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DISEASES

Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 12 (1–2), pp. 1–2 (1977)

Interactions of Pesticides and Microorganisms

To commemorate the 25th anniversary of the Hungarian Microbiological Society, a Congress was held at the Hungarian Academy of Sciences in Budapest, 6–10 September, 1976.

One of the sessions was on "The Interaction of Pesticides and Microorganisms" joining to the European international meetings (Gent, 1970; Budapest, 1970; Wroclaw, 1974 etc.) aimed at discussing the approaches in these problems.

The Editorial Board of Acta Phytopathologica offered to devote a double issue of the 12th volume to the 16 papers written by 33 authors of eight countries.

Many important questions were discussed in these papers from ecological, soil and production biological, environmental and of course plant protectional points of view. The "total" number of microorganisms, bacteria, fungi, algae their species composition, their physiological groups, the survival of rhizobia in the soil, the legume–rhizobium symbiosis, the antibiotic activity of a *Bacillus*, the degradation of pesticides affected by pesticides, pesticide interactions and the combined application of pesticides and fertilizers are dealt with in the articles. The effect of microorganisms on pesticides should be stressed as their effect on phytoxicity of herbicides, detoxification and decomposition of pesticides and so on.

During the edition of papers for Acta Phytopathologica we received the sad news that one of the authors J. P. VOETS died on the 25th December, 1976 in Gent.

This being his last work we are deeply moved and we wish to pay tribute to him by remembering him here.

Professor J. P. VOETS was born on July 16, 1923. He graduated as agricultural engineer at the University of Gent in 1945. He obtained his Ph. D. degree in 1962 at the University of Lille, France. From 1945 to 1947 he was Director of the Central Laboratory of the Ministry of Economic Affairs at Brussels. He became attached in 1947 to the Department of General and Industrial Microbiology at the Faculty of Agriculture of the University of Gent. In 1961 he was nominated professor and in 1966 he became director of the Department of General and Industrial Microbiology. In 1971 he was elected as Chairman of the Interfaculty Center for Environmental Studies at the University of Gent.

Prof. VOETS was a member of several learned societies, author of two textbooks on microbiology in Dutch, author and co-author of numerous scientific publications in the field of food, industrial and environmental microbiology and special advisor to the Belgian Ministry of Environmental Policy and to the Commission on Food Additives of the European Common Market.

We remember him also as an excellent organizer of the "Action des pesticides et herbicides sur la microflore et la faunule du sol biodégradation tellurique de leurs molécules" colloque international, held in Gent, May 1970, and the editor of Proceedings of this Meeting (Meded. Fac. Landbouwwet, Gent 35.2).

We are convinced that this collection of papers will contribute to clarifying the rather complex problems of pesticide action.

M. KECSKÉS
President of the Agricultural and
Industrial Microbiological Section
of the Hungarian Microbiological
Society

Acta Phytopathologia Academiae Scientiarum Hungaricae, Vol. 12 (1—2), pp. 3—11 (1977)

Bioassay of Herbicides by Bioluminescence

By

Y. T. TCHAN and C. M. CHIOU*

Department of Microbiology, University of Sydney, Sydney, New South Wales, 2006, Australia

Bioluminescence of photobacteria is used to design herbicide bioassay techniques. Two microbial physiological activities were involved in establishing these methods.

(a) Direct toxic effect of acrolein to bacteria.

(b) Interference of oxygen production by photosynthesis inhibiting herbicides and the measurement of oxygen by bioluminescence.

The sensitivites of all these methods are high (ranging from 0.001 ppm 0.1 ppm) and are suitable for field applications.

It is widely recognized that the monitoring of herbicides in soil and in water is essential in avoiding pollution in natural environment. Furthermore a systematic application of herbicides under technological surveillance would not only prevent unwanted side effects but also provide economical basis for agricultural production (PILLAY and TCHAN, 1972; BOWMER *et al.*, 1974). Such monitoring would require rapid assay methods and recently a specific rapid bioassay method for photosynthesis inhibiting herbicides was reported (TCHAN *et al.*, 1975). However that method has certain limitations in field application. This paper reports new improved methods applicable not only to photosynthesis inhibiting herbicides, but also to other herbicides which do not directly affect photosynthesis.

Materials and Methods

Organisms and culture methods

Luminous bacteria – *Photobacterium phosphoreum*, strain T_3 isolated from an Australian fish *Tripterophycis intermedius*, was used in these experiments (TCHAN *et al.*, 1975). *Dunaliella tertiolecta*, a marine flagellate alga was kindly supplied by Professor A. D. BROWN of Wollongong University.

(a) The SWYP medium for luminous bacteria T_3 and the standardization of the culture technique was as described earlier (TCHAN *et al.*, 1975).

* Holder of Loxton Scholarship of the Faculty of Agriculture.

(b) *D. tertiolecta* was cultivated in 100 ml Johnson's medium (JOHNSON *et al.*, 1968) in 500 ml conical flask in a light room illuminated by 40W "Gro-Lux" fluorescent tubes.

Assessment of cell concentration

Concentration of bioassay organisms was routinely determined by optical density (O.D.) using a cuvette with 1 cm light path. (700 n.m. for luminous bacteria and 662 n.m. for algal cells).

Determination of light emission

The light emission was determined by a photomultiplier "RCA" 931 housed in a compartment (PM) with its aperture facing a mirror mounted in a rotor. The sample was placed in the sample compartment (S) with its aperture positioned at 90° to the photomultiplier's. The light emission was directed to the photomultiplier by the rotating mirror and measured on a "Riken Denshi" recorder. The intensity was finally expressed either as a voltage or as a percentage of the control (see Fig. 3 Bioluminescence measurement position).

Maintenance of luminous bacteria for constant light emission

When the culture reached its maximum light emission, it could be stored at 5°C for at least 2 days. When required, the stock culture was diluted with an equal volume of fresh medium and incubated at 20°C with agitation for 30 min (TCHAN *et al.*, 1975).

Herbicides

Acrolein (2-propenal)
Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea)
Neburon (1-n-butyl-3-(3,4-dichlorophenyl)-1-methylurea)
Atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine)
Simazine (2-chloro-4,6-bis(ethylamino)-S-triazine)
Bromacil (5-bromio-3-*sec*-butyl-6-methyluracil)

Results

Direct and indirect methods were used to bioassay different types of herbicides.

(A) *Bioassay of acrolein*

This bioassay is based on the toxicity of acrolein to luminous bacteria. Several parameters were studied before the final procedure was adapted.

Effect of the duration of contact between acrolein and bacteria T_3 on light emission:

(*i*) One ml of acrolein solution in saline was mixed with 4 ml of T_3 culture (O.D. = 0.30) to give the following final concentrations of acrolein, 0, 1 ppm, 2 ppm and 3 ppm. After a period contact time the bottles were shaken for 10 seconds and light measurements were carried out immediately by a photomultiplier and was found to decrease continuously for 35 minutes and then stabilized for up to 60 minutes.

Concentration of T_3 cells and their sensitivity to acrolein

(*i*) The sensitivity of T_3 to acrolein was influenced by the ratio between the number of bacterial cells and the concentration of the herbicide. Acrolein in saline and T_3 (O.D. = 0.28) was mixed in the following ratios:

T_3/acrolein: 4/1, 3/2, 2/3, 1/4.

The final concentration of acrolein was maintained at 1 ppm and the volume at 5 ml. The light emission was determined at regular time intervals as shown in Fig. 1.

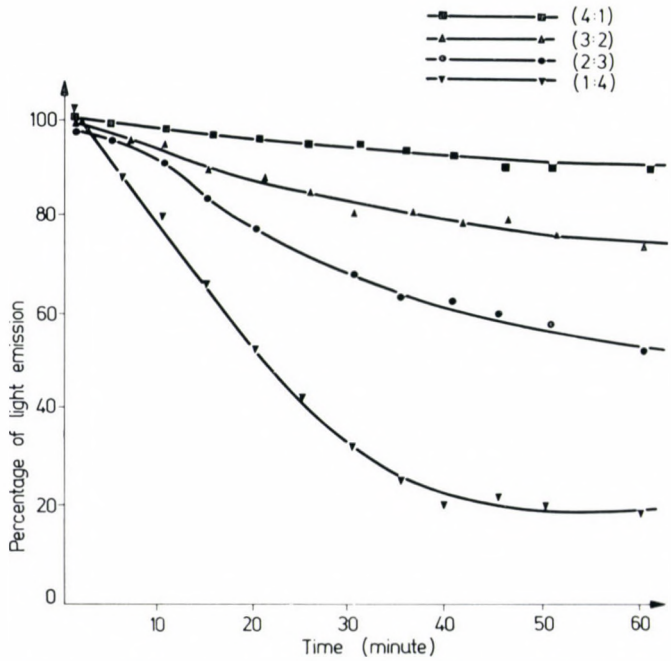

Fig. 1. Influence of contact time of acrolein on light emission

Using the ratio 1/4, the sensitivity of T_3 to acrolein was established (Fig. 2).

The method was considered as suitable for the estimation of acrolein in the range of 0.005 – 1 ppm.

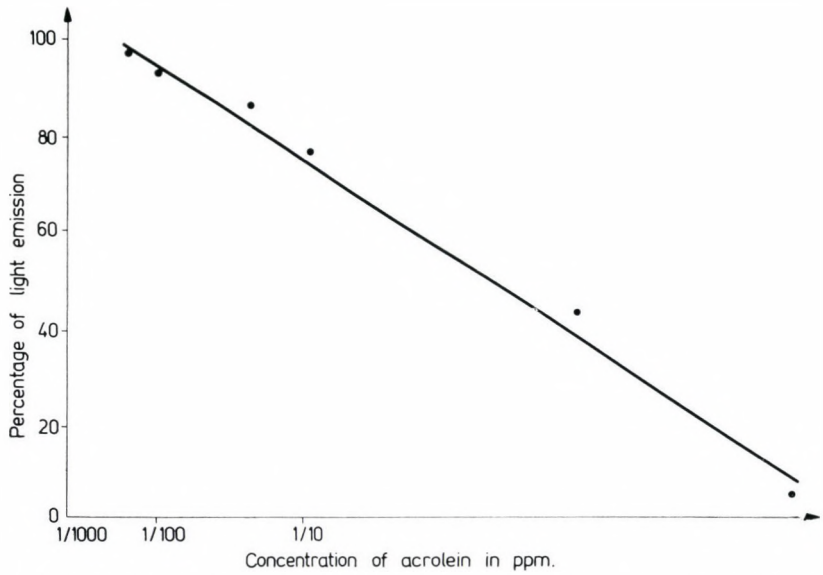

Fig. 2. Relationship between the concentration of acrolein and light emission by T_3

Adopted procedure – 1 ml of T_3 is added to 4 ml of acrolein solution of appropriate concentration. After mixing, the suspension is kept for 35 minutes then shaken for 10 seconds with a "Vortex" mixer before the light emission is measured with a photomultiplier.

(B) *Bioassay of photosynthesis inhibiting herbicides* (diuron, neburon, atrazine, simazine and bromacil)*

The herbicides interfere with oxygen evolution which can be quantitated by measuring the oxygen dependent emission of luminescence by bacteria.

The experiments described below are valid for all photosynthesis inhibiting herbicides listed above. Diuron and bromacil were chosen to illustrate the techniques.

Luminous bacteria were mixed with the algae in the presence of herbicides under test. A McCartney bottle of 7 ml capacity was filled with the mixture and stopped with a rubber stopper to exclude all air from the bottle. This was achieved

* Footnote: T_3 was not sensitive to herbicides under investigation and had no bearing at the concentrations of herbicides (up to 10 ppm) used in our experiments.

by inserting a hypodermic needle through the rubber stopper before pushing it into the neck of the fully filled bottle. The excess liquid escaped through the needle with a complete exclusion of all air. The needle was then withdrawn. Such a mixture will exhaust all the dissolved oxygen within 30 minutes as indicated by the cessation of its light emission. The bottle was placed in the sample compartment (S) ready to be assayed. The assay mixture was exposed to light from a microscope lamp (100 W) directed onto it by the mirror, for various periods of time

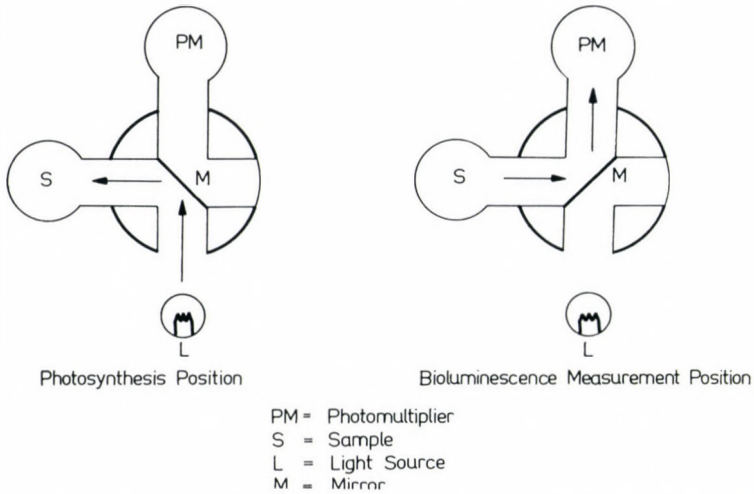

Fig. 3. Position of mirror for photosynthesis and bioluminescence measurement

to allow photosynthesis to proceed (Fig. 3, oxygen production position). After the required period of illumination, the mirror was rotated to direct the emitted light to the photomultiplier (Fig. 3, light measurement position). The intensity of bioluminescence was measured on the recorder.

Bioassay of diuron

Several parameters were studied for the standardization of the procedure.

(*i*) Duration of illumination of the algae and light emission by T_3.

A mixed culture in the proportion 1 : 1 (T_3: *D. tertiolecta (Dt)*; $OD_{T3} = 0.28$; $OD_{Dt} = 0.30$) was used (Table 1 summarizes the results).

Two minutes of illumination gave the maximum light emission by the photobacterium. However 30 sec was selected as operational exposure time for the following reasons:

1. it gave reproducible results;
2. it allowed repetitive measurement without prolonging the time for oxygen consumption.

Table 1

Influence of duration of illumination on light emission and oxygen consumption

Duration of illumination (second)	12	30	60	120	180	300
Light emission (volt)	1.61	1.68	1.86	3.37	3.22	2.86
Time required to consume the produced oxygen (second)	20	35	80	90	100	120

The light emission became constant after several repetitive operations using a 30 sec exposure, the estimation could be completed within 20 min (compared to over one hour if 2 min exposure is used).

(*ii*) Optimum proportion of T_3: *D. tertiolecta.*

It was important to determine:

1. the minimum time for the consumption of the initially dissolved oxygen in the mixture;

2. the intensity of light emission by T_3 using oxygen produced through photosynthesis when different proportions of T_3 and algae were used.

Table 2

Influence of T_3/*D. tertiolecta* ratio on consumption of initial oxygen and light emission
($OD_{T_3} = 0.28$)
($OD_{Dt} = 0.30$)

T_3/*D. tertiolecta*	1/9	3/7	5/5	7/3	9/1
Time required for consumption of initial oxygen (minute)	90	20	15	7	4
Light emission after 30 sec illumination (volt)	0.07	0.3	0.46	0.36	0.27

The proportion of 1 : 1 was the best compromise since it took only 15 min to consume the initial oxygen but gave maximum light emission after 30 sec exposure to light.

(*iii*) Proportion of test organisms to the herbicide solution.

The measurement of the herbicide activity was influenced by the quantity of microorganisms as shown in Table 3.

The best result was given by one part of herbicide solution to nine parts of test organism suspension.

Adopted procedure – Nine ml of an equal volume mixture of T_3 (OD_{T_3} = = 0.28) and *D. tertiolecta* ($OD_{Dt} = 0.30$) is mixed with 1 ml of diuron in saline solution. The mixture is transferred to a McCartney bottle of 7 ml capacity and

Table 3

Influence of the quantity of microbial population on light emission in the presence of diuron (0.05 ppm)

Ratio: (Volume) Herbicides / Microorganisms	1/9	5/5	7/5	9/1
Light emission in volts	0.15	0.09	0.02	0.006

sealed by a rubber stopper. The bottle is kept in the dark until light emission ceases. After exposure to the light for 30 seconds the light emission is measured. The operation is repeated until a constant reading is obtained. The light emission is expressed as a percentage of the control. The sensitivity of the system to diuron is shown in Fig. 4.

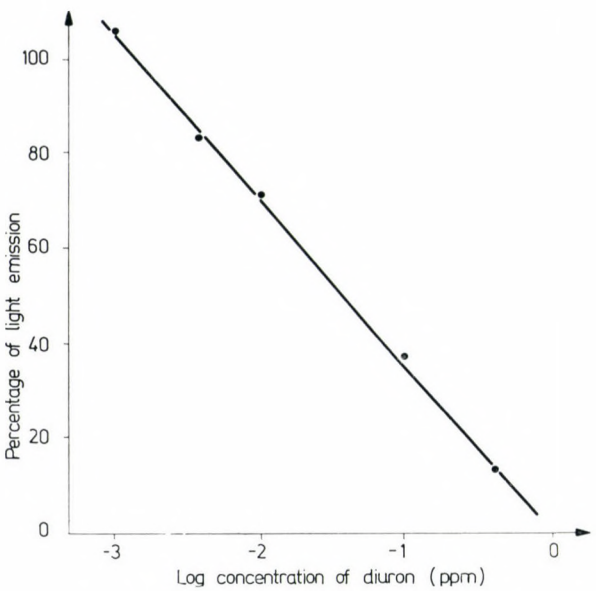

Fig. 4. Sensitivity of T_3/Dt to diuron

For bromacil the sensitivity established under similar conditions was found to be inadequate. The age of culture was investigated in order to ascertain its influence on sensitivity of algae to bromacil. It was found that there was a gradual increase in inhibition of oxygen evolution with age of the culture. A 10-day old culture gave adequate response to bromacil for bioassay and the order of 0.005 ppm can then be measured (see Fig. 5).

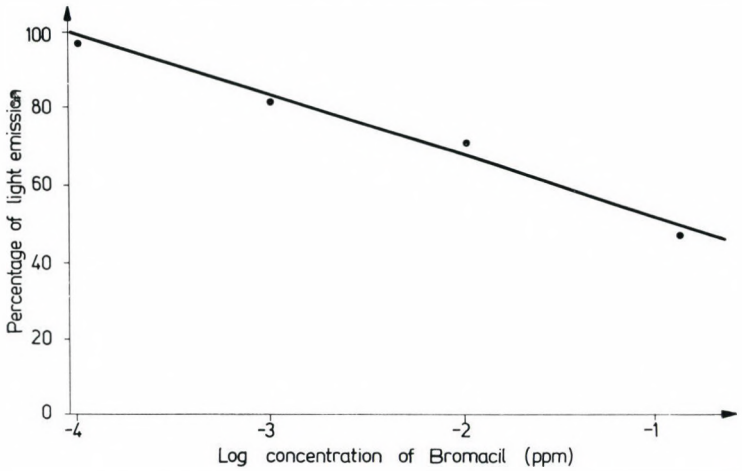

Fig. 5. Sensitivity of T_3/Dt to Bromacil

Discussion

One of us had discussed earlier, the bioassay methods of herbicides and their application to agriculture (TCHAN, 1971). The advantage of using bioluminescenc for the bioassay of photosynthesis inhibiting herbicides was recently introduced (TCHAN *et al.*, 1975). The shortcoming of that technique resided in the use of a vacuum pump which was cumbersome but necessary because of the incompatibility of the test alga (*Chlorella* isolated from soil) with the SWYP medium (TCHAN *et al.*, 1975). This imposed the physical separation of the two components (the alga and T_3 in the SWYP medium). This difficulty has been overcome in the present method by using *D. tertiolecta* which is highly sensitive to the herbicides under investigation and tolerates SWYP medium, thus allowing the presence of two biotic components in the same vessel. The oxygen in the system is exhausted by their respiration when kept in the dark. This has considerably simplified the manipulation and avoided the danger of damage to algal cells by high vacuum (TCHAN *et al.*, 1975).

The sensitivity of the new techniques is high for most photosynthesis inhibiting herbicides (see Table 4).

Compared to the earlier method (TCHAN *et al.*, 1975) its sensitivity was equal or better, but the operation is much simpler. It thus allows the handling of large numbers of samples and replications. The elimination of the vacuum pump should facilitate greatly the operational procedures and has shown to be advantageous in field trials.

The bioassay of acrolein using luminous bacteria is new. Compared to another bioassay method using *Euglena* (BOWMER *et al.*, 1974), the present method

Table 4

Sensitivity of T_3/*D. tertiolecta* system of different photosynthesis inhibiting herbicides

Herbicides	Sensitivity range (in ppm)
Diuron	0.001 – 1
Neburon	0.05 – 1
Atrazine	0.05 – 2
Simazine	0.1 – 4
Bromacil	0.005 – 1

is better and faster. The sensitivity has been increased by more than 10-fold (0.1 ppm with *Euglena* to 0.005 ppm with T_3). Since the measurement is made in the presence of air, a large number of samples can be prepared for estimation. With some minor modifications, the technique can be adopted for use with many other sensitive light detecting instruments such as liquid scintillation counter or spectrophotometer. Furthermore the T_3 is not sensitive to the degradation products of acrolein and thus can also be used in conjunction with colorimetric methods for the study of degradation of acrolein (BOWMER *et al.*, 1974).

The specificity of the method to acrolein can be further confirmed by determining the light emission in aerated and non-aerated samples. Aeration removes the volatile acrolein and the increased light emission is thus due to the removal of this toxic substance.

Acknowledgement

The authors wish to thank Mrs. Ilze Dalins and Dr. K. Y. Cho for their valuable criticism and constructive discussion in the preparation of the manuscript. Our acknowledgement is extended to the Rural Credit Development Fund of Australia, the Water Research Foundation of New South Wales and the University of Sydney Research Grant for their financial assistance in this project. The financial support given by the Loxton Scholarship to Mr. C. M. Chiou is likewise acknowledged.

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The Effect of Herbicides on the Antibiotic Activity of *Bacillus subtilis* 160*

By

Z. KRĘŻEL and D. LESZCZYŃSKA

Department of Microbiology, Agricultural Academy of Wrocław, Poland

The effect of 1 ppm of chloropham and 10 ppm of Venzar on the antibiotic activity of *Bacillus subtilis* 160 was examined. As microbiological tests the phytopathogenic strains of *Pseudomonas lachrymans* and *Erwinia carotovora* were used.

The stimulation of antibiotic activity of *Bacillus subtilis* 160 by the CIPC was found with the methods of disc, cup plate and turbidimetrically. Venzar, however, failed to exert such an effect on the strains under examination. The results were confirmed by *in vivo* plant tests in micropot experiments.

In our experiments on the effect of herbicides some changes in bacterial morphology and physiology could be seen (BALICKA and KRĘŻEL, 1963, 1969, 1970), (KRĘŻEL and LESZCZYŃSKA, 1970, 1972). Particular attention was paid to urea and carbamate herbicides; linuron, monolinuron and chloropham in the doses of 10, 50, 100 ppm had an inhibitory action on the antibiotic activity of the tested bacterial strains. The effect of lower doses of these herbicides was different in that the stimulation of the antibiotic production was more frequent. That was the reason for the studies on the influence of the herbicides on relationships between saprophytic and phytopathogenic bacteria.

Material and Methods

The effect of Venzar (3-Cyclohexyl-5,6-trimethylenuracil) and chloropham (Isopropyl-N-(3-chlorphenyl)-carbamate) on the antibiotic activity of the strain *Bacillus subtilis* 160 was examined. The strain had been isolated from the phyllosphere of cucumber and its resistance to herbicides was measured. The incubation time of bacterial culture was 9 days at 28°C, as that time proved to be optimal for antibiotic production. The cultural media was cabbage extract (KRASILNIKOW, 1966) and potato extract containing CIPC (chloropham) and Venzar in the doses of 1 and 10 ppm, respectively.

The antibiotic activity was determined by cup plate and disc methods and turbidimetrically (OBOJSKA and OSTROWSKA, 1963). As test microorganisms *Erwinia*

* Financed by the Grant No. FG-Po-249 USA Department of Agriculture.

carotovora 1008 (from the Collection of Microorganisms of Brno) and *Pseudomonas lachrymans* (own collection) were used; they were incubated on the potato and cabbage extracts without herbicides.

The size of inhibition areas of test bacteria produced by *Bacillus subtilis* cultures pointed to the effect of herbicides on the antibiotic activity of bacteria.

Another test was based on the growth intensity of test bacteria in liquid medium infected with various dilutions of filtrate of *Bacillus subtilis* cultures (1 : 1, 1 : 2, 1 : 4, 1 : 8, 1 : 16), the growth was measured turbidimetrically.

Some plants were also used to find out changes in the activity of *Bacillus subtilis* due to the action of herbicides, there were potato chips and cucumber plants. The experiments were performed as follow.

a) Potato chips were saturated with the culture of *Bacillus subtilis* or its filtrates during 24 hours and then infected with *Erwinia carotovora*. The presence or absence of necrosis pointed to the antibiotic activity of *Bacillus subtilis*.

b) Pot experiments with growing cucumber plants used as test were carried out in Knopp media and in sterile sand (BALICKA and KRĘŻEL, 1970). Synthetic medium or sand was saturated with *Bacillus subtilis* culture and then plants were sprayed with the suspension of *Pseudomonas lachrymans* or *Erwinia carotovora* cultures, and placed in humid chamber. Some modification of that method was applied, namely the plants sprayed threefold with filtrate of *Bacillus subtilis* culture at 24-hour intervals and afterwards sprayed with *Pseudomonas lachrymans* or *Erwinia carotovora* suspension; incubation in humid chamber. The reaction of plants to pathogenic bacteria was expressed by a number of necrotic spots on the leaves, after 7 days of incubation.

The penetration of antibiotic substances into the plant tissues was checked by disc method the fragments of stalk and leaves were placed on the solid medium inoculated with *Pseudomonas lachrymans* or *Erwinia carotovora*. The size of inhibited growth areas indicated the presence of antibiotic substances in the plants.

Results and Discussion

Strains *Erwinia carotovora* and *Pseudomonas lachrymans* were resistant to the herbicides in the doses used. *Bacillus subtilis* 160 exhibited some susceptibility on herbicides; its growth was decreased to 14% in presence of 1 ppm of CIPC in liquid medium and to 3% of 10 ppm of Venzar.

The antibiotic activity of *Bacillus subtilis* against *Erwinia carotovora* has been found to be similar to that of *Pseudomonas lachrymans*. The size of inhibition areas of their growth on solid medium was 16–17 mm. The spectrum of antibiotic activity measured turbidimetrically is given in Table 1. The antibiotic substances were of polypeptide nature.

Saturation of potato chips with filtrate of *Bacillus subtilis* followed by inoculation with *Erwinia carotovora* entirely inhibited its growth (Fig. 1).

Table 1

Spectrum of antibiotic activity of *Bacillus subtilis* 160

Dilution	Growth inhibition in per cent	
	Erwinia carotovora	*Pseudomonas lachrymans*
Undiluted 2.5 mg of dry weight of cells	100	100
1 : 1	60	60
1 :	30	30
1 : 4	20	30
1 : 8	20	17
1 : 16	15	20
1 : 32	12	15
1 : 64	10	8

Bacillus subtilis was found to be subject to changes under the effect of 1 ppm of CIPC. After 9 days of incubation the filtrate of the strain proved to be more active against *Pseudomonas lachrymans* and *Erwinia carotovora*. This could be confirmed by the methods of disc, cup plate and turbidimetrically, as well a by means of plant test (*in vitro*) in micropot experiments. *Bacillus subtilis* incubated in medium with 1 ppm of CIPC was capable of reducing the infection of plants with pathogenic bacteria to the degree higher than that observed in control culture; in such cases the percentage of healthy plants was increased from 7 to 12

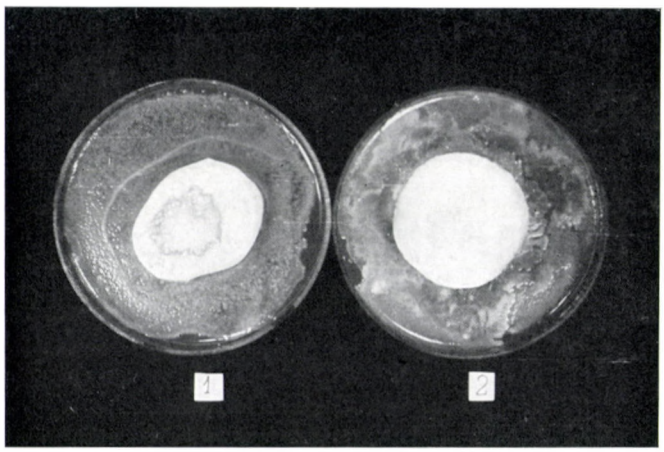

Fig. 1. Effect of antibiotic substances produced by *Bacillus subtilis* 160 on *Erwinia carotovora* growth. 1 — Growth of *Erwinia carotovora* on the potato chips; 2 — growth of *Erwinia carotovora* on the potato chips saturated with filtrate of *Bacillus subtilis* 160 culture

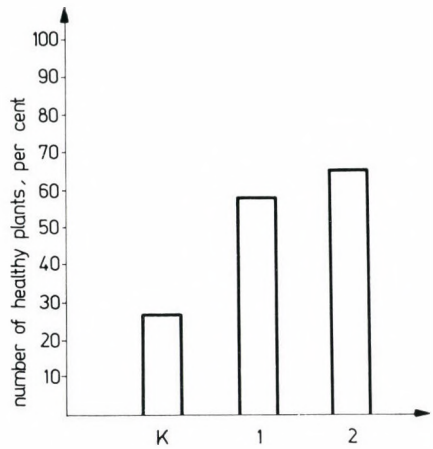

Fig. 2. Infection of cucumber leaves by *Erwinia carotovora*. K — infected by *Erwinia carotovora*; 1 — sprayed with *Bacillus subtilis* culture and then infected by *Erwinia carotovora*; 2 — sprayed with *Bacillus subtilis* culture preincubated in presence of 1 ppm of CIPC

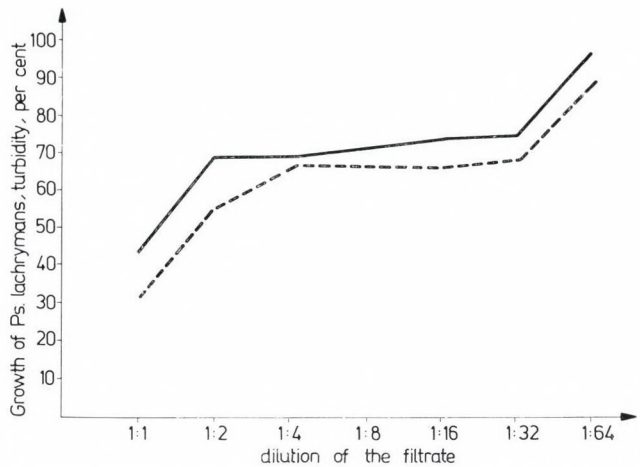

Fig. 3. Effect of Venzar on the antibiotic activity of *Bacillus subtilis* 160. 1 — Growth of *Pseudomonas lachrymans* in the medium with *Bacillus subtilis* filtrate; 2 — growth of *Pseudomonas lachrymans* in the medium with filtrate of *Bacillus subtilis* preincubated in presence of 10 ppm of Venzar

(Fig. 2). Venzar in the dose of 10 ppm did not affect the antibiotic activity of *Bacillus subtilis* 160 (Fig. 3).

The stimulating effect of some herbicides (CIPC, linuron, monolinuron) in the doses of 5 ppm on the antibiotic activity of *Streptomyces griseus* and *Streptomyces antibioticus* was already dealt with in our previous papers (KRĘŻEL

and LESZCZYŃSKA, 1970; KRĘŻEL and KOSINKIEWICZ, 1972). Both strains produce antibiotics of polypeptide nature. The present paper confirms the possibility of a stimulating effect of low doses of CIPC (1 ppm) on the production polypeptide antibiotics by *Bacillus subtilis*.

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Effect of Long-term Systemic Herbicide Application on *Rhizobium* Survival in Soil

By

HANA MAREĆKOVÁ and MARTA HUSÁROVÁ[1]

Research Institute of Crop Production, Prague—Ruzyně, ČSSR, [1]Research Institute of Maize, Trnava, ČSSR

The influence of long-term treatment of soil by herbicides on survival of natural population of nodule bacteria *Rhizobium meliloti* and *Rhizobium trifolii* has been estimated in comparison to untreated plots. The estimates of viable *Rhizobia* in soil were carried out by the method of Brockwell or indirect "Plant Infection" count.

The steadily increasing use of protective chemical preparations, especially of herbicides makes it necessary to investigate their possible unfavourable effect on soil microflora.

In our previous studies (VINTIKOVÁ *et al.*, 1965) we investigated the sensitivity of nodule bacteria from our culture collection of the species *R. leguminosarum*, *R. phaseoli*, *R. japonicum*, *R. lupini*, *R. trifolii* and *R. meliloti*, which comprised practically all species occurring in our country, to several herbicides directly on solid medium by the agar diffusion method.

A lot of investigations have been done since in this field. A very detailed study of the problem of *Rhizobia* sensitivity and resistance to pesticides has been carried out by BORBÉLY and KECSKÉS (1972), KECSKÉS and VINCENT (1973), KECSKÉS (1972) with a survey of the effect of about one hundred herbicides on the main species of the genus *Rhizobium* under laboratory conditions.

The growth of *R. trifolii* in the medium containing atrazine and *linuron* has has been followed for some weeks by GROSSBARD (1973), a stimulation by the rate of 25 ppm has been found there. The author supposes the survival of *Rhizobium* in soils free from the respective host plant for a short period under exposure of low doses of herbicides applied to non leguminous plants.

Similar conclusions of the possible survival of *Rhizobia* were reached by KECSKÉS and co-workers.

Methods and Discussion

We found the possibility of testing the survival of *R. trifolii* and *R. meliloti* population under natural field conditions. For this reason we used the soil sample from the plots of the field trials with maize monoculture of the Research Institute of Maize in Trnava. This trial was established in the year 1962 on degraded cherno-

zem, the herbicides have been applied since 1963. The annual herbicide rate was 4 kg of Zeazin, in 1964 7 kg of Ramrod was added. The main subject of investigation carried out by HUSÁROVÁ (1972) was the accumulation of mineral nitrogen after 6 to 8 years application.

For our investigation of the *Rhizobia* survival we received the soil samples in 1974, that was 11 years of continual herbicide application. The assessment of viable *Rhizobium* cell counts in natural soil samples brings some difficulties, as there is no selective medium for the direct agar plate counts. The only possible method is the indirect "Plant infection" counts used by several authors and summarized in VINCENT's Manual (1970). This method depends on the ability of a specific *Rhizobium* to produce nodules on the host legume and assumes that a single Rhizobium cell added to the test plant leads to a sufficient population in the root surroundings to cause nodulation. The numbers of tubes with nodulated plants can then be used to determine the likely number of *Rhizoma* in suspension under test.

The procedure of BROCKWELL (1963) of the estimates of "Most Probable Number" is based on fivefold dilution steps.

After evaluation of the experiment according to the nodulated plant number and according to the soil dilution we received the following results. In the untreated control soil sampled at the beginning of spring we found 151,000 cells of *R. trifolii* per 1 g and 26,000 cells in the herbicide treated samples, that is a slight decrease in the presence of herbicide. In *R. meliloti* experiments an increase of cells in herbicide treated soil samples was recorded in comparison to untreated ones, 2180 cells/g in control soil to 71,000 cells/g in herbicide treated soil. In this case rather a low number of *R. meliloti* cells was stimulated.

Table 1

Natural population of *R. trifolii* and *R. meliloti* in herbicide treated and control soil samples

		Cell number/g	
		Control soil	Herbicide treated soil
I	*R. trifolii*	∅ 151,000 (58,000–1,132,000)	∅ 26,900 (10,346–69 940)
	R. meliloti	∅ 2180 (838–5668)	∅ 71,000 (27,307–184,600)
II	*R. trifolii*	∅ 410,000 (15,768–1,066,000)	∅ 135,000 (51,923–351,000)
	R. meliloti	∅ 145,000 (55,769–377 000)	∅ 25,200,000 (9,692,300–63,520,000)

I spring
II summer

Similar results as to the slight depression of *R. trifolii* and rather a pronounced stimulation of *R. meliloti* by triazine preparation were received from soil sampled at the beginning of summer, only the actual rhizobia cell numbers were higher.

Conclusions

From this experiment we can conclude that the survival of *R. trifolii* and *R. meliloti* in soil free from the specific host plant was fairly good.

The findings that *R. trifolii* was inhibited and *R. meliloti* stimulated by triazine herbicides does not exclude the results of our previous laboratory experiments with several collection strains that the susceptibility or resistance was more a character of the individual strain than of the whole species. Organisms that are stimulated by the respective herbicide would after a long period of its application prevail over the natural population in soil.

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Effect of Some Pesticides on the Nodulation and Nitrogen Fixation of Infected Broad Bean Plants with Root Rot and Wilt Diseases

By

S. H. SALEM,* A. S. HAMED,** L. ZOHDY** and A. F. SAHAB**

* Faculty of Agriculture, Zagazig University, Zagazig, Egypt
** National Research Center, Dokki, Cairo, Egypt

The effect of some pesticides on the nodulation and nitrogen fixation of broad bean plants *Vicia faba* infected with root rot and wilt diseases was studied in pot experiments. The study revealed the following conclusions.

Inoculation of the soil with either *Fusarium oxysporum* or *Rhizoctonia solani* inhibited the nodulation and symbiotic N-fixation processes of broad bean plants.

Application of different insecticides namely Nuvacron and Dipterex affected also the symbiotic nitrogen relationship of the plants.

Application of fungicide "Dithan A — 40" as soil treatment to the infected soil to combat pathogenic fungi stimulated the symbiotic N-fixation of the plants.

Cultivation of broad bean plants at the application time of herbicide "Atrazine" inhibited the nodulation and the N-fixation processes of the plants. On the other hand cultivation of the plants 30 days after herbicide application did not affect the symbiotic N-fixation, due to its degradation by the soil microorganisms.

Broad bean plant is always subjected to infection with root rot and wilt diseases that alter the metabolic process causing high damage in the yield of the plants. The mentioned diseases may affect the symbiotic N-fixation process induced by *Rhizobium* and the leguminous plants. Some investigators have shown that the presence of pathogenic fungi in the rhizosphere of leguminous plants affect the nodulation process. LUGAUSKAS (1963) indicated that *Penicillium*, *Fusarium*, *Rhizopus* and *Alternaria* predominated in the rhizosphere of clover and lupin affected nodulation. CHNONKAR and SUBBA-ROA (1966) found that the nodule mycoflora of *Trifolium alexandrinum* comprised species of *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizoctonia* and *Trichoderma*. The latter affected the nodulation of clover plants.

Application of pesticides to control different pests in leguminous plants is common nowadays. This might also represent another factor affecting the symbiotic relationships between *Rhizobium* and leguminous plants. Some pesticides, however, were found to stimulate rather than inhibit the soil microflora. The stimulating effect of insecticides and herbicides on nodulation was recorded by some investigators such as ANDERSON and BAKERS (1950), ABOU EL FADL and

FAHMY (1958). They found that 2,4-D and DDT had a stimulatory effect on the formation of nodules of some leguminous plants. TAHA *et al.* (1966) and (1972) showed also a stimulatory effect of Dipterex on the formation of effective nodules of broad bean, lentil and Egyptian clover. On the other hand, other investigators showed that herbicides and insecticides might inhibit *Rhizobia* or nodulation process. Among them were PAYNE and FAULTS (1947), NILSSON (1957) and BRAITHWAITE *et al.* (1958). TAHA *et al.* (1966) in their investigation on CIPC, found that it completely inhibited nodulation in both broad bean and clover.

As little data were available on the effectiveness of *Rhizobium* inoculation of the infected leguminous plants treated with pesticides, it seemed desirable to investigate the effect of pesticide application on the nodulation and symbiotic N-fixation of the infected broad bean plants with root rot and wilt diseases.

Materials and Methods

Pot experiments were carried out using fertile clay loamy soil from the National Research Center farm. Pots of 20 cm diameter were filled with 2 kg soil for each. The pots were divided into 3 sets. On the first set, the pots were inoculated with the main pathogens either *Fusarium oxysporum* or *Rhizoctonia solani*. In the second set, the soil was treated with different pesticides. In the third set, the soil was treated with both the main pathogen and the pesticide. On this basis, the different treatments could be represented as follows:

1 – Soil without inoculation and without pesticide applications (control).
2 – Soil inoculated with the main pathogen for root rot disease *(Rhizoctonia solani)*.
3 – Soil inoculated with the main pathogen for wilt disease *(Fusarium oxysporum)*.
4 – Soil inoculated with the main pathogen + pesticide.

Inoculation of the soil with the pathogenic Fungi

Soil infection was carried out by sand – barley medium (25 gm clean sand, 75 gm barley and enough water to cover the mixture), which was inoculated beforehand with each particular fungi. The flasks were incubated at 25°C for two weeks.

Soil was inoculated with the fungal inoculum at a rate of 4% of soil weight. The infected soils were thereafter watered and mixed thoroughly for one week to ensure the even distribution of the inoculated fungi.

In the control treatment the soil was mixed with the same amount of sterilized sand mixture alone.

Inoculation of broad bean seeds with Rhizobium

Broad bean seeds (variety Giza 2) were soaked for 1 hour in 3 days old liquid culture of effective strain of *R. leguminosarum*.

Each pot was sown with 7 seeds of inoculated broad bean. 15 days after sowing the plants were thinned to leave 4 plants for every pot. The plants were irrigated every 2 days with distilled water.

Application of pesticides

Different pesticides were used in this investigation namely insecticides (Nuvacron and Dipterex) fungicide (Dithan A–40) and herbicide such as Atrazine. Their chemical structure and concentration used in the investigation were as follows.

1 – *Insecticides:*

Nuvacron 40%: *cis* (2-methyl-carbamyl-1-methyl-vinyl) diethyl phosphate.
Dipterex 80%: 0,0-dimethyl-1-hydroxy-2 trichloroethyl phosphate.
(For insecticides 1 and 2 field application rates were used) 1 field application rate 2.0 lit/fed.

2 – *Fungicide:*

Dithan A–40%, 93% disodium-ethylene-bis-dithiocarbamate (one field application rate 20 kg/fed).

3 – *Herbicide:*

Atrazine 50%: 2-chloro-4-ethylamino, 6-iso-propylamino-s-triazine (one field application rate 1.5 kg/fed*).

The insecticides and fungicide were sprayed by automizer 2 days after inoculation of the soil with the pathogenic fungi.

The herbicide, Atrazine was used in one concentration but cultivation of broad bean seeds was adopted at different times after Atrazine application namely 0, 10, 20 and 30 days. This was done to study the effect of cultivation time after the herbicide application on the symbiotic nitrogen fixation process.

After 49 days, the nodules on every plant root system were counted on both the secondary and the main roots. Dry weight of the whole plants was also determined. Total nitrogen was determined in dried plants using the modified micro-kjeldahl method as a criterion of nitrogen fixation.

Results and Discussion

I – *Insecticides*

The nodulation density, dry weight and total nitrogen of broad bean plants as affected by inoculation with the pathogenic fungi for root rot disease and

* Feddan = 4200 m^2.

application of insecticides are presented in Table (1). Data showed that the highest nodulation density on the secondary roots was observed on the control plants where neither inoculation of the soil with the pathogenic fungi nor application of insecticides were used. Dry weight of the plants and total nitrogen showed also the same general trend.

Table 1

Nodulation, dry weight and total nitrogen of infected broad bean plants as influenced by insecticide applications

Treatments	Number of nodules/plant			Dry weight, g/plant	Total nitrogen, mg/plant
	Main root	Sesondary roots	Total nodules/ plant		
Untreated (control)	18.0	71.0	89.0	2.027	43
Soil inoculated with;					
Fusarium. Oxy f. fobae	16.0	30.0	46.0	1.426	30
Rhizoctonia solani	32.0	48.0	80.0	0.756	7
Soil applied with;					
Nuvacron I	12.0	49.0	61.0	1.288	26
Nuvacron II	7.0	48.0	56.0	1.340	28
Dipterex I	6.0	55.0	61.0	1.737	21
Dipterex II	26.0	65.0	91.0	2.162	40
Soil treated with;					
Fus. oxy. + Nuv. I	18.0	45.0	63.0	1.281	27
Fus. oxy. + Nuv. II	18.0	28.0	46.0	1.412	22
Fus. oxy. + Dipt. I	19.0	30.0	49.0	1.513	26
Fus. oxy. + Dipt. II	16.0	29.0	45.0	1.538	28
Rhiz. sol. + Nuv. I	11.0	39.0	50.0	0.812	16
Rhiz. sol. + Nuv. II	10.0	31.0	41.0	1.181	14
Rhiz. sol. + Dipt. I	14.0	28.0	42.0	1.240	16
Rhiz. sol. + Dipt. II	8.0	27.0	35.0	1.826	40

"I" one field application rate
"II" double field application rate

Inoculation of the soil with either *Fusarium oxysporum* or *Rhizoctonia solani* depressed the nodulation densities, the dry weight and the nitrogen content. Inoculation of the soil with *Rhizoctonia* was superior in this respect since it gave very low values of dry weight and nitrogen contents of the plants, in spite of the formation of high numbers of nodules on the root system. It seemed that root rot disease caused by *Rhizoctonia solani* prevented the roots from absorbing the different nutrients especially those playing an important role in the symbiotic N-fixation. In addition, infection of the plants with *Rhizoctonia* might cause damage in the root system reducing the root area and consequently the nodulation process.

Application of insecticides to the soil also reduced the values of symbiotic nitrogen fixation as compared with those of the control. However, application of Dipterex in the second concentration slightly increased the nodulation density and the dry weight but decreased the nitrogen content of the plants compared with that of the control. In fact, it stimulated the symbiotic N-fixation compared with that of the infected plants.

Application of insecticides in the different concentrations to the inoculated soil with the pathogenic fungi showed also lower values of symbiotic nitrogen fixation.

In general, the results showed that either inoculation of the pathogenic fungi or application of insecticides inhibited the symbiotic N-fixation. However, application of the mentioned insecticides to the inoculated soil with the pathogenic fungi inhibited the values in case of *F. oxysporium* but stimulated the values in case of *Rhizoctonia solani*. It seems that the damage in plants infected with *F. oxysporum* occurred early enough since *Fusarium* infects directly the vascular system of the plants. Therefore, application of insecticides in the latter case represented an additional factor inhibiting the nodulation process besides that occurring due to *Fusarium* infection. On the other hand, *Rhizoctonia* infects the plants by means of the cortex and epidermis causing rot disease, and application of insecticides in this case might be beneficial for the symbiotic N-fixation. The organophosphorus insecticides' degradation by the rhizospheric soil microorganisms might act as a stimulatory factor for the nodule formation and symbiotic N-fixation. This explanation supported the results obtained by TAHA *et al.* (1972) who found that Dipterex in normal field rates stimulated high numbers of effective nodules and high rate of nitrogen fixation for broad bean plants.

II – *Fungicide*

Data of the nodulation density and the symbiotic N-fixation of broad bean plants as influenced by inoculation of pathogenic fungi and application of fungicide Dithan A – 40 are presented in Table 2. Nodulation density, dry weight and nitrogen content of the control plants gave relatively high figures compared with those of other treatments. Inoculation of the soil with the main pathogen either *Fusarium* or *Rhizoctonia* depressed the nodulation density, dry weight and nitrogen content of the plants. Dithan A – 40 (Fungicide) however, slightly reduced the mentioned values of broad bean plants. Application of Dithan A – 40 to the inoculated soil with either *Fusarium* or *Rhizoctonia* stimulated the symbiotic nitrogen fixation in broad bean plants. This was especially clear in case of *Rhizoctonia* inoculation. This could be explained by the fact, that Dithan as fungicide combats the pathogenic fungi from invading the plants and the inoculated *Rhizobium* finds its way into healthy plants to form effective nodules and consequently high nitrogen fixation takes place. This is in line with the results of DUGGA (1935), APPLEMAN (1941) and KERNKAMP (1948), who showed that application of fungicides in field practice caused no deleterious effect on *Rhizobium*

Table 2

Nodulation, dry weight and total nitrogen of infected broad bean plants as influenced by Dithan A-40 application

Treatments	Number of nodules/plant			Dry weight, g/ plant	Total nitrogen, mg/plant
	Main root	Secondary root	Total nodules, per plant		
Untreated soil	23	64	87	1.625	25
Soil inoculated with;					
Fusarium oxysporum	4	11	15	0.700	17
Rhizoctonia solani	4	16	20	0.800	18
Soil applied with;					
Dithan A—40	8	40	48	1.175	21
Soil treated with;					
Fus.oxy. + Dithan	42	36	78	1.175	30
Rhiz.sol. + Dithan	28	61	89	1.750	40

present in the soil and nodulation of legume plants. Therefore, it is recommended to use Dithan for controlling root rot disease of broad bean plants infected with *Rhizoctonia* since it stimulated the symbiotic N-fixation.

III — *Herbicide*

Data in Table 3 show the nodulation density, dry weight and nitrogen content of inoculated broad bean plants as influenced by Atrazine application. The highest values of dry weight and nitrogen fixed symbiotically were obtained in the control treatment.

Inoculation of the soil with either *Fusarium* or *Rhizoctonia* inhibited the mentioned values. This could be due to the infection of the root system causing root rot or wilt disease that might affect the absorption system of plants for different nutrients. The latter might also affect the symbiotic nitrogen fixation. Cultivation of broad bean, just after Atrazine application, in soil previously inoculated with the pathogenic fungi greatly inhibited the symbiotic N-fixation. Similar inhibitory effect of s-triazine herbicide on soil microorganisms, was recorded by GORLENKI *et al.* (1969) and BAKALIVANOV (1971). Cultivation of broad bean, 30 days after application of the herbicide, relatively reduced the inhibitory effect on symbiotic N-fixation. This could be attributed to the degradation of the herbicide by soil microorganisms decreasing its toxicity on *Rhizobium* viability and consequently on the nodule formation and N-fixation. Such conclusions confirm the results of HARRIS *et al.* (1968) who found that Atrazine, compound is degraded by soil microorganisms. AGUNDIS and BEHRENS (1966) reported that 95% of Atrazine was lost in a month.

From the above discussion it can be concluded that inoculation of the soil with the pathogenic fungi specific for root rot and wilt diseases inhibited indirectly

Table 3

Nodulation, dry weight and total nitrogen of infected broad bean as influenced by Atrazine application

Treatments	Number of nodules			Dry weight g/plant	Total nitrogen mg/plant
	Main root	Secondary roots	Total nodules per plant		
Untreated soil	23	83	106	1.593	30
Soil inoculated with;					
Fusarium oxysporum	18	66	84	1.268	24
Rhizoctonia solani	11	12	23	1.230	24
Soil treated with Atrazine at;					
cultivation time after Atrazine application: 0	8	16	24	0.988	18
cultivation time after Atrazine application: 10 days	12	15	27	0.915	8
cultivation time after Atrazine application: 20 days	8	21	29	0.925	9
cultivation time after Atrazine application: 30 days	24	24	48	1.305	23
Soil treated with Fus. and Atrazine at;					
0	9	11	20	1.118	22
10 days	12	12	24	0.990	17
20 days	14	19	33	1.138	22
30 days	16	27	43	1.200	
Soil treated with Rhiz. and Atrazine at;					
0	13	14	27	1.040	16
10 days	25	23	48	1.045	21
20 days	35	23	58	0.940	17
30 days	37	75	112	1.325	24

the symbiotic N-fixation processes of broad bean plants. Application of insecticides at cultivation time exerted also the same effect on the N-fixation. Fungicide application (Dithan A – 40) however, is recommended for controlling root rot and wilt diseases since it had no effect on the symbiotic N-fixation of the plants. Cultivation of broad bean at application time of Atrazine inhibited nodulation and N-fixation processes of the plant. On the other hand, cultivation of the plants 30 days after herbicide application did not affect the symbiotic N-fixation due to its degradation by the soil microorganisms.

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The Influence of Pyrazon, Ethofumesate and Metamitron on the Soil Microbiota

By

J. P. VOETS, M. O. ANGEROSA IMAS, H. GODDEERIS and W. VERSTRAETE

Laboratory of General and Industrial Microbiology, University of Gent, Coupure 533, 9000 Gent, Belgium

Field studies on the microbiology of sugar beet soils have indicated that pyrazon and ethofumesate tend to inhibit the soil microbiota and its activities. To verify this observation, dose effect experiments were set up in the laboratory with the latter herbicides as well as with a third herbicide, i.e. metamitron.

Three doses were examined: zero, a high field application rate and twenty times the high field application rate. The following parameters were investigated: the numbers of bacteria, fungi and cellulolytic microorganisms; the phosphatase, urease and saccharase activity; the soil respiration rate and finally the soil nitrification rate. In addition the Michaelis-Menten constants for the soil phosphatase were determined. The results obtained indicate that in general the three herbicides do not damage significantly the soil microbiota. However, a temporary inhibition of the respiration and nitrification rate was noted for all three herbicides at their field application rate. The results furthermore indicate that all three herbicides are biodegradable and that metamitron stimulates the soil urease activity.

Sugar beet crops most generally receive intensive applications of herbicides and insecticides. Several pesticide treatment systems are currently in use. In Belgium, particularly the following two systems are widely applicated:

System 1: a. di-allate 1.4 kg/ha
b. pyrazon 2.5 kg/ha
c. phenmedipham 1.0 kg/ha + wetting agent 1.0 kg/ha

System 2: a. cycloate 2.5 kg/ha + lenacil 0.35 kg/ha
b. pyrazon 1.25 kg/ha
c. phemedipham 1.0 kg/ha + wetting agent 1.0 kg/ha

a: before sowing; b: pre-emergence; c: post-emergence.

The effect of some the most important pesticide treatment systems for sugarbeet crops on the soil microbial and enzymatic constitution has been studied during the period 1972–1974 (LIVENS *et al.*, 1974; VERSTRAETE and VOETS, 1974). These studies revealed that the impact of these treatment systems generally is not pronounced at the level of the individual soil microbial and enzymatic characteristics. However, when the effects of these treatment systems are evaluated by means of a cumulative rank index, it appears that several systems indeed alter considerably the soil microbiology. In addition, these field studies indicated that pyrazon and ethofumesate, both at the application rate of 4 kg active substance per ha, tended to inhibit the soil microbiota and its activities.

This paper reports on a detailed study with regard to the influence of the latter two herbicides. In addition, a herbicide recently introduced on the market i.e. metamitron was included in the research project.

In contrast to the previous studies, this project dealt with specific chemicals only and not with overall pesticide treatment systems. Furthermore, the three chemicals are only investigated under laboratory conditions by means of pot experiments.

Materials and Methods

The soil used had the following characteristics. Texture : sandy loam (11% clay, 60% silt, 29% sand); % organic C: 1.17; pH_{H_2O}: 5.8; and CEC: 10. The soil was carefully homogenized and brought to 75% of its field capacity. Subsequently, aliquots of 2.0 kg wet soil were filled in 2.0 l plastic pots. The aliquots were given their respective treatments and incubated at 22°C. At regular intervals distilled water was added to adjust evaporation losses.

The following treatments were examined.

A.1. *Pyrazon* (2-phenyl-5-amino-4-chloro-3(2H)pyridazinone)
- a. soil as such; control
- b. soil treated at a rate corresponding to 4.0 kg pyrazon a.s./ha
- c. soil treated at a rate corresponding to 80.0 kg pyrazon a.s./ha

A.2. *Ethofumesate* (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate)
- a. soil as such; control
- b. soil treated at a rate corresponding to 2.0 kg ethofumesate a.s./ha
- c. soil treated at a rate corresponding to 40.0 kg ethofumesate a.s./ha

A.3. *Metamitron* (4-amino-3-methyl-6-phenyl-1,2,4-triazine-5(4,4)-on)
- a. soil as such; control
- b. soil treated at a rate corresponding to 10 kg metamitron a.s./ha
- c. soil treated at a rate corresponding to 200 kg metamitron a.s./ha

Series b corresponds to a high field application rate. The second dose (series c) surpasses the first one with a factor 20. The purpose of this dose is to verify whether accidental spills or overapplications could have a harmful effect on the microbiota.

The three herbicides were applied in their commercial form. Pyrazon is manufactered by B.A.S.F., ethofumesate by Fisons and metamitron by Bayer.

Total numbers of bacteria were determined on a medium with the following composition: beef extract 3.0 g, peptone 5.0 g, agar 18 g, aqua dest. 1.0 l. Fungi were enumerated on Martin's agar (1950). The cellulolytic microorganisms were counted on the following medium: KH_2PO_4 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g; $FeSO_4$ 0.01 g, $MnSO_4$ 0.01 g, NH_4NO_3 0.3 g, powdered cellulose 10.0 g, tap water 1.0 l, agar 10.0 g, pH 6.7. The saccharase, phosphatase and urease enzymatic activities of the soil were assayed according to HOFMANN und HOFFMANN (1966). The Vm and Km-characteristics of the respective enzymatic activities

were measured as described by TABATABAI and BREMNER (1971). The soil respiration activity was determined according to JENKINSON and POWLSON (1976). To study the nitrogen transformations in the soils 215 mg urea was added to a 0.5 kg soil sample. The sample was subsequently incubated at room temperature. This addition corresponds with a sample concentration of 200 parts $N/10^6$ and to a field application of about 300 kg N/ha. At regular intervals, subsamples of 10 g soil were withdrawn and analyzed for their ammonium, nitrite and nitrate content by steam destillation (BREMNER and KEENEY, 1965).

Results and Discussion

The results of the microbial analyses are summarized in Table 1 and those of the enzymatic analyses in Table 2. The results were statistically analyzed by the *t*-test on paired observations (Table 3). It appears that the pesticides have only a minor impact on the microbial populations and enzymatic activities examined. Pyrazon tends to reduce the number of bacteria at its highest application rate but exhibits otherwise no striking effects. Ethofumesate appears to affect slightly the cellulolytic organisms and the urease activity. Metamitron finally tends to enhance the phosphatase activity at the normal application rate but it inhibits the latter activity at the higher dose. The most remarkable trend is that this herbicide seems to stimulate the soil urease activity. The latter finding is not too surprising in view of the triazin-one moiety in the metamitron molecule and suggests that ureolytic bacteria actively participate in the breakdown of this herbicide.

To verify whether the soil enzymatic affinity was altered by the herbicides, the enzymatic procedures as described by HOFMANN und HOFFMANN (1966) were performed for various substrate concentrations. The results obtained were linearized according to the Michaelis – Menten equation. The corresponding Vm and Km-values thus obtained for the phosphatase are given in Table 4. Pyrazon appeared to decrease both the Vm and the Km constants. Ethofumesate exhibited only a minor effect. Metamitron finally tended to increase both Vm and Km. In general, the shifts observed are small and probably of no pedological significance.

The soil respiration activity is schematized in Fig. 1. Quite striking is the fact that all soils of the b-series have the lowest respiration activity while all soils of the c-series exhibit the highest values. The results of the b-series suggest that all three herbicides at their normal application rate reduce the overal microbial metabolism with 5 – 10 per cent.

The results of the c-series can best be explained in the following way: the herbicides, when applied at a high rate, induce a selection of resistant microbial populations. The latter populations subsequently metabolize the xenobiotic chemicals which gives rise to the increased CO_2-production. These results are most interesting because they suggest that the products examined are not recalcitrant.

Table 1

Influence of the individual herbicides on some microbial groups (log N/g moist soil)

Microbial group	Time after treatment											
	0			1			3			8 weeks		
	a*	b	c	a	b	c	a	b	c	a	b	c
Pyrazon												
Total bacteria	6.628**	6.389	6.322	7.267	7.290	7.161	7.477	7.519	7.352	7.699	7.580	7.176
Total fungi	3.618	3.550	3.585	3.568	3.667	3.677	3.813	3.836	3.792	3.623	3.720	3.618
Cellulolytic microorganisms	6.041	5.954	6.021	5.813	6.279	6.161	6.267	6.322	6.097	5.623	5.597	5.677
Ethofumesate												
Total bacteria	7.728	7.580	7.916	7.462	7.568	7.767	7.736	7.699	7.703	7.477	7.342	7.243
Total fungi	4.591	4.618	4.677	4.342	4.255	4.301	4.290	4.217	4.144	4.079	4.431	4.342
Cellulolytic microorganisms	6.455	6.462	6.470	6.312	6.371	6.230	6.279	7.021	7.120	6.978	6.875	6.778
Metamitron												
Total bacteria	8.512	8.000	8.267	7.407	7.585	7.556	6.778	6.836	6.653	7.362	7.362	7.312
Total fungi	4.658	4.491	4.505	4.470	4.653	4.658	4.648	4.415	4.407	3.898	3.886	3.866
Cellulolytic microorganisms	4.423	4.477	4.462	6.021	5.093	6.230	5.371	5.380	5.161	6.144	6.097	6.217

* a : control sample; b : normal application rate; c : normal application rate $\times 20$

** all data are the average of two repetitions

Table 2

Influence of the individual herbicides on some enzymatic activities (mg product formed/100 g dry soil/3 h)

Enzymatic activity	Time after treatment											
	0			1			3			8 weeks		
	a*	b	c	a	b	c	a	b	c	a	b	c
Pyrazon												
Urease	23.23**	24.77	23.89	20.36	25.98	25.61	27.41	22.72	23.37	22.59	24.40	24.92
Phosphatase	27.27	27.27	29.45	24.04	28.36	28.36	26.18	26.18	24.04	26.18	26.18	28.36
Saccharase	20.11	17.93	18.09	18.45	19.38	18.53	18.48	20.34	18.26	19.05	20.42	19.30
Ethofumesate												
Urease	19.16	19.16	21.29	19.16	19.16	20.13	19.62	16.74	21.76	21.39	18.27	19.06
Phosphatase	26.82	29.78	29.78	29.78	30.56	29.61	28.67	30.56	30.89	37.32	35.03	31.66
Saccharase	19.08	1983	19.35	21.71	20.40	18.93	19.87	20.28	22.46	19.42	15.00	16.16
Metamitron												
Urease	15.53	18.69	20.24	14.54	14.86	16.08	16.35	20.61	19.53	9.36	10.19	10.25
Phosphatase	35.07	38.10	31.60	38.10	39.40	36.80	33.34	37.23	33.77	26.47	25.79	24.42
Saccharase	19.53	19.12	20.55	21.45	17.73	15.52	16.50	16.20	15.67	16.95	17.77	15.30

* a : control sample; b : normal application rate; c : normal application $\times 20$

** all data are the average of two repetitions

Table 3

Statistical evaluation of the results obtained for the treatments a, b and c for three herbicides

Parameter	a versus b	a versus c
Pyrazon		
Bacteria	n.s.	a > c**
Fungi	n s	n.s.
Cellulolytic microorganisms	n.s.	n.s.
Urease	n.s.	n.s.
Phosphatase	n.s.	n.s.
Saccharase	n.s.	n.s.
Ethofumesate		
Bacteria	n.s.	n.s.
Fungi	n.s.	n.s.
Cellulolytic microorganisms	n.s.	a > c*
Urease	a > b*	n.s.
Phosphatase	n.s.	n.s.
Saccharase	n.s.	n.s.
Metamitron		
Bacteria	n.s.	n.s.
Fungi	n.s.	n.s.
Cellulolytic microorganisms	n.s.	n.s.
Urease	a < b*	a < c**
Phosphatase	a < b*	a > c*
Saccharase	n.s.	n.s.

n.s. : not significant
* : $p \leq 0.20$
** : $p \leq 0.10$

Table 4

Michaelis–Menten constants for the soil phosphatase activity

Treatment	Vm*	Km**
Pyrazon		
* 3 weeks		
a	93	733
c	83	675
* 8 weeks		
a	100	1054
c	76	733
Ethofumesate		
* 3 weeks		
a	93	888
c	96	733
* 8 weeks		
a	83	803
c	104	888
Metamitron		
* 3 weeks		
a	69	675
c	100	767
* 8 weeks		
a	76	964
c	111	992

* Vm : mg product formed/100 g dry soil/15 h
** Km : mg substrate/1 reaction mixture

The outcome of the nitrification experiments is represented in Fig. 2. The results clearly illustrate that all herbicides and in particular metamitron slow down the nitrification process. However, the inhibition is not drastic nor long-lasting.

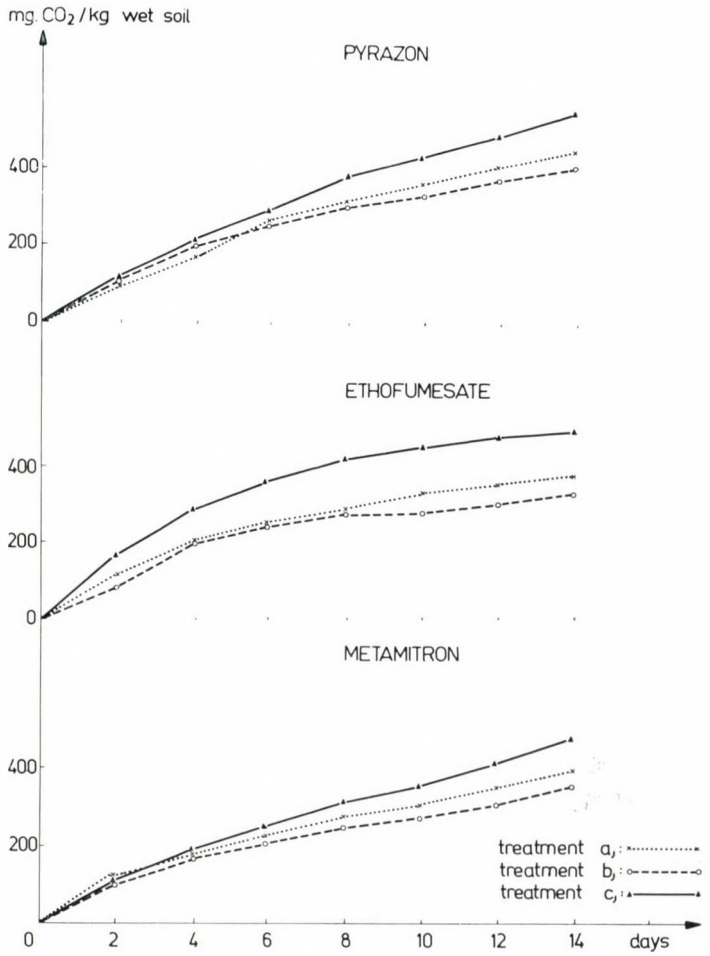

Fig. 1. CO_2 production of the three treatments for the different pesticides. Treatment a: × — — ×; treatment b: o — — o; treatment c: △ — — △

From the various experiments, it appears that none of the herbicides examined exhibits any drastic or irreversible influences on the soil microbiota or its activities. Hence, these results did not confirm the tendencies observed for the field trials in which systematically herbicide treatment systems comprising pyrazon or ethofumesate exhibited a negative influence on the soil microbiota. It must be remembered however that the laboratory investigations performed consist

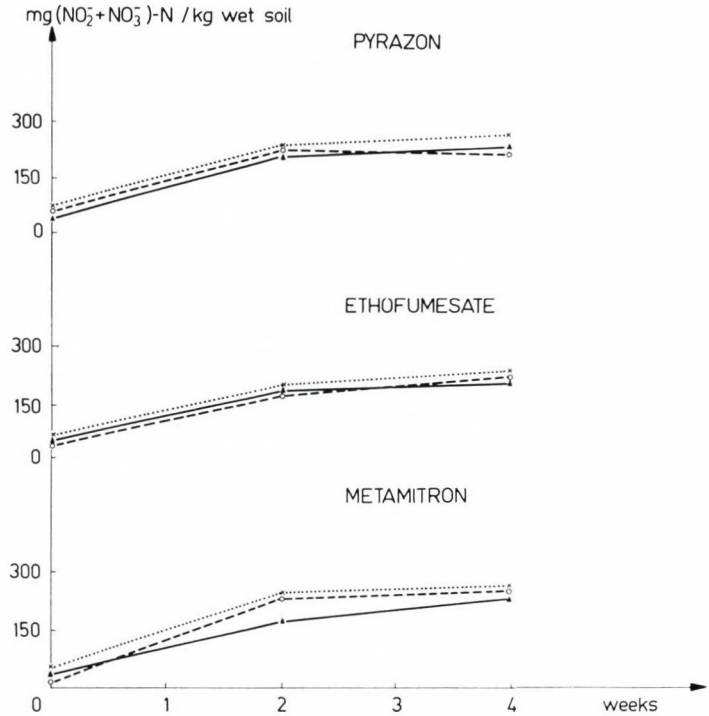

Fig. 2. Influence of the different pesticides on the nitrification rate. For the symbols: see Fig. 1

of a herbicide and a microbiota component only, while the field experiments include also a plant component. It is possible that the herbicides particularly effect the microbiota-plant interaction. To verify the latter hypothesis, field trials were set up and followed during the growing season 1976. The results obtained (STRYCKERS *et al.*, 1977) indicated that the soil microbiota is indeed clearly inhibited in the soils of sugar beet crops treated with pyrazon. This effect was temporary, however. As to ethofumesate, no consistent effect was noted in these field trials and this herbicide therefore can not any longer be considered as inhibitive towards the soil microbiota. Finally, the results of the metamitron field trials confirmed those of the laboratory results, that is a definite but short-lasting inhibition of the major microbial groups and their activities.

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The Effect of Herbicides on Soil Bacteria Belonging to Certain Physiological Groups

By

B. HELMECZI

Agricultural University of Debrecen, Department of Soil Science and Microbiology, Hungary

In 1974 and 1975, within the framework of smallplot field experiments, the effect of 10 herbicides — Afalon 20 EC, Akticon 90 WP, Eptan SH 202, urea Statecide 65 WP, Kartex "A", Kartex "B", Propachlor 40 EC, Satecide 65 WP, Sutan 80 EC, Terbutrine 50 EC — and their combinations, was investigated with regard to the changes in the count of aerobic N_2-fixing, aerobic cellulose decomposing, ammonifying and nitrifying bacteria living in soils.

The experiments were set in pseudomycelial chernozem soil with maize plants arranged in Latin rectangles and random blocks. The number of bacteria were counted by applying Pochon's method — with certain modifications — samples taken in spring, summer and autumn. On the basis of the results thus obtained, the following statements can be made.

(1) Among the herbicides and herbicide combinations applied in 1974 there was not any that inhibited the increase in bacteria belonging to all the four physiological groups; there was even only one that decreased the bacterial counts in three physiological groups (Kartex "A" 10 kh/ha). As against this among the seven herbicide combinations applied in 1975 there were four that decreased the bacterial count of four physiological groups.

(2) The bacteria belonging to the four physiological groups behaved differently, and was sensitive to different extents to the investigated herbicides. Occasionally, stimulating effects also occurred.

(3) Among the bacteria belonging to the four investigated physiological groups the nitrifying bacteria had the greatest degree of sensitivity to the herbicides examined. The counts — in comparison with the control bacteria — decreased considerably in all the treatments and in both years.

(4) The aerobic cellulose decomposing bacteria were the least sensitive to herbicides. With these bacteria, the herbicide combinations (with the exception of Kartex "A" 10 kg/ha) applied to soil in 1974 had a stimulating effect, their number in comparison with that of the control increased to a small extent. The aerobic N_2-fixing and ammonifying bacteria in relation to sensitivity to the examined herbicides had a middle place.

As a result of a rapid development in the chemical industries, the various possibilities of chemical weed killers came into existence in opposition to the weed killing which had initially been carried out exclusively in a mechanical way. Formerly, the application of agents with total effect was generally wide spread, while recently — in the last 30—40 years — that of the selective and superselective agents was more general, and chemical weed killers developed parallel with this.

Chemical weed killers, which in the early years extended only to a few of the plant stands cultivated, from the 40s on – in Hungary rather from the 50s – considerable proportions, assumed spread over ever more plant cultures, and in certain cultures it even became an exclusive method (for example, in grain crops). The application of herbicides changed the whole agrotechniques and it has become a fact that today plant cultivation without weed killing chemicals would almost be threatened with failure.

Simultaneously with the spread in the application of weed killers (but in general that of chemicals as well) the view also arose that their application on a large scale might cause undesirable environmental pollution and environment protections problems.

Starting from the fact that part of the chemicals applied in plant protection (fungicides, insecticides, etc.) also gets into soils – and the herbicides are directly applied to soils – the question arose what effects these would have on the living world of soils. True, a considerable part of them – after exerting the favourable effect – decomposes into harmless compounds; part of them, however, persists in the soil or is incorporated in the plants.

Thus it can be supposed that after a certain time the quantity of chemicals that remained beyond a definite threshold value may disturb to a smaller or larger extent the biological balance or may even upset it.

What has been said above was the point of departure of certain researchers who as early as the 40s set the effect of herbicides on soil microorganisms as the aim of their investigations. At the beginning the investigations proved that the herbicides do not inhibit the microorganisms of the soil even after applying several times higher doses than was actually done in practice. Later on, Hungarian researchers – VIRÁG (1958), VIRÁG *et al.* (1960) – and others also came to similar conclusions.

Today information on a larger scale is already available in this field, nevertheless, the results which have so far been obtained are not yet in agreement even at present. In the meantime it also turned out that not only herbicides have an effect on the microorganisms of soil but also the latter have a reciprocal effect on herbicides as well. The literature related to the subject covers such a large sphere that it is almost impossible to survey it. A great help is provided in orientation by the theme documentation of MANNINGER (1967a, 1967b, 1972). It is very charasteristic that the theme documentations published in 1967 present the summary of 201 works, while that from 1972 contains the summary of 426 more remarkable works. This difference is even more conspicuous if we also consider that the material of the two theme documentations published in 1967 was selected in the one case from works published in 1944–1965, while in the other from 1905–1965 – and in their great majority from works published between 1950 and 1965 – while at the same time the material of the theme documentation published in 1972 consisted of the products of merely four years (1968–1971).

From the figures it can be seen that the number of the related investigations increases parallel with the spread of applying the chemicals, and that the number

of published papers is rapidly growing. We also have a nearly one decade's research experience in this field.

The results summed up in the theme documentations mentioned above, and also those published in the various papers, have been used in our examinations.

The pesticide effects on soil microorganism have been dealt with by many researchers also in Hungary. The following works have been studied and referred to by us in detail: HELMECZI (1965), KECSKÉS (1969a, b), KISS (1966), KISS (1965), PÁNTOS *et al.* (1964), SZABÓ (1964), SZEGI (1970) and TAKÁTS (1965).

The results of the examinations have been summarized by HELMECZI (1974). From the investigations carried out so far, no uniform and relatively final conclusions can be drawn, in spite of the great number of these investigations. An explanation of the difficulties of drawing uniform inferences may be found in the complex and variable nature of soil biological events, as well as in the multifariousness of the herbicides and microorganisms examined, together with the differences in the methods applied to the investigations.

Studying the relationship between herbicides and soil microorganism is also such a field where the permanent appearance of newer and newer herbicides makes further research justifiable still for a long time. This was kept in mind when we aimed to make the effect of recently applied herbicides on soil bacteria the subject of a continual series of investigations. In the present paper we furnish data on part of our investigations.

Conditions, Material and Method of the investigations

Our examinations – in laboratory and field conditions – related to herbicide effects on soil bacteria are based on more than a decade's research work. The examinations were directed at the determination of (i) quantitative changes in soil bacteria belonging to certain physiological groups (aerobic N_2-fixing, aerobic cellulose decomposing, aerobic-proteolytic and nitrifying soil bacteria); (ii) various processes (cellulose decomposition, proteolysis) taking place through the activity of the various soil microorganisms; (iii) the quantity of the nitrogen fixed by N_2-fixers.

In the present paper the results of those examinations will be described that were carried out in recent years (1974, 1975) on the basis of experiments set in field conditions, the results characterize the quantitative changes which ensued under the effect of herbicides in the soil bacteria belonging to the four physiological groups mentioned above.

The field experiments were arranged in pseudomycelial chernozem soil treated 10 times in 1974, and again 8 times in 1975, in a random block and Latin rectangles by repeating the treatments 5 times in each case. The treatments are summarized in Tables 1 a and b.

Table 1

Treatments of soils with herbicides

No. of treatment	a) (1974) Herbicide combinations	Dose kg/ha
1.	Control	—
2.	Satecide 60 WP (propachlor)	8.0
	Terbutrine 50 WP (terbuthryne)	3.0
3.	Satecide 60 WP	6.0
	Terbutrine 50 WP	5.0
4.	Satecide 60 WP	8.0
	Terbutrine 50 WP	5.0
	Akticon 90 WP (atrazine)	1.5
5.	Tricomb (unknown)	10.0
6.	Tricomb	15.0
7.	Kartex "A" (propochlor)	10.0
8.	Kartex "B" (promethryne + monolinuron)	10.0
9.	Satecide 60 WP	8.0
	Akticon 90 WP	1.5
10.	Carbamide Satecide 60 WP	8.0
	Akticon 90 WP	1.5

No.	b) (1975) Herbicide combinations	Dose kg/ha or l/ha
1.	Carbamide Satecide 65 WP	8.0
	Akticon 90 WP	1.5
2.	Tricomb	10.0
3.	Satecid 65 WP	8.0
	Akticon 90 WP	1.5
4.	Carbamide Satecide 65 WP	8.0
	Terbutrine 50 WP	3.0
	Akticon 90 WP	1.5
5.	Propachlor 40 EC/S 4201	10.0
	Sutan EC/S 4201 (buthylate)	1.5
6.	Propachlor 40 EC/S 4201	10.0
	Eptan EC/S 4202 (EPTC)	1.5
7.	Carbamide Satecid 65 WP	8.0
	Afalon 20 EC (linuron)	3.0
8.	Control	—

The experimental plants were MVSC – 380 hybrid maize in both cases. The green crop in 1974 was autumn wheat, and again in 1975 it was pea. Sowing of the maize took place on 18 April, 1974 and on 21 April, 1975 – in a pattern where rows and roots were at a distance of 70×40 cm from each other. The herbicide treatment of the soil was carried out in both cases on the day of sowing. The herbicide treatments were carried out with sprayer by plots so that the quantity of chemicals needed for 100 m^2 was sprayed with 10 litres of water.

The herbicides (Table 1a, 1b) which were used during the experiments were obtained from the North-Hungarian Chemical Works (Sajóbábony).

For the purposes of the microbiological examinations samplings were taken 3 times a year (after spraying the chemicals in spring, summer and autumn), from the upper 20 cm layer of the soil. Samplings were carried in sterile conditions and collected in sterile vessels. The samples were homogenized. The bacterial counts belonging to the various physiological groups were determined from the homogeneous samples by using POCHON's method (1954) – with slight modifications. In the knowledge of the soil moisture content, the bacterial counts were recalculated for dry soils.

Results of the Experiments and their Evaluation

Within the frameworks of a short publication no possibility arises for the presentation of the results in detail. When describing the results we follow the system – even if this is not satisfactory from all viewpoints – of indicating the mean values of bacterial counts determined at various points of time, according to physiological groups and by years; Table 2 contains the averages of the results of 1974, and again Table 2 shows those of 1975.

