSKAYA and SHIROKOV (1961) reported that incidence of grey mould of strawberry could be controlled by epiphytic mycoflora.

Application of foliar sprays containing the spores of certain plant surface saprophytes or bacterial cells are known to control incidence of certain disease (BHATT and VAUGHAN, 1963; SINGH and SINHA, 1962). LEBEN (1964) isolated several bacteria from cucumber seedlings grown under controlled conditions. One of these isolates (A-180) was found to show significant decrease in the incidence of anthracnose disease. There are, thus, sufficient evidences to prove that

plant surface saprophytes may effectively control plant pathogens in certain conditions.

Antifungal factors produced by a fungus can also have an important role by their activity against the other colonizers. WEINDLING (1934) noted that *Gliocladium fimbriatum* was an active antagonist against *Rhizoctonia solani* and some other fungi. He and EMERSON (1936) isolated a compound termed gliotoxin from *Trichoderma lignorum* cultures. This compound was found to be an effective control of overed smut of barley, bunt of wheat and *Helminthosporium* leaf spot of oats. The first use of antibiotic under field conditions was reported by ZAUMEYER et al. (1953), who showed that halo-blight *(Pseudomonas phaseolicola)* of beans could be easily eradicated by streptomycin spray. VÖRÖS et al. (1957) have demonstrated that streptomycin-treated potato plants become resistant to *Phytophthora infestans* due to the induction of plant phenol oxidase system by the applied antibiotic, resulting in quinone-production in tissues at concentrations sufficient to inhibit the pathogen.

In addition to leaking antibiological factors and nutrients some fungi have been known to release growth hormones also. DIEM (1971) has reported several bacteria and yeasts, whereas BUCKLEY and PUGH (1971) have reported species of *Cladosporium*, *Aureobasidium* and *Epicoccum* which are capable of producing auxin-like substances. However, the extent of the physiological effects due to these substances and the limits of the amounts of their production in natural conditions, are not known.

Occasionally plant surface pathogens may bring about morphological variations in the epidermal cells. For instance, MEYER (1955) reported that stomata in the inflorescences of *Raphanus sativus* infected with *Peronospora parasitica* undergo enormous multiplications. This multiplication could involve the guard cells as well as the subsidiary cells or the guard cells alone.

The phenomenon of mycoparasitism also may play an important role in maintaining the ecological niche on the plant surface. For instance, *Ciccinibolus cesatii* is known to parasitize the hyphae and conidiophores of *Erysiphe graminis*, *C. ewonyi-japonici* parasitises *Oidium ewonyi-japonici*, *C. asteris* parasitises uredo- and teleutosori of several rusts, and, *Tuberculina* spp. attack the *Uredinales*. KEENER (1954) and BOURRIGUET (1946) have reported that *Cladosporium aecidiicola* grows in the aecia of *Puccinia conspicua* and *Verticillium hemileiae*. PON et al. (1954) reported a *Xanthomonas* sp. parasitic on uredia of cereal rusts.

Chemical Factors in Relation to Plant Surface Interactions

LATHAM and WATSON (1966; 1967) have demonstrated that the presence of certain crop residues results in disease reduction in onions as a result of antibiotic activities among the soil pathogens and other microorganisms in the crop-

residues. WRIGHT (1956) showed that the straw fungi and those associated with viable seeds were capable of producing antibiotic substances. SAITO (1958, 1965) reported that the hymenomycetes on beech litter, produced substances that were antifungal as well as antibacterial. But, the fungi on recently fallen leaves did not exhibit much antibiotic activity (SAITO, 1966). WEBSTER and DIX (1960) have suspected that the difference in the fungal colonization on the upper and lower internodes may be due to the presence of some inhibitors in the lower internodes.

