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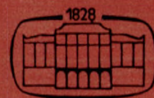

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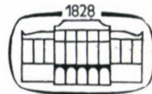

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1983

Contents

DISEASES

Alkenals, volatile defense substances in plants, their properties and activities
Horst Lyr and Lothar Banasiak 3
Polyfactorial thehory of plant immunity
I. Szepessy 13
Isolation of phytoalexins from germinating seeds of groundnut (Arachis hypogaea)
T. Narayanaswamy and *A. Mahadevan* 33
Some factors in the development of resistance of groundnut seedlings to damping-off caused by Rhizoctonia solani
M. N. Reddy and *A. S. Rao* 37
Changes in phenolic acids in groundnut leaves infected with Puccinia arachidis Speg.
M. N. Reddy 45
Drechslera rostrata a destructive mycoparasite of Rhizopus nigricans
R. C. Gupta, *R. L. Fotedar* and *K. N. Pandey* 49
Multiplication of Xanthomonas campestris pv. oryzicola in sensitive and resistant rice cultivars
M. Rai, *P. R. Reddy* and *R. Sridhi* 57
The effect of 2,4-D on the petiole tissues of sunflower (Helianthus annuus L.)
F. Herdi 63
Characteristics of the electro-blot radioimmunoassay (EBRIA) in relation to the identification of plant viruses
D. D. Shukla, *I. J. O'Donnell* and *K. H. Gough* 79
Aggregationseigenschaften des Nelkenringflecken-Virus in vitro
T. Kühne, *E. Proll* und *D. Reichenbächer* 85
Untersudhungen zur Stabilität des Nelkenringflecken-Virus
T. Kühne und *K. Eisbein* 101
Empfindliche Verfahren zur quantitativen Virusbestimmung am Beispiel des Gerstenstreifenmosaik-Virus (GSMV)
W. D. Ostermann und *R. M. Leiser* 111
New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses XVIII. Concluding remarks
J. Horváth 121

PESTS

Mass occurrence of Yponomeuta(= Hyponomeuta) padellus Linné (Lep. Hyponomeutidae) in 1982 at Hévíz
Gy. Sáringer, *J. Horváth* and *K. Zsoár* 163

PESTICIDES CHEMISTRY

Effect of chlorflurenol on the yield and technological value of red pepper (Capsicum annuum L.)
S. Lukasik, B. Achremowich, D. Kulpa and *T. Fraczek* 165

DISEASES

Characteristics of the protein A-immunosorbent electron microscopic technique (PAISEM) for detecting plant virus particles
D. D. Shukla and *K. H. Gough* 173
Serologische Untersuchungen mit dem cucumber mosaic virus (Gurkenmosaik-Virus). IV. Verwandtschaftliche Beziehungen zu einem Stamm des peanut stunt virus
J. Richter und *D. Reichenbächer* 187
A new pepper strain of tomato mosaic virus
G. Csillėry, I. Tóbiás and *J. Ruskó* 195
Population dynamict of aphids, vectors of maize dwarf mosaic virus and aphid resistance of some maize hybrids
I. Milinkó, Zs. Rakk and *Gy. Kovács* 201
Reaction of Physalis species to plant viruses VII. Additional data on the virus susceptibility of Physalis minima L.
J. Horváth 209
The role of some plants in the ecology of cucumber mosaic virus with special regard to bean
J. Horváth 217
Effect of single and double infection with Myrothecium verrucaria and common bean mosaic virus (CBMV) on dry weight and chemical constituents of bean plants
M. A. Amer, M. El-Hammady and *F. Abo El-Abbas* 225
Viruses of vegetable plants in Hungary and some of their properties
J. Horváth and *L. Beczner* 237
Effect of rust infection on lipid composition in leaves fo groundnut (Arachis hypogaea L.)
G. Rama Gopal and *P. Sreenivasulu* 255
Some biochemical aspects of host-pathogen interactions in Pythium stalk rot of maize: I. Role of toxin, pectolytic and cellulolytic enzymes in pathogenesis
E. A. Sadik, M. M. Payak and *S. L. Mehta* 261
Cross-recative antigens among Fusarium oyysporum f. sp. lupini and its host and non-host plants of unrelated families, Papilionaceae and Gramineae
M. Rataj-Guranowska and *I. Wiatroszak* 271
Effect of nitrogen supply on downy mildew development in sunflowers grown in perlit culture
S. A. Mohamed and *F. Virányi* 281
Trichoderma species in Hungary
L. Vajna 291
Overwintering of Diaporthe helianthi, a new destructive pathogen of sunflowers in Hungary
J. Vörös, J. Léránth and *L. Vajna* 303
„Protoplast killer" extracted from tobacco leaves inoculated with bacteria
Z. Klement, R. Gáborjányi, G. Somlyai and *É. Visnyovszky* 307
The influence of MCPA on the vascular tissue in the leaf petioles of sunflower (Helianthus annuus L.)
F. Herdi 313

Contents

DISEASES

Alkenals, volatile defense substances in plants, their properties and activities
HORST LYR and LOTHAR BANASIAK 3
Polyfactorial theory of plant immunity
I. SZEPESSY 13
Isolation of phytoalexins from germinating seeds of groundnut *(Arachis hypogaea)*
P. NARAYANASWAMY and A. MAHADEVAN 33
Some factors in the development of resistance of groundnut seedlings to damping-off caused by *Rhizoctonia solani*
M. N. REDDY and A. S. RAO 37
Changes in phenolic acids in groundnut leaves infected with *Puccinia arachidis* Speg.
M. N. REDDY 45
Drechslera rostrata a destructive mycoparasite of *Rhizopus nigricans*
R. C. GUPTA, R. L. FOTEDAR and K. N. PANDEY 49
Multiplication of *Xanthomonas campestris* pv. *oryzicola* in sensitive and resistant rice cultivars
M. RAI, P. R. REDDY and R. SRIDHARI 57
The effect of 2,4-D on the petiole tissues of sunflower (*Helianthus annuus* L.)
F. HERDI 63
Characteristics of the electro-blot radioimmunoassay (EBRIA) in relation to the identification of plant viruses
D. D. SHUKLA, I. J. O'DONNELL and K. H. GOUGH 79
Aggregationseigenschaften des Nelkenringflecken-Virus in vitro
T. KÜHNE, E. PROLL und D. REICHENBÄCHER 85
Untersuchungen zur Stabilität des Nelkenringflecken-Virus
T. KÜHNE und K. EISBEIN 101
Empfindliche Verfahren zur quantitativen Virusbestimmung am Beispiel des Gerstenstreifenmosaik-Virus (GSMV)
W.-D. OSTERMANN und R.-M. LEISER 111
New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses XVIII. Concluding remarks
J. HORVÁTH 121

PESTS

Mass occurrence of *Yponomeuta (= Hyponomeuta) padellus* Linné (Lep. *Hyponomeutidae*) in 1982 at Hévíz
GY. SÁRINGER, J. HORVÁTH and K. ZSOÁR 163

PESTICIDES CHEMISTRY

Effect of chlorflurenol on the yield and technological value of red pepper (*Capsicum annuum* L.)
S. ŁUKASIK, B. ACHREMOWICZ, D. KULPA and T. FRĄCZEK 165

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 18 (1—3), pp. 3—12 (1983)

Alkenals, Volatile Defense Substances in Plants, Their Properties and Activities

Horst Lyr and Lothar Banasiak

Institute for Plant Protection Research, Academy of the Agricultural Sciences, 1532 Kleinmachnow, GDR

Alkenals and alkenols (2-hexenal, 2-hexenol) are produced by most plants. According to the results described here they may have a natural function as defense substances as gaseous phytoalexins. The broad biocidal spectrum is based on an unspecific reaction mainly with membrane bound proteins, therefore the appearance of resistance is improbable. The more easily synthesizable derivative 2-ethylhexenal and its acid have a strong antifungal activity which makes them useful as conservation means for moist plant materials (grains, straw, hay, silage). The regulation of production of 2-hexenal in plants is discussed.

There exists an extensive literature on plant defense substances, especially on phytoalexins and other secondary plant products which hinder insects or other invading organisms from attacking higher plants.

Very few compounds could be used till now as models for synthetic pesticides. The most famous example are the "pyrethroids" derived from pyrethrum of *Chrysanthemum cinerariaefolium.* But among fungicides no modern product was elaborated by analogous synthesis from natural antifungal products.

In spite of the large literature on phytoalexins as cell constituents pretty little is known on volatile compounds produced by plants, which is mainly due to methodical difficulties. But it is known, that some plants are able to produce very effective volatile compounds as for instance hydrocyanic acid (*Sorbus* species and other plants) (Schlösser, 1980), mustard oils *(Cruciferae)*, garlic extract *(Allium sativum)* (Tansey, 1975) which have strong antimicrobial activities.

During former work on nitrogen fixation in *Robinia pseudoacacia* we made some observations on insecticidal volatile compounds from this species which were confirmed by the publication of Schildknecht and Rauch (1961). They identified the volatile compound, which this tree excretes through the transpiration stream in considerable amounts (3 $\mu g/m^3$) to the surrounding air, as trans-hex-2-en-1-al (2-hexenal) (Fig. 1). Its presence was described by Major et al. (1960, 1963), also in *Ginkgo biloba* and *Ailanthus glandulosa.*

Meanwhile several publications demonstrated that this compound is widely distributed in green plants, for example as an odour component in green fruits (cucumbers, tomatoes, apples, beans, bananas) (Drawert et al.; 1966, Tressel and Egewert, 1973). After short homogenization of fruits the level of 2-hexenal increased within 10 min from 0.02–3.2 mg/kg up to 6.6–40.4 mg/kg. Hatanaka et al. (1978) compared 40 plant species regarding their enzymatic activity in formation of 2-hexenal and found high activity in green leaves of Sphenosida, Pthe-

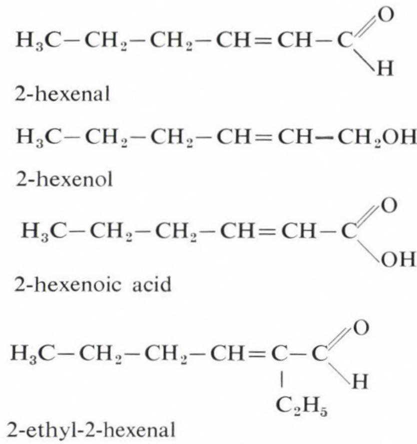

Fig. 1. Structural formulae of 2-hexenals and corresponding alcohol and acid

ropsida, Theaceae and Leguminosae, whereas vegetables, fruits and Monocotyledonae had lower activities.

Correlated with the enzymatic activities were the corresponding concentrations of 2-hexenal and 2-hexenol, which had been lower in winter than in summer time.

The pathway of biosynthesis of 2-hexenal was elucidated by several groups (Sekiya et al., 1979) (Fig. 2). The key enzyme is a membrane-bound lipoxygenase which is located in chloroplasts and probably also in mitochondria (Mc Leod

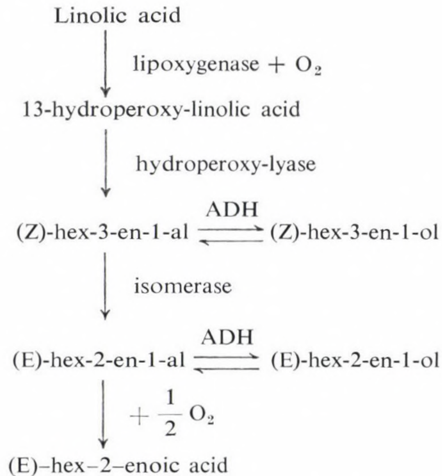

Fig. 2. Scheme for the biogenesis of hexenal and hexenol in plants (ADH = alcohol dehydrogenase)

and Pikk, 1979; Halangh et al., 1977). Substrates are unsaturated fatty acids (linolic and linolenic acid) from which in several enzymatic steps trans-2-hexenal is produced. It is either exudated by the transpiration stream or further metabolized. By reduction mainly cis-3-hexenol ("leaf alcohol") and n-hexanol are generated, whereas by oxidation the corresponding acids.

Materials and Methods

Chemicals

2-Butenal was purified from technical product by destillation. 2-Pentenal, 2-hexenal and 2-heptenal were synthesized according to Hoaglin and Hirsh (1950) by vinylether condensation. 2-Ethyl-2-hexenal was prepared from n-butyraldehyde by aldolcondensation (Häusermann, 1951).

The corresponding alcohol 2-ethyl-2-hexenol was obtained by $LiAlH_4$-reduction of the aldehyde (Green and Hickinbotton, 1957), the 2-ethyl-2-hexenoic acid was prepared according to Pummerer and Smidt (1957).

For experimental purposes we used further the 2-hexenal acetals. They were prepared in common manner by acetalization of the corresponding aldehyde with triethyl-orthoformate or ethylene glycol.

Propionic acid used for comparison is commercially available.

Application to host-parasite combinations

Seedlings were grown in moisture sand up to a height of about 10–12 cm. Then the seedlings of barley (sort "Astacus") were infected with spores of *Erysiphe graminis*, or wheat seedlings (sort "Strubes Dickkopf") with Puccinia triticina uredospores. The infected seedlings were put into glass vessels of adequate volume. Just before closing the vessels gas tight various amounts of the test substances were brought into the vessels and evaporated by slightly heating the drops from outside, so that the calculated concentration within the gas volume of the vessel was reached. The closed vessels were kept at 25°C under continuous light and after some days the number of sporulating pustulae was counted and compared with that of the controls without addition of 2-alkenals. The time of contact with the test compounds was 24 h, thereafter the vessels were opened. A similar procedure was used to test the activity of 2-ethyl-2-hexenal and corresponding alcohol and acid in the gas atmosphere against various fungi, but the vessels were kept in dark. The diameter of growth of the test fungi on open malt agar dishes (2% malt) was used as measure for the activity of the tested compounds related to the controls without test substances. The time of evaluating depended on the growth velocity of the fungi and lay between three and seven days. The malt agar dishes had been inoculated by mycelium pieces, 1 cm in diameter. Insects and mites were put into vessels of a volume of 2 l. Each vessel contained 100 animals. The test substances were applied as described above. The vessels were closed gas tight and opened after 24 h. Mortality was counted and related to controls without test substances.

Grain kernels with different moisture contents were placed into glass vessels each containing 500 g of the seeds. The test substances had been sprayed on the seeds, which after mixing were filled into the vessels which were closed tight with plastic foil. After some time from each vessel 100 kernels were sterile replaced and layered on malt agar dishes. After four days at 25°C the infection rate was counted.

Results

We investigated comparatively the activity of unbranched alkenals with 3–7 carbon atoms regarding their biological activity in host/parasite combinations of barley *(Erysiphe graminis)* and wheat *(Puccinia triticina)*. Although vapour pressure, reactivity and some other properties are different and the compounds were externally applicated and not internally generated, it seemed interesting to analyze their activity especially in host/parasite combinations as a model as close as possible to natural conditions. Certain uptake into the plant tissue could be assumed because in higher concentrations phytotoxicity was apparent. Uptake is especially involved in the curative test (application 24 h after infection). Phytotoxicity was calculated by the number of killed or severely damaged leaves or plants. It was expressed as PLD_{50}. The relation of the effective doses on plants and fungi (PLD_{50}/FED_{50}) is expressed as quotient (Q).

Q was in all tests higher than 1 although to a different extent.

The results are demonstrated in Table 1. As it can be seen, the rust is more sensitive than mildew in this test, although the contrary could be expected because the mycelium of *Erysiphe* has a more direct contact to the surrounding gaseous phase than rusts after infection. Some experiments indicated a certain systemic

Table 1

Fungicidal effect of 2-alkenals of different chain lengths over the gaseous phase on the host-parasite combinations

	Puccinia triticina/ Wheat		*Erysiphe graminis*/ Barley	
	FED_{50}	Q	FED_{50}	Q
2-Butenal	0.09	4.5	0.08	4.8
2-Pentenal	0.02	111	0.77	3.4
2-Hexenal	0.09	52.6	0.22	6.3
2-Heptenal	0.06	19.6	0.04	35.7
2-Hexenal diethylacetal	0.16	33.3	0.94	5.9
2-Hexenal ethylenacetal	0.07	66.6	0.12	38.5

ED_{50} values in μg/ml

$$Q = \frac{PLD_{50}\ \text{(plant)}}{FED_{50}\ \text{(fungus)}}$$

activity when the compounds were added with the water to the sand in which the seedlings were grown. This indicates that an internal curing effect can be caused by these compounds.

Other tests with *Dicotyledons* revealed that the phytotoxic level of the alkenals is too near to the fungitoxic level, so that it is not reasonable to use these compounds as pesticides by application in a gaseous phase in green houses. Tables 2 and 3 demonstrate the insecticidal and acaricidal effect on some species. The effectivity is not so high as in phosphor-organic insecticides, but still considerable especially on flies and mites. Larger beetles are less sensitive than smaller ones. An inhibition of bacterial growth could also be demonstrated, therefore the compounds can be classified as biocidal although their effectivity differs.

Because 2-hexenal is not easy to synthesize, we used in further investigations the related compound 2-ethylhexenal, which is commercially available.

In several tests fungi proved to be very sensitive against this compound. Therefore we compared the effect of 2-ethyl-2-hexenal, 2-ethyl-2-hexenol and 2-ethyl-2-hexenoic acid on various species which are common in nature on various plant materials (grains, straw, hay, silage).

Figure 3 shows some results. The application over the gaseous phase is 300 times more effective than an addition of these compounds to the agar medium. The comparison with propionic acid by the same method revealed, that the aldehyde and the alcohol had a higher activity whereas the corresponding 2-ethyl-2-hexenoic acid was the most effective.

As some fungi are very dangerous because of their ability to form mycotoxins, which cause severe problems in breeding animals in agriculture, we tested the effect of special formulations of 2-ethyl-2-hexenal for the microbicidal effect

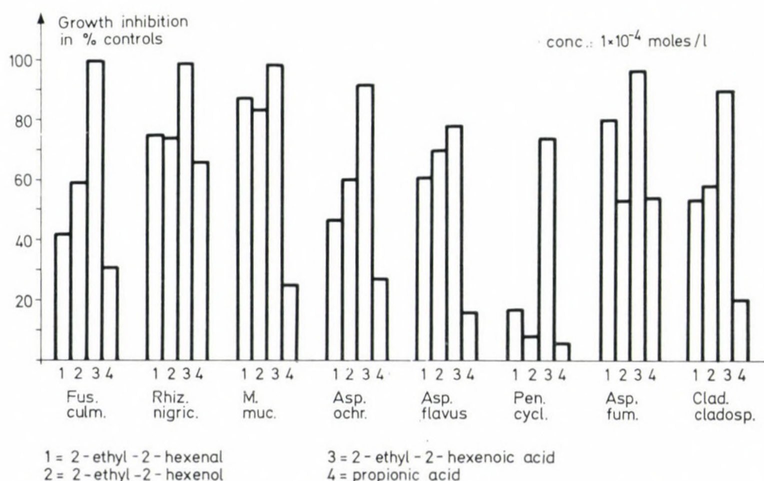

Fig. 3. Fungicidal activity of 2-ethyl-2-hexenal, corresponding alcohol and acid, and propionis acid in the gaseous atmosphere against various fungi

on the microflora of freshly harvested grain kernels. The moisture content laid between 18 and 24%. The results of some experiments in the laboratory are summarized in Table 4. It demonstrates, that it is possible to eliminate to a great extent fungal or bacterial activity for several months. Other experiments on

Table 2

Insecticidal effect of 2-alkenals over the gaseous phase on various insects after an application time of 24 h (final concentration 5×10^{-5} mol/l)

	Mortality, %						
	Oryzaephilus surinamensis	*Tribolium confusum*	*Sitophilus granarius*	*Myzus persicae*	*Tetranychus urticae*	*Trialeurodes vaporaiorum*	*Musca domestica*
2-Pentenal	65	0	0	23	57	—	100
2-Hexenal	51	0	0	74	72	100	100
2-Ethyl-2-hexenal	88	1	4	100	100	100	100

Table 3

Acaricidal effects of 2-ethyl-2-hexenal on mite *(Tyrophagus putresventiae)* (Schr.) in wheat and its ovicidal effect (dose 5 ml/kg wheat)

Time after application weeks	Mortality of adults %	Mortality of eggs %	Mortality of hatched larvae %
2	100	40	100
4	100	10	100
6	97.7	—	—
8	87.1	—	—
10	80.2	—	—
12	72.9	—	—
18	24.6	—	—

Table 4

Effect of 2-ethyl-2-hexenal for preserving wheat seeds with a moisture content of 22% (dose: 4 ml/kg)

Time after application weeks	Yeast and racterial infection fate	Fungal infection rate
1	4	6
12	1	0
Untreated control	100	100

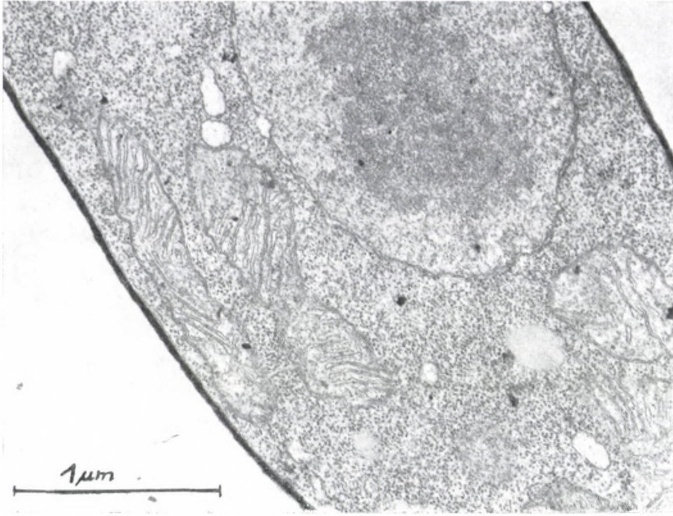

Fig. 4. Ultrastructure of hyphae of Mucor mucedo, control (phot. Dr. Casperson)

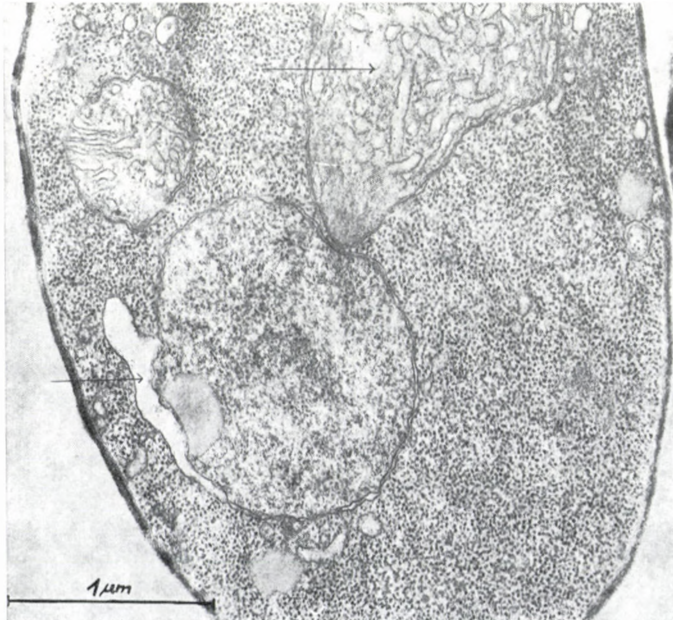

Fig. 5. Ultrastructural changes in mitochondria and nucleus membrane in Mucor mucedo 2 hours after application of 2-ethyl-2-hexenal (phot. Dr. Casperson)

a larger scale showed, that it is possible to store rye, wheat or barley without technical drying for several months as fattening food for pigs and broilers without any harm for the animals.

No investigations on the mechanism of action of this group of substances exist till now. Electron microscopical investigations revealed, that in *Mucor mucedo* mainly the membrane system is affected (Figs 4 and 5). This is understandable by the hydrophobic properties of these compounds. But they differ considerably in activity from saturated aldehydes of the same structure. According to Nyman (1969) the latters exhibited at concentrations of 8 to 12 μg/ml no fungicidal effect, but even stimulated the growth of *Diposascus aggregatus*. We obtained similar results.

It can be assumed that the degradation of 2-hexenal and analogously that of 2-ethyl-2-hexenal is performed as part of the acid metabolism. One main way should be a hydration to the saturated compound and an oxydation to the corresponding acid, which is easily metabolizable in the fatty acid pathway. A branching in the molecule does not hinder this pathway, but decreases the velocity. But as "vinylogous" compounds in contrast to the saturated derivatives (Nandi, 1977) there exist two reactive electrophilic centres, which interact with nucleophilic compounds such as amines, amino acids, alcohols, phenols, sulfhydryl compounds. This seems to be the basis for the antimicrobial activity, where probable membrane bound hydrophobic proteins are mainly affected. An example is the chemical reaction of 2-ethyl-2-hexenal with glutathione pointed out spectrophotometrically. The further degradation of such combined products is not yet investigated. By this mechanism of action no problems of acquired resistance in microorganisms are to be expected which is an important fact as well as for natural compounds as for synthetic pesticides.

Conclusions

The results demonstrate that 2-hexenal, 2-ethyl-2-hexenal and derivatives are very active biocidal compounds with a broad spectrum of activity. The biosynthesis of 2-hexenal as breakdown product from unsaturated fatty acids in plants is strongly increased by a mechanical damage of tissues or during ageing processes. Of importance is the fact that the key enzyme lipoxygenase stands in plants under hormonal control of cytokinins (Grossmann and Lesheim, 1978; Wetterau et al., 1978), which repress the enzyme activity in a still unknown manner. Therefore a connection exists between senescence and linoleic acid oxidation in chloroplasts, which can be observed by the yellowing of the leaves in the autumn. According to the famous papers of Mothes (1960) cytokinins counteract the senescence process or even can reverse it, i.e. the balance between degradation and synthesis of fatty acids is influenced, probably through lipoxygenase activity. The phytochrome system seems to be also involved in the regulation of lipoxygenase activity (Oelze-Karow and Mohr, 1976). Low levels of 2-hexenal seem always to exist within the plant system, as the early work of Schildknecht and Rauch (1961)

demonstrated, although probably in different levels in various plant species or families. If we take into consideration that microorganisms invading a plant have not such an optimal food situation as in pure cultures on malt agar, much lower doses than under our test conditions can already impair growth and development. With obligate parasites an accumulation of cytokinins at the infected area suppresses lipoxygenase activity and the formation 2-hexenal which already at low doses can eliminate rusts or mildew fungi, as our results demonstrate.

Therefore it is possible that the almost sterile interior of a plant body may be achieved by low levels of alkenals within the intercellular system of the plant tissue. The fact that *Robinia pseudoacacia* has almost no leaf diseases and no insect pests is due to the high level of alkenals in this plant cannot be explained.

It is fascinating that insects seem to have adapted to this situation by using hexenal and hexenol as olfactory orientation help to find their host plants. According to Visser (1979) electroantennograms of *Leptinotarsa decemlineata* revealed that among 44 natural volatile compounds trans-2-hexenol had the strongest effect, cis- and trans-3-hexenol a very good and trans-2-hexenal a medium effect. The C_6 chain length was optimal and within one ml air still 1.2×10^8 molecules of trans-2-hexenol could be perceived. This demonstrates that in phytophagous insects these compounds are a constituent of the odour bouquet which signalize the colour "green".

In higher animals a connection seems to exist also between ageing and lipoxygenase activity which is measurable by pentane or hexane determination in the respiration air (Sharma, 1977; Horton, 1977).

Therefore this process and its regulation seems to be of general interest in plants as well as in animals.

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Polyfactorial Theory of Plant Immunity

I. Szepessy

Department of Plant Pathology, University of Agricultural Sciences, H-4032 Debrecen, Hungary

Plant immunology is one of the new rapidly developing branches of biology. A great deal of knowledge considering this field has already been gathered, but a comprehensive, overall theory has not been established yet. The author's intention is to sum up knowledge concerning this field and unite the conclusions that can be drawn into a comprehensive theory.

This new theory has two important features: (i) in the development of plant immunity concurrent action of a great number of factors and mechanisms takes part and (ii) these factors and mechanisms considering their fundamental principle are the same as the ones functioning in human and animal organisms for similar reasons and with corresponding purpose.

Studying the history of plant immunology one notices that as soon as a researcher or a group had discovered a significant, important factor, proved its role and at the same time believed that the action of this factor ensures immunity of the plant against one or another infectious disease.

This effort can be traced up till now. Since the discovery of the significance of the cuticule thickness or the role of certain physiological features the phytoalexins, or after getting acquainted with interferons, the latter, always the currently actual factor became the most important one. Overestimation or negation of the importance of hypersensitive reactions is an especially good example. There is an important difficulty in the development of the comprehensive plant immunological theory, namely that the terminology and technical terms belonging to the subject are consistently mixed up (e.g. immunity — resistance or pathogenity — virulence).

In this paper an attempt is made to elaborate a uniform system in this respect as well.

Connections of plant protection and immunology

Studying the biological effects and economic importance of plant diseases one can be convinced of their grave negative effects concerning all mankind. The sums are staggering even if data of a smaller territorial unit (country, production area) are studied. By this fact we are obliged to find every possibility, to use all the ways and means to reduce and eliminate the losses, respectively.

Studying the methods of plant healing first of all it must be stated that two possibilities, two feasible ways are at our disposal in the fight against plant diseases:

1. we protect our plants and ourselves from the pathogens of diseases destroying them or keeping them off from our agricultural plants.

2. such plants are grown, and the characteristics of plants are so directed that they themselves are to combat the offensive pathogens, that in the fight between the host-organism and the pathogen under given environmental conditions the host-plant should overcome.

Expressing the parallel in other words: in the first case it is the man, the grower who protects his plants against the offensive of the pathogens and their pathogenic effects, whereas in the other case it is the plant that defends itself against infection and stops the extensive spread of the pathogens, due to its morphological features and functional ability.

The two above mentioned ways of protection against pathogens attacking plants nowadays are becoming independent disciplines, independent subjects in education.

Our knowledge gathered around the first possibility is summed up in *plant protectionology* (phytophylakology), whereas the aims, methods, knowledge of the way mentioned in the second place are summed up in *plant immunology* (phytoimmunology).

On the basis of the above stated the subjects of the two disciplines can be defined as follows: In plant protectionology those procedures and chemicals are dealt with, by the help of which the pathogens can be destroyed and kept off from our agricultural plants, whereas in plant immunology those possibilities are thrown light upon, those ways and relations are aimed to be revealed by the help of which our agricultural plants can be raised without diseases, even in the presence of the pathogens and their attack.

Changes of the Content in the Concept of "Plant Immunity"

In the course of time extremely great differences have been found in the development of the concept of "plant immunology". At the turn of the century, as soon as one or another factor of immunity from diseases had become known, immunity was explained by this single factor. Earlier the anatomical-morphological features (Cobb, 1892) seemed the most important. Later the physiological qualities were emphasized (Ward, 1902). After Köhler (1929) a quite interesting interpretation of the concept was spread in plant pathology, that prevailed e.g. in our country until recent years. According to Köhler "immunity" means that between a given pathogen and a determined host-organism (e.g. corn rust and grapes) not any biological connection is established. This formulation, however, involves a lot of risks. On the one hand, it is a debatable point whether there was biological contact between the two organisms in those cases when visible disease did not occur. If there was biological contact, where did the process stop? On the other hand this theory is harmful as well, since it hides the most important question: – to stick to the example – why cannot corn rust infect grapes?

It must be mentioned in this place that some specialists want to reserve the technical term "plant immunity" for formation of antibody in plant pathology as well. However, Gäumann (1951) himself – who also belongs to this trend – says that even if plant organisms produce specific antibodies we cannot have great hopes regarding their practical importance in the future either. However, plant organisms have innumerable such characteristics and a great many products are produced in their life-functions, which are capable of developing immunity from diseases. On this basis not only the changes in the host-plant after infection are included in the category of immunity by most of the specialists investigating plant immunity, but also the characteristics, inherently existing in the plant, not specific, having an active or passive effect on the pathogen.

During the later progress of this discipline a great number of factors taking part in the development of immunity became known. Thus, similarly to human immunology – though with some difference – two tendencies were differentiated in plant immunity as well.

One of them is the narrower interpretation of the concept "plant immunity". According to this only those factors belong to the category of immunity, that become active in the plant organism after the infection under the influence of pathogenic action. Immunity is claimed by this tendency in an extreme case to be built up exclusively on specific antibodies, produced in the organism under the influence of the infecting agent.

Category of "plant immunity" is interpreted by the other tendency many-sidedly, in a much broader sense. By this latter interpretation the factors, already existing in host-plants "sui generis" before infection, having an effect against the parasite and its harmful action, respectively, are also included in this concept.

It has already been mentioned above that in defining the concept of plant immunity we also accept the broader interpretation which otherwise is nearest its original meaning (immunity = security, exemption).

Beyond the related it is necessary to emphasize that we agree with that point of view according to which immunity is a state, a mutual relation. Therefore the term "immune-state" will also be used and suggested.

This term includes, that it is not a single and steady characteristic, but a temporary changing state brought about, developed by a multifactorial process. Innumerable factors take part in the development of this state, but in its changes (strengthening or weakening) as well.

Three large groups of factors can be distinguished in this state as well as in the outbreak and rapid increase of plant diseases:

1. host-plant,
2. pathogen and
3. external environmental factors.

If immunity is accepted to be the desirable state to reach, it becomes clear, that all the three factors play determining role in its development. It would be a mistake to pick mechanically out one of the three factors and explain the state of immunity with its characteristics and changes, respectively.

Therefore identification of the terms resistance and immunity is wrong and even harmful. Resistance is one of the important factors, it is the characteristic of the host-plant (resistentia=resistance, ability to resist).

Later on we shall see, that the peculiarities of the pathogen as well as changes of the external factors play an important role in developing the immune-state. According to this resistance is not identical with immunity and is not even its stage, but a factor of the development of immunity.

Definition of the concept of "plant immune-state"

We start from the above mentioned, when all the factors taking part in the development of the immune-state are studied. If historical development is taken for our basis we can come to the conclusion that immunity develops as a complex, total effect of several factors.

The first living beings fed on those organic substances in a heterotrophic way, that had accumulated until the appearance of living beings on the Earth under the sterile conditions existing up to that time. Naturally, after appearance of living beings and their rapid breeding this reserve became exhausted and the fight between the species began in the struggle for life. Under such conditions only those living beings (in most cases individuals) could survive that developed a defensive system against the invaders.

These defences are various, they rest upon different morphological or functional grounds, but their task is the same: stopping the invaders, preventing the offensive, prevention of disadvantageous (destroying) effect, namely maintenance of the state of immunity. In fact, entities, standing on different stages of evolutional development try to maintain this state, to bring about different defensive structures in one or another way.

However the task is identical in any case: to develop the immunity of the host-organism.

On this question Mechnikov (1903) writes the followings:

"Lack of sensibility against infection developed in the most ancient times. Immunity is of the same ancient origin as disease. The simplest and primary organisms were forced to carry on a persistent struggle for life. They struggle with the living beings so as to eat them or to prevent becoming a prey to them."

Such life-and-death struggle is carried on between green plants and their parasites as well. The struggle is settled according to the fact which fighting party has stronger weapons.

As it is classically expressed by Nobecourt (1928): " . . .strictly speaking, immunity or sensibility is nothing else, than the favourable or unfavourable consequence for the host-organism of the conflict between the attacking devices of the parasite and the defensive mean of the plant." To this statement, – that we think right in its grounds – we can only add, that the factors of the environment influence the outcome of the mutual fight indisputably and to a great extent.

This statement was justified by experimental results of the last 50–60 years beyond all doubt.

On this basis the concept can be defined as follows: Immunity means the superiority of the defence of a given host-organism over the effects of the offensive means of a certain pathogen.

Thus plant immunity denotes such relation of forces, such state that guarantees superiority of the defence of the host-plant over the offensive means of the pathogenic organism under given environmental conditions.

Process of Development of Immune State

It comes from the above that "immunity" or as it was stated more distinctly the "immune-state" is not a given, steady position or quality. It is much rather the outcome of the fight between the pathogen and the host-organism during the disease, the state developed under the influence of the stronger weapons of the host-organism.

In most cases – naturally – it is the pathogen that proves stronger. In these cases the host-organism cannot maintain its immunity, it becomes diseased, immune-state does not develop.

So as to get acquainted more distinctly with the process and factors of the development of immunity, the possibilities of the pathogen and the host-organism fighting against it, its characteristics, "weapons", one must thoroughly deal with the process of the disease, with the pathogenesis.

Pathogenesis

The disease is a process consisting of several stages in which a long row of changes comes one after another. In the succeeding stages different characteristics of the pathogen and the host-plant play an outstanding role.

Likewise, those effects are also different, which are exerted by the factors of the external environment on the important characteristics of the two "players" in the different phases.

Conditions of development of the disease

In order that a disease could develop between a given host-plant and a pathogen a certain "relationship" of the two organisms, their inclination towards each other is necessary. The quality of the host-plant is denoted by affinity (inclination, relationship, contestableness), whereas that peculiarity which directs the outbreak and process of the disease on the part of the pathogen is called pathogenity.

Either affinity of the host-plant in question towards the given parasite or pathogenity of the pathogen in point for the host-plant is absent disease cannot

develop. The effect of the external environmental factors can lead to the same result as well by influencing either affinity of the host-plant or pathogenity of the pathogen in a negative direction. Consequently when a pathogen meets a host-organism some characteristics of it help the pathogen (affinity). Some other characteristics of the host-plant, however, act just in the opposite direction, aim at forcing back and fighting down the invader. These characteristics are denoted by the term capability of resisting or resistance.

On the basis of the above it is clear, that pathogenity as well as affinity and resistance are in the forefront of the plant immunological investigations. Namely if we succeed in diminishing or preventing assertion of pathogenity or if resistance is successfully increased to the disadvantage of affinity, a great step can be taken on the way of developing of immune-state.

Accordingly on the basis of the above it is indispensable to get acquainted with the contents of the mentioned concepts.

Pathogenicity of the pathogen

A totality of those characteristics of the pathogen are meant by pathogenity or capability of causing diseases by means of which its host-plant can be attacked and harmful changes leading to physiological as well as economic damage (i.e. diseases) can be brought about. The definition accepted in medicine also emphasizes the above characteristics: That sui generis feature of a genus or species is meant by pathogenity in consequence of which it is able to bring on disease. This characteristic is hereditary and constant.

It follows from the above definitions that a microorganism can be considered a pathogen (it possesses pathogenity) if it can infect a certain host-organism.

This capability of causing diseases, however, depends on the existence and effectiveness of several characteristics of the pathogen. Thus pathogenity itself is a multifactorial characteristic. Some well-known examples can be referred to in support of this statement. No pathogen is known that possesses an absolute pathogenity and is able to infect every host-organism. The pathogens make a selection, i.e. they have a capability of specialization by means of which their pathogenity is effective only in case of certain host-plants or host-plant-groups. Thus one of the important components of capability to cause diseases is specialization. There are essential differences between the pathogens also in that respect as they penetrate into their host-plant. Entities of one species are able to break through the undamaged dermal tissue by active mechanical force, whereas some other species can penetrate into the inner tissues only through the existing natural openings. This capability of penetration (invasivity) is also a part of pathogenity.

There are great differences between occurrence and gravity etc. of diseases brought on by certain pathogens. Thus, pathogenity is a complex feature of the pathogen. The component characteristics are hereditary features (specialization, means of penetration etc.).

On this bases it can be stated that pathogenity of microorganisms bringing on plant diseases is a hereditary characteristic. A practicing pathologist finds that there are great differences between the changes brought about by one and the same pathogen. In one case the disease breaking out leads to grave consequences, whereas in the other case it shows mild process. Its causes can be summed up as follows: A pathogen species must not be considered a genetically clear line, as a group of entities, corresponding to each other in every sense. In the same way as the species of macroorganisms, those of microorganisms are also heterogeneous populations of a group showing an inordinate quantity of (genetical, physiological, morphological etc.) differences. There may be essential differences, – besides others, – in pathogenity between certain groups (e.g. between biotypes). The differences in outer appearance and gravity etc. of the disease can be traced back to other reasons as well. Such phenomenon can be observed in case of occurrence of an identical biotype as well. Since genetical information does not determine the capability to cause diseases of a constant (fixed) value, rather determines the limits of strength and direction of pathogenity. It depends – on the one hand – on the development of external environmental factors, on the other hand – on the degree of affinity and resistance of the host-organism how many of the limited possibilities can be realized after the meeting of the host-plant and the pathogen.

The stronger the pathogenicity of a given pathogen compared with the resistance of the host-plant attacked, the graver outbreak of the disease can be expected. In reverse: a pathogen possessing determined pathogenicity will bring on graver disease in case of host-organism having less (less effective) resistance. It comes from the above relations that pathogenicity of the pathogen is effective only if it is stronger than the resistance of the host-plant. Otherwise the host-plant prevents, retains, fights off the pathogen by asserting its strong resistance. Thus the condition of the outbreak of the disease on the part of the pathogen is that its pathogenicity should be stronger in given circumstances than resistance of the host-plant attacked.

Resistance and affinity of the host-organism

That capability of the living organism is generally meant by resistance, by means of which it is able to resist external, harmful influences. An extremely great number of varieties of resistance is known (chemical resistance, cold resistance, drough tolerance etc.). Within this, totality of those characteristics of the host-plant are meant by pathological resistance by the help of which it is able to prevent the attacking pathogen from exerting its pathogenity. In other words, all those features of the host-organism are included in the category of pathological resistance, that act against the attack of the pathogen, its spreading over in the organism and exertion of its pathogenic effect. It also follows from this that resistance of the host-organism – similarly to the pathogenity of the pathogen – is a complex, extremely complicated multifactorial feature.

That important fact must be inevitably kept in view that there is no absolute resistance, namely it can never be said, that a certain host-plant (species) resists every pathogen, in every circumstance.

It can be stated that every host organism possesses such peculiarities, which try to assure defence against a given pathogen. However, in most cases these characteristics are weak, too weak to retain the pathogen.

Thus in the first place that question must be considered what factors influence relativeness of resistance, fluctuation of its effectiveness.

This relativeness exists within the plant itself and in comparison of its characteristics, respectively. Since beside resistance every host-organism has such peculiarities that – can be expressed – act with opposite sign, namely in the direction of susceptibility, affinity. Accordingly the characteristics of the host-organism in connection with the pathogen can be divided into two groups.

One of them is destined for reception, promotion of spreading over, whereas the other one is on the contrary for prevention of the pathogen and fighting it off (resistance).

Both affinity and resistance can appear in various forms. The main point is that resistance can prevail only if its general effect proves stronger than affinity.

Relativeness of resistance is seen also in such sense that its effectiveness depends on the strength of the pathogenity of the current parasite. It means that under given resistance the host-organism can successfully fight off a pathogen of weaker pathogenity, whereas the same plant can be defeated by a pathogen having a stronger capability of causing diseases. Finally, thirdly relativeness of the concepts "resistance" and "resistant" is proved also by the fact that even a given host-plant does not behave in the same way towards a given pathogen under different conditions. Thus it seems that resistance – more precisely – a group of resistantial features can range within certain limits both in effect and strength.

The characteristic of the host-plant to resist a pathogen in different phases of the pathogenesis rests on hereditary grounds. It has also been found that effectiveness of these peculiarities can range within certain limits. To be more precise: every host-organism inheritably possesses such characteristics, by the help of which it can resist any pathogen within certain limits.

It depends on the external environmental factors what strength the resistential characteristic ranging within predetermined limits will have. It depends on the pathogenity (related to this resistance) of the attacking pathogen and on the affinity of this host-plant shown towards the attacking pathogen in point, whether or not this resistance occurring with determined strength will be effective.

Thus, to sum it up it can be said, that the result of the meeting of a pathogen and a host-plant is influenced by a very great number of factors. Disease can break out only if pathogenity of the parasite in question proves stronger than resistance of the host-organism under given environmental conditions.

Phases of pathogenesis and variation of power relations during the course of the disease

Pathogenity of the pathogen as well as resistance and affinity of the host-organism are very complicated multifactorial features. It became clear after a closer study that both the pathogen and the host-organism possess a whole series of such characteristics that influence the development of the disease.

Meanwhile a great number of technical terms arouse and it is necessary to get acquainted more closely with their contents so as to understand the further correlations. It must be said, however, that unfortunately the meaning of concepts has got mixed up to some extent in the course of time.

For the sake of better orientation we must follow the course of the disease, the pathogenesis. If the disease is considered as a continuously varying mutual relation of the host-organism and the pathogen, we find that the different characteristics of the two fighting parties become predominant in the different stages. The course of the disease, the pathogenesis consists of several stages. If the way of the parasite is followed, the outbreak and development of the disease pass through the following phases:

1. Formation of the inoculum
2. Transmission of the inoculum on the host-plant
3. Invasion
4. Infection
5. Incubation
6. Morbid-stadium

During the last stage (6. Morbid-stadium) of the course of the disease reproductive increase of the pathogens takes place e.g. in case of fungi formation of spores in great quantities. This latter process helps – on the one hand – the development of the symptoms, whereas on the other – it produces the inoculum of the next course of disease. This correlation means that pathogenesis is a recurring process, returning into itself. The phases follow each other in determined order, then the new pathological process is closely connected with the previous one. On this basis it seems right to illustrate the pathological process (pathogenesis) with two lines, running parallel to each other and describing spiral form

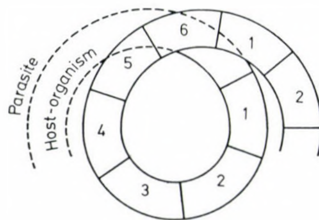

Fig. 1. Depiction of the pathogenesis. Phasis of the pathological process: 1. Formation of the inoculum 2. Transmission of the inoculum on the host-plant 3. Invasion. 4. Infection 5. Incubation 6. Morbid stadium

(Fig. 1.). The outer line symbolizes the parasite, whereas the inner one the host-organism. Thus the first stage (1. formation of the inoculum) of the given pathological process is directly connected with the last phase of the previous pathological process, reflecting reality.

Formation of the inoculum

The virulent pathogen (virus, mycoplasma, bacterium) or its virulent organ, capable of infection (in case of fungi) is called inoculum.

In this phase the host-plant and the pathogen are not in touch. In the first place it is the task of plant protection to prevent formation of inoculum, to liquidate, to destroy the sources of infection.

According to our starting point formation of inoculum already takes place in the morbid stadium of the previous pathological process.

The relation of the previous host-plant and the inoculum developing on or in it will be exposed in greater detail when analyzing the morbid-stadium.

Transmission of the inoculum

From the sources of infection the inoculum gets to the host-plant in a passive or possibly active way. One of the important tasks of plant protection is to keep off the pathogens from our agricultural plants, beside destroying them. There is the best possibility to do it just in the stage of inoculum transmission. All these take place before the meeting of the two components, therefore there are not immunological correlations involved yet.

Invasion

Invasion (penetration, getting in) is the process, during which the pathogen or its special organ gets in, penetrates into the host-organism in a passive or an active way. If we ourselves promote this process for experimental purposes this action is called inoculation (ingestion, "injection"). This work is also called – incorrectly – "artificial infection". Among the organisms causing plant disease active invasion is very common, namely penetration after "breaking open" connected with growth and exertion of strength. Such phenomenon practically never occurs in human and veterinary medicine. It is important for us in an immunological respect because for this reason "retarding force", resistance of the outer walls (cuticula, etc.) is of great importance. Thus the pathogen touching the host-plant must get in the host-organism in some way. The capability of penetration of the parasite is called invasivity. It means the totality of such charcteristics, by the help of which the pathogen can overcome obstacles raised by the host-organism.

It must be known about invasivity, that it is effective against a wide range of host-plants in case of all pathogens namely each parasite can get in, penetrate

into more host-organisms than it is able to infect, bring a disease later. Accordingly invasivity is one of the important components of pathogenity, that aims at overcoming obstacles raised against penetration.

The host-plant really has a great number of such characteristics, by the help of which it tries to prevent penetration, invasion of the parasite. These peculiarities are called comprehensively retardation. It is shown in Figure 2,

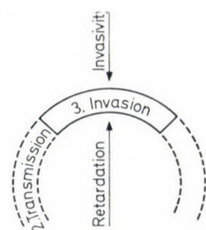

Fig. 2. In the phase of invasion invasivity — on the part of the pathogen — and retardation — on the part of the host-organism — fight each other

that in the phase of invasion invasivity – on the part of the pathogen – and retardation – on the part of the host-organism – fight.

The great importance of the characteristics that can be included in retardation gives reason for introduction of this new technical term into plant immunology. After the meeting of a parasite and a host-organism in most cases the pathological process stops because the host-plant prevents the penetration already. We find similar circumstances also when studying the natural defence of human and animal organisms.

Human body very often gets into contact with such microorganisms that can bring on disease. In most cases these pathogenic organisms, however, cannot penetrate into the host-organism, cannot carry out invasion. There can be on the one hand anatomical-morphological, and on the other chemical reasons for it. The first obstacle is the skin, which proves an excellent preventive factor with its stratified, often keratinizing epithelium. The slightly acid reaction (pH 6) of the skin surface also belongs here, for most of the bacteries like alkaline medium. Of the chemical components of retardation lyzozim can be mentioned – as an example – having positive bactericidal effect. To come back to retardation of plants it can be stated that a wide range of components is here as well. Moreover, retardation is a much more significant, important peculiarity in case of plants than in case of human or animal organisms.

Invasion of a parasite, got on the surface of the host-plant can be influenced by a great number of factors. In most cases the pathological process already stops in the phase of invasion, for such substances are excreted and diffused by the host-plant on the surface (cuticularical excretion) that prevent germination of the pathogen, or its penetration into the inner tissues. They are components of chemical retardation.

If the host-plant could not destroy the parasite which is on its surface by its excreted chemical substances or prevent it from germination, the latter tries to penetrate into the macroorganism. However, certain anatomical, morphological peculiarities of the host-plant can prevent it. The thickness of the epithelium (cuticula, periderma) as well as the form and function of the stomas are most widely studied of these. Pilosity, time and rhythm of opening of flowers, vax-cover etc. also belong here.

These components are summed up as mechanical retardation.

Infection

Infection is the process during which the parasite establishes biological contact with the host-plant and uses substances of the host-plant for its own nourishment and building up new entities.

Infection is closely connected with invasion and it lasts only a very short time. Apart from this it is the most important phase of the disease, the beginning of the contact proper.

For this purpose the pathogen must have all those peculiarities that enable it to establish contact with a certain host-plant to use substances of the latter so as to rebuild its own body. This peculiarity is said infectivity (capability of infection). Infectivity is a characteristic, acting on chemical, primarily enzymatical bases.

Infectivity is closely connected with specialization of the parasites. In this respect phylogenetical specialization and ontogenetical specialization are differentiated. By the first we mean, that each parasite can infect only certain plants — family, genus, species, variety. The other term means, that a given parasite is able to attack its host-plant only in a certain stage of the latter.

The host-organism sets its own axeny against infectivity of the parasite (Fig. 3). Thus in the stage of infection infectivity of the parasite and axeny of the host-organism fight each other.

Factors of axeny are found in the host-organism before infection as well and independently of the infection and they exert their influence against the pathogen preventing its infectivity and its effort to establish biological contact.

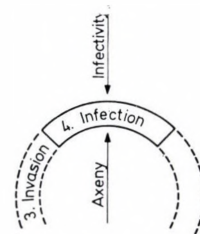

Fig. 3. In the stage of infection infectivity of the parasite and axeny of the host-organism fight each other

In human organism there can also be such substances (e.g. normal antibodies, properdine) that are present before penetration of the pathogen and they exert their bactericidal influence after the penetration of the microorganism, prevent the establishment of the biological contact, the infection.

Axeny appears in two different forms in the plant world. In case of active axeny the host-organism "offers" the "guest" such substance which destroys the latter. In other words, the green plant (host-organism) contains substances having an active toxic effect on the parasite. These substances are called comprehensively prohibitives.

In case of passive axeny the host-organism does not give the guest such nutriment that it would need further on, and in this way it makes it impossible for "the guest to stay any longer", and this latter is destroyed for lack of indispensable nutriment.

Incubation

Strictly speaking, incubation (latency) is the period lasting from the infection until the first visible symptom. One of the most important processes of the development of the disease takes place in this phase, namely spreading over of the pathogen in the host-plant, called generalization. After infection, i.e. having infected the suitable host-plant the parasite tries to spread over in it, to break down the substances of the macoorganism so as to rebuild its own body of them. This capability of the pathogen is called aggressivity.

Thus, aggressivity means that the pathogen can live in the infected host-plant or on its surface, it can fight off the obstacles raised by the host-plant against its nutrition and spreading over, it can grow and multiply on the infected plant or in its inner tissues.

In case of lacking aggressivity the pathogen is never able to develop the disease to the morbid-stadium.

Considering the extent of spreading over during incubation the pathogens can be divided into two large groups: local and diffuse parasites.

Eath pathogen has certain capability of selection as regards the place of spreading (hystological specialization, organographical specialization).

So as to measure the degree of aggressivity the length of the period of incubation is used. While studying this it is shown how much difference can be between the certain pathogens as regards aggressivity. At the same time it is clearly noticeable, what a decisive effect the external environmental factors have on aggressivity. Infection brings about changes in the life of the host-plant as well. The infected macroorganism always vigorously reacts after being infected by any pathogen. Its normal metabolism is disturbed, disorders break out in the physiological processes, the biochemical balance breaks up. The disease is accompanied by these changes and usually are the characteristics of it. A large group of reactions beginning under the influence of infection is directed against the

invading pathogen or its toxic substances (toxins). These processes are summed up under the comprehensive name of "immune reactions".

In connection with this, the capability of the host-plant to bring into action and carry out counter-reactions after infection, under the influence of the infection is called: immune-reactivity.

Thus, in the fifth phase of the pathogenesis, in the stage of incubation aggressivity of the pathogen and immune-reactivity of the host-organism are

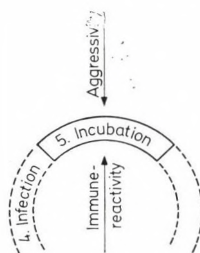

Fig. 4. In the phase of the incubation aggressivity of the pathogen and immune-reactivity of the host-organism are opposed to each other

opposed to each other (Fig. 4). The capacity of immune-reactions of each macro-organism – man, animal, plant – is hereditary. Exertion of capability and manifestation of reaction is influenced by a great number of factors.

It is a common peculiarity of every immune-reaction to become active only after infection as a response to the attack of the pathogen, directed against the parasite.

The picture is not so uniform if we want to classify immune-reactions keeping in view the place of process of different immune reactions, the biochemical changes connected with the reaction, the specifity of the reactions.

The grouping in (Table 1) tries to compare the plant immune reactions to the phenomena known in human immunology. It is true that the animal and plant organisms are on different stages of development and they essentially differ from each other in structural and functional characteristics as well. The development is common, however, and the effort can be seen beyond any doubt in case of both groups that one organism as well as the other tries to fight off, neutralize or destroy the parasite invaded. The character and aim of the struggle carried on by the help of immune reactions are completely identical.

Morbid-stadium

The morbid-stadium is characterized by occurrence of symptoms i.e. the development of the disease. Thus, this phase lasts from the appearance of the first symptom (sign) till the destruction, death or recovery of the host-organism. During the morbid-stadium reproductive multiplication of the pathogen takes place for example in case of fungi formation of spores in great quantities. It is

important not only as a symptom, but it also means the first stage of the next, newer pathological process (1. formation of the inoculum). Considering the whole pathological process the pathogen can win the struggle only if it also possesses the capability to bring about harmful changes in the infected and invaded plant accompanied by symptoms, physiological disorders, grave damage, in some cases decay – besides its characteristics, so far enumerated (invasivity, infectivity,

Table 1

Immune-reactions of the plant organism, comparing to the process of similar function of human and animal organisms

Defensive reactions starting under the influence of the infection	
in plant organisms	in human and animal organisms
Plasmatical reactions	Phagocytosis
Hypersensitive reactions	Non-specific allergic process, idiosyncrasy
Formation of phyto-alexine	Formation of alexines, non-specific antibodies in the blood
Formation of interferons	Formation of interferons
Premunity	Premunity or infectious immunity
?	Immunogenity, specific capability of reaction, coming into action under the influence of an antigen

aggressiveness). This characteristic of the pathogen is said virulence. In other words: the pathogen with strength sufficiently superior to the host-organism attacked even in the last phase of pathogenesis is virulent.

Change, strengthening or weakening of virulence is often seen within one or another species of pathogens. The degree of damage, strength of symptoms, in some cases the proportion of mortality (death, decay) can also change. This circumstance can be attributed to genetic changes of the pathogen on the one hand, but the changed immunological behaviour of the host-organism and very often the different effects of the external environmental factors can also be the cause.

There are several ways of determining the degree of virulence. The degree of virulence can be expressed in percentage by means of the following formula:

$$v\,\% = \frac{s \times 100}{i}$$

Where: v = virulence,
s = number of plants showing symptoms,
i = number of plants, brought into connection with the pathogen, probably infected.

In a qualitative sense the pathogen possesses stronger virulence that is able to bring on a disease accompanied by graver consequences, possibly quick decay:

$$M\,\% = \frac{m \times 100}{s}$$

Where: M = mortality (decay),
m = number of plants destroyed,
s = number of plants, showing symptoms.

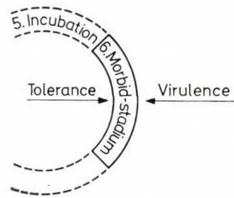

Fig. 5. In the morbid-stadium virulence of the pathogen and tolerance of the host-organism are against each other

The economic damage is also taken into account when determining the strength of virulence.

The differences in virulence between the races (e.g. biotypes) of each pathogen species can be measured by the same methods. The effect of the parasite develops in its full depth in the last phase of the pathogenesis (6. morbid-stadium). But this effect can still be neutralized by the host-plant. This characteristic of it asserts itself somehow by tolerating the pathogen, carrying it in itself without damage. Just for the above this characteristic is called tolerance.

Accordingly in the morbid-stadium virulence of the pathogen and tolerance of the host-organism are against each other (Fig. 5).

Under capability of tolerance totality of such peculiarities is meant by means of which the host-plant prevents either the occurrence and development of the symptoms or the physiological damage itself after the pathogen has spread over in it.

At the present time little is known about the biological, chemical basis of tolerance. Opinions differ in judging economic importance of tolerance. Tolerance is considered a valuable characteristic by most of the specialists. If the disease does not do damage, protection is not needed against it. In many cases achievement of tolerance against one or another disease is set as an aim of improvement. Work in these lines has already conduced to a great number of useful results. We ourselves join to that minority, which submits tolerance to thorough judgement. The tolerant plant plays the same role as a bacilli-carrier in ahuman community.

Pathological x-value

It comes from the above that it is necessary for the pathogen to have a slightly more attacking force in every stadium than the defensive potential of the host-organism for the normal course of the pathological process Table 2. Since, the pathogen, as an attacking party directs the progress of the pathogenesis. If the defensive strength of the host-organism exceeds the attacking force of the parasite, the pathological process stops.

In connection with this several questions arise. The first one is superior strength of what degree should the pathogen possess against resistance of the host-plant. It is not indifferent either whether or not this mutual relation changes the different stages of pathogenesis of one and the same disease. So as to throw more light upon relations, existing in this respect, the concept of pathological x-value is used.

According to this that minimal "additional strength", "superior strength" which the attacker needs for bringing on disease of the host-plant and developing it fully is called pathological x-value. If affinity of a host-organism towards a given pathogen is strong, explicit, than this parasite needs only minimal "additional strength" to carry on the pathogenesis. In this case it can be said that the two organisms "stand near to each other" (Fig. 6).

Table 2

Role of the immunologically important characteristics of the pathogen and the host-organism in different phases of the pathogenesis

Stages of the pathogenesis	Pathogenity of the pathogen	Resistance of the host-organism
1. Formation of inoculum	—	—
2. Transmission of inoculum	—	—
3. Invasion	Invasivity	Retardation a. chemical b. mechanical
4. Infection	Infectivity	Axeny a. active b. passive
5. Incubation	Aggressiveness	Immune-reactivity a. plasmatic reaction b. hypersensitive reaction c. formation of phytoalexine d. formation of interferon e. premunity
6. Morbid-stadium	Virulence	Tolerance

It also happens that affinity of the host-plant is slight towards a given pathogen, i.e. the two opposing parties are "far off from each other" (Fig. 7). Disease can occur, pathogenesis can progress also in this case, but an incomparably greater pathological x-value is needed on the part of the pathogen.

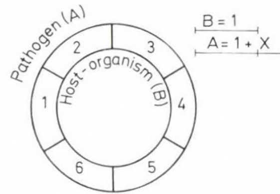

Fig. 6. If affinity of a host-organism towards a given pathogen is strong the two organisms "stand near to each other"

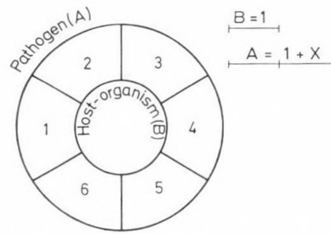

Fig. 7. If affinity of host-plant is slight towards a given pathogen, the two opposing parties are "far off from each other"

In practice e.g. this statement appears in such a way that it is very difficult to improve resistant varieties against the narrowly specialized pathogens (the parasite and the pathogen stand near to each other, the pathological x-value is low).

One must not think, however, that the pathological x-value depends only on the specialization, and it is an important factor only in the stage of infection.

The pathological x-value is a decisive factor in the last four phases of the pathogenesis, it assures the progress of the disease. In other words: the pathogenesis can stop, cease at any stage if "additional strength" of the parasite can be compressed below the pathological x-value. This aim can be achieved also by diminishing "superior strength" of the pathogen or increase defensive strength of the host-organism, or "keeping off the two organisms from each other" by controlling the environmental conditions.

It must be emphasized, that the pathological x-value is not a given characteristic of one party or another, but a ratio number. It means the necessary "superior strength" of a given pathogen against a certain host-plant, without which "superior strength" the pathological process cannot progress.

The morbid balance

Studying the role of the pathological x-value several conclusions can be drawn.

In order that a pathological process can proceed from the beginning to the end, it is necessary for the pathogen and the host-plant to maintain relation near to a certain state of balance, to live beside each other, though in permanent fight.

This special balance differs from the state of balance taken in a physical sense in the fact that one of the parties, namely the attacker, the parasite possesses some determined additional strength (pathological x-value). Since it leads forth the pathogenesis by the help of the latter. It follows also from this additional strength, that when plotting the pathogenesis, the external, longer line represents the pathogen.

If in a certain stage of the pathogenesis the state of balance breaks up in favour of the host-plant, the disease ceases or rather its further progress stops. Converting the thesis it can be said that the disease takes its normal course only if special relation of forces exists between the two struggling parties always ensuring sufficient superior strength for the attacker. This relation accompanying the pathogenesis is called aequilibritas morbi, morbid balance or balance of the disease.

The relation of the struggling parties in case of morbid balance is characterized by permanent fight. In the struggle both parties must find newer and newer weapons to defeat the opponent. Thus as long as the disease lasts (i.e. there is no recovery or destruction) this special state of balance definitely exists.

One more conclusion can be drawn from the simultaneous study of the concepts of "pathological x-value" and "morbid-balance": in each phase of the pathogenesis the pathogen needs different pathological x-values so as to maintain the morbid-balance depending on affinity of the host-organism in this stage.

Conclusions

The parasite can bring on disease of the macroorganism only if it has the capability of pathogenity. On the other hand the host-organism opposes its resistance against it. Its components show analogy with those defences human and animal organisms are in the possession of.

Components of pathogenity and resistance are outlined in Table 2.

Both characteristics are hereditary, extremely complicated and complex.

The course of the plant disease is assured by superior strength of the pathogen -- pathological x-value –, possessed by the latter against the host-organism.

During the pathological process the two struggling parties are in such a state of equilibrium – morbid-balance, which is characterized by certain superior strength of the pathogen.

If in any stage of pathogenesis capability of protection of the host-organism becomes stronger than capability of bringing on disease of the parasite, the process stops, relation of forces beneficial for the host-organism, immune-state develops.

It is more beneficial biologically as well as economically if the immune state develops at the earliest possible stage of the pathogenesis.

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Isolation of Phytoalexins from Germinating Seeds of Groundnut (*Arachis hypogaea*)*

P. NARAYANASWAMY and A. MAHADEVAN

Centre of Advanced Study in Botany, University of Madras, Madras-600005, India

Phytoalexins were produced by groundnut seeds of cultivar TMV 7 challenged with *Rhizoctonia bataticola* or native microflora. Extract from the infected seeds inhibited spore germination of *Alternaria alternata*, *Curvularia spicata* and *Helminthosporium oryzae* and mycelial growth of *C. spicata* and *H. oryzae*. Seeds incubated in spore suspension of *C. spicata* released phytoalexins into the medium which completely inhibited the spore germination of *C. spicata* and *H. oryzae*. The phytoalexins were identified with *cis*- and *trans*-isomers of 3,5,4'-trihydroxy-4-isopentenyl stilbene which inhibited the spore germination of *C. spicata*. Two other phytoalexins toxic to germ-tube growth of *A. alternata* and *C. spicata* have not been identified.

Isolation of phytoalexins was reported from germinating seeds of leguminous plants by using seed germination technique, first proposed by KEEN (1975) and confirmed in our laboratory (GNANAMANICKAM, 1979). KEEN found that germinating seeds of *Arachis hypogaea* variety American produced phytoalexins when infected by native microflora. The phytoalexin proved to be *cis*- and *trans*-isomers of 3,5,4'-trihydroxy-4-isopentenyl stilbene (KEEN and INGHAM, 1976). We investigated the production of phytoalexins both in the extract and diffusate of groundnut seeds challenged with *Rhizoctonia bataticola* and native microflora as well as with spore suspension of *C. spicata*. The results are presented below.

Seeds of cultivar TMV 7 soaked in water were cut into 2—3 mm pieces and placed in a moist chamber, 26 °C. Thick mycelial suspension of *R. bataticola* prepared from 7-day-old culture was added to the seeds and thoroughly mixed. In another experiment, the seeds were allowed to be colonized by the native microflora. Heat killed seeds and seeds inoculated with heat killed mycelium were kept as controls. Observation was made on the 5th day of incubation.

Fungi that colonized the seed surface were *Aspergillus flavus*, *A. niger* and *Mucor* spp., and a few other non-identified species. After 5 days of incubation, the seeds became yellowish brown in colour. Control seeds killed before incubation did not show any change in colour.

Infected seed extract at 4 g/ml completely inhibited the spore germination of *C. spicata* and *H. oryzae*, even at the end of 24 h. Control seed extract was slightly toxic. Furthermore, the extract inhibited the elongation of germ-tube of *C. spicata* and *H. oryzae* by 48% and 45%, respectively, on the 10th day of incubation.

Fifty g seeds were surface sterilized with 0.1% $HgCl_2$ and washed thoroughly in sterile distilled water. They were placed in a sterile Petri dish (15 cm diam.) and

* Memoir No. 361 of CAS in Botany

inoculated with spore suspension (10^6 spores/ml) of *C. spicata*. Control seeds received similar quantity of sterile distilled water. This was incubated for 5 days and the leachate was collected. Seeds were washed thrice with 15 ml of 90% alcohol; it was filtered through Whatman No.41 filter paper and concentrated on a water bath, 45 °C. The residue was dissolved in 5 ml of 30% alcohol to represent 1 ml of the extract for 10 g of seeds and assayed against spore germination of *C. spicata* and *H. oryzae*.

The diffusate when tested at 5 g/ml completely inhibited the spore germination of *C. spicata* and *H. oryzae*. Is was diluted and further assayed. Germination of *H. oryzae* spores was delayed by the diffusate even at 5% concentration (Table 1). However, with prolonged incubation, the spores germinated except in 80% con-

Table 1

Dilution end point of seed phytoalexin assayed against *H. oryzae* and *C. spicata*

Incubation h	Concentration of diffusate, %									
	5		10		20		40		80	
	H.o.	C.s.	H.o.	C.s.	H.o.	C.s.	H.o.	C.s.	H.o.	C.s.
	Per cent inhibition									
2	100	0 (38)	100	0 (88)	100	100	100	100	100	100
6	0 (52)	0 (45)	0 (58)	0 (54)	0 (64)	0 (65)	0 (81)	0 (78)	100	100
12	0 (23)	0 (24)	0 (50)	0 (32)	0 (66)	0 (37)	0 (81)	0 (52)	100	100

( ) = germ tube inhibition
H. o. = *H. oryzae*
C. s. = *C. spicata*

Table 2

Properties of seed phytoalexins

Spot No.	Colour under UV	Colour with DSA	Rf value	Spore germination assay			
				A. alternata		*C. spicata*	
				Per cent inhibition			
1	blue	bright yellow	0.14	100		100	
2	blue	bright yellow	0.28	100		100	
3	light blue	yellow	0.36	0	(32)	0	(12)
4	—	pink	0.53	0	(50)	0	(80)

( ) = inhibition of germ-tube length

centration of the diffusate. *C. spicata* spores germinated and developed germ-tube in 10% concentration of the diffusate. Concentrations up to 40% delayed the germination and substantially reduced the germ-tube development of the germinated spores.

The crude extracts of control and inoculated seed were chromatographed on the plates coated with Silica gel G. The chromatoplates were developed in ethyl acetate-benzene-methanol-water (100:80:20:20, v/v). Fluorescence under UV,

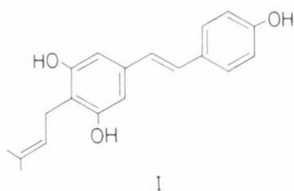

Fig. 1

colour with diazotized sulphanilic acid (DSA) and R_f values were noted. Six spots were detected of which two were present only in the infected seeds. Of the two, one with R_f 0.32 was bluish fluorescent under UV. It was eluted in ethanol and purified by tlc using chloroform-methanol (100:3 v/v) solvent. Four spots were detected. Fluorescence, colour with DSA, R_f values and biological activity of the substances are presented in Table 2.

The substance with R_f value of 0.14 in the second solvent gave λ max (EtOH) 202 nm whereas the substance with R_f0.28 gave λ max (EtOH) 327 nm. These two substances were identified as *cis*- and *trans*-isomers of 3,5,4'-trihydroxy-4-isopentenyl stilbene (I) on the basis of UV spectral characteristics and co-chromatograph with authentic substances.

The four spots were eluted in ethanol, evaporated to dryness, dissolved in 2 ml of 30% ethanol and assayed against spore germination of *A. alternata* and *C. spicata*. The spots 1 and 2 completely inhibited the spore germination of the fungi. Substances 3 and 4 were, however, less inhibitory.

As early as 1971, SHANMUGAM claimed that the kernels of groundnut produced phytoalexin like substances which inhibited the sclerotial germination and mycelial growth of *Macrophomina phaseoli*. In this study we showed that groundnut seeds when inoculated with *R. bataticola* readily accumulated phytoalexins which were identified as *cis*- and *trans*-isomers of 3,5,4'-trihydroxyisopentenyl stilbene. Two more phytoalexins were detected but their identification remains to be made. Also the native microflora of seeds induced the synthesis of phytoalexins in germinating seeds. The phytoalexins inhibited the spore germination of and germ-tube elongation of *A. alternata*, *C. spicata* and *H. oryzae* and mycelial growth of *C. spicata* and *H. oryzae*.

Extracts from uninoculated seeds partially inhibited the spore germination and germ-tube growth of *C. spicata* and *H. oryzae*. According to KALAICHELVAN

(1980), preformed inhibitory substances "prohibitins" in groundnut included p-coumaric acid, homogentisic acid and a phenol which inhibited fungi and bacteria.

Plants release a variety of substances toxic to microorganisms and this is an important aspect of defense of germinating seeds (MAHADEVAN, 1982). During germination, groundnut seeds leached gentisic acid, vanillic acid, p-coumaric acid, salicylic acid, quercetin and a few unidentified phenols (KALAICHELVAN, 1980) and 4 phytoalexins. The diffusate was toxic to a varietyof bacteria and a few fungi.

The stilbene phytoalexins are exceptionally toxic to *H. oryzae*, the causal pathogen of brown spot disease on rice. Attempts therefore may be made to exploit the stilbenes in the control of *H. oryzae*.

Acknowledgement

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Some Factors in the Development of Resistance of Groundnut Seedlings to Damping-off Caused by *Rhizoctonia solani*

M. N. Reddy and A. S. Rao*

Department of Botany, S. V. University, Tirupati, 517 502, India

Studies on the effect of age of the seedlings at inoculation time, anatomy and biochemical constituents, of the hypocotyls at different age intervals were carried out in an attempt to determine the factors that are responsible for the resistance of seedlings to infection by *R. solani*. Basing on these observations the possible reasons for the observed resistance of the seedlings with age are discussed.

Studies on damping-off of groundnut caused by *Rhizoctonia solani* Kuhn revealed that the seedlings become resistant with increasing age (Reddy, 1976). This paper presents results aimed at the elucidation of some of the possible mechanisms associated with this seemingly regular decline in susceptibility of the seedlings with age.

Materials and Methods

Healthy seeds of groundnut (*Arachis hypogaea* L.) Var TMV2 were surface sterilised and sown, in steamed soil, in seed pans. The sowing period was staggered so that the seedlings of 7, 10, 13, 16, 19, 20 and 21 days after germination were available on the same day. They were transplanted into an inoculum-soil mixture with 10% of *R. solani* culture (grown on 3% oatmeal – sand medium for 10 days) in 6" pots, observed for disease occurrence and development and percentage recorded. Three replicate pots were used for each age group, each containing four seedlings.

For histological observations, the hypocotyl bits of different ages were chemically fixed in formalin-acetic acid – alcohol (FAA), dehydrated in tertiary butyl alcohol (TBA) series and embedded in paraffin (Jensen, 1962). Embedded tissues were sectioned (15 μ thick) with a rotary microtome and sections affixed to slides with egg-albumin adhesive. The sections were passed through a regular xylol – alcohol series down to water and stained with an aqueous solution of safranin – fast green, mounted in Canada balsam and examined.

*Present address: Professor and Head, Department of Botany, Nagarjuna University, Guntur.

The hypocotyl regions of 21-day old seedlings were analysed for reducing and non-reducing sugars, starch, total nitrogen, protein nitrogen and soluble nitrogen, total phenols and *ortho*-dihydric phenols, as described earlier (Reddy and Rao, 1978).

Results

The change in susceptibility of groundnut seedlings with increasing age, to *R. solani* was determined, in order to establish the most suitable age for infection and the fate of susceptibility with increasing age of the seedling. The results are presented in Table 1. The disease intensity declined with increasing age of the seedling at inoculation from 7 to 21 days after sowing. Seven and ten day old seedlings were extremely susceptible, resulting in almost cent per cent damping-off. Resistance developed from sixteenth day onwards and the seedlings at 20 and 21 day-old become completely resistant. The 21 day old seedlings do not seem to be infected because hypocotyl lesions do not develop in them. The period in which this resistance developed was actually associated with expansion and maturation of the hypocotyl. The hypocotyl makes its appearance as a fleshy, succulent and smooth tissue. With age, rapidly loses its fleshy nature and becomes thin, hard, dry and its colour also changes from pure white to cream yellow and later on to brown. Seven day old seedlings are highly susceptible and only in this case various lesion maturation stages could be differentiated as already described (Reddy and Rao, 1978). The lesion appearance on the hypocotyl occurs in about 48 h after inoculation. In the rest of the cases, there was merging of two or more stages and also prolonged time taken for the lesion appearance.

The anatomy of hypocotyls of seedlings of various ages showed an interesting pattern in internal morphology as seen from figures in Plate I. In the early or younger stages major cross section of the hypocotyl was occupied by a cortex of homogeneous, thin walled loosely arranged parenchyma cells with intercellular spaces.

The stele occupies only a small portion. With the increasing age the area occupied by the stele formed major portion of the hypocotyl, due to its expansion

Table 1

Effect of seedling age at inoculation with *R. solani* on hypocotyl lesions of groundnut

	Age at inoculation, dasy						
	7	10	13	16	19	20	21
Seedlings with infection on hypocotyl, %**	100*	96	58	46	10	6	0

* Each figure is an average of three replicates
** Significant at 1% level

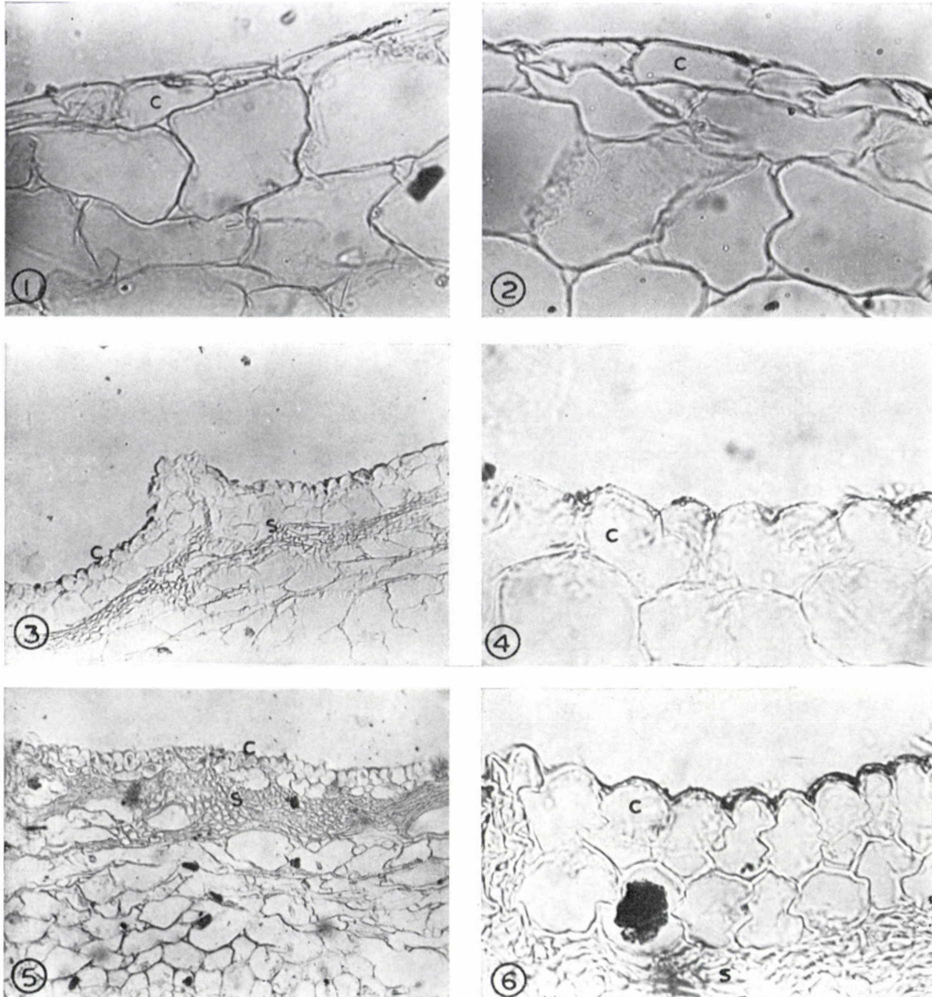

Plate I

Histology of hypocotyls of healthy groundnut seedlings during development. Main emphasis is on surface layers and cortical regions.

1. T. S of hypocotyl of 7 days old seedling. Note the nature of epidermal layer (c) (+450)
2. T. S of 13 days old seedling hypocotyl (× 700)
3. T. S of 16 days old seedling hypocotyl. Note the enlargement of surface layers (c), deposition of cuticle and initiation of formation of crushed cortical zone (S) (× 150)
4. A magnified view of the cells (c) of epidermal layer (× 150)
5. T. S of hypocotyl of 20 days old seedling. Note further enlargement of epidermal cells (c), deposition of cuticle and further increase in the thickness of crushed cortical zone (S) (× 125)
6. A magnified view of epidermal (S) and hypodermal layers and a portion of crushed zone (S) (× 475)

Table 2

Certain biochemical constituents of hypocotyls at various stages of seedling development

Constituent	Quantity in mg/g fresh wt. at various intervals					
	7	9	12	16	18	21
Reducing sugars	4.21	4.62	4.15	3.99	3.96	3.32
Non-reducing sugars	9.51	9.23	7.62	7.25	6.63	6.40
Starch	29.50	34.16	31.16	28.16	23.83	22.96
Total nitrogen	2.83	2.93	3.40	3.40	3.66	3.42
Protein nitrogen	1.59	1.39	1.26	1.22	1.06	2.35
Soluble nitrogen	1.24	1.54	2.14	2.18	2.60	0.95
Total phenols	2.36	2.63	3.03	3.26	3.33	4.26
Ortho-dihydric phenols	0.10	0.12	0.17	0.20	0.33	0.42

* Each figure is an average of three replicates

and the cortical region being transformed into a compact tissue of crushed cells. These crushed cells appear as shrunken cells without any contents. The crushing of cortical cells started from 16th day onwards and as age increased to 20 or 21 days it became several layered thick (Plate I – 3, 5) and even the hypodermis disappeared.

A characteristic change was also observed in the epidermal layer with increasing age (Plate I, Figs 1 to 6). In the early stages the epidermal cell was thin walled and horizontally elongated, but as age increased it attained a longitudinally elongated barrel shape with thickened walls. In the early stages the cuticle was either absent or too thin to be noticed, but as time progressed its thickness increased (Plate I, Figs 1 to 6). The coarse or dry and hard nature of the aged hypocotyls may be due to these changes in the epidermal layer and those observed in the internal tissues.

The results of a study of the biochemical constituents in 21 day old hypocotyls followed the same trend as observed in the healthy seedling hypocotyls at 7, 9, 12, 16 and 18 days old reported elsewhere (Reddy and Rao, 1978). Carbohydrate fractions further decreased, phenolics increased and nitrogen fractions fluctuated (Table 2).

Discussion

It is evident from the results that the resistance of seedlings to infection by *R. solani* gradually increases with age. Similar resistance with advancing age to infection by *R. solani* has been reported for several other hosts (Carrera, 1951; Bateman and Lumsden, 1965). The reasons for this seemingly regular decline

in susceptibility of hosts with increasing age are far from clear and various possible reasons have been postulated to explain this. The principle of Yarwood (1967) that "resistance is the rule rather than the exception in nature" implies that every plant has some mechanism for resisting most of the microorganisms in its environment. A given host may possess multiple features for resisting the attack of microorganisms.

The increased resistance of seedlings to *R. solani* with age has been attributed to several factors like conversion of pectin to calcium pectate which cannot be destroyed by the fungus (Bateman and Lumsden, 1965), physical and chemical nature of the cuticle (Martin, 1964; Wang and Pinckard, 1973) or prevention of diffusion of substances from within the plant, hence suppressing the formation of appressoria on the stem surfaces of older seedlings (Kerr and Flentje, 1957; Flentje, 1959). The importance of infection cushions in penetrating the tissues and initiation of disease has been well established (Dodman and Flentje, 1970; Weinhold and Motta, 1973).

The epidermis as a whole composed of the cuticle and outer epidermal cell wall was also known to act as a defense barrier (Martin, 1964). The activity of the fungus was assumed to be on pectic compounds of the constituent parenchymatous tissue of the young seedlings. The capacity of *R. solani* to produce pectic enzymes during pathogenesis is well known (Barker and Walker, 1962; Van Etten et al., 1967).

The results indicate that the young seedling hypocotyl possess completely parenchymatous tissue which is gradually being replaced by a few layered crushed compact tissue. There were also considerable changes in the epidermal layer. A gradual decrease in the hypocotyl exudates, loss of several compounds and lack of formation of any infection cushions with age was recorded (Reddy, 1980). Further, an increase in the quantity of epicuticular waxes of hypocotyls and some qualitative differences of the components with age were also observed (Reddy and Rao, 1980).