The number of ammonifying and aerobic N_2-fixing bacteria is given in thousands and to one decimal place, and again that of the nitrifying and aerobic cellulose decomposing bacteria to be found only in smaller quantities is given rounded to two places of decimals. It is well observable in the Table that – with the exception of the aerobic N_2-fixers – in the soil examined in 1974, the bacterial count was considerably smaller than that in the samples examined in 1975. One of the explanations of this is that although the examinations were carried out in pseudomycelial chernozem soil in both years – in the farm of the Agricultural University of Debrecen – the area in which the 1975 experiments were carried out was considerably better supplied with organic matter than the other.

In treatment No. 1, which appears in Table 2, no herbicide was applied – as is shown by Table 1a (control); the plots were hoed.

The herbicide combinations, in comparison with the control, decreased the number of ammonifying bacteria in the case of all the treatments. The inhibition was the greatest in treatment No. 7 (Kartex A 10 kg/ha), where the numerical decrease reached 49%. The most moderate inhibition was found in treatment No. 8 (Kartex B 10 kg/ha), where it amounted to altogether 5%. The number of ammonifying bacteria in the average of the 9 treatments showed a 25% decrease in comparison with that of the control.

According to the data of the 1975 experiments (Table 2), the ammonifying bacteria showed different sensitivities to the various herbicide combinations. Among the herbicide combinations (Table 1b) applied there were even such (carbamide Satecid 65 WP + Afalon 20 EC 8 + 3 kg/ha) under the effect of which the number of these bacteria considerably increased. At the same time,

Table 2

The number of bacteria belonging to the various physiological groups in the soil (thousand/g soil)

Treatments	Ammonifying bacteria	Aerobic N_2-fixing bacteria	Nitrifying bacteria	Aerobic cellulose decomposing bacteria
		1974		
1	57.4	59.3	2.72	0.13
2	33.2	44.2	0.61	1.09
3	54.2	410.1	0.32	0.24
4	52.9	71.3	0.41	0.21
5	42.3	139.2	0.34	0.19
6	51.1	139.4	0.62	0.16
7	29.3	116.7	1.12	0.11
8	54.5	231.9	0.73	0.47
9	38.7	180.5	0.91	0.18
10	32.7	289.8	1.33	0.26
		1975		
1	1211.8	32.5	4.51	1.02
2	116.9	205.5	9.62	1.83
3	+ 836.3	56.5	4.14	1.46
4	260.4	41.9	6.61	0.55
5	929.6	41.1	1.92	0.18
6	1433.6	38.6	4.73	1.49
7	3442.0	49.8	1.71	5.11
8	1424.2	392.4	32.90	1.48

some of the treatments, especially No. 2 (Tricomb 10 kg/ha) and No. 4 (Carbamide Satecid 65 WP + Terbutrine 50 WP + Akticon 90 WP 8 + 3 + 1.5 kg/ha) showed a great degree of inhibition. The extent of the inhibition reached nearly 33% average (treatments Nos 1, 2, 3, 4 and 5), in comparison with that of the control.

According to the data from 1974, the number of aerobic N_2-fixing bacteria – disregarding one exception – increased to a smaller or greater extent in comparison with the control. Here the exception was treatment No. 2 (Satecide 60 WP + Terbutrine 50 WP 8 + 3 kg/ha), where the number of bacteria decreased to a rather significant extent (26%) in comparison with the control. As against the herbicides applied in 1975, the aerobic N_2-fixers also showed a greater sensitivity (Table 2).

According to the data of Table 2, the greatest degree of inhibition manifested itself in the case of nitrifying bacteria. The number of these, under the effect of the herbicides applied in both years of the experiments, decreased

very considerably, in comparison with the control. The degree of inhibition was the greatest in treatment No. 3 (Satecid 60 WP + Terbutrine 50 WP 6 + 5 kg/ha) of the investigations carried out in 1974, where the number of nitrifying bacteria dropped to nearly one decimal place, in comparison with the control. The number of nitrifying bacteria according to the 1974 investigations decreased to the smallest extent by the herbicide combination (Carbamide Satecide 60 WP + Akticon 90 WP 8 + 1.5 kg/ha) applied in treatment No. 10, but the inhibitory effect reached a nearly 50% value even here.

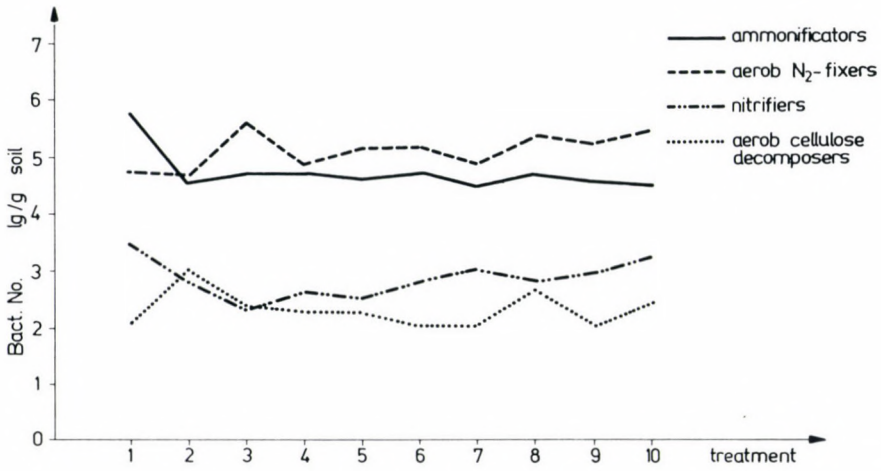

Fig. 1. Herbicide effects on the quantitative changes in soil bacteria (average values of 1974)

According to Table 2, the aerobic cellulose decomposing bacteria showed the smallest degree of sensitivity to the herbicides and herbicide combinations applied in the experiment. By the data of the 1974 experiments, the number of aerobic cellulose decomposing bacteria in the herbicide treatments – with one exception – increased in comparison with the that of the control. Exceptionally, even though not to a great extent (15%), the number of aerobic cellulose decomposing bacteria also decreased in treatment No. 7 (Kartex "A" 10 kg/ha). By the data of the 1975 experiments, a greater degree of inhibition on the activity of aerobic cellulose decomposing bacteria was exerted by the herbicide combination (Propachlor 40 EC + Sután EC 10 + 1.5 kg/ha) applied in treatment No. 5. As can be seen from Table 2, the bacteria belonging to the four different physiological groups behaved differently towards the herbicides applied during the investigations, that is they showed a sensitivity of varying degrees. In 1974 there was not any among the materials of treatments which inhibited the reproduction of the bacteria belonging to the four physiological groups; and there was even only one (Kartex "A" 10 kg/ha) that decreased the number of bacteria belonging to

three physiological groups. As against this, in the treatments even of a smaller number applied in 1975 there were four such (Table 1b: Nos 1, 3, 4 and 5) that decreased the bacterial number in the four physiological groups. Thus, it can be inferred that the herbicide combinations which were applied in 1975 exerted a more uniform inhibitory effect on the bacteria belonging to the four physiological groups than the herbicide combinations applied in 1974.

In the interests of easier surveying and of emphasizing the tendencies, the results of the investigations are shown in Figs 1 and 2.

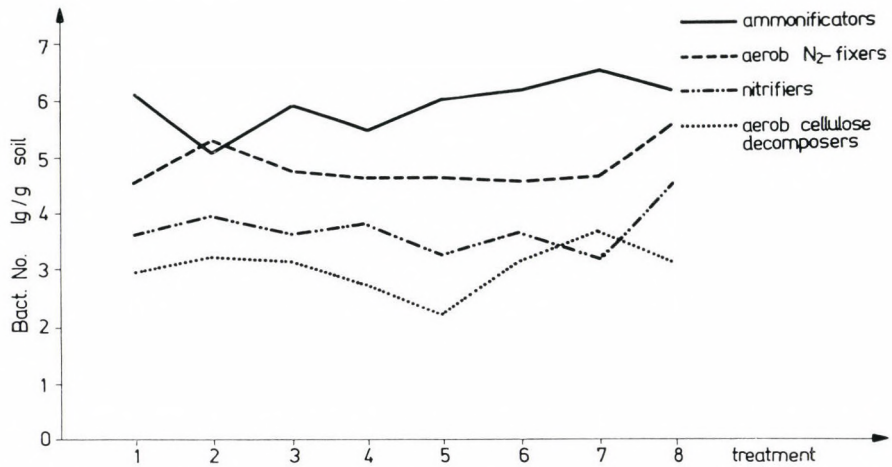

Fig. 2. Herbicide effects on the quantitative changes in soil bacteria (average values of 1975)

In several of the publications there were such statements that during soil microbiological investigations the testing of activity leads to a more reliable result than do quantitative determinations. This was our point of departure when parallel with determining the bacterial numbers we carried out also activity investigations (cellulose decomposition, protein decomposition, N_2-fixing ability) in field experiments and under laboratory conditions, as has already been indicated under the title "The conditions, material and method of the investigations". Our results obtained during the activity examinations support rather well the inferences drawn in the present paper, and which will be dealt with in detail in a following study.

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Interactions of the Herbicide Propanil and a Metabolite, 3,4-Dichloroaniline, with Blue-Green Algae

By

S. J. L. WRIGHT, ANNE F. STAINTHORPE and J. D. DOWNS

School of Biological Sciences, University of Bath, Bath, England

At concentrations below those used for weed control, propanil significantly inhibited the photoautotrophic growth of several blue-green algae, including N_2-fixing species common in soil and rice fields. Sensitivity to propanil was affected by growth conditions. *Gloeocapsa alpicola* (unicellular) was the most propanil sensitive of the algae tested. Low concentrations (< 0.03 ppm) of propanil slightly stimulated some algae, intermediate concentrations (< 0.2 ppm) reduced growth in all species and caused cellular abnormalities in some, whilst concentrations above 5 ppm stopped photosynthesis and prevented algal growth. The metabolite 3,4-dichloroaniline (DCA), formed in algal cultures, was far less inhibitory than propanil to algal growth. Propanil addition to growing cultures of *A. cylindrica* caused a phase of lysis which was followed by recovery on continued incubation.

Propanil (3',4'-dichloropropionanilide) is a contact herbicide used for weed control in rice at application rates ranging up to 6.7 kg/ha (PATEL, 1972; RAY, 1973), giving up to *ca.* 12 ppm in the soil or water. It acts as an inhibitor of photosynthesis, especially of the Hill reaction (GOOD, 1961; CAMPER and MORELAND, 1965) and is therefore a potential toxicant of oxygen-evolving algae, including blue-green algae. Propanil is not persistent in the field, so the rate of degradation and the toxicity of products are important in considering the possible effects on non-target organisms.

3,4-Dichloroaniline (DCA), the primary product of propanil degradation in plants (STILL, 1968), soils (BARTHA and PRAMER, 1967; BARTHA, 1968), rice paddy water (DEUEL, 1975) and microbial culture (LANZILOTTA and PRAMER, 1970), is also formed by the action of sunlight on dilute aqueous solutions of propanil (MOILANEN and CROSBY, 1972).

It is important to determine the interactions of soil and aquatic microbes with pesticides which enter their environment. This is particularly relevant in the case of blue-green algae, which contribute in various ways to soil fertility (SHIELDS and DURRELL, 1964) and which are known to fix atmospheric N_2 in temperate and tropical soils and rice paddy fields (OKUDA and YAMAGUCHI, 1952; WATANABE, 1962; HENRIKSSON, 1971), so enhancing productivity. Increased rice yields

have been obtained by inoculating paddy irrigation water with blue-green algae (WATANABE *et al.*, 1951) and by the use of algae as a fertilizer (WATANABE, 1962).

Growth of the green alga *Chlorococcum aplanosporum* in soil was reduced by 1 ppm propanil, with 2.5 ppm causing an estimated 50% inhibition (SHARABI, 1969). The same author found that at 5 ppm propanil the growth of *C. aplanosporum* in culture solution was prevented, but at concentrations between 0.5 and 1 ppm initial inhibition was followed by recovery. WRIGHT (1972) reported 50% inhibition of growth of the green alga *Chlorella pyrenoidosa* by 0.18 ppm propanil. Growth and nitrogen content in the N_2-fixing blue-green alga *Tolypothrix tenuis* were adversely affected by the propanil formulation Stam at 0.18 ppm and chlorophyll synthesis was reduced by 1.8 ppm (HAMDI *et al.*, 1970). IBRAHIM (1972) reported that 0.1 ppm propanil (as Stam) inhibited growth and N_2-fixation in *T. tenuis* and *Calothrix brevissima*, whereas 0.01 ppm propanil was slightly stimulatory and 10 ppm prevented growth completely.

This paper reports investigations of the interactions of propanil and its metabolite DCA with several species of blue-green algae, including N_2-fixing types commonly found in soil and rice fields.

Materials and Methods

Blue-green algae

The cultures used in these studies were obtained from the Culture Centre of Algae and Protozoa (C.C.A.P.), Cambridge, England. They were: *Anabaena cylindrica* Lemmerman (1403/2a)*; *Anabaena variabilis* BORNET et FLAHAULT (1403/8); *Nostoc muscorum* Agardh (1453/12)*; *Nostoc entophytum* Bornet et Flahault (1453/14)*; *Tolypothrix tenuis* Kützing (1482/3a)* and *Gloeocapsa alpicola* Bornet (1430/1). N_2-fixing algae, typical of soil and rice paddies, are marked* and C.C.A.P. reference numbers are given in parenthesis. With the exception of *A. variabilis* and *G. alpicola*, all cultures were initially axenic.

Culture conditions

The mineral salts medium D, containing H_5 microelements solution (KRATZ and MYERS, 1955) was used. For nitrogen-fixing conditions, nitrates in the medium were replaced by Na_2SO_4 and $CaCl_2 \cdot 2H_2O$. Media were sterilized at 121°C for 15 min and had a pH of 7.8. Algal stock cultures were maintained on agar (1% w/v) slopes of KRATZ and MYERS medium under illumination at room temperature.

Two methods were used for liquid cultures.

(a) Shake cultures: Cultures (50 ml) were shaken (110 osc./min) in 250 ml Erlenmeyer flasks at 30°C in an orbital shaker-incubator (Gallenkamp), with illumination (*ca.* 3000 lux) from white fluorescent lights above. The inoculum

(5 ml) was taken at 72 h from exponential phase cultures, homogenized by magnetic stirring before removing samples.

(b) Sparged cultures: The method was based on that previously used (WRIGHT, 1972) for culturing green algae. Cultures (20 ml) were incubated in large tubes under illumination (*ca.* 4000 lux) at 30°C in a transparent water bath. Media were inoculated with 0.5 ml cells, concentrated two-fold from exponential phase cultures (48 to 72h), and aerated by bubbling CO_2: air (2.5%: 97.5%) at 10 ml/min.

Growth of filamentous algae was measured by dry weight of washed cells, using the whole culture for each sample, and of the unicellular *G. alpicola* by absorbance at 550 nm.

The method for testing algal growth in the presence of soil was modified from that of PILLAY and TCHAN (1972). A spot inoculum of a suspension of algal cells (concentrated 20-fold from an exponential phase culture) was placed on the centre of a 11 mm diameter disc cut from a membrane filter (0.45 μm grade). This was placed on a 13 mm diameter paper antibiotic assay disc (Whatman) which was then placed on the levelled surface of soil or sterilized soil (20 g) which had been evenly moistened with 8.5 ml water or propanil solution, in a glass Petri dish. Triplicate samples in each dish were incubated under illumination at room temperature for up to 14 days and growth of algae on the membrane filter measured by dry weight.

Herbicide

Purified propanil was kindly supplied by Rohm & Haas Co., Philadelphia, U.S.A. and a formulation (250 g active ingredient/l) was donated by the A.R.C. Weed Research Organization, England.

Stock solutions were prepared by shaking excess propanil (80 mg) in 400 ml sterile growth medium at room temperature for 3 days. The solution was then resterilized by membrane filtration. The resulting concentration of propanil, determined by u.v. absorbance (250 nm) and reference to standards, was *ca.* 100 ppm. Propanil solutions, stored in the dark at 4°C, were discarded if found to contain any DCA. DCA solutions (*ca.* 200 ppm) were prepared in the same way as for propanil and the concentration checked by the procedure described below.

The required propanil or DCA concentration ranges for experiments were obtained by dilution of stock solutions with sterile growth medium. Formulated propanil was diluted in the medium to give required levels of the active ingredient after membrane filtration. All treatments were duplicated in each experiment,

3,4-Dichloroaniline assay

The procedure was based on that of EL-DIB (1971) for diazotization of the aromatic amine and coupling with 1-naphthol. The pink-purple colour was read

at 510 nm using 2 or 4 cm cuvettes. Samples (15 ml) were assayed after precipitation of salts with 0.3 ml 3 N NaOH and removal by filtration to prevent dye-adsorption by hydroxides at the final stage of the assay.

Photosynthesis

Photosynthetic O_2-evolution was measured using an oxygen electrode (RANK BROS, Cambridge, England). Algal cells in exponential growth were concentrated five-fold in fresh medium and 3 ml algal suspension, together with 1 ml 0.3 M carbonate/bicarbonate buffer, were placed in the perspex chamber of the electrode. Photosynthesis rates were determined from recorder traces of duplicate samples during light saturation for a few minutes with propanil addition and also at various intervals after adding propanil in the dark.

Results

Algal growth in liquid cultures

Growth in sparged medium was more rapid than in shaken medium. Fastest-growing cultures were *A. cylindrica* and *T. tenuis* in complete medium, both with doubling times of 10h and 40h in sparged and shaken cultures, respectively. *A. cylindrica* gave higher yield under N_2-fixing conditions than in standard medium. In shaken cultures the medium reached pH 9 after 96 h, whilst in sparged cultures the pH stabilized at 7.8 after 24 h, following an initial drop to pH 7.2. Growth curves of the algae were not affected by such changes.

Effect of propanil and DCA on algal growth

At 5 ppm, propanil prevented the growth of all algae in liquid medium. For detailed studies a range of propanil concentrations up to 1 ppm were used. Growth was measured in all samples in each experiment at a time when the control cultures were in late exponential phase (72 h, sparged; 96 h, shaken) and values expressed as % of the control. From plots relating % of control growth to inhibitor concentration, the levels of propanil causing 50% and 90% reduction in growth were derived (Table 1), together with 50% reduction values for DCA.

DCA was far less inhibitory than propanil to all algae. *A. cylindrica* was less sensitive to propanil under N_2-fixing conditions than when grown in complete medium. The unicellular *G. alpicola* was more sensitive than filamentous algae to propanil. *T. tenuis* was more sensitive to both inhibitors in sparged cultures and *A. cylindrica* showed a similar trend with DCA, but not with propanil. Slight stimulation (10%) occurred in the presence of up to 0.03 ppm pure propanil in *A. cylindrica* cultures (in both N_2-fixing and complete medium conditions) and in *N. entophytum* treated with similar concentrations of formulated propanil.

Table 1

Inhibition of growth of blue-green algae by propanil and DCA

Alga	Growth conditions	Propanil (ppm)		DCA (ppm)
		50% redn.	90% redn.	50% redn.
T. tenuis	shaken, complete medium	0.2	>1.0	>6
T. tenuis	sparged, complete medium	0.13	>0.5	3
A. cylindrica	sparged, complete medium	0.16	0.28	0.7
A. cylindrica	shaken, complete medium	0.09	>0.4	2.3
A. cylindrica	shaken, N_2-fixing	>0.25	N.T.	2.3
N. entophytum	shaken, complete medium	0.17	>1.0	>6
N. muscorum	sparged, complete medium	0.08	0.4	N.T.
A. variabilis	sparged, complete medium	0.48	1.0	N.T.
G. alpicola	sparged, complete medium	0.005	0.015	N.T.

N.T. (not tested)

Formulated propanil inhibited growth of *A. cylindrica*, *T. tenuis* and *N. entophytum* similarly; the concentrations causing 50% inhibition being almost 2× those obtained with pure propanil.

Addition of propanil (0.05 to 0.2 ppm) to *A. cylindrica* shake cultures in early exponential phase immediately inhibited growth and caused lysis of some cells, with a decline in growth curves. The extent and duration of lysis was related to propanil concentration; times required for growth to recover to original values were 38 h (0.05 ppm), 48 h (0.1 ppm) and 72 h (0.2 ppm). Growth of apparently unaffected cells proceeded so that the lysis phase gave way to renewed phases of growth at slightly lower rates than in the untreated controls.

No inhibition of growth of *A. cylindrica*, *T. tenuis* or *N. entophytum* was detected on the filter discs in the presence of soil or sterilized soil treated with up to 5 ppm propanil.

Effect of propanil on photosynthesis

Photosynthetic O_2-evolution was immediately stopped on addition of 8 ppm propanil to *A. cylindrica*, *T. tenuis* and *N. entophytum; A. cylindrica* being the most sensitive. The ability to recover photosynthetic activity in the light, after periods of propanil treatment in the dark, varied among species. Highest recovery was shown by *T. tenuis* (33% of control rate), *N. entophytum* recovered 29% and *A. cylindrica* only 8%.

Morphological and cytological effects

Microscopic examination (phase-contrast) were made over 4 days to compare untreated cultures with those treated with propanil at 0.13, 0.2, 0.3 and 5 ppm.

1. A. cylindrica

Cells in propanil inhibited cultures appeared smaller and more densely packed with granules. In propanil treated standard and N_2-fixing cultures the trichomes were longer, and akinetes and heterocysts more numerous and larger, than in control cultures. At 5 ppm, propanil caused bleaching and cell lysis.

2. A. variabilis

Observations were similar to those recorded for *A. cylindrica* in standard medium.

3. N. entophytum

Propanil caused aggregation into macroscopic clumps. These contained long motile trichomes of cells, with no evidence of the characteristic short hormogone or terminal heterocyst differentiation which occurred in controls.

4. T. tenuis

The most noticeable effect of propanil was an increase in trichome length and tendency to aggregate and tangle with reduced tendency for terminal heterocyst formation. Cells in treated cultures appeared darker in colour with less granulation than controls.

Breakdown of propanil

Preliminary experiments established that DCA was formed when propanil (5 ppm) was added to sparged or shaken algal cultures during growth. No DCA was formed from propanil in uninoculated illuminated medium, neither did algal extracellular products react in the diazotization-dye coupling assay. Subsequent experiments with dense axenic and non-axenic cultures of some algae indicated that the latter system was particularly active in forming DCA and that the bacterial contaminants were also active when free of algae. The most active system was a non-axenic *N. entophytum* culture, which formed 1.85 ppm DCA from 5 ppm propanil in 92 h. Axenic *T. tenuis* cultures produced 1 ppm DCA after 96 h.

The stability of DCA in algal cultures was investigated in separate experiments in which a range of DCA concentrations were incubated, shaking, under illumination for 96 h. Under these conditions the DCA levels fell only slightly in uninoculated medium. However, in the presence of algae, especially *A. cylindrica* (the most sensitive to DCA), the DCA levels in the supernatant medium were markedly reduced (Table 2). DCA could not be detected in the algal cell pellet, but this may have been due to inadequate extraction.

Table 2

DCA concentrations (ppm) before and after illuminated incubation in algal cultures

Control		Supernatants from algal cultures (92h)		
Oh	92h	*A. cylindrica*	*N. entophytum*	*T. tenuis*
1.0	1.0	0.64	0.77	0.85
2.0	1.9	N.T.	N.T.	N.T.
2.5	2.4	1.0	N.T.	N.T.
3.0	2.6	1.2	1.82	1.87
5.0	4.7	N.T.	3.2	2.8

N. T. (not tested)

Discussion

The algae differed in susceptibility to propanil, and culture conditions had an influence. For convenience and reproducibility, shaken cultures are preferred and the lower rate of growth in such cultures may be considered more relevant to natural growth rates. The relatively low yield of *A. cylindrica* in shake cultures may be due to the use of an oscillation speed above that regarded as optimal for this organism in shake culture (FOGG *et al.*, 1973). In current experiments with blue-green algae, cultures are shaken at 90—95 osc./min, which allows more rapid growth of filamentous types.

In both culture systems 50% inhibition of growth of all but one of the algae was caused by less than 0.2 ppm propanil, with 5 ppm preventing any growth. Such concentrations, well within the limits of practical application rates, are comparable with those reported for propanil inhibition of some blue-green algae (HAMDI *et al.*, 1970; IBRAHIM, 1972) and coccoid green algae (SHARABI, 1969; WRIGHT, 1972), suggesting on this evidence a similar order of sensitivity in filamentous prokaryotic and unicellular eukaryotic algae. The stimulation of some algae by low doses of propanil are similar to those reported by IBRAHIM (1972). Coccoid blue-green algae seem to be most sensitive, agreeing with our observations on susceptibility of different algae to other herbicides (WRIGHT *et al.*, unpublished).

Although it is not possible to ascribe significance to the apparent lower toxicity of formulated propanil owing to the possibility of slight inaccuracy in defining the absolute concentration of active ingredient in dilute samples, the result is perhaps contrary to expectation.

The lack of inhibitory effect in the presence of propanil treated soil is consistent with other reports (SHARABI, 1969; IBRAHIM, 1972), even though in this system the algae were not in direct contact with the soil. The lack of toxicity was not attributed to microbial degradation of propanil since sterilized soil gave the same result; it may, however, be related to a lack of sensitivity of the method used or to adsorption or photochemical inactivation in the soil.

In view of its known activity as an inhibitor of the Hill reaction in plant photosynthesis, it is not surprising to find that propanil immediately inhibits photosynthetic O_2-evolution in micro algae. Other workers have reported propanil inhibition of chlorophyll synthesis in micro algae (SHARABI, 1969; HAMDI *et al.*, 1970). Inhibition of photosynthesis would seem to be the primary mode of action of propanil on blue-green algae and SHARABI (1969) noted that inhibition of chlorophyll synthesis preceded changes in cell numbers in a green alga.

Another example of the rapid action of propanil was the marked lysis induced when it was added to *A. cylindrica* cultures. This, like the effect on photosynthesis is probably related to the ease and rapidity of propanil entry into algal cells (SHARABI, 1969). Although it was the only blue-green alga tested for the effect of adding propanil during algal growth, the post-lysis recovery of *A. cylindrica* is similar to that observed with *Chlorella* cells following a decline in cell numbers after adding 0.5 to 2 ppm propanil (WRIGHT & WALKER, unpublished data) and also for recovery of *C. aplanosporum* following inhibition (but no reported lysis) by 0.5 to 1 ppm propanil (SHARABI, 1969). Lysis of some cells and "recovery" of the culture suggest uneven distribution of sensitivity in the population.

Whilst propanil was stable in uninoculated media, the metabolite DCA was formed in algal cultures, especially those which became non-axenic through bacterial contamination. DCA is a reported propanil metabolite in different moulds (LANZILOTTA and PRAMER, 1970; WRIGHT and FOREY, 1972) and was also formed by the green alga *Chlorella* (WRIGHT and WALKER, unpublished). The low toxicity of DCA, relative to propanil, towards blue-green algae, is constitent with its lower toxicity to green algae (SHARABI, 1969; WRIGHT and WALKER, unpublished) and also with other reports on the detoxication of phenylamide herbicides as represented by their conversion to aniline compounds (CLARK and WRIGHT, 1970; WRIGHT, 1972). Recovery from propanil inhibition in *A. cylindrica* may be connected with DCA formation, but direct evidence has not yet been obtained. Also, the relatively low toxicity and non-persistence of DCA in algal cultures may be due to complexing with algal cells or extracellular slime, as occurs with humic materials in plants (YIH *et al.*, 1968) and soil (BARTHA, 1971). Another possibility is the formation of 3,3',4,4'-tetrachloroazobenzene (TCAB), which is known to occur in sunlight – exposed propanil solutions (MOILANEN and CROSBY, 1972), and which was not inhibitory to algae (SHARABI, 1969).

No consistent pattern was evident in the morphological or cytological changes in propanil treated algae. Granulation of cells and heterocyst frequency in *T. tenuis* decreased in the presence of propanil, whereas the proportion of akinetes and heterocysts increased in *A. cylindrica*, especially under N_2-fixing conditions. The latter observation may be linked with the relative resistance of *A. cylindrica* to propanil in such conditions in view of the spore-like nature of akinetes and the lack of photosystem II and Hill reaction activity in heterocysts (FOGG *et al.*, 1973), which also contain the nitrogenase. Increased heterocyst

frequency in pesticide (insecticide)-treated blue-green algae was also noted by SINGH (1973).

Although only one alga, *A. cylindrica*, was grown under N_2-fixing conditions in these studies, its lower sensitivity to propanil in this state is interesting in consideration of the possible significance of propanil and DCA interference with beneficial N_2-fixation by algae in rice fields. The effect of propanil on algal N_2-fixation is an aspect which we now propose to investigate more fully, together with studies on the fate of propanil and DCA in algal cultures.

Acknowledgements

The authors are indebted to Dr. A. D. Dodge who advised on the use of the O_2-electrode and to Mr. A. P. Walker for the use of some experimental data.

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The Effect of Microorganisms on Phytotoxicity of Herbicides. Part V. Interaction of some Microorganisms with Betanal*

By

N. BALICKA and Z. KRĘŻEL

Department of Microbiology, Agriculture Academy of Wrocław, Poland

The interaction of epiphytic bacteria and Betanal was examined. The selected bacterial strains were determined to be producers of substances inhibiting test plants and therefore our investigation indicated the interaction of two negative agents affecting the plants. Change in phytotoxicity of Betanal due to the presence of bacterial metabolites was found to depend on the way of their application. When the metabolites were introduced into plants through roots before spraying with Betanal then the effect of herbicide was reinforced. The foliar application of Betanal with bacterial metabolites brought about no change in herbicide activity. The substances produced by bacteria render the plants more susceptible to the harmful effect of Betanal.

Betanal is a herbicide used for foliar application in beet cultures. Phendipham (N-methoxycarbonylaminophenyl-N-3-methylphenylcarbamate) is an active substance in this herbicide; it is soluble in water in doses of 10 mg/litre and hydrolyzes to methyl N-3-hydroxyphenylcarbamate, 3-methylalanine and CO_2 (KOSSMAN, 1970). According to the information of the producer (SCHERING INFORMATION BETANAL, 1973) Betanal loses about 50% of its activity after 25–45 days in the soil and in the sugar beet tissue 20–40% after 8 days (KASSEBEER, 1969). The time of Betanal degradation in weed tissue is much longer and that is the reason for the Betanal selectivity (VAN DER ZWEEP *et al.*, 1969). The rate of Betanal degradation depends strongly on environmental pH; in neutral pH 50% of herbicide disappears after 10 minutes, but with pH 4 it persists for about 135 days. Betanal inhibits photosynthesis in the plants and causes disturbance in permeability of the chloroplast wall.

The foliar application of Betanal raises the question: whether the direct contact of herbicides with the microorganisms of the phyllosphere may be the reason for some change in herbicide phytotoxicity. The aim of this paper was to examine various forms of interaction between microorganisms and herbicides followed by change in the phytotoxicity of the latter (BALICKA 1975a; BALICKA *et al.*, 1975b and 1975c).

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Material and Methods

200 strains of bacteria and yeasts from the leaves of sugar beet and some weeds were collected. Their susceptibility to Betanal was studied with disc method after JOHNSON (1960) and thread method after KUHFUSS (1950). Phytotoxic effect of Betanal was determined by biological tests of *Sinapis alba*. The biological testing was performed in different ways.

1) Simultaneous introduction of herbicide and bacterial metabolites into the plants; a) Betanal in the dose of 1 ppm was suspended in the supernatant of bacterial culture and sprayed over the seedlings, b) seedlings were sprayed with bacterial culture incubated in the medium with Betanal (micropot experiment) in the dose of 1 ppm.

2) Pretreatment of plants with bacterial metabolites and then foliar application of Betanal; bacterial metabolites were introduced into seeds by soaking, or into plants by foliar spraying, or by saturating the growth medium (sand) in micropot experiment. Betanal was used in the dose of 1 ppm.

Of the 200 strains isolated, 12 bacterial strains resistant to Betanal and affecting the phytotoxicity of Betanal were selected. Some of them produced substances inhibiting test plants.

Results

There was an increased Betanal phytotoxicity due to the presence of bacteria from genus *Enterobacteriaceae* No. 206. The strain produced phytotoxic substances which killed about 50% of tested plants when introduced through seeds or roots. Foliar treatment resulted in death in only 10% of plants. So, the interaction of herbicides and bacterial metabolites depended on the ways of their application; when the metabolites had been introduced into plants b e f o r e spraying with Betanal then the effect of herbicide was more marked.

Such an effect was obtained by soaking the seeds (Fig. 1), or saturating the ground with bacterial metabolites (Fig. 2). The correlation coefficient in these cases was very high – 0.99. Foliar application of Betanal suspended in the bacterial supernatant killed the plants (Fig. 3), but that was the effect of herbicide, only. Foliar treatment of bacterial metabolites followed by Betanal (after 24 hours) also enhanced its inhibitory effect on plants, but the correlation coefficient was lower – 0.95 (Fig. 4). Betanal and bacteria during its incubation could be detected because of fluctuation of Betanal in the temperature of 28–30°C and in neutral medium.

The increase in Betanal phytotoxicity due to the presence of some bacteria may be due to various mechanisms. One of them might be analogous to the carrier of herbicides which affect cell wall permeability and enhance the penetration of active substances into plant tissue (KOSINKIEWICZ *et al.*, 1975). Another possibility may involve binding of herbicide and bacterial metabolites to the compounds more

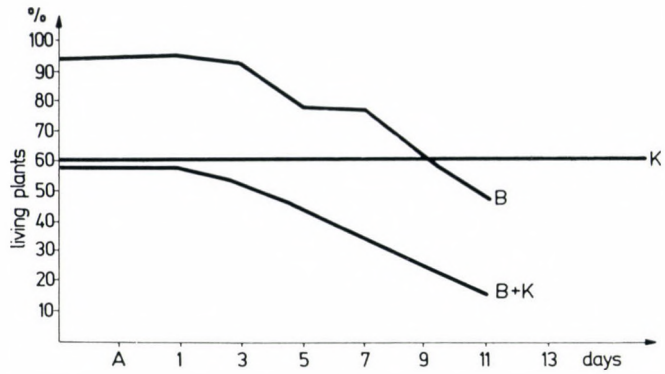

Fig. 1. Interaction of Betanal and bacterial culture. K — seeds soaked with the bacterial metabolites, B — seedlings sprayed with Betanal, K + B — seeds soaked with the bacterial metabolites, and seedlings sprayed with Betanal. Correlation coefficient — 0.99

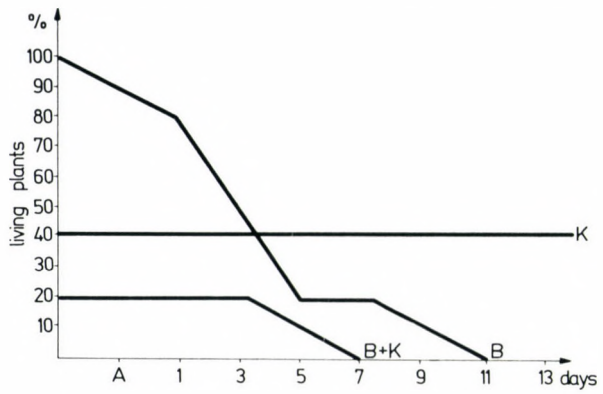

Fig. 2. Interaction of Betanal and bacterial culture. Micropot experiment. K — sand saturated with the bacterial culture, B — seedlings sprayed with Betanal, K + B — sand saturated with the bacterial culture and seedlings sprayed with Betanal. Correlation coefficient — 0.95

toxic than the individual component. In our case, both the mechanisms proposed are questionable because simultaneous application of herbicide with bacterial culture gives no effect. Yet, the bacterial metabolites made plants more sensitive to Betanal, probably due to the specific influence on the physiological processes.

We assumed that the polysaccharides produced by bacteria were responsible for such an effect (KRĘŻEL *et al.*, 1975); some sugars e.g. raffinose and maltose demonstrated the tendency to increase the phytotoxicity of Betanal. The attempt to combine the herbicide with the polysaccharides of bacterial origin from some *Enterobacteriaceae* cultures (*Klebsiella oxytoca* and *Escharichia coli*) confirms our assumption that the increase of Betanal phytotoxicity was 30% and 80%, respectively. But we have not obtained the same result with the strain No. 206 and therefore the problem has not yet been fully solved.

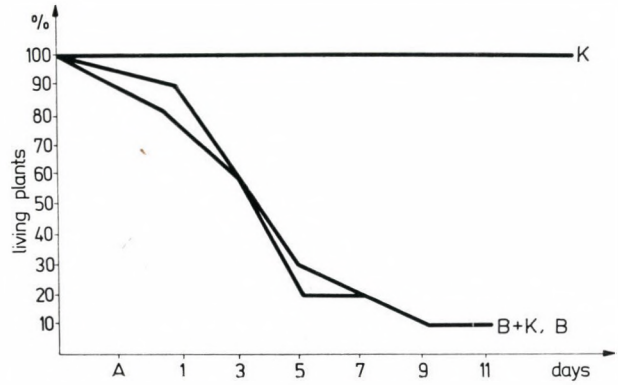

Fig. 3. Interaction of Betanal and bacterial culture. K — seedlings sprayed with the supernatant of the bacterial culture, B — seedlings sprayed with Betanal, K + B — seedlings sprayed with Betanal suspended in supernatant of the culture

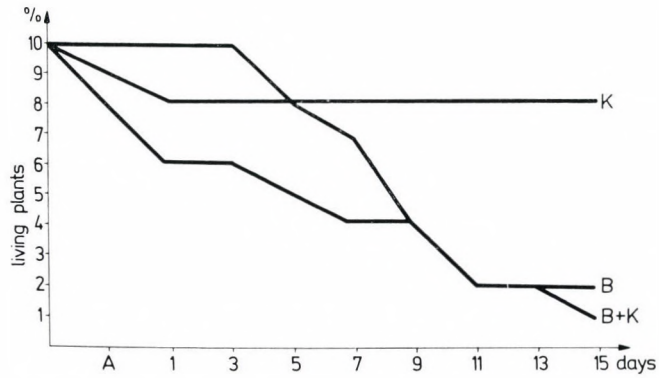

Fig. 4. Interaction of Betanal and bacterial culture. Micropot experiment. K — seedlings with the supernatant of the bacterial culture, B — seedlings sprayed with Betanal, K + B — seedlings sprayed with the supernatant of the bacterial culture followed with Betanal (after 24 hours). Correlation coefficient — 0.95

Conclusion

The experiments show that the increase of Betanal phytotoxicity in the presence of bacteria was not the simple summary effect of two phytotoxic agents (herbicide and metabolites of bacteria). It is highly probable that substances produced by the bacteria and penetrating through seeds or roots into plant tissue render them more susceptible to a harmful effect on Betanal and that is the reason for the increase of herbicide phytotoxicity.

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Detoxication of the Herbicide Propachlor by Certain Microscopic Fungi

By

V. RANKOV and B. VALEV

Vegetable Crops Research Institute "Maritsa", Plovdiv, Bulgaria

The participation of ten microscopic fungi from *Penicillium*, *Aspergillus*, *Fusarium* and *Trichoderma* in detoxication of the herbicide propachlor has been studied in a model experiment carried out for 120 days under controlled conditions (temperature 28°C and soil humidity 65%). The trial was conducted with alluvial meadow soil: 1. nonsterilized, 2. sterilized, 3. non-sterilized + 200 ml Czapek's nutrient liquid medium per 1000 g soil, 4. sterilized + nutrient medium. The residual herbicide amounts were determined by test culture — brewing barley.

The results show that the microscopic fungi from *Penicillium (P. liliacinum, P. ciclopium, P. pupurogenum, P. sclerotiorum)*. *Aspergillus (Asp. niger, Asp. flavus)* and *Trichoderma viride* quicken the degradation of the herbicide propachlor and on account of this they participate in its detoxication.

The ways of detoxication of herbicides in soil are physical-chemical, chemical and biological (AUDUS, 1960; KEARNEY and KAUFMAN, 1969; Anonymous, 1970). However more and more investigations show that soil microorganisms are the decisive factor for decomposition of herbicides (AUDUS, 1960; ILIN, 1965; KEARNEY *et al.*, 1967; KEARNEY and KAUFMAN, 1969; Anonymous, 1970).

The different herbicides are the object of decomposition by the soil microorganisms which use the latter for food and as a source of energy. Various soil microorganisms take part in the detoxication of herbicides. With regard to this the microscopic fungi count for a great deal. According to JENSEN (1959) delapon is degradated by the microscopic fungus *Trichoderma viride*. HILL's (1956) studies show that soil fungi from the species *Penicillium* and *Aspergillus* are capable of using monuron as the only source of nitrogen. Our investigations (RANKOV-1968), conducted *in vitro* with more than 60 species of soil microorganisms (bacteria, actinomyces and microscopic fungi) show that some strains from *Aspergillus (Asp. niger, Asp. flavus)*, *Penicillium (P. notatum, P. clavigerum)* can use the herbicides monolinuron and linuron as a sole nitrogen source. According to BAKALIVANOV (1972) some microscopic fungi as Asper*gillus terreus*, *Penicillium conescens*, *Trichoderma lignorum* decompose some 90% of the herbicides introduced in the nutrient medium, the inactivation of herbicide simazine being weaker than that of atrazine. According to FLETCHER (1960) by BURGES and RAW (1967), the microscopic fungi from *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma* can decompose simazine and atrazine, whereas simeron is decomposed by *Aspergillus*.

With regard to this a number of other herbicides are reported (BURGES and RAW, 1967; CHARPANTIER and POCHON, 1962; KALININ and MERJINSKII, 1965; KEARNEY *et al.*, 1967; Anonymous, 1970).

Material and Methods

The possibility for decomposition of the herbicide propachlor N-isopropyl-chloracetanilide by certain microscopic fungi in a model experiment has also been studied. According to KAUFMAN *et al.* (1971), propachlor is metabolised by *Fusarium oxysporum*, by which metabolite 2-hidroxy-N-isopropylacetanilide has been found. The herbicide propachlor is widely applied by some vegetable crops (VELEV and RANKOV, 1975) for weed control and on account of this, studying the possibilities for its detoxication is of interest. According to MAIER-BODE, (1971) in the recommended doses for practice (4.0 – 4.5 kg/ha) propachlor has a persistence of about two months in the soil. This period is longer in light soils (FETVADJIEVA, 1973) and in increased doses (LUOBENOV, 1971).

The model experiment was carried out under controlled conditions (temperature 28°C and soil humidity 65%) in pots of 1 kg in four treatments. Alluvial meadow soil with light mechanical composition, sandy loam soil with comparatively low humus content – 1.8% with soil reaction – pH 6.8 – 7.0 (in water).

Ten strains of soil microscopic fungi from *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma* were used. The fungi were cultivated on 200 ml Czapek's liquid nutrient medium is Erlenmayer flasks of 500 ml for 8 days at 28°C. The mycelium obtained from one flask, after drying and lightly rubbing flat is mixed with the soil from one pot of 1000 g. The experiment with each of the microscopic fungi was carried out to the following scheme: 1. non-sterilized soil, 2. sterilized soil at temperature 160°C for 3 hours, 3. non-sterilized soil, moistened with 200 ml CZAPEK's nutrient medium, 4. sterilized soil + moistening with nutrient medium. The addition of nutrient medium was aimed at improving the nutritive regime and the conditions for the development of the strains applied in the soil. The same schemes of soil were used as controls without adding mycelium from microscopic fungi. Having in mind the number of strains and the adopted scheme of soil use, we should mention that the experiment was conduced in 44 treatments. The herbicide was applied to the soil at a dose of 6.7 ppm at the start of the experiment.

The residual herbicide amounts were determined according to Reisler's method (KEARNEY and KAUFMAN, 1969) by using brewing barley as test culture – indicator, which was proved to be very susceptible to this herbicide. Soil samples are analyzed dynamically in the course of 120 days.

Results

Data presented in Fig. 1 show that after application of mycelium from microscopic fungi of *Penicillium (Pen. lilacinum, Pen. cyclopium, Pen. purpurogenum, Pen. sclerotiorum)* on the 30th day the herbicide was completely decom-

posed, or there were only trace remains left; in all treatments the residual values were below 0.42 ppm, generally below 0.15 – 0.20 ppm. The results of the treatments with nutrient medium application are similar. The more vigorous development of the test culture and some of the treatments is explained with the herbicide traces left which stimulate the plant growth.

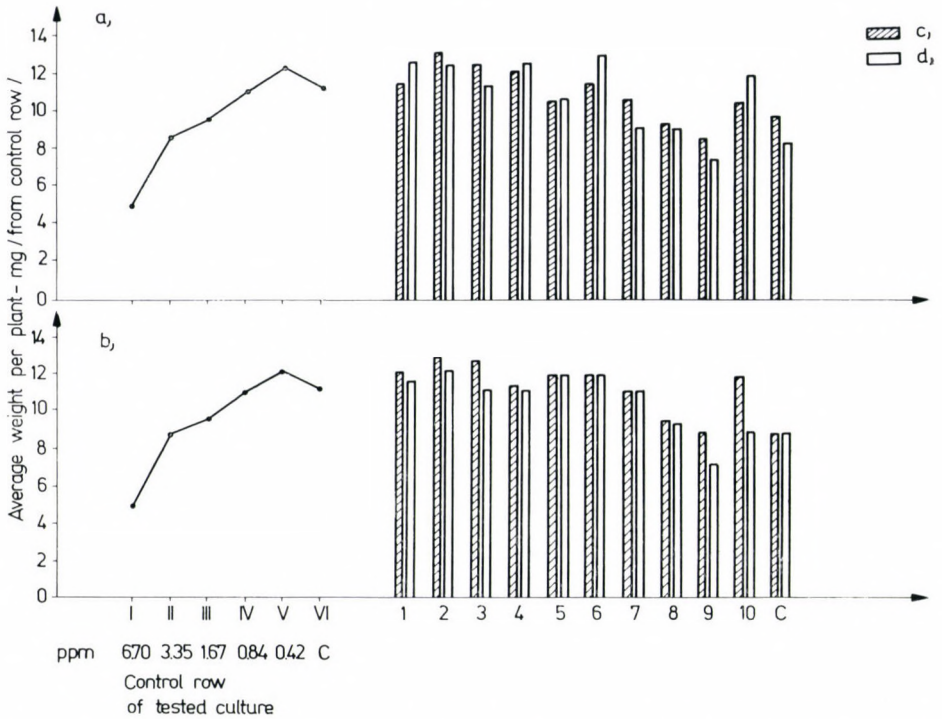

Fig. 1. Residual amounts from the herbicide propachlor on the 30th day after its application in soil. a) Soil, b) soil + nutrient medium, c) non-sterilized soil, d) sterilized soil. Microscopic fungi: 1. *Penicillium lilacium*, 2. *Penicillium cyclopium*, 3. *Penicillium purpurogenum*, 4. *Penicillium sclerotiorum*, 5. *Aspergillus niger*, 6. *Aspergillus flavus*, 7. *Fusarium moniliforme*, 8. *Fusarium sambucinum*, 9. *Trichoderma lignorum*, 10. *Trichoderma viride*. C — control without mycelium from microscopic fungus

Herbicide residual amounts after mycelium application from *Aspergillus (Asp. niger, Asp. flavus)* are insignificant. But it is found that they are significant after mycelium application from *Fusarium (F. moniliforme, F. sambucinum)* — approximately 1.67 ppm or about 25% from the herbicide is not decomposed. This is more apparently expressed by application of this mycelium in sterilized soil where one can not expect herbicide decomposition by other soil microorganisms. The highest residual amount is noted after application of mycelium from *Trichoderma lignorum* in sterilized soil — almost 5 ppm, or about 74% of the

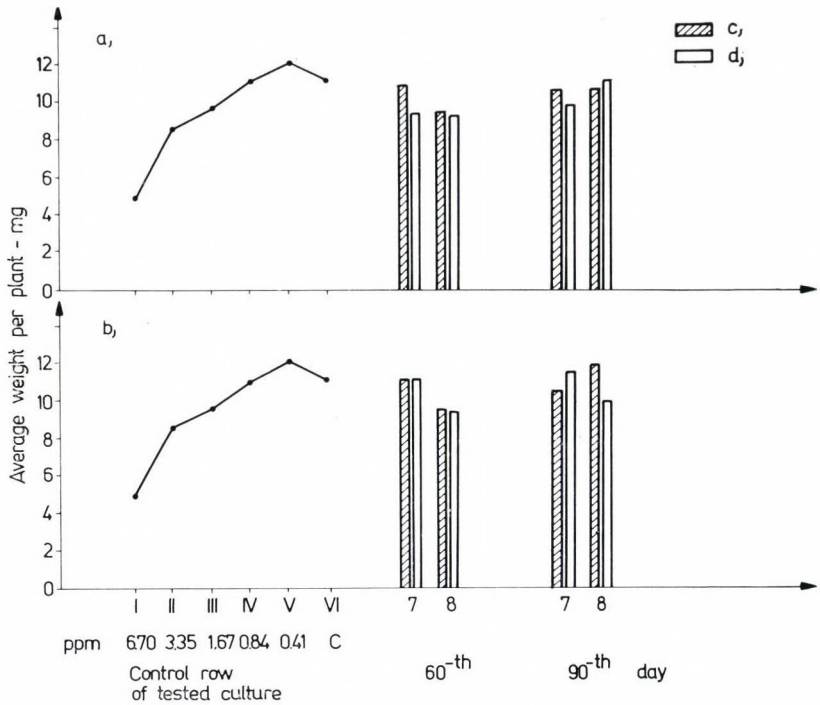

Fig. 2. Residual herbicide amounts in certain treatments of the experiment on 60th day of its application in soil. a) Soil, b) soil + nutrient medium, c) non-sterilized soil, d) sterilized soil. Microscopic fungi: 7. *Fusarium moniliforme*, 8. *Fusarium sambucinum*

applied herbicide quantity. Application of nutrient medium in soil does not cause changes too. However, with application of mycelium from this microscopic fungus in non-sterilized, ordinary soil, the residual amounts were 3.2 ppm. They were considerably lower after mycelium application from *Trichoderma viride*.

Higher residual herbicide quantities were determined in the control soil too — 1.55 ppm. The highest amounts were in sterilized soil — 3.35 ppm, or about 50% of the applied quantity. Considerably lower residual amount of herbicide in non-sterilized soil is due to the effect of the microorganisms which accelerate its decomposition. A similar result was obtained for the residue content in the control after adding nutrient medium — 3.0 ppm.

No residual herbicide amounts were obtained on the 60th day in most of the treatments. On account of that in Fig. 2 are shown only the treatments in which mycelium was applied from microscopic fungi but residual amounts are still to be determined. Higher herbicide amounts are found after mycelium from *Fusarium sambucinum* — 2.30 ppm in comparison with *Fusarium moniliforme*. Moistening of soil (sterilized and non-sterilized) with nutrient medium lead to essential

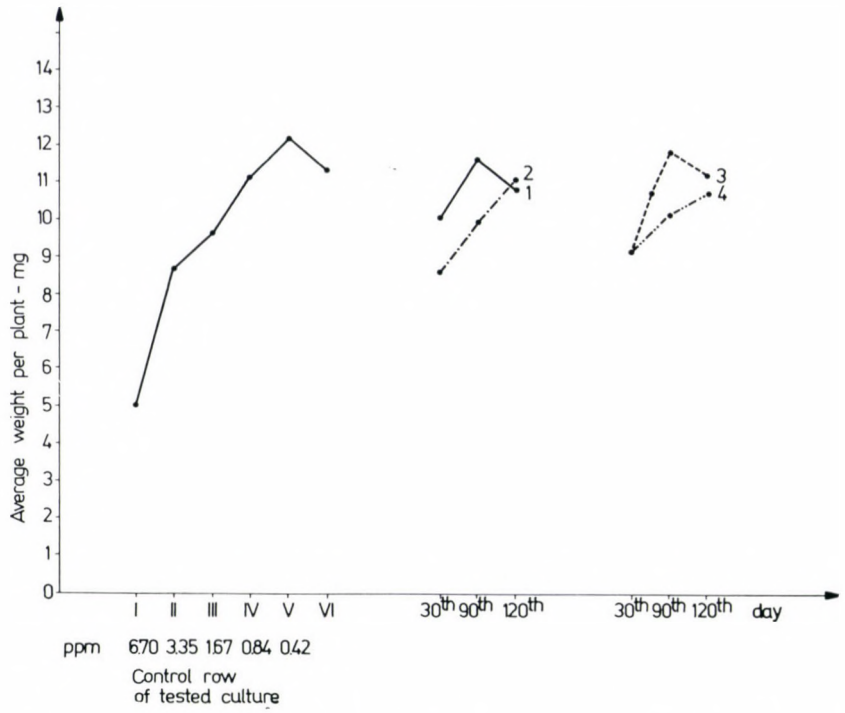

Fig. 3. Residual herbicide amounts in the control treatments of the experiment. 1. Non-sterilized soil, 2. sterilized soil, 3. non-sterilized soil + nutrient medium, 4. sterilized soil + nutrient medium

changes in the content of residual herbicide amounts. Low herbicide amounts (traces), or none are determined on the 90th day.

Decomposition of herbicide in the control treatment is similar to this.

Comparing the four cases of herbicide decomposition in soil without mycelium application from microscopic fungi, it is obvious that in sterilized soil the decomposition is much slower than that in non-sterilized one. No herbicide residues are found in the sterilized soil until the 120th day.

Conclusions

The microscopic fungi from *Penicillium (P. lilacinum, P. cyclopium, P. purpurogenum, P. sclerotiorum)*, *Aspergillus (Asp. niger, Asp. flavus)* and *Trichoderma viride*, accelerate decomposition of the herbicide propachlor, thus taking part in its detoxication.

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Some Data on the Decompositon of Dinitro-*o*-Cresol by Microorganisms

By

MÁRIA NEHÉZ, ANNA PÁLDY and A. SELYPES

Institute of Public Health and Epidemiology, Medical University of Szeged, Hungary

The pollution of our environment by pesticides is one of the basic problems of public health nowadays. The quicker the decomposition of a pesticide is, the more advantageous it is from hygienic aspects. From this point of view we investigated the decomposition of dinitro-*o*-cresol (DNOC), the effective agent of the pesticide 'Krezonit E' several bacteria. In our experiments we chose *E. coli*, *P. vulgaris*, *B. cereus*, and *B. subtilis* which can be found in the soil and easily cultivated in laboratories. It has turned out that all of these bacteria have an effect on the decomposition of especially at 37°C, the optimal temperature for these bacteria. During the investigations it emerged, whether these bacteria affect the toxicity of the pesticide during te decomposition, and to what extent the metabolites are toxic. Therefore by intraperitoneal administration to white mice we stated the LD_{50} before and after the microbiological decomposition of DNOC by the method of LITCHFIELD and WILCOXON. The DNOC quantity of the solution was evaluated by the PARKER method. Accoding to our results during the microbiological decomposition of DNOC toxic metabolite or metabolites are not formed.

There are more than 1000 different pesticides available in the world (20). In Hungary the number of pesticides provisionally or permanently permitted is 289, the number permitted for experimental use is 76 (Permitted pesticides 1976). The hygienic and toxicological effect of various pesticides is important. The pollution of our enviroment by pesticides is one of the basic problems of public health nowadays.

Dinitro-*o*-cresol (DNOC) is a fenol derivative belonging to the organic insecticides and herbicides, a yellow powder dissolving at 85.8°C, not so well in water but quite well in organic solvent. It is the oldest insecticide in use since the turn of the century. Under the name Antinonnin it has been kept on the market by Bayer since 1892. In France G. Truffaut and J. Pastac discovered in 1932 that DNOC can also be used as a contact herbicide. It retards the development of dicotyledonous plants while not effecting others sprouting into ears. It may cause serious poisoning to those exposed to inhaling powder particles or spray drops when handling it. The tolerable maximum of its fume concentration in the air is 0.1 mg/m^3 (2). Its solution is easily absorbed from the intestine and even through the skin. It excretes in the urine partly in an unchanged and partly in a reduced form (one of the nitro groups turning into an amino group). In case of chronic

absorption, excretion is not complete, some cumulation may be observed. The action mechanism of DNOC causes a disorder in the oxidative phosphorylation processes of the intermediary metabolism. It separates the oxidation and the phosphorylation, prevents the building up of high energy content phosphate esters as a result of oxidation, and obstructs the forming of adenosintriphosphate. The processes of oxidation are hightened but the energy released during oxidation is not put to use, it is transformed into heat. The body temperature may eventually rise even to 40°C. The basal metabolism may rise to 10–12 times of its normal state owing to the effect of toxic doses. Their toxicity decreases with cold but increases with heat. The climatic situation may also influence the toxic effects (4, 5, 6, 12, 22, 24, 25).

Pesticides containing dinitro-*o*-cresol used in Hungary are Krezonit E and Novenda. The effective agent in the former is 50 per cent and in the latter 25 per cent. Krezonit E, the subject of our investigation is a concentration of 0.7–0.9% when used as insecticide. When it is used as a herbicide the concentration is 2.8–3.8 kg/ha, and when only for defoliation 520–700 litre/ha is in the 1.0–1.5% solution. From the aspect of public health, the prohibition period for consuming treated foodstuff is 10 and 60 days respectively. The tolerated residue of the agent is 0.1 mg/kg (7, 14, 24). The longer the persistence of the pesticide applied is, the higher the possibility of chemical exposition will be. The quicker the decomposition of a pesticide is, the more advantageous it will be from hygienic aspects. Part of the pesticides that gets into the soil is metabolized, to a certain extent by the microflora in it. Disulphoton, for instance, oxidates and decomposes only to a limited extent in the soil, whereas Triallat and Dikobenil does not decompose in sterilized soil (1, 3, 6, 8, 9, 16, 18, 19, 21, 23).

1. The microbiological decomposition of dinitro-*o*-cresol

Starting from the conception mentioned above, the first question we posed in our investigation was the following: to what extent is the decomposition of the effective agent of Krezonit E (DNOC) dependent on the action of Gram-negative *E. coli*, *P. vulgaris* and Gram-positive *B. Cereus*, *B. subtilis*.

Material and Methods

For the experiment we used a 12-hour bouillon cultivation of *E. coli* and *P. vulgaris* and a spore infected 6–8-hour boullion cultivation of *B. cereus* and *B. subtilis* containing 50.10^6 bacteria and 20 γ DNOC per ml. One part of these cultivations was incubated at room temperature, at 22°C, and the other at 37°C. The determination of the DNOC content was done after 4, 24, 48, 96 and 144 hours by Parker's method (17) in three parallel photometric measurements. The experiments were repeated twice, and when evaluating the results, the standard

deviation was determined. Simultaneously with these measurements, the chemical decomposition of DNOC was controlled under sterile conditions, incubated for 22 days in sterile distilled water and then in a solution of sterile bouillon.

Results

Our results show that each strain of the bacteria used has an effect on the decomposition of DNOC, especially at 37°C, the optimum temperature for these bacteria.

But in sterile distilled water and sterile bouillon the chemical decomposition of DNOC during the 22-day period of observation did not occur (15).

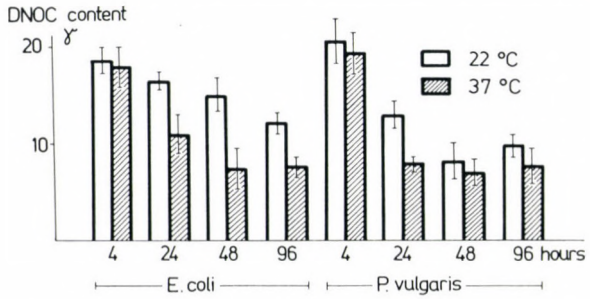

Fig. 1. Microbiological metabolization of DNOC by the effect of *E. coli* and *P. vulgaris*

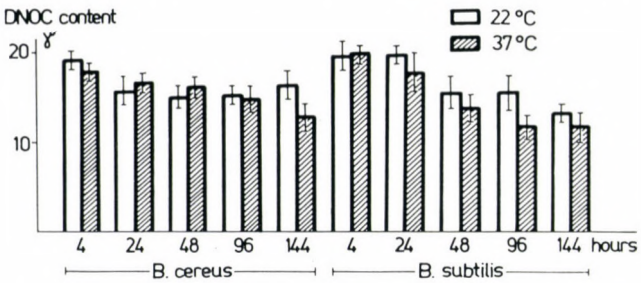

Fig. 2. Microbiological metabolization of DNOC by the effect of *B. cereus* and *B. subtilis*

Table 1

DNOC content after 22 days' incubation

DNOC containing solution (20 γ)	Incubated on 22°C		Incubated on 37°C	
	DNOC content γ	σ	DNOC content, γ	σ
Sterile destilled water	20.0	±0.9	20.2	±1.0
Sterile bouillon	19.2	±0.9	19.6	±0.8

II. The microbiological decomposition from the point of view of toxicity

During the investigation the question arose, whether these bacterial strains affect the toxicity of the pesticide during metabolization, and to what extent the metabolites are toxic. Therefore by intraperitoneal injection to white mice the LD_{50} value was determined before and after the microbiological decomposition of DNOC.

Material and Method

For the experiment non-pure strain, male white mice were used weighing 25 – 30 g each. The death rate of the animals following the intraperitoneal injection was observed in 24 hours, 48 hours and a week. The 24-hour death rate did not change during further observation. The LD_{50} value expressed in DNOC content was determined on the basis of the 24-hour death rate by the method of LITCHFIELD and WILCOXON.

For investigating the effect of microbiological metabolization, a 12-hour bouillon cultivated colony of the named bacterial strains was used. The experiment was set in such a way that, after adding a solution of sterile bouillon and Krezonit E, the starting concentration of DNOC should be 500 γ/ml. Half of this solution containing the bacteria was filtrated with Seitz filter right after the measurings, inactivated according to need, and intraperitoneally injected into the mice. Five doses were prepared expressed in DNOC effective agent value increasing from 20 mg/kg to 40 mg/kg and administered per dose to 10 – 10 mice. Following this the LD_{50} value was determined as described above. This gave the data obtained before the microbiological metabolization. The other half of the solution containing the bacteria, after 2- and 4-day incubation at 37°C, was subjected to the same examination. This gave the data obtained after the microbiological decomposition. At the intraperitoneal injections administered after decomposition, the decrease of DNOC concentration owing to decomposition was not taken into account because the ml/mouse weight of the material injected after decomposition was identical with the 500 γ/ml solution before decomposition. Thus the LD_{50} values obtained in this way – evaluated by the method of LITCHFIELD and WILCOXON – occurred only as a shift of the curve in the region of the co-ordinate in relation to the values obtained before microbiological metabolization. In the Table these values are marked with.* The ml/mouse weight of the solution injected after microbiological decomposition was identical with the one injected before metabolization, however, the DNOC content was 28 – 58% lower than before microbiological metabolization.

The extent of the microbiological decomposition of DNOC or rather the DNOC quantity of the solution was evaluated by the *Parker* method, 3 – 3 parallel photometric measuring being applied in every case (17). The LD_{16} and LD_{84} values were also determined in every case.

Table 2

Changing of the DNOC content by the effect of the microbiological metabolization
DNOC content of the solutions used for the i.p. injection of mice

Bacterium	Before	After	
	the microbiological metabolization γ/ml		per cent
E. coli	500	360	72
P. vulgaris	500	280	56
B. cereus	500	210	42
B. subtilis	500	330	66

Results and Discussion

The LD_{50} values expressed in DNOC content before microbiological metabolization, and in relation with these the LD_{50} values obtained after microbiological decomposition are shown in Fig. 3 and Table 3.

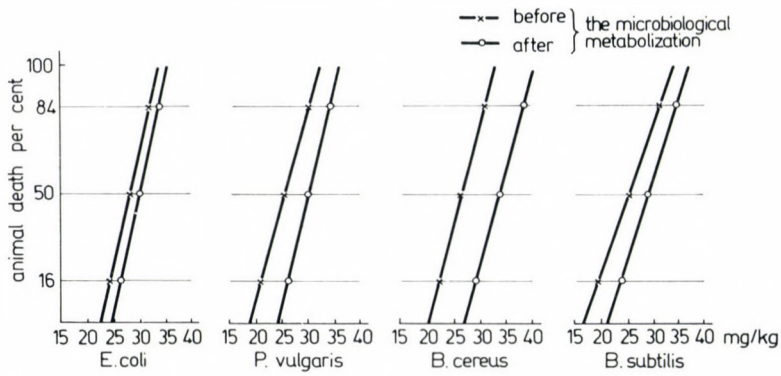

Fig. 3. The changing of LD_{16}; LD_{84} by the effect of microbiological metabolization

Table 3

LD_{50} results effected by the microbiological metabolization

Bacterium	Before		* After	
	the microbiological metabolization			
	LD_{50} mg/kg	Confidence limits, mg/kg	LD_{50} mg/kg	Confidence limits, mg/kg
E. coli	28.8	26.5—31.3	30.0	28.3—31.8
P. vulgaris	25.7	23.4—28.3	30.0	28.3—31.8
B. cereus	26.8	24.6—29.2	33.7	31.6—35.9
B. subtilis	25.2	22.5—27.0	29.1	26.9—31.4

The death values obtained after microbiological decomposition shifted toward the higher dose region. The LD_{50} values that shifted to the higher value region, in number and also graphically, illustrate well that after the microbiological decomposition caused by the bacteria used, the toxicity of the solutions of Krezonit E containing DNOC decreased, and this corresponds approximately to the per cent decrease of the DNOC content owing to the action of decomposition. The results of the investigations suggest that during the decomposition of the pesticide Krezonit E with its dinitro-*o*-cresol content exposed to the action of Gram-negative *E. coli*, *P. vulgaris* and of Gram-positive *B. cereus* and *B. subtilis*, less toxic metabolite or metabolites develop. It is known from the literature that one species of *Pseudomonas* and one species of *Arthrobacter* decomposes DNOC probably by oxidation–reduction. In pure cultures of these bacteria 2,3,5-trihydroxytoluene was found as an intermediate product before the ring cleavage. The reaction series resulting in the hydroxidated product was different with these two species of bacteria. During the decomposition of *Pseudomonas* an intermediate product, 3-methyl-5-amino-catechol was formed, owing to the reduction of the corresponding nitro component, the 3-methyl-5-nitro catechol. The removal of the nitro group is probably due to the action of enzymes (26). The bacteria use the metabolites as C and N sources for their own building material (1, 11). *Corynebacterium simplex* decomposes DNOC to a colourless matter and nitrite ion (10). In this investigation the process through which DNOC was decomposed by the above named bacteria was not studied. This problem needs further investigation.

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Xenobiotics and Soil Microbiota Affected by Xenobiotic Interactions. III. 2,4-D-Na and the Species Composition of Fungi in a Chernozem

By

Cs. Dobolyi, Zs. Pásztor and M. Kecskés

National Research Institute of Hygiene, Budapest; Research Institute of Soil Sciences and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary

In the presence of atrazine, the decomposition rate of 2,4-D-Na was slower. The number of microscopic fungi decreased in the first three weeks but increased at the fourth week of the incubation period in chernozem with forest residues soil type investigated in laboratory (at 50% of maximum water capacity and 26–28°C).

On the effect of experimental high doses of 2,4-D-Na and 2,4-D-Na + atrazine the occurrence of aspergilli and fungi having dark hyphae or some melanoide pigmentation organ decreased, penicillia and fungi having hyaline colonies increased. The last group involved a lot of yeasts (pathogenous fungi) 211 fungal isolates belonging to 45 species of 30 genera were identified in the control soil, and their occurrence in the different treatments was observed.

Using pesticides as well as combinations (Kaufman, 1970; 1972) in the intensive agricultural production, many interactions can occur among the pesticides in the soil. So for example dalapon and insectides (disulphoton phorate, carbaryl) applied together increase the phytotoxicity of dalapon towards *Avena sativa* (Nash, 1967). In the presence of atrazine and insecticides (DDT, Diazinon, parathion, carbifuran) the mortality of *Drosphila melanogaster* increases (Lichenstein *et al.*, 1973) this synergetic inhibition depends on the environmental factors (Liang and Lichenstein, 1974).

Microbial degradation of dalapon has been found to decrease by amitrol (Kaufman *et al.*, 1970). The enzyme system of soil microbes hydrolizing the phenylcarbamates is competitively inhibited by methylcarbamate pesticides (Kaufman, 1970). Moreover the PCMC decomposition was inhibited by chlorporpham (Priest and Stephens, 1975). In the case of propanil and solan Bartha (1969) reported hybrid products as a result of interactions.

Many combination of 2,4-D-Na and atrazine (Dikonirt + Hungazin PK), 2,4-D- and atrazine containing combinations (Nitrikol, Hungaria K 64, Hungaria Viratol) and other phenoxy derivatives + atrazine combinations are used in Hungary.

Continuing our work on 2,4-D and atrazine (Kecskés *et al.*, 1975; Pásztor *et al.*, 1977, etc.) we wanted to provide some data on the decomposition of 2,4-D-Na and the fungal microflora affected by 2,4-D-Na and atrazine interactions in soil ecosystems.

Materials and Methods

0.01, 0.1 and 1% technically pure 2,4-D-Na as well as 0.1% atrazine were added to chernozem with forest residues and they were mixed homogeneously (SZÜCS, 1963). Water was added also to 50% of the maximal water capacity of soil and the samples were incubated at 26°C for 28 days. The herbicide residues were determined by gas chromatography (methylester, electron capture detector). The effect of herbicides, herbicide interactions on mycoflora was followed by soil suspension dilution technique, using corn meal agar with chlorocide; the number of colonies was counted and the isolates were identified.

On the basis of colony morphology and pigmentation and the frequency of occurrence of the fungi in the differently treated soils, the fungi were divided into four groups: Aspergilli, Penicillia, "hyaline fungi" and fungi with dark colony or dark organs.

Results and Discussion

More residues of the 0.01% and 0.1% 2,4-D-Na were found than in the soil samples treated only with 2,4-D-Na on the inhibitory effect of atrazine addition. In the soils treated only with 0.01% 2,4-D-Na or in those containing 0.1% atrazine too the 2,4-D-Na content decreased under 1% of the original quantity (Fig. 1). In the case of the very high 2,4-D-Na doses, the residues determined were nearly the same with atrazine and without the addition of atrazine.

The number of the "total" fungal elements which is not the same as the number of cells present because the shorter or longer fragments of the fungal hyphae represent only one colony-forming unit, was 128.500 in the ecosystems without herbicide treatments on the effect of 2,4-D-Na (Fig. 2). In the function

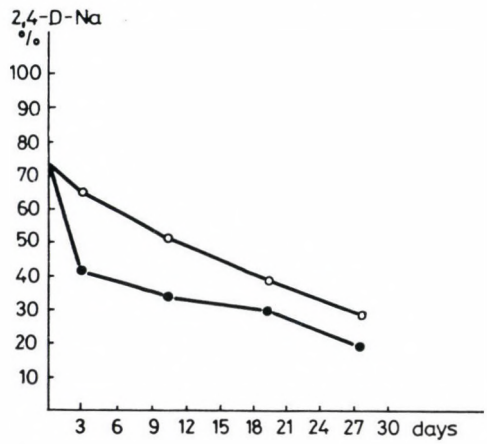


Fig. 1. The effect of atrazine on the decomposition of 0.1% 2,4-D-Na (unshaded circles: 2,4-D-Na) (shaded circles: 2,4-D-Na + atrazine)

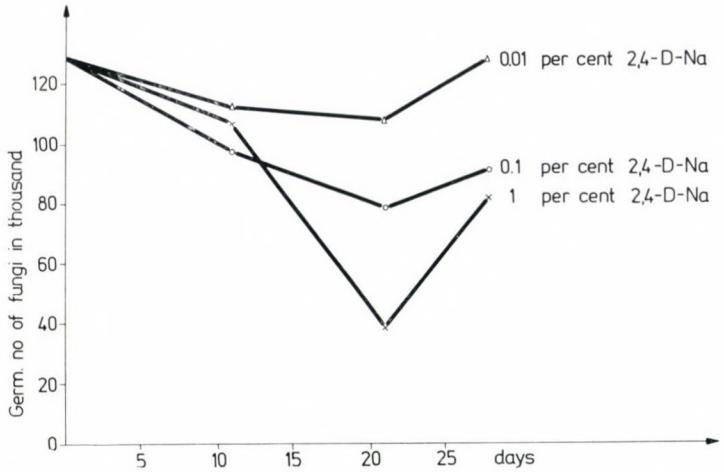


Fig. 2. Number of "total" fungal elements affected by different doses of 2,4-D-Na

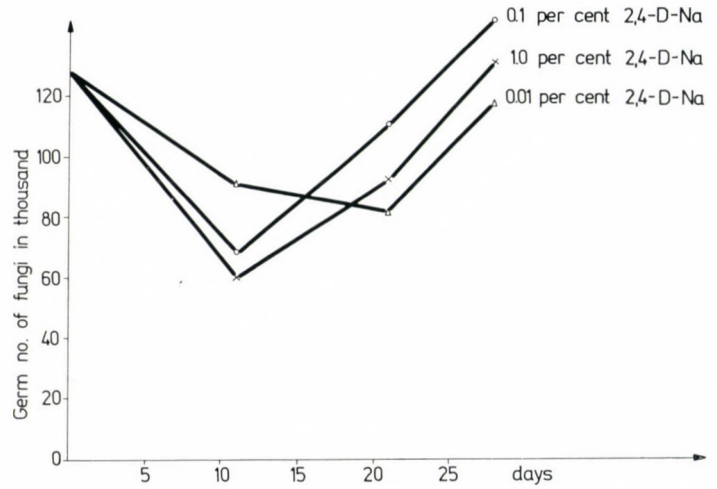


Fig. 3. Number of total fungal elements affected by different doses of 2,4-D-Na + atrazine

of the applied concentration the number of fungi decreased in the first three weeks and increased at the fourth week of the incubation period.

At the minimum on the 21st day, on the effect of the 0.01% 2,4-D-Na, the number of the fungal elements decreased to 91% in the case of the 0.1% and 1% herbicide doses by 67% and 30% respectively.

The increase of the fourth week is very significant, nearly the original quantity was registered. This tendency was more emphasized with the addition of 0.1% atrazine (Fig. 3). The minimum of fungi was noticed on the 11th day of incubation.

When the 0.1, and 1 % 2,4-D-Na and 0.1 % atrazine were added to the soil together the "total" number of fungi exceeded in number the control on the 28th day, and in the case of the 0.01 % 2,4-D-Na samples (where the atrazine was present and in one order of magnitude more quantity) was nearly the same as the control. The pro anal. 2,4-D affected the mycoflora essentially in the same way as the 2,4-D-Na, but it was less toxic in the first days and later the increase of the number of fungal elements was more moderate.

Table 1

Fungal species occurring in chernozem with forest residues soil

Acremonium kiliense	Pestalotia sp.
Acremonium ochraceum	Phialophora sp.
Actinomucor repens	Phoma sp.
Alternaria alternata	Rhizoctonia sp.
Aspergillus (6 species)	Saccharomyces marxianus
Aureobasidium pullulans	Sclerotium sp.
Botrytis cinerea	Sphaeropsis sp.
Cladosporium herbarium	Spicaria violacea
Coniothyrium sp.	Stachybotrys atra
Cryptococcus albidus var. diffluens	Stemphylium botryosum
	Stysanus stemonitis
Custingophora olivacea	Torulopsis candida
Fusarium oxysporum	Trichoderma viride
Fusarium solani	Verticillium falcatum
Fusidium sp.	Verticillium sp.
Mucor racemosus	Volutella fructi
Paecilomyces vanioti	
Penicillium (8 species)	

The species composition of the intact soil was established with the identification of 211 isolates; 45 species belonging to 30 genera were identified (Table 1). Observing the frequency of occurrence of the fungal species together it was found that the decrease and increase of the total number of fungi could be characterized

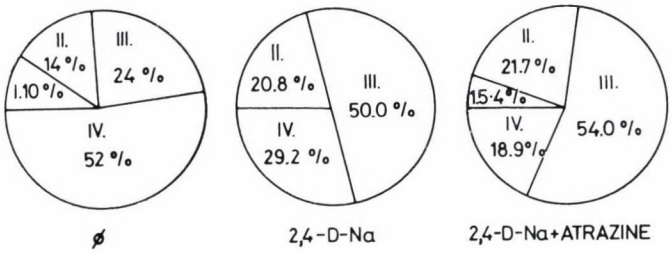
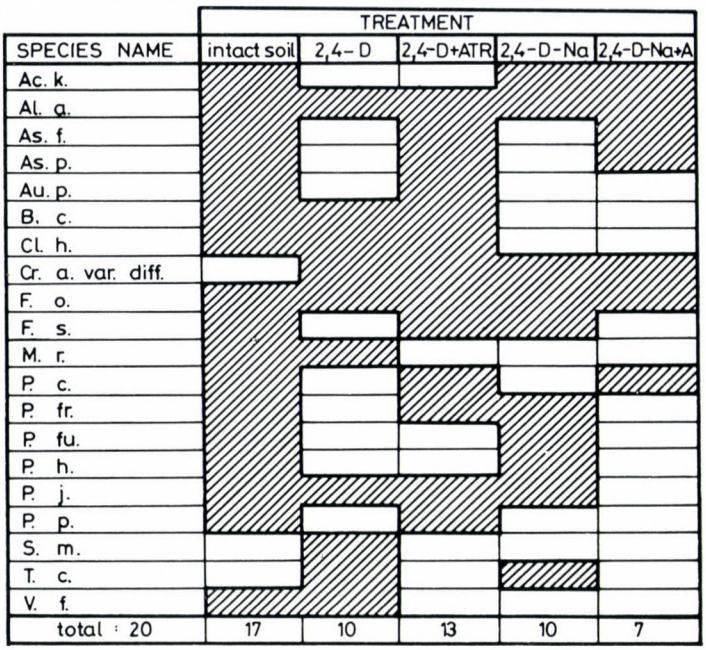


Fig. 4. Ratio of different groups of fungi affected by 1000 ppm 2,4-D-Na + 1000 ppm atrazine

Table 2

The effect of 2,4-D + atrazine as well as 2,4-D-Na + atrazine on the occurrence of fungal species in chernozem exosystems



Shaded = the presence of the species
Unshaded = the absence of the species
AC = Acremonium kiliense
Al.a. = Alternaria alternata
As.f. = Aspergillus fumigatus
As.p. = Aspergillus puniceus
B.c. = Botrytis cinerea
Cl.h. = Cladosporium herbarum
Cr.a. var. diff. = Cryptococcus albidus var. diffluens
F.o. = Fusarium oxysporum
F.s. = Fusarium solani
M.r. = Mucor racemosus
P.C. = Penicillium cyclopium
P.fr. = Penicillium frequentans
P.fu. = Penicillium funiculosum
P.h. = Penicillium herque
P.j. = Penicillium janthinellum
P.p. = Penicillium purpurogenum
S.m. = Saccharomyces marxianus
T.c. = Torulopsis candida
V.f. = Verticillium falcatum

with the change of the relative and absolute number of special groups having different colony morphology and pigmentation. The ratio of these groups changed in the presence of initial doses of 1000 ppm 2,4-D-Na and 1000 ppm atrazine after 28 days' incubation period in the following way (Fig. 4). *a) Aspergilli* representing 10% of the mycoflora of intact chernozem soil were not discovered in the samples treated with 2,4-D-Na and on the effect of 2,4-D-Na + atrazine their occurrence decreased markedly. *b) Penicillina* occurring in 14% ratio originally were found three times more on the effect of herbicide treatments.