GROVER (1971) reported that spore germination and appressoria formation in *Colletotrichum piperatum* were two independent processes which could be controlled by altering the nutrient balance. He found that the washed spores of *C. piperatum* germinated and produced appressoria *in vitro* in the presence of glucose, sucrose and thiamine, but, in water on glass slides as well as on leaf, stem and green pepper fruits, only spore germination without appressoria formation was possible. On the other hand, germination as well as appressorium formation could be easily achieved on the surface of red pepper fruits. He had also demonstrated that amino acids and amides had an inhibitory action against spore germination and appressoria formation. Although low concentrations of sucrose alone could induce these two processes, sucrose supplemented with other nutrients showed no response. Appressorium formation is also known to be influenced by contact stimulus (PURDY, 1958, DICKINSON, 1949), presence of carbohydrates (PURDY, 1958), presence of zinc (PAVGI and DICKSON, 1961) and in the presence of a zinc-gelatin medium (BROYLES, 1955). HURD-KARRER and RODENHISER (1947) reported that rust appressoria were formed on nutrient agar supplemented with glucose, phosphates and other nutrients but not on water agar indicating the role of nutrients in appressoria development.

KLINCARE et al. (1971) demonstrated that pretreatment of seeds of some agricultural plants by Mercuran, 50 per cent TMTD [Thiram; tetramethylthiurum disulphide; $(CH_3)_2NCSSSCSN(CH_3)_2$] and streptomycin prior to sowing resulted in a remarkable decrease in the epiphytic microflora of both aerial and underground parts. On the other hand, similar treatment by some trace elements resulted in increase of epiphytic microorganisms. KLINCARE et al. (1971) suggested that this might have been due to the secretion of vitamins, auxins and gibberellin-like substances by epiphytic bacteria which in turn, might have stimulated seed germination and shoot growth of the plants, bringing about the variations in other epiphytic microbes. STOTT (1971) studied the effect of Fungex and Captan on the leaf surface saprophytes and suggested that the implications of such studies could be very useful in understanding the plant surface niche.

Incidence of *Botrytis cinerea* has been noticed to increase with the increase of organic fragments on the fruit surfaces and, in the presence of pollen on bean leaves, it results in a marked increase in its growth and sporulation (CHOU and PREECE, 1968). A similar instance was reported by FOKKEMA (1968; 1971) wherein he demonstrated that artificial inoculation of pollen onto the rye leaves resulted in enhancing the growth of *Cladosporium herbarum* and *Helminthosporium sati-*

vum. In addition, he could obtain similar results in moist chambers supplemented with pollen and conidia of these two fungi indicating the importance of pollen as the substrate for these fungi.

The spores which have insufficient levels of endogenous nutrients need additional supply of nutrients for germination and growth. BROWN (1922) demonstrated that growth and infection by *Botrytis cinerea* on petals of *Cereus* and *Gloxinia*, and on leaves of broad bean, was enhanced with natural exudates and with added nutrients. PURDY (1958) reported that *Sclerotinia sclerotiorum* caused infections on clover and lettuce only with added carbon source. LAST (1960) and DEVERALL and WOOD (1961) found that sugars added to infection drop caused increased infection of *Botrytis fabae* on broad bean. This has been proved by ORELLANA and THOMAS (1962) who have shown that susceptibility of castor bean to *Botrytis cinerea* depends upon the differences in the amount of sugar in the leachates of different varieties.

The mature plants of *Cicer arietinum* are found to be resistant to *Mycosphaerella rabiei* whereas young plants are susceptible. HAFIZ (1952) has suggested that this is because of malic acid production by glandular hairs on the leaves of mature plants. SHARMA and SINHA (1971) demonstrated that leaf exudates from susceptible *Sorghum* varieties were less effective against anthracnose fungus *(Colletotrichum graminicola)* than those from resistant varieties. CHAND and WALKER (1964) also reported a similar instance wherein they have demonstrated that *Pseudomonas lachrymans* multiplies more rapidly in the exudates of a susceptible variety of cucumber than in those from a resistant variety. Exudates from young leaves are more effective in causing inhibition than those from mature leaves (SHARMA and SINHA, 1971; KERR and FLENTJE, 1957; KOSUGE and HEWITT, 1964 and SINHA, 1965). The leaf exudates collected at different timings from the leaves of the same age also exhibit great variations in their degree to cause fungal inhibition (SHARMA and SINHA, 1971).