The nutritional deficiency with increasing age may be having some direct effect on the performance of the pathogen. A continuous diminution of carbohydrate fractions with age, may be having some relation with the resistance as also reported by some workers (Graigner, 1956; Hunter and Guinn, 1968). The phenolic compounds have long been considered to play an important role in disease resistance. Some authors have observed a definite correlation between increase in phenolic compounds and increase in resistance (Hunter, 1974). The increase observed in the present study may not be so high as to account for the total resistance of the seedlings.

The resistance may be said to have been governed by more than one factor and that it cannot be explained on the basis of any one single factor. Morphological, structural as well as physiological factors may be said to be responsible. The evidence for the first two are many when compared to the third one. The changes in various physiological constituents are naturally associated with the actively growing period of the plants. It may be concluded that the resistance of

groundnut seedlings with advancing age is mostly due to the morphological and structural changes that take place during growth. The mechanism involved may be any one or more of the factors like the physical and chemical nature of the surface layers, conversion of some compounds to resistant compounds, development of mechanical tissues and/or loss of exudation of nutrients from within the host and prevention of formation of infection cushions.

Acknowledgements

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Changes in Phenolic Acids in Groundnut Leaves Infected with *Puccinia arachidis* Speg

M. N. Reddy

Department of Botany, S. V. University, Tirupati-517 502, India

Phenolic acid metabolism of rust-infected groundnut leaves was studied at various stages of disease development. There was no qualitative change in the phenolic acids. Almost all the phenolics showed increase due to infection and the increase was more conspicuous in some of the compounds. The significance of these results is discussed.

Groundnut rust (*Puccinia arachidis* Speg.) is one of the most economically important diseases resulting in serious losses of the crop. Much attention has been paid in the recent past to study various aspects of biology and pathology of this pathogen. Except for a few reports (Subrahmanyam et al., 1976; Reddy and Rao, 1976; Siddaramaiah et al., 1979; Reddy and Ramagopal, 1982) not much information is available on the physiology of infected plants. The present paper deals with the effect of rust infection on phenolic acid metabolism in groundnut leaves.

Materials and Methods

Healthy and rust (*Puccinia arachidis* Speg.) — infected groundnut (*Arachis hypogaea* L. Var. TMV 2) leaves were used for analyses. The method of raising plants, type of inoculation and sampling were same as described in one of the previous papers (Subrahmanyam et al., 1976). A known quantity of healthy and infected tissues at different stages of disease development was chopped into pieces and extracted for phenolic acids according to the method of Bate-Smith (1954). The method of separation of individual phenolic acids by 2-dimensional paper chromatography, their identification and quantitative estimation were same as described elsewhere (Reddy and Rao, 1975, 1978).

Results

The spectrum of phenolic acids assayed from healthy and infected tissues are presented in Table 1. There was no qualitative change in phenolic acids due to infection. Altogether ten phenolic acids were detected in both healthy and infected tissues. In healthy tissues the quantities of different phenolics fluctuated during the sampling period. On the other hand infection resulted in the tremendous

Table 1

Effect of rust-infection on phenolic acid content (μg/g fresh wt.)* of healthy and infected groundnut leaves at various stages of disease development

Compound	*Stage 1 (5)*+		*Stage 2 (8)*		*Stage 3 (10)*		*Stage 4 (12)*		*Stage 5 (15)*	
	H	I	H	I	H	I	H	I	H	I
Trans-Caffeic acid	17.0	18.5	22.5	35.0	25.0	45.0	54.0	115.0	35.0	155.0
Chlorogenic acid	11.5	12.5	19.0	22.5	17.5	95.0	16.0	145.0	12.5	139.0
Cis-Caffeic acid	5.5	6.0	6.0	40.0	5.0	72.5	6.5	117.0	5.5	106.0
Cis-o-Coumaric acid	1.5	2.0	2.5	5.0	2.5	7.5	2.0	9.0	2.5	15.0
p-Hydroxybenzoic acid	2.5	2.0	3.5	7.5	3.0	7.0	4.0	17.5	3.5	16.5
Trans-p-Coumaric acid	1.5	2.5	2.5	7.5	2.0	5.5	2.5	7.0	2.0	6.5
Cis-Ferulic acid	1.5	1.0	2.0	5.0	1.5	5.5	1.5	6.5	2.0	15.0
Vanillic acid	1.5	1.5	3.0	3.5	2.5	7.5	2.0	7.0	2.0	6.0
Trans-Ferulic acid	1.5	2.0	2.5	2.5	2.0	5.5	2.5	7.5	2.0	9.0
Unidentified	2.0	1.5	2.5	25.0	2.0	30.0	2.5	22.5	2.0	35.0
Total (μg)	46.0	49.5	66.0	153.5	63.0	281.0	93.5	454.0	69.0	503.0
Total in % of control	100	107.6	100	232.6	100	446.1	100	485.6	100	729.1

H = Healthy; I = Infected
* Each figure is an average of three replicate samples
+ Figures in parentheses indicate the days after inoculation

augmentation of some of the phenolics and a general increase of the others. The nature of change varied with the progress of the disease and rapid increase occurred as lesions matured. An overall consideration of the data reveal that the phenolic acid metabolism of the host significantly changes as a result of infection.

Discussion

Alterations in the phenolic acid metabolism due to infection by a wide range of fungal pathogens have long been recognised (Farkas and Kiraly, 1962; Kosuge, 1969). Infection generally results in a more or less unspecific shift towards increased aromatization of the host metabolism (Farkas and Kiraly, 1962). The accumulation of various phenolics detected in the present investigation suggests an enhanced aromatic metabolism due to infection. This accumulation in the infected tissues may be due to one or more factors suggested in various host-pathogen interactions like release from glycosides or glycosidic esters by enzymatic activity of the pathogen or the host (Noversoke et al., 1964; Pridham, 1965) or enhanced synthesis by the host (Neish, 1964) or their production by the pathogen (Farkas and Ledingham, 1959).

The increase of phenolics in the infected tissues reveals that they may play an important role in inducing resistance against further invasion of the pathogen as they may be involved in the metabolism of the plants either with their oxidative products toxic to the pathogen (Kosuge, 1969) or with their direct influence on other metabolic processes (Zenk and Muller, 1963). Several workers have studied

the fungitoxic activity of various phenolics on several fungi and were also attributed in killing the host cell and inhibiting further growth of the parasite and hence lesion limitation (see Cruickshank and Perrin, 1964). It would be of particular interest to study the effect, of all those phenolics which have shown tremendous increase in the present investigation, on the pathogen by way of their effect on spore germination, initial stages of disease expression and final development of lesions. The increase may definitely be having some significance in the disease development. It is also important to study the alterations in the enzymes involved in phenolic acid metabolism for better understanding of the accumulation of various phenolics. Some of these studies are in progress.

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Drechslera rostrata a Destructive Mycoparasite of *Rhizopus nigricans*

R. C. Gupta, R. L. Fotedar and K. N. Pandey

Department of Botany, Kumaun University, Almora 263 601, India

In plate cultures, *Drechslera rostrata* attacked different morphological structures of *Rhizopus nigricans*. Frequent coiling, penetration and coagulation were recorded as a result of hypogeal interference reaction. The parasitic activity of *D. rostrata* was noticed for the first time on *Rhizopus nigricans*. Nine other fungal species isolated from the seeds of *Eleusine coracana* also exhibited exhibited various hyperparasitic reactions against *R. nigricans*.

In recent years studies on hyperparasitic interactions have received considerable impetus due to its importance and implications in biological control and competitive survival of the pathogen in the absence of suitable host (van Den Heuvel, 1970; McBride, 1971; Ikediugwu and Webster, 1960a, b; Ikediugwu et al., 1970). In artificial cultures fungicolous fungi have been recorded frequently and significant changes such as coiling, penetration, morphological alterations etc. have been observed in the interacting organisms during hyphal interference reactions. Shafie and Webster (1979) have studied *Curvularia* species as parasites of *Rhizopus* and other fungi. However, Jager et al. (1979) have discussed the possibility of using mycoparasites in biological control of plant pathogens. *Drechslera rostrata* and *Rhizopus nigricans* were recorded frequently from one year stored seeds of *Eleusine coracana. D. rostrata* was found to be a detrimental seed-borne pathogen causing severe seedling blight of *Eleusine coradana* which is an important crop of western Himalaya. There is a paucity of information about antagonistic interactions among seed-borne fungi (Tandon et al., 1979; Shafie and Webster, 1979). The present investigation was designed to quantify our knowledge on hyperparasitic activity of *Drechslera rostrata* against *Rhizopus nigricans.*

Materials and Methods

Seeds of *Eleusine coracana* were collected from different villages of Almora, Kumaun Himalaya. Seed mycoflora was screened by standard blotter method (Ista, 1966). The mycoparasitic activity of *D. rostrata* and nine other less frequent fungi viz., *Curvularia lunata, Alternaria alternata, A. tenuis, Aspergillus flavus, A. niger, Cladosporium cladosporioides, C. herbarum, Mortierlila substilissima,* and *Fusarium equiseti,* was studied against *Rhizopus nigricans* following the method of Skidmore and Dickinson (1976). Colony interaction was studied by inoculating the opposing colonies in pairs, 3 cm apart on potato dextrose agar (pH 5.6)

in sterilized petri dishes of 9 cm diam. Single or dual inoculated agar block cultures served as control. Hyphal interference between the test fungi was observed on pieces of deplasticised cellophane placed in PDA in sterilized petri dishes. The opposing test fungi were inoculated approximately 2 cm apart in three replicates. After the colonies met together microscopic observation was made in small squares of cellophane cut from the intermingling growth region. These squares were mounted on glass slides in cotton-blue and lactophenol mixture. The change in permeability was examined by mounting the squares in 70% (w/v) glucose solution and water.

Results and Discussion

According to the key designed by Porter (1924) and modified by Dickinson and Boardman (1971), colony interaction between *Drechslera rostrata* and *Rhizopus nigricans* was assigned to group three, where intermingling growth of the test fungi was observed, however, *D. rostrata* the antagonist grew into the opposed fungal colony. The type of colony interaction between *R. nigricans* and other nine fungi has been shown in Table 1. Initially the growth of *R. nigricans* was very fast but later on due to the attack by *D. rostrata* the growth of colony was retarded and restricted. The hyphae of the antagonist continued growing into that of the host. *D. rostrata* was observed to be the most destructive parasite of *R. nigricans* amongst all the test antagonists.

Hyphal interference studies indicate changes in permeability of the susceptible – fungus *R. nigricans*. The antagonised cells failed to show plasmolysis emphasizing destructive or necrotrophic nature of parasitism by *D. rostrata*. Different types of interference reactions were observed of which the coiling phenomenon was much pronounced (Fig. 1). In some instances intense coiling indicates strong antagonistic behaviour of the parasite. A few workers have suggested that the coiling may be effected due to thigmotrophic and/or chemotrophic factors (Ikediugwu and Webster, 1970; Dennis and Webster, 1971). Many of the recent investigations have evidenced that most of the phycomycetous fungi – with wider hyphal diameter – are very much susceptible to the hyperparasites with narrower hyphae (Durell, 1966; Gupta et al., 1979; Dennis and Webster, 1971). Penetration of the host hyphae by *D. rostrata* was noticed frequently (Figs 2, 3). After penetration the infection mycelium traversed the lumen of a part or the entire length of the host mycelium. The internal parasitic mycelium branched and sometimes short branches formed appressorium like structures which penetrated the wall of the host from inside by developing fine hypha (Fig. 4). This result gives an evidence that *D. rostrata* might be producing some cell wall degrading enzymes. In a few instances tuberculate structures were observed in the antagonised hyphae. The internal infection hypha also attacked the sporangiophores and sporangia of *R. nigricans* (Figs 5, 6, 7) where the branching behaviour was quite significant. Formation of conidiophore of the myco-

Table 1

Parasitic activity of fungal isolates from seeds of *Eleusine coracana* on *Rhizopus nigricans*

Antagonists	Colony interaction	
	Hyphal interaction	Grades
Drechslera rostrata	c, p, co	3
Curvularia lunata	p	1
Alternaria alternata	c, p	1
A. tenuis	v, c	1
Aspergillus flavus	c	2
Aspergillus niger	v	2
Cladosporium cladosporioides	—	1
C. herbarum	p	1
Mortierella subtilissima	—	3
Fusarium equiseti	p	3

c-coiling; p-penetration; co-coagulation; v-vacuolation
Grades: 1-Mutual intermingling growth; 2-Growth ceases to overgrown fungus; 3-Overgrowth by opposite fungus

parasite on the host hypha emphasized necrotrophic nature of the former (Fig. 8). Other fungal species also showed various types of reactions against *R. nigricans* but they were not as significant as those of *D. rostrata* (Table 1). The counteraction of the host hyphae resulted into distortion and swellings in the infection hyphae. Ultimately, the host hyphae were lysed and replaced by the parasitic hyphae (Fig. 9). Various workers (Barnett and Binder, 1973; Dennis and Webster, 1971; Skidmore and Dickinson, 1976) have also reported similar types of mycoparasitic

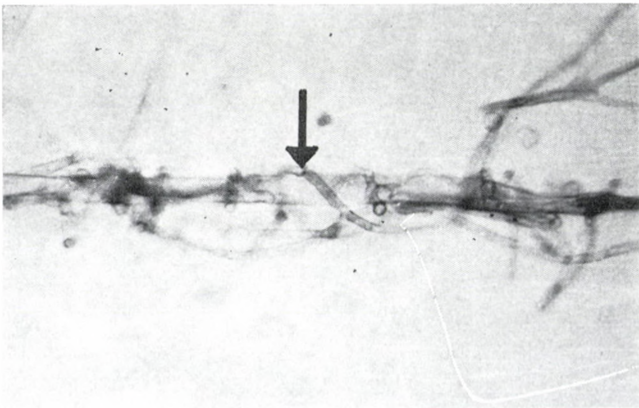


Fig. 1. Coiling of *Rhizopus nigricans* hypha by *Drechslera rostrata*

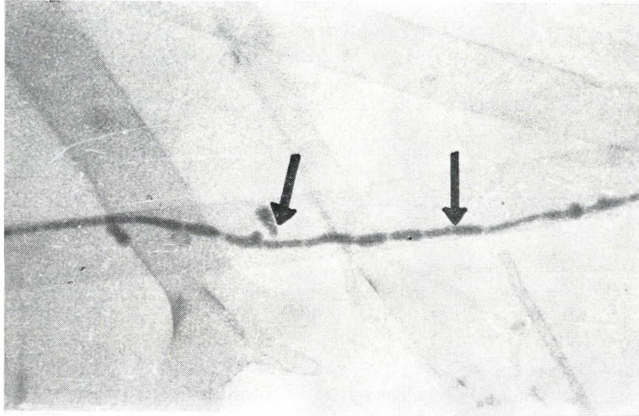


Fig. 2. Penetration by *D. rostrata* into the lumen of *R. nigricans* showing branching

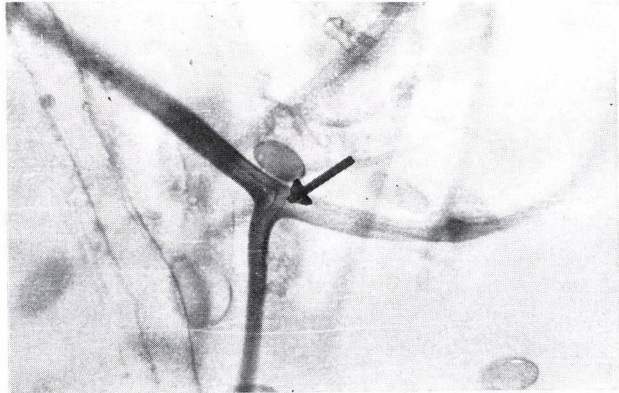


Fig. 3. Penetration and branching of *D. rostrata* hyphae inside *R. nigricans*

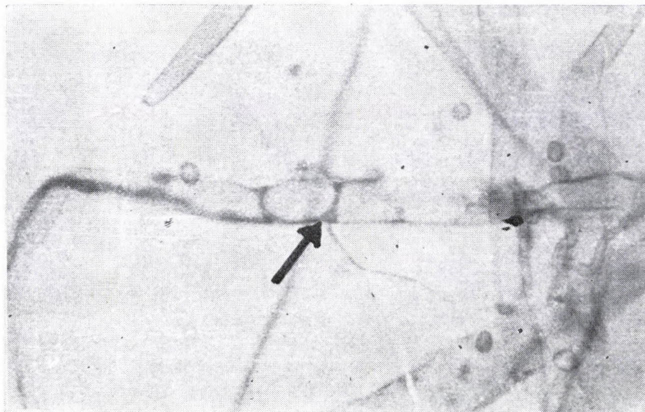
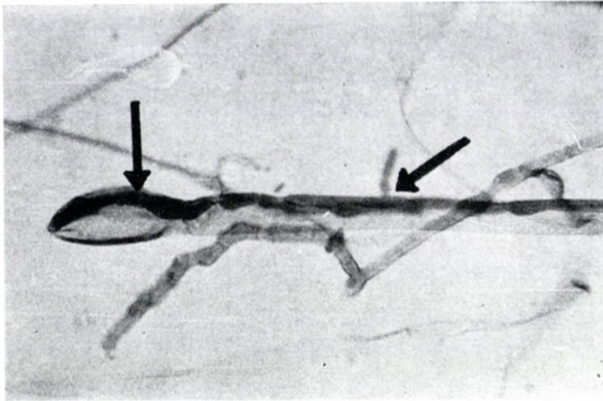
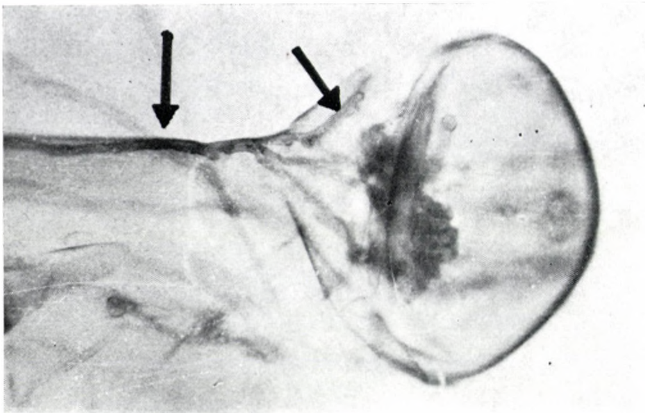
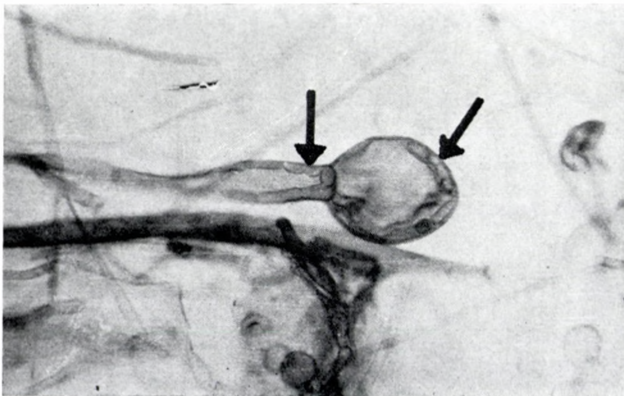


Fig. 4. Infection hypha of *D. rostrata* coming out of *R. nigricans*







Figs 5, 6, 7. Penetration of sporangiophore and sporangium of *R. nigricans* by the mycoparasite

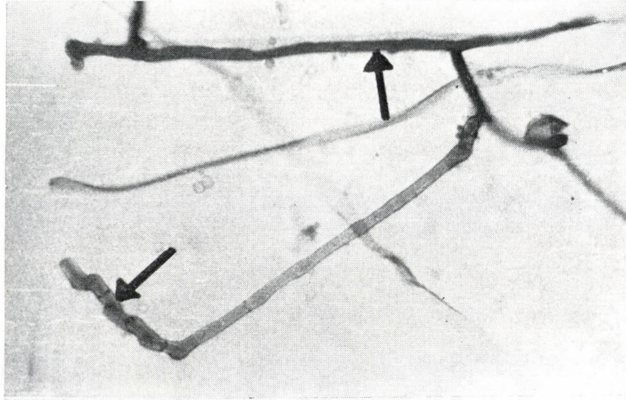


Fig. 8. Penetration and formation of conidiophore of *D. rostrata* on *R. nigricans*

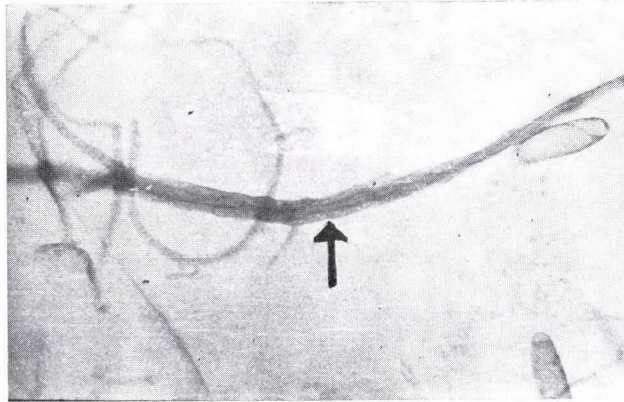


Fig. 9. Host hypha replaced by the hyphal development of the mycoparasite

interactions. *R. nigricans* has been reported to be parasitised by a number of fungi (Gupta and Tandon, 1976; Rai et al., 1977) but *D. rostrata* is hereby reported to be a new mycoparasite of *R. nigricans* for the first time. This mycoparasitic adaptability of *D. rostrata* provides an additional tool for survival, perennation and maintenance of its inoculum potential particularly in absence of suitable host which might help in occurrence of disease of *Eleusine coracana.*

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Multiplication of *Xanthomonas campestris* pv. *oryzicola* in Sensitive and Resistant Rice Cultivars

MUNNESHWAR RAI,[1] P. RANGA REDDY and R. SRIDHARI[2]

Department of Plant Pathology, Central Rice Research Institute, Cuttack
753 006, India

The internal bacterial population of leaf streak pathogen of rice *(Xanthomonas campestris* pv. *oryzicola)* in sensitive (cv. IR 8) and resistant (cv. NC 1281) host tissues was composed of two different types of cells capable of producing large mucoid and small translucent colonies. Translucent colony forming cells of the bacterium was more in number in the resistant tissues than in sensitive tissues. Although the occurrence of mucoid colony forming cells of the bacterium was more in sensitive tissues until five days after inoculation, translucent colony forming cells were more than the mucoid colony forming cells during later stages. Bacterial cells of both the colony types produced typical lesions 48 h after inoculation on the leaves of cultivar IR 8 while the resistant cultivar NC 1281 produced the symptoms 72 h after inoculation with the cells of translucent colony types. However, when inoculated with the cells of mucoid colony type the symptom expression by the resistant cultivar was delayed.

Multiplication of pathogenic bacteria in sensitive and resistant cultivars of several host plants has been examined. These investigations have concluded that although pathogenic bacteria persist and multiply appreciably in tissues of both sensitive and resistant cultivars, their population declines with time, especially in resistant tissues (Allington and Chamberlain, 1949; Diachun and Troutman, 1954; Scharen, 1959; Chand and Walker, 1964; Cafati and Saettler, 1980); hypersensitive reaction of host cells is associated with abrupt inhibition of bacterial multiplication (Klement et al., 1964); lower number of bacterial cells are required to cause necrosis in resistant than in susceptible tissues (Stall and Cook, 1966) and that the accumulation of antibacterial substances in the diseased tissue (O'Brien and Wood, 1973) might be related to the reduction in bacterial population (Cafati and Saettler, 1980). We studied the multiplication of *Xanthomonas campestris* pv. *oryzicola* (Fang et al.) Dye, the causal organism of bacterial leaf streak of rice in a sensitive and a resistant cultivar and present evidence for the natural occurrence of large mucoid (wild) and small translucent (mutant) colony forming bacterial cells in host tissues and for their variation in virulence patterns.

[1] Present address: Department of Plant Pathology, Chandrasekhar Azad University, Kanpur, India

[2] To whom correspondence should be addressed

Materials and Methods

Plant material and inoculation

The fully expanded top leaves of 45-day-old plants of rice cultivars IR 8 sensitive and NC 1281 resistant to the bacterium were inoculated by smearing the leaves with a piece of muslin cloth soaked previously in a cell suspension (ca. 10^9 cells/ml) of 48-h-old culture of *X. campestris* pv. *oryzicola* (isolate from naturally infected rice cultivar IR 8) maintained on potato-sucrose agar slopes. The isolate when plated on potato-sucrose agar medium gave rise to two distinct colony types (large mucoid and translucent colonies). The large mucoid colonies were used to prepare the bacterial suspension for the inoculation.

Assessment of bacterial population

The multiplication of the bacterium in the inoculated leaves of both the sensitive and resistant cultivars was monitored daily until eight days after inoculation. The leaves were harvested and portions of 2 cm bits containing the inoculated region were cut and surface sterilized in 0.1% mercuric chloride solution followed by several washings in sterile distilled water. The tissue was disintegrated under aseptic conditions and suspended in 10 ml of one per cent peptone-water. After about 30 min, appropriate serial dilutions were made and 0.1 ml of the suspension was spread on potato-sucrose agar medium in Petri dishes. The plates were incubated at 28 ± 2 °C for 4 days. The number and type (mucoid and translucent) of colonies were counted at the end of the incubation period. Populations of the bacterium is expressed as colony-forming units (CFU) per 10 ml of the suspension in log numbers. The results represent the average of three replications.

Pathogenicity tests

To test the pathogenicity of translucent and mucoid colonies originated from sensitive and resistant tissues, each of these isolates cultured independently on potato-sucrose agar slopes was inoculated onto cultivars IR 8 and NC 1281 separately as described above. The incubation period required for the appearance of initial symptoms and the host reaction (six days after inoculation) was recorded.

Results

Initial symptoms appeared as small water-soaked lesions in the inoculated leaves of sensitive cultivar IR 8 within 48 h and the length of the lesions increased gradually assuming characteristic pale yellow translucent streaks. Within 4–5 days, bacterial exudate in the form of small granules appeared on the surface of the lesions. On the other hand, brown necrotic lesions without water-soaking appeared in resistant cultivar NC 1281 only between 72 and 96 h after inoculation.

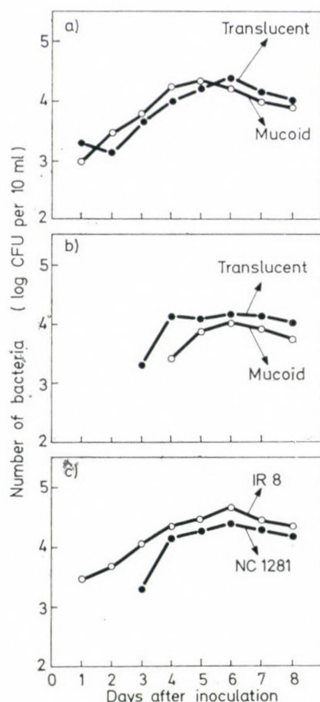

Fig. 1. Internal population of *Xanthomonas campestris* pv. *oryzicola* forming translucent and mucoid colony types of sensitive IR 8 (1-A) resistant NC, 1281 (1-B) and total population in cultivars IR 8 and NC 1281 (1-C). Leaf samples consisting of 2 cm leaf bits containing the inoculated region were disintegrated singly in 10 ml of peptone-water and the suspension after suitable dilution was used to assess the population. Values represent colony-forming units (CFU) in log number per 10 ml suspension and are averages of three replications

These lesions developed slowly without any brown bacterial exudation. Significant difference was noticed in the size of the lesions between the sensitive and resistant cultivars. The lesion length (mean of 100 lesions) recorded six days after inoculation was 5 to 25 mm in the sensitive cultivar IR 8 while it was 2 to 5 mm in resistant NC 1281.

The bacterial cells isolated from the leaves of both sensitive and resistant cultivars formed two distinct colony types: large mucoid and small round translucent colonies. Both the types of colonies were raised and yellow in colour. However, the rate of bacterial multiplication and the proportion in the number of large and small colonies recovered from the infected tissues of the cultivars markedly varied. The sensitive cultivar favoured a rapid multiplication of the pathogen and the bacterium could be recovered from the leaves even 24 h after inoculation (Fig. 1*a*). The rate of bacterial multiplication gradually increased until six days after inoculation and declined thereafter in this cultivar. The number of large colonies were more in the suspension from the sensitive cultivar up to

Table 1

Infection pattern of translucent and mucoid colonies of *Xanthomonas campestris* pv. *oryzicola* in sensitive (IR 8) and resistant (NC 1281) cultivars. Observations on disease reactions were recorded six days after inoculation

Source of bacterial isolates	Colony types	Incubation period (h)		Disease reaction	
		IR 8	NC 1281	IR 8	NC 1281
IR 8	Translucent	48	72	Susceptible	Susceptible
	Mucoid	48	72—96	Susceptible	Resistant
NC 1281	Translucent	48	772	Susceptible	Susceptible
	Mucoid	48	72—96	Susceptible	Resistant

five days after inoculation. In subsequent observations, however, the number of small translucent colonies was greater than the large mucoid colonies. In contrast, the bacterium could be reisolated from the resistant cultivar only 72 h after inoculation (Fig. 1*b*). Bacterial isolations from the infected resistant tissues yielded only translucent colonies 72 h after inoculation while both translucent and mucoid colonies were isolated from 96 h after inoculation onwards. The initial retardation in the build up of the pathogen in these tissues has not only slowed down the symptom appearance but also considerably reduced its severity. The number of translucent colonies dominated over the mucoid colonies in the suspensions obtained from resistant cultivar until 8 days after inoculation. Nevertheless, the total number of colonies, in general, was significantly more in the sensitive than in the resistant cultivar (Fig. 1*c*).

When the bacterial cells from large and small colonies obtained from suspensions derived from cultivars IR 8 and NC 1281 were inoculated separately on these cultivars, sensitive cultivar was readily infected and produced typical water-soaked symptoms of the disease 48 h after inoculation irrespective of the colony type and its origin (Table 1). On the contrary, it took 72 to 96 h to produce lesions by resistant cultivar when inoculated with the bacterial cells of large colonies isolated from either of the cultivars. However, the resistant cultivar produced lesions only 72 h after inoculation when inoculated with bacterial cells from small translucent colonies isolated from either of the cultivars.

Discussion

The occurrence of mucoid large, mucoid small and translucent colonies in cultures of a vascular pathogen of rice *X. campestris* pv. *oryzae* on artificial media has been reported (Irri, 1977; Reddy and Kauffman, 1977). Based on the fact that mucoid colonies of *X. campestris* pv. *oryzae* were more virulent than the translucent colonies in producing leaf blight, the loss of virulence during storage of the bacterium in culture has been attributed to the occurrence of translucent colonies (Reddy and Kauffman, 1977). In contrast, our results with *X. campestris* pv. *oryzicola* show that the bacterial cells of translucent colony obtained from the

infected tissues are more virulent than the cells of mucoid colony. The development of highly virulent bacterial cells in the host tissues that produce translucent colonies in culture might have been induced by the defence products of the host. This probability appears to be more since *X. campestris* pv. *oryzicola* parasitises the synthetic parenchymatous tissues. The delayed response of the sensitive cultivar to accumulate the prohibitins or phytoalexins or both might explain the increase in the population of translucent colonies in the sensitive tissues five days after inoculation.

Contrary to the existing view (Klement and Goodman, 1967) that the uncongenial (nonpathogenic) bacterium induces rapid formation of atypical symptoms (hypersensitive reaction), the response of the resistant cultivar to produce characteristic brown necrotic spots similar to hypersensitive reaction induced by the cells of mucoid colony types was considerably delayed. Resistant tissues take longer to express the disease symptoms than susceptible in response to bacterial infection (Cafati and Saettler, 1980). Presumably, the delay is due to the build up of bacterial population to reach a threshold level to induce tissue necrosis since, the bacterium multiplies at slow pace in the resistant tissues.

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The Effect of 2,4-D on the Petiole Tissues of Sunflower (*Helianthus annuus* L.)

F. Herdi

Research Institute for Plant Protection, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

The effect of three concentrations of Dikonirt D containing 2,4-D amine active ingredient were studied on the petioles of sunflower. The cell wall thickness of the vascular bundle, phloem parenchyma cell and tracheas were used as reference parameters. I studied the shape of the petiole, the position of the bristles, the number of the cell layers in the laminar collenchyma of the petiole surfaces, the number of vascular bundles, the numbers of the cell layers in the bundle sheath, phloem parenchyma and the cambium, the numbers of tracheae and the arrangement of the tracheids.

The cell walls in the bundle sheath showed decreasing thickness; it was most serious at the 7250 ppm concentrations. Cell wall thickness decreased with time in the treatments with 145 and 7250 ppm while the opposite was observed at 1450 ppm.

Similar trends were observed in the phloem parenchyma except the 7250 ppm where no change in relation to the first sampling was observed. The tracheal wall thickness did not change at 145 ppm and increased in the 1450 and 7250 ppm treatments. A characteristic symptom was the multiplication of the petiole vascular bundles: the existing ones segregated and new ones differentiated from procambium foci. The radial arrangement of the tracheas and tracheids became irregular in the treated plants.

From these observations it was concluded that the herbicide was harmful to the petiole vascular bundles at all the three concentrations used. These damages were: cell wall thickening, the multiplication of the vascular bundles, the irregular arrangement of the tracheae and tracheids, the increase in the numbers of tracheids and the ramification of the vascular bundles.

Cereals are regularly treated with hormone-based herbicides. The spraying is often performed from the air when the herbicide drifting can cause damages in the neighbouring dicotyledonous crops.

The hormone-based herbicides cause damages on the whole plants, both on the parts above and under the soil surface. Deformations occur (Ubrizsy and Gimesi, 1969) such funneliform leaves (Buhl, 1958; Ubrizsy, 1962), interlacing (Way, 1962; 1963a; 1964; Kiermayer cit. Audus, 1964), in the grapevine nettle-like growth and ginkgo-shaped leaves are found (Ubrizsy, 1962; Szatala, 1967). Deformations were observed on the crop (Andersen, Bachthaller, Hanf cit. Ubrizsy, 1962; Ubrizsy and Gimesi, 1969; Terpó, Pomogyi and Terpó, 1971; Way, 1963; 1963a; 1964a; Ubrizsy, 1962) and on the roots (Arlt and Feyerabend, 1973; Buhl, 1958; Way, 1962; 1963a; 1964). Sunflower is very sensitive to modern herbicides. These herbicides cause disturbances in cell differentiation (Watson, 1948); intensive cell divisions were observed in meristematic tissues and in the

shoot phloem by Eames (1951). The deleterious effect of the 2,4-D on the cambium, endodermis, embrional pericycles and phloem was demonstrated by Swanson (cit. Klingman, 1963) and Beal (cit. Audus, 1964).

Damages in the phloem, cambium and the neighbouring parenchyma were observed as consequences of dichlobenyl and dicamba treatments (Pate et al., 1965). Deformations in root and shoot xylem and phloem was observed by Mayer (1970). Eanes (1949) reported abnormal, elongated vascular bundles, decrease in phloem quantity, decrease in the growth of metaxylem vascular elements. Bradley (cit. Audus, 1964) found an increase in the petiole diameter caused by additional xylem and phloem growth. The changes in the cell wall thickness and structure was observed by Gorter and Gifford (cit. Audus, 1964).

Herdi (1979) studied the vascular bundles (VB) in the petiole of the beetroot and found a dicamba effect of cell wall thickening in the bundle sheath (BS) and angular collenchyma, the deformation and multiplication of the VBs. Sorokin et al. (1965) found that IAA and 2,4-D caused hyperplasia and xylem discontinuity in bean shoots.

Material and Methods

Sunflower seedlings were used (Vniimk 6541 variety) sown 12 May, treated 12 June when they were about 12 cm high. Dikonirt D containing 2,4-D active ingredient was sprayed at 145, 1450 and 7250 ppm with untreated control. Leaf

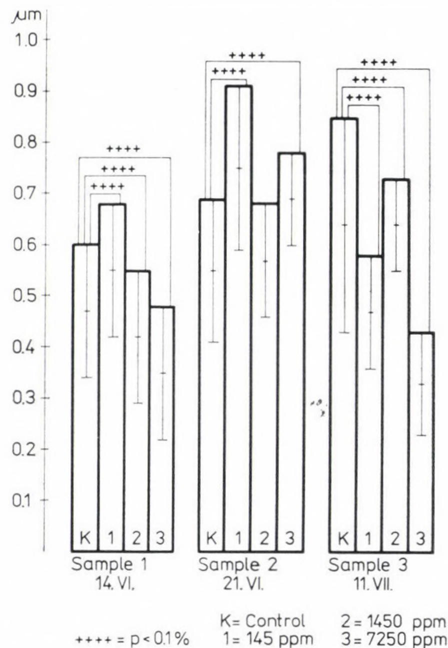

Fig. 1. The effect of 2,4-D on the bundle sheath cell wall thickness

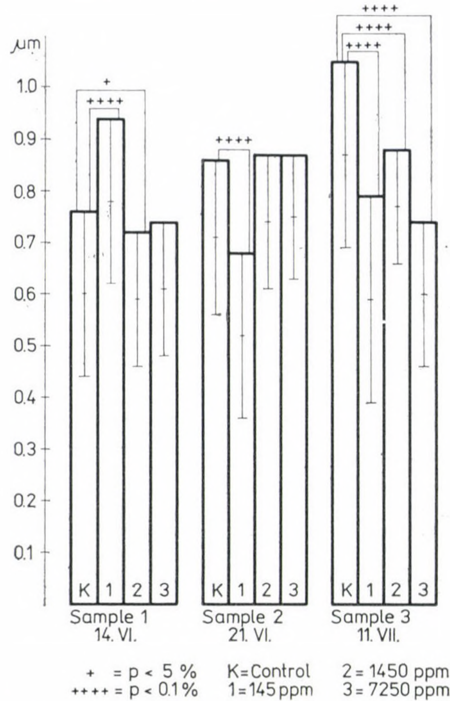

Fig. 2. The effect of 2,4-D on the phloem cell wall thickness

samples were taken 14 and 21 June, 11 July. The leaf samples were stored in 40% ethyl alcohol. Sections were made from the central part of the petioles using an MC-2 sliding microtome with a KTOC-2 electric freezing equipment. Staining was made with Ehrlich's acidic haematoxylin. I studied the petiole shape, the number of cell layers in the collenchyma, the multi-cell bristles, the VB numbers, within VBs I measured the cell wall thickness of BS and parenchyma cells as well as of tracheids.

I counted the numbers of cambium cell layers, and observed the increase in VB numbers and their arrangement in the petiole.

Results

Tissue deformations are shown in Figs 1–3.

Untreated sample, 14 June

The petiole was triangle-shaped, bifacial, the convex surface slightly undulated. The laminar collenchyma was found on both the convex and the concave surface of the petiole, and contained 2–3 cell layers and 4–5 cell layers at the petiole edges.

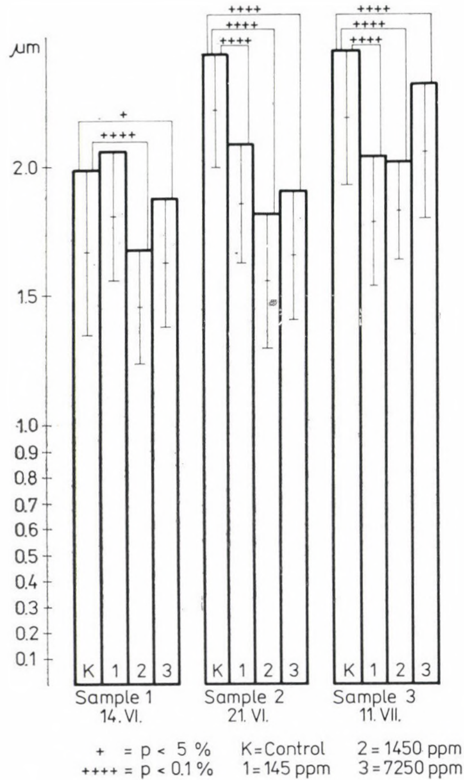

Fig. 3. The effect of 2,4-D on the tracheal cell walls

The epidermal multi-cell bristles were scattered on both petiole surfaces. There were 12–15 of collateral open VBs in the petiole (Fig. 6*a*), relatively distant from each other (Fig.4*a*) three of them were conspicuously larger than the others.

BS contained 4–7 cell layers and one layer of amylaceous sheath above the phloem. 2–3 cell layer of BS surrounded the VB at the xylem and at both edges. The cambium contained 3–4 cell layers, the tracheas and tracheids were radially arranged. In longitudinal sections, the VBs were parallel with the petiole margins, no ramification was found.

Untreated sample, 21 June

Petiole was similar to the 1st sample; 3–4 cell layers of laminar collenchyma were found on the concave surface while three on the convex surface which increased to 7 above the central VB. The multi-cell bristles were distributed similarly, with slightly more on the concave surface.

There were 12–15 VBs, three of them larger than the others (Fig. 6*b*), their arrangement was also similar (Fig. 4*b*). The BS contained 4–7 cell layer and one

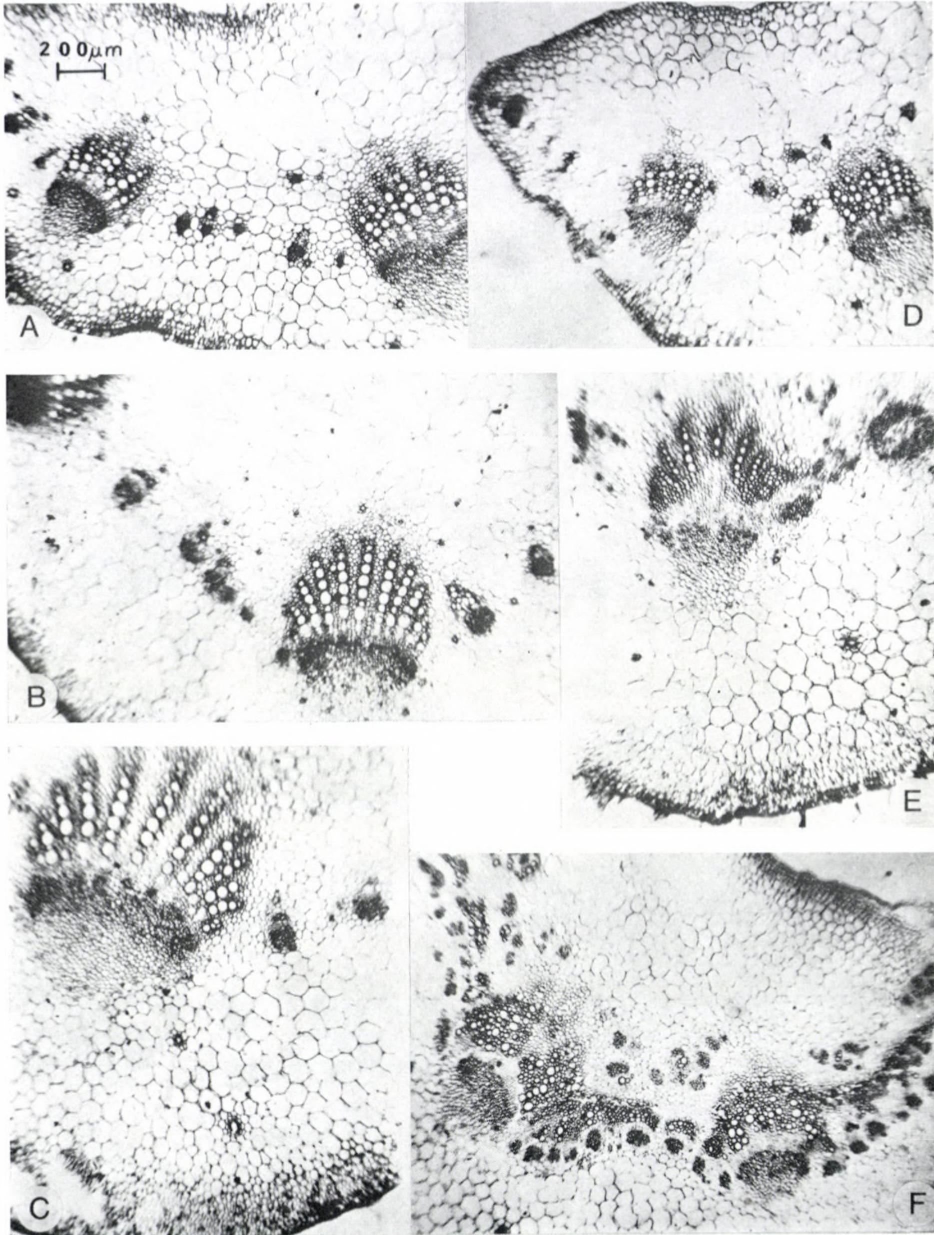

Fig. 4. The arrangement of the vascular bundles in the control and 2,4-D treated petioles. *a– c* control; *d f* 145 ppm. *a, d* 1st sample, 14 June; *b, e* 2nd sample, 21 June; *c, f* 3rd sample, 11 July. The figures are on the same scale

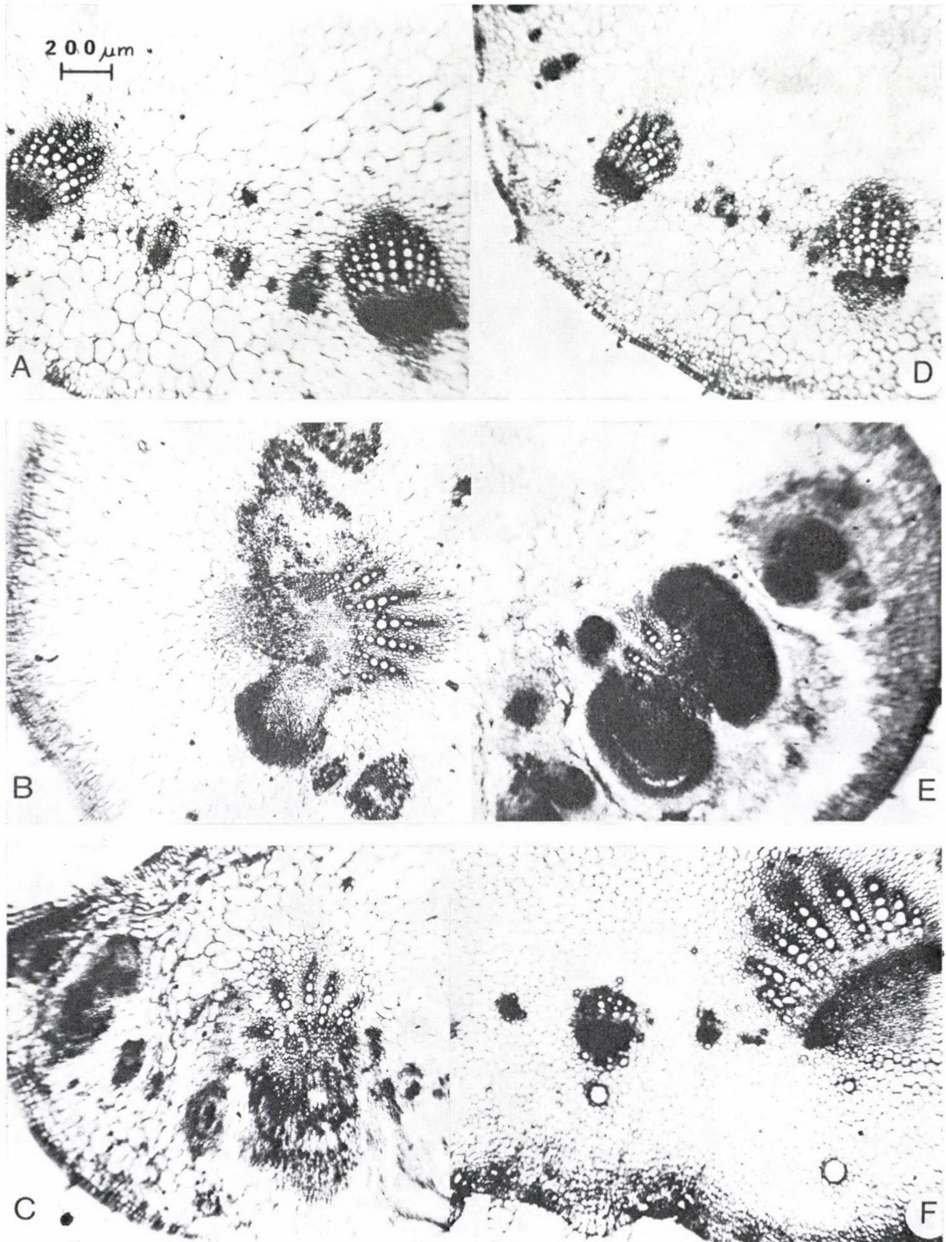

Fig. 5. The arrangement of the vascular bundles in the 2,4-D treated petioles. *a–c* 1450 ppm. *d–f* 7250 ppm. *a*, *d* 1st sample, 14 June; *b*, *e* 2nd sample, 21 June; *c*, *f* 3rd sample, 11 July. The figures are on the same scale

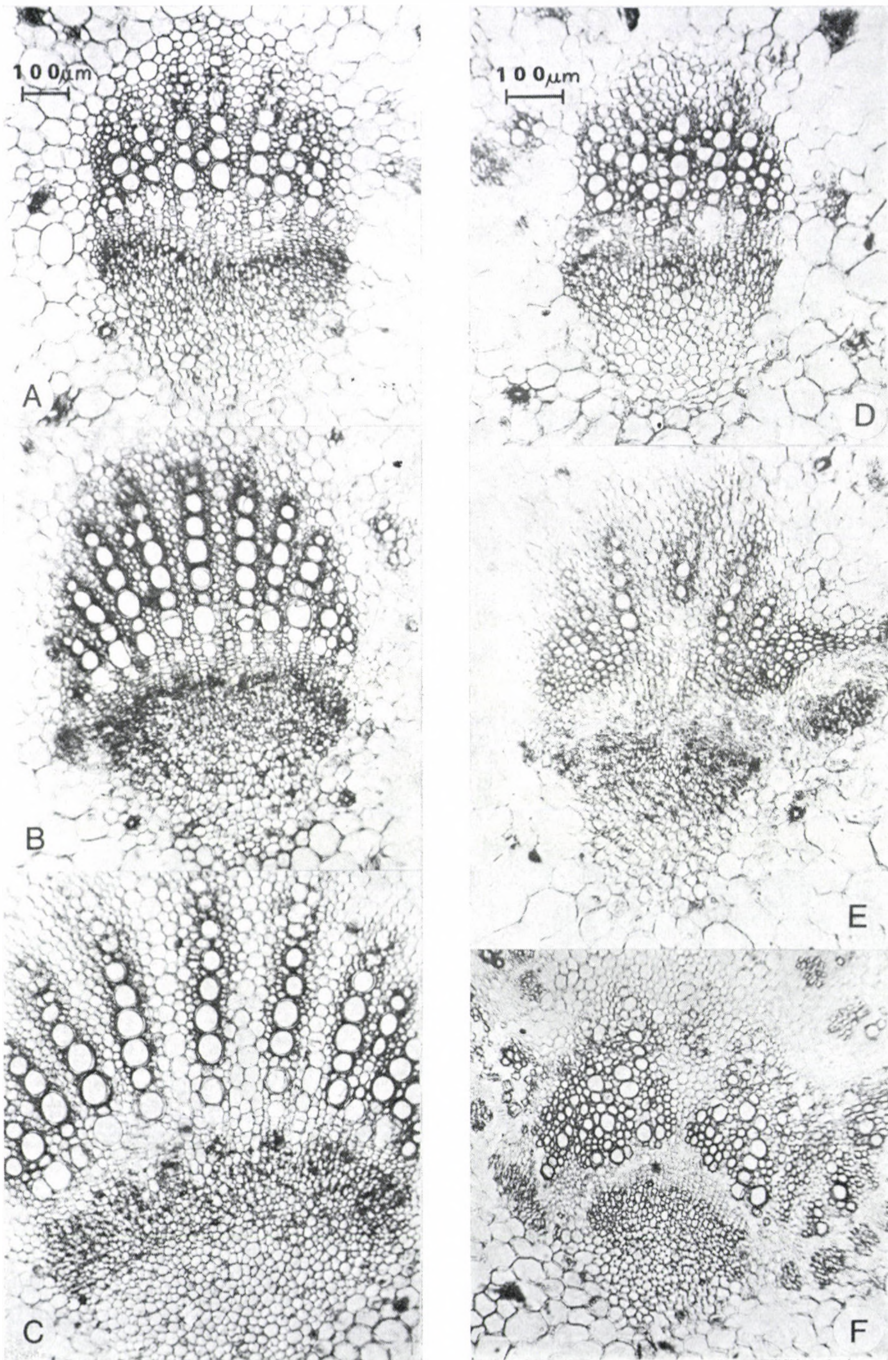

Fig. 6. Vascular bundle structure in the petiole and the effect of 145 ppm. 2,4-D on this structure. *a—c* control; *d—f* 145 ppm. *a, d* 1st sample, 14 June; *b, e* 2nd sample, 21 June; *c, f* 3rd sample, 11 July. All plates except D are on the same scale

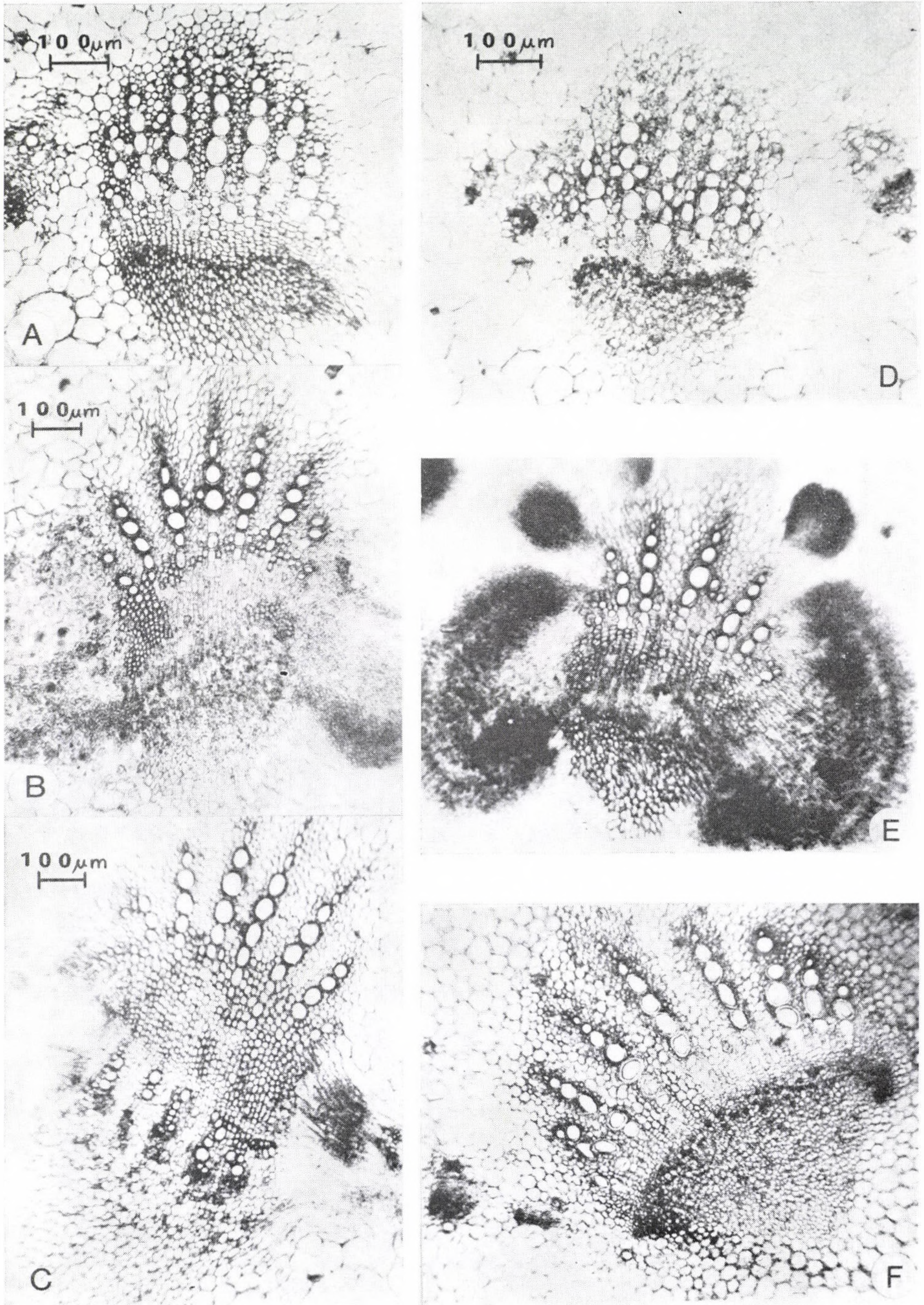

Fig. 7. The effect of 2,4-D on the petiole structure and vascular bundle *a–c* 1450 ppm. *d–f* 7250 ppm. *a*, *d* 1st sample, 14 June; *b*, *e* 2nd sample, 21 June; *c*, *f* 3rd sample, 11 July. *c*, *e* and *f* are on the same scale

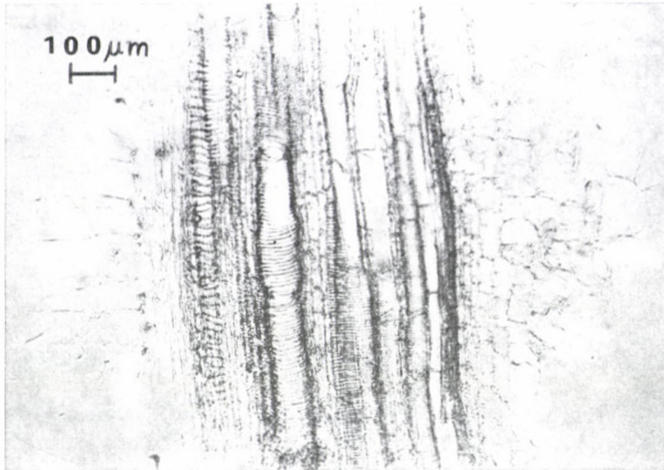

Fig. 8. The structure of the control petiole, longitudinal section. 2nd sample, 21 June

layer of amylaceous sheath above the phloem; 2–3 cell layers of BS surrounded the VB at the xylem and at both edges. The cambium contained 4–6 cell layers, the tracheas and tracheids were radially arranged.

In longitudinal sections (Fig. 8) the VBs were similar to the 14 June sample.

Untreated sample, 11 July

The petiole was similar to the 1st sample; with 4–6 cell layers of laminar collenchyma on the concave, 5–8 on the convex surface. The multi-cell bristles were scattered individually, their distance to each other was larger on the convex surface.

The VB number was 16–18, three of them larger than the others (Fig. 6*c*), with a similar arrangement (Fig. 4*c*). The BS contained 6–10 cell layers and a one-cell thick layer of amylaceous sheath above the phloem while only 2–4 cell layers thickness was found at the xylem and at both edges.

The cambium was 4–6 cell layer thick. The tracheas and tracheids (T and T) were radically arranged.

The VBs were not ramificating.

The effect of 145 ppm Dikonirt-D, 14 June

The petiole shape was similar to that of the control. The laminar collenchyma was 2–3 cell layer thick on both surfaces, increasing to 4–5 layers at the edges. The multi-cell bristles were scattered individually, distant from each other.

12–14 VBs were found, three of them larger than the others (Fig. 6*d*), their arrangement was similar to that of the control (Fig. 4*d*).

The BS was 4–8 cell thick above the phloem plus the amylaceous sheath while only 1–2 layers found at both sides and at the xylem. The cell wall of BS cell slightly thickened (Fig. 1), the phloem parenchyma cell walls thickened conspicuously (Fig. 2).

There was a slight thickening in the trachea walls (Fig. 3). The cambium contained 4–6 cell layers.

The T and T were mainly radially arranged but some of them became scattered (Fig. 6*d*).

VB multiplication was seen around the large VBs via differentiation from procambium foci. No ramification in the tracheas was found.

The effect of 145 ppm Dikonirt-D, 21 June

The concave petiole surface was strong undulated; the laminar collenchyma was 3–4 cell layer thick, and 4–6 at the edges. On the convex surface, 4–6 cell layers were found and angular collenchyma appeared.

The multi-cell bristles were singly scattered on the convex but were more densely packed on the concave surface.

18–21 VBs were found, three of them large, the others of medium or small size (Fig. 6*e*). The VBs were closer to each other than in the control.

The BS was 4–8 cell layers thick above the phloem plus the amylaceous sheath, while less thick, 2–4 cell layers at the xylem and at both sides.

The BS cell walls grew thicker (Fig. 1). The phloem parenchyma cells walls grew thinner than the control and the 14 June sample (Fig. 2).

The tracheal cell walls were thinner than in the control (Fig. 3). The cambium was 8 – 10 cell layers thick with insular tracheas and tracheids behind them. Most VBs contained T and T forming a layer which was multicell-layer thick; behind this, the T and T were radially arranged (Fig. 6*e*).

The VBs seriously multiplicated via both segregation and differentiation. The VBs formed a nearly continuous layer (Fig. 4*e*) with some local parenchyma layer only.

VB ramification was first found in this sample.

The effect of 245 ppm Dikonirt-D, 22 July

Both petiole surfaces were slightly undulated. The laminar collenchyma consisted of 4–5 cell layers on the concave and 4–6 cell layers on the convex surface; there were 5–8 cell layers at the petiole edges.

The multi-cell bristles were scattered on both surfaces. Above the three conspicuously larger VBs, many smaller VBs forming a continuous layer was found (Fig. 4*f*); in this continuous layer, the counting of VBs was not possible.

I found 5–10 cell layers of BS plus the amylaceous sheath above the phloem with 2–3 cell layers at the xylem and laterally.

The BS cell walls were seriously thinner than in the control and in the 21 June sample (Fig. 1).

The phloem parenchyma cell walls were also thinner (Fig. 2).

The tracheal cell walls (Fig. 3) were slightly thinner than those of the control or the 21 June sample. The cambium was 6–8 cell layers thick with a nearly continuous T and T layer. The T and T arrangement was radial in a few cases only (Fig. 6*f*), they were mostly grouped or continuously arranged.

The VB multiplication via segregation of the existing ones and differentiation of new ones from procambium foci was so intensive that a nearly continuous layer was found. I found no ramification here.

The effect of 1450 ppm Dikonirt-D, 14 June

The concave petiole surface was slightly undulated, the laminar collenchyma was 2–3 cell layers thick on the concave surface while 4–5 on the convex one and 4–6 at the petiole edges.

The multi-cell bristles were scattered on both the convex and the concave surfaces; more was found on the convex side.

The VB number was 20–23 with a control-like size distribution (Fig. 7*a*) and arrangement (Fig. 5*a*). The BS was 4–9 cell layer thick above the phloem, 1–2 layer laterally and at the xylem. The amylaceous sheath was found at the phloem. The BS (Fig. 1) and the phloem parenchyma (Fig. 2) cell walls were slightly thinner than the control and the 145 ppm 14 June sample.

A similar relationship was found in the tracheal cell walls (Fig. 3).

The cambium contained 5–9 cell layers.

The T and T were radially arranged (Fig. 7*a*). VB multiplication took place mainly via differentiation from cambium foci. No VB ramification was found.

The effect of 1450 ppm Dikonirt-D, 21 June

The concave petiole surface was more undulated than in the 1st sample, the convex one was slightly undulated. The laminar collenchyma cell layer thickness was 3–4 at the concave surface, 3–5 at the convex and 4–6 laterally.

The multi-cell bristles were nearer to each other on both surfaces than in the 1st sample. Some of the bristles on the convex surface were of more than one cell thick.

There were 19–22 VBs in control-like size distribution. The BS above the phloem was 6–10 cell layer thick plus the amylaceous sheath; the BS was 2–3 cell layer thick at the xylem and laterally. The BS (Fig. 1) and phloem parenchyma (Fig. 2) cell wall thickness was nearly identical with the ones in the control and slightly more thick than in the 1st 1450 ppm sample.

The tracheal cell walls (Fig. 3) was more thin than in the control but more thick than in the 1st 1450 ppm sample.

The cambium layer was 4–8 cell layer thick.

T and T majority was radially arranged but there were ones scattered and clumped. VBs were very close to each other (Fig. 5*b*), moderately increased in

numbers via segregation of the existing ones. In the VBs between the cambium and the differentiated xylem elements some imperfectly differentiated cells were observed (Fig. 7*b*) which was a sign that xylem differentiation was stopped by the 1450 ppm Dikonirt-D. There were ramifications observed as in the 2nd 1450 ppm sample (Fig. 7*b*).

The effect of 1450 ppm Dikonirt-D, 11 July

The petiole was deformed, curved, nearly rectangle in cross-sections; both surfaces slightly undulated. The laminar collenchyma was 3–5 cell layer thick on the concave surface while 4–6 cell layers thick on the convex one and laterally.

The multi-cell bristles were distributed not so close to each other on either surface; some of them were of more-than-one cell thick.

The VB numbers reached 23–26, four of them was larger than the others. There was a 4–8 cell layers thick BS and one layer of amylaceous sheath above the phloem. The BS was only 2–3 cell layer thick round the other parts of the VB.

The cell wall thickness of the BS (Fig. 1), the phloem parenchyma (Fig. 2) and the tracheas (Fig. 3) grew thinner than in the control but thicker than in the previous 1450 ppm sample. The cambium layer was 4–6 cell layers thick.

T and T were radially arranged.

The VB numbers slightly increased via segregation. VBs were close to each other (Fig. 5*c*). The VBs consisted of small numbers of radially arranged T and T next to the cambium layer. In the next zone non-differentiated xylem elements with thick walls were found then a new layer of radially arranged T and T (Fig. 7*c*). Most of the VBs were ramificated.

The effect of 7250 ppm Dikonirt-D, 14 June

Both petiole surfaces were slightly undulated. The laminar collenchyma cell layer thickness was 2–3 at the concave surface 3–4 at the convex one and 4–6 at the petiole edges.

More bristles were found on the concave surface; they were scattered, some of them being more-than-one cell thick.

The VB numbers were 14–16, three of them were of larger size (Fig. 7*d*), all in control-like arrangement (Fig. 5*d*). There were 4–6 cell layers of BS and one layer of amylaceous sheath above the phloem, 1–2 cell layers above the xylem and laterally. The BS cell walls were far more thin than in the control (Fig. 1). The difference in the phloem cell and tracheal cell walls was much less (Figs 2 and 3).

The cambium was 3–6 cell layers thick.

T and T were radially arranged, some of them were scattered around the radial elements (Fig. 7*d*).

The VBs slightly multiplicated via differentiation from procambium foci. No VB ramification was found.

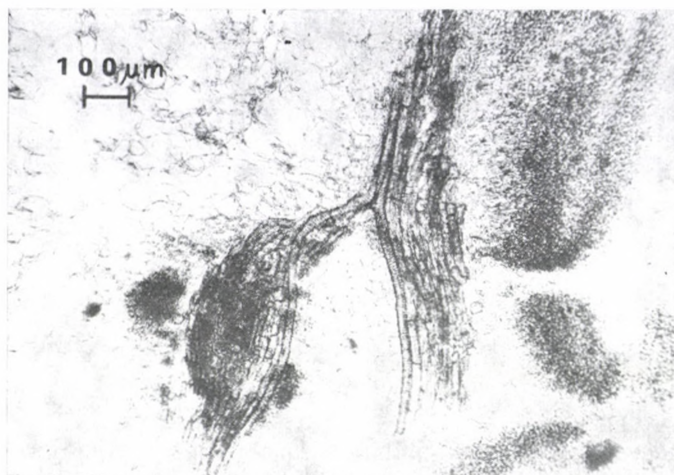

Fig. 9. Vascular bundle ramification in the petiole; the effect of 7250 ppm 2,4-D treatment. Longitudinal section, 2nd sample, 21 June

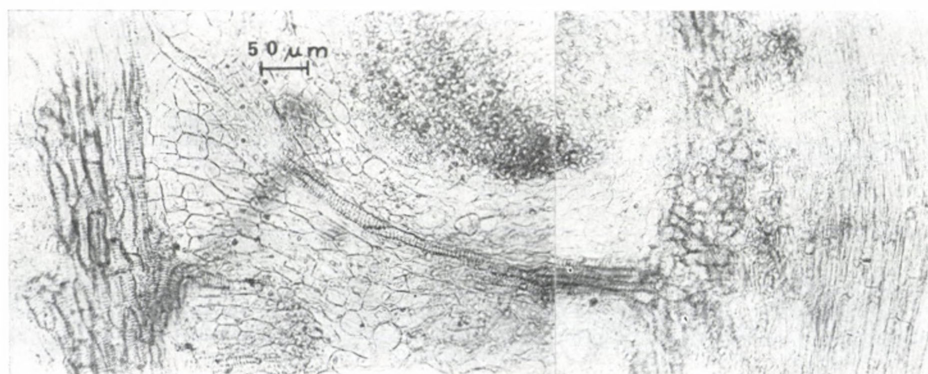

Fig. 10. Vascular bundle ramification; the effect of 7250 ppm 2,4-D treatment. 2nd sample, 21 June. Longitudinal section

The effect of 7250 ppm Dikonirt-D, 21 June

The petiole was seriously deformed, curved. The laminar collenchyma was 2–3 cell layers thick on the concave surface, 3–4 cell layers thick on the convex one and 4–5 laterally. The multi-cell bristles were more numerous on the concave surface than on the convex one where some bristles were more-than-one cell thick.

There were 12–14 VBs, four of them large, the majority was of medium size and 1–2 only were small like in the control. The BS was 4–8 cell layers thick above the phloem, 2–3 cell layers thick at the xylem. The BS cell walls (Fig. 1) and the

phloem parenchyma (Fig. 2) grew more thick than in both the control and the 7250 ppm, 1st sample. The tracheal cell walls were much more thin than in the control while slightly more thick than in the 7250 ppm, 1st sample (Fig. 3).

The cambium layer was 4–8 cell thick.

T and T were radially arranged (Fig. 7*e*) with some elements scattered among them.

VB multiplication was slight via differentiation from procambium foci. VBs were close to each other (Fig. 5*e*), strong ramificated (Figs 7*e*, 9, and 10).

The effect of 7250 ppm Dikonirt-D, 11 July

Both petiole surface were strong undulated with laminar collenchyma of 6–8 cell layers on the concave surface and 8–11 cell layers on the convex one and at the petiole edges.

The multi-cell bristles were found in large numbers on both surfaces, close to each other many of them were more-than-one cell thick on the convex surface.

The VB numbers were 20–23, three of them much larger, the others were of medium or small size. The BS consisted of 10–18 cell layers of very small cells above the phloem (Fig. 7*f*). The BS was 3–4 cell layer thick laterally and at the xylem. The BS cell walls (Fig. 1) and the phloem parenchyma cell walls (Fig. 2) were much more thin than in the control and in the 2nd 7250 ppm sample.

The tracheid walls thickness differed slightly from the control (Fig. 3); the tracheal cell walls were more thin than in the control but more thick than in the 2nd 7250 ppm sample.

The cambium was 5 – 8 cell layers thick.

T and T were radially arranged.

VB multiplication took place via differentiation from procambium foci, especially around the large VBs. VBs were found distant from each other (Fig. 5*f*) in a control-like arrangement. The large number of BS cells and the small BS and phloem parenchymal cell size were characteristic of the VBs. VBs did not ramificate.

The above concentrations of Dikonirt-D was harmful to the petiole of the sunflower mainly the VBs. The BS and phloem parenchyma cell wall thickness was most influenced by the 7250 ppm concentration. For the tracheal cell walls the 1450 ppm was most harmful. The 1450 ppm seriously depressed the differentiation of the xylem elements and caused VB ramification.

In conclusion, the 1450 ppm was most harmful to the petiole structure; the 7250 ppm had no such effects as expected in relation to the 1450 ppm treatment.

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Characteristics of the Electro-blot Radioimmunoassay (EBRIA) in Relation to the Identification of Plant Viruses

D. D. Shukla, I. J. O'Donnell and K. H. Gough

Division of Protein Chemistry, CSIRO, 343 Royal Parade, Parkville (Melbourne), Victoria 3052, Australia

The Electro-Blot Radioimmunoassay (EBRIA) detected plant viruses at amounts as low as 0.5 ng. The aminophenylthioether paper was found more efficient than the nitrocellulose sheet in binding the plant virus coat proteins. Antisera prepared in response to intact particles and to LiCl-isolated coat protein of a virus, although identical in their sensitivities in the homologous situations (0.5 ng), gave the same lower sensitivity (50 ng) when tested under the heterologous conditions. The technique is very convenient for establishing relationships between strains of plant viruses as coat protein of several strains can be transferred to one paper and the paper then sequentially probed with different antisera. EBRIA is also very economical with respect to the amount of antiserum required for probing; 5 μl antiserum is sufficient to probe a paper containing eight virus samples.

In a previous paper (O'Donnell et al., 1982) we reported the use of a new technique, the Electro-Blot Radioimmunoassay (EBRIA) (Renart et al., 1979; Bittner et al., 1980), for detecting plant viruses. The technique involved sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) of virus infected plant sap, electrophoretic transfer of protein bands onto activated paper (Electro-Blot), probing the viral coat protein band with specific antiserum, and then detection of the immune complex with ^{125}I-labelled protein A (Radioimmunoassay). The major advantage of the technique was that it detected the viruses by two important stable properties: serological specificity and molecular weight of the viral coat protein.

In this paper we have examined the detection limit of EBRIA using known amounts of purified viruses, and have compared the performance of antisera to whole virus preparation with that raised against isolated viral coat protein. We have also compared the performance of the readily available nitrocellulose sheet with aminophenylthioether paper (APT), and have demonstrated the applicability of the technique for distinguishing between strains of plant viruses by sequential probing of the transferred proteins with different antisera.

Materials and Methods

The viruses used in the various experiments were Erysimum latent virus (ELV), a tymovirus (Shukla et al., 1980), four strains of sugarcane mosaic virus (SCMV), a potyvirus: Johnson grass (JG), sugarcane (SC), sabi grass (Sabi) and Queensland blue couch grass (BC) (Gough and Shukla, 1981) and a common

strain of tobacco mosaic virus (TMV) (Shukla and Gough, 1979). The antisera to ELV, TMV and the four SCMV strains were the same as described previously (Shukla and Gough, 1979; Gough and Shukla, 1980; O'Donnell et al., 1982). The antiserum to the LiCl-isolated ELV coat protein (Shukla et al., 1980) was produced in a rabbit by a series of five injections over a period of 12 weeks. The protein (250 μg protein/250 μl H_2O/injection) was emulsified with an equal volume of Freunds complete adjuvant (first two injections) or Freunds incomplete adjuvant (last three) and injected intramuscularly into each hind leg and subcutaneously into several sites on the neck each time. The blood was collected three weeks after the last injection. The antiserum had a titre of 1 : 32 in immunodiffusion tests.

The four SCMV strains were tested as purified preparations as well as infected sap from sweetcorn. Therefore, it was necessary to absorb the antisera to these four strains against healthy extracts of sweetcorn to prevent anti-host protein immune complexes interfering with the virus detection. This was done using a CNBr-Sepharose column as described previously (O'Donnell et al., 1982). The ELV and TMV antisera were not absorbed against host proteins because only purified preparations of these viruses were used in the tests.

For determining the limit of virus detection by EBRIA, purified preparations of ELV, TMV and SCMV-JG were prepared at 0.5 mg virus/ml Tris-glycine buffer (O'Donnell et al., 1982). For determining the cross reactivities between the four SCMV strains, purified virus preparations at 0.1 mg virus/ml Tris-glycine buffer and also leaf extracts from sweetcorn infected with the strains for three weeks were used. The leaf extracts were prepared as described previously (O'Donnell et al., 1982). Coat proteins of ELV, SCMV-Sabi and TMV were prepared using LiCl (Gough and Shukla, 1981). Approximately 0.5 mg coat protein was dissolved in one ml of Laemmli sample buffer (Laemmli, 1970) and the solution heated for 3 min in a boiling water bath. 10 μl of either virus or the LiCl-isolated coat protein preparations was loaded in each slot of the gels. The conditions for SDS-PAGE were as described by O'Donnell et al. (1982).

The APT paper (approximately 12 × 14 cm) was prepared according to the method of Reiser and Wardale (1981) and was diazotized according to instructions of Alwine et al. (1977). The nitrocellulose sheet was purchased from Bio-Rad. Transfer of protein bands onto ATP paper and the subsequent probing and reprobing with antisera was according to O'Donnell et al. (1982). The same procedure was used for transfer of proteins to nitrocellulose sheet, but for immunological detection of these proteins the procedure of Towbin et al. (1979) was followed.

Results

From Fig. 1A it can be seen that while Coomassie blue staining gave a detection limit of 500 ng for the purified ELV preparation after SDS-PAGE, the EBRIA detected the virus at amounts as low as 0.5 ng (Fig. 1). Identical sensitivities were found for SCMV and TMV.

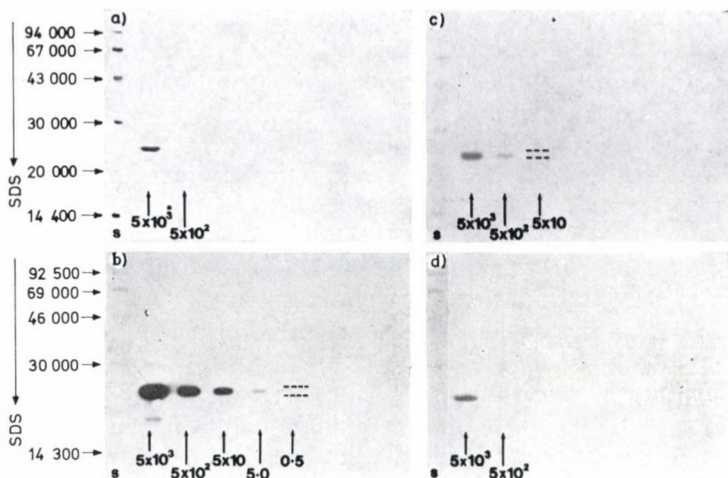

Fig. 1. Coomassie blue-stained gel pattern (a) and autoradiographs (b, c, d) of a purified Erysimum latent virus (ELV) preparation (10 μl samples of a series of 10-fold dilutions from the initial concentration of 0.5 mg virus/ml was loaded) after SDS-PAGE. After electrophoresis, the protein bands on duplicate gels were either stained with Coomassie blue or transferred to aminophenylthioether (APT) or nitrocellulose sheet. The paper was then probed with antisera and ^{125}I-labelled protein A. The dotted lines in b) and c) mean that the band was visible on the original autoradiograph. b) The APT paper probed with an antiserum to intact particles of ELV. c) The APT paper reprobed with an antiserum to LiCl-isolated ELV coat protein. d) ELV protein bands bound to nitrocellulose sheet and probed with ELV antiserum to intact particles. s) in A is Pharmacia standards, and in b, c, d it is ^{14}C Amersham standards. *) represents the amount of virus (in ng) detected

Reprobing of the APT paper shown in Fig. 1B with different quantities (2.5 to 50 μl) of the ELV antiserum to intact virus particles showed that the detection of viral coat protein bands is relatively insensitive to the amounts of antiserum except at the lowest amount (2.5 μl) which resulted in decreased sensitivity. Antiserum amounts greater than 25 μl increased the nonspecific background.

When the APT papers containing the LiCl-isolated coat protein of ELV, SCMV-Sabi and TMV were probed with antisera prepared in response to intact virus particles, the coat proteins reacted positively with their respective antisera in each case. As expected, there was no cross-reaction between coat proteins of these three unrelated viruses and their antisera. The antiserum prepared in response to the LiCl-isolated coat protein of ELV gave a detection limit of 0.5 ng with the LiCl-isolated coat protein bands. However, when the coat protein antiserum (titre 1 : 32) was used to reprobe the APT paper containing ELV bands (Fig. 1B), the detection was obtained only up to 50 ng amount (Fig. 1C) compared to the 0.5 ng detection limit obtained with the intact virus antiserum. Similarly, the antiserum to intact ELV particles (titre 1 : 4096) reacted with the

LiCl-isolated coat protein bands only up to 50 ng amount. This may be due to the differences in antigenic specificity of the LiCl- and SDS-treated coat proteins and the antisera to intact virus particles and isolated coat protein (Moghal and Francki, 1976).

When ELV protein band from duplicate gels were transferred onto APT paper and nitrocellulose sheet and probed with the antiserum to intact virus particles, the nitrocellulose gave a lower sensitivity (500 ng, Fig. 1D) than the APT paper (0.5 ng, as in Fig. 1B).

When the leaf extract and purified preparations of the four SCMV strains were run in SDS-PAGE, the former gave a complicated Coomassie blue-stained gel pattern (Fig. 2A, a, b, c, d) whereas only one band was observed with the latter with each of the four strains (Fig. 2A, e, f, g, h). When the protein bands from the duplicate gel were transferred onto APT paper and probed with a mixture of antisera to the four strains, only one prominent band, the viral coat protein, was visible with the whole leaf extract as well as with the purified preparations (Fig. 2B). Cleaning and reprobing of the APT paper with the SCMV-JG antiserum gave positive reaction only with the JG coat protein bands (Fig. 2C, a, e). Reprobing the paper separately with antisera to the SC, BC and Sabi

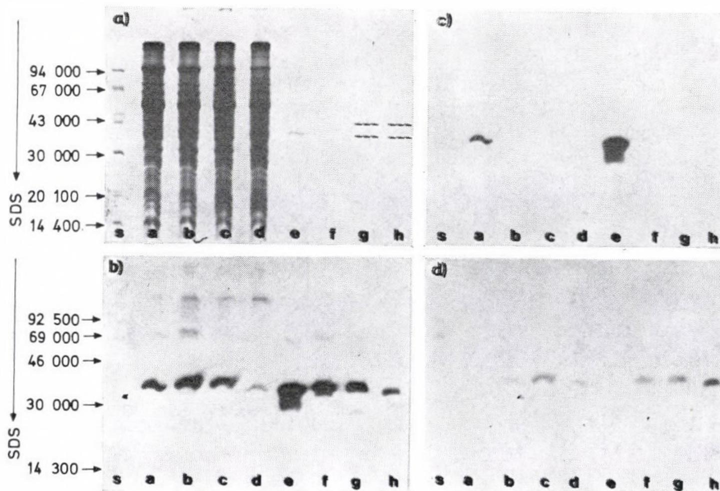

Fig. 2. Coomassie blue-stained gel pattern (a) and autoradiographs (b, c, d) of proteins from sweetcorn infected with JG (a), SC (b), BC (c) and Sabi (d) strains of sugarcane mosaic virus, and of purified preparations (0.1 mg/ml) of JG (e), SC (f), BC (g) and Sabi (h) after separation on SDS-PAGE. 10 μl of each virus sample was loaded per slot of the gels. After electrophoresis the proteins on duplicate gels were either stained with Coomassie blue or transferred to aminophenylthioether paper. The paper was then probed with antisera and ^{123}I-labelled protein A. b) The paper probed with a mixture of antisera to the four strains. c) The paper reprobed with antiserum to JG strain. d) The paper reprobed with antiserum to Sabi strain. s) in a) is Pharmacia standards, and in b, c and d) it is ^{14}C Amersham standards.

strains gave an autoradiograph pattern as shown in Fig. 2D, thus demonstrating that these three strains are related to each other and are distinct serologically from the JG strain.

Discussion

The present results confirm our previous findings that EBRIA is a highly sensitive technique (O'Donnell et al., 1982) and demonstrate that it is capable of detecting plant viruses at amounts as low as 0.5 ng. The APT paper was found more efficient than the nitrocellulose sheet, even though the latter has been reported to have a much higher capacity for binding proteins (Towbin et al., 1979; Legocki and Verma, 1981). The lower efficiency of the nitrocellulose sheet with plant viruses may be related to its lower binding capacity for low molecular weight proteins. Furthermore, proteins transferred to nitrocellulose sheets can not be cleaned and reprobed satisfactorily (Burnette, 1981; I. J. O'Donnell, unpublished results). In contrast, the APT papers can be cleaned and reprobed with different antisera (Renart et al., 1979). We have satisfactorily probed one APT paper containing proteins 18 times.