The change was more emphasized in the other two groups of fungi representing more than 75% of the "total" fungi counted: *c)* Fungi having hyaline colonies (Moniliaceae, yeasts etc.) occurring in the 24% of control soil increased in 2,4-D-Na treatments to 50% and in the presence of 2,4-D-Na and atrazine even more (54%). *d)* Groups of fungi having dark hypha or some organs with melanoid pigmentation that is "dark colony" fungi (Ascomycetes with fruiting bodies, Sphaeropsidales, Melanconiales, Mycelia Sterilia with dark sclerotium, Dematiaceae) which occurred by more than 50% in the control soil decreased on the effect of 2,4-D-Na from 52% to 29% and much more decrease was observed on the interaction of 2,4-D-Na and atrazine (19%). As it was mentioned earlier comparative investigations were carried out using analytically pure 2,4-D instead of 2,4-D-Na and the results were near to the above description.

To evaluate the method used by us we have to mention DOMSCH (1970) in that the dilution techniques for microflora do not give us the information most appropriate for the analysis of soil metabolic patterns because we could receive much about the population of resting spores but virtually nothing about active mycelia. New techniques are undoubtedly needed however, in spite of this we found that it may be supposed that there are our results with the old technique indicated significant effects of fungi and some higher role of microscopic fungi in the decomposition or detoxication of atrazine and 2,4-D in soil. Though there are data in the literature on microscopic fungi decomposing these active ingredients separately in axonic cultures and in the soil too (KAUFMAN and BLAKE, 1969, 1970 and others: KECSKÉS, 1976), the effect of their interactions on soil ecosystems needs some further investigations.

As regards the effect of interaction of 2,4-D-Na and atrazine on the species decomposition in chernozem soil it is also remarkable from many ecological points of view because of the relationship of pathogenic forms and their antagonists as well as well as the species diversity and other aspects too.

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Xenobiotics and Soil Microbiota Affected by Xenobiotic Interactions. IV. Decomposition of Linuron in the Presence of Other Pesticides in Two Soil Types

By

M. KECSKÉS and T. CSERHÁTI

Research Institute of Soil Sciences and Agricultural Chemistry of the Hungarian Academy of Sciences and Research Institute for Plant Protection, Budapest, Hungary

The decomposition of linuron was studied in the presence of gamma BHC, diazinon zoocides, TMTD and the Hg-content Falisan fungicides, propachlor, simazine,) methoprothryne + simazine (Gesaran), promethryne + simazine (Camparol 1803n herbicides as well as gamma BHC + TMTD as disinfectants in carbonaceous browir forest soil and chernozem with forest residues soil containing 60% water of the maximum water capacity at 26–28°C.

Among them the gamma BHC and diazinon decreased its decomposition speed in both soil types. TMTD and herbicides did not inhibit the decomposition at all.

Linuron N-(3,4-dichloro-phenyl)-N'-methoxy-N'-methylurea is a world wide applied representative of the disubstituted urea derivatives. Linuron is also the active ingredient of Afalon and Linuron 50 used in Hungary but it occurs in combinations too: MEDEX as well as the NIKE Combi combinations (the last one permitted only for experimental purposes).

Its favourable effect in Lupinus cultures was also proved by us (KECSKÉS, *et al.*, 1972).

The data concerning the decomposition of linuron in soil are very different (BARTHA and PRAMER, 1969; MAJUMDAR, 1969; SAVAGE, 1973; GEISSBÜHLER and GUTH, 1970; MORRIS and PENNEY, 1971; STECKO, 1972; SAVAGE, 1972) e.g. according to MORRIS and PENNEY (1971) it persisted for more than one year, in contrast with this SAVAGE (1972) found that linuron disappeared in 112 days. Naturally the decomposition of linuron is influenced to a great extent by the biotic and abiotic ecological factors among them we suppose the other pesticides present in the soil too.

This study aims to investigate the linuron decomposition in the presence of other pesticides.

Materials and Methods

30–30 ppm gamma BHC (Lindan = No. 2) and diazinon (Basudin = No. 3) zoocides, 30–30 ppm TMTD (Thiram = No. 4) and Hg-content (Falisan = No. 5) fungicides, 15–15 ppm Lindan + TMTD (No. 6) as disinfectants 5–5 ppm propachlor (Ramrod = No. 7), simazine (Gesatop 50 = No. 8), methoprometh-

ryne + simazine (Gesaran = No. 9), promethryne + simazine (Camparol 1803 = No 10) herbicides were added to soils containing 10 ppm pro anal. linuron and besides these soil samples with 10 ppm linuron served separately as a control. The soils (carbonaceous brown forest soil of Gödöllő, chernozem with forest residues of Martonvásár) with pesticides were incubated at 26–28°C at 60% of their maximum water capacity. Linuron content of the samples taken from these soils was determined with electron capture gas chromatography at the beginning of incubation and after 1, 2, 6, 10, 18 and 26 weeks.

Results

The change of linuron content of soils in time was described as a function of $y = a + bx$ where the $y = \log C$, the log. of the linuron concentration and x = the investigation time (in weeks).

The r values characteristic for the goodness of fit further the standard deviation of the slope that is variation coefficient were determined from which the weighted mean variation coefficient of the whole experiment series could be calculated.

The parameters of the regression lines of the first order reaction characterizing the decomposition of linuron can be seen in Table 1 from which it is obvious

Table 1

Decomposition of linuron in soils in the presence of pesticides in the first 10 weeks

Soil	Treatment	$b \times 10^3$	Goodness of fit %	Decomp. %	
				1 week	2 weeks
Chernozem with forest residues	1	5.68	99	18	22
	2	4.42	95	9	17
	3	4.74	90	0	10
	4	6.08	98	12	18
	5	5.39	95	2	7
	6	4.08	98	6	16
	7	5.27	98	11	20
	8	5.17	99	19	22
	9	5.81	90	15	24
	10	5.42	95	16	22
Carbonaceous brown forest soil	1	5.19	98	22	26
	2	4.49	99.9	2	18
	3	3.88	98	3	15
	4	4.92	98	16	28
	5	4.73	98	2	8
	6	4.29	90	2	11
	7	4.79	99	19	22
	8	4.92	99	22	27
	9	4.71	99.9	20	23
	10	4.65	99.9	20	22

that the decomposition until the 10th week could be followed at 95% level of significance with first order reaction in 85% of the cases.

The weighted mean variation coefficient was found to be 14.27%. The size of this could be attributed to the different inhibitory capacity of pesticides present in the soil (added by us), which in time decreased in most of the tested pesticides. But the decomposition speed of linuron significantly decreased in both soils on the effect of gamma BHC and diazinon (Figs 1 and 2, treatments 2, 3, 6).

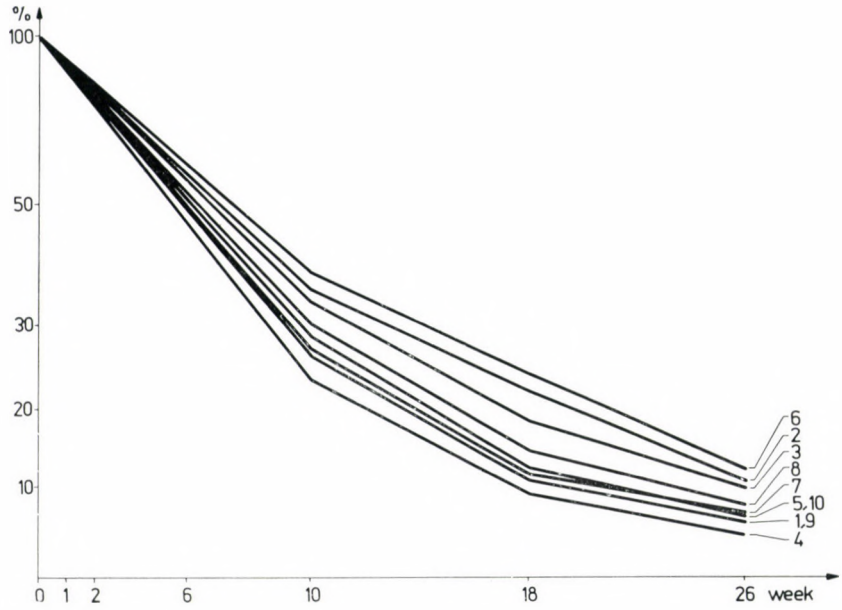

Fig. 1. Decomposition of linuron at 26–28°C in the presence of different pesticides in chernozem with forest residues soil.

1. 10 ppm pro anal. linuron; 2. 10 ppm pro anal. linuron + 30 ppm Lindane; 3. 10 ppm pro anal. linuron + 30 ppm Basudin; 4. 10 ppm pro anal. linuron + 30 ppm Thiram; 5. 10 ppm pro anal. linuron + 30 ppm Falisan; 6. 10 ppm pro anal. linuron + 15 Lindane + 15 ppm Thiram; 7. 10 ppm pro anal. linuron + 5 ppm Ramrod; 8. 10 ppm pro anal. linuron + 5 ppm Gesatop 50; 9. 10 ppm pro anal. linuron + 5 ppm Gesaran; 10. 10 ppm pro anal. linuron + 5 ppm Camparol 1803

After the first 10 weeks the rate of decomposition cannot be followed by the equations applied till now. The rates of breakdown decrease, their relative order remains unchanged. After 26 weeks of incubation 5–10% of the original linuron content was present in the soil.

The initial negative effect of the Hg containing Falisan – presumably because of Hg adsorption – later was not detectable.

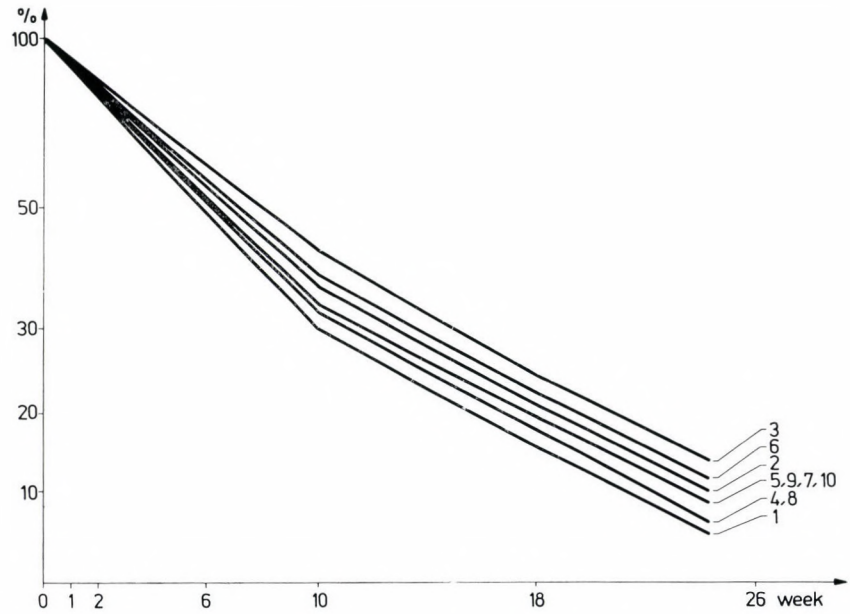

Fig. 2. Decomposition of linuron in the presence of different pesticides in carbonaceous brown forest soil. Pesticide doses were the same as Fig. 1

In the case of these doses which could occur in practice too we have to point out the relatively "favourable" effect of TMTD on the decomposition of linuron (Figs 1, 2 treatments No 4).

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Xenobitics and Soil Microbiota Affected by Xenobiotic Interactions. V. Decomposition of N-phenyl-phthalamine Acid in Soils with Disinfectants

By

M. GOMBOS, GY. HUBER, GY. PFEIFER and M. KECSKÉS

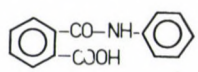
Research Institute of Heavy Chemical Industry, Veszprém and Research Institute of Soil Sciences and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary

"High" (0.01 and 0.1 %) doses of N-phenyl-phthalamine acid (NEVIKI 7106 = Nevirol 20 WP regulator) decreased the "total" number of microbes only very slightly and only in the first 2–3 weeks at 26–28°C and at 60 % of maximum water capacity of different soils (blown sand, rust coloured brown forest soil, chernozem with forest residues and "Kotu" soil). Though the "huge" doses (1.0 and 3.0 %) decreased it markedly, but it only lasted for 4–6 weeks. The "high" doses decomposed in chernozem with forest residues soil very quickly. The decomposition of "huge" doses in different soils was more slow, but more than 50 and 75 % of them could not be detected already after the first week. The decomposition of the 1 % doses of regulator was more markedly inhibited in the carbonaceous brown forest and "Kotu" soils in the presence of TMTD and Lindane than diazinon.

The production of N-meta-tolil-phthalamine acid was first reported by TINGLE and BRENTON (1909): HOFFMANN and SMITH (1949) published data on its hormone effect. As regards its regulator and the yield increasing effect the licences of the U. S. Rubber Company (U. S. Rubber Co., 1951, 1955) provide ample evidence. According to these the N-meta-tolil-phthalamine acid until 1000 ppm concentration has a hormone effect, above 2000 ppm a fungicide and herbicide effect. More detailed examinations were carried out with tomato (TEUBNER and WITTWER, 1955; 1957, SHIBASAKI and INAGAKI, 1962) lime bean (WADDINGTON and TEUBNER, 1963) as well as with cherry (QUENTIN *et al.*, 1957).

On the basis of the previous glasshouse experiments, among the synthethized derivatives of N-meta-tolil-phthalamine acid the N-phenyl-phthalamine acid (NEVIKI 7106 = Nevirol 20 WP, Fig. 1) proved to have a "regulator effect" comparable and commensurable to the basic compound (KOVÁCS *et al.*, 1974).

Before it was permitted to be used in Hungarian agricultural practice its effect on the quantitative change of the microbes and its degradation in the eco-



Mw = 241.250

Fig. 1. N-phenyl-phthalamine acid (NEVIKI 7106 = NEVIROL 20 WP)

systems of different Hungarian soil types parallelly with the regulator effect investigations were studied (OTT *et al.*, 1976). These tests contribute to the investigation series aimed at studying the effect of pesticide on soil microorganisms and the decomposition of xenobiotics (KECSKÉS and DOBOLYI, 1976; PÁSZTOR *et al.*, 1977; DOBOLYI *et al.*, 1977; KECSKÉS and CSERHÁTI, 1977).

These tests also belong to those investigations (KECSKÉS, 1976a) on the basis of which we proposed to complement the officially prescribed previous tests of pesticides with a "soil microbiological test minimum" system (KECSKÉS, 1976a; 1976b) before permitting their use in practice.

Materials and Methods

Soils belonging to different genetical groups and having different humus and organic matter content etc. (Table 1) were chosen for the investigations:

Table 1

Investigations on some characteristics of studied soils

Type of soil	Humus content, %	Carbonate content, %	A_K*	Salt content, %	Moisture content at sampling, %	pH		Loss of ignition	Mineral composition
						H_2O	KCl		
Blown sand	0.26	6.54	—	—	0.35	8.5	8.3	7.47	quartz, small chlorit
Rust coloured brown forest soil	0.57	0.47	—	—	0.58	7.5	7.1	1.98	quartz
Chernozem with forest residues	1.97	1.31	44	0.02	3.16	8.2	7.4	6.68	quartz, chlorit
"Kotu"	5.29	9.11	36	0.09	8.77	8.5	8.1	18.85	quartz, dolomite clorit

* Upper limit of plasticity determined by the method of Arany

from the skeleton soils the blown sand (Izsák) from the brown forest soils the rust coloured brown forest soil (Őrbottyán) from the chernozems the chernozem with forest residues (Martonvásár) as well as from the bog soils the "Kotu" (Bugyi).

0.01, 0.1 "high", 1.0 and 3.0% "huge" doses of pro anal. N-phenyl-phthalamine acid was added to the soil having 60% of the maximum water capacity and the samples were incubated at 26–28°C for 35 days.

The "total" number of microorganisms was determined with dilution technique (FEHÉR, 1953) and the residues of the regulator were measured too (OTT *et al.*, 1976).

Results and Discussion

Investigations were carried out firstly with fertile chernozem soil which was studied by us many times (KECSKÉS, 1976a) and in this way we could compare the results with others received during our other pesticide tests.

As it is seen from Fig. 2 the 0.01 and 0.1% concentration decreased the "total" number of microorganisms only very slightly but the 1% and 3% doses caused significant depressions though this effect decreased not parallely but tended with the decomposition speed of the regulator (Fig. 3). The smallest dose applied by us from which much less could drop to the soil in practice already after the second week could not differ from the control essentially.

The other three soil types were studied with respect to quantitative changes of microbes and further to determine if the decomposition takes place easily not only in fertile chernozem. On the effect of the 1% high dose of regulator the "total" number of microorganisms in the "Kotu" soil (Fig. 4) was relatively smaller than in the rust coloured brown forest and the chernozem with forest residues soils but at the 4th and 5th week this difference already evened out.

A similar tendency was observed when the 3% dose was used (Fig. 5), but there was no great difference among the three soils mentioned earlier. As regards the "total" number of microbes a marked depression was noticed in the blown sand.

The decomposition of the "high" and "huge" doses of N-phenyl-phthalamine acid was also studied in the presence of 0.01 and 1% TMTD and gamma BHC (T + L = Thiram + Lindane) and 0.01 and 1% diazinon (B = Basudin) in sandy (Gödöllő) and "Kotu" soil (Bugyi).

As it is demonstrated in Fig. 6 the speed of the decomposition was quick especially in the heavy "Kotu" soil with big organic material content and rich soil microflora. After four weeks the regulator could not be detected in this soil in spite of the presence of soil disinfectants.

On comparing the decomposition speed of N-phenyl-phthalamine acid in the two investigated soils in the "Kotu" soil already after the first week 86% less regulator residues were found. In the presence of diazinon (Basudin) 47% and of Thiram + Lindane, 68% residues were detected of the original amount of the regulator (Figs 7 and 8).

The disinfectants inhibited only the decomposition of the "huge" (1.0 and 3.0%) doses of N-phenyl-phthalamine acid.

It was concluded that N-phenyl-phthalamine acid could be used in practice in much higher doses than it is normally applied and in the presence of other pesticides (disinfectants) without the danger of contamination in the soil and it would not be harmful over a long period of time for the soil microbiota.

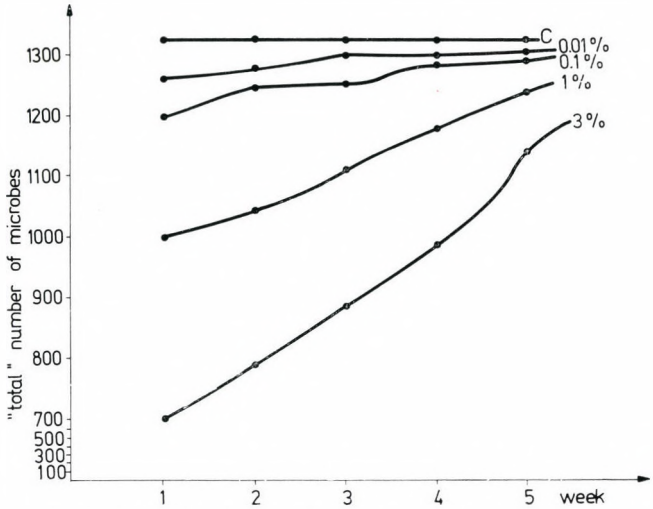


Fig. 2. The effect of N-phenyl-phthalamine acid on the "total" number of microbes in chernozem with forest residues soil

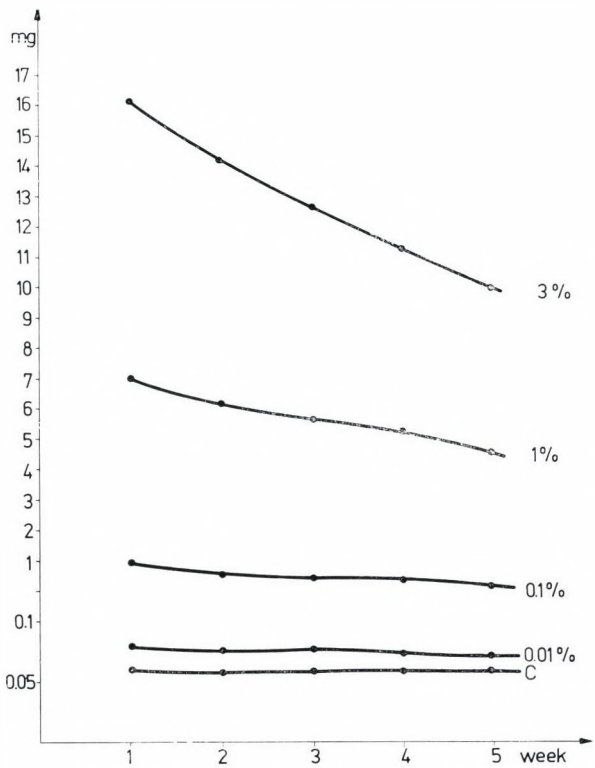


Fig. 3. Decomposition of N-phenyl-phthalamine in chernozem with forest residues soil

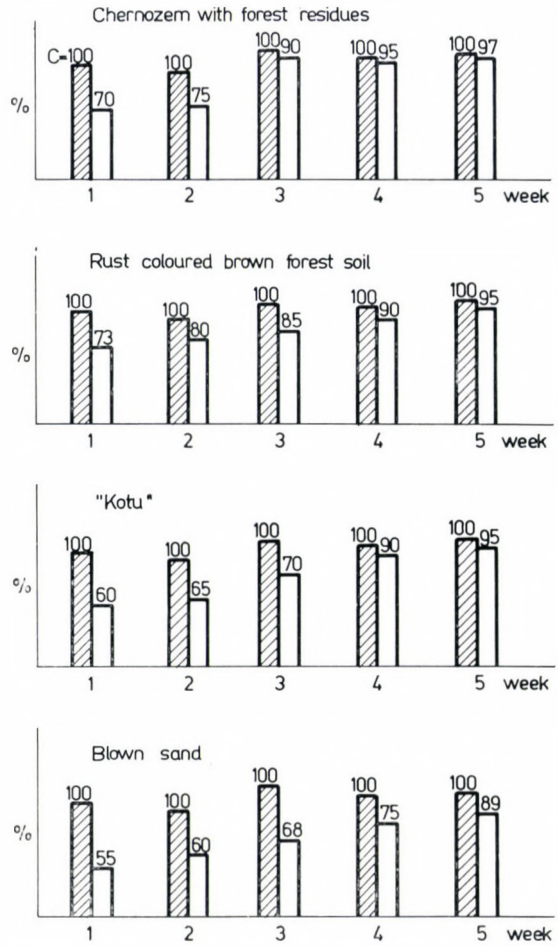


Fig. 4. The effect of 1% N-phenyl-phthalamine acid on the "total" number of microbes in different soils

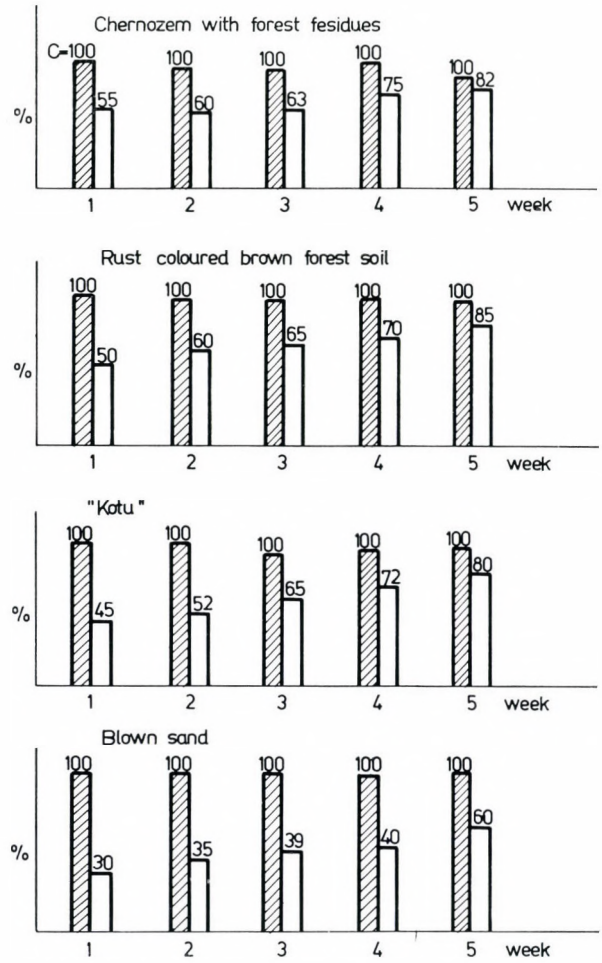


Fig. 5. The effect of 3% N-phenyl-phthalamine acid on the "total" number of microbes in different soils

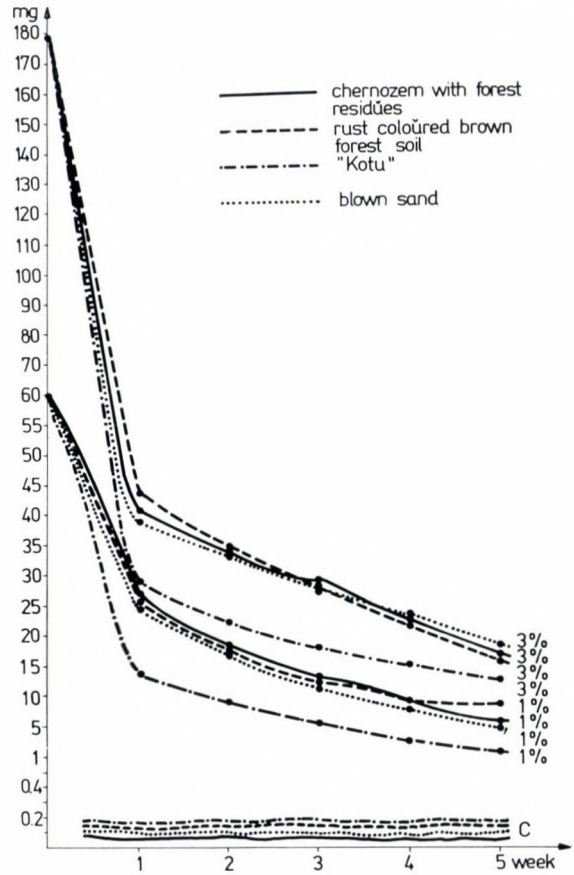


Fig. 6. Decomposition of N-phenyl-phthalamine acid in different soils

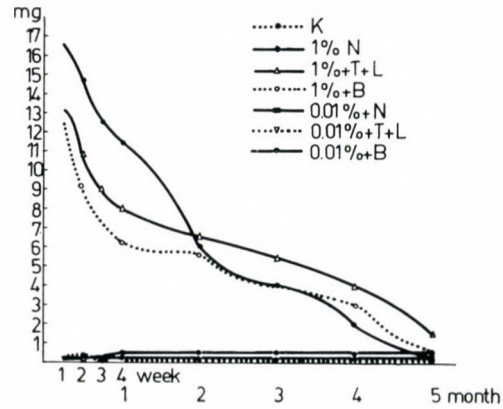


Fig. 7. Decomposition of N-phenyl-phthalamine acid in the presence of TMTD + gamma BHC (T + L) as well Basudin (B) in carbonaceous brown forest soil

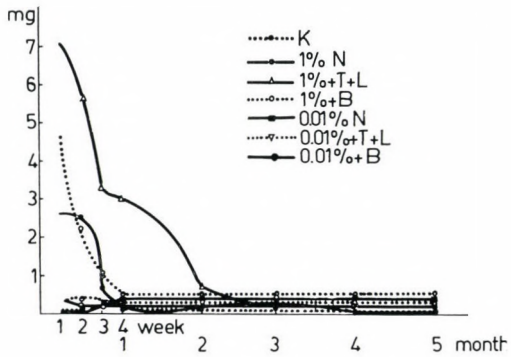


Fig. 8. Decomposition of N-phenyl-phthalamine acid in the presence of TMTD + gamma BHC (T + L) as well as Basudin (B) in "Kotu" soil

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Relationship Between Soil Microflora and Afalon and Patoran Herbicides Under Fertilizer Application

By

D. BAKALIVANOV and S. HLEBAROVA

"N. Poushkarov" Institute of Soil Science, Sofia, Bulgaria

The influence of afalon (linuron — $C_9H_{10}Cl_2N_2O_6$) and patoran (methobromuron — $C_9H_{11}B\,N_2O_2$) herbicides on soil microorganisms used in applications of 200 g of active substance per dka in field experiments with sunflower on chernozem-smolnitsa. This influence was studied through fertilizer application in two levels respectively N-12 kg/dka, P-8 kg/dka, K-12 kg/dka and N-24 kg/dka, P-24 kg/dka, K-24 kg/dka. Detoxication of the preparation was observed as well. The analyses were carried out in dynamics respectively on the 5th, 30th and 90th day after the soil treatment. It was determined that the herbicides applied some inhibiting effect on soil microorganisms, which was more clearly expressed at the beginning of the experiment. Later on this influence grew weaker and stopped on the 90th day after the treatment with most of the studied groups of microorganisms. Detoxication mostly took place (about 3/4) for 90 days after the introduction of preparations in the soil.

Fertilizer application that was used weakens the inhibiting effect of herbicides on the studied groups of soil microorganisms and also adds to the increase of their detoxication especially when higher rates are applied.

Carbamide herbicides afalon and patoran are greatly applied in the agriculture of Bulgaria (LJUBENOV, 1972). Together with their good herbicide properties, some authors have reported on the inhibiting effect of afalon on different groups of soil microflora and microfauna (RANKOV, 1968; SANOCZKA-WOLOSZYN and WOLOSZYN, 1970, WEGSZYN, 1970). On the other hand the research work of MIKLASZEWSKI (1975) shows that the same preparation weakly activized cellulose-decomposing microorganisms in ordinary rates of application while bigger rates caused an inhibiting effect. Data for patoran herbicide in this respect are rather scarce which makes it necessary to be studied.

As the soil is creating favourable conditions for the development of soil microflora under fertilizer application and the inhibiting effect of some herbicides is easily overcome (BAKALIVANOV *et al.*, 1975), we studied the development of some groups of soil microorganisms under different rates of application and use of the herbicides mentioned in order to determine the conditions decreasing and eliminating the undesirable consequences of their usage. We studied also the process of detoxication of the preparations under the same conditions.

Material and Methods

Afalon and patoran preparations, produced by Höesh BRD and "Ciba Geigy" Basel Schweiz were used in a rate of 200 g/dka of active substance in field experiments with sunflower. The experiments were carried out in the experimental plot of "N. Poushkarov" Institute of Soil Science, Sofia, on chernozem-smolnitsa. There were two levels of application in the experimental scheme:

1. N – 12 kg/dka, P – 8 kg/dka, K – 12 kg/dka; 2. N – 24 kg/dka, P – 24 kg/dka, K – 24 kg/dka. The analyses on soil microorganisms were carried out through inoculation of diluted soil suspensions on nutrient medium treated with meat peptone agar (MPA) for soil bacteria, starch ammonium agar for actinomyces, the medium of Czapek for microscopic fungi, the medium of Ejbi for Azobacter. The total biological activity of soil (respiration) was studied by Carl Zeiss interferometer through CO_2 discharge from soil samples composed of glucose and ammonium sulphate, incubated under 27°C for 20 hours. The determination of the residual quantities of herbicides was assessed by a Pye Unicam gas chromatograph.

All analyses were carried out periodically every 5, 30 and 90 days and 150 days after the soil treatment for the residual quantities.

Results and Discussion

The research on total biological activity of soil indicated certain inhibiting effect of afalon and patoran herbicides. In Fig. 1a and b it is better expressed in the case with the first herbicide. The experiments carried out by MANNINGER and SZÁVA (1972), for the conditions of some Hungarian soils did not show any particular differences in the respiration rate of the soils treated with afalon. This

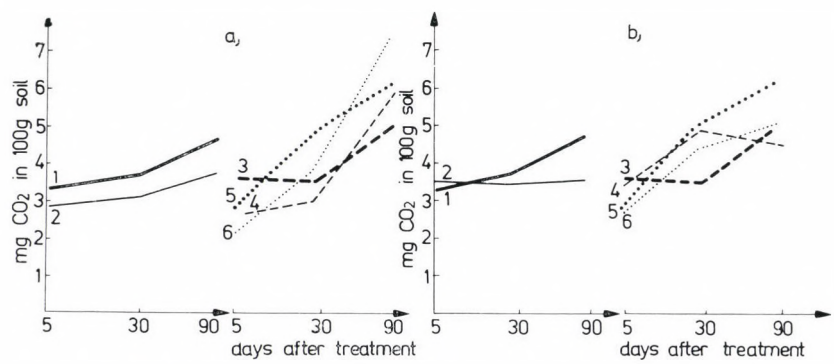

Fig. 1a. Influence of afalon on respiration rate in soil. 1. Without fertilizers, 2. without fertilizers + afalon, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ – afalon, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ – afalon
Fig. 1b. Influence of patoran on respiration rate in soil. 1. Without fertilizers, 2. without fertilizers + patoran, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + patoran, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + patoran

can be due to the experiments' treatment and the methodics applied. The depressing influence of afalon in the treated variants in our experiments did not show in the later investigations when even increase in the quantity of the released CO_2 (on the 90th day) had been determined. Such increase was observed on the 30th day after the herbicide introduction in the variants with patoran + low rates of application. This demonstrates that the agrotechnics used, i.e. fertilizer application, has a considerable influence on the biological activity of the herbicides mentioned.

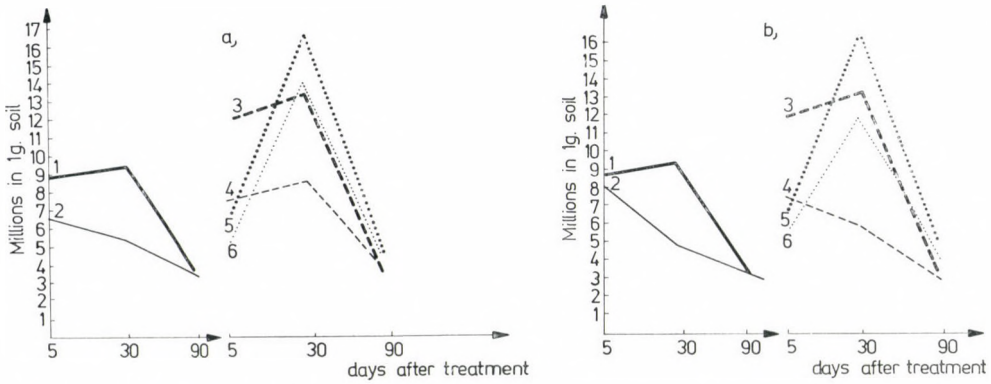

Fig. 2a. Influence of afalon on soil bacteria. 1. Without fertilizers, 2. without fertilizers + afalon, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + afalon, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + afalon
Fig. 2b. Influence of patoran on soil bacteria. 1. Without fertilizers, 2. without fertilizers + patoran, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + patoran, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + patoran

Herbicides show a certain depressing effect on the quantity of soil bacteria which is better expressed at the beginning of the experiment (Fig. 2a and b). In this way on the 5th day after the treatment the quantity of these microorganisms in the variant "afalon without application" is nearly 1/3 less than that in the control and in the variant "patoran without application" it is about 1/4 less. The inhibition mentioned can be determined also in the analyses of the 30th day. Towards the 90th day the quantity of the bacteria of the treated variants becomes similar to that of the control, which may be due to the decrease of toxicity of the preparations which is a result of their decomposition. The research work of RANKOV (1972) for the conditions of alluvial-meadow soil near Plovdiv notices the same tendencies of afalon's influence but certain stimulation effect of the afalon towards the end of the vegetation period can be also observed. The latter can be a result of the considerable differences in the soil on which the experiment had been carried out in comparison with the chernozem-smolnitsa which was the subject of our research. In this case the author mentioned explains this stimulation with the application of the herbicide as a source of nitrogen. But this can also be due to the stimulating activity of some of the products from the preparation's decomposition. With smolnitsa, due to its strong adsorption, it may

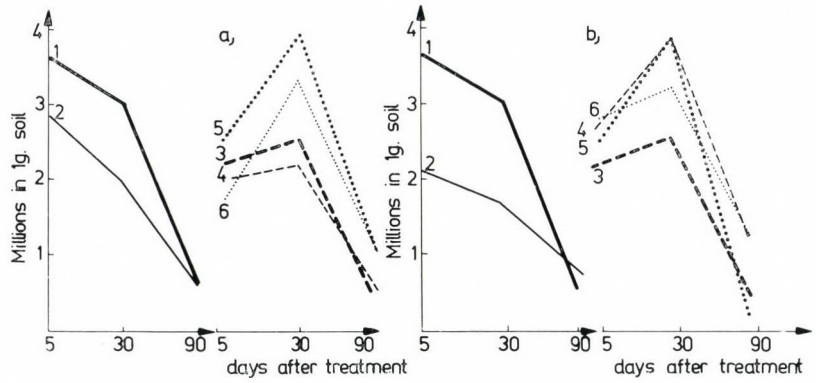

Fig. 3a. Influence of afalon on soil actinomycetes. 1. Without fertilizers, 2. without fertilizers + afalon, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + afalon, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + afalon
Fig. 3b. Influence of patoran on soil actinomycetes. 1. Without fertilizers, 2. without fertilizers + patoran, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + patoran, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + patoran

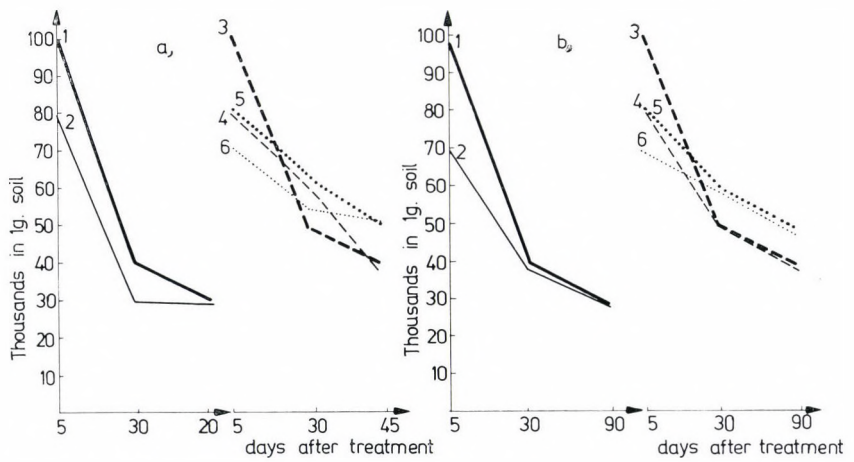

Fig. 4a. Influence of afalon on soil fungi. 1. Without fertilizers, 2. without fertilizers + afalon, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + afalon, 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + afalon
Fig. 4b. Influence of patoran on soil fungi. 1. Without fertilizers, 2. without fertilizers + patoran, 3. $N_{12}P_8K_{12}$, 4. $N_{12}P_8K_{12}$ + patoran 5. $N_{24}P_{24}K_{24}$, 6. $N_{24}P_{24}K_{24}$ + patoran

be that these products are blocked which can be the reason for lack of stimulating effect. On the other hand, the activity of the preparations is closely connected with the agrotechnical conditions used and the application. Data shown on the same figure also demonstrate that the improvement of nutrient regime in soil through increase of the quantity of the main nutrient elements N, P and K, appears as a main precondition for the growth of the population of soil bacteria. With

higher rates of application their quantity is more weakly influenced by the herbicides used and is quickly recovered to norma[1]. As we can see later on, this is due to their increased activity which causes also increase in the detoxication of the preparations. For this reason the higher rate of mineral application causes a considerable decrease of the depressing effect of the preparations.

The herbicides studied also had an inhibiting effect on soil actinomyces which was strongly expressed in the plots with patoran. The analyses demonstrated a decrease in the quantity of these microorganisms on the 5th and 30th day after the treatment in the plots with afalon and especially in these with patoran without application. Towards the 90th day after the treatment no difference between the controls and the treated variants was observed. The use of fertilizers with these microorganisms also contributes to the decrease of the inhibiting effect of the preparations in comparison to the untreated plots. In the variants with patoran with lower rate of application there are found more actinomyces in comparison with the respective control.

As regards soil microscopic fungi data in Fig. 4a and 4b also prove an inhibiting effect of the preparations. Patoran's activity is a considerable one but is mainly expressed at the beginning of the experiment when a decrease with about 1/3 of the microscopic fungi is determined in these plots. Fertilizer application with these microorganisms also helps the decrease in the inhibiting effect of the preparations. Even in the plots with afalon + low rate of application an increase of the fungi on the 30th day after the treatment is established.

Studies on free nitrogen fixation bacteria "*Azotobacter*" also show that they decrease under the influence of afalon and increase under that of patoran (Table 1).

Table 1

Influence of afalon and patoran on the germination of soil particles with *Azotobacter*

	5 days after treatment	30 days after treatment	90 days after treatment
Control without application	58	75	57
+ $N_{12}P_8K_{12}$	79	76	69
+ $N_{24}P_{24}K_{24}$	78	80	71
Afalon without application	39	79	55
+ $N_{12}P_8K_{12}$	49	83	68
+ $N_{24}P_{24}K_{24}$	62	85	73
Patoran without application	80	80	63
+ $N_{12}P_8K_{12}$	97	92	76
+ $N_{24}P_{24}K_{24}$	87	93	72

Notice: Figures express the percentage of soil particles germinated with colonies of *Azotobacter*.

Data in this Table demonstrate that the effect is strongest at the beginning of the experiment. Towards the 30th day after the treatment certain stimulating effect of both preparations is observed, which is better expressed as regards the size and pigmentation of the colonies especially under the influence of afalon (Fig. 5). Fertilizer application scarcely changes the influence of the preparations. The stimulating effect of the herbicides can be due to the application of these preparations

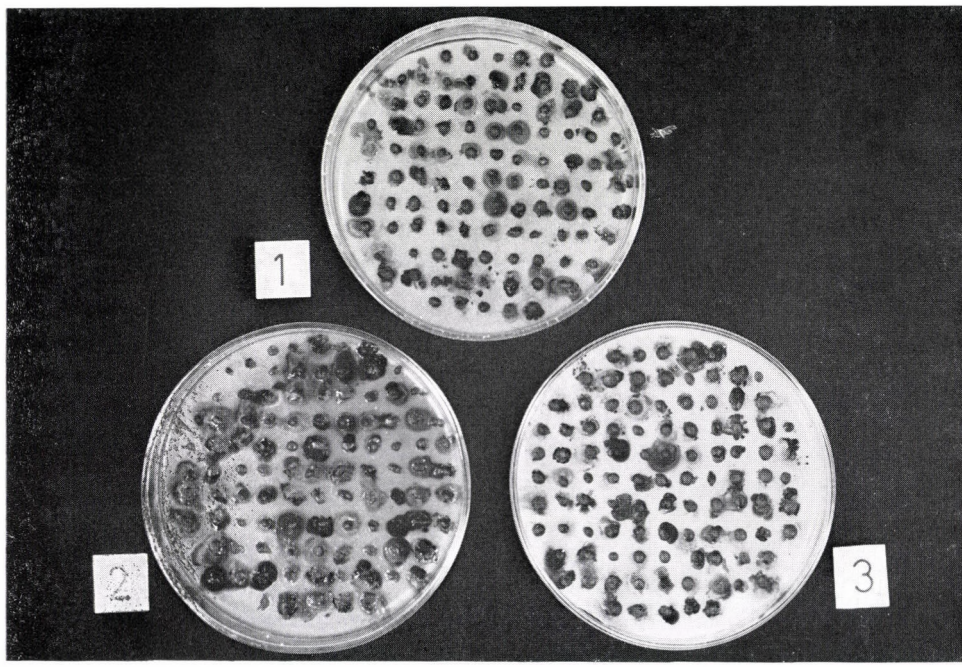

Fig. 5. Influence of afalon and patoran on colonies of *Azotobacter* on the 30th day after the soil treatment. 1. Control, 2. Afalon, 3. Patoran

as a nutrient source on the one hand (RANKOV, 1968) and to the morphological characteristics of these bacteria on the other hand and to their thick cell cover and mucous-producing ability which decreases their penetrating activity. In this case the small quantity of penetrating preparation can have a stirring effect which acts as a stimulation.

The effect of afalon and patoran herbicides with plants is well studied, but the mechanism of this activity with microorganisms has not been throughly examined. To a certain extent the case may concern an indirect activity of the preparations. Under their influnce a great number of weeds are destroyed and soil microconditions are changed as regards roots' excretions, temperature, evaporation, etc. For this reason a decrease in the quantity of soil microorganisms can take place as it was proved for other herbicides (RAIZER *et al.*, 1970).

Detoxication of afalon and patoran takes place mainly during the first 3 months (Fig. 6a and b). For example, 47.5% of the primary quantity of afalon and 50% of that of patoran had been determined one month after the soil treatment while after three months this quantity was 36.3% and 35%. Certain speeding of the detoxication process is observed in the variants with fertilizer application which can be due to the increase of the quantity of soil microflora and enzyme activity in the soil. Higher rate of fertilizer application with afalon and patoran

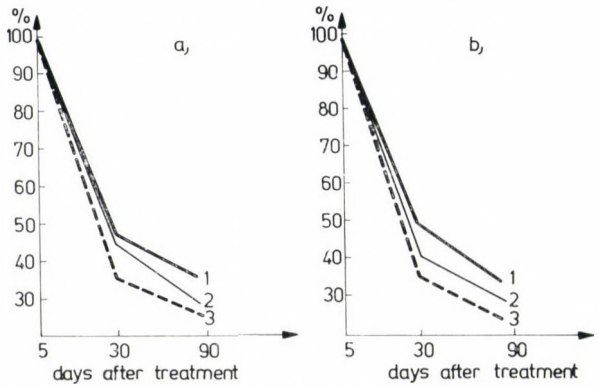

Fig. 6a. Residue (in %) of afalon in soil. 1. Without fertilizers, 2. $N_{12}P_8K_{12}$, 3. $N_{24}P_{24}K_{24}$
Fig. 6b. Residue (in %) of patoran in soil. 1. Without fertilizers, 2. $N_{12}P_8K_{12}$, 3. $N_{24}P_{24}K_{24}$

helps the speeding up of their decomposition, while the lower rate of application has a weaker influence on the process of detoxication of both preparations. This is the reason for their quantity to be 8–10% less in these variants in comparison to the untreated control.

Conclusion

1. Carbamide herbicides afalon and patoran cause certain decrease in the quantity of soil bacteria, actinomyces and microscopic fungi with most of the analyses. Preparations' activity is more clearly expressed at the beginning of the treatment and usually stops after the 90th day.

2. Under the conditions of a field experiment detoxication of these herbicides takes place mainly (about 2/3) till the 90th day after the soil treatment.

3. Fertilizer application weakens the inhibiting effect of herbicides on the soil microorganisms studied and also helps the speeding of their detoxication.

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Effect of a Few Herbicides on the Microbiological Decomposition of Corn Stalk

By

B. TÓTH

University of Agricultural Sciences, Keszthely, Hungary

As an experiment soil we had brown forest soil in the neighbourhood of Keszthely. The carbondioxide productive capacity of the experimental soil was measured under laboratory conditions, using various doses of Rhamrod Aktikon, Afalon and Hungaria K-64 herbicides and those of chemical fertilizers, with and without any corn stalks.

It has been found that the dose of herbicides used in practice hindered CO_2 production considerably only if it is applied in 100—400 times higher concentration, however this hindering effect can be strongly reduced by adding an adequate dose of nutritive material supplement (100—500 mg N/200 g soil).

The doses of Hungaria K-64 and Aktikon used in practice stimulated the CO_2 production of the soil, under the experimental conditions. The doses of Rhamrod and Afalon applied have no appreciable effect on the CO_2 quantity, produced at the decomposition of corn stalks.

Every year 90—160 cwt/ha corn stalks get into the field, the main parts of which being cellulose, hemicellulose, and lignin. To promote the microbiological decomposition of this large quantity of plant residues, is our common interest.

Material and Method

Laboratory model experiments were carried out to study the effect of Hungaria K—64, Aktikon, Afalon and Rhamrod herbicides, applied in various doses, on CO_2 production of the soil when corn stalk was being decomposed in the soil. The herbicide treatment, at a constant level of K and P, was combined with N-nutrient supplement of various doses.

The soil used for the experiment was a Ramann type brown forest soil in the neighbourhood of Keszthely, having the following parameters:

pH_{KCl} 6.51
pH_{H_2O} 6.92
total organic material 2.47%
total N-content 0.37 ×
K-content 24.4 mg K_2O/100 g soil
P-content 13.8 mg P_2O_5/100 g soil.

Soil samples of 200 g each in air-dried condition were added to each pot, and the water content of soils was established for 60% maximum water capacity. The carbondioxide production was tested according to the method described by SZEGI (1962), for 20 weeks at 28°C.

The following treatments were used in the experiment:

1. 200 g soil
2. 200 g soil + 4 g corn stalk
3. 200 g soil + 4 g corn stalk + N_1, N_2, N_3, N_4 N-doses,
   where N_1 = 20 mg N/pot,
   N_2 = 100 mg N/pot,
   N_3 = 500 mg N/pot,
   N_4 = 1000 mg N/pot.
4. 200 g soil + 4 g corn stalk + H_1, H_2, H_3, H_4, H_5 herbicide doses,
   where H_1 = 1 mg of herbicide/pot,
   H_2 = 5 mg of herbicide/pot,
   H_3 = 50 mg of herbicide/pot,
   H_4 = 100 mg of herbicide/pot and
   H_5 = 400 mg of herbicide/pot.
5. 200 g soil + 4 g corn stalk + N_1 + various herbicide doses.
6. 200 g soil + 4 g corn stalk + N_2 + various herbicide doses.
7. 200 g soil + 4 g corn stalk + N_3 + various herbicide doses.
8. 200 g soil + 4 g corn stalk + N_4 + various herbicide doses.

The N-supplement was added in the form of ammoniumsulphate. The quantity of CO_2, released from soil was measured every two weeks.

Results and Discussion

1. The presence of corn stalk in the untreated soil considerably increases the ability of the soil to produce CO_2 (from 1021 mg to the total 2921 mg CO_2).

2. The total amounts of carbon dioxide, produced during the experimental period of 20 weeks are given in Tables 1–4.

Table 1

The total quantity of CO_2, produced with various doses of N-supplement and Hungaria K-64 herbicides (mg CO_2/pot)

	$N\varnothing$	N_1	N_2	N_3	N_4
$H\varnothing$	2921	3641	3808	4481	4699
H_1	3016	3810	4015	4900	4720
H_2	3410	3870	4083	4870	4522
H_3	2514	3273	3620	4036	4447
H_4	2008	2325	2801	4093	4210
H_5	612	1908	1912	3420	3850

Table 2

The total quantity of CO_2, produced with various doses of N-supplement and Aktikon herbicides (mg CO_2/pot)

	N∅	N_1	N_2	N_3	N_4
H∅	2921	3641	3808	4481	4699
H_1	3242	3913	4016	4625	4420
H_2	3007	3735	4052	4235	4035
H_3	2245	2826	3410	3640	3712
H_4	1337	1635	2240	3660	2975
H_5	785	1210	1823	2088	3030

Table 3

The total quantity of CO_2, produced with various doses of N-supplement and Afalon herbicides (mg CO_2/pot)

	N∅	N_1	N_2	N_3	N_4
H∅	2921	3641	3808	4481	4699
H_1	2618	3701	3900	4384	4418
H_2	2710	3602	3735	4018	4460
H_3	2001	3110	3204	3390	3408
H_4	1781	1941	2073	2138	2604
H_5	987	1644	1802	1711	2020

Table 4

The total quantity of CO_2, produced with various doses of N-supplement and Rhamrod herbicides (mg CO_2/pot)

	N∅	N_1	N_2	N_3	N_4
H∅	2921	3641	3808	4481	4699
H_1	2870	3706	3924	4516	4536
H_2	2775	2987	3710	4201	4301
H_3	2016	2705	3060	3601	3410
H_4	1230	1825	2118	2087	2020
H_5	1081	1038	1260	1120	1415

The following conclusions can be drawn based on the data of Tables 1–4:

In the samples without any herbicide treatment, the increasing doses of N resulted in an increase of the biological activity of soil, up to 500 mg N per 200 g soil dose, but not after it.

Of the 4 herbicides tested, the doses of Hungaria K–64 and Aktikon as applied under field conditions (1–5 mg of herbicide per pot), in all the N doses applied, slightly increased the CO_2 production of the soil. At lower doses of

Rhamrod and Afalon, no significant differences were found as compared to the control.

The greatest stimulative effect was found with Hungaria K – 64, at 5 mg per pot dose, without any N-supplement. In this treatment, as compared to the control, the total 116.7 per cent CO_2 production was observed.

The greatest depression was also noted when 400 mg of Hungaria K – 64 per pot was applied without any N-supplement. In this case, the CO_2 production of soil reached only 20.9 per cent as compared to the control.

The increasing doses of N partly eliminate the depressive effect of herbicides. When using a 500 mg N per pot rate nutrient supplement, even the highest dose of herbicide resulted in only a 26 per cent decrease in CO_2 production.

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Perforation of Conidia of *Cochliobolus sativus* by Soil Amoebae

By

KENNETH M. OLD

Department of Biological Sciences, Dundee University, Dundee, Scotland

When pigmented spores of fungi are placed into natural soils from Europe and North America they commonly are perforated and lysed. Holes may be simple perforations 0.2–0.5 μm diam or larger holes 0.6–6.0 μm diam. The latter are initiated by the formation of annular depressions in the wall surface. The larger holes are caused by giant amoebae of the order *Leptomyxida* Pussard which attack the spores, perforate the walls and digest the cell contents. The process of perforation has been studied in culture and recorded photographically.

Successful soil-borne plant pathogenic fungi must be able to survive in the absence of the host plant by forming some type of resting propagule. Saprophytes face a similar problem of survival when substrates are locally exhausted. Some of the most effective survival structures are pigmented spores or sclerotia. The pigment is often a melanin compound located in the cell wall, and is considered to play a role in conferring resistance to biodegradation on fungal propagules. BULL (1970) and KUO and ALEXANDER (1967) have shown that cell wall lysing enzymes are inhibited by melanins. OLD and ROBERTSON (1970b) and CLOUGH (1975) showed with *Cochliobolus sativus* and *Thielaviopsis basicola* that spores of hyaline isolates of these fungi survived for only a few days in soil. Wild type pigmented isolates survived much longer. The deposits of pigment in the spore wall of *C. sativus* also conferred resistance to chitinase and snail gut enzyme (OLD and ROBERTSON 1970b).

Despite their inherent resistance to biodegradation, pigmented spores eventually undergo breakdown in soil. This may be facilitated by puncture of the melanized wall layers. REISINGER (1972) showed that mites could puncture conidia of *Helminthosporium spiciferum* allowing colonization of the spore lumina by soil bacteria. OLD (1967) showed that conidia of *C. sativus* became perforated by holes 2–4 μ diameter allowing invasion of the spores by a wide range of soil microorganisms. The non-melanized wall components were completely digested leaving an empty perforated spore shell (OLD and ROBERTSON, 1970a; WONG and OLD, 1974). This phenomenon has been called perforation lysis and the subject has recently been reviewed (OLD and WONG, 1976).

Perforation lysis

Perforation lysis has been recorded for a variety of fungi including *C. sativus*, *T. basicola* (CLOUGH and PATRICK, 1972), *Alternaria tenuis*, *Curvularia protuberata*, *Stemphyllium dendriticum*, *Cladosporium* sp. and *Stachybotrys atra* (OLD and WONG, 1976). Perforation is caused by a component of the soil microbiota (OLD and WONG, 1976) and has been found in spores incubated in soils from Scotland, Canada and Holland. The agent of perforation is probably generally distributed in soils. Perforations range from 0.2 – 6.0 μ diameter. The smaller holes 0.2 – 0.5 μ are simple holes in the spore wall. The larger holes 0.5 – 0.6 μ diameter appear to develop from annular shaped depressions in the spore wall. These depressions and incomplete holes are often found side by side with completed perforations. Perforated conidia of *C. sativus* and chlamydospores of *T. basicola* are shown in Figs 2 and 3. Hyaline conidia, either mutant isolates or immature conidia do not become perforated but undergo generalised lysis and are extensively colonized by bacteria (Fig. 5).

It is apparent then that micoorganisms are present in many natural soils which able to penetrate the barrier offered by the pigmented spore wall. Once breached the spores are rapidly colonized, and several microbial species can play a part in the decomposition of the cell wall residues.

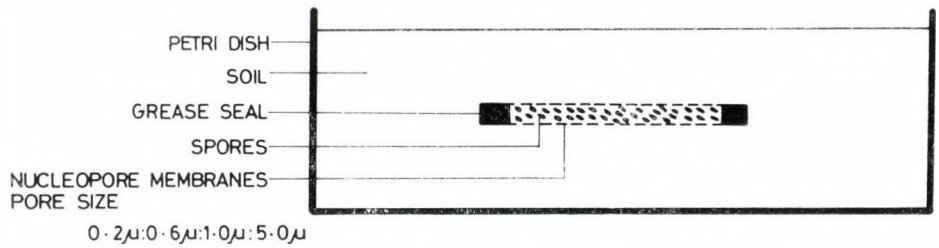

Fig. 1

Nature of the perforating agents

For several years attempts have been made to identify the causal agents. These attempts and proposals as to the nature of the agents are discussed by OLD and WONG (1976) and OLD and PATRICK (1976). It was proposed that the small holes less than 0.5 μ diameter approximately could be caused by direct penetration of the spore wall by bacteria aligned end on to the fungus surface. However, no explanation could be given for the origin of the larger holes and annular depressions in the spore wall.

The key to the discovery of the agent causing the large holes was a technique devised by OLD and WONG (1976) and used to good effect by OLD and PATRICK (1976). The method is shown diagramatically in Fig. 1. Spores are placed into natural soil, but access to them can only be gained by soil microorganisms passing

through pores in "Nuclepore" membranes. By using different membrane pore sizes, the dimensions of the perforating agent can be inferred (OLD and PATRICK, 1976).

Results showed that the agent was either a bacterium of cell diameter less than 1.0 μ, or was an organism with variable dimensions, possibly a soil amoeba. By recovering the membranes bearing perforated conidia and associated microflora and incubating them with fresh aqueous suspensions of conidia of *C. sativus*, OLD (1977) has shown that the large holes and annular depressions are caused by giant soil amoebae. The perforated conidia shown in Fig. 4 were the result of culturing spores with amoebae *in vitro*. The holes and annular depressions formed are identical to those shown in Fig. 2, produced in natural soil.

Activities and properties of the lytic amoebae

After 2 weeks incubation at 25°C, 10 aqueous suspensions of conidia of *C. sativus* inoculated with membranes bearing perforated conidia and associated microflora all contained large numbers perforated conidia, and large numbers of active amoebae. Subcultures were made to Cruikshank tissue culture chambers (Sterilin, Teddington, Middlesex ENGLAND) and a photographic record prepared of the progress of perforation (OLD, 1977). The amoebae (Fig. 6) are commonly branched cells of several hundred microns extent. They flow toward and engulf whole conidia. About 4–5 hours after contact is made the conidium is usually entirely lysed (Figs 7–11). Internal septa disappear and perforations appear in the spore walls. Several holes may be found in a single conidium.

Particles of spore wall debris, often disc shaped and corresponding in size to the holes in the spore, are ingested by the amoebae. This debris is either egested as the active amoebae flows across the substratum or is retained within the protoplast during encystment. When the cyst germinates the cell wall debris is left inside the empty cyst wall (OLD, 1977) Figs 12–14.

The identity of the amoebae has not yet been ascertained. They appear to be closely related to *Leptomyxa reticulata* Goodey, a group of giant soil amoebae described by SINGH (1948) as being commonly found in agriculture soils. The amoebae readily encyst in soil and in culture with fungus spores. Cysts may form within lysed conidia. The amoeba apparently feeds on the spore by eroding annular depressions in the spore wall and, perhaps by a combination of mechanical and enzymatic activity breaks the spore wall in a circular manner. The details of this mechanism need further investigation. There seems to be no doubt that the giant amoebae are responsible for the large holes and annular depressions in the spore walls of *C. sativus*. So far conidia of two other species, *Alternaria tenuis* and *Cladosporium* sp. have been incubated with the amoebae. Spores were perforated and lysed, and it seems likely that the amoebae can attack a wide range of fungal species.

The smaller holes reported in the walls of *C. sativus* and *T. basicola* (OLD and PATRICK, 1976) have not been seen when conidia are attacked by amoebae in

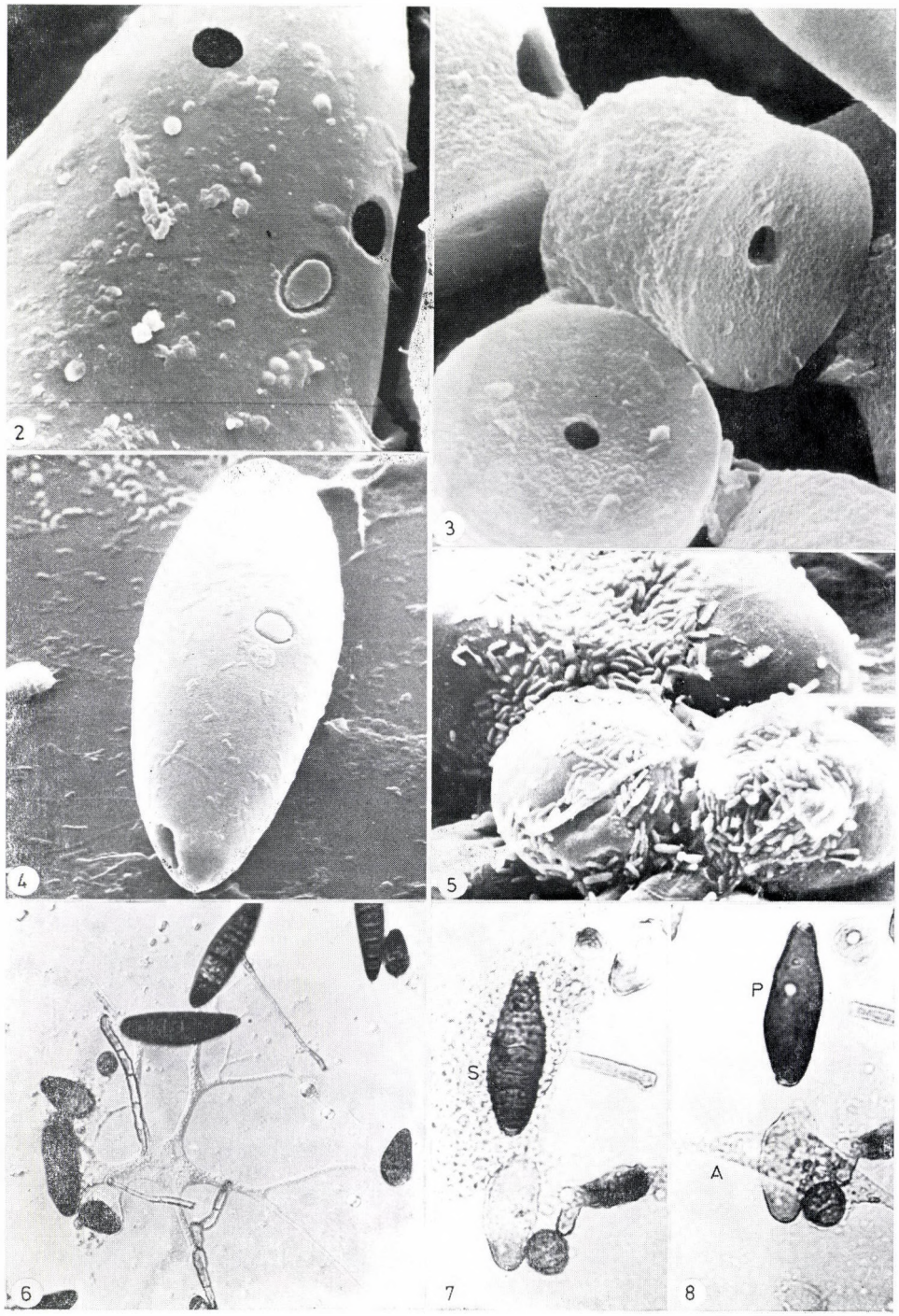
2
3
4
5
6
7
S
8
P
A

Fig. 2. Conidium of *Cochliobolus sativus* recovered from natural soil perforated by two holes and showing an annular depression (×8000)
Fig. 3. Chlamydospores of *Thielaviopsis basicola* recovered from natural soil showing perforations (×9000)
Fig. 4. Conidium of *C. sativus* perforated by amoebae in culture. Note the hole and annular depression in the spore wall (×2000)
Fig. 5. Hyaline conidia of *C. sativus* recovered from natural soil showing colonization by bacteria and general erosion (×2000)
Fig. 6. Giant amoeba attacking conidia of *C. sativus* in culture (×1000)
Fig. 7. Conidium of *C. sativus* engulfed by amoeba. Note that septa (S) are intact (×1500)
Fig. 8. Same conidium as shown in Fig. 7. The amoeba (A) has left the conidium empty, perforated (P) and lacking septa (×1500)

culture. It is possible that these are quite distinct from the larger holes and may be caused by bacterial erosion of the spore walls. Further work is needed to establish the identity of the agent causing the smaller holes.

This recent discovery of the relationship between a common soil amoebae and the resting spores of plant pathogenic and saprophytic fungi offers intriguing possibilities for future research. It may be possible to control soil borne or seed borne diseases caused by fungi by inoculating soil or plant parts with these antagonistic organisms. Also the role of amoebae in the microbial balance soil should be further explored.

Note added in proof: The giant amoeba belongs to the family *Vampyrellidae* in the order *Proteomyxida*.

Fig. 9. Time 0 hr. One conidium is engulfed by the amoeba but is intact
Fig. 10. Time 3 hr. One conidium is perforated and lysed and another is engulfed
Fig. 11. Time 8 hr. Both conidia are perforated and lysed the amoeba has divided and formed two cysts
Fig. 12. Excystment. Time 0
Fig. 13. Excystment. Time 10 min to 15 min
Fig. 14. Excystment, Time 20 min to 25 min. Note that the dense (D) material (remains of fungal cells digested by the amoeba) is left in the empty cyst

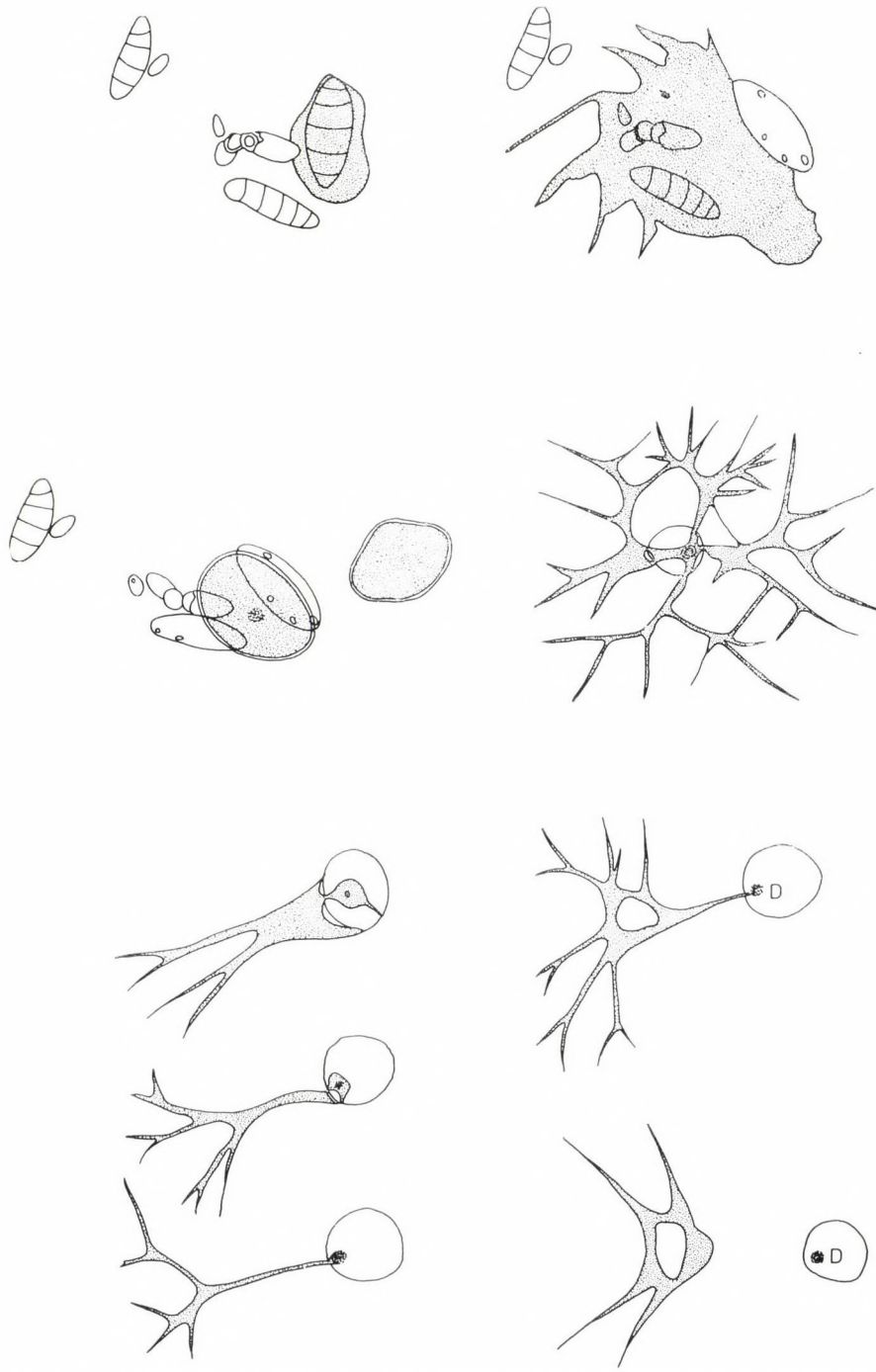

Plate 2 Drawings of amoebae and conidia from microscopical observations. Figs 9—14 (× 800 approx.)

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Brief Statement on Hungarian Research on the Interactions of Pesticides, Microorganisms and Higher Plants

By

M. KECSKÉS

Research Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary

Since the beginning of the 1950ies, pesticides have been used in great amounts in Hungary. Their quantity and number increased mainly in the next two decades (MATYÓ and KECSKÉS, 1973; 1974). It has been estimated that 32,610 tons of active ingredient were used already in 1975 and presumably there will be 36% more applied in 1980 (Mezőgazdasági és Élelmezésügyi Minisztérium, Erdőrendezési Főosztály, 1976). In 1976, 292 officially permitted pesticide formulations as well as 76 permitted for experimental purposes (made from more than 150 active ingredients, Engedélyezett növényvédőszerek, 1976) were available in Hungary and the expected number of pesticides will be approximately 800 in 1980. As regards the pesticide value per ha of cultivated area, Hungary ranks 6—7th among the countries all over the world (KONKOLY, 1977).

FEHÉR (1954) was the first to focus attention on the microbial decomposition of herbicides. UBRIZSY (1974) urged the investigation of interactions of pesticides and microorganisms in the 1960ies and early 70ies.

We will try to give a short outline citing research carried out in this field during the last 25 years in Hungary. Altogether 114 works have appeared so far including 14 reviews, bibliographies and 100 papers containing data of original research work and besides these 6 other papers dealing with pedological aspects of pesticides.

As regards the 98 original papers there are Ph. D., C. Sc. and D. Sc. dissertations and prizewinning competition works, 51 of them published in Hungarian (most of them with foreign language summaries) from these nearly half of them in foreign languages (mainly in English) one third of them in Hungarian journals and books, and one quarter of them have appeared abroad.

The bibliography of the original papers published until 1972 is listed already (KECSKÉS, 1977c) but about 60% of them were prepared later. There are good reviews of international herbicide literature in Ph. D. dissertation and review articles of TAKÁTS (1966; 1967a, b) and some reports on the interaction of herbicides and microorganisms (VIRÁG, 1967a; 1973a, b and MANNINGER, 1967a, b) as well as on herbicides and insecticides (TIMÁR, 1963) and about the pesticides (MANNINGER, 1972) too. There are citations concerning Hungarian research especially with respect to protection of the environment by HELMECZI (1974).

A discussion paper by VIRÁG (1967b) on soil algae should be mentioned, furthermore an introductory lecture and review by SZEGI (1974, 1976).

The survey of the international literature, Hungarian research and the synthesis of our work carried out from 1964 up till now, is presented in a D. Sc. dissertation and thesis (KECSKÉS, 1976d, e) and in addition in several synopses of our investigations (KECSKÉS, 1973a, 1976c, 177a, b).

The original papers reported so far are grouped according to the "classical" order of pesticides as follows:

Fungicides

The effect of seven fungicides (seed dressers) on Rhizobium leguminosarum and its symbiosis with Vicia sativa was studied in laboratory, light room, glasshouse and field experiments (KECSKÉS and VINCENT, 1969a, b, c; 1973a). The rhizobia affected by other fungicides (KECSKÉS, 1970) fungicides and microelements and the legume–rhizobium symbiosis (ELEK and KECSKÉS, 1972) comparative investigations of Ceresan and TMTD in different Hungarian soil types (KECSKÉS and SZÜCS, 1974); fungicides and rhizobium inoculation in recultivation area (KECSKÉS, 1976a) were studied. A survey of soil and rhizobiological effects of TMTD (KECSKÉS, 1977a) as well as a forum article of the compatibility of fungicide treatment and rhizobium inoculation of vetch seeds (KECSKÉS and VINCENT, 1973b) and other practical conclusions (KECSKÉS, 1973b, c), furthermore a synopsis of the vetch–rhizobium symbiosis affected by fungicides (KECSKÉS, 1972b) have been reported.

BAKONDI-ZÁMORY and GÄRTNER (1977) as well as KISS, PAPP, GÄRTNER and BAKONDI (1977) recently published data on the effect of fungicides on Rhizobium japonicum and its symbiosis with Glycine max. The genetical studies of benomyl on streptomycete (BUDAY, KISS, GERGELY and ÖCSÉNYI, 1973) and the work of SZENDE (1977) were carried out with captan and rhizobia. The decomposition of technical TMTD and Thiram preparations in the soil as well as their effect on the change of the "total" number of microbes and different groups of soil microorganisms were also observed (KECSKÉS, 1975; KECSKÉS and SCHMIDT, 1976).

Zoocides

Relatively few publications have appeared in connection with zoocides. The effect of Lindan, Dyfonat and Basudin on the glucose assimilation, the N-fixation and intermedier metabolism of Azotobacter agile and A. chroococcum (SALEM and GULYÁS, 1971) further the root mass, hay-yield, N-content and number of root nodules of Trifolium pratense and Medicago sativa affected by the same insecticides was studied by SALEM, SZEGI and GULYÁS (1971).

The effect of gamma-BHC on rhizobia, rhizobium–legume symbiosis (and other zoocides), the persistence of gamma-BHC (KECSKÉS, 1973a; KECSKÉS, 1975;

KECSKÉS and BALÁZS, 1977) have appeared in summarizing works: (KECSKÉS, 1975; 1976c; 1977b); the effect of gamma-BHC, diazinon and menazone on soil algal synusiums (P. KOMÁROMY and KECSKÉS, 1977a) and the trichlorfone, dimethoate, phosphamidon, prothoate on the growth of a few bacteria and microscopic fungi (KECSKÉS and DOBOLYI, 1977) and the zoocide–herbicide interactions. These effects will be discussed below. The decomposition of diazinon in "natural", "recultivation" and culture ecosystems was observed too (KECSKÉS, HARGITAI, FARKAS and TÓTH, Á., 1977).

Herbicides

As regards the herbicides the situation is much better, as there are Ph. D. thesis (VIRÁG, 1958b; TAKÁTS, 1966; KISS, M., 1966a), citations and data in C. Sc. and D. Sc. theses too (HELMECZI, 1965a, b; SZEGI, 1973a, b). Summarizing research works concerning the action of herbicides on the growth of different soil bacteria in axenic cultures (KECSKÉS, 1972a, 1974) and the effect of certain herbicides like Gramoxone on symbiotic and non-symbiotic N-fixing bacteria (MANNINGER, BAKONDI-ZÁMORY and TAKÁTS, 1972; SZEGI, GULYÁS, MANNINGER, BAKONDI-ZÁMORY, 1974), the Hungazin DT (simazine) on the N-fixation of Azotobacter. Moreover 2,4-D and related compounds on rhizobia and different microbes (KECSKÉS, 1973a; KECSKÉS, NAGY, KECSKÉS, É. and KOVÁCS, 1973; KECSKÉS, SZÜCS, BALÁZS, 1973; 1976) were studied too. Genetical investigations conducted with herbicides with respect to Rhizobium meliloti its phages and other bacteria (SZENDE, 1974; 1977). Observations on herbicides (KISS, M., 1966a, b; BUDAY, KISS, M., GERGELY and ÖCSÉNYI, 1973; GERGELY, 1973) and pesticides (BUDAY and KISS, M., 1973; GERGELY and KISS, M., 1977) on ray fungi were reported of which many investigations of genetical nature can be found.

In works of TAKÁTS (1966), PÁNTOS, GYURKÓ, TAKÁTS and VARGA (1962), GYURKÓ, VARGA and TAKÁTS (1964), PÁNTOS, GYURKÓ and TAKÁTS (1964a, b) there are many data on the herbicidal sensitivity of bacteria, ray fungi, microscopic fungi (mycorrhizal ones) and protozoa, furthermore on the utilization of some herbicides as C and N sources for pseudomonads and streptomyceta. Data on the effect of some herbicides on soil microflora, microfungi were published by VIRÁG (1958a, b; 1959, 1964), VIRÁG and MÁRTON (1962). KISS reported (1966; 1967) about the herbicide sensitivity and quantitative and qualitative occurrence of soil algae on the effect of herbicides. The diquat-dibromide sensitivity of some soil algae was also studied (P. KOMÁROMY and KECSKÉS, 1977b). Recently some data on the effect of herbicides on different physiological groups of soil bacteria appeared in HELMECZI's paper (1977).

The herbicide sensitivity of cellulose decomposing microscopic fungi and cellulose decomposition influenced by herbicides (SZEGI, 1970, 1972, 1973a, b; SZEGI and GULYÁS, 1971; SZEGI, GULYÁS and FAWAZ, 1972) have also been published as well as the effect of Afalon (linuron) and Convulan (p-chlorophenoxy acid-i-propylesther + atrazine) on the respiration of soil microorgan-

isms (MANNINGER and SZÁVA, 1972). With respect to CO_2 production of the decomposition of maize stalks in the presence of herbicides it was studied by TÓTH, B. (1977).

SZABÓ (1964, 1967) reported on the effect of some herbicides on the root nodulation of Pisum and its rhizospheric microflora.

The effect of 108 herbicides on Rhizobium lupini and its symbiosis with Lupinus albus and L. luteus was studied by us in laboratory and field experiments for more than seven years from biological and agricultural points of view (BORBÉLY and KECSKÉS, 1972; KECSKÉS, BORBÉLY, F. and BORBÉLY, I., 1978; KECSKÉS, BORBÉLY, F. and ELEK, 1972; 1975; summarizing works: KECSKÉS, ELEK, BORBÉLY, I. and BORBÉLY, F., 1972a; 1972b; 1973, connected with protein change of lupins: ELEK, BORBÉLY, I., BORBÉLY, F. and KECSKÉS, 1974a; 1974b, in connection with weed mass: BORBÉLY, F., BORBÉLY, I., ELEK and KECSKÉS, 1976, practical aspects: BORBÉLY, I., BORBÉLY, F., ELEK and KECSKÉS, 1972, 1973, 1974).