Concentrated leaf washings of woodland trees have been shown to inhibit the germ tube growth in *Botrytis cinerea* (TOPPS and WAIN, 1957). Leaf surface-phenols could inhibit the development of *Venturia inaequalis* and *V. pirina* on apple and pear leaves respectively (KIRKHAM, 1954). MARTIN and JUNIPER (1970) reported that an ether soluble fraction of apple leaf-surface-wax caused the inhibition of *Podosphaera leucotricha*. Resistance of *Ginkgo* leaves to fungal diseases has been suggested to be associated at least in part with the cuticular wax (JOHNSTON and SPROSTON, 1965). HEATHER (1967) suggested that resistance of *Eucalyptus bicostata* leaves to *Phaeoseptoria eucalypti* was due to the hydrophobic property of the leaf surface wax. BLAKEMAN (1971) has suggested that products of pollution on leaf surfaces may also restrict the growth of pathogens, e.g. incidence of black spot *(Diplocarpon rosae)* on roses is found to be eliminated in highly polluted areas.

BLAKEMAN (1971) found that pretreatment of leaves with decenylsuccinic acid resulted in enhancing the production of leachable sugars and amino acids.

He suggested that these chemicals, in turn, caused an increase in spore germination and infection on the leaf surface. SOL (1967), on the other hand, demonstrated that if roots of broad bean plants were supplied with ammonium as source of nitrogen instead of nitrate, it resulted in higher levels of leachable sugars and amino acids which in turn enhanced the growth of leaf surface colonizers.

Antibiotic substances produced by one microorganism may also interact with other colonizers. MORGAN (1963) showed that filtrates from three bacterial cultures could cause the lysis of rust uredospores. Even autoclaved culture filtrate from *Bacillus pumilus* could bring about the uredospore lysis, showing that antifungal substance was not an enzyme. LEBEN (1964) also showed that a cell free filtrate of a cucumber bacterium could cause the inhibition of *Colletotrichum lagenarium*.

PURNELL (1971) has performed studies on the effect of washing the leaves of *Brassica napus* and then inoculating them with the conidia of *Erysiphe cruciferarum*. In general, spore germination and appressorium formation was 10 per cent higher on the leaves as compared to that on glass slides indicating the presence of some stimulating factor for this behaviour on the leaf surface. Production of primary hyphae was poor in winter months. About 20 per cent higher hyphal production over the unwashed controls was recorded in conidia on leaves inoculated 5 days after the date of washing. Similar inoculations later than or before 5 days after washing of leaves revealed a reduction in hyphal production on washed leaves as compared to that on unwashed leaves. Similar results were obtained when the *in vitro* effect of successive washings from the washed leaves was studied against the conidia of *Botrytis cinerea*. He (1971) has concluded that hyphal growth in these cases is controlled by two factors. One is probably carbohydrate in nature which appears in sufficient amounts only 5 days after the date of washing. The other is an inhibitor which appears gradually and is effective only after about 12 days of washing, resulting in a subsequent decrease in spore germination and hyphal production.

Several systemic fungicides are now used to control the incidence of some pathogens e.g. dimethirimol controls the incidence of powdery mildew, *Sphaerotheca fuliginea*, benomyl is effective against apple scab, *Venturia inaequalis*, and carboxin prevents the spread of *Exobasidium vexans* on tea. In assessing the role of surface application of fungicides, the situation becomes complex due to the nature of interaction between the host, the pathogen, the phylloplane fungi and the fungicides. On the whole, the mechanism of penetration of the pathogen by a fungicide is an unsolved problem (GOTTLIEB and SHAW, 1970 and MCCALLAN, 1967). WAIN and WILKINSON (1946) and ARMAN and WAIN (1958) have suggested that exudates from the fungal spores themselves and from the leaves may dissolve the deposited fungicide and render it available to the fungus.

BURCHILL and COOK (1971) and BURCHILL et al. (1965) suggested that the inhibition of perithecial development of *Venturia inaequalis* on 5 per cent sprayed, and urea dipped apple leaves was due to the growth stimulation of *Fusarium* spp.,

Cladosporium herbarum and *Alternaria alternata* on them. They found that the microflora of non-treated leaves was dominated by Gram-positive organisms and by pigmented or chromogenic forms, many of which had stimulatory effect on *V. inaequalis in vitro*. Urea-treated leaves, however, showed the predominance of Gram negative, non-chromogenic flora including fluorescent pseudomonads comprising several Gram negative bacteria antagonistic to *V. inaequalis in vitro*. They (1971) have also reported that the apple leaf flora before leaf-fall comprises some microorganisms which stimulate perithecial production in *V. inaequalis*. This was indirectly proved by the fact that perithecial inhibition occurred if leaf flora was removed by sodium hypochloride solution at the time of leaf-fall. However, the workers are not sure that the organisms antagonistic to perithecial development are present on the leaf surface before leaf-fall, or they spread onto fallen leaves from soil.