EBRIA is very convenient for establishing serological relationships between strains of plant viruses as demonstrated here with the four SCMV strains. Protein of several strains or viruses can be transferred on one paper and the paper then sequentially probed with different antisera. The present EBRIA results with the four SCMV strains corresponded very well with the results of our recent investigation of these strains by Protein A-immunosorbent electron microscopy (Shukla and Gough, 1979; 1984).

EBRIA has proved to be very economical with respect to the amount of antiserum required for probing; 5 μl antiserum is sufficient to probe a paper of 12×14 cm containing 8 virus samples. Our results also suggest that even low-titred antisera can be used with success in EBRIA.

Acknowledgements

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Aggregationseigenschaften des Nelkenringflecken-Virus in vitro

Thomas Kühne, Eckhard Proll und Dieter Reichenbächer

Institut für Phytopathologie Aschersleben der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik
432 Aschersleben, Theodor-Roemer Weg 4, DDR

The influence of temperature, composition of medium and virus concentration on reversible aggregation of virions of carnation ringspot virus (CRSV) in vitro was investigated by measuring the transmission of CRSV-solutions at $\lambda = 400$ nm. In 0.01 M phosphate buffer, pH 5.0 at 5.0 mg CRSV/ml particle aggregation could already be observed at 35°C, in diluted samples (0.15 mg CRSV/ml) only at 71°C. At low virus concentration the aggregation was favoured by the presence of NaCl or KCl (1.0 M) as well as $MgCl_4$ (0.1 M) and was inhibited by sucrose (20% w/v). Under the same conditions the particles formed aggregates, which did not dissociate upon cooling to 4°C, if the buffer contained $EDTA-Na_2$ (0.01 M). Contrary to this observation the chelating agent did not markedly influence the reversible aggregation at 35°C and 5.0 mg CRSV/ml. Before and after heating the sedimentation coefficient and the buoyant density in CsCl of the CRSV-particles were determined. As a mean of the obtained results it was concluded *(i)* that the aggregation of the virions is mainly caused by hydrophobic interactions and *(ii)* that virions at high concentration (5.0 mg CRSV/ml) aggregate without altering their structure whereas there is a necessity for modifying the capsid to enable the CRSV-particles to aggregate at lower concentration.

Das Nelkenringflecken-Virus (carnation ringspot virus, CRSV) ist ein kleines isometrisches Pflanzenvirus der Dianthovirus-Gruppe (Matthews, 1981). Nach Tremaine u. a. (1976) können gereinigte, konzentrierte CRSV-Suspensionen (7,0 mg Virus/ml) einer temperaturabhängigen Trübung unterliegen. Als Ursache hierfür werden die Aggregation der Partikeln genannt. Die Autoren unterschieden and Hand dieser Eigenschaft drei Stämme des Virus. Der Stamm A lag bereits nach der Reindarstellung (pH 5,0) zum großen Teil in Form stabiler Aggregationsprodukte aus 12, 23, 34, 45 oder 56 Partikeln vor. Im Unterschied dazu enthielten die Suspensionen der Stämme R und N im gleichen Medium bei 4 °C nur monomere Virusteilchen. Letztere lagerten sich bei Temperaturerhöhung auf 25 °C bzw. 40 °C jedoch zu großen, nicht näher definierten Aggregationsprodukten zusammen, die eine sichtbare Trübung der Präparate hervorriefen. Wurde die Temperatur der Proben wieder auf 4 °C gesenkt, zerfielen die entstandenen Strukturen, und die Präparate wurden wieder klar.

Mit dem vorliegenden Beitrag sollen dieses spezifische Verhalten des CRSV eingehender charakterisiert und die Ursachen hierfür diskutiert werden.

Material und Methoden

Reindarstellung des CRSV

Das Untersuchungsobjekt war ein aus Nelkenpflanzen gewonnenes CRSV-Isolat der American Type Culture Collection, das auf Phaseolus vulgaris L. (‚Pinto') vermehrt wurde. Die Ernte der Primärblätter erfolgte 4 bis 6 Tage nach ihrer mechanischen Inokulation. 100 g Blattmaterial wurden mit 300 ml, 0,01 M Kalium-Natrium-Phosphatpuffer unter Zusatz von 0,001 M $MgSO_4$, pH 5,0 (Puffer I) und 100 ml Chloroform homogenisiert und anschließend niedertourig zentrifugiert. Die wäßrige Phase wurde mit 1%iger Trichloressigsäure auf pH 5,0 eingestellt und 1 h bei 4 °C inkubiert. Nach dem Abtrennen der unlöslichen Bestandteile durch niedertourige Zentrifugation wurde der klare Überstand ultrazentrifugiert (120 min, 80 000 · g), das Sediment nachfolgend in 40 ml Puffer I suspendiert und das Präparat durch niedertourige Zentrifugation geklärt. Daran schloß sich eine zweite Ultrazentrifugation des Überstandes (90 min, 110 000 · g) an. Das Sediment wurde in 12 ml Puffer I resuspendiert und über Nacht gegen das gleiche Lösungs- medium dialysiert. Der letzte Schritt bestand in einer Rohrzucker-Dichtegradien-tenzentrifugation des partiell gereinigten CRSV-Präparates (10–50% Rohrzucker Gew./Vol. in Puffer I, 180 min, 65 000 · *g* bei 5°C). Der Becherinhalt wurde mit Hilfe eines Durchflußphotometers Uvicord II (LKB Produkter, Schweden) fraktioniert und der Rohrzucker vom Virus durch Gelfiltration über Epidex B 2 abgetrennt. Das gereinigte CRSV wurde in Puffer I bei 4°C aufbewahrt. Um es in ein anderes Lösungsmedium zu überführen, wurde ein Teil eines solchen Viruspräparates mit dem jeweiligen Puffer auf die gewünschte Konzentration verdünnt und mindestens 18 h bei 4°C gegen den 50fachen Überschuß des gleichen Puffers dialysiert.

Die Konzentration des CRSV wurde spektrophotometrisch unter Verwendung seines dekadischen Extinktionskoeffizienten $E_{260\,nm}^{0,1\,\%} = 6.46\ cm^2/mg$ bestimmt (Kalmakoff u. Tremaine, 1967).

Photometrische Messungen

Die Transmission der CRSV-Suspensionen wurde bei einer Wellenlänge von $\lambda = 400$ nm mit Hilfe eines Spektralkolorimeters vom Typ Spekol mit temperierbarem Extinktionsmeßansatz EK 5 und Zusatzverstärker Spekol ZV (VEB Carl Zeiss Jena/DDR) gemessen. Nach Anschluß des Gerätes an ein Thermostatenbad konnte verfolgt werden, wie sich die Transmission der Viruspräparate bei gegebener Temperatur in der Zeit änderte. Zu diesem Zweck wurde zunächst die Lichtdurchlässigkeit bei Zimmertemperatur gemessen. Danach wurden die leeren Glasküvetten 20 min im Meßansatz temperiert und anschließend mit der Virus- bzw. Pufferlösung — beide waren zuvor 30 s im Wasserbad auf die Versuchstemperatur erwärmt worden — gefüllt. Die Küvetten wurden mit Glasdeckeln verschlossen. Der erste Transmissionswert (Zeitpunkt t = 0) wurde zwei

Minuten, nachdem die Probentemperierung im Wasserbad begonnen worden war, abgelesen. Die Messung wurde fortgesetzt, bis sich die eingetretene Trübung der Virussuspension nicht mehr änderte. Sodann wurde der Wassermantel des Meßansatzes entleert und die Transmission der Proben bei ihrer langsamen Abkühlung auf Zimmertemperature bzw. 4°C verfolgt.

Analytische Ultrazentrifugation

Für die analytische Ultrazentrifugation stand eine präparative Ultrazentrifuge des Modells L 5–75 mit pre.-UV-Scanner (λ = 278 nm) der Firma Beckman (USA) zur Verfügung. Die Sedimentationskoeffizienten wurden nach der graphischen Methode von ELIAS (1961) bestimmt und auf Normalbedingungen umgerechnet. Desweiteren wurde die Schwebedichte des CRSV in Caesiumchlorid ermittelt, wobei das Tomatenzwergbusch-Virus (tomato bushy stunt virus, TBSV)* mit $\rho 25_{CsCl}$ = 1,348 g/cm^3 (Mayo u. Jones, 1973) als Marker diente. Das Caesiumchlorid (Serva, BRD) war stets in Puffer I gelöst.

Immunelektrophorese

Versuche hierzu erfolgten nach der von Reichenbächer u. a. (1976) beschrebenen Methode. Die Versuchsbedingungen sind in der Legende zur Abbildung 1 angegeben.

Ergebnisse

Die Partikeln des CRSV tragen in Puffer I eine positive Gesamtladung, die durch Erhöhung des pH-Wertes auf pH 6,5 reduziert wird (Abb. 1).** Obwohl zwischen ihnen dadurch repulsive Kräfte wirken, können diese überwunden und Aggregationsprodukte gebildet werden. Hinweise dafür, daß auch die Virions dieses Isolates leicht aggregieren, wurden bereits im Verlaufe der Virusreindarstellung erhalten. Die Abbildung 2 zeigt eine Rohrzucker-Dichtegradiente mit mehreren lichtstreuenden Zonen und sein Fraktionierungsprofil. Das CRSV konnte serologisch und elektronenoptisch in der Hauptbande und allen Nebenbanden nachgewiesen werden. Das legt den Schluß nahe, daß letztere durch Aggregationsprodukte des Virus gebildet worden waren. Im weiteren sollte die Fähigkeit der Virions des CRSV zur Zusammenlagerung in vitro eingehender untersucht werden.

Es sei an dieser Stelle vorausgeschickt, daß die zur Anwendung gelangten Puffersysteme das Virus bei Zimmertemperatur nicht nachweisbar veränderten.

* Das TBSV war auf Celosia argentea L. vermehrt und nach der für das CRSV beschriebenen Methode gereinigt worden.

** Sedimentationskoeffizient und Durchmesser der Virions waren in beiden Medien gleich.

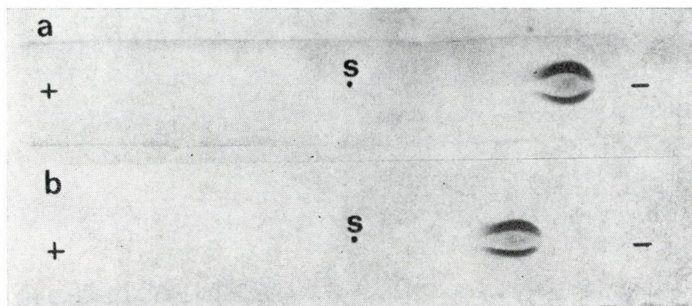

Abb. 1. Immunopherogramme des CRSV zum Nachweis der unterschiedlichen Wanderung der Viruspartikeln in Abhängigkeit vom pH-Wert des Lösungsmediums, S = Startpunkt der Probe, Elektrodenpuffer: 0,01 M Phosphatpuffer mit 0,001 M $MgSO_4$ a = pH 5,0; b = pH 6,5 Stromstärke: 10 mA/Palette, Spannung: 7,5 V/cm, Dauer: 2 h, Temperatur: 25°C

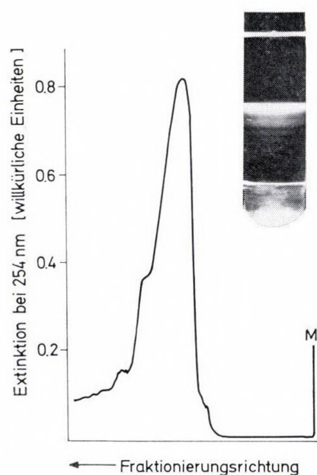

Abb. 2. Rohrzucker-Dichtegradient mit partiell gereinigtem CRSV, Fraktionierungsprofil bei 254 nm, Zentrifugationsbedingungen: 3 h, 65 000 · g, 5°C, SW 27-Rotor

Einfluß der Temperatur auf die Virusaggregation

Bei einer gegebenen Viruskonzentration (c = 0,21 mg/ml) aggregierten die Partikeln in Puffer I um so schneller, je höher die Versuchstemperatur war (Abb. 3). Dabei wurde in jeder Variante (56, 59, 63 bzw. 66°C) ein Transmissionswert (T') erreicht, der bei der entsprechenden Temperatur nicht mehr wesentlich unterschritten wurde. Er war bei 56°C am größten, bei 66°C am kleinsten und kann als Parameter für den Grad der Aggregation angesehen werden. Der Kurvenverlauf in Abbildung 3 deutet darauf hin, daß sich in der Suspension eine Art Gleichgewicht zwischen monomeren Viruspartikeln und polymeren Strukturen

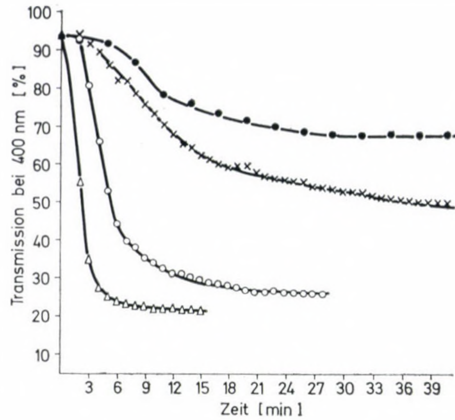

Abb. 3. Transmission einer CRSV-Suspension bei 400 nm in Abhängigkeit von der Zeit und der Versuchstemperatur, Schichtdicke: 1,0 cm, CRSV (0.21 mg/ml) in 0,01 M Phosphatpuffer mit 0,001 M $MgSO_4$ und 1,0 M NaCl, pH 5,0, . . . 56°C, x x x 59°C, 0 0 0 63°C, Δ Δ Δ 66°C

einstellt, das durch Temperaturerhöhung zugunsten der Aggregationsprodukte verschoben werden kann. Nach dem Abkühlen auf Zimmertemperatur wiesen die Proben wieder eine Lichtdurchlässigkeit von 94% auf.

Einfluß der Viruskonzentration

Wie aus Abbildung 4a hervorgeht, lagerten sich die Virions in Puffer I bei 71°C um so schneller zusammen, je höher ihre Konzentration war. Prämisse für diese Versuche war, daß die Transmission einer Virussuspension in gleichem Maße von der Länge des Lichtweges (d) und der Viruskonzentration (c) abhängt, so daß das Produkt d · c für alle Proben den gleichen Ausgangswert annahm. Die Transmissionswerte nach dem Abkühlen betrugen in der Probe mit der höchsten Konzentration 85%, in der mittleren Variante 80% und in der dritten 76%.

Bei einer CRSV-Konzentration von 5,0 mg/ml waren bereits 35°C ausreichend, um die Lichtdurchlässigkeit innerhalb weniger Minuten von 57%* auf ca. 0% absinken zu lassen. Auch hier verschwand die Trübung bei nachfolgender Abkühlung auf 4°C (T' = 53%).

Einfluß der Wasserstoffionenkonzentration

Die Wasserstoffionenkonzentration des Lösungsmediums beeinflußte das Aggregationsverhalten des CRSV sichtbar. So lagerten sich die Partikeln in 0,01 M Phosphatpuffer mit 0,001 M $MgSO_4$, pH 6,0 deutlich schneller zusammen

* Der geringe Transmissionswert resultiert aus der starken Opaleszenz von CRSV-Suspensionen in diesen Konzentrationsbereich.

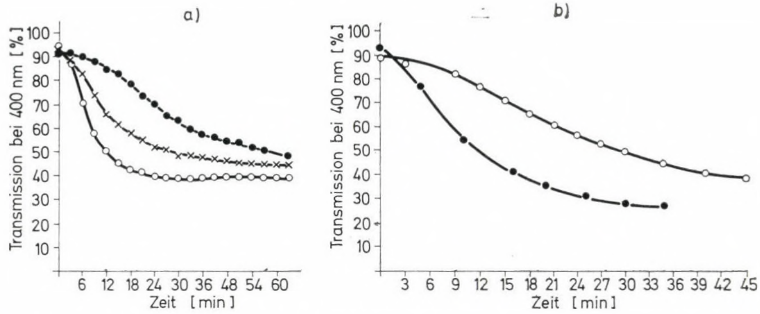

Abb. 4. Transmission einer CRSV-Suspension bei 400 nm und 71°C in Abhängigkeit von der Zeit bei verschiedenen pH-Werten und unterschiedlichen Viruskonzentrationen, a = CRSV in Puffer I . . . 0,07 mg/ml, Schichtdicke 3,0 cm, x x x 0,21 mg/ml, Schichtdicke 1,0 cm, 0 0 0 0,42 mg/ml, Schichtdicke: 0,5 cm, b = Schichtdicke 2,0 cm, CRSV in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$, 0 0 0 pH 5,0, . . . pH 6,0

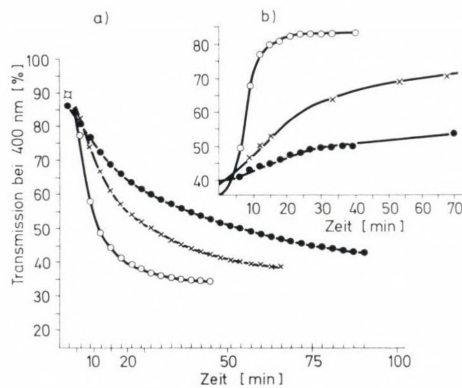

Abb. 5. Abnahme und Zunahme der Transmission einer CRSV-Suspension bei 400 nm in Abhängigkeit von der Zeit und der Viruskonzentration, CRSV in 0,01 M Phosphatpuffer mit 0.001 M $MgSO_4$, pH 6,0, . . . 0,07 mg/ml, Schichtdicke 3,0 cm, x x x 0,10 mg/ml, Schichtdicke 2,0 cm, 0 0 0 0,21 mg/ml, Schichtdicke 1,0 cm, a = konstante Temperatur 71°C, b = nachfolgende langsame Abkühlung der Präparate von 71 auf 25°C

als bei pH 5,0 (Abb 4b). Wiederum war eine Beziehung zwischen der Trübungsgeschwindigkeit und der Viruskonzentration offensichtlich (Abb. 5). Im Unterschied zur pH 5,0-Variante erreichte jedoch nur die Probe mit der höchsten CRSV-Konzentration nach dem Abkühlen ihre ursprüngliche Lichtdurchlässigkeit wieder zurück.

Einfluß der Ionenstärke

Wie schnell und in welchem Umfang sich die Virusteilchen zusammenlagerten, hing in starkem Maße auch von der Ionenstärke des Puffersystems ab. Aus der Abbildung 6 ist zu ersehen, daß eine geringe Neutralsalzzugabe (0,1 M

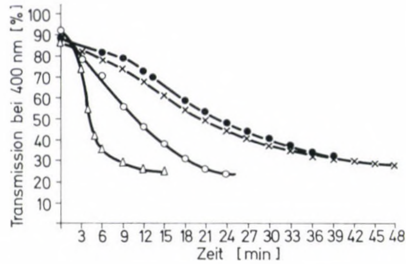

Abb. 6. Transmission einer CRSV-Suspension bei 400 nm und 71°C in Abhängigkeit von der Zeit und dem Lösungsmedium. Schichtdicke 2,0 cm, CRSV in 0,01 M Phosphatpuffer, pH 5,0, x x x ohne Zusätze, . . . mit 0,1 M NaCl, 0 0 0 mit 0,1 M $MgCl_2$ Δ Δ Δ mit 1,0 M NaCl (66°C)

NaCl) zu 0,01 M Phosphatpuffer pH 5,0 den Aggregationsprozeß nicht stimulierte. Wurde dagegen 1.0 M NaCl bzw. KCl eingesetzt, trat die Trübung der Virussuspension (c = 0,15 mg Virus/ml) bei 71°C so schnell ein, daß sie sich photometrisch nicht verfolgen ließ und daher bei 66°C gearbeitet werden mußte. Bei Anwesenheit von 0.1 M $MgCl_2$ lagerten sich die Virions ebenfalls schneller zusammen als im Phosphatpuffer ohne Zusätze.

Alle Präparate wurden während der nachfolgenden Abkühlung wieder klar.

Einfluß von Saccharose und Dinatriumdiamintetraacetat (EDTA-Na_2)

Enthielt der Puffer I 20% Rohrzucker, so änderte sich die Lichtdurchlässigkeit der CRSV-Präparate (c = 0,15 mg Virus/ml) bei 71°C nicht; erst bei 76°C war eine sehr langsame Abnahme zu beobachten (von T' = 91% auf 76% in 125 min). Bei pH 6,0 verlief der Prozeß wiederum beschleunigt (von T' = 92% auf 29% in 125 min). In beiden Fällen blieb die umgekehrte Reaktion jedoch aus, was die Vermutung nahe legt, daß sich die CRSV-Partikeln entweder irreversibel zusammengelagert hatten oder ein Teil von ihnen bereits degradiert war.

Eine irreversible Trübung der CRSV-Proben (c=0,15 mg Virus/ml) wurde bei 71°C auch dann beobachtet, wenn im Puffer I alle bivalenten Kationen durch EDTA-Na_2 (0,01 M) gebunden waren (von T' = 93% auf 35% in 60 min); etwa 50% der Viruspartikeln blieben unter den genannten Bedingungen in monomerer Form erhalten. Interessanterweise aggregierten die Virions in einer Konzentration von 5,0 mg/ml im gleichen Medium bei 35°C reversibel (von T' = 59% auf 3% in 50 min, Endwert bei 4°C betrug 59%).

Nachweis der Virusaggregation mit elektronenoptischen und hydrodynamischen Verfahren

Der sichere elektronenoptische Nachweis von Aggregationsprodukten der CRSV-Partikeln in vitro gelang nicht, da auch das unbehandelte Virus bei der elektronenmikroskopischen Präparation ausgedehnte Flächen unmittelbar anein-

anderliegender Virions bilden konnte. Infolge der Schrumpfung der Mikrotröpfchen während des Trocknungsprozesses auf dem Netzobjektträger konnten ähnliche Effekte selbst bei hochverdünnten DRSV-Präparaten beobachtet werden.

Der Sedimentationskoeffizient des auf 71°C erwärmten CRSV ($S_{20,w}$ = 127 bis 129 S) unterschied sich nicht signifikant von dem Wert, den wir für das native Virus ermittelt hatten ($S_{20,w}$ = 129,1 ± 1,8 S) Andererseits enthielten die temperaturbehandelten Präparate in der Regel zusätzliche Komponenten, die in den unbehandelten CRSV-Proben fehlten. So sind in der Abbildung 7 neben der monomeren Virusfraktion mit $S_{20,w}$ = 128 S noch zwei Fraktionen mit $S_{20\text{-}w}$ = = 185 S bzw. 227 S zu erkennen. Ihr Anteil an der Gesamtabsorption der Probe war unterschiedlich und hing u. a. vom Lösungsmedium ab, in dem die Virusaggregation stattgefunden hatte. Die 227 S-Komponente erschien nur selten so deutlich. Gewöhnlich war ihre Konzentration für die Ausbildung einer Absorptionsbande (boundary) unzureichend. Nach 48stündiger Inkubation der Virusprobe bei 4°C hatte sich ihr Sedimentationsverhalten nicht verändert; bei Verdünnung des Systems blieb der prozentuale Anteil der drei Fraktionen ebenfalls konstant. Das berechtigt zu dem Schluß, daß es sich hierbei um relativ stabile Aggregationsprodukte des CRSV handelt; sie beeinflussen die Lichttransmission der Suspension nicht wesentlich und müssen deshalb deutlich kleiner sein als die polymeren Strukturen, die sich bei 71°C bilden. Die Division ihrer Sedimentationskoeffizienten durch den S-Wert des monomeren CRSV ergab durchschnittliche Quotienten von 1,5 bzw. 1,8, die mit den aus der Theorie abgeleiteten Größen 1,52 für Dimere bzw. 1,87 für Trimere aus kugelförmigen Teilchen übereinstimmen (Tremaine u. a., 1976) und durch die Werte für Aggregationsprodukte anderer isometrischer Pflanzenviren bestätigt werden (Bradish u. Crawford, 1960; Markham, 1962; Kassanis u. Woods, 1968; Kassanis u. Lebeurier, 1969; Kassanis u. a., 1973; Piazzolla u. a., 1977a; Verduin, 1978).

Die Schwebedichte von temperaturbehandelten CRSV-Partikeln sollte weiteren Aufschluß darüber geben, ob und in welchem Umfang ihre reversible Aggregation mit einer Veränderung ihrer Struktur einherging. Zu diesem Zwecke wurden die CRSV-Präparate mit 0,15 mg Virus/ml bzw. 5,0 mg Virus/ml auf

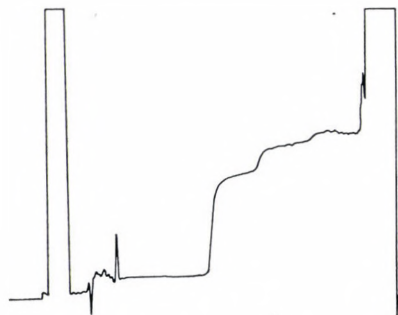

Abb. 7. Sedimentationsbild des CRSV in 0,1 M NaCl-Lösung bei pH 5,0 nach 40 min Inkubation bei 76°C, Drehzahl: 24 700 U/min, Sedimentationsrichtung: →

Tabelle 1

Schwebedichten ($\rho\ 25_{CsCl}$ des CRSV nach reversibler Aggregation bei unterschiedlichen Bedingungen

Aggregationsbedingungen			Schwebedichte ρ^{25}_{CsCl} g/cm³
Viruskonzentration mg/ml	Lösungsmedium	Temperatur °C	
5,0	Puffer I*	35	1,358**
5,0	Puffer I mit 0,01 M EDTA—Na_2	35	1,358**
0,15	Puffer I mit 1,0 M NaCl	71	1,357**
0,15	Puffer I	71	1,348***

* Puffer I entspricht 0,01 M Phosphatpuffer mit 0,001 M $MgSO_4$, pH 5,0
** 2 Werte
*** 6 Werte

eine Konzentration von 0,03 mg Virus/ml verdünnt, so daß nach dem Mischen mit der CsCl-Stammlösung ein Endwert von 0,015 mg Virus/ml erreicht wurde. Die Tabelle 1 faßt die Resultate zusammen.

Bezogen auf den von Mayo u. Jones (1973) ermittelten Dichtewert des TBSV ($\rho 25_{CsCl}$ = 1,348 g/cm³), wurde für das native CRSV eine mittlere Schwebe-

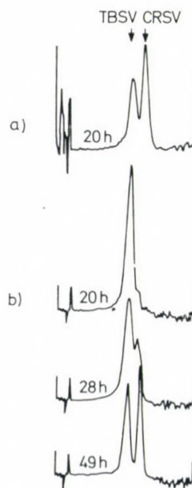

Abb. 8. Absorptionsbild nach Gleichgewichtszentrifugation im CsCl-Dichtegradienten bei 39 400 U/min und 25°C, Rotor An—A—P (Beckman), a = unbehandeltes CRSV in Puffer I nach 20 h Zentrifugation b = CRSV in Puffer I nach 1 h Inkubation bei 71°C und unterschiedlicher Zentrifugationsdauer

dichte von $\rho 25_{CsCl} = 1.3576 \pm 0,0005$ g/cm^3 bestimmt. Wie aus der Tabelle 1 hervorgeht, veränderte sich die Schwebedichte des CRSV in konzentrierten Präparaten infolge reversibler Aggregation in keinem der beiden Lösungsmedien. War hingegen der gleiche Prozeß in verdünnten Suspensionen bei 71°C abgelaufen, blieb der Dichtewert nur in einem Puffersystem mit hoher Ionenstärke konstant. Anderenfalls bildeten die CRSV-Partikeln nach 20stündiger Zentrifugation im CsCl-Gradienten mit dem TBSV eine gemeinsame Bande (Abb. 8); das obere Absorptionsbild (a) zeichnet die Position des unbehandelten Virus. Bemerkenswert ist die Tatsache, daß sich bei fortdauernder Zentrifugation die CRSV-Fraktion langsam von der TBSV-Bande trennte. Dieser Prozeß wurde durch kontinuierliche Bestrahlung des Zelleninhaltes mit dem UV-Licht des Scanners wesentlich beschleunigt. Er fand seinen Abschluß, nachdem die Partikeln einen Dichtebereich von 1,357–1,358 g/cm^3 erreicht hatten.

Diskussion

Mit Hilfe von Transmissionsmessungen und hydrodynamischen Verfahren konnte der Nachweis erbracht werden, daß sich die Partikeln des CRVS in vitro bei erhöhter Temperatur zusammenlagern können. Ergebnisse der Rocket-Immunelektrophorese bestätigten diesen Befund. Danach reduziert sich unter Bedingungen, die die Virusaggregation fördern, die Gesamtzahl der für die Antikörper verfügbaren antigenen Determinanten auf der Kapsidoberfläche (Reichenbächer u. a., 1981).

Die Fähigkeit der Virions zu aggregieren, wird durch die Struktur des Kapsidproteins bestimmt. In Anlehnung an Heukeshoven u. Dernick (1981) läßt sich aus der Aminosäurezusammensetzung des CRSV-Hüllproteins (Kalmakoff u. Tremaine, 1967) und der von Bull u. Breese (1974) veröffentlichten Skala der Hydrophobizität der Aminosäuren die sogenannte relative Hydrophobizität berechnen. Ihr Wert ist mit 1,23 größer als entsprechende Angaben für eine Reihe anderer Proteine (Übersicht in Heukeshoven u. Dernick, 1981). Dabei ist zu berücksichtigen, daß auf diese Weise nur die mittlere Hydrophobizität des gesamten Proteinmoleküls eingeschätzt werden kann. Mit großer Wahrscheinlichkeit existieren an der Oberfläche einer Proteinuntereinheit des CRSV Bereiche, die eine noch wesentlich höhere Hydrophobizität aufweisen, da die Primärstruktur von Proteinen im allgemeinen das »Verstecken« aller apolaren Aminosäureseitenketten im Molekülinneren verhindert (Klotz, 1970; Tanford, 1973; Ponnuswamy u. a., 1980; Kanehisa u. Tsong, 1980). Infolgedessen wäre die Neigung der CRSV-Partikeln erklärbar, sich dem Kontakt mit dem polaren Wasser zu entziehen und Aggregationsprodukte zu bilden.

Ähnliche Polymerisationsreaktionen sind am Beispiel des Hüllproteins (Doppeldiskus) verschiedener Stämme des Tabakmosaik-Virus (tobacco mosaic virus, TMV) ausführlich untersucht worden (Lauffer, 1975; Lauffer u. Shalaby, 1980; Shalaby u. Lauffer, 1980a, b).

Die Experimente am CRSV offenbarten die aus der Theorie zu erwartenden und am TMV-Protein bereits nachgewiesenen Effekte, wonach die Zusammenlagerung der Virions durch erhöhte Temperatur, Ionenstärke und Viruskonzentration stimuliert wird. Im Unterschied zum Kapsidprotein des TMV aggregierten die CRSV-Partikeln bei pH 6,0 in stärkerem Maße als bei pH 5,0, was u. a. auf deren verringerte positive Gesamtladung im weniger saueren Medium zurückgeführt werden kann.

Die Untersuchungen ergaben weiterhin Anhaltspunkte dafür, daß es sich bei der Trübung der CRSV-Suspension nicht in jedem Falle um eine Zusammenlagerung nativer Virions handeln kann. Darauf deutet z. B. die verringerte Schwebedichte der Partikeln nach ihrer reversiblen Aggregation in Puffer I bei 71°C (Variente 1). Der Dichtewert blieb vergleichsweise unverändert, wenn sich die Virusteilchen in konzentrierten Suspensionen bei 35°C zusammengelagert hatten (Variante 2). Beide Erscheinungen können folgendermaßen diskutiert werden:

Geht man davon aus, daß sich die Aggregationsreaktion in vereinfachter Form durch die Gleichung

$\Delta F = \Delta H - T \cdot \Delta S$ (1)
ΔF = Veränderung der freien Energie (kJ/mol)
ΔH = Veränderung der Enthalpie (kJ/mol)
ΔS = Veränderung der Entropie (kJ/mol · K)
T = absolute Temperatur des Systems (K)

beschreiben läßt (Lauffer, 1975), so ist ihr spontaner Ablauf nur unter der Bedingung $\Delta F < 0$ im System möglich. Andererseits ist bekannt, daß die Überführung einer unpolaren Verbindung (Kohlenwasserstoff) aus der wäßrigen in die unpolare Phase mit einer Enthalpieerhöhung ($\Delta H > 0$) einhergeht (Tanford, 1973). Demzufolge muß die Aggregation des CRSV mit einem großen Entropieeffekt ($\Delta S \gg 0$) verbunden sein, und die Gesamtzahl apolarer Oberflächenstrukturen im System kann als Maß für das Bestreben des Virus angesehen werden, bei einer gegebenen Temperatur polymere Strukturen zu bilden. In der Variante 2 sind daher schon 35°C ausreichend, um trotz repulsiver Kräfte $T \cdot \Delta S > \Delta H$ und somit $\Delta F < 0$ zu erfüllen. Unter den Bedingungen der Variante 1 verhindern die geringe Viruskonzentration (kleiner Entropiefaktor) und die abstoßenden Kräfte ein Zusammengehen der Teilchen auch bei $t \geq 35$°C; obwohl sich das Produkt $T \cdot \Delta S$ proportional mit steigender Temperatur verändert, wird $\Delta F < 0$ nicht erreicht. Nach Oakenfull u. Fenwick (1977) verstärken sich hydrophobe Wechselwirkungen nur bis zu einer Temperatur von ca. 55°C. Die CRSV-Suspension trübt sich bei einer Konzentration von 0,15 mg Virus/ml jedoch erst bei ca. 70°C, in einem Temperaturbereich also, in dem die meisten Proteine denaturieren. Es ist daher wahrscheinlich, daß sich auch im CRSV-Virion bestimmte Abschnitte der Proteinuntereinheiten lockern, ähnlich wie Piazzolla u. a. (1977b) für das Chicoree-Gelbscheckungs-Virus (chicory yellow mottle virus, ChYMV) nachwiesen. In einem solchen Prozeß könnten zusätzliche apolare Aminosäureseitengruppen freigelegt werden, die bislang im Molekülinneren lokalisiert waren. Der

potentielle positive Entropiebetrag (ΔS) im System würde sich erhöhen; die Voraussetzung für die Virusaggregation wäre gegeben. Wird der Temperaturfaktor nach erfolgter Reaktion verringert (Abkühlung) dissoziieren die polymeren Strukturen. Der native Zustand des Hüllproteins und damit der Virions wird zunächst jedoch nicht wieder erreicht. Außer einer veränderten Schwebedichte deuten folgende Befunde auf einen solchen Umstand hin:

– EDTA-Na_2 beeinflußte die reversible Aggregation in konzentrierten Viruspräparaten (5 mg Virus/ml) nicht, verursachte dagegen unter den Bedingungen der Variante 1 die Irreversibilität der Reaktion und die signifikant geringeren Sedimentationskoeffizienten der verbliebenen monomeren Viruspartikeln.
– Die Zusammenlagerung der Virions in Präparaten mit 0,15 mg Virus/ml wurde durch Saccharose deutlich gehemmt. Letztere erhöht nach Back u. a. (1979) die Wasserstruktur, verstärkt auf diese Weise vorhandene hydrophobe Wechselwirkungen und müßte demzufolge eine Umorientierung apolarer Aminosäureseitenketten aus der »organischen Phase« im Inneren der Proteinuntereinheit in die wäßrige Phase an der Kapsidoberfläche erschweren. Die erst bei 76°C eingetretene Trübung der CRSV-Suspensionen kann als Bestätigung dessen betrachtet werden. Andererseits ist zu erwarten, daß die infolge der Temperaturbehandlung zwischen den Partikeln entstandenen hydrophoben Wechselwirkungen auch unterhalb 76°C in hohem Maße erhalten bleiben; die beobachtete Irreversibilität des Prozesses in saccharosehaltigem Puffer steht damit in gutem Einklang. (An dieser Stelle sei auch an die Stabilität der Virusaggregate im Rohrzucker-Dichtegradienten erinnert.)
– Bei pH 6,0 und 71°C lagerten sich die Partikeln schneller und weniger reversibel zusammen als bei pH 5,0. Als Ursache hierfür ist möglicherweise eine Lockerung der CRSV-Struktur anzusehen, auf die in einer späteren Publikation eingegangen wird. Zwar wurden morphologische Veränderungen der Virions erst bei pH 7,0 nachgewiesen (Kühne u. Eisbein); sie könnten jedoch durch erhöhte Temperatur auch bei einem geringeren pH-Wert induziert werden. Ein ähnliches Verhalten ist für das Trespenmosaik-Virus (brome mosaic virus, BMV) geschildert (Incardona u. a., 1973). Man kann mit einiger Sicherheit annehmen, daß in einer gelockerten CRSV-Struktur mehr apolare Bereiche des Hüllproteins wasserexponiert sind als im nativen Virus. Somit wäre neben der verringerten Oberflächenladung ein weiterer die Aggregation stimulierender Faktor vorhanden.

Die Abbildung 5 stützt unseres Erachtens die Hypothese, daß es von der Anzahl oberflächenlokalisierter hydrophober Seitenketten abhängt, in welchem Umfang die Konformation des Hüllproteins modifiziert werden muß, um eine Aggregation der CRSV-Partikeln zu ermöglichen. Während bei einer Konzentration von 0,21 mg/ml offenbar nur geringfügige Strukturänderungen erforderlich

sind und der Prozeß daher ebenso reversibel verläuft wie bei pH 5,0, scheint die Aggregation der Partikeln bei einer Konzentration von 0,07 mg/ml in großem Umfang über Wechselwirkungen zwischen freigelegten Aminosäuregruppen realisiert zu werden. Bei pH 5,0 waren die Transmissionswerte nach dem Abkühlen der Präparate in ähnlicher Weise – wenn auch absolut gesehen geringer – abgestuft.

Auf der Grundlage der vorgestellten Resultate läßt sich weiterhin einschätzen, daß das von uns untersuchte Virusisolat in seinen Aggregationseigenschaften den von Tremaine u. a. (1976) beschriebenen CRSV-N und CRSV-R näher steht als dem bei Zimmertemperatur irreversibel aggregierendem Stamm CRSV-A. Es ist davon auszugehen, daß die Autoren wahrscheinlich nur das Zusammengehen strukturell nicht modifizierter Partikeln beobachteten (CRSV-Konzentrationen von 7 mg/ml und höher bei Temperaturen von maximal 40°C deuten darauf hin).

Abschließend sei erwähnt, daß eine temperaturinduzierte, reversible Trübung der Präparate auch im Verlaufe der Reindarstellung des blueberry shoestring virus beobachtet wurde (Ramsdell, 1979).

Zusammenfassung

Mit Hilfe von Transmissionsmessungen ($\lambda = 400$ nm) wurde untersucht, welchen Einfluß Temperatur, Lösungsmedium und Viruskonzentration auf die reversible Aggregation der Virions des carnation ringspot virus (CRSV) in vitro ausüben. In 0,01 M Phosphatpuffer, pH 5,0 mit 5 mg CRSV/ml konnte die Aggregation bereits bei 35°C beobachtet werden, in verdünnten CRSV-Suspensionen (0,15 mg/ml!) erst bei 71°C. Natrium- oder Kalciumchlorid (1,0 M) sowie Magnesiumchlorid (0,15 M) stimulierten den Prozeß; in Gegenwart von Saccharose (20%) aggregierten die Partikeln (0,15 mg CRSV/ml) bei 71°C nicht. Durch Zusatz von EDTA$-$Na$_2$ lagerten sich die Virions bei 71°C und 0,15 mg CRSV/ml irreversibel, bei 35°C und 5 mg CRSV/ml hingegen reversibel zusammen. Die Viruspartikeln wurden vor und nach der Temperaturbehandlung durch hydrodynamische Untersuchungen (Sedimentationskoeffizient, Schwebedichte in Caesiumchlorid) charakterisiert. Aus den Befunden wird gefolgert, daß (1) die Aggregation der Virions des CRSV vorrangig durch hydrophobe Wechselwirkungen bedingt ist und daß (2) dieser Prozeß in Abhängigkeit von den äußeren Bedingungen mit Veränderungen der Virusstruktur verbunden sein kann.

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Untersuchungen zur Stabilität des Nelkenringflecken-Virus

Thomas Kühne und Klaus Eisbein

Institut für Phytopathologie Aschersleben der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik 432 Aschersleben, Theodor-Roemer-Weg 4 DDR

The stability of virions of carnation ringspot virus (CRSV) in 0,01 M phosphate buffer was analysed in the range of pH 5,0–8,0 by means of analytical ultracentrifugation and electron microscopy. It was shown that at pH 5,0 NaCl (0,1 M) or EDTA-Na_2 (0,01 M) do not influence on sedimentation coefficient ($S_{20,w}$ = 129 S), buoyant density (ρ^{25} = 1.358 g/cm^3) and diameter (d = 31 nm) of the particles. Between pH 7,0 and pH 7,5 the structure of the virus capsid becomes disturbed what is indicated by particle swelling in the presence of NaCl ($S_{20,w}$ = 117 S, d = 35 nm) and their partially disassembling in the presence of EDTA—Na_2. Evidence is given that pH-depending interactions between protein subunits and interactions with participation of divalent cations are the major forces in stabilizing the architecture of CRSV-particles.

Das Nelkenringflecken-Virus (carnation ringspot virus, CRSV) ist ein kleines isometrisches Pflanzenvirus der Dianthovirus-Gruppe (Matthews, 1981). Es wurde von Kassanis (1955) erstmalig aus Nelkenpflanzen isoliert. Sein Genom ist geteilt; es setzt sich aus zwei Hauptkomponenten (ssRNS 1–1,5 · 10^6, ssRNS 2–0,5 · 10^6) und einer nur in sehr geringer Konzentration vorhandenen ssRNS 3 (0,6 · 10^6) zusammen (Tremaine et al., 1975, Henriques and Morris, 1979). Die Virushülle besteht aus 180 identischen Proteinuntereinheiten mit einer Molekülmasse von ca. 38 000 (Kalmakoff and Tremaine, 1967). Die nativen CRSV-Partikeln bilden bei der isopyknischen Zentrifugation in Caesiumchlorid (CsCl) eine Bande bei einer Dichte von ρ^{25} = 1,358 g/cm^3 (Kühne et al.). Sie sedimentieren in schwach saurem Medium einheitlich mit $S_{20,w}$ = 129 S (Tremaine and Ronald, 1976, Kühne et al.). Ihre Sedimentationsgeschwindigkeit ist pH-abhängig; sie kann im Neutralbereich durch Zugabe von NaCl oder EDTA-Na_2 zum Viruspräparat verringert werden (Tremaine and Ronald, 1976).

Untersuchung zur Stabilität phytopathogener Viren in vitro sollen Aufschluß darüber geben, welche Arten von Bindungen Proteinuntereinheiten und Nukleinsäure im Virion zusammenhalten. Solche Informationen helfen z. B., die Reinigung und Aufbewahrung von Viren zu optimieren, Virusarten oder -stämme miteinander zu vergleichen (Kaper, 1973) oder Rückschlüsse auf molekulare Abläufe im Infektionsprozeß zu ziehen (Durham, 1978). Unsere Experimente waren darauf gerichtet, die Wirkung von Neutralsalzen auf die Virions des CRSV im Bereich pH 5,0 bis pH 8,0 eingehender zu untersuchen und die Bedeutung bivalenter Kationen für die Stabilisierung der Partikeln einzuschätzen.

Material und Methoden

Virusvermehrung und -reindarstellung

Die Untersuchungen erfolgten an einem Nelkenisolat des CRSV der American Type Culture Collection. Das mechanisch übertragbare Virus wurde auf Phaseolus vulgaris L. ‚Pinto' erhalten und vermehrt und nach einer von Kühne et al. beschriebenen Methode gereinigt. Als Lösungsmedium diente 0,01 M K-Na-Phosphatpuffer mit 0,001 M $MgSO_4$, pH 5,0 (Puffer I). Um diesen Puffer I gegen ein anderes Medium auszutauschen, wurde das Ausgangspräparat (c = 1 mg Virus/ml) mit dem jeweiligen Puffer auf die gewünschte Konzentration ($c \leq 0{,}2$ mg Virus/ml) verdünnt und mindestens 18 h bei 4°C gegen den 50fachen Überschuß des gleichen Puffers dialysiert. Sollten derartige Proben wieder in das Puffersystem I überführt werden, erfolgte eine Dialyse (48 h) bei mehrmaligem Wechsel des Dialysepuffers.

Analytische Ultrazentrifugation

Die Sedimentationsversuche wurden in einer präparativen Ultrazentrifuge des Modells L 5–75 mit prep. UV-Scanner (λ = 278 nm) (Beckman/USA) unter Verwendung von Doppelsektorzellen (12 mm Schichtdicke) durchgeführt. Die Sedimentationskoeffizienten errechneten wir nach der Methode von Elias (1961), wobei wir den Anstieg der erhaltenen Geraden mit der Methode der kleinsten Quadratsummen optimierten. Die Viskosität der Lösungen bestimmten wir mit einem Schnellviskosimeter K 4 nach Seide-Deckert (Firma Deckert/DDR). Der Wert des partiellen spezifischen Volumens wurde mit $\bar{v} = 0{,}693$ cm^3/g (Kalmakoff and Tremaine, 1967) angenommen.*

Bei der Bestimmung der Schwebedichte in CsCl diente das Tomatenzwergbusch-Virus (tomato bushy stunt virus, TBSV) als Marker ($\lambda^{25} = 1.348$ g/cm^3, Mayo and Jones, 1973). Um einer Dissoziation der CRSV-Partikeln durch das Schwermetallsalz vorzubeugen, wurden die Präparate vor der Zentrifugation im Verlaufe von 24 h bei Zimmertemperatur mit Formaldehyd (2%) fixiert. Anschließend wurden sie mit Puffer I verdünnt und mit einer Lösung von Caesiumchlorid (Serva/BRD) in Puffer I versetzt, so daß die CsCl-Ausgangskonzentration 475 bis 485 mg/ml betrug.

Elektronenmikroskopie

Alle CRSV-Proben wurden vor der Präparation mit Formaldehyd (2%) fixiert. Die Negativkontrastierung nach Brenner und Horne (1959) erfolgte mit Natriumwolframat (4%), die Auswertung der Präparate mit einem Elektronenmikroskop vom Typ JEM 100 B (Jeol/Japan).

* Es ist ein theoretischer, summarischer Wert, so daß Ungenauigkeiten der ermittelten Sedimentationskoeffizienten zwangsläufig sind. Versuche zur experimentellen Bestimmung von $\bar{v}$ schlugen fehl, da die Viruspartikeln in konzentrierten Suspensionen (c = 6 mg/ml) bereits bei 20°C in erheblichem Maße aggregiert waren.

Ergebnisse

Hydrodynamische Eigenschaften

Für das native CRSV wurde ein mittlerer Sedimentationskoeffizient von 129,1 ± 1,8 S und eine mittlere Schwebedichte in CsCl von 1,3576 ± 0.0005 g/cm³ bestimmt. Um den Einfluß des Lösungsmediums auf die Struktur der Partikeln zu verdeutlichen, wurden beide Kenngrößen nach Dialyse der Proben gegen folgende 3 Puffersysteme ermittelt:

A – 0,01 M K-Na-Phosphatpuffer + 0,001 M $MgSO_4$
B – 0,01 M K-Na-Phosphatpuffer + 0,001 M $MgSO_4$ + 0,1 M NaCl
C – 0,01 M K-Na-Phosphatpuffer + 0,01 M EDTA-Na_2

Jedes System wurde auf die pH-Werte 5,0; 7,0; 7,5 und 8.0 eingestellt. In den Abbildungen 1, 2 und 3 sind an ausgewählten Beispielen das Sedimentationsverhalten und die Position der CRSV-Bande im CsCl-Gradienten wiedergegeben. Die Tabelle 1 faßt die Resultate zusammen. Danach wurden die Sedimentationseigenschaften der Partikeln im Puffersystem A nicht wesentlich von der Wasserstoffionenkonzentration beeinflußt. Die Schwebedichte wurde hingegen mit steigendem pH-Wert deutlich größer (Abb. 1). Im Puffersystem B blieb der S-Wert bei pH 5,0 und 7,0 unverändert; im schwach basischen Medium (pH 7,5 und 8,0) war er dagegen mit $S_{20,w}$ = 117 S deutlich geringer. Trotz der veränderten Sedimentationseigenschaften erschien das Viruspräparat bemer-

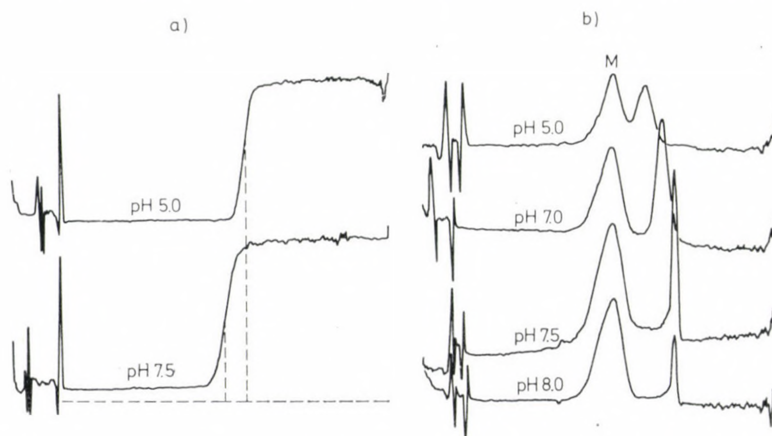

Abb. 1. Veränderung des Sedimentationsverhaltens des CRSV in Abhängigkeit vom Lösungsmedium
a – Sedimentationsbild des CRSV in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$, Drehzahl: 20 100 U/min, Sedimentationszeit: 20 min, Sedimentationsrichtung →.
b – Absorptionsbild nach Gleichgewichtszentrifugation im CsCl-Dichtegradienten in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$, pH 5,0 bei 25°C und 39 300 U/min, CRSV nach Inkubation in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$ mit unterschiedlichen pH-Werten, Viren mit 2% Formaldehyd fixiert, M – Marker (TBSV)

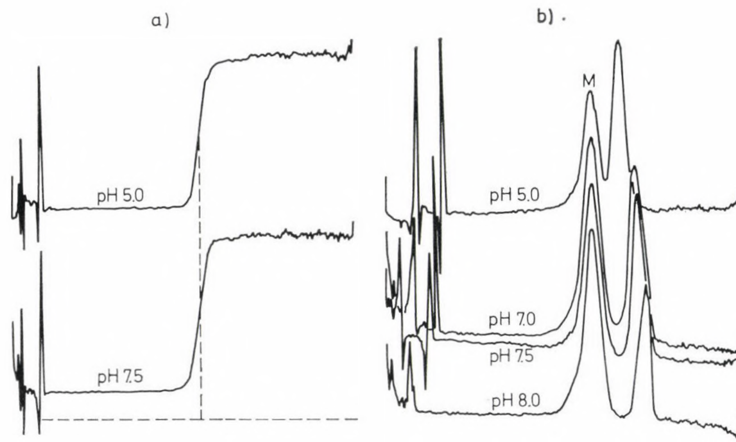

Abb. 2. Veränderung des Sedimentationsverhaltens des CRSV in Abhängigkeit vom Lösungsmedium

a — Sedimentationsbild des CRSV in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$ + 0,1 M NaCl, Drehzahl: 18 600 U/min,Sedimentationszeit: 35 min, Sedimentationsrichtung →
b — Absorptionsbild nach Gleichgewichtszentrifugation im CsCl-Dichtegradienten in 001 M Phosphatpuffer + 0,001 M $MgSO_4$, pH 5.0 bei 25°C und 39 100 U/min, CRSV nach Inkubation in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$ + 0,1 M NaCl mit unterschiedlichen pH-Werten, Viren mit 2% Formaldehyd fixiert, M — Marker (TBSV)

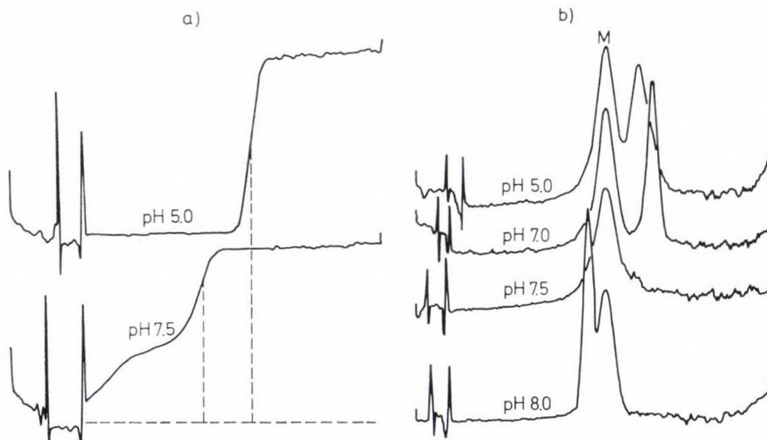

Abb. 3. Veränderung des Sedimentationsverhaltens des CRSV in Abhängigkeit vom Lösungsmedium

a — Sedimentationsbild des CRSV in 0.01 M Phosphatpuffer + 0,01 M EDTA, Drehzahl: 17 300 U/min, Sedimentationszeit: 35 min, Sedimentationsrichtung →
P — Absorptionsbild nach Gleichgewichtszentrifugation im CsCl-Dichtegradienten in 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$, pH 5,0 bei 25°C und 39 000 U/min, CRSV nach Inkubation in 0,01 M Phosphatpuffer + 0,01 M EDTA mit unterschiedlichen pH-Werten, Viren mit 2% Formaldehyd fixiert, M — Marker (TBSV)

kenswert homogen, was durch den steilen Anstieg der Absorptionsbande (boundary) in der Abbildung 2 a belegt wird. Bei der Gleichgewichtszentrifugation im CsCl-Gradienten registrierten wir wiederum eine pH-abhängige Verlagerung der Partikeln in dichtere Bereiche. Ungeklärt bleibt jedoch die Tatsache, daß die Bande bei pH 8,0 stets nur von einem Teil der eingesetzten CRSV-Menge gebildet wurde (Abb. 2b). Was das Virus im Puffersystem C suspendiert, so reagierten die Partikeln auf den Übergang von pH 7,0 auf pH 7,5 noch wesentlich empfindlicher als im System B. Die Abbildung 3a zeigt das Sedimentationsbild eines heterogenen Präparates, dessen größte Komponenten mit einem mittleren Koeffizienten von 95 bis 100 S sedimentierten. Die Schwebedichte der Partikeln bei pH 5,0 war wiederum unverändert. Wie auch in Gegenwart zweiwertiger Kationen hatte sich die CRSV-Bande bei pH 7,0 von der TBSV-Bande etwas entfernt. Die heterogen sedimentierenden Zustandsformen (pH 7.5 und pH 8.0) waren dagegen im Dichtebereich des Markers bzw. noch näher am Meniskus lokalisiert (Abb. 3b).

Im folgenden wurden die Präparate aus den 12 angeführten Lösungsmedien gegen Puffer I rückdialysiert. Durch Bestimmung ihrer Sedimentationskoeffizienten wollten wir erste Hinweise erhalten, in welchem Maße die Veränderungen in den hydrodynamischen Eigenschaften reversibel waren. Dabei zeigte sich, daß alle Präparate, die vor der Rückdialyse S-Werte von $S_{20,w} \geq 128$ S aufwiesen, auch danach vom nativen Virus nicht zu unterscheiden waren. Die CRSV-Suspensionen im Puffersystem C (pH 7,5 und pH 8,0) waren nach der Rückdialyse stets trübe und in ihrer Zusammensetzung sehr heterogen. Sie enthielten überwiegend

Tabelle 1

Sedimentationskoeffizienten und Schwebedichten in CsCl des CRSV in verschiedenen Puffersystemen

Puffersystem*	Hydrodyn. Parameter	pH-Wert			
		5,0	7,0	7,5	8,0
A	$S_{20,w}$ (S)	129	130	131	129
	ρ_{25}(g/cm³)	1,358	1,361	1,362	1,363–1,364
B	$S_{20,w}$ (S)	128	129	117	117
	ρ_{25}(g/cm³)	1,358	1,363	1,366	1,367
C	$S_{20,w}$ (S)	129	129	97	97
	ρ_{25}(g/cm³)	1,360	1360	~ 1,348	~1,342

* Angaben zur Zusammensetzung im Text

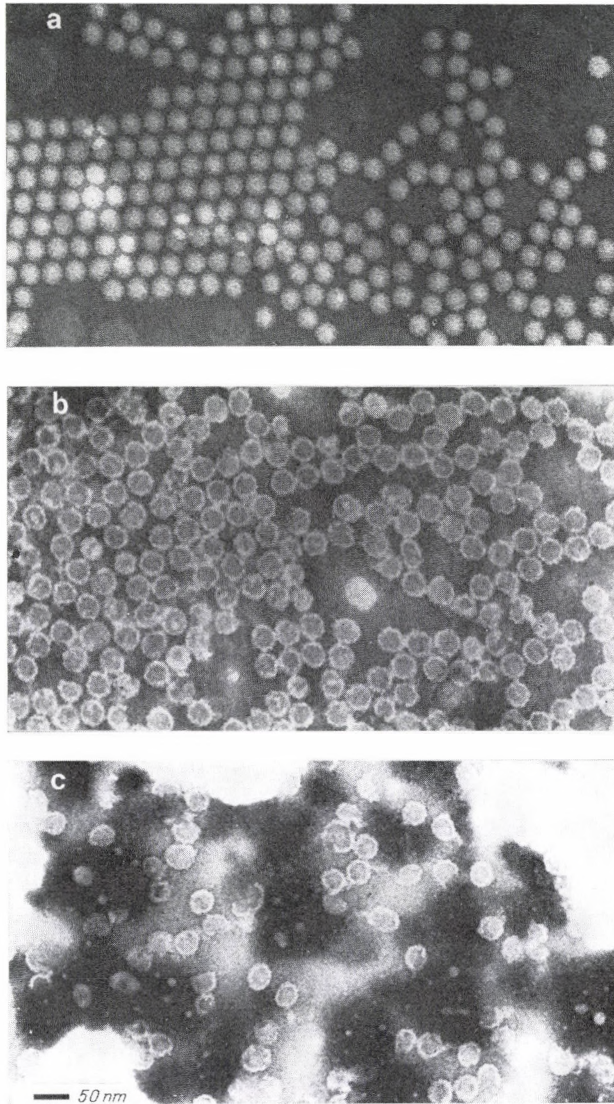

Abb. 4. Einfluß des Lösungsmediums auf das elektronenoptische Erscheinungsbild des CRSV
a — 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$, pH 5,0
b — 0,01 M Phosphatpuffer + 0,001 M $MgSO_4$ + 0,1 M NaCl, pH 7,5
c — 0,01 M Phosphatpuffer + 0,01 M EDTA, pH 7,5, Präparate fixiert mit Formaldehyd (2%) und kontrastiert mit Natriumwolframat (4%), Vergrößerung: 140 000 : 1

hochmolekulare Bestandteile, die bereits bei einer Drehzahl von 10 000 U/min sehr schnell sedimentierten. Die 117-S-Partikeln gingen in Puffer I in eine schneller sedimentierende Form über ($S_{20,w}$ = 123 bis 126 S), ohne ihren ursprünglichen S-Wert wieder zu erreichen.

Elektronenmikroskopie

Die elektronenoptischen Befunde stützen die Ergebnisse der hydrodynamischen Versuche. So waren in Präparaten des nativen CRSV in Form und Größe einheitliche isometrische Partikeln mit einem Durchmesser von 31 nm zu erkennen (Abb. 4a). Im Puffersystem B bei pH 7,5 und pH 8,0 erschienen die Teilchen deutlich verändert, waren jedoch noch relativ einheitlich (Abb. 4b). Letzteres steht im Einklang mit dem steilen Anstieg der Absorptionsbande im Sedimentationsgeschwindigkeitslauf der 117-S-Form. Ihr mittlerer Durchmesser betrug 35 nm; alle Partikeln waren im Unterschied zum nativen Virus vom Kontrastierungsmittel durchdrungen. Die CRSV-Präparate im Puffersystem C bei pH 7,5 und pH 8,0 erschienen im Elektronenmikroskop ebenso heterogen wie bei der Sedimentation in der analytischen Ultrazentrifuge. Neben vielen beschädigten oder zerfallenen Teilchen waren auch unregelmäßige, »aufgeblähte« Formen mit einem maximalen Durchmesser von 42 nm zu erkennen (Abb. 4c).

Diskussion

Die Untersuchungsergebnisse zeigen, daß NaCl (0,1 M) und EDTA-Na_2 (0,01 M) die Stabilität der CRSV-Partikeln im pH-Intervall 5,0 bis 8,0 wesentlich beeinflussen können. In Lösungen geringer Ionenstärke (Puffersystem A) waren der Sedimentationskoeffizient und der Teilchendurchmesser über den gesamten pH-Bereich unverändert, jedoch nahm die Schwebedichte der Partikeln in CsCl mit steigendem pH-Wert deutlich zu.

In NaCl-haltigem Puffer (System B) verringerte sich der S-Wert zwischen pH 7,0 und pH 7,5 sprunghaft von 129 S auf 117 S; gleichzeitig wurde ein Anschwellen der Partikeln von 31 nm auf 35 nm beobachtet. Auch erhöhte sich ihre Schwebedichte mit steigendem pH-Wert. Die Tatsache, daß sie in geschwollenem Zustand Kontrastierungsmittel aufgenommen hatten, läßt eingedrungene Cl^-- oder Cs^+-Ionen als Ursache für die größere Dichte vermuten. Rowlands et al. (1971), Matthews (1974) und Gingery (1976) diskutieren Schwebedichtedifferenzen eines Viruspräparates in unterschiedlichen Puffern auf gleiche Weise. Möglicherweise sind die höheren ρ-Werte der CRSV-Partikeln im Puffersystem A bei pH $\geq$ 7 ebenfalls durch einen solchen Vorgang bedingt. Voraussetzung dafür wären Veränderungen in der Virusstruktur, die durch den Übergang von pH 5,0 auf pH 7,0; 7,5 und 8.0 induziert werden und den Ionen das Eindringen ermöglichen; S-Wert und elektronenoptisches Erscheinungsbild der Partikeln werden aber nicht nachweisbar beeinflußt. Gleiches gilt für das CRSV im Puffersystem C bei pH 7,0.

Im schwach basischen Bereich wurden die Virusteilchen durch EDTA-Na_2 sehr stark destabilisiert. Es fällt auch hier die sprunghafte Veränderung zwischen pH 7,0 und pH 7,5 auf, während die weitere Erhöhung der pH-Wertes vur noch geringe Auswirkung auf die Partikeln hatte. Die kleineren Dichtewerte im System C können noch nicht erklärt werden. Es wäre jedoch denkbar, daß die Bande durch Partikeln gebildet wurde, die bereits einen Teil ihrer Nukleinsäure verloren hatten.

Wie unsere Befunde belegen, sind die Virions des CRSV im schwach sauren Medium (pH 5,0) überaus stabil. Selbst bei gleichzeitiger Einwirkung von NaCl (1,0 M) und EDTA-Na_2 (0,01 M) blieben die hydrodynamischen Eigenschaften der Partikeln unverändert (Kühne, unveröffentlicht). Mit Erhöhung des pH-Wertes des niedermolaren Puffers wird die Virusstruktur leicht gelockert und kann bei pH 7,5 durch Neutralsalze (0,1 M NaCl) oder Chelatbildner (0,01 M EDTA-Na_2) weiter destabilisiert werden. Auf ähnliche Weise reagiert eine ganze Reihe isometrischer RNS-haltiger Pflanzenviren, von denen Bromoviren (Bancroft, 1970, Adolph, 1975, Durham et al., 1977, Pfeiffer, 1980) und Sobemoviren (Wells and Sisler, 1969, Sehgal and Hsu, 1976, Hull, 1977) hinsichtlich ihrer Stabilität besonders intensiv untersucht wurden. Es gilt heute als gesichert, daß die Partikeln dieser Viren durch ein Kräftespektrum zusammengehalten werden, dessen Hauptkomponenten pH-abhängige Bindungen zwischen ihren Proteinuntereinheiten, salzlabile Ionenbindungen zwischen den Untereinheiten und der RNS sowie Wechselwirkungen unter Beteiligung zweiwertiger Kationen darstellen. Die vorliegenden Ergebnisse deuten auf ähnliche Verhältnisse beim CRSV hin. So existieren im leicht sauren Medium offensichtlich feste Bindungen zwischen den Untereinheiten der Proteinhülle, die die Integrität der Partikeln bei Einwirkung von NaCl und EDTA-Na_2 garantieren. Im Neutralbereich werden diese Bindungen gelockert oder gelöst, so daß das Neutralsalz vorhandene Ionenbindungen aufheben kann, was sich in einem Anschwellen der Partikeln äußert. Wesentlich empfindlicher reagieren die Virusteilchen bei pH $\geq$ 7.5 auf den Entzug aller zweiwertigen Kationen durch EDTA-Na_2. Den Wechselwirkungen unter Beteiligung solcher Ionen kommt daher offenbar eine größere Bedeutung bei der Stabilisierung der CRSV-Struktur zu als den salzlabilen Bindungen. Hydrophobe Wechselwirkungen zwischen den Proteinuntereinheiten, wichtigster Stabilisierungsfaktor der Tymoviren (Kaper, 1975), sind für das CRSV wenig wahrscheinlich, da hohe Ionenstärken (1,0 M NaCl) bei pH 7,5 zum Zerfall der Partikeln führen (Kühne, in Vorbereitung).

Vergleicht man CRSV und Trespenmosaik-Virus (brome mosaic virus, BMV) hinsichtlich ihres Verhaltens in vitro, wird deutlich, daß zweiwertige Kationen für die Struktur der CRSV-Partikeln von größerer Bedeutung sind. Während die mit EDTA-Na_2 behandelten Virions des BMV bei pH > 7 ihren Durchmesser nur um etwa 10% vergrößern (Pfeiffer, 1980), zerfällt der CRSV unter den gleichen Bedingungen bereits teilweise. Das Südliche Bohnenmosaik-Virus (southern bean mosaic virus, SBMV) und das TBSV sind dagegen weniger pH-empfindlich als das CRSV, denn ihre Partikeln können bei pH $\geq$ 7,5 nur

dann in eine geschwollene Form übergehen, wenn ihnen durch EDTA-Na_2 alle bivalenten Kationen entzogen werden (Hsu et al., 1976, Krüse et al., 1981).

Mit unseren Befunden können wir die von Tremaine und Ronald (1976) publizierten Angaben zur Stabilität des CRSV und ihren Ursachen bestätigen und ergänzen.

Zusammenfassung

Mit Hilfe der analytischen Ultrazentrifugation und der Elektronenmikroskopie wurde die Stabilität der Virions des Nelkenringflecken-Virus (carnation ringspot virus, CRSV) im pH-Bereich 5,0 bis 8,0 in 0,01 M Phosphatpuffer untersucht. Es zeigte sich, daß bei pH 5,0 Sedimentationskoeffizient ($S_{20,w}$ = 129 S), Schwebedichte in Caesiumchlorid (ρ^{25} = 1,358 g/cm^3) und Durchmesser der Partikeln (d = 31 nm) durch NaCl (0,1 M) oder EDTA-Na_2 (0,01 M) nicht verändert werden. Zwischen pH 7,0 und pH 7,5 lockert sich die Struktur des Viruscapsids, so daß die Teilchen unter Einwirkung von NaCl in eine geschwollene Form übergehen ($S_{20,w}$ = 117 S, d = 35 nm). Sind bei pH 7,5 die zweiwertigen Kationen im Viruspräparat durch EDTA-Na_2 gebunden, beginnt der Partikelzerfall. Als stabilisierende Kräfte in den Virions des CRSV werden pH-abhängige Wechselwirkungen zwischen den Proteinuntereinheiten und Bindungen unter Beteiligung zweiwertiger Kationen diskutiert.

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Empfindliche Verfahren zur quantitativen Virusbestimmung am Beispiel des Gerstenstreifenmosaik-Virus (GSMV)

Wolf-Dieter Ostermann und Robert-Matthias Leiser

Institut für Phytopathologie Aschersleben der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik, 432 Aschersleben, Theodor-Roemer-Weg 4, DDR

The usefulness of two methods — the radial immunodiffusion test (RIDT) and the polyacrylamide gel electrophoresis in the presence of sodium dodecyl-sulphate (SDS-PAGE) — was investigated fort the estimation of virus concentrations in the case of barley stripe mosaic virus (BSMV) by using the area of the precipitation halos in the case of RIDT and the area or the height of the peaks, respectively, in the case of SDS-PAGE. By means of double logarithmic evaluation we have found in both cases a linear relationship between the area and the virus concentration over the whole investigated concentration range (from 512 to 1 μg BSMV per ml). Both methods are simple, rapid and accurate and can be used for the estimation of virus concentrations in the crude sap of infected plants.

Ein wesentlicher Schritt zum möglichen Verständnis von Resistenzmechanismen der Pflanze gegen eine Virusinfektion ist die exakte Kenntnis des Virusgehaltes im Verlaufe der Infektion. Zur quantitativen Bestimmung der Viruskonzentration bei Infektion von Gerste mit dem Gerstenstreifenmosaik-Virus (GSMV) liegen bisher nur wenig Angaben vor. Pring und Timian (1969), Pring (1971) sowie Palomar und Brakke (1976) ermittelten die Konzentration auf dem Umwege über die Infektiositätsbestimmung durch Übertragung des extrahierbaren Virus auf einen systemischen Wirt (Gerste ‚Black Hulles' C. I. 666). Ein geeigneter Lokalläsionswirt für das GSMV ist nicht bekannt (McKinney und Greeley, 1965). Palomar und Brakke (1976) verwendeten als Konzentrationsmaß außerdem die Fläche der bei der Dichtegradientenzentrifugation des gereinigten Virus erhaltenen Peaks nach spektrophotometrischer Registrierung. Von Stanarius (1980) wurde die von Slack und Shepherd (1975) ursprünglich als qualitativer Test eingeführte Technik der Radialimmundiffusion unter Verwendung von dissoziiertem Virus (D-Protein) als quantitative Bestimmungsmethode eingesetzt.

In der vorliegenden Arbeit wird eine Weiterentwicklung der dort beschriebenen Arbeitsweise vorgestellt, indem die Präzipitate mit einem Proteinfarbstoff angefärbt werden, wodurch sich die Auswertung vereinfacht und die Empfindlichkeit weiter gesteigert wird. Weiterhin wird die Eignung der Peakhöhe bzw. -fläche der GSMV-Hüllprotein-Bande nach SDS-PAGE als Konzentrationsmaß untersucht. Für beide Bestimmungsmethoden werden verschiedene Formen der graphischen Auftragung zur Ermittlung der Eichgeraden diskutiert.

Material und Methoden

Virus und Antiserum

Die Virusreinigung, die Virusspaltung und die Herstellung von Antiserum gegen pyrrolidinabgebautes, formaldehydfixiertes Virus (D-Protein) wurde nach Slack und Shepherd (1975) vorgenommen. Für die Herstellung der Eichkurven wurde gereinigtes Virus mit einer Konzentration von 0.51 mg GSMV/ml in einer geometrischen Reihe bis auf 0.001 mg/ml verdünnt. Die Ermittlung der Ausgangskonzentration erfolgte spektrophotometrisch mit Hilfe des von Lane (1974) angegebenen Extinktionskoeffizienten $\varepsilon_{260\,\mathrm{nm}}^{1,0\,\mathrm{cm}}$ von 2,7 für eine Lösung mit der Konzentration 1 mg/ml.

Herstellung der Agargele und Durchführung des Radialimmundiffusionstestes (RIDT)

Geeignet verdünntes (1 : 9) D-Protein-Antiserum wurde mit dem gleichen Volumen 1.5%igem Agar Noble (Difco Laboratories, Detroit, Michigan, USA) in 0.1 M Trish-HCl-Puffer pH 7.2(+ 0.85% NaCl) versetzt. Der Agar war zuvor im Dampftopf verflüssigt und ebenso wie das Antiserum auf 50°C gebracht worden. 20 ml antiserumhaltige Agarlösung wurden auf entfettete und mit 1%igem Agar vorbeschichtete Glasplatten (125 cm^2) aufgebracht, die sich auf einer nivellierten Unterlage befanden. Zur Sicherung eines langsamen und damit gleichmäßigen Erstarrungsvorganges waren die Platten vor dem Begießen ebenfalls erwärmt worden. Nach dem Erstarren wurden die Antigendepots ausgestanzt (Durchmesser 3 mm) und jeweils 10 μl Antigenlösung eingefüllt. Das zu charakterisierende Antigen (Virus bzw. Extrakt) war zuvor mit dem gleichen Volumen einer 5%igen wäßrigen Pyrrolidinlösung versetzt worden. Die Reaktionszeit betrug 3–4 Tage bei Zimmertemperatur in einer feuchten Kammer. Anschließend erfolgte Färbung und Entfärbung nach Weeke (1973). Als Proteinfarbstoff diente Coomassiebrillantblau R-250 (Ferak, Berlin-West).

SDS-Polyacrylamid-Gelelektrophorese (SDS-PAGE)

Die SDS-PAGE erfolgte nach Laemmli (1970) mit 11,25 bzw. 3% Acrylamid im Trenn- bzw. Sammelgel. Gefärbt wurde mit 0,025% Coomassiebrillantblau R-250 in Methanol-Wasser-Essigsäure (5 : 5 : 1 Vol.), entfärbt im gleichen Lösungsmittelgemisch. Die densitometrische Registrierung geschah wie bei Hofferek u. a. (1973) beschrieben, wobei allerdings der Standard-Kompensationsschreiber G1B1 des VEB Carl Zeiss Jena durch den Kompensationsschreiber K 201 mit Integrationsvorrichtung ersetzt wurde. Bei dem verwendeten Registrierpapier mit 15 Integrationslinien entsprechen 10 Integrationseinheiten einer Fläche von 1 cm^2.

Resultate

RIDT

Die optimale Antiserumkonzentration ergibt sich einerseits aus dem Bestreben, möglichst große Präzipitathöfe zu erhalten (wobei die Präzipitatfläche umgekehrt proportional der Antiserumkonzentration ist), und andererseits aus der Tatsache, daß eine gewisse, für eine ausreichende Anfärbung notwendige

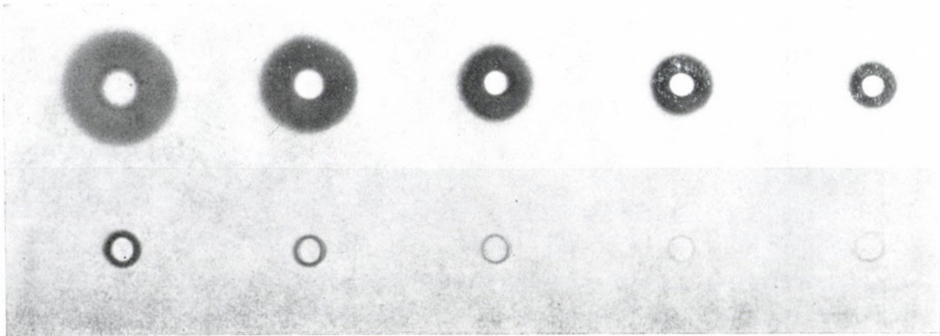

Abb. 1. Mit Coomassiebrillantblau R-250 angefärbte RID-Platte. Der Agar (0,75 %) enthält Antiserum gegen GSMV-D-Protein. In die Antigenlöcher wurden von links oben nach rechts unten je 10 μl einer geometrischen Verdünnungsreihe von mit Pyrrolidin abgebautem GSMV gegeben: 0,512—0,246— ... — 0,002—0,001 mg/ml. (Das Foto ist zusammengesetzt aus zwei Aufnahmen für die Verdünnungsstufen 1—5 bzw. 6—10)

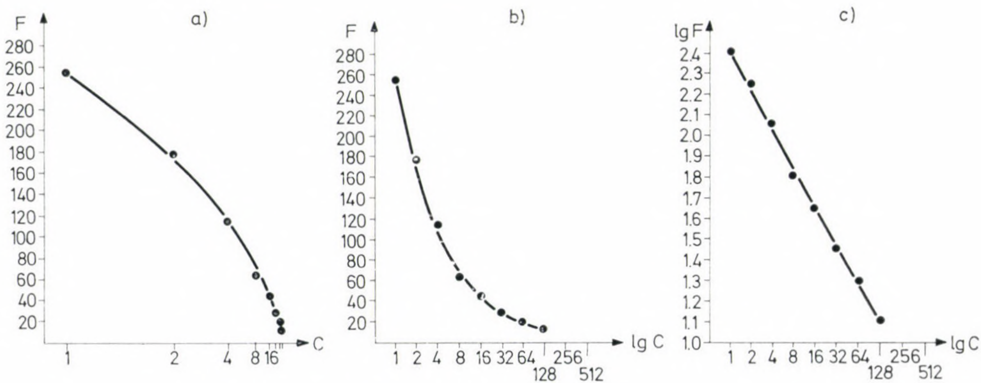

Abb. 2. Beziehung zwischen der RID-Peakfläche und der Konzentration* bei unterschiedlicher graphischer Darstellung. A: F—c, B: F—lgc, C: lgF—lgc

* *Fußnote zur Erklärung* (footnote for the explanation) Die Konzentrationswerte auf der Abszisse sind dimensionslos, da nicht die Konzentrationen selbst aufgetragen sind, sondern die numerischen bzw. logarithmischen reziproken Werte der geometrischen Verdünnungsreihe. 1, 2, ... 256, 512 bedeutet also 1, 1/2, ... 1/256, 1/512 der Ausgangskonzentration von 0,512 mg/ml.

Präzipitatmenge natürlich nicht unterschritten werden darf. Mit dem von uns erhaltenen Antiserumpool wurde bei einer 1 : 9-Verdünnung das in Abbildung 1 dargestellt Reaktionsbild erhalten. Wenn man als die wesentliche, das Präzipitat charakterisierende Größe seine Fläche ansieht, findet man in der Literatur drei Varianten der graphischen Auswertung des Zusammenhanges zwischen der Fläche F (mm^2) und der Konzentration c (mg/ml): F – c, F – lgc, lgF – lgc. Alle

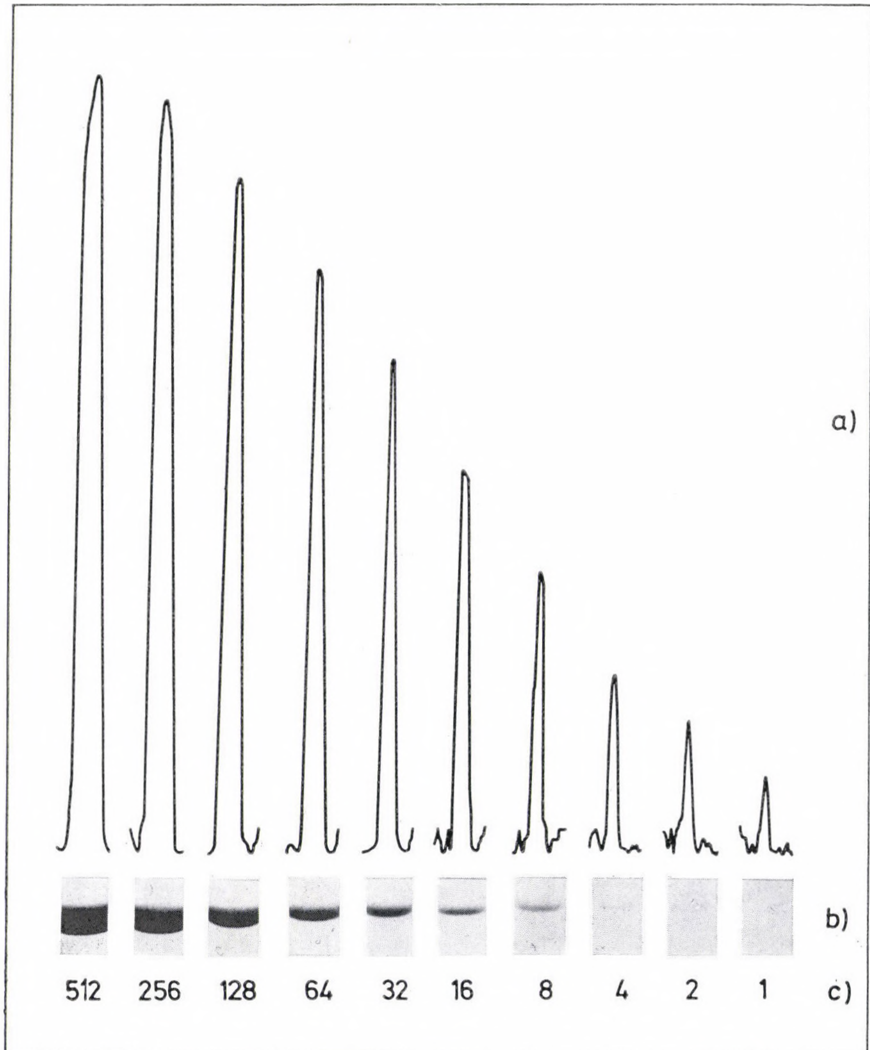

Abb. 3. SDS-PAGE des GSMV im diskontinuierlichen Tris-HCl-System von LAEMMLI. a und b: Ausschnitte aus dem Densitogramm bzw. dem Elektropherogramm nach Anfärbung mit Coomassiebrillantblau R-250. c = Konzentration der aufgetragenen Lösung in μg/ml

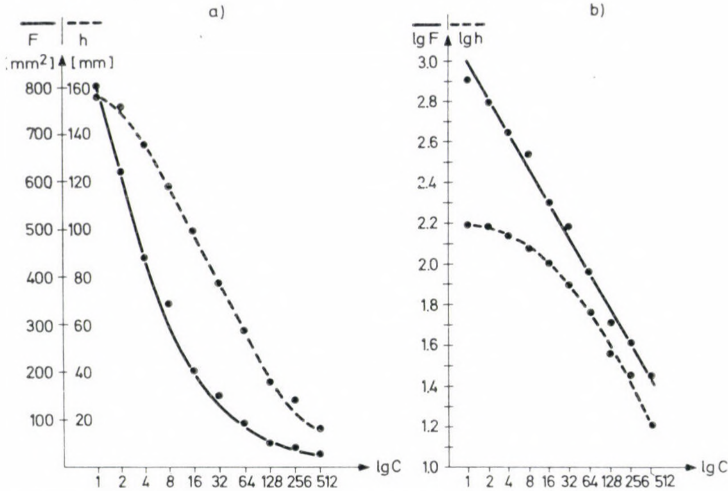

Abb. 4. Beziehung zwischen der SDS-PAGE-Peakfläche bzw. -Peakhöhe und der Konzentration** bei unterschiedlicher graphischer Darstellung. A: F bzw. h—lgc, B: lgF bzw. lgh—lgc

drei Möglichkeiten sind in Abbildung 2 dargestellt. Aus der Abbildung 2A wird ersichtlich, daß die von Mancini u. a. (1965) beschriebene und von Richter u. a. (1976) sowie Stanarius (1980) bestätigte Linearität beim Auftragen der Flächen gegen die Konzentration unter unseren Bedingungen nur für begrenzte Konzentrationsabschnitte gilt. Beim Erfassen mehrerer Zehnerpotenzen ergibt sich näherungsweise ein parabelförmiger Zusammenhang. Die Auftragung F – lgc (Abb. 2b) erscheint günstiger beim Erfassen großer Konzentrationsunterschiede, führt aber ebenfalls zu einem parabelförmigen Zusammenhang. Sehr deutlich wird bei dieser Form der Darstellung die Nachweisgrenze, die bei etwa 4 μg GSMV/ml liegt. Die doppeltlogarithmische Auftragung (lgF – lgc, Abb. 2c) führt über den gesamten untersuchten Konzentrationsbereich zu einem linearen Zusammenhang. Der Nachteil, von den Logarithmen erst auf die numerischen Werte umrechnen zu müssen, sollte dadurch aufgewogen werden, daß sich Zwischenwerte wesentlich sicherer ablesen lassen als bei nichtlinearer Abhängigkeit. Mit Blattextrakten anstelle von gereinigtem Virus zu arbeiten, ist nicht problematischer, da wir mit einem monospezifischen Antiserum (gegen das GSMV-Hüllprotein) arbeiten, so daß andere Antigene im Extrakt nicht stören.