As regards the decomposition of herbicides little has been done. Mainly the decomposition of pro anal. and commercial preparations of 2,4-D and 2,4-D-Na were studied (KECSKÉS, SZÜCS and BALÁZS, 1973; KECSKÉS, 1975; KECSKÉS, BALÁZS and SCHMIDT, 1975 etc.), but there are investigations on the decomposition of DNOC in axenic cultures (NEHÉZ, PÁLDY and SELYPES, 1977), further on diuron and treflan (KECSKÉS, 1975) on atrazine (MANNINGER, GÄRTNER, BAKONDI-ZÁMORY and SOÓS, 1975, 1977) and on N-phenyl-phthalamine acid (GOMBOS, PFIFER and KECSKÉS, 1977). The persistence of Isocil DuPont 997 and Venzar (KECSKÉS, GOMBOS, HORVÁTH and POZSÁR, 1974) as well as the relative biological stability of 6-methyluracil end-product (HORVÁTH, KECSKÉS, POZSÁR, 1975) were studied too.

Pesticide interactions

The works on the effect of pesticide interactions as their effects will be quoted separately here on the growth of axenic cultures (pesticide combinations: KECSKÉS, 1972a; 1974, insecticides + linuron: KECSKÉS and DOBOLYI (1977), herbicide interactions: PÁSZTOR, DOBOLYI and KECSKÉS, 1977a) on the qualitative and quantitative changes of soil microbiota (DOBOLYI, PÁSZTOR and KECSKÉS, 1977a, b; KECSKÉS, BORBÉLY, F. and BORBÉLY, I., 1977; P. KOMÁROMY and KECSKÉS, 1977a; PÁSZTOR, DOBOLYI and KECSKÉS, 1978a, DOBOLYI, PÁSZTOR and KECSKÉS 1978 as well as on the decomposition of pecticides in soil (DOBOLYI, PÁSZTOR and KECSKÉS, 1977a, b; CSERHÁTI and KECSKÉS, 1977; GOMBOS, HUBER, PFEIFER and KECSKÉS, 1977; KECSKÉS and CSERHÁTI, 1977; PÁSZTOR, DOBOLYI and KECSKÉS, 1978).

We mention here also the effect of the high N-doses with the application of herbicides on the root nodulation of Medicago sativa (KECSKÉS, SZALKAI and PEKÁRY, 1975).

In connection with the above mentioned problems investigations were carried out on the adsorption of herbicides in the soil like Gramoxone (SZEGI,

MARENKO and GULYÁS, 1973) and linuron (CSERHÁTI, VÉGH and KECSKÉS, 1977a, b) Buvinol and its components (atrazine + 2,45-TE) as well as their migration in the soil (STEFANOVITS and TOMKÓ, 1972; 1976) further on the complex metabolism of Klorinol (2,45-TE) and Buvinol (PECZNIK and KAMPFL, 1976) too.

Finally we should like to mention our efforts to introduce the "Soil microbiological test minimum" as a previous test system (KECSKÉS, 1976b, d, e) beside the officially prescribed investigations before permitting the pesticide formulations on the market.

Here we also refer to the use of nodulation forms of legumes described by us (KECSKÉS and VINCENT, 1969a) as well as other efforts (CSERHÁTI, VÉGH and KECSKÉS, 1978) to detect the pesticide effect on soil microbiota.

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Edited by Z. KIRÁLY

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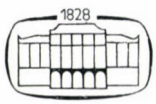

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The Role of Ethylene and Abscisic Acid in TMV-induced Symptoms in Tobacco

By

K. W. BAILISS,[1] E. BALÁZS[2] and Z. KIRÁLY[2]

[1] Department of Plant Sciences, Wye College (University of London) Wye, Nr. Ashford, Kent TN25 5AH, U.K.

[2] Department of Pathophysiology, Research Institute for Plant Protection, H—1525 Budapest, P.O. Box 102, Hungary

Xanthi-*nc* tobacco leaves pre-treated with ABA or with Ethrel exhibited senescence and increased the number of TMV local lesions. Changes in local lesion numbers were not associated with similar changes in recoverable infective TMV.

Endogenous ABA progressively increased in leaves as TMV lesions developed.

When BYMV-infected leaves of *Tetragonia expansa* were exposed to ethylene, necrotic spots developed in the normally chlorotic lesions. An inhibitor of ethylene action, namely CO_2, was able to inhibit the development of chlorotic lesions in infected *Tetragonia* leaves.

In a systemic host–pathogen combination (cultivar Samsun infected by TMV) both Ethrel and ABA caused dwarfing in healthy and in infected plants. Regarding the effect of ABA, it is proposed that an ABA-induced stimulation of ethylene release exists and this would be directly involved in the reduction of extension growth.

It is well known that abscisic acid (ABA) (e.g. BALÁZS *et al.*, 1973) and ethylene (e.g. BURG, 1962) can accelerate senescence when applied exogenously to plants and that enhanced senescence is often associated with the development of virus-induced local lesions and systemic symptoms. Virus-induced necrotic lesions may be a consequence of enhanced leaf senescence. This hypothesis is supported by data which show that local lesion formation is suppressed when juvenility is extended but increased when tissue senscence is promoted (e.g. KIRÁLY and SZIRMAI, 1964; KIRÁLY *et al.*, 1968; BALÁZS *et al.*, 1973). Increased ethylene production during local lesion development is well documented and there is evidence which points to the possible involvement of ethylene in virus localisation and/or concomitant host necrosis (BALÁZS *et al.*, 1969; NAKAGAKI *et al.*, 1970; GÁBORJÁNYI *et al.*, 1971; and PITCHARD and ROSS, 1975). In addition, BALÁZS *et al.* (1973) suggested a possible involvement of ABA by showing that the aging effect of ABA increased TMV lesion production and multiplication in tobacco.

Work with viruses which systemically invade host plants has shown that host ethylene production may be unaffected (BALÁZS *et al.*, 1969), or associated with virus-induced premature senescence (NAKAGAKI *et al.*, 1970). Increased ethylene production has also been correlated with cucumber mosaic virus (CMV)-induced stunting of cucumber hypocotyls and epinasty of cucumber cotyledons (MARCO *et al.*, 1976; LEVY and MARCO, 1976). Little attention has been paid

to the possible role of ABA in virus-induced stunting but BAILISS (1977) found no significant difference in the endogenous ABA content of healthy and CMV-infected cucumber cotyledons and leaves.

This paper reports further studies on the role of ethylene and ABA in virus-induced symptoms.

Materials and Methods

Viruses and hosts. Tobacco plants (*Nicotiana tabacum* cvs. Samsun, Xanthi and Xanthi-*nc*) were grown under glasshouse conditions and used at the 8–10 leaf stage. Experiments on chemically-induced stunting were made with two month old tobacco plants. The Ul strain of TMV was cultured in *N. tabacum* cv. Samsun and the virus purified using a modification of the methods described by FRAENKEL-CONRAT (1966) and GOODING and HEBERT (1967). Infectivity was determined by inoculating tobacco leaves (cv. Xanthi-*nc*) and counting the number of lesions produced. In some experiments chlorotic lesions were induced in leaves of *Tetragonia expansa* by infection with bean yellow mosaic virus (BYMV).

Chemical treatments. Aqueous solutions of ABA at concentrations of 1, 10 and 100 μg/ml were applied to tobacco leaves by either spraying half-leaves of attached leaves twice daily or by injection (KLEMENT, 1963). Ethrel (2-chloroethyl-phosphonic acid) (Amchem P. A., USA), dissolved in 0.06 *M* phosphate buffer (pH 6.5) to give a concentration of 200 μg/ml was sprayed daily onto half-leaves. Plants were exposed to CO_2 and ethylene by removing them from the soil and placing them into nutrient solution under three litre capacity bell jars. Ethylene gas or CO_2 was bubbled through water into the bell jar until a 1% concentration was reached.

Ethylene determination. Plant ethylene production was estimated by collecting the gas from plants placed under bell jars with a continuous air stream in 0.25 *M* mercuric perchlorate in 2 *M* $HClO_4$ (YOUNG *et al.*, 1952) and the amount estimated by gas chromatography after releasing the ethylene by the addition of 2 *N* hydrochloric acid (BALÁZS *et al.*, 1969).

ABA determination. A modification of the methods of LENTON *et al.* (1971) and BAILISS (1977) was used to estimate the endogenous ABA content of plant tissue. Leaf tissue (*c.* 30 g fresh weight) was immersed immediately after sampling in 300 ml chilled 80% methanol and 1.5 g sodium bicarbonate added to the extract. The material was left at −15°C for five hours and then homogenized in a Waring blender at 4°C. The homogenate was left overnight at 4°C then centrifuged at 5000 *g* for 30 min at 4°C. The supernatant was reduced to the aqueous phase in a rotary evaporator at 40°C, adjusted pH 3.5 with 2 *N* HCl and extracted three times with equal volumes of diethyl ether. Bulked ether extracts were extracted three times with equal volumes of 5% aqueous sodium bicarbonate and, after adjusting the pH to 3.5, the combined aqueous extracts were extracted three times with equal volumes of diethyl ether. Combined ether extracts were evapo-

rated to dryness with a rotary evaporator, re-dissolved in a small volume of 96% ethanol and streaked onto Whatman No. 1 chromatography paper or Silufol 254 (Merck) thin-layer chromatography plates. Two solvent systems were used for further separation; a) *n*-butanol : ammonia : propanol : water, 2 : 1 : 6 : 2, and b) ethyl acetate : formic acid : benzene, 100 : 2 : 50. Authentic ABA markers were run on all chromatograms and their position located under UV light. Zones corresponding to the markers were eluted with ethanol and the ethanol evaporated off *in vacuo* in small vials. A 0.1 ml aliquot of Regisil reagent (Regis, Chicago, USA) (*bis*-trimethyl-silyl-trifluoroacetamide dissolved in 1% trimethyl-chlorosilane) was added to each vial. After sealing, the vials were placed in a water bath and exposed to 100°C for 10 min or longer. All determinations were made with a Packard Series 7400 chromatograph equipped with flame ionization detectors and with 60 cm × 4 mm glass columns containing Chromosorb G (60–80 mesh) coated with 1.5% SE 30. The column temperature was 200°C and the N_2 carrier gas flow rate was 48 ml/min.

Results

The effect of Ethrel and ABA-induced senescence on lesion numbers and virus infectivity. Half leaves of Xanthi-*nc* tobacco were sprayed with ABA and Ethrel (an ethylene producing agent) for varying periods before the entire leaves were

Table 1

The effect of Ethrel and ABA on local lesion production and TMV infectivity in Xanthi-*nc* tobacco

Experiment		Period of treatment (days)	Lesion number (per cent of untreated control leaves)*	Infectivity of ultracentrifuged extracts (per cent of untreated control leaves)*
ABA	I.	18–20	197.1**	120.9
	II.	14–17	153.8	113.1
	III.	10–13	126.6	103.9
Ethrel	I.	8–9	198.3	119.9
	II.	5–7	168.1	111.5
	III.	3–4	145.3	103.1

Each experiment was repeated 5 times. In each replicate 8 plants with 6–8 leaves were used. The ultracentrifuged extracts were assayed on 20 leaves of Xanthi-*nc* by half leaf comparisons.

* The correlation between lesion number and infectivity of the ultracentrifuged extract was significant ($P < 0.05$).

** Mean number of lesions per half leaves for the water-sprayed control was 138. ABA (100 μg/ml) or Ethrel (200 μg/ml in phosphate buffer pH 6.5) was sprayed onto half-leaves twice daily

inoculated with TMV. In a series of experiments such treatments increased the numbers of local lesions in the treated half-leaves compared with water-sprayed control half-leaves. The size of the increase in lesion numbers depended on the length of the treatment period. In every experiment the infectivity of ultracentrifuged leaf extracts was also determined (cf. BALÁZS *et al.*, 1976). Table 1 illustrates the results of typical experiments. Ethrel and ABA-induced increases in lesion numbers were pronounced after the longer treatment periods. However, the infectivity of ultracentrifuged extracts was always less than was expected on the basis of lesion production (Table 1). The data suggested that Ethrel and ABA treatment decreased the infectivity of the lesions which developed. This was further examined by determining the infectivity of virus recoverable from 100 lesions removed with a 2 mm diameter cork borer from the treated half-leaves and compared with 100 lesions similarly removed from the control half-leaves. The results (Table 2) confirmed that infectivity was reduced in lesions tested from treated leaves.

Table 2

Infectivity of TMV in lesions after Ethrel and ABA treatment

Experiment	Lesion number	
	control half-leaves	treated half-leaves
Ethrel	89	73
Abscisic acid	97	75

48 h after infection with TMV 100 lesions were cut from the leaves with a cork borer (2 mm in diameter), ground in 3 ml buffer pH 6.9 and assayed on Xanthi-*nc*.

Plants were sprayed twice daily with 200 ppm Ethrel and 100 ppm abscisic acid respectively, for 4 days. The ratio of lesion numbers in control and treated half-leaves was 131 : 100. The experiment was repeated ten times; the data are the means of 10 experiments

The possibility was considered that the chemicals used might have a direct effect on TMV infectivity. Therefore ethylene gas was bubbled through a purified preparation of TMV (100 μg/ml) for 1 h at 22°C. Air was bubbled through a second aliquot of the purified preparation to serve as a control. There was no difference in the infectivity of the two preparations as evidenced by lesion counts made on inoculated Xanthi-*nc* leaves. It was shown previously that ABA had no effect on the infectivity of TMV when mixed with the virus *in vitro* (BALÁZS *et al.*, 1973).

Endogenous ABA content of TMV-infected Xanthi-nc leaves. As ABA treatment affected lesion production it was decided to investigate the endogenous ABA content of healthy and infected half-leaves. Half-leaves of tobacco cv. Xanthi-*nc* were inoculated with TMV and the corresponding half-leaves with water. Samples were taken from inoculated and control half-leaves 24, 48 and 72 h after inocula-

tion and the ABA content estimated. The results (Table 3) showed a progressive increase in endogenous ABA in infected compared with control half-leaves. The rise in endogenous ABA was correlated with the appearance and expansion of local lesions.

Table 3

The endogenous ABA content of Xanthi-*nc* tobacco leaves infected or uninfected with TMV

Time between inoculation and sampling (h)	ABA content of half-leaves (μg/kg) fresh weight	
	control	infected
24	36	43
48	36	52
72	40	79

Half-leaves of Xanthi-*nc* plants (6—8 leaf stage) were inoculated with TMV. Twenty-four, 48 and 72 h after inoculation the infected and control half-leaves were collected separately and the ABA content estimated. Ten plants were used at each sampling time

The effect of ethylene and CO_2 on BYMV-induced symptoms in Tetragonia expansa. The effect of ethylene on symptom expression was further studied in a virus–host interaction resulting in the formation of chlorotic lesions. *T. expansa* plants were placed in bell jars containing 1 % CO_2 or 1 % ethylene two days after inoculation with BYMV for four days. Plants exposed to CO_2 failed to develop typical chlorotic lesions; the plants showed symptomless infection. BYMV-infected plants exposed to ethylene developed local necrotic spots within the chlorotic lesions. This effect was best shown in the older mature leaves. Necrotization could also be induced by spraying inoculated leaves with 1000 ppm Ethrel twice daily for four days after inoculation with BYMV.

The effect of ABA and Ethrel on the growth of healthy and TMV-infected Samsun tobacco. Further studies on the role of ABA and ethylene on virus symptom expression were made using systemically infected tobacco plants. Both ABA and Ethrel treatments caused further stunting in infected plants (Table 4). Untreated infected plants were stunted compared with control plants. The extension growth of plants sprayed once or twice daily virtually ceased. It was considered that the effects shown after ABA treatment could involve ABA-induced stimulation of ethylene release rather than a simple direct effect of ABA. Therefore, healthy Samsun leaves were injected with a range of ABA concentrations and control plants injected with water. As shown in Table 5, ABA treatment enhanced ethylene production and the magnitude of ethylene release depended on the concentration of ABA supplied.

Table 4

The effect of ABA and Ethrel on stem length and leaf fresh weight of healthy and TMV-infected Samsun tobacco

Plant material	Stem length (cm)	Leaf weight (g)
Healthy Samsun	12.8 ± 1.0	5.7 ± 0.5
Healthy Samsun plus ABA (100 μg/ml)	6.0 ± 1.0	3.7 ± 0.5
TMV-infected Samsun	5.6 ± 0.9	2.5 ± 0.6
TMV-infected Samsun plus ABA (100 μg/ml)	3.3 ± 0.5	2.0 ± 0.4
Healthy Samsun	14.3 ± 0.8	—
Healthy Samsun plus Ethrel (200 μg/ml)	6.4 ± 0.7	—
TMV-infected Samsun	6.0 ± 0.9	—
TMV-infected Samsun plus Ethrel (200 μg/ml)	3.1 ± 0.5	—

Plants were measured one month after inoculation with TMV and the data are the mean of 20 measurements. Plants were inoculated when two months old with a purified preparation of TMV (120 μg/ml) by using a glass rod. No abrasive was added to the inoculum. The ABA treatment was applied by spraying twice daily for one month after inoculation. The Ethrel treatment was applied similarly but once daily

Table 5

The effect of ABA on ethylene production in healthy Samsun tobacco plants

ABA treatment (μg/ml)	Ethylene production (μl/24 h/g tissue) time after treatment	
	24—48 h 2 days	48—72 h 3 days
0	1.5	1.5
1	1.7	1.9
10	1.6	2.9
100	4.2	5.8

ABA was injected with a hypodermic syringe into the tobacco leaves. Control plants were injected with water

Discussion

Pre-treatment of tobacco (cv. Xanthi-*nc*) leaves with ABA and Ethrel induced senescence and increased the number of TMV local lesions visible to the naked eye. These results contrasted markedly with those obtained by BALÁZS *et al.* (1976) who pre-treated tobacco leaves with kinetin and found senescence was delayed and TMV lesion numbers decreased. In both experiments, however, changes in lesion numbers were not associated with similar changes in recoverable

infective TMV. Results reported here for Ethrel and ABA treatment support the possibility that virus replication is reduced in lesions which are formed. In experiments in which kinetin caused a reduction in lesion numbers there was a concomitant increase in single necrotic cells which, in terms of virus infectivity, probably compensated for the decrease in macro lesions (BALÁZS *et al.*, 1976). The demonstration that kinetin, ABA and ethylene treatments affect lesion numbers strengthens earlier observations (GÁBORJÁNYI *et al.*, 1971) that plant growth regulators are probably involved in local lesion development.

The role of ethylene in lesion development has been considered to be a consequence of enhanced host tissue senescence caused or followed by ethylene production (BALÁZS *et al.*, 1969; NAKAGAKI *et al.*, 1970; GÁBORJÁNYI *et al.*, 1971; PITCHARD and ROSS, 1975). ABA may also be involved for it accelerates leaf senescence in Xanthi-*nc* tobacco (BALÁZS *et al.*, 1973) and evidence is presented here that endogenous ABA progressively increases in tobacco leaves as TMV lesions develop, ABA treatment increases ethylene production in Samsun tobacco and, as discussed above, exogenous ABA treatment increases lesion production.

The precise roles of ABA and ethylene in virus-induced necrosis remain unclear. However, the experiments reported with BYMV infection of *Tetragonia expansa* provide further information regarding the role of ethylene. BYMV-induced chlorotic lesions represent localized areas of intense tissue senescence without necrosis. BYMV infection stimulates ethylene production (GÁBORJÁNYI *et al.*, 1971) and indicates tissue necrosis may not be a prerequisite for enhanced ethylene production. However, when infected plants were exposed to ethylene, necrotic spots developed in the chlorotic lesions whereas exposure to CO_2 (an inhibitor of ethylene action) inhibited chlorotic lesion development. Clearly ethylene is involved in chlorotic lesion formation and perhaps in the necrotization process, although the latter may be very much dependent on the ethylene concentration supplied and the time when the plant is exposed to the gas relative to inoculation.

In the systemic TMV/tobacco cv. Samsun system both Ethrel and ABA treatments caused dwarfing in healthy and infected plants. As ABA treatment increased ethylene production, it is possible that ethylene may have the more direct effect on extension growth. However, further work is required to clarify the roles of ethylene and ABA in virus-induced effects on extension growth, particularly in view of, for example, the absence of endogenous ABA imbalance in CMV-stunted cucumber (BAILISS, 1977) and the variable effects of systemic infections on host ethylene production (e.g. BALÁZS *et al.*, 1969; MARCO *et al.*, 1976).

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Some Observations on Virus-Induced Local Lesions by Transmission and Scanning Electron Microscopy

By

M. AUGUSTA FAVALI, G. G. CONTI* and MARIA BASSI**

Istituto di Scienze Botaniche, Università di Milano, via G. Colombo 60, 20133 Milano, Italy

The early modifications undergone by the epidermal cells of leaves of *Vigna sinensis* and *Nicotiana glutinosa* after mechanical inoculation were studied by transmission electron microscopy. Immediately after inoculation the outer wall of the epidermal cells showed electron-lucid areas, sometimes reaching the plasmalemma. Electron-lucid blebs were also seen just under the cuticle. The cytoplasm of the epidermal cells was highly vacuolated. The alterations of the cell wall were no longer visible 6 hr after inoculation, while cytoplasmic vacuolation persisted.

The formation of local lesions was followed by scanning electron microscopy. Small depressions were visible on the leaf surface as early as 24 hr after inoculation. These depressions were due to the collapse of the epidermal and palisade cells. Five days after inoculation the thickness of the leaf in the lesion region was reduced by half. The tracheids of the veins bordering the lesions were pervious 24 hr after inoculation, but were semi-obliterated 3 days after inoculation.

Abrasion of the leaf surface is often a necessary prerequisite for successful mechanical inoculation of plant viruses. The consequences of this injury on the surface of the epidermal cells have been extensively studied (HERRIDGE and SCHLEGEL, 1962; MUNDRY, 1963; BRANTS, 1964; 1965; 1971; YARWOOD and FULTON, 1967; GEROLA *et al.*, 1969; CONTI and LOCCI, 1972; DUAFALA and NEMANIC, 1974), but little information is available on the modifications brought about inside these cells. It is generally maintained that the treatment wounds the cuticle and cell wall in such a way as to expose the cell membrane and allow the pinocytotic uptake of virus particles; or that also the cell membrane is broken and the virus particles are allowed to penetrate directly into the cytoplasm.

Since in a previous work (GEROLA *et al.*, 1969) we demonstrated that after carborundum treatment small blebs appear under the cuticle of the external wall of the epidermal cells, we have now tried to see if these cells showed alterations that might be ascribed to the wounding of the leaf surface, and that might be assumed to facilitate the penetration of virus particles. Besides, since scanning electron microscopy has been applied with success to the study of the inoculated

* Gruppo di Ricerca Virus e Virosi delle Piante, C.N.R., c/o Istituto di Patologia Vegetale, Università di Milano.

** Istituto di Botanica, Università di Messina.

leaf surface (CONTI and LOCCI, 1972; DUAFALA and NEMANIC, 1974), we have applied this technique to the study of the development of local lesions after mechanical inoculation.

Materials and Methods

Plants of *Nicotiana glutinosa* and *Vigna sinensis*, grown in a greenhouse at 22°, were mechanically inoculated with a purified TMV suspension (0.3 μg/ml), using Carborundum (600 mesh) as an abrasive.

Transmission electron microscopy

Small pieces of the leaf blade were taken from both untreated and inoculated leaves, immediately after inoculation and after 6 hr, 24 hr, 3 days and 5 days later. They were fixed in phosphate-buffered 3% glutaraldehyde, pH 6.9, at 4° for 2 hr, post-fixed in phosphate-buffered 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop 1A.

Scanning electron microscopy

Pieces of about 1 × 1 cm, including a secondary vein, were cut out of the leaf blade of both untreated and inoculated leaves, at the same time intervals as above. After fixation in phosphate-buffered 3% glutaraldehyde, pH 6.9, a few pieces were embedded in agar and cut transversally with a tissue chopper for the observation of their internal structure, while others were kept as such for the study of the leaf surface. All the samples were post-fixed in phosphate-buffered 1% osmium tetroxide for 24 hr, dehydrated in ethanol and critical point dried with CO_2 using amyl acetate as an intermediate fluid. The samples were coated with carbon and gold in a high vacuum evaporator on a rotating-tilting stage, and observed with a Jeol scanning electron microscope, type JSM U3.

Results

Transmission electron microscopy

Immediately after inoculation, the epidermal cells showed modifications both in their external wall and in the cytoplasm. The external wall had rarefied areas, most often localized immediately under the cuticle (Fig. 1), but sometimes also extending through the whole thickness of the wall and reaching the plasmalemma (Fig. 3). Over these rarefied areas the cuticle was either broken or extremely thinned. The rarefied areas took also the form of vesicles, which pushed the cuticle outwards (Fig. 2). The cytoplasm of the epidermal cells was full of vacuoles of

various size, which gave it a swollen appearance (Fig. 4), but the plasmalemma did not show alterations. The nucleus and cytoplasmic organelles did not seem affected. The underlying palisade cells retained their normal aspect.

Six hours after inoculation, the blebs and rarefactions of the outer wall of the epidermal cells were no longer visible, and the wall had resumed its normal aspect. Only in a few cases remains of the former blebs could be traced as tiny bulges (Fig. 5). In the later stages, the volume of the epidermal cells was progressively reduced owing to the gradual reduction of the cell vacuole. After 5 days, the epidermal cells were completely collapsed (Fig. 6).

Figs 1, 2, 3 and 4. Transmission electron micrographs (TEM) of epidermal cells of leaves of *Vigna sinensis* immediately after mechanical inoculation

Fig. 1. The outer cell wall shows an extended rarefied zone (arrow), immediately under the cuticle which is extremely thinned. × 15,000

Fig. 2. Blebs in the outer cell wall, which have an electron lucid content and push the cuticle outwards. × 15,000

Fig. 3. Rarefied areas in the outer cell wall. Two of these areas are in close contact with the plasmalemma (arrows) × 12,000

Fig. 4. Epidermal cell with vacuolated cytoplasm. The cell organelles appear intact. The outer cell wall shows rarefied areas just under the cuticle (arrow) × 9,000

Fig. 5. TEM. Epidermal cell of *V. sinensis* 6 hr after inoculation. In the outer cell wall two tiny bulges are visible (arrows) most probably remnants of former blebs. The cytoplasm is still vacuolated. × 30,000

Fig. 6. TEM of a section of *Nicotiana glutinosa* 5 days after inoculation. The epidermal cell is collapsed; the outer cell wall has a normal appearance. × 20,000

Fig. 7. Scanning electron micrograph (SEM) of a section through the leaf blade of *N. glutinosa* 5 days after inoculation. On the left, a vein (V) surrounded by healthy tissues. On the right, a portion of a lesion (L). Note that in the lesion area the epidermal and mesophyll cells, as well as the hairs, are collapsed × 100

Fig. 8. SEM. Surface of a leaf of *V. sinensis* 1 day after inoculation. Small depressions are visible scattered at random (arrows). × 100

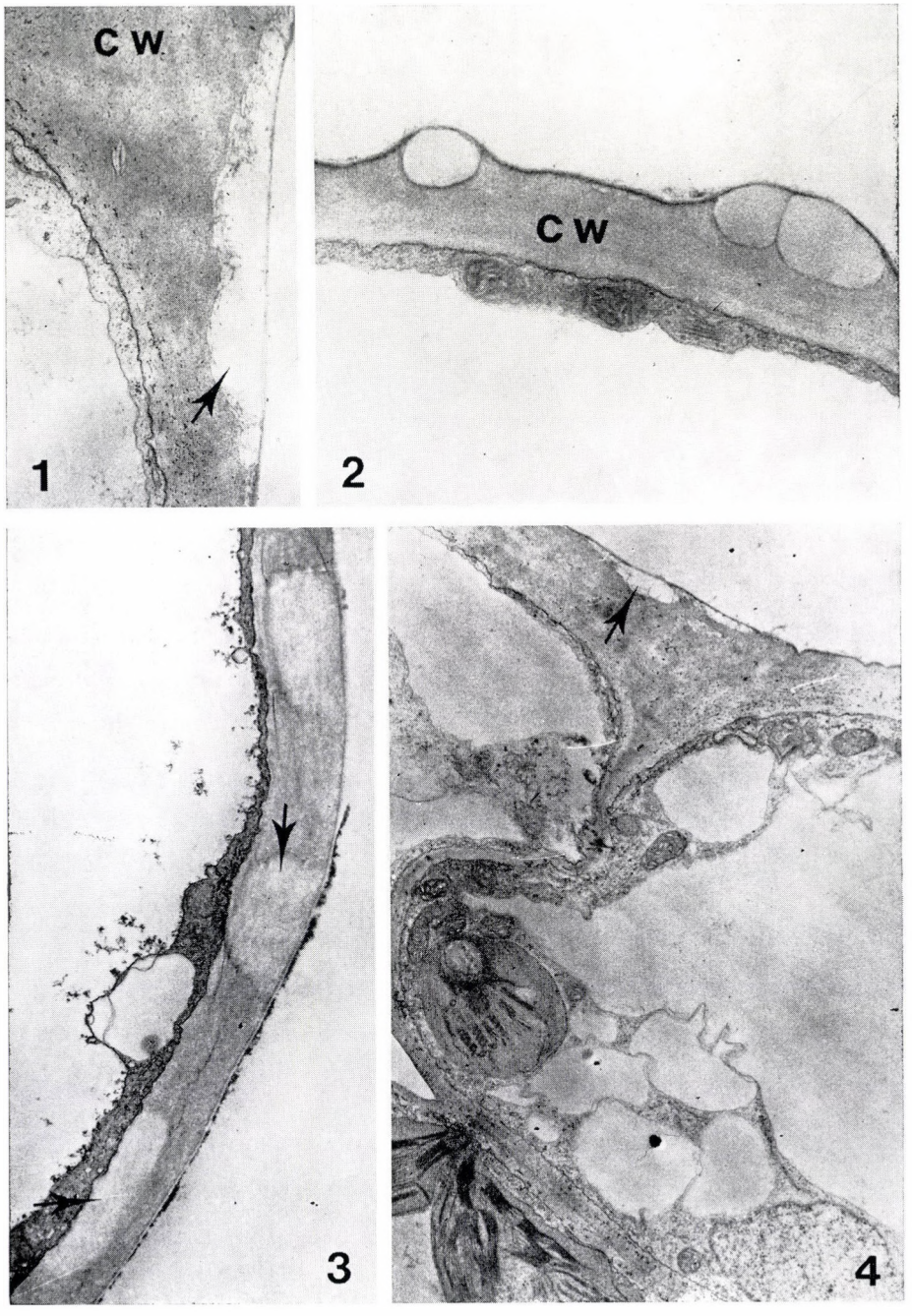
Fig. 9. SEM. Surface of a leaf of *N. glutinosa* 5 days after inoculation. A fully mature lesion is visible. The epidermal cells, hairs and stomata are collapsed. × 50

Fig. 10. SEM. Leaf of *N. glutinosa*. Section through a leaf vein in a lesion of 1 day. The tracheid lumen (t) is pervious. Chloroplasts are visible in the spongy parenchyma (arrows). × 1,000

Fig. 11. SEM. Leaf of *N. glutinosa*. Section through a leaf vein in a lesion of 3 days. The tracheid lumen (t) is partially occluded by plugs (arrows). × 1,000

Fig. 12. SEM. Section of a healthy leaf of *N. glutinosa*. The epidermis (E) and the palisade layer (P) and spongy parenchyma (S) are well preserved. Chloroplasts are clearly recognizable (arrows). × 500

Fig. 13. SEM. Section through a 5-day lesion in a leaf of *N. glutinosa*. Note that the epidermis (E) and palisade layer (P) are much flattened and the spongy parenchyma (S) has wide lacunae. Chloroplasts are no loger recognizable. × 500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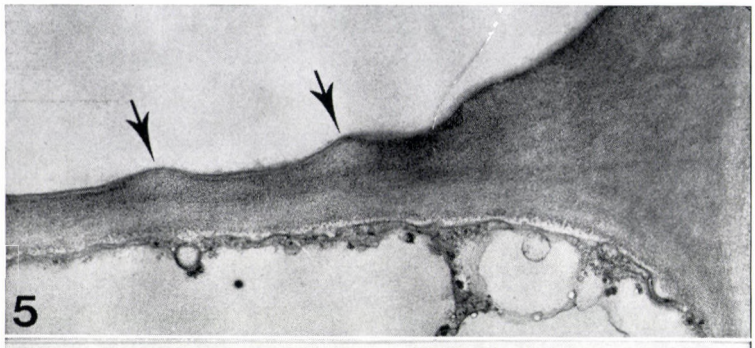
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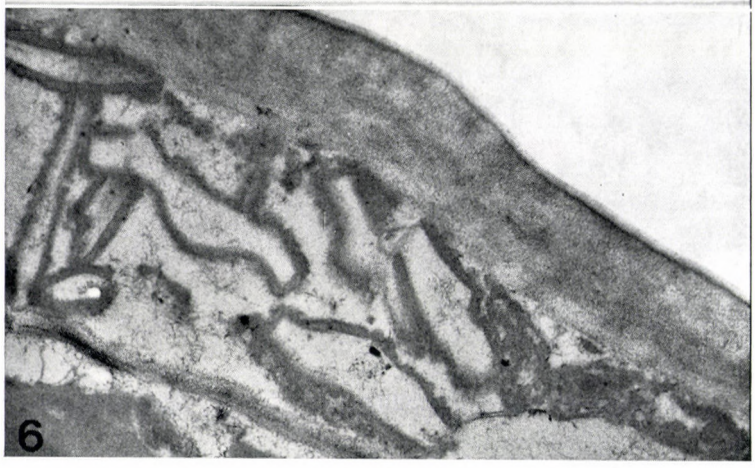
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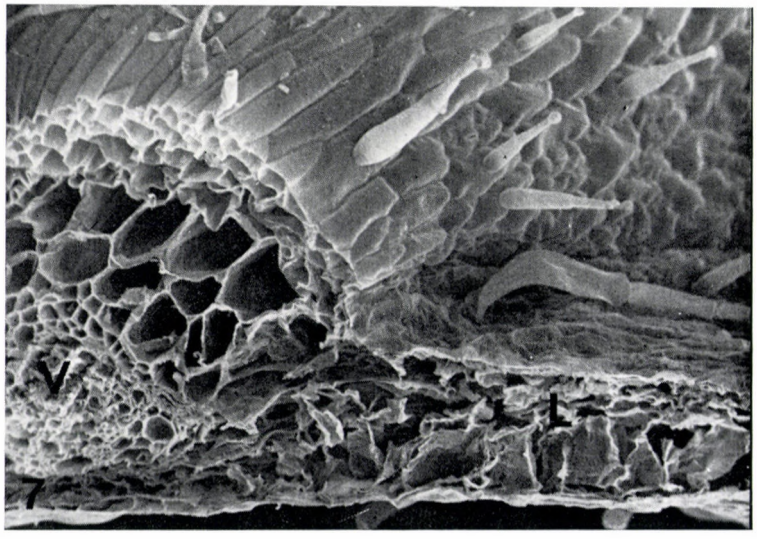
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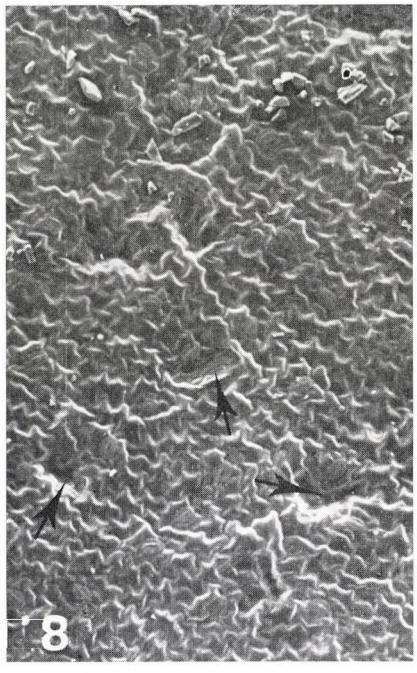

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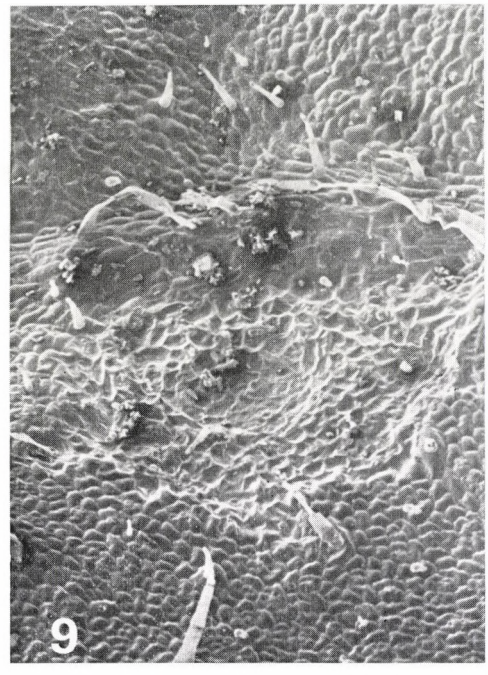

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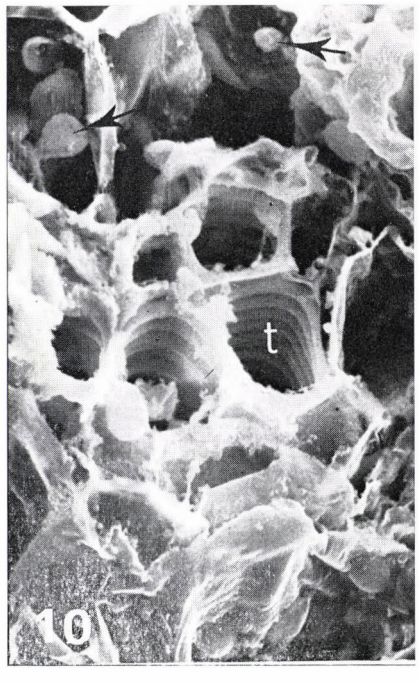

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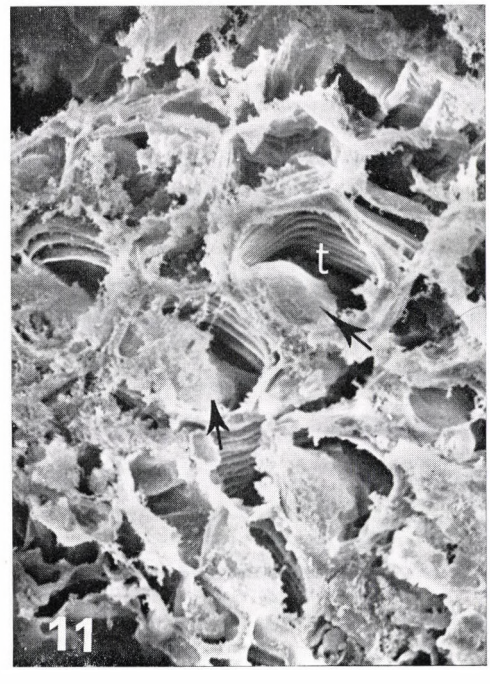

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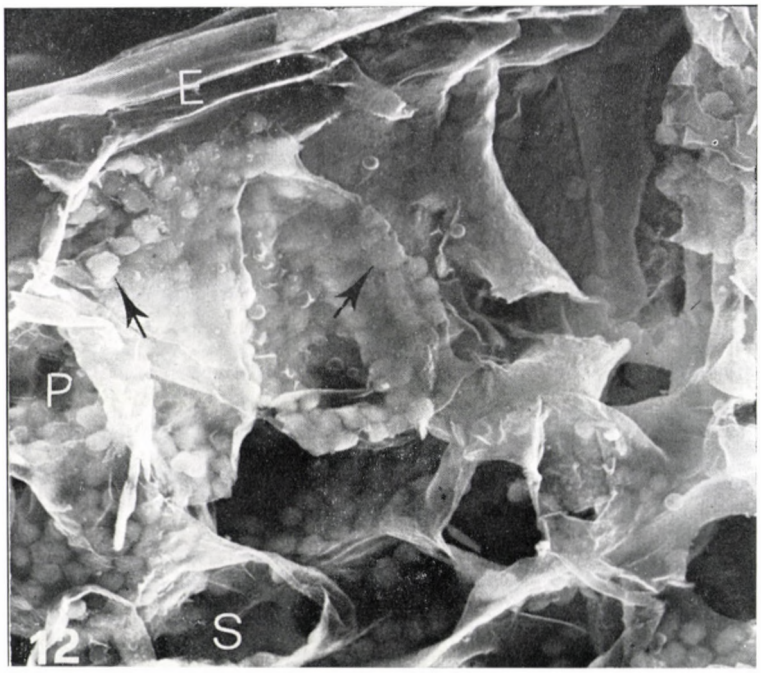
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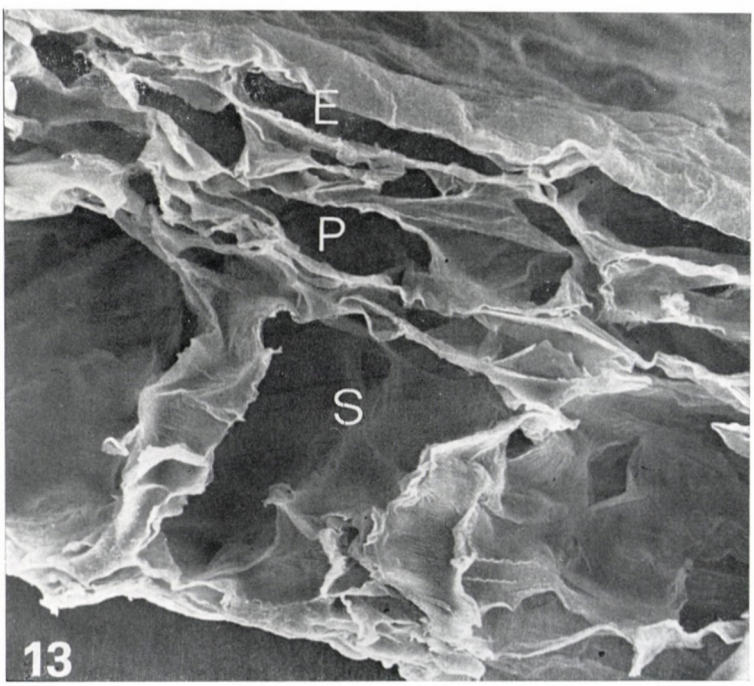
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Scanning electron microscopy

Immediately after inoculation and 6 hr later, the epidermal cells appeared turgid, and the only signs left by carborundum rubbing were scratches of the cuticle, in the case of hairless leaves such as those of *V. sinensis*, ando also broken hairs in the case of *N. glutinosa*.

After 24 hr, small depressions were detectable scattered at random on the leaf surface (Fig. 8), and the epidermal cells as well as the stomata appeared collapsed. In transversal section, the mesophyll cells showed sightly collapsed walls, while the veins retained their normal aspect (Figs 7 and 10).

In the later stages, i.e. after 3 and 5 days, the lesions were enlarged (Fig. 9), and when observed in transversal section showed strongly collapsed epidermal and mesophyll cells (Fig. 13). In particular, the thickness of the palisade layer was reduced by half (compare Fig. 13 with Fig. 12), while the spongy parenchyma showed wide lacunae (Fig. 13). Chloroplasts, which were clearly visible in untreated leaves (Fig. 12), were not detectable. The veins situated right at the border of the lesions showed a number of tracheids with partially occluded lumen (Fig. 11).

Discussion

The changes brought about by carborundum point to a rapid induction of a water imbalance in the epidermal cells, with an increased afflux of water in their cytoplasm and wall. Here water seems to gather in delimited areas, situated immediately under the cuticular areas injured by carborundum. It may be suggested that this focal hydration provides an easy pathway for the virus particles to reach the plasmalemma, which, however, does not seem directly affected by the wounding. Our results therefore suggest that the use of an abrasive favours a partial exposure of the cell membrane by creating rarefied, hyperhydrated areas in the cell wall, and in this way facilitates the contact between virus particles and plasmalemma. These phenomena are transient, and are followed by a dehydration of both the epidermal and underlying palisade cells.

Our findings, therefore, support the old hypothesis by BENDA (1956), that rubbing of the leaf surface induces the output and successive reabsorption of cellular exudates, and strengthen the suggestion by BALDACCI and BETTO (1963), that virus penetration could be achieved by an "interfibre mechanism".

The examination of the leaf surface by scanning electron microscopy confirmed that carborundum treatment causes only very superficial wounding, and that the epidermal cells collapse only when the infective centres are well established, i.e. 24 hr after inoculation. An important point is that scanning electron microscopy allows the exact localization of focal point of infection at this short time interval, when other techniques fail to detect them.

The development of the lesions can also be followed, by and large, by scanning electron microscopy. Particularly evident are the collapse of the palisade

cells and the disappearance of cytoplasmic organelles in the stages where cytoplasmic degeneration takes place (WEINTRAUB and RAGETLI, 1964; HAYASHI and MATSUI, 1965; ISRAEL and ROSS, 1967; DA GRAÇA and MARTIN, 1975 and 1976). Also the obliteration of the tracheids at the boundary between the lesion and the surrounding tissues can be easily detected. In a previous research by transmission electron microscopy (FAVALI *et al.*, 1974), complete obliteration of the tracheids was found when the lesions were fully developed. We have now demonstrated that tracheid occlusion begins at an earlier stage, and can be detected before the lesions become fully necrotic.

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The skilful technical assistance of Mr. R. Bonecchi, "Centro Gino Bozza", Politecnico, Milano, and of Mrs. Maria Teresa Spro cati, Gruppo Virus e Virosi delle Piante del C.N.R., Milano, is gratefully acknowledged.

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Heat-induced Local Lesions with High Peroxidase Activity in a Systemic Host of TMV

By

E. BALÁZS, B. BARNA and Z. KIRÁLY

Research Institute for Plant Protection, H—1525 Budapest, P.O. Box 102, Hungary

Local necroses were induced in leaves of virus-infected Xanthi tobacco, a systemic host for TMV, by hot-water-treatment (50°C for 40 sec) 2—5 days after inoculation. Peroxidase activity was augmented during necrogenesis. The increased perioxidase isozymes were present also in the control samples, however, enzyme activities were very high in the necrotic parts of the leaf. The heat-induced necroses and the parallelly augmented peroxidase could not stop systematization of the virus. Neither tissue necrosis, nor increased peroxidase activity are responsible for virus localization, i.e. for host resistance.

The role of hypersensitive necrosis in plant disease resistance accompanied by augmented peroxidase and polyphenoloxidase activities has been a disputed question in the past decade (cf. GOODMAN *et al.*, 1967). The primary role of the hypersensitive response was questioned in the case of fungus diseases (BROWN *et al.*, 1966; SEEVERS *et al.*, 1972; KIRÁLY *et al.*, 1972; ÉRSEK *et al.*, 1973; BARNA *et al.*, 1974; MAYAMA *et al.*, 1975; TANI *et al.*, 1975) as well as in diseases caused by bacteria (KIRÁLY *et al.*, 1977). In resistance to viral infections an additional importance has been attributed to peroxidase enzyme because of its supposed role in the systemic acquired resistance (SIMONS and ROSS, 1970). However, the latest results reported on the absence of a relationship between peroxidase activity and plant viral resistance (BIRECKA *et al.*, 1975; WESTSTEIJN, 1976). Recently FOSTER and ROSS (1975a, b) called attention to the possibility of induction of tissue necrosis in the systemically infected Turkish tobacco leaves. In this case the host plant remained systemic to TMV in spite of the appearance of necrotic lesions.

The aim of our work was to measure the activity of peroxidases in the development of this "artificial necrosis" and have information on the eventual role of peroxidase in virus localization, e.g. in resistance to the virus.

Materials and Methods

Tobacco plants (*Nicotiana tabacum* L. cv. Xanthi) were grown under normal greenhouse conditions and used for virus inoculation in the 6—8 leaf stage. The U_1 strain of tobacco mosaic virus (TMV) was cultured in *Nicotiana tabacum*

L. cv. Samsun. Tobacco leaves showing the typical disease symptoms of TMV were ground (1 g leaf per 10 ml 0.01 *M* phosphate buffer) with a pestle and mortar and the homogenate was used to inoculate the plants. No abrasive was added to the inoculum. All of the half leaves of tobacco plants were inoculated. Forty-eight hours after inoculation the lower half of leaves was immersed into hot water (50°C for 40 seconds) (FOSTER and ROSS, 1975a). After treatment the plants were put into the greenhouse. All experiments were repeated ten times using 6 – 8 plants.

Leaf material for the assay of peroxidase activity was taken from four parts of each leaf; namely (a) absolute control, (b) heat treated, (c) systemically infected, (d) systemically infected and heat treated parts (cf. Fig. 1). Using a cork-borer, samples were taken, 3 g each, and were homogenized with quartz sand in ice-cooled mortar and pestle in 12 ml 0.2 *M* acetate buffer pH 5.6. The slurry was centrifuged at 6000 *g* for 20 min at 0°C. The supernatant was used for the assay of peroxidase activity. Peroxidase activity was measured spectrophotometrically in a Unicam SP 800 spectrophotometer. Pyrogallol was used as a substrate. The enzyme extracts occurred in the optimal concentration (0.2 ml) in the final volume of 3 ml reaction mixture. Measurement was made at 430 nm, and enzyme activities were expressed as the per cent increase in absorption between 15 and 45 seconds after the enzyme extract was added. Data were related to the untreated and uninoculated control.

Peroxidase activity was expressed both on a fresh weight basis and on a protein basis. For determining the protein content the method of LOWRY *et al.* (1951) was used. An aliquot of the extracts was used for polyacrylamide gel electrophoresis. Amounts of 100 – 200 μg protein were layered on gel. The gel electrophoresis of Davis (1964) was followed at pH 8.3. Samples were run in 0.01 *M* tris-glicin buffer (30 min 2 mA and 120 min 5 mA per tube). The gels were soaked in benzidine saturated 0.2 *M* acetate buffer, pH 5.6 and to the reaction mixture H_2O_2 was added (GÁBORJÁNYI *et al.*, 1973).

Peroxidase activities were measured immediately, 1, 2 and 3 days after the heat treatment, during the heat-induced local lesion development.

Results and Discussion

Using the technique of FOSTER and ROSS (1975a) we were able to induce necroses by hot water treatment in TMV-infected but symptomless half leaves of Xanthi, a compatible host for TMV (Fig. 1). As is seen, the local necroses appeared only on the TMV-inoculated and heat-treated parts of the half leaves. The treatment consisted of immersing the upper part of leaves into hot water of 50°C for 40 seconds, 2 – 5 days after inoculation. Necrotic lesions appeared 24 – 48 hours after the hot water treatment. During this time period the activity of peroxidase enzyme was measured and compared to the uninoculated but heat-treated control (Fig. 2). Neither the systemic infection, nor the hot water treat-

ment induced significant changes in activity of peroxidase during that time period. On the other hand, peroxidase activity was gradually augmented during necrogenesis. Increase in peroxidase activity in local lesion hosts as a result of virus infection is well known from the literature (FARKAS *et al*,. 1960; LOEBENSTEIN and LINSEY, 1961; ROSS, 1961a, b; FARKAS and STAHMANN, 1966; SOLYMOSY *et al.*, 1967; NOVACKY and HAMPTON, 1968; CHANT and BATES, 1970; SIMONS and ROSS,

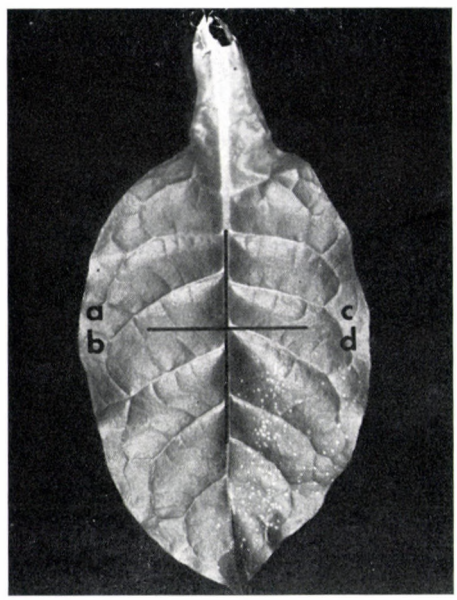

Fig. 1. Induction of necrotic lesions in "systemic Xanthi" tobacco leaf inoculated with TMV by heating the leaves in hot water at 50°C for 40 sec, two days after inoculation; (a) Uninoculated, untreated with heat; (b) uninoculated, heat-treated; (c) inoculated with TMV, untreated with heat; (d) inoculated with TMV and treated with hot water

1970; VAN LOON and GEELEN, 1971; WOOD and BARBARA, 1971; GÁBORJÁNYI *et al.*, 1973; BIRECKA *et al.*, 1975; VEGETTI *et al.*, 1975; VAN LOON, 1976; WESTSTEIJN, 1976).

Peroxidase samples from the heat-induced "artificial necroses" were electrophoretized. It was found that the so-called new peroxidase isozymes increased during the necrogenesis (Fig. 3). However, if we used enough quantities of samples, as was recommended by BIRECKA *et al.* (1975), it was clearly shown that the new peroxidases are present in all of the samples including the control ones. The activity of these isozymes was very high in the necrotic parts of leaves as compared to the controls. This means that the development of "artificial necroses" is also related to augmented peroxidase activity. This type of necroses could not stop virus movement. It was found that systemic distribution of the virus occurred in

the leaves, in spite of the development of local lesions. By this way we successfully repeated the findings of FOSTER and ROSS (1975a, b).

As a conclusion of these experiments we suggest that both peroxidase activity and the hypersensitive necroses (local lesion) are consequences, and not causes, of host resistance to viral infection. Similar conclusions have been made recently

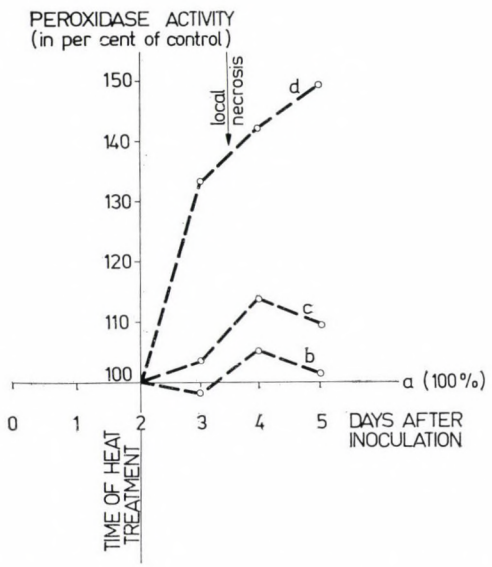

Fig. 2. Activity of peroxidase enzyme in per cent of uninoculated and non-heat-treated control leaf part (a). (b): Uninoculated, treated in hot water at 50°C for 40 sec two days after inoculation with TMV. (c): Virus-inoculated, untreated with hot water. (d): TMV-inoculated and hot water-treated

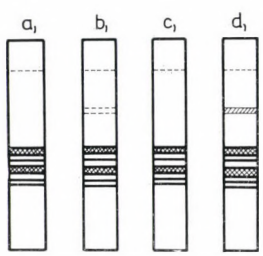

Fig. 3. Diagramatic representation of the distribution of the major peroxidase isozyme components following electrophoresis of soluble protein extracts from (a): uninoculated, untreated with hot water; (b): uninoculated, treated with hot water at 50°C for 40 sec two days after inoculation with TMV; (c): virus-inoculated, untreated with hot water; (d): inoculated with TMV and treated with hot water

in relation to host resistance to fungal infections (BROWN *et al.*, 1966; SEEVERS *et al.*, 1972; KIRÁLY *et al.*, 1972; ÉRSEK *et al.*, 1973; BARNA *et al.*, 1974; MAYAMA *et al.*, 1975; TANI *et al.*, 1975).

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Rhabdovirus-like Particles Associated with Cow-parsnip Mosaic

By

Z. POLÁK, O. KRÁLÍK and J. LIMBERK

Department of Plant Pathology, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha

Bacilliform particles of about 265 × 90 nm were found in ultrathin sections as mostly globular aggregates in nucleus and within the perinuclear space of leaf parenchyma cells of naturally mosaic diseased cow-parsnips (*Heracleum sphondylium* L.) or in leaves and flower petals of manually inoculated and infected parsleys.

In 1963 a disease of cow-parsnip, *Heracleum sphondylium* L. ssp. *australe* (Hartm.) Ahlfv. has been found in three weed associations on the Greater Prague territory. That disease causes mild chlorosis or flavescence in the first leaf, later

Fig. 1. *Heracleum sphondylium* L. with symptoms of disease caused by the rhabdovirus

deformations and decoloration which consist of bright yellow flecking expanding from the centre of the leaf blade along the main veins. The cause proved to be a sap transmissible virus (POLÁK, 1966). A virus isolate from *H. sphondylium* ssp. *sibiricum* (L.) Simk found in SW Finland, carrying similar symptoms, appeared to be identical with that from Prague on the basis of host range and symptom response (POLÁK, 1968).

Material and Method

The cow-parsnip virus isolate from 1963 has been kept since in parsley (*Petroselinum hortense* Hoffm.). For comparison the virus was in 1973 reisolated from diseased cow-parsnip plants from the original locality where it has been found before.

The inoculum was prepared immediately before use. The grinding medium for all manual inoculation experiments was distilled water. As an abrasive celite (0.2% w/v) was added after grinding or the plants were dusted with 500-mesh carborundum powder before being inoculated. Plants were held at least four weeks after inoculation for final reading of symptoms.

For concentration of the virus leaves of infected parsley plants (80 – 100 g) were harvested and soaked in one half volume/weight of 0.3 *M* glycine buffer adjusted to pH 8.5 (CROWLEY, 1967) which contained 1.10^{-4} 2-mercaptoethanol and incubated about 30 minutes. The infectious juice was extracted from the homogenate and emulsified by addition of Freon 113 (1/3 the volume of the buffer) by stirring 20 minutes on a magnetic stirrer. The emulsion was centrifuged at 10,000 rpm for 10 minutes. The aqueous extract was removed and centrifuged at 30,000 rpm for 120 minutes (Spinco rotor 30) and the virus-containing pellet was suspended in 0.05 *M* glycine at pH 8.5 and centrifuged again by one low and one high speed run (10,000 rpm/10 min and 45,000 rpm/90 min in Spinco rotor Ti 50). Resulting pellet was suspended in 0.01 *M* glycine buffer at pH 8.3.

Leaf dip and concentrated preparations were investigated in Tesla BS 413 and JEM 100B electron microscopes on carbon-backed Formvar coated grids for negative staining which was stained with 2% dodeca tungstophosphoric acid (PTA) adjusted to pH 7.2 with potassium hydroxide, 1% uranyl acetate, 1% uranyl formate and 1% ammonium molybdate adjusted to pH 6.5 by ammonium hydroxide.

Ultrathin sections were prepared both from leaves of naturally infected cow-parsnips and leaves and flower petals of manually infected parsleys. Small pieces of plant tissue were fixed in 6.25% glutaraldehyde in 0.1 *M* phosphate buffer pH 7.3 by means of vacuum technique (MILNE, 1966), postfixed in 2% osmium tetroxide, during dehydration stained with 0.5% uranyl acetate and transferred for embedding into Durcupan (Fluka). Sections were cut on a Reichert ultramicrotome using diamond Ge-Fe-Ri knife and examined with JEM 100B microscope.

Virion measurements were made on photographs of ultrathin sectioned material which were taken at three various magnifications of the microscope

Results and Discussion

Suitability of parsley as an experimental host for concentration of the virus and particle morphology studies was judged on the basis of comparison with the original host, cow-parsnip.

When dilutions of sap from diseased cow-parsnip plants were rubbed on leaves of parsley seedlings, seldom more than 50% of parsleys became infected. Similar inoculation of parsley seedlings with parsley infectious sap diluted up to 1 : 16 generally resulted in nearly 100% infection. Local lesion counts from *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste and Reyn. leaves were significantly higher after rubbing with infectious sap from diseased parsleys then with juice extracted from cow-parsnip plants. Because stability of symptoms throughout the year, especially under the glasshouse temperature conditions, was better too parsley was chosen not only for maintainance of the virus but also as the experimental host for further investigations.

Attempts to concentrate the virus were made always from parsley leaves carrying well developed symptoms at about three to four weeks after manual inoculation. The best results were achieved with the above mentioned procedure using glycine buffer at pH 8.5. Infectivity of resuspended resulting pellet varied from 60 to 85% (as shown by manual inoculation of the concentrate on to leaves of healthy parsley seedlings immediately after finishing the preparation). The infectivity and stabilitv of the concentrate was not enhanced when mercaptoethanol was substituted by 0.01 *M* $ZnCl_2$ (CROWLEY, 1967) or 0.01 *M* $MgCl_2$.

Our effort to visualize virions of the studied virus both from leaf dips, homogenates and concentrates using negative EM-techniques did not bring satisfactory results.

Activity of proteolytic enzymes used to be blamed for inactivating some rhabdoviruses and also organic solvents readily destroy the envelope of the particle (FRANCKI, 1973). However, infectivity has been shown to be unaffected by pancreatic ribonuclease (ATCHISON *et al.*, 1969) and hence it appears that the viral RNA is well protected. That seems to be a reasonable explanation for our failure to visualize the virus which anyway retains its infectivity for short period of time 24 hours being *in vitro*.

On the other hand electron microscopy of ultrathin sections revealed inclusions composed of bacilliform particles in mostly globular aggregates. All the particles were found in the nucleus and especially within the perinuclear space preferably of parenchyma cells.

Complete bacilliform particles measured 265 × 90 nm (the measures represent the mean value obtained from dimensions of 121 virions remeasured at three various magnifications of the microscope).

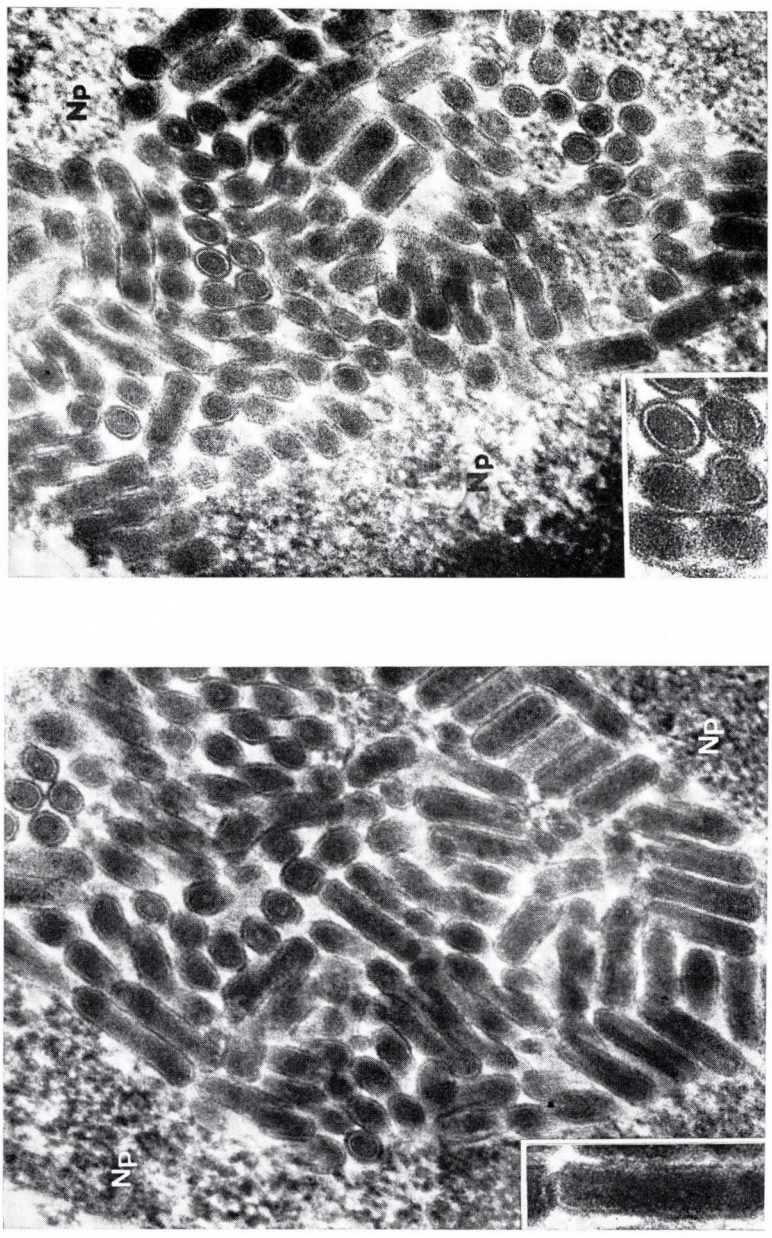

Fig. 2. and Fig. 3. Rhabdovirus particles in nucleus of parsley parenchyma leaf cells (longitudinal and transversal section of virions).
Np = nucleoplasma

Virions in longitudinal position seem to consist of an electron dense core and external membrane surrounding loosely the particle. They are cylindrical in shape with two hemisperical ends. Surface projections in the external membrane and bullet shaped particles were not observed. Some particles in transverse section show that the virion consists of two distinct layers which correspond to viral membrane and nucleocapsid with a central canal. Surface projections in transverse sections were also not visible.

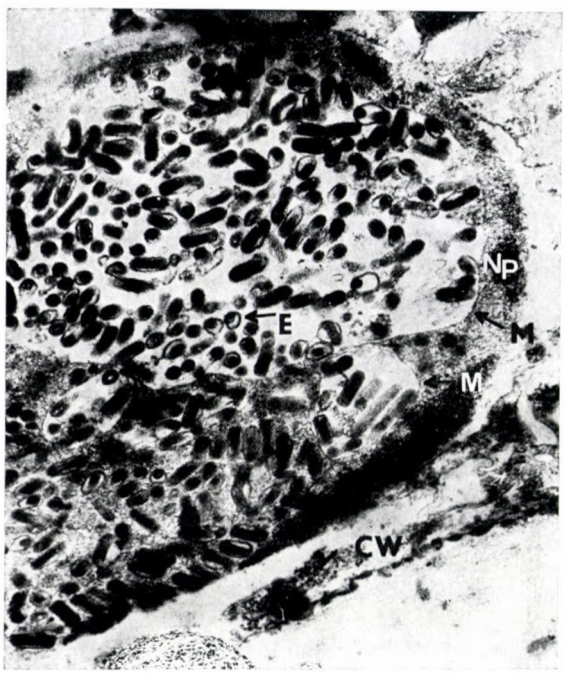

Fig. 4. Virions in a flower petal cell of parsley. The picture shows envelopes very loosely attached to the inner body of particles. Np = nucleoplasma; M = membrane (of inclusion); E = envelopes of virions; CW = cell wall

Other type of virus-like particles besides bacilliform ones was not found.

The phytopathogenic rhabdoviruses, viruses being bacilliform in shape are transmitted mainly by hoppers and aphids; few of them – for instance – Gomphrena virus (KITAJIMA and COSTA, 1966), potato yellow dwarf virus (MACLEOD *et al.*, 1966), lettuce necrotic yellows wirus (HARRISON and CROWLEY, 1965) broccoli necrotic yellows wirus (HILLS and CAMPBELL, 1968), eggplant mottled dwarf virus (MARTELLI, 1969), lucerne enation mosaic virus (cf. FRANCKI, 1973), sonchus yellow net virus (CHRISTIE *et al.*, 1974) and rhabdovirus of Cynara (RUSSO *et al.*, 1975) are transmitted mechanically.

Manually transmissible rhabdovirus-like entity described in this paper carrying biological properties shown by POLÁK (1966) differs from the cow-parsnip

mosaic virus described by WOLF (1972), and from other viruses described from cow-parsnip as cellery mosaic virus (WOLF, 1972) and parsnip mosaic virus (MURANT *et al.*, 1970) both being filamentous in shape and parsnip yellow fleck virus (MURANT and GOOLD, 1968) with isometric virions.

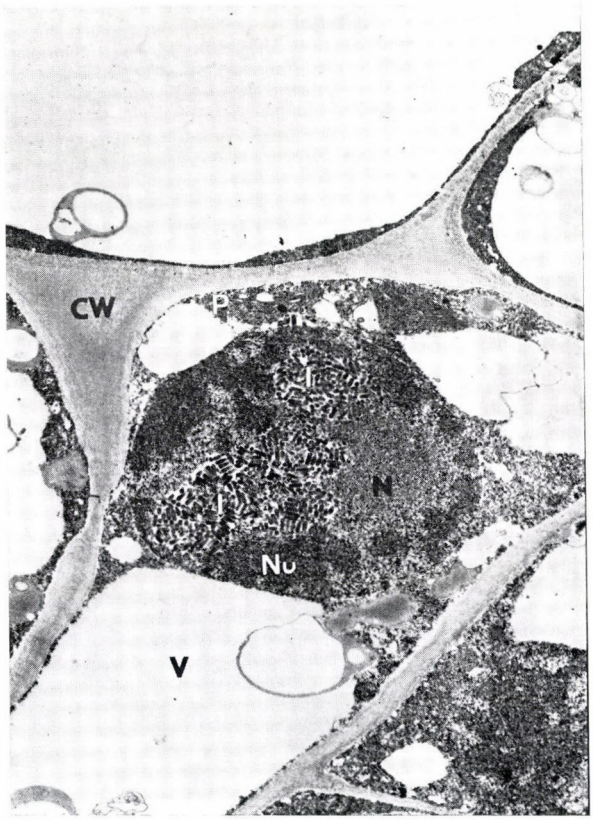

Fig. 5. Cell of cow-parsnip leaf parenchyma with a rhabdovirus inclusion in the nucleus. N = nucleus; Nu. = nucleolis; I = inclusion; V = vacuole; P = plastide; CW = cell wall

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Effect of Watermelon Mosaic Virus on the Yield of *Cucurbita pepo**

By

BHARTI BHARGAVA

Department of Botany, University of Gorakhpur, Gorakhpur, India

In field experiments, early infection with watermelon mosaic virus caused greater loss in yield of *Cucurbita pepo* but the loss was much less by late infection. Three different strains of WMV varied in their effect on the yield. Spread of infection in the crop could be prevented by regular spray of 0.03 per cent folidol E 605.

Watermelon mosaic virus (WMV) has been reported from different parts of the world on a number of cucurbits. In India, it was first reported by BHARGAVA and JOSHI (1960) and other authentic reports about its occurrence are by SINGH (1964), JAGANATHAN and RAMAKRISHNAN (1971), NAGARAJAN and RAMAKRISHNAN (1971) and TEWARI (1972). Recently BHARGAVA (1974) found it naturally occurring on cultivated varieties of *Benincasa hispida*, *Trichosanthes dioica*, *Lagenaria vulgaris*, *Cucumis sativus* and two wild cucurbits *viz. Coccinia grandis* and *Momordica dioica*. THOMAS (1971) in Australia and DEMSKI and CHALKLEY (1972) in U.S.A. have reported yield losses in different cultivars of pumpkin and squash due to WMV infection. The present study was made to determine the effect of disease on yield of *Cucurbita pepo* infected with WMV during two different growth periods.

Material and Methods

Cucurbita pepo was planted in a randomized block with three replications. Three plots (3×5.50 metres) were laid out and 30 plants were sown in each plot in six rows, 75 cm apart, each row having five plants with a distance of 50 cm between each. The plants were divided into three lots, plants in the first lot were inoculated with a strain of WMV (WMV-Td isolated from *Trichosanthes dioica*), 14 days after germination; in the second lot 28 days after germination and the third lot was left uninoculated as control. The plants were sprayed with 0.03 per cent folidol E 605, once every week to control insect pests and reduce the chances of

* This work formed a part of the thesis approved for Ph. D. degree of Gorakhpur University.

spread of the disease to control plants during the experimental period. Watering of the plants was done every third day. The time of flowering and fruiting was noted regularly in each case and fruits were plucked and weighed as they matured.

In another plot 10 plants each of *C. pepo* were inoculated at an early stage (14 days after germination) with three different strains of WMV, *viz.* WMV-Td, WMV-Bh and WMV-LvU the latter two being isolated from *Benincasa hispida* and *Lagenaria vulgaris* respectively, and similar observations were made.

Observations and Results

Inoculated plants showed infection in the usual time of 8–10 days. All the control plants remained healthy throughout the experimental period. The number of plants bearing female flowers and fruits was much reduced due to early infection and less so by late infection (Table 1).

Table 1

Effect of WMV-Td strain infection on flowering and fruiting of *C. pepo*

Treatment	Plot	Number of plants out of 10 inoculated	
		Flowered (0)	Fruited
Control	I	9	9
	II	8	8
	III	8	8
Early inoculation	I	3	1
	II	3	1
	III	4	2
Late inoculation	I	9	8
	II	6	5
	III	6	5

Table 2

Effect of WMV-Td strain infection on the yield of *C. pepo*

Treatments	Number of fruits harvested			Total No. of fruits harvested	Average weight of fruits (g)
	Plot I	Plot II	Plot III		
Control	27	24	24	75	1269
Early inoculation	2	2	3	7	205
Late inoculation	24	15	15	54	947

The fruits on infected plants were reduced both in number and size, the reduction being greater in early infected plants (Table 2). It was further noted that the fruits obtained from infected plants did not show much distortion but had much thicker skin.

Results of infection with different strains of WVM (Table 3) showed variation in their effect on the yield of *C. pepo;* the maximum reduction being by WMV-LvU.

Table 3

Effect of different strains of WMV on flowering and fruiting of *C. pepo*

	Strains of WMV		
	WMV-Td	WMV-Bh	WMV-LvU
Number of plants inoculated	10	10	10
Number of plants flowered (♀)	4	5	3
Number of plants fruited	3	4	2

Discussion

CONROY (1965) reported that flower formation and fruit set in tomatoes infected with tomato yellow top virus in the seedling stage were severly reduced. He and other workers (SIEVERT, 1971; SMITH, 1967) have reported time of infection as an important factor in determining the loss of yield in other crops. Among the cucurbits FLETCHER *et al.* (1969) showed that early infection by cucumber green mottle virus resulted in loss in yield while late infection had little effect. In his experiments with WMV, THOMAS (1971) reported that cucumber suffered no reduction in yield from either early or late infection with WMV, but other cucurbits behaved differently, and there was a yield reduction after early infection but not after late infection. DEMSKI and CHALKLEY (1972), however, found that losses in summer squash from infection by WMV occurred at all stages, although early infection caused the greatest yield loss. The greatest detrimental effect of WMV was on marketability when there was a 90 per cent loss of saleable fruits regardless of time of infection. In the present study the loss in yield depended on the duration of viral infection in plants and the yield was much reduced in early infected plants. The insecticidal spray of 0.03 per cent folidol E 605 controlled the spread of infection to healthy plants thus preventing the loss. It is therefore recommended that infection should be prevented by regular sprays with an insecticide during early stages of growth.

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Viruses in Natural Infections of Yellow Lupin (*Lupinus luteus* L.) in Poland

I. Bean yellow mosaic virus

By

I. FRENCEL and H. POSPIESZNY

Institute of Plant Genetics of the Polish Academy of Sciences,
Laboratory of Disease Resistance, Poznan, Poland

A continued review of the occurrence and propagation of viruses in natural infections of yellow lupin in cultivations in Poland has been started. In the years 1973—1974 has been stated that the bean yellow mosaic virus (BYMV) appeared in 84% (56 virus isolates out of 64 all isolates obtained). Five different types of representatives of BYMV have been isolated, the greater part represented the typical strain of this virus.

In Poland, fodder varieties of yellow lupin undoubtedly belong to the more valuable fodder plants. Apart from traditional cultivations for green forage at present increases the tendency of an economic utilization of seeds of this plant. At the same time originates an economic problem of virus diseases.

Virus diseases of yellow lupin cause a considerable decrease of seed yield and thereby are one of the chief causes of a variable faithfulness of yield. Agrotechnic methods used for the prevention of virus diseases are not always efficient. Hence, the trend of plant breeders toward the integration of fight methods and the introduction of resistant plants is quite well-founded.

The problem of virus diseases of yellow lupin in Poland has induced us to undertake complex investigations on resistance of this plant. In this connexion a very essential importance have etiological problems: local differentiation of pathogenic agents, frequency of occurrence, evolution of strains of viruses. A detailed interpratation of etiology in a course of many years comparative investigations should give information on the dynamics of the pathogenic potential and its evolutionary traits.

Accepting the assumption that different climatic-soil conditions of the cultivation of yellow lupin in Poland may exert an influence on the differentiation of the occurrence of viruses in natural infections, in the years 1973—1974 the harvest of samples of plant material suspected of virus infection from 10 different places of geographically distinct regions was performed. In the first series of harvest plants were collected chiefly from experimental plots situated in stations of the Main Centre of Investgations of Varieties of Cultivated Plants (COBORU — Slupia Wielka), and only occasionally from cultivations of other origin; 64 virus isolates have been obtained as designed for further, detailed identification.

The collection of virus isolates from natural infections we intend systematically continue during a period at least of some years, taking into account in

further series only plant material from Polish seed plantations of yellow lupin. The results of investigations shall be published successively.

The most widespread virus disease of yellow lupin in countries of central Europe is the so-called "Lupin Narrow Leaft" BŁASZCZAK, 1963), before known as "Yellow Lupin Mosaic". This disease was described for the first time in Germany (MERKEL, 1929).

Though at first the incitants of Lupin Narrow Leaf were searched even among ecological factors (MANNINGER and MOLNÁR, 1946; NÉMETH, 1956; KREYBIG, 1958), however most views have maintained that this is a disease of virus origin. According to MASTENBROEK (1942) Lupin Narrow Leaf is caused by a virus determined as lupinus virus 1. STEVENICK (1957) considers that this disease is incited by a pea mosaic virus (PV_2), when ZSCHAU (1962) have identified the Bean yellow mosaic virus (BYMV).

The etiology of Lupin Narrow Leaf was also investigated in Poland. KSIĄŻEK (1962) maintains the above mentioned results of STEVENICK, but BŁASZCZAK (1963) has identified local isolates of BYMV in the region of West Poland.

In this paper has been given the characterisics of isolates of BYMV obtained from natural infections of yellow lupin as well as their relative frequency of occurence in Poland.

Materials and Methods

Virus isolates have been obtained by means of isolation on test plants, from ill plants of yellow lupin collected in summer months.

Biological tests were performed under greenhouse conditions. Plants were inoculated mechanically with juice from ill plants, diluted with water. Macroscopic symptoms were observed as a rule in the case of 10 to 20 inoculated plants.

Physical properties of isolates have been determined in juice from infected pea and as a test plant was used red goosefoot (*Chenopodium amaranticolor* C. and Rm). The measurement of virus particle peak lengths was performed directly from electron microscopic negatives (10,000 ×) in comparison with the standard (TMV = 300 nm long) using a binocular. Microscopic preparations from crude sap were prepared by a "dip" method and coloured by means of phosphotungstic acid (PTA, 2%, pH 7.2).