DUNN et al. (1971) reported that leaf washings of 5 plant species they studied *in vitro*, contained a factor which inhibited the fungicidal activity of ETD (ethylene thiurum disulphide) to *Alternaria brassicicola*. This factor in leaf exudates was identified as D-glucose and the observation was further proved by the fact that glucose pretreatment rendered *A. brassicicola* spores resistant to ETD toxicity. This is in agreement with TRIPATHI and GOTTLIEB (1965) and GOTTLIEB and TRIPATHI (1968) who proved that glucose was the most important carbohydrate metabolite involved in the fungal spore germination. DUNN et al. (1971) have also suggested the possible mechanism of ETD inactivation as follows: glucose present on plant surface enters into spores and stimulates their oxidative enzymes active in respiratory and biosynthetic processes. Fungicide concentrations without exogenous glucose do not cope up with this enzyme activity and in turn get metabolized by the fungus to non-toxic compounds. But whether such phenomenon occurs on the plant surfaces *in vivo*, is yet to be determined.

BIEDERMANN and MÜLLER (1952) demonstrated that water soluble exudates of vine leaves could reduce the toxicity of copper fungicides to *Alternaria tenuis*. In addition, L-histidine, L-cysteine and DL-methionine could inhibit the fungicidal activity of sodium dimethyldithiocarbamate (DEKHUIJZEN, 1964). The fungitoxicity of Zineb to *Aspergillus niger* was enhanced in the presence of leaf exudates of vine, potato, sugar-beet and apple. Soluble copper extracts were less toxic to *A. tenuis* spores than the equivalent amounts of copper applied as cupric sulphate. This has been attributed to detoxification of Zineb by the components of leaf exudates (HISLOP, 1966).

As mentioned earlier, the phylloplane organisms are capable of producing hormones, for example, *Azotobacter* and *Beijerinckia* produce indole acetic acid and gibberellins (VANCURA, 1961) which in turn influence the growth of other colonizers. Certain phylloplane fungi also produce auxins (BUCKLEY and PUGH, 1971 and VALADON and LODGE, 1970). BHAT et al. (1971) have demonstrated that the presence of leaf saprophytes, especially the nitrogen fixers, on mulberry, sandal and *Dolichos*, is beneficial for the well-being of the plants. JUMP (1938), on the

other hand, has reported that forking in red pine needles is caused due to production of some phytohormone by *Aureobasidium pullulans* which colonizes green pine needles.

RUINEN (1956) has reported that a fern, *Drymoglossum piloselloides*, attached to the leaves of plants in Indonesia is fed by the nutrients secreted by the leaf cuticle. FLENTJE, DODMAN and KERR (1963) have given a direct evidence that exudates from radish stems and cotyledons stimulate the formation of infection cushions during attack by *Thanatephorus cucumeris*. SHARP (1965), on the other hand, demonstrated the inhibition of spores of *Puccinia striiformis* on wheat leaves and suggested that it was due to the presence of inhibitory secretions on the leaf surface. These observations, thus, support the view that leaf surfaces carry the components which are stimulatory to some, but inhibitory to other organisms.

Even the preformed substances present in "surface layers", which are termed "phytoncides" may be of importance in microbial colonization. For instance, potato varieties resistant to *Streptomyces scabies* contain higher amounts of chlorogenic acid in their peels than in those of the susceptible ones. GRIFFITHS (1968) reported that gentisic acid which has been isolated from the surface tissues of the members of 73 angiosperm families, was potentially an antifungal compound. Several bacteriostatic volatile and non-volatile fractions have been isolated from extracts of *Allium sativum* and *A. cepa* (ABDULLAEVA, 1962). TOKIN (1960) reported that the phytoncides of onion and horse-radish could bring about effective control of barley smut and of seed borne diseases of cotton. In contrast to these reports, BARNES and WILLIAMS (1960) have found that the infection by *Venturia inaequalis* of apple fruits results in the production of higher amounts of phenolic compounds in the epidermal tissues. Thus, many a time, the components of surface layers may be the result of microbial activity.