SDS-PAGE

Die Gestalt der Banden im Gel sowie ihrer Peaks im Densitogramm in Abhängigkeit von der Konzentration zeigt die Abbildung 3. Wie beim RIDT sollte die Fläche die eigentlich charakteristische Größe sein; bei diesen Peaks wie auch bei den »Rockets« der Elektroimmundiffusion arbeitet man jedoch

** Siehe Fußnote zu Abb. 2.

häufig auch mit den Gipfelhöhen, weshalb beide Größen in Abhängigkeit von der Konzentration gezeigt werden. Da die erfaßbare Konzentration kleiner als beim RIDT und nichtlogarithmisch schwierig darzustellen ist, wurde lediglich die halb- bzw. doppeltlogarithmische Darstellung gewählt. Beide sind in Abbildung 4 dargestellt. Bei halblogarithmischer Auftragung (F bzw. h – lgc, Abb. 4a) ergibt sich für die Fläche die gleiche Kurvenform wie beim RIDT, während sich für die Peakhöhe über einen weiten Konzentrationsbereich eine lineare Beziehung ergibt, von der nur die Werte bei sehr niedrigen und sehr hohen Konzentrationen abweichen. Dementsprechend ergibt sich eine stark gekrümmte Kurve bei doppeltlogarithmischer Auftragung (Abb. 4b). Anders dagegen bei der Fläche: hier resultiert aus der lgF/lgc – Auftragung wie beim RIDT eine Gerade. Problematischer als beim RIDT ist die Viruskonzentrationsbestimmung in Extrakten, da die Ermittlung der Höhe und insbesondere der Fläche der Hüllproteinbande durch eng benachbarte Banden erheblich erschwert wird. Man kann sich jedoch zunutze machen, daß das Hüllprotein unter geeigneten Pufferbedingungen eine höhere thermische Stabilität besitzt als die meisten der Blattproteine. 10 min Behandlung bei 60°C führt zu einem Proteinmuster, aus dem sich die Hüllprotein-Bande deutlich heraushebt, wie es aus Abbildung 5 ersichtlich ist.

Nachweisempfindlichkeit

Sämtliche Versuche zur Eignung und zum Vergleich beider Methoden wurden mit den gleichen Virusverdünnungen im Bereich 512 bis 1 μg/ml durchgeführt. Mit dem RIDT lassen sich 4 μg/ml sicher erfassen, mit der SDS-PAGE

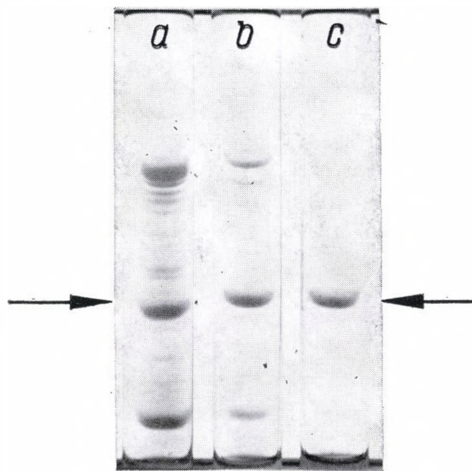

Abb. 5. Proteinmuster des 15 000 · g-Überstandes eines Gerstenblattextraktes nach Extraktion mit 0,1 M Tris-HCl-Puffer pH 8,0 (OXELFELT, 1972) und SDS-PAGE. Die Pfeile kennzeichen die Lage des GSMV-Hüllproteins. a: ohne Temperaturbehandlung, b und c: mit Temperaturbehandlung des Extraktes (10 min 55°C bzw. 10 min 60°C)

1 μg/ml. Da die Auftragsmenge bei der SDS-PAGE jedoch 4mal so hoch war (5 bzw. 20 μl Antigenlösung), erscheinen beide Methoden zunächst als gleich empfindlich. Berücksichtigt man jedoch, daß bei der SDS-PAGE mit 20 μl nur 1/10 der möglichen Auftragsmenge (200 μl) ausgenutzt wurde, sollte sich die Nachweisgrenze hierbei theoretisch bis auf 100 ng/ml senken lassen. Versuche zum Erreichen der unteren Nachweisgrenze wurden jedoch nicht durchgeführt.

Diskussion

Voraussetzung dafür, daß die serologisch bzw. gelelektrophoretisch erfaßte Konzentration an Virus-Hüllprotein als Maß für die Viruskonzentration verwendet werden kann, ist, daß kein überschüssiges Hüllprotein produziert wird. Vorangehende SDS-gelelektrophoretische Versuche (Ostermann, in Vorbereitung) an durch Differentialzentrifugation erhaltenen Zellfraktionen hatten gezeigt, daß die Bande des GSMV-Hüllproteins ausschließlich in der bei 100 000 · g sedimentierenden mikrosomalen Fraktion erscheint und insbesondere nicht in der cytoplasmatischen (löslichen) Fraktion nachweisbar ist. Damit unterscheidet sich das GSMV grundlegend z. B. vom TMV, bei dem eine ausgeprägte Akkumulation von überschüssigem Hüllprotein stattfindet (Oxelfelt, 1972), und bietet die Möglichkeit, im oben genannten Sinne mit dem Hüllprotein zu arbeiten.

Für beide Bestimmungsmethoden wurde bei doppeltlogarithmischer Auftragung (lgF – lgc) eine lineare Beziehung erhalten, mit der sich große Konzentrationsunterschiede exakt beschreiben lassen. Innerhalb kleiner Konzentrationsbereiche führt auch die arithmetische Auftragung zu einer quasilinearen Beziehung. Hierbei müßte durch eine Vielzahl von Meßpunkten jedoch abgesichert werden, in welchem Bereich die Linearität gilt. Durch Transformation in ein doppeltlogarithmisches System kann man auch die von O'Farrell (1975) gefundene Beziehung zwischen der Proteinmenge je Spot und der Spotgröße bei zweidimensionaler Proteinelektrophorese linearisieren, was für die breite Anwendbarkeit des Verfahrens spricht.

Die von uns erhaltene, konkav gekrümmte Beziehung zwischen der Fläche und der Konzentration entspricht dem Zustand, wie ihn Mancini u. a. (1965) bei nicht ausreichender Diffusionszeit erhalten haben. Für ein Protein der Größe des GSMV-Hüllproteins sollte eine Diffusionszeit von 3 Tagen nach Mancini u. a. (1965) jedoch ausreichend sein, geringe Schwankungen um die Normzeit von 3 Tagen blieben auch ohne Einfluß auf das Resultat. Wesentlich verlängerte Zeiten wurden allerdings nicht untersucht. Die Tatsache, daß die elektrophoretischen Verfahren (SDS-PAGE, O'Farrell-Technik) zu den gleichen Abhängigkeiten bezüglich der Fläche führen, spricht nach unserer Meinung ebenfalls gegen eine unzureichende Diffusionszeit beim RIDT. Möglicherweise spielen hierbei aber auch Pufferzusammensetzung und pH-Wert sowie Agarqualität und -konzentration eine wesentliche Rolle.

Zum Problem der quantitativen Densitometrie angefärbter Proteine im Polyacrylamid-Gel sind vergleichsweise wenig Arbeiten bekannt. Von Fishbein

(1972) und Davis u. a. (1974) stammen methodische Abhandlungen, während Bennett und Scott (1971) sowie Bauwe (1979) das Verfahren auf die quantitative Bestimmung des Fraktion-I-Proteins (Ribulose-1,5-biphosphat-Carboxylase) anwendeten. Alle diese Arbeiten beschreiben eine Linearität zwischen der Peakfläche und der Konzentration im Bereich sehr niedriger Konzentrationen und weisen auf das Abweichen von der Linearität bei höheren Konzentrationen hin. Dementsprechend erscheinen die von uns vorgelegten Abhängigkeiten allgemeingültiger, was nicht ausschließt, auch bei der SDS-PAGE in kleineren Konzentrationsbereichen mit anders gearteten Beziehungen zu arbeiten.

Zusammenfassung

Am Beispiel des Gerstenstreifenmosaik-Virus (GSMV) wurde die Eignung des Radialimmundiffusionstestes (RIDT) sowie der SDS-Polyacrylamidgelelektrophorese (SDS-PAGE) für die Viruskonzentrationsbestimmung untersucht. Als Konzentrationsmaß dienten die Fläche der Diffusionshöfe beim RIDT sowie die Fläche bzw. die Höhe der densitometrisch erhaltenen Peaks nach SDS-PAGE. Durch doppeltlogarithmische Auftragung wurde bei Verwendung der Flächen in beiden Fällen eine lineare Beziehung über den gesamten untersuchten Konzentrationsbereich (512 bis 1 μg GSMV/ml) gefunden. Beide Methoden können zur quantitativen Virusbestimmung im Rohsaft infizierter Pflanzen verwendet werden.

Danksagung

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New Artificial Hosts and Non-Hosts of Plant Viruses and their Role in the Identification and Separation of Viruses XVIII. Concluding Remarks

J. HORVÁTH

University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P. O. Box 71, Hungary

The last part of the publication series gives a survey of the results of experiments carried out in the past years. The investigations on new artificial hosts and non-hosts of plant viruses, and on their role in the identification and separation of viruses covered 24 viruses belonging to 9 virus groups and 2 monotypic groups. In the course of inoculation experiments 456 species of 66 genera belonging to 17 plant families, so far unknown as host plants of viruses were detected. Of the experimental plants 248 were first tested in the literature of virology, while the rest (208 plants) first proved to be indicator plants for certain viruses.

In our experiments 246 plants of 58 genera from 21 plant families were newly pointed out as resistant. Of the resistant plants 111 were first tested in plant virological experiments.

Between the 456 plants and 24 viruses studied 1312 new compatible host-virus relations were detected; 597 of them were locally, 234 of systemically and 481 of locally and systemically. In the new host-virus relations important role is played by the virus susceptibility of perennial herbaceous and woody plants. Out of the 1312 new susceptible host-virus relations 13.1 per cent were latent infection.

In further experiments 664 incompatible host-virus relations were found between 246 plants and 23 viruses. Of these plants particularly important are the ones that can be used as genetic donors in breeding for resistance to viruses.

The new virus hosts and resistant plants have important functions as dichotomous separators, reciprocal separators, semiseparators and satellite separators in the differentiation of the viruses studied. Among the viruses and plants included in the experiments 9558 combinations (possibilities and variations, respectively) of virus separation were established.

In seventeen papers of a series of studies on new artificial hosts and new artificial non-hosts of plant viruses as well as their role in the identification and separation of viruses published between 1977 and 1982 (see Horváth 1977–1982 in the Literature) account was given of the results of experiments carried out in earlier years. The present, final part of the study series summarizes and completes the results attained.

On the basis of foreign and Hungarian papers published on the subject in the last six years it can be unambiguously established that the host plants of viruses, the virus resistant plants, the genetic donors, and the host-ranges of new viruses are invariably central subjects of research. Of these papers those dealing with new viruses and host plants described for science, with new host-virus relations detected in the gene centres as well as with the demonstration of compatible and

incompatible relations (cf. Fribourg, 1977; Fribourg et al., 1977*a*, *b*; Salazar and Harrison, 1977, 1978; Sasaki and Shikata, 1978; Beczner and Devergne, 1979; Bem and Murant, 1979; Falk et al., 1979; Kenten and Jones, 1979; Adams and Barbara, 1980; Beczner and Vassányi, 1980; Bos et al., 1980; Horváth and Besada, 1980; Horváth et al., 1980; Jones et al., 1980; Rana et al., 1980; Hollings and Horváth, 1978, 1981; Hammond, 1981; Hammond and Hull, 1981; Horváth, 1981*b*, *c*, *d*; 1982*c*; 1983*a*, *b*, *c*; 1984; Horváth and Beczner, 1982*a*; Mowat, 1982 and others) are of outstanding importance.

Further, interesting are those recently published studies which give comprehensive surveys of viruses possessing wide host ranges (e.g. Christie and Crawford, 1978; Weissenfels et al., 1978; Douine et al., 1979; Horváth, 1980, 1982*d*) and of the virophilic and/or virophobic plants that play an important role in the isolation, identification and separation of viruses (Matsumoto and Hiruki, 1980; Horváth and Beczner, 1982*b*, *c*; Hammond, 1982; Hein, 1983).

The rich content of papers and manuals published on the ecology of viruses and on plants playing a role in virus ecology in the last years clearly shows that the investigation of relations between viruses and plants and the exploration of relations have never so far been more intensive than today (cf. Harrison, 1977; Scott and Bainbridge, 1978; Tresh, 1978, 1980*a*, *b*, 1981*a*, *b*; Tanaki et al., 1979; Palti and Kranz, 1980; Quiot, 1980; Gibbs, 1981; Maramorosch and Harris, 1981; Bos, 1981, 1982; Horváth, 1982*e*; Plumb and Tresh, 1982).

These facts encouraged us to give a summarizing survey of our own research results in the present paper.

Virus Groups and Viruses

Our experiments were carried out with 23 viruses belonging to nine virus groups and two monotypic groups, as well as with the broad bean wilt virus, a so-called "*ungrouped*" virus dealt with in the publication XVII (Table 1). At the same time when our publication on the broad bean wilt virus ("*ungrouped*" virus) appeared, another study of Matthews (1982) related with the classification and nomenclature of viruses became known, in which the broad bean wilt virus was described as a possible member of the comovirus group. The comovirus group (radish mosaic virus as member) was treated in part XII of our publication series (Horváth, 1979*f*) when the proper place of the broad bean wilt virus was still unknown.

The individual viruses within the potyvirus group were discussed in different subdivisions. It was made possible by an excellent study by Edwardson (1974), though we did not always follow Edwardson's (ibid.) suggestions. According to the results of our own experiments (Horváth et al., 1976, 1979; Horváth, 1979*a*), electron microscope examinations revealed that both *Malva* vein clearing virus and celery mosaic virus show, in all the preparations, pinwheels, scrolls together with laminated aggregates. Accordingly, and following the classification proposed

Table 1

Virus groups and viruses[1]

Virus groups[1]	Viruses
Tobra	Tobacco rattle virus (TRV)
Tobamo	Tobacco mosaic virus (TMV)
	Tomato mosaic virus (ToMV)
Potex	Potato virus X (PVX)
	Potato aucuba mosaic virus (PAMV)
Carla	Potato virus M (PVM)
	Potato virus S (PVS)
Poty (Subdivision-I)	Bean common mosaic virus (BCMV)
	Celery mosaic virus (CeMV)
	Malva vein clearing virus (MVCV)
Poty (Subdivision-II)	Bean yellow mosaic virus (BYMV)
	Lettuce mosaic virus (LMV)
Poty (Subdivision-III)	Potato virus Y (PVY)
	Turnip mosaic virus (TuMV)
	Watermelon mosaic virus (WMV)
Cucumo	Cucumber mosaic virus (CMV)
Tymo	Turnip yellow mosaic virus (TYMV)
	Belladonna mottle virus (BMV)
Como	Radish mosaic virus (RMV)
Nepo (CLRV Subgroup)	Cherry leaf roll virus (CLRV)
Nepo (TRSV Subgroup)	Tobacco ring spot virus (TRSV)
Monotypic (Almo)	Alfalfa mosaic virus (AMV)
Monotypic (Tobanecro)	Tobacco necrosis virus (TNV)
Ungrouped virus	Broad bean wilt virus (BBWV)

[1] Matthews (1982) in his paper classified the broad bean wilt virus, as possible member, to the Comovirus group.

by Edwardson (1974), the above mentioned two viruses may be better included in subdivision-III and not (as proposed by Edwardson, ibid.) in subdivision-I of the potyvirus group.

On discussing subgroups of the nepovirus group we took the classification proposed by Martelli et al. (1978) for basis.

Artificial Hosts and Non-hosts

Host plants

In the course of inculation experiments we have tested 456 species and varieties, respectively, of 66 genera (Table 2) belonging to 17 plant families. Of the plants included in the experiments 248 were test plants first described in the literature of virology, while the rest (208 plants) proved new hosts to viruses studied by us.

Table 2

Investigated plant families and genus

Family	Genus[1]
Aizoaceae	*Aptenia* (1), *Tetragonia* (4)
Amaranthaceae	*Amaranthus* (37), *Gomphrena* (2)
Caryophyllaceae	*Cucubalus* (1), *Melandrium* (3), *Saponaria* (1), *Silene* (4)
Chenopodiaceae	*Beta* (2), *Chenopodium* (20, *Obione* (1)
Commelinaceae	*Commelina* (5), *Tinantia* (1)
Compositae (Asteraceae)	*Carthamus* (1), *Helianthus* (12), *Lactuca* (11), *Zinnia* (5)
Cruciferae (Brassicaceae)	*Brassica* (4), *Bunias* (1), *Capsella* (1), *Cheiranthus* (1), *Crambe* (7), *Diplotaxis* (3), *Rorippa* (1)
Cucurbitaceae	*Bryonia* (2), *Colocynthis* (1), *Cucumis* (1), *Cucurbita* (9), *Cyclanthera* (2), *Lagenaria* (2)
Fabaceae (Leguminosae, Papilionaceae)	*Amorpha* (1), *Galega* (3), *Phaseolus* (56), *Vicia* (4), *Vigna* (1)
Geraniaceae	*Erodium* (6), *Geranium* (11)
Labiatae (Lamiaceae)	*Ocimum*(6)
Malvaceae	*Abutilon* (1), *Althaea* (1), *Anoda* (2), *Gossypium* (1), *Hibiscus* (3), *Kitaibelia* (1), *Lavatera* (2), *Malope* (1), *Malva* (10), *Malvastrum* (1), *Napeae* (1), *Sida* (2), *Sidalcea* (1), *Urocarpidium* (1)
Papaveraceae	*Papaver* (1)
Scrophulariaceae	*Paulownia* (2), *Pentstemon* (19)
Solanaceae	*Atropa* (1), *Browallia* (7), *Capsicum* (19), *Cestrum* (1), *Datura* (20), *Lycium* (12), *Lycopersicon* (12), *Nicotiana* (32), *Petunia* (5), *Physalis* (11), *Scopolia* (1), *Solanum* (47)
Umbelliferae (Apiaceae)	*Ammi* (2), *Cicuta* (1)
Tropaeolaceae	*Tropaeolum* (1)

[1] Numbers of the investigated species in the mentioned genus are in parentheses.

Non-host plants

In the framework of our investigations we have studied 246 species and varieties, respectively, of 58 genera (Table 3) belonging to 22 plant families. Of the test plants 111 were first used in virological experiments, and the other plants were first found to be artificial non-hosts (resistant or immune plants) to various plant viruses.

New Host-virus Relations

Between the 456 plants and 24 viruses included in the experiments 1312 new host-virus relations were pointed out (Table 4). In the new host-virus relations important role is played by the perennial herbaceous and woody plants, which

Table 3

Investigated plant families and genus

Family	Genus[1]
Aizoaceae	*Aptenia* (1), *Tetragonia* (3)
Amaranthaceae	*Gomphrena* (1)
Basellaceae	*Basella* (1)
Caryophyllaceae	*Saponaria* (2), *Silene* (6), *Vaccaria* (1), *Viscaria* (1)
Chenopodiaceae	*Beta* (3), *Chenopodium* (6), *Obione* (1)
Cistaceae	*Helianthemum* (1)
Commelinaceae	*Commelina* (5), *Rhoeo* (1), *Tinantia* (1)
Cruciferae (Brassicaceae)	*Brassica* (2), *Cheiranthus* (1), *Crambe* (7), *Diplotaxis* (1), *Rorippa* (1)
Compositae (Asteraceae)	*Centaurea* (1), *Helianthus* (13)
Cucurbitaceae	*Bryonia* (2), *Citrullus* (1), *Cucumis* (1), *Cucurbita* (2), *Luffa* (1)
Ericaceae	*Leiophyllum* (1)
Fabaceae (Leguminosae, Papillionaceae)	*Cassia* (1), *Cyamopsis* (1), *Galega* (2), *Phaseolus* (41), *Vicia* (4), *Vigna* (2)
Geraniaceae	*Erodium* (6), *Geranium* (11)
Labiatae (Lamiaceae)	*Ballota* (1), *Ocimum* (3)
Nolanaceae	*Nolana* (2)
Polygonaceae	*Emex* (1)
Scrophulariaceae	*Paulownia* (2), *Pentstemon* (8)
Solanaceae	*Atropa* (1), *Browallia* (5), *Capsicum* (2), *Cestrum* (1), *Datura* (8), *Lycopersicon* (6), *Nicandra* (1), *Nicotiana* (18), *Petunia* (6), *Physalis* (11), *Solanum* (24), *Trechobaetes* (1)
Tropaeolaceae	*Tropaeolum* (5)
Umbelliferae (Apiaceae)	*Ammi* (2)
Urticaceae	*Urtica* (1)
Vitaceae	*Vitis* (1)

[1] Numbers of the investigated species in the mentioned genus are in parenthesis.

as prognostic hosts have a great part in the circulation of various viruses in nature. Of the new host-virus relations between perennial herbaceous plants and viruses the following are of particular importance: *Cucubalus baccifer*, *Galega bicolor* – alfalfa mosaic virus; *Cicuta virosa* – celery mosaic virus; *Silena tatarica*, – cucumber mosaic virus; *Crambe armena*, *C. cordifolia*, *C. hispanica*, *C. orientalis*, *C. tatarica*, *Geranium pyreanicum* – tobacco mosaic virus; *Helianthum atrorubens*, *H. cernuus*, *H. decapetalus*, *H. giganteus*, *H. gosse-serratus*, *H. maximilliani*, *H. mollis*, *H. salicifolius*, *H. tomentosus*, *H. trachelifolius*, *Pentstemon gracilis*, *P. humilis*, *P. tubiflorus* – tobacco ring spot virus; *Helianthus mollis* – turnip mosaic virus; *Galega hartlandii* – alfalfa mosaic and broad bean wilt viruses; *Geranium sibiricum* – cucumber mosaic and tobacco mosaic viruses; *Pentstemon murrayanus* – tobacco mosaic and tomato mosaic viruses; *Pentstemon glaucus* –

Table 4

New susceptible plants of viruses

Plants[1]	Family[2]	Viruses[3]
Abutilon indicum[0]	Mal	MVCV
Althaea sinensis[0]	Mal	MVCV
Amaranthus angustifolius[0]	Ama	AMV, CMV, PVX, TMV
A. ascendens[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. atropurpureus[0]	Ama	AMV, CMV, PVM, PVX, TMV
A. aureus	Ama	AMV, CMV, PVX, PVY, TMV
A. bouchoni[0]	Ama	AMV, CMV, PVM, PVX, TMV
A. caracu	Ama	AMV, CMV, PVM, PVX, TMV
A. caudatus	Ama	PVX, TMV
A. caudatus L. cv. *Atripurpureus*[0]	Ama	AMV, CMV, PVM, PVX PVY, TMV
A. chlorostachys[0]	Ama	AMV, CMV, PVX, PVY, TMV
A. chlorostachys f. *strictus*[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. chlorostachys var. *powelli*[0]	Ama	AMV, CMV, PVX, TMV
A. cruentus	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. deflexus	Ama	CMV, PVX, TMV
A. deflexus var. *rufescens*[0]	Ama	CMV, PVX, TMV
A. dubius	Ama	AMV, CMV, PVM, PVX, TMV
A. emarginatus[0]	Ama	AMV, CMV, PVX, PVY, TMV
A. gangetucus var. *multicolor*[0]	Ama	AMV, CMV, PVM, PVX, TMV
Anoda dilleniana[0]	Mal	MVCV, TRSV, WMV
Anoda lavateroides[0]	Mal	MVCV
Aptenia cordifolia	Aiz	CLRV TMV, TNV, ToMV, TRSV
Atropa bella-donna	Sol	PVM, PVS, TNV, ToMV
Beta lomatogona	Chen	CLRV, CMV, ToMV
B. macrocarpa	Chen	AMV, CLRV, CMV, PVX, ToMV, TRSV, TRV, TNV
Brassica adpressa	Cru= Brass	RMV, TYMV
B. campestris	Cru= Brass	RMV
B. carinata	Cru= Brass	RMV
B. nigra	Cru= Brass	RMV
Browallia americana	Sol	BMV, CLRV
B. cordata[0]	Sol	BMV, CMV, PAMV, PVX, RMV, TMV, ToMV TRSV
B. demissa	Sol	BMV, CMV, PAMV, PVX, PVY, ToMV, TRSV, RMV

(Continued 1)

Plants[1]	Family[2]	Viruses[3]
B. graminifolia[0]	Sol	BMV
B. grandiflora	Sol	BMV, CMV, PAMV, PVX, PVY, RMV, TMV, ToMV, TRSV
B. roezli[0]	Sol	BMV, CMV, CLRV, PAMV, PVX, TMV, ToMV, TRSV, RMV
B. viscosa	Sol	BMV, CMV, PAMV, RMV, TMV, ToMV, TRSV
Bryonia alba	Cuc	TNV, ToMV
B. dioica	Cuc	TNV, ToMV
Bunias orientalis	Cru = Brass	RMV, TuMV, TYMV
Capsella grandiflora[0]	Cru = Brass	CMV, RMV, TYMV
Capsicum annuum	Sol	ToMV
C. annuum cv. *Bogyiszlói vastaghúsú*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Cecei édes*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum var. *cerasiforme*[0]	Sol	PAMV, PVY
A. graecizans	Ama	AMV, CMV, PVX
A. hypochondriacus[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. hypochondriacus cv. *Monstrosus*[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. leucocarpus[0]	Ama	CMV, PVM, PVX
A. lividus	Ama	CMV, PVX
A. mantegazzianus[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. oleraceus[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. paniculatus	Ama	CMV, PVM, PVX, PVY, TMV
A. paniculatus cv. *Roter Dom*[0]	Ama	AMV, CMV, PVM, PVX, TMV
A. paniculatus cv. *Roter Paris*[0]	Ama	AMV, CMV, PVX, TMV
A. paniculatus cv. *Sanguineus nanus*[0]	Ama	AMV, CMV, PVX, TMV
A. paniculatus var. *flavus*[0]	Ama	AMV, CMV, PVM, PVX, TMV
A. patulus	Ama	PVX, PVY, TMV
A. retroflexus	Ama	PVM, TMV
A. speciosus[0]	Ama	AMV, CMV, PVM, PVX, TMV
A. spinosus	Ama	AMV, CMV, PVM, PVX, TMV
A. sylvestris[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
A. tricolor[0]	Ama	PMV
A. tricolor cv. *Malten Fire*[0]	Ama	AMV, CMV, PVX, PVY, TMV

(Continued 2)

Plants[1]	Family[2]	Viruses[3]
A. viridis[0]	Ama	AMV, CMV, PVM, PVX, PVY, TMV
Ammi majus	Umb=Api	BYMV, CMV
A. visnaga[0]	Umb=Api	AMV, BBWV, BYMV, CeMV, CLRV, CMV, TRV, WMV
Amorpha fruticosa	Fab=Legu=Pap	AMV
C. annuum cv. *Csokros csüngő*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Csokros felálló*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Csokros felálló* 11[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Dokomlási 2710*[0]	Sol	PAMV, PVY
C. annuum cv. *Hatvani hajtatási*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Kalocsai E-15*[0]	Sol	PAMV, PVY
C. annuum cv. *Kalocsai felálló*[0]	Sol	PAMV, PVY
C. annuum cv. *Korai halvány-zöld*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Kovácsházi hajtatási*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Magyar kincs*[0]	Sol	CMV, PAMV, PVX, TMV
C. annuum cv. *Markgärtner*[0]	Sol	PAMV
C. annuum cv. *Maritza*[0]	Sol	PAMV, PVX, PVY, TMV
C. annuum cv. *Sonnenpreis*[0]	Sol	PAMV, PVX, PVY, TMV
C. annuum cv. *Tétényi hajtatási zöld*[0]	Sol	CMV, PAMV, PVX, TMV
C. praetermissum[0]	Sol	BBWV
Carthamus lanatus[0]	Comp=Aster	LMV
Cestrum parqui[0]	Sol	BMV
Cheiranthus cheiri	Cru=Brass	TRSV
Chenopodium amaranticolor	Chen	ToMV
C. ambrosioides var. *ambrosioides*[0]	Chen	PVM
C. ambrosioides var. *anthelminticum*	Chen	PVM
C. aristatum[0]	Chen	PVM
C. botrys	Chen	AMV, PVX, PVY
C. capitatum	Chen	AMV, PVX, PVY, TMV
C. ficifolium	Chen	PVM
C. foetidum	Chen	AMV, PVX, PVY
C. foliosum	Chen	PVM
C. giganteum	Chen	PVM, PVX, PVY
C. glaucum	Chen	PVM
C. hybridum	Chen	PVM, PVS
C. murale	Chen	PVX, TMV
C. opulifolium	Chen	PVM, PVS
C. polyspermum	Chen	PVM, PVS
C. quinoa	Chen	PVY, TMV
C. quinoa f. *rubescens*	Chen	PVY, TMV, TNV
C. quinoa f. *viridescens*[0]	Chen	PVM, PVS, PVX, PVY, TMV, TNV

(Continued 3)

Plants[1]	Family[2]	Viruses[3]
C. rubrum	Chen	PVM, PVS
C. vulvaria	Chen	PVM
Cicuta virosa[0]	Umb=Api	CeMV
Colocynthis vulgaris	Cuc	CMV
Commelina clandestina[0]	Com	CLRV, CMV, TMV, TNV, ToMV, TRSV
C. coelestis	Com	CLRV
C. communis	Com	CLRV, TMV, TNV, ToMV, TRSV
C. graminifolia[0]	Com	CMV, CLRV, TMV, TNV, ToMV, TRSV
C. tuberosa[0]	Com	CLRV, CMV, TMV, TNV, ToMV, TRSV
Crambe abyssinica	Cru=Brass	RMV, TNV, TYMV
C. armena[0]	Cru=Brass	TMV
C. cordifolia[0]	Cru=Brass	TMV
C. hispanica[0]	Cru=Brass	TMV
C. maritima	Cru=Brass	TMV
C. orientalis[0]	Cru=Brass	TMV
C. tataria[0]	Cru=Brass	TMV
Cucubalus baccifer[0]	Cary	AMV
Cucumis myriocarpus	Cuc	PVX, TMV, TNV, ToMV, TRSV
Cucurbita andreana	Cuc	CMV
C. pepo var. *aurantiformis*[0]	Cuc	CMV
C. pepo convar. *clypeata*[0]	Cuc	CMV
C. pepo convar. *oblonga*[0]	Cuc	CMV
C. pepo convar. *patissonina* f. *radiata*[0]	Cuc	CMV, TMV, TNV, ToMV, TRSV, WMV
C. pepo convar. *piriformis*[0]	Cuc	CMV
C. pepo convar. *pomiformis*[0]	Cuc	CMV
C. pepo var. *subrotunda*[0]	Cuc	CMV
C. pepo var. *verrucosa*[0]	Cuc	CMV
Cyclanthera explodens	Cuc	CMV
C. pedata	Cuc	CMV
Datura aegyptiaca	Sol	TMV
D. arborea	Sol	TMV
D. carolinianum[0]	Sol	AMV, BMV, PAMV, TMV
D. ceratocaula[0]	Sol	AMV, CMV, PAMV, PVX, TMV, TRSV
D. chlorantha	Sol	AMV, BMV, CMV, TMV, ToMV, TRSV, TRV
D. fastuosa cv. *Alba*[0]	Sol	AMV, BMV, CMV, PAMV, PVX, TMV, ToMV, TRSV, TRV
C. gigantea	Sol	AMV, BMV, CMV, PAMV, PVX, TMV, TMV, ToMV, TRV
D. godronii cv. *Minka*[0]	Sol	AMV, BMV, CMV, PAMV, PVX, TMV, TNV, ToMV, TRSV, TRV

(Continued 4)

Plants[1]	Family[2]	Viruses[3]
D. inermis	Sol	AMV, CMV, TMV, TNV, ToMV, TRSV, TRV
D. innoxia	Sol	TMV, TNV, ToMV, TRSV
D. leichardtii	Sol	AMV, BMV, CMV, TMV, TNV, ToMV, TRSV, TRV
D. metel	Sol	PAMV, TNV, ToMV
D. metel var. *inermis*[0]	Sol	BMV, PAMV, PVX, TMV, TNV
D. metel var. *muricata*[0]	Sol	AMV, BMV, PAMV, PVX, PVY, TMV, TNV
D. meteloides	Sol	TNV, ToMV, TRSV, TRV
D. quercifolia	Sol	BMV, TMV, ToMV, TRSV, TRV
D. rosei[0]	Sol	AMV, BMV, CMV, PAMV, PVX, TMV, TNV, ToMV, TRSV, TRV
D. stramonium var. *chalybea*	Sol	BMV, PAMV, PVX, TMV, TNV
D. stramonium f. *inermis*[0]	Sol	BMV, PAMV, PVX, TMV, TNV
D. tatula[0]	Sol	BMV, PVX, TMV, TNV
Diplotaxis erucoides	Cru=Brass	RMV, TYMV
D. tenuifolia	Cru=Brass	RMV
D. viminea[0]	Cru=Brass	CMV, RMV, TYMV
Erodium ciconium[0]	Ger	CMV, PVX, RMV, TMV, TNV, ToMV
E. cicutarium	Ger	CLRV, RMV, TMV, TNV, ToMV
E. gruinum	Ger	CMV, RMV, TMV, TNV, ToMV
E. malacoides[0]	Ger	CLRV, CMV
E. manescavi[0]	Ger	CMV, RMV, TMV, TNV
E. moschatum	Ger	CLRV, CMV, PAMV, RMV, TMV, TNV, ToMV
Galega bicolor[0]	Fab=Legu=Pap	AMV
G. hartlandii[0]	Fab=Legu=Pap	AMV, BYMV
G. officinalis	Fab=Legu=Pap	BCMV
Geranium columbianum[0]	Ger	TMV
G. cristatum[0]	Ger	TMV
G. dissectum	Ger	CMV, PVX, TMV
G. lucidum[0]	Ger	TMV
G. molle	Ger	TMV
G. pratense	Ger	TMV
G. pusillum[0]	Ger	TMV
G. pyrenaicum[0]	Ger	TMV
G. robertianum	Ger	TMV
G. rotundifolium	Ger	TMV
G. sibiricum[0]	Ger	CMV, TMV
Gomphrena decumbens[0]	Ama	BBMV, BYMV, CMV, LMV, PVM, PVS, PVX, TMV, TNV, ToMV, TRSV

(Continued 5)

Plants[1]	Family[2]	Viruses[3]
G. diffusa[0]	Ama	BBWV
Gossypium barbadense[0]	Mal	MVCV
Helianthus annuus cv. *Csakinszkij*[0]	Comp=Aster	TuMV
H. annuus cv. *Iregi csíkos*[0]	Comp=Aster	TuMV
H. atrorubens[0]	Comp=Aster	TRSV
H. cernuus[0]	Comp=Aster	TRSV
H. decapetalus[0]	Comp=Aster	TRSV
H. giganteus[0]	Comp=Aster	TRSV
H. grosse-serratus[0]	Comp=Aster	TRSV
H. maximiliani[0]	Comp=Aster	TRSV
H. mollis[0]	Comp=Aster	TRSV, TuMV
H. salicifolius[0]	Comp=Aster	TRSV
H. tomentosus[0]	Comp=Aster	TRSV
H. trachelifolius[0]	Comp=Aster	TRSV
Hibiscus cannabinus	Mal	MVCV
H. manihot	Mal	AMV, BMV
H. sabdariffa[0]	Mal	MVCV
Kitaibelia vitifolia	Mal	MVCV
Lactuca altaica	Comp=Aster	LMV
L. quercina[0]	Comp=Aster	LMV
L. sativa cv. *Aranysárga kőfej*[0]	Comp=Aster	LMV
L. sativa cv. *Budai hajtató*[0]	Comp=Aster	LMV
L. sativa convar. *incocta* var. *capitata Cazard Grosser Gelber*	Comp=Aster	LMV
L. sativa cv. *Keményfejű*[0]	Comp=Aster	LMV
L. sativa cv. *Május királya*[0]	Comp=Aster	LMV
L. sativa cv. *Soroksári*[0]	Comp=Aster	LMV
L. sativa cv. *Szombathelyi*[0]	Comp=Aster	LMV
L. sativa cv. *Téli vajfej*[0]	Comp=Aster	LMV
L. tatarica[0]	Comp=Aster	LMV
Lagenaria leucantha	Cuc	WMV
L. siceraria var. *cugurda*[0]	Cuc	CMV, WMV
Lavatera ambigua[0]	Mal	MVCV
L. arborea	Mal	WMV
Lycium australe[0]	Sol	PVX, PVY, TMV, TNV, TRSV
L. barbarum	Sol	BMV, CMV, TRSV
L. carolinianum[0]	Sol	AMV, BMV, CMV, PVX, PVY, TRSV
L. chinense	Sol	BMV, CMV, PVX, TRSV, TRV
L. europaeum	Sol	BBWV, BMV, CMV, PAMV, PVX, PVY, TMV, TNV, ToMV, TRSV, TRV
L. flexicaule[0]	Sol	AMV, BMV, CMV, PVX, PVY, TMV, TRSV
L. halimifolium	Sol	BMV, TRSV

(Continued 6)

Plants[1]	Family[2]	Viruses[3]
L. horridum[0]	Sol	AMV, BBWV, BMV, CMV, PAMV, PVX, PVY, TMV, TRSV
L. mediterraneum	Sol	BBWV
L. pallidum[0]	Sol	BBWV
L. ruthenicum	Sol	AMV, BBWV, BMV, CMV, PAMV, PVX, PVY, TMV, ToMV, TRSV
L. turcomanicum[0]	Sol	AMV, BMV, CMV, PAMV, PVX, PVY, TMV, TRSV
Lycopersicon esculentum cv. *Kecskeméti 363*[0]	Sol	CMV, TMV, ToMV
L. esculentum cv. *Kecskeméti konzerv*[0]	Sol	CMV, TMV, ToMV
L. esculentum cv. *Pécs gyöngye*[0]	Sol	CMV, TMV, ToMV
L. esculentum cv. *Red Cherry*	Sol	PVM
L. glandulosum	Sol	PVM, PVS
L. hirsutum	Sol	PVM, PVS
L. humboldtii	Sol	PVM
L. peruvianum	Sol	PVM, PVS
L. pimpinellifolium	Sol	PVM
L. pyriforme	Sol	PVM
L. racemiflorum[0]	Sol	PVM
L. racemigerum	Sol	PVM
Malope trifida	Mal	MVCV
Malva alcea	Mal	WMV
M. borealis	Mal	CMV, MVCV, WMV
M. crispa	Mal	TRSV
M. meluca[0]	Mal	MVCV
M. moschata	Mal	CMV
M. neglecta	Mal	CMV, WMV
M. pusilla	Mal	AMV, CMV, ToMV, TRSV, WMV
M. silvestris	Mal	WMV
M. silvestris var. *mauritiana*[0]	Mal	MVCV
M. verticillata	Mal	AMV, CMV, TRSV
Malvastrum capense[0]	Mal	MVCV
Melandrium album	Cary	AMV
M. rubrum[0]	Cary	AMV
M. silvestre[0]	Cary	AMV, CMV
Napeae dioica[0]	Mal	MVCV
Nicotiana acuminata	Sol	PVY
N. attenuata	Sol	AMV, BMV, PVX, PVY
N. auriculata[0]	Sol	AMV, BMV, PVX, PVY
N. chinensis	Sol	BMV, CLRV, CMV, PAMV, TNV, ToMV, TRSV, TRV, TuMV
N. debneyi	Sol	PVY
N. exigua	Sol	BMV, PVY

(Continued 7)

Plants[1]	Family[2]	Viruses[3]
N. glutinosa	Sol	ToMV
N. goodspeedii	Sol	BMV, PVY
N. knightiana	Sol	BMV, CLRV, CMV, PAMV, TNV, ToMV, TRSV, TRV
N. langsdorffii	Sol	PVX
N. occidentalis	Sol	BMV, CLRV, PVY, RMV, TRV, TuMV
N. paniculata	Sol	BMV, PVY
N. plumbaginifolia	Sol	PVX, PVY
N. quadrivalvis	Sol	BBWV, BMV, CLRV, CMV, PAMV, PVY, ToMV, TRV
N. repanda	Sol	PVY
N. sanderae	Sol	PVY
N. solanifolia	Sol	PVX, PVY
N. sylvestris	Sol	BMV
N. tabacum cv. *Ambalema*	Sol	PAMV, PVY
N. tabacum cv. *Bel 61–10*	Sol	BMV, PAMV, PVX, PVY, TMV, ToMV, TRSV
N. tabacum cv. *Debreceni*[0]	Sol	CMV, PVY, TMV
N. tabacum cv. *Érdi*[0]	Sol	CMV, PVY, TMV
N. tabacum cv. *Hevesi*[0]	Sol	CMV, PVY, TMV
N. tabacum cv. *Hicks-Fixed A2-426*[0]	Sol	PAMV, PVX, PVY, TMV
N. tabacum cv. *I. 787*[0]	Sol	PVY
N. tabacum cv. *Kerti*[0]	Sol	CMV, PVY, TMV
N. tabacum x *Nicotiana glutinosa*[0]	Sol	PVY
N. tabacum cv. *sanderae*[0]	Sol	PVY
N. tabacum cv. *Szabolcsi*[0]	Sol	CMV, PVY
N. tabacum cv. *Szuloki*[0]	Sol	PVX, PVY
N. tabacum cv. *Xanthi-nc*	Sol	PAMV, PVX, PVY, TNV, ToMV, TRSV, TRV
N. texana	Sol	PVX, PVY
Obione sibirica[0]	Chen	AMV, CLRV, CMV, PVM, PVS, PVY, RMV, TMV, TNV, ToMV, TRSV, TuMV
Ocimum basilicum	Lab=Lami	ToMV, TRSV
O. canum	Lab=Lami	AMV, BBWV, CMV, PAMV, PVX, TMV, ToMV, TRSV, TRV
O. gratissimum[0]	Lab=Lami	BBWV
O. sanctum[0]	Lab=Lami	AMV, BBWV, CMV, PAMV, PVX, TMV, ToMV, TRSV, TRV
O. selloi[0]	Lab=Lami	BBWV
O. viride[0]	Lab=Lami	BBWV
Papaver orientale	Pap	TuMV
Paulownia fargesii[0]	Scrop	CMV, PAMV, PVX, TMV, ToMV, TRSV, TRV

(Continued 8)

Plants[1]	Family[2]	Viruses[3]
P. tomentosa	Scrop	PAMV, TMV, TNV, ToMV, TRSV
Pentstemon alpinus[0]	Scrop	AMV, PAMV, TMV, ToMV, TRSV
P. attenuatus[0]	Scrop	PAMV, TMV, ToMV, TRSV
P. calycosus[0]	Scrop	PAMV, TMV, ToMV, TRSV
P. cardinalis[0]	Scrop	CLRV, PAMV, TMV, ToMV, TRSV
P. digitalis	Scrop	TRSV
P glaucus[0]	Scrop	TMV, TRSV
P. gracilis[0]	Scrop	TRSV
P. grandiflorus	Scrop	CLRV, ToMV
P. hirsutus[0]	Scrop	TMV, ToMV, TRSV
P. humilis[0]	Scrop	TRSV
P. laevigatus[0]	Scrop	CLRV, PAMV, TMV, ToMV, TRSV
P. murrayanus[0]	Scrop	TMV, ToMV
P. ovatus[0]	Scrop	PAMV, TMV, ToMV, TRSV
P. pubescens[0]	Scrop	CLRV, TMV, TNV, ToMV, TRSV
P. stenopetalus[0]	Scrop	CLRV, TMV, TRSV
P. tubiflorus[0]	Scrop	TRSV
P. utahensis[0]	Scrop	CLRV, TMV, ToMV, TRSV
P. venustus[0]	Scrop	CLRV, TMV, ToMV, TRSV
P. whippleanus[0]	Scrop	PAMV, TMV, ToMV, TRSV
Petunia atkinsiana[0]	Sol	AMV, BMV, CMV, PAMV, PVY, TMV, TNV, TRSV TuMV
P. axillaris	Sol	AMV, PAMV, PVY, TMV, TNV, TRSV
P. hybrida cv. *Rose de Haven amélioré*[0]	Sol	AMV, BMV, CMV, PAMV, PVY
P. parviflora[0]	Sol	AMV, BMV, CMV, PAMV, PVY
P. violacea	Sol	AMV, BMV, PAMV, TMV, TNV, TuMV
Phaseolus caffer[0]	Fab=Legu=Pap	BCMV
P. ricciardianus[0]	Fab=Legu=Pap	AMV
P. vulgaris cv. *Aladin*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Andrásbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Annelise*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Barnabab*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Cardinal*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Caroline*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Cukorbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Fehér gyöngy*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Fehér középbab*[0]	Fab=Legu=Pap	PVM
P. vulgaris cv. *Fertődi 5*[0]	Fab=Legu=Pap	AMV, PVM, TMV

(Continued 9)

Plants[1]	Family[2]	Viruses[3]
P. vulgaris cv. *Fertődi 23*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Fullcrop*	Fab=Legu=Pap	PVM
P. vulgaris cv. *GN 59*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *GN 123*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Harkovszkaja*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Icar Fundulea 51*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Icar Fundulea*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Japán gyöngybab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Kanizsai csíkos-bab*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Kentucky Wonder*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Kereskedelmi hosszú fürjbab*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Kinghorn Wax*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Kompolti gyöngy-bab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Korai fürj*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Kőbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Középbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Májbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Michelite*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Michigan*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Moldovszkaja*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Olomucka Zelenoluska*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Őrségi cseresznye-bab*[0]	Fab=Legu=Pap	AMV, PVM, TMV,
P. vulgaris cv. *Perlicska*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Pinto*	Fab=Legu=Pap	PVM, TNV
P. vulgaris cv. *Prinzess*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Processor*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Red Kidney*	Fab=Legu=Pap	AMV, BCMV, CLRV, PVM, TNV
P. vulgaris cv. *Refugee*	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Robust*	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Saxa*	Fab=Legu=Pap	TMV
P. vulgaris cv. *Soproni lapos*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Szegedi fehér*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Sztepnaja*	Fab=Legu=Pap	PVM
P. vulgaris cv. *Tápiószelei barna*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Tápiószelei fürj*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Tápláni fekete cirádás fürjbab*[0]	Fab=Legu=Pap	AMV, PVM
P. vulgaris cv. *Tápláni*	Fab=Legu=Pap	AMV, PVM
nagyszemű cseresznyebab[0]	Fab=Legu=Pap	TMV
P. vulgaris cv. *Tendergreen*		
P. vulgaris cv. *Tétényi cukorbab*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Tétényi gyöngybab*[0]	Fab=Legu=Pap	AMV, PVM, TMV

(Continued 10)

Plants[1]	Family[2]	Viruses[3]
P. vulgaris cv. *Tétényi fehér középbab*[0]	Fac=Legu=Pap	AMV, PMV, TMV
P. vulgaris cv. *Tétényi középbab*[0]	Fab=Legu=Pap	AMV, TMV
P. vulgaris cv. *Tétényi nagyszemű fehér*[0]	Fab=Legu=Pap	AMV, PVM, TMV
P. vulgaris cv. *Wade*	Fab=Legu=Pap	AMV, PVM, TMV
Physalis aequata	Sol	PAMV, PVY, TMV, TNV, TRSV, TRV
P. alkekengi	Sol	PAMV
P. curassavica[0]	Sol	BBWV
P. ixocarpa	Sol	CMV, PAMV, TMV, TNV, TRSV
P. minima	Sol	BBWV
P. peruviana	Sol	AMV, CMV, PAMV, TMV, TRSV
P. peruviana var. *macrocarpa*[0]	Sol	AMV, CMV, PAMV, PVY, TMV, TRSV
P. philadelphica	Sol	CMV, PAMV, TRSV
P. pruinosa	Sol	AMV, CMV, PAMV, PVY, TMV, TRSV
P. pubescens	Sol	PAMV, TRV
P. viscosa	Sol	CMV, PVX, TNV, TRV
Rorippa islandica	Cru=Brass	ToMV
Saponaria cerastioides[0]	Cary	CMV
Scopolia lurida[0]	Sol	AMV
Sida nepaea[0]	Mal	MVCV
S. rhombifolia	Mal	MVCV
Sidalcea malvaeflora[0]	Mal	MVCV
Silene armeria	Cary	BBWV
S. dichotoma	Cary	AMV
S. pendula	Cary	AMV, CMV
S. tatarica[0]	Cary	CMV
Solanum acroscopium	Sol	TMV
S. ajanhuiri[0]	Sol	TMV
S. berthaultii	Sol	TMV
S. boliviense[0]	Sol	TMV
S. brachycarpum[0]	Sol	TMV
S. brevicaule[0]	Sol	TMV
S. canasense[0]	Sol	PVY, TMV
S. capsicastrum	Sol	CLRV, PAMV, PVM, PVS, TNV, ToMV, TRSV
S. chacoense	Sol	TMV
S. demissum A6-hybrid	Sol	CMV, PVM, PVS
S. demissum 530-Hybrid[0]	Sol	PVX, PVY
S. demissum S. hybrid[0]	Sol	PVX
S. ehrenbergii[0]	Sol	TMV
S. etuberosum[0]	Sol	TMV
S. famatinae	Sol	PVY, TMV
S. gourlayi	Sol	TMV

(Continued 11)

Plants[1]	Family[2]	Viruses[3]
S. guerreroense	Sol	TMV
S. hjertingii[0]	Sol	TMV
S. hougasii[0]	Sol	TMV
S. jamesii	Sol	TMV
S. kurtzianum	Sol	TMV
S. leptophyes[0]	Sol	TMV
S. medians[0]	Sol	TMV
S. megistacrolobum	Sol	TMV
S. ochroleucum	Sol	BMV, CLRV, PAMV, PVM, PVS, TMV, TNV, ToMV, TRSV, TRV
S. pseudocapsicum	Sol	CLRV, TNV, ToMV, TRV
S. rostratum	Sol	BMV, CMV, TMV, TNV, ToMV, TRSV
S. sanctae-rosae	Sol	TMV
S. simplicifolium	Sol	TMV
S. sucrense	Sol	TMV
S. tarijense	Sol	TMV
S. tuberosum cv. *59/558*[0]	Sol	PVY
S. tuberosum cv. *Aranyalma*[0]	Sol	AMV
S. tuberosum cv. *Auriga*	Sol	AMV
S. tuberosum cv. *Axilia*	Sol	AMV
S. tuberosum cv. *Edgecote Purple*[0]	Sol	PVY
S. tuberosum cv. *Früka*	Sol	AMV
S. tuberosum cv. *Gülbaba*[0]	Sol	AMV
S. tuberosum cv. *Jowisz*	Sol	AMV
S. tuberosum cv. *Kisvárdai rózsa*[0]	Sol	AMV
S. tuberosum cv. *Korai rózsa*[0]	Sol	AMV
S. tuberosum cv. *Opal*	Sol	AMV
S. tuberosum cv. *Osa*	Sol	AMV
S. tuberosum cv. *Somogyi kifli*[0]	Sol	AMV
S. tuberosum cv. *Somogyi korai*[0]	Sol	AMV
S. tuberosum cv. *Somogyi sárga*[0]	Sol	AMV
S. verrucosum	Sol	TMV
Tetragonia crystallina[0]	Aiz	BYMV, CLRV, CMV, LMV, PVX, RMV, TMV, TNV, ToMV, TRSV, TRV, TuMV, WMV
T. echinata	Aiz	BBWV, BYMV, CLRV, CMV, LMV, PVX, RMV, TMV, TNV, ToMV, TRSV, TRV, TuMV, WMV
T. eremaea[0]	Aiz	LMV
T. tetragonoides	Aiz	CLRV, RMV, ToMV
Tinantia erecta	Com	AMV, BYMV, CLRV, PVY, TMV, TNV, ToMV, TRSV, TRV, TuMV
Tropaeolum minus cv. *Cherry rose*[0]	Trop	BBWV, TuMV

(Continued 12)

Plants[1]	Family[2]	Viruses[3]
Urocarpidium peruvianum[0]	Mal	MVCV
Vicia faba cv. *Dornburger*[0]	Fab=Legu=Pap	BYMV
V. faba cv. *Dreifachweise*[0]	Fab=Legu=Pap	BYMV
V. faba cv. *Erfurter Gewöhnliche*[0]	Fab=Legu=Pap	BYMV
V. faba cv. *Hangdown*[0]	Fab=Legu=Pap	BYMV
Vigna catjang[0]	Fab=Legu=Pap	AMV, BCMV, PVM, PVS, TMV, TNV, TRSV, TRV
Zinnia haageana	Comp=Aster	LMV
Z. haageana cv. *Cocarde*[0]	Comp=Aster	LMV
Z. pumila[0]	Comp=Aster	LMV
Z. tenuiflora[0]	Comp=Aster	LMV
Z. verticillata[0]	Comp=Aster	LMV

[1] Plants marked [0] are new experimentally species in plant virology.

[2] Aiz, *Aizoaceae;* Ama, *Amaranthaceae;* Cary, *Caryophyllaceae;* Chen, *Chenopodiaceae;* Com, *Commelinaceae;* Comp=Aster, *Compositae (Asteraceae);* Cru=Brass, *Crucuferae (Brassicaceae);* Cuc, *Cucurbitaceae;* Fab=Legu=Pap, *Fabaceae (Leguminosae, Papillionaceae);* Ger, *Geraniaceae;* Lab=Lami, *Labiatae (Lamiaceae);* Mal, *Malvaceae;* Pap, *Papaveraceae;* Scrop, *Scrophulariaceae;* Sol, *Solanaceae;* Umb=Api, *Umbelliferae (Apiaceae);* Trop, *Tropaeolaceae.*

[3] AMV, alfalfa mosaic virus; BBWV, broad bean wilt virus; BCMV, bean common mosaic virus; BMV, belladonna mottle virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; LMV, lettuce mosaic virus; MVCV, *Malva* vein clearing virus; PAMV, potato aucuba mosaic virus PVM, potato virus M; PVS, potato virus S; PVX, potato virus X; PVY, potato virus Y; RMV, radish mosaic virus; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; ToMV, tomato mosaic virus; TRSV, tobacco ring spot virus; TRV, tobacco rattle virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV, watermelon mosaic virus.

tobacco mosaic and tobacco ring spot viruses; *Pentstemon stenopetalus* – cherry leaf roll, tobacco mosaic and tobacco ring spot viruses; *Amaranthus deflexus* var. *rufescens* – cucumber mosaic, tomato mosaic and tobacco ring spot viruses; *Pentstemon hirsutus* – tobacco mosaic virus, tomato mosaic virus and tobacco ring spot virus; *Pentstemon utahensis*, *P. venustus* – cherry leaf roll, tobacco mosaic, tomato mosaic and tobacco ring spot viruses; *Erodium manescavi* – cucumber mosaic, radish mosaic, tobacco mosaic and tobacco necrosis viruses; *Pentstemon attenuatus*, *P. calycosus*, *P. ovatus*, *P. whippleanus* – potato aucuba mosaic, tobacco mosaic, tomato mosaic and tobacco ring spot viruses; *Pentstemon alpinus* – alfalfa mosaic, potato aucuba mosaic, tobacco mosaic, tomato mosaic and tobacco ring spot viruses; *Pentstemon cardinalis*, *P. laevigatus* – cherry leaf roll, potato aucuba mosaic, tobacco mosaic, tomato mosaic and tobacco ring spot viruses; *Pentstemon pubescens* – cherry leaf roll, tobacco ring spot viruses; *Pentstemon pubescens* – cherry leaf roll, tobacco mosaic, tobacco necrosis,

tomato mosaic and tobacco ring spot viruses; *Physalis peruviana* var. *macrocarpa* – alfalfa mosaic virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus Y, tobacco mosaic virus and tobacco ring spot virus; *Lycium turcomanicum* – alfalfa mosaic, belladonna mottle, cucumber mosaic, potato aucuba mosaic, potato X, potato Y, tobacco mosaic, and tobacco ring spot viruses; *Tinantia erecta* – alfalfa mosaic, bean yellow mosaic, cherry leaf roll, potato Y, tobacco mosaic, tobacco necrosis, tomato mosaic, tobacco ring spot, tobacco rattle and turnip mosaic viruses. From a virological point of view not less important are those perennial woody plants new in plant virology which have proved susceptible to some viruses listed below (e.g. *Lycium australe* to potato X, potato Y, tobacco mosaic, tobacco necrosis and tobacco ring spot viruses; *L. flexicaule* to alfalfa mosaic, belladonna mottle, cucumber mosaic, potato X, potato Y, tobacco mosaic and tobacco ring spot viruses; *L. horridum* to alfalfa mosaic, broad bean wilt, belladonna mottle, cucumber mosaic, potato aucuba mosaic, potato X, potato Y, tobacco mosaic and tobacco ring spot viruses; *Paulownia fargesii* to cucumber mosaic, potato aucuba mosaic, potato X, tobacco mosaic, tomato mosaic, tobacco ring spot and tobacco rattle viruses).

Perennial woody plants known in plant virology but not yet known in the following host-virus relations are also very important (e.g. *Amorpha fruticosa* – alfalfa mosaic virus; *Datura arborea* – tobacco mosaic virus; *Lycium barbarum* – belladonna mottle, cucumber mosaic and tobacco ring spot viruses; *L. chinense* – belladonna mottle, cucumber mosaic, potato X, tobacco ring spot, and tobacco rattle viruses; *L. europaeum* – broad bean wilt, belladonna mottle, cucumber mosaic, potato aucuba mosaic, potato X, potato Y, tobacco mosaic, tobacco necrosis, tomato mosaic, tobacco ring spot and tobacco rattle viruses; *L. halimifolium* – belladonna mottle and tobacco ring spot viruses; *L. ruthenicum* – alfalfa mosaic, broad bean wilt, belladonna mottle, cucumber mosaic, potato aucuba mosaic, potato X, potato Y, tobacco mosaic, tomato mosaic and tobacco ring spot viruses; *Paulownia tomentosa* – tobacco mosaic, tobacco necrosis, tomato mosaic and tobacco ring spot viruses). An analysis of the experiment results reveals that of the 1312 new host-virus relations 597 were of locally, 234 of systemically and 481 of locally and systemically (Table 5). Most of the new host-virus relations were detected (see Table 5) in the case of tobacco mosaic virus (205), alfalfa mosaic virus (140), cucumber mosaic virus (131), potato virus M (103), and tobacco ring spot virus (91). Further, remarkable is the fact that for some viruses new susceptible plant families have been described (e.g. *Aizoaceae* and *Geraniaceae* for potato virus X, *Geraniaceae* and *Labiatae* or *Lamiaceae* for potato aucuba mosaic virus, *Commelinaceae* for potato virus S, *Commelinaceae* and *Umbelliferae* or *Apiaceae* for bean yellow mosaic virus, and *Geraniaceae* for cherry leaf roll virus).

As to the new host-virus relations some other experiment results are also worth being called attention to. Of the new local i.e. theoretically important hosts the species *Amaranthus* and *Chenopodium* and the cultivars *Phaseolus vulgaris*, while among the new systemically (potato virus M) susceptible hosts the

Table 5

The compatible host-virus relations and their types

Viruses[1]	Host-virus reactions			Sum total	Literature
	Local	Systemic	Local and systemic		
TRV	11	—	19	*30*	Horváth (1978*a*)
TMV	121	—	84	*205*	Horváth (1978*b*)
ToMV	44	2	29	*75*	Horváth (1978*b*)
PVX	57	21	20	*98*	Horváth (1978*c*)
PAMV	6	15	50	*71*	Horváth (1978*c*)
PVM	90	10	3	*103*	Horváth (1978*d*)
PVS	8	4	3	*15*	Horváth (1978*d*)
BCMV	—	2	2	*4*	Horváth (1979*a*)
BYMV	3	6	2	*11*	Horváth (1979*b*)
CeMV	—	1	1	*2*	Horváth (1979*a*)
MVCV	—	19	—	*19*	Horváth (1979*a*)
LMV	4	17	—	*21*	Horváth (1979*b*)
PVY	32	40	8	*80*	Horváth (1979*c*)
TuMV	3	8	3	*14*	Horváth (1979*c*)
WMV	2	5	6	*13*	Horváth (1979*c*)
CMV	44	29	58	*131*	Horváth (1979*d*)
BMV	10	1	37	*48*	Horváth (1979*e*)
TYMV	—	—	6	*6*	Horváth (1979*e*)
RMV	10	1	14	*25*	Horváth (1979*f*)
CLRV	1	11	22	*34*	Horváth (1979*g*)
TRSV	25	4	62	*91*	Horváth (1979*h*)
AMV	65	29	46	*140*	Horváth (1981*a*)
TNV	54	—	1	*55*	Horváth (1982*a*)
BBWV	7	9	5	*21*	Horváth (1982*b*)
Sum total	*597*	*234*	*481*	*1312*	

[1] TRV, tobacco rattle virus; TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; PVX, potato virus X; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; BCMV, bean common mosaic virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; MVCV, *Malva* vein clearing virus; LMV, lettuce mosaic virus; PVY, potato virus Y; TuMV, turnip mosaic virus; WMV, watermelon mosaic virus; CMV, cucumber mosaic virus; BMV, belladonna mottle virus; TYMV, turnip yellow mosaic virus; RMV, radish mosaic virus; CLRV, cherry leaf roll virus; TRSV, tobacco ring spot virus; AMV, alfalfa mosaic virus; TNV, tobacco necrosis virus; BBWV, broad bean wilt virus.

Lycopersicon species have diagnostical importance in the first place. The *Lycopersicon* species are also important as virus production hosts of potato virus M and potato virus S. *Gossypium barbadense* and *Hibiscus sabdariffa* as new hosts to *Malva* vein clearing virus are economically very important plants. Among the plants susceptible to potato virus Y special importance is attached to some new virus susceptible but *Peronospora tabacina* resistant plants (e.g. *Nicotiana*

Table 6

Latent infection in the local, local and systemic and systemic host-virus relations

Viruses[1]	Local	Local and systemic[2]	Systemic
		Host-virus relation	
TRV	—	—/—	—
TMV	6	23/27	—
ToMV	4	7/7	—
PVX	2	5/5	1
PAMV	—	17/27	13
PVM	—	—/—	1
PVS	—	1/—	4
BCMV	—	—/—	—
CeMV	—	—/—	—
MVCV	—	—/—	—
BYMV	—	—/—	—
LMV	—	—/—	—
PVY	—	—/2	—
TuMV	—	—/—	1
WMV	—	—/—	—
CMV	1	3/22	2
TYMV	—	3/5	—
BMV	—	—/—	—
RMV	5	—/1	—
CLRV	—	1/—	—
TRSV	—	4/1	—
AMV	—	2/3	—
TNV	6	1/—	—
BBWV	—	2/3	—
Sum total	*24*	*69/103*	*22*

[1] TRV, tobacco rattle virus; TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; PVX, potato virus X; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; BCMV, bean common mosaic virus; CeMV, celery mosaic virus; MVCV, *Malva* vein clearing virus; BYMV, bean yellow mosaic virus; LMV lettuce mosaic virus; PVY, potato virus Y; TuMV, turnip mosaic virus; WMV, watermelon mosaic virus; CMV cucumber mosaic virus; TYMV, turnip yellow mosaic virus; BMV, belladonna mottle virus; RMV, radish mosaic virus; CLRV, cherry leaf roll virus; TRSV, tobacco ring spot virus; AMV, alfalfa mosaic virus; TNV, tobacco necrosis virus; BBWV, broad bean wilt virus.

[2] Numerator shows the latent infection of the non-inoculated or subsequently developed leaves (systemic susceptibility), denominator shows the latent infection of the inoculated leaves (local susceptibility).

exigua, *N. goodspeedii*, *N. tabacum* cv. *Resistant Hicks Fixed A2—426*) as virus production hosts. Of the cucumber mosaic virus susceptible plants very important are the new prognostical phanerophyta and hemikryptophyta hosts (e.g. *Amaranthus deflexus*, *Commelina tuberosa*, *Erodium manescavi*, *Geranium sibiricum*,

Lycium spp., *Malva moschata*, *Paulownia fargesii*, *Physalis peruviana*, *Silene tatarica*). After Schmelzer (1966) – the first to report a monocotyledonous plant *(Commelina coelestis)* as systemic host to cherry leaf roll virus –, we have detected further four monocotyledonous hosts *(Commelina clandestina*, *C. graminifolia*, *C. tuberosa* and *Tinantia erecta)* of cherry leaf roll virus. Special attention is worth being paid to a member of *Amaranthus*, *Obione*, *Vigna*, *Cucubalus*, *Datura*, *Galega*, *Lycium*, *Melandrium*, *Ocimum*, *Pentstemon* species as well as to *Solanum tuberosum* varieties which as host plants of alfalfa mosaic virus are new to science (see Table 4). Remarkable is the relatively large number of latent host-virus relations, although it is a well-known fact (cf. Weil, 1961; Gibbs, 1979; Shukla et al., 1980 and others) that wild plants often get infected in a latent way or show but slight symptoms after infection. Of the 597 local host-virus relations 24, of the 234 systemic host-virus relations 22 were symptomless, while of the 481 local and systemic host-virus relations 172 were latent (Table 6). The occurrence

Table 7

Latent (symptomless) virus infection on various new host plants after artificial inoculation by different viruses

Viruses[1]	In local host-virus relations
TMV	*Cucurbita pepo* convar. *patissonina* f. *radiata*
	Erodium ciconium
	E. cicutarium (E. cicutifolium)
	E. gruinum
	E. manescavi
	E. moschatum
ToMV	*E. ciconium*
	E. cicutarium
	E. gruinum
	E. moschatum
PVX	*Beta macrocarpa (B. vulgaris* ssp. *macrocarpa)*
	Cucumis myriocarpus
CMV	*Solanum rostratum*
RMV	*Erodium ciconium*
	E. cicutarium
	E. gruinum
	E. manescavi
	E. moschatum
TNV	*Atropa bella-donna*
	Erodium ciconium
	E. cicutarium
	E. gruinum
	E. manescavi
	E. moschatum

[1] TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; PVX, potato virus X; CMV, cucumber mosaic virus; RMV, radish mosaic virus; TNV, tobacco necrosis virus.

Table 8

Latent (symptomless) virus infection on various new host plants after artificial inoculation by different viruses

Viruses[1]	In local and systemic host-virus relations[2]
TMV	*Commelina clandestina*
	C. communis (C. coelestis)
	C. graminifolia
	C. tuberosa
	Crambe armena
	C. cordifolia
	C. hispanica
	C. maritima
	C. orientalis
	C. tatarica
	Geranium cristatum
	G. columbianum
	G. dissectum
	G. lucidum
	G. molle
	G. pratense
	G. pusillum
	G. pyreanicum
	G. robertianum
	G. rotundifolium
	G. sibiricum
	Lycopersicon esculentum cv. *Kecskeméti 363**
	L. esculentum cv. *Kecskeméti Konzerv**
	Nicotiana tabacum cv. *Debreceni**
	N. tabacum cv. *Kerti**
	Solanum rostratum
	Tinantia erecta (T. fugax)
ToMV	*Commelina clandestina*
	C. communis (C. coelestis)
	C. graminifolia
	C. tuberosa
	Rorippa islandica
	Solanum rostratum
	Tinantia erecta
PVX	*Browallia cordata**
	*Br. demissa (Br. americana)**
	*Br. grandiflora***
	*Br. roezli**
	Erodium ciconium
	*Ocimum canum***
	*O. sanctum***
	Physalis viscosa
PAMV	*Browallia cordata*
	*Br. demissa (Br. americana)**
	*Br. grandiflora**
	*Br. roezli**

(Continued 1)

Viruses[1]	In local and systemic host-virus relations[2]
	*Br. viscosa**
	Erodium moschatum
	Nicotiana knightiana
	*Ocimum canum***
	*O. sanctum***
	Pentstemon alpinus
	P. attenuatus
	P. calycosus
	P. cardinalis
	P. laevigatus
	P. ovatus
	P. whippleanus
	Petunia atkinsiana
	P. axillaris
	P. hybrida cv. *Rose de Haven amélioré*
	P. parviflora
	P. violacea
	*Physalis aequata**
	*Ph. alkekengi (Ph. franchetti)**
	*Ph. ixocarpa**
	*Ph. peruviana**
	Ph. peruviana var. *macrocarpa**
	*Ph. philadelphica**
	*Ph. pruinosa**
	*Ph. pubescens**
PVS	*Solanum ochroleucum***
PVY	*Browallia grandiflora**
	*Physalis pruinosa**
CMV	*Beta lomatogona***
	B. macrocarpa (B. vulgaris ssp. *macrocarpa)***
	*Browallia cordata**
	*Br. roezli**
	*Capsella grandiflora**
	Diplotaxis viminea
	*Erodium ciconium**
	*E. gruinum**
	*E. malacoides**
	*E. manescavi**
	*E. moschatum**
	Lycopersicon esculentum cv. *Kecskeméti 363**
	L. esculentum cv. *Kecskeméti konzerv**
	L. esculentum cv. *Pécs gyöngye**
	*Nicotiana chinensis**
	*N. knightiana**
	*N. quadrivalvis**
	N. tabacum cv. *Debreceni**
	N. tabacum cv. *Érdi**
	N. tabacum cv. *Hevesi**
	N. tabacum cv. *Kerti**

(Continued 2)

Viruses[1]	In local and systemic host-virus relations[2]
	N. tabacum cv. *Szabolcsi**
	*Ocimum canum**
	*O. sanctum**
TYMV	*Brassica adpressa***
	*Bunias orientalis**
	*Capsella grandiflora**
	*Crambe abyssinica**
	Diplotaxis erucoides
	D. viminea
RMV	*Diplotaxis erucoides**
CLRV	*Beta lomatogona***
TRSV	*Gomphrena decumbens***
	*Helianthus maximiliani***
	*H. tomentosus***
	Solanum rostratum
AMV	*Beta macrocarpa (B. vulgaris* ssp. *macrocarpa)***
	Pentstemon alpinus
	Physalis peruviana var. *macrocarpa**
	*P. pruinosa**
TNV	*Cucurbita pepo* convar. *patissonina* f. *radiata***
BBWV	*Ocimum gratissimum**
	O. selloi
	O. viride

[1] TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; PVX, potato virus X; PAMV, potato aucuba mosaic virus; PVS, potato virus S; PVY, potato virus Y; CMV, cucumber mosaic virus; TYMV, turnip yellow mosaic virus; RMV, radish mosaic virus; CLRV, cherry leaf roll virus; TRSV, tobacco ring spot virus; AMV, alfalfa mosaic virus; TNV, tobacco necrosis virus; BBWV, broad bean wilt virus.

[2] Plants marked one asterisk are only locally latent, and plants marked two asterisks are only systemically latent in the local and systemic host-virus relations. Plants without asterisk(s) are equally locally and systemically latent.

of latent host-virus relations was about 13.1 per cent (172 latent infections of 1312 susceptible host-virus relations).

Latent local host-virus relations were pointed out between 6 viruses and 24 plants (Table 7). Particularly remarkable is the latent local susceptibility of *Erodium* spp. and *Cucurbita pepo* convar. *patissonina* f. *radiata* to tobacco mosaic virus, and of *Solanum rostratum* to cucumber mosaic virus. Local and systemic relations were found to be latent between 14 viruses and 118 plants (Table 8). In the systemic host-virus relation latent infection was detected with 6 viruses and 22 plants (Table 9). Special attention should be paid to those plants (e.g. *Ocimum canum*, *O. selloi*, *Solanum ochroleucum*, *Beta macrocarpa*, *Brassica adpressa*, *Beta lomatogona*, *Gomphrena decumbens*, *Helianthus maximilliani*, *H. tomentosus*, *Cucurbita pepo* convar. *patissonina* f. *radiata*, *Atropa bella-donna*,

Table 9

Latent (symptomless) virus infection on various new host plants after artificial inoculation by different viruses

Viruses[1]	In systemic host-virus relations
PVX	*Geranium dissectum*
PAMV	*Datura carolinianum*
	D. ceratocaula
	D. fastuosa cv. *Alba*
	D. gigantea (D. tatula)
	D. godronii cv. *Minka*
	D. rosei
	D. stramonium var. *chalybea (D. tatula)*
	D. stramonium f. *inermis*
	Nicotiana tabacum cv. *Ambalema*
	N. tabacum cv. *Bel 61—10*
	N. tabacum cv. *Hicks Fixed A2—426*
	N. tabacum cv. *Xanthi-nc*
	N. texana
PVM	*Atropa bella-donna*
PVS	*A. bella-donna*
	Lycopersicon glandulosum
	L. hirsutum
	L. peruvianum
TuMV	*Tinantia erecta (T. fugax)*
CMV	*Geranium dissectum*
	G. sibiricum

[1] PVX, potato virus X; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; TuMV, turnip mosaic virus; CMV, cucumber mosaic virus.

Tinantia erecta, *Geranium dissectum*, *G. sibiricum*, *Lycopersicon glandulosum*, *L. hirsutum*, *L. peruvianum*, *Nicotiana tabacum* cv. *Xanthi-nc* etc.) as showing latent systemic infection (see Tables 8 and 9).

New Non-host Virus Relations

Among the host-virus relations between the 246 plants and 23 viruses included in the experiments 664 were found to be incompatible (Table 10). In the course of the *Malva* vein clearing virus tests resistant plants have not been detected. In the new incompatible host-virus relations those plants are of special importance which can be reckoned with as resistance sources (genetic donors). Among the genetic donors outstanding role is played by *Solanum acaule*, *S. cardiophyllum* and *S. vernei* which are hypersensitive to tobacco mosaic virus, a pathogen of potato. A singular plant, *Phaseolus vulgaris* No. 2316, is considered to be important

Table 10

New resistant plants against different viruses

Plants[1]	Family[2]	Viruses[3]
Ammi majus	Umb=Api	BMV, PVY, TuMV
A. visnaga[0]	Umb=Api	BCMV, BMV, PVY, RMV, TuMV
Aptenia cordifolia	Aiz	BCMV, CMV, PAMV, PVY, RMV, TYMV
Atropa bella-donna	Sol	BCMV, BYMV, CLRV, RMV, TRSV, TuMV, TYMV, WMV
Ballota foetida	Lab=Lami	WMV
Basella rubra[0]	Basel	CeMV
Beta lomatogona	Chen	BMV, PVY
B. macrocarpa	Chen	BCMV, BYMV, PVY, TuMV, TYMV, WMV
B. trigyna	Chen	TuMV, WMV
Brassica adpressa	Cru=Brass	WMV
B. chinensis	Cru=Brass	WMV
Browallia cordata[0]	Sol	AMV, BCMV, PVM, PVS, PVY, TYMV
B. demissa	Sol	BCMV, PVM, PVS, TYMV
B. grandiflora	Sol	AMV, BCMV, PVM, PVS, TYMV
B. roezli[0]	Sol	AMV, BCMV, PVM, PVS, PVY, TYMV
B. viscosa	Sol	AMV, BCMV, PVM, PVS, TYMV
Bryonia alba	Cuc	AMV, BCMV, CLRV, PAMV, PVX, PVY, RMV, TRSV, TuMV, TYMV
B. dioica	Cuc	AMV, BCMV, CLRV, PAMV, PVX, PVY, RMV, TRSV, TuMV, TYMV
Capsicum annuum	Sol	WMV
C. annuum cv. *Markgärtner*[0]	Sol	TuMV
Cassia tora	Fab=Legu=Pap	CLRV
Centaurea phrygia[0]	Comp=Aster	PVY
Cestrum parqui[0]	Sol	PVX
Cheiranthus cheiri	Cru=Brass	BYMV, PAMV, PVM, PVS, PVX, PVY, TNV, ToMV, WMV
Chenopodium amaranticolor	Chen	BMV
C. anthelminticum	Chen	PVM
C. bonus-henricus	Chen	PVM
C. botrys	Chen	PAMV
C. foetidum	Chen	PVM
C. murale	Chen	PVY
Citrullus lanatus	Cuc	CLRV
Commelina clandestina[0]	Com	BCMV, BYMV, PAMV, PVM, PVS, PVY, RMV, TRV, TuMV, TYMV
C. coelestis	Com	PAMV

(Continued 1)

Plants[1]	Family[2]	Viruses[3]
C. communis	Com	BCMV, BYMV, PVM, PVS, PVY, RMV, TRV, TuMV, TYMV, WMV
C. graminifolia[0]	Com	BCMV, BYMV, PAMV, PVM, PVS, PVY, RMV, TRV, TuMV, TYMV, WMV
C. tuberosa[0]	Com	BCMV, BYMV, PAMV, PVM, PVS, PVY, RMV, TRV, TuMV, WMV
Crambe abyssinica	Cru=Brass	CMV, PAMV, PVX, PVY
C. armena[0]	Cru=Brass	CMV, PAMV, PVX, RMV, TYMV
C. cordifolia[0]	Cru=Brass	CMV, PAMV, PVX, PVY, RMV, TYMV
C. hispanica[0]	Cru=Brass	CMV, PAMV, PVX, PVY, RMV, TYMV
C. maritima	Cru=Brass	CMV, PAMV, PVX, PVY, RMV, TYMV
C. orientalis[0]	Cru=Brass	CMV, PAMV, PVX, PVY, RMV, TYMV
C. tatarica[0]	Cru=Brass	CMV, PAMV, PVX, PVY, RMV, TYMV
Cucumis myriocarpus	Cuc	BCMV, CLRV, CMV, PAMV, PVM, PVS, PVY, RMV, TYMV
Cucurbita pepo	Cuc	CLRV
C. pepo convar. *patissonina*[0] f. *radiata*	Cuc	AMV, BCMV, BMV, BYMV, CLRV, PAMV, PVM, PVS, PVX, PVY, TRV, TuMV
Cyamopsis tetragonoloba	Fab=Legu=Pap	PVY
Datura arborea	Sol	PVY
D. fastuosa[0]	Sol	PVY
D. godronii[0]	Sol	BCMV, CeMV, CLRV, PVY, RMV, TYMV, WMV
D. rosei[0]	Sol	CeMV, WMV
D. stramonium	Sol	BCMV, WMV
D. stramonium var. *chalybea*	Sol	BCMV, BYMV
D. stramonium f. *inermis*[0]	Sol	CeMV, BCMV, BYMV, PVY
D. tatula[0]	Sol	BCMV, BYMV, PVY
Diplotaxis viminea[0]	Cru=Brass	PVX
Emex spinosa[0]	Poly	BBWV
Erodium ciconium[0]	Ger	BCMV, BMV, PVY, TRSV, TYMV, WMV
E. cicutarium	Ger	BCMV, PVY, TRSV, TYMV, WMV
E. gruinum	Ger	BCMV, BMV, PVX, PVY, TRSV, TYMV
E. malacoides[0]	Ger	BMV, WMV
E. manescavi[0]	Ger	BCMV, BMV, PVX, PVY, TRSV, TYMV

(Continued 2)

Plants[1]	Family[2]	Viruses[3]
E. moschatum	Ger	BCMV, BMV, PVX, PVY, TRSV, TYMV
Galega hartlandii[0]	Fab=Legu=Pap	PVM, PVS
G. officinalis	Fab=Legu=Pap	CLRV, PVM, PVS
Geranium columbianum[0]	Ger	CMV, PAMV, PVY
G. cristatum[0]	Ger	CMV, PAMV, PVY
G. dissectum	Ger	PAMV, PVY
G. lucidum[0]	Ger	CMV, PAMV, PVY
G. molle	Ger	CMV, PAMV, PVY
G. pratense	Ger	CMV, PAMV, PVY
G. pusillum[0]	Ger	CMV, PAMV, PVY
G. pyrenaicum[0]	Ger	BMV, CMV, PAMV, PVY
Geranium robertianum	Ger	BMV, CMV, PAMV, PVY
G. rotundifolium	Ger	BMV, CMV, PAMV, PVY
G. sibiricum[0]	Ger	BMV, PAMV, PVY
Gomphrena decumbens[0]	Ama	BCMV, BMV, CeMV, CLRV, PAMV, PVY, RMV, TYMV, WMV
Helianthemum nummularium	Cist	BMV
Helianthus atrorubens[0]	Comp=Aster	BMV
H. californicus[0]	Comp=Aster	BMV
H. cernuus[0]	Comp=Aster	BMV
H. decapetalus[0]	Comp=Aster	BMV
H. doronicoides[0]	Comp=Aster	BMV
H. giganteus[0]	Comp=Aster	BMV
H. grosse-serratus[0]	Comp=Aster	BMV
H. maximiliani[0]	Comp=Aster	BMV
H. mollis[0]	Comp=Aster	BMV
H. organophyllus[0]	Comp=Aster	BMV
H. salicifolius[0]	Comp=Aster	BMV
H. tomentosus[0]	Comp=Aster	BMV
H. trachelifolius[0]	Comp=Aster	BMV
Leiophyllum buxifolium[0]	Eri	CMV, WMV
Luffa cylindrica	Cuc	WMV
Lycopersicon esculentum cv. *Red Cherry*	Sol	PVS
L. humboldtii	Sol	PVS
L. pimpinellifolium	Sol	PVS
L. pyriforme	Sol	PVS
L. racemiflorum[0]	Sol	PVS
L. racemigerum	Sol	PVS
Nicandra physaloides	Sol	PVS
Nicotiana acuminata	Sol	PVM, PVS
N. alata	Sol	PVM, PVS
N. chinensis	Sol	CeMV, PVM, PVS, RMV, TYMV, WMV
N. fragrans	Sol	PVM, PVS
N. glutinosa	Sol	CLRV
N. knightiana	Sol	CeMV, PVM, PVS, RMV, TYMV, WMV

(Continued 3)

Plants[1]	Family[2]	Viruses[3]
N. langsdorffii	Sol	PVM, PVS
N. longiflora	Sol	PVM, PVS
N. occidentalis	Sol	CeMV, TYMV
N. paniculata	Sol	PVM, PVS
N. plumbaginifolia	Sol	PVM, PVS
N. quadrivalvis	Sol	CeMV, PVM, PVS, TYMV, WMV
N. rustica	Sol	PVM
N. sanderae	Sol	PVM, PVS
N. solanifolia	Sol	PVM, PVS
N. sylvestris	Sol	PVS
N. tabacum cv. *Bel 61—10*	Sol	CeMV, PVM, PVS, TYMV, WMV
N. tabacum cv. *Xanthi-nc*	Sol	PVM, PVS
Nolana paradoxa[0]	Nol	LMV
N. protrata[0]	Nol	LMV
Obione sibirica[0]	Chen	BCMV, CeMV, TYMV
Ocimum basilicum	Lab=Lami	CeHV, CLRV, PVM
O. canum	Lab=Lami	BCMV, CeMV, CLRV, LMV, PVM, PVS, PVY, RMV, TYMV, WMV
O. sanctum[0]	Lab=Lami	CeMV, CLRV, PVM, PVS, PVY, RMV, TYMV, WMV
Paulownia fargesii[0]	Scrop	AMV, BCMV, CLRV, PVM, PVS, PVY, RMV, TYMV
P. imperialis	Scrop	BCMV, CLRV, PVM, PVS, PVY, RMV, TYMV
Pentstemon alpinus[0]	Scrop	CMV, PVY
P. attenuatus[0]	Scrop	CMV, PVY
P. calycosus[0]	Scrop	CMV, PVY
P. cardinalis[0]	Scrop	CMV, PVY
P. hirsutus[0]	Scrop	CMV
P. laevigatus[0]	Scrop	CMV, PVY
P. ovatus[0]	Scrop	CMV, PVY
P. whippleanus[0]	Scrop	CMV, PVY
Petunia atkinsiana[0]	Sol	CeMV, PVM, PVS, RMV, TYMV, WMV
P. axillaris	Sol	PVM, PVS, RMV, TYMV, WMV
P. hybrida	Sol	CLRV, PVM
P. hybrida hort. cv. *Rose de Haven amélioré*[0]	Sol	CLRV, PVM, PVS, RMV
P. parviflora[0]	Sol	CLRV, PVM, PVS, WMV
P. violacea	Sol	PVM, PVS, RMV, TYMV, WMV
Phaseolus aureus	Fab=Legu=Pap	PAMV, PVY
P. lunatus	Fab=Legu=Pap	BMV
P. vulgaris cv. *Annelise*	Fab=Legu=Pap	TMV, PVS
P. vulgaris cv. *Barnabab*[0]	Fab=Legu=Pap	TMV, PVS
P. vulgaris cv. *Caroline*	Fab=Legu=Pap	TMV
P. vulgaris cv. *Cordon*	Fab=Legu=Pap	PVS
P. vulgaris cv. *Falomiteana*	Fab=Legu=Pap	PVS

(Continued 4)

Plants[1]	Family[2]	Viruses[3]		
P. vulgaris cv. *Fertődi 5.*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Fullcrop*	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *GN 59.*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *GN 123.*	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Harkovszkaja*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Icar Fundulea 416*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Japán gyöngybab*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Kanizsai csíkosbab*[0]	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Kentucky Wonder*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Kereskedelmi hosszú fürjbab*[0]	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Kinghorn Wax*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Korai fürj*[0]	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Michelite*	Fab=Legu=Pap	PVS		
P. vulgaris No. *2316*[0]	Fab=Legu=Pap	BCMV		
P. vulgaris cv. *Olomucka Zelenoluska*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Őrségi cseresznyebab*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Perlicska*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Pinto*	Fab=Legu=Pap	BMV,	PVS,	ToMV
P. vulgaris cv. *Prinzess*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Processor*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Red Kidney*	Fab=Legu=Pap	BMV, RMV,	CMV, TMV,	PAMV, PVS, ToMV
P. vulgaris cv. *Refugee*	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Robust*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Soproni lapos*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Sztepnaja*	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Tápiószelei barna*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Tápiószelei fürj*[0]	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Tápláni fekete 'cirádás' fürjbab*[0]	Fab=Legu=Pap	PVS,	TMV	
P. vulgaris cv. *Tápláni nagyszemű cseresznyebab*[0]	Fab=Legu=Pap	TMV		
P. vulgaris cv. *Tétényi cukorbab*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Tétényi gyöngybab*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Tétényi középbab*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Tétényi nagyszemű fehér*[0]	Fab=Legu=Pap	PVS		
P. vulgaris cv. *Wade*	Fab=Legu=Pap	PVS		
Physalis aequata	Sol	BCMV, PVS, RMV, TMV, TYMV		
P. alkekengi	Sol	BCMV, PVM, PVS, RMV, TYMV		
P. angulata	Sol	BCMV, PVM, PVS, RMV, TNV, TYMV		
P. floridana	Sol	BCMV, PVM, RMV, TYMV		
P. ixocarpa	Sol	BCMV, PVM, PVS, RMV, TYMV		

(Continued 5)

Plants[1]	Family[2]	Viruses[3]
P. peruviana	Sol	BCMV, PVM, PVS, RMV, TNV, TYMV
P. peruviana var. *macrocarpa*[0]	Sol	BCMV, PVM, PVS, RMV, TNV, TYMV
P. philadelphica	Sol	BCMV, RMV, TYMV
P. pruinosa	Sol	BCMV, PVM, PVS, RMV, TNV, TYMV
P. pubescens	Sol	BCMV, RMV, TYMV
P. viscosa	Sol	BCMV, PVS, RMV, TYMV
Rhoeo discolor	Com	BMV
Rorippa islandica	Cru=Brass	BMV
Saponaria cerastioides[0]	Cary	WMV
S. officinalis	Cary	CLRV
Silene armeria	Cary	BMV
S. conica[0]	Cary	BMV
S. dichotoma	Cary	BMV
S. gallica[0]	Cary	BMV
S. pendula	Cary	BMV
S. tatarica[0]	Cary	BMV
Solanum acaule	Sol	TMV
S. acroscopium	Sol	TuMV
S. ajanhuiri[0]	Sol	TuMV
S. boliviense[0]	Sol	TuMV
S. brachycarpum[0]	Sol	TuMV
S. brevicaule[0]	Sol	TuMV
S. brevidens[0]	Sol	TuMV
S. capsicastrum	Sol	BCMV, BYMV, RMV, TuMV, TYMV
S. cardiophyllum	Sol	TMV
S. chacoense	Sol	TuMV
S. demissum A6-hybrid	Sol	BMV
S. demissum Redd 530-hybrid[0]	Sol	BMV
S. demissum Stamm S-hybrid[0]	Sol	BMV
S. ehrenbergii[0]	Sol	TuMV
S. gourlayi	Sol	TuMV
S. hjertingii[0]	Sol	TuMV
S. hougasii[0]	Sol	TuMV
S. infundibuliforme[0]	Sol	TuMV
S. leptophyes[0]	Sol	TuMV
S. ochroleucum	Sol	BCMV, BYMV, RMV, TuMV, TYMV
S. rostratum	Sol	BCMV, CLRV, RMV, TYMV
S. simplicifolium	Sol	TuMV
S. stoloniferum	Sol	TuMV
S. vernei	Sol	TMV, TuMV
Tetragonia crystallina[0]	Aiz	AMV, BCMV, BMV, CeMV, PVM, PVS, PVY, TYMV
T. echinata	Aiz	BCMV, BMV, CeMV, PVM, PVS, TYMV

(Continued 6)

Plants[1]	Familyi[2]	Viruses[3]
T. tetragonoides	Aiz	BCMV
Tinantia erecta[0]	Com	BCMV, CMV, PAMV, PVS, RMV, TYMV
Trechonaetes sativa[0]	Sol	TRSV
Tropaeolum majus	Trop	PVY
T. minus	Trop	BMV, WMV
T. minus cv. *Cherry rose*[0]	Trop	WMV
T. peltophorum[0]	Trop	BMV, WMV
T. peregrinum	Trop	BMV, WMV
Urtica urens	Urt	BYMV
Vaccaria segetalis[0]	Cary	BMV
Vicia faba cv. *Dornburger*[0]	Fab=Legu=Pap	PVY
V. faba cv. *Dreifachweise*[0]	Fab=Legu=Pap	PVY
V. faba cv. *Erfurter Gewöhnliche*[0]	Fab=Legu=Pap	PVY
V. faba cv. *Hangdown*[0]	Fab=Legu=Pap	PVY
Vigna catjang[0]	Fab=Legu=Pap	ToMV
V. sinensis var. *sesquipedalis*[0]	Fab=Legu=Pap	BMV, ToMV
Viscaria vulgaris	Cary	BMV
Vitis vinifera	Vita	CLRV

[1] Plants marked [0] are new experimentally plan ts in plant virology.