Results

1. Experimental host range. 56 isolates have represented the BYMV. Biological tests have shown a differentiation between isolates and have enabled the separation of 5 different groups (Table 1).

Experimental host range has been presented in Table 2.

Table 1

Differentiation into groups of isolates of BYMV collected in Poland on the basis of biological tests

Locality	Groups and number of BYMV isolates					Total number
	I	II	III	IV	V	
Krościna Mała	4	1				5
Marianowo	3	2				5
Masłowice	3	2		1	1	7
Młochów	2	2	2	1		7
Nikutowo	4	1				5
Poznań	2	2		1		5
Prusin	4	1			1	6
Przecław	4	2			1	7
Słupia Wielka	3	1		1		5
Wiatrowo	3	1			1	5
Altogether	32	15	2	4	4	57

The detailed description of symptoms on some tests plants are as follows:

Lupinus albus L. "Kali" – isolates of the I and II group caused local necrotic spots on cotyledons and leaves. All isolates attacked systemically white lupin. Plants infected by isolates of the I and II groups generally turned brown and withered. Other isolates caused in different intensity the occurrence of little necrotic spots, especially in the case of isolates of the III and IV groups, and then the youngest leaves withered. Plants showed a strong inhibition of growth, further leaves – often deformed – showed mosaic. Some plants during the subsequent period withered.

Lupinus angustifolius L. "Obornicki" – isolates of the I and II groups caused comparatively quick decay of plants. Isolates of the III and IV groups attacked blue lupin more mildly than isolates of the I and II groups; plants showed an inhibition of growth; deformed leaves with symptoms of mosaic in the low parts of plants, withered and fell away. Afterwards part of plants withered. Isolates of the V group caused on leaves only symptoms of mosaic.

Lupinus luteus L. "Bas" – all isolates attacked this plant systemically causing symptoms characteristic for Lupin Narrow Leaf; at first mosaic of leaves and then a claw-like arrangement and narrowing of leaflets as well as an inhibition of growth.

Pisum sativum L. "Kujawski wczesny" – only an isolate of the IV group caused sometimes local necrotic spots. All isolates attacked pea systemically, causing mosaic. The intensity of symptoms was dependent on a great degree from the environment conditions, what do not exclude the statement of certain differences, which however are with difficulty perceptible under uncontrolled greenhouse conditions. Two cultivars of pea – "Karabałyjskij" and "Cud Ameryki" were not attacked.

Table 2

Results of the test on experimental hosts range for the isolates of BYMV

Host	Isolates of BYMV				
	I	II	III	IV	V
Leguminosae					
Lathyrus odoratus	−, S	−, S	−, S	−, S	−, S
Lupinus albus	L, S	L, S	−, S	−, S	−, S
Lupinus angustifolius	−, S	−, S	−, S	−, S	−, S
Lupinus luteus					
Bas	−, S	−, S	−, S	−, S	−, S
Bałtyk	−, S	−, S	−, S	−, S	−, S
Wista	−, S	−, S	−, S	−, S	−, S
Phaseolus vulgaris					
Canellini	L, S	L, S	L, S	L, S	L, −
Saxa	L, S	L, S	L, S	L, S	L, −
Wiejska	L, S	L, S	L, S	L, S	L, −
Pisum sativum					
Kujawski Wczesny	−, S	−, S	−, S	(L), S	−, S
Karabałyjskij	−, −	−, −	−, −	−, −	−, −
Cud Ameryki	−, −	−, −	−, −	−, −	−, −
Trifolium incarnatum	−, S	−, S	−, S	−, S	−, S
Trifolium pratense	−, S	−, S	−, S	−, S	−, S
Trifolium repens	−, −	−, −	−, −	−, −	−, −
Other					
Cucumis sativus	−, −	−, −	−, −	−, −	−, −
Chenopodium amarant.	L, S	L, S	L, S	L, S	L, −
Chenopodium quinoa	L, S	L, −	L, S	L, −	L, −
Lactuca sativa	−, −	−, −	−, −	−, −	−, −
Nicotiana tabacum	−, −	−, −	(L), −	−, −	−, −

Explanations: L = local infection
S = systemic infection
− = absence of symptoms, absence of infection
( ) = infection did not appear in each case

Phaseolus vulgaris L. "Canellini", "Saxa" and "Wiejska" – all isolates caused a local infection in a form of chlorotic spots. Except an isolate of the V group all other isolates attacked bean systemically, causing at first chlorotic spots and then mosaic often deformation of leaves as well as tips of plants.

Trifolium pratense L. "Hruszowska" – all isolates attacked red clover sytemically, causing mosaic of leaves of different type. Isolates of the I and V groups attacked red clover most intensely, causing green-yellow mosaic; isolates of the II and IV groups caused light-green mosaic and an isolate of the III group – a faint dark-green mosaic.

Vicia faba L. "Nadwiślański" – sometimes an isolate of the II group caused not numerous, local chlorotic spots, other isolates did not cause visible local lesions. All isolates caused systemically a mosaic of leaves.

Chenopodium amaranticolor Coste and Reyn – an isolate of the V group caused only local chlorotic spots, other isolates, except a similar local infection, attacked red goosefoot also systemically. At first appeared chlorotic spots and during the later period a deformation of leaves and the whole tips of plants.

Chenopodium quinoa Willd. – all isolates caused on that plant local, chlorotic spots and an isolate of the III group even necrotic spots. Only isolates of the I and III groups attacked *Ch. quinoa* systemically, causing at first not numerous, chlorotic spots and then a deformation of leaves and tips of plants (especially in the case of an isolate of the III group) as well as a fall of leaves.

On the ground of detailed observations of macroscopic symptoms has been given the list of test plants biologically differentiating individual isolates of BYMV (Table 3).

Table 3

Biological differences among isolates of BYMV on differential test plants

Test plant	BYMV isolates				
	I	II	III	IV	V
Chenopodium amaranticolor	L, S	L, S	L, S	L, S	L, –
Chenopodium quinoa	L, S	L, –	L, S	L, –	L, –
Lupinus albus	L, S	L, S	–, S	–, S	–, S
Phaseolus vulgaris	L, S	L, S	L, S	L, S	L, –

Explanations: L = local injection
S = systemic infection
– = absence of infection

The interpretation of results of own investigations have been compared with the identification of isolates of BYMV against the background of literature, accepting at the same time the classification of the strans of BYMV according to Bos *et al.* (1974) (Table 4).

Table 4

Identification of local isolates of BYMV in comparison to the present literature. Classification of strains according to Bos (*et al.*)

Local isolates of BYMV from yellow lupin (group)	Consistence of interpretations		Reference
	Isolates of BYMV – typical strain (Code)	Isolates of BYMV – yellow pea mosaic virus (Code)	
I	L 1		Błaszczak (1963); Bos *et al.* (1974)
II	E – 212		Bos *et al.* (1974)
III	LP – 1		Own investigation
IV	R_g ; B_{25}		Błaszczak (1963); Bos *et al.* (1974), resp.
V		E – 198	Bos *et al.* (1974)

2. Physical properties. Physical properties of individual isolates of BYMV are in general similar and comparatively they do not differ considerably from data of literature (BŁASZCZAK, 1966; BOS, 1970; EVANS, 1973) (Table 5).

Table 5

Physical properties of isolates of BYMV in plant sap

Isolates of BYMV	Thermal inactiv. in C°	Duration *in vitro* in days	Dilution
I	60—65	3—6	10^{-3}—10^{-4}
II	60—65	6—9	10^{-3}—10^{-4}
III	65—70	9—12	10^{-4}—10^{-5}
IV	60 65	6—9	10^{-4}—10^{-5}
V	55—60	3—6	10^{-3}—10^{-4}

3. Electron microscopy. Preliminary measurements of particles length (performed for 60 particles) in the case of an isolate of the III group has amounted about 830 nm. For comparison, the particles length of some isolates of BYMV according to determinations of other authors has amounted from 800 to 840 nm (BOS *et al.*, 1974).

Discussion

Above 80% of virus isolates which have been obtained from yellow lupin in cultivations in Poland in the years 1973—74 has represented BYMV. Biological differentiation among isolates of BYMV in most cases reduced to the previously recognized isolates of strains (Table 4): isolates of the I group fulfil biological properties of the isolate coded "L—1" (BŁASZCZAK, 1963; BOS *et al.*, 1974), isolates of the II group are consistent with characterization of the isolate coded "E 212" (BOS *et al.*, 1974) and isolates of the IV group can be compared with the isolate "B 25" (BOS, 1970) and "R g" (BŁASZCZAK, 1963) whereas isolates of the V group are consistent with the isolate coded "E 198" (BOS *et al.*, 1974). But biological properties of the isolates of the III group are not entirely consistent with till now described isolates of BYMV. This suggests the finding of a new local biotype, which symbolically has been coded "LP—1".

Accepting the classification of BYMV strains according to BOS *et al.* (1974) it has been stated that most of local BYMV isolates from yellow lupin occurring in Poland represent a typical strain of BYMV and only occasionally occur isolates of the yellow pea mosaic strain.

All investigated isolates of BYMV attacked yellow lupin causing symptoms of a disease characteristic for the Lupin Narrow Leaf disease.

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New Artificial Hosts and Non-Hosts of Plant Viruses and their Role in the Identification and Separation of Viruses

I. Historical Review

By

J. HORVÁTH

Research Institute for Plant Protection, H–1525 Budapest, P.O. Box 102, Hungary

In this part of his publication series the author deals with the importance of plants in virus identification, virus separation (virus differentiation), breeding for virus resistance, and in virus epidemiology, and gives a historical review of research results concerning the virus hosts reported among members of the lower groups of the flora (bacteria, algae, fungi and lichens) as well as the highest ones (mosses, ferns, gymnosperms and angiosperms). He presents the chronological order for a wide host range of viruses and gives a general survey of the role of virophilic and virophobic plant families, genera and species in virus identification and virus separation.

Introduction

The research of virus host range and virus differentiation – reckoned by the science of virology from the famous test plant experiments of JOHNSON (1925) and SMITH (1931) – enriched the world of science with invaluable scientific and practical results in the past half of this century. In the last several years the interest in virus hosts and sources of virus resistance has a real renaissance. It is not easy to say what gave the impulse to this sudden revival of interest. However, it is certain that the study of viruses from theoretical and practical points of view is not possible without plants. A basic precondition of studying the viruses is to know the relationships between plants and viruses as well as the systems they are found in and originate from.

Bacteria and other microorganisms are known to have in some cases a favourable effect on their hosts; the plant viruses, on the other hand, cause more or less serious diseases, and the plants, as fundamental objects, are indispensable in acquiring a knowledge of these diseases and the pathogens causing them. A minor part of the plant viruses multiply in various insects too (e.g. arboviruses, propagative viruses), but most of them perish without the living plant cell.

Knowledge of the most important characteristics of viruses, such as e.g. infectivity (MAYER, 1886), filterability (IWANOWSKY, 1892), latency (JOHNSON, 1925), mutation (MCKINNEY, 1926), serological activity (DVORÁK, 1927; PURDY,

1928), premunity (McKINNEY, 1929; THUNG, 1931), localization (HOLMES, 1929), crystallization (STANLEY, 1935), variability (JENSEN, 1936), persistency and non-persistency (WATSON and ROBERTS, 1939), infectivity of nucleic acids (GIERER and SCHRAMM, 1956), reconstitution (FRAENKEL-CONRAT, 1956), relationship between propagative and animal viruses (MARAMOROSCH, 1955; SHIKATA and MARAMOROSCH, 1967), recombination (BEST and GALLUS, 1955), propagation of bacterium viruses (phages) in higher plants (SANDER, 1964), as well as the application of various examination methods (isolation, differentiation, identification, measuring of biological activity, chemical-, physical-morphological examination of the properties of virus particles, or determination of genetic features, etc.) were also made possible by living plants as virus receptors and virus receptor indicators, respectively.

The host reactions and symptoms as well as virus host ranges changing according to the virus–host relations are highly important from the point of view of virus diagnosis and differentiation (separation). Beside the invaluable diagnostic importance of electron microscopy and serology the virus host plants (indicator plants) have retained – and will certainly retain in the future too – their significance. As it is known the electron microscope and serological examinations of many viruses encounter difficulties. In most cases not only the cheapest but also the most reliable identification of viruses is made possible by the indicator plants. In spite of the perfection of electron microscope and serological methods one of the most dangerous and economically most harmful pathogen of potato (potato virus Y) can – even today – be most reliably identified with the hybrid test plant *Solanum demissum* A6 (leaflet method; cf. HORVÁTH, 1966c; ZSCHÜTTIG and HORVÁTH, 1968). Latter plant is also suitable to point out the polyphagous cucumber mosaic virus with (HORVÁTH and POCSAI, 1972a). As regards the importance of test plants HOLLING (1966) the well-known English plant virologist wrote: "Test plants alone cannot give a reliable identification of a newly isolated virus nor, on present evidence, can they form any basis for a natural virus classification. They do, however, offer the most sensitive and reliable means of detecting virus – over one hundred times more sensitive than serological tests or electron microscopy and five hundred times more so than density-gradient centrifugation with several polyhedral viruses."

Plants also have an enormous importance in finding out the relations between the virus strains (cross protection). It was e.g. with the help of indicator plants suitable to point out various virus strains that we established the relationships between potato virus Y strains and isolates, and made the discovery – important also for the practice – that a cross protection reaction existed between the Hungarian and German cucumber mosaic virus isolates (HORVÁTH, 1969g; HORVÁTH *et al.*, 1974b).

Plants play an important role in throwing light upon virus epidemiological problems and correlations as well. However, to know the virus epidemiological correlations not only naturally or spontaneously infected plants must be detected; so-called artificial virus hosts and virus resistant plants identified under experi-

mental conditions are also indispensable. Namely, in many cases the detection of naturally occurring virus reservoirs is due to earlier found artificial virus hosts (prognostic hosts) which on the basis of the results of challenges by artificial inoculation "prognostized" the natural virus hosts. In many cases, namely, the new artificial host plants, as prognostic hosts, called attention to virus reservoirs spontaneously occurring in the nature. We may mention as an example the first occasion of finding in Hungary a natural infection of cucumber mosaic virus in *Paulownia imperialis* (cf. HORVÁTH, 1973b, 1975h).

Furthermore, the artificial virus hosts, besides their prognostic importance, are indispensable in virus identification and differentiation. The virus resistant plants (non hosts), on the other hand, are of invaluable significance in breeding for virus resistance and in virus differentiation.

The work of breeding for virus resistance is aimed all over the world at spotting the resistance genes of wild plant species and using this knowledge in practice; the basis of this work is also formed by the investigations into the virus host plant ranges. This widely extended scientific work is equally important both from the theoretical and practical points of view. Of the investigations made in Hungary the ones aimed at pointing out the resistance of wild *Lycopersicon* species to tobacco mosaic virus (MILINKÓ, 1958, 1961, 1962, 1964), potato virus M and potato virus S (HORVÁTH, 1971b, 1972a, 1973c, d), of wild *Solanum* species to potato pathogenous viruses (HORVÁTH, 1966c, 1968d, 1970a; SÁRVÁRI, 1967) and tobacco mosaic virus (HORVÁTH, 1968a, f, 1971a), of wild *Medicago* species to alfalfa mosaic virus (BECZNER and LÁSZLÓ, 1972) are particularly important. The research work carried out in Hungary to determine the virus susceptibility of various cultivated plants (bean, potato, tobacco, pepper, tomato) included as crossing partners in the work of breeding for resistance (cf. SZIRMAI, 1954; HORVÁTH, 1967a, 1969b, m, 1971b, 1972a, d, e, 1973c; BECZNER and HORVÁTH, 1969, 1973, 1974; SZIRMAI, 1970; HORVÁTH and BECZNER, 1972, 1973a; KOVÁCS and HORVÁTH, 1973; GÁSPÁR, 1974) is also significant and has led to numerous new results important from a practical point of view as well. Of the recent investigations carried out abroad are the studies on the relationship between the wild *Solanum* species and cultivated potato varieties on the one hand, and cucumber mosaic virus on the other hand deserve special attention (SCHMELZER and SPAAR, 1975).

Plants are of importance not only in virus diagnosis and differentiation, or in pointing out epidemiological correlations or breeding for virus resistance, but also the produced hosts, the new artificial hosts play an essential role in the quantitative changes of viruses occurring in the given plant, which has great significance in the serology too. In the course of examining the virus donor hosts from the point of view of virus production – analysed mainly in the relation of tobacco mosaic virus to certain plants – it was found that in *Physalis floridana* the amount of tobacco mosaic virus is about two thousand times more than in bean plants (LINDNER *et al.*, 1961). The highest virus production (10 per cent of the dry matter) has been pointed out so far in *Physalis floridana* and *Nicotiana*

tabacum plants. Potato virus X attained 2 per cent in tomato, turnip yellow mosaic virus 0.6 per cent in cabbage, alfalfa mosaic virus 0.13 per cent in tobacco relative to the dry matter weights of the examined plants (cf. MATTHEWS, 1970; YARWOOD, 1971a). Thus, a further task of the virus–host range research is to detect plants which from the point of view of virus production are the most favourable.

In spite of the very fast rate of progress in the field of researches on the virus–host range and virus differentiation, seemingly simple but fundamentally important questions have remained unexplained. Science has not answered the question why certain viruses (polyphagous viruses) have a wide, while others (oligophagous viruses) a narrow range of hosts. The above mentioned basic properties of the polyphagous and oligophagous viruses are important from both the theoretical and practical points of view. Another question left unanswered by the science is why certain plants (virophilic plants) are susceptible to many, while others (virophobic plants) only to a few viruses. This behaviour of the virophilic and virophobic plants needs explanation in many respects of the host–virus relations. The fact, that of the about 250 thousand plant species belonging to the virologically best known and economically most important angiosperms (cf. WILSON and LOOMIS, 1962) the number of those with known reactions to viruses is still relatively low, must urge the virus researchers to try to reveal the so far unknown correlations between plants and viruses. The expected results will contribute to a better knowledge of correlations between viruses and plants to the extent they are able to clarify the so far unknown or not sufficiently known plant–virus relations.

The analysis of earlier studies on the virus–host ranges and a survey of their genetic models shows no close correlation between the taxonomic – phylogenetic place and virus susceptibility of plants (cf. BALD and TINSLEY, 1967a, b, c, 1970; HOLLINGS, 1959a; SCHMELZER, 1961). In spite of this, taxonomic correlations recently found in the host ranges of some virus groups (cf. WATSON and GIBBS, 1974) call attention to the necessity of a thorougher analysis, apart from theoretical points of view, is of primary importance in solving problems of virus taxonomy, diagnosis, differentiation and epidemiology, and seeking out new sources of virus resistance.

For several years – as it is known – the disappearance of certain plant species and cenoses, and the change of the floristic composition – the synantropization – of the plant cenoses have accelerated, evidently causing substantial, though so far hardly revealed changes in the host – virus relations too. To study the action of quantitative, qualitative and spatial changes of the infection chain (natural hosts) in the flora and in the different agricultural cultivation systems is also the task of the virus–host range research.

The results attained in Hungary in the last fifteen years in connection with the identification of prognostic virus hosts, virus diagnostic and virus differentiating plants as well as sources of virus resistance form an integral part of the international research work in this subject. Our investigations are aimed partly to establish artificial host – virus relations with prognostic, diagnostic and differ-

entiating functions, and to find sources of virus resistance which is important in the work of breeding for virus resistance both from the theoretical and practical points of view.

By describing the prognostic virus hosts, diagnostic and differentiating plants, resistant plants and sources of resistance we wish to disclose such correlations between plants and viruses which are new and important for plant virology all over the world.

In the first part of our study "*New artificial hosts and non-hosts of plant viruses and their role in the identification and separation of viruses*" – planned to be published in twelve successive papers – we give a historical survey of the hosts of plant viruses found in the lower as well as the highly developed groups of the flora. In the second publication general remarks and proposals concerning our own experimental work will be presented. In the 3rd to 12th papers the new artificial hosts and non-hosts of the tobravirus, tobamovirus, potexvirus, carlavirus, potyvirus, cucumovirus, tymovirus, comovirus, brobwilvirus *(nomen provisoricum)* and monotypic groups, respectively, as well as the possibilities of virus separation are discussed.

Members of the lower groups of the Plant Kingdom

1. Bacteria (Schizomycophytes)

Some twenty years after the discovery of the phytopathogenic tobacco mosaic virus TWORT (1915), an English physician-bacteriologist noticed in his Staphylococcus culture an infectious acute disease which caused the disintegration of the culture. The infecting agent was later found to be filterable and transferable from culture to culture. TWORT *(ibid.)* published his observations in the "Lancet", one of the most famous English scientific journals; among his theories as to the nature of the infecting agent he mentioned the virus theory too: "*. . .the agent of the disease might be a virus that grows on and destroys the micrococci it infects.*"

F. W. TWORT's scientific line was followed in the work of FELIX D'HÉRELLE Canadian physician-bacteriologist at the Pasteur Institute, Paris. In the course of studying the *Shiga* dysentery bacillus belonging to the *Shigella* group D'HÉRELLE found an anti-dysenteric microbe that he called bacteriophage or bacterium virus (D'HÉRELLE, 1917, 1921, 1922, 1926).

Until those days the research of phages (BURNET, 1927, 1934; SCHLESINGER, 1933, 1934; DELBRÜCK, 1946; WILLIAMS and FRASER, 1953; STOLP, 1956; KLEMENT and KIRÁLY, 1957; KLEMENT and LOVAS, 1959; KLEMENT, 1959; ADAMS, 1959; LWOFF, 1953, 1959; KLEMENT and LOVREKOVICH, 1960; KELLENBERGER, 1961; MAHLER and FRASER, 1961; STENT, 1963; BENDET, 1963; LOVREKOVICH and KLEMENT, 1965; EISENSTARK, 1967; WATANABE and AUGUST, 1967; VALENTINE *et al.*, 1969; RALPH, 1969; CUMMINGS *et al.*, 1970; HOHN and HOHN, 1970; SALSER,

1971; CAIRNS *et al.*, 1972; SEMANCIK *et al.*, 1972; DUNLEAVY and URS, 1973; ALFÖLDY and NÁSZ, 1974) has pointed out phages and viruses in many bacterium hosts belonging to various genera (e.g. *Pseudomonas*, *Xanthomonas*, *Vibrio*, *Rhizobium*, *Chromobacterium*, *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Corynebacterium*, *Echerichia*, *Aerobacter*, *Klebsiella*, *Erwinia*, *Salmonella*, *Shigella*, *Pasteurella*, *Brucella*, *Hydrogenomonas*, *Photobacterium*, *Caulobacter*, *Azotobacter*, *Agrobacterium*, *Flavobacterium*, *Serratia*, *Proteus*, *Plague*, *Bordetella*, *Mallomyces*, *Hemolytic*, *Listeria*, *Actinomyces*, *Chondrococcus*, *Spirochaeta*, *Saprospira*, *Hemophilus*, *Bacillus*, *Clostridium*, *Mycobacterium*, *Streptomyces*, *Nocardia*), and established their most important physical, chemical and biological properties.

From a morphological point of view the phages can be divided into tailed, icosahedron and filiform phages. Their dimensions are rather different (cf. ADAMS, 1959). The phages of serie *Echericia coli* T, recently called myovirus too (e.g. myovirus e-T4), which contain double-stranded deoxyribonucleic acid (dsDNA, D/2 or 2-DNA) are the best known ones. The cubic symmetrical head part of the myoviruses (phages T1, T2, T3, T4, etc.) is some 100 nm in diameter, to which a long contractile tail of about 100 nm is attached. The capside of the head, the tail and the basal lamina are composed of antigenic nature, head- and tail-specific protein. On the basis of their nucleic acid contents we distinguish phages containing single-stranded DNA (coliphage fd = inovirus), double-stranded DNA (lipid phage PM2 = lipovirus p-PM2), and recently ones containing ribonucleic acid (RNA) (e.g. coliphage f2 = masculovirus e-f2) (LOEB and ZINDER, 1961; RALP, 1969; WILDY, 1971). The polyhedric head of the bactriophage of *Pseudomonas phaseolicola* ∅6 containing double-stranded RNA is 60 nm in diameter. The phage particle contains 13 per cent RNA, 62 per cent protein and 25 per cent lipoid (VIDAVER, KOSKI, VAN ETTEN, 1973 *cit.* WOOD, 1973).

In the course of examinations made in Hungary in connection with the tailed phage of *Xanthomonas phaseoli* var. fuscans it was found that the head of the phage was 72 nm in diameter, the tail 200 nm long and 25 nm thick (KLEMENT and LOVAS, 1959).

Some phages are strictly host specific (cf. ARBER, 1968); this property renders it possible to typify the phages. By means of this procedure bacteria belonging to the same serotype can be differentiated according to the range of their phages. A bacterium of a certain phage type is only dissolved by the given phage. There are phages which have wide host ranges (polyvirulent phages); they can be used to point out relationships. As for the possibilities of using phages in the practice we refer here to the papers of KLEMENT and LOVAS (1957, 1959) and KLEMENT and LOVREKOVICH (1960); they found that the four most important bacterial pathogens of beans (*Corynebacterium flaccumfaciens*, *Pseudomonas phaseolica*, *Xanthomonas phaseoli*, *X. phaseoli* var. fuscans) which are symptomatologically hardly – if at all – separable – could be identified with the help of phages. Further possibilities of differentiation were reported later by LOVREKOVICH and KLEMENT (1965) between *Xanthomonas vesicatoria* strains infecting tomato and

pepper plants, as well as by ECHANDI and SUN (1973) for *Corynebacterium michiganense*.

The relationships between bacteria and the viruses of the highest plants (cf. KLEMENT *et al.*, 1966; LOEBENSTEIN and LOVREKOVICH, 1966; LOVREKOVICH *et al.*, 1968; HORVÁTH, 1972c) – which may be manifest *vice versa* – suggest that the appearance of bacterial and virus diseases both in antagonistic an synergistic forms may even occur spontaneously in the nature. Another reason why further studies on the relationship between bacteria and viruses are important is that there are evidences of a simultaneous transmission of the two pathogens by grafting and mechanically (BASIT *et al.*, 1970). Furthermore, bacteria are important not only because they cause many human, animal and plant diseases, but also because they are hosts of bacterial viruses or bacteriophages, which are models of extreme significance in virology, genetics, biophysics, biochemistry and molecular biology (cf. SANDER, 1964).

2. *Algae*

The about fifty thousand morphologically highly diversified alga species (in Hungary some three thousand species have been identified so far) escaped the attention of the virus researchers for a long time. Phage-like viruses associated with prokaryotic, blue-green alga have been well documented both structurally and biochemically (see reviews by BROWN, 1972; PADAN and SHILO, 1973). The investigations of SAFFERMAN and MORRIS (1963) American researchers – nearly fifty years after the discovery of the bacterial viruses and bacteriophages, respectively – pointed out that the species of *Lyngbya*, *Plectonema* and *Phormidium* played an important role as virus hosts. The first prokaryotic virus isolated from the above three genera was given the name LPP–1 virus on the basis of the initial letters of the names and the order of isolation. In the course of subsequent investigations further viruses from other filamentous species (e.g. *Oscillatoria princeps*, *Clyndrospermum* sp., *Anabaenopsis raciborskii*, *A. circularis*, *Raphidiopsis indica*, *Anabaena* sp., *Nostoc muscorum*) and unicellular hosts (e.g. *Microcystis aeruginosa*, *M. pulverea*, *M. muscicola*, *Synechococcus elongatis*, *Anacystus nidulans*, *Synechococcus cedrorum*) have been described.

At present, however, little is known about viruses in cells of eukaryotic algae. The first observation of virus-like particles within cells of eukaryotic alga occurred during a study on the red alga *Sirodotia* (cf. LEE, 1971). On the basis of a summarizing work by BROWN (1972), PIENAAR (1976), HOFFMANN and STANKER (1976), WOLF (1977) it can be established that further eukaryotic algal viruses occur in the following alga species: *Chlorella pyrenoidosa*, *Oedogonium* sp., *Scenedesmus armatus*, or the aquatic phycomycete *Aphelidium*, *Chorda tomentosa*, *Cyanophora* sp., *Porphyridium*, *Pylaiella*, *Ectocarpus*, *Chrysochromulina*, *Coccolithus*, *Hydrurus*, *Aulacomonas*, *Uronema*, *Platymonas*, *Pyramimonas*, *Cylindrocapsa*, *Stigeoclonium*, *Coelochaete*, *Radiofilum*, *Hymenomonas*, *Micromonas*, *Cryptomonas*, *Skuja* (freshwater red alga). On the basis of the available data the

so far described many highly variable prokaryotic viruses which contain DNA and resemble morphologically bacteriophages rather than viruses of higher plants (except for the SM – 1 virus isolated from species of *Synechococcus elongatis* and *Microcystis seruginosa*, cf. SAFFERMAN and MORRIS, 1967, as well as the *Oscillatoria* virus isolated from the species *Oscillatoria princeps* which resembles the tobacco mosaic virus, cf. UEDA, 1965) can be placed in three groups as regards their structural and morphological characteristics. The first group includes the viruses isolated from *Synechococcus elongatis* and *Microcystis seruginosa* species (LPP – 1, LPP – 2, SM – 1) which have short and thick non-contractile tails. In the second group viruses with rigid contractile tail sheaths are found (N – 1, a virus isolated from the species *Nostoc muscorum*; virus AS – 1 isolated from *Anacystis nidulans* and *Synechococcus cedrorum*).

The virus S – 1, recently isolated from the blue-green alga *Synechococcus elongatis*, belongs to the third group which has a long and rigid, non-contractile tail (LUFTIG and HASELKORN, 1967; SAFFERMAN *et al.*, 1969; ADOLPH and HASELKORN, 1971; SAFFERMAN *et al.*, 1972; MACKENZIE and HASELKORN, 1972; ADOLPH and HASELKORN, 1973).

The above three groups of DNA-containing prokaryotic viruses known and described so far need completion on the basis of the most recent highly remarkable results of research.

In most instances, the virus-like particles in eukaryotic algae have been shown to have a five- or six-sided, polygonal profile in sectional view. The virus particles from various algal hosts range in size from about 40 to 240 nm in diameter (cf. HOFFMANN and STANKER, 1976). Non-polygonal virus-like particles have also been reported in eukaryotic algal cells (MATTOX *et al.*, 1972).

In a paper published lately GIBBS *et al.* (1975) and GIBBS and HARRISON (1976) give account of a tobamovirus isolated from the green alga *Chara corallina* var. corallina (*Chara corallina* virus) which differs from the so far isolated algal viruses not only in having single-stranded RNA content (molecular weight: 2.3×10^6), but also in its serological relationship with, and morphological similarness to the tobacco mosaic virus (18×532 nm). A classification of *Chara corallina* virus (CCV) and 68 other tobamovirus isolates, whose coat protein composition is known, showed that CCV is distant from all, but is most closely related to the cucurbit tobamoviruses (see SKOTNICKI *et al.*, 1976).

3. Fungi

The first observations suggesting that soil fungi found on the roots of virus infected plants are capable of virus transmission were made four and a half decades ago. This theory remained, however, unproved for a long time, although the relation between *Thielaviopsis* (*Milowia*) *basicola* and the tobacco necrosis virus could be pointed out (SZIRMAI, 1939).

The relations between fungi and viruses were experimentally proved in the 1960's. At that time three authors reported virus transmission by fungi indepen-

dently (*Olpidium* sp.): HIDAKA (1960) for tobacco stunt virus, TEAKLE (1960) for tobacco necrosis virus and CAMPBELL *et al.* (1961) for lettuce big-vein virus. These results gave a great impetus to the research work, and since that time fungi (*Plasmodiophorales*, *Chytridiales*, *Perisporiales*) were reported as virus vector (cf. GROGAN and CAMPBELL, 1966; YARWOOD, 1971b; HORVÁTH, 1972c), and later other fungi as virus hosts have attracted the attention of scientific workers dealing with viruses.

Today it would undoubtedly be difficult to say whether it was the results of the fungal vector researches that started and stimulated the investigations which after 1962 attained so significant results in the course of fungal virus host studies.

The first experimental proof of fungus-virus host relations was produced when GANDY (1960) pointed out in experiments that the serious disease of the cultivated 2-spored mushroom (*Agaricus bisporus*, syn.: *Psalliota bispora*) could be transmitted by the mycelium of the fungus, and when GANDY and HOLLINGS (1962) as well as HOLLINGS (1962) verified that the extremely severe disease first described by SINDEN and HAUSER (1950) in the United States of America as "Die back", "La Franche" "watery stripe", "brown", then in England as "X", and in Holland by the name "afsterwings", and now known all over the world, could be traced back to virus infection. Electron microscope examinations have revealed that the infectious disease is brought on with the separate or joint participation of isometric (25 nm, 29 nm, 35 nm and 50 nm) and anisometric bacilliform (19×50 nm) and rod-shaped viruses (HOLLINGS, 1962; HOLLINGS *et al.*, 1963; DIELEMAN-VAN ZAAYEN, 1969; HOLLINGS and STONE, 1971; HOLLINGS, 1972; SPIRE, 1972; LUISONI, 1972; DIELEMAN-VAN ZAAYEN, 1972a). Five years after the first evidence of fungus-virus host relations had been given DIELEMAN-VAN ZAAYEN (1967) in Holland pointed out the occurrence of viruses in *Ascomycetes*, then two years later – in Holland again – the occurrence of tobacco mosaic virus in phycomycetes (*Pythium*) was also proved (BRANTS, 1969). Of conidial fungi (*Fungi Imperfecti*, *Deuteromycetes*) virus was first found in *Penicillium stoloniferum* (ELLIS and KLEINSCHMIDT, 1967). From that time on the presence of various viruses have been pointed out in many *Penicillium* and *Aspergillus* species (BANKS *et al.*, 1968, 1969, 1970) as well as in unicellular *Phycomycetes* fungi (SCHNEPF *et al.*, 1970; AIST and WILLIAMS, 1971).

On the basis of a review of the available literary data we can say that numerous species of *Myxomycetes*, *Phycomycetes*, *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* can be regarded as important virus hosts (BLATTNÝ and PILÁT, 1957; BLATTNÝ, 1966; BRANTS, 1969; DIELEMAN-VAN ZAAYEN *et al.*, 1970; NIENHAUS, 1971; HOLLINGS and STONE, 1971; ALBOUY and LAPIERRE, 1971; SPIRE, 1971; FERAULT *et al.*, 1971; WOOD *et al.*, 1971; LHOAS, 1972; DIELEMAN-VAN ZAAYEN, 1972a, b; LUISONI, 1972; BORDER, 1972; BOZARTH, 1972; HOOPER *et al.*, 1972; ALBOUY and LAPIERRE, 1972; RATTI and BUCK, 1972; VOLKOFF *et al.*, 1972; SPIRE *et al.*, 1972; METITIRI and ZACHARIAN, 1972; YARWOOD and HECHT-POINAR, 1973; BLATTNÝ *et al.*, 1973; WOOD and BOZARTH, 1973; RAWLINSON *et al.*, 1973; SANSING *et al.*, 1973; MOFFITT and LISTER, 1973; RAWLINSON and MACLEAN,

1973; DUNKLE, 1974; WOOD *et al.*, 1974; PASSMORE and FROST, 1974; NIENHAUS, 1974 report in letter, HUTTINGA *et al.*, 1975; GIBBS and HARRISON, 1976; LEMKE, 1977; WOLF, 1977). According to our knowledge viruses have been pointed out so far in the following fungi:

Myxomycetes: Plasmodiophora brassicae.

Phycomycetes: Pythium sp., *Mucor* sp., *Rhizopus* sp., *Choanephora* sp., *Synchephalastrum* sp.

Ascomycetes: Saccharomyces carlsbergensis, S. cerevisiae, Ophiobolus graminis, Neurospora crassa, Hypoxylon sp., *Diplocarpon rosae, Daldinia* sp., *Peziza ostracoderma* (syn.: *Plicaria fulva), Erysiphe graminis, Gaeumannomyces graminis, Sphaerotheca lanestris, Endothia parasitica.*

Basidiomycetes: Agaricus bisporus, A. campestris, Armillaria mellea, Boletus* sp., *Cantharellus infundibuliformis, Hypholoma* sp., *Laccaria amethystina, L. laccata, Lentinus edodes, Polyporus* sp., *Ustilago maydis, Coelosporium asterum, C. madiae, Uromyces phaseoli* var. typica, *U. phaseoli* var. vignae, *U. fabae.*

Deuteromycetes (Fungi Imperfecti): Alternaria tenuis, Arthrobotrys sp., *Aspergillus flavus, A. foetidus, A. glaucus, A. niger, Aspergillus* sp., *Botrytis* sp., *Candia* sp., *Tilletiopsis* sp., *Cephalosporium acremonium, Cephalosporium* sp., *Chrysosporium* sp., *Fusarium moniliforme, Fusarium* sp., *Gliocladium* sp., *Gliomasti* sp., *Helminthosporidum mydis, H. oryzae, H. victoriae, Kloeckera* sp., *Mycogone perniciosa, Paecilomyces* sp., *Penicillium brevi-compactum, P. chrysogenum, P. citrinum, P. cyaneo-fulvum, P. funiculosum, P. multicolor, P. notatum, P. stoloniferum, P. variabile, Penicillium* sp., *Periconia circinata, Piricularia oryzae, Rhizoctonia solani, Sclerotium cepivorum, Scopulariopsis* sp., *Spicaria* sp., *Stemphylium botryosum, Trichothecium* sp., *Verticillium* sp.

On the basis of the investigations made by YARWOOD and HECHT-POINAR (1973) it can be supposed that – as suggested by the biological test plant experiments – *Erysiphe polygoni, Frommea obtusa* var. duchesneae, *Kunkelia nitens, Phragmidium* sp., *Phyllactinia corylea, Puccinia iridis, P. oxalidis, P. pelargonii, zonalis* and *Uromyces polygoni* are also virus hosts. Recently it has become known that the 36 nm diameter isometric virus pointed out in *Armillaria mellea* causes the yellowness of spruce (*Picea excelsa*, syn.: *P. abies*) (BLATTNÝ *et al.*, 1973).

The fifteen years old fungal virus host researches have enriched the science of virology with many new results. According to our present knowledge 60 different viruses have been pointed out so far in some 80 fungus species – with the experiment results of YARWOOD and HECHT-POINAR (1973) taken into account.

According to the results of analyses performed so far the mycoviruses contain double-stranded RNA (cf. LEMKE and NASH, 1974; DUNKLE, 1974). As regards their morphology they are generally isometric (25–50 nm). Exceptions are:

* In recent experiments carried out by VAN ZAAYEN (1976) *Agaricus bitorquis* proved to be immune against mushroom virus.

a 200 nm diameter virus isolated from *Phycomycetes* fungi, which is similar to the iridescent viruses (cf. KRIEG, 1973), furthermore a rod-shaped virus (17 × 350 nm, 18 × 120 nm) isolated from *Peziza ostracoderma*, *Agaricus bisporus*, *Mycogone perniciosa*, *Coleosporium asterum*, *C. madiae*, *Erysiphe graminis*, *Sphaerotheca lanestris*, *Uromyces phaseoli* var. typica, *U. phaseoli* var. vignae, *U. fabae*, *U. polygoni*; a filamentous virus (13 × 500 nm) characteristic of the potexvirus group, isolated from *Boletus edulis*; a phage-type virus (head = 80 × 90 nm, tail = 70 nm in length) isolated from *Saccharomyces carlsbergensis* (VOLKOFF and WALTERS, 1970); a bacilliform virus (19 × 50 nm) isolated from *Agaricus bisporus*, and a spiral symmetry virus of 65 nm diameter isolated from *Agaricus bisporus* (HOLLINGS, 1962; DIELEMAN-VAN ZAAYEN and TEMMINK, 1968; ALBOUY, 1972; LUISONI, 1972; HUTTINGA *et al.*, 1975). Attempts to point out a serological relationship between viruses isolated so far from *Agaricus bisporus* and those of higher plants have failed so far. The mycoviruses – like the viruses of the highest plants – often are found in a complex form in various fungus species. E.g. viruses separable both serologically and on the basis of differences in RNA qualities were found to occur in *Penicillum stoloniferum* (BUCK and KEMPSON-JONES, 1970; BOZARTH *et al.*, 1971) and *Aspergillus foetidus* (RATTI and BUCK, 1972). It is remarkable that the fungi as mycovirus hosts, similarly to the hosts of viruses infecting higher plants, contain viruses in different quantities. In 1 g dry matter of fungal mycelium they generally occur in a quantity of 0.01 μg. The *Penicillium chrysogenum* and *P. stoloniferum* in which the amount of virus is as much as 1 mg are exceptions (BOZARTH *et al.*, 1971; WOOD and BOZARTH, 1972).

On the basis of the research results obtained so far it has been established that virus infected fungi cause much more serious diseases in cultivated plants than the virus-free individuals of the same fungus species do (BOZARTH, 1972). These results show that fungal virus hosts have an extremely important role in the theoretical and practical virus research (see LEMKE, 1977).

As a scientific prodrome we mention here that recently a mycoplasm has been found to occur in the aquatic fungus *Aphanomyces astaci* (HEATH and UNESTAM, 1974); as a consequence of infection by the mycoplasm the fungus is no longer able to produce zoospores.

4. Lichens

As it is well known lichens are symbiotic combinations of algae and fungi, or occasionally of hypomycetes and uni- or multicellular blue-green algae (gonidia, *Cyanophyceae*, *Chlorophyceae*) owing to their special structure they cannot be regarded as separate plants; they are so-called complex thalloid plants, qualitatively new organisms. Lichens become easily acclimatized to various climatic conditions; they are found in tropical and polar regions, at sea-level and in snow-covered mountains alike. The number of species known at present is about twenty thousand. In Hungary some thousand species of lichens are known. They all belong to the class of *Ascolichenes*.

According to our knowledge lichens as virus hosts are not known. However, we are of the opinion that the virus host nature of many epiphytal species of *Ascolichenes* (*Hypogymnia physodes*, *Parmelia sulcata*, *P. aipolia*, *Evernia prunastri*) known in Hungary – which contain *Ascomycetes* of virus host nature too – is not excluded in spite of the fact that our earlier attempts to transmit virus from lichens to virophilic plants (e.g. *Chenopodium* species, *Cucumis sativus*, *Gomphrena globosa*, etc.) with carborundum-spatula-buffer technique were unsuccessful. Nevertheless, the unsuccessful attempt – supposed to be in connection with special acidic compounds produced by the lichens as well as with the presence of various pigments as possible inhibitors – does not exclude the occurrence of viruses in lichens closely connected with virus infected plants or even insects. For this very reason, in the future research one has to pay increased attention to the investigation of lichens as possible virus hosts and reservoirs.

The higher plants of the flora

1. Mosses (Bryophyta)

Of the highest cauline or telomic (*Cormophyta*, *Telomophyta*) plants of the flora the mosses (*Bryophyta*) are not known to be infected by viruses. It must be added, however, that up to now the mosses have escaped the attention of the virologists. Considering that the number of moss species is rather high (nearly 600) in Hungary, and at their habitats many cultivated, wild and woody plants known to be susceptible to viruses are found – some of which (e.g. *Bromus tectorum*) usually are in symbiosis with *Syntrichia ruralis*. cf. HORTOBÁGYI, 1974) – it can be supposed that a study on mosses as possible virus hosts might contribute many new results to our present knowledge of the host ranges of viruses.

2. Ferns (Pteridophyta)

The ferns (*Pteridophyta*) – these autotrophic plants with stems, leaves and roots – are widely distributed in various parts of the world. To our knowledge there are some twelve thousand species of them on the Earth, but only 45 in Hungary.

The available data concerning the virus susceptibility of ferns are relatively few. Of the classes of *Lycopsidae*, *Sphenopsidae* and *Pteropsidae* it is only in the latter that we know about virus infection. In the course of electron microscope examinations of mosaic, leaf narrowing and leaf-edge undulation in *Phyllitis scolopendrium* – a member of the family *Polypodiaceae* which occurs in the mountains of Bükk and Mátra (Hungary) too – HULL (1968) isolated a $135-320 \times 22$ nm rod-shape, mechanically transmissible and supposedly soil borne virus partly characteristic of the tobamovirus group. The virus isolated in England (Botanic Garden, Cambridge University, Cornwall) was given the name harts tongue fern

mottle virus. The virus was mechanically easily transmitted to *Dryopteris filix-mas*, but the diagnostic test plants best known and most frequently used in plant virology (*Cucumis sativus*, *Gomphrea globosa*, *Nicotiana clevelandii*, *N. tabacum*, *Vigna unguiculata*) proved to be resistant to virus inoculation. In the course of his investigations HULL (1968) found rod-shape virus particles in further *Pteridophyte* plants: *Pellaea falcata*, *Polystrichum falcatum*, *Pteris cretia* var. albolineatum, *P.* "*Childsii*". Attention is worth being paid to the experiments performed by BLATTNÝ (1970) in Czechoslovakia and Yugoslavia; in *Pteridum aquilinum* plants he found spherical virus particles which caused a serious disease and ultimate destruction of plants.

Not much later the investigations of HULL and BLATTNÝ (*loc. cit.*) were followed by reports from CHEO and GERARD (1971) as well as from CHEO (1972) on experiments carried out in the course of studying the artificial host–virus relation of ferns and tobacco mosaic virus. They pointed out that of the twenty ferns artificially inoculated with tobacco mosaic virus seven species and two varieties (*Davallia mariesii*, *Nephrolepsis exaltata*, *Pellaea viridis*, *Platycerium bifurcatum*, *Polypodium augustifolium*, *P. polycarpon*, *P. subauriculatum* cv. Knight., *P. vulgare* cv. Columbianum, *Pteris umbrosa*) were susceptible.

In the course of recent investigation involving *Polypodium vulgare* and *Dryopteris filix-mas* plants occurring in the medium height mountain-range of the Rhineland (Bonn, Eifel) a so far unknown 785 nm long and 12–14 nm wide flexible, mechanically and aphid (*Myzus persicae*) transmissible virus has been found which according to its characteristic features belongs to the potyvirus group (NIENHUS *et al.*, 1974). With the data published by HULL (1968), HEINZE (1968), CHEO apd GERARD (1971), CHEO (1972) and NIENHUS *et al.* (1974) as well as WOLF (1977) taken into account, some 16 species and varieties, respectively, have been proved to be susceptible to viruses.

The results obtained concerning the virus susceptibility of *Pteridophytes* furnish sound proof of a close link and a phylogenetic relationship between plants and viruses.

3. Gymnosperms

Literary data on the virus infection of seed or flowering plants belonging to the gymnosperms (spermatophytes) – just as in the case of *Pteridophytes* – have been available for a relatively short time. In the course of investigations restricted to *Coniferopsides* YARWOOD (1959a, b), a well-known American virologist was the first to point out the susceptibility of *Pinus silvestris* to tobacco necrosis virus and arabis mosaic virus under natural conditions. These first results of investigations gave an impetus to the European virus research work. As a result seven gymnospermous plants (*Chamaecyparis lawsoniana*, *Picea excelsa*, *P. sitchensis*, *Pinus monticola*, *P. nigra*, *P. silvestris*, *P. mungo*) were reported to be susceptible to viruses (ČECH *et al.*, 1961; SVOBODOVÁ, 1963; HARRISON, 1964; PENO and POPOVIČ, 1965; SCHMELZER *et al.*, 1966; BIDDLE and TINSLEY, 1968; BLATTNÝ,

1970). Further symptomatological lesions (yellowing, spotting, asymmetric growth) possibly characteristic of virus infection have been observed in *Taxus baccata*, *Pinus mungo*, *P. silvestris*, *Picea pungens* and *P. abies* (SCHMELZER *et al.*, 1966; SCHMELZER *et al.*, 1969; SCHMELZER and WOLF, 1971).

4. Angiosperms

The relations between angiospermous plants and viruses are the best known of all. Considering that members of this division are cultivated plants while others are wild, in the research of host–virus relations the emphasis has been laid on the angiospermous plants.

Some hundred of over 600 viruses and virus strains, respectively (cf. MARTYN, 1968, 1971) known at present have been pointed out to occur in the most important 12 plants used for human nutrition: rice, wheat, maize, beet, sugar-cane, potato, sweet potato, manioc, bean, soybean, coconut and banana. Besides the above plants some vegetable plants highly important from an economic point of view (e.g. pepper, tomato, cucumber, patisson, lettuce, etc.) are known as natural hosts of numerous viruses. The virus–host researches have revealed extremely noteworthy data – particularly for the last twenty years – concerning the importance of terrestrial and aquatic weeds (MACCLEMENT and RICHARDS, 1956; HEIN, 1956, 1967a, b, 1959; WILSON, 1969; MURANT, 1970; TOMLINSON *et al.*, 1970; DUFFUS, 1971; TINSLEY, 1971; GRACIA and FELDMAN, 1972; CHESSIN and LESEMANN, 1972; ZETTLER and FREEMAN, 1972; FELDMAN and GRACIA, 1972a, b; COOPER and HARRISON, 1973; HORVÁTH and SZIRMAI, 1973; DINOOR, 1974) as well as woody and ornamental plants (SCHMELZER, 1962a, b, c, d, 1966; SCHMELZER and SCHMIDT, 1968; SCHMELZER, 1970, 1974; HORVÁTH, 1973b; HORVÁTH *et al.*, 1974c; HORVÁTH and SZIRMAI, 1975) as earlier perfectly unknown, primarily perennial virus reservoirs taking part in the infection chain. Recent results obtained in this field have provided important data of practical and scientific value for solving epidemiological problems much discussed by the virologists.

Viruses of wide host ranges

In the subject of virus–host relations the first larger comprehensive work – in a chronological order – was written on the artificial or experimental host-range of the turnip mosaic virus. In artificial inoculation experiments TOMPKINS *et al.* (1938) found 43 species and varieties, respectively, of 12 plant families susceptible, and 53 species of 26 families resistant to infection by turnip mosaic virus.

Papers published by PRICE (1940) and HOLMES (1946) dealt with the reactions (susceptibility, resistance) of some 300 and 310 plant species, respectively, to tobacco necrosis virus, cucumber mosaic virus, cucumber green mottle mosaic virus, tobacco ring spot virus, tomato ring spot virus and tobacco etch virus.

The works of USCHDRAWEIT and VALENTIN (1956a, b, 1957) revealed that in Europe many ornamental plants are spontaneous hosts to cucumber mosaic virus, tobacco rattle virus and turnip mosaic virus. Many of the examined plants belong to the family *Cruciferae* (*Brassicaceae*).

In the research of virus–host ranges outstanding results were attained by the late Dr. K. SCHMELZER German virologist; he first excited interest with his paper on the host range of tobacco rattle virus (SCHMELZER, 1957a). In the course of artificial inoculation experiments including 539 plants he found 35.6 per cent of the inoculated plants to be systemic, and 35.1 per cent to be local host plants. The rest (29.3 per cent) can be regarded as resistant to infection by tobacco rattle virus. In the course of the examinations many species of the families *Solanaceae* and *Scrophulariaceae* have proved to be particularly susceptible. Plants belonging to the families *Rosaceae* and *Gramineae* (*Poaceae*), on the other hand, have been found to be resistant to the virus.

Two years later SCHMELZER (1959) published the artificial host range of beet mosaic virus. Of 316 plants 60 were found to be systemic, and 46 local hosts of the virus. The rest – 210 plants – resistant to virus inoculation are of importance in virus differentiation. In the course of artificial inoculation experiments species belonging to the families *Chenopodiaceae* and *Hydrophyllaceae* proved to be the most susceptible, though susceptible plants were found in the families *Papaveraceae*, *Caryophyllaceae* and *Fabaceae* (*Leguminosae*, *Papilionaceae*) as well.

In an extensive study on the host range of nasturtium ring spot virus (syn.: broad bean wilt virus) SCHMELZER (1960, 1970) examined many species of 22 plant families and detected 122 plants susceptible to the virus; in a later work (SCHMELZER, 1974) he increased this number by further 53 susceptible plant species. The author called attention to further susceptible families (*Basellaceae*, *Rubiaceae*) which are not familiar to virologists. Information on new host plants of the polyphagous virus are found in the work of HORVÁTH and SZIRMAI (1975).

On the host range (determined after the examination of 49 species in eight families) of six potato pathogen viruses (potato virus X, potato virus Y, potato virus S, potato virus M, potato aucuba mosaic virus, potato virus A) belonging to different virus groups detailed data and a critical evaluation are published in one of our earlier works (HORVÁTH, 1964).

In the middle of the sixties a study of some extension was published on the host ranges of further ten viruses. HOLLINGS and STONE (1964) spotted 28 new host plants of carnation mottle virus. The effect of carnation ring spot virus on 51 plants was described in a later publication of HOLLINGS and STONE (1965b). New important data on the host plants of *Pelargonium* leaf curl virus, tomato bushy stunt virus, broad bean mottle virus, carnation mottle virus, carnation ring spot virus, *Cymbidium* ring spot virus, raspberry ring spot virus, tobacco necrosis virus and turnip crinkle virus are found in another paper by HOLLING and STONE (1965a).

In the nepovirus group tobacco ring spot virus was the first of which a

detailed host-range study was published. On the basis of the work of DEZEEUW (1965) it can be established that some 246 species of 54 plant families are susceptible to virus inoculation. In the course of experiments carried out in the past several years we pointed out further plants susceptible to tobacco ring spot virus in the genera: *Paulownia*, *Physalis*, *Ocimum* and *Browallia* (HORVÁTH, 1973g, 1974b, c, e, 1975a, b, c, d).

The results attained until 1966–1967–1968 in the research of virus host ranges were summarized and published in two outstanding works without which orientation in this field would has not been possible. THORNBERRY (1966) in his book "*Index of plant virus diseases*" enumerated 2215–6675 host–virus relations in 101 plant families, supplied with literary references – in a regrettable way without a bibliography and only from English speaking countries. SCHMELZER and WOLF (1971) publish 2353–7641 host–virus relations in their book "*Wirtspflanzen der Viren und Virosen Europas*". The works of THORNBERRY and of SCHMELZER and WOLF (*op. cit.*) have given a new impetus to research.

According to a recent work – in a chronological order – summing up the host range of tomato spotted wilt virus (BEST, 1968), the range of hosts of this virus includes 160 dicotyledonous species of 29-, and 6 monocotyledonous species of 5 families.

According to a comprehensive study of HORVÁTH (1969h) the potato virus Y is a pathogen of some 126 plants. Some of them are particularly important in experimental virology owing to their resistance against downy mildew of tobacco (*Peronospora tabacina*) and susceptibility to virus (HORVÁTH, 1967e, 1969c, HORVÁTH and BECZNER, 1968). The virus susceptible plants mostly belong to the family *Solanaceae*. As a result of recent investigations carried out in Hungary the host range of potato virus Y has been substantially widened (HORVÁTH, 1969c, 1970b, 1972b, 1974a, b, c, 1975a, b, c, d).

According to BENNETT (1971) the hosts of beet leaf curl virus are found in 300 species of 44 families. Only dicotyledonous plants are known to be susceptible to the virus, monocotyledonous plants have not proved to be susceptible so far. The virus susceptible plants are mostly found in the families *Caryophyllaceae*, *Cruciferae* (*Brassicaceae*), *Chenopodiaceae*, *Fabaceae* (*Leguminosae*, *Papilionaceae*), *Compositae* (*Asteraceae*) and *Solanaceae*.

Among the virus pathogens of plants the host range of alfalfa mosaic virus – the best known polyphage – includes 500 species in 60 plant families (BECZNER and HORVÁTH, 1968; HULL, 1969; CRILL *et al.*, 1970; BECZNER and HORVÁTH, 1972; BECZNER and SCHMELZER, 1972a, b; HORVÁTH and BECZNER, 1973b; BECZNER, 1973a, b; SCHMELZER *et al.*, 1973). Spontaneous susceptibility to alfalfa mosaic virus has recently been pointed out in two Mediterraneous *Cistus* species (*C. populifolius*, *C. monspeliensis*) unknown so far in virology (SCHMELZER, 1974).

Investigations into the host range of a new virus (*Erysimum* latent virus) isolated in the course of an extensive study of viruses in plants belonging to the family *Cruciferae* (*Brassicaceae*) have revealed that the virus is a pathogen of 40 species in 19 families. Besides the *Cruciferae* (*Brassicaceae*) plants belonging

to the families *Cucurbitaceae*, *Fabaceae* (*Leguminosae*, *Papilionaceae*), and *Solanaceae* are susceptible in the first place (SHUKLA and SCHMELZER, 1972).

On the basis of an excellent work written of the host ranges of three virus pathogens of beet (DUFFUS, 1973) it can be established that 146 species from 23 plant families are susceptible to beet western yellows virus, 121 species from 5 families to beet yellows virus and 37 species from 14 families to beet pseudo yellows virus. The three viruses are pathogens first of all to various species in the families *Chenopodiaceae*, *Amaranthaceae*, *Aizoaceae*, *Cruciferae* (*Brassicaceae*), *Compositae* (*Asteraceae*) and *Solanaceae*.

In the course of investigations concerning the host range of cucumber mosaic virus – a wide-spread pathogen of many monocotyledonous and dicotyledonous plants, well-known for its polyphagous nature all over the world – further 72 cruciferous ornamental and wild plants have been found to be hosts of the virus (SHUKLA and SCHMELZER, 1973).

According to a comprehensive work recently written on the host range of potato spindle tuber virus (viroid, reviewed by DIENER, 1974) – a pathogen that has lately attracted the interests of virologists' – plants susceptible to the virus are mostly found in the families *Boraginaceae*, *Campanulaceae*, *Caryophyllaceae*, *Compositae* (*Asteraceae*), *Convolvulaceae*, *Dipsacaceae*, *Scrophulariaceae* and *Solanaceae*. According to the investigations 232 plants have proved to be virus hosts (SINGH, 1973).

Of about sixteen rhabdoviruses known so far some (e.g. lettuce necrotic yellows virus, potato yellow dwarf virus) are pathogens to dicotyledonous plants, while others (e.g. wheat streak mosaic virus, rice transitory yellowing virus, northern cereal mosaic virus, barley yellow striate mosaic virus, winter wheat mosaic virus) to monocotyledonous ones only. Of the two economically most harmful rhabdoviruses (potato yellow dwarf virus and lettuce necrotic yellows virus) the host plants of the former are found in 46 species of eight families, while those of the latter in 15 species of four families (FRANCKI, 1973). Hosts of potato yellow dwarf virus are encountered mainly among *Cruciferae* (*Brassicaceae*), *Fabaceae* (*Leguminosae*, *Papilionaceae*), and *Solanaceae*, while those of lettuce necrotic yellows virus in the family *Compositae* (*Asteraceae*).

Works published in the last several years include a study by BAKKER (1974) on rice yellow mottle virus. The excellent dissertation gives account of 18 local and 16 systemic virus hosts found among 248 inoculated species of the families *Gramineae* (*Poaceae*), *Cyperaceae*, *Typhaceae*, *Araceae* and *Commelinaceae*. The rest – 214 plants – are not hosts to the virus. Furthermore, 25 species of ten families of dicotyledonous plants also have proved to be resistant against the virus.

According to the most recent results of investigations concerning the viruses of ornamental and arboraceous plants the experimental host range of elm mottle virus has been completed by further 82 species of 26 plant families (cf. SCHMELZER, 1974). With these new results – and an earlier work by SCHMELZER (1969) also taken into consideration – 148 species from 31 plant families can be regarded as susceptible to viruses.

According to a paper written on the host plants of brome mosaic virus (bromovirus group) the hosts of the virus are mainly found among *Gramineae* (*Poaceae*). Of the examined 162 susceptible plants 140 belonged to species of the family *Gramineae* (*Poaceae*). In the families *Amaranthaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Fabaceae* (*Leguminosae*, *Papilionaceae*), *Rosaceae* and *Solanaceae* some 22 susceptible plants have been detected (LANE, 1974).

In the course of examining the host plants of soybean mild mosaic virus, a recently described spherical, 26–27 nm diameter virus transmissible by aphids and seed (so-called soil-borne virus) TAKAHASHI, TANAKA and TSUDA (1974) inoculated 76 species from 14 plant families. They found that 43 of the 76 inoculated plants showed systemic, and 9 local symptoms as a response to the virus inoculation.

In our recent investigations (HORVÁTH, 1975h; HORVÁTH *et al.*, 1975b) we pointed out 17 new artificial host plants for the cosmopolite and polyphagous cucumber mosaic virus, and in another paper (HORVÁTH *et al.*, 1975a) described eleven new watermelon mosaic virus hosts. We consider the recently discovered 28 differentiating plants suitable to separate the above two viruses which often occur together in a complex form to be of particularly important.

When examining the host plants of tomato mosaic virus, a wide-spread pathogen of tomato crops in Hungary, we found some 26 new artificial and experimental hosts, respectively, in the families *Aizoaceae*, *Amaranthaceae*, *Cruciferae* (*Brassicaceae*), *Cucurbitaceae*, *Geraniaceae*, *Labiatae* (*Lamiaceae*), *Scrophulariaceae* and *Solanaceae* (MAMULA *et al.*, 1974). Investigations into the host range of cherry leaf roll virus spontaneously occurring in Hungary first in *Sambucus nigra* have revealed eleven new so far unknown nepovirus hosts; it is especially remarkable to point out new hosts like *Ammi visnaga*, *Aptenia cordifolia*, *Browallia* spp. and *Commelina* spp. which are hardly – if at all – known in plant virology (HORVÁTH, 1974e; HORVÁTH *et al.*, 1974c).

According to two outstanding works published in the last years the host plants of 60 exactly identified viruses in the potyvirus group, 23 provisionally identified viruses and 15 virus strains include some 1187 plant species and varieties (EDWARDSON, 1974a, b). On the basis of the mentioned paper it can be established that certain plant families are particularly susceptible (after the family name we give in brackets the number of viruses which are pathogens of the plant family in question): *Chenopodiaceae* (44), *Fabaceae* (*Leguminosae*, *Papilionaceae*) (37), *Solanaceae* (36), *Aizoaceae* (25), *Amaranthaceae* (23), while others (e.g. *Balsaminaceae*, *Boraginaceae*, *Violaceae*) only to one or another of the 98 tested viruses. The highest susceptibility to the different viruses is found in the following plant species (the number of viruses to which the plant in question is susceptible is given in brackets): *Chenopodium amaranticolor* (36), *Ch. quinoa* (36), *Nicotiana tabacum* (30), *Phaseolus vulgaris* (27), *Tetragonia expansa* (25), *Gomphrena globosa* (24), *N. clevelandii* (24). Particularly polyphagous viruses belonging to the potyvirus group are (the number of susceptible plants is given in brackets): turnip mosaic virus (175), watermelon mosaic virus (119), potato virus Y (115), bean yellow mosaic virus (104), bean common mosaic virus (64), celery mosaic virus (10).

The role of virophilic and virophobic plant families, genera and species in the identification and separation of viruses

On the basis of a review of the more important papers and handbooks written on the host–virus relations as well as from our own research results it can be established that some plant families (e.g. *Chenopodiaceae*, *Scrophulariaceae*, *Solanaceae*, etc.) are highly susceptible to various viruses, while others are hardly affected by them. In the work of virus isolation the so-called virophilic plants (*Chenopodium amaranticolor*, *Cucumis sativus*, *Gomphrena globosa*, *Nicotiana clevelandii*, *N. megalosiphon*, *Ocimum basilicum*, *O. canum*, *Physalis floridana*, *Torenia fournieri*, *Tetragonia tetragonoides*, etc.) which are susceptible to many viruses are indispensable. On the other hand, there are plants (e.g. *Crucianella stylosa*, *Helianthemum chamaecistus*, *Lythrum salicaria*, *Ruta graveolens*) susceptible to a comparatively small number of viruses; they are called relative virophobic plants (HOLLINGS, 1956, 1969a; SCHMELZER, 1961; HOLLINGS and STONE, 1963, 1965c; LOVISOLO, 1966; THORNBERRY, 1966; BOS, 1967; YARWOOD and FULTON, 1967; HORVÁTH, 1970b, 1974b, c, 1975a, d).

The virophilic *Nicotiana clevelandii* was found by HOLLINGS (1959b) to be a host plant of 41 viruses. It is remarkable that *Nicotiana clevelandii* is susceptible to viruses non pathogenic to other plant species of the family *Solanaceae*. *Chenopodium amaranticolor* is one of the most useful virophilic plants known in plant virology, suitable first of all to point out polyhderic viruses. It is known to be an excellent indicator of many viruses, and can be used in quantitative analyses too (HOLLINGS, 1956, 1957). Similar role is played by *Chenopodium quinoa* (HORVÁTH, 1962, 1964) which differs from *Ch. amaranticolor* being primarily used for pointing out rod-shaped- and filamentous viruses on the one hand, and having a lower inhibitor content, on the other. *Chenopodium hybridum* is susceptible to all viruses belonging to the bromovirus group (LANE, 1974) and can therefore be regarded as a special bromovirus indicator. Of the plants recently found to be virophilic *Tetragonia tetragonoides* an artificial host to some 50 viruses, *Emilia sagittata* to 32, and *Torenia fournieri* to 31 viruses (HOLLINGS and STONE, 1963, 1965c; KLINKOWSKI, 1977). According to our recent experiments other virophilic *Tetragonia* species (*T. crystallina*, *T. echinata*) less known in virology – similarly to *T. tetragonoides* – play an important role in the isolation and identification of various viruses (HORVÁTH, 1973f; HORVÁTH and SZIRMAI, 1973; MAMULA *et al.*, 1974). Of the *Physalis* species *Ph. floridana* is an indicator of about 45 viruses (HORVÁTH, 1970b, 1974b), and for many viruses not only as the most important receptor plant but also as a production host (TANAKA and IMADA, 1974).

Beside the virophilic and relative or absolute virophobic plants the bridge-plants or intermediary virus hosts (e.g. *Chenopodium foetidum*, *Cucumis sativus*, *Gomphrena globosa*, *Stellaria media*) have lately been of special interest. Apart from their virus receptivity their importance lies in the fact that compared to the original donor virus host (source) they have a lower virus inhibitor content and thereby facilitate virus isolation with those virophilic hosts from which it would be

otherwise difficult – if at all – possible owing to the high inhibitor content of the original donor host (GENDRON and KASSANIS, 1954; KASSANIS, 1955; SCHMELZER, 1957b, 1959, 1961).

Beside the bridge host plants the screening or differentiating (separating) plants (differentiators, separators) are of invaluable significance. The viruses are known to occur in nature most often in a complex form (cf. HORVÁTH, 1962, 1963, 1968g; HORVÁTH and BECZNER, 1973a; HORVÁTH *et al.*, 1974a, 1975a, b), which – besides exaggerating the internationally more or less unsolved problem of their control – increases the difficulties of identification and isolation.

In the science of plant virology differentiating the viruses arose already in the so-called symptomatological phase of virology. Yet, the separation of plant viruses did not initially rely on symptomatology – due certainly to an insufficient knowledge of virus–host relations –, it departed instead from a physico-chemical basis. There are, even now, publications which describe first of all physico-chemical methods for virus separation. We mention here the early investigations of ALLARD (1918), JOHNSON (1929) and ALLINGTON (1938). They found sodium permanganate, silver nitrate, phenol and mercury chloride suitable for the separation of various viruses (e.g. cucumber mosaic virus and potato virus X). FREEMAN (1935) reported the separation of a latent mosaic virus complex by changing the hydrogen ion concentration. ALLINGTON (1938) also reported the use of chemicals in the separation of viruses. He separated potato virus X from cucumber mosaic virus by means of a solution containing given concentrations of silver nitrate and mercury chloride. He also separated tobacco mosaic virus from tobacco ring spot virus using a method based on the difference in tolerance to phenol and sodium permanganate between these two viruses. Tobacco mosaic virus is inactivated by 4 per cent phenol whereas tobacco ring spot virus remains infectious after treatment with phenol of the same concentration. 0.5 per cent sodium permanganate has no deleterious effect on tobacco mosaic virus whereas it inactivates tobacco ring spot virus. Relatively recent investigations include experiments aimed at determining differences of pH tolerance in various viruses and its applicability in virus separation (FREEMAN, 1935; STANLEY and WYCKOFF, 1937; SNELL, 1938; KAUSCHE, 1938a, b; MATTHEWS, 1970; DUNCAN and BRUENING, 1971; HAVIAROVÁ and VALENTA, 1972; PROLL *et al.*, 1972). KAUSCHE (1938a, b) has demonstrated that different plant viruses exhibit varying degrees of sensitivity to pH. He found that potato virus X was inactivated under acidic, and tobacco mosaic virus under alkaline conditions. The tolerance of plant viruses to alcohol is also varying to which JOHNSON (1929) already called attention nearly half a century ago. Differences in alcohol tolerance between the tobacco necrosis virus strains have recently been pointed out by SZIRMAI (1961, 1964). Data on the use of chemicals in virus separation can also be found in publications issued not so long ago. KLINKOWSKI and SCHMELZER (1957) gave account of a successful separation of potato virus Y from cucumber mosaic virus by means of a 0.7 per cent solution of potassium bichromate. Publications of similar content are less and less in number. This probably can be explained by the fact that in the meantime the

knowledge of host–virus relations has become more complete and the differentiation of viruses by means of suitable plants (separators) is more efficient and reliable.

Of the different physical properties the thermal inactivation point, *in vitro* longevity, and dilution end point come in the first place in the course of virus differentiation. Of these methods controlled heat treatment is relatively reliable in the separation of viruses with different and known heat tolerances. However, we should like to note here that strains of the same virus species may have different thermal inactivation points (HORVÁTH, 1966a, b, 1976b, c) which may even be altered by environmental factors (light, temperature, etc.) acting on the host. For this very reason differentiation on the basis of differences in heat tolerance can be carried out only in the case of viruses with known and constant properties, and even then only under controlled conditions. Differentiation on the basis of *in vitro* storability and dilutability is a very doubtful method, since these properties are in close correlation with the virus concentration (SCHRAMM, 1952) – which is known to change frequently. Differentiation on the basis of the physical properties of viruses – as pointed out in our earlier papers (cf. HORVÁTH, 1967d, 1968g, 1969b, h) – is in most cases unreliable, as it is known that the environmental conditions influence the properties of the viruses by changing the physiological state of the host plant. For this very reason, differentiation on the basis of the physical properties of viruses can be regarded as an appropriate method only under controlled conditions and with the necessary control tests performed (HORVÁTH, 1969i).

As seen from the above listed major works various methods are available for the separation of plant viruses. Some of them are, however, rather uncertain or circumstantial, and is not therefore widely used in virology. Virologists prefer, and almost exclusively apply such methods which make use of another characteristic feature of the separator plants, namely, that they are susceptible to some viruses (local, systemic, local and systemic susceptibility), while resistant to others. Viruses possessing different host specificities, and plants of different virus susceptibilities enable the differentiation of a single virus in a single plant (semiseparators), or the separation of two viruses in the same plant (dichotomous separators). It is due to this important quality of the differentiating plants that today reliable methods involving separator plants are available for the separation of a number of virus complexes (HORVÁTH, 1967d).

JOHNSON (1925) and later KÖHLER (1933) were the first authors to report the separability of potato virus X and potato virus Y in *Datura stramonium*, since this plant is systemically susceptible to potato virus X and resistant to potato virus Y. It was more than four decades ago that SMITH (1931) introduced the term "*filter plant*", which since then has been generally used in virology. CADMAN (1958) pointed out the fact that potato ring necrosis virus gets localized in certain host plants (*Chenopodium amaranticolor*, *Phaseolus vulgaris*, *Nicotiana glutinosa*) and systematized in others (*Petunia hybrida*, *Datura stramonium*, *Nicotiana sylvestris*). This discovery makes it possible to choose host plants locally susceptible

to one and systemically to the other virus, or susceptible to one and resistant to the other virus, depending on the virus partners. Some years ago LOVISOLO (1959, 1961, 1966) gave account of *Ocimum basilicum* as being not only a good virus indicator but also suitable for virus differentiation. Similar results were obtained by HORVÁTH (1975b) and HORVÁTH and BECZNER (1973b) concerning *Ocimum canum*. *Crambe* species belonging to the family *Cruciferae* (*Brassicaceae*) have also proved to be good differentiator plants (THORNBERRY and PHILIPPE, 1965; HORVÁTH, 1969j, 1972f; HORVÁTH *et al.*, 1973). The results of studies on virus differentiation enable today the researchers to separate many virus complexes (reviewed by HORVÁTH, 1962, 1966, 1967d, 1968b, g, 1969a, b, e, i, 1974c; HORVÁTH *et al.*, 1975b). Determination of many new separator plants is of particular importance. In the past several years we spotted many plants suitable for virus differentiation in the following genera: *Browallia* (HORVÁTH, 1968c, 1974e), *Chenopodium* (HORVÁTH, 1968e, 1969a, 1970c; HORVÁTH and DE BOX, 1972), *Physalis* (HORVÁTH, 1970b, 1974b, c, 1975a, c, d), *Lycium* (HORVÁTH, 1972b; BECZNER and HORVÁTH, 1972), *Lycopersicon* (HORVÁTH, 1973c, d), *Geranium* (HORVÁTH, 1973e), *Paulownia* (HORVÁTH, 1973g), *Bryonia* (HORVÁTH, 1973i), *Phaseolus* (HORVÁTH, 1973j), *Ocimum* (HORVÁTH, 1975b; HORVÁTH and BECZNER, 1973b), *Erodium* (HORVÁTH, 1974d), *Tinantia* (HORVÁTH, 1975e), *Cucumis*, *Gomphrena* and *Solanum* (HORVÁTH, 1975f), *Amaranthus* (HORVÁTH, 1975g, 1976a, b). These new results of investigation contribute to the differentiation of viruses occurring most often in a complex form in nature, as well as to the separation of authentic virus species.

As a result of the rapid progress of inhibitor research it is now possible to separate plant viruses by means of inhibitors occurring in certain plants (cf. BHULLOR, 1965). However, we should like to point out here that leaves of different sequence on the same plant are not only differently susceptible to a given virus (cf. HORVÁTH, 1969d, f, k, l, 1973a; HORVÁTH and POCSAI, 1972b), but the quantities of inhibitors contained by them are also different (HORVÁTH, 1973h).

Even these few literary references show that the investigation of virus separation has made a great progress especially during the last several years. In this respect outstanding results have been attained by the Hungarian virus research. The most serious problem in virus separation is represented by the fact that according to our present knowledge there is no virus specialized to a single plant, and that the virophilic plants – highly suitable for the isolation of unknown viruses – cannot be used for separation purposes.

Furthermore, it is of extreme importance in virus differentiation that the viruses can be transmitted from one plant to another by mono-, bi- and polyfactorial methods (cf. HORVÁTH, 1972c). Various methods are available for the separation of viruses transmissible by insect vectors, other vectors with sucking or chewing mouth organs, and in other ways (vegetative propagation organs, *Cuscuta* species, lower fungi, roots, seeds, pollen) (cf. HORVÁTH, 1966c, 1967d). We should like to point here to the increased danger represented by the fact that tobacco mosaic virus pathogenic to potato – the natural occurrence of which has been established in Hungary too – can be transmitted with the vegetative

reproduction organs of potato plants infected by the virus (HORVÁTH, 1970a), and the same stands also for alfalfa mosaic virus (HORVÁTH, *ined).*

Virus transmission in a persistent (circulative) or non-persistent (stylet-borne) way – first formulated by WATSON and ROBERTS (1939) – has proved to be a key issue in separating single viruses from complexes. It is a fundamental and indispensable technique in separating circulative- and stylet-borne viruses. The discovery of vector specificity has enabled the separation of not only virus species but also of virus strains (cf. HORVÁTH, 1972c); this is of special importance because the separation of different strains of the same virus by means of differentiator plants encounters in most cases difficulties (HORVÁTH, 1966a, b, 1967b, c).

The increasing distribution and multiplication of known or so far unknown plant viruses – irrespective to geographical borders, mostly as a result of human activity (cf. YARWOOD, 1970) – indicate that the economic importance of viruses is continously growing and plants are playing an increasing role in virus identification and differentiation, in the further exploitation of the possibilities of breeding for resistance against viruses, as well as in the linkage of the infection chain important in virus epidemics, and finally, in the clarification of the epidemiological correlations.

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New Artificial Hosts and Non-Hosts of Plant Viruses and their Role in the Identification and Separation of Viruses

II. General Remarks and Proposals on the Experimental Work

By

J. HORVÁTH

Research Institute for Plant Protection, H—1525 Budapest, P. O. Box 102, Hungary

In his second publication the author deals in detail with the classification and denomination of viruses and viruses strains (isolates) included in his experiments, with the possibilities of symbolization, with test plants, with the role of donor plants, the methods of artificial inoculation, the compatible and incompatible host–virus relations, with reisolation and inoculation, with the theoretical bases of virus separation and disjuction, respectively, as well as their practical possibilities and recent terminology, then presents a symbolic system for the most important properties characteristic of the host–virus relations and suggests the use of a host–virus cryptogram built on a pentamer information system.

Introduction

Plant virology has been studied for about eighty years for many aspects, by different methods and competence and with varying possibilities. Every line of research has contributed surprising results to this branch of science. Investigations on the artificial virus hosts and virus resistant plants are extremely important both from the theoretical and practical points of view. Beyond their prognostic significance the artificial virus hosts play an important role in the identification and separation of viruses. Plants resistant to viruses, on the other hand, are invaluable in breeding for virus resistance and in virus differentiation.

It was in the fifties that attention was turned to this field of plant virology research in Hungary (cf. SZIRMAI, 1950, 1951, 1952, 1954, 1957, 1958a, b; MILINKÓ, 1952, 1956, 1958, 1961, 1962, 1964; SOLYMOSY, 1958, 1960; PETRÓCZI, 1959). In the second half of the sixties investigations on virus prognostics, diagnostics, differentiation and resistance became more intensive in Hungary. The description of several prognostic artificial hosts, identification of numerous new virus diagnostic and virus differentiating plants and detection of sources of virus resistance are due to these investigations (reviewed by SZIRMAI, 1971 and HORVÁTH, 1976).

Our results attained in the last one and a half decade concerning the identification of prognostic virus hosts and virus resistant sources form an integral part of the Hungarian and international research work. The aim was, on the one hand, to point out artificial host–virus relations which have a function in the prognosis, diagnosis and separation of viruses, on the other hand, to find virus resistance sources important – both practically and theoretically – in the work of breeding for virus resistance.

In the course of studying the artificial host–virus relations some theories have been formed and ideas thrown up that we should like to mention in the present publication along with proposals made for the first time on some of them. Some of the most important ones are: the proposal on a uniform system of symbols for viruses, virus strains and isolates, interpretation and introduction of a new terminology in virus separation, and the possibility of symbolic description of the most important features characterizing the host–virus relation in the so-called host–virus cryptogram.

Present paper is aimed at explaining the above mentioned ideas and giving proposals on them, as well as presenting the methods and giving general information about the experiments that will be described in successive publications.