In certain cases, self-inhibitory and self-stimulatory products of microorganisms themselves, may also be of great importance. For instance, self-inhibition of germinating rust spores occurs due to the production of some volatile substances (ALLEN, 1955, FORSYTH, 1955). However, the activity of these inhibitors varies depending upon the spore concentration, storage conditions, and the storage period. HOYER (1962) has demonstrated that washings of spores of *Puccinia recondita* contain a germination inhibitor which is not specific, and, depending upon the periodicity of its production, inhibits the spore germination of different *Puccinia* species to different degrees. He found that maximum concentration of this inhibitor was in winter and a minimum in summer. BHOWMIK and PRASADA (1963) and SINHA (1965) have also reported a self-inhibitory factor in germinating uredospores of *Puccinia sorghi* and *P. penniseti*, respectively.

Microbiology of Degradation of Plant Organs

In general, the first sign of senescence of the plant organs is the loss of chlorophyll, fresh weight, water and total nitrogen content, with differences depending upon the plant species and the organ concerned. According to SIMON (1967), senescence of intact leaves may be progressive e.g. in cucumber, synchronous e.g. in leaves from deciduous trees fallen in autumn, or overall, involving the whole plant, usually following flowering. In the case of detached leaves, senescence will depend upon the conditions of incubation e.g. in light, dim light or in dark. Physiological changes in the senescing plant parts result in great variations in the microflora (DICKINSON, 1965; 1967; HUDSON, 1962; 1971; HOGG and HUDSON, 1966; HERING, 1965; FRIEND, 1965 and BADDELEY, 1971).

The specificity of the colonizers is controlled by the chemical composition of the plant substrate. SIU (1951) has shown that *Cladosporium herbarum*, *Alternaria tenuis*, and *Epicoccum nigrum* have a high cellulolytic activity. REESE and LEVINSON (1952) and HANCOCK et al. (1964) have demonstrated that *Botrytis cinerea* is also an active cellulose decomposer. It has also been found that different species and even different isolates of the same species differ in their degree of cellulose decomposition. For instance, HOGG (1966) reported that three isolates of *A. tenuis* from *Fagus* leaves differed remarkably in their degree of pectolytic activity. In the studies on artificial inoculation of γ-irradiated leaves by *Aureobasidium pullulans*, *C. herbarum* and *Mycena galopus*, HERING (1967) found that dry weight loss caused by the former two was only 2 to 4 per cent whereas by the latter 15–20 per cent showing its high rate of cellulose decomposition. The reports that *A. pullulans* is non-cellulolytic (REESE and LEVINSON, 1952 and SIU, 1951), have been contradicted by FRANKLAND (1969) who showed that *A. pullulans* caused about 7 per cent loss of dry weight in about 6 months of similarly treated leaves. This evidence suggests that *A. pullulans* is capable of decomposing at least some cellulose and lignin as well as soluble carbohydrates.

DICKINSON and MORGAN-JONES (1966) studied the colonization of pycnidial fungi on moribund parts of *Halimione portulacoides*, such as yellowed leaves, dispersed propagules and dead stem parts. They compared this mycoflora with that on the corresponding organs of intact plants and reported that pycnidial fungi were not important in the decay of *Halimione*-tissues. WAKSMAN, UMBREIT and CORDON (1939) demonstrated that thermophilic fungi were very important in the decomposition of stable manure. Our studies (1972) have shown that *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., and *Fusidium* spp. are among the active decomposers of cotton leaves whereas *Phoma* spp. (pycnidial forms) are relatively less active. The predominance of *Phoma* spp. in dilution plates and only rarely so in the moist chambers supports this fact.

Recent work has shown that different fungi differ in their ability to decompose different cell constituents of leaf-litter. LINDEBURG (1944) reported that *Mycena galopus* could decompose 37 per cent of the dry matter of beech litter in 6

months at 25°C. For oak litter, he found that the decomposition by *M. galopus* was close to 15–20 per cent of the dry matter. HERING (1967) observed that *Mycena galopus* and *Collybia personata* caused the greatest decomposition of oak leaf-litter.