[2] Aiz, *Aizoaceae;* Ama, *Amaranthaceae;* Basel, *Basellaceae;* Cary, *Caryophyllaceae;* Chen, *Chenopodiaceae;* Cist, *Cistaceae;* Com, *Commelinaceae;* Comp=Aster, *Compositae (Asteraceae);* Cru=Brass, *Cruciferae (Brassicaceae);* Cuc, *Cucurbitaceae;* Eri, *Ericaceae;* Fab=Legu=Pap, *Fabaceae (Leguminosae, Papilionaceae);* Ger, *Geraniaceae;* Lab=Lami, *Labiatae (Lamiaceae);* Nol, *Nolanaceae;* Poly, *Polygonaceae;* Scrop, *Scophullariaceae;* Sol, *Solanaceae;* Umb=Api, *Umbelliferae (Apiaceae);* Urt, *Urticaceae;* Trop, *Tropaeolaceae;* Vita, *Vitaceae.*

[3] AMV, alfalfa mosaic virus; BBWV, broad bean wilt virus; BCMV, bean common mosaic virus; BMV, belladonna mottle virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; LMV, lettuce mosaic virus; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; PVX, potato virus X; PVY, potato virus Y; RMV, radish mosaic virus; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; ToMV, tomato mosaic virus; TRSV, tobacco ring spot virus; TRV, tobacco rattle virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV, watermelon mosaic virus.

as a source of resistance of its hypersensitivity to bean common mosaic virus. On the basis of their hypersensitive reactions *Solanum acroscopicum*, *S. ajanhuiri*, *S. boliviense*, *S. brachycarpum*, *S. brevicaule*, *S. brevidens*, *S. ehrenbergii*, *S. gouriayi*, *S. hjertingii*, *S. hougasii*, *S. infundibuliforme*, *S. leptophyes* proved to be highly important sources of resistance to potato virus Y.

The potato virus Y immunity of *Solanum chacoense*, *S. simplicifolium*, *S. stoloniferum* and *S. vernei* is of particular significance. A survey of the incompatible and resistant host-virus relations (Table 11) reveals that the largest number

Table 11

New incompatible host-virus relations

Viruses[1]	Number of the resistant plants	Literature
TRV	*5*	Horváth (1978a)
TMV	*22*	Horváth (1978b)
ToMV	*5*	Horváth (1978b)
PVX	*16*	Horváth (1978c)
PAMV	*33*	Horváth (1978c)
PVM	*53*	Horváth (1978d)
PVS	*86*	Horváth (1978d)
BCMV	*51*	Horváth (1979a)
BYMV	*14*	Horváth (1979b)
CeMV	*17*	Horváth (1979a)
LMV	*3*	Horváth (1979b)
PVY	*65*	Horváth (1979c)
TuMV	*31*	Horváth (1979c)
WMV	*35*	Horváth (1979c)
CMV	*29*	Horváth (1979d)
BMV	*51*	Horváth (1979e)
TYMV	*57*	Horváth (1979e)
RMV	*44*	Horváth (1979f)
CLRV	*23*	Horváth (1979g)
TRSV	*9*	Horváth (1979h)
AMV	*9*	Horváth (1981a)
TNV	*5*	Horváth (1982a)
BBWV	*1*	Horváth (1982b)
Sum total	*664*	

[1] TRV, tobacco rattle virus; TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; PVX, potato virus X; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; BCMV, bean common mosaic virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; LMV, lettuce mosaic virus; PVY, potato virus Y; TuMV, turnip mosaic virus; WMV, watermelon mosaic virus; CMV, cucumber mosaic virus; BMV, belladonna mottle virus; TYMV, turnip yellow mosaic virus; RMV, radish mosaic virus; CLRV, cherry leaf roll virus; TRSV, tobacco ring spot virus; AMV, alfalfa mosaic virus; TNV, tobacco necrosis virus; BBWV, broad bean wilt virus.

of resistant plants have been found in the case of infection by potato virus S (86), potato virus Y (65), turnip yellow mosaic virus (57), potato virus M (53) and bean common mosaic virus (51).

New Hosts and Non-hosts as Separators of Viruses

It is not only in determining the host ranges of viruses, identifying the viruses and exploring the scopes of virus epidemiology, virus geography and virus ecology that the new host plants and new resistant plants play an outstanding

Table 12

New hosts and non-hosts as separators of different viruses

Viruses[1]	Number of suitable plants for separation	Possible combinations of separation by different viruses[2]	Literature
TRV	26	*154*/3, 5–9, 11, 13–24	Horváth (1978a)
TMV	133	*583*/1, 3–9, 11, 13–24	Horváth (1978b)
ToMV	68	*441*/1–2, 4–9, 11, 13–24	Horváth (1978b)
PVX	66	*313*/1–3, 5–9, 11, 13–24	Horváth (1978c)
PAMV	86	*419*/1–4, 6–9, 11, 13–24	Horváth (1978c)
PVM	105	*737*/1–5, 7–9, 11, 13–24	Horváth (1978d)
PVS	94	*739*/1–6, 8–9, 11, 13–24	Horváth (1978d)
BCMV	53	*610*/1–7, 9, 11, 13–24	Horváth (1979a)
CeMV	17	*239*/1–8, 11, 13–16, 18–24	Horváth (1979a)
MVCV	1	*3*/1–2, 15	Horváth (1979a)
BYMV	29	*190*/1–8, 13–24	Horváth (1979b)
LMV	5	*52*/1–9, 13, 15–22, 24	Horváth (1979b)
PVY	130	*639*/1–9, 11, 14–24	Horváth (1979c)
TuMV	25	*218*/1–9, 13, 15–24	Horváth (1979c)
WMV	35	*358*/1–9, 11, 13–14, 16–24	Horváth (1979c)
CMV	91	*416*/1–9, 11, 13–15, 17–23	Horváth (1979d)
TYMV	60	*658*/1–7, 11, 13–16, 18–24	Horváth (1979e)
BMV	74	*330*/1–9, 11, 13–17, 19–24	Horváth (1979e)
RMV	62	*576*/1–9, 11, 13–18, 20–24	Horváth (1979f)
CLRV	51	*403*/1–9, 11, 13–19, 21–24	Horváth (1979g)
TRSV	77	*393*/1–9, 11, 13–20, 22–23	Horváth (1979h)
AMV	81	*381*/1–9, 11, 13–21, 23–24	Horváth (1981a)
TNV	59	*575*/1–9, 11, 13–22, 24	Horváth (1982a)
BBWV	21	*131*/1–9, 11, 13–23	Horváth (1982b)

[1] TRV, tobacco rattle virus (1); TMV, tobacco mosaic virus (2); ToMV, tomato mosaic virus (3); PVX, potato virus X (4); PAMv, potato aucuba mosaic virus (5); PVM, potato virus M (6); PVS, potato virus S (7); BCMV, bean common mosaic virus (8); CeMV, celery mosaic virus (9); MVCV, *Malva* vein clearing virus (10); BYMV, bean yellow mosaic virus (11); LMV, lettuce mosaic virus (12); PVY, potato virus Y (13); TuMV, turnip mosaic virus (14); WMV, watermelon mosaic virus (15); CMV, cucumber mosaic virus (16); TYMV, turnip yellow mosaic virus (17); BMV, belladonna mottle virus (18); RMV, radish mosaic virus (19); CLRV, cherry leaf roll virus (20); TRSV, tobacco ring spot virus (21); AMV, alfalfa mosaic virus (22); TNV, tobacco necrosis virus (23); BBWV, broad bean wilt virus (24).

[2] Possible combinations of virus separation (in numerator) and the separable viruses (in denominator). See the numbered viruses in the foot-note 1.

role, they are also very important in separation the different viruses and isolation authentic viruses. In the case of the 24 viruses included in the experiments 9558 separation combinations were pointed out (Table 12). The number of plants suitable for separation was the largest (see Table 12) in the case of tobacco mosaic virus (133), potato virus Y (130), and potato virus M (105), while the separation combinations (possibilities) were the richest with potato virus S (739), potato

Table 13

Impossible separations between different viruses by the newly investigated experimental plants[1]

Investigated viruses	Not separable viruses
TMV, PVX, MVCV, LMV	TRV
MVCV, LMV	TMV
MVCV, LMV	ToMV
MVCV, LMV	PVX
MVCV, LMV	PAMV
MVCV, LMV	PVM
MVCV, LMV	PVS
MVCV, LMV	BCMV
MVCV, LMV, TYMV	CeMV
ToMV, PVX, PAMV, PVM, PVS, BCMV, CeMV, BYMV, LMV, PVY, TuMV, CMV, TYMV, BMV, RMV, CLRV, TRSV, AMV TNV, BBWV	MVCV
CeMV, MVCV, LMV	BYMV
MVCV, BYMV, TuMV, TNV	LMV
MVCV, LMV	PVY
MVCV, BYMV, LMV	TuMV
MVCV, LMV	WMV
MVCV, LMV, BBWV	CMV
BCMV, CeMV, MVCV, LMV	TYMV
MVCV, LMV	BMV
MVCV, LMV	RMV
MVCV, LMV	CLRV
MVCV, LMV, BBWV	TRSV
MVCV, LMV	AMV
MVCV, LMV	TNV
MVCV, LMV	BBWV

[1] Abbreviated viruses: AMV, alfalfa mosaic virus; BBWV, broad bean wilt virus; BCMV, bean common mosaic virus; BMV, belladonna mottle virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; LMV, lettuce mosaic virus; MVCV, *Malva* vein clearing virus; PAMV, potato aucuba mosaic virus; PVM, potato virus M; PVS, potato virus S; PVX, potato virus X; PVY, potato virus Y; RMV, radish mosaic virus; TMV, tobacco mosaic virus; TNV, tobacco necrosis virus; ToMV, tomato mosaic virus; TRSV, tobacco ring spot virus; TRV, tobacco rattle virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV, watermelon mosaic virus.

virus M (737), turnip yellow mosaic virus (658), potato virus Y (639), bean common mosaic virus (610), tobacco mosaic virus (583), radish mosaic virus (576) and tobacco necrosis virus (575).

The new experimental plants studied by us made it possible to separate 15 from 21-, 5 from 20-, 3 from 19- and 1 from 3 viruses (see Table 12). Between

the viruses essential differences were shown in separability. According to Table 13 in the case of the viruses and plants studied by us lettuce mosaic virus can be separated from 19, *Malva* vein clearing virus from 3 viruses, but not a single virus is separable from either lettuce mosaic virus or *Malva* vein clearing virus. The extension of investigations into the host-virus relations renders it possible to describe plants suitable for separation (separators) be which even those viruses as have so far been difficult to differentiate will be separable. The spontaneous appearance of new host-virus relations in nature (e.g. *Phaseolus vulgaris* and cucumber mosaic virus) makes it necessary to carry on studying the possibilities of virus separation. One of the important tasks is e.g. the reliable separation of cucumber mosaic virus – a pathogen recently occurring in *Phaseolus vulgaris* in Hungary too (cf. Schmidt and Horváth, 1982; Horváth and Beczner, 1983; Horváth et al., 1983) – from other virus pathogens of bean (e.g. bean common mosaic virus, bean yellow mosaic virus).

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Mass Occurrence of Yponomeuta *(=Hyponomeuta) padellus* Linné (Lep., *Hyponomeutidae*) in 1982 at Hévíz

Short communication

GY. SÁRINGER, J. HORVÁTH and K. ZSOÁR

University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P. O. Box 71, Hungary

On 9 June 1982 the leaves of *Prunus avium* Mill., shrubs and tress found in large numbers in the wet gallery forest surrounding the lake of Hévíz (Western Hungary) were totally nibbled off by the larvae of *Y. padellus* L. The branches and trunks of the tress and shrubs were webbed by the larvae so perfectly that they gave the impression of being covered by a plastic film of silves colour. The continuous web could be removed in one piece from the branches and trunks and could have even been cut into stripes by scissors. Most branches were entwinded by a gauzy curtain of web. At the sites of branching, and particularly on the ground round the trunk thousands, or even tens of thousands of spindle-shaped pupae were set close side by side in a sac of web. Larvae no longer existed at that time, all individuals of the population were in pupal stage.

We collected a large number of pupae for rearing them under laboratory conditions. The hatching of adults in the laboratory began on 20 June and lasted 15 days; 87.5 per cent of the adults swarmed in the first five days. The adults were identified by Dr. László Gozmány and Dr. András Vojnits, lepidopterologists at the Museum of Natural Sciences, Budapest.

In the course of the laboratory rearing an extremely large number of parasites were also identified, with two *Tachimidae* species, namely *Bessa parallela* Meigen and *Zenillia dolosa* Meigen (det.: Dr. Mihányi F.) among them. The former infected 32.1, the latter 28.3 per cent of the pupae.

Of the species *Z. dolosa* Meigen we have to note that according to our present knowledge it has been a rare species in Hungary; in the collection of the Museum of Natural Sciences (Budapest) altogether 4 specimens have been kept so far. With the Hévíz material the collection has become much richer.

From about 13.4 per cent of the pupae so far unidentified species belonging to the family *Ichneumonidae* hatched too. According to Martouret (in: Balachowsky, 1966) *Pimpla examinator* F. and *P. maculator* F. *(Ichneumonidae)* parasitize the *Y. padellus*, while Schwenke (1978) writes about *Pimpla turionella* L. as the most frequent *Ichneumonidae* parasite of *Y. padellus*.

After discovering the damage at Hévíz we followed the population with attention. We found that the moths had all swarmed by 25 June, and the trees and shrubs resumed sprouting. At the end of June a considerable number of leaves

could be seen already on the trees. At that time (early in the afternoon) moths sitting motionless on the trunks and branches were observed.

Mass occurrences of *Y. padellus* of an extent like that either in orchards or in forests have not been recorded in Hungary. Moreover, its known feed plants do not even include the *Prunus avium* Mill. In the forest entomology of Győrfi (1957) *Prunus spinosa* L., *Crataegus* sp., *Mespilus germanica* L., *Pyrus* sp., wild cherry and *Salix* spp. are listed among the feed plants of *Y. padellus*. Schwenke (1978) mentions the *Prunus* and *Crataegus* species as its feed plants. Balás and Sáringer (1982) write about its "living mainly on plum-trees, though occurring on other stone fruits and on pomiferous plants, thorn-bush and sloe-bush too".

According to Jablonowski (1912), Kovács (1939) and Kadocsa (1943) in Hungary it develops a single generation a year.

Bognár and Huzián (1979) give account of severe foliar damages as seldom occurring in Hungary. The foliar damages of plum-trees that Jablonowski (1912) refers to may have been more or less serious.

About the causes of gradation little is known for the time being. According to Schwenke (1978) mass reproduction of *Y. padellus* occurs every 10 years or even with longer intervals.

As to the mass occurrence at Hévíz, the development of the large number of larvae was probably made possible by the favourable conditions of the ecological factors, first of all of temperature and precipitation. In 1982 the summer was dry. The favourable weather conditions at the time of egg laying by the adults as well as during the overwintering period of young larvae may also have played some role. The winter of 1981–82 was relatively mild. The individual numbers in the following years will certainly be influenced by the extent of parasite infestation which was 73.8 per cent in 1982, as well as by the winter temperature of 1982–83. The latter can be regarded as positively mild, therefore from a gradological point of view the trend of the individual number of *Y. padellus* L. at Hévíz will be worth being followed with attention in 1983 too.

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Effect of Chlorflurenol on Yield and Technological Value of Red Pepper (Capsicum Annuum L.)

S. Łukasik,[1] B. Achremowicz,[2] D. Kulpa,[2] and T. Frączek[2]

It was found that treatment with chlorflurenol individual flowers and whole plants of pepper in full blooming caused an increase of the total number and weight of fruit. However, individual fruit from plants treated with chlorflurenol had a smaller weight than fruit from control plants. Under the influence of chlorflurenol fruit maturity time was shortened from 3 to 5 days as compared with control plants. On the plants treated with chlorflurenol seedless fruit were developed. In fruit from plants treated with chlorflurenol increased the content of vitamin C, raw protein, dry matter, total extract and total sugar. Chlorflurenol caused the decreased content β-carotene in pepper fruit.

Chlorflurenol has already been used in the production of pickling cucumbers (Schneider 1974). It seemos that there is a possibility to use this compound in tomato production, especially with the once-over machine harvest technique for processing industry (Rudich and Rabinowitch, 1974).

In this paperan an attempt was made to use chlorflurenol to increase the yield of sweat pepper. Technological value of obtained fruit was evaluated to utilize it in processing.

Materials and Methods

The experiments on the Poznańska Słodka variety were carried out in the years 1978 and 1980 in the greenhouse (Lublin-Felin).

In 1978 the temperature in the greenhouse (non-heated) was very unfavourable for the growth and crop of pepper and it oscillated between 5–15°C at night and 10–30°C during the day. And in 1980 the air temperature in the greenhouse was on the normal level and was 15–18°C at night and 22–28°C during the day. The plants were grown in Mitscherlich's pots filled with hotbed soil in the period from April to September.

Chlorflurenol (CME 74 050 P) was applied in solutions having the concentration 0.25; 0.50 and 1.00 ml/l. In 1978 individual fully open flowers were immersed one time in chlorflurenol successively with their blooming and in 1980 the whole plants in full bloom were sprayed with the preparation. 0.001% Tween 80 was

[1] Institute of Horticultural Production, Agricultural Academy, 20-069 Lublin, ul. Leszczyńskiego 7, Poland

[2] Institute of Chemistry and Agricultural Technology, Agricultural Academy, 20-033 Lublin, ul. Akademicka 13, Poland

given to the chlorflurenol solution. The control plants were treated only with Tween 80.

The experiments were arranged in completely randomised design. Each combination in 1978 included 10 plants and in 1980–26 plants. Paper labels were put on peduncles with the hormonization date during chlorflurenol treatment of individual flowers. Fruit from both series of experiments were harvested individually as they matured, determining their weight and date of harvest. On the basis of the data of chlorflurenol treatment and the harvest date, the number of days elapsing from flowers hormonization to the fruit harvest were calculated for all the fruit in 1978.

In the cross-sected fruit the colour of the flesh and extent of chamber filling with seeds were determined.

In the pepper fruit obtained in 1980 from the plants treated with chlorflurenol and control ones, the content of dry matter, total extract, total sugar, raw protein, vitamin C, β-carotene, fibre and raw ash were determined. Six analyses were carried out for all the chemical components except in each combination (3 times from two first fruit harvests). Raw protein was determined in 4 repetitions (2 times from two first harvests). 3–4 fruit were taken for each analysis. The dry matter was determined in the desiccator at 105°C. The total extract was determined with the help of refractometer. The total content of sugar was determined with Samogyi-Nelson's method, and raw protein with Kiejldhal's method. Tilman's method was used to determine vitamin C and β-carotene was examined according to Polish Standards-PN 71/1 7510 (Charłampowicz, 1966). The fibre was determined with Hemmberg-Stohman's method and total ash with sample burning.

The results of the total number and weight of fruit and the results of the chemical analyses were statistically estimated with the help of Duncan's multiple range test.

Results and Discussion

The highest total number of fruit obtained with chlorflurenol application in solutions with the concentration 1.00 ml/l (1978) and 0.25 ml/l (1980). In comparison with the control plants, the number of fruit in the discussed combinations increased on one plant on the average, in 1978 it increased 6.3 fruit and in 1980 – 4.3 fruit. The increase of the total number of fruit was also found in other experiments after one time treatment of flowering plants with methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate in the 1.00 mg/l solutions (Łukasik and Hortynska, 1977). Under the influence of this compound the number of fruit of greater weight was increased in comparison to the control plants. As a result of this commercial and total yield were higher. Similarly in tomatoes treated with chlorflurenol methyl ester increased the total and early yield of fruit (Łukasik, 1977). In these experiments, however, in spite of a considerable increase of fruit number in plants treated with chlorflurenol, no essential increase of total weight

of fruit was noticed. It was caused by the fact that in plants treated with chlorflurenol there were many fruit of the smallest weight (Table 2).

The highest total weight of fruit was obtained with chlorflurenol concentration 0.50 ml/l. There was an increase of one plants, on the average, in this combination in comparison with the control plant; in 1978 the increase was over 61 g and in 1980 over 37 g. In the remaining combinations, in spite of the increase of the total number of fruit, the total fruit weight was on the level of the control plants weight (Table 1).

Under the influence of chlorflurenol there was time shortening of fruit maturity. Treating flowers with solutions of 1.00 ml/l the fruit matured 5 days earlier than in the control plants. Using concentration of 0.25 ml/l the fruit were harvested 3 days earlier than in the control combination (Table 3). The increase of early yield was also noticed when the developed fruit of pepper were treated with etephon (Łukasik and Hortynska, 1976) and with methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate (Łukasik and Hortynska, 1977).

The fruit on the plants treated with chlorflurenol were similar to the control fruit-red coloured. At higher concentrations of chlorflurenol a slight deformation of fruit was noticed. A similar deformation of fruit at high concentrations of morphactin was noticed in tomatoes (Łukasik, 1975).

Under the influence of chlorflurenol developed seedless fruit. The highest percentage of parthenocarpic fruit in the total number of fruit was noticed when chlorflurenol was used in concentration 1.00 ml/l (Table 4). It should be stressed that the development of parthenocarpic fruit was noticed in frequent earlier experiments under the influence of morphactins; on pepper (Jayakaran, 1973), tomatoes (Schneider, 1964; 1970; Łukasik and Huszcza, 1974) and cucumbers (Robinson et al., 1971; Cantlifee et al., 1972; Cantlifee, 1974).

Table 1

Effect of chlorflurenol applied to individual blooming flowers (1978) and to whole plants in bloom (1980) on the yield of Poznańska Słodka variety pepper. Average for one plant

Concentration of chlorflurenol ml/l	Years			
	1978		1980	
	Total number of fruit, pieces[2]	Total weight of fruit, g[1]	Total number of fruit, pieces[3]	Total weight of fruit, g[1]
0.00	2.9	67.0	6.7	267 9
0.25	6.2	114.2	10.4	281.7
0.50	6.5	128.7	9.8	305.7
1.00	9.2	78.3	8.4	256.7

[1] Differences nonsignificant
[2] Differences are significant at $D_{0.05} = 1.09-1.18$
[3] Differences are significant at $D_{0.05} = 1.09-1.19$

Table 2

Percentage of fruit of different weight in total number of fruit in Poznańska Słodka variety pepper treated with chlorflurenol in solutions of different concentration in 1978 and 1980

Concentration of chlorflure-nol, ml/l	Weight of single fruit, g							
	0–19		20–34		35–50		> 50	
	1978	1980	1978	1980	1978	1980	1978	1980
0.00	51.72	18.13	31.03	17.03	17.24	33.52	0.00	31.32
0.25	61.29	48.33	30.64	20.45	8.06	14.87	0.00	16.36
0.50	52.31	47.25	46.15	14.28	1.54	11.35	0.00	27.11
1.00	46.34	38.71	46.34	24.88	7.32	14.28	0.00	22.12

Table 3

Average number of days from chlorflurenol treatment of single flowers to fruit harvest in Poznańska Słodka variety pepper in 1978

Concentration of chlor-flurenol ml/l	Average number of days to maturity of pepper fruit
0,00	68,9
0.25	65.6
0.50	64.2
1.00	63.3

Table 4

Percentage of parthenocarpy in total number of fruit in Poznańska Słodka variety pepper treated with chlorflurenol at different concentrations in 1978 and 1980

Concentration of chlor-flurenol ml/l	Percentage of partheno-carpy	
	1978	1980
0.00	0.0	3.9
0.25	45.8	74.0
0.50	44.6	66.7
1.00	58.5	75.6

The highest amount of dry matter was in the pepper fruit collected from plants treated with chlorflurenol at lowest concentration (0.25 ml/l). In comparison with the control plants the content of the dry matter in the discussed combination increased about 2%. Similarly, fruit collected from plants treated with chlorflurenol at concentration 0.50 and 1.00 ml/l contained 1.15 and 0.60% dry matter more than in the control combination (Table 5).

Table 5

Effect of chlorflurenol on the content of some chemical constituents in fruit of Poznańska Słodka variety pepper treated with different chlorflurenol concentrations

Compounds	Concentration of chlorflurenol, ml/l			
	0.00	0.25	0.50	1.00
Total extract, %[1]	6.60	7.55	7.38	7.30
Dry matter, %[1]	6.65	8.40	7.80	7.25
Total sugar, %[1]	5.40	5.83	6.25	6.10
Raw protein, %[2]	0.73	1.25	1.19	1.20
Fibre, %[1]	1.52	1.18	1.41	1.40
Total ash, %[1]	0.76	0.76	0.69	0.62
Vitamin C, mg%[3]	110.55	119.10	132.08	135.82
B-carotene, mg%[4]	0.94	0.87	0.86	0.40

[1] Differences nonsignificant
[2] Differences are significant at $D_{0,05} = 0.08-0.90$
[3] Differences are significant at $D_{0,05} = 7.79-8.42$
[4] Differences are significant at $D_{0,05} = 0.17-0.19$

In fruit harvested from plants treated with chlorflurenol a higher content of total extract (about 1%) and higher content of total sugar (about 0.50%) were noticed. However, the content of fibre in fruit treated with chlorflurenol was slightly lower than in the control fruit.

It should be stressed that together with the chlorflurenol concentration increase there was an essential increase of vitamin C. Thus, in fruit collected from plants treated with chlorflurenol at 1.00 and 0.50 ml/l the content of vitamin C was about 25 and 21 mg% higher than in the control fruit (Table 5). The higher content of vitamin C was found in seedless fruit of tomatoes treated with chlorflurenol methyl ester (Łukasik et al., 1978).

The highest amount of raw protein was in pepper fruit treated with chlorflurenol. And the difference in the content of raw protein was proved to be statistically significant (Table 5).

The content of total ash in pepper fruit was on the same level as in the control. Chlorflurenol decreased considerably β-carotene content in pepper fruit (Table 5).

Conclusions

The following conclusions are based on the data of the experiments.

- Treatment with chlorflurenol of the individual flowers and whole plants of pepper in full blooming increased considerably of the number of fruit (Table 1).

- Individual fruit from plants treated with chlorflurenol had a smaller weight than fruit from control plants. So, the total weight of fruit from plants treated with chlorflurenol was only slightly higher than in control plants (Tables 1 and 2).
- Under the influence of chlorflurenol fruit maturity time was shortened from 3 to 5 days as compared with control plants (Table 3).
- On plants treated with chlorflurenol seedless fruit were developed. Percent of parthenocarpy of fruit was highest at chlorflurenol concentration 1 ml/l (Table 4).
- In fruit from plants treated with chlorflurenol increased statistically significant the content of vitamin C (Table 5).
- Chlorflurenol in all combinations caused statistically significant increase of raw protein content in pepper fruit (Table 5).
- In fruit harvested from plants treated with chlorflurenol higher content of dry matter, total extract and total sugar than in control fruit was noticed (Table 5).
- Content of raw ash in fruit was on the control combination level (Table 5).
- Chlorflurenol decreased the content of β-carotene in pepper fruit (Table 5).

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Contents

DISEASES

Characteristics of the protein A-immunosorbent electron microscopic technique (PA-ISEM) for detecting plant virus particles
D. D. Shukla and K. H. Gough 173
Serologische Untersuchungen mit dem cucumber mosaic virus (Gurkenmosaik-Virus). IV. Verwandtschaftliche Beziehungen zu einem Stamm des peanut stunt virus
J. Richter und D. Reichenbächer 187
A new pepper strain of tomato mosaic virus
G. Csilléry, I. Tóbiás and J. Ruskó 195
Population dynamics of aphids, vectors of maize dwarf mosaic virus and aphid resistance of some maize hybrids
I. Milinkó, Zs. Rakk and Gy. Kovács 201
Reaction of *Physalis* species to plant viruses VII. Additional data on the virus susceptibility of *Physalis minima* L.
J. Horváth 209
The role of some plants in the ecology of cucumber mosaic virus with special regard to bean
J. Horváth 217
Effect of single and double infection with *Myrothecium verrucaria* and common bean mosaic virus (CBMV) on dry weight and chemical constituents of bean plants
M. A. Amer, M. El-Hammady and F. Abo El-Abbas 225
Viruses of vegetable plants in Hungary and some of their properties
J. Horváth and L. Beczner 237
Effect of rust infection on lipid composition in leaves of groundnut (*Arachis hypogaea* L.)
G. Rama Gopal and P. Sreenivasulu 255
Some biochemical aspects of host-pathogen interactions in *Pythium* stalk rot of maize: I. Role of toxin, pectolytic and cellulolytic enzymes in pathogenesis
E. A. Sadik, M. M. Payak and S. L. Mehta 261
Cross-reactive antigens among *Fusarium oxysporum* f. sp. *lupini* and its host and non-host plants of unrelated families, *Papilionaceae* and *Gramineae*
M. Rataj-Guranowska and I. Wiatroszak 271
Effect of nitrogen supply on downy mildew development in sunflowers grown in perlit culture
S. A. Mohamed and F. Virányi 281
Trichoderma species in Hungary
L. Vajna 291
Overwintering of *Diaporthe helianthi*, a new destructive pathogen of sunflowers in Hungary
J. Vörös, J. Léránth and L. Vajna 303
"Protoplast killer" extracted from tobacco leaves inoculated with bacteria
Z. Klement, R. Gáborjányi, G. Somlyai and É. Visnyovszky 307
The influence of MCPA on the vascular tissue in the leaf petioles of sunflower (*Helianthus annuus* L.)
F. Herdi 313

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Characteristics of the Protein A-Immunosorbent Electron Microscopic Technique (PA-ISEM) for Detecting Plant Virus Particles

D. D. Shukla and K. H. Gough

Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization, Parkville (Melbourne), Victoria 3052, Australia

This paper describes the optimum conditions for Protein A-immunosorbent electron microscopic technique (PA-ISEM) for detecting plant virus particles. Protein A was found effective only at concentrations between 0.01 to 0.5 mg/ml with an optimum of 0.01 mg/ml. The optimum antiserum coating dilution was found to lie between 1 : 40 and 1 : 80 for three virus-antibody combinations tested although the titre of the antisera ranged from 1 : 512 to 1 : 4096. This indicated that the optimum antiserum coating dilution for PA-ISEM is not dependent on the antiserum titre. Obviously, a convenient dilution of 1 : 50 is appropriate for any virus-antibody combination. A 5 min adsorption of Protein A to the grid and a 10 min reaction of Protein A to antibody were found optimum for the technique; prolonged incubation of these two reactants caused a reduction in the number of particles trapped. A 10 to 15 min reaction time for antibody and virus is sufficient to detect most plant viruses; an extended reaction time of 60 to 120 min is required for only those viruses which are known to occur in extremely low concentration in plants, e.g. luteoviruses. The optimum decoration titre of an antiserum was found to be half of its titre obtained in microprecipitin or agar gel double diffusion tests. A decoration time of 10 min at the optimum decorating dilution gave satisfactory results. Protein A plus antiserum coated grids can be stored for 12 months or more at −20 °C and still retain most of their activity. The conclusions drawn from the present work have been summarized in the form of a protocol designed for successful application of the technique in the diagnosis of plant viruses.

In two previous papers (Shukla and Gough, 1979; Gough and Shukla, 1980), we reported a modified immunosorbent electron microscopic technique (ISEM) for detecting elongated and isometric insect and plant viruses, which involved precoating electron microscope grids with Protein A (a wall protein of *Staphylococcus aureus*) before coating them with the specific antiserum (PA-ISEM). The technique has since been used successfully by several workers for the detection of animal, human, insect and plant viruses (Lesemann and Paul, 1980; Milne, 1980; Van Regenmortel et al., 1980; Nicolaeiff et al., 1980, 1982; Anderson and Gibbs, 1981; Makkouk et al., 1981; Obert et al., 1981; Polatnick and Wool, 1981; Paliwal, 1982; Van Balen, 1982). Apart from its high sensitivity, the PA-ISEM has been found to have the following advantages (Lesemann and Paul, 1980; Makkouk et al., 1981) over the conventional Derrick-ISEM (Derrick, 1973; Milne and Luisoni, 1977): firstly, it gives a low non-specific trapping of particles with normal sera, and therefore, the degree of specific binding by the antisera can

be clearly demonstrated; secondly, it allows the detection of weak heterologous reactions; and thirdly, low-titered antisera can be used with success.

In order to increase the sensitivity and efficiency of the PA-ISEM further, we have examined the various parameters so that optimum conditions for the technique are established. The parameters investigated include concentration of Protein A, dilution of antiserum, adsorption time of Protein A onto the grid, reaction time of antibody with Protein A and virus with antibody, different types of support films, stains and buffer systems, and stability of the Protein A plus antiserum-treated grids during storage.

Materials und Methods

Viruses

The viruses used in the various experiments were a common strain of tobacco mosaic virus (TMV), Johnson grass strain of sugarcane mosaic virus (SCMV), Erysimum latent virus (ELV) (Shukla and Gough, 1979; Gough and Shukla, 1980), and an isolate of potato leafroll virus (PLRV) collected in Victoria (O'Donnell et al., 1982). Most of the experiments were performed using SCMV (a potyvirus) as a model system. The viruses were cultivated in an insect-proof glasshouse in Turkish tobacco (TMV), sweetcorn cv. Iochief (SCMV), chinese cabbage (ELV) and *Physalis floridana* Rydb. (PLRV). Virus extracts were prepared from systemically infected leaves from plants infected for about four weeks (sweetcorn and *P. floridana*) or three to six months (tobacco and chinese cabbage) by grinding the leaf tissue in a pestle and mortar in 1 ml/g (PLRV), 10 ml/g (SCMV) or 1000 ml/g (TMV and ELV) of 0.1 M-phosphate buffer, pH 7.0 (PB).

Antisera

The antisera to ELV, SCMV and TMV were those described previously (Shukla and Gough, 1979; Gough and Shukla, 1980). PLRV antiserum was obtained from Dr. R. Stace-Smith (Canada) and had a titre of 1 : 1024 in agar gel double diffusion tests (Rowhani and Stace-Smith, 1979). It reacted positively with the PLRV isolate in the Electro-Blot Radioimmunoassay and PA-ISEM (O'Donnell et al., 1982). Unless otherwise stated, the antiserum dilution (prepared in PB) used for coating grids and decorating the virus particles was 1 : 100.

Grid holder

A grid holder (Fig. 1) was designed to hold 12 pairs of forceps in a slanting position for ease of washing. The main section of the apparatus consists of a piece of polyvinyl chloride (PVC), size 32 cm × 1.2 cm, with a 0.5 cm channel cut into it. A piece of silicon tubing is glued inside the channel to grip the forceps handle. The channel is held at an angle of 30° to the horizontal by a piece of PVC, 10.5 cm in height, at each end.

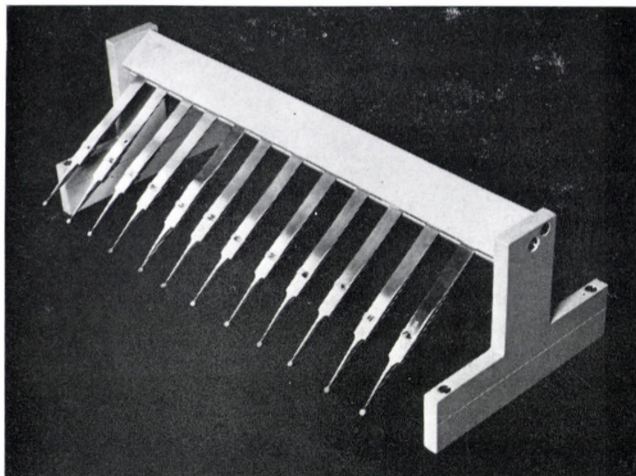

Fig. 1. The grid holder described in the text

Processing of the grids

The methods for preparing and processing the grids were essentially the same as described previously (Shukla and Gough, 1979). Solutions of Protein A and bovine serum albumin were prepared in PB. The grids were processed in the laboratory at about 20 °C. The various parameters were tested for determining the optimum conditions for the technique by using the standard procedure (Shukla and Gough, 1979) and changing one parameter each time. In experiments designed to determine the optimum reaction time for Protein A, antiserum or virus the grid holder was placed in a humid chamber made of perspex, and a beaker of water was placed in the chamber to maintain a relative humidity of about 90%. For testing the stability of Protein A plus antiserum treated grids, freshly prepared grids were coated with Protein A and antisera to ELV, SCMV and TMV. They were washed with 20 drops each of PB and water, drained and air dried. The grids were then placed in a grid box, and the box was wrapped in a plastic bag and stored at −20 °C. The activity of the stored grids was tested at different intervals up to 12 months.

Counting of virus particles

Particles were counted at magnifications of 15 000 (SCMV and TMV) or 54 000 (ELV and PLRV) in five fields of view on randomly selected squares on each of the two grids per treatment. The mean was calculated and multiplied by the Relative Area Factor, calculated for the electron microscope used, to obtain the number of particles per 1000 μm^2 area (Roberts, 1980).

Results

Support films

Grids with the three different types of support films, namely Butavar (0.25% in chloroform), Formvar (0.2% in ethylene dichloride) and Parlodion (2% in amyl acetate), attached similar numbers of SCMV particles. Formvar and Butavar films proved to be mechanically very stable whereas grids with Parlodion film had many broken squares, suggesting its unsuitability for ISEM where several rounds of washing are required to get rid of cell components and salts. Increasing the concentration of Parlodian to 2.5 and 3.0% produced no significant improvement.

Protein A concentration

To determine its optimum concentration for the technique, Protein A was tested at various concentrations in two different experiments (0.01 to 5.0 mg/ml – Table 1, 0.0001 to 2.0 mg/ml – Table 2) using SCMV as the test virus. Bovine serum albumin diluted at the same concentrations as that of Protein A was used as control in the second experiment (Table 2). In both the experiments 0.01 mg/ml

Table 1

Effect of protein A concentration on the number of sugarcane mosaic virus particles[a]

Protein A concentration mg/ml	Number of particles[b]
0.01	4963 ±222
0.05	4162 ±70
0.1	4118 ±190
0.5	3628 ±229
1.0	756 ±99
2.0	690 ±116
5.0	690 ±149

[a] Per 1000 μm^2 area of grids coated with Protein A plus antiserum.
[b] Mean and standard deviation of 5 areas on each of two grids per treatment.

Table 2

Effect of concentration of Protein A and bovine serum albumin (BSA) on the number of sugarcane mosaic virus particles[a]

Treatment	Number of particles[b] at concentration, mg/ml					
	0.0001	0.001	0.01	0.1	1.0	2.0
Protein A	311 ±75	267 ±72	3561 ±229	2604 ±184	400 ±123	378 ±93
BSA	422 ±84	445 ±104	289 ±66	178 ±55	89 ±28	155 ±47

[a] Per 1000 μm^2 area of grids.
[b] Mean and standard deviation of 5 areas on each of two grids per treatment.

Protein A trapped the maximum number of SCMV particles. The concentrations below 0.01 mg/ml and above 0.5 mg/ml yielded almost the same number of particles as obtained with BSA.

Antiserum coating-dilution

In a previous paper we demonstrated that, irrespective of the antiserum titre or virus used, PA-ISEM traps the maximum number of particles when grids are coated with antiserum dilution of 1 : 100 or less (Gough and Shukla, 1980). To determine the optimum dilution, we tested three viruses using antisera with titres ranging from 1 : 512 to 1 : 4096 at six different antiserum concentrations between undiluted and 1 : 100. The maximum number of SCMV and TMV particles was obtained at 1 : 40 antiserum dilution and that of ELV at antiserum dilution of 1 : 80 (Table 3).

Table 3

Effect of antiserum dilution on the number of sugarcane mosaic virus (SCMV), tobacco mosaic virus (TMV) and Erysimum latent virus (ELV) particles[a]

Virus	Sap dilution	Antiserum titre	Undiluted	Number of particles[b] at antiserum dilution				
				1 : 20	1 : 40	1 : 60	1 : 80	1 : 100
SCMV	1 : 10	1 : 512	4029 ±194	3940 ±229	5342 ±309	5164 ±234	3828 ±375	3227 ±232
TMV	1 : 1000	1 : 2048	3895 ±269	5409 ±434	7857 ±811	6611 ±610	6700 ±607	3828 ±418
ELV	1 : 1000	1 : 4096	14 347 ±1 651	47 551 ±3 829	51 943 ±4 219	82 937 ±5 308	101 303 ±4 782	84 372 ±4 954

[a] Per 1000 μm^2 area of grids coated with Protein A plus antiserum.
[b] Mean and standard deviation of 5 areas on each of two grids per treatment

Reaction time for Protein A, antibody and virus

From Table 4 it is evident that the optimum time for adsorption of Protein A onto the grid is 5 min. There was an insignificant gradual decrease in the number of SCMV particles when the Protein A adsorption time was lengthened. The optimum reaction time for antibody and Protein A was found to be 10 min (Table 4).

Table 4

Effect of reaction time of Protein A, antiserum and virus on the number of sugarcane mosaic virus particles[a]

Reactants	Number of particles[b] after the reaction time, min				
	5	10	15	30	60
Protein A	4585 ±248	4541 ±204	4474 ±194	4162 ±184	3917 ±222
Antiserum	4362 ±181	4852 ±183	4763 ±209	4452 ±272	4496 ±294
Virus	2448 ±171	4606 ±119	5275 ±168	5809 ±199	8013 ±537

[a] Per 1000 μm^2 area of grids coated with Protein A plus antiserum.
[b] Mean and standard deviation of 5 areas on each of two grids per treatment.

The number of particles trapped after 5 min incubation was slightly lower than that obtained at 10 min. Extended antibody-Protein A reaction time did not increase the number of SCMV particles. However, there was a progressive increase in the number of SCMV particles when the antibody-virus reaction time was lengthened; the maximum allowed 60 min trapped the maximum number of particles. The difference between the antibody-virus reaction times of 5 and 60 min was a roughly 3-fold increase in the particle number (Table 4).

Antiserum dilution for decoration of virus particles

When five different dilutions of SCMV antiserum ranging from 1 : 20 to 1 : 1000 were used to decorate SCMV particles using preimmune serum as a control, complete decoration of the particles was obtained up to 1 : 200 antiserum dilution and some decoration was still visible at 1 : 500, but 1 : 1000 did not decorate the particles at all (Fig. 2). When SCMV particles were decorated for 2, 5, 10 and 15 min using antiserum dilution of 1 : 200, the amount of decoration increased up to 10 min but a 15 min decoration time did not increase the width of the particles.

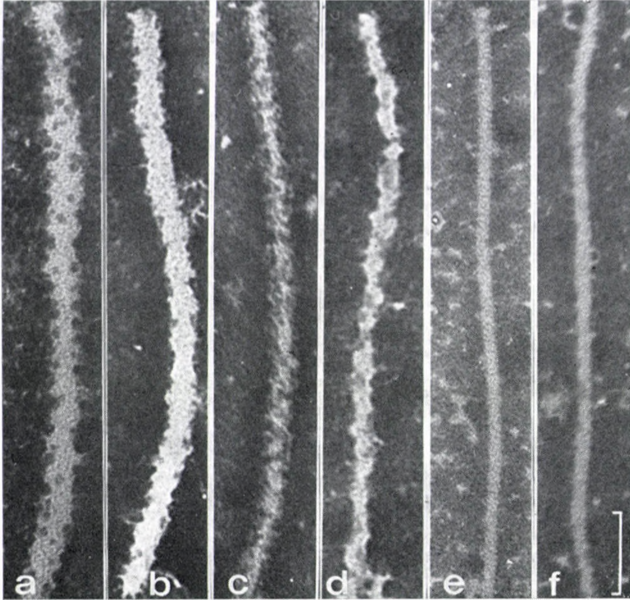

Fig. 2. Particles of sugarcane mosaic virus, decorated by different dilutions of homologous antiserum and stained with 2% aqueous phosphotungstic acid, pH 7.0: (a) 1 : 20, (b) 1 : 100, (c) 1 : 200, (d) 1 : 500, (e) 1 : 1000 and (f) 1 : 50 of a preimmune serum. The bar represents 100 nm

Stains

To find a suitable stain for the technique, 2% aqueous solutions of the three commonly used stains, ammonium molybdate, pH 6.0 (AM), phosphotungstic acid, pH 7.0 (PTA) and uranyl acetate, pH 4.5 (UA) were tested on TMV particles. After a 2 min incubation with UA and PTA good staining of the particles was achieved. However, AM stained the particles very poorly after this incubation time, and even after 5 and 10 min it did not equal the 2 min-staining of UA and PTA. There was no significant increase in the contrast of the particles when the staining time for PTA and UA was increased to 5 and 10 min. With PTA and AM more stain deposited on the sides of the grid squares after prolonged staining This caused the support film to break.

Buffers

To find out if different buffer systems could prevent the non-specific adsorption of cell component interfering with the detection of isometric viruses, we compared the results obtained with PB, PB plus 4% polyethylene glycol (M.Wt. 6000), PB plus 0.4 M sucrose (Derrick and Brlansky, 1976), phosphate-buffered saline (PBS, 0.02 M-phosphate containing 0.15 M-NaCl, pH 7.0), PBS-Tween (PBS with 0.05% Tween 20) and Tris buffer (0.05 M, pH 7.0) using the PLRV isolate

as the test virus. There was no significant difference in the number of PLRV particles and also in the non-specific adsorption of the cellular components with different buffer systems used (data not shown). PB is probably the best buffer for ISEM. Tris has the inherent problem of reacting with copper grids after long contact, and like PB, it precipitates with UA (Milne and Luisoni, 1977; Paliwal, 1977). High NaCl concentrations in buffers have been found to cause clumping of virus particles (Beier and Shepherd, 1978). Addition of sucrose, Tween 20, or polyethylene glycol did not reduce non-specific adsorption, at least in our experiments with PLRV.

Stability of Protein A plus antiserum treated grids

Table 5 shows that there was virtually no difference in the number of ELV and SCMV particles trapped on the freshly prepared grids and on grids stored for one month whereas, in the case of TMV, a reduction in the particle number of

Table 5

Number of sugarcane mosaic virus (SCMV), tobacco mosaic virus (TMV) and Erysimum latent virus (ELV) particles[a] on Protein A plus antiserum coated grids stored at −20 °C for different durations

Virus	Treatment	Number of particles[b] after storage, month				
		1	2	3	7	12
SCMV	Fresh	4897 ±314	3784 ±140	4563 ±225	4207 ±191	3517 ±221
	Stored	4963 ±245	3517 ±211	3940 ±132	3227 ±234	2292 ±104
	Activity Retained	100%	93%	86%	78%	65%
TMV	Fresh	2402 ±135	3494 ±206	6811 ±314	4029 ±170	3561 ±187
	Stored	2292 ±109	3361 ±137	6321 ±376	3628 ±151	3116 ±211
	Activity Retained	95%	96%	93%	90%	88%
ELV	Fresh	76 336 ±8 013	92 120 ±5 009	111 346 ±4 873	126 558 ±2 913	130 575 ±6 470
	Stored	76 623 ±5 717	86 667 ±4 501	102 599 ±4 219	122 540 ±5 835	126 271 ±6 116
	Activity Retained	100%	94%	93%	97%	97%

[a] Per 1000 μm^2 area of grids.
[b] Mean and standard deviation of 5 areas on each of two grids per treatment.

about 5% occurred after this storage time. After 2 months storage there was a progressive decrease in the number of SCMV and TMV particles as the storage time was lengthened. After 12 months grids tested with SCMV and TMV retained 65% and 88% of their activity, respectively. A decrease also in the number of ELV particles occurred after a storage of 2 months and beyond, but it was not a progressive decrease. With ELV, grids retained 97% of their activity after the 12 month storage period.

Discussion

The results presented in this paper demonstrate several variables, from preparation of grid to staining of virus particles, which can have significant effects on the results obtained with PA-ISEM. Some parameters like type of grid (copper, nickel, copper coated with palladium and copper coated with rhodium) and different buffer systems appeared to have no influence on the technique; type of support films had only qualitative effect; whereas factors like Protein A and antiserum concentrations, reaction time of Protein A, antibody and virus proved to be critical for the technique.

Protein A was found to be effective only at concentrations between 0.01 to 0.5 mg/ml with an optimum of 0.01 mg/ml. Its ineffectiveness at very low concentrations, 0.001 mg/ml and below, may be due to insufficient Protein A molecules on the grid to trap all the antibody molecules in the antiserum. However, it was surprising to note that higher Protein A concentrations, 1.0 mg/ml and above, trapped only the same number of particles as obtained with non-specific adsorption. One explanation for this effect could be that saturation of grids by Protein A molecules at high concentration may have rendered the Protein A binding sites inaccessible to antibody molecules, and thus very few or no antibody molecules would have bound to the grid, resulting in minimal virus particle trapping beyond non-specific adsorption.

Antiserum coating dilution was another factor of crucial importance for the technique. For the three virus-antibody combinations tested (ELV, SCMV and TMV) optimum dilution was found to lie between 1 : 40 and 1 : 80 although the titre of the antisera ranged from 1 : 512 to 1 : 4096. This indicated that the optimum antiserum coating dilution for the PA-ISEM is not dependent on the antiserum titre, as has also been found with the Derrick-ISEM where it ranges from 1 : 1000 to 1 : 2000 (Gough and Shukla, 1980). Obviously, a convenient dilution of 1 : 50 should be appropriate for any virus-antibody combination with PA-ISEM, unless, perhaps, the antiserum titre is very low. In one experiment where a 1 : 50 dilution of the SCMV antiserum was compared with its purified γ globulin at about the same concentration as present in the 1 : 50 dilution of whole antiserum, no difference was found either in the number of SCMV particles trapped (5030 and 4941, respectively) or in the amount of contaminant present on the grids suggesting that purified γ globulins have no advantage over whole antiserum in the PA-ISEM.

The dilution of a virus extract to be used in PA-ISEM has to be a compromise based on the content of a particular virus in the extract. Although we have not investigated this question in detail, our experience with different viruses suggests that a 1 : 1 dilution would be appropriate for viruses with extremely low concentration (luteoviruses – D. D. Shukla, unpublished). 1 : 10 for viruses with low concentration (potyviruses – Shukla and Gough, 1979; Gough and Shukla, 1980; D. D. Shukla and K. H. Gough, unpublished), 1 : 100 for viruses with moderate particle content (alfalfa mosaic virus, broad bean wilt virus, cucumoviruses, ilarviruses – Shukla and Gough, 1983) and 1 : 1000 for viruses known to occur in high concentration (tobamoviruses, tymoviruses – Shukla and Gough, 1979; Gough and Shukla, 1980).

A 5 min adsorption of Protein A to the grid and a 10 min reaction of antibody to Protein A were found optimum for the technique. Prolonged incubation of these two reactants caused a reduction in the number of particles trapped. The same results were obtained also with the Derrick-ISEM when the SCMV antiserum was incubated on the grids from 5 to 60 min (results not shown). On the other hand, we observed a progressive increase in the number of SCMV particles when the virus incubation time was lengthened; the maximum allowed 60 min trapped the maximum number of particles. These results suggest that the optimum reaction time for SCMV may be more than 60 min, although we have regularly found that a 10 min incubation with this virus is sufficient for its detection by PA-ISEM (Shukla and Gough, 1979; Gough and Shukla, 1980; and this report). On the basis of our experience with the technique, it can be said that a 10 to 15 min virus incubation time would be sufficient to detect most plant viruses; an extended incubation time of 60 min or more is required only for those viruses which are known to occur in extremely low concentration in plants, e.g. luteoviruses.

We have found as did Milne and Lesemann (1978) that the particle decoration titre of an antiserum is similar to the titre determined by microprecipitin or agar gel double diffusion tests. Additionally, we have found that the decoration titre of an antiserum gives only a partial decoration of the particles. Therefore, we recommend that for decoration purposes the antiserum should be diluted to half of its microprecipitin or agar double diffusion titre. For instance, the SCMV antiserum with a microprecipitin titre of 1 : 512 will have a decoration titre of about 1 : 500 and an optimum decorating dilution around 1 : 250, which we correctly obtained. This was found true also with ELV and TMV. A decoration time of 10 min at optimum decorating dilution will give satisfactory results.

UA was found best among the three stains investigated. It distributed evenly on grid squares and gave good particle contrast. However, its use in the decoration experiments was found unsatisfactory. The extent of particle decoration at different antiserum dilutions could not be determined directly even at high magnification when this stain was used, and it was necessary that the particles be photographed. PTA proved much better in this respect.

In a previous paper (Gough and Shukla, 1980), we reported that Protein A plus antiserum-coated grids can be stored for up to 6 months at 4 °C. Our present

results show that Protein A plus antiserum-coated grids can be stored for 12 months or more at −20 °C and still retain most of their activity. This makes the technique more efficient and convenient to use in field diagnosis of plant viruses. As suggested by Paliwal (1977), workers at field stations with no electron microscope facility can use the technique if they are supplied with pretreated grids, and the prepared grids then sent to a laboratory with such a facility for examination.

The simple and inexpensive grid holder designed by us (Fig. 1) was found very convenient for grid processing. Grids held in a slanting position at about 30° angle were easy to wash. A filter paper placed underneath the apparatus absorbed the buffer and water coming from the grids after washings.

On the basis of the conclusions of the present report the following protocol can be followed for the successful detection of plant viruses in a variety of situations using PA-ISEM.

(1) Place 5 to 15 μl (alternatively a drop from the pasteur pipette) of 0.01 mg/ml Protein A in 0.1 M – phosphate buffer, pH 7.0 (PB), onto 0.2% Formvar (0.25% Butavar alternatively) – carbon coated and glow-discharged 400 mesh copper or nickel grids held in forceps preferably in a grid holder (Figure 1), incubate for 5 min, then drain using the corner of a piece of filter paper;

(2) Add 5 to 15 μl of PB-diluted antiserum (1 : 50 irrespective of the virus-antibody combination), incubate for 10 min, wash with 20 or more drops of PB (using Pasteur pipette) and then drain;

(3) Place 5 to 15 μl of PB-diluted virus extract (1 : 1 to 1 : 1000), incubate for 10 to 120 min, wash with 30 or more drops of PB, then drain (the dilution of virus extract and the incubation time will depend on the virus concentration of extracts, e.g. luteoviruses – 1 : 1 and 60 – 120 min, potyviruses 1 : 10 and 10 to 15 min, ilaviruses and broad bean wilt virus – 1 : 100 and 10 min, tobamovirus and tymoviruses – 1 : 1000 and 10 min);

(4) Decorate virus particles by adding to the grid 5 to 15 μl of PB-diluted antiserum (dilute to half of the titre obtained in either agar gel double diffusion test or microprecipitin test), incubate for 10 min, wash with 30 drops of PB and 30 or more drops of distilled water, and then drain;

(5) Stain the grids with 5 drops of 2% uranyl acetate, pH 4.5 (or 2% phosphotungstic acid, pH 7.0 if particle decoration is desired), incubate for 2 min, then drain and air dry;

(6) Do not allow the grids to dry except after staining. Use a humid chamber if incubation time exceeds 15 min. When using stored Protein A plus antiserum-coated grids (pretreated grids can be stored for more than 12 months at −20 °C), active the grid by placing a drop of PB on the grid and incubate for 5 min, then drain; the subsequent steps then follow the standard procedure. All steps are performed at laboratory temperature around 20° to 25 °C.

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Serologische Untersuchungen mit dem Cucumber Mosaic Virus (Gurkenmosaik-Virus)

IV. Verwandtschaftliche Beziehungen zu einem Stamm des peanut stunt virus

JOHANNES RICHTER und DIETER REICHENBÄCHER

Institut für Phytopathologie Aschersleben der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik, 4320 Aschersleben, Theodor-Roemer-Weg DDR

Two methods were used to estimate the extent of cross-reactivity between the serological groups N (= ToRS) and U (= DTL) of cucumber mosaic virus and the "clover blotch" strain of peanut stunt virus (PSV-CB). From the reaction end points of antisera in agar gel double diffusion tests the serological differentiation indices of reciprocal tests (RT-SDI values) were calculated as follows: 4.6 (CMV-U and PSV-CB), 4.7 (CMV-U and PSV-CB) and <1 (CMV-N and CMV-U), respectively. Moreover, the height (h) of the precipitation peaks was measured using the rocket immunoelectrophoresis. In comparative tests a good correspondence between the quotient $h_{heterologous}/h_{homologous}$ and the SDI values could be indicated.

With the double-antibody sandwich method of the enzyme-linked immunosorbent assay (ELISA) the homologous viruses (CMV or PSV) can be detected only. However, enzyme conjugates prepared with antibodies to one of the serological groups of CMV enable the detection of both groups.

Das cucumber mosaic virus (CMV) und das peanut stunt virus (PSV) sind Vertreter der Cucumovirus-Gruppe, die serologisch miteinander verwandt sind. Eine Differenzierung beider Viren gelingt mit Hilfe des Agargel-Doppeldiffusionstestes, wenn Formaldehyd-stabilisierte, gereinigte Viruspräparationen als Testantigen verwendet werden (Devergne und Cardin, 1975 u. 1976). Mit der gleichen Methode ist auch eine Differenzierung beider Viren in serologische Gruppen (Serogruppen) bzw. Serotypen möglich. Beim CMV sind zwei Gruppen zu unterscheiden, die mit ToRS bzw. N und DTL bzw. U bezeichnet werden (Devergne und Cardin, 1973; Richter u. a., 1972b und 1975; siehe auch Richter, 1982). Vom PSV wurden bisher 5 serologisch unterscheidbare Stämme beschrieben (Devergne und Cardin, 1976; Beczner und Devergne, 1979; Richter u. a., 1979b), für die die Bezeichnungen PSV-V, PSV-W, PSV-Tp, PSV-CB (= "clover blotch virus") und PSV-RoM (= "robinia mosaic virus") in Gebrauch sind bzw. hiermit vorgeschlagen werden.

Exakte Angaben zum Grad der serologischen Verwandtschaft zwischen CMV und PSV wurden bisher nur in begrenztem Umfange mitgeteilt (Devergne und Cardin, 1975; Beczner und Devergne, 1979), wobei der serologische Differenzierungsindex (SDI) nach Van Regenmortel und Von Wechmar (1970) als Maß

für die Kreuzreaktivität verwendet wurde. Wir führten daher mit verschiedenen Methoden Untersuchungen mit dem Ziel durch, exakte Angaben über die verwandtschaftlichen Beziehungen zwischen CMV jund PSV zu erhalten, über die im folgenden berichtet wird. Als Versuchsobjekte dienten je ein Vertreter der beiden Serogruppen des CMV sowie das CB-Isolat des PSV.

Material and Methoden

Antigene. Die Stämme CMV-N/I und CMV-U/Sz (siehe Richter u. a., 1975) sowie PSV-CB (Musil u. a., 1975; Richter u. a., 1979b) wurden auf Nicotiana tabacum L. cv. 'Samsun' bzw. Zuchtstamm 'Bel 61-10'vermehrt, nach Proll und Richter (1972) gereinigt und anschließend mit 2% Formaldehyd fixiert (Richter u. a., 1972*a*). Die Bestimmung der Viruskonzentration erfolgte spektrophotometrisch unter Verwendung des Extinktionskoeffizienten von $E^{0,1\%}_{1cm}$ von 5,0 bei 260 nm (Francki u. a., 1966).

Antiseren. Als Versuchstiere dienten Kaninchen, die mit fixiertem Virus injiziert wurden. Die Tiere erhielten eine intravenöse Startinjektion und 4 Wochen darauf eine intramuskuläre Booster-Injektion. Die für die Untersuchungen verwendeten Antiseren wurden im Abstand von 4 bis 30 Wochen danach aus den Versuchstieren gewonnen. Diese erhielten zwischendurch in der 8. Woche eine zweite intramuskuläre Booster-Injektion. Insgesamt wurden jeweils 4 Tiere pro Antigen verwendet.

Serologische Methoden. Zur Ermittlung des homologen und Kreuzreaktionsvermögens der Antiseren wurden 3 verschiedene Methoden verwendet.

Im Agargel-Doppeldiffusionstest (1% Difco Special Agar-Noble in aqua dest. unter Zusatz von 0.3% Natriumazid) wurden die Antiserumtiter gegen die FA-stabilisierten Viren in einer Konzentration von 1 mg/ml ermittelt. Die Berechnung der SDI-Werte, der durchschnittlichen SDI-Werte und der reziproken SDI-Werte (RT-SDI) erfolgte nach Paul u. a. (1980).

Die Rocket-Immunelektrophorese (RIEP) erfolgte – wie bei Reichenbächer u. a. (1979) für Tobamoviren beschrieben – auf Glasplatten (9 × 6,5 cm), die mit 0.8% Agarose (Serva, Heidelberg) in 0,05 M Phosphatpuffer, pH 8,0, beschichtet wurden. Die optimale Antiserumkonzentration im Gel wurde in Vorversuchen ermittelt, sie variierte in Abhängigkeit vom Antiserum zwischen 1 und 12% bei einem Gesamtgelvolumen von 15 ml. In die Antigendepots wurden jeweils 10 μl einer Lösung mit 1 mg Virus/ml gegeben. Weitere methodische Einzelheiten siehe Reichenbächer u. a. (1978). Die Vermessung der Höhe der Präzipitat-Peaks erfolgte an Dauerpräparaten nach Anfärbung mit Coomassie Brilliant Blue R 250. Die Einzelversuche wurden in 4 bis 5facher Wiederholung durchgeführt.

Für den enzyme-linked immunosorbent assay (ELISA) wurden die Doppelantikörper-Sandwich-Methode (double-antibody sandwich method) in der Modifikation von Richter u. a. (1979a) eingesetzt. Zu den Versuchen wurde je ein

Antiserum gegen CMV-N/I, CMV-U/Sz und PSV-CB verwendet. Die Gewinnung der Immunglobuline sowie ihre Konjugation mit alkalischer Phosphatase (Reinheitsgrad 1, Boehringer, Mannheim) erfolgten nach Clark und Adams (1977).

Resultate

Tabelle 1 enthält die durchschnittlichen SDI-Werte für die geprüften Paare. Den Berechnungen wurden die SDI-Werte von Antiseren aus jeweils 4 Tieren zugrundegelegt, die 6, 12, 18, 24 und 30 Wochen nach einer intramuskulären Zweitinjektion (siehe Material und Methoden) gewonnen wurden.

Tabelle 1

Durchschnittliche SDI-Werte zwischen 3 Isolaten aus der Cucumovirus-Gruppe

Antiserum \ Virus	CMV-N/I	CMV-U/Sz	PSV-CB
CMV-N/I	0	<1	4,4
CMV-U/Sz	<1	0	4,3
PSV-CB	4,8	5,1	0

Aus den in Tabelle 1 aufgeführten Werten lassen sich die durchschnittlichen SDI-Werte aus den reziproken Testen (RT-SDI) wie folgt ermitteln: <1 (CMV-N/I und CMV-U/Sz), 4,6 (CMV-N/I und PSV-CB) sowie 4,7 (CMV-U/Sz und PSV-CB).

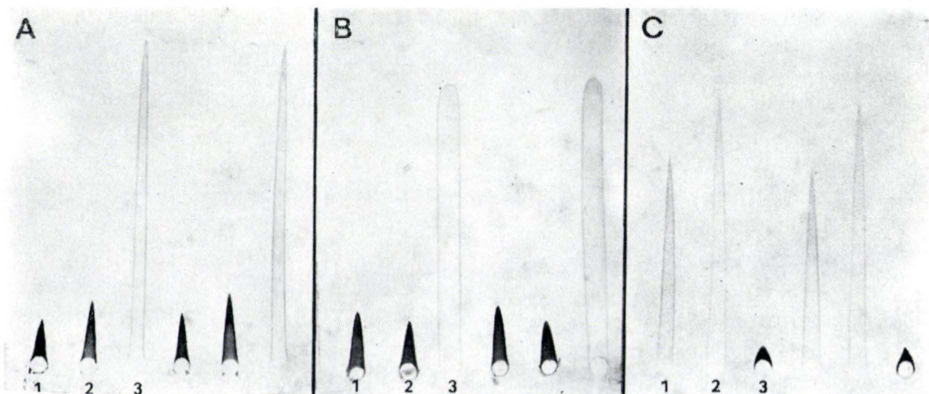

Abb. 1. Differenzierung von 3 Isolaten aus der Cucumovirus-Gruppe mit der Rocket-Immunelektrophorese. A — Antiserum CMV-N; B — Antiserum CMV-U; C — Antiserum PSV-CB; 1 — CMV-N; 2 — CMV-U; 3 — PSV-CB. Jede Gelplatte enthält eine Wiederholung bei gleicher Reihenfolge der Virusproben

Tabelle 2

Vergleich der durchschnittlichen SDI-Werte mit den in der Rocket-Immunelektrophorese ermittelten Gipfelhöhen (h) zur Bewertung der Kreuzreaktivität zwischen 3 Isolaten aus der Cucumovirus-Gruppe

Virus / Antiserum	CMV-N/I		CMV-U/Sz		PSV-CB	
	∅ SDI	$\frac{h_{heterolog}}{h_{homolog}}$	∅ SDI	$\frac{h_{heterolog}}{h_{homolog}}$	∅ SDI	$\frac{h_{heterolog}}{h_{homolog}}$
CMV-N/I	0	1,0	<1	1,2	3,6	4,1
CMV-U/Sz	<1	1,2	0	1,0	4,8	5,0
PSV-CB	3,7	3,3	6,4	8,9	0	1,0

Für die Differenzierungsversuche mit Hilfe der Rocket-Immunelektrophorese wurden 5 Antiseren gegen jedes Virusisolat aus jeweils einem Kaninchen verwendet, die 4, 8, 12, 16 bzw. 24 Wochen nach der intramuskulären Zweitinjektion gewonnen wurden. Bei der Testung der homologen und heterologen Antiserum-Virus-Kombinationen zeigten sich charakteristische Unterschiede in der Intensität der Präzipitate sowie der Höhe der Präzipitations-Peaks (Abb. 1). Über ein Ausmessen der Peak-Höhen (h) können die Unterschiede zwischen den geprüften Isolaten quantitativ erfaßt werden, vorausgesetzt, daß deren Konzentration gleich ist. Als Maß für die Kreuzreaktivität der Antigene dient der Quotient $\frac{h_{heterolog}}{h_{homolog}}$ (Reichenbächer u. a., 1979).

In Tabelle 2 sind diese Werte den durchschnittlichen SDI-Werten gegenübergestellt, die parallel dazu ermittelt wurden.

Eine Mittelwertbildung für die reziproken Paare ergibt für den Quotienten $\frac{h_{heterolog}}{h_{homolog}}$ Werte von 1,2 (CMV-N/I und CMV-U/Sz), 3.7 (CMV-N/I und PSV-CB) bzw. 7,0 (CMV-U/Sz und PSV-CB), wobei nur im letzteren Falle eine größere Differenz zwischen den beiden Einzelwerten besteht. Die entsprechenden Werte für den RT-SDI lauten 1, 3,7 und 5,6.[1] Es zeigte sich somit eine bemerkenswert gute Übereinstimmung zwischen beiden Werten, die das auf der R-IEP fußende Verfahren als Alternative zur Bestimmung der SDI-Werte über das Austitern der Antiseren mit den homologen und heterologen Reaktionspartnern ausweist.

Im ELISA wurde je ein Antiserum gegen CMV-N/I, CMV-U/Sz und PSV-CB mit einer Verdünnungsreihe der drei gereinigten und FA-stabilisierten Viren im Konzentrationsbereich von 1 ng/ml bis 10 μg/ml (Verdünnung in Zehnerpotenzen) getestet. Die zur Enzymkonjugation verwendeten Antiseren reagierten im Agargel-Doppeldiffusionstest deutlich mit dem heterologen Virus. Für die beiden CMV-Seren wurden mit dem homologen Virus Titer von 1 : 4096 bzw. 1 : 2048

[1] Die Abweichungen gegenüber den in Tabelle 1 aufgeführten Werten sind dadurch zu erklären, daß letztere an einem umfangreicheren Material ermittelt wurden.

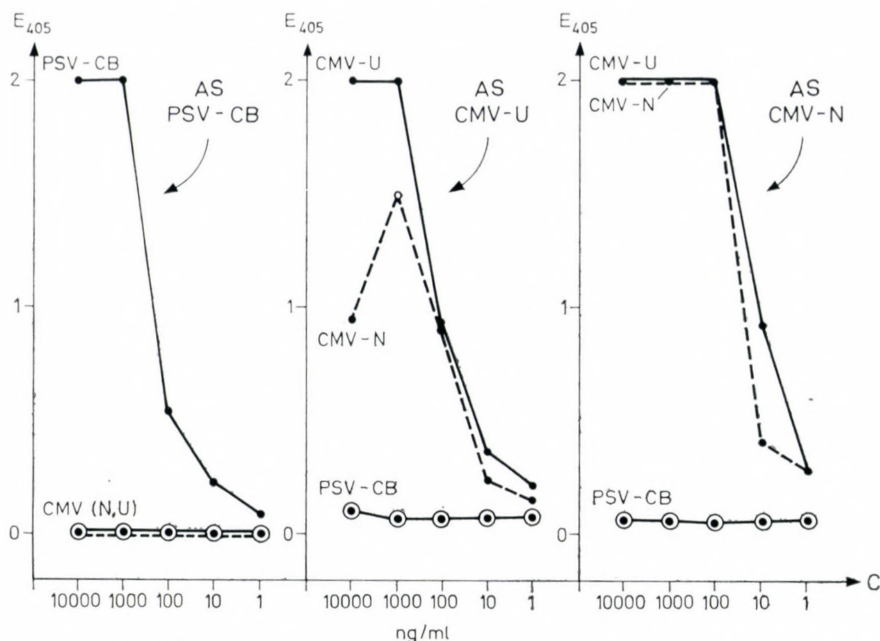

Abb. 2. Nachweis von 3 Isolaten aus der Cucumovirus-Gruppe mit dem ELISA (double antibody sandwich method). Coating der PVC-Blister: 4 h/37 °C (1 μg IgG/ml); Inkubation der gereinigten, FA-stabilisierten Antigene: 18 h/6 °C; Inkubation der Enzym-Konjugate: 4 h/37 °C (Verdünnung 1 : 1000). AS = Antiserum; Abkürzungen für die Viren siehe Text

und für das PSV solche von 1 : 128 ermittelt, während die entsprechenden Werte für das PSV-Antiserum 1 : 1024 bzw. 1 : 32 betrugen.

Aus Abb. 2 ist ersichtlich, daß a) keines der Antiseren mit dem heterologen Virus reagierte und b) jedes der CMV-Antiseren zum Nachweis der beiden Serogruppen (N und U) geeignet war. Unterschiede im Reaktionsvermögen zwischen beiden Antigenen traten nur beim Antiserum gegen CMV-U innerhalb eines bestimmten Konzentrationsbereiches auf. Dies ist insofern hervorzuheben, als sich die Doppelantikörper-Sandwich-Methode (= „direkter" ELISA) durch eine hohe Spezifität auszeichnet und in einer Reihe von Fällen mit einem Antiserum gegen einen bestimmten Virusstamm eng verwandte Stämme mit einem SDI-Wert von <2 nicht mehr nachgewiesen werden können (Koenig, 1978, 1981; van Regenmortel u. Burckard, 1980).

Diskussion

Die Ergebnisse bestätigen übereinstimmend, daß zwischen den beiden Serogruppen des CMV eine enge und zwischen diesen und dem PSV (in unseren Untersuchungen repräsentiert durch das „clover blotch"-Isolat aus Trifolium repens L.)

eine entferntere serologische Verwandtschaft besteht. Bei einem Wert von 4,6 bzw. 4,7 für den RT-SDI zwischen den beiden Serogruppen des CMV auf der einen und unserem PSV-Isolat auf der anderen Seite sowie bei Berücksichtigung der Tatsache, daß sich beide Viren im Wirtskreis und in der Symptomatologie unterscheiden, ist es gerechtfertigt, von unterschiedlichen Viren zu sprechen (siehe dazu Hamilton u. a., 1981).

Die Anwendung der RIEP zur Differenzierung kreuzreagierender Virusantigene, die erstmals von Reichenbächer u. a. (1979) vorgenommen wurde, hat sich im vorliegenden Falle erneut bewährt, wobei die Brauchbarkeit des Quotienten $\frac{h_{\text{heterolog}}}{h_{\text{homolog}}}$ als Index durch Vergleiche mit den SDI-Werten erhärtet werden konnte. Es ist zu betonen, daß in beiden Fällen die Testung einer größeren Anzahl von Antiseren notwendig ist, um repräsentative Werte zu erhalten. Der Ermittlung des SDI liegt die Bestimmung des Antiserumtiters zugrunde, die an geometrischen Verdünnungsreihen durchgeführt wird. Die ermittelten Werte können um ± 2 Verdünnungsstufen schwanken und sind somit mit einer hohen Ungenauigkeit behaftet. Im Gegensatz dazu können die Gipfelhöhen bei der RIEP stufenlos abgelesen werden. Versuche mit einer Verdünnungsreihe von homologem Virus (CMV-N/I) und einer ausgewählten Antiserumverdünnung haben gezeigt, daß innerhalb eines bestimmten Konzentrationsbereiches eine lineare Beziehung zwischen Viruskonzentration und Gipfelhöhe besteht. Es müßten allerdings weitere Versuche durchgeführt werden, um die Genauigkeit des Verfahrens exakt einschätzen zu können. Aggregationen des Antigens, die die Ergebnisse beeinflussen können (Reichenbächer u. a., 1981), traten bei den geprüften, FA-stabilisierten Viruspräparationen nicht auf. –

Die Möglichkeit, CMV und PSV mit Hilfe des direkten ELISA sicher zu differenzieren, wurde in jüngster Zeit auch von van Regenmortel (1981) aufgezeigt. Die Tatsache, daß unterschiedliche CMV-Stämme mit Antiserum gegen ein einzelnes Virusisolat erfaßt werden können (siehe auch Devergne u.a., 1978) hat insofern praktische Konsequenzen, als der direkte ELISA zur Routinediagnose des CMV im Rohsaft infizierter Pflanzen empfohlen werden kann. Erste positive Erfahrungen aus unserem Labor liegen vor.

Zusammenfassung

Es wurden zwei unterschiedliche Methoden angewendet, um das Ausmaß der Kreuzreaktivität zwischen den beiden serologischen Gruppen (N (= ToRS) bzw. U (= DTL) des cucumber mosaic virus (CMV) und dem "clover blotch"-Stamm des peanut stunt virus (PSV-CB) zu ermitteln. Anhand der im Agargel-Doppeldiffusionstest ermittelten Serumtiter wurden die serologischen Differenzierungsindizes bei Reziproktestungen (RT-SDI-Werte) wie folgt errechnet: 4,6 (CMV-N und PSV-CB), 4,7 (CMV-U und PSV-CB) und 1 (CMV-N und CMV-U). Außerdem wurde die Höhe (h) der Präzipitations-Peaks in der Rocket-Immunelektrophorese ermittelt. Vergleichende Untersuchungen zeigten eine gute Übereinstimmung zwischen dem Quotienten $\frac{h_{\text{heterolog}}}{h_{\text{omolog}}}$ und den SDI-Werten.

Mit Hilfe der Doppelantikörper-Sandwich-Methode des enzyme-linked immunosorbent assay (ELISA) konnte nur das homologe Virus (CMV oder PSV) erfaßt werden. Dagegen ermöglichen Enzymkonjugate, die mit Antikörpern gegen eine der beiden serologischen Gruppen (CMV-N oder CMV-U) hergestellt wurden, den Nachweis beider Gruppen.

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A New Pepper Strain of Tomato Mosaic Virus

G. CSILLÉRY, I. TÓBIÁS and J. RUSKÓ

Vegetable Crops Research Institute, Budatétény, Hungary

A new strain of tomato mosaic virus (ToMV-Ob) was isolated from pepper in Óbuda, Hungary. The virus is regarded as a new strain of ToMV on the basis of host-plant reactions and serological relations. This virus is considered of special interest for peppers breeders because it infects peppers containing L^1 allele of TMV resistance gene systemically. In contrast to other tobamoviruses this ToMV-Ob strain systemically infects both *Nicotiana tabacum* cv. Xanthi-nc and *Nicotiana glutinosa,* carrying the N gene for TMV resistance.

A new disease of pepper (*Capsicum annuum* cv. Soroksári hajtató) was found in greenhouses of Óbuda State Farm, Budapest, in 1978. The upper leaves of the diseased plants were completely yellow or yellow spotted (Fig. 1). The symptoms were unlike any known viral disease of pepper and suggested to be a mineral or hormonal deficiency.

We inoculated some test plants with crude extracts of diseased leaves. Symptoms of the test plants suggested a highly infectious agent belonging to the tobamovirus group. We made some investigations to establish the distribution of the disease. The disease was also found on pepper in greenhouses of Óbuda, Szegvár and Soroksár.

Strains of TMV are infectious on pepper (Greenleaf et al., 1964, Feldman and Orenianer, 1972; Rast, 1979; Burgyán et al., 1978; Selassie et al., 1981).

Further investigations were made to prove whether the causative agent of the yellow fleck disease of pepper is a new member of the tobamovirus group or it is only a strain (Gibbs, 1977). After a preliminary investigation this virus was named tomato mosaic virus-Ob (ToMV-Ob) (Csillléry and Ruskó, 1980.), because it causes local lesions on *Nicotiana sylvestris* without systemic infection (Hollings and Huttinga, 1976).