Classification, denomination and symbols of viruses and virus strains (isolates) and major literary sources

Owing to various alternatives existing in the taxonomy of viruses more than one possibilities were offered in classifying and denominating the viruses, virus strains and isolates. The hierarchic system elaborated by HOLMES (1948) could not be followed because of the host-changing arboviruses. The neoclassic system, or neoclassic LHT-system (cf. LWOFF, HORNE and TOURNIER, 1962) – which with new criteria set up contains new taxonomic consequences – has also become out-of-date by now. The present requirements are no longer fulfilled by the system earlier suggested by us with minor alterations either (HORVÁTH, 1967c). As opposed to the hierarchic and neoclassic systems GIBBS, HARRISON, WATSON and WILDY (1966) elaborated a new, so-called antihierarchic system, called also GHWW- or VAC-system (verancular name and cryptograms). This system is based on a cryptogram completing the vulgar English name of the virus, which consists of writing down with symbols the four most important pairs of features characteristic of the virus. The symbol pairs are: type of nucleic acid/strandedness of nucleic acid, molecular weight of nucleic acid (in millions)/percentage of nucleic acid in infective particles, outline of particle/outline of "nucleocapsid" (the nucleic acid plus the protein most closely in contact with it), kinds of host infected/kinds of vector (reviewed by GIBBS, 1969; ANONYMOUS, 1970; HORVÁTH, 1972a, c, e). In our present series of publication we have followed the VAC-system. As regards the names and cryptograms of viruses and virus groups we adopted the principles

of the works of MARTYN (1968, 1971), GIBBS (1969), HARRISON *et al.* (1971), ANONYMOUS (1971), WILDY (1971), HORVÁTH (1972e), WOOD (1973), BELLETT *et al.* (1973), and of the publications "Descriptions of Plant Viruses" issued under the editorship of GIBBS, HARRISON and MURANT (1970–1975).

We have completed the virus groups suggested by HARRISON *et al.* (1971) (tobravirus, tobamovirus, potexvirus, carlavirus, potyvirus, cucumovirus, tymovirus, comovirus, nepovirus, bromovirus, tombusvirus, caulimovirus, monotypic groups) with a new group, the so-called brobwilvirus (broad bean wilt virus group). The new group (nomen provisoricum) shares many properties with the comovirus (or cowpea mosaic virus) group but differs from it in having aphid vectors, a wide rather than narrow host range and much more unstable particles. The name "brobwilvirus group" suggested by us to be provisionally accepted was given to the group because the broad bean wilt virus, earlier isolated and described in Hungary belongs to this group (HORVÁTH and SZIRMAI, 1975).

Classification, denomination, symbols of viruses, virus strains and isolates included in our series of publications as well as the relevant literature are shown in Table 1. The eleven virus groups, 22 viruses, 89 isolates listed in the Table as subjects of investigations in the past one and a half decade include those viruses whose recent, so far unknown natural host plants have been pointed out in Hungary, and whose occurrence in Hungary has been first established by us. Here we express our thanks to all who made their virus cultures available for our experiments (see Table 1) rendering thereby a comparison between Hungarian and foreign virus strains and isolates possible.

I should like to point out here that the symbolic designation of viruses, virus strains and isolates has been uniform neither in the international literature nor in our earlier works. We should like to make up for this deficiency – similarly to our last work (cf. HORVÁTH *et al.*, 1975b, c). Therefore we should make the suggestion of a three-parted symbolic designation of virus species, virus strain and virus isolate. The first symbol would be the abbreviation of the English name of the virus species as used in the international literature (e.g. turnip mosaic virus = TuMV). The second symbol – separated by a hyphen from the first one – would represent by the least possible letters the English name of the virus strain (e.g. ordinary strain = O). The third symbol – separated by a virgule from the second one – would indicate the isolate and consist of letters referring to the name of the plant (from which the virus was isolated), or to the country (where the isolation took place), or to other properties (e.g. Papaver = P, Hungary = H). For example, by the above method the ordinary strain of turnip mosaic virus isolated from Papaver somniferum would be written down by symbols in the following way: TuMV-O/P. In case the strain of the virus isolate cannot be established or is not yet known, then a virgule instead of a hyphen should be put between the virus species and virus isolate. For example, the symbol of the Tm isolate of broad bean wilt virus (BBWV) of unknown strain isolated from *Tropaeolum majus* is: BBWV/Tm (see Table 1).

Table 1

Classification, nomenclature and symbols of viruses, virus strains and isolates

Group[1]	Name	Cryptogram	Symbol	References
Tobravirus	Tobacco rattle virus	R/1 : 2.3 + 0.6 — 1.3/5 : E/E : S/Ne	TRV/H	Horváth (1967 *ined.*, 1972e, 1976)
			TRV/Lisse	Maat (1963)
			TRV-G/1	Waś (1974)
Tobamovirus	Tobacco mosaic virus	R/1 : 2/5 : E/E : S/*	TMV-U1	Siegel and Wildman (1954)
	Tomato mosaic virus	R/1 : 2/5 : E/E : S/*	ToMV-D/H	Horváth and Beczner (1973), Mamula *et al.* (1974)
Potexvirus	Potato aucuba mosaic virus	R/* : */5 : E/E : S/Ap	PAMV/MO937	Horváth (1964)
			PAMV/AO937	Horváth (1964)
			PAMV/SS	Horváth (1972d, g)
			PAMV—GW/104	Hollings (1959)
	Potato virus X	R/1 : 2.1/6 : E/E : S/(Fu)	PVX/G	Horváth and Beczner (1968)
Carlavirus	Potato virus M	*/* : */* : E/E : S/Ap	PVM/SS62	Horváth (1964)
			PVM/Spatz	Horváth and Hinfner (1964)
			PVM/Bie	Horváth (1971), Horváth and de Bokx (1972)
			PVM/UD	Horváth (1972f)
	Potato virus S	*/* : */* : E/E : S/Ap	PVS/KRO936	Horváth (1964)
			PVS/L	de Bokx (1970), Horváth (1972b)
			PVS/Yss	de Bokx (1970), Horváth (1972b)
			PVS/Fort	de Bokx (1970), Horváth (1972b)
			PVS/E	de Bokx (1970), Horváth (1972b)

Potyvirus	Bean common mosaic virus	*/* : */* : E/E : S/Ap	BCMV/F23	Horváth (1973b), Kovács and Horváth (1973)
	Bean yellow mosaic virus	*/* : */* : E/E : S/Ap	BYMV-S	Provvidenti and Granett (1974)
			BYMV/RM	Horváth (1974 *ined.*, 1976)
	Potato virus Y	*/* : */* : E/E : S/Ap	PVY-C/EP	Horváth (1966a)
			PVY-N/Adg43	Horváth (1966b)
			PVY-N/BdN	Horváth (1966b)
			PVY-N/Bie	Horváth (1966b)
			PVY-N/CSW	Horváth (1966b)
			PVY-N/Ine	Horváth (1966b)
			PVY-N/Lü72	Horváth (1966b)
			PVY-N/Lü86	Horváth (1966b)
			PVY-N/PK	Horváth (1966b)
			PVY-N/L	Horváth (1966b)
			PVY-N/LL	Horváth (1966b)
			PVY-N/N	Horváth (1966b)
			PVY-N/P	Horváth (1966b)
			PVY-N/R	Horváth (1966b)
			PVY-N/W	Horváth (1966b)
			PVY-N/UM	Horváth (1966b)
			PVY-N/Von	Horváth (1966b)
			PVY-R/Gie	Horváth (1967a)
			PVY-R/M3	Klinkowski and Schmelzer (1957), Horváth (1967a)
			PVY-An/Lü85	Horváth (1967b)
			PVY-An/Epe	Horváth (1967b)
			PVY-An/Rs188	Horváth (1967b)
	Watermelon mosaic virus	*/* : */* : E/E : S/Ap	WMV-G/PW	Horváth *et al.* (1974a, 1975b)
	Turnip mosaic virus	*/* : */* : E/E : S/Ap	TuMV-O/A11	Horváth *et al.* (1975d)
			TuMV-O/P	Horváth and Besada (1975)

Table 1 (continued)

Group[1]	Name	Cryptogram	Symbol	References
			TuMV-O/HS	JURETIĆ *et al.* (1976)
			TuMV-C/JN	JURETIĆ *et al.* (1976)
			TuMV-C/K30	JURETIĆ *et al.* (1976)
	Celery mosaic virus	*/* : */* : E/E : S/Ap	CeMV/P	HORVÁTH *et al.* (1976a)
			CeMV/Ag	HORVÁTH *et al.* (1976a)
			CeMV/Dc	HORVÁTH *et al.* (1976a)
			CeMV/Pc	HORVÁTH *et al.* (1976a)
Cucomovirus	Cucumber mosaic virus	R/1 : 1/18 : S/S : S/Ap	CMV-G/R	HORVÁTH (1969), HORVÁTH and HINFNER (1969a, b)
			CMV-W	SKIEBE and SCHMELZER (1967)
			CMV-G/P	HORVÁTH (1973a)
			CMV-G/E	HORVÁTH and SZIRMAI (1973)
			CMV-G/EN	HORVÁTH and SZIRMAI (1973)
			CMV-G/Ac	HORVÁTH *et al.* (1975a)
			CMV-G/Ad	HORVÁTH *et al.* (1975a)
			CMV-G/Ae	HORVÁTH *et al.* (1975a)
			CMV-G/Am	HORVÁTH *et al.* (1975a)
			CMV-G/Aq	HORVÁTH *et al.* (1975a)
			CMV-G/Sc	HORVÁTH *et al.* (1975a)
			CMV-G/PC	HORVÁTH *et al.* (1975c)
			CMV-G/Cir	HORVÁTH *et al.* (1967b)
Tymovirus	Turnip yellow mosaic virus	R/1 : 1.9/34 : S/S : S/Cl	TYMV/HB	HORVÁTH *et al.* (1973)
			TYMV/HS	JURETIĆ *et al.* (1973)
			TYMV/Y65	MAMULA (1968)
	Belladonna mottle virus	R/1 : 2.0/37 : S/S : S/Cl	BMV/H	HORVÁTH *et al.* (1976c)

Comovirus	Radish mosaic virus	R/1 : 1.3/26 + 2.2/34 : S/S : S/Cl	RMV/M	Mamula *et al.* (1972)
			RMV/HS7	Horváth *et al.* (1973)
			RMV/HB9	Juretić *et al.* (1973)
			RMV/HZ	Štefanac and Mamula (1972)
			RMV/B	Mamula *et al.* (1972)
			RMV/G	Mamula *et al.* (1972)
			RMV/N	Mamula *et al.* (1972)
			RMV/Sa	Mamula *et al.* (1972)
Nepovirus	Cherry leaf roll virus	R/* : */* : S/S : S/Ne	CLRV-Sr/YN	Horváth *et al.* (1974b)
			CLRV-Sr/CR	Horváth *et al.* (1974b)
	Tobacco ring spot virus	R/1 : 2.2/40 : S/S : S/Ne	TRSV/Y-49	Demski and Jellum (1965), Demski *et al.* (1971), Demski and Harris (1974)
Brobwilvirus[2]	Broad bean wilt virus	R/1 : */33 : S/S : S/Ap	BBWV/Tm syn.: NRSV/Tm	Horváth and Szirmai (1975)
			BBWV/HZ syn.: NRSV/HZ	Horváth (1976)
Monotypic	Tobacco necrosis virus	R/1 : 1.5/19 : S/S : S/Fu	TNV-f	Szirmai (1964)
	Alfalfa mosaic virus	R/1 : 1.3 + 1.1 + 0.9/18 : U/U : S/Ap	AMV-CaL/SK	Horváth (1963)
			AMV–L/K2	Beczner (1972)
			AMV-S/N1	Beczner (1972)
			AMV-CaL/KE	Horváth (1976)

[1] Potato aucuba mosaic virus (potexvirus group), turnip mosaic virus, celery mosaic virus (potyvirus group) and cherry leaf roll virus (nepovirus group) are possible members of the virus groups written between brackets (cf. Harrison *et al.* 1971).

[2] Nomen provisoricum *(non al.)*

Experimental plants

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In the course of studying the artificial host–virus relations we have examined 464 plant species, varieties, forms, convarieties, cultivars and hybrids (of which 227 are new in the literature of plant virology) belonging to 22 plant families for virus relations not studied by other so far. In the present paper we are listing the names synonyms and families or all plants included in our successive publi-

cations. The synonyms stand after the names of plants e.g.: *Alliaria petiolata* (M. B.) Cavara et Grande; syn.: *A. officinalis* Andrz. In some exceptional cases the names are found in reverse order. The family names – as it is known – end in *-aceae* put after the name of one of the family's genera. In exceptional cases when both the old and the new, standard name can be used both family names are indicated with a sign of equality between them (e.g. *Cruciferae = Brassicaceae)*. The family names in an abbreviated form shown below are written after the names and synonyms, respectively, and authors of plants – with a colon to separate them: Aiz (*Aizoaceae*), Ama (*Amaranthaceae*), Basel (*Basellaceae*), Cary (*Caryophyllaceae*), Chen (*Chenopodiaceae*), Cist (*Cistaceae*), Com (*Commelinaceae*), Comp=Aster (*Compositae*, *Asteraceae*), Cru=Bras (*Cruciferae*, *Brassicaceae*), Cuc (*Cucurbitaceae*), Eri (*Ericaceae*), Fab=Legu=Pap (*Fabaceae*, *Leguminosae*, *Papilionaceae*), Ger (*Geraniaceae*), Lab=Lami (*Labiatae*, *Lamiaceae*), Mal (*Malvaceae*), Pap (*Papaveraceae*), Scrop (*Scrophulariaceae*), Sol (*Solanaceae*), Trop (*Tropaeolaceae*), Umb=Api (*Umbelliferae*, *Apiceae*), Urt (*Urticaceae*), Vita (*Vitaceae*).

Of plants examined for artificial host-virus relations and listed below those with "°" indices (e.g. *Amaranthus angustifolius* Lam., syn.: *A. graecizans* L.°) are first dealt with in the literature of plant virology, and in this sense can be regarded as plants new to science.

Amaranthus angustifolius Lam.; syn.: *A. graecizans* L.°: Ama
- *ascendens* Lois.; syn.: *A. lividus* L. var. *ascendens*°: Ama
- *atropurpureus* Roxb.; syn.: *A. hybridus* L.°: Ama
- *aureus* F. G. Dietr.; syn.: *A. paniculatus* L. var. *flavus*: Ama
- *bouchoni* Thell.°: Ama
- *caracu* Zucc. et Steud.; syn.: *A. hypochondriacus* L.: Ama
- *caudatus* L.: Ama
- – L. cv. *Atripurpureus;* syn.: *A. caudatus* L. var. *sanguineus*°: Ama
- *chlorostachys* Willd.; syn.: *A. hybridus* L.°: Ama
- – Willd. f. *strictus* (Willd.) Thell; syn.: *A. chlorostachys* Willd. f. *leucocarpus* (S. Wats.) A. T. Hunziker°: Ama
- – Willd. var. *powelli* (S. Wats.) Priszter°: Ama
- *cruentus* L.; syn.: *A. paniculatus* L.: Ama
- *deflexus* L.: Ama
- – L. var. *rufescens* (Godr.) Thell°: Ama
- *dubius* Mart.: Ama
- *emarginatus* Salzm. ex Moq.; syn.: *A. lividus* L. var. *ascendens*°: Ama
- *gangeticus* L. var. *multicolor* hort.; syn.: *A. tricolor* L.°: Ama
- *graecizans* L.; syn.: *A. graecizans* L. var. *sylvestris* (Vill.) Aschers., *A. sylvestris* Desf.: Ama
- *hypochondriacus* L.°: Ama
- – L. cv. *Monstrosus*°: Ama

Amaranthus leucocarpus S. Wats.; syn.: *A. chlorostachys* Willd. var. *leucocarpus* (S. Wats.) A. T. Hunziker°: Ama
— *lividus* L.; syn.: *A. lividus* L. var. *ascendens* (Lois.): Ama
— *mantegazzianus* Passerini°: Ama
— *oleraceus* L.; syn.: *A. lividus* L. var. *oleraceus* (L.) Thell.°: Ama
— *paniculatus* L.; syn.: *A. cruentus* L.: Ama
— — L. cv. *Roter Dom*°: Ama
— — L. cv. *Roter Paris*°: Ama
— — L. cv. *Sanguineus nanus*°: Ama
— — L. var. *flavus* (L.) Thell.; syn.: *A. aureus* Regel°: Ama
— *patulus* Bert.; syn.: *A. hybridus* ssp. *cruentus* var. *patulus* Thell.: Ama
— *retroflexus* L.: Ama
— *speciosus* Sims.; syn.: *A. paniculatus* L. f. *speciosus* (Sims.) Voss°: Ama
— *spinosus* L.: Ama
— *sylvestris* Desf.; syn.: *A. graecizans* L. var. *sylvestris* (Vill.) Aschers.°: Ama
— *tricolor* L.: Ama
— — L. cv. *Malten Fire*°: Ama
— *viridis* L.; syn.: *A. lividus* L. var. *ascendens* (Loiss.) Thell.°: Ama
Ammi majus L.: Umb=Api
— *visnaga* (L.) Lam.°: Umb=Api
Amorpha fruticosa L.: Fab=Legu=Pap
Anoda dilleniana Cav.°: Mal
Aptenia cordifolia (L.) Schwantes: Aiz
Atropa bella-donna L.: Sol
Ballota foetida Lamk.: Lab=Lami
Basella rubra L.°: Basel
Beta lomatogona Fisch. et Ma.: Chen
— *macrocarpa* Guss.; syn.: *B. vulgaris* L. ssp. *macrocarpa* Guss.: Chen
— *trigyna* W. et K.: Chen
Brassica adpressa Boiss.: Cru=Bras
— *campestris* L. em. Hartm.; syn.: *Br. rapa* L. em. Metzger ssp. *campestris* (L.) Clapham: Cru=Bras
— *carinata* A. Braun: Cru=Bras
— *chinensis* L.: Cru=Bras
— *nigra* (L.) Koch; syn.: *Br. sinapoides* Roth, *Sinapis nigra* L.: Cru=Bras
Browallia americana L.: Sol
— *cordata* G. Don°: Sol
— *demissa* L.; syn.: *Br. americana* L.: Sol
— *graminifolia* Grah.°: Sol
— *grandiflora* Grah.: Sol
— *roezli* Nichols.°: Sol
— *viscosa* H. B. et K.: Sol
Bryonia alba L.: Cuc
— *dioica* Jacq.; syn.: *Br. cretica* L. ssp. *dioica* (Jacq.) Tutin: Cuc

Bunias orientalis L.: Cru = Bras
Capsella grandiflora Boiss.°: Cru = Bras
Capsicum annuum L.: Sol
– – L. cv. *Bogyiszlói vastaghúsú*°: Sol
– – L. cv. *Cecei édes*°: Sol
– – L. var. *cerasiforme*°: Sol
– – L. cv. *Csokros csüngő*°: Sol
– – L. cv. *Csokros felálló I.*°: Sol
– – L. cv. *Csokros felálló II.*°: Sol
– – L. cv. *Dokomlási 2710*°: Sol
– – L. cv. *Hatvani hajtatási*°: Sol
– – L. cv. *Kalocsai E – 15*°: Sol
– – L. cv. *Kalocsai felálló*°: Sol
– – L. cv. *Korai halványzöld*°: Sol
– – L. cv. *Kovácsházi hajtatási*°: Sol
– – L. cv. *Magyar kincs*°: Sol
– – L. cv. *Maritza*°: Sol
– – L. cv. *Markgärtner*°: Sol
– – L. cv. *Sonnenpreis*°: Sol
– – L. cv. *Tétényi hajtatási zöld*°: Sol
Cassia tora L.: Fab = Legu = Pap
Centaurea phrygia L.°: Comp = Aster
Cestrum parqui L'Hérit.°: Sol
Cheiranthus cheiri L.: Cru = Bras
Chenopodium amaranticolor Coste et Reyn.; syn.: *Ch. giganteum* D. Don: Chen
– *ambrosioides* L. var. *ambrosioides*°: Chen
– – L. var. *anthelminticum* (L.) A. Gray: Chen
– *anthelminticum* L. syn.: *Ch. ambrosioides* L.: Chen
– *aristatum* L.; syn.: *Teloxys aristata* (L.) Moq.°: Chen
– *bonus-henricus* L.: Chen
– *botrys* L.: Chen
– *capitatum* (L.) Aschers.: Chen
– *ficifolium* Sm.; syn.: *Ch. album* L. var. *ficifolium* (L.) G. F. W. Mey.: Chen
– *foetidum* Schrad.; syn.: *Ch. schraderianum* Roem. et Schult.: Chen
– *foliosum* (Moench) Aschers.; syn.: *Blitum virgatum* L.: Chen
– *giganteum* D. Don; syn.: *Ch. amaranticolor* Coste et Reyn.: Chen
– *glaucum* L.: Chen
– *hybridum* L.: Chen
– *murale* L.: Chen
– *opulifolium* Schrad.: Chen
– *polyspermum* L.: Chen
– *quinoa* Willd.: Chen
– – Willd. f. *rubescens* (Moq.) Hunziker: Chen
– – Willd. f. *viridescens* (Moq.) Hunziker°: Chen

Chenopodium rubrum L.; syn.: *Blitum rubrum* (L.) C. A. Mey.: Chen
— *vulvaria* L. Chen
Cicuta virosa L.°: Umb=Api
Citrullus lanatus (Thunb.) Mansfeld; syn.: *C. vulgaris* Schrad., *Colocynthis citrullus* (L.) Kuntze: Cuc
Colocynthis vulgaris Schrad.: Cuc
Commelina clandestina Mart.°: Com
— *coelestis* Willd.; syn.: *C. communis* L.: Com
— *communis* L.; syn.: *C. coelestis* Willd.: Com
— *graminifolia* H. B. et K.°: Com
— *tuberosa* L.°: Com
Crambe abyssinica Hochst. ex R. E. Frees: Cru=Bras
— *armena* N. Busch.°: Cru=Bras
— *cordifolia* Stev.°: Cru=Bras
— *hispanica* L.°: Cru=Bras
— *maritima* L.: Cru=Bras
— *orientalis* L.°: Cru=Bras
— *tataria* Sebeók°: Cru=Bras
Cucubalus baccifer L.°: Cary
Cucumis myriocarpus Naud.: Cuc
Cucurbita andreana Naud.: Cuc
— *pepo* L.: Cuc
— — L. var. *aurantiformis*°: Cuc
— — L. convar. *clypeata* Alef.°: Cuc
— — L. convar. *oblonga* Alef.°: Cuc
— — L. convar. *patissonina* Greb. f. *radiata* Nois.°: Cuc
— — L. convar *piriformis* Alef.°: Cuc
— — L. convar. *pomiformis* Alef.°: Cuc
— — L. var. *subrotunda*°: Cuc
— — L. var. *verrucosa* L.°: Cuc
Cyamopsis tetragonoloba (L.) Taub.: Fab=Legu=Pap
Cyclanthera explodens Naud.: Cuc
— *pedata* Schrad.: Cuc
Datura aegyptiaca Vesl.; syn.: *D. fastuosa* L.: Sol
— *arborea* L.: Sol
— *carolinianum* L.°: Sol
— *ceratocaula* Ort.°: Sol
— *chlorantha* Hook.; syn.: *D. humilis* Desf.: Sol
— *fastuosa* L. cv. *Alba*°: Sol
— *gigantea* hort.; syn.: *D. tatula* L.: Sol
— *godronii* Danert cv. *Minka*°: Sol
— *inermis* Jacq.; syn.: *D. stramonium* L. f. *inermis*: Sol
— *innoxia* Mill.: Sol
— *leichardtii* F. Muell.: Sol

Datura metel L.: Sol
– – L. var. *inermis*°: Sol
– – L. var. *muricata*°: Sol
– *meteloides* DC.: Sol
– *quercifolia* H. B. et K.: Sol
– *rosei* Safford°: Sol
– *stramonium* L.: Sol
– – L. var. *chalybea* Koch: Sol
– – L. f. *inermis* (Juss.) Koch°: Sol
– *tatula* L.; syn.: *D. stramonium* L. var. *chalybea* Koch°: Sol
Diplotaxis erucoides DC.: Cru=Bras
– *tenuifolia* (Jusl.) DC.: Cru=Bras
– *viminea* (L.) DC.°: Cru=Bras
Erodium ciconium (L.) L'Hérit. ex Ait.°: Ger
– *cicutarium* (L.) L'Hérit. ex Ait.: Ger
– *gruinum* (L.) L'Hérit. ex Ait.: Ger
– *malacoides* Willd.°: Ger
– *manescavi* Coss.°: Ger
– *moschatum* (L.) L'Hérit. ex Ait.: Ger
Galega bicolor Hausskn.°: Fab=Legu=Pap
– *hartlandii* Clarke°: Fab=Legu=Pap
– *officinalis* L.: Fab=Legu=Pap
Geranium cristatum Stev.°: Ger
– *columbinum* L.°: Ger
– *dissectum* Jusl.: Ger
– *lucidum* L.°: Ger
– *molle* L.: Ger
– *pratense* L.: Ger
– *pusillum* Burm.°: Ger
– *pyrenaicum* Burm.°: Ger
– *robertianum* L.: Ger
– *rotundifolium* L.: Ger
– *sibiricum* L.°: Ger
Gomphrena decumbens Jacq.°: Ama
Helianthemum nummularium (L.) Dun.: Cist
Helianthus annuus L. cv. *Csakinszkij*°: Com=Aster
– – L. cv. *Iregi csikos*°: Comp=Aster
– *atrorubens* L.°: Comp=Aster
– *californicus* DC.°: Comp=Aster
– *cernuus* Benth. et Hook.°: Comp=Aster
– *decapetalus* L.°: Comp=Aster
– *doronicoides* Lam.°: Comp=Aster
– *giganteus* L.°: Comp=Aster
– *grosse-serratus* Mart.°: Comp=Aster

Helianthus maximiliani Schrad.°: Comp=Aster
— *mollis* Lam.°: Comp=Aster
— *organophyllus* Torr. et Gray°: Comp=Aster
— *salicifolius* A. Dietr.°: Comp=Aster
— *tomentosus* Mchx.°: Comp=Aster
— *trachelifolius* Mill.°: Comp=Aster
Hibiscus manihot L.: Mal
Lagenaria leucantha Rusby: Cuc
— *siceraria* (Mol.) Standl. var. *cugurda*°: Cuc
Lavatera arborea L.: Mal
Leiophyllum buxifolium (Berg.) Ell.°: Eri
Luffa cylindrica (L.) Roem.: Cuc
Lycium australe F. Muell.°: Sol
— *barbarum* L.; syn.: *L. halimifolium* Mill.: Sol
— *carolinianum* Walt.°: Sol
— *chinense* Mill.: Sol
— *europaeum* L.: Sol
— *flexicaule* Pojark.°: Sol
— *halimifolium* Mill.; syn.: *L. barbarum* L.: Sol
— *horridum* Thunbg.°: Sol
— *ruthenicum* Murr.: Sol
— *turcomanicum* Turcz.°: Sol
Lycopersicon esculentum Mill. cv. *Kecskeméti 363*°: Sol
— — Mill. cv. *Kecskeméti konzerv*°: Sol
— — Mill. cv. *Pécs gyöngye*: Sol
— — Mill. cv. *Red Cherry*: Sol
— *glandulosum* C. H. Mull.: Sol
— *hirsutum* H. B. et K.: Sol
— *humboldtii* Dun.: Sol
— *peruvianum* (L.) Mill.: Sol
— *pimpinellifolium* Jusl.: Sol
— *pyriforme* Dun.: Sol
— *racemiflorum* Dun.°: Sol
— *racemigerum* Lange: Sol
Malva alcea L.: Mal
— *borealis* Wallm.: Mal
— *crispa* L.; syn.: *M. verticillata* L. var. *crispa* L.: Mal
— *moschata* L.: Mal
— *neglecta* Wallr.: Mal
— *pusilla* Sm.: Mal
— *silvestris* L.: Mal
— *verticillata* L.: Mal
Melandrium album (Mill.) Garcke: Cary
— *rubrum* (Weig.) Garcke; syn.: *M. silvestre* (Schkuhr) Röehl.°: Cary

Melandrium silvestre (Schkuhr) Röehl.; syn.: *M. rubrum* (Weig.) Gracke°: Cary
Nicandra physaloides (L.) Gaertn.: Sol
Nicotiana acuminata Hook.: Sol
- *alata* Link. et Otto: Sol
- *attenuata* Torr. ex S. Wats.: Sol
- *auriculata* Bert.°: Sol
- *chinensis* Fisch.: Sol
- *debneyi* Domin: Sol
- *exigua* Wheeler: Sol
- *fragrans* Hook.: Sol
- *glutinosa* L.: Sol
- *goodspeedii* Wheeler: Sol
- *knightiana* Goodspeed.: Sol
- *langsdorffii* Weinm.: Sol
- *longiflora* Cav.: Sol
- *occidentalis* Wheeler: Sol
- *paniculata* L.: Sol
- *plumbaginifolia* Viv.: Sol
- *quadrivalvis* Pursh.: Sol
- *repanda* Willd.: Sol
- *rustica* L.: Sol
- *sanderae* W. Wats.; syn.: *N. alata* Link. et Otto × *N. forgetiana* Sander: Sol
- *solanifolia* Walp.: Sol
- *sylvestris* Speg. et Comes: Sol
- *tabacum* L. cv. *Ambalema*: Sol
- – L. cv. *Bel 61–10*: Sol
- – L. cv. *Debreceni*°: Sol
- – L. cv. *Érdi*°: Sol
- – L. cv. *Hevesi*°: Sol
- – L. cv. *Hicks-Fixed A2–426*°: Sol
- – *tabacum* L. cv. *I. 787*°: Sol
- – L. cv. *Kerti*°: Sol
- – L. × *Nicotiana glutinosa* L.°: Sol
- – L. cv. *sanderae*°: Sol
- – L. cv. *Szabolcsi*°: Sol
- – L. cv. *Szuloki*°: Sol
- – L. cv. *Xanthi-nc*: Sol
- *texana* L.: Sol

Obione sibirica (L.) Fisch.; syn.: *Atriplex sibirica* L.°: Chen
Ocimum basilicum L.: Lab=Lami
- *canum* Sims.: Lab=Lami
- *sanctum* L.°: Lab=Lami

Papaver orientale L.: Pap
Paulownia fargesii Franch.°: Scrop

Paulownia tomentosa (Thunb.) Steud.; syn.: *P. imperialis* S. et Z.: Scrop
Pentstemon alpinus Torr.°: Scrop
— *attenuatus* Dougl.°: Scrop
— *calycosus* Small.°: Scrop
— *cardinalis* Woot. et Standl.°: Scrop
— *digitalis* Nutt.: Scrop
— *glaucus* Grah.°: Scrop
— *gracilis* Nutt.°: Scrop
— *grandiflorus* Nutt.: Scrop
— *hirsutus* (L.) Willd.°: Scrop
— *humilis* Nutt.°: Scrop
— *laevigatus* Ait.°: Scrop
— *murrayanus* Hook.°: Scrop
— *ovatus* Dougl.°: Scrop
— *pubescens* Soland.°: Scrop
— *stenopetalus* Howell°: Scrop
— *tubiflorus* Nutt.°: Scrop
— *utahensis* A. Nels°: Scrop
— *venustus* Dougl.°: Scrop
— *wippleanus* A. Gray.°: Scrop
Petunia atkinsiana Don.°: Sol
— *axillaris* (Lam.) BSP.: Sol
— *hybrida* hort.: Sol
— — — cv. *Rose de Haven amélioré*°: Sol
— *parviflora* Juss.°: Sol
— *violacea* Lindl.: Sol
Phaseolus aureus Roxb.; syn.: *Ph. mungo* L.: Fab=Legu=Pap
— *caffer* Haberle; syn.: *Vigna capensis* Walp.°: Fab=Legu=Pap
— *lunatus* L.: Fab=Legu=Pap
— *ricciardianus* Ten.°: Fab=Legu=Pap
— *vulgaris* L. cv. *Aladin*: Fab=Legu=Pap
— — L. cv. *Andrásbab*°: Fab=Legu=Pap
— — L. cv. *Annelise*: Fab=Legu=Pap
— — L. cv. *Barnabab*°: Fab=Legu=Pap
— — L. cv. *Cardinal*: Fab=Legu=Pap
— — L. cv. *Caroline*: Fab=Legu=Pap
— — L. cv. *Cordon*: Fab=Legu=Pap
— — L. cv. *Cukorbab*°: Fab=Legu=Pap
— — L. cv. *Falomiteana:* Fab-Legu-Pap
— — L. cv. *Fehér gyöngy*°: Fab-Legu-Pap
— — L. cv. *Fehér középbab*°: Fab=Legu=Pap
— — L. cv. *Fertődi 5.*°: Fab=Legu=Pap
— — L. cv. *Fertődi 23.*°: Fab=Legu=Pap
— — L. cv. *Fullcrop*: Fab=Legu=Pap

Phaseolus vulgaris L. cv. *GN 59.*: Fab=Legu=Pap
— — L. cv. *GN 123.*: Fab=Legu=Pap
— — L. cv. *Harkovszkaja*: Fab=Legu=Pap
— — L. cv. *Icar Fundulea 51.*: Fab=Legu=Pap
— — L. cv. *Icar Fundulea 416.*: Fab=Legu=Pap
— — L. cv. *Japán gyöngybab*°: Fab=Legu=Pap
— — L. cv. *Kanizsai csíkosbab*°: Fab=Legu=Pap
— — L. cv. *Kentucky Wonder*: Fab=Legu=Pap
— — L. cv. *Kereskedelmi hosszú fürjbab*°: Fab=Legu=Pap
— — L. cv. *Kinghorn Wax.*: Fab=Legu=Pap
— — L. cv. *Kompolti gyöngybab*°: Fab=Legu=Pap
— — L. cv. *Korai fürj*°: Fab=Legu=Pap
— — L. cv. *Kőbab*°: Fab=Legu=Pap
— — L. cv. *Középbab*°: Fab=Legu=Pap
— — L. cv. *Májbab*°: Fab=Legu=Pap
— — L. cv. *Michelite*: Fab=Legu=Pap
— — L. cv. *Michigan*: Fab=Legu=Pap
— — L. cv. *Moldvoszkaja*: Fab=Legu=Pap
— — L. *No. 2316*°: Fab=Legu=Pap
— — L. cv. *Olomucka Zelenoluska*: Fab=Legu=Pap
— — L. cv. *Őrségi cseresznyebab*°: Fab=Legu=Pap
— — L. cv. *Perlicska*: Fab=Legu=Pap
— — L. cv. *Pinto*: Fab=Legu=Pap
— — L. cv. *Prinzess*: Fab=Legu=Pap
— — L. cv. *Processor*: Fab=Legu=Pap
— — L. cv. *Red Kidney:* Fab-Legu-Pap
— — L. cv. *Refugee*: Fab=Legu=Pap
— — L. cv. *Robust*: Fab=Legu=Pap
— — L. cv. *Saxa*: Fab=Legu=Pap
— — L. cv. *Soproni lapos*°: Fab=Legu=Pap
— — L. cv. *Szegedi fehér*°: Fab=Legu=Pap
— — L. cv. *Sztepnaja*; Fab=Legu=Pap
— — L. cv. *Tápiószelei barna*°: Fab=Legu=Pap
— — L. cv. *Tápiószelei fürj*°: Fab=Legu=Pap
— — L. cv. *Tápláni fekete 'cirádás' fürjbab*°: Fab=Legu=Pap
— — L. cv. *Tápláni nagyszemű cseresznyebab*°: Fab=Legu=Pap
— — L. cv. *Tendergreen*: Fab=Legu=Pap
— — L. cv. *Tétényi cukorbab*°: Fab=Legu=Pap
— — L. cv. *Tétényi fehér középbab*°: Fab=Legu=Pap
— — L. cv. *Tétényi gyöngybab*°: Fab=Legu=Pap
— — L. cv. *Tétényi középbab*°: Fab=Legu=Pap
— — L. cv. *Tétényi nagyszemű fehér*°: Fab=Legu=Pap
— — L. cv. *Wade*: Fab=Legu=Pap
Physalis aequata Jacq.: Sol

Physalis alkekengi L.; syn.: *Ph. franchetti* Mast.: Sol
— *angulata* L.: Sol
— *floridana* Rydb.: Sol
— *ixocarpa* Brot.: Sol
— *peruviana* L.: Sol
— *peruviana* L. var. *macrocarpa*°: Sol
— *philadelphica* Lam.: Sol
— *pruinosa* L.: Sol
— *pubescens* L.: Sol
— *viscosa* L.: Sol
Rhoeo discolor Hance: Com
Rorippa islandica (Oeder) Borb.: Cru=Bras
Saponaria cerastioides Fisch.°: Cary
— *officinalis* L.: Cary
Scopolia lurida Dun.°: Sol
Silene armeria L.: Cary
— *conica* L.°: Cary
— *dichotoma* Ehrh.: Cary
— *gallica* L.°: Cary
— *pendula* L.: Cary
— *tatarica* (L.) Pers.°: Cary
Solanum acaule Bitt.: Sol
— *acroscopium* Ochoa: Sol
— *ajanhuiri* Juz. et Buk.°: Sol
— *berthaultii* Hawkes: Sol
— *boliviense* Dun.°: Sol
— *brachycarpum* Corr.°: Sol
— *brevicaule* Bitt°: Sol
— *brevidens* Phil.°: Sol
— *canasense* Hawkes°: Sol
— *capsicastrum* Link: Sol
— *cardiophyllum* Lindl.: Sol
— *chacoense* Bitt°: Sol
— *demissum* Lindl. *A6-hybrid*: Sol
— — Lindl. *Redd. 530-hybrid*°: Sol
— — Lindl. *Stamm S-hybrid*°: Sol
— *ehrenbergii* (Bitt.) Rydb.°: Sol
— *etuberosum* Lindl.°: Sol
— *famatinae* Bitt. et Wittm.: Sol
— *gourlayi* Hawkes: Sol
— *guerreroense* Corr.: Sol
— *hjertingii* Hawkes°: Sol
— *hougasii* Corr.°: Sol
— *infundibuliforme* Phil.°: Sol

Solanum jamesii Torr.: Sol
- *kurtzianum* Bitt. et Wittm.: Sol
- *leptophyes* Bitt.°: Sol
- *medians* Bitt.°: Sol
- *megistacrolobum* Bitt.: Sol
- *ochroleucum* Bast.: Sol
- *pseudocapsicum* L.: Sol
- *rostratum* Dun.: Sol
- *sanctae-rosae* Hawkes: Sol
- *simplicifolium* Bitt.: Sol
- *stoloniferum* Schlechtend: Sol
- *sucrense* Hawkes: Sol
- *tarijense* Hawkes: Sol
- *tuberosum* L. cv. *59/558*°: Sol
- – L. cv. *Aranyalma*°: Sol
- – L. cv. *Auriga*: Sol
- – L. cv. *Axilia*: Sol
- – L. cv. *Edgecote Purple*°: Sol
- – L. cv. *Früka*: Sol
- – L. cv. *Gülbaba*°: Sol
- – L. cv. *Jowisz*: Sol
- – L. cv. *Kisvárdai rózsa*°: Sol
- – L. cv. *Korai rózsa*°: Sol
- – L. cv. *Opal*: Sol
- – L. cv. *Osa*: Sol
- – L. cv. *Somogyi kifli*°: Sol
- – L. cv. *Somogyi korai*°: Sol
- – L. cv. *Somogyi sárga*°: Sol
- *vernei* Bitt. et Wittm.: Sol
- *verrucosum* Schlechtend.: Sol

Tetragonia crystallina L'Hérit.°: Aiz
- *echinata* Ait.: Aiz
- *tetragonoides* (Pall.) O. Ktze; syn.: *T. expansa* Murr.: Aiz

Tinantia erecta (Jacq.) Schlechtend.; syn.: *T. fugax* Scheidw.°: Com

Trechonaetes sativa Miers°: Sol

Tropaeolum majus L.: Trop
- *minus* L.: Trop
- – L. cv. *Cherry rose*°: Trop
- *peltophorum* Benth.°: Trop
- *peregrinum* L.: Trop

Urtica urens L.: Urt

Vaccaria segetalis (L.) Scop.°: Cary

Vicia faba L. cv. *Dornburger*°: Fab=Legu=Pap
- – L. cv. *Dreifachweise*°: Fab=Legu=Pap

Vicia faba L. cv. *Erfurter Gewöhnliche*°: Fab=Legu=Pap
— — L. cv. *Hangdown*°: Fab=Legu=Pap
Vigna catjang Walp.; syn.: *V. cylindrica* Skeels°: Fab=Legu=Pap
— *sinensis* (L.) Savi: Fab=Legu=Pap
— — L. var. *sesquipedalis* (L.) Fruwirth°: Fab=Legu=Pap
Viscaria vulgaris Bernh.: Cary
Vitis vinifera L.: Vita

Donor plants as virus sources

In the course of artificial inoculation experiments inocula (tissue saps) containing viruses were obtained from previously virus inoculated plants known to be virus propagating hosts of the respective viruses. The different virus inocula were obtained from the following virus sources or propagating hosts: alfalfa mosaic virus (*Nicotiana tabacum*), bean common mosaic virus (*Phaseolus vulgaris* cv. *Red Kidney*), bean yellow mosaic virus (*Phaseolus vulgaris* cv. *Red Kidney*), belladonna mottle virus (*Nicotiana glutinosa*, *Nicotiana tabacum*), broad bean wilt virus (*Gomphrena globosa*, *Nicotiana tabacum*, *Tropaeolum majus*), celery mosaic virus (*Ammi majus*, *Ammi visnaga*), cherry leaf roll virus (*Nicotiana tabacum*), cucumber mosaic virus (*Cucumis sativus*, *Nicotiana tabacum*), potato aucuba mosaic virus (*Nicotiana glutinosa*), potato virus M (*Lycopersicon esculentum*, *Solanum tuberosum*), potato virus S (*Solanum tuberosum*), potato virus X (*Nicotiana tabacum*), potato virus Y (*Nicotiana tabacum*), radish mosaic virus (*Brassica rapa* var. *rapa*), tobacco mosaic virus (*Nicotiana tabacum* cv. *Samsun*), tobacco necrosis virus (*Phaseolus vulgaris* cv. *Red Kidney*), tobacco rattle virus (*Nicotiana tabacum*), tobacco ring spot virus (*Nicotiana tabacum* cv. *Xanthi-nc*), tomato mosaic virus (*Nicotiana tabacum* cv. *Samsun*), turnip mosaic virus (*Brassica rapa* var. *rapa*), turnip yellow mosaic virus (*Brassica rapa* var. *rapa*), watermelon mosaic virus (*Cucurbita pepo* convar. *patissonina* f. *radiata*).

Artifical inoculation

Leaves of previously virus inoculated virus sources and production hosts, respectively, were smashed in a porcelain mortar generally with the same amount of tap or distilled water added, and the tissue sap thus obtained – and in certain cases filtered through a sterile nylon screening cloth – was rubbed onto the leaves of young experimental or test plants with abrasive-spatula technique. As abrasive 500–600 mesh carborundum was used which was applied to the leaves of the test plants by means of a special blower. After the inoculation the plants were sprayed with tap water.

In the vector transmission experiments wingless *Myzus persicae* aphids were used. The aphids were bred on virus-free plants (*Brassica rapa* var. *rapa*). In the case of stylet-borne viruses the aphids were starved for 3–4 hours before the virus transmission experiments; after the starvation period they were placed

for 8 – 10 minutes onto the virus source. After the virus-uptake feeding the aphids were transferred for a few minutes to the young test plants to be examined. After the infection feeding they were killed with the insecticide Phosdrin.

Plant—virus relationships

The alternative relationship between test plants and viruses is expressed in the compatibility and incompatibility. In determining compatibility and incompatibility the interpretation generally used in plant virology was taken as basis.

In a compatible test plant – virus relation the experimental or test plant is host to the virus. In the case of a compatible test (host) plant – virus connection the inoculated plant responds in most cases with visible, manifest symptoms to the inoculation. The reactions (symptoms) of the inoculated plants are either local or systemic according to whether they appear on inoculated leaves of the plant or on non-inoculated young leaves usually formed after the inoculation. In some host–virus combinations local and systemic symptoms may jointly occur.

In some cases the compatible test (host) plant – virus connection is not expressed by visible, manifest symptoms, but remains latent (symptomless). The latent susceptibility may be either local or systemic, or both local and systemic.

In a special case of the host–virus relation the virus introduced into the host plant multiplies, the host plant becomes diseased through normergic (plasmatic) or hyperergic (amputative or necrogenic hypersensitivity) defence reactions tries to get rid of the virus. In our papers – in contrast with some authors – this host–virus relation is also considered to be compatible for the very reason that the virus multiplies in the plant whereby the plant is an absolute host of the virus.

After the appearance of a certain compatible host–virus relation recovery, a so far unexplained phenomenon, followed in some cases by recurrence.

In the case of an incompatible test plant – virus "relation" connection cannot, for different reasons, be established; disease cannot develop, the plant is no host to the virus. Incompatibility may be caused by the insufficient aggressivity of the virus and/or by the resistance of the plant. In literature the ability of the plant to resist pathogens is generally called resistance.

In our earlier papers written in English and Hungarian – similarly to the English literature – the term immunity was used to express the highest absolute degree of resistance. By the immunity of plants we understand that the virus inoculated plant shows no symptoms of disease after inoculation, does not become disordered, and from the inoculated plant (either from inoculated leaves, or from those formed after the inoculation, or from other parts of the plant) virus cannot be reisolated to indicator plants, or transmitted to compatible indicator plants susceptible to grafting. We thought it necessary to use the term immunity in the above sense in the Hungarian literature as well because the different interpretations of resistance often gave rise to misunderstanding. Considering, however,

that in the relationship of plants and viruses the term immunity is objectionable from an immunological point of view, in this and the successive publications we shall use the expressions resistant and resistance, respectively, in the above sense of immune and immunity.

Of the test plants artificially inoculated with virus the ones found resistant to a certain virus and thus possibly important as crossing partners in the work of breeding for virus resistance are called virus resistant sources, while the others not susceptible to viruses simply resistant plants.

Some of the resistance sources gave hypersensitive responses to inoculation, while others proved resistant. The hypersensitive plants were given the symbol HR, the resistant plants were marked with R.

Reisolation and inoculation

Some of the artificially inoculated plants may remain symptomless following the inoculation even if the plant is otherwise susceptible to the virus. To detect latent infections we performed reisolation–inoculation tests. Tissue saps were prepared both from inoculated labelled leaves of the inoculated plants and from non-inoculated or subsequently developed ones – previously surface disinfected in a 2 per cent solution of NaOH then washed with a jet of water –, and rubbed with abrasive-spatula technique onto indicator plants which provided the quickest and most reliable way of pointing out the virus by local lesions. In the course of the reisolation–inoculation tests viruses were pointed out by using the following indicator plants: *Chenopodium amaranticolor*, *Phaseolus vulgaris* (alfalfa mosaic virus), *Phaseolus vulgaris* cv. *Red Kidney* (bean common mosaic virus), *Chenopodium amaranticolor*, *Phaseolus vulgaris* cv. *Red Kidney*, *Tetragonia tetragonoides* (been yellow mosaic virus), *Datura stramonium*, *Nicotiana glutinosa*, *Nicotiana tabacum* (belladonna mottle virus), *Chenopodium quinoa*, *Nicotiana glutinosa*, *Phaseolus vulgaris* (broad bean wilt virus), *Chenopodium amaranticolor* (celery mosaic virus), *Chenopodium amaranticolor*, *Nicotiana tabacum* cv. *Bel 61–10* (cherry leaf roll virus), *Chenopodium amaranticolor*, *Cucumis sativus*, *Tetragonia tetragonoides* (cucumber mosaic virus), *Capsicum annuum* (potato aucuba mosaic virus), *Chenopodium amaranticolor*, *Solanum rostratum* (potato virus M), *Chenopodium amaranticolor*, *Solanum rostratum* (potato virus S), *Gomphrena globosa* (potato virus X), *Lycium* sp., *Solanum demissum* A6-hybride (potato virus Y), *Brassica rapa* var. *rapa* (radish mosaic virus), *Nicotiana glutinosa*, *Nicotiana tabacum* cv. *Xanthi-nc* (tobacco mosaic virus), *Phaseolus vulgaris* cv. *Red Kidney*, *Tetragonia tetragonoides* (tobacco necrosis virus), *Chenopodium amaranticolor*, *Phaseolus vulgaris* (tobacco rattle virus), *Chenopodium amaranticolor* (tobacco ring spot virus), *Nicotiana glutinosa*, *Nicotiana tabacum* cv. *Xanthi-nc* (tomato mosaic virus), *Brassica rapa* var. *rapa*, *Nicotiana tabacum* (turnip mosaic virus), *Brassica rapa* var. *rapa* (turnip yellow mosaic virus), *Chenopodium amaranticolor*, *Lavatera trimestris* (watermelon mosaic virus).

The literature often does not make distinction between indicator and test plants; therefore we should like to underline again that the indicator plants show well-known characteristic symptoms, possibly local lesions soon after the virus inoculation, while a test plant is that one whose response to a given virus is not fully – if at all – known, or in its way to be established.

Basic principles and practical way of disjoining (separating) plant viruses

Separation of a single virus or a number of viruses from a virus complex containing two or more viruses, and the purposeful selection of separator plants and elaboration of the logical sequence of separation are called virus disjunction. In the course of virus disjunction (see Fig. 1) acceptor-indicator plants suitable to receive and point out viruses are required. The acceptor-indicator plants may be primary, secondary and tertiary separators or filter plants. Plants suitable for virus separation, which in the course of the so-called step-wise virus separation are of extreme importance and which enable the simultaneous separation of two viruses from virus complexes including three, four, five etc., viruses, are called primary separators. Plants suitable for virus separation, next after the primary separators in the course of the so-called "step-wise" or "multistage" virus sepa-

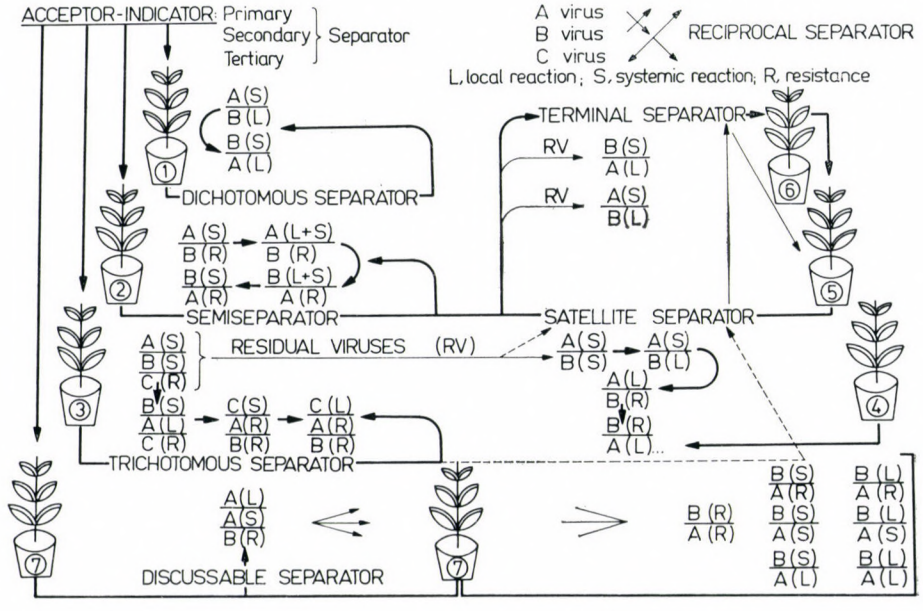

Fig. 1. Steps for disjunction (separation) of plant viruses

ration, which have the role of separating so-called residual viruses inseparable by the primary separators are called secondary separators. Tertiary separators are those plants which are suitable to separate residual virus(es) inseparable by either primary or secondary separators.

The virus disjunction is based on the fact that the plants are locally susceptible to some viruses and systemically to others, or susceptible to certain viruses and resistant to others (reciprocal separators). The dichotomous separators which play an important role in virus separation are plants showing different type and incompatible (local or systemic) susceptibilities to two, definite viruses and are thus suitable for the simultaneous differentiation of two viruses. A large proportion of the virus separators are not suitable for the simultaneous differentiation of two viruses. The so-called semiseparator plants are only suitable to separate one of two virus components. The other virus component can be differentiated with so-called satellite separators which when applied parallel to the semiseparators enable the differentiation of residual viruses. Plants suitable to differentiate the last two residual viruses in a virus complex including several viruses are called terminal separators.

From the point of view of virus separation the trichotomous separators possess advantageous properties. They are plants suitable to differentiate two of three virus components simultaneously, and eliminate the third one (through resistance).

A part of the separators described in the world literature is regarded as discussable separators being unreliable as regards their responses to virus inoculation, and therefore unfit for the reliable separation of viruses.

Symbols for host–virus connection

When discussing the new artificial virus host plants and artificial host–virus relations, respectively, as well as the new resistant plants we shall deal with the local, the systemic, and the local and systemic host–virus relations, as well as with the resistant plants and resistance sources – if we have succeeded in finding such plants at all – separately for the sake of a clearer comprehension of the subject. Owing to the different nature of host–virus relations we have applied symbols by which the most important features of the host–virus relations can be expressed (written down) in the shortest possible form. In studying the artificial host–virus connection we have elaborated a new symbolic signalling or information system built up with the five most important factors taken into consideration. The pentamer information system covers the following aspects: (1) test plant, (2) virus, (3) way of virus transmission, (4) reaction types expressing the relationship between test plant (host) and virus, and (5) suitability or unsuitability for virus separation of test plants and hosts, respectively, and of resistant plants. The information system contains a total of 28 signals of information by which all

characteristics indispensable for knowing the host–virus relations can be symbolically expressed. The 28 signals of information can be written down with the so-called host–virus cryptogram that we are the first to suggest.

Information system and information

1. Test plant: name, synonym, unknown in plant virology (from a scientific point of view a new experimental plant), new host plant, new resistant plant, the family it belongs to, form of life.

2. Virus: name, synonym, cryptogram (if the name and cryptogram of the virus is not separately indicated).

3. Way of virus transmission:* transmission by grafting, mechanical transmission, transmission by vectors.

4. Reaction types expressing the relationship between test plant (host) and virus: latent local susceptibility, latent systemic susceptibility, latent local and latent systemic susceptibility, manifest local susceptibility, manifest systemic susceptibility, manifest local and manifest systemic susceptibility, recovery, recurrence, hypersensitive reaction, resistance.

5. Suitability or unsuitabity for virus separation of test plants and hosts, respectively, and of resistant plants: plant suitable for separation, plant unsuitable for separation (discussable separator).

Information and its symbols

To represent the different properties symbols found on Hungarian and English typewriters alike, as well as their combinations are used.

1. Information on test plants, and its symbols

Plant name: e.g. Tinantia erecta
Synonym: Tinantia fugax
If unknown in plant virology: o (put after the name of the plant as an exponent)
New host plant: + (after the name of the plant as exponent)

* Considering that of the methods of virus transmission we only applied grafting and mechanical transmission in our experiments, and only used aphids as virus vectors, therefore the present paper as well as the subsequent series of publications only contain information symbols concerning these three ways of virus transmission.

For other vectors taking part in virus transmission (reviewed by HORVÁTH 1972e) the following symbols are suggested: VeBeetl (beetles), VeBug (bugs), VeButt-Moth (butterflies and moths), VeEar (earwigs), VeFli (flies), VeGrass (grasshoppers), VeLea (leafhoppers) VeLe-Beetl (leaf beetles), VeMit (mites), VeNe (nematodes), VePsy (psyllids), VeSca-In (scale insects), VeSlu-Sna (slugs and snails), VeThr (thrips), VeWand-Sca (wandering scale), VeWhi (whiteflies), VeWood (woodlice).

For other ways of virus transmission of importance for the host–virus relation the following symbols are suggested: Cu (Cuscuta), Fu (fungus), Po (pollen), R (root), Se (seed).

New resistant plant: ⊠ (after the name of the plant as exponent)

Family it belongs to: e.g. Com (short form of the family *Commelinaceae;* abbreviations of other family names are found in the section "*Experimental plants*")

Form of life: H (perennial, hemikryptophyton), Th (annual, therophyton), TH (biennial, hemitherophyton), Ph (woody plant, phanerophyta). If no data have been available on the form of life, or the existing ones are contradictory, this circumstance is expressed by a "?". In establishing the form of life for the experimental plants we relied on the work by Soó and Kárpáti (1968), and on the five-volume flora work of Soó (1964–1973). In obtaining information about the form of life of plants invaluable help was offered by Dr. Sz. Priszter and Dr. O. Borsos (Hortus Botanicus Universitatis Hungariae, Budapest), to whom I repeatedly express my gratitude and thanks.

2. *Information on the virus, and its symbols*

Name of virus: e.g. TuMV = turnip mosaic virus. Viruses included in our experiments are written down in the following short forms: AMV (alfalfa mosaic virus), BBWV (broad bean wilt virus; syn.: NRSV, nasturtium ring spot 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), 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; syn.: PSMV [potato stem mottle virus]), TuMV (turnip mosaic virus; syn.: CBRV [cabbage black ring virus]), TYMV (turnip yellow mosaic virus), WMV (watermelon mosaic virus).

Synonym: CBRV = cabbage black ring virus, or CBRSV = cabbage black ring spot virus

Cryptogram: */* : */* : E/E : S/Ap

3. *Information on the way of virus transmission, and its symbols*

Virus transmission by grafting: G

Mechanical virus transmission: M

Virus transmission by vectors: e.g. VeAp-sb (virus transmission by aphids in non-persistent or stylet-borne manner)

4. *Information on the types of reaction expressing the relationship between test plant (host) and virus, and its symbols*

Latent local susceptibility: /–

Latent systemic susceptibility: –/

Latent local and latent systemic susceptibility: –/–

Manifest local susceptibility: /+
Manifest systemic susceptibility: +/
Manifest local and manifest systemic susceptibility: +/+
Recovery: ry
Recurrence: re
Hypersensitive reaction: HR
Resistance: R

5. *Information on the suitability or unsuitability for virus separation of test plants and hosts, respectively, and of resistant plants; and its symbols*

Plant suitable for virus separation: ⊟. Capital letters beside this symbol refer to the virus which is not pathogenic either locally or systemically to the plant in question (e.g. ⊟ AMV, CMV), or in a given local host–virus relation is only systemically (e.g. ⊟PVX), and a given systemic host–virus relation only locally (e.g. ⊟ TRV) pathogenic. If a plant is resistant to a given virus, it may be locally, systemically, or locally and systemically susceptible to another, or even more than one viruses. In this case the resistant plant can be used as separator to separate such viruses as it is susceptible to (e.g. a plant resistant to a given virus is locally susceptible to BCMV: ⊟/BCMV; or a plant resistant to a given virus is systemically susceptible to BYMV: ⊟ BYMV/; or a plant resistant to a given virus is locally and systemically susceptible to CLRV: ⊟ CLRV/CLRV. The minus sign beside ⊟ (⊟ −) means that according to our present knowledge in the case of the given viruses the plant in question is not suitable for differentiation.

Plant unsuitable for virus separation: ⊞. As regards certain host–virus relations there were sometimes differences in our own data or those earlier published by others (e.g. a plant that we have found to be susceptible or resistant to a certain virus is – according to the literary data – resistant or susceptible, respectively).

To mark plants with a behaviour contradictory to our own experiments, as well as those unsuitable for virus differentiation (discussable separators) we use the symbol ⊞; capital letters and figures found beside this symbol refer to the virus and to the literary source found in the references in which the contradictory information about the virus and the plant is contained (e.g. ⊞ CMV12 means that according to the work found in the references under the serial number 12 the cucumber mosaic virus (CMV) – not withstanding the result of our experiments – cannot be reliably separated with the given plant).

Host-virus cryptogram

The pentamer information system and its 28 pieces of information (with the signs // separating the individual information systems, and with the comma separating the different pieces of information) can be written down in a so-called host–virus cryptogram: e.g. *Tinantia erecta* (*T. fugax*)$^{\bigcirc, +, \boxtimes}$: Com, H, TH, Th, Ph, ? // TuMV, syn.: CBRV, */* : */* : E/E : S/Ap // G, M, VeAp-sb // /−, −/, −/−, /+, +/, +/+, ry, re, HR, R, ⊟, ⊠.

The above cryptogram – which contains all members of the information even the contradictory ones (e.g. + or ⊠) – represents an actually non-existing host–virus relation, and is aimed at showing the way of writing down the different properties in a cryptogram.

The symbolic information system (host–virus cryptogram) that we shall use in the subsequent publications has been built up with the most important – though not all – aspects taken into consideration. Its further improvement would by all means be justified; here we think first of all of the importance of taking into account the environmental factors in the pre- and postinoculation stages, but it is also necessary to know the phenophases of test plants and concentrations of viruses, and complete the information systems with these data.

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Inhibition of Bacterial Multiplication in Incompatible Host-Parasite Relationships in the Absence of the Hypersensitive Necrosis

By

Z. KIRÁLY,[1] MÁRIA HEVESI and Z. KLEMENT

Department of Plant Pathology, University of Missouri, Columbia, Mo., and Research Institute for Plant Protection, 1525 Budapest, P. O. Box 102, Hungary

The multiplication of *Pseudomonas pisi* and *P. syringae* within incompatible tobacco leaf tissues is suppressed in comparison with the compatible *P. tabaci*, but this inhibition seems to be separate from the appearance of the hypersensitive necrosis. The low rate of multiplication of bacteria in incompatible host–parasite relationships remained similar when either hypersensitive necrosis developed or when it was inhibited by albumin infiltrated into the intercellular space of the leaf. In the tobacco leaves not treated with albumin that were inoculated with either *P. pisi* or with *P. syringae*, multiplication of the bacterium did not decrease at the time of appearance of the visible hypersensitive necrosis (7—9 h), rather it continued to increase for a relatively long time (24—72 h), and only then leveled off. This also supports the suggestion that the basic resistance phenomenon is not causally related to tissue necrosis.

In the plant pathology literature, it has been generally contended that tissue necrosis, associated with the hypersensitive reaction of plants, is involved in resistance against pathogenic fungi (WARD, 1902; STAKMAN, 1915), viruses (HOLMES, 1929) and against bacteria (KLEMENT *et al.*, 1964). It was suggested that the growth or multiplication of pathogens is restricted in plant tissues which react in a hypersensitive manner, and pathogens are inhibited, damaged, or even killed in collapsing tissues that later become necrotic. Recently, however, it was found that tissue necrosis and phytoalexin production are a consequence, rather than the cause, of resistance to diseases caused by rust pathogens and by *Phytophthora infestans* (KIRÁLY *et al.*, 1972; ÉRSEK *et al.*, 1973). Supporting this view, MAYAMA *et al.* (1975) came to the conclusion that "the hypersensitive response in rust disease is not a determinant for incompatible reactions". They claimed that hypersensitivity may be a stress symptom, and only incidental to incompatible host–parasite interactions. A similar conclusion was reached by TANI *et al.* (1975). It was also recently shown that environmental and chemical stress treatments can induce necrotic lesions in plants inoculated with viruses that normally do not cause local lesion necrosis, in other words hypersensitive reaction in those hosts (FOSTER and ROSS, 1975). Necrotic lesions induced in compatible hosts by stress do not prevent systemic virus movement, thus necrotic lesion formation and virus localization do not seem to be causally related. With respect to bacterial diseases,

[1] Visiting Professor, Department of Plant Pathology, University of Missouri, Columbia, Mo., USA.

a preliminary report by one of us (KIRÁLY, 1974) also reported on the non-association of suppressed bacterial replication with the hypersensitive response. Multiplication of *Pseudomonas pisi* in an incompatible host was inhibited even if the hypersensitive response did not develop in that host. Hypersensitive necrosis in the tobacco leaves was suppressed or fully inhibited by treatment with albumin, yet the incompatible bacterium multiplied at a low rate and reached a low stationary phase population level, just as in leaves wich developed the hypersensitive response.

In this paper we report on the multiplication of population of another incompatible bacterium, *P. syringae*, in an incompatible host when the development of the hypersensitive response was prevented. The multiplication of population is compared with a compatible bacterium wherein early or hypersensitive necrosis does not occur.

Materials and Methods

Tobacco plants (*Nicotiana tabacum* L. cultivar Samsun) were grown under greenhouse conditions. Fully matured leaves from plants of the 7–10 leaf stage were used for experiments. The injection infiltration method of KLEMENT (1963) was used for introduction of both bacterial suspensions or albumin solutions into intercellular space.

Bacteria [*Pseudomonas pisi* Sackett, *P. syringae* Van Hall, *P. tabaci* (Wolf and Foster) Stevens and *P. fluorescens* (Flugge) Migula] were grown for 24 h on nutrient agar at 28°C. Inocula were prepared from bacteria washed from the surface of the nutrient agar, and adjusted with a densitometer. Bacterial cell number was verified by standard plate count procedure. Tobacco leaves were usually injected with a 5×10^6 cells/ml suspension that, in most instances, induced confluent hypersensitive tissue necrosis in the cases of incompatible host–parasite relationships. Leaves infiltrated with water served as controls. Bacterial populations in the tissue were determined by the dilution-plate technique at different times after inoculation.

Human serum albumin (Phylaxia Institute, Budapest) at one, two or three per cent concentrations were injected into the leaves by the above-mentioned procedure before or after the inoculation with bacteria. In the latter case it was important to wait for a certain time (ca. 30 min) until the water previously infiltrated along with the bacteria evaporated.

For the determination of necrosis on a cellular level, leaves were infiltrated with a 1 per cent aqueous solution of Evans blue (Fisher Scientific Company, St. Louis) at various intervals after inoculation and were examined by light microscopy (TURNER and NOVACKY, 1974).

Results

The presence of hypersensitive necrosis does not influence the multiplication of populations of incompatible bacteria in leaf tissue. It was known from earlier observations that the multiplication of bacteria in incompatible host tissues ceases at about the same time that the hypersensitive tissue collapse becomes visible. Therefore, the cause-and-effect relationship between the development of tissue

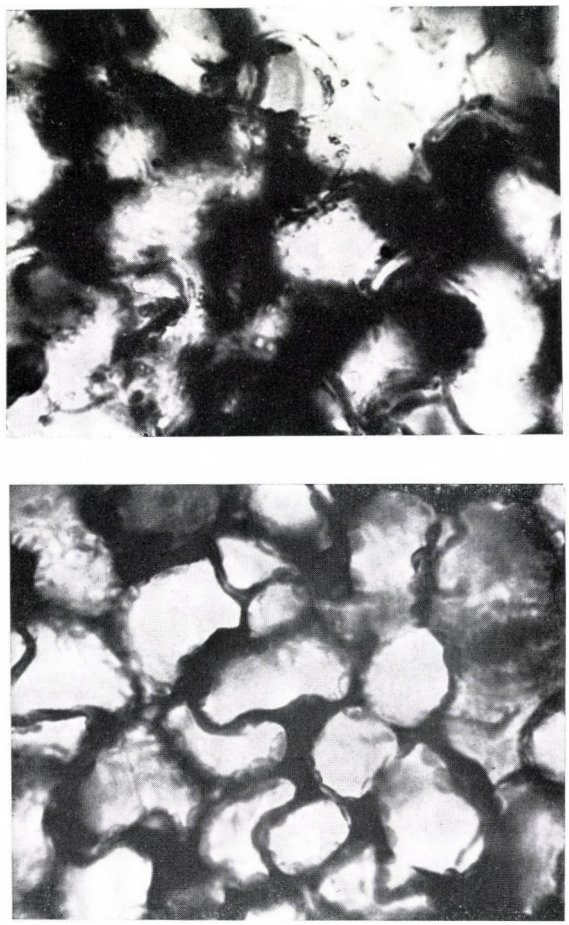
Fig. 1. Inhibition of tissue necrosis induced by *Pseudomonas syringae* in Samsun tobacco leaf by the infiltration of albumin into the intercellular space. Both half leaves were injected with a 5×10^6 cells/ml bacterial suspension. Then, after 30 min, the right half leaf was injected with a 1% solution of human serum albumin

necrosis and the inhibition of bacterial multiplication was regarded as an obvious fact. In these early investigations a high concentration of inoculum was used for the induction of the hypersensitive reaction, i.e. 10^8 bacterial cell/ml (KLEMENT *et al.*, 1964).

Our objectives were to induce the typical hypersensitive leaf necrosis with a much lower concentration of inoculum and, in some experiments, to inhibit the development of tissue necrosis by the application of albumin (Figs 1, 2A, 2B) and to determine the rate of multiplication of *Pseudomonas pisi* and *P. syringae* in tobacco leaf tissues. The inoculum contained 5×10^6 bacterial cell/ml which induced confluent necrosis in the injected leaf. Albumin was applied immediately

after inoculation with the bacterium as a one, two, or three per cent solution (cf. GÁBORJÁNYI *et al.*, 1974) in most experiments. Without albumin the hypersensitive necrosis developed in Samsum tobacco leaves seven hours after inoculation with *P. pisi* or eight to nine hours after inoculation with *P. syringae*.

As a result of incompatible host–parasite relationship, *P. pisi* multiplied at a low rate to a low stationary phase population level, both in leaves with the hypersensitive response and in which the development of the necrosis was inhibited



A

B

Fig. 2. Inhibition of cell necrosis induced by *Pseudomonas syringae* in leaves of Samsun tobacco. Inoculum and albumin were applied as indicated under Fig. 1. Both half leaves were then infiltrated with a 1% aqueous solution of Evans blue at the beginning of the formation of necrosis in the half non-treated with albumin. After a 15 min equilibrium period, leaf discs, 12 mm in diameter, were examined by light microscopy. A: Cell necrosis in albumin non-treated half leaf. B: Inhibition of necrosis as a result of treatment with albumin

by treatment with albumin (Fig. 3). In this experiment the leaves were injected with albumin and then immediately after evaporation they were challenged with the bacterium. It is apparent that the multiplication of bacteria in the untreated control continued after the appearance of the hypersensitive necrosis (7 h) until 24 h after inoculation.

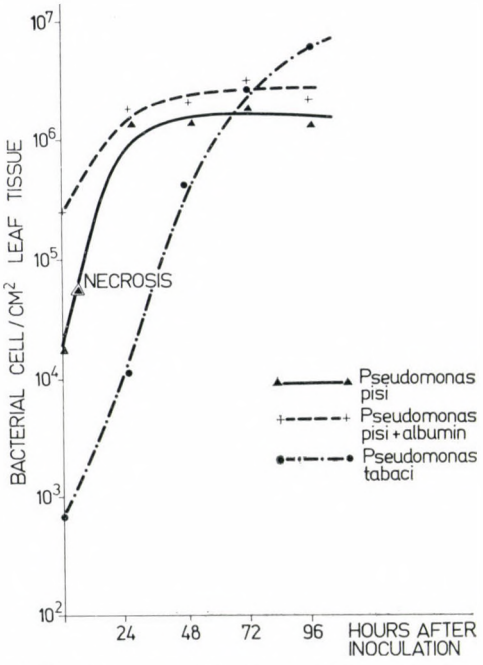


Fig. 3. Multiplication of *Pseudomonas pisi* in Samsun tobacco leaf, in which the development of the hypersensitive necrosis was inhibited by albumin. The multiplication of *P. tabaci* in Samsun tobacco leaf (a compatible host–pathogen combination) is compared to that of *P. pisi*. Inoculum and albumin were applied to the half leaves as indicated under Fig. 1 except that the inoculum of *P. tabaci* contained only 10^5 cell/ml. Leaf discs (1 cm^2) were cut at different intervals and macerated in 0.1 ml sterile physiological saline per disc. From the suspension a tenfold dilution series was set up. The cell number of 1 cm^2 leaf surface was determined with the plate-count technique at different times after inoculation

The results with *P. syringae*, another incompatible bacterium in tobacco, show a similar pattern (Fig. 4). The typical hypersensitive tissue collapse appears 8–9 h after inoculation with this bacterium; however, the multiplication of the bacterium continues to increase until 48–72 h. The inhibition of multiplication of bacteria seems to be independent from the development of the hypersensitive response. This idea is also supported by the fact, that growth ceases after 48–72 h even if the development of the necrosis is suppressed by the application of albumin. Basically, there was no difference between bacterial multiplication with the devel-

opment of the hypersensitive response, or without it. In the experiments with *P. syringae*, the leaves were always inoculated first with the bacterium and then, after evaporation, treated with albumin. Thus, the observed inhibition of bacteria by a pre-treatment with albumin was ruled out.

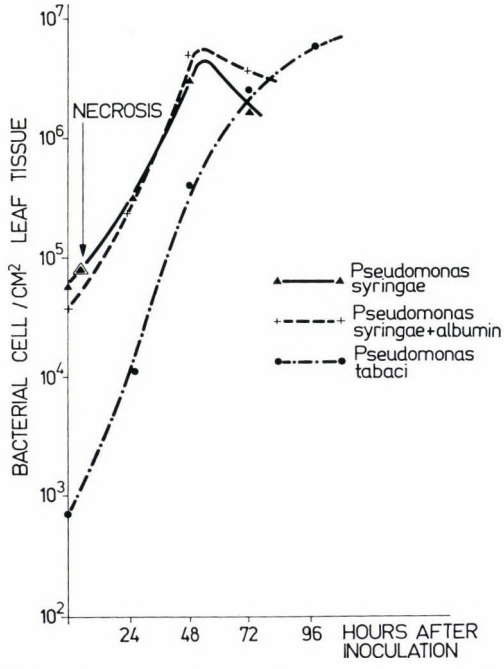


Fig. 4. Multiplication of *Pseudomonas syringae* in Samsun tobacco leaf in which the development of the hypersensitive necrosis was inhibited by albumin. The multiplication of *P. tabaci* in Samsun tobacco leaf (a compatible host–pathogen combination) is compared to that of *P. pisi*

As is seen from Figures 3 and 4, the population of bacteria in incompatible hosts increases by 1 or 2 logs and then levels off. It was of interest to compare growth rate to that of the bacterium in a compatible host (e.g. *P. tabaci* in tobacco). It is seen in both Figs 3 and 4 that the multiplication of *P. tabaci* in its compatible host, which is naturally free from visible early necrosis of leaf tissue, is significantly greater revealing a 4 log increase. The initial inoculum level in this instance is relatively low, 10^5 cells/ml.

Albumin does not inhibit multiplication of bacteria

It was important to know whether or not albumin inhibited the multiplication of bacteria in the leaf intercellular space, and whether this was the reason for the low rate of multiplication in the incompatible host. Evidence has shown

Table 1

The influence of albumin on the multiplication of *Pseudomonas syringae in vitro* in comparison with the standard bouillon medium[a]

	Bacterial cell/ml after 48 h
Bouillon	7×10^8
Albumin, 1%	2.5×10^8
Synthetic medium + albumin, 1%	3×10^8

[a] The initial inoculum contained 10^3 bacterial cell/ml. The bacteria were grown on 28°C. Composition of the synthetic medium: KH_2PO_4, 1 g; NaCl, 0.2 g; $MgSO_4 \cdot 7\,H_2O$, 0.2 g; distilled water, 1000 ml; pH 7.0. Albumin was sterilized by filtration through a G5 filter

Table 2

Influence of albumin on the multiplication of *Pseudomonas fluorescens* in tobacco leaf intercellulars

Time after inoculation	Bacterial cell number/ml	
	P. fluorescens	P. fluorescens + 1% albumin
0 h	3×10^4	3×10^4
24 h	3.3×10^4	8.0×10^4
48 h	1.0×10^5	1.2×10^5
72 h	8.0×10^4	1.0×10^5

Leaves were injected with a 5×10^6 cell/ml bacterial suspension, then, after 30 minutes, one group of leaves were injected with a 1% solution of human serum albumin. Leaf discs (1 cm^2) were cut at different intervals and macerated in 0.1 ml sterile physiological saline per disc. From the suspension a tenfold dilution series was set up. The cell number of 1 cm^2 leaf surface was determined with the plate-count technique at different times after injection.

that albumin *increased* the multiplication of *P. syringae in vitro*. Table 1 compares the rate of multiplication of this bacterium in bouillon, in 1% albumin and in a synthetic medium containing 1% albumin. After 48 h the bacterial numbers were determined turbidimetrically. In all of the three media *P. syringae* multiplied to a high level and albumin supported bacterial growth. This indirect evidence rules out the possibility that albumin inhibited bacterial growth in the intercellular space. Probably, it contributes to the nutrients available for the bacteria in the intercellular space, although preliminary experiments with *P. fluorescens in vivo* did not show increased multiplication of this saprophyte in tobacco leaves (Table 2).

Discussion

Multiplication of bacterial pathogens within incompatible leaf tissues is suppressed in comparison with the compatible ones. This seems to be a well established fact, as was shown by ALLINGTON and CHAMBERLAIN (1949) as well as by KLEMENT *et al.* (1964). However, our results call the attention to the non-association of hypersensitive necrosis with the low rate of multiplication of incompatible bacteria in the resistant tobacco leaf tissues. Necrosis may be incidental to the low rate of multiplication as the latter remains suppressed with or without necrosis. A compatible host/parasite relationship, *P. tabaci* in tobacco, was characterized by a high rate (4 logs) of multiplication and with a delayed appearance of necrosis. That the albumin prevented necrosis in the hypersensitive host, was demonstrated both on tissue and cellular levels (Figs 1 and 2A, 2B). It was apparent that in the inoculated resistant leaves, that were not treated with albumin, the multiplication of the bacterium did not decrease at the time of appearance of the visible hypersensitive tissue collapse (7–9 h), rather it continued to increase for an extended period of time (24–72 h). This supports the view that low rate of bacterial multiplication, which characterizes the resistance phenomenon, is not causally related to tissue necrosis.

The theory of TURNER and NOVACKY (1974) on the relationship between plant cell death and bacterial multiplication may explain our results. According to them the multiplication of bacteria introduced into tobacco leaves at inoculum doses lower than 5×10^6 cell/ml, is correlated with a capacity to cause cell death in leaves. Instead of confluent necrosis only isolated plant cell death occurred at these low levels of inoculum. Some incompatible bacterial species (*P. pisi*, *P. syringae*) were more effective in causing plant cell death than some other (*P. tomato*, *P. phaseolicola*, *Erwinia amylovora*) and, consequently, their multiplication was also greater. On the other hand, the saprophytic *P. fluorescens* did not induce plant cell death nor was bacterial multiplication recorded in leaves. Mechanical damage to plant cells causing cell death immediately increased the growth of this saprophyte in tobacco leaves. Some stresses applied to the leaves also permitted multiplication of the saprophyte and the development of tissue necrosis (LOVREKOVICH and LOVREKOVICH, 1970). Similarly, when pathogenic and saprophytic bacteria were inoculated together into bean leaves, the saprophyte population was stimulated by the release of nutrients induced by the pathogen (YOUNG and PATON, 1972). Supposedly, the non-visible dead cells caused by the pathogen contributed to the increased multiplication of the saprophyte. Thus, plant cell death apparently increases multiplication of bacteria. From his recent experiments on the development of bacterial populations *in vivo*, YOUNG (1974) also concluded that damage to host cell-membrane permeability, allowing leakage, supported bacterial growth.

In the light of these results we suggest that plant cell or tissue necrosis, caused by inoculum concentrations not higher than 5×10^6 cells/ml, do not inhibit the multiplication of bacteria in the incompatible host. Rather, factor(s), other

than the necrosis appears to be responsible for the low rate of bacterial multiplication in the resistant plant (cf. GOODMAN *et al.*, 1976; HUANG *et al.*, 1975; RUDOLPH, 1976).

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Cell Contact Recognition Versus Toxin Action in Induction of Bacterial Hypersensitive Reaction

By

Z. KLEMENT

Research Institute for Plant Protection,
1525, Budapest P. O. Box 102, Hungary

The question, whether there is a toxin produced by bacteria that would play a role in the induction of hypersensitive reaction (HR) was investigated by an indirect method.