CHANG (1967), in his *in vitro* studies showed that within three weeks, at 45°C, *Chaetomium thermophile* could cause a breakdown of over 40 per cent of the total weight of wheat straw. HENSEN (1957) has reported that *Humicola insolens* is a cellulose decomposer whereas *Sporotrichum thermophile* is capable of decomposing hemicellulose and pectin. GREGORY et al. (1963) have studied the changes in microbial population in relation to changes in nitrogen content, sugar content, lipids and ash in the hay. These studies, thus, support the hypothesis that different microbes are responsible for decomposition of different substrates.

GARRETT (1956) has proposed that a fungus capable of high saprophytic ability has a high growth rate, good enzyme equipment, higher capacity to produce antibiotic toxins and a higher tolerance to antibiotics. PUGH and WILLIAMS (1968), during their studies of fungal colonization on *Salsola kali*, have added two more characteristics to this list. viz. the ability of the fungus to possess protective mechanisms against high light intensity, for example, presence of melanin pigments, and perhaps the ability to utilize high light energy. According to NICOT (1960), the pigmentation of mycelium, spores, pycnidia and perithecia, presence of thick membranes, high rate of sporulation (multicellular spores) and a tendency for aggregation of the chlamydospores are the means for protection against desiccation and strong light. PUGH and WILLIAMS (1968) have found that the fungi isolated from the aerial parts of *Salsola kali* possess these characteristics but those isolated from buried parts lack several of them. The leaf-litter and phylloplane fungi are also reported to have these characteristics (WEBSTER, 1956; HUDSON and WEBSTER, 1958; PUGH, 1958; HUDSON, 1962; KENDRICK and BURGES, 1962; HOGG and HUDSON, 1966; DICKINSON, 1965; FRIEND, 1965 and LAST and DEIGHTON, 1965). In addition, LAST and DEIGHTON (1965) suggested that carotenoid pigments are also responsible for resistance to UV-radiation and for enhanced energy reception.

SAITO (1965) has divided the fungi involved in beech-litter decomposition into two major groups: lignin decomposing hymenomycetes and the non-lignin decomposing fungi (microfungi, cup-fungi and wood rotting fungi). He (1960) has described that the first fungi to become associated with the beech leaves are the microfungi, when the flare-up of these declines, the hymenomycetes become predominant and here again, recolonization by microfungi may take place due to the accumulation of the available nutrients as the hymenomycetes develop. This recolonization is influenced by competitive saprophytic ability of the fungi which in turn is controlled by temperatute.

Earlier, SAITO (1958) had shown that several fungi had better growth on the sterilized leaves as compared to their growth on natural or contaminated beech leaves. These contaminated leaves, if sterilized and tested, behaved in the same

way as did the natural sterilized leaves. He (1966) has also demonstrated that re-colonization of fungi is easier on a substrate in the laboratory as compared to that in the field conditions. It has been suggested to be due to unfavourable environmental conditions coupled with competition among other microorganisms. This is supported by the observation that the basidiomycetes do not grow in the presence of competitors.

It is now well established that among the soil microbes, food specialization results in the development of several ecological niches within any given habitat. SAITO (1966) has demonstrated that in the establishment of a microbial community, even if no new colonizers invade, the early colonizers disappear when the available food gets exhausted (unless new substrates are added). Thus, in a microbial community, appearance of a new competitor does not seem to be prime cause to replace the earlier colonizers. SAITO (1966) has repeated the observations of GARRETT (1956) and ROBINSON (1967) proving thereby that the first colonizers are the phycomycetes. These are followed by the ascomycetes, the deuteromycetes, and the basidiomycetes in a successive order. WEBSTER (1956, 1957) has also described a similar mycoflora in the three localities of cocksfoot tussocks.

In our studies (1972) on *Gossypium* leaves, in dilution plates, the members of the *Mucorales* were recorded frequently on the decomposing leaves. The reason for this deviation, is, however, unknown. In moist chambers, on the other hand, no sugar fungi were recorded on any of the leaf types investigated. It might have been perhaps due to the lack of nutritionally available sugars in all the leaf types (however, the presence of sugars in green leaves cannot be denied). KREUTZER (1965), during his studies on microbial succession in soil, has also reported some deviations from the normal sequence described above. KAMAL and SINGH (1970) have explained that occurrence of *Mucorales* on the decomposing leaves may have been due to the production of sugars as by-products during decomposition of cellulose and lignin. FRANKLAND (1966) has also reported a succession of saprophytic fungi on the petioles of *Pteridium aquilinum* wherein cellulose and lignin decomposers predominate before the appearance of sugar fungi.