During the preliminary study it became obvious that ToMV-Ob strain could systemically infect *Nicotiana tabacum* cv. Xanthi-nc (Fig. 2) and *Nicotiana glutinosa.* To eliminate possibilities of mixed infection with other tobamoviruses, the ToMV-Ob strain was maintained on *Nicotiana tabacum* cv. Xanthi-nc. Only the systemically infected top leaves of plants were used for further investigations.

Test plants were grown in an insect-proof greenhouse at 18–26 °C or in a temperature-controlled light chamber. Mechanical inoculations were made with freshly prepared sap (pestle and mortal) diluted approximately 2 : 1 with 0.06 M phosphate buffer, pH 7, using carborundum 500 mesh as an abrasive. The test plants used are listed in Table 1.

Fig. 1. Systemic symptoms of tomato mosaic virus-Ob strain on Capsicum annuum cv. Soroksári hajtató

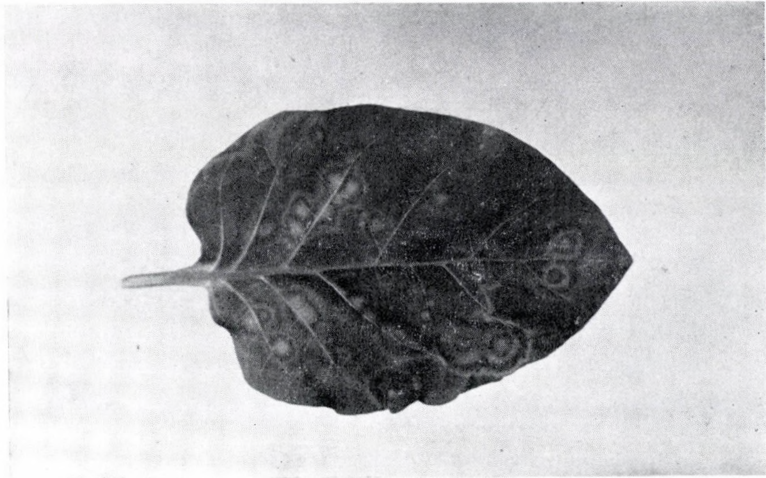

Fig. 2. Systemic symptoms on Nicotiana tabacum cv. Xanthi-nc infected with tomato mosaic virus-Ob strain

Symptomatology. Symptoms observed corresponded to those that were described for ToMV in general, except the symptoms on *Nicotiana tabacum* cv. Xanthi-nc and *Nicotiana glutinosa*. The expression of symptoms on these plants depended on temperature. Tobacco plants were grown in a temperature-controlled light chamber at 18–20 °C 26 – 28°C and 30–32 °C. The ToMV-Ob strain was compared with TMV-U1 and ToMV-Sd strains (Burgyán et al., 1978). The results are

Table 1

Results of host range tests with ToMV-Ob

Chenopodium quinoa	L/—
Datura stramonium	L/—
Plantago major	L/—
Nicotiana sylvestris	L/—
Nicotiana glutinosa	L/S
Nicotiana tabacum cv. Xanthi-nc	L/S
Nicotiana tabacum cv. Samsun	(L)/S
Nicotiana rustica	L/S
Petunia hybrida	L/—
Lycopersicon esculentum cv. K 509	l/S
Capsicum annuum	
cv. Javított Cecei (L*)	l/S
cv. D. Cecei (L[1])	L/S
cv. Fehérözön (L[1])	L/S
cv. Keystone Resistant Giant (L[1])	L/S
Capsicum chinense	
cv. "Mishme" (L[1])	L/S
cv. PI 315008 (L[3])	L/—
cv. PI 159236 (L[3])	I /—

L*, L[1] and L[3] = Alleles of TMV resistance gene
L = local symptoms
S = systemic symptoms
l = symptomless local infection
— = no infection, as demonstrated by back inoculation
( ) = not always observed

Table 2

Comparison of symptoms caused by different tobamovirus strains depending on the temperature

Plants	ToMV-Ob			TMV-U$_1$			ToMV-Sd		
	18–20	26–28	30–32	18–20	26–28	30–32	18–20	26–28	30–32
	°C								
Nicotiana tabacum cv. Xanthi-nc	L/s	L/S	L/S	L/—	L/—	L/S+	L/—	L/—	L/S+
Nicotiana glutinosa	L/s	L/S	L/S	L/—	L/—	L/S+	L/—	L/—	L/S+

L = local symptoms
S = systemic symptoms
s = symptomless systemic infection
— = no infection, as demonstrated by back inoculation
+ = indicated plants are died

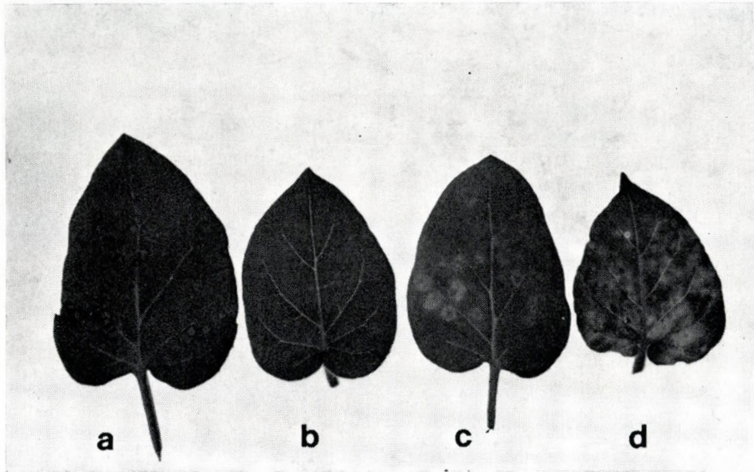

Fig. 3. Local (a) and systemic (b) symptoms at 18–20 °C. Local (c) and systemic (d) symptoms at 26–28 °C on *Nicotiana glutinosa* infected with tomato mosaic virus-Ob strain

given in Table 2. *Nicotiana tabacum* cv. Xanthi-nc and *Nicotiana glutinosa* plants infected with ToMV-Ob at 18–20 °C showed necrotic local lesions as well as systemic latent infections. On the other hand, at higher temperature, at 26–28 °C and 30–32 °C, the inoculated leaves showed only yellow spots or ring-like spots without any necrosis, and clear, dark yellow mosaic symptoms on the systemically infected ones (Fig. 3). Formation of necrotic symptoms depended on temperature: they developed only below 26 °C. Symptoms observed in plants inoculated with TMV-U_1 and ToMV-Sd, corresponded to those described for *TMV in general.* Other interesting property of ToMO-Ob is that this strain invades systemically *Capsicum annuum* cv. Fehérözön; cv. Keystone Resistant Giant which contains the L^1 allele of TMV resistance gene. In contrary *Capsicum chinense* PI 159236 and PI 315008 and other accessions were resistant, producing necrotic local lesions (Boukema, 1980; Csilléry and Ruskó, 1980.) These accessions contain a new allele of TMV resistance gene described by Boukema (1980) as an L^3 allele.

Table 3

Homologous and heterologous titers of different Tobamo isolates

Antigens	Antisera		
	ToMV-Ob	ToMV-D/H	TMV-U_1
ToMV-Ob	512 (0)	128 (2)	32 (6)
ToMV-D/H	8 (6)	512 (0)	1024 (1)
TMV-U_1	2 (8)	512 (0)	2048 (0)

Serological differential indexes are in brackets

Serology. In order to establish the serological relationship among ToMV-Ob and other tobamoviruses, our strain was compared in microprecipitin serological test with TMV-U1 and ToMV-D/H strains that are type members of tobacco and tomato mosaic virus group, respectively (Burgyán et al., 1978). Antisera and antigens were prepared as it was described previously (Tóbiás et al., 1982; Burgyán et al., 1978). The titers were estimated in microprecipitin reactions under paraffin oil (van Slogteren, 1954). Results are given in Table 3. Serological differential index (SDI) of TMV-U1 and ToMV-D/H varied between 0 and 1, demonstrating that they are closely related. ToMV-Ob differs from both TMV and ToMV strains and the serological differential index varied between 6 and 8, while the reciprocal SDI varied between 2 and 6.

Discussion

On the basis of host plant reactions ToMV-Ob is considered as a new strain of tomato mosaic virus. It is distinctly related to ToMV-D/H and TMV-U1, type members of the tomato and tobacco mosaic virus group, respectively. Hamilton et al. (1981) advised for establishing the taxonomic status of a new isolate that if the SDI with several antisera was greater than three and there were little differences in the host range the new isolate could be considered as a new strain. On the basis of our symptomatological and serological results the new virus isolated from pepper ToMV-Ob is a new pepper strain of tomato mosaic virus.

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Population Dynamics of Aphids, Vectors of Maize Dwarf Mosaic Virus and Aphid Resistance of Some Maize Hybrids

I. Milinkó, Zsuzsanna, V. Rakk and Gy. Kovács

Department of Plant Protection, Agricultural University, H-8361 Keszthely, P. O. Box 71, Hungary

The authors studied at Keszthely in 1980 and 1981 the population dynamics of aphids, known as vectors of maize dwarf mosaic virus (MDMV) and the resistance of some maize hybrids to aphids. In the investigated plots in both years the aphid species *Rhopalosiphum padi* L., *Metopolophium dirhodum* Walk. and *Macrosiphum avenae* Fabr. dominated. Also the species *Rhopalosiphum maidis* Fitch and *Aphis fabae* Scop. occurred, but in lower individual density. The aphids colonized the studied maize hybrids to a different degree. This pre-formed aphid resistance, as one of the indirect means of controlling the virus, may be of importance in the integrated control of MDMV.

The maize dwarf mosaic virus (MDMV) is one of the most severe pathogens of maize both in Hungary and on world scale, causing grave losses both in yield quantity and quality. It has been known in Hungary since about 20 years (Szirmai and Paizsné, 1963) and has spread since to the whole area of Hungary as compared to the first place of occurrence in Baja in South Hungary (Milinkó, 1978).

As regards the annual and intercyclic dynamics of MDMV epidemics, the virus overwinters in the soil, in rhizomes of systemically infected *Sorghum halepense* Pers. plants. The aphids may take up the virus from the Sorghum halepense plants sprouting in the spring and transmit it to young maize plants in an area of 3–4 Km in diameter. The transmission is non-persistent, stylet-borne. The extent of epidemics is determined therefore by the following factors:

- number and proximity of primary sources of infection,
- meteorological factors, which affect the development of aphic vectors,
- the gradation of the aphids themselves,
- the susceptibility or resistance of the maize hybrids grown in the region.

These factors also determine the measure of economic damage to be expected and the possibilities of control (Milinkó, 1974, 1977, 1978, 1979, 1980 Peti, 1979; Milinkó et al., 1979; Baranyai, 1980; Milinkó and Baranyai, 1981; Milinkó et al., 1981).

In our studies described in the present paper the aim was the following:

- to establish the aphid vectors of MDMV in the Keszthely area (Müller, 1961);

- to establish the population dynamics of vectors in different years, i.e. in years with and without MDMV epidemics (Kozma, 1980; Vidosné and Milinkó, 1981);
- to establish the resistance or susceptibility of perspectivic maize hybrids to aphids (Rhodes and Luckmann, 1967; Dicke and Guthrie, 1978);
- to compare all these informations with studies on resistance to virus (Milinkó et al., 1982).

Materials and Methods

Population dynamics studies

The study of population dynamics of possible MDMV vectors was carried out in the years 1980 and 1981 in the Keszthely Region of the Bak Experimental Farm, belonging to the Keszthely Agricultural University. The large-scale maize stands investigated belonged to the variety PX 20. The surveys were made every 7–10 days in both years from the end of May or beginning of June. At each date 10–10 survey sites were selected and on each site 5–5 plants were chosen at random and subjected to a detailed study. The percentage of plants infested by aphids was determined and the extent of infestation estimated by using the Banks-scale. So each aphid species received a score number per plant according to the following scale values:

0 = there is no aphid colonization
1 = sporadically occurring individuals
2 = small colonies
3 = large, but not continuous colonies
4 = large, continuous aphid colonies

The aphids found in the surveys were collected, preserved in alcohol and determined to species in the laboratory. The data gained were depicted in diagrams to illustrate the population dynamics of aphid species established.

Aphid-resistance studies

These studies were made in both years in the Keszthely Station of the Research Institute of Cereal Growing. The stands contained experimental hybrids grown in small plots, in four repetitions. Five plants per plot i.e. 20 plants per hybrid were investigated for aphid infestation. Besides the infestation percentage the plants were also scored by using the Banks scale for individual density. Based on these data the hybrids were ranged into categories of resistance or susceptibility. No virus resistance could be evaluated in this experiment as no spontaneous virus infection occurred, due to the absence of virus reservoir *Sorghum halepense* plants.

Results

Population dynamics studies

In the period of aphid colonization the maize plants were in 1980 in the three- or four-leaf stage. The first alate aphids appeared then, in the first days of June; after the appearance of the first aphids, their individual number gradually increased. The more pronounced increase started after mid-June and by the end of June smaller or larger colonies were found on nearly 100 per cent of the plants. In 1981, at the time of the first survey (24 May) no aphids were found yet on the plants. The first colonization, beginning at the 6–7-leaf stage of maize by the beginning of June, was followed by a rapid increase in individual density. By 20 June nearly all plants were infested and the peak of the individual curve appeared between 29 June and 6 July.

At the site of studies in both years the species *Rhopalosiphum padi* L. and *Metopolophium dirhodum* Walk. played a dominant role. *Macrosiphum avenae* Fabr. was also present continuously during the vegetation period but in much

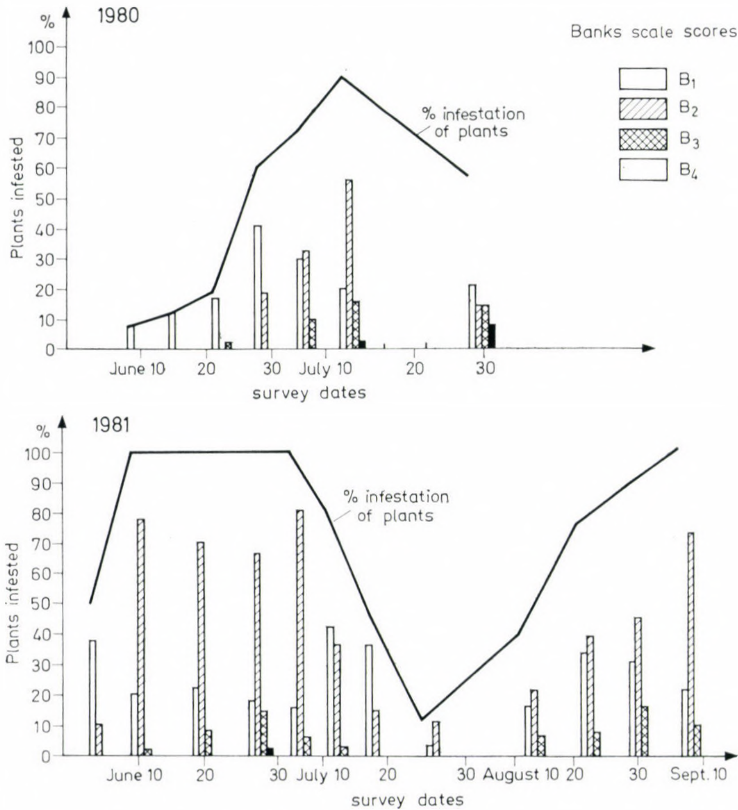

Fig. 1. Population dynamics of *Rhopalosiphum padi* L. on maize in 1980 and 1981 in Keszthely

lower individual numbers than the aforementioned. The species *Rhopalosiphum maidis* Fitch and *Aphis fabae* Scop. appeared in 1980 sporadically and in 1981 more regularly, but in even lower individual densities than the others mentioned above.

The population dynamics of the first mentioned three species are shown in Figs 1, 2 and 3. The graphs depict the percentages of plants infected by aphids, the columnar diagrams show the individual densities of the different aphid species, as indicated by by the Banks scale values. As shown in Figures, the intensive increase in population densities started in 1980 about three weeks later than in 1981. As well known, in that year also the vegetation periods of most crop plants were delayed by nearly three weeks, amont them those of cereals which serve as first secondary hosts for many aphids. From the cereals the aphids usually fly to maize only if these become too mature to comply with their nutrient requirements.

In 1980 the usual mid-summer heat wave was not pronounced and also the vegetative growth of the maize was delayed; also the collapse of the aphid grada-

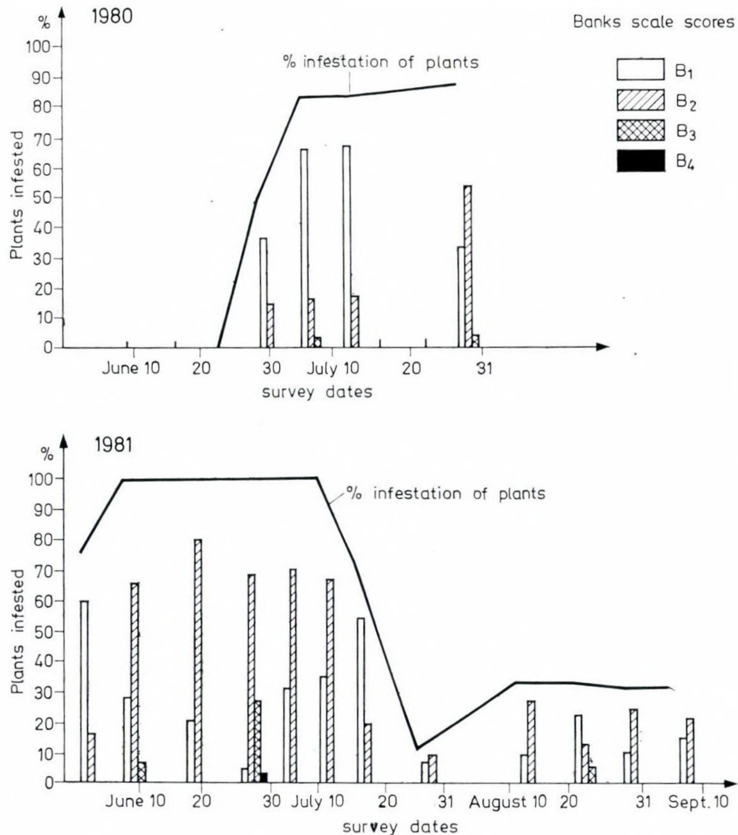

Fig. 2. Population dynamics of *Metopolophium dirhodum* Walk. on maize in 1980 and 1981 in Keszthely

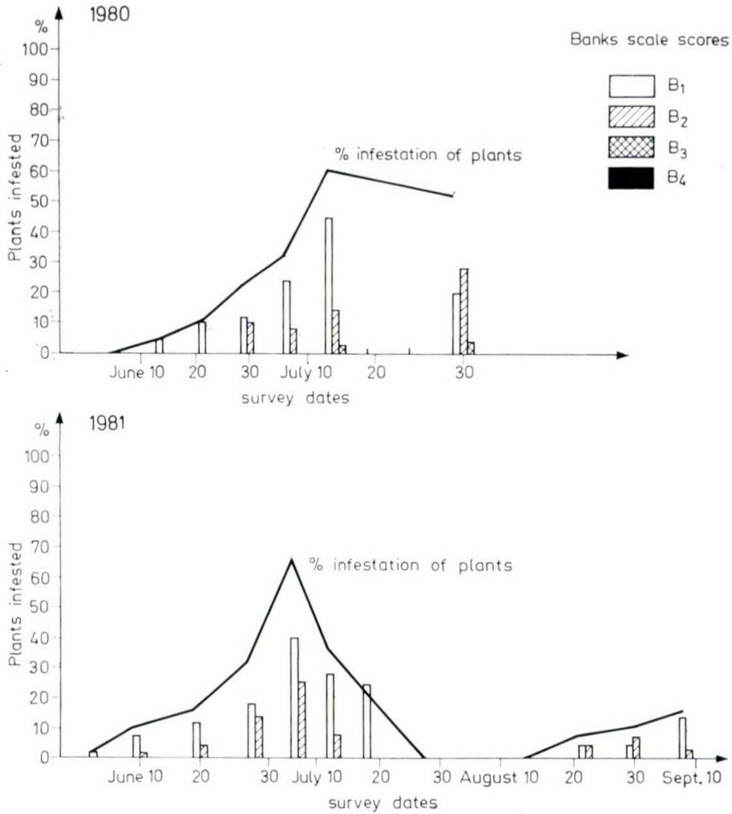

Fig. 3. Population dynamics of *Macrosiphum avenae* Fabr. on maize in 1980 and 1981 in Keszthely

tion was observed at a later time than in the year following. In 1981 the dry, hot weather in July did not present optimal conditions for the multiplication of aphid. Also the vegetative development of maize was earlier terminated, so the gradation collapsed much faster than in the previous year.

Studies on aphid-resistance

In the two years 22 maize hybrids were studied for differences in aphid infestation (percentages infested) and in the size of colonies. The experimental hybrids belonging to the maturation groups FAO 100 and FAO 600 as well as the Hybrid Anjou 256 SC were very heavily infested. Both the percentages of infestation and the individual densities of aphids were the lowest on the hybrids Sze SC 369, Sze MSC 378, Pioneer 3709 SC and JX 92 SC. The hybrids Pioneer 3780 MSC, Mv SC 580, Pioneer 3906 SC, Sze SC 444 and Sze DC 488 showed moderate infestations, as shown in Tables 1 and 2. From among them, the hybrids Sze MSC 378,

Table 1

The aphid infestation of different maize hybrids (Keszthely, 1980)

FAO No.	Hybrid	Percentage of aphid infestation			
		B_1	B_2	B_3	B_4
100	BC 188 MSC	40	30	10	20
	BC 85343	40	30	15	15
	BC 9–109	35	25	25	15
200	Anjou 256 SC	50	35	5	5
	MSC 3279	55	30	5	—
300	Sze TC 344	50	35	5	—
	Sze MSC 378	50	25	5	—
	Sze SC 369	50	20	—	—
	JX 92 SC	50	30	5	—
400	Pioneer 3709 MSC	50	25	—	—
	Mv SC 429	55	30	5	—
500	Pioneer 3780 MSC	50	30	5	—
	Mv SC 580	50	35	5	—
600	DC 3538	50	35	—	—
	14 621	40	30	20	10
	14 622	50	30	15	5

Table 2

The aphid infestation of different maize hybrids (Keszthely, 1981)

FAO No.	Hybrid	Percentage of aphid infestation			
		B_1	B_2	B_3	B_4
200	Anjou 256 SC	—	5	80	15
	Sze TC 255	5	40	55	—
	MSC 3279	10	45	45	—
300	Pioneer 3906 SC	30	55	25	—
	Sze SC 369	25	45	30	—
400	Sze SC 444	—	25	75	—
	Mv SC 429	—	5	95	—
	Sze DC 488	—	—	90	10
500	Sze MSC 515	—	5	90	5
	Sze TC 505	—	5	80	15

Sze SC 369 and JX 92 SC were found in other experiments resistant to virus while the hybrids Mv SC 580 and Pioneer 3780 MSC showed a moderate susceptibility to MDMV (Milinkó et al., 1982).

Most maize hybrids showing a reduced aphid infestation also displayed a good stalk stability. It seems therefore that the MDMV resistance of these hybrids may be explained by the presence of a preformed resistance i.e. firm tissue structure.

Conclusions

According to our present knowledge, it is the weakest point of the integrated control against MDMV is the still uncompletely solved control of the virus reservoir *Sorghum halepense* plants (Milinkó, 1974). Another problem, that the chemical control measures applied against the virus vector aphids harm the members of the beneficial fauna (Baranyai, 1980; Milinkó and Baranyai, 1981). So the establishment of dominant aphid species, the knowledge of their bionomics and the recording of their population dynamics may serve as basis for future control methods.

The MDMV resistance demonstrated in 1981 in nine maize hybrids (Milinkó et al., 1982) may be due at least for the most part to an aphids resistance, which, in turn is based upon a preformed resistance (stable tissue structure, thick cuticle etc.). This seems to be supported by the MDMV-resistant hybrids Sze SC 369, Sze MSC 378 and JX SC 92 as well moderately susceptible hybrids Mv SC 580 and Pioneer 3780 MSC.

By this type of co-ordinated, complex investigations the virus resistance of crops may be interpreted or even improved in the future. This may also serve for a substantial improvement in the integrated control of MDMV.

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Reaction of *Physalis* Species to Plant Viruses VII. Additional Data on the Virus Susceptibility of *Physalis minima* L.

J. Horváth

Institute of Plant Protection, University of Agricultural Sciences, H-8361 Keszthely, P. O. Box 71, Hungary

In the course of experiments concerning the virus susceptibility of *Physalis minima* L., a species relatively little known in the literature of plant virology, we found the plant in question to be locally and systemically susceptible to 11 viruses (alfalfa mosaic virus, *Arabis* mosaic virus, belladonna mottle virus, cucumber mosaic virus, *Melandrium* yellow fleck virus, tobacco rattle virus, tobacco ring spot virus, tomato aspermy virus, tomato mosaic virus, tomato ring spot virus, turnip mosaic virus) and systemically susceptible to one virus (broad bean wilt virus). The same experimental plant proved resistant to nine viruses (bean common mosaic virus, bean yellow mosaic virus, carnation ring spot virus, cauliflower mosaic virus, celery mosaic virus, cucumber green mottle mosaic virus, radish mosaic virus, turnip yellow mosaic virus, watermelon mosaic virus).

The compatible and incompatible new host-virus relations play an important role in the identification and differentiation of the above-mentioned viruses. The possibility of differentiating the cucumber mosaic virus strains recently found to occur in bean plants *(Phaseolus vulgaris* L.) from various virus pathogens of bean (e.g. bean common mosaic virus, bean yellow mosaic virus) by means of *Physalis minima* plants deserves particular attention.

As pointed out in our earlier publications related with the susceptibility of *Physalis* species to plant viruses there is a very strong affinity between the different *Physalis* species and viruses. According to our present knowledge responses of about 26 *Physalis* species and one variety to 90 viruses have been disclosed so far. In earlier publications 62 new host-virus relations and some 67 cases of resistance revealed in our experiments for 12 *Physalis* species and a variety were described (cf. Horváth, 1970, 1974*a,b*, 1975*a,b*, 1981). Among the *Physalis* species, *Physalis minima* is relatively less known for virus susceptibility. As far as we know at present the plant under discussion is only susceptible to tobacco mosaic virus, potato virus A, potato virus X and potato virus Y (cf. Palm and Jochems, 1924; Stelzner and Schwalb, 1943; Horváth, 1970). In India it was a few years ago that *Physalis minima* as a well-known and dangerous weed plant was found to be a host to cucumber mosaic virus (Joshi and Dubey, 1976). Considering that data on the virus susceptibility of *Physalis minima* are few in number, in our recent experiments we set the aim of supplying further information concerning the virus susceptibility of the plant in question.

Materials and Methods

In our experiments young *Physalis minima* plants were inoculated with 21 plant viruses to which the responses of *Physalis minima* had not been known so far (Table 1). As to the maintenance of the viruses, the methods of inoculation, the re-isolation of viruses from the inoculated and non-inoculated or subsequently developed leaves and the test or indicator plants detailed data can be found in our earlier publications (cf. Horváth, 1974*a*, 1976, 1977, 1981). In the experiments described in this paper newly investigated viruses (tobacco rattle virus, tomato ring spot virus) were kept in *Nicotiana tabacum* L. cv. *Xanthi-nc*. These viruses were also re-isolated from inoculated and non-inoculated leaves of infected *Physalis* plants and tested on *Chenopodium amaranticolor* Coste et Reyn. and *Ch. quinoa* Willd. indicator plants.

Table 1

Viruses inoculated in the experiments

Viruses	Strains or isolates	Literature
Alfalfa mosaic virus	K_2, MvS70	Beczner (1972)
Arabis mosaic virus	Phil. 2	Schmelzer (1968, 1974)
Bean common mosaic virus	F23	Horváth (1973)
Bean yellow mosaic virus	S, RM	Horváth (1976)
Belladonna mottle virus	H	Horváth et al. (1976)
Broad bean wilt virus	Tm, HZ	Horváth and Szirmai (1975), Horváth (1976)
Carnation ring spot virus	IPA[1]	Fritzsche and Schmelzer (1967)
Cauliflower mosaic virus	DH	Horváth et al. (1979, 1980*a*)
Celery mosaic virus	P	Horváth et al. (1976)
Cucumber green mottle mosaic virus	IPA[1]	Schmelzer (1967)
Cucumber mosaic virus	EN, U/246, U/58-77F3, W	Horváth and Szirmai (1973), Horváth (1976), Schmidt and Horváth (1982)
Melandrium yellow fleck virus	LB	Hollings et al. (1978) Hollings and Horváth (1981)
Radish mosaic virus	HS7	Horváth et al. (1973)
Tobacco rattle virus	H	Horváth (1976)
Tobacco ring spot virus	D	Horváth (1976)
Tomato aspermy virus	Tm12	Horváth et al. (1980*b*)
Tomato mosaic virus	H	Horváth and Beczner (1973), Mamula et al. (1974), Juretić et al. (1977)
Tomato ring spot virus	Bonn	F. Nienhaus (1979)
Turnip mosaic virus	A11	Horváth et al. (1975*a*)
Turnip yellow mosaic virus	HB	Horváth et al. (1973)
Watermelon mosaic virus	PW	Horváth et al. (1975*b*)

[1] Institute of Phytopathology, Aschersleben, GDR

Results and Discussion

In our experiments *Physalis minima* was found to be locally and systemically susceptible to 11 viruses, systemically susceptible to one virus, and resistant to further 9 viruses (Table 2). The pathological features caused by alfalfa mosaic virus, cucumber mosaic virus and turnip mosaic virus in the compatible host-virus relations are particularly remarkable from a symptomatological point of view. The responses of *Physalis minima* plants inoculated with local (K_2) and systemic (MvS70) strains of alfalfa mosaic virus, respectively, showed essential differences as regards the intensity of symptoms. The K_2 virus strain produced slight systemic symptoms (mosaic), while the systemic virus strain MvS70 induced a very severe systemic mosaic and leaf deformations reminding of symptoms caused by herbicides. On the basis of the results of experiments the local and systemic strains of alfalfa mosaic virus – originally differentiated by *Phaseolus vulgaris* plants (cf. Beczner, 1973; Horváth and Beczner, 1982) – seem to be separable in *Physalis minima* plants as well. Owing to the short time of incubation (4–5 days), and because of its strong affinity to alfalfa mosaic virus *Physalis minima* is an important test plant and a remarkable prognostic host. Among the strains of cucumber mosaic virus symptomatological differences could also be pointed out. In comparison to the W strain the EN strain caused much slighter symptoms. It is worthy of attention that the strains of cucumber mosaic virus pathogens of legumes recently isolated from bean plants in Hungary proved equally pathogenic for *Physalis minima* plants. The symptomatological differences between cucumber mosaic virus strains – pointed out in our recent experiments with *Physalis minima* plants to – are well known in the literature of virology (cf. Corbett and Sisler, 1964; Smith, 1972; Welvaert and Samyn, 1974; Horváth, 1974c, 1976; Gibbs and Harrison, 1976; Klinkowski, 1977*a,b,c*; Bos, 1978; Francki et al., 1979; Kaper and Waterworth, 1981). *Physalis minima* as a natural host to cucumber mosaic virus (cf. Joshi and Dubey, 1976) is of a great importance from an ecological point of view as well.

The turnip mosaic virus, a pathogen occurring all over the world, produced characteristic chlorotic-necrotic lesions on the inoculated leaves of *Physalis minima* plants after four days of inoculation. On the 10th days following the inoculation intensive systemic spottedness, mosaic and leaf deformation were observed. The strong affinity between *Physalis minima* and turnip mosaic virus has a prognostic importance.

According to our investigations *Physalis minima* – like *Physalis curassavica* L., a species that has recently become known from the literature of plant virology (cf. Horváth, 1981) – proved a latent systemic host to *Melandrium* yellow fleck virus.

Physalis minima was found to be resistant to nine viruses (see Table 2). On the basis of compatible and incompatible host-virus relations *Physalis minima*, in addition to its virus diagnostic- and virus prognostic role proved an important virus separator as well (Table 3). It play a particularly important role in the case

Table 2

Reaction of *physalis minima* L. to some viruses

Viruses	Symptoms[1]	Results of the reisolation of the investigated viruses
Alfalfa mosaic virus	IL: necrotic rings NIL: mosaic, leaf deformations	IL: positive NIL: positive
Arabis mosaic virus	IL: chlorotic and necrotic lesions NIL: severe mosaic	IL: positive NIL: positive
Bean common mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Bean yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Belladonna mottle virus	IL: necrotic rings NIL: chlorotic and necrotic rings, leaf deformations	IL: positive NIL: positive
Broad bean wilt virus	IL: no symptoms NIL: vein clearing, mosaic, recovery	IL: negative NIL: positive
Carnation ring spot virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cauliflower mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Celery mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber green mottle mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Cucumber mosaic virus	IL: chlorotic, necrotic lesions NIL: severe mosaic, leaf deformations	IL: positive NIL: positive
Melandrium yellow fleck virus	IL: necrotic lesions NIL: no symptoms	IL: positive NIL: positive
Radish mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Tobacco rattle virus	IL: necrotic lesions NIL: yellow spots	IL: positive NIL: positive
Tobacco ring spot virus	IL: necrotic rings NIL: necrotic lesions and leaf drop	IL: positive NIL: positive
Tomato aspermy virus	IL: chlorotic-necrotic lesions NIL: mosaic	IL: positive NIL: positive
Tomato mosaic virus	IL: necrotic lesions NIL: necrotic lesions, leaf drop	IL: positive NIL: positive
Tomato ring spot virus	IL: necrotic lesions NIL: mosaic, leaf deformations, stunting	IL: positive NIL: positive

(Table 2, continued)

Viruses	Symptoms[1]	Results of the reisolation of the investigated viruses
Turnip mosaic virus	IL: chlorotic and necrotic lesions NIL: mosaic, leaf deformations	IL: positive NIL: positive
Turnip yellow mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative
Watermelon mosaic virus	IL: no symptoms NIL: no symptoms	IL: negative NIL: negative

[1] IL: Inoculated leaves; NIL: non-inoculated leaves, or subsequently developed leaves

of such viruses as often occurring in a complex form (e.g. cucumber mosaic virus + celery mosaic virus, cucumber mosaic virus + watermelon mosaic virus, turnip mosaic virus + radish mosaic virus, turnip mosaic virus + turnip yellow mosaic virus and others). The possibility of separating cucumber mosaic virus from bean common mosaic virus and bean yellow mosaic virus, respectively, by means of *Physalis minima* plants deserves special attention. It is a well known fact that the appearance of cucumber mosaic virus beside bean common mosaic virus and bean yellow mosaic virus in bean plants has lately become more and more frequent. In our opinion *Physalis minima* plays an important role in differentiating cucumber mosaic virus strains isolated from bean plants in the United States of America, France, Iran, Japan, Spain, the German Democratic Republic, Iraq and Hungary and described as pathogenic for legumes (reviewed by Horváth, 1983), as well as

Table 3

Separation of different viruses with *Physalis minima* L.

Viruses	
Separated	Eliminated
Alfalfa mosaic virus	Bean common mosaic virus
Arabis mosaic virus	Bean yellow mosaic virus
Belladonna mottle virus	Carnation ring spot virus
Broad bean wilt virus	Cauliflower mosaic virus
Cucumber mosaic virus	Celery mosaic virus
Melandrium yellow fleck virus	Cucumber green mottle mosaic virus
Tobacco rattle virus	Radish mosaic virus
Tobacco ring spot virus	Turnip yellow mosaic virus
Tomato aspermy virus	Watermelon mosaic virus
Tomato mosaic virus	
Tomato ringspot virus	
Turnip mosaic virus	

bean common mosaic virus and bean yellow mosaic virus. The two strains of cucumber mosaic virus isolated from bean plants in Hungary (U/246, U/58-77F3) were found equally pathogenic to *Physalis minima* plants.

On the basis of experiments performed so far with *Physalis minima* it can be established that this plant is susceptible not only to tobacco mosaic virus (Palms and Jochems, 1924), potato virus A, potato virus X and potato virus Y (Stelzner and Schwalb, 1943) but – according to our investigations – to further eleven viruses too. With the recent experimental data the number of compatible host-virus relations disclosed between *Physalis minima* plants and viruses has risen to 16.

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The Role of Some Plants in the Ecology of Cucumber Mosaic Virus with Special Regard to Bean[1]

J. Horváth

Institute of Plant Protection, University of Agricultural Sciences, H-8361 Keszthely, P. O. Box 71, Hungary

The aphid-, seed- and mechanically transmissible cucumber mosaic virus (CMV), as the type member of the cucumovirus group, is the most important pathogen of plants in Hungary. The biologically and serologically different isolates of CMV can be divided into two serogroups (U or D and N or To). The different isolates of CMV occurred in a wide host range. On the basis of the data of the summarizing study it can be established that the natural hosts of CMV cover some 476 plant species of 67 families, the artificial ones 536 plant species of 53 families.

The weed plants, e.g. *Stellaria media* and *Echinocystis lobata* are most important in the ecology of CMV in Hungary. The seeds of the above-mentioned CMV-infected plants can transmit the virus year by year. In the last year we demonstrated that the perennials *Circaea lutetiana*, *Amaranthus deflexus*, the tree *Paulownia tomentosa*, the shrub *Lycium barbarum* and the overwintering *Brassica napus* are very important in the ecology of CMV. The natural occurrence of a new, seed-transmitted legume strain of CMV (CMV-U) on beans is very important in the ecology of the virus. The investigations on the relationship and the interaction between the legume strain of CMV and the other bean pathogenic viruses (e.g. bean common mosaic virus, BCMV; bean yellow mosaic virus, BYMV etc.) on beans are in progress.

Plant viruses like other microorganisms have the faculty of reproducing, spreading and surviving under favourable conditions. In possession of this faculty the viruses, as pathogens of various plant diseases, are of great importance in the ecosystem and pathosystem. Virus ecology is concerned with the study of how a virus is perpetuated under natural conditions. The survival of the virus is the result of a complex interaction of different factors, such as viruses, hosts, vectors, environment and agricultural practices (Table 1). The most important role in virus ecology, except vectors (e.g. aphids, leaf-hoppers, nematodes etc.) which are able to transmit viruses from weeds to crop plants and from crops to weeds, is undoubtedly played by those plants which, due to their susceptibility to viruses, enable the latter to survive under natural conditions and prevent the infection chain from breaking. Harrison (1981) in his paper also distinguished "Wilpad" (wild plant-adapted) viruses that seem particularly well adapted for survival in wild plants and "Culpad" (cultivated plant-adapted) viruses that seem to thrive best in cultivated plants. Viruses (e.g. cucumber mosaic virus) in several groups (cucumovirus,

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Table 1

Different factors in virus ecology

Viruses	Hosts	Vectors	Environment	Agricultural practice
Concentration and stability Spread in the host Caused disease Host range Strain	Affinity to virus Life cycle Natural form of growing Propagation Suitable to feeding of vectors	Activity and frequency Migration	Soil and clima Wind Temperature Humidity	Fertilization Time of showing Cultivation Size of the field (table) Density of plants Time of growing Rotation of crops Vicinity

potyvirus, rhabdovirus, tymovirus) occur both in communities of wild plants and in crops. In this respect CMV is a unspecialized virus.

The interesting questions of the natural survival and circulation of viruses, the problems of virus ecology and virus geography have been discussed in many papers for the last thirty years (cf. Hein, 1953; MacClement and Richards, 1956; Schwarz, 1959; Swenson, 1968; Broadbent, 1969; Matthews, 1970; Murant, 1970; Tomlinson et al., 1970; Duffus, 1971; Häni, 1971; Cooper and Harrison, 1973; Tomlinson and Walker, 1973; Dinoor, 1974; Thresh, 1974; 1976, 1978, 1980*a*,*b*, 1981*a*,*b*; Gibbs and Harrison, 1976; Horváth, 1976, 1977*a*,*b*, 1982; Berger, 1977; Harrison, 1977, 1978, 1981; Zitter, 1977; Bos, 1978, 1981; Schmelzer, 1980; Quiot, 1980; Adlerz, 1981; Carter, 1981; Conti, 1981; Jones, 1981; Gibbs, 1981; Randles et al., 1981).

In the present paper we should like to give an account of several wild and cultivated plants that play an important role in the ecology of CMV in Hungary, and to call attention to such artificial new hosts as recently found susceptible to CMV and may be important as prognostic virus hosts.

Cucumber Mosaic Virus and Its Hosts

On the basis of data of previous studies it can be established that the natural hosts of CMV cover some 476 plant species of 67 families, the artificial ones 536 plant species of 53 families (cf. Horváth, 1979, 1980*a*). In Hungary CMV belongs to the most important viruses. Those isolated so far from a large number of plants (e.g. *Circaea lutetiana*, *Cucurbita pepo*, *Cucumis sativus*, *Echinocystis lobata*, *Paulownia tomentosa*, *Phaseolus vulgaris*, *Scopolia carniolica*, *Stellaria media*, *Capsicum annuum*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *Lactuca sativa*, *Asclepias syriaca* etc.) are of the D or U and To or N serotypes (cf. Richter et al., 1972; Devergne, 1975; Horváth and Beczner, 1982*a*; Smith and Horváth, 1982).

It is remarkable that the CMV strains isolated so far from the family *Leguminosae* belong without exception to the D or U serotype, while among those isolated from various other plant families CMV strains of either serotype have occurred (cf. Horváth, 1982). In the ecology of CMV in Hungary a highly important role is played by trees, overwintering- and perennial plants as well as by those annual and biannual plants the seeds of which are suitable for CMV transmission, or which are favourable feed plants for virus vectors.

Natural Hosts

Trees and shrubs

Among the trees and shrubs *Paulownia tomentosa* and *Lycium barbarum* have a great part in the circulation and natural survival of CMV (Horváth, 1972, 1973*a*, 1975*a*,*b*; Horváth et al., 1976). Both are important feed plants for various aphid vectors (e.g. *Myzus persicae*) and thereby highly suitable virus reservoirs.

Overwintering plants

According to our investigations *Brassica napus* gets infected with CMV in the late summer- and autumn months from weed plants through aphids as virus vectors (Horváth, 1969, 1980*b*). The virus having overwintered in rape plants becomes a source of infection for various perennial and annual plants in spring. Besides its high significance as a feed plant of *Myzus persicae*, one of the most active virus vectors, the rape is of importance as a virus reservoir as well. As a consequence of the increasing production of rape the virus pathogens of *Brassica*, including CMV, are expected to spread at a faster rate.

Perennial plants

Of the perennial plants *Circaea lutetiana* (family: *Onagraceae*) and *Amaranthus deflexus* (family: *Amaranthaceae*) are worth of attention (Horváth, 1976; Horváth et al., 1976, 1981, 1982 unpublished). The demonstration of CMV in declared protected *Circaea lutetiana* in Hungary is of special interest. According to our opinion this perennial plant is a highly important virus reservoir, and plays a decisive role in causing the earlier described CMV infections of *Echinocystis lobata* and some horticultural plants occurring at the same site (Horváth and Szirmai, 1973).

Annual plants

From an ecological point of view those annual plants are of importance in the first place whose generative or vegetative reproduction organs are suitable for CMV transmission. According to literary data in the seed-borne transmission of

CMV important role is played by the *Stellaria*, *Cerastium*, *Spergula* and *Echinocystis* (Tomlinson and Carter, 1970; Horváth and Szirmai, 1973; Horváth, 1975*a,b*, 1976; Neergaard, 1977). In the course of investigations made at home we have found that in the epidemiology of CMV *Echinocystis lobata* and *Stellaria media* are of the greatest importance.

It is recently that the so-called legume strain of CMV has been pointed out to occur in Hungary; according to our present knowledge it is found in *Phaseolus vulgaris* (Schmidt and Horváth, 1982), *Nicotiana tabacum* (Beczner and Burgyán, 1982) and *Lupinus polyphyllus* (cf. Horváth and Beczner, 1982*a*). Of the above plants it is undoubtedly the bean that has the greatest importance, since the legume strain of CMV is known to be transmissible by the seeds of bean plants (cf. Bos and Maat, 1974; Meiners et al., 1977). According to the data of Schmidt et al. (1980, 1981*a,b*) the loss of yield caused by virus infection may even be as much as 38%. In the course of studying the *Phaseolus* – CMV relation we found 52 *Phaseolus* varieties and a number of less or hardly known *Phaseolus* species (e.g. *P. aconitifolius*, *P. coccineus*, *P. lunatus*, *P. mungo* etc.) to be equally susceptible to infection by CMV (Horváth and Beczner, 1982*b,c,d*). Knowing the CMV susceptibility of *Phaseolus* species is important not only from a virus ecological point of view; they may serve as valuable basic material in breeding for virus resistance. The virus susceptibility of annual and perennial *Phaseolus* species – some 200 in number – found in the tropical, subtropical and temperature zones, which have a great share in human nutrition, is highly important not only from the point of view of virus ecology and virus geography; it has considerable economic aspects as well. The 38% yield loss caused by CMV (cf. Schmidt et al., 1980, 1981*a,b*) may become catastrophic if the 22 million ha production area of bean (Anonymous, 1975) gets infected with CMV easily transmissible by bean seeds. The very rapid spreading of the legume strain of CMV in bean plants is indicated by the fact that in our current year's experiments the occurrence of CMV in bean crops was sometimes as high as 80%.

Artificial Hosts

In the course of artificial inoculation experiments we have found many new plants susceptible to infection by CMV (cf. Horváth, 1976, 1979, 1983). Among these artificial hosts trees and shrubs as well as biannual and perennial plants are of special importance (Table 2). The genetically determined and experimentally demonstrated virus susceptibility of plants may supply highly significant data concerning the ecology and geography of viruses, of CMV in present case. In this respect the *Lycium* species – known also to be important feed plants for virus vectors (aphids) – are in all probability highly significant (Horváth, 1972, 1976; Beczner and Horváth, 1972). In consequence of a favourable interaction between the ecological factors (e.g. virus – host – vector) an infection chain difficult to survey properly may develop.

Table 2

Artificial hosts as prognostical plants of cucumber mosaic virus

Natural form of growing or life cycle	Plants	References
Trees	*Paulownia fargesii*	Horváth (1973*b*, 1976)
Shrubs	*Lycium chinense*	Horváth (1976)
	L. barbarum	Horváth (1976)
	L. europaeum	Horváth (1972, 1976)
	L. flexicaule	Horváth (1976)
	L. horridum	Horváth (1972, 1976)
	L. ruthenicum	Horváth (1972, 1976), Beczner and Horváth (1972)
Perennial	*Commelina tuberosa*	Horváth (1976, 1979)
	Erodium manescavi	Horváth (1974)
	Geranium sibiricum	Horváth (1973*c*)
	Malva moschata	Horváth (1976, 1979)
	Melandrium sylvestre	Horváth (1976, 1979)
	Physalis peruviana	Horváth (1976, 1979)
	Silene tatarica	Horváth (1976, 1979)
Biannual	*Malva pusilla*	Horváth (1976, 1979)
	M. verticillata	Horváth (1976, 1979)
	Silene spp.	Horváth (1976, 1979)

Conclusions

In the ecology of viruses the plants and vectors undoubtedly have a major part; yet, we should like to emphasize that with a view to a better understanding of ecological, epidemiological and geographical correlations, and the knowledge of other ecological factors the international co-operation of virologists is – in our opinion – highly important or even indispensable.

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Effect of Single and Double Infection with *Myrothecium verrucaria* and Common Bean Mosaic Virus (CBMV) on Dry Weight and Chemical Constituents of Bean Plants

M. A. Amer, M. El-Hammady and F. Abo El-Abbas

Faculty of Agriculture, Ain Shams University Cairo, Egypt

Single and double infections with CBMV and *Myrothecium verrucaria* markedly decreased the dry weight of bean leaves. Single infection with the virus or double infection with both pathogens decreased total carbohydrate content. On the contrary, the fungus stimulated carbohydrate accumulation as compared with control plants. Virus infection increased total nitrogen content of inoculated leaves above healthy ones followed by combined inoculation. On the contrary, fungal inoculation diminished greatly total nitrogen percentage.

The viral inoculation created the content of conjugated form of phenyl-alanine, glutamic acid, arginine and alanine which reflected itself in double inoculation. Also, the reduction in the conjugated form of methionine, iso-leucine and tyrosine in case of fungal inoculation reflected itself on the double inoculation.

Under field conditions, cultivated plants may be infected with more than one pathogens. Mixed infection with different viruses and different fungi have been studied by some authors (Nitzany, 1966; Tuboly et al., 1970; Magyarosy and Hancock, 1974; El-Hammady et al., 1975; and Besada, 1978). In such studies, attention was mainly focussed on the pathological interactions.

The aim of the present work was to study the physiological effects of viral and fungal infection on bean plants. Due to their importance, common bean mosaic virus and *Myrothecium verrucaria* served as models in the present experiments. Effect of single and double infection with these two pathogens on dry weight, carbohydrate content, total nitrogen and amino acids of bean plants was studied.

Materials and Methods

The causal fungus of bean leaf spot disease (*Myrothecium verrucaria* Ditmar ex. Fr.) was kindly provided by the Plant Pathology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Common bean mosaic virus (CBMV) was originally isolated from naturally diseased bean plants (cv. Contender). The virus was identified on the basis of its host range, mode of transmission and physical properties.

Under greenhouse conditions, *Phaseolus vulgaris* cv. Contender seeds were sown in 30 cm diameter pots filled with clay soil. The plants were thinned to two

plants in each pot. The growing plants were grouped into four groups. Each group contained 30 pots. The groups were:

1. Inoculated with CBMV when the plants were 23 days old.
2. Inoculated with *Myrothecium verrucaria* when the plants were 30 days old and reinoculated by the fungus every 7 days.
3. Inoculated with CBMV followed by *M. verrucaria* at the time of inoculation of the previous groups.
4. Not inoculated (control).

Samples of plant leaves were taken from the previous treatments to determine dry weight at 70 °C and to be analyzed for carbohydrate, total nitrogen and amino acid content.

The first sample was taken when the plants were 33 days old. Sampling was repeated every week, six times. Ten plants were collected for each particular treatment.

Reducing sugars, total sugars and total carbohydrate were determined using the method reported by Shaffer and Hartman (1921).

Total nitrogen was determined colorimetrically according to the method described by Naguib (1969).

The free amino acids were extracted in 70% ethanol. The residue of dried leaves was used for the determination of conjugated amino acids. For extraction of conjugated amino acids, the residue was hydrolysed by 6 *N* HCl at 90 °C for 24 h.

One dimensional ascending paper chromatography was carried out for qualitative and quantitative determination of amino acids using two systems successively. The running solvents were *n*-butanol : acetic acid : water. The first was in ratio: 4 : 1 : 5 (v : v : v) and the second in 4 : 1 : 1 (v : v : v) (Block, 1958). Amino acids were rendered visible by spraying with 0.2 ninhydrin in acetone (w/v) (Smith, 1958; and Marble et al., 1959). The concentration of amino acids was calculated as mg/100 g dry weight of each sample (Hussein, 1973). Free and conjugated amino acids were divided into four groups according to Cohen (1967).

Results and Discussion

Dry weight

The results of the present work (Fig. 1) revealed that leaf dry weight of healthy plants was much higher than that of the inoculated ones reaching 5% level of significance during 54–68 days after sowing. *Myrothecium* inoculation also created significant increase in the dry weight in the same period above viral and doubly infected plants. Sirry et al. (1974) indicated that, *Myrothecium* infection decreased dry weight of bean plants. Confirming the reduction obtained with viral infection, Allam and Abo El-Ghar (1970), Allam et al. (1973) reported that the virus infection reduced the dry weight in infected plants below healthy ones.

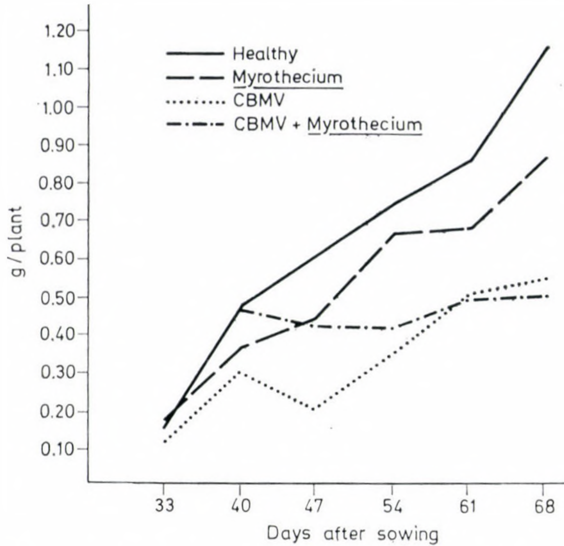

Fig. 1. Effect of single and double infection with *Myrothecium* and CBMV on the dry weight of Contender bean leaves (in g per plant)

Carbohydrate content

Regarding the effect of infection on the seasonal changes of total carbohydrate (Fig. 2), it could be noticed that a reduction in carbolydrates occurred during the flowering period extended 47–54 days after sowing for single infection with CBMV or combined with *Myrothecium* in comparison with only one sample (47 days after sowing) of healthy plants. Moreover, the reduction in reducing sugars was detected after this date till the end of experiment concomitant with an increase in total sugars. The reverse was true during the period prior this time since the reduction in total sugars was associated with an increase in the reducing form particularly with double infection, a phenomenon that indicates the transformation of the latter form to reducing sugars to provide their demands which was not detected either for healthy or fungal infection. Here again, as previously mentioned, viral infection showed a depressive effect for total carbohydrate (both forms). Such behaviour was also detected with double infections for total carbohydrate but not for reducing and total sugars. In this respect, Fawzy (1973) and El-Wafaei (1976) reported that the viral infection decreased the carbohydrate content of plants.

On the other hand, fungal infection stimulated total carbohydrate accumulation as well as reducing and total sugars as compared with the control. These findings are in agreement with Sirry et al. (1975). They indicated an increase in carbohydrate in inoculated plants. Zayed (1972) reported that the fungal infection increased the total carbohydrate content. In addition Shaw (1961); Zaki and Miro-

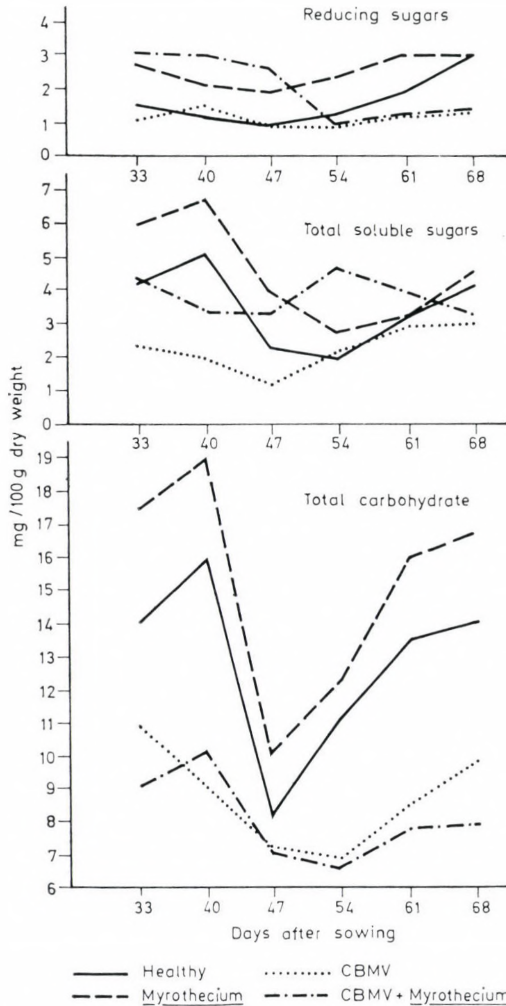

Fig. 2. Effect of single and double infection with *Myrothecium* and CBMV on the reducing sugars (R. S.), total soluble sugars (T. S. S.) and total carbohydrate (T. C.) of Contender bean leaves (g/100 g dry weight)

cha (1964), reported that, metabolites such as C^{14}-labeled sucrose, glucose, ribose and xylose have been shown to accumulate in infected plants.

It could be concluded that in case of double infection *Myrothecium* failed to initiate higher carbohydrate content of these plants when they were firstly inoculated with CBMV and later with *Myrothecium*. So first infection with CBMV altered the stimulatory effect of subsequent fungal infection in increasing total carbohydrate. Confirming these phenomena, the data* of fungal growth on media extract-

* Unpublished work.

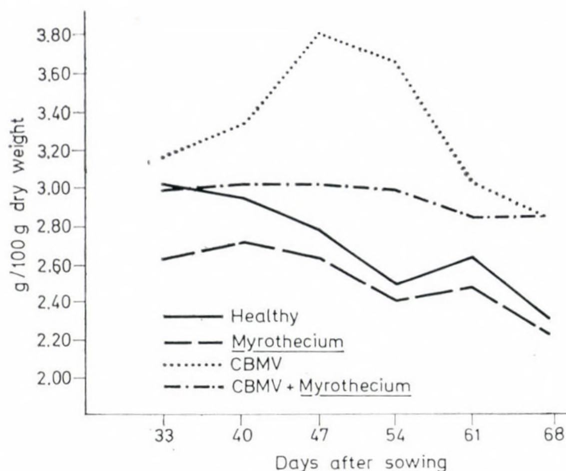

Fig. 3. Effect of single and double infection with *Myrothecium* and CBMV on the total nitrogen of Contender bean leaves (g /100 g dry weight)

ed from viral infected plants and those of leaf spotting with *Myrothecium* on viral inoculated leaves showed the same effect. On the other hand, TMV-infectivity on differential hosts was reduced when the leaves were prior treated with sap of mildewed squash plants.

It seems that antagonistic effect between these two pathogens could occur not only via their pathological interaction but also in their physiological action on the host (El-Hammady et al., 1975).

Total nitrogen

As for total nitrogen content (Fig. 3), viral infection predisposed the leaves of bean plants to increase nitrogen content above healthy as well as the ones inoculated with *Myrothecium* or those with double infection. This owe much to the reduction in total carbohydrate concentration which resulted in dry matter accumulation. Fungal infection diminished greatly total nitrogen and this associated with high carbohydrate content. On the other hand, double infection almost gave moderate and somewhat stable trend compared with the two single infection as well as control. Hence, such behaviour could be discussed as antagonistic affect between these two pathogens. It could be stated that different mosaic type viruses increased the total nitrogen in different plants (El-Wafaei, 1976 and El-Hammady et al., 1976). On the other hand, fungal infection was reported to decrease the total nitrogen content. Higazy (1968) found that the percentage of nitrogen content per plant was decreased in broad bean plants infected with *Botrytis fabae*. In addition, Raafat et al. (1970) reported the same effect with stem rust on wheat.

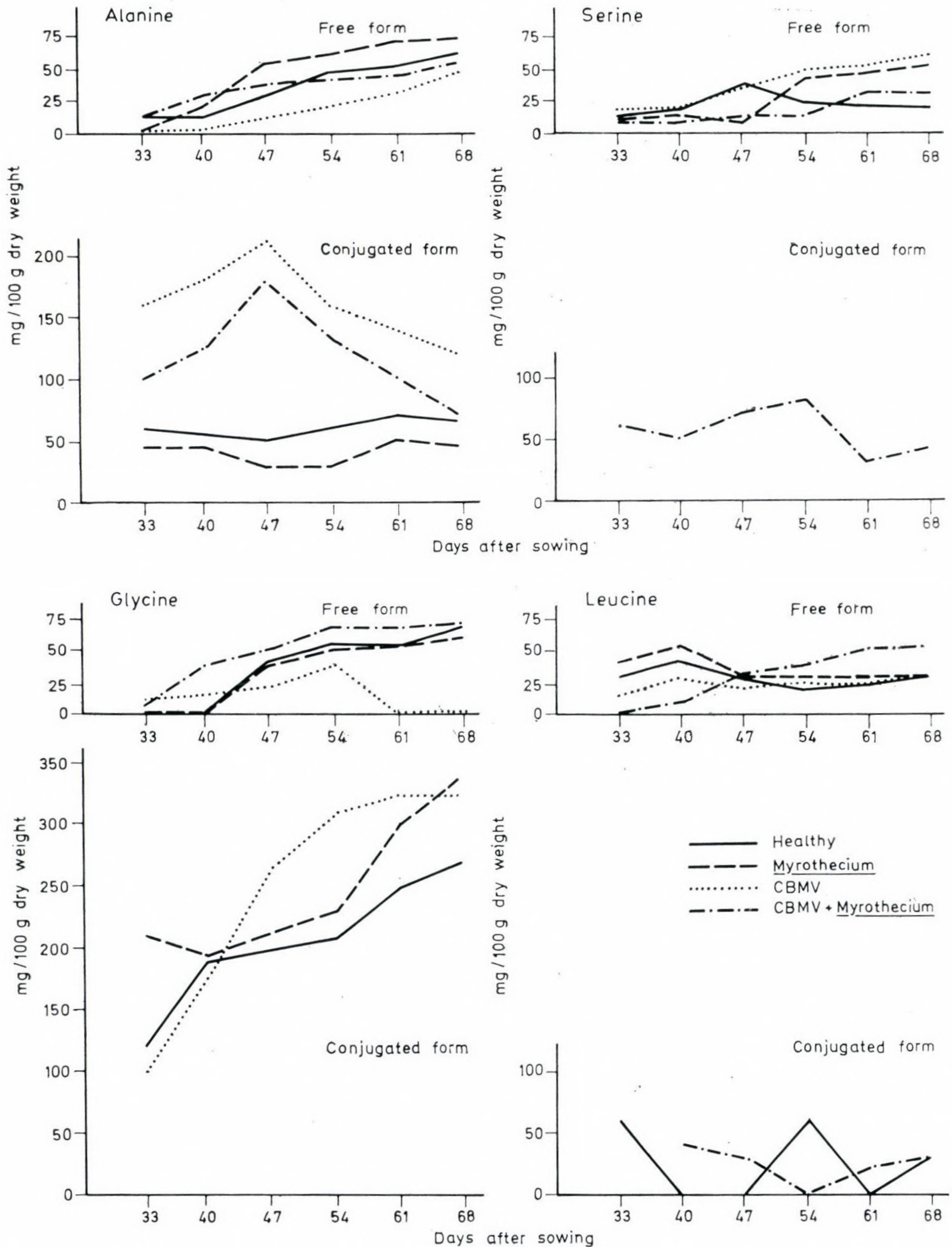

Fig. 4. Effect of single and double infection with *Myrothecium* and CBMV on free and conjugated amino acids (I: Pyruvate) of Contender bean leaves (mg/100 g dry weight)

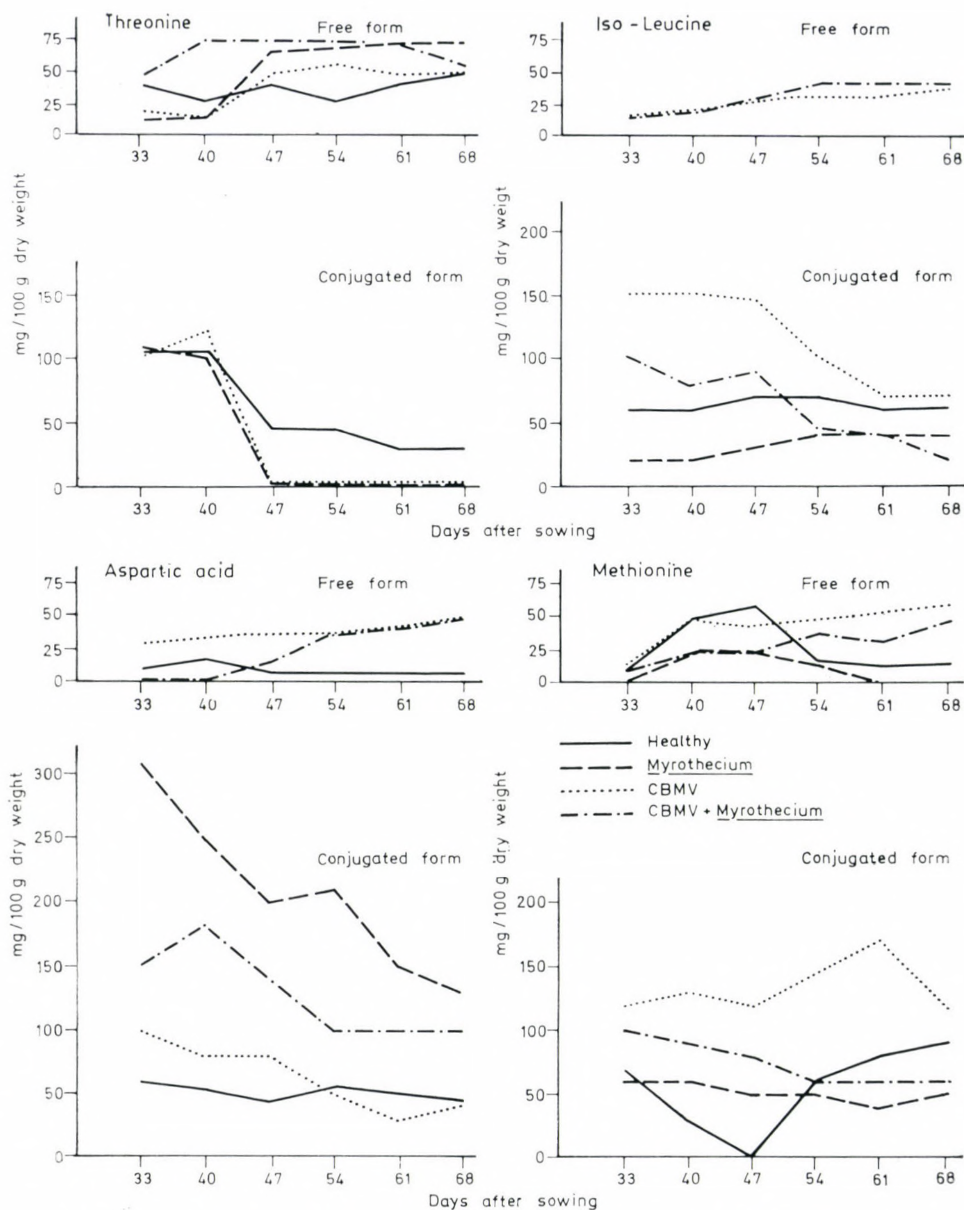

Fig. 5. Effect of single and double infection with *Myrothecium* and CBMV on free and conjugated amino acids (II: Oxaloacetate) of Contender bean leaves (mg/100 g dry weight)

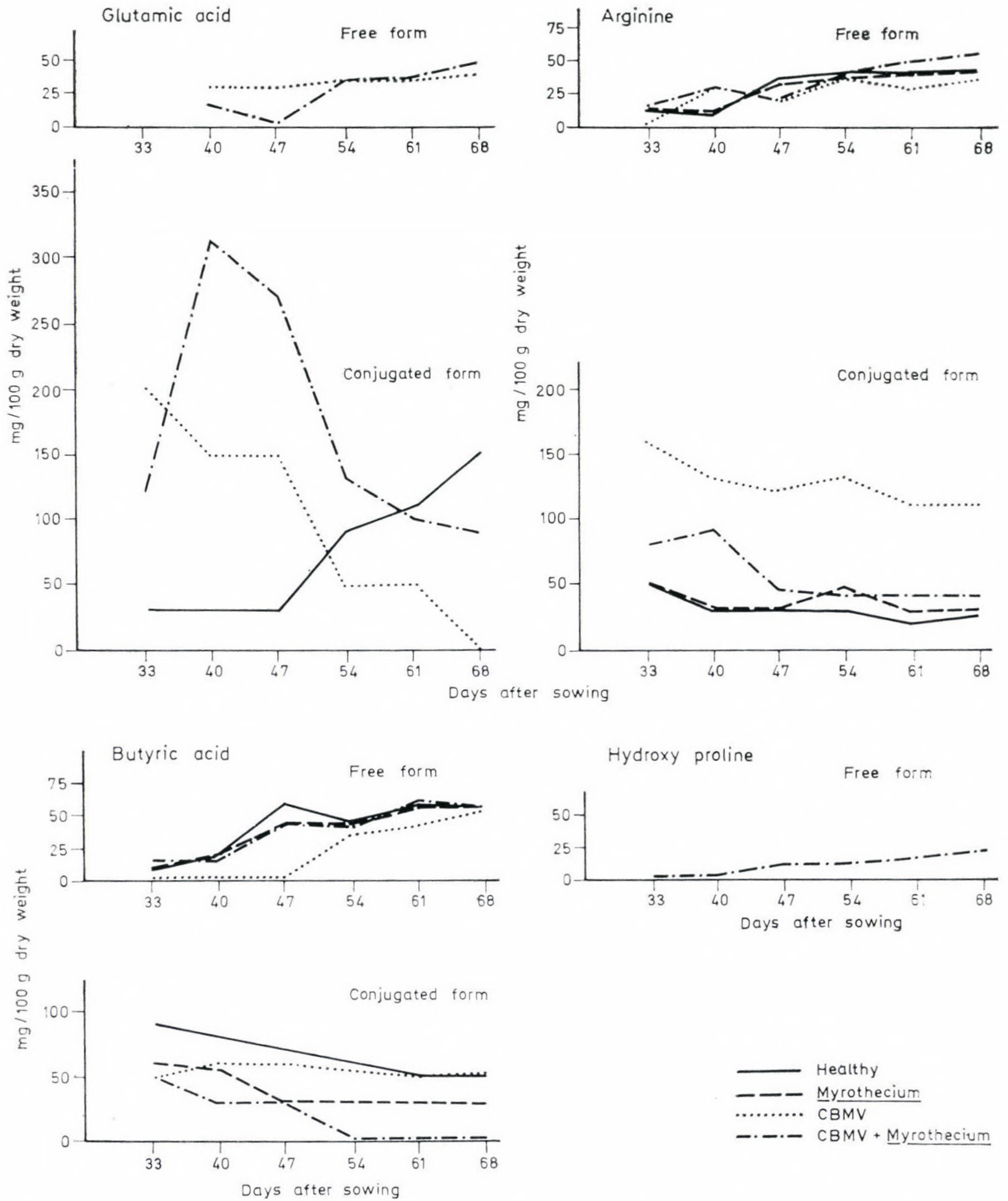

Fig. 6. Effect of single and double infection with *Myrothecium* and CBMV on free and conjugated amino acids (III. ketoglutarate) of Contender bean leaves (mg/100 g dry weight)

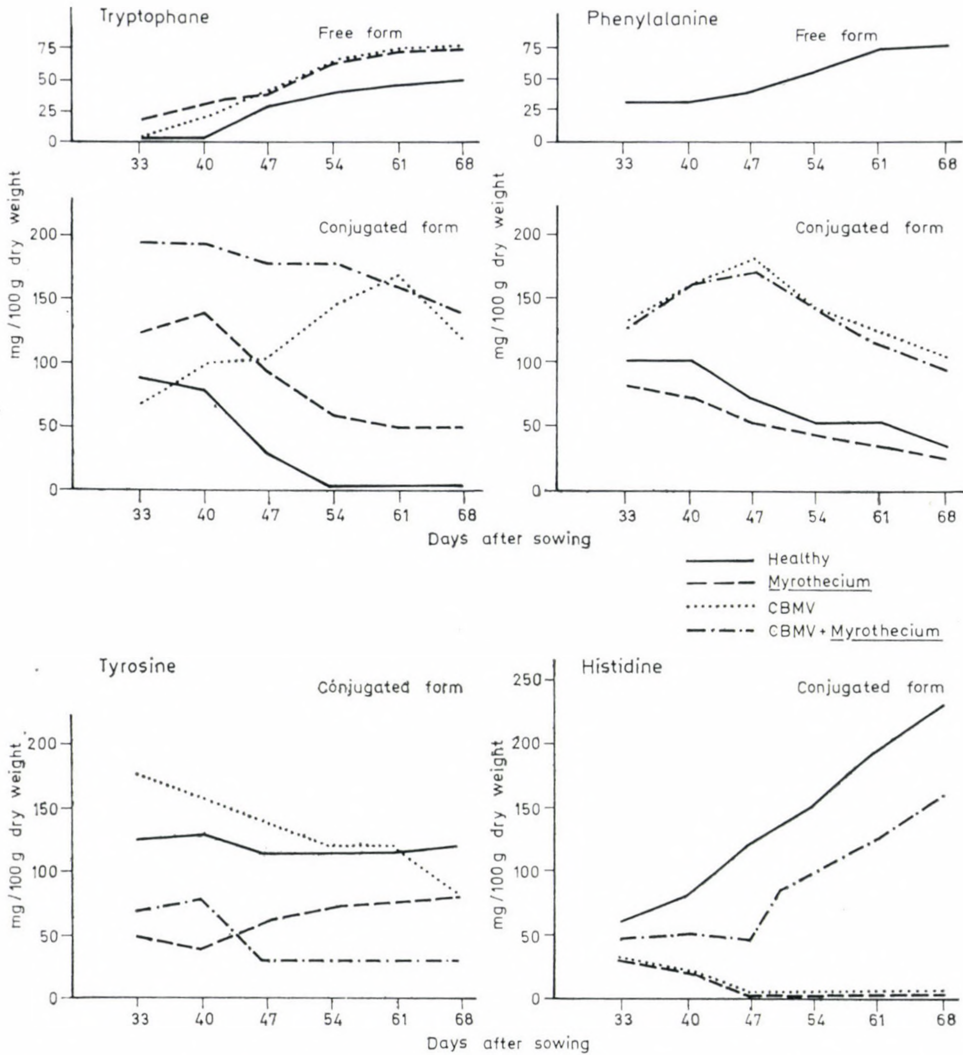

Fig. 7. Effect of single and double infection with *Myrothecium* and CBMV on the amino acids content (IV: Aromatic group) of Contender bean leaves (mg/100 g dry weight),

Amino acids

Regarding amino acid content (Figs 4–7) the different infections induced an increase in the conjugated form of tryptophane as compared to the control, whereas reduced histidine, butyric acid and threonine as well as the free form of phenylalanine. The latter was hindered completely. In this respect, Hussein (1973) found that fungal infection increased tryptophane of infected plants. Viral infec-

tion also increased the content of conjugated form of phenylalanine, glutamic acid, arginine and alanine which was seen in case of the double infection. This was true for the free form of methionine and aspartic acid. Fife (1956) and Bozarth and Diener (1963) found that glutamic acid increased in plants after viral infection. Fife (1956) and Jaiswall and Bhatio (1971) showed the same effect for arginine. Selman et al. (1961) also found that alanine content increased after virus infection. Fife (1956) and Porter (1959) recorded an increase in aspartic acid of virus infected plants.

On the other hand, the reduction in the conjugated form of methionine, iso-leucine and tyrosine in case of fungal infection was experienced in the double infections.

However, both viral infection and double infection stimulated the free form of glutamic acid and iso-leucine as well as the hydroxy proline. Double infection stimulated the conjugated form of serine and leucine as compared to the other treatments.

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Viruses of Vegetable Plants in Hungary and Some of Their Properties[1]

J. Horváth and L. Beczner

Institute of Plant Protection, University of Agricultural Sciences, H-8361 Keszthely, P. O. Box 71, and Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

Investigations on viruses isolated from the following vegetable plants have been carried out: bean (alfalfa mosaic, bean common mosaic, bean yellow mosaic, cucumber mosaic viruses), pea (alfalfa mosaic, bean yellow mosaic, broad bean leafroll, clover yellow vein, pea enation mosaic viruses), pepper (alfalfa mosaic, broad bean wilt, cucumber mosaic, potato virus X, potato virus Y, tobacco mosaic, tomato aspermy, tomato mosaic viruses), tomato (alfalfa mosaic, cucumber mosaic, potato virus X, potato virus Y, tobacco mosaic, tomato aspermy, tomato mosaic viruses), *Brassica* spp. (cauliflower mosaic, cucumber mosaic, radish mosaic, turnip mosaic, turnip yellow mosaic viruses), celery (celery mosaic, cucumber mosaic viruses), cucumber (cucumber mosaic, watermelon mosaic viruses), patisson (cucumber mosaic, watermelon mosaic viruses) and lettuce (cucumber mosaic, lettuce mosaic viruses).

The characterization of viruses resulted in several new findings such as new test plants for differentiation and separation, and new indicator plants, new intermediary strains of viruses, as well as the determination of new host-virus relations and source of resistance important for breeding.

In recent years the most important task has been to clear up the serological relationships within morphological groups like tobamo-, cucumo-, como-, and potyviruses using comparative serological methods. Emphasis has lately been laid on the bean yellow mosaic, cauliflower mosaic, lettuce mosaic viruses and the legume strain of cucumber mosaic virus, as well as on the peanut stunt virus and the pepper strain of tomato mosaic virus.

Vegetable growing has intensively improved for some years in Hungary and has now an about 5% share in the total agricultural production. The vegetable production exceeds by far the home requirements so nearly half of it is exported. The export of fresh and processed vegetables of good quality represents a high value in our foreign trade.

The virus diseases have a great influence on the vegetable production; they cause damages of different extent depending on species, variety, growing conditions (glasshouse or field conditions), factors influencing the activity of virus vectors (e.g. aphids). In the last 15 years the viruses caused serious problems in the production of pepper, tomato, cucumber, lettuce, onion, cabbage, bean, pea, broadbean and recently patisson in Hungary.

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The clearing up of the etiology of "újhitűség", a virus disease of pepper more than 40 years ago (Szirmai, 1941) started a continuous and systematic virological research work in Hungary. The primary goal of this work has been to study the etiology of and work out the way of protection against virus diseases menacing the cultivation of plants.

During the last four decades many works published on the etiology of virus diseases of pepper (Szirmai, 1941; Petróczi, 1956; Solymosy, 1958, 1960; Solymosy and Szalay-Marzsó, 1962; Szalay-Marzsó and Solymosy, 1961–1962), tomato (Milinkó, 1956, 1961; Solymosy, 1964; Horváth and Beczner, 1973; Beczner and Horváth, 1974; Horváth, 1968), cucumber, melon and watermelon (Molnár, 1963; Molnár and Schmelzer, 1964), bean (Németh, 1954), lettuce (Szirmai, 1957) and onion (Szirmai, 1958), as well as vegetable pathogen viruses of cruciferae plants (Mamula et al., 1972; Horváth et al., 1973; Juretić et al., 1973; Horváth et al., 1975*a*) and potato (Horváth, 1963, 1966*a*,*b*, 1967*a*,*b*).

Table 1

Viruses isolated from vegetable plants in Hungary

Viruses[1]	Vegetable plants
AMV	bean, pea, pepper, tomato
BBWV	pepper
BCMV	bean
BYMV	bean, pea
CaMV	*Brassica* spp., cabbage
CeMV	celery, parsley, carrot, parsnip
CMV	bean, *Brassica* spp., cabbage, celery, cucumber, lettuce, pepper, patisson, tomato
CYVV	pea
LMV	lettuce
PEMV	pea
PVX	pepper, tomato
PVY	pepper, tomato
RMV	*Brassica* spp.
TAV	pepper, tomato
TMV	pepper, tomato
ToMV	pepper, tomato
TuMV	*Brassica* spp.
TYMV	*Brassica* spp.
WMV	cucumber, patisson

[1] AMV, alfalfa mosaic virus; BBWV, broad bean wilt virus; BCMV, bean common mosaic virus; BYMV, bean yellow mosaic virus; CaMV, cauliflower mosaic virus; CeMV, celery mosaic virus; CMV, cucumber mosaic virus; CYVV, clover yellow vein virus; LMV, lettuce mosaic virus; PEMV, pea enation mosaic virus; PVX, potato virus X; PVY, potato virus Y; RMV, radish mosaic virus; TAV, tomato aspermy virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV, watermelon mosaic virus.

These papers were followed by ones dealing with more detailed investigations and characterization of already known and new viruses isolated from vegetable and other hosts, with studies on the serological characteristics, host range, differentiative and separative hosts etc. These works were summarized in reviews by Szirmai (1971), Horváth (1976, 1977*a*) and Beczner (1980).

In this paper we wish to give a general survey of the work done in Hungary mainly in the last decade with viruses of bean, pea, broadbean, pepper, tomato, lettuce, celery, cucumber, patisson and those of species belonging to the *Brassica* genus. We also would like to outline the trends of virological research work in Hungary.

The viruses isolated from the above mentioned vegetable plants are summarized in Table 1.

Virus Diseases of Leguminous Vegetable Plants

We are still lagging behind with the systematic investigation and description of viruses infecting bean, pea, lentil and broadbean though for some years more and more investigations have been carried out in this field. From leguminous plants the following viruses were isolated so far:

Alfalfa mosaic virus (AMV)

It is extremely widespread in Hungary in lucerne and in *Trifolium* species (Beczner, 1968*a*, 1972; Beczner et al., 1974). It infects bean (Hosszú et al., 1975) pea and *Vicia* sp. (Beczner, unpublished). The occurrence of AMV in bean and pea is sporadical, but in broadbean is of great significance. The virus has two pathotypes, one of them causes necrotic local lesions to bean (AMV-N), the other causes systemic infections (AMV-S). Both pathotypes were found in broadbean, while bean and pea plants were infected only with AMV-S. Of the weak, middle and severe strains of AMV (cf. Beczner, 1973, 1978) the occurrence of the first one was the most frequent. No quantitative serological differences were found between the pathotypes.

Bean common mosaic virus (BCMV)

It was described as occurring in *Phaseolus vulgaris* by Németh (1954), Beczner (1968*b*), Kovács and Horváth (1973) as well as Horváth (1976, 1979*c*). Being transmissible by seeds, it frequently occurs in white and spotted beans (*Phaseolus vulgaris* cv. *Fürj* and *Tétényi fehér*) popular in home gardens. In bean varieties cultivated in large farms (e. g. *Phaseolus vulgaris* cv. *Valja*, *Maxidor*, *Cherockee*) the virus rarely occurs, if at all.

Bean yellow mosaic virus (BYMV)

The virus was described in bean, lupinus, white clover, red clover and *Gladiolus* by Beczner (1968*b*, 1970, 1978), Beczner and Gáborjányi (1968), Kovács and Horváth (1973), Horváth (1976, 1979*d*), respectively. The viruses were characterized first of all on a symptomatological basis after Bos et al.'s system (1974). The Hungarian isolates can also be divided into bean mosaic, pea common mosaic and pea necrotic strains. Of the isolates the BYMV-*Lupinus* strain is remarkable. It was isolated from *Lupinus albus*, *L. angustifolius*, *L. luteus*, *L. mutabilis*, *Glycine max* and *Phaseolus vulgaris*, and systematically infects the *Chenopodium amaranticolor* and *C. quinoa* plants. These plants are – among others – the differentiating plants of BYMV and clover yellow vein virus (CYVV). The BYMV systemically infects the *Chenopodium amaranticolor* and locally the *C. quinoa* plants, while with CYVV it is the other way round. The two viruses significantly differ in serological and pathological characteristics (Beczner et al., 1976; Lindsten et al., 1976; Bos et al., 1977). On the base of serological properties and cytoplasmic inclusion the *Lupinus* strain was considered to be a special strain of BYMV (Beczner, 1970; Beczner et al., 1982*a*). For the comparison of the strains occurring in Hungary about 70 isolates have been collected from different hosts and antisera produced against a few representatives of different pathotypes.

Clover yellow vein virus (CYVV)

The virus was collected from pea, red and white clover during a work of surveying the occurrence and importance of BYMV strains and those of more or less related viruses in comparative studies. From a virus identification point of view it is of great importance that in fifty per cent of the cases the virus isolated from infected white clover was only CYVV, and in the other 50% BYMV. It has to be mentioned that according to some authors BYMV cannot infect *Trifolium repens* plants (Bos et al., 1977; Lindsten et al., 1976).