By this method it was possible to decide the existence or the absence of an unstabile bacterial toxin in tobacco leaf tissue, in its original milieu, without extraction of presumed toxic material.

Experiments on rinsing of a previously inoculated area with streptomycin solution show that no toxic metabolites responsible for the induction of the HR were produced by *Pseudomonas pisi* in the incompatible Xanthi tobacco plant. It is suggested that rather the contact between living bacterial cells and plant cell is important for recognition of incompatibility and induction of the HR.

Most phytopathogenic bacteria which cause leaf spot diseases, induce hypersensitive necrosis in an incompatible host plant (KLEMENT, 1963; KLEMENT *et al.*, 1964). The rapid cell collapse of the incompatible plant develops during the first 24 h, usually by 6–12 h after inoculation. KLEMENT and GOODMAN (1967) have shown that only living bacterial cells are able to induce the hypersensitive reaction (HR). The induction of host cell collapse by the bacterium requires no more than 1–3 h, and after this time, live bacterial cells are not necessary for the development of necrosis (KLEMENT, 1971).

In contrast with these results GARDNER and KADO (1972) indicated that a compound isolated from *Erwinia rubrifaciens* reproduced symptoms of the HR. Similarly, SEQUEIRA and AINSLIE (1969) reported that a cell fraction of *Pseudomonas solanacearum* also caused necrosis similar to the HR in tobacco leaves. Later however, this report was not confirmed (SEQUEIRA, 1976).

Representing another point of view, LOVREKOVICH *et al.* (1970) demonstrated that bacterial multiplication in the leaf intercellular space gave rise to ammonia accumulation and an increase in pH which caused tissue necrosis. However, the investigations of O'BRIEN and WOOD (1973) refuted these results.

COOK and STALL (1969) suggested that certain volatile products of bacteria might be associated with the HR in plants.

According to some hypothetical ideas proposed, the bacterial cell disrupts the integrity of host cell-wall in a way which finally results in the collapse of the infected host cell (SEQUEIRA, 1976). However, the original question – what is

the inducer of bacterial HR or of the rapid cell necrosis? – remained unanswered. SEQUEIRA (1976) on the basis of many investigations concluded that "the bacterium produces a highly unstable toxin that damages host membranes only when released in close proximity to the cell wall".

In our work presented here, investigations using an indirect method were carried out to decide whether a bacterial toxin was responsible for induction of the HR.

Materials and Methods

The pea-pathogen *Pseudomonas pisi* Sackett and tobacco (*Nicotiana tabacum* cv. Xanthi) were used as the incompatible host–parasite combination. Tobacco plants had 6–8 leaves when used.

The culture of *P. pisi* was grown on nutrient agar medium at 28°C for 24 h. Cells of the bacterium were suspended in sterile tap water and 10^8 or 10^9 cells/ml were infiltrated in a portion (ca 1 cm^2) of each intercostal area at the same time (KLEMENT, 1963). The inoculated areas (central area) were immediately marked with a nontoxic ink. The central previously inoculated areas of the half-leaves were injected with a 1000 μg/ml streptomycin solution and those of the opposite (control) half-leaves with sterile water at 30 or 60 min intervals. By means of this method the bacterial cells or presumed toxic metabolite(s) were pushed and spread from the central intercellular spaces into the neighbouring tissues. This outer zone was also marked.

With this method it should be possible to determine the presence or absence of a bacterial toxin in its original milieu without conducting extractions for the presumed material.

Effects of the treatments were determined the following day after the challenge injection of water or streptomycin solution both in the central and in the outside area.

Results and Discussion

The occurrence of HR was observed both in the central and in the outside areas of the half-leaves when intercellular spaces of the central areas were rinsed with water during the first 5 h after inoculation. By this experiment it was established that there must be bacterial cells or toxic material in large enough quantity to produce the HR in the outside areas too. If the treatments with water were conducted 6 or more hours after inoculation, only the central areas became necrotic, and the neighbouring tissue in the outer zone remained symptomless.

In the opposite half-leaves where bacteria were killed by streptomycin in the previously inoculated areas, (at different intervals) no HR developed in the outside areas. The HR did appear however, in the central areas if the treatments

with streptomycin were carried out at least 2 h after inoculation (Table 1). By making comparisons between the results of water and streptomycin treatments, the following conclusions can be made.

If a toxic metabolite responsible for the tissue necrosis (HR) were produced by the bacteria in the intercellular spaces, the HR would have developed in the outside area after the bacteria were killed in the central area and washed out into the neighbouring zones.

Since necrosis in the outside zones occurred only when the central areas were rinsed with water it is supported that the inducer of HR is bound to the live bacterial cell. It would seem from other experiments that the multiplication of the bacterium or at least metabolically active bacterial cells are necessary for HR-induction. O'BRIEN and KLEMENT (unpublished work) have shown that bacteria lysed by bacteriophage, as well as the live but infected ("ill") bacterial cells were unable to induce HR. ERCOLANI (1970) did not observe HR on tomato plant inoculated with a histidine-requiring mutant of *P. syringae* (incompatible combination) because this strain was not able to multiply in tomato leaves in the absence of histidine. However, this mutant recovered its capacity for HR induction if histidine was introduced to the plant. Recent work of DURBIN and KLEMENT (1977) showed that HR did not occur if the multiplication of incompatible bacteria was inhibited at 37°C. On the other hand, pathogens which were able to multiply at 37°C did cause the HR at this temperature.

In the presence of streptomycin, the occurrence of HR could be observed only in the central area if the treatments were completed less than 2 h after inoculation. These results agree with previously published experiments (KLEMENT, 1972), according to which, no HR develops if the bacteria are killed in the plant tissue during the induction time. Accordingly, when the pathogen was killed after the induction period, then HR appeared. Since the induction period of *P. pisi*

Table 1

Appearance of the HR in the central areas and outside zones on tobacco leaves

Time of rinsing of the previously inoculated area*	Water		Streptomycin	
	central area	outside zone	central area	outside zone
0 h	+	+	−	−
1 h	+	+	−	−
2 h	+	+	+	−
3 h	+	+	+	−
4 h	+	+	+	−
5 h	+	+	+	−
6 h	+	−	+	−
7 h	+	−	+	−

*The central areas were inoculated with *Pseudomonas pisi* and washed out with water and streptomycin, respectively

in tobacco leaf is 1.5—2 h, it is easy to understand the appearance of HR in the central areas after 2 h.

It was interesting, that HR appeared in the central areas, but not in the outside ones if treatments with water were carried out more that 5 h after inoculation, though there were enough live bacterial cells (10^5 cell/cm^2) in the outside areas as well. This question is connected with another series of experiments which will be published elsewhere.

In the central areas the early symptoms of tissue collapse became visible by 5—7 h after inoculation. However, no HR was detected in the outside areas after rinsing of the previously inoculated areas either with water or with streptomycin. During the development of tissue necrosis in the inoculated central zone, more and more tobacco cells were necrotized. The cytotoxic material(s) which penetrated from the collapsed host cells into the intercellular spaces and washed out into the neighbouring tissues, did not cause tissue necrosis there. From this result the conclusion can be drawn that these cytotoxic materials are unable to penetrate through the plasmalemma of the healthy plant cells. This fact suggests that the same factor is not responsible for HR-induction as for the establishment of cell collapse.

The thought may be raised that a toxic bacterial metabolite is diluted by rinsing of the previously inoculated central zone and so it has lost its effectiveness. However, the fact is, that the minimal bacterial number of *P. pisi* able to induce confluent tissue necrosis is 5×10^6 cells/ml and 200 times more bacterial cells (10^9 cells/ml) were used throughout this experiment, therefore the toxic bacterial metabolites also had to be in a relatively high concentration. On the contrary, the intercellular fluid of central areas was only 2—3 times diluted by washing out of these areas.

To sum up these results it can be concluded that toxic bacterial metabolites are not responsible for the induction of the HR, rather the contact between the living bacterial cell and the plant cell is important for recognition of incompatibility.

It seems, that the cell contact recognition is connected or coincides with multiplication of bacterial cells and that the multiplication of bacteria is the essential factor of the HR induction.

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An Improved Method for Detecting Systemic Infection of Sunflower Seedlings Caused by *Plasmopara halstedii*

By

F. VIRÁNYI

Research Institute for Plant Protection, H-1525 Budapest P. O. Box 102

An improved evaluation technique was elaborated for detecting any kind of infection of sunflower downy mildew. Based on five categories of symptom expression the following system was used: *a.* evaluation of sporulation on cotyledons of plants 8—10 days after inoculation by *Plasmopara halstedii*; *b.* survey of systemic leaf-symptoms on true leaves of 3 weeks old plants; *c.* counting of plants showing damping off; *d.* evaluation of lesion appearance on hypocotyls removed from soil; *e.* evaluation of sporulation on hypocotyls without any symptoms; *f.* histological examination of non-sporulated hypocotyls.

Downy mildew, caused by *Plasmopara halstedii* (Farlow) Berl. et de Toni, is one of the most serious diseases of oilseed sunflower. The recent enlargement of its cultivation has been accompanied by an increased incidence of downy mildew and has prompted the evaluation of sunflower cultivars and lines for resistance. The selecting work in the field, which is generally used, often involves difficulties since natural inoculum levels vary, and weather conditions are changing from year to year. The use of laboratory methods seems to be more reliable. In the latter case plants are usually tested for susceptibility or resistance by artificial inoculation then induced sporulation. This method was believed totally correct until COHEN and SACKSTON (1974) revealed the latent infection of sunflower with *P. halstedii*. This finding challenged the validity of selecting work based only on fungus sporulation on the plants, as the pathogen is able to remain in a latent form within the host exhibiting no symptoms at all.

For this reason an attempt was made by the author to improve a laboratory technique for detecting any kind of infection of *P. halstedii* in sunflower seedlings.

Materials and Methods

Test plants and source of inoculum

Seeds of sunflower cultivars (VNIIMK 6540, Csakinszkij, Peredovik, GK—70, GK—71, 516/a, and 816/b) each possessing susceptibility to downy mildew were obtained from Breeding Stations of Szeged and of Iregszemcse, Hungary.

Seedlings for inoculation were grown from seed that had been surface sterilized with 2 per cent chlorogenium solution, washed thoroughly in sterile distilled water, then germinated on moist filter paper in Petri dishes for two days.

The inoculum of *P. halstedii* was obtained from field collections and maintained on the cultivar "VNIIMK 6540". In order to obtain the inoculum these plants were taken in a saturated atmosphere overnight at about 18–20°C and the zoosporangia formed were washed down by gently shaking the detached cotyledons for some minutes in twice distilled water. The spore suspension was filtered through several layers of cheesecloth and its concentration was adjusted to 5×10^4 zoosporangia/ml by diluting with bidistilled water.

Inoculation technique

Inoculations with *P. halstedii* were performed by the whole seedling immersion method (WSI) which was originally described by MOLDAVAN and ZHIVILO (1965), then modified by COHEN and SACKSTON (1973). The inoculated seedlings were planted in pasteurized soil, 5 per pot, and maintained on greenhouse benches at 18–28°C. At least 30 seedlings of each cultivar were inoculated and the experiments were replicated many times.

Evaluation of systemic infection

Sporulation on cotyledons. Eight to 12 days after inoculation the plants were covered with polyethylene bags to provide a saturated atmosphere at 20°C for 16 hours. The intensity of sporulation was assessed visually by a numerical scale from 0 to 4 (COHEN and SACKSTON, 1973). Plants with fungal sporulation were considered systemically infected and removed (Fig. 1).

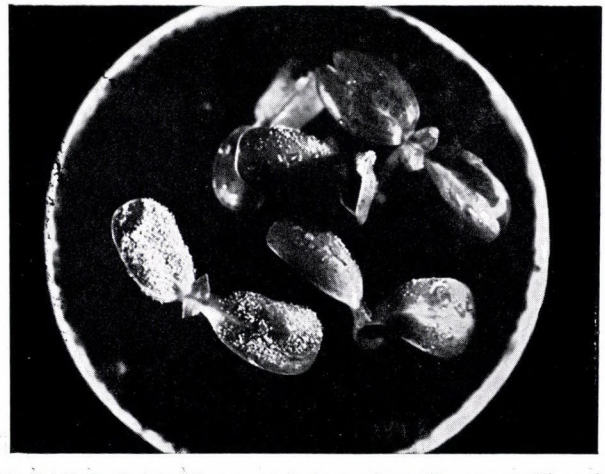

Fig. 1. Sporulation of *P. halstedii* on cotyledons of sunflower seedlings inoculated by the WSI method

Determination of true leaf symptoms. The number of seedlings with symmetrical chlorosis, typical of this disease, was determined 3 weeks after planting. At the same time those plants showing damping off (GOOSSEN and SACKSTON, 1968) were also counted.

Evaluation of symptoms on hypocotyls. Rest of the plants was removed from the soil and examined symptomatologically. Seedlings which showed lesions on their hypocotyls were recorded as diseased ones (Fig. 2).

Sporulation on hypocotyls. Underground parts of the plants (except roots) that seemed to be healthy were cut to five pieces, first washed in tap water, then

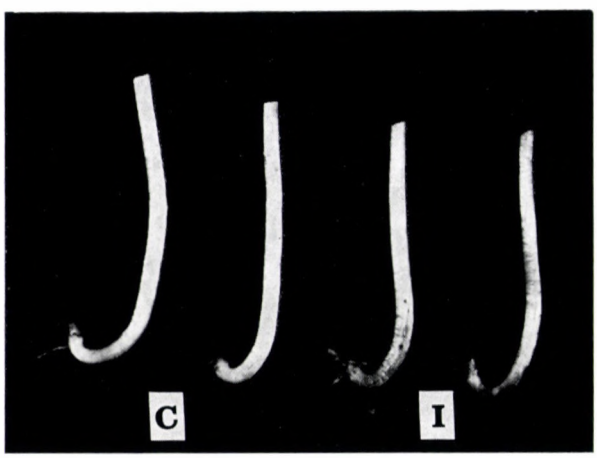

Fig. 2. Hypocotyl lesions on sunflower seedlings inoculated with *P. halstedii* (i = inoculated, c = noninoculated control)

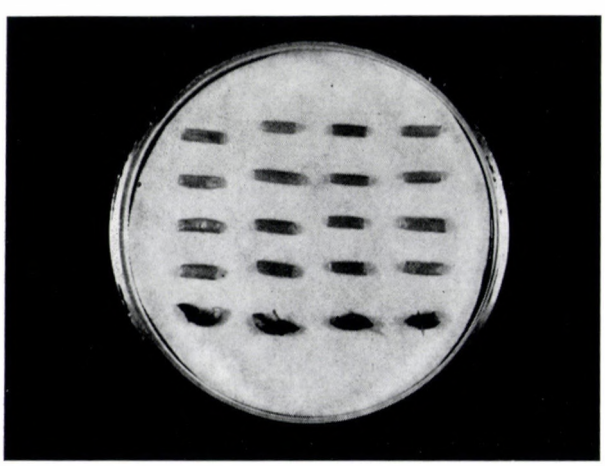

Fig. 3. Incubation of hypocotyl pieces for fungal sporulation

in sterile distilled water, placed onto moist filter paper in Petri dishes, and incubated at 19°C in darkness for 2 days (Fig. 3). Sporulation was detected by means of a stereomicroscope.

Histological examination. If zoosporangia did not appear on some of the cotyledon pieces, they were fixed in formol–acetic-alcohol (FAA) mixture according to WILLER (1970) for histological work. Freehand sections from the fixed material were stained with 0.05 per cent cotton blue in lactophenol. Under light microscope it was easy to recognize the hyphae and haustoria of *P. halstedii*, if present (Figure 4).

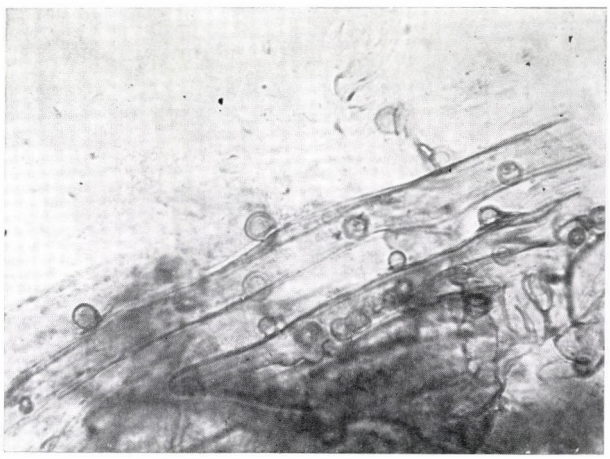

Fig. 4. Hyphae and haustoria of *P. halstedii* in sunflower hypocotyl tissue (×500)

Results and Discussion

The whole seedling inoculation technique proved to be suitable for providing a uniform infection load to all the sunflower cultivars tested. This method involved only one difficulty, i.e. the diversity of seed germination among the cultivars. To avoid this, seeds of the cultivars GK−70 and GK−71 were germinated at 26°C, while those of the other cultivars at room temperature.

As a rule, susceptibility of sunflower to downy mildew is ascertained by placing the plants in a saturated atmosphere before assessing fungus sporulation (PANCHENKO, 1965; ZIMMER, 1972, 1974). In our experiments, however, the incidence of plants with sporulation on the cotyledons varied from time to time ranging from 20 to 80 per cent of the inoculated seedlings. This result led us to the idea of searching for more dependable methods. It was obvious to employ some other symptom expressions for evaluating systemic infection. It is known from the literature that *P. halstedii* causes leaf chlorosis, extending in a symmetrical pattern along the main veins (NOVOTELNOVA, 1966), damping off (GOOSSEN and SACKSTON, 1968), as well as hypocotyl lesions (COHEN and SACKSTON, 1973).

Table 1

Response of suflower seedings to downy mildew on the basis of various symptom expressions

Cultivars	Per cent of plants with					Per cent of all plants infected
	sporulation on cotyledon	leaf symptom	damping off	hypocotyl lesion	sporulation on hypocotyl	
VNIIMK 6540	71	4	1	12	6	94
Csakinszkij	66	10	2	2	10	90
Peredovik	43	5	2	23	14	87
GK-70	76	3	1	2	8	90
GK-71	66	8	2	4	8	88
516/a	73	10	1	1	6	91
816/b	82	8	1	3	4	98

During their study on the factors affecting *P. halstedii* infection, COHEN and SACKSTON (1973) considered the inoculated plants to be systemically infected, when the leaf chlorosis appeared. According to our greenhouse experiments the appearance of leaf chlorosis mainly depends on the light conditions, as well as on the cultivars tested (Table 1). The data obtained also underline the importance of investigating various symptoms of sunflower downy mildew in the selecting work for disease resistance.

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Effect of Ethrel* on the Phenolics of Mung (*Phaseolus aureus*) Infected by *Rhizoctonia solani*

Short communication

By

Y. K. ARORA and K. L. BAJAJ**

Department of Biochemistry Punjab Agricultural University, Ludhiana, India

Infected hypocotyls of *P. aureus* were treated with 50 ppm of Ethrel. Samples were taken at 24, 48, 72 and 96 hours interval. Total phenolic contents were determined at each interval and compared with those of infected samples. Ethrel treatment resulted in about two-fold increase in the total phenolic contents.

Mung bean is an important seed legume and dampling-off of mung caused by *Rhizoctonia solani* Kuhn. is one of the most serious diseases of this protein rich crop. Though some of the phenolics in their oxidized forms have been implicated in resistance mechanism (FARKAS and KIRÁLY, 1962), the effect of the growth hormones on the phenolics of mung have not been critically studied. Among plant growth hormones ethrel is a "broad spectrum" physiological agent in plant metabolism and it causes diverse morphological and physiological effects. In order to elucidate the biochemical attributes of resistance and susceptibility, shifts brought about in the total phenolics of diseased hypocotyls under the stress of fungal infection and diseased hypocotyls sprayed with ethrel were studied in the present investigations.

Mung bean variety ML—1 seeds were germinated in sterilized garden soil in iron trays (18″ × 12″) and plant grown indoor where temperature varied from 25° to 30°C. The inoculum was prepared from one week old mycelial mats of *Rhizoctonia solani* grown on potato dextrose agar (PDA) slants at 20°C (BOOTH, 1971). The mycelial mats were fragmented in a Waring blender for one minute and diluted to 20 ml/mat (VAN ETTEN *et al.*, 1967). After 72 hours of growth, hypocotyls were inoculated by dribbling the mycelial suspension over the hypocotyls at the rate of 100 ml/tray. After 12 hours of inoculation, hypocotyls were sprayed with 50 ppm solution of ethrel. Hypocotyls (nonsprayed and sprayed) were removed at suitable intervals for quantitative analysis. Hypocotyls were dried and powdered in a pestle mortar. 0.5 g of the powdered sample of hypocotyls was refluxed with 75 ml of absolute alcohol for two hours. The extracted material

* (2-chloroethyl)-phosphonic acid.

** Present address: Department of Vegetable Crops, Landscaping & Floriculture, Punjab Agricultural University, Ludhiana (India)

was centrifuged. The supernatant was taken and evaporated under vacuum on a steam bath. The residue was dissolved in methanol and the volume made up to 10 ml. For total phenolic estimation an aliquot of 0.5 ml of extract was diluted to 7 ml. The contents were well mixed and 0.5 ml Folin – Denis reagent then added and thoroughly shaken again. Exactly after three minutes, 1.0 ml of saturated sodium carbonate was added and the volume made up to 10 ml with good mixing. After one hour, the absorption of blue coloured complex was measured spectrophotometrically at 725 nm. Total phenols were quantified on the basis of standard curve prepared by using tannic acid as a standard.

Ethrel spray on the inoculated hypocotyls caused about two fold increase in the total phenolic constituents (Table 1).

Table 1

Changes of total phenols in infected and ethrel sprayed hypocotyls

Sampling time after infection (hours)	Total phenol percentage on dry weight basis	Sampling time after ethrel spray (hours)	Total phenol percentage on dry weight basis
24	2.28	24	4.00
48	2.00	48	4.00
72	1.82	72	3.76
96	1.84	96	3.44

High concentrations of phenolic compounds appear to be due to increased channelling of precursors towards phenols synthesis under the action of this growth hormone. This may be due to the accumulation of the intermediates of carbohydrate breakdown which are effectively used for the synthesis of phenolic compounds. Increase of polyphenol oxidase activity have been observed (SRINIVASAN *et al.*, 1973). Increased polyphenol oxidase activity results into increased quantity of quinones which are invariably involved in the resistance mechanism (BHULLAR *et al.*, 1972). Since increased level of polyphenols results into increased resistance towards fungal infection (CHOPRA *et al.*, 1974), the present investigations show that ethrel, in addition to its physiological effects (PRATT and GOESCHL, 1969; KRISHNAMOORTHY, 1970), may presumably be playing a vital role in conferring resistance to mungbean by *Rhizoctonia solani*.

Acknowledgements

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Changes in the Amino Acid Patterns of Soybean and Pea Seeds Due to Infection by *Cephalosporium* sp.

By

M. A. ABD-EL-REHIM, S. H. MICHAIL and E. A. ABU ELGASIM

Faculty of Agriculture, University of Alexandria, Egypt

Amino acid patterns of soybeans cv. Hardey and peas cv. Little Marvel seed samples, healthy and inoculated with *Cephalosporium* sp. clarified that certain free amino acids were depleted, namely isoleucine, β-alanine, glycine and arginine in inoculated soybean seeds, and β-alanine only in inoculated pea seeds. However, certain free amino acids were detected in inoculated seeds only. These were phenylalanine, threonine and citrulline in soybeans, and aspartic acid besides an unidentified amino acid in pea seeds. Combined amino acid patterns of soybeans and pea seeds revealed that infection resulted in depletion of arginine and phenylalanine respectively. Moreover, leucine, isoleucine and two unidentified amino acids were synthesized in pea seeds.

Throughout the course of seed health testing of 19 soybean cultivars, it was found that 11 cultivars showed *Cephalosporium* sp. growth on blotter and the percentage incidence ranged from 1—16% culminating in cv. Hardey. In this regard it was intended to examine the changes in amino acid patterns due to infection by *Cephalosporium* sp. on its respective and irrespective hosts. Therefore, two seed samples representing cvs. Hardey of soybeans and Little Marvel of peas were selected for assessment.

Materials and Methods

Four grams of oven-dried (105°C) milled seeds were taken for analysis. Extraction of free and combined amino acids was carried out according to the method adopted by HAIS and MACEK (1963). For these studies, one dimensional chromatograms were made using the descending technique. The solvent used for amino acid separation consisted of *n*-butyl alcohol/glacial acetic acid/distilled water (4 : 1 : 1 v/v/v); to which formic acid (85%) was added in the ratio of 1 : 100 by volume. Development of amino acid spots was accomplished as recommended by BLOCK *et al.* (1958). The developing amino acid spots were identified according to their R_f values and the location of the standards.

Results

The free amino acid patterns in healthy and inoculated soybean and pea seeds are presented in Table 1 which elucidate the following.

Healthy soybean seed samples that served as check treatment revealed the presence of certain free amino acids namely, isoleucine, β-alanine, glycine and arginine that were lacking in the inoculated seed sample.

Inoculated soybean seed sample contained phenylalanine, threonine and citrulline which were not detected in healthy seeds.

Aspartic acid and an unidentified amino acid were detected in inoculated pea seeds, whereas β-alanine was detected in healthy seeds only.

The combined amino acid patterns recorded in Table 2 demonstrate the following.

Healthy soybean seeds that served as check contained equal number of combined amino acids as the inoculated seed sample.

Arginine was detected in healthy soybean seed sample whereas asparagine was found in the inoculated seed sample only.

Table 1

Free amino acids detected in soybean and pea seeds inoculated by *Cephalosporium* sp.

Amino acids	SCF	STF	PCF	PTF
Isoleucine	+	−	−	−
Phenylalanine	−	+	−	−
Valine	+	+	−	−
Unidentified	−	−	+	+
Unidentified	+	+	−	−
α-Aminobutyric acid	+	+	−	−
Unidentified	+	+	−	+
β-Alanine	+	−	+	−
Threonine	−	+	+	+
Glutamic acid	+	+	+	+
Glycine	+	−	−	−
Citrulline	−	+	−	−
Aspartic acid	−	−	−	+
Arginine	+	−	−	−
Asparagine	+	+	+	+
Unidentified	−	−	+	+
Unidentified	+	+	−	−
Cystine	−	−	+	+

+ = detected. − = not detected. SCF = soybean check treatment free amino acids. STF = soybean treatment free amino acids. PCF = peas check free amino acids. PTF = peas treatment free amino acids

Table 2

Combined amino detected in soybean and pea seeds inoculated by *Cephalosporium* sp.

Amino acids	SCC	STC	PCC	PTC
Unidentified	−	−	−	−
Leucine	−	−	−	+
Isoleucine	−	−	−	+
Phenylalanine	+	+	+	−
Valine	+	+	+	+
Proline	+	+	+	+
β-Alanine	+	+	+	+
Glutamic acid	+	+	+	+
Aspartic acid	+	+	+	+
Arginine	+	−	+	+
Asparagine	−	+	−	−
Unidentified	+	+	+	+
Unidentified	−	−	−	+

+ = detected. − = not detected. SCC = soybean check combined amino acids. STC = soybean treatment combined amino acids. PCC = peas check combined amino acids. PTC = peas treatment combined amino acids

Phenylalanine was assessed in healthy pea seeds whereas leucine, isoleucine and two unidentified amino acids were detected in inoculated pea seeds.

Discussion

The amino acid patterns of soybean and pea seeds inoculated with *Cephalosporium* sp. indicated the depletion of certain free amino acids, as a result of infection, namely isoleucine, β-alanine, glycine and arginine in inoculated soybean seeds and β-alanine only in inoculated pea seeds. Likewise, combined amino acid patterns in inoculated soybean and pea seeds revealed consumption of arginine and phenylalanine as compared with non-inoculated seeds. These results are in line with the findings of ROHRINGER (1957), who found differences in free amino acids between healthy wheat seedlings and those infected with *Puccinia graminis* f. sp. *tritici*, but no change was found in the combined amino acids after protein hydrolysis. MCCOMBS and WINSTEAD (1964) detected changes in sugars and amino acids of cucumber fruits infected with *Pythium aphanidermatum*. SEGHAL and BOONE (1964) reported that most protein amino acids in strawberry leaves infected with multiplier disease were considerably diminished compared to healthy leaves. VAN ANDEL (1966) suggested that decrease in amino acids content in infected tissue could be attributed to the utilization by the pathogen or enzyme activities involved in the amino acids metabolism, which is often increased in diseased tissue. On the other hand, certain free amino acids were detected in the inoculated

seeds only, indicating their synthesis by either the pathogen or as a result of seed protein degradation. These amino acids were phenylalanine, threonine and citrulline in inoculated soybean seeds and aspartic acid and an unidendified amino acid in pea seeds. Asparagine was also synthesized in inoculated soybean seeds, and two unidentified amino acids, leucine and isoleucine were found in inoculated pea seeds. These findings are substantiated by several investigators. PATEL and WALKER (1963) reported that bean plants susceptible to infection by halo-blight organism showed increase in ornithine, histidine, methionine, asparagine, glutamine, β-alanine and lysine in inoculated leaves. WELKLEE *et al.* (1967) found that metabolic changes induced by cucumber mosaic virus in resistant and susceptible strains of cowpea resulted in increase of most of the amino acids, especially asparagine that was recovered two days after inoculation. HARPAZ and APPLEBAUM (1961) noticed abnormal accumulation of asparagine in plants infected by maize dwarf virus in comparison with healthy plants of the same age. HANKS and FELDMAN (1964) indicated that infected roots of citrus by *Radopholus similis* had approximately 100–400% more total free amino acids than roots of the corresponding healthy plants. ABD-EL-REHIM *et al.* (1974) detected asparagine accumulation in tomato fruits infected by *Geotrichum candidum* and *Alternaria alternata*.

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Comparative Studies on Pectolytic and Cellulolytic Enzyme Activities of Two Isolates of *Alternaria porri*

By

E. H. WASFY, S. H. MICHAIL, H. M. ELAROSI and M. A. SALEM

Faculty of Agriculture, University of Alexandria, Egypt

In vitro and *in vivo* tests, PME and PMG showed higher activity in the virulent isolate of *Alternaria porri*, the causal agent of onion scald disease, than the avirulent isolate. PMTE was not detected in both isolates. Cellulase activity of the virulent isolate was higher than that of the avirulent one *in vivo* and the reverse *in vitro*.

It was previously reported that *A. porri*, the causal agent of onion scald disease (EL-HELALY *et al.*, 1966), frequently produced sectors on PDA medium in corporated with certain fungicides such as Dithane Z–78 and Hercules 3944. Pure cultures from such sectors were obtained in order to make a comparative study with respect to their pectolytic and cellulolytic enzymes activities which may give some interpretation for their differences in pathogenicity. Therefore, two isolates were obtained and studied for that purpose. No records on pectolytic and cellulolytic enzymes of *A. porri* have been found in the available literature.

Materials and Methods

Medium size onion bulbs of Giza 6 cultivar were artificially inoculated with each one of the two isolates of the fungus.

Enzyme assays

Modified Czapek–Dox liquid medium containing 1% pectin and 0.5% carboxymethyl cellulose (CMC) as carbon sources was used for determination of pectolytic and cellulolytic enzyme activities, respectively. The medium was adjusted at pH 6 by citric phosphate buffer and poured in 100 ml flasks at the rate of 25 ml/flask. The two isolates of *A. porri* were cultured on that medium and incubated at room temperature (24–31°C). Pectolytic and cellulolytic enzymes were determined every two days up to 20 days for pectolytic and cellulolytic enzymes.

The pectolytic and cellulolytic enzymes were also determined in tissues of healthy and artificially inoculated onion bulbs. Crude enzyme extraction from the healthy and inoculated bulbs were prepared by a modified method of NANSON (1955). Five ml of phosphate buffer at pH 7 for pectolytic enzymes and pH 4

for cellulolytic enzymes were added to 30 g of healthy tissues or 20-day-old artificially inoculated tissues, then mixed in a waring blender for 30 seconds. The mixture was strained through layers of sterile muslin cloth, and the extracts were stored at 5°C.

Polymethyl-galacturonase "PMG" assay

PMG activity was determined adopting the method suggested by TALBOYS and BUSCH (1970), using a mixture consisting of 1.2% pectin, and 0.2 *M* NaCl. Two ml of the culture filtrate or extract were added to 8 ml of the reaction mixture (containing 4 ml pectin solution and 4 ml citrate phosphate buffer at pH 6) in test tubes, and were incubated for $2^1/_2$ hours at room temperature (29°C). The viscosity of the reactants was determined using the capillary OSTWALD viscometer. Enzyme activity was estimated as a percentage of the reduction in the viscosity of the reaction mixture, compared to the boiled culture filtrate (control). The reduction in viscosity was calculated by using the following equation:

$$\text{Per cent reduction in viscosity} = \frac{\text{Tt} \times 100}{\text{Tc}} .$$

Tt = time necessary for the volume of a given treatment to pass through the capillary tube of the viscometer.

Tc = time necessary for the same volume of the control treatment to pass throug the viscometer.

Pectinmethyl-esterase "PME" assay

PME was determined by the method suggested by SMITH (1958) after making some modifications. Reaction mixture consisted of 0.5 g pectin, 0.58 g NaCl, 2.5 ml 0.05% bromothymol blue solution, and the total volume was completed to 100 ml with distilled water. The reaction mixture was adjusted at pH 7 by adding 0.1 *N* NaOH and getting the green colour. PME activity in 2 ml culture filtrate or extract was determined after mixing with 8 ml of the reaction mixture in a test tube and the colour of the indicator was readjusted to green with 0.1 *N* NaOH. Tubes containing the reaction mixtures were incubated at room temperature (29°C) for 4 hours, then titrated with 0.01 *N* NaOH. Enzymes activity was expressed as micro equivalent of the free carboxylic groups liberated/min/ml culture filtrates. Control treatment was done similarly except that the culture filtrate was previously boiled.

Pectinmethyl-transeleminase "PMTE" assay

PMTE activity was determined according to the method suggested by TALBOYS and BUSCH (1970) using 4 ml of a mixture consisting of 0.1% pectin and 0.125×10^{-3} *M* $CaCl_2$ which was adjusted at pH 9 by adding 4 ml carbonate-bicarbonate buffer. Two ml of the culture filtrate or extract were mixed in test

tubes with 8 ml of the reaction mixture. The tubes were incubated for 24 hours at room temperature (27–31°C). Enzyme activity was measured by the direct spectrophotometric method, using a Unicam SP 500 spectrophotometer at 235 nm wave length and expressed as the change in transmission of the reaction mixture. Control treatment was done similarly except that the culture filtrate was previously boiled.

Cellulase assay

Cellulase activity was determined by the method suggested by HANCOCK *et al.* (1964) after making some modification, using a reaction mixture consisting of 6 ml 1.2% CMC and adjusted at pH 5 with 2 ml citrate phosphate buffer. Two ml of culture filtrate or extract were added to the 8 ml of the reaction mixture in test tubes and were incubated at room temperature (28°C) for 3 hours. The viscosity of the reactants was determined using the capillary OSTWALD viscometer. Enzyme activity was estimated as a percentage of the reduction in viscosity of the reaction mixture compared to the control. Control treatment was done similarly except that the culture filtrate was previously boiled.

Results

Two isolates of *A. porri* representing two degrees of virulence, i.e. virulent and avirulent, were tested for PMG, PME, PMTE and cellulase activities.

I – *In vitro tests*

1. Polymethyl-galacturonase "PMG"

The two tested isolates differed in their ability to produce PMG at different ages of the culture (Fig. 1). Virulent isolate showed the highest PMG activity in most tested ages while the avirulent isolate was the lowest in this respect. Differences in enzyme activity between the two isolates were prominent.

2. Pectinmethyl-esterase "PME"

Enzyme activity of avirulent isolate appeared later than virulent one (Fig. 2). No enzyme activity was detected before 8 days of inoculation in case of both isolates. Virulent isolate showed the highest PME activity at all tested ages while avirulent isolate was the lowest one in this regard.

3. Pectinmethyl-transeleminase "PMTE"

Tests for PMTE of the two isolates of the fungus at different ages starting from 2 days and up to 20 days showed negative results which suggested that the enzyme was lacking.

4. Cellulase

Enzyme activity of avirulent isolate showed the highest cellulase activity at all tested ages while the virulent isolate was the lowest one in this respect (Fig. 3).

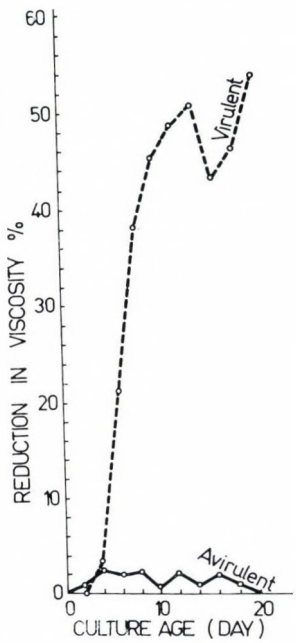

Fig. 1. Effects of the age of cultures on the activity of PMG in culture filtrate of *Alternaria porri* isolates

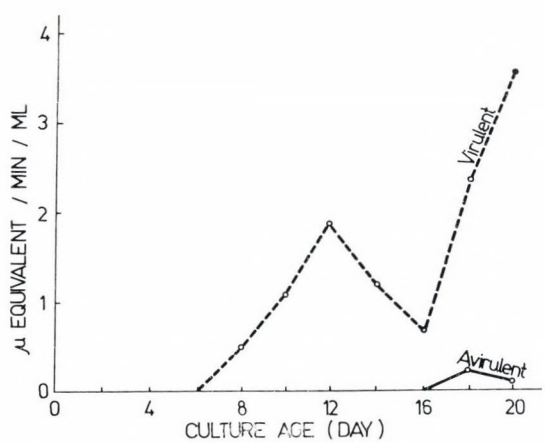

Fig. 2. Effect of the age of cultures on the activity of PME in culture filtrate of *Alternaria porri* isolates

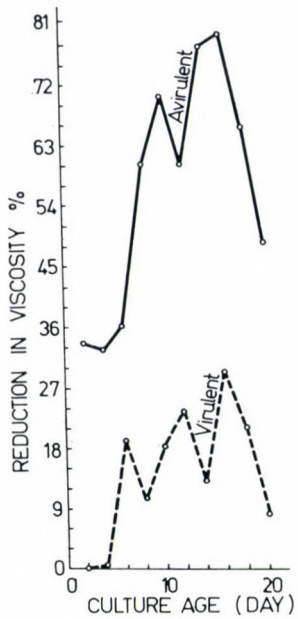

Fig. 3. Effect of the age of cultures on the activity of cellulase in culture filtrate of *Aiternaria porri* isolates

II — *In vivo tests*

The activity of all tested enzymes, after 20 days of inoculation, in tissues infected with the virulent isolate was higher than their activity in tissues inoculated with avirulent isolate or in healthy tissues (Table 1). The activity of all tested enzymes was more or less equal in both healthy tissues and tissues inoculated with the avirulent isolate. However, differences in the activity of the forementioned enzymes in bulb tissues inoculated with virulent or avirulent isolate were prominent.

Table 1

Pectolytic and cellulolytic enzyme activities of extracts of bulb tissues infected with virulent and avirulent isolates of *A. porri*, after 20 days of inoculation

Treatment	Enzyme			
	PMG[1]	PME[2]	PMTE[3]	Cellulase
Uninoculated tissues (control)	2.81	1.13	—	1.50
Tissues inoculated with avirulent isolate	2.56	1.31	—	2.93
Tissues inoculated with virulent isolate	53.53	2.94	—	93.79

[1] Reduction in viscosity %
[2] μ equivalent/min/ml
[3] Transmission (%)

Conclusion

In vitro and *in vivo* tests, the virulent isolate showed higher activity than the avirulent isolate in both PME and PMG. In case of cellulase, the virulent isolate showed higher activity than the avirulent one *in vivo* tests, and the reverse *in vitro* tests. Positive or negative relations between the virulence of the fungi and the pectolytic and cellulolytic enzymes activity is extensively documented in the literature. The present work is in line with such findings. In onion, many pathogens showed this trend such as *Botrytis cinerea* and *B. squamosa* (HANCOCK *et al.*, 1962); *B. squamosa* (HANCOCK *et al.*, 1964); *B. allii*, *B. cinerea* and *B. squamosa* (HANCOCK *et al.*, 1964); *Pyrenochaeta terrestris* (KEEN and HORTON, 1966) and *Sclerotium cepivorum* (ABDEL-RAZIK, 1970).

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Degradation of Cellulose and Lignin in Malformed Mango Inflorescence by *Fusarium moniliforme* var. *subglutinans* Wr. et Rg.

By

N. C. CHATTOPADHYAY and B. NANDI*

Department of Botany, University of Burdwan, Burdwan, West Bengal, India

Fusarium moniliforme var *subglutinans* Wr. et Rg. causes extensive malformations of young inflorescence of mango plant which finally fail to fruit. During the development of the disease considerable amount of cellulose and lignin have been found to be degraded. The losses of these two structural components in malformed inflorescence of "Himsagar" and "Bombai" cultivars of mango have been investigated on the basis of quantitative estimation following standard methods. In healthy tissues of both cultivars the cellulose and lignin contents are about 60 % and 30 % of the dry tissues respectively. Considerable differences in the degradation of different components of cellulose of the two cultivars have been noticed. From the standpoint of chemical degradation the 'Bombai' cultivar proves to be somewhat more susceptible than the other.

Literatures on the utilization of cellulose (SIW, 1951; SCHEFFER and COWLING, 1966) and lignin (DUNCAN, 1960; LEVI and PRESTON, 1965; VAN VLIET, 1954) by wood-decaying fungi are more frequent than those by fungal pathogens during stages of disease development in plants. In many plant diseases, different fungi cause graded degrees of degradation of these two important structural components of perennial plants. Cellulose of host cell walls is known to be attacked through liberated cellulose-breaking enzymes of the fungal pathogen ultimately causing collapse of the host cells (BATEMAN, 1963, 1964; HUSAIN and DIMOND, 1960; HANCOCK and MILLAR, 1965). As regards lignin degradation, besides the efficient white rot type of wood-rotting *Basidiomycetes*, many other fungi are capable of decomposing ligning containing plant materials (KIRK, 1971). It is, however, still a question as to what extent phytopathogenic fungi can attack lignin and whether such ability has any influence to cause disease. In the present investigation, the changes in cellulose and lignin contents of malformed tissues of mango inflorescence as a result of infection by *Fusarium moniliforme* var. *subglutinans* Wr. et Rg., have been worked out. The host (*Mangifera indica* L.), producing one of the most important fruits in Indian subcontinent, when attacked by the pathogen shows development of malformed inflorescences that fail to fruit almost completely and thus result in tremendous economic loss.

* Present address: Institute of Physiological Botany of the University of Uppsala S—751 21 Uppsala (Sweden).

Materials and Methods

F. moniliforme var. *subglutinans* was isolated from malformed mango inflorescences collected from plantations in and around Burdwan, West Bengal, India, and its identification was confirmed by IARI, New Delhi. Healthy mango plants of popular "Himsagar" and "Bombai" cultivars in pots at the time of flowering were inoculated with conidial suspension of the fungus and kept for a period of 30 and 45 days. After the periods, tissues from infected malformed regions were dried and ground into fine powder (40 mesh) for quantitative estimation of cellulose and lignin.

Isolation and quantitative estimation of cellulose in healthy and malformed inflorescence of the two cultivars were done following TAPPI standard (1954) and COWLING (1961). Holocellulose was taken as residue remaining upon successive pre-extraction of 100 mg of powdered host tissue with ethanol-benzene, ethanol and hot water to remove extraneous substances followed by a succession of chlorination and monoethanol-amine extraction to remove lignin. The holocellulose was then washed several times in ethanol and ethyl ether, kept at 35°C for at least 2 hours to remove ether and finally dried to constant weight.

The isolated holocellulose was treated with 17.5% aqueous NaOH when hemicellulose was dissolved but the alpha-cellulose remained insoluble and was separated. The beta-cellulose was precipitated by acidifying the alkaline hemicellulose solution while the gamma-cellulose remained soluble in the acidic solution. The percentage yield of alpha- and beta-cellulose was determined by dry weight. The gamma-cellulose was calculated by subtracting the percentage of the alpha- and beta-fractions from the percentage of holocellulose in the moisture-free original sample.

The procedure adapted for quantitative estimation of lignin was essentially that of SAEMAN *et al.* (1954) by removing the total carbohydrates through hydrolysis with 72.01% (w/v) H_2SO_4 and then condensing the lignin to an insoluble residue which was then measured gravimetrically.

Results and Discussion

It is evident from the Table 1 that the cellulose content in healthy tissues is about 60% of the dry weight in both the host cultivars being slightly higher in "Himsagar" than that in "Bombai". It is also evident that the cellulose content in malformed tissues decreases as a result of loss during disease development. In both cultivars, all the three fractions of cellulose show simultaneous decrease. KELMAN and COWLING (1965) presented evidence where the pathogen was capable of causing significant degradation of alpha- and hemicellulose in *Pseudomonas solanacearum*-infected tomato stem. Many fungal pathogens of plants are known to produce enzymes which not only degrade soluble cellulose substrates but also hydrolyze the more complex forms of cellulose. These organisms often produce

Table 1

Amount of different components of cellulose in healthy and malfored inflorescences of two cultivars of mango after 45 days of infection by *F. moniliforme* var. *subglutinans*

Host cultivar	Cellulose content[1]							
	Healthy tissue (mg/100 mg)				Loss in malformed tissue (%)			
	a	b	c	d	a	b	c	d
Himsagar	61.4	40.1	13.3	8.0	18.6	18.8	19.1	18.0
Bombai	58.0	42.0	11.0	5.0	22.5	21.7	20.8	25.0

[1] Mean of three replicates
a = Holocellulose; b = alpha-cellulose; c = beta-cellulose; d = gamma-cellulose

Table 2

Amount of lignin in healthy and malformed inflorescences of two cultivars of mango after 30 and 45 days of infection by *F. moniliforme* var. *subglutinans*

Host cultivar	Lignin content[1]		
	Healthy tissue (mg/100 mg)	Loss in malformed tissue (%)	
		30 days	45 days
Himsagar	32.3	15.0	23.4
Bombai	37.3	17.5	26.5

[1] Mean of three replicates

a C_1 cellulase which is responsible for the initial attack on the more ordered forms of cellulose and a C_x cellulase which completes the degradation to soluble sugars. During the course of pathogenesis in the present study different components of cellulose are degraded simultaneously by a team of enzymes possibly acting as both invasive and digestive agents. However, considerable differences are evident in the differential degradation of various components of cellulose in the two host cultivars. GOTO and OKABE (1959) reported similar results of differential degradation in two hosts by two phytopathogenic species of *Xanthomonas*. It is also interesting to note that "Himsagar" cultivar with slightly higher available cellulose shows slightly lower degradation. This seems to corroborate the earlier findings (CHATTOPADHYAY and NANDI, 1977) of higher degree of resistance of "Himsagar" cultivar as evident from the higher frequencies of disease in "Bombai" cultivar in nature.

Table 2 shows that in both cultivars the lignin contents in healthy tissues are more than 30% of the total dry weight. Of the two, "Bombai" cultivars shows higher values than the "Himsagar". The lignin contents also show considerable

decrease in infected tissues of both host cultivars within 30 days during disease development. The loss increases with advancement of the disease. GULYÁS (1967) reported destruction of about 20% of the lignin in wheat straw by cultures of *Fusarium* sp. Effective decomposition of 10—65% phenol lignin from liquid medium by *Fusarium lactis*, *F. nivale* and many other unidentified fusaria was reported by FISCHER (1953). Thus, from the stand point of chemical degradation of the host tissues the test fungus proves to be very efficient and causes significant interference not only in the normal physiological process of the host (CHATTOPADHYAY and NANDI, 1977) but also acts on the main structural components of the host which finally leads to the formation of the characteristic malformations of the infected inflorescences.

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Analysis of Symptoms Caused by *Fusarium graminearum* Schwabe and its Relation to Powdery Mildew Infection in Wheat

By

Á. MESTERHÁZY* and A. K. ROWAISHED**

*Cereal Research Institute, Szeged, Hungary
**University of Aden, Yemen, PDR

Infections carried out at different times did not result in a similar vertical response.

The rank of the varieties according to the parameters considered changed from parameter to parameter. Most of the varieties do not give uniform reaction ranks to the different parameters. Therefore, resistance tests cannot be based on one single parameter, results calculated on this basis do not give the information needed in most of the cases. We suggest therefore taking more symptoms into account and calculate an average index by the mean of ranks.

Powdery mildew reaction influences significantly the susceptibility of wheat to *Fusarium graminearum*, this is valid for both infection type and infection severity. Its effect is strongest just after flowering, decreasing later. The effect is more expressed on the primary symptoms than for the yield and 1000 kernel weight.

We were unsuccessful in establishing a seedling–adult stage relationship in resistance to *Fusarium*. In the future this phenomenon should be investigated with plant material having resistance of the same level to powdery mildew and possibly to other diseases.

Regarding this and other influencing factors, the natural and artificial infection data do not correspond to the genetical background of *Fusarium* resistance. This may be a source of the inconsistent results obtained in some breeding programs.

The detailed analysis of the parameters shows that field data correlate highly significantly with each other. The level of significancy is, however, higher using the data of the first infection. Similarly highly significant correlations are counted for the greenhouse data, but they are somewhat lower than expected on the basis of earlier results.

The natural infection shows significant correlations with most of the field parameters, e.g. the field inoculation corresponds well to the natural infection.

The resistance of wheat to *Fusarium* diseases has worldwide importance because of the increasing damages caused by this genus. In Hungary the most important species in wheat are *F. graminearum* (BÉKÉSY and HINFNER, 1971; MESTERHÁZY, 1974) and *F. culmorum* and *F. avenaceum* (MESTERHÁZY, 1974a). Beside these other *Fusarium* species also occur in the investigated population, but they seem to be relatively unimportant (BÉKÉSY and HINFNER, 1971; MESTERHÁZY, 1974a).

Many investigations were carried out on wheat resistance to *Fusarium* over the past 80 years. ARTHUR (1891) first recorded differences in susceptibility to fusarial head blight. A number of research workers tried to find a source of resistance (ATANASOFF, 1920; MCINNES and FOGELMAN, 1923; SCOTT, 1927; HANSON *et al.*, 1940; SCHROEDER and CHRISTENSEN, 1963; PURSS, 1971; NAKAGAWA, 1955; NAKAGAWA *et al.*, 1967), but only differences in susceptibility were recorded. Our unpublished results also support this statement. In experiments with the same plant material the interannual correlations are low and the reason for this has not been evaluated until now (SCOTT, 1927; MESDAG, 1976). The highest correlations were produced by HANSON *et al.* (1940) with the value of $r = 0.67$ and $r = 0.64$, generally about $r = 0.50$, but this is exceptional.

As resistant plant material does not exist, recently more attention has been payed to the symptom expression and yield components (BOCKMANN, 1967; FOCKE, 1974; MESDAG, 1976; FEEKES, 1976; MESTERHÁZY, 1975a), because tolerance can be established only by help of these data. No detailed analysis has been published, however, on the relations of different symptoms and yield components. Therefore this was the main task of this paper. Another task has been the investigation of the powdery mildew effect on *Fusarium* infection, as we supposed that the low interannual correlations are caused at least in part by the different disease development caused by other causing agents.

Material and Methods

Twenty varieties and lines of winter wheat were tested for resistance to *Fusarium graminearum* infection. Various infection methods were tested, e.g. field and greenhouse inoculation. The field inoculation was carried out in different times; 2–5 days after 50% flowering, than one and two weeks after the first inoculation.

Infection material. The isolates (No. 1373, 831 and 1717) of *F. graminearum* Schwabe originated from our culture collection, identified by the author (senior), all of them with high aggressiveness. Earlier investigations showed that they affect similarly the wheat plants of various varieties, therefore the suspensions from them could be mixed. As this procedure may change the aggressiveness of the suspension, the aggressiveness of the mixture was checked (MESTERHÁZY, 1975b).

Production of inoculum. The bubble-breeding method was used as described by MESTERHÁZY (1974b). In short, liquid Czapek–Dox medium was placed in an Erlenmeyer flask provided with a glass tube and the apparatus and medium was sterilized together in an autoclave for an hour at 0.8 atmosphere. Then they were inoculated and supplied with sterile air bubbled through the medium for a week at room temperature. Then the ready suspension was homogenized for 20 seconds in a blendor. The aggressiveness of the suspensions was controlled by the laboratory method (see later) in order to assure that the plants were infected

with material of relatively uniform aggressiveness. The check variety was Bezostaya – 1 for this purpose.

Field infection. The first inoculation was carried out on the 23rd May 1975, 2 – 5 days after 50% flowering. Earlier investigations (MESTERHÁZY, unpublished) showed that this difference does not influence the results of infection. At each infection time we infected a separate set of 3 × 20 ears of uniform size by spraying them with 5 ml suspension for 20 ears. Care was taken to produce a uniform inoculation for each ear and spikelet. The method was based essentially on SCHROEDER's and CHRISTENSEN's (1963) method. After inoculation the ears were covered with plastic bags for 24 hours to provide high humidity. Control ears were treated by the same way for each infection time except without *Fusarium*. The second inoculation was carried out on the 30th of May, and the third on the 6th of June.

Two weeks after inoculations the amount of disease was estimated using an 0 – 4 scale, that is, 0 = no infection, 1 = 25, 2 = 50, 3 = 75 and 4 = 100% of the spikelets are infected.

After harvest (full ripening) the following evaluation was carried out: 1000 kernel weight, yield (from each replications 10 uniform ears were selected), seed number/ear, furthermore ratio of killed seed after 4 and 7 days from the beginning of the experiment on PCNB medium according to PAPAVIZAS (1967). From each replication 50 seeds were checked. Surface sterilization was accomplished by a Neomagnol solution (1 g/100 ml dist. water).

Greenhouse infection. The method is based on MESTERHÁZY (1975a). 15 seeds of each replications were sown in clay pots containing sand. From each variety three control and four infected pots were sown. After surface sterilization the seeds were submerged for 24 hours in distilled water and suspension of *F. graminearum*, respectively. The pots were filled up to 2/3rd volume with sterile sand, their surface was irrigated with 20 ml of distilled water and *Fusarium* suspension, respectively. This doubled infection resulted in uniform disease development. After this, the seeds were covered with sterile sand and irrigated by distilled water. On the 6th day 20 ml Knop solution per pot was added. Shoot length (mm), root length (mm), root infection (%), number of killed plants and dry matter content per pot were determined on the 20th day after sowing.

Laboratory infection. This method was used to check the aggressiveness of the suspensions. In this method a double layer filter paper was submerged into a suspension of the fungus and placed into Petri dihes of 100 – 120 mm diameter. On this infestated paper 25 seeds were sown after surface sterilization as mentioned before. Seeds germinated on filter paper moistened with distilled water served as controls. Incubation took place at 25°C, and counting of killed plants was done on the 4th, 6th and 8th day.

Evaluation of the powdery mildew infection. In 1975 only powdery mildew occurred in epidemic form, therefore we have considered it as a factor potentially influencing the *Fusarium* results. Four types of infection were distinguished.

0 = immune, the plants are free of infection

2 = poorly developed small pustules developed with low density, resistant

5 = pustules are relatively well developed with medium density, moderately resistant

8 = pustules are large, richly sporulating, their density is high, susceptible.

The infection severity was evaluated on a 0 – 8 scale, according to the international practice. It has been, however, modified, since the traditional scale does not take the important role of the flag leaf and the ear as producers of assimilates (IBRAHIM and ELENEIN, 1977) well enough into consideration.

0 = the plants are free of infection
1 = the pustules occur on the lowest leaves and stem
2 = the infection reached about the half height of the plants
3 = all leaves are infected except the flag leaf
4 = the first pustules occur on the flag leaf
5 = about 50% of the flag leaf area is infected
6 = the flag leaf area is covered by pustules almost completely
7 = some pustules occur on the ears
8 = the ears show heavy infection.

The scale has been evaluated by the senior author based on yellow rust evaluation presented by LINE *et al.* (1972).

Evaluation of the natural infection by F. graminearum. Seeds of the uninfected controls were surface sterilized and placed on PCNB agar. The developed colonies were transferred to PDA agar and identified according to the BOOTH (1971) manual. The number of killed seeds by different *Fusarium* species was also recorded. As we were interested in the natural infection caused by *F. graminearum*, for further analysis only these seeds were considered which were infected by this species.

Results and Discussion

1. Field experiment. Tables 1 and 2 show the 1000 kernel weight and the yield data. The most severe infection, as indicated by both parameters was caused by the first inoculation, for which the average performance was less than 50% of the second one. As for the behaviour of individual varieties, the infection gave variable results, e.g. the rank of the best varieties changed with successive infections for all parameters investigated. This is important as tolerance is evaluated according to the 1000 kernel weight or yield data differences. As a consequence therefore, the data have only limited indirect value for breeding purposes, since the time of inoculation differently influences the performance of individual varieties. The first infection gave the greatest differences. Later infections caused less harm, and no significant difference was shown here. To better characterize the performance of the varieties, we used the means of the three inoculations. These averages are listed in Table 3. Regarding the different parameters the varieties do not perform similarly. If we list them in ranks from 1 – 20 (the first is the best, the 20th is the worst) we verify this statement (Table 4). Only some of the varieties

Table 1

Data of 1000 kernel weight of wheat varieties infected by *Fusarium graminearum* at three different inoculation times.
(Data represent the average of three replications)

Variety	Infection time			Mean
	1st	2nd	3rd	
	in the percentage of the control			
I/1	28.97	67.67	73 97	56.87
I/3	31.47	78.23	100.83	70.17
I/5	19.54	73.82	69.18	54.18
I/6	31.40	86.52	85.44	67.95
I/7	26.92	81.95	87.63	65.50
I/8	29.75	73.43	70.80	57.99
I/9	48.75	71.20	66.11	62.00
I/10	43.51	89.08	69.44	67.50
I/11	47.75	77.05	74.48	66.43
II/1	21.68	56.49	85.90	54.68
II/2	51.76	80.23	79.55	70.51
II/3	18.61	70.43	72.61	53.88
II/4	30.57	72.13	75.12	59.27
II/5	28.87	47.62	67.12	47.87
II/6	71.62	78.96	64.32	71.63
II/7	24.11	59.02	64.39	49.17
II/8	45.61	65.94	52.32	54.62
II/10	38.01	78.13	76.59	64.24
II/11	32.13	69.28	73.73	58.37
II/12	8.82	76.10	66.96	50.62
Mean	33.99	72.66	73.87	60.18

LSD 0.1%	Between varieties	16.37
	Between infections	6.33
	Between combinations	28.37

such as II/3 or II/10 show a relatively uniform performance. Therefore we need to know the general performance of the varieties to gain greater security in the resistance estimations. We think that the average of various parameter ranks shows clearly the differences between varieties.

2. Greenhouse experiment. Data for this experiment (Table 5 for natural data, Table 6 for ranks) also show the different demonstration of different parameters for most of the varieties. However, we can evaluate the general reaction in this case by means of the average of the parameter ranks, too. The performance of the varieties is more uniform here as shown by the lower LDS values, but there are varieties, too, for which the behaviour is very inconsistent.

What can be the sources of the variability for the ranks of the varieties found in both experiments? We suppose that one of them may be the different disease

Table 2

Data of the yield of wheat varieties infected by *F. graminearum* at three different inoculation times. (Data represent the average of three replications)

Variety	Infection time			Mean
	1st	2nd	3rd	
	in the percentage of the control			
I/1	23.28	54.47	56.03	44.60
I/3	28.42	71.96	63.58	54.65
I/5	18.34	62.93	53.42	44.89
I/6	25.07	81.92	73.67	60.22
I/7	18.28	67.59	61.46	47.44
I/8	29.14	59.74	43.01	43.96
I/9	38.90	58.84	55.56	51.10
I/10	41.57	97.00	44.88	61.15
I/11	38.45	66.13	63.61	56.67
II/1	19.41	42.94	80.82	47.72
II/2	44.11	69.10	66.06	59.22
II/3	18.91	60.29	49.75	43.65
II/4	30.11	65.48	69.44	55.01
II/5	23.42	28.99	46.34	32.92
II/6	34.65	79.57	58.16	57.46
II/7	18.60	52.35	49.22	40.05
II/8	41.84	51.44	43.45	42.57
II/10	42.76	64.14	48.07	51.65
II/11	29.39	60.20	70.14	53.24
II/12	9.72	79.35	47.45	45.51
Mean	28.72	63.52	56.75	49.65

LSD 0.1%	Between varieties	20.61
	Between infections	7.98
	Between combinations	35.71

development for individual varieties. Some varieties such as I/1 react very severely to relatively small infections on the field or we can observe the opposite reaction at the variety I/3. The same is true also for the greenhouse experiment. Where the symptom development is rapid, we can observe severe damages for yield, 1000 kernel weight in the field or dry matter content, root length, shoot length or plant height in the greenhouse. A slower development of the disease results at the same primary symptoms, i.e. No. of infected seeds or root infection higher yield in both the field and greenhouse. We think therefore that tolerance can be determined not only from the field experiment, but also from the greenhouse data. It is now a new research task to investigate this problem and determine whether or not it has an importance for breeding, i.e. whether or not it is possible to forecast tolerance in the field on the basis of greenhouse data.

Table 3

Performance of the varieties in the field infected by *Fusarium graminearum*. Data represent the mean of the three infections

Variety	1000 kernel weight	Yield	Ear infection on the field scale 0—4	No. of the *Fusarium* colonies	No. of the killed seeds on the 4th day	No. of the killed seeds on the 7th day	No. of the killed seeds by natural *Fusarium* infection in the control
	in % of the check						
I/3	56.87	44.60	1.66	14.7	12.3	13.7	2
I/3	70.17	54.65	1.88	23.3	10.0	11.3	3
I/5	54.18	44.89	1.66	22.0	14.3	14.7	4
I/6	67.95	60.22	1.77	17.7	13.0	14.7	5
I/7	65.50	47.44	1.66	21.3	17.7	18.3	6
I/8	57.99	43.96	1.88	21.7	15.3	16.0	5
I/9	62.00	51.10	1.88	21.3	13.7	14.7	2
I/10	67.50	61.15	1.77	9.7	8.7	10.0	1
I/11	66.43	56.67	1.66	14.3	10.7	12.0	2
II/1	54.68	47.72	2.10	16.0	13.7	13.7	4
II/2	70.51	59.22	1.66	15.0	10.7	13.0	4
II/3	53.88	43.65	1.88	17.7	14.7	16.3	11
II/4	59.27	55.01	1.77	15.0	11.3	13.3	5
II/5	47.87	32.92	2.33	15.7	11.7	13.7	4
II/6	71.63	57.46	1.33	19.0	11.7	13.	4
II/7	49.17	40.05	1.77	22.7	15.3	15.3	3
II/8	54.62	42.57	1.88	20.0	10.7	11.7	4
II/10	64.24	51.65	1.44	15.0	8.0	10.0	2
II/11	58.37	53.24	1.88	14.0	7.7	10.7	3
II/12	50.62	45.51	1.88	15.0	13.3	14.0	5
Mean	60.18	49.65	1.79	17.3	11.7	13.3	4.0
LSD 0.1%	16.37	20.61	0.75	1.29	1.23	1.22	—

3. Comparison of the two experiments. If we compare the average ranks of the two experiments we find that there is no correlation ($r = 0.0967$) between the field and greenhouse results. Earlier findings (MESTERHÁZY, 1975a; MESTERHÁZY, unpublished) show that varieties like Bezostaya−1 and Rannaya−12 infected by a number of different isolates of various *Fusarium* species react similarly in both the seedling and adult stages. Consequently we supposed that in this case similar results should also have been observed. We were very interested in finding some explanation for the discouraging results obtained here with the different varieties. The answer to this question is important as it concerns the relationship of seedling and adult stage resistance. So far nobody has been successful in finding a relationship useful for breeding. The importance of the relationship is, namely, that in the case of good agreement a selection process could be evaluated on the basis of the seedling selection to improve adult stage resistance.

Table 4

Performance of the varieties in the field infected by *Fusarium graminearum*. Data represent the ranks from 1 to 20 based on Table 3. (1 = best, 20 = worst performance)

Variety	1000 kernel weight	Yield	Ear infection on the field scale 0—4	No. of the *Fusarium* colonies	No. of the killed seeds on the 4th day	No. of the killed seeds on the 7th day	No. of the killed seeds by natural infection in the check	Mean
	in % of the check							
I/1	13	15	5	4	11	11	3.5	8.93
I/3	3	7	15	20	4	4	7	8.57
I/5	16	14	5	18	15	15	11.5	13.50
I/6	4	2	9.5	11.5	12	15	16.5	10.07
I/7	7	12	5	15.5	20	20	19	14.07
I/8	12	16	15	17	19	18	16.5	16.21
I/9	9	10	15	15.5	16.5	15	3.5	12.07
I/10	5	1	9.5	1	3	1.5	1	3.14
I/11	6	5	5	3	6	6	3.5	4.93
II/1	14	11	19	10	14	11	11.5	12.93
II/2	2	3	5	6.5	6	7	11.5	5.86
II/3	17	17	15	11.5	16.5	19	20	16.57
II/4	10	6	9.5	6.5	8	8.5	16.5	9.29
II/5	20	20	20	9	9.5	11	11.5	14.43
II/6	1	4	1	13	9.5	8.5	11.5	6.93
II/7	19	19	9.5	19	18	17	7	15.50
II/8	15	18	15	14	6	5	11.5	12.07
II/10	8	9	2	6.5	2	1.5	3.5	4.64
II/11	11	8	15	2	1	3	7	6.71
II/12	18	13	15	6.5	13	13	16.5	13.57
Mean	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.50

LSD 0.1%	Between varieties	6.76
LSD 1%	Between varieties	5.24
LSD 5%	Between varieties	3.96

For us, apart from the developmental differences, the differences in susceptibility to other diseases seem to be the most important. In the greenhouse only *Fusarium* is present under nearly sterile conditions, other diseases do not influence the reaction to *Fusarium*. On the contrary, in the field a number of other diseases occur, some of them produce even epiphytotics, which may influence the reaction to *Fusarium*. To investigate this, we evaluated the field plots two weeks after the second infection for powdery mildew and other diseases. In 1975 only powdery mildew was epidemic, therefore we concentrated to this disease.

4. Investigation of the effect of powdery mildew. The influence of the powdery mildew on *Fusarium* infection is shown in Table 7. Correlation coefficients were calculated between *Fusarium* results and powdery mildew infection. The consequences are as follow.

Table 5

Performance of the wheat varieties infected by *Fusarium graminearum* in the greenhouse. Data represent the mean of 4 replications related to the control in %

Variety	Shoot length	Root length	Root infection	Dry matter content	Emergence
I/1	0.64	0.33	100.00	2.50	6.66
I/3	15.49	3.46	96.52	26.16	26.78
I/5	7.20	1.89	95.78	11.13	30.00
I/6	1.74	0.11	93.85	7.39	5.77
I/7	1.75	1.17	97.50	8.09	8.93
I/8	5.05	3.09	98.21	27.21	51.79
I/9	5.18	4.11	96.06	11.02	36.67
I/10	1.62	1.30	99.98	15.18	31.67
I/11	2.45	2.96	92.29	28.99	24.44
II/1	4.39	3.40	97.44	16.67	41.67
II/2	11.33	6.47	90.59	19.39	28.57
II/3	2.50	1.31	98.12	20.40	21.13
II/4	0.00	0.00	95.00	0.00	0.00
II/5	2.41	0.72	93.08	33.34	23.08
II/6	1.57	2.63	99.33	31.55	18.34
II/7	1.60	0.97	95.45	4.89	10.71
II/8	2.61	0.77	100.00	4.89	16.07
II/10	1.71	0.37	99.33	15.20	21.43
II/11	0.74	0.52	99.98	6.77	15.00
II/12	2.04	1.32	99.98	19.39	20.00
Mean	3.10	1.84	96.91	15.51	21.95
LSD 0.1%	9.79	5.52	25.98	35.23	38.50
LSD 1%	7.58	4.28	20.12	27.27	35.10

1. Both infection type and infection severity increase the predisposition and the susceptibility to *Fusarium* attack. 2. The strongest influence is recorded for the first infection just after flowering. Later infections are less influenced, because more developed seeds are less sensitive and the possibility for an influence is lower. This is valid for all of the parameters investigated. 3. Primary symptoms are more influenced than secondary ones, here the correlation coefficients are significantly higher and less of decreasing tendency than for the yield components. This means that the results of the artificial inoculation methods are of limited value for selection purposes or determining differences between varieties, because the result is a resultant of a number of influencing agents. This is not the problem of inoculation technique, but it is a general problem being present in every host–pathogen relationship, both under natural or artificial conditions. This does not mean that artificial inoculation methods are not valuable, but the results should be really understood considering all

Table 6

The ranks of the varieties according to their different parameters of the greenhouse experiment

Variety	Shoot length	Root length	Root infection	Dry matter content	Emergence	x
I/1	18	18	19.5	19	19	18.70
I/3	2	4	9	5	6.5	5.30
I/5	4	8	7	12	8.5	7.90
I/6	13	19	4	15	17	13.60
I/7	12	12	11	14	18	13.40
I/8	6	6	13	2	1	5.60
I/9	5	3	8	13	3.5	6.50
I/10	15	9.5	16	10	5	11.10
I/11	1	1	1	1	2	1.20
II/1	7	5	10	9	3.5	6.90
II/2	3	2	2	7.5	6.5	4.20
II/3	9	11	12	6	11.5	9.90
II/3	9	11	12	6	11.5	9.90
II/4	20	20	5	20	20	17.00
II/5	10	15	3	3	10	8.20
II/6	19	7	14	4	13	11.40
II/7	16	13	6	17.5	14	13.30
II/8	8	14	19.5	17.5	15	14.80
II/10	14	17	15	11	11.5	13.70
II/11	17	16	17.5	16	16	16.50
II/12	11	9.5	17.5	7.5	8.5	10.30

LSD 0.1%	4.95
1%	3.51
5%	2.46

important influencing agents. We do not think that this is the only agent influencing inoculation results by *Fusarium*, but in our case this was considered to be the most important. We suppose that the different reaction of the varieties to other diseases may be the reason or one of the reasons of the inconsistent results presented in the literature (SCOTT, 1927; MESDAG, 1976).

We emphasize therefore that the relationships between seedling and adult stage resistance should be studied with varieties being nearly or completely free of other diseases, or controlled chemically without an effect on *Fusarium* disease development.

5. Relationships between the parameters. The different symptoms were evaluated in detail to detect the relationships between them. The analysis was conducted using the data of the first infection, where the disease incidence was the most severe and using the average data of the three field inoculations (Table 8). The most important consequences are as follow. 1. The field parameters are closely connected with each other, the decrease in yield and 1000 kernel weight

Table 7

Effect of powdery mildew infection on the performance of wheat to *Fusarium graminearum*

Infection		1000 kernel weight	Yield	Ratio of the killed seeds after 4 days	Ratio of the killed seeds after 7 days	Ratio of seeds infected by *F. graminearum*
Infection type	1st inf.	−0.422*	−0.431*	0.666***	0.639***	0.597***
	2nd inf.	−0.155	−0.168	0.491***	0.420*	0.466**
	3rd inf.	−0.100	−0.106	0.284	0.431	0.216
	Mean[1]	−0.297	−0.363	0.601***	0.588***	0.610***
Infection severity	1st inf.	−0.291	−0.387*	0.551***	0.526***	0.493***
	2nd inf.	−0.095	−0.145	0.578***	0.551***	0.560***
	3rd inf.	0.267	0.049	0.344	0.326	0.314
	Mean[1]	−0.072	−0.262	0.618***	0.608***	0.659***

*P = 10%
**P = 5%
***P = 1%

[1] This mean has been calculated by the averages of the three infections, not by considering the correlation coefficients.

is due to the *Fusarium* infection. The relationships are more close in the case of the first infection. This confirms our earlier results (MESTERHÁZY, 1975). The greenhouse parameters also show close relationships, but the level of the significance is somewhat less than observed at the field data. 2. The correlation coefficients between natural infection and the other parameters, presented in column 7, show close relationships in most of the cases. This means that this method developed in our laboratory gives good agreement with the natural infection and can replace the natural infection in breeding work. Considering that the correlations were counted between parameters influenced with powdery mildew, we concluded that both artificial and natural infection are influenced by powdery mildew. Therefore the natural infection is not of absolute value and is not connected as closely to the genetical background as often supposed.

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Table 8

Correlation analysis of the field and greenhouse results

Symptoms	Field evaluation					Greenhouse evaluation						
	1000 kernel weight	Yield	Field evaluation	No. of the killed seeds after		No. of the killed seeds by *F. graminearum*		Shoot lenght	Root lenght	Root infection	Dry matter content	Emergence
				4 days	7 days	infected	control					
	1	2	3	4	5	6	7	8	9	10	11	12
A) Field data represent the results of the first infection												
1	—	0.778^{a}	-0.418^{e}	-0.733^{a}	-0.700^{a}	-0.696^{a}	−0.351	−0.170	0.377	−0.118	0.220	0.064
2	—	—	−0.304	-0.782^{a}	-0.784^{a}	-0.758^{a}	-0.457^{d}	0.201	0.316	−0.117	0.084	0.173
3	—	—	—	0.404^{e}	0.357	0.436^{e}	0.145	0.300	0.096	0.114	0.061	0.272
4	—	—	—	—	0.959^{a}	0.970^{a}	0.554^{c}	0.161	0.025	−0.199	0.072	0.072
5	—	—	—	—	—	0.933^{a}	0.533^{c}	0.097	−0.017	−0.171	−0.139	−0.075
6	—	—	—	—	—	—	0.520^{c}	0.213	0.066	0.111	−0.088	0.100
7	—	—	—	—	—	—	—	0.004	0.106	−0.010	0.075	−0.151
8	—	—	—	—	—	—	—	—	0.727^{a}	−0.337	0.344	0.467^{d}
9	—	—	—	—	—	—	—	—	—	-0.427^{e}	0.455^{d}	0.650_{b}
10	—	—	—	—	—	—	—	—	—	—	−0.209	0.002
11	—	—	—	—	—	—	—	—	—	—	—	0.548_{c}
B) Field data represent the results of the three field infections as means												
1	—	0.927^{a}	-0.588^{b}	−0.317	−0.274	−0.348	−0.231	0.326	0.393^{e}	0.160	0.178	0.012
2	—	—	-0.519^{c}	-0.452^{d}	-0.420^{e}	-0.551^{c}	−0.174	0.161	0.283	−0.135	−0.023	−0.053
3	—	—	—	0.135	0.093	0.134	0.144	0.096	0.023	−0.156	0.121	0.269
4	—	—	—	—	0.945^{a}	0.975^{a}	0.497^{d}	−0.032	0.098	−0.106	−0.065	0.118
5	—	—	—	—	—	0.929^{a}	0.659^{b}	0.033	−0.014	−0.162	−0.064	−0.099
6	—	—	—	—	—	—	0.441^{e}	−0.024	0.058	−0.078	−0.068	0.070
7	—	—	—	—	—	—	—	−0.204	−0.094	−0.088	0.018	−0.151
8	—	—	—	—	—	—	—	—	0.727^{a}	−0.337	0.344	0.467
9	—	—	—	—	—	—	—	—	—	0.427^{e}	0.455^{d}	0.549_{b}
10	—	—	—	—	—	—	—	—	—	—	−0.209	0.002
11	—	—	—	—	—	—	—	—	—	—	—	0.548_{c}

^{a} P = 0.1%, ^{b} P = 1% ^{c} P = 2% ^{d} P = 5% ^{e} P = 10%

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Powdery Mildews on *Verbena X Hybrida*

By

GYÖNGYVÉR SZ. NAGY* and KLÁRA G. ALBERT

*Research Institute for Plant Protection 1525 Budapest, P. O. Box 102, and Research Institute for Fruitgrowing and Ornamentals, Budapest

The powdery mildew disease of *Verbena X hybrida* is caused by two species, *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* in Hungary. The identification of pathogens was carried out chiefly on the basis of the conidial states. Cleistothecia of *S. fuliginea* were found only. On the basis of the wall-cells of cleistothecia and the results of cross inoculations this species proved to be identical with *S. fuliginea*. The other species, *E. cichoracearum* is a strongly specialized form for Verbena.

Powdery mildew on *Verbena X hybrida* was observed in the experimental fields and glasshouse of the Research Institute for Fruitgrowing and Ornamentals, in Budapest. The lines of the cv. "Hungaria", improved by dr. Z. KOVÁCS, Head of the Department for Ornamentals, were infected in different extent. During the first observations only the conidial state, known as *Oidium verbenae* THÜM. et BOLLE, was found. On the basis of literature data this imperfect state may belong to *Erysiphe polyphaga* HAMM., *Sphaerotheca fuliginea* (SCHLECHT. ex FR.) POLLACCI, *S. verbenae* SAVULESCU et NEGRU or *Microsphaera ferruginea* ERIKSSON (BLUMER, 1967). These powdery mildew species are known on other *Verbena* species, too. Besides the above mentioned mildew species even *Erysiphe cichoracearum* DC. ex MÉRAT emend. SALMON has been published on this host from various parts of the world (HIRATA, 1966).

Powdery mildew of *Verbena* was described from Hungary by MOESZ (1939) and UBRIZSY (in UBRIZSY and VÖRÖS, 1966) as *Oidium verbenae* on *Verbena chamaedryfolia* (Balatonszemes, Budapest, Mátraháza). FOLK (in MARTINOVICH *et al.*, 1975) published the powdery mildew of *Verbena* spp. also as *Oidium verbenae*.

Thus the identity of the powdery mildew of *Verbena* has not been known in Hungary. This paper deals with the identification of the powdery mildew on *Verbena X hybrida*.

Material and Methods

At first the morphological characteristics of the conidial state (development, form and germination of conidia, shape of germ tube and appressoria, presence or lack of fibrosine bodies in conidia) were investigated (SZ. NAGY, 1970). The

following steps were to search for cleistothecia and to investigate their morphological characters (number of asci and ascospores and the shape of the cleistothecial wall-cells (BLUMER, 1967).

Cross inoculations were carried out between cucumber and *Verbena* with conidia of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* in laboratory by means of leaf disc method and under glasshouse conditions on potted plants (SZ. NAGY, 1972).

Results

The white mildew cover appears on the upper and lower surfaces of the leaves and on other green parts of the plants. The first symptoms could be find in gardens in July, and the plants are damaged until the end of the vegetation period. Powdery mildew is present on plants in glasshouses throughout the year. Powdery mildew on "Hungaria" and other cultivars of *Verbena X hybrida* was found in public gardens of Budapest, too. *V. rigida*, a frequently planted *Verbena* species in gardens, was not affected by mildew in 1976.

Identification on the basis of the imperfect state

Two types of conidial form were found. The one type of the conidia contains fibrosine bodies, the other one does not. But the conidia of both types develop in chains (5–6 conidia/day).

The conidia with fibrosine bodies are elliptical. Their sizes are: $28-31 \times 15-21\ \mu$ ($23-36 \times 13-25\ \mu$, $29.29 \times 18.28\ \mu$ in mean), length/width is 1.62. The germ tubes, starting from the ventral part of the conidia, are mostly simple, slightly curved, sometimes forked, which latter phenomenon is exclusively characteristic to *S. fuliginea* (HIRATA, 1942).

Conidia without fibrosine bodies are cylindrical. Their sizes are $28-31 \times 15-18\ \mu$ ($23-39 \times 13-23\ \mu$, $29.16 \times 16.39\ \mu$ in mean), L/W = 1.78. The germ tubes, starting from one of the corners of conidia, are simple, straight. The appressoria are club-shaped. This conidial state belongs to the *Erysiphe cichoracearum*-type (Euoidium) (HIRATA, 1942).

Identification on the basis of the perfect state

Cleistothecia were found on the leaves of *Verbena X hybrida* "Hungaria" on the 29th of October, 1975. Their size is $87-110\ \mu$ ($76-130\ \mu$) in diameter. The appendices are mycelium-like. One ascus with 8 ascospores develops in each cleistothecia. The appearance of the wall-cells of cleistothecia agrees with those of *S. fuliginea* on cucumber.

Inoculation tests

Powdery mildew colonies developed on leaf discs both of cucumber and *Verbena* under laboratory conditions, when they were inoculated with conidia of *S. fuliginea* from cucumber or *Verbena*

But the rate of the growth and the development of the mildew was influenced by the origin of the inocula. The growth was slightly faster and the conidia formed earlier when the leaf discs of *Verbena* was inoculated with mildew from *Verbena* or cucumber from cucumber, than in the case of inoculation *Verbena* from cucumber or cucumber from *Verbena*. Artificial inoculations in glasshouse with *S. fuliginea* were also successful on both cucumber and *Verbena* plants. Moreover some spontaneous infections were observed in a glasshouse where healthy *Verbena* plants were planted among cucumber plants infected by *S. fuliginea*.

Inoculations with *E. cichoracearum* were less successful. Although the conidia germinated on the leaf discs of both cucumber and *Verbena*, but colonies developed only on the *Verbena* leaf discs when the conidia originated from *Verbena* and on the cucumber leaf discs when the conidia originated from cucumber. *E. cichoracearum* colonies developed on *Verbena X hybrida* leaves under glasshouse conditions even if the inoculum originated from cucumber, but the development of the mildew was slower on *Verbena* than on cucumber plants.

Discussion

Investigations on the basis of the conidial state proved that two powdery mildew species infect *Verbena X hybrida* in Hungary.

Erysiphe polyphaga and *Microsphaera ferruginea*, mentioned by BLUMER (1967) as the pathogens on *Verbena* spp., are out of question, because the name *Erysiphe polyphaga* is not valid and not right (SZ. NAGY, 1975b); and the colour of mycelia and colonies of *M. ferruginea* is light brown and conidia of *Microsphaera* spp. are formed singly. Whereas the powdery mildews on *Verbena* form white cover on the host and the conidia develop in chains. This latter species has not been found since its publication by ERIKSSON in 1882–1884 (BLUMER, 1967).

The powdery mildew with conidia containing fibrosine bodies belongs to the genus *Sphaerotheca*, and it may be *S. fuliginea* or *S. verbenae*. The morphological characters of the conidial state, however entirely agrees with *S. fuliginea*. The only morphological difference, given by BLUMER (1967) between these two *Sphaerotheca* species is the shape of the wall-cells of the cleistothecia, although this difference is not reported in the original description of *S. verbenae* (SAVULESCU and NEGRU, 1953). The size of the cleistothecia studied by us is nearer to those of *S. verbenae* (83–100 μ), but the shape of the wall-cells agrees with those of *S. fuliginea* on cucumber. The cross inoculation tests in laboratory and in glass-

house and the spontaneous infection also proved that this powdery mildew on *Verbena X hybrida* is identical with *S. fuliginea.*

In the case of the other mildew species we have to depend on the conidial state, since cleistothecia have not been found. This imperfect state is of *Erysiphe cichoracearum*-type and it agrees in its morphological characteristics with those of *E. cichoracearum* on other plants (cucurbits, Aster, Phlox etc.) (Sz. NAGY, 1970, 1975a). Inoculations with *E. cichoracearum* from cucumber were successful on *Verbena X hybrida* under glasshouse conditions, but not in the laboratory. The conclusion can be drawn, that the other powdery mildew infecting *Verbena X hybrida* is *Erysiphe cichoracearum*, but it is a strongly specialized form for this host.

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Studies on the Rust of *Majorana hortensis* Mnch. in Egypt

By

ABDEL-HAMID M. TARABEIH

Department of Plant Pathology, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt

Outbreak of sweet marjoram rust have recently been prevalent in El-Kanater El-Khieria near Cairo. Symptoms of the disease and the morphological features of the causal organism are reported here for the first time in Egypt. Effect of the pathogen on the fresh weight and percentage of volatile oil in the leaves are also reported. The use of an effective fungicide for the chemical control of the disease is suggested.

Sweet marjoram (*Origanum majorana* L. or *Majorana hortensis* Mnch.) is one of the medicinal and aromatic plants. The occurrence and cultivation is known from different parts of the world, U.S.S.R., China, Germany, Czechoslovakia, Hungary, France, Tunis, U.S.A. etc.

Sweet marjoram has been cultivated in Egypt since 1952 as a perennial plant for foreign markets. In industry, the oil is used in perfumes, soaps and some liquors.

In the survey of EL-HELALY *et al.* (1966) on plant diseases in U.A.R. and their causative organisms, the marjoram rust has not been recorded. This rust may reduce the crop yield by up to 70%. It is the aim of this paper to report some observations on the disease, to outline certain suggestions for its control and to give a concise description of the causative organism.

The rust of marjoram has been recorded by many authors. DURRIEU (1956) reported *Puccinia menthae* on *Origanum*, GUYOT (1953) cited *Puccinia rubsaameni*, and MEHTIEVA (1959) recorded *P. menthae* on *Origanum vulgare*.

Symptoms of the disease and characteristics of the causative organism

The rust was observed for the first time in Egypt in August 1972, when it was found on some sweet marjoram plants grown from seedlings and obtained from the Medicinal and Aromatic Experimental Station of the Ministry of Agriculture at El-Kanater El-Khieria, near Cairo.

Symptoms of marjoram rust become visible early in October and November. The pathogen produces brown uredosori 0.5 to 1 mm in diameter. Uredia are noticed on the lower surface of the leaf and are consequently surrounded by yellow halo (Fig. 1). Uredia are also observed on the infected stems. Teleutospores could

not be detected. Uredospores are one celled, light brown, thick walled, warty, oblong to ovate 21.3–28.4 × 17.75–21.3 μ (av. 23.8 × 19.5 μ) (Fig. 2).

The rust noticed to bring about a partial or compelete premature defoliation and a general loss of vigor of the affected plants.

Fig. 1. Naturally infected sweet marjoram leaves showing uredosori surrounded by yellow halos

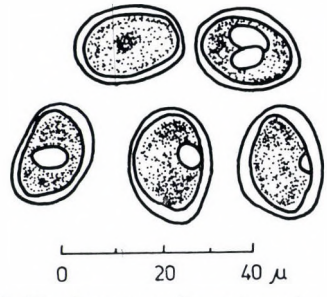

Fig. 2. Uredospores of sweet marjoram rust

Chemical control of sweet marjoram rust

For the control of the disease, certain available commercial fungicides were tried as foliage spray. Plants were sprayed twice per month from the time of detection of the first signs of the disease. Each plant received 100 ml of the fungicidal solution. Control plants were sprayed with water. The fungicides used were as follow.

Benomyl (50% methyl-1-buthylcarbamyl)-2-benzimidazole carbamate), E.I., Du Pont de Nemours and Co., Wilmington, Delaware, U.S.A.

Dithane M−22 (80% manganese ethylene bisdithiocarbamate), Bayer Germany.

Dithane M−45 (80% zinc and manganese ethylene bisdithiocarbamate), Filital, Industrie, Chimiche, Italy.

The effect of the fungicides on the disease development was estimated on the basis of the fresh weight of the plants and the oil content of the leaves.

Table 1

Efficiency of certain fungicides in controlling marjoram rust, indicated as fresh weight in gm and percentage of oil in the leaves

Fungicide	Concentration %	Fresh weight in gm	% of volatile oil
Check	none	16.0	0.07
Benlate	0.05	43	0.67
	0.10	76	0.82
Dithane M-22	0.3	30	0.55
	0.5	50	0.75
Dithane M-45	0.3	21	0.50
	0.5	25	0.51

To determine the oil content of the leaves, plants were cut 12 cm high above the ground level. This was carried out in order to allow for better development of new branches of the plants for the second cut. Oil was extracted using a steam distillation apparatus (SLEEM, 1973) (Table 1).

Data in Table 1. reveal that:

The rust of sweet marjoram reduces both the fresh weight of the plants and the volatile oil content of the affected leaves.

Benlate, in a 0.1% concentration proved to be the best fungicide for the control of the disease. This treatment also prevents the reduction of oil content of the leaves. Dithane M−22 in a 0.5% concentration was somewhat less effective.

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Pycnidial Induction in *Macrophomina phaseolina*

By

S. H. MICHAIL, M. A. ABD-EL-REHIM and E. A. ABU ELGASIM

Faculty of Agriculture, University of Alexandria, Egypt

The pycnidial state, *Macrophomina phaseolina* was successfully produced by culturing the sterile state, *Sclerotium bataticola* on water-agar-leaf medium at 20°C under alternating 12 hours on/off cycles of NUV and darkness for 7—10 days.

The imperfect state of *Macrophomina phaseolina* (Tassi) Goid (= *Sclerotium bataticola* Taub.) was encountered in seed samples of four soybean cultivars namely: Bossier, Bragg, Clark and Hark, plated on potato dextrose agar (PDA) medium. Although pycnidia of this fungus had been observed on many hosts (KULKARNI *et al.*, 1962; MATHUR, 1967 and KULKARNI and PATIL, 1968), yet they are not produced in conventional media. The fungus may, however, sporulate on special media (LUTTRELL, 1946 and ASHWORTH, 1959). In the present investigation, the water-agar-leaf medium suggested by SRINIVASAN *et al.* (1971) and CHIDAMBRAM and MATHUR (1975) was adopted. Mycelial discs from the growing margins of 48 hours old cultures of *S. bataticola* were transferred to autoclaved leaf bits (2—3 cm long) of wheat, barley, maize and *Agrostis semiverticillata* placed on 1.5% water-agar medium in pyrex petri dishes. The dishes were incubated at 20°C under 12 hours alternating cycles of near ultraviolet light (NUV) and darkness. Observations for pycnidial production were recorded every two days using the stereobinocular microscope. Pycnidia were observed after 7 days on the leaf bits of wheat, barley and maize, and after 10 days on *A. semiverticillata*. Pycnidia (Fig. 1) appeared as raised, grey to black bodies. Pycnidia were erumpent, oval to globular with a distinct ostiole, $80-200 \times 75-185$ μ (av. 140×130 μ). Pycnidiospores 1-celled, hyaline, elongate, $10-24 \times 6-8$ μ (av. 17×7 μ). These measurements fairly coincide with those reported by KULKARNI *et al.* (1968) on *M. phaseolina*.

The numbers of pycnidia produced on different substrates were assessed by agitating vigorously 5 ml of sterile tap water with pycnidia on each of the tested leaf bits. The density of the resulting pycnidia suspension was determined by the aid of haemocytometer, and the average numbers of pycnidia per 1 ml were calculated. The average numbers of pycnidia produced on wheat, barley, maize and *A. semiverticillata* were 125, 95, 95 and 55 respectively.

The simplicity of this procedure favours its potential usefulness in the study of sterile and non-sporulating fungi. The autoclaved leaf bits being transparent

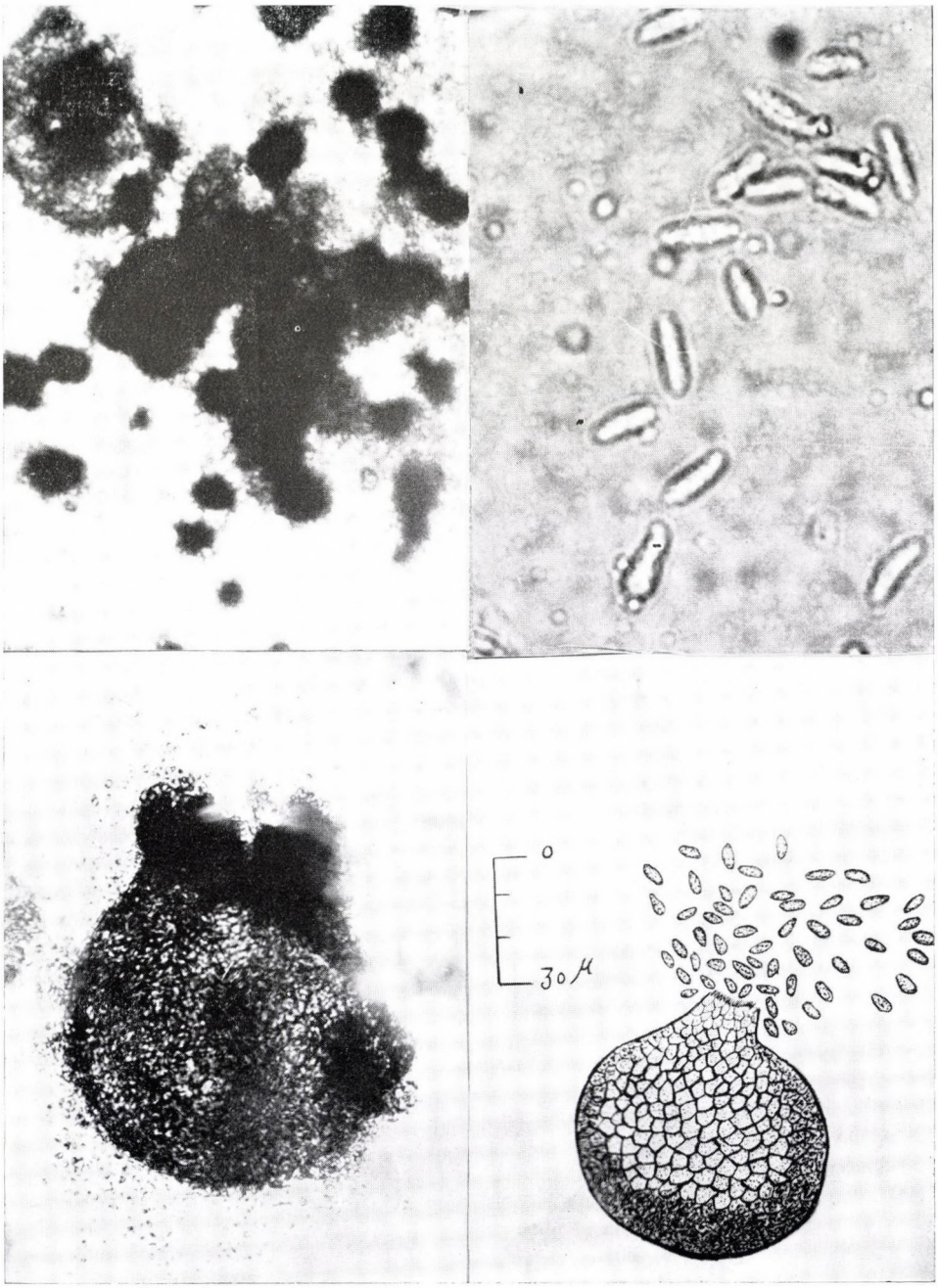

Fig. 1. Pycnidia and pynidiospores of *M. phaseolina;* photomicrograph of (upper left × 77) pycnidia (larger bodies) and sclerotia (small bodies), (upper right × 355) pycnidiospores, (lower left × 268) pycnidium and pycnidiospores; (lower right) camera lucida drawing of pycnidium and pycnidiospores

facilitate direct observation of the sporulating structures under the stereobinocular as well as the compound microscope. The results obtained in this study confirm the work of SRINIVASAN *et al.* (1971) and CHIDAMBRAM and MATHUR (1975), that leaves of Graminae are favorable substrata for sporulation of certain fungi. *Alternaria porri* (Ellis) Cif., the onion scald fungus in Egypt, which remained non-sporulating for years in spite of the different methods used to induce its sporulation, recently furnished profuse sporulation by culturing it on autoclaved pieces of *A. semiverticillata* young leaves placed on water-agar after 15 days on 20°C under alternating 12 hours on/off cycles of NUV and darkness (MICHAIL *et al.*, 1976).

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Root-Region Actinomycete Flora of Coriander*

By

O. P. MALL

Botany Department Science College Vikram University, Ujjain, India

Quantitative estimation of rhizosphere and rhizoplane microflora of two coriander varieties has revealed greater root influence on actinomycete and bacteria than on fungi. Out of these two varieties, local variety is very susceptible to *Fusarium* wilt while other variety MP 5365 is moderately resistant for the wilt. In rhizosphere 16—22 actinomycete species were recorded in both varieties. Actinomycete population was less in seedling, reached maximum at vegetative and flowering stages and then dropped to negligible at seed stage of the plant. After maturation of seeds, the frequency of certain actinomycetes increased manyfold. Predominant actinomycete species recorded from rhizosphere were *Streptomyces kitasawaensis*, *S. griseobrunneus*, *S. erythrochromogenes*, *S. albosporeus*, *S. pseudogriseolus* and *Micromonospora* species. Besides these species, the vegetative and flowering stage of MP 5365 were dominated by *S. cyaneus* and *S. bikiniensis* and those stages of local variety by *S. wedmorensis*. Further manyfold increase of *S. kitasawaensis*, *S. bikiniensis*, *S. erythrochromogenes*, *S. olivaceum* and *Streptomyces* spI in MP 5365 and that of only *S. kitasawaensis* and *S. wedmorensis* in local variety was recorded at harvest stages of the plants. These differences in the actinomycete flora of two varieties at vegetative and flowering stages are of significance in the establishment of the pathogen in the infection-court while differences at harvest stage are significant in the survival of the pathogen after crop maturation. In rhizoplane *S. flavochromogenes* and *S. gougeroti* were more noticeable and *S. erythrochromogenes*, *S. albosporeus* and *Micromonospora* sp. were not recorded.

A large number of data has accumulated to establish positive root influence on the flora around the root (KATZNELSON, 1960; LOCHHEAD, 1959). The root influence is maximum at the root surface and diminishes with the increasing distance. Real picture of the root influence would, therefore, emerge by quantitative and qualitative estimation of rhisoplane (CLARK, 1949; KATZNELSON, 1960). But most of the studies on estimation of root influence have taken into account only bacterial and fungal population of rhizosphere. Actinomycetes constitute an important part of root-region microflora but only a few papers have appeared on its behaviour. In view of scanty information on these aspects this work of quantitative and qualitative estimation of rhizosphere and rhizoplane actinomycete population of coriander was undertaken.

Material and Method

Two varieties of coriander a local and an improved MP 5365 were employed for this study. The local variety was very susceptible and MP 5365 was comparatively resistant to the *Fusarium wilt*. The plants were sown in earthen pots having block cotton soil and the pots were kept under field conditions (the experiments were done from September to December when mean maximum temperature varied 30–30°C and mean minimum temperature varied 16–19°C). Samples were drawn after four days of emergence of seedling and subsequent samples were drawn at four days interval till the plants were twenty days old (in this way five samples were taken, the first sample constituted the seedling stage and average data from second to fifth reading constituted vegetative stage). After twenty days the interval for sampling was increased to ten days and this was continued till the crop was ripe for harvest. (Average data from sixth and seventh samples constitute flowering stage, followed by seed and harvest stage respectively.) In this way all the data collected were represented in five stages of plant development viz., seedling, vegetative, flowering, seed and harvest in all the tables.

For control soil one pot was left without sowing and four samples were taken from this and only two average reading is given in the tables as there was not much variation in the soil microflora. For sampling of rhizosphere and control soils and for rhizoplane methods outlined by KATZNELSON (1960) were followed. Suitable dilutions of soil suspensions and root homogenate were plated separately on soil-extract agar for actinomycetes and bacteria and on peptone-dextrose-rose bengal-streptomycin agar for fungi. Colonies appearing on the agar plates were counted and their actual numbers were calculated. Actinomycete colonies were transferred and maintained on soil-extract semisolid agar deeps. Identification of actinomycetes was done on the basis of morphological and physiological characteristics following WAKSMAN (1962).

Results

Rhizosphere – R : S ratios of bacteria, actinomycetes and fungi of two varieties of coriander is presented in the Table 1. R : S ratio was maximum on actinomycetes followed by bacteria and minimum on fungi. In seedlings rhizosphere effect was slight and increased 3–5 times during vegetative growth. At flowering stage the ratio was almost same but decreased considerably during seed stage. In the last sampling when the seeds were mature and the plants were ready for harvest another increase in the population was recorded which was more significant in bacteria. Comparing the R : S values of two coriander varieties variety MP 5365 in general showed slightly higher values in bacteria and fungi. In the case of actinomycetes local variety showed a higher R : S ratio.

Rhizoplane – Population on the root surface is expressed as number per gram of moist root and is represented in the Table 2. Here trend of population

Table 1

Rhizosphere ratio and incidence expressed as 10^6/g of soil of Bacteria, Actynomicetes and Fungi in rhizosphere of local and MP 5365 varieties of Coriander at different stages of plant development

Variety	Organism	Seedling	Vegetative	Flowering	Seed	Harvest
MP 5365	Bacteria					
	R	53.08	300.8	39.38	90.50	1205.0
	S	70.50	69.15	63.4	59.0	59.0
	R : S	1<	4.31	4.62	1.53	20.4
MP 5365	Actinomycetes					
	R	4.04	18.3	19.52	5.5	2.34
	S	6.0	4.5	4.5	6.0	6.0
	R : S	1<	1< – 6.1	4.25	1<	1<
MP 5365	Fungi					
	R	90.72	136.33	126.62	105.80	141.84
	S	61.32	56.98	52.09	51.78	51.78
	R : S	1.12	1.75	2.95	1.98	2.64
Local	Bacteria					
	R	162.08	344.66	348.6	150.00	1360.0
	S	105.2	102.82	109.35	118.2	118.2
	R : S	1.54	3.33	3.4	1.2	11.5
Local	Actinomycetes					
	R	2.30	17.75	20.7	2.40	16.00
	S	2.00	3.0	4.4	4.80	4.80
	R : S	1.15	5.8	4.7	1<	3.3
Local	Fungi					
	R	161.80	199.93	234.09	119.02	113.60
	S	121.9	115.5	101.43	101.90	101.90
	R : S	1.13	1.66	2.04	1.10	1.22

R — rhizosphere soil
S — control soil
R : S — rhizosphere ratio
1 < — less than one

Table 2

Rhizoplane microflora in two varieties of *Coriander* (Numbers in 10,000/gram of moist root)

Variety	Organism	Seedling	Vegetative	Flowering	Seed	Harvest
MP 5365	Bacteria	540.0	276.0	239.5	283.0	780.0
	Actinomycetes	35.0	15.8	23.0	40.2	240.0
	Fungi	0.2	0.3	2.9	4.1	19.4
Local	Bacteria	758.5	443.0	536.2	857.5	1080.0
	Actinomycetes	25.2	18.5	16.7	17.5	100.0
	Fungi	0.1	0.8	3.4	4.5	33.6

Table 3

Percentage occurrence of *Actinomycetes* in rhizosphere and rhizoplane of *Coriander* variety MP 5365 at various stages of development

Name of species	Control soil	Rhizosphere soil					
		Seedling	Vegeta-tive	Flower-ing	Seed	Harvest	Control soil
Micromonospora parva	8.3	—	5.2	3.0	—	—	19.3
Micromonospora sp.	3.3	—	2.4	0.4	—	—	4.3
Streptomyces aureus	0.8	—	1.4	7.1	—	—	20.0
S. albosporeus	0.7	—	1.4	7.1	—	—	1.2
S. bikiniensis	8.3	—	11.8	3.6	—	8.4	5.8
S. cinnamonensis	—	—	0.6	2.5	—	—	—
S. cyaneus	—	—	0.4	7.1	—	—	8.1
S. erythrochromogenes	—	—	4.9	7.4	—	10.5	—
S. filamentosus	0.7	—	1.5	2.1	—	—	0.5
S. flavochromogenes	—	—	1.9	5.0	—	—	—
S. griscobrunneus	9.1	12.5	0.3	2.2	—	—	8.1
S. griseochromogenes	—	10.4	4.1	3.6	—	—	—
S. kitasawaensis	16.6	59.4	45.7	30.9	27.2	32.7	20.8
S. lucensis	—	—	0.4	3.6	—	—	—
S. pseudogriseolus	0.5	9.9	3.0	3.5	—	—	2.5
S. olivaceus	—	—	0.3	5.5	—	8.4	—
Strepromyces Sp. I.	—	—	1.5	7.1	72.4	38.7	1.2
Streptomyces Sp. III	0.3	6.5	12.4	—	—	—	—
		Rhizoplane					
Strepromyces flavochromogenes		14.3	16.6	7.8	—	—	
S. kitasawaensis		14.3	14.3	30.0	—	—	
S. pseudogriseolus		14.3	7.2	7.8	—	—	
S. gougeroti		28.6	7.2	15.6	—	12.3	
S. bikiniensis		—	19.3	7.8	—	12.3	
Streptomyces Sp. I		28.6	40.8	33.5	100.0	73.8	

was entirely different from that of rhizosphere. The root influence in all stages was maximum on bacteria followed by actinomycetes and fungi. Population of actinomycetes was high in seedlings and then slightly decreasing with the age of plants but rising again with the maturation of seeds. In contrast with the rhizosphere actinomycetes on the root surface were poorly represented. As regards difference in two varieties MP 5365 showed higher number of actinomycetes as compared to local variety.

Trend of species of actinomycetes in rhizosphere and rhizoplane — Tables 3 and 4 record trend of actinomycete population in rhizosphere and rhizoplane of two coriander varieties. In the rhizosphere of both the varieties 16—22 species of actinomycetes were recorded. Rhizosphere of the local variety though having higher R : S ratio was inhabited by fewer number of species. In the rhizosphere of seedlings of both the varieties *S. kitasawaensis* and *S. griseobrunneus* occurred

Table 4

Percentage occurrence of *Actinomycetes* in rhizosphere and rhizoplane of *Coriander* variety local at various stages of development

Name of species	Control soil	Rhizosphere soil					
		Seedling	Vegeta-tive	Flower-ing	Seed	Harvest	Control soil
Micromonospora parva	—	21.5	7.1	4.8	100.0	—	—
Micromonospora sp.	—	8.6	6.8	7.2	—	—	—
Streptomyces aureus	10.0	—	1.4	—	—	—	9.5
S. albosporeus	2.5	4.3	3.7	4.8	—	—	3.2
S. cinnamonensis	12.0	—	—	—	—	—	9.6
S. cyaneus	—	1.4	—	—	—	—	—
S. erythrochromogenes	—	—	1.3	2.4	—	—	—
S. filamentosus	—	8.6	18.6	19.2	—	—	—
S. griseobrunneus	10.0	8.6	—	9.6	—	—	12.8
S. gougeroti	—	—	1.6	—	—	—	—
S. kitasawaensis	50.0	40.0	37.4	38.4	—	62.5	46.0
S. naganishi	—	—	0.7	7.2	—	—	—
S. olivaceus	—	—	1.7	—	—	—	—
S. pseudogriseolus	5.0	0.8	1.5	2.4	—	—	4.3
S. wedmorensis	—	3.4	6.5	—	—	37.8	—
Streptomyces sp. 4	10.0	3.0	13.1	4.9	—	—	15.2
		Rhizoplane					
S. flavochromogenes		—	48.0	24.3	—	57.1	
S. kitasawaensis		—	32.1	48.0	—	28.6	
S. pseudogriseolus		—	19.6	—	—	—	
S. griseobrunneus		—	—	29.3	—	14.3	

in higher percentage. In addition, *Streptomyces pseudogriseolus* in MP 5365 and *S. filamentosus* and *Micromonospora* species in the local variety were also present. Number of species colonizing the rhizosphere increased during vegetative growth and remained unchanged up to the flowering stage. Predominant species in these stages in both the varieties were *Streptomyces kitasawaensis*, *S. griseobrunneus*, *S. erythrochromogenes*, *S. albosporeus*, *S. pseudogriseolus*, *Streptomyces* sp. III and IV and *Micromonospora* species. Additional dominant species were *Streptomyces cyaneus* and *S. bikiniensis* in MP 5365 and *S. wedmorensis* in the local variety. Population of actinomycetes dropped considerably at seed stage but at harvest stage *Streptomyces kitasawaensis*, *S. bikiniensis*, *S. erythrochromogenes*, *S. olivaceus* and *Streptomyces* sp. I in MP 5365 and *S. kitasawaensis* and *S. wedmorensis* in local variety showed manyfold increase.

In rhizoplane actinomycete flora was considerably reduced and only 6 and 3 species were recorded in MP 5365 and local variety respectively. Trend of population on the root surface was comparatively homogenous than rhizosphere. The dominant species of rhizosphere were more uniformly represented in the rhizo-

plane except *Streptomyces flavochromogenes* which was noticeable in both the varieties. Similarly *Streptomyces gougeroti* appeared in good percentage in variety MP 5365.

Discussion

Taxonomic studies on actinomycete flora have received little attention. In most of these studies emphasis has been on their antagonistic properties. First attempt on actinomycete flora in the rhizosphere is that of ROUATT *et al.* (1951) and AGNIHOTRUDU (1955) isolated *Streptomyces griseus*, *S. erythrochromogenes* and species of *Nocardia* and *Micromonospora* from rhizosphere of wilt resistant pigeon pea plants. RHEM (1961) found noticeable population of *Streptomyces antibioticus*, *S. diastaticus*, and *Actinomyces chromogenes* in rhizosphere of barley plants. RANGASWAMI and VASANTHARAJAN (1962), on the other hand, found no rhizosphere effect on any specific taxonomic group of actinomycete. In the present study a positive root influence which was more significant on the root surface was recorded in both the coriander varieties. It was also noticeable in this study that stimulation of actinomycetes was almost similar to that of bacterial flora. Typical actinomycete flora of coriander rhizosphere constitute *Streptomyces kitasawaensis*, *S. giseobrunneus*, *S. erythrochromogenes*, *S. albosporeus*, *S. pseudogriseolus* and other species of *Streptomyces* and *Micromonospora*. Population in the rhizoplane was constituted by *Streptomyces kitasawaensis*, *S. griseobrunneus* and *S. filamentosus*. As mentioned earlier variety MP 5365 was moderately resistant to wilt disease and also showed increased population of actinomycetes, this is in accord with the observation on pigeon pea. Further it may also be recalled here that certain actinomycetes from the rhizosphere and rhizoplane of coriander were also found to be highly antagonistic by the author. These actinomycetes may play a very significant role in the establishment of the pathogen in the infection court at the time of disease incidence.

Manyfold increase in the microflora in root-region at harvest stage was an important observation made in this study. During developing stage of plant root-region has been considered an infection court for the successful pathogenesis. Similarly interactions developing at senescence stage of root could be of immense significance in the survival of pathogens.

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Damping-off of *Araucaria excelsa* Seedlings in Egypt

By

ABDEL-HAMID M. TARABEIH

Department of Plant Pathology, Faculty of Agriculture University of Alexandria, Alexandria, Egypt

Rhizoctonia solani, *Fusarium oxysporum* and *F. solani* were recorded for the first time in Egypt to cause damping-off of *Araucaria excelsa*, R. Br. seedlings.

Araucaria excelsa, R. Br. (Norfolk-Island-Pine) is an upright evergreen coniferous tree. It is grown as small, symmetrical, potted plants largely for Christmas scales.

Few *Araucaria* seeds can germinate; many factors are responsible for reduction of seed germination, one of them is due to fungal infection.

The purpose of this paper is to identify the pathogens causing losses to seed germination and seedlings.

In Egypt no previous work on the fungal effect on seed germination of *Araucaria* has been reported (EL-HELALY *et al.*, 1966).

YOUNG (1948) recorded *Rhizoctonia* on *Araucaria cunninghamii* causing root rot in forest nursery in South Quensland. SIMMONDS (1952) reported that *Sclerotium rolfsii* caused severe losses in one-year old hop pine (*Araucaria cunninghamii*) seedling at Como forestry nursery and he added that the seedling root rot observed on this host in several forestry nurseries was associated with a *Fusarium* sp. which could reproduce the disease in pot inoculation experiments. HOLMES and BUSZEWICZ (1969), cited that Batko in England recorded *Monochaetia* spp. on *Araucaria araucana* seed.

For the purpose of isolation, naturally diseased seeds and seedlings (Fig. 1) of *Araucaria excelsa*, were carefully dug out, washed thoroughly, surface sterilized by dipping in 0.1% mercuric chloride for 3 minutes, rinsed in sterile water, cut into small pieces, placed on potato dextrose agar (PDA) medium and then incubated at room temperature (23—27°C).

The isolation experiments revealed the presence of one species of *Rhizoctonia* and two species of *Fusarium*; the isolated fungi were purified and incubated on PDA slants for further studies.

For testing the pathogenicity of the isolated fungi viz. *Rhizoctonia solani*,* *Fusarium oxysporum** and *F. solani** pots filled with autoclaved sandy-loam soil were inoculated separately with the test organisms, watered daily, and five sterilized *Araucaria* seeds were used for each pot.

Fig. 1. Naturally infected seed and seedlings of *Araucaria excelsa*

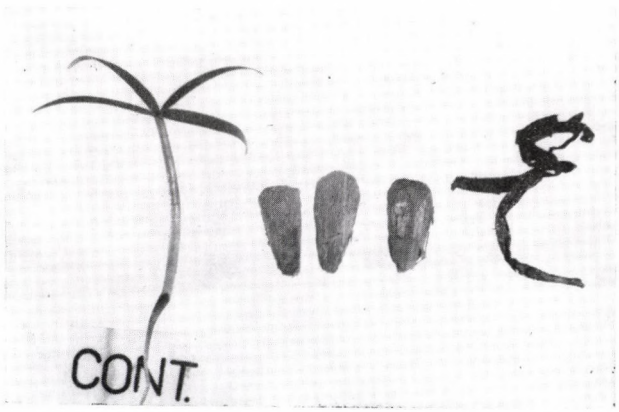

Fig. 2. Healthy seedlings (control), seeds and collapsed seedling artifically infected with *Rhizoctonia solani*

Table 1

Effect of soil inoculation by *Rhizoctonia solani*, *Fusarium oxysporum* and *F. solani* separately on seed germination and seedling development of *Araucaria excelsa*

Tested fungus	Pre-emergence damping-off, %	Damped-off of seedling, %	Healthy seedling, %
Check	5	0.0	95
R. solani	60	20	20
F. oxysporum	30	5	65
F. solani	25	3	72

Identity of the isolated fungi was kindly verified by the Commonwealth Mycological Institute at Kew, England.

The data in Table 1 reveal that:

In general, *R. solani* was highly pathogenic to *Araucaria* seed and seedlings as it reduced seed germination and incited damping-off of seedlings.

Pathogenicity of each of *F. oxysporum* and *F. solani* was, however, moderate on *Araucaria* seed and seedlings.

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Histological Investigations of the Gonads of some Lepidopterous Species Having Reduced Reproduction Elicited by Short Photoperiod

By

KATALIN V. DESEŐ,* BLANKA BENETTOVA-REZÁBOVÁ** and V. LANDA**

*Centro di Studio di Fitofarmacia, Università di Bologna (Italy),
**Entomological Institute of ČSAV, Prague (Bohemia)

Histological investigations showed that short photoperiod during egg, larval and pupal stages elicits changes in the gonads both in fermales and males. Changes were observed in the follicular epithelium cells of the half-mature ovocytes and that of the walls of the paired accessory glands of males. A big difference in the size of ovocytes in sequence in an ovariole and ovosorption in the newly emerged females were attributed also to the effect of a short photoperiod during pre-adult development. In males the length of the paired accessory glands and the colour of the fluid in them were also influenced.

Competition between eggs during maturation, and the existence of a "defence mechanism" are indicated.

In certain lepidopterous species fecundity is decreased after diapause attributed to weight loss during diapause (see reviewed by DESEŐ, 1973a). However, experiments carried out under laboratory and field conditions with the codling moth (*Laspeyresia pomonella* L. (Tortr.)), the plum fruit moth (*Grapholitha funebrana* Tr. (Tortr.)) and the oriental fruit moth (*L. molesta* Busck (Tortr.)), demonstrated that short photoperiod during pre-adult development without inducing diapause decreased the number of ovipositing females and partly also their fecundity. When the larval diapause occurs, physiological changes seem to increase since after diapause a large percentage of the adults did not even mate (DESEŐ and SÁRINGER, 1970; DESEŐ, 1973a, b). Topical application of Farnesylmethylether improved to a small extent both the receptivity of females and the vigour of males (DESEŐ, 1971, 1973a).

In other lepidopterous species PARKER and THOMSON (1927), NISKITSUTSUJJ-UWO (1953), SANTA and OTUKA (1955) and CLOUTIER and BECK (1963) studied the induction of diapause by short photoperiod and noted that the size of the testes decreased already before larvae were induced to enter diapause. Furthermore, HANSEN and HARWOOD (1968) observed degenerative changes both in the testes and ovaries of the codling moth after diapause. DESEŐ (1970), DESEŐ and SÁRINGER (1970) reported differences between the ovarioles of *G. funebrana*; in post-diapause females the vitellogenesis was either blocked or was slower than in the following summer generation. The ovarioles sometimes looked like those present in females immediately after egglaying. The percentage of females with non blocked vitello-

genesis was about the same as the percentage of females with normal fecundiy (15%).

As females in the autumn have the greatest weight, but their fecundity is about the same as in post-diapause females in spring of lower weight (DESEŐ, 1973b) and, as short-day photoperiod itself induces a decrease in the size of testes without diapause (DESEŐ, 1973a) suggests that it would be worthwhile to look for histological differences in the gonads of adults (1) emerging after diapause, (2) developing under short-day conditions without dapause and (3) exposed to a long-day photoperiod.

Materials and Methods

A population of *G. funebrana* from southwestern Hungary was examined histologically after diapause. The emerging females were kept in jars supplied with cotton, soaked in a 5—10% honey water solution, with or without plum leaves for olfactory stimuli, with or without males. Eleven females at a variety of ages (prior to mating, after mating and during ovideposition) were killed, cheked for spermatozoa and their ovaries were prepared for histological observation.

Histological studies were made also on the gonads from 24 postdiapause females and that of 20 males of *G. funebrana*, originating from different Hungarian orchards. From *L. pomonella* 28 postdiapause females and 18 males, collected in different Hungarian orchards, were histologically investigated. The adults of both species were kept together five days long and fed with honey water solution. After that period they were prepared for histological work. This was repeated with the first summer generation of *L. pomonella* as well.

Furthermore, two times 5 females and 5 males of laboratory population of *L. pomonella* (originating from Yakima (Washington) USA) and those of *L. molesta* (Hungarian laboratory population) were also investigated histologically. One group was allowed to enter and complete diapause, the other not. Diapause was inhibited by high temperature (DESEŐ, 1973a, b; 1974; 1975a, b).

The dissected ovarioles and the whole abdomen of the males were fixed in the cold Carnoy' solution (fixative) for 24 hours and transferred into paraffine through butanol. 4 μl thick sections were stained by Pappenheim's staining solution. The section were then mounted into Canada balsam.

Results

A) The gonads of the females, developing under short-day conditions and, when allowed, with an abnormal reproductive activity, showed the following changes indipendently that they completed or not diapause.

1. Resorption of full grown eggs is commonly seen in the females that lay no eggs, or only a few (Figs 1 and 2). However, the same can be observed in females

developed under long-day conditions after normal egglaying but with some mature eggs in the abdomen.

2. In the eggs half filled with yolk the cells of the follicular epithelium bordering the ovocyte are sometimes small, with relatively small nuclei, giving a thin outlook to the follicular epithelium bordering the ovocyte (Fig. 3). In females, developing under long-day conditions, these cells are yet always columnar and tightly packed with relatively large nuclei. The follicular epithelium therefore seems to be thick at this stage of vitellogenesis. However, thin follicular epithelium in the eggs half filled with yolk can occur also in long-day females after egglying.

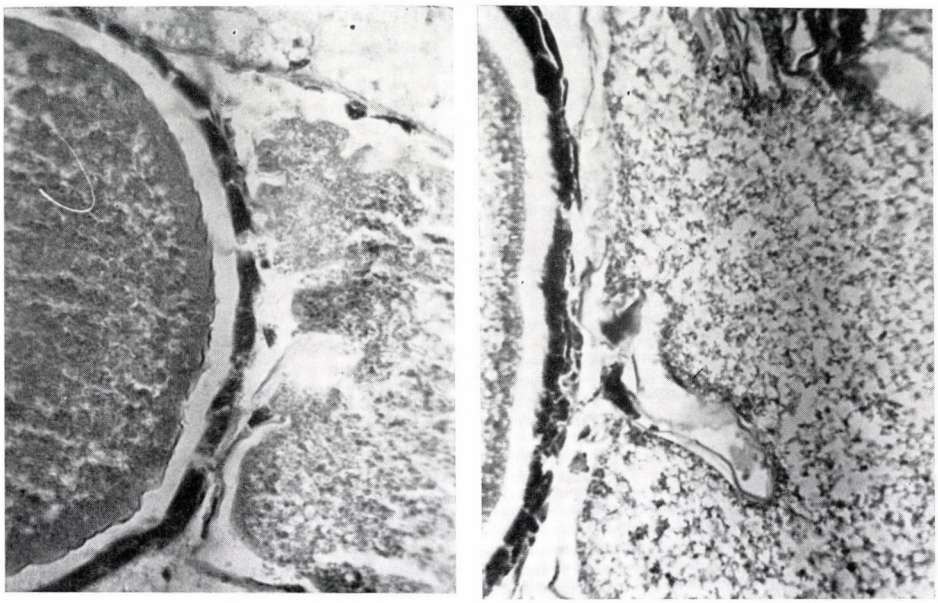
Furthermore, in the follicular epithelium cells of short-day females can be observed sometimes more nuclei than one (Figs 4, 5, 6, 7). If there can be made whatever a comparison with other species as *Habrobacon juglandis* Ashmead (CASSIDY and KING, 1972; KING and CASSIDY, 1973), we had an impresson that the dividing of the cells of the follicular epithelium is stopped in an earlier vitellogenetical stage than normally, but the ovocites continue to grow further. It could have an interest to mention that in *Musca domestica* similar changes in the follicular epithelium cells were elicited by the chemosterilant: 2,7,11-trimethyl 7,11-dihydr-dichlor-2 dodeceneric acid (REZEBOVA and LANDA, 1974).

3. Pronounced differences can be observed sometimes in the size of mature eggs and that of the following anterior eggs in the ovariole that has not yet finished vitellogenesis. In such ovarioles the ripe eggs can be two times the size of the adjacent, developing eggs. This phenomena was observed only in some cases, furthermore was observed a single case in codling moth female after egglaying , but interestingly, it can be elicited by topical application of 1/μg Farnesyl-methylether on *G. funebrana* females (DESEŐ, unpublished data).

A further observation which suggests that the eggs does not mature in sequence, one after the other, in these microlepidopterous species, can be demonstrated by Fig. 8. Therefore we have the supposition that a part of the eggs in a single ovariole are ripening together in the same time, and from this group an anterior, younger egg can earlier become full grown than a posterior older one.

Although short photoperiod as well as ageing or chemosterilants can elicit similar abnormalities in the ovarioles of females, there is a basical difference between the effects of them. Whereas short photoperiod elicits changes usually in a single ovariole the other ones effect all ovarioles.

B) In the case of males the following changes were observed. In males developed under short-day conditions the length of the paired accessory glands were about the two-third or half of that of the long-day males. The walls of these glands are thin, the cells are small with relatively small nuclei (Figs 9, 10). In long-day males, however, these walls are thick and consist of tightly packed, elongated cells with large nuclei (Figs 11, 12). The walls of the accessory glands in short-day males seem to be similar to that of the depleted glands in *Aedes egypti* L. observed by DAPPLES *et al.* (1974). However, thin walls in the accessory glands could be found also in long-day males after more matings usually, but even than, a part of these long glands showed always thick walls.



Figs 1, 2. Signs of ovosorption in the newly emerged codling moth after deveoping under SD conditions with or without diapause

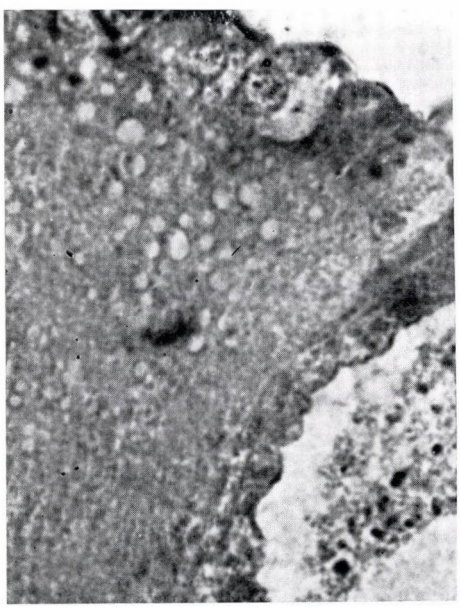
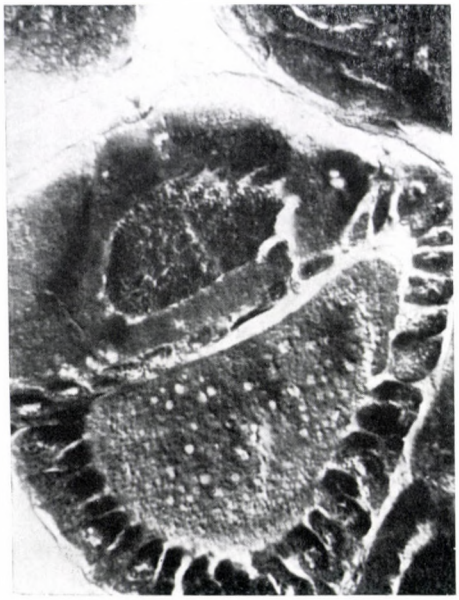
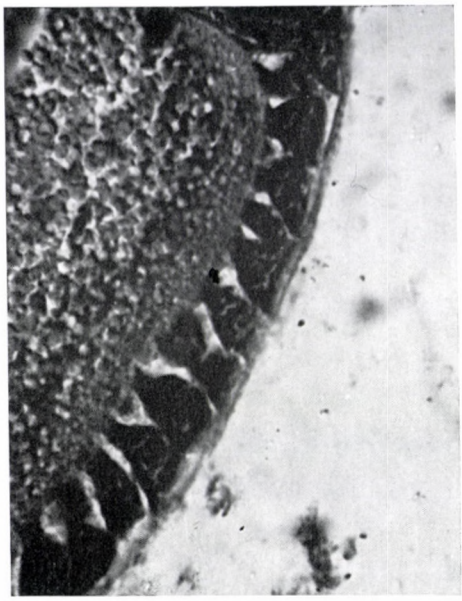
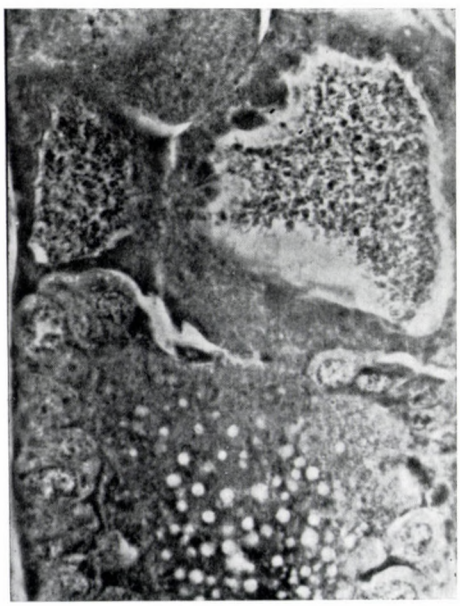
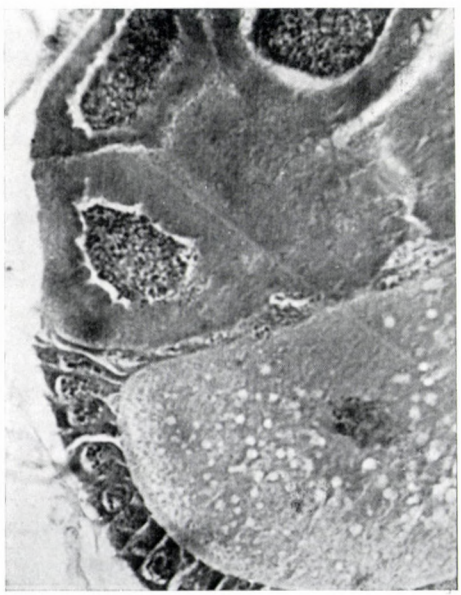


Fig. 3. Thin cells of follicular epithelium in case of ovocytes of earlier vitellogenetical stages (*L. pomonella* L.)









Figs 4, 5, 6, 7. Multinucleated follicular epithelium cells in SD females with or without diapause. (*L. pomonella* L.)

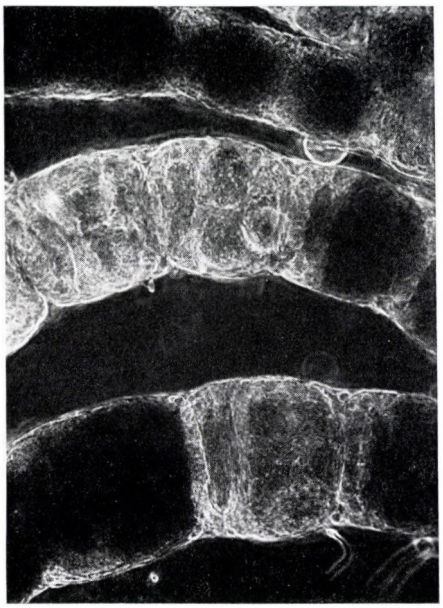
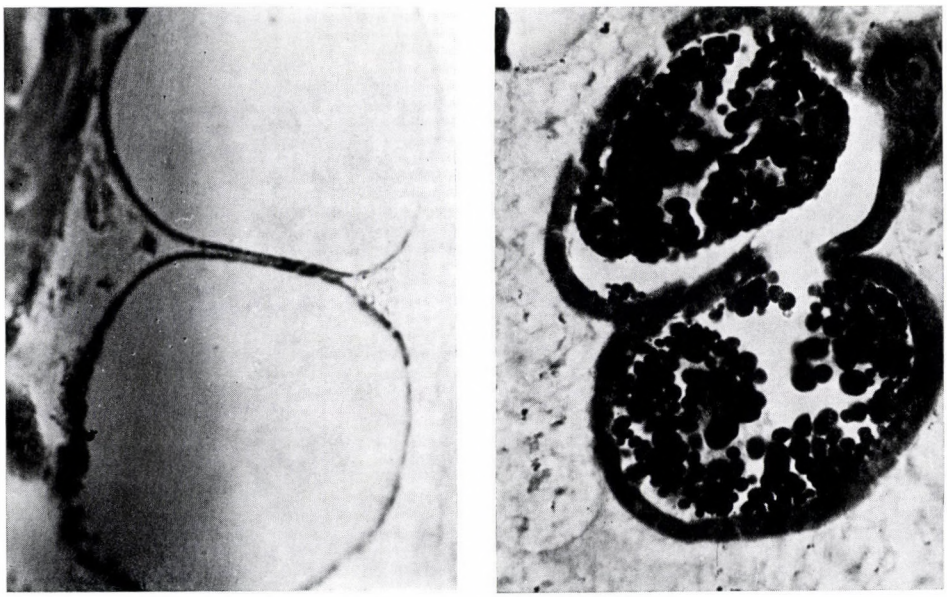
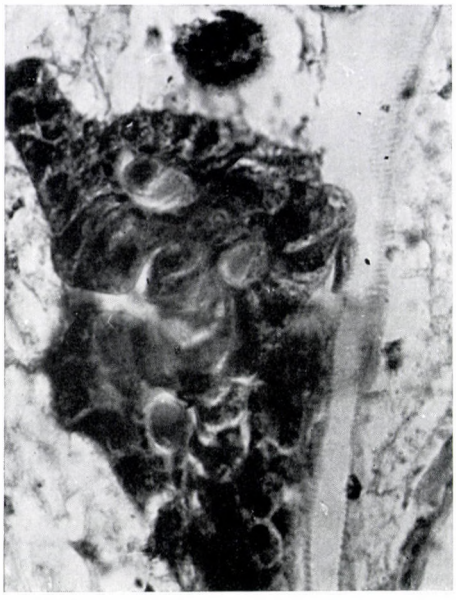


Fig. 8. Different amount of the depletion of materials in the ovarioles of *C. molesta* Busck



Figs 9, 10. Dissections of the paired accessory glands of codling moth males after developing under SD conditions



Figs 11, 12. Dissections of the paired accessory glands of codling moth males after developing under LD conditions

Fig. 13. Spermatheca of a SD female of codling moth full with spermatozoa

Furthermore, in short-day males (independently that they completed or not diapause) the fluid of the accessory glands was different of that in long-day males. Namely in long-day males this fluid was white and thick, whereas in short-day males that was transparent. Abnormalities in testes, concerning the sperms and sperm-bundles were not detected.

In case of females that mate but did not lay eggs, the supposingly eupyrene sperm bundles could be observed in the receptaculum seminis (Fig. 13).

Discussion

The histological investigation showed that ovosorption, thin, sometimes multinucleic follicular epithelium cells, and partial ovogenesis could be observed in females which did not mate, or which after mating did not oviposit, or laid only few eggs. All these changes, like in males the short accessory glands, the thin wall of these glands and the transparent fluid in them were observed in old adults after mating or egglaying, or after the treatments of certain chemicals. So, these changes, which appear after the development under short-day conditions, can be elicited by other factors as well. However there seem to be a basical difference between the effect of the short photoperiod on the gonads of females and that of the other factors mentioned above. Namely, whereas short photoperiod may elicit changes in one single ovariole other factors influence all of them. However, the histological change in a single ovariole may be one reason of the decreased fecundity even if we consider the changes in the gonads as the consequence of the changed metabolism.

Our data on the number of eggs and afterwards on the histology of these individual females suggest that the ovarioles discharge their eggs in a defined sequence and therefore, if vitellogenesis in one is slowed down, the other will store the mature eggs, until the egg from the slower ovariole is not laid. So the process of egglaying is blocked practically. Whether in these females there is a lack in nutrients necessary for normal vitellogenesis or there is an inhibited transportation of materials into the ovocyte, we do not know.

However in some females with decreased fecundity or no egglaying after mating, the role of the males must be taken in consideration. Although in histological section there could be seen a large amount of sperms in the ducts of females, the lack, or decrease of some compounds produced by the male accessory glands can not be excluded, suggested by the aberrations in the accessory glands described above.

The question arises what is the cause of the impaired development of the gonads? We have seen that thin, multinucleated follicular epithelium cells, partial vitellogenesis, ovosorption and inhibited or blocked vitellogenesis appear with ageing or, may be elicited by the topical application of chemosterilants of different type. So, the reason of these histological changes might be a certain physiological weakness (as regards the signs of ageing), or the lack of a supposed defense mech-

anism (as regards the changes elicited also by chemicals) which had to be triggered by the adversely changing environment in the autumn (DESEŐ and SÁRINGER, 1975a, b).

However in the case of the old adults, the reserve materials disappear from the body; the weight decreases. In our species the short photoperiod did not decrease the weight of the adults and they showed always large amount of reserves. Although we do not exclude the possibility of a certain physiological weekness in muscles and nerves observed by other authors (ILYINSKAYA, 1969; SCHOONEVELD, 1970), we incline to accept the other explanation.

Namely like in the case of the chemosterilants some individuals treated do not show the signs of a changed metabolism, so adults, having developed under short-day conditions, might have "normal" fertility. We suppose that in case of these specimen a "defense mechanism" is triggered by the extarnal factors, which is able to protect the insect against the toxic effect of the chemicals, or against the maleffect of the short photoperiod. Earlier observations (DESEŐ and SÁRINGER, 1975a) seem to confirm this supposition: different combinations of photoperiod and temperature influence the reproduction in different extent. With other words: there seem to exist an optimal combination of short photoperiod and temperature in accordance with the intrinsic factor of the insect when the reproduction is not influenced. That is the case at which "defense mechanism" is supposed to be at work. We suppose, furthermore that non-optimal combinations of factors mentioned above, would elicit changes in different degrees in the metabolism; so, irreparable histological aberrations in the gonads.

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Photoperiodic and Temperature Effects on Rate of Development and Diapause in the Green Stink Bug, *Nezara viridula* L. (*Heteroptera: Pentatomidae*)

By

M. ALI* and M. A. EWIESS**

* Plant Protection Dept., Fac. Agric., Al-Azhar University, Nasr City, Cairo, A. R. Egypt
** Plant Protection Dept., Fac. Agric., Cairo University, Giza, A. R. Egypt

There were noticeable differences in the rate of development, survival rate and diapause incidence among *Nezara viridula* L. individuals reared at different temperatures and photoperiods. The optimum temperature for the greatest rate of development and survival of immature stages was 25°C. Long and short photoperiods influenced the rate of development at 20°C and 25°C ,however, this effect completely disappeared at 30°C.

At 25°C, photoperiods of 10 and 11 hours per day induced diapause in large percentage of adults; intermediate photoperiod of 12 hours per day induced 28.6% diapause while long photoperiod of 14 hours per day suppressed the induction of diapause. The critical photoperiod is approximately 12 hours light per day. The stage or stages sensitive to photoperiod inducing diapause, or conversely initiating maturation of gonads was determined to be the fourth nymphal stage. The present paper deals with information about rate of development, daily rate of increase in adult body weight, diapause incidence and maturation of gonads as influenced by different temperature and photoperiods conditions.

The green stink bug, *Nezara viridula* L. is a common polyphagous insect. It was primarily known as a pest of cotton in Egypt (KAMAL, 1937). Recently, this pest is widely distributed in different parts of the world and becomes a serious pest of various economic crops. The range of geographical distribution of *Nezara viridula* L. covers temperate and tropic areas, including south eastern Asia and Africa (MITCHEL and MAU, 1969). In field, the insect concerned was found feeding on cabbage, rice, maize, okra, citrus, tobacco, groundnut and several species of beans (Phaseolus) and vigna (CORPUZ, 1969). The bug sucking often kills plants and disseminates the wound fungus, *Rhizopus nigricans*, which causes internal rot (VAN HEERDEN, 1933).

Detailed life history and behavioural studies of *N. viridula* L. under field conditions have been reported by WATSON (1934) and KIRITANI (1966, 1971); and under laboratory conditions by KARIYA (1961). This species may have three overlapping generations or two complete generations a year with a partial third (VAN HEERDEN, 1933; KAMAL, 1937; and KIRITANI, 1971). It overwinters as inactive adults. Overwintering takes place among the leaves of ever-green trees. Autumn migration from breeding place to hibernacula lasts almost for 4 months

from middle September to middle January in the following year. Adults seemed to feed on food plants more than one week before arriving at hibernacula and males likely begin autumn migration earlier than females. Spring migration from hibernacula to food plants, occurs during the period of late March to early April (KIRITANI and HOKYO, 1970).

This paper presents data on the influence of different temperatures and photoperiods on rate of development, survival of immature stages and rate of increase in adult body weight. It also to determine the sensitive stage to photoperiodic effects and to verify the role of photoperiod as an environmental factor in regulating development and inducing diapause.

Material and Methods

Bugs were collected from cotton fields by a sweeping net at Mosttorod region (about 5 km from Cairo). They were transported in plastic bottles with perforated caps and were kept in air conditioned room with a temperature of 25°C ± 0.5°C.

For different purposes, bugs were reared in half-kilogram baby-milk containers. Green pods were used as food supply for feeding immatures and adults.

The influence of temperature and photoperiod on rate of development, survival of immature, weight of adults and maturation of gonads was determined at 20°, 25° and 30°C with photoperiods (light/dark) L/D: 14/10, 13/11, 12/12, 11/13 and 10/14 hours. The eggs used in the present experiments were obtained from stock culture which was maintained at 25°C ± 0.5°C and 14-hours photophase.

The rearing units and their contents were daily examined. Food was changed every 24 hours. At the same time, the durations of the various developmental stages, nymphal mortality, adult weight and sex ratio were recorded.

The diapause of adults in *Nezara viridula* L. had been used to evaluate the developmental state of the gonads. In order to distinguish the diapause incidence, 20–30 days old adults were dissected. The criteria employed to diagnose diapaused or nondiapaused status in this experiment were (1) failure of a female to oviposite within certain period after emergence and copulation with a male and (2) the condition of ovaries in females and accessory glands in males and the degree of fat tissue development. Females which had ripe ovarioles, large vitellarium with at least one oocyte and produced eggs were suspected to be nondiapaused. The diapausing females have ovarioles with an undeveloped vitellarium associated with the presence of the moderate to large amount of fat body tissue.

To establish the stage or stages of development responsiveness to stimuli that determine diapause or nondiapause induction, eggs, various nymphal instars and adults of different ages were transferred from the condition which had been determined to allow nondiapause development (25°C and 14 hours photophase),

to other which had been determined to induce diapause (25°C and 10 hours photophase). Control treatments were reared continuously without transfer. The number of diapaused bugs of each treatment at the end of the experiment was determined by dissection and examination of reproductive gonads, fat body tissue and alimentary canal on the 30th day after adult emergence.

Results and Discussion

Egg hatchability and incubation period

The percentages of hatched eggs varied according to temperature and rearing conditions (Table 1). At 20°C, the percentages of hatched eggs exerted no significant differences and ranged between 82.1% and 83.3%. Eggs preserved at 25°C resulted in 95% to 100% hatchelings.

Incubation period was significantly longer at 20°C than at 25°C or 30°C. Thus, the mean incubation periods were 9.2, 5.0 and 5.0 days at 20°, 25° and 30°C, respectively. No significant variations in either the percentages of hatched eggs or in incubation periods (in days) were observed between treatments of 10-hours photoperiod compared with treatments of 14-hours photoperiod at equivalent temperature. This agrees with McMULLEN' (1967) results on *Coccinella novemnotata* Herbst.

Duration of nymphal stages

The mean number of days required by nymphs to complete their development varied widely from photoperiod and temperature to the other (Table 1). The rate of development of all immature stages was greatest at 30°C and least at 20°C. The greatest increase of growth rate per 5°C temperature increment occurred between 20° and 25°C, and the least between 25°C and 30°C.

The effect of photoperiod on the developmental rate was particularly evident at 20°C and 25°C. An increase in photoperiod at each of these two temperatures resulted in significant reduction of the time required for nymphal development. The decrease in days required for nymphal development with photoperiod increase indicate that a long photophase provides a long feeding period that in turn enhances the metabolic functions and accelerates the development.

With regard to mortality of immature stages, it was noticed that it did not coincide with temperature increase or decrease. Low mortality was obtained at 20°C, the lowest at 25°C, while the highest occurred at 30°C (Table 2).

While photoperiod greatly influenced the rate of development, it did not exert conspicuous effect on mortality of immatures at an equivalent temperature.

Generally, nymphs of first and second instars showed less mortality at low temperature than at higher one. This is well illustrated by the fact that at 25°C and 30°C, a higher percentages of nymphs died during first and second stadia

Table 1

Percentage of egg hatchability and rate of development of *N. viridula*, in days,

Temp. °C	Photoperiod (hrs) light/dark	No. of nymphs tested	No. of eggs tested	% egg hatchability	Mean incubation period (days)
20°	10/14	145	119	82.1	9.2 ± 0.3
	14/10	120	100	83.3	9.3 ± 0.1
25°	10/14	100	96	96.0	5.0 ± 0.0
	11/13	160	160	100.0	5i2 ± 0.2
	12/12	289	248	85.8	5.0 ± 0.0
	13/11	160	160	100.0	4.7 ± 0.6
	14/10	100	95	95.0	5.0 ± 0.4
30°	10/14	80	70	87.5	5.0 ± 0.1
	14/10	100	85	85.0	5.0 ± 0.1

than during the fourth and fifth. This would indicate that under field conditions, nymphs could be more readily in the first instars if a high environmental temperature prevailed at nymphal ecdysis. Conversely a uniformly cool autumn would be particularly harmful in the fourth and fifth stadia, especially if such temperature prevailed to the end of September.

The number of female and male bugs which developed from nymphs reared at different constant temperatures and photoperiods (Table 2) revealed that it was neither affected by temperature nor by photoperiod. The sex ratio was approximately 1 : 1. At 20°C, the number of males slightly surpassed that of females, however, this noticeable difference was of no significance.

The results obtained by PHILOGÉNE and BENJAMIN (1971) on *Neodiprion swainei* Medd. are contradicted with the present results since they found that an increase of photoperiod was accompanied by increase of time required for larval development. On the other hand, HODEK (1957), MCMULLEN (1967) and ALI (1971) found that no differences in rate of development of *Coccinella septempunctata* L., *Coccinella novemnotata* Herbst and *Subcoccinella 24-punctata* L. respectively were observed between treatments of long photoperiod compared with treatments of short one.

Weight of adult

Table 3 presents weights of the newly emerged bugs (females and males), weights of the same bugs after elapsing of 20 days and the daily rate of body increase when reared at different photoperiods and temperatures. Female and male bugs which resulted from nymphs reared at low temperature (20°C) were heavier than those developed at 25°C and 30°C. No great variations in weights of newly emerged bugs could be detected with treatment of long photoperiod

when reared at constant temperatures and photoperiods

Mean duration of instars (days)					Mean total development time (days)
1st	2nd	3rd	4th	5th	
13.5 ± 0.9	11.2 ± 2.5	14.3 ± 1.1	13.8 ± 1.7	9.0 ± 1.5	71.0 ± 1.0
12.0 ± 0.2	9.3 ± 0.6	9.3 ± 1.1	9.8 ± 1.0	9.0 ± 1.0	58.7 ± 0.1
5.5 ± 0.7	6.5 ± 0.3	7.5 ± 0.7	7.8 ± 0.2	8.8 ± 0.3	41.1 ± 0 7
4 5 ± 0 1	5 5 ± 0.03	7.0 ± 0.0	7.3 ± 1.0	8.3 ± 0.8	37.8 ± 0.7
4.5 ± 0.05	5.5 ± 0.01	6.0 ± 0.0	7.5 ± 0.1	8.3 ± 2.0	36.8 ± 1.4
5.0 ± 0.0	6.0 ± 0.0	5.5 ± 0.1	6.0 ± 0.4	7.8 ± 1.4	35.0 ± 1.0
4.3 ± 0.3	6.0 ± 1.6	6.3 ± 1.3	6.3 ± 1.1	7.5 ± 1.0	35.4 ± 1.5
5.0 ± 0.0	5.0 ± 0.6	5.0 ± 0.0	5.5 ± 0.3	6.5 ± 0.3	32.0 ± 0.5
4.5 ± 0.3	4.8 ± 0.2	4.8 ± 0.3	4.8 ± 0.3	5.8 ± 0.3	29.7 ± 0.7

Table 2

Effects of temperature and photoperiod on survival rate of immatures and sex ratio in *Nezara viridula* L.

Temp.	Photoperiod (hour) light/dark	No. of nymphs tested	% Total mortality	% Emerged bugs		Sex ratio
				females	males	female : male
20°C	10/14	119	51.0	40	60	1 : 1.5
	14/10	100	60.7	45	55	1 : 1.2
25°C	10/14	96	17.7	50	50	1 : 1 0
	11/13	160	20.3	42	58	1 : 1.0
	12/12	248	24.6	45	55	1 : 1.2
	13/11	160	21.3	50	50	1 : 1.0
	14/10	95	22.7	50	50	1 : 1.0
30°C	10/14	70	48.0	50	50	1 : 1.0
	14/10	85	59.4	50	50	1 : 1.0

(14 hour) compared with treatment of short photoperiod (10-hour) at equivalent temperature except those of 20°C.

The daily rate of increase in body weight of bugs that spent 20 days after emergence under the forementioned rearing conditions showed obvious increase with the increase of photoperiod. The rate of increase was significantly affected by photoperiodic regimens. There was apparent increase in body weight of adults at 25°C rather than at 30°C. The great differences between bugs, particularly females, reared under long and short photophases illustrate the importance and the role of day length as an environmental factor regulating development in this insect. These results also indicate that, under field conditions bugs which occur

Table 3

Changes in body weight of *N. viridula* adults as influenced by different temperatures and photoperiods

Temp. °C	Photo-period (hours) light/dark	Mean weight of newly emerged adult (mg)		Mean weight of 20 days old adult (mg)		Daily rate of increase in adult body wt. (mg)	
		female	male	female	male	female	male
20°	10/14	171.8 ± 3.8	142.1 ± 8.0	203.0 ± 15.8	149.2 ± 5.1	1.56	0.36
	14/10	155.3 ± 3.5	142.6 ± 6.8	217.3 ± 23.9	157.6 ± 6.5	3.10	0.78
25°	10/14	148.1 ± 8.1	123.2 ± 10.5	187.2 ± 6.4	160.8 ± 2.5	1.96	1.88
	11/13	162.5 ± 11.7	137.1 ± 9.2	182.6 ± 7.1	153.6 ± 7.2	1.01	0.83
	12/12	87.0 ± 2.3	76.0 ± 3.4	154.5 ± 2.5	119.5 ± 4.6	3.38	2.17
	13/11	106.2 ± 6.3	101.0 ± 6.4	181.3 ± 11.6	146.8 ± 9.8	3.76	2.29
	14/10	140.5 ± 6.9	114.2 ± 5.1	232.0 ± 22.6	153.6 ± 4.6	4.56	1.97
30°	10/14	158.1 ± 16.1	125.0 ± 5.4	185.5 ± 4.5	150.4 ± 5.4	1.37	1.27
	14/10	152.7 ± 7.2	137.8 ± 16.5	197.1 ± 16.6	148.3 ± 9.3	2.52	0.53

in June would be heavier than those found in September due to the presence of long-day in the first case, while short-day and low temperature would be prevailing in the other case. The light weights of females developed under short photophase could be explained by the fact that such conditions suppress or inhibit the maturation of gonads and the development of oocytes. Similar findings were reported by BEARDS and STRONG (1966) in *Lygus hesperus* Knight.

Influence of photoperiod on diapause induction

The effects of various photoperiods on the incidence of diapause at a rearing temperature of 25°C are given in Table 4, and Figure 1. The shape of the curve is typical for long-day insects which usually enter diapause in response to short photophases.

Table 4

Percentages of diapausing bugs under the influence of different photoperiods at 25°C

Photoperiod l/d hrs	No. of dissected bugs	% of diapausing bugs
10/14	40	100.0 ± 0.0
11/13	18	96.3 ± 2.5
12/12	20	58.6 ± 3.6
13/11	26	23.8 ± 1.4
14/10	34	0.0 ± 0.0

Under the conditions tested, it was noticed that the effective range of photophases was between 10 and 14 hours. Bugs exposed to long photoperiod (L/D: 14/10 hrs) developed without diapause and laid fertile eggs, while short photoperiod (L/D: 10/14 hrs) induced 100% diapausing adults. Intermediate percentage of diapause incidence was obtained with 12 hours photophase.

The sensitivity of *N. viridula* L. individuals to photoperiodic effects was demonstrated by complete absence of diapause under 14 hours photophase, while a 13 hours photophase evoked 23.8 per cent diapause. Under short photoperiods, in the majority of bugs the gonads did not develop well and remained without oocytes even after long period (more than 30 days).

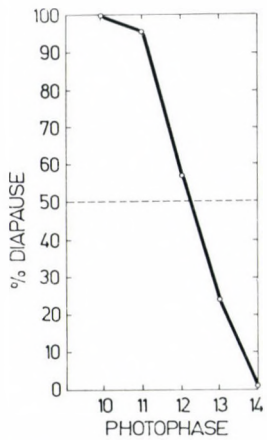

Fig. 1. The relationship between photophase and diapause incidence among 30-day-old *Nezara viridula* L. adults reared at 25°C

The critical photoperiod lies, therefore, between 13/11 and 12/12 hours light/dark. This result coincides with that denoted by BEARDS and STRONG (1966) in *Lygus hesperus* Knight, however, ALI and SÁRINGER (1975) found that the critical photophase of alfalfa ladybird, *Subcoccinella 24-punctata* L. lies between 14 and 15 hrs daily light. It is, however, necessary to state that the critical photophase may be influenced by other factors, so that the precise estimation of critical photoperiod is difficult to determine.

According to the previos discussion, it can be concluded that the green stink bug *Nezara viridula* L. belongs to long-day insects and its diapause is photoperiod-dependent phenomenon.

Photoperiod has been recognized for a number of years as one of the chief environmental factors regulating diapause in insect species. In the case of a species closely related to *N. viridula* L., HONEK (1969) stated that the exposure of *Aelia acuminata* (L.) to short-day or darkness (0—15 hrs light) induced diapausing bugs, while in the long-day (16 hrs light and more hours light) bugs developed without diapause.

Influence of photoperiod on maturation of gonads

Female and male bugs were separately dissected after they had been exposed to long and short photoperiods for 30 days. The examination of dissected females showed great variation in both size and measurements of ovarioles of diapausing and non-diapausing females. Non-diapausing females had ripe ovarioles: their vitellarium was large, with at least one oocyte, the pedicellus was yellow and females oviposited fertile eggs. The diapausing females had ovarioles with an undeveloped vitellarium, the pedicellus was colourless and consequently could not oviposite (Fig. 2–1, 2).

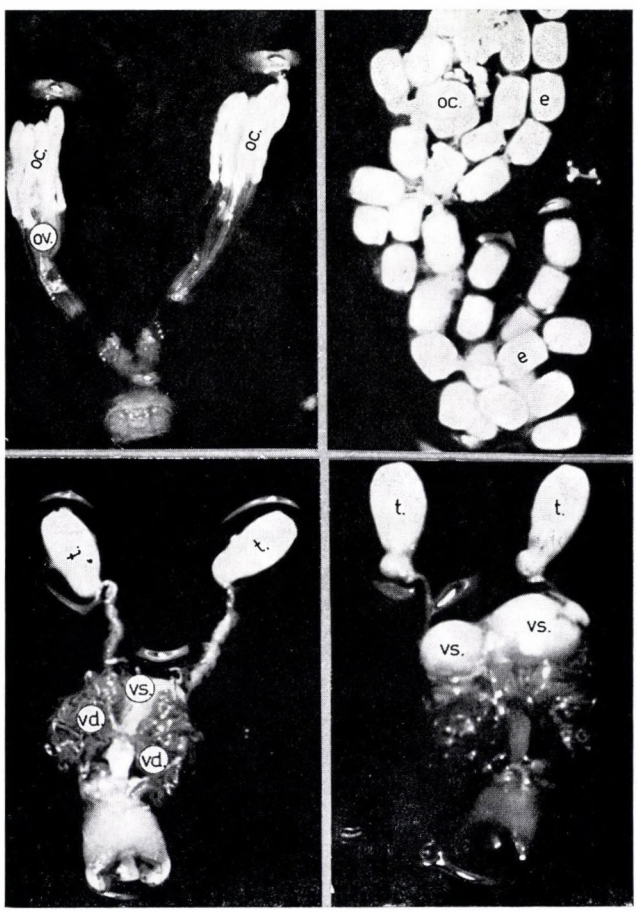

Plate 1. Response of the green stink bug (*Nezara viridula* L.) adults to long and short photoperiods, as illustrated by gross dissection 1: Ovary of diapausing female; 2: ovary of non-diapausing female; 3: testis of diapausing male; 4: testis of nondiapausing male; Ov: ovary; Oc: oocyte; e: egg; t: testis; vs: vesicula seminalis; vd: vasa deferentia

The examination of male gonads revealed no great differences between tests of long photoperiod-reared males compared with short photoperiod-reared males, however, vesicula seminales of males exposed to short photophase was apparently smaller than that of males kept under long photophase (Plate 1). The present data are in agreement with results on *Lygus hesperus* Knight (BEARDS and STRONG, 1966) and *Subcoccinella 24-punctata* L. (ALI, 1974).

Results presented here sustain the influence of photoperiod foregoing explained. Photophases longer than 12 hours daily light were favourable for oogenesis and oocytes development, while photophases shorter than 11 hours daily light resulted in complete arrest of bug activity and ceased or inhibited the maturation of gonads, especially in females.

Sensitive stage for photoperiodic effects

The treatment of first, second and third nymphal instars with long-day regimens was found to be without effect if after that time the bugs were kept under short-day condition. All the resulting adults remained non-reproductive and 100% entered diapause as adults (Table 5).

On the other hand, among nymphs transferred in fourth and fifth instars, only 80% and 31%, respectively, entered diapause as adults. These results indicate that the exposure of fourth and fifth nymphal stages to long photoperiod stimulated reproduction in adults and diapause incidence reduced to nearly 0%. Consequently, it is evident that the previous stages are the most responsive stages for induction of diapause, or conversely the initiation of gonads maturation.

Table 5

Diapause conditions of *N. viridula* L. population at a constant temperature of 25°C, in combination with 14/10 and 10/14 hours photoperiods

No.	Nymphal instars						Adult age (in days)					No. of tested bugs	Diapause %
	Eggs	N_1	N_2	N_3	N_4	N_5	0	5	10	15	20		
1	– –											24	0
2	—— –											49	100
3	—— —— –											32	100
4	— — —— –											36	100
5	—— —— —— —— –											40	90
6	—— —— —— —— —— –											52	31
7	—— —— —— —— —— —— – – – – – – – – – – – – – – – – – –											44	0
8	—— —— —— —— —— —— —— —— – – – – – – – – – – – – – – –											40	0
9	—— —— —— —— —— —— —— —— —— —— – – – – – – – – – – – –											27	0
10	—— —— —— —— —— —— —— —— —— —— —— —— – – – – – – – – –											52	0
11	—— —— —— —— —— —— —— —— —— —— —— —— —— —— – – – – – –											30	0
12	– –											28	100

—— 14-hours of light/10 hours of darkness
– – – – – – – 10-hours of light/14 hours of darkness

The present data seem to be in line with those reported by HODEK (1971) who stated that the larvae of *Pyrrhocoris apterus* L. (Heteroptera), at least for the 12 and especially the last 7 days before adult ecdysis, are the sensitive stages for induction or inhibition of diapause. BEARDS and STRONG (1966) also emphasized that diapause in *Lygus hersperus* Knight (Heteroptera) is a function of photoperiod and that, under natural conditions, the photoperiod apparently exerts its diapause-inducing influence during the sensitive nymphal stage.

HONEK (1969) suggested that the effect of short photoperiod in the course of larval development in *Aelia acuminata* L. (Heteroptera : Pentatomidae) has no substantial influence on induction of diapause, however, his results seem to be incomplete since no experimental data on the larval sensitivity to increase or decrease in day length are given.

The purpose of determining the sensitive stage to photoperiodic effects is to know the possibility of its nullification and consequently preventing insect from entering diapause and exposing it to different environmental severities. The fact connected with this is that in some insect species it is more difficult to prevent diapause, in others, to induce it.

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Extraction of Natural Antifeedants from the Fruits of *Amorpha fruticosa* L.

By

M. A. GOMBOS and K. GASKÓ

Research Institute of Heavy Chemical Industry, Veszprém

From the extract of fruits of *Amorpha fruticosa* L. three biologically active compounds were isolated. In spite of the fact that the compounds isolated were not identical in the different years and localities it was established that the biological activity was carried by the identical molecular structure. In course of the investigations these compounds