SAITO (1958) demonstrated that more amount of beech leaves was decomposed by basidiomycetes as compared to that by filamentous fungi. He found that when from beech leaves, water or alcohol benzene soluble fraction was removed, the filamentous fungi did not grow on the remaining constituents although the basidiomycetous fungi were not affected. If the remaining constituents were further treated with hydrochloric acid, basidiomycetes lost the capacity to grow completely on it but filamentous fungi could grow, though slightly. SAITO (1958) has suggested that the basidiomycetes are finally decomposed by bacteria.

NYKVIST (1963) has reported that during leaching and decomposition of various litters, sucrose is the most frequent, although xylose, fructose and glucose are also detected. SAITO (1965) found that the cold water extract obtained from leaves infected with *Trichoderma viride* and *Piptoporus betulinus* contained little or no glucose at all. It indicated that probably the glucose of these leaves had

already been consumed by these fungi. In addition, HENDERSON and FARMER (1955) have reported that many soil fungi decompose the phenolic compounds related to lignin. GERD (1956) has stated that phenolic and their related compounds in litter are very important as regards the growth of a microorganism. However, according to BURGES (1965), phenolic compounds act as inhibitors of bacterial growth during microbial succession.

CHANG (1967) has recognized three groups of fungi involved in the decomposition of wheat straw compost: primary colonizers (the sugar fungi), thermophilic fungi (fungi capable of utilizing celluloses and hemicelluloses) and the mesophilic fungi. He describes that these fungi succeed one another with the changing temperature of the composts. PRAKASH and SAKSENA (1952) studied the capacity of twenty-two fungi to decompose paddy and bajra straws. They have demonstrated that the rate of straw decomposition by fungi has neither a relation with change in pH of the straw nor with oxidase activity of the fungi, but they have found a good correlation between the total carbon decomposed and the total loss in weight of the straw. For instance, maximum loss in weight of the straw was recorded in the case of *Penicillium luteum* but maximum total carbon decomposition was recorded in the case of *Chateomium* sp.

Microbial succession on the so-called natural litter has been extensively worked out and the chemical changes in the decomposing litter have been related to the participating mycoflora (SAITO, 1957; BURGES, 1958 and CARRE, 1964). SAITO (1966) has correlated the chemical and microbiological analysis both in field and in laboratory conditions. In parallel with the previous data, he has found that the fungi are the primary decomposers of the forest litter, saprophytic bacteria and actinomycetes being subsidiary.

Conclusions

The account presented above indicates that the plant surface provides a very complex ecological niche comprising a variety of non-pathogenic and pathogenic microbes dependent upon the substrates controlled by the host specific micro- and macro-environments. The interplay of all the components of this niche involves interactions and competitions resulting in the establishment by some and failure of other microorganisms. The degradation of different plant organs is influenced by the surface colonizers in conjunction with biochemical and physical factors prevailing thereupon.

The mechanism of leaching onto the plant surfaces and the factors associated with it should be explored extensively and there is ample scope for research in this field. A critical relationship between the pollution and the plant surface microflora needs to be studied in detail. Microclimate of the plant surface, especially in relation to the energy exchange phenomenon, should be dealt with extensively. An understanding of mechanisms of microbial colonization of different

plant organs can give a clue as to which of the microbes are really responsible for furthering or decreasing the economical value of the particular crop. Investigations on the phenomenon of senescence followed by decomposition in relation to the successive colonizers will positively give an understanding about the organisms which take the most active part in decomposition.

The mechanism of production of stimulatory and inhibitory principles by different plant surface microbes and their effect on the associated entities is to be understood. Selective use of fungicides, fertilizers and other biologically important chemicals against the selective group of colonizers should be investigated in detail. The most important aspect would be to search for a good combination of cultural methods for such studies because so far no single method is known which can give satisfactory results from all points of view.

Once we know all this, we find the difficulty in making field assessments of the *in vitro* results. For instance, if microbiological control of a disease is to be employed on a commercial scale, the extent of inhibitory interactions occurring on the plant surface under natural conditions should be studied very critically. It is only the future research which will enable us to manipulate the plant surface microbes in a predetermined manner.

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