Broad bean stain virus (BBSV), Pea seedborne symptomless virus (PSbSV)-strain

While purifying the pea seedborne mosaic virus (PSbMV) Kowalska and Beczner (1980) found that the virus culture became contaminated with a comovirus, the so-called PSbSV. The isolate was identified as a separate virus on the basis of pathological, serological characteristics as well as because it was transmissible by pea seeds. The detailed serological comparison showed that the virus was closely related to the BBSV, the pea green mottle (PGMV), and the pea mild mosaic (PMMV) viruses, which were found under similar circumstances (cf. Beczner et al., 1980*a*). According to the qualitative serological comparison the PMMV (see Clark, 1972) and the *Glycine* mosaic virus (GMV, see Bowyer et al., 1980) are serologically identical and occupy an intermediary position between BBSV and PSbSV (Beczner et al., 1980*a*). The investigations pointed out the prob-

lems of identification of viruses closely related and or belonging to the same serogroup. The BBSV strain occurred in Hungary only in pea varieties imported from Poland (cvs. *Nora* and *Cüd-Ameryki)* due to seed-transmission.

The significance of occurrence of BBSV is increasing in case of complex infection, for the practically latent infections can be the source of severe symptoms; mosaic, malformation and stunting.

Pea seed-borne mosaic virus (PSbMV)

Under natural conditions it has not been found in Hungary so far. It was found in some cases in experimental seed samples originated from Poland (Kowalska, 1979). According to the serological comparison the Polish and American isolates of PSbMV proved to be serologically identical (Kowalska and Beczner, unpublished data).

Broad bean leaf roll virus (BBLRV)

The Hungarian occurrence of the virus was established in lucerne by Beczner et al. (1973). This year infection of a higher percentage has been estimated in broadbean in the North-Eastern part of the country. Further characterization of the virus is in progress.

Cucumber mosaic virus (CMV)

In spite of the fact that CMV belongs to the most common and important viruses in Hungary (cf. Horváth, 1969*a*, 1975, 1976, 1979*a*, 1980*b*; Beczner et al., 1978) the isolation of legume-pathogen strains started in 1980 from *Phaseolus vulgaris* (Schmidt and Horváth, 1982), *Nicotiana tabacum* (Beczner and Burgyán, 1982) and *Lupinus polyphyllus*. Considering the pathobiological properties, the bean, tobacco and *Lupinus* isolates belonging to the CMV-D sero- and pathotype described by Devergne (1975) known also as CMV-U strain in Richter's system (Richter et al., 1972). Only the isolate CMV-Nt80/3 showing weak vein banding and recovery in tobacco are entirely different from the others. The bean isolates were isolated from *Phaseolus vulgaris cv. Anna* and *Bergçold* × *Lada* hybrid plants (cf. Schmidt and Horváth, 1982). The resistance investigations carried out in 1982 showed that all the 52 bean cultivars tested were susceptible to the infections, there were differences only in sensitivity between cultivars (Horváth and Beczner, 1982).

Pea enation mosaic virus (PEMV)

Occurrence of PEMV in Hungary is recorded in annual reports of the Plant Protection Institute (PPI), Budapest. In the 1977 survey of pea-fields this virus was the most frequently isolated one. On the basis of symptomatological characteri-

zation the Hungarian isolates were identical with the type-strain of PEMV. Out of the 58 pea and 8 *Vicia faba* cultivars none was resistant to the virus. The wide occurrence of the virus in the *Vicia faba* and *Pisum sativum* cultures makes further investigations necessary. The *Pisum sativum* cv. *Rajnai törpe* proved to be a good propagative host of PEMV. The purification of the virus by the method of Izadpanah and Shepherd (1966) was relatively successful, though the titer of the antiserum prepared remained low, 1 : 32.

Virus Diseases of Solanaceous Vegetable Plants

Because of the similarity of virological problems, the viruses infecting *Capsicum annuum* and *Lycopersicon esculentum* will be discussed together. The virus infections of these cultures cause the greatest losses of production in Hungary year by year. In tomato and pepper the following viruses occur:

Alfalfa mosaic virus (AMV)

The infection of tomato and pepper in Hungary is common, the characteristic yellow mosaic pattern of pepper and the systemic necrotic spots of tomato have been mentioned by many authors (cf. Horváth, 1968, 1969*b*; Kuroli, 1969; Sum, 1975; Hosszú et al., 1975; Tóbiás et al., 1978; Salamon et al., 1980*a*). In the provocative inoculation experiments AMV-N and AMV-S strains equally infected all pepper (Beczner and Horváth, 1969) and tomato (Beczner and Horváth, unpublished data) cultivars without any differences is resistance to the virus. The steady 30–50% infection rate of alfalfa stocks means a significant source and pressure of infection for the vicinity of alfalfa fields (Hosszú et al., 1975).

Cucumoviruses

The cucumber mosaic virus (CMV) is one of the most important pathogens in Hungary (cf. Horváth, 1976, 1979*a*). The investigations were focussed mainly on the extreme variability of symptoms. Experiments in the last decade have led to the following conclusions: complex infections in pepper are very frequent, the CMV is usually accompanied by potato virus Y (PVY), AMV, tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) and in certain places sporadically with broad bean wilt virus (BBWV), tomato aspermy virus (TAV) and potato virus X (PVX). The same stands for tomato except that BBWV has not been isolated from tomato. The virus complexes can be separated with differentiating and separating plants (Horváth, 1969*b*, 1976; Beczner et al., 1978; Salamon et al., 1980*a*,*b*). The CMV isolates show large variability in pathology, and can be grouped on the basis of symptoms in *Nicotiana tabacum* cv. *Xanthi-nc* and *N. glutinosa* as well as in *Phaseolus vulgaris*. These characteristics might be completed with serological properties (Beczner et al., 1978; Tóbiás and Maat, 1982b). Some virus strains be-

longing to the CMV-To serogroup caused etch ring on inoculated leaves of *Xanthi-nc* tobacco, followed by mild mosaic and a fast recovery; others caused strong leaf narrowing on *Xanthi-nc* tobacco and mild mosaic, small rosetting and dwarfing in *Nicotiana glutinosa*. The isolates of CMV-D serogroup caused chlorotic lesions or latent infection on inoculated leaves of *Xanthi-nc* tobacco, while the systemic symptom appeared in an extremely severe form of mosaic, leaf deformation, vein banding, blistering, spots. Recovery was rare. The systemic symptoms in *Nicotiana glutinosa*, mosaic and malformation were also very severe. The isolate causes sy stemic infection (CMV-*Legume* strain) to bean that belongs to this serotype. The variability of CMV strains has already been pointed out; the fern-leaf diseases of tomato can be produced by certain CMV strains by themselves in pure cultures (Horváth and Beczner, 1973; Beczner and Horváth, 1974; Horváth, 1976).

The occurrence of TAV in pepper fields is of special interest (cf. Salamon et al., 1980*a*). It is surprising how narrow the natural host range of this cucumovirus is, and previously it was found in pepper and tomato cultivated under glass (Solymosy, 1964; Paludan, 1967).

The analysis of peanut stunt virus (PSV) widespread in Hungary (Beczner et al., 1978; Beczner and Devergne, 1979), *Robinia* mosaic virus (RoMV, cf. Beczner and Burgyán, 1982, unpublished) and that of CMV and TAV isolates made the comparative serological characterization of cucumoviruses possible. The relationships of cucumoviruses are partly confirmed, partly completed by the results of gel-diffusion, rocket-immunoelectrophoresis and ELISA serological investigations (cf. Beczner et al., 1982*a*).

Tobamoviruses

The tobacco mosaic virus (TMV) is one of the most important virus pathogen of pepper and tomato in Hungary (Szirmai, 1941; Milinkó, 1961). Now its occurrence in the field is scarce, attributive to the hygienic seedling growing and the cultivation of more resistant cultivars. In case of cultivation under glass it is of greater importance because of the accumulation of inoculum. During the detailed characterization of tobamoviruses we found that beside the common strain of TMV, tomato mosaic virus (ToMV) also occurred in Hungary (Mamula et al., 1974; Horváth, 1976; Juretić et al., 1977; Burgyán et al., 1978, 1980; Salamon et al., 1980*a,b*; Csilléri and Ruskó, 1980*a,b*). Burgyán et al. (1978, 1980) compared TMV and ToMV isolates from pepper, tomato, tobacco and *Solanum dulcamara* in symptomatological and serological experiments. All the TMV strains were serologically entirely identical with the TMV-Ul, while the five ToMV isolates causing only local lesions in *Nicotiana sylvestris*, belonged to four serotypes. On the basis of the pathological properties and pronounced serological differences demonstrated in immundiffusion, microprecipitin, rocket-electrophoresis and ELISA test we tend to consider TMV and ToMV as different viruses. Considering the distant serological relationship, the *Solanum dulcamara* (Sd) strain of ToMV possibly represents a separate virus (Burgyán et al., 1980; Beczner et al., 1980*b*; Salamon

and Beczner, 1982). The strain is remarkable, because isolates serologically identical with ToMV-Sd, but of different pathotypes have been found in several places in Hungary since 1979 (Csilléri and Ruskó, 1980*a*, 1982; Salamon and Beczner, 1982; Tóbiás and Maat, 1982*a*). The ToMV-Ob standard isolates and the LB and XII isolates separated from a virus complex thought to be also ToMV-Ob culture systemically infected the pepper cultivars homozygous for the L^1 resistance gene (i.e. *D-Cecei, Fehérözön)* as well as the *Nicotiana glutinosa* and *N. tabacum* cv. *Xanthi-nc* tobacco plants under normal glasshouse conditions (18–22 °C; Csilléri and Ruskó, 1982; Salamon and Beczner, 1982; Tóbiás and Maat, 1982*a*).

In the case of tomato, intensive research work has been carried out to study the application of attenuated virus strains as a means of cross-protection. Gáborjányi and his co-workers (1980) and Burgyán and Gáborjányi (1982) found, that the virus content in the spontaneously infected plants increased rapidly. The multiplication of the mild strain was about half of the control and of the severe strain. The virus content in "vacinated" plants remained at the same level as in the plants infected only with the mild strain. The development of cross-protection was in connection with the inhibition of virus multiplication in doubly infected plants. The breakdown of the cross-protection was not followed by changes in virus content. During this study the presence of virus having mixed coats was demonstrated serologically in tomato plants cross-protected with a mild strain (MII-16) of ToMV against a severe Ul strain of TMV (Burgyán and Gáborjányi, 1982).

Potato virus Y (PVY)

This virus regularly occurs in Hungary in pepper and tomato (Horváth, 1968, 1979*b*; Kuroli, 1969; Salamon et al., 1980*a,b*; Tóbiás and Molnár, 1981). Horváth (1966*a,b,c,d*, 1967*a,b,d)* studied the C-, N-, R- and Anomalous strainsof PVY. The importance of PVY has increased for some years in Hungary, as the occurrence of the so-called tobacco veinal necrosis strain (R) has become more frequent because of the cultivation of susceptible and very sensitive *Cocer* tobacco cultivars. In recent years the so-called potato tuber necrotic ringspot has caused problems in potato cultivation; its pathogen is thought to be the strain R of PVY (Beczner et al., 1982*b*, 1983).

Potato virus X (PVX)

The occurrence of PVX in pepper and tomato plantations is sporadical (Horváth, 1969*b*; Beczner, 1979; Salamon et al., 1980*a*; Tóbiás and Molnár, 1981).

Virus Diseases of *Brassica* spp. Vegetable Plants

Viruses pathogenic to cruciferous plants and their natural hosts are listed in Table 2 (cf. Horváth et al., 1981*a*).

Table 2

Natural occurrence of viruses on cruciferous plants in Hungary

Viruses[1]	Natural cruciferous hosts
CaMV	*Brassica rapa* var. *rapa*
	B. oleracea convar. *botrytis*
CMV	*B. napus*
RMV	*B. rapa* var. *rapa*
TuMV	*Alliaria petiolata*
	Brassica rapa var. *rapa*
	B. oleracea convar. *botrytis*
	B. oleracea convar. *capitata*
TYMV	*B. rapa* var. *rapa*

[1] CaMV, cauliflower mosaic virus; CMV, cucumber mosaic virus; RMV, radish mosaic virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus.

Cucumber mosaic virus (CMV)

CMV caused severe damages to *Brassica napus* (Horváth, 1969*a*, 1980*c*). Beczner and co-workers (1978) stated that both the To and D serotypes of CMV occurred on *Brassica napus*.

Radish mosaic virus (RMV)

According to the results obtained from agar-gel double diffusion and intragel absorption tests, the Hungarian isolates of RMV recovered from turnip plants *(Brassica rapa* var. *rapa)* were identical with the European isolate but differed from the American type strain of the virus (Mamula et al., 1972; Horváth, 1976, 1979*e*).

Turnip yellow mosaic virus (TYMV)

This virus was isolated also from spontaneously infected turnip plants, and the different Hungarian isolates of TYMV were found to be serologically related to, but not identical with the Yugoslav isolate, which is identical with the type strain of the virus (Horváth et al., 1973; Juretić et al., 1973).

Turnip mosaic virus (TuMV)

The natural hosts of TuMV in Hungary are *Alliaria petiolata*, *Brassica rapa* var. *rapa*, *B. napus*, *B. oleracea* convar. *botrytis*, *B. oleracea* convar. *capitata*. The different isolates of TuMV collected were found to be related to the so-called cabbage or ordinary strain of the virus (see Horváth et al., 1975*a*; Horváth, 1976, 1979*b*; Juretić et al., 1976).

Cauliflower mosaic virus (CaMV)

Horváth and co-workers (1980) described the natural occurrence of CaMV in turnip and cauliflower plants. The host range investigations carried out with three strains of the virus showed that all the three were closely related and almost identical with the original CaMV isolate named Cabbage B isolate (cf. Horváth et al., 1980). One of the above mentioned three CaMV isolates, which was isolated from cauliflower plants (cv. *Hoshima helios*), was also used in the comparative cytology tests. In turnip plants *(Brassica rapa* cv. *Just Right)* the two strains induced the same symptoms. In the Cabb-S infected cells the viroplasms contained large amounts of electron dense material and small amounts of virions in contrast to the Cabb-D/H infected cells, where there were large amounts of virions and less electron dense material (Balázs et al., 1981; Xiong et al., 1982).

Virus Diseases of Cucurbitaceous Vegetable Plants

In Hungary two viruses, the cucumber mosaic virus (CMV) and the watermelon mosaic virus (WMV) were isolated from *Cucumis sativus*, *C. melo*, *Citrullus lanatus*, *Cucurbita maxima*, *C. pepo* and *C. pepo* convar. *patissonina* (patisson) plants (Molnár, 1963, 1965; Molnár and Schmelzer, 1964; Horváth et al., 1975*b*,*c*, 1976; Horváth, 1976, 1979*a*,*b*; Bródszky, 1975; Tóbiás and Velich, 1982).

Cucumber mosaic virus (CMV)

Both serotypes of CMV occur in *Cucurbitaceae* in Hungary, but the CMV-D serotype does so more frequently. Therefore this serotype has got the sign U (Ungarn = Hungary) in Dr. Schmelzer's virus collection. Most recent vectorological and infection dynamical experiments carried out with cucumber plants have shown that the occurrence of CMV is about threefold compared to that occurrence of watermelon mosaic virus (WMV, cf. Basky, 1982). The rate of virus infection of *Cucurbitaceae* may reach 100% at the end of the vegetation period and it is in close correlation with the great number of aphids on these species (Basky, 1982). The spread of virus falls regularly to the second half of July.

Watermelon mosaic virus (WMV)

It was from watermelon, musk melon and squash that the virus was first isolated and described in Hungary (Molnár 1963; Molnár and Schmelzer, 1964). Earlier and later isolations of WMV from patisson and cucumber all belonged to the G (general) strain of the virus (cf. Horváth et al., 1975*c*; Horváth, 1976, 1979*b*). As it is known, the WMV-G differs from the WMV-S first of all in its host range not being restricted to the family *Cucurbitaceae* but infecting some 17 families including e.g. *Chenopodiaceae*, *Euphorbiaceae*, *Leguminosae*, *Malvaceae*

etc. (cf. Horváth et al., 1975*c*). The virus does not spread by seeds. It is remarkable that the overwintering host plant of the virus has not been found so far in Hungary, inspite of the fact that this virus, appearing each year and reaching important infection's rate is being investigated systematically. The host range of WMV cited also in the literature is very narrow (cf. Horváth, 1979*b*).

Virus Diseases of Lettuce *(Lactuca sativa)*

Viruses infecting lettuce have been studied by Szirmai (1957), Horváth et al. (1979) in Hungary. Lettuce mosaic virus (LMV) – because of its seed-transmission – is more frequent in lettuce than CMV. Out of 37 isolates from lettuce, 29 proved to be LMV and 6 to be CMV, and in two cases complex infections were found (Horváth et al., 1981*b*). Good summarizing papers were published in

Table 3

Some new results of host range investigations

Plant species	Plant reaction for virus inoculation[1]	
	Susceptible	Resistant
Ammi majus	BYMV, CMV	PVY, TuMV
Cucumis myriocarpus	TMV, ToMV, PVX, TRSV, TNV	BCMV, CMV, RMV TYMV
Cucurbita andreana	CMV	
Cyclanthera pedata	CMV	
Lactuca altaica	LMV	
L. quercina	LMV	
L. tatarica	LMV	
Lagenaria siceraria	CMV, WMV	
Ocimum sanctum	TRV, TMV, ToMV, PVX, CMV, TRSV, AMV, BBWV	CeMV, CLRV, LMV, PVM, PVY, TYMV, WMV
Phaseolus caffer	BCMV	
P. riccardianus	AMV	
Tatragonia crystallina	TRV, TMV, ToMV, PVX, BYMV, LMV, TuMV, WMV, CMV, RMV, CLRV, TRSV, TNV	PVM, PVS, AMV, BCMV, CeMV, TYMV

[1] AMV, alfalfa mosaic virus; BBWV, broad bean wilt virus; BCMV, bean common mosaic virus; BYMV, bean yellow mosaic virus; CeMV, celery mosaic virus; CLRV, cherry leaf roll virus; CMV, cucumber mosaic virus; LMV, lettuce mosaic virus; PVM, potato virus M; PVS, potato virus S; PVX, potato virus X; PVY, potato virus Y; RMV, radish mosaic virus; TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; TRSV, tobacco ring spot virus; TRV, tobacco rattle virus; TuMV, turnip mosaic virus; TYMV, turnip yellow mosaic virus; WMV, watermelon mosaic virus.

Hungary on the viruses pathogenic to lettuce (cf. Horváth, 1980*a*), on their isolation and identification (Horváth et al., 1981*b*), as well as on the natural and experimental host plants of LMV and CMV (Horváth, 1979*d*, 1980*b*).

Virus Diseases of Celery (*Apium graveolens* convar. *dulce*), Parsley (*Petroselinum crispum*), Carrot (*Daucus carota*) and Parsnip (*Pastinaca sativa*)

The celery mosaic virus (CeMV) was isolated from celery, parsley, carrot and parsnip plants (Horváth et al., 1976). Szürke (1979) laid emphasis on the complex infection and reported that the rate of virus infection of 29 cultivars varied between 1 to 58%.

The Results of Host Range Investigations

Studying the viruses infectious to vegetable plants detailed host range and symptomatological investigations have been carried out. As a result, a series of new host plants have been found, several of which are demonstrated in Table 3. In his D.Sc. thesis Horváth (1976) describes the results of host plant experiments with 22 viruses belonging to 11 different virus groups. Our own investigations in recent years have led to the identification of about 1263 new host-virus relations and 640 plants resistant to it, as well as 6442 combinations of possible virus separations.

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Effect of Rust Infection on Lipid Composition in Leaves of Groundnut (*Arachis hypogaea* L.)

G. RAMA GOPAL and P. SREENIVASULU

Department of Botany, S. V. University, Tirupati-517 502 (A. P.), India

Phospholipids and glycolipids of rust-infected leaves were quantitatively determined and compared with the lipids of healthy leaves. The content of total lipid, total lipid sugar, the ratio of total lipid sugar to lipid phosphorus, individual glycolipids and phosphatidyl glycerol were less in diseased leaves whereas the total lipid phosphorus content was unchanged and the remaining individual phospholipids varied slightly. The results are discussed in relation to yellowing of leaves during disease development-

Groundnut is an important oilseed crop, and India is the World's largest producer of groundnuts. Loss due to diseases is one of the most important factors reasoned for low yields. Among them rust (*Puccinia arachidis* Sperg.) of groundnut is a destructive disease in many groundnut growing areas of India (Subrahmanyam et al., 1979). Some of the biochemical changes in rust-infected groundnut leaves have already been reported (Subrahmanyam et al., 1976; Reddy and Rao, 1976; Subrahmanyam et al., 1978; Siddaramaiah et al., 1979) and the present investigation is intended to study the changes in lipids associated with disease development in groundnut leaves as they are the important constituents of biological membranes.

Materials and Methods

Groundnut (var. TMV-2) plants were raised in garden soil in earthen pots. The leaves were inoculated and sampled as per the procedure of Subrahmanyam et al. (1976). The leaves from rust-infected and corresponding healthy plants were analysed at stages 2, 3 and 4 out of 5 stages — (1) no visible symptoms (5 days after inoculation); (2) small yellowish green and circular lesions (8 days after inoculation); (3) orange-red pustules (10 days after inoculation); (4) pustules dark brown and coalesced to irregular patches, and general yellowing of the leaves (12 days after inoculation); and (5) leaf tissues around the infection sites became necrotic and dried out in irregular patches (15 days after inoculation).

Total lipids from leaves were extracted according to the procedure of Hoppe and Heitefuss (1974*a*). The lipid residues were dissolved in chloroform and stored at 0 °C until used. The individual lipid components were separated by TLC on silica gel G plates (0.5 mm) prepared as described by Bajwa and Sastry (1974). The plates were activated at 110 °C for 30 min and were developed in chloroform-methanol-acetic acid-water (170 : 25 : 25 : 3, vol./vol.) as the solvent system.

After chromatography the spots were visualized by exposure to iodine and identified by standards simultaneously. The identity of the spots was further confirmed by spraying with phospholipid, glycolipid and ninhydrin reagents (Skipski and Barclay, 1969).

Dry weight of lipid extract was determined gravimetrically after keeping the lipid residue in a vacuum desiccator over KOH until constant weight was reached. Lipid phosphorus was determined as described by Bartlett (1959). For the determination of phosphorus in lipid spots on TLC plates, the spots were scraped into test tubes and processed essentially as described above except that the tubes were centrifuged at 1000 rpm for 10 min to sediment silica gel before colour measurement. For lipid sugar estimation, aliquots of lipid extract were evaporated to dryness and hydrolyzed for 1 h at 50 °C with 1 ml of 0.5 N NaOH in 90% methanol. The samples were taken to dryness under compressed air and further hydrolyzed with 1 ml of 2 N H_2SO_4 for 2.5 h in a boiling water-bath. One ml of chloroform was then added to the samples and mixed to partition pigments. Sugar in the aqueous layer was estimated by the phenol-H_2SO_4 method as described by Roughan and Batt (1968) using galactose as standard. Sugar in lipid spots separated on TLC plates was determined essentially as described above but without prior hydrolysis.

Results

The content of total lipid and lipid sugar, which reflects mainly glycosyl glycerides were less in rust infected leaves when compared to healthy leaves (Table 1). The quantity of lipid phosphorus remained almost unaltered except at stage 2 where it was slightly more in diseased leaves (Table 1). The ratio of lipid sugar to lipid phosphorus was considerably reduced in the diseased leaves at all stages of disease development.

Table 1

Total lipid composition of healthy (H) and rust infected (RI) groundnut leaves at various stages of disease development

Stage	Nature of leaf	Total lipid		Lipid sugar		Lipid phosphorus		Lipid sugar
		Content mg/g fresh leaf	Variation	Content μg/g fresh leaf	Variation	Content μg/g fresh leaf	Variation	Lipid phosphorus
2	H	22.50		968.13		143.45		6.75
	RI	21.00	−6.67	691.87	−28.54	158.66	+10.64	4.36
3	H	19.75		1085.40		160.51		6.76
	RI	16.66	−15.65	636.06	−41.41	161.62	+0.62	3.94
4	H	21.85		1096.52		155.28		7.06
	RI	17.50	−19.91	782.34	−28.67	155.77	+0.30	5.02

[Values given are average of triplicate determinations; variation was expressed as per cent increase (+) or decrease (−) over healthy]

Table 2

Glycolipid composition of healthy (H) and rust infected (RI) groundnut leaves at various stages of disease development

Stage	Nature of leaf	MGD*		DGD*		SL*		MGD : DGD : SL (based on sugar content)
		Content	Variation	Content	Variation	content	Variation	
2	H	535.72		267.86		115.39		1 : 0.50 : 0.22
	RI	340.91	−36.37	200.32	−25.21	96.81	−16.10	1 : 0.59 : 0.28
3	H	569.72		226.19		113.11		1 : 0.39 : 0.19
	RI	282.15	−50.40	167.11	−26.12	102.67	−9.23	1 : 0.59 : 0.36
4	H	576.92		198.53		138.01		1 : 0.34 : 0.24
	RI	257.00	−55.46	179.67	−9.50	106.45	−22.87	1 : 0.69 : 0.41

* MGD, monogalactosyl diglyceride; DGD, digalactosyl diglyceride; SL, sulphoquinovosyl diglyceride

[Values expressed as μg sugar/g fresh leaf are average of 3 samples; variation was expressed as per cent increase (+) or decrease (−) over healthy]

A comparison of the glycolipid composition between healthy and diseased leaves (Table 2) shows that the content of monogalactosyl diglyceride was the most severely reduced. All the three glycolipids were reduced at all stages of disease development. The ratios of the three predominant chloroplast glycolipids tended to be same at stage 2 but it was more in the diseased tissue at stages 3 and 4.

While the total phospholipid content remained nearly the same in healthy and diseased leaves, the phospholipid composition was slightly altered during disease development (Table 3). Phosphatidyl glycerol, the main phospholipid of the chloroplast, is reduced particularly at stages 3 and 4 in diseased leaves. The remaining phospholipids were slightly more in diseased leaves at stages 2 and 4 but PC, PE and DPG were less at stage 3 when compared to healthy leaves.

Discussion

Biological membranes are diffusion barriers and govern all permeability processes. Rust-infected plant tissue showed an increase in membrane permeability (Hoppe and Heitefuss, 1974). Since phospholipids and glycolipids are important functional constituents of biological membranes, we investigated these lipids during pathogenesis. The decline in glycolipid and PG concentration coincided with the decrease in chlorophyll content of the infected leaves (Subrahmanyam et al., 1976), indicated a specific damage more probably to the chloroplast than other plasma membranes. The chloroplast lipids consist nearly exclusively of MGD, DGD, SL and PG. Damage to chloroplasts in rust-infected tissue has been reported by several authors (Shaw, 1963) and can possibly lead to cell decompart-

Table 3

Phospholipid composition of healthy (H) and rust infected (RI) groundnut leaves at various stages of disease development

Stage	Nature of leaf	PI*		PC*		PG*		PE*		DPG*	
		Content	Variation	Content	Variation	Content	Variation	Content	Variation	Content	Variation
2	H	22.34		46.67		43.85		21.21		21.88	
	RI	26.93	+20.55	50.55	+8.31	44.12	+0.85	23.21	+9.43	22.78	+4.11
3	H	28.00		53.34		49.21		25.53		26.27	
	RI	29.97	+7.04	51.26	−4.01	44.41	−9.75	23.27	−8.85	22.68	−13.67
4	H	25.67		50.57		49.04		23.09		22.48	
	RI	26.86	+4.64	53.53	+5.85	43.75	−10.79	25.64	+11.04	24.06	+7.03

* PI, phosphotidylinositol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DPG, diphosphatidyl glycerol

[Values expressed as μg phosphorus/g fresh leaf are average of 3 samples; variation was expressed as per cent increase (+) or decrease (−) over healthy]

mentation. Similar changes in chloroplast lipids and chlorophylls were also reported in *Phaseolus vulgaris* leaves infected with *Uromyces phaseoli* (Hope and Heitefuss, 1974*a*) and groundnut leaves infected with groundnut chlorotic spot virus (Sreenivasulu et al., 1977). In the infected tissue slight changes were observed in the concentration of PC, PE, PI and DPG, and a decrease in these lipids might have been compensated or overcompensated by the fungal lipids as suggested by Hoppe and Heitefuss (1974*a*). We observed a decrease in those lipids, which may not be present in the fungus. These results might be explained by an enhanced degradation of all plant membrane lipids in the diseased tissue. Thus, yellowing of leaves in diseased plants is probably due to degradation of the chloroplast lipids and pigments. Higher chlorophyllase activity in virus diseased plants showing chlorotic symptoms has been reported (Sreenivasulu and Nayudu, 1978). It would be interesting to study whether glycosyl glyceride hydrolyzing enzymes are similarly enhanced in diseased tissue.

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Some Biochemical Aspects of Host-Pathogen Interactions in Pythium Stalk Rot of Maize: I. Role of Toxin, Pectolytic and Cellulolytic Enzymes in Pathogenesis[1]

E. A. SADIK,[2] M. M. PAYAK and S. L. MEHTA

Division of Mycology & Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India

Culture filtrates of *Pythium aphanidermatum* were able to induce wilt as well as cause softening of the cut ends of tomato shoots indicating the presence of toxic metabolites and hydrolytic enzymes. Toxic substances were found to be heat-labile and mos-active at pH 6.5. Cell-free culture filtrates reduced the length, fresh and dry weight of roots of maize seedlings. The growth reduction varied with the virulence of isolates.

Analysis of culture filtrates of the four isolates showed absence of pectin methylesterase (PME) production. Polygalacturonase (PG) and/or depolymerase (DP) activity, however, was observed on potato broth medium especially with pectin + glucose as carbon source but not on synthetic medium. All the isolates produced cellulases in vitro. Their capacity to produce cellulases was much greater than that to produce pectolytic enzymes.

Highly virulent isolates produced more toxin, pectolytic and cellulolytic enzymes than the moderately and weakly virulent ones. Furthermore, differences in virulence between the isolates could possibly be due to their variability in toxin, pectolytic and cellulolytic enzymes production.

Pythium aphanidermatum (Edson) Fitz.* incites Stalk Rot of Maize – a major disease in India. The disease symptoms suggested the possibility of involvement of metabolites such as toxin(s), pectolytic and cellulolytic enzymes in the process of pathogenesis. The production of toxins by plant pathogens in vivo as well as in vitro and their role in the process of pathogenesis has received a good deal of attention (Friend and Threlfall, 1976). Toxin production by species of *Pythium* such as *P. irregulare* (Brandenburg, 1960; Vukovits, 1953 and Martin, 1964) and *P. ultimum* (Takahashi, 1951) has been recorded. However, there are no reports of the production of toxin by *P. aphanidermatum* or *P. butleri*.

The in vitro production of pectolytic enzymes by *P. debaryanum* and of pectolytic and cellulolytic enzymes by *P. aphanidermatum* both in vitro and in vivo has been shown (Gupta, 1956; Winstead and McCombs, 1961). Results of our study on in vitro production of toxin, pectolytic and cellulolytic enzymes by maize isolates of *P. aphanidermatum* differing in virulence are presented here.

[1] Part of Ph. D. thesis submitted by E. A. Sadik to the Post-Graduate School, Indian Agricultural Research, New Delhi.

[2] Present Address: Botany Department, Faculty of Agriculture, EL Mansoura University, EL Mansoura, Egypt.

* Also identified as *P. butleri* Subram.

Materials and Methods

Four isolates (Pa_3 and Pa_4 highly virulent, Pa_1 moderately virulent and Pa_2 weakly virulent) used in this study, were grown at 28 °C in 250 ml Erlenmeyer flasks, each containing 100 ml of potato dextrose broth supplemented with 0.1% yeast extract (Difco). After growth for 10 days, the contents were filtered through absorbent cotton and then through Whatman's No. 1 filter paper. The culture filtrates so obtained were tested for their ability to induce wilt on 5 to 6-week old tomato cuttings (cv. Pusa Ruby). Water and uninoculated medium were used as controls. Two replicates were taken for each treatment. Cell-free culture filtrates were also tested for their effect on maize seedlings using a new modification of the method followed by Sadik (1973) and by Litzenberger (1949). The technique being a novel one has been fully described under 'Results'.

The isolates were grown in 250 ml conical flasks, each containing 100 ml of the basal medium (composition: 3 g, potassium dihydrogen phosphate; 2 g, potassium dibasic phosphate; 0.5 g, magnesium sulphate; 2 g, asparagine; 1 g, yeast extract and water to make 1 l). In addition, the carbon sources were 10 g glucose or 5 g glucose + 5 g cellulose or 10 g cellulose powder, for studies on cellulolytic enzymes. For pectolytic enzymes, 5 g or 2.5 g glucose +2.5 g or 5 g of citrus pectin were used as carbon sources. The ability of the fungus to produce pectolytic enzymes was also determined by growing on potato broth supplemented with 0.1% yeast extract. The carbon sources were 20 g glucose or 10 g glucose + 10 g citrus pectin per litre of potato broth. The pH was adjusted to 4.5 for polygalacturonase (PG) and/or depolymerase (DP) and to 7.0 for pectin methylesterase (PME) and to 6.0 for cellulases. The sterilized media were inoculated and cultures were incubated at 29 °C for 10 days. The contents were filtered through Whatman's No. 1 filter paper. The pH of culture filtrates was adjusted with HCl to 4.5 for PG and/or DP assay, to 7.0 for PME assay and to 6.5 for cellulolytic enzyme assay.

Pectin methylesterase assay

It was assayed by the method described by Matta and Dimond (1963) by determining the amount of alkali required to neutralize the carboxyl groups freed due to hydrolysis of ester linkage in pectin solution.

Polygalacturonase and/or depolymerase assay

These were determined using the viscometeric method (Alexander, 1954). The activity was expressed as percentage of reduction in viscosity of pectin solution after 30 min incubation at 30 °C, calculated according to the formula of Kerteesz (1951). All measurements were made by recording the time of flow in Oswald's viscometer containing 5 ml substrate (1% citrus pectin solution) buffered at pH 4.5 with 0.02 M citrate buffer and 5 ml of the culture filtrate.

Cellulase assay

It was determined by measuring the reduction in viscosity of 1% carboxymethyl cellulose (CMC, Sodium salt) solution in Ostwald's viscometer pipettes and expressed as percentage of reduction in viscosity of CMC solution after 20 min incubation at 30 °C. Five ml of the substrate (1% CMC) adjusted to pH 6.5 with phosphate buffer (0.02 M) and mixed with 5 ml of culture filtrate were used in all analyses. Two tests with two replicates each were conducted for all assays. Values reported are averages.

Results

Toxin studies

Culture filtrates of all the four isolates induced wilting in tomato cuttings. However, the period required for the appearance of the first sign of wilting differed significantly. It was 5.45, 6.45, 3.55 and 3.20 hr. with the culture filtrates of Pa_1, Pa_2, Pa_3 and Pa_4 respectively. Wilt symptoms started to appear at the lower leaves and spread gradually upwards (Fig. 1). After 18 h, necrosis set in around the veins of leaflets and stem collapsed at or near the cut ends of tomato cuttings. The stem collapse was greater with culture filtrates of isolates Pa_3 and Pa_4 followed by Pa_1 and Pa_2. Wilting indicated that the pathogen was producing some kind of toxic metabolite and the stem collapse suggested the presence of hydrolytic enzymes in the culture filtrates.

Effect of pH

The pH of culture filtrates was adjusted to 5.0, 6.5 and 8.0 and then assayed for toxin activity. The activity in all the isolates was highest at pH 6.5 than at pH 8.0 or 5.0.

Effect of heating

Heating of culture filtrates at 50, 75, 100 and 121 °C for 15 min caused significant reduction in their ability to induce wilt symptoms as compared to the unheated culture filtrates. Greater reduction occurred with increase in temperature. Culture filtrates at 121 °C showed very slight wilting in tomato cuttings even after 36 h. Although, wilt symptoms did appear in culture filtrates heated to 75° and 100 °C, stem collapse did not occur. This shows that the fungus produces a wilt-inducing metabolite which is heat labile. The metabolite(s) responsible for the stem collapse are also inactivated. However, the toxic metabolite is able to withstand higher temperatures than those metabolites which are responsible for stem collapse.

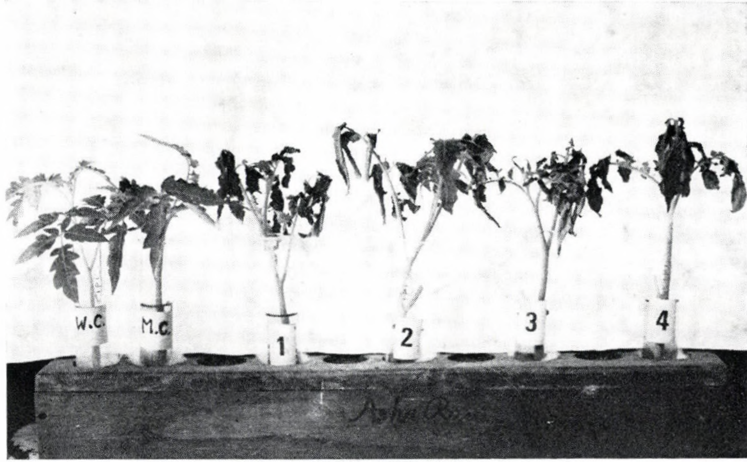

Fig. 1. Wilt symptoms in tomato cuttings induced by culture filtrates of four isolates after 18 h. WC, in water; MC, in uninoculated medium; 1–4 in culture filtrates of Pa_1, to Pa_4, respectively

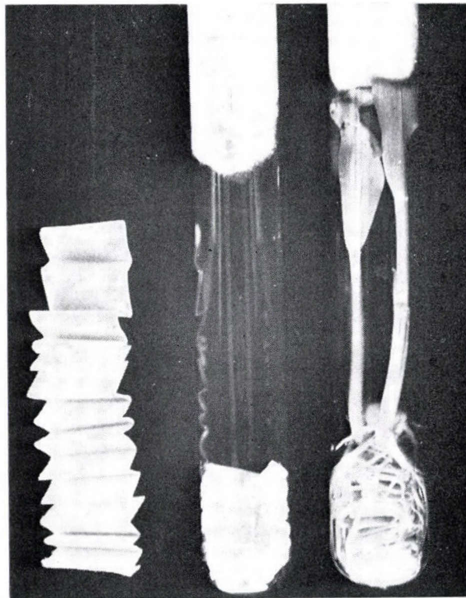

Fig. 2. Technique for determining the effect of culture filtrates on growth of maize seedlings. a, Folded filter paper strip. b, Test tube containing folded filter paper strip. c, Maize seedling growing on folded filter paper moistened with storile water

Effect of culture filtrates on the growth of maize seedlings

A modified method described herein (Fig. 2) was adopted. Cell-free culture filtrates (7.5 ml) were transferred to each of the sterilized test tubes (22 mm in diameter) containing about 30 layers of folded Whatman's No. 1 filter paper strips (2 × 46 cm); 3 cm deep (Fig. 2). Each tube was seeded with two surface-sterilized germinated maize seeds of cv. CM 500 and incubated at 28 °C. Uninoculated medium and sterile water were used as controls. For each treatment, five tubes were used as replicates. After incubation for five days, the seedlings were pulled out and the length of plumule and primary root as well as colour, fresh and dry weight of these structures were recorded. Data (Table 1 and Fig. 3) indicate marked reduction of root length. Fresh and dry weights of roots were also reduced. Reductions were greater in culture filtrates of isolates Pa_1, Pa_3 and Pa_4 followed by isolate Pa_2. The colour of the roots also changed from normal white to brown. The length of plumule grown in culture filtrates was greater over those grown in the uninoculated medium. Fresh and dry weights of plumule were not significantly affected. No change in the plumule colour was detected in any of the treatments. These results show that certain metabolites in the culture filtrates adversely affect the growth of maize seedlings as judged by reduction in root development.

Pectinmethylesterase

No pectin methylesterase (PME) was produced in the culture filtrates by any of the isolates when grown on both synthetic and semi-synthetic media.

Table 1

Effect of culture filtrates on the growth of maize seedlings

Treatment	Root				Plumule		
	Length cm	Weight of two seedlings, g		Discolouration	Length cm	Weight of two seedlings, g	
		Fresh	Dry			Fresh	Dry
Sterile water	20.0	0.640	0.060	No	15.4	1.422	0.125
Uninoculated medium	13.5	0.483	0.060	No	13.3	1.196	0.122
Culture filtrates of isolate Pa_1	6.6	0.350	0.041	Browning	15.4	1.269	0.123
Culture filtrates of isolate Pa_2	8.3	0.413	0.052	Browning	14.1	1.258	0.124
Culture filtrates of isolate Pa_3	6.1	0.321	0.040	Browning	14.2	1.257	0.128
Culture filtrates of isolate Pa_4	6.1	0.322	0.040	Browning	14.1	1.213	0.130
L. S. D. 5%	1.7	0.066	0.008		0.5	0.098	0.010

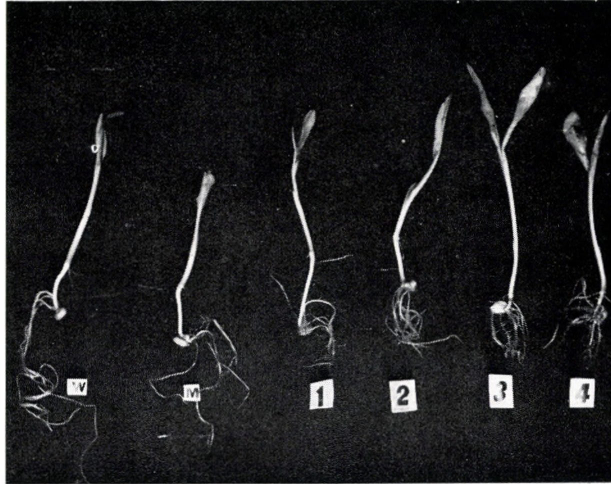

Fig. 3. Effect of culture filtrates on growth of maize seedlings. W, in water; M, in uninoculated medium; 1–4 = in culture filtrates of four isolates

Polygalacturonase and/or depolymerase

Culture filtrates from synthetic medium were not able to reduce the viscosity of citrus pectin solution, whereas those from potato broth medium reduced the viscosity to a slight extent (Table 2). Isolates Pa_4 and Pa_3 produced more polygalacturonase (PG) and/or depolymerase (DP) followed by isolate Pa_1. Isolate Pa_2 showed least PG and/or DP activity in all media having different sources of carbon. Data also show that the activity of PG and/or DP was much less in the culture filtrates of basal medium (potato broth) containing pectin or glucose as carbon source and more in medium having glucose + pectin.

Table 2

Production of polygalacturonase and/or depolymerase in culture filtrates (potato broth) of *P. aphanidermatum* isolates

Isolate	% reduction in viscosity of 1% citrus pectin solution		
	Carbon source		
	Pectin	Glucose	Pectin + Glucose
Pa_1	0.53	1.60	6.54
Pa_2	0.20	0.54	2.75
Pa_3	1.30	2.64	7.08
Pa_4	1.20	2.88	8.00
L.S.D. 5%			1.12

Cellulolytic enzymes

Data on production of cellulases by the four siolates on media having different carbon sources are presented in Table 3. All the isolates produced cellulases in vitro. However, isolates Pa_4 and Pa_3 showed greater activity followed by isolate Pa_1 on media with cellulose + glucose. Isolate Pa_2 exhibited least activity in media

Table 3

Production of cellulytic enzymes in culture filtrates of *P. aphanidermatum* isolates

Isolate	% reduction in viscosity of 1% CMC solution		
	Carbon source		
	Cellulose	Glucose	Cellulose + Glucose
Pa_1	6.30	23.01	26.57
Pa_2	9.03	14.60	22.73
Pa_3	7.60	23.33	28.68
Pa_4	10.14	23.95	29.37
L.S.D. 5%			1.45

with cellulose + glucose Isolate Pa_2 exhibited least activity in media with cellulose + glucose or with glucose only as carbon sources but in medium having cellulose only it showed more activity than isolates Pa_1 and Pa_3. It can also be observed that more cellulases were produced in medium containing cellulose + glucose followed by the one having glucose. All isolates produced least amount of cellulases on medium with cellulose as sole source of carbon.

Discussion

In the present study, in vitro production of toxin as well as hydrolytic enzymes by *P. aphanidermatum* has been shown. Culture filtrates from highly virulent isolates induced wilt in three to three-and-half hours as compared to moderately and weakly virulent isolates which required five-and-half to six-and-half hours. This difference was statistically significant. It indicates that the capacity for toxin production differs from isolate to isolate. The production of toxin by other species of *Pythium* has also been shown (Brandenburg, 1950; Vukovits, 1963 and Martin, 1964). The toxic metabolites from the culture filtrates were found to be heat labile and their ability to induce wilt in tomato cuttings was highest at pH 6.5. The toxin from *P. irregulare* has also been reported to be thermolabile (Martin, 1964).

The effect on maize seedlings consisted of reduction in root growth as judged by linear measurement as well as by fresh and dry weight determinations. Such

effects have been observed by Brandenburg (1950) and Martin (1964) with *P. irregulare*, Comstock et al. (1972) with *Phyllosticta maydis* and Sadik (1973) with *Cephalosporium maydis*.

The new modified technique for studying the effect of culture filtrates on growth of maize seedlings has the following advantages over the use of absorbent cotton wool (Sadik, 1973) and Sand (Litzenberger, 1947): (*i*) observations with regard to contamination as well as root growth can be made easily during incubation period, (*ii*) the layered filter paper strips supply and maintain the requisite amount of moisture, (*iii*) the seedlings can be easily manipulated with intact roots in injury-free condition and (*iv*) the filter paper strips being inert have no effect on the activity of the culture filtrates.

Pythium aphanidermatum from maize did not show pectin methylesterase activity in the culture filtrates. This is in agreement with the finding of Winstead and McCombs (1961) and Chakravarty (1965). The present fungus isolates behave in the same way as did the one used by Winstead and McCombs (1961) with respect to inability of their culture filtrates to reduce the viscosity of citrus pectin solution. On the other hand, culture filtrates derived from potato broth were able to reduce the viscosity of citrus pectin to some extent. It suggests that possibly the fungus has specific preferences for particular substrate(s) to secrete PG and/or DP. The four isolates employed in the present study showed statistically significant differences in the production of PG and/or DP when grown on potato broth with pectin + glucose as carbon source. Also the isolates having greater virulence showed higher enzymatic activity. This kind of association has also been observed by Friedman (1962) in *Erwinia carotovora* and by Vidhyasekaran (1978) in *Helminthosporium nodulosum*.

All the isolates produced cellulases in vitro; cellulase production was greater in medium containing cellulose + glucose followed by the one having glucose, while the medium with cellulose alone, produced the least amount of cellulases. This indicates that cellulolytic enzymes are constitutive rather than adaptive enzymes. Highly virulent isolates produced more cellulases than did weakly pathogenic ones, suggesting involvement of cellulolytic enzymes in pathogenesis. A similar kind of association of virulence with high cellulase production has also been demonstrated in *Colletotrichum linicolum* (Klug and Threinen, 1957), *C. lagenarium* (Henry and Garber, 1967), *Fusarium oxysporum* f. *callistephi* (Horst, 1965) and *Pseudomonas solanacearum* (Kelman and Cowling, 1965). Furthermore, *P. aphanidermatum* has also been shown to have greater capacity to produce cellulolytic than pectolytic enzymes.

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Cross-reactive Antigens Among *Fusarium oxysporum* f. sp. *lupini* and its Host and Non-host Plants of Unrelated Families, *Papilionaceae* and *Gramineae*

Maria Rataj-Guranowska and I. Wiatroszak

Institute of Plant Genetics, Polish Academy of Sciences, 60-479 Poznań, Poland

Soluble antigens from several strains of five *Fusarium* species and from three plant species of *Papilionaceae*, two of *Gramineae*, and one of *Cruciferae* were compared for the presence of cross-reactive antigens (CRA) by means of the ager-gel double diffusion technique.

When fungal antigens were reacted with plant antisera CRA were found among all the *Papilionaceae* species and *Fusarium* species and between *Gramineae* plants and *Fusarium graminearum*. When plant antigens were reacted with fungal antisera CRA were detected among lupin (host plant, but not susceptible variety), soybean (non-host), Triticale (non-host) and *Fusarium oxysporum* f. sp. *lupini*. CRA shared by fusaria and plants are associated with compatible as well as incompatible host–parasite systems.

The presence of cross-reactive antigens (CRA) has been demonstrated in many plant – parasite systems (e.g. Doubly et al., 1960; Schnathorst and Devay, 1963; Wimalajeewa and Devay, 1971). The protein sharing between plants and microorganisms has been of special interest because of its relevance to the compatible host – parasite relationship (Dazzo and Hubbell, 1975; Devay and Adler, 1976).

However, there were instances where the compatibility or incompatibility could not be explained on the basis of the common antigen theory (Palmerly and Callow, 1978; Barna et al., 1978). In some cases, where phytopathogenic *Fusarium* species were involved in such studies the significance of CRA was not clear and the data obtained were inconsistent (Fedotova, 1948; Fedotova, 1970; Abd-el-Rehim and Hansen, 1970; Charudattan and Devay, 1972).

Fusarium species were isolated from the vascular tissues of wilted lupin plants. Lupins, belonging to ten different species were in general infected by *Fusarium oxysporum* and *Fusarium solani* (85% of the isolations yielded these two species); *Lupinus luteus* (L.) was mainly infected by *F. oxysporum* (74%), while the other two lupin species, *Lupinus albus* (L.) and *Lupinus angustifolius* (L.) were frequently infected by *F. solani* (53 and 39%, respectively).

The present study was made to determine the presence and significance of common antigens among five *Fusarium* species and their host and non-host plants of unrelated families. Among the plant species investigated lupin and soybean *(Papilionaceae)*, maize and Triticale *(Gramineae)*, and rape *(Cruciferae)* were involved.

Materials and Methods

Fungi

Single spored isolates were used in serological tests. *Fusarium oxysporum* Schl. f. sp. *lupini* Snyd. et Hans. isolates Nos F 3, F 80, F 100, F 111, F 227, *Fusarium solani* (Mart.) Sacc. f. sp. *lupini* Weimer isolate No. F 288, and *Fusarium culmorum* (W. G. Smith) Sacc. isolate No. F 269 were obtained from our own culture collection. *Fusarium oxysporum* f. sp. *lupini* race 2 isolate No. IMI 141125, race 3 isolate No. IMI 141142, and *Fusarium oxysporum* var. *redolens* (Wollenw.) Gordon isolate No. IMI 250086 were kindly supplied by Dr. C. Booth (Commonwealth Mycological Institute, Kew, England). *Fusarium graminearum* Schwabe isolate No. 12377 was obtained from Dr. E. Lewartowska (Institute of Plant Genetics, Poznań).

Preparation of fungal antigens

Antigen No. 1, for initial immunization was prepared as follows: microconidia were obtained from 2-day-old shaken culture, grown on CMC medium (Cappellini and Peterson, 1965). Conidia were harvested by filtration through three layers of cheesecloth, then centrifuged at 4000 *g* for 20 min at 4 °C. Microconidia were stirred in 2% phenol for 20 min and washed two times in 0.85% NaCl. The sediment was resuspended in small amount of sterile distilled water and freeze-dried.

Antigen No. 2 was prepared according to the method used by Hornok (1979). Fungi were grown in the Czapek medium (50 ml of medium in 250 ml Erlenmeyer flasks) at 24 °C for 3 days in the dark. The mycelium was harvested by filtration, washed three times with distilled water, dried between paper towels and freeze-dried. The frozen mycelium was ground in a mortar and extracted with distilled water (1 ml per 1 g wet weight of the mycelium) for 1 h. The homogenate was centrifuged at 5000 *g* for 30 min at 4 °C. The supernatant was recentrifuged at 60 000 *g* for 60 min at 0 °C. Protein was determined by the tanine micromethod (Mejbaum-Katzenellenbogen, 1955) with human serum albumine as the standard. Antigen No. 2 was stored in small samples at −20 °C.

Preparation of fungal antisera

A modification of the method described by Hornok and Szécsi (1977) was applied. Two groups of ten 6-week-old female CFLP mice (from the Laboratory Animal Production Institute, Gödöllő, Hungary) were injected intraperitoneally at weekly intervals. The first and the second injection consisted of 0.5 mg spores in 100 μl of saline (antigen No. 1) and 100 μl of Freund's incomplete adjuvant (DIFCO). The following four injections consisted of 3 mg protein extract (antigen No. 2) in 100 μl of water and 100 μl of Freund's incomplete adjuvant.

Two days after the last injection mice received 5×10^6 Ehrlich Lettre ascites tumour cells (supplied by the Department of Pathological Anatomy, Semmelweis

Medical University, Budapest) in 0.5 ml physiological saline. Eight to ten days later animals with swollen abdomen were killed and the ascitic fluid was collected. The fluids of 10 mice were pooled and the cells were removed by centrifugation. Immunoglobulins from the immune ascitic fluids were isolated by ammonium-sulphate precipitation.

Preparation of plant antigens

Ten g of dry seed samples were homogenized in a mortar, extracted three times with 10 ml of precooled benzene to remove lipids. The resulting powder was then extracted with 0.1 M phosphate buffer, pH 7.6, containing 2.5% NaCl and 3 mM EDTA. The whole was passed through cheesecloth and centrifuged at 6000 *g* for 20 min at 0 °C. The protein content of the extracts was adjusted to 15 and 30 mg ml^{-1}.

Preparation of plant antisera

Antisera produced to plant seed albumins were kindly supplied by members of the Institute of Plant Genetics, Poznań. Albumins were purified according to the method described by Johnson and Fairbrothers (1965); a long-term immunization of White New Zealand rabbits was carried out according to the schedule proposed by Clausen (1969).

Antisera to seed albumins of yellow lupin (*Lupinus luteus* L.) cv. Popularny and cv. Afus, soybean (*Glycine max.* L.) cv. Warszawska, rape (*Brassica napus* L.) cv. Janpol, maize (*Zea mays* L.) s-80 line, and Triticale No. 94 line were used in the experiments.

Pathogenicity tests

Before serological tests, pathogenicity tests were carried out by inoculating host and non-host plants with *Fusarium* isolates. Yellow lupin cultivars, Popularny and Afus, field susceptible and field resistant were not checked.

Ten plants were infected with each fungal isolate. Plants were grown in plastic pots, filled with sterile soil, in the glasshouse. *Fusarium* isolates were grown in stationary cultures in Czapek medium at 24 °C. Six-week-old plants were inoculated by pouring a 3-day-old solution culture of the fungus into holes made around the base of the plants with a knife. Symptoms were evaluated after 4–6 weeks.

Ouchterlony double-diffusion tests

Agar gel plates were prepared from 1% agar (Difco Noble) in 0.05 M barbital buffer, pH 8.6, containing 0.02% sodium azide. Wells cut in the agar were 1.5 mm deep and 8 mm in diameter; the distance between the wells was 5 mm. Plates

were incubated at room temperature for 2 days. Agar-gel plates were stained in a solution containing 0.05% Crocein Scharlach MOO (Fluka), 0.04% Coomassie brilliant blue G 250 (Serva), 27% ethanol and 10% acetic acid.

Results and Discussion

Pathogenicity tests

On the basis of disease symptoms different host-parasite relationships were determined (Table 1). Two lupin cultivars, Popularny and Afus showed no symptoms when inoculated with *F. graminearum*, a species never been reported to attack lupins in Poland (Zgórkiewicz, 1969; Rataj-Guranowska, 1981).

Rape, a plant known to decrease *Fusarium* population in the soil (Lacicowa, personal communication) was 'immune' to all fusaria tested. As expected, soybean was 'immune' to most *Fusarium* species pathogenic to lupins. These results confirmed those of Armstrong and Armstrong (1964), who found, that *F. oxysporum* f. sp. *lupini* was non-pathogenic to soybean (cv. Yelredo), and fusaria inducing wilt disease of soybean did not infect lupins.

No symptoms were detected in Triticale and maize in all combinations, except for those where *F. culmorum* and *F. graminearum* were involved. Both species are important pathogens of *Gramineae* (Booth, 1971), the former attacks a wide range

Table 1

Pathogenicity of *Fusarium* isolates to different plant species and cultivars

Fusarium species	Origin	Plants				
		Lupin cv. Popularny	Soy-bean	Triti-cale	Maize	Rape
F. oxysporum f. sp. *lupini* race 2 IMI 141125	lupin	0	–	–	+–	–
F. oxysporum f. sp. *lupini* race 3 IMI 141142	lupin	0	–	–	+–	–
F. oxysporum f. sp. *lupini* F 3	lupin	0	–	–	+–	–
F. oxysporum f. sp. *lupini* F 80	lupin	0	–	–	+–	–
F. oxysporum f. sp. *lupini* F 100	lupin	0	–	–	–	–
F. oxysporum f. sp. *lupini* F 111	lupin	0	–	–	+–	–
F. oxysporum f. sp. *lupini* F 227	lupin	0	–	–	–	–
F. oxysporum var. *redolens* IMI 250086	pea	0	–	–	+–	–
F. solani f. sp. *lupini* F 288	lupin	0	–	–	–	–
F. culmorum F 269	lupin	0	+–	+–	+–	–
F. graminearum 12377	corn	–	–	+	+	–

+ = severe symptoms; +– = moderate symptoms; – = no symptoms; 0 = not tested

of plants, also belonging to *Papilionaceae*, e.g. lupins (Rataj-Guranowska, 1981). As expected, *F. culmorum* was moderately pathogenic to all plant species used in these tests, except for rape.

Serological tests

Results of the double-diffusion tests are demonstrated in Fig. 1. Protein extracts from seeds gave 4–8 precipitin bands when reacted with plant antisera in homologous combinations. When antisera towards yellow lupin cv. Popularny (susceptible host), yellow lupin cv. Afus (resistant host) and soybean cv. Warszawska ('immune' host) were reacted with antigen preparations of *F. oxysporum* f. sp. *lupini* race 3 (IMI 141142) and *F. oxysporum* f. sp. *lupini* isolates Nos F 3, F 80, F 111 (from *L. luteus*) and F 227 (from *L. angustifolius*), at a protein con-

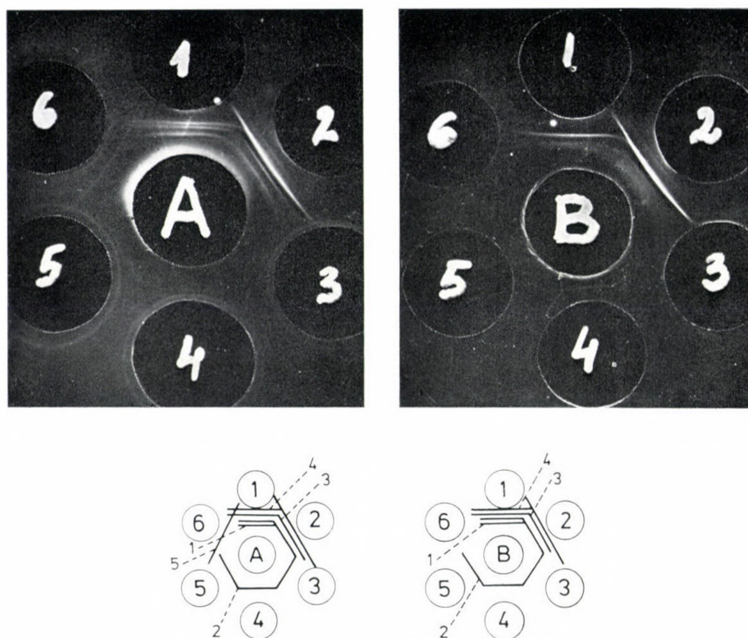

Fig. 1. Gel-diffusion plates and their diagrammatic representations showing characteristic precipitin lines in heterologous reactions. The wells contained 80 μl of antigen and 100 μl of antisera samples. Symbols: A = antigen preparation from *F. oxysporum* f. sp. *lupini;* 1 = antiserum against *F. oxysporum* f. sp. *lupini* race 2; 2 = antiserum against *F. oxysporum* f. s.p *lupini* race 3; 3 = antiserum against soybean; 4 = antiserum against lupin (susceptible cv.); 5 = antiserum against lupin (resistant cv.); 6 = antiserum against Triticale. B = antiserum against *F. oxysporum* f. sp. *lupini* race 3; 1 = antigens from *F. oxysporum* f. sp. *lupini* race 2; 2 = antigens from *F. oxysporum* f. sp. *lupini* race 3; 3 = antigens from Triticale; 4 = antigens from soybean; 5 = antigens from lupin (resistant cv.); 6 = antigens from lupin (susceptible cv.). (Threefold concentrated immune mouse ascitic fluid and unconcentrated rabbit antisera were used.)

Table 2

The presence of CRA when fungal antigens were reacted with plant antisera

Antigens	Protein concentration, mg ml^{-1}	Antisera					
		L-S	L-R	S	M	T	R
F. oxysporum f. sp. *lupini* race 2 IMI 141125	4	−	−	−	−	0	−
	30	+	+	+	−	+	−
F. oxysporum f. sp. *lupini* race 3 IMI 141142	4	+	+	+	−	0	−
	30	+	+	+	−	+	−
F. oxysporum f. sp. *lupini* F 3	4	+	+	+	−	0	−
F. oxysporum f. sp. *lupini* F 80	4	+	+	+	−	+	−
F. oxysporum f. sp. *lupini* F 100	4	−	+	−	−	0	−
	30	+	+	+	−	0	−
F. oxysporum f. sp. *lupini* F 111	4	+	+	+	−	0	−
	30	+	+	+	−	0	−
F. oxysporum f. sp. *lupini* F 227	4	+	+	+	−	0	−
	30	+	+	+	−	0	−
F. solani f. sp. *lupini* F 288	4	−	−	−	−	0	−
	30	+	+	+	−	0	−
F. oxysporum var. *redolens* IMI 250086	4	−	−	−	−	0	−
	30	+	+	+	−	0	−
F. culmorum F 269	4	−	−	−	−	−	−
	30	+	+	+	−	−	−
F. graminearum 12377	4	−	−	−	+	+	−
	30	+	+	+	+	+	−

L-S = lupin, susceptible host (cv. Popularny); L-R = lupin, resistant host (cv. Afus); S = soybean; M = maize; T = Triticale; R = rape
+ = precipitin line present; − = precipitin line absent; 0 = not tested

centration of 4 mg ml^{-1}, 1–3 precipitin lines were obtained (Table 2). Using antigen preparations of higher protein concentration (30 mg ml^{-1}) CRA were also found when the above-mentioned antisera were reacted with antigen preparations of *F. oxysporum* f. sp. *lupini* race 2 (IMI 141125) and a *F. oxysporum* f. sp. *lupini* isolate (No. L 100 from *Lupinus albus*) and all the other *Fusarium* species either pathogenic or not to *Papilionaceae* species. These results may be explained by the lower concentration of the CRA in the latter fungi.

When the antigen preparation from *F. culmorum* (F 269) was reacted with antisera against maize and Triticale a very faint precipitin line was observed. One distinct line was detected when *F. graminearum* (12377) antigens were reacted with antisera towards maize and Triticale. These antisera did not react with antigens of *F. oxysporum* f. sp. *lupini*, *F. oxysporum* var. *redolens* and *F. solani* isolates.

The antiserum towards rape did not react with any of the different fungal antigen preparations.

When antigens from *F. oxysporum* f. sp. *lupini* race 3 (IMI 141142) were reacted with the antiserum towards Triticale a single precipitin line, not identical with the others was obtained.

Antigen preparations from *F. oxysporum* f. sp. *lupini* strains gave 4–5 precipitin lines in homologous reactions. Other *Fusarium* species had at least one common antigen with *F. oxysporum* f. sp. *lupini*. The main precipitin line common in *F. oxysporum* f. sp. *lupini* race 2 and 3 was shared by all the other *Fusarium* species, namely *F. oxysporum* var. *redolens*, *F. solani*, *F. graminearum* and *F. culmorum*. The last two species are taxonomically distant from the other three. In serotaxonomical studies *F. graminearum* and *F. culmorum* were found to be closely related to each other (Hornok, 1980).

When plant antigens were reacted with antisera to *F. oxysporum* f. sp. *lupini* race 2 (IMI 141125) and race 3 (IMI 141142) precipitin lines were present in certain combinations. Antiserum prepared against *F. oxysporum* f. sp. *lupini* race 3 (IMI 141142) proved to be more reactive. CRA were detected when this antiserum was reacted with proteins from yellow lupin cv. Afus (resistant host), soybean cv. Warszawska ('immune' host) and Triticale (non-host). Surprisingly, CRA were not detected when proteins from yellow lupin cv. Popularny (susceptible host) were used as antigen sample (Table 3).

CRA were detected among all the pathogenic *F. oxysporum* f. sp. *lupini* isolates and lupin cultivars, either susceptible or not to *Fusarium* wilt disease. Similarly, Charudattan and Devay (1972) found that an antigen, which cross-reacted with *F. oxysporum* f. sp. *vasinfectum* was present in resistant as well as susceptible cotton lines. This antigen was later isolated and characterized as a protein-carbohydrate complex (Charudattan and Devay, 1981).

CRA were also found among yellow lupin and its different pathogens, *F. oxysporum*, *F. solani* and *F. oxysporum* var. *redolens*. Similar results were reported

Table 3

The presence of CRA when plant antigens were reacted with fungal antisera

Antigens	Protein concentration, mg ml^{-1}	Antisera	
		F. oxysporum f. sp. lupini race 2	*F. oxysporum* f. sp. lupini race 3
Lupin cv. Popularny	15	—	—
	30	—	—
Lupin cv. Afus	15	—	+
	30	+	+
Soybean	15	+	+
	30	+	+
Maize	15	—	—
	30	—	—
Triticale	15	—	+
	30	+	+
Rape	15	—	—
	30	—	—

+ = precipitin line present; — = precipitin line absent

by Devay et al. (1981), who found CRA among *Thielaviopsis basicola* and *F. oxysporum* f. sp. *vasinfectum*, and their common host plant, the cotton.

In our study CRA were found to be "family-specific" being present between *Papilionaceae* plants and *Fusarium* species either in compatible or incompatible combinations. Palmerley and Callow (1978) also found so-called "family-specific" CRA among tomato, potato, tobacco and *Phytophthora infestans* race 4.

An unexpected result was the presence of CRA between *F. graminarum* and the non-host lupin cultivars, as well as between Triticale and non-pathogenic *Fusarium* species isolated from *Papilionaceae* plants. These results are inconsistent with those of Charudattan and Devay (1972), who did not find CRA between *Fusarium moniliforme* and the non-host cotton. These rather controversial results might be explained, if we consider the observations of Armstrong and Armstrong (1941), who found non-host plants as carriers of wilt-causing *Fusarium* species.

Our results contradict those of Fedotova (1970) and Abd-el-Rehim and Hashem (1970), who suggested that common antigens may be indicators of plant host-parasite compatibility. However, it is still possible that CRA contribute to tolerant, compatible host-parasite interactions. On the other hand, recent interpretation of the serological cross-reactions by Lane and Koprowski (1982), based on experiments with monoclonal antibodies indicates that CRA may have no biological value in many cases.

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Effect of Nitrogen Supply on Downy Mildew Development in Sunflowers Grown in Perlit Culture

S. A. MOHAMED and F. VIRÁNYI

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102. Hungary

Sunflower seedlings (cv. GK-70) inoculated with *Plasmopara halstedii*, the downy mildew pathogen, were grown in sterile perlit and supplied with Hoagland's solution as nutrient containing various amounts of nitrogen either in form of nitrate, or ammonia. The incidence and severity of systemic infection by *P. halstedii* was assessed two times during a 3-week period using a disease index (DI) to be developed for this purpose.

The optimum level of NO_3-nitrogen observed to minimize downy mildew infection was at 284 and 420 ppm, the former being equal to standard solution. Moreover, these nitrogen rates also favoured the development of sunflowers, while others, i.e. 70, 630 and 1050 ppm of NO_3, significantly decreased plant height. The severe stunting of sunflowers treated with either low, or high level of nitrate are probably due to both downy mildew infection and unfavourable nitrogen supply.

In case of NH_4-nitrogen treatments, however, the number of plants without infection increased with increasing amount of NH_4 from 70 up to 284 ppm, while adding more ammonia proved to be strongly phytotoxic. Similarly to treatments with NO_3, the optimum growth of the inoculated seedlings occurred at 284 ppm of NH_4, whereas a contradictory tendency was evident among uninoculated sunflowers.

Downy mildew of sunflower caused by *Plasmopara halstedii* (Farlow) Berlese et de Toni is a widespread and serious disease wherever sunflower (*Helianthus annuus* L.) is grown (Leppik, 1962; Novotelnova, 1966). Severely affected plants which reach maturity yield little or no viable seed. Losses were estimated to be up to 50% of the potential seed yield in some fields in the United States (Zimmer, 1971) and also in Hungary (Kurnik et al., 1976). Although there are, at present, efficient control measures, such as resistance genes and systemic fungicides, available against this disease, we do not know anything about the role of fertilizers in changing susceptibility or resistance of sunflowers to *P. halstedii*. Furthermore, there are only a very few and even conflicting reports on the effect of host nutrition on other downy mildews, in particular those of *Gramineae* (Singh et al., 1970; Frederiksen et al., 1973; Yamada and Aday, 1977), and all these data refer to field experiments.

In this paper we report about studies on the effect of source and amount of nitrogen supply on susceptibility of young sunflowers to *P. halstedii* infection.

Materials and Methods

The sunflower cultivar GK-70, highly susceptible to downy mildew was used throughout these experiments. Inoculation of the pregerminated seeds was made as described earlier (Virányi, 1977) by using a sporangium suspension (5×10^4 per ml) of the Hl isolate of *P. halstedii*. The inoculated seedlings were planted in sterile perlit, 5 per pot, and were grown under various conditions, in the greenhouse, in a growth chamber, or in a phytotron (Fisons model 600 G3/THTL), respectively. At least 20 seedlings per treatment were used and the experiments were repeated three times.

Table 1

Chemical composition of nutrient solutions to obtain different levels of NO_3 or NH_4 nitrogen (Cheo et al., 1952)

Salts	Molarity of stock solutions	Amount (ml) of stock solution per liter of final solution				
		NO_3 ppm				
		70	284*	420	630	1050
$Ca(NO_3) \cdot 4\,H_2O$	2.5	—	3.02	2.0	2.8	2.0
KNO_3	2.0	2.75	2.52	2.5	2.5	2.5
$NaNO_3$	6.0	—	—	2.5	5.0	—
NH_4NO_3	6.0	—	—	—	—	5.0
KCl	3.0	—	—	—	—	—
KH_2PO_4	1.0	1.0	1.3	1.0	1.0	1.0
$MgSO_4 \cdot 7\,H_2O$	2.0	1.0	0.97	1.0	1.0	1.0
$CaCl_2 \cdot 2\,H_2O$	4.0	1.25	—	—	—	—
$(NH_4)_2SO_4$	2.0	—	—	—	—	—
$CaHPO_4 \cdot 2H_2O$	1.0	—	—	—	—	—
NaCl	5.0	8.5	—	—	2.0	2.0
Microelements		1.0	1.0	1.0	1.0	1.0
		NH_4 ppm				
		35	70	140	210	284*
$Ca(NO_3) \cdot 4\,H_2O$	2.5	—	—	—	—	—
KNO_3	2.0	—	—	—	—	—
$NaNO_3$	6.0	—	—	—	—	—
NH_4NO_3	6.0	—	—	—	—	—
KCl	3.0	—	—	—	—	—
KH_2PO_4	1.0	0.81	0.81	0.81	0.81	0.81
$MgSO_4 \cdot 7\,H_2O$	2.0	1.0	1.0	1.0	1.0	1.0
$CaCl_2 \cdot 2\,H_2O$	4.0	—	—	—	—	—
$(NH_4)_2SO_4$	2.0	0.65	1.3	2.6	3.9	5.1
$CaHPO_4 \cdot 2\,H_2O$	1.0	0.5	0.5	0.5	0.5	0.5
NaCl	5.0	—	—	—	—	—
Microelements		1.0	1.0	1.0	1.0	1.0

* Standard Hoagland's solution

Table 2

Assessment key for the severity of downy mildew infection in sunflower seedlings

Disease symptom	Affected host tissue	Score
Sporulation on root-neck	root-neck	1
Sporulation on hypocotyl	root-neck and hypocotyl	2
Sporulation on cotyledon(s)	root-neck, hypocotyl and cotyledons	3
Sporulation on epicotyl	root-neck, hypocotyl, cotyledons and lower part of stem	4
Sporulation on true leaves or leaf chlorosis	total plant	5

Nutrient supply. A Hoagland's solution modified by Cheo et al. (1952) served as nutrient containing various amounts of nitrate or ammonia and microelements (Table 1). Freshly made solutions of each treatment having the same osmotic value were supplied to corresponding pots, 70 ml per each every day. Twice-distilled water was furthermore added as required.

Evaluation of the incidence and severity of systemic infection. For calculating the incidence of downy mildew infection, the number of plants showing disease symptoms in all treatments were counted. To make differences between treatments concerning severity of the disease, or in other words, to observe the extent of fungal invasion within the host as affected by nutrients, an improved evaluation system has been developed on the basis of previous methods (Virányi, 1977; Virányi and Bartha, 1981). This new assessment is based on a scale from 1 to 5, each representing a degree of infection, i.e. severity of the disease as it is shown in Table 2. Every plant to be assessed at two times (at cotyledonary and four-leaf stages) was scored and then an average disease index (DI) per treatment was calculated as follows:

$$DI = \frac{X + 2X_1 + 3X_2 + 4X_3 + 5X_4}{5N} \times 100$$

where $X \ldots X_4$ = numbers of plants in each category
N = total number of plants evaluated.

In addition, height of sunflower seedlings at three-week stage was also recorded.

Results and Discussion

Effect of NO_3-nitrogen. Experimental data obtained under different conditions, either in the greenhouse, or in a growth chamber, highly correspond and demonstrate that the optimum level of NO_3-nitrogen observed to minimize downy

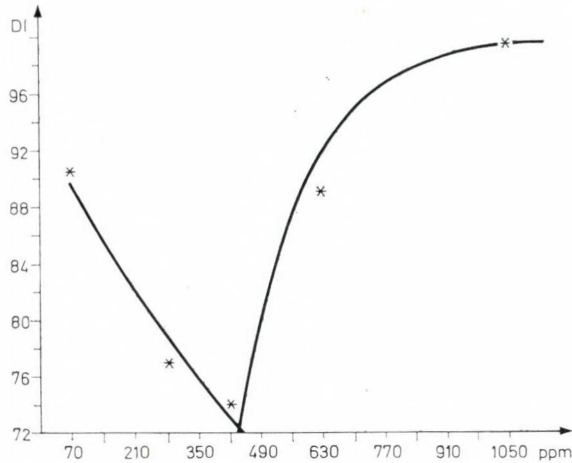

Fig. 1. Effect of NO_3-nitrogen on the severity of downy mildew in sunflower grown in the greenhouse

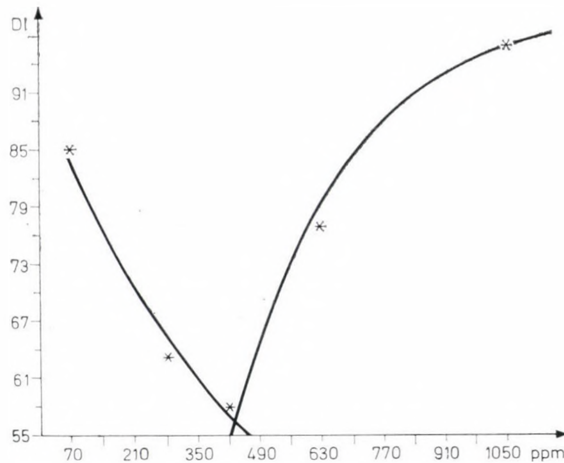

Fig. 2. Effect of NO_3-nitrogen on the severity of downy mildew in sunflower grown in a growth chamber

mildew infection equals to, or exceeds the amount in standard solution (Figures 1 and 2). As it is shown, changes in disease severity, i.e. in DI value can be characterized by two single curves, the first being a hyperbole which is followed by a saturation type curve. The fact that plants grown at 420 ppm of NO_3 showed the least infection by *P. halstedii*, is difficult to explain because this contrasts with the general meaning that increasing the amount of nitrogen fertilizer is associated with increased susceptibility of host plants to obligate parasites (Király, 1976). From

the data shown in Figs 1 and 2 it is also clear that the sunflower seedlings supplied with either high or low amounts of NO_3 respond quite similarly to these unfavourable conditions, suggesting that suboptimal level of nitrogen or even an overdose of it equally favour downy mildew development. In any case, all these results

Fig. 3. Differences in plant height of mildewed sunflowers, cv. GK-70, as affected by nitrate supply 21 days after seeding (greenhouse experiment)

Fig. 4. Differences in plant height of uninoculated sunflowers as affected by nitrate supply 21 days after seeding (greenhouse experiment)

Fig. 5. Differences in plant height of mildewed sunflowers, cv. GK-70, as affected by nitrate supply 21 days after seeding (growth chamber experiment)

Fig. 6. Differences in plant height of uninoculated sunflowers as affected by nitrate supply 21 days after seeding (growth chamber experiment)

obtained in our experiments with NO_3-nitrogen can be considered as preliminary ones and require more detailed investigations.

The nitrogen supplied in form of nitrate affects not only disease development but also plant growth. As it is shown in Table 3 and 4 and in Figs 3 to 6, those

seedlings treated with 284 or 420 ppm of NO_3 exhibited more rapid growth and were higher than plants receiving lower or higher amount of nitrate. Thus, sunflowers either infected or not, responded similarly to nitrogen treatment showing the same growing tendency in every experiment.

Effect of NH_4-nitrogen. Alterations in the severity of downy mildew infection in case of NH_4-supply are illustrated in Figs 7 and 8. As it is seen from the logistic curve, the young sunflowers exhibit opposite reactions to ammonia than did to nitrate, at least in our experimental conditions. Though the phytotoxic effect of NH_4 over 284 ppm prevented us from investigating this form of nitrogen at higher rates, the significant decrease of disease severity due to increasing NH_4 from 70 up to 284 ppm, the latter being equal to standard solution, seems to be very conspicuous. At present we do not know how ammonia can reduce downy mildew

Table 3

Changes in plant height (cm) of sunflower seedlings in relation to NO_3-supply and *Plasmopara halstedii* infection (greenhouse experiment)

Treatment ppm	Inoculated	Control
70	11.9	25.3
284	11.9	29.6
420	14.4	34.3
630	11.0	28.3
1050	9.3	23.0
LSD (P = 0.05)	2.2	1.7

Table 4

Changes in plant height (cm) of sunflower seedlings in relation to NO_3-supply and *Plasmopara halstedii* infection (growth chamber experiment)

Treatment ppm	Inoculated	Control
70	10.7	17.9
284	11.3	18.7
420	13.1	20.0
630	9.9	15.6
1050	7.6	13.6
LSD (P = 0.05)	2.1	2.3

Table 5

Changes in plant height (cm) of sunflower seedlings in relation to NH_4-supply and *Plasmopara halstedii* infection (growth chamber experiment)

Treatment ppm	Inoculated	Control
35	5.8	14.3
70	6.1	11.3
140	6.9	11.0
210	6.9	10.0
284	8.8	7.4
LSD (P = 0.05)	—	1.6

Table 6

Changes in plant height (cm) of sunflower seedlings in relation to NH_4-supply and *Plasmopara halstedii* infection (phytotron experiment)

Treatment ppm	Inoculated	Control
35	4.6	18.0
70	5.0	17.9
140	5.5	16.6
210	7.3	15.8
284	7.6	15.3
LSD (P = 0.05)	1.1	1.9

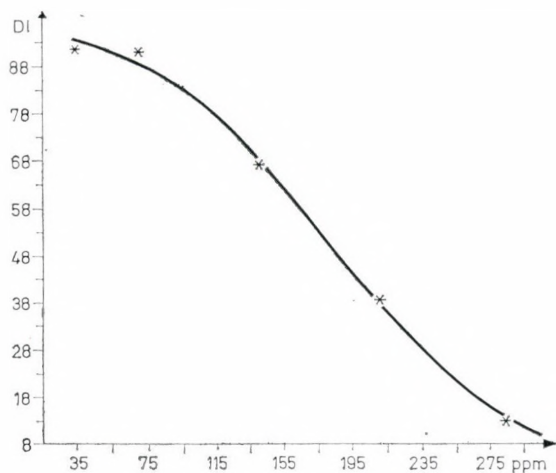

Fig. 7. Effect of NH_4-nitrogen on the severity of downy mildew in sunflower grown in a growth chamber

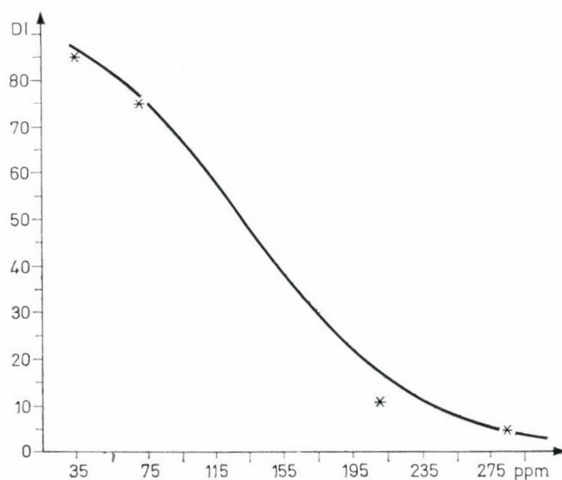

Fig. 8. Effect of NH_4-nitrogen on the severity of downy mildew in sunflower grown in a phytotron

infection but it seems likely that fungal development is inhibited through the changes in host metabolism, rather than by altered ion concentration of the system.

Similarly to treatments with NO_3-nitrogen, there was a close correlation between NH_4-supply and plant growth (Tables 5 and 6). The best result in average plant height was obtained with inoculated seedlings receiving 284 ppm of NH_4,

Fig. 9. Growth of sunflowers in relation to ammonia treatments in a growth chamber experiment (control plants behind)

Fig. 10. Growth of sunflowers in relation to ammonia treatments in a phytotron experiment (control plants behind)

whereas a contradictory tendency was evident among uninoculated sunflowers (Figs 9 and 10).

Our preliminary observations suggest that nitrogen fertilizer either in form of nitrate or of ammonia may reduce the incidence and severity of downy mildew in young sunflowers when applied after inoculation with *P. halstedii*. These data, however, do not explain the mechanism of this phenomenon, the factors either conditioning a relative resistance of the host, or inhibiting fungal invasion. By all means, further experiments are needed to clarify these problems.

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Trichoderma Species in Hungary

L. VAJNA

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

On the bases of morphological and cultural studies of a great number of isolates from different substrates five species of *Trichoderma* genus were identified in Hungary. These are: *T. koningii* Oud., *T. aureoviride* Rifai, *T. harzianum* Rifai, *T. longibrachiatum* Rifai and *T. viride* Pers. ex S. F. Gray. Morphological and cultural features of five isolates of these species are given.

It is well known that species belonging to the genus *Trichoderma* are of great biological and economical importance. There are hundreds of papers about their role in the soil microbiology, and in the biological control of wide range of plant pathogenic fungi. In spite of this there is almost no data about their occurrence in Hungary. The only species published in Hungary is *Trichoderma koningii* Oud. (Vörös, 1957).

During the last two years we have got some isolates of *Trichoderma* for identification, and at the same time we have isolated *Trichoderma spp.* from dead apple twigs and from dead wood of oak. Studying the Hungarian literature about the *Trichoderma* species it turned out that four species identified by us are new data for Hungary. For this reason we describe one isolate for each identified species using the accepted system for this genus published by Rifai, M. A. in 1969. (Rifai, 1969).

Materials and Methods

The isolates used in our investigations have been obtained from the Research Institut of Agrochemistry and Soil Sciences, Budapest (isolates: T. 7/4; T. 32; T. 6) and from the local station of Plant Protection and Agrochemistry, Miskolc (T.-M) and some of them isolated by us (T. NKI).

Isolations were made by bringing into culture colonies growing naturally on dead wood of apple twigs, oak wood and cork-wood.

To study the morphological and cultural characteristics of isolates was used medium PDA, pH 7.0. Cultures in Petri dishes (diam. 9 cm) were incubated in Phytotron at 27 °C with alternate lightening (12 h/day). Morphological characters of isolates in culture were observed daily, and the radial growth was measured at the same time each day.

Microscopic examination was carried out in microscopic mounts made from young conidial zones of 7–8–9 day-old cultures by taking out small pieces of tufts. Slides were stained with cotton blue in lactic acid. For microscopic measurements a × 100 oil immersion lens was used. Microphotography was carried out by an Opton photomicroscope.

Results

Investigating 26 isolates of *Trichoderma* we have identified five species aggregatum. These are as follows: *Trichoderma koningii* (isolate T. 32); *T. harzianum* (is. T. NKI); *T. viride* (is. T-M); *T. aureoviride* (is. T. 6) and *T. longibrachiatum* (is. T. 7/4).

The morphological and cultural characteristics of the identified isolates are described below.

Trichoderma koningii Oud. aggr. (isolate T. 32)

The radial growth of colonies is rapid, about 10–12 mm daily. The surface at first is smooth, later the formation of aerial hyphae makes it slightly hairy. The colour changes from white to whitish green later dark green. No discolouration of the medium takes place. Formation of intercalar and terminal clamidospores were mentioned. Conidiophores formed in ring-like zones in rather loose tufts. In two week old colonies these zones become less obvious because of conidia formation in wide areas outside the primary zones (Fig. 1). The conidiophores are much branched. The main branch put out several side branches in groups of two or three. The length of side branches is increased with distance from the apex of the main branch making a pyramidal branching system. On side branches numerous smaller lateral branches are formed similar to its bearer.

The phialides are nine-pin-shaped, typically they stand at a wide angle to their bearer in false verticils up to five (Fig. 2). The phialospores are mostly elliptic-subcylindrical (Fig. 3), smooth walled, pale green, 3.2×2.4 μm (2.3–$4.6 \times$ $\times 1.7$–3.4 μm).

The described T. 32 isolate was isolated from cork-wood.

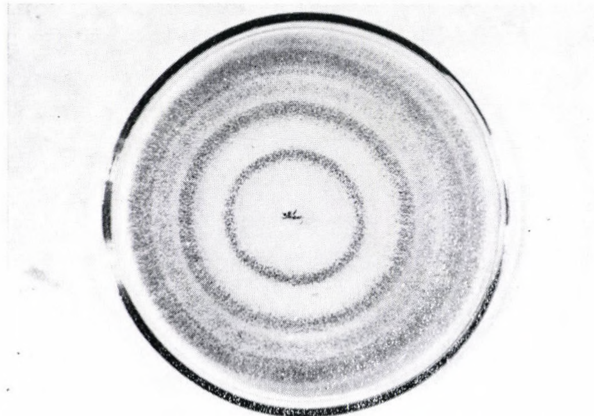

Fig. 1. Two-week-old culture of *T. koningii* Oud.

T. aureoviride Rifai aggr. (isolate T. 6)

The radial growth of colony is rapid, about 10–12 mm/day. The surface at first is almost smooth, watery-white, aerial hyphae formation is poor. On the 5th–6th day ring-like conidial zone formation can be observed and the medium

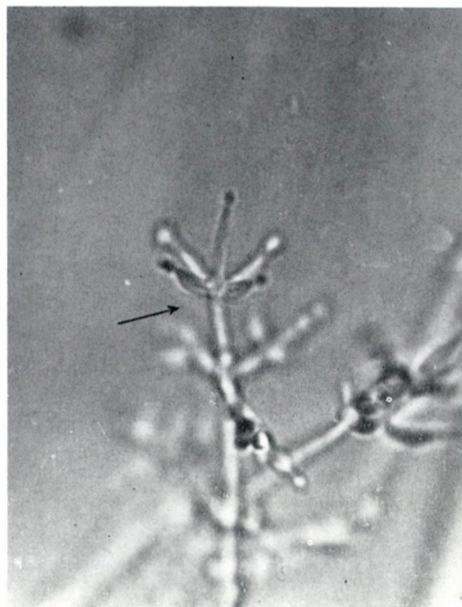

Fig. 2. Fals verticil formation consisting of 4 phialides (arrow) at the apex of main branch of *T. koningii* Oud.

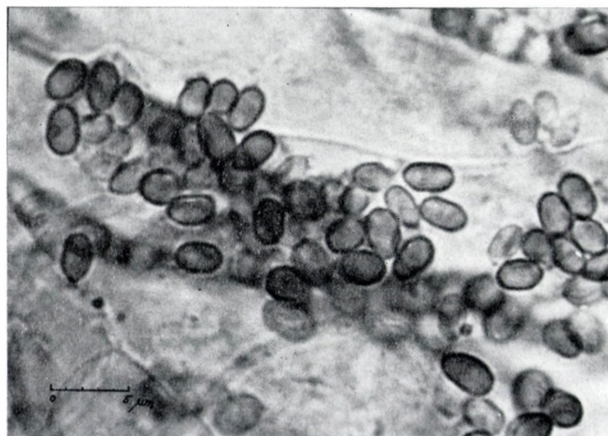

Fig. 3. Elliptic–subcylindrical phialospores of *T. koningii* Oud.

has a yellowish colouration. Intensive fructification begins at first at the marginal zone. After two weeks the colony has very slight zones with loose conidial tufts, and on the whole surface small tufts are formed (Fig. 4). The fructification makes the colonies grass-green, and the medium becomes brownish-yellow which is due to the presence of needle-shaped, golden yellow crystals.

Conidiophores are slender, side-branches typically arise in groups of two or three, and these side branches put out further smaller lateral branches (Fig. 5) making a conifer-like branching system.

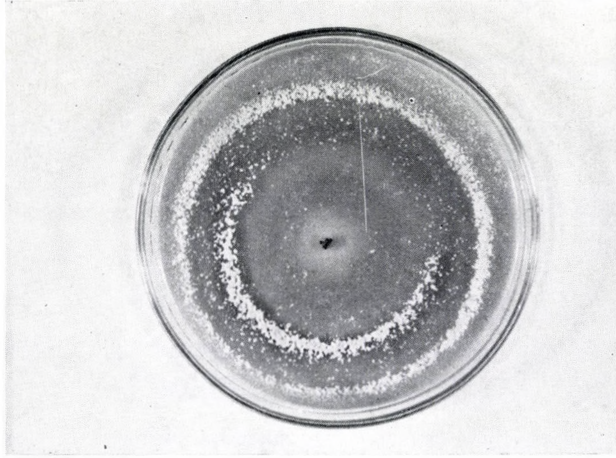

Fig. 4. Two-week-old culture of *T. aureoviride* Rifai

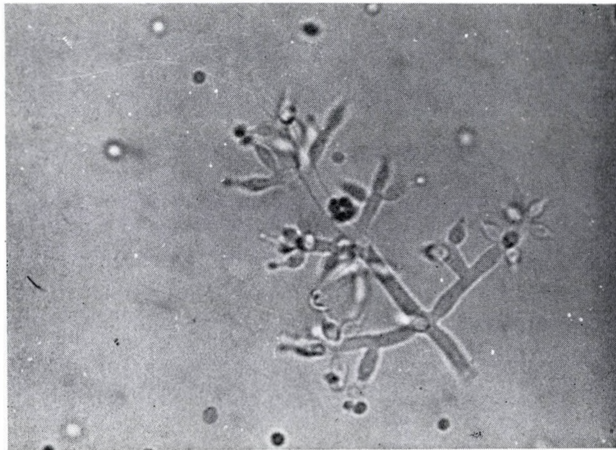

Fig. 5. Conidiophore with phialides of *T. aureoviride* Rifai

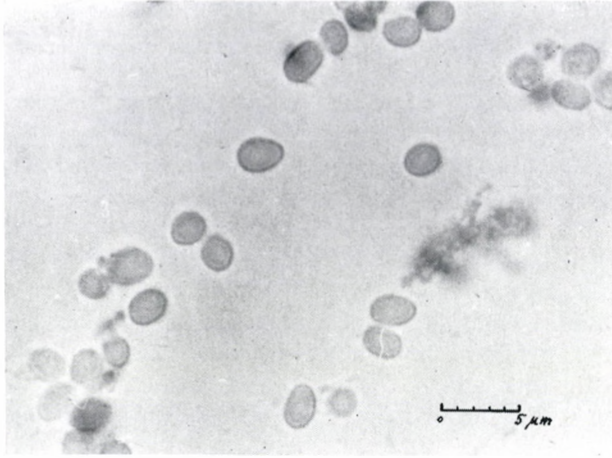

Fig. 6. Obovate-ellipsoidal phialospores of *T. aureoviride* Rifai

The phialides are bottle-shaped, arise in groups of two or three, typically forming a false verticil. The phialospores are smooth-walled obovate-ellipsoidal often with a distinct truncate base, 2.7 × 2.0 μm (1.1–4.6 × 1.1–3.4 μm) (Fig. 6).

The isolate T. 6 was brought into culture from cork-wood.

T. harzianum Rifai aggr. (isolate T. 26)

The radial growth of colony is rapid, 10–12 mm/day. First 2–3 days colonies are watery white with aerial hyphae formation. Fructification begins at the 3th day at the centre of colonies. Intensive fructification appears in very distinct

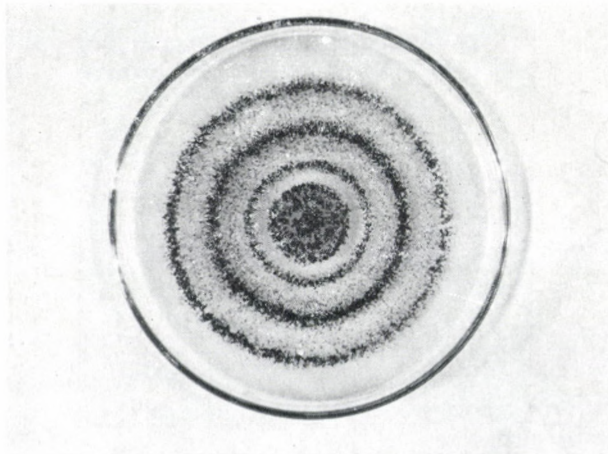

Fig. 7. Two-week-old culture of *T. harzianum* Rifai.

bright green (later dull green) zones in form of rather compact tufts (Fig. 7). Discolouration of the medium does not take place. The culture has a characteristic coconut odour.

The branching system of conidiophores are very similar to pyramidal branching of *T. koningii* and *T. aureoviride*. The main branches put out side-branches in groups of two or three, (Fig. 8, arrows), sometimes singly. The lower side-branches form lateral branches similar to their bearers. The length of side branches increase with distance from the apex of the main branch.

The whole shape of typical conidiophores is pyramidal (Fig. 8). Phialides are skittle-shaped, they arise in false verticils immediately beneath the branch-terminating phialides, and singly and irregularly along the side-branches. The phialospores are subglobose, smooth-walled, pale-green, 3.0×2.8 μm (2.4–$4.0 \times$ $\times 2.0$–3.0 μm) (Fig. 9).

The isolate T. 26 is brought into culture from dead bark of oak.

T. longibrachiatum Rifai aggr. (isolate T. 7/4)

The radial growth of colony is very fast, about 14 mm/day. On the 5th day after inoculation colonies grew to the edge of the Petri dishes. Colonies during the first 3 days are white, at the centre small light green tufts are forming, later on the

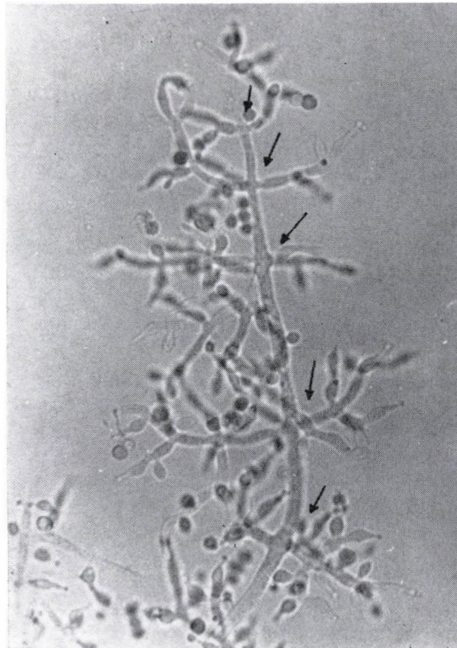

Fig. 8. Conidiophore of *T. harzianum* Rifai, side-branches forming in groups of two or three (arrows)

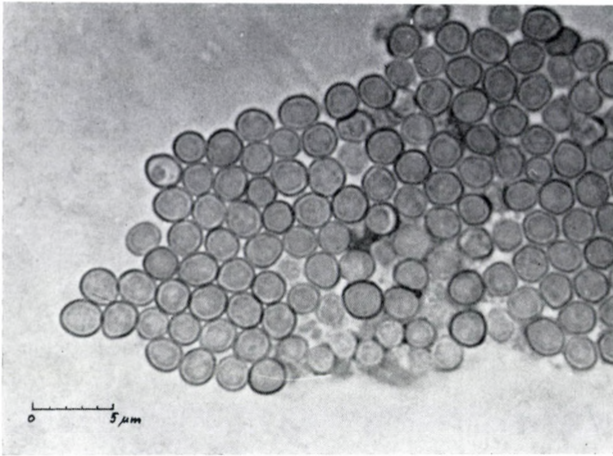

Fig. 9. Subglobose, smooth-walled phialospores of *T. harzianum* Rifai

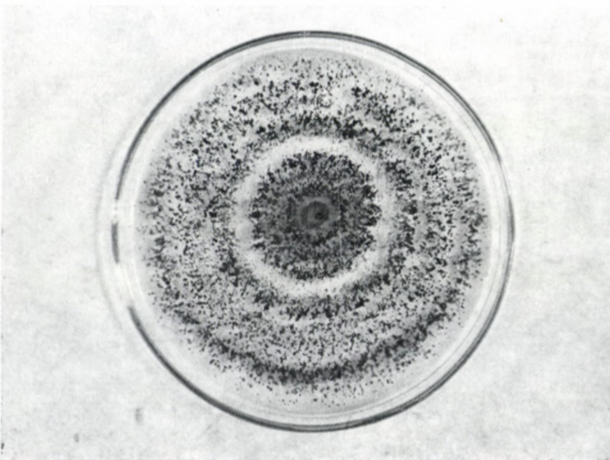

Fig. 10. Two-week-old culture of *T. longibrachiatum* Rifai

5th day colonies become zonate and fructification appears on the whole surface predominantly in ring-like zones but small loose tufts are formed between zones too (Fig. 10). Colonies after two weeks are covered with dark-green tufts, zonation is not distinct, the medium remains uncoloured.

Conidiophores have a very simple branching system in comparison with *T. koningii*, *T. aureoviride* or *T. harzianum*. The main branches put out irregularly and singly only a few side branches, standing at right angles to their bearer. Side branches are relatively short and rarely form further side branches (Fig. 11).

Phialides are bottle-shaped, formed mostly singly and irregularly. Phialospores are broadly ellipsoidal, subcylindrical often with truncate bases, pale green, 4.0×2.0 μm (3.4–5.0×1.5–3.0 μm) (Fig. 12).

The isolate was brought into culture from cork-wood.

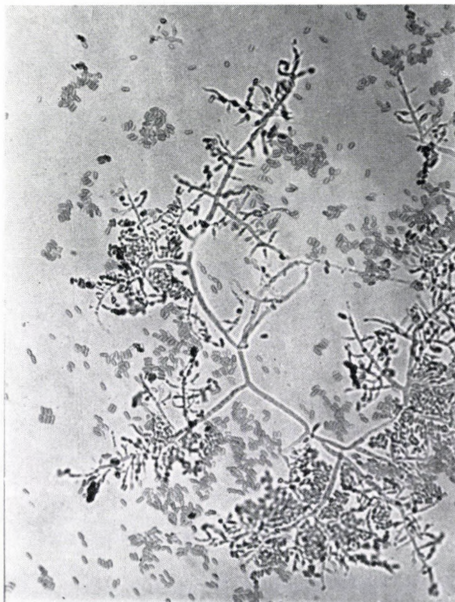

Fig. 11. Conidiophores of *T. longibrachiatum* Rifai

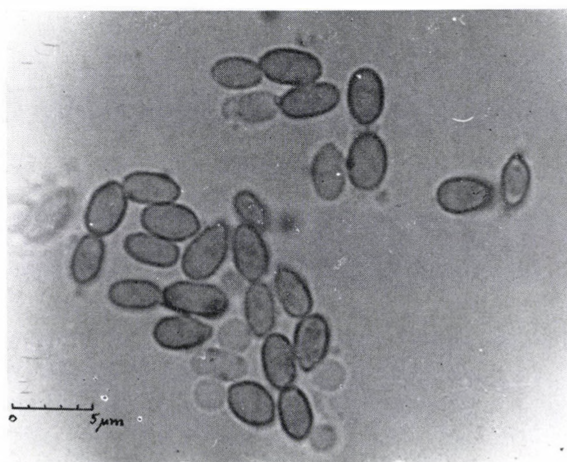

Fig. 12. Broadly ellipsoidal, subcylindrical phialospores of *T. longibrachiatum* Rifai

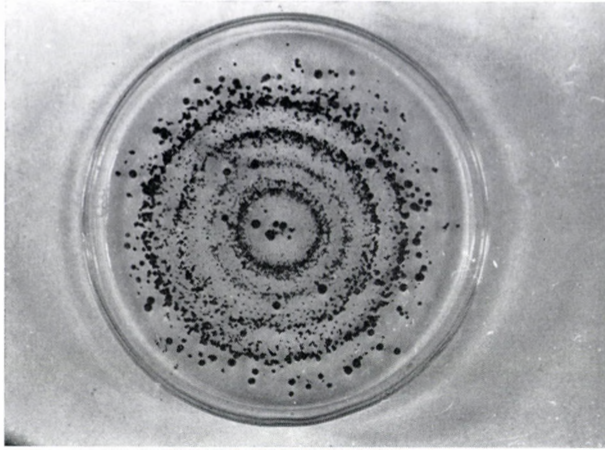

Fig. 13. 18-day-old culture of *T. viride* Pers. ex S. F. Gray

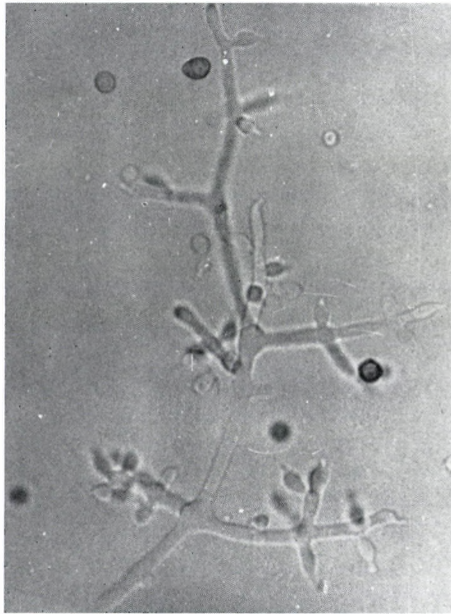

Fig. 14. Conidiophore of *T. viride* Pers. ex S. F. Gray

T. viride Pers. ex. S. F. Gray (isolate T-M)

The radial growth of colony is quite slow, about 6–8 mm/day. Colonies at the first 3–4 days are white with poor aerial hyphae formation. On the 6–7th day distinct zones appear and at the central ring-like zone pale-green fructification

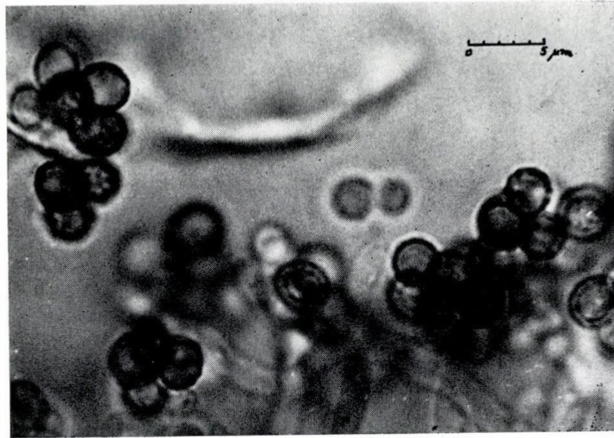

Fig. 15. Phialospores of *T. viride* Pers. ex. S. F. Gray with a distinct ornamentation

begins. In two-week old cultures dull-green fructification can be observed, and between zones the surface of the medium is covered with white, white-green tufts (Fig. 13).

The medium remains uncoloured, and has a distinct coconut odour. The conidiophores arise in compact tufts which form ring-like zones and have an irregular pyramidal branching system. The main branches of conidiophores put out several side branches which arise singly or in groups of two or three (Fig. 14). The length of the side branches is increasing with distance from the apex of their bearers. Small lateral branches arise on larger side branches.

Phialides arise in false verticils, consisting of not more than two or three phialides, or often in opposite pairs or singly.

The phialospores are subglobose, globose, sometimes broadly ellipsoidal with a distinct ornamentation on their surface, green-coloured, 3.7×3.4 µm (2.5–5.0×2.5–4.5 µm) (Fig. 15).

The isolate was brought into culture from dead wood of oak.

Conclusions

In the present study five *Trichoderma* species or species-aggregations were identified from different plant substrates in Hungary. These are: *Trichoderma koningii* Oud. aggr., *T. aureoviride* Rifai aggr., *T. harzianum* Rifai aggr., *T. longibrachiatum* Rifai aggr. and *T. viride* Pers. ex. S. F. Gray.

Identification of different isolates was possibile only on the bases of evaluation and integration of all important morphological characters of conidiophores and cultural features of colonies.

We consider that the system and keys to the identification given by Rifai (1969) is still a good base for mycologists working on this genus.

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Overwintering of *Diaporthe helianthi*, A New Destructive Pathogen of Sunflowers in Hungary

J. VÖRÖS, JUDIT LÉRÁNTH and L. VAJNA

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

During the study on the overwintering of *Diaporthe helianthi* (anamorph: *Phomopsis helianthi*) from October 1981 till June 1982 the fungus remained viable and infective only on infected stem pieces that overwintered above the soil surface. No pathogen was reisolated from, and artificial inoculations were negative with stem pieces buried in the ground 5, 15 or 30 cm deep. This result suggests that by careful and proper ploughing of the infected plant residues into the soil will prevent the overwintering of the pathogen, and eliminate the primary inoculum from infected areas.

At the end of the vegetation period in 1981 a new sunflower disease appeared in the south-eastern part of Hungary. Symptoms, characteristics of the pathogen, as well as distribution of the disease in 1981 in Hungary have been already published (Németh et al., 1981). The pathogen proved to be identical with *Diaporthe helianthi* Muntanola-Cvetkovic, Mihaljcevic et Petrov (anamorph: *Phomopsis helianthi*) (Muntanola-Cvetkovic et al., 1981). *Diaporthe perithecia* have developed on artificially inoculated sunflower stems, under glasshouse conditions. Both α and β conidia were detected in July 1982 in pycnidia that developed on infected and overwintered stem pieces.

During a survey of various sunflowers in 1981 all varieties of hybrids and breeding materials proved to be susceptible to the new pathogen. At present no dependable chemical control of the disease is available. For this reason one of the most important problems to be solved is how to eliminate the pathogen from infected fields. This paper summarizes the results obtained during the study on the owerwintering of *Diaporthe helianthi*.

Materials and Methods

Diaporthe helianthi-infected sunflower stems of various origin were collected in September 1981. Three stem pieces of each group, 20 cm long ,were placed into plastic net bags. Two bags of each were buried in the ground 5, 15 and 30 cm deep, while two additional bags were left on the soil surface on October 12, 1981. In addition, whole infected sunflower stems were left in standing position in the experimental garden.

Reisolation of the pathogen was carried out on 2% malt extract containing Czapek agar plates.

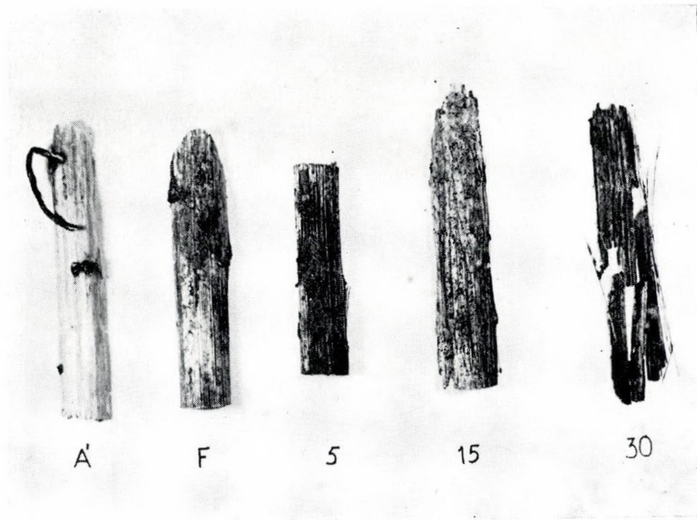

Fig. 1. Overwintered sunflower stem pieces after 7 months. A = in standing position; F = on the soil surface; 5 = 5 cm deep in the soil; 15 = 15 cm deep in the soil; 30 = 30 cm deep in the soil

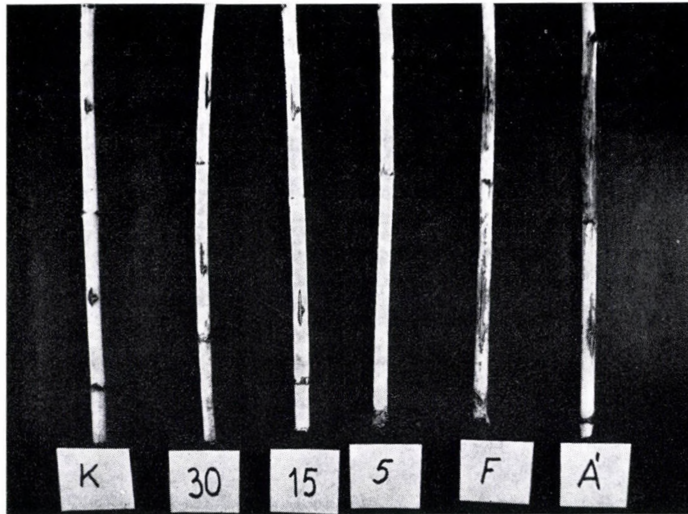

Fig. 2. Sunflower stems, artificially inoculated with overwintered stem tissue pieces, infected by *Diaporthe helianthi*. K = Control; 30 = inoculum from infected stem overwintered 30 cm deep in the ground; 15 = inoculum from infected stem overwintered 15 cm deep in the soil; 5 = inoculum from infected stem overwintered 5 cm deep in the soil; F = inoculum from infected stem overwintered on the soil surface; A = inoculum from infected stem overwintered in standing position above the soil surface

Artificial inoculations were made on IH 49 sunflower plants grown in a phytotrone. At flowering, small wounds (cuts) were made on the stems (2 cuts/plant). Small pieces (4 × 2 mm) of infected stem tissues were placed on the wounds, covered by wet cotton, and by aluminium foil. Covers were removed 5–7 days after inoculation. The first symptoms appeared in 7–10 days, and complete death of the inoculated plant occurred after 3–4 weeks.

Results

Infected sunflower stem pieces were removed from the soil and from the soil surface for the reisolation of the pathogen on May 14, 1982. When observing the overwintered material, bark tissues with pycnidia were visible only on stem pieces left on the soil surface and on stems left in standing position. No bark and pycnidia remained on stem pieces buried 5 and 15 cm deep in the ground. Stem pieces at 30 cm deep almost completely disorganized during the winter (Fig. 1).

Diaporthe helianthi was reisolated only from standing stems and from stem pieces left on the soil surface. No pathogen was detectable from stem pieces which overwintered 5, 15 and 30 cm deep in the soil.

Artificial inoculations were carried out with overwintered diseased stem tissue pieces on June 4, 1982. Disease symptoms developed only on sunflowers inoculated with infected stem tissues which overwintered above the soil surface. Sunflower plants inoculated with stem tissue pieces from materials which overwintered 5, 15 and 30 cm deep in the soil remained healthy (Fig. 2).

Discussion

Diaporthe helianthi (anamorph: *Phomopsis helianthi*) is a new, destructive pathogen of sunflowers in Hungary. Neither resistant varieties or hybrids, nor dependable chemical control procedures are available at present. Elimination of the primary inoculum from infected fields seems to be the only and most economical measure to prevent the disease. The results presented here suggest that proper and careful ploughing of infected plant debris will prevent the overwintering of the pathogen in infected areas.

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"Protoplast Killer" Extracted from Tobacco Leaves Inoculated with Bacteria

Z. Klement, R. Gáborjányi, G. Somlyai and Éva Visnyovszky

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

Tobacco protoplasts were killed by extracts from the leaves of *Nicotiana tabacum* L. Xanthi-*nc* a cultivar of tobacco responding hypersensitively to *Pseudomonas syringae* pv. *pisi*. Toxic activity of the substance appeared just before development of the hypersensitive necrosis and it was related to density of the inoculum. Leaf extracts originating from tobacco inoculated with either saprophytic *P. fluorescens* or compatible *P. s.* pv. *tabaci* did not result significant protoplast death. However, the extract killed protoplasts when it was prepared from tobacco inoculated with high concentrations of *P. s.* pv. *tabaci* (more than 2×10^{-8} cell ml^{-1}) required to cause confluent necrosis as early as HR appears.

A "protoplast killer" so termed by Pierpoint (1983) was extracted by Hooley and McCarthy (1980) from the leaves of the hypersensitive host *Nicotiana tabacum* L. cv. Xanthi-*nc* that had been inoculated with tobacco mosaic virus (TMV). The substance was obtained from tobacco leaves before lesions became necrotic and killed healthy tobacco mesophyll protoplasts. The toxicity of leaf extracts was related to density of the local lesions; moreover, it was not produced in infected leaves of systemic hosts. The factor was also not produced from leaves with chemically-induced lesions.

Hypersensitive necrosis on tobacco leaves induced by various phytopathogenic bacteria has been reported by Klement et al. (1964). Visible necrotic lesions similar to local necrotic spots caused by viruses do not form when low concentrations of bacteria are used nevertheless individual plant cells react hypersensitively (Turner and Novacky, 1974). The number of dead plant cells is positively correlated with the number of bacterial cells in the inoculum. Tobacco leaf tissue inoculated with more than 10^7 bacteria ml^{-1} causes confluent necrosis of that region of the leaf panel that was inoculated. It has been shown that toxic bacterial metabolites are not responsible for the hypersensitive necrosis (Klement, 1977) but contact between the bacterium and plant cell is necessary to initiate an apparent autolytic rapid cell collapse associated with HR (Stall and Cook, 1978). With the injection infiltration method (Klement, 1963) all cells of leaf panel react simultaneously by 7–10 hs after the inoculation. This has provided an ideal laboratory procedure with which to investigate physiological changes during the development of necrosis in a synchronized *in vivo* system.

It has been suggested by Klement et al. (1977) and Klement (1982) that both the development of plant cell death in hypersensitive and normosensitive hosts are similar after the induction of necrosis. However, the formation and develop-

ment of this apparently self-destructive process is not fully understood (Keen and Holliday, 1982; Klement, 1982).

In this study we have attempted to present evidence that a toxic substance originates from the necrotically responding tobacco tissues that were previously inoculated with the incompatible *Pseudomonas syringae* pv. *pisi*, the compatible *P. syringae* pv. *tabaci* and a saprophytic *P. fluorescens*.

Materials and Methods

Protoplasts were prepared from 10–12 week-old plants of *Nicotiana tabacum* L. cv. Xanthi-*nc*. The surfaces of the leaves were desinfested with sodium hypochloride solution and the lower epidermis was peeled before the enzyme treatment. Protoplasts were isolated from the palisade parenchyma tissue according to the two-step procedure of Takebe et al. (1969). Mannitol in 0.45 M was used instead of 0.7 M. One ml samples of $1–4 \times 10^5$ freshly isolated and washed protoplasts were mixed with equal volumes of leaf extracts. To determine the per cent of killed protoplasts the samples were stained with Evan's blue and the mortality was calculated according to the procedure of Hooley and McCarthy (1980). Determinations of survival were made after the protoplasts were incubated together with leaf extracts at room temperature for 2–4 hos.

Leaf extracts; Intervenial regions of fully expanded tobacco leaves were injected by the suspensions of *Pseudomonas syringae* pv. *tabaci* (compatible) *P. syringae* pv. *pisi* (incompatible) and *P. fluorescens* (saprophytic) bacteria. Two, four and six hours after the inoculation the leaves were harvested and homogenized in an ice-cold mortar with equal amount of 0.45 M mannitol (pH 5.2). The homogenates were centrifuged at 5000 *g* for 15 min. The samples were frozen at −20 °C overnight, then thawed and collected in Eppendorf tubes and centrifuged for 10 min at 10 000 rpm. One ml of this leaf extract was added to the protoplast suspension.

Results and Discussion

There are inherent difficulties in keeping leaf protoplasts alive at reasonably high percentages in culture medium. The percentage of protoplasts killed by extracts of inoculated leaves varied in our experiments between 30–65%, however, the general trend of the data showed that the percentage of killed protoplast was significantly higher in cultures inoculated with extracts made from hypersensitively reacting leaves than from non-inoculated controls.

Some steps of the development of HR in tobacco leaves inoculated with an incompatible *P. s.* pv. *pisi* have been reported (Klement et al. 1972; Essenberg, 1973). One and half hours are required for induction of necrosis by the pathogen and 7–8 hs after inoculation tissue collapse occurs. The biochemical changes during the symptomless latent period, between the induction of HR and tissue collapse are not well known (Klement, 1982). In order to determine the time course of appearance of toxicity of leaf extracts during the development of HR, samples

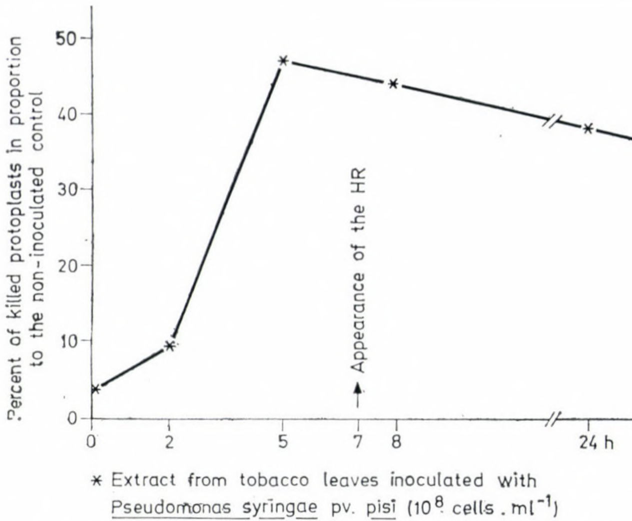

Fig. 1. The time course of appearance of toxicity of leaf extracts during the development of hypersensitive reaction (HR)

were obtained from bacterially inoculated leaves at 0, 2, 5, 8 and 24 h after the inoculation with 10^8 cells ml^{-1} of *P. s.* pv. *pisi* (Fig. 1). The toxicity of extracts was initially detected at 5 h after the inoculation and a few hours before the HR (necrosis) appeared. After the appearance of necrosis the toxicity of leaf extracts decreased moderately. These results agree with the experiment of Holley and McCarthy (1980) who noted that the toxicity of extracts in virus infected host appeared before tissues became necrotic.

The number of necrotized host cells depends on the number of the incompatible bacterium used for induction of HR (Turner and Novacky, 1974). Confluent necrosis of leaf tissue developed where the concentration of inoculum was more than 5×10^6 cells ml^{-1}. Therefore, the relationship of toxic activity of extracts to the concentration of the inoculum was also investigated (Table 1). Over the range 10^5 to 10^7 cells ml^{-1} of the inoculum the toxicity increased with bacterial number.

Table 1

The percentage of killed protoplast incubated with extracts which were prepared from tobacco leaves inoculated with various number of *Pseudomonas syringae* pv. *pisi* by 6 h after the inoculation

Number of the bacterium cells · ml^{-1}	10^5	10^6	10^7
Percentage of killed protoplasts	40	45	65

In three separate experiments the toxic activity of extracts prepared from tobacco leaves which were inoculated with the incompatible *P. s.* pv. *pisi* with the compatible *P. s.* pv. *tabaci* and with saprophytic *P. fluorescens* were investigated respectively. In these experiments 10^5, 10^6 and 10^7 cells ml^{-1} bacteria were used for inoculation of leaves. Samples were taken 2, 4, 6 h after the inoculation. As we have previously mentioned *P. s.* pv. *tabaci* at these concentrations does not cause necrosis during the 2–6 h duration of the experiment. In all instances only the extracts made from hypersensitively reacting leaves were able to kill protoplasts in high percentages (Fig. 2). Leaf extracts originating from tobacco inoculated with either *P. s.* pv. *tabaci* or *P. fluorescens* did not result significant protoplast death. Klement et al. (1977) have shown that the local necrosis in susceptible tobacco caused by *P. s.* pv. *tabaci* is not a consequence of toxic metabolites of the pathogen, but it is the result of an autolytic process induced by the bacterium. Our data suggest that the development of necrosis in both the hypersensitive and susceptible tobacco may be due to the same induced autolysis. The difference between hypersensitive and normosensitive necrosis may only be due to the number of bacteria necessary for the induction of host cell collapse. According to Turner and Novacky (1974) as few as 1–10 cells of *P. s.* pv. *pisi* can induce collapse in one mesophyll

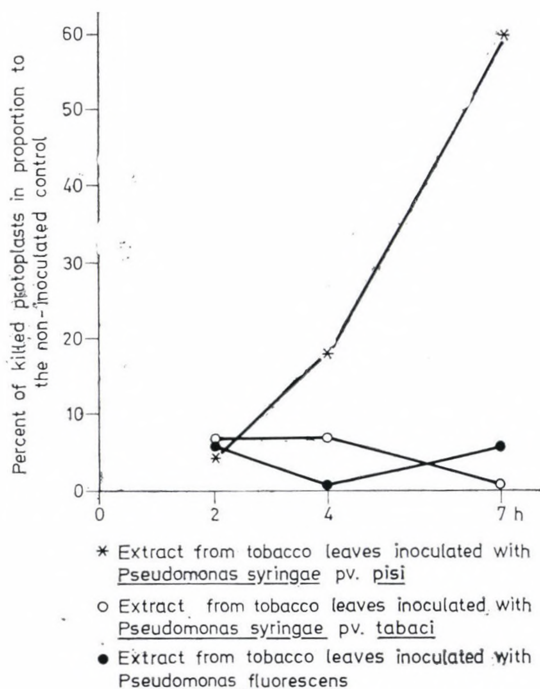

Fig. 2. Toxic activity of extracts prepared from tobacco leaves inoculated with compatible, incompatible and saprophytic bacteria (10^7 cells ml^{-1})

cell of the non-host tobacco but 50–100 cells of *P. s.* pv. *tabaci* per one tobacco cell are necessary for the induction of cell death in susceptible host (Klement et al. 1977). These findings were the basis for investigation of the toxicity of leaf tissue inoculated with high number of *P. s.* pv. *tabaci*. It has been calculated that in tobacco-*P. s.* pv. *tabaci* system more than 10^8 cells ml^{-1} are required to induce confluent necrosis 7–8 h after the inoculation. Extracts also killed protoplasts when they were prepared from susceptible tobacco inoculated with high concentrations of *P. s.* pv. *tabaci* cells. This result appears to confirm our contention that there is a similarity in the development of necrosis in susceptible and resistant or nonhost plants.

Our results also show that toxic substance/s are formed during the development of necrosis induced by bacteria as well as virus. We emphasize that these toxic substances have been formed before the appearance of visible necrosis. Therefore we think that the toxic activity of extracts is not a product of the necrotized tissue, but rather may be the cause of tissue necrosis.

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The Influence of MCPA on the Vascular Tissue in the Leaf Petioles of Sunflower (*Helianthus annuus* L.)

F. Herdi

Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P. O. Box 102, Hungary

The histological alterations caused by MCPA (2-methyl-4-chloro-phenoxy-acetic acid) in sunflower leaf petioles were studied at three different dates and by the use of three concentrations. The 5000 ppm concentration damaged the plants to an extent that they were killed by the time of third sampling (July 11th), so samples could be taken only at the two earlier dates (June 14th and June 21st).

To evaluate the effect of MCPA the thickness of cell walls was measured in the bundle sheaths, phloem cells and tracheae. Also the form ofleaf petioles, the distribution of plant hairs, the number of cells in the lamellar collenchyma layer both on the convex and concave sides of the petioles and the number of vascular bundles were considered. In the latter the number of cell layers in the bundle sheaths, phloem and cambium tissues and the distribution of tracheae and tracheids was established.

The walls of bundle sheaths and phloem cells became considerably thinner by the time of the third sampling in the 100 ppm treatment, as compared to the control. In the 1000 ppm treatment only a slight thickening was noted in the cell walls and in the 5000 ppm treatment there were hardly any differences found between the first and third sampling.

The tracheal walls were thinner than those of the control in all treatments and at all sampling dates. There were no significant differences resulting from the treatments, as the comparison of the first and third samplings indicated.

A multiplication of the vascular bundles may be mentioned as a characteristic symptom which was caused either by the division of existing bundles or by the development of new ones from procambium strands.

In the treated plants the tracheae and tracheids showed within the vascular bundles a scattered, random distribution, contrary to the radial distribution of the same elements in the control.

It can be concluded that MCPA damaged some elements of the vascular syssem in all the three concentrations used. The alterations appeared as thickening of the cell walls, multiplication of vascular bundles and as scattered distribution of tracheae and tracheids.

The herbicides with hormonal effect used in the chemical weed control of cereal crops are usually applied by aeroplane or helicopter. The drift of herbicides to neighbouring cultures may cause severe damage, even destruction of sensitive crops, especially of dicotyledonous plants.

The hormonal herbicides act both on the aerial plant organs and on the parts hidden in the soil and cause different malformations (Ubrizsy and Gimesi, 1969). In the shoots deformations, distortions and flattening occur, the leaves become either funnel-shaped (Buhl, 1958; Ubrizsy, 1962) or adhesion occurs (Way, 1962, 1963*a*, 1964; Kiermayer cit. in Audus, 1964). In case of grapevine typical fan-leaf

and gingko-leaf deformations are observed (Ubrizsy, 1962; Szatala, 1967). Other types of alterations and deformations were reported by Andersen, Bachthaller and Hanf (cit. in Ubrizsy, 1962), Ubrizsy and Gimesi (1969) Terpó and Pomogy-Terpó (1971), Way (1963, 1963*b*, 1964*a*). Also on the fruits (Ubrizsy, 1962) and roots (Arlt and Feyerabend, 1973; Buhl, 1958; Way, 1962, 1963*a*, 1964) were severe deformations observed. Sunflower is very sensitive to hormonal herbicides (Ubrizsy, 1962).

The hormonal-type herbicides may exert an especially heavy damage at the time of cell differentiation (Watson, 1948). Eames (1951) observed a very intensive cell proliferation in the differentiating tissues and in the phloem vascular elements, caused by this type of preparations.

The same preparations also strongly influence the formation of cell walls. Upon the effect of 2,4-D the most conspicuous changes were observed in the cambial tissue, endodermis, embryonal pericycle and phloem (Swanson, cit. in Klingman, 1963; Beal, cit. in Aufus, 1964). As a result of dichlobenyl and dicamba treatment the damage on the phloem, cambium and adjacent parenchyma was observed (Pate et al., 1965). Meyer (1970) observed the damage in phloem and xylem elements in the stalk and root, caused by picloram and 2,4,5-T preparations.

Eames (1949) observed an abnormal development in the vascular bundles which became elongated; at the same time a decrease in phloem quantity and a retarded growth of metaxylem vessels was noted. Bradley et al. (cit. in Audus, 1976) noticed an increase in the leaf petiole diameter, caused by the increase of secondary xylem and phloem elements. Changes in the cell wall formation were noted by Gorter and Gifford (cit. in Audus, 1964).

Herdi (1979) observed after a dicamba treatment the thickening of cell walls in the bundle sheaths and phloem, simultaneously with deformations and proliferations in the vascular bundles of red beet leaf petioles. Sorokin et al. (1965) reported on hyperplasia and defective development of xylem in bean shoots treated with IAA and 2,4-D.

Materials and Methods

The experiments were carried out on sunflower plants (variety VNIIMK 6541), sown on May 12th; the herbicide treatment was made on June 12th when the plants were 10–12 cm high and in the true leaf stage 2.

The preparation used in the experiment was a technical-grade MCPA (2-methyl-4-chloro-phenoxy acetic acid), sprayed onto the plants.

The plants received the following treatments:

MCPA 100 ppm
MCPA 1000 ppm
MCPA 5000 ppm

Untreated control

The samples were taken at three dates:

First sampling June 14th
Second sampling June 21th
Third sampling July 11th

As a result of 5000 ppm MCPA treatment the plants died by the third sampling date, so in this case only the first and second samples could be investigated. The samples were collected in the morning hours and fixed in 40% alcohol. The sections were made from the middle portion of leaf petioles, by using an MC-2 type sled microtome provided with a KTOC-2 freezer device. The sections were stained by acidic haematoxyline according to the method of Ehrlich.

In course of the studies we investigated the shape of the petiole, the number of cell layers in the collenchyma and measured the thickness of cell walls in the bundle sheath and phloem and tracheae. Also the number of cell layers in the cambium, the multiplication of vascular bundles and their distribution within the leaf petiole was noted. The data gained were subjected to a statistical analysis (double *t* test).

Results

The histological alterations found in the leaf petioles and vascular bundles at three different dates and caused by three MCPA dosages are shown in Figs 1–7. The plants treated with the highest dosage (5000 ppm MCPA) died by the time of the third sampling, so here only the data of the 1st and 2nd sampling could be evaluated.

Table 1

Changes in the number of cell layers of lamellar collenchyma in sunflower leaf petioles treated with MCPA

Treatment	Date of sampling (1978)	Number of collenchyma cell layers on the		
		concave side	convex side	edges
Control	June 14th	2–3	2–3	4–5
	June 21st	3–4	3	4–7
	July 11th	4–6	5–8	6–9
100 ppm	June 14th	3–4	4–5	4–7
	June 21st	4–5	5–7	5–9
	July 11th	4–6	6–7	6–9
1000 ppm	June 14th	2–3	3–4	3–5
	June 21st	2–3	2–4	3–5
	July 11th	1–3	2–4	2–4
5000 ppm	June 14th	2–3	2–4	3–6
	June 21st	2–3	3–4	3–6

First sample of untreated control (June 14th)

The leaf petiole is obtusely triangular, bifacial, the convex side is slightly undulated.

Both on the convex and concave side of the leaf petiole lamellar collenchyma tissue can be found (Table 1). On both sides of the petiole on the epidermis multicellular hairs are singly scattered.

In the leaf petiole collateral open vascular bundles are found (Fig. 6A), characteristic for sunflower. The bundles are placed to some distance from each other, their distribution is shown in Fig. 4A; from the 12–15 bundles three are large, the others considerably smaller.

Above the phloem a bundle sheath and one cell layer starchy sheath is found (Table 2); the vascular bundle is surrounded by the bundle sheath, laterally and from the xylem side.

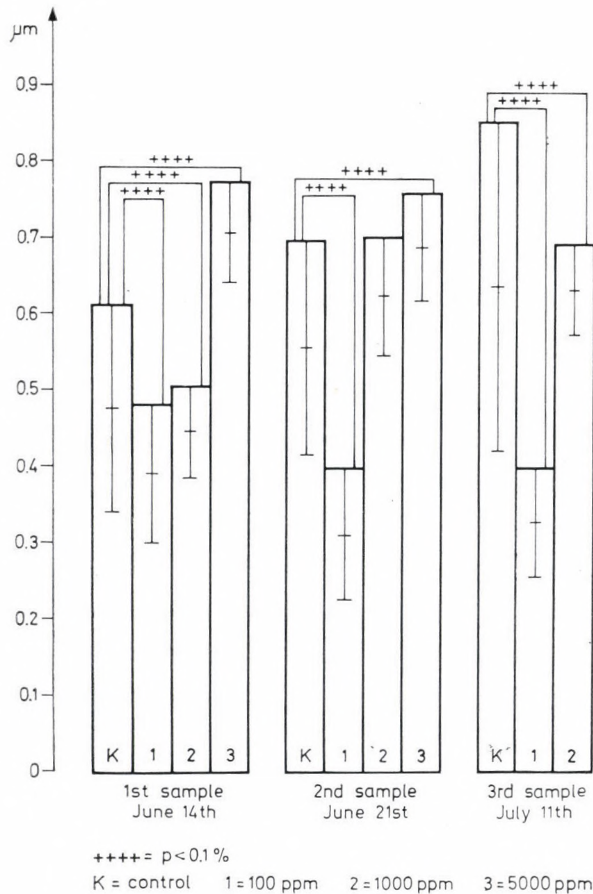


Fig. 1. Changes in the wall thickness in the bundle sheath as a result of MCPA treatment

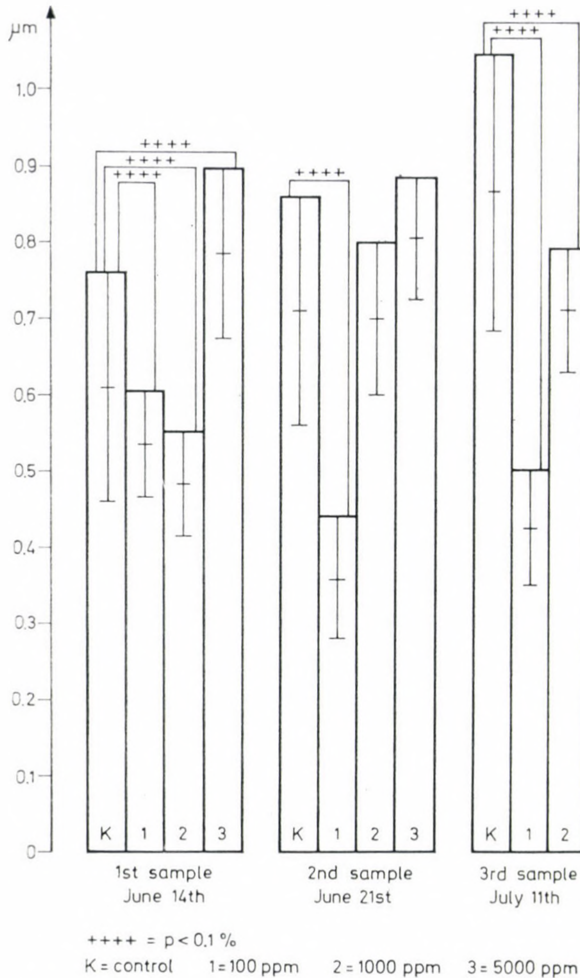


Fig. 2. Changes in the wall thickness of phloem cells as a result of MCPA treatment

The number of cell layers (appearing on the sections as cell rows) of the cambium is shown in Table 2. The tracheae and tracheids are radially distributed. In the vascular bundles no branching is observed.

Second sample of untreated control (June 21st)

The leaf petiole resembles to the one in the first sample, the number of lamellar collenchyma layers is shown in Table 1. The multicellular plant hairs are scattered singly both on the concave and convex side; on the latter their number is somewhat higher.

There are 12–15 vascular bundles of which three are large (Fig. 6B) and the others are much smaller.

The distribution of the bundles (Fig. 4B) resembles to that in the first sample. In the bundle above the phloem a bundle sheath (Table 2) and one layer of starchy sheath is found. The sheath surrounding the bundle consists of 2–3 cell layers.

The cell row numbers of the cambium are shown in Table 2.

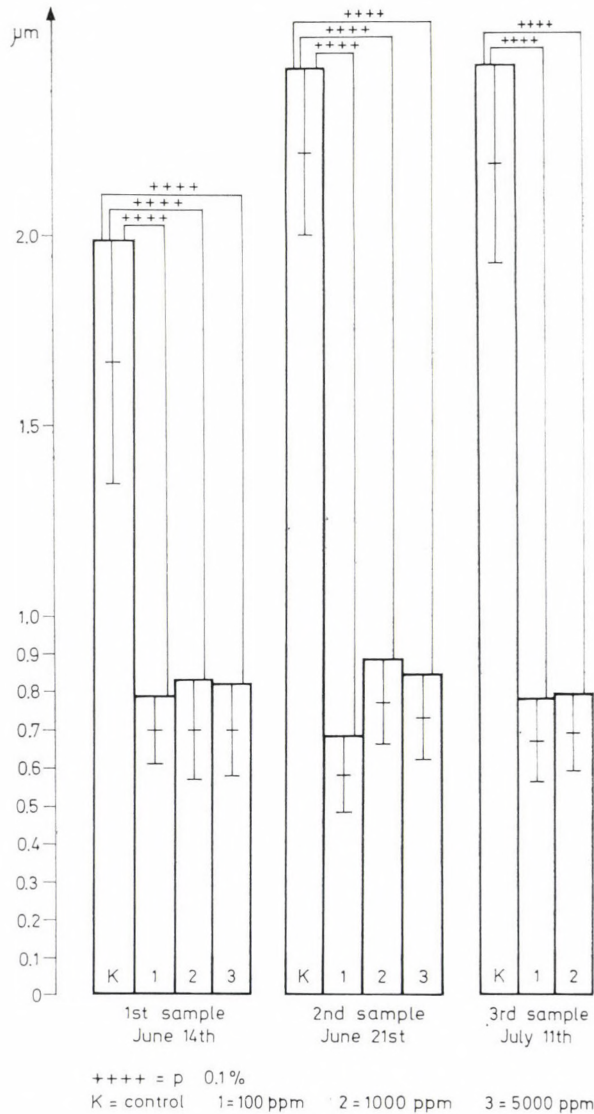


Fig. 3. Changes in the wall thickness of tracheae as a result of MCPA treatment

Table 2

Changes in the number of cell layers in the bundle sheath and cambium in sunflower leaf petioles treated with MCPA

Treatment	Date of sampling (1978)	Number of cell layers in the		
		bundle sheath		cambium
		above the phloem	laterally and from the xylem side	
Control	June 14th	4–7	2–3	3–4
	June 21st	4–7	2–3	4–6
	July 11th	6–10	2–4	4–6
100 ppm	June 14th	7–11	2–4	4–7
	June 21st	6–8	2–4	6–9
	July 11th	7–13	2–6	3–7
1000 ppm	June 14th	4–9	2–3	4–6
	June 21st	5–8	1–2	6–8
	July 11th	4–9	1–2	4–6
5000 ppm	June 14th	4–6	2–3	3–6
	June 21st	3–6	1–2	4–7

The tracheae and tracheids are radially distributed and in the vascular bundles no branching is observed.

Third sample of untreated control (July 11st)

The petiole resembles to the one of the first sample, the number of cell rows in the lamellar collenchyma on the concave and convex side is shown in Table 1.

The multicellular hairs are singly distributed on both sides of the petiole; while they are placed to a greater distance from each other on the convex side, they are more densely situated on the concave one, sometimes near to each other.

There are 16–18 vascular bundles, three of which are large (Fig. 6C) and the others are much smaller. The distribution of vascular bundles is similar to the earlier samples (Fig. 4C).

In the vascular bundle above the phloem a bundle sheath (Table 2) and a layer of starchy sheath cells is found. The number of cell layers of the bundle sheath surrounding the bundle laterally and from the side of the xylem as well as the number of cell layers in the cambium are shown in Table 2.

The tracheae and tracheids are radially distributed.

The transport (vascular) bundles do not branch.

First sample of 100 ppm MCPA treatment (June 14th)

The shape of the petiole resembles to the control, the convex side is slightly undulated.

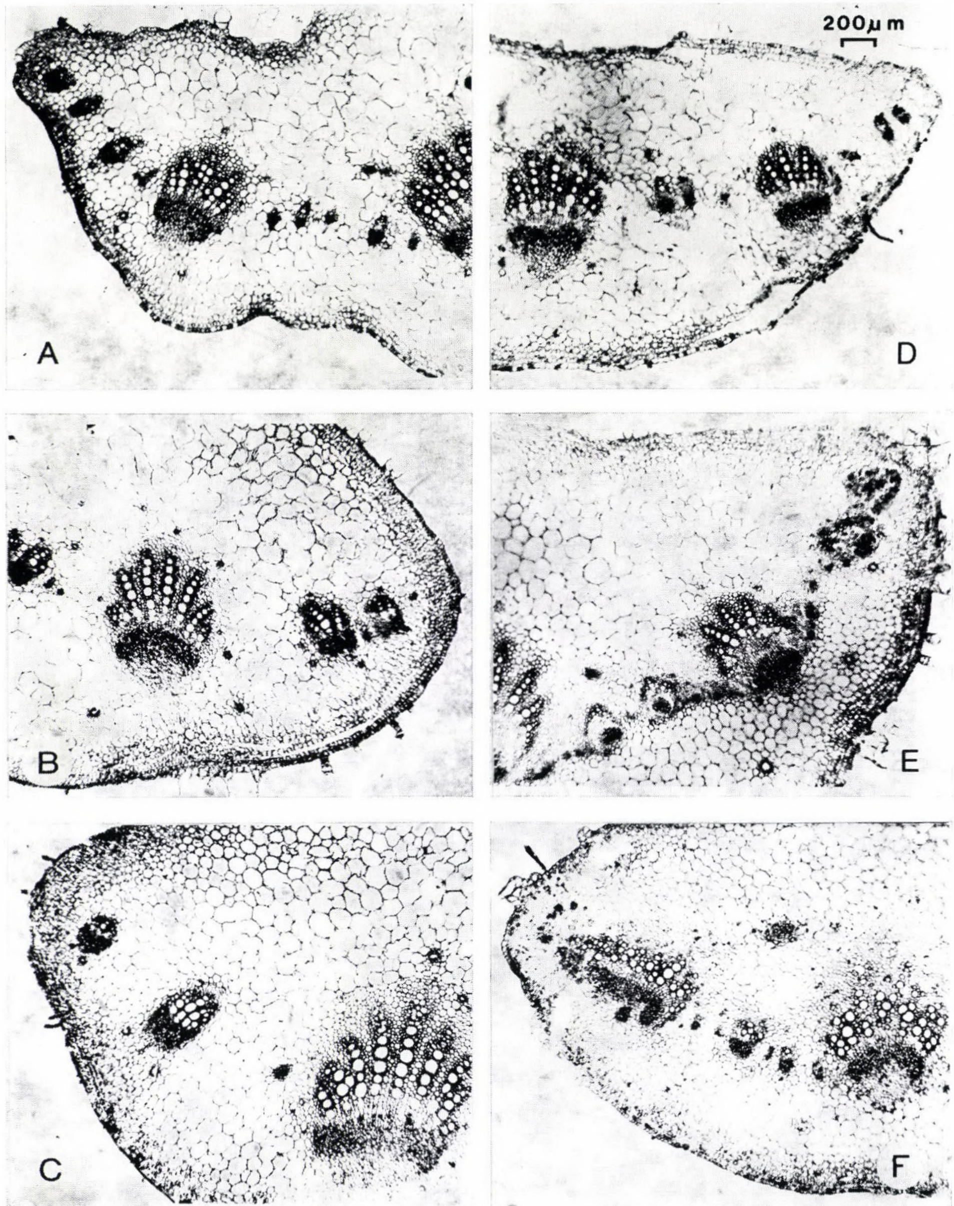


Fig. 4. Distribution of vascular bundles in the control and MCPA-treated leaf petioles A–C = control; D–F = 100 ppm; A, D = 1st sample (June 14th); B, E = 2nd sample (June 21st); C, F = 3rd sample (July 11th). All figures in the same scale

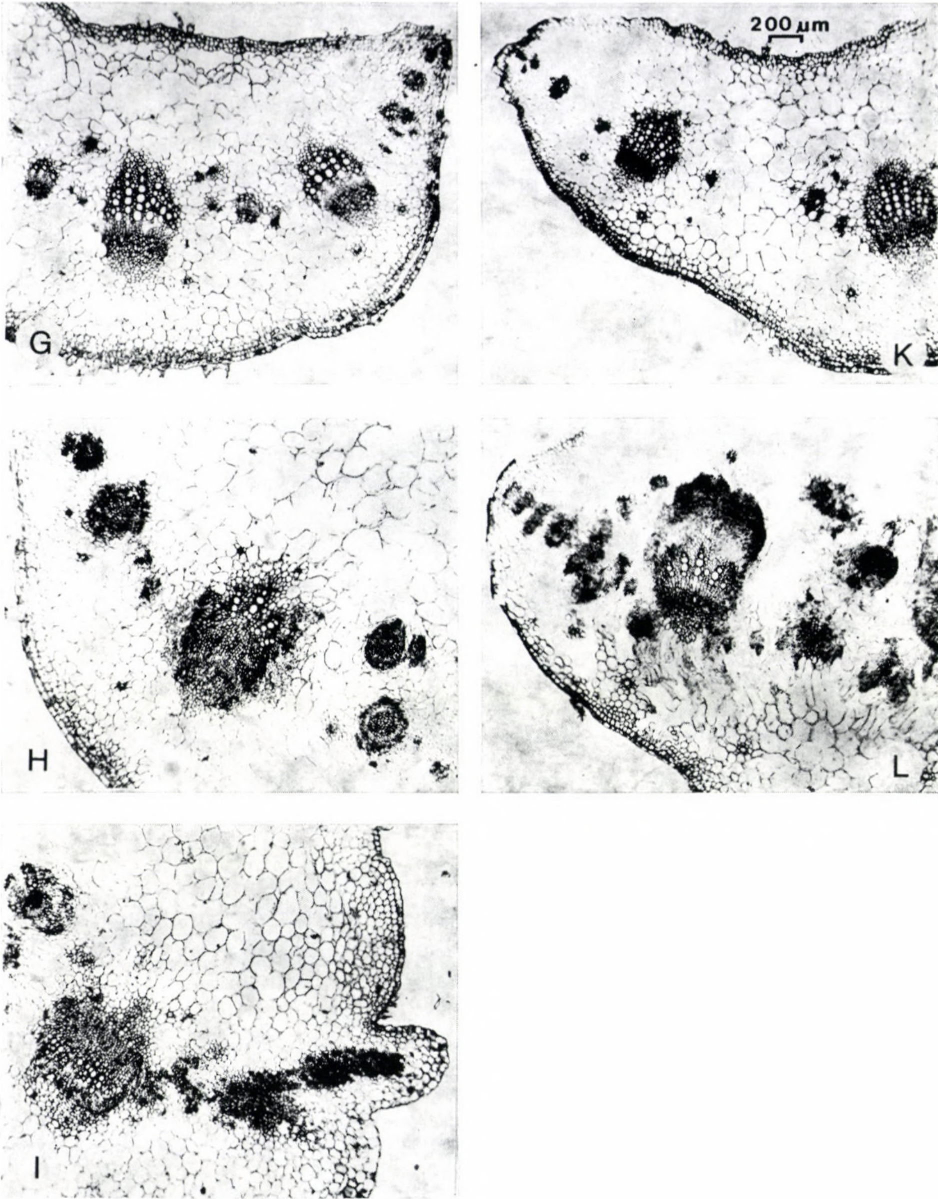


Fig. 5. Distribution of vascular bundles in the leaf petioles of MCPA-treated plants G–I = 1000 ppm; K–L = 5000 ppm; G, K = 1st sample (June 14th); H, L = 2nd sample (June 21st); I = 3rd sample (July 11th). All figures in the same scale

On both sides of the petiole a lamellar collenchyma layer is found (Table 1) and on the convex side also angular collenchyma occurs in some places.

The multicellular hairs are placed singly on both sides, well distanced from each other; on the convex side some of the hairs has a thickness of more than one cell rows.

There are 14–17 vascular bundles of which three are of large diameter (Fig. 6D), the others are smaller. The bundles are placed and distributed in the leaf petiole as in the control (Fig. 4D).

The number of cell layers in the bundle sheath and cambium is shown in Table 2.

The tracheae and tracheids are radially distributed, in some cases interspersed with irregular ones.

The cell walls of the sheath are considerably thinner compared to the ones of the control (Fig. 1) and in this case the first sample shows thinner walls than the other two. Also in the case of phloem cells a considerable difference from the control cells can be observed regarding the wall diameter (Fig. 2); there is a small difference only to the 1000 ppm sample.

Also regarding the cell wall thickness of tracheae there is a great difference to the control (Fig. 3) but the differences are negligible among the treated samples.

The multiplication of the bundles is not significant and resulted from the appearance of new ones from procambium strands.

In the vascular bundles no branching is noted.

Second sample of 100 ppm MCPA treatment (June 21st)

The shape of the petiole resembles to the control, slightly undulated on both sides.

The lamellar collenchyma layer (Table 1) contains on the convex side considerably more angular collenchyma elements than in the first sample.

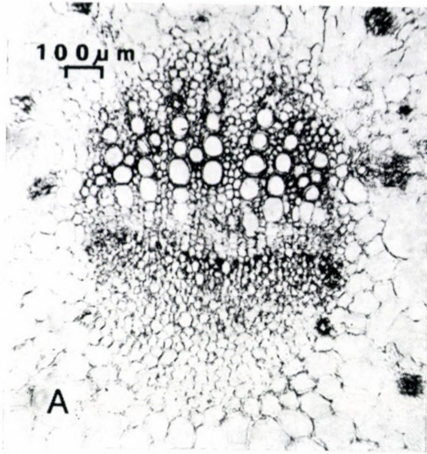
The multicellular hairs are placed singly, well distanced from each other on the concave side; they are also singly distributed on the donvex side but more densely placed. On the convex side some of the hairs has a thickness of more than one cell rows. From among the vascular bundles three are large (Fig. 6E); the number of bundles can not be established as these form a continuous layer in the petiole (Fig. 4E).

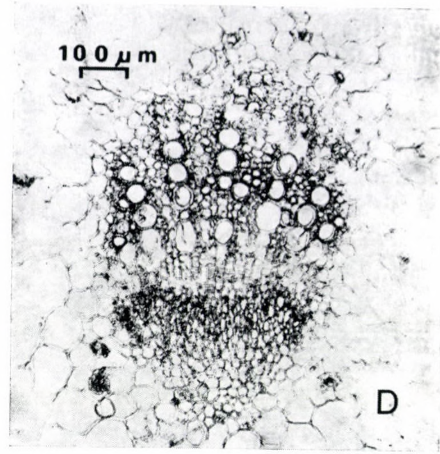
The number of cell layers in the bundle sheath and cambium is shown in Table 2.

The distribution of tracheae and tracheids is radial, with locally occurring irregularities.

→

Fig. 6. Histological changes in the vascular bundles of control and MCPA-treated leaf petioles A–C = control; D–F = 100 ppm; A, D = 1st sample (June 14th); B, E = 2nd sample (June 21st); C, F = 3rd sample (July 11st). The scales of figures A, B, E and F are the same


100 µm
A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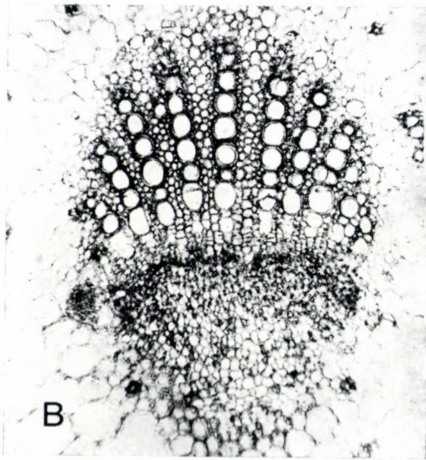
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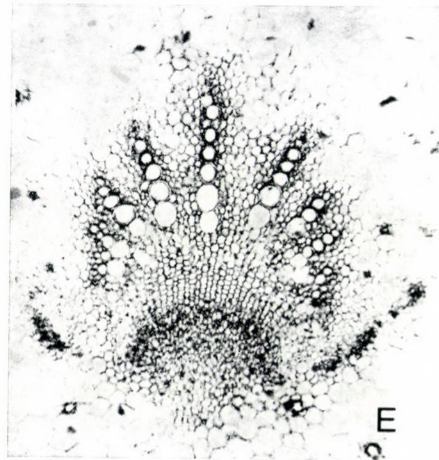
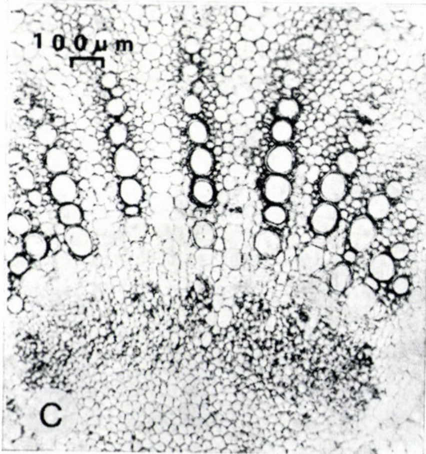
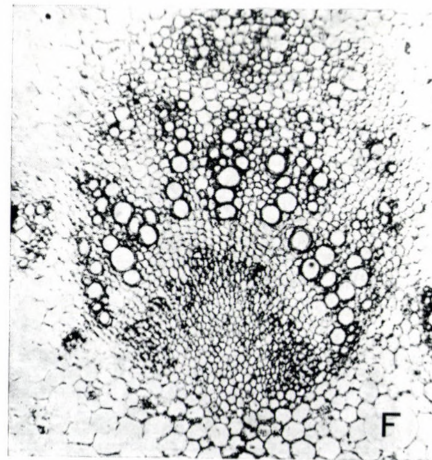

E


100 µm
C


F

The walls of the bundle sheath cells (Fig. 1) became considerably thinner if compared to the control but also compared to the values of the first sample of 100 ppm treatment. The cell walls are the thinnest in this sample regarding all treatments and samples. Also the most conspicuous reduction in cell wall diameter was noticed in the phloem cells (Fig. 2) in this sample, differing from all other treatments.

Regarding the thickness of tracheal walls (Fig. 3) also here the greatest difference from the control is observed while it is minimal compared to the other treatments.

The multiplication of the vascular bundles takes place partly by the division of already existing ones and by the formation of new bundles from procambium strands.

No branching of vascular bundles appears.

Third sample of 100 ppm MCPA treatment (July 11st)

The shape of the petiole resembles to that of the control, however, the concave side is highly, while the convex side slightly undulated.

The lamellar collenchyma layer (Table 1) contains many angular collenchyma elements, both on the concave and convex side.

The multicellular hairs are placed on both sides singly, well distanced from each other.

The number of vascular bundles (of which three are large, as shown in Fig. 6F) can not be established as they form in some places a continuous layer (Fig. 4F).

The number of cell rows in the bundle sheath and cambium is shown in Table 2.

The distribution of tracheids and tracheae is radial like in the treated samples dealt with above, with irregularly placed tracheae occurring in some places.

The walls of the bundle sheath cells (Fig. 1) are very thin compared to the control and to the 1000 ppm MCPA treatment. Similarly, the cells of the phloem considerably differ from the control (Fig. 2) and from the sample of 1000 ppm MCPA treatment.

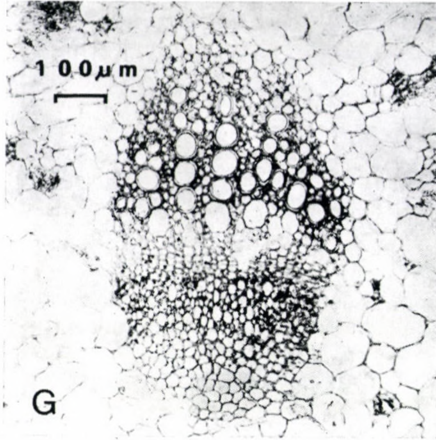
The wall of the tracheae is considerably thinner than the ones in the control (Fig. 3) but show no significant differences from the third sample of the 1000 ppm MCPA treatment.

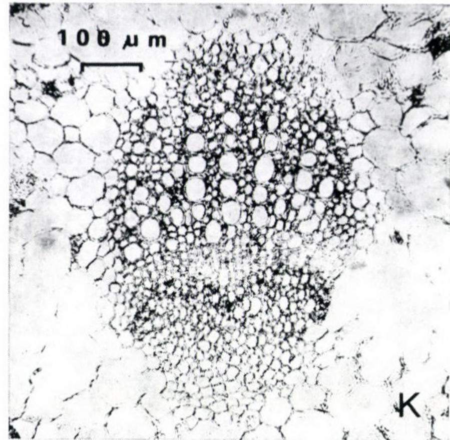
The multiplication of vascular bundles takes place mainly by the formation of new ones from procambium strands but also many already existing ones divide.

No branching of vascular bundles appears.

→

Fig. 7. Histological changes in the vascular bundles of leaf of petioles. MCPA-treated plants G–I = 1000 ppm; K–L 5000 ppm; G, K = 1st sample (June 14th); H, L = 2nd sample (June 21st); I = 3rd sample (July 11th)


100 µm
G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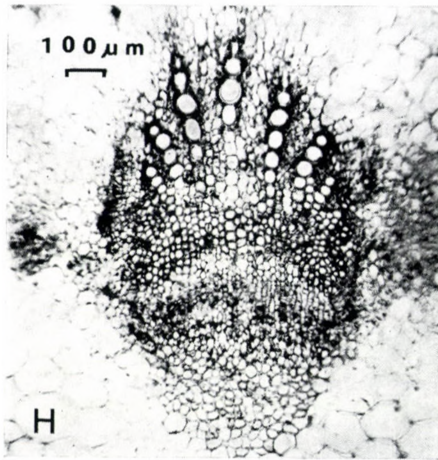
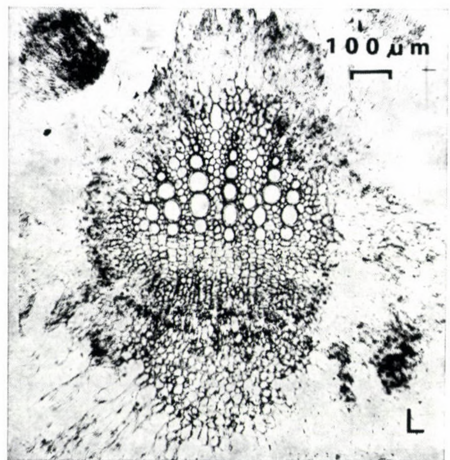
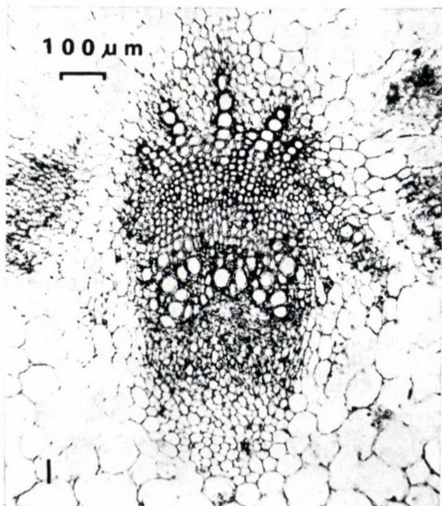
K


100 µm
H


100 µm
L


100 µm
I

First sample of 1000 ppm MCPA treatment (June 14th)

The shape of the petiole resembles to that of the control, slightly undulate on the concave side.

In the lamellar collenchyma layer (Table 1) angular collenchyma elements can be found only occasionally and on the convex side.

The multicellular hairs are placed singly and well distanced both on the concave and convex side; on the latter some of the hairs is built up from more than one cell layer.

There are 24–28 vascular bundles, three of which are large (Fig. 7G), the others are considerably smaller. Their distribution in the leaf petiole resembles to that in the control (Fig. 5G).

The number of cell layers in the bundle sheath and cambium is contained in Table 2.

The tracheae and tracheids are radially distributed.

The cell walls of the vascular bundles (Fig. 1) are thinner than the ones of the control and of the 5000 ppm MCPA treatment, but slightly thicker than the cells of the 100 ppm treatment. The wall of phloem cells (Fig. 2) is thin as compared both to the control and the other treatments.

The walls of tracheae are considerably thinner than the ones of the control (Fig. 3) but do not significantly differ from the other treatments.

The multiplication of vascular bundles takes place mostly from procambium strands, but occasionally also by the division of vascular bundles.

No branching of the vascular bundles is observed.

Second sample of 1000 ppm MCPA treatment (June 21st)

The shape of the petiole resembles to the control, the concave side is slightly undulated.

The lamellar collenchyma layer (Table 1) contains on both sides only few angular collenchyma elements.

The multicellular hairs are placed on both sides singly, apart from each other; on the convex side some hairs consist of more than one cell layers.

The number of vascular bundles is 17–21, three of which are large (Fig. 7H), the others are small; their placement resembles to the control (Fig. 5H). Beside the median bundle a group of other boundles is noted.

The number of cell layers in the vascular bundle and cambium is contained in Table 2.

The tracheae and tracheids are radially arranged.

The walls of vascular bundle cells (Fig. 1) shows an insignificant thickening compared to the control, but a considerable thickening compared to the 100 ppm treatment. The cell walls are, however, still thinner than in the 5000 ppm treatment. The walls of phloem cells (Fig. 2) are somewhat thinner than the ones of

the control and the 5000 ppm treatment, but still noticeably thicker than in the 100 ppm treatment.

In case of tracheae (Fig. 3) the cell wall is considerably thinner than in the control but still thicker than in the other treatments.

As regards the multiplication of vascular bundles, the division of existing ones is more common but also the formation of new ones from procambium strands can be noted.

It is characteristic for the large vascular bundles that between the cambial tissue and the radially distributed tracheae and tracheids a layer consisting of undifferentiated, thick-walled xylem elements is inserted.

No branching of vascular bundles is noted.

Third sample of 1000 ppm MCPA treatment (July 11th)

The petiole resembles to the control, both the convex and concave sides are slightly undulated.

The lamellar collenchyma layer (Table 1) contains only on the convex side sporadically some angular elements.

The multicellular hairs appear on both sides singly and interspaced. On the concave side some hairs consist of more than one row of cells.

The number of vascular bundles can not be established because they form a nearly continuous layer (Fig. 5I), but three larger bundles are clearly distinct from the others (Fig. 7I).

The number of cell layers in the vascular bundles and cambium is contained in Table 2.

Interspersed between the radially arranged tracheae and tracheids some irregularly placed elements may be found.

The cell walls of the bundle sheaths (Fig. 1) are thinner than the control but considerably thicker than in the sample of 100 ppm treatment.

The phloem cells (Fig. 2) are similar to the bundle sheath cells, as regards wall thickness.

The walls of tracheae (Fig. 3) are considerably thinner than the ones of the control while the difference from the 100 ppm sample is insignificant.

The multiplication of vascular bundles takes place mostly by the formation of new ones from procambium strands, the division of existing bundles is less frequent.

In the larger vascular bundles beneath the cambium layer irregularly placed tracheae and tracheids form a nearly continuous layer; beneath this layer a considerable amount of undifferentiated xylem elements is found, consisting of thick-walled cells. Only beneath this layer are placed the radially arranged tracheae and tracheids.

No branching of the vascular bundles appears.

First sample of 5000 ppm MCPA treatment (June 14th)

The leaf petiole resembles in shape to the control, the concave side is slightly undulated. The lamellar collenchyma contains on the convex side a large amount of angular elements (Table 1).

The multicellular hairs are placed singly and with great distances on both sides of the petiole; cells consisting of more than one cell row are found only on the convex side.

There are 22–26 vascular bundles, of which three are large (Fig. 7K), the others considerably smaller; their distribution within the petiole resembles to that of the control (Fig. 5K).

The number of cell layers in the sheath bundle and cambium is contained in Table 2.

The tracheae and tracheids are radially arranged, sometimes interspersed with the same elements, but irregularly placed.

The cell walls in the bundle sheaths (Fig. 1) are considerably thicker than in the control or in the other treatments; this applies to the formation of phloem cell walls, too (Fig. 2).

The walls of tracheae (Fig. 3) are much thinner than those of the control, but the difference to the other samples is negligible.

The multiplication of the bundles takes place prominently from procambium strands, the formation of new ones by division of existing bundles is subordinate.

No branching of vascular bundles can be noted.

Second sample of 5000 ppm MCPA treatment (June 21st)

The shape of petiole resembles to the one of the control, the concave side is slightly undulated.

The lamellar collenchyma layer (Table 1) does not contain angular collenchyma elements either on the concave or convex side.

The multicellular hairs are placed on both sides singly, with great distances; on the convex side some hairs consist of more than one cell layers.

The number of vascular bundles can not be established as they form a nearly continuous layer (Fig. 5L); the three large bundles (Fig. 7L) are clearly distinct from the others.

The number of cell layers in the bundle sheath and cambium is contained in Table 2.

Tracheae and tracheids occur in some places interspersed between radially arranged tracheids and tracheae.

The walls of bundle sheaths (Fig. 1) are thicker than the ones of the control and other treatments. The same applies for the thickness of phloem cell walls (Fig. 2).

The walls of tracheae (Fig. 3) is very thin compared to the control, the measure of diameter decrease is nearly identical with the one observed in other treatments.

The multiplication of vascular bundles takes place primarily by formation of new ones from procambium strands.

The larger vascular bundles show a marked branching.

Discussion

Based on the experimental results it could be established that MCPA damaged the vascular bundles in all the three dosages applied. It considerably altered the thickness of cell walls both in the bundle sheath, the phloem and in the tracheae. The most conspicuous alterations as regards wall thickness could be observed in the bundle sheath and phloem cells in the 100 ppm MCPA treatment. As a result of 1000 ppm the walls of bundle sheath and phloem became somewhat thicker as compared to the data of the first sampling. In the 5000 ppm treatment a thickening of cell walls was noted in the bundle sheaths and phloem compared both to the control and the other treatments. The walls of tracheae suffered a considerable decrease in diameter in all treatments, compared to the control.

As a result of 1000 ppm treatment a large amount of undifferentiated xylem elements appeared within the vascular bundles.

As a final conclusion it can be established that the highest difference in the wall thickness of bundle sheath, phloem cells and tracheae appeared in the 100 ppm treatment as compared to the control. But as regards the whole structure of the leaf petiole and vascular bundle, the highest damage was done by the 1000 and 5000 ppm treatments which manifested itself in the alteration of distribution and in the multiplication of the bundles. In case of 5000 ppm the whole petiole became deformed and distorted.

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VOLUME 28 NOS 1–2

CONTENTS

Babos, K.–Bermudez, I. R.–Cumana, L. J. C.: Xylotomic examination of some Venezuelan Capparis species, II
Bizot, M.–Pócs, T · East African Bryophytes, V
Borhidi, A.: Studies in Rondeletieae (Rubiaceae) III. The genera Rogiera and Arachnothryx
Dave, Y. S.–Rao, K. S.: Cambial activity in Mangifera indica L.
Fernandez, M. Z.–Borhidi, A.: Rubiaceas cubanas II–III
Hably, L.: Egerian (Upper Oligocene) macroflora from Verőcemaros (Hungary)
Kiss, T.: Aspects and types of competition between lichen species in epiphytic communities
Maróti, M.–Rudolf, J.–Bognár, J.–Pozsár, B. I.: In vitro plantlets from potato shoot segments
Potoczki, Á.–Szőke, É.–Verzár-Petri, G.: Growth dynamics of the callus tissues of petal and ovary origin of the Datura innoxia Mill.
Reyes, D. M.: El género Diplasiolejeunea en Cuba
Suba, J.–Kiszely-Vámosi, A.–Légrády, Gy.–Orbán, S.: Examination of the photosynthetic fixation $^{14}CO_2$ on bryophyte and lichen species
Szilágyi, L.: Meiosis in anther-derived polyhaploid of Nicotiana interspecific hybrid
Szujkó-Lacza, J.: Developmental morphology of Armeniaca vulgaris Lam. (Rosaceae)
Vales, M.–Borhidi, A.–Del-Risco, E.: Anatomia de la madera de Myricaceae en Cuba; Consideraciones ecológicas
Vánky, K.–Gönczöl, J.–Tóth, S.: Review of the Ustilaginales of Hungary
Verzár-Petri, G.–Ladocsay, T.–Oroszlán, P.: Differentiation and production of cannabinoids in tissue cultures of Cannabis sativa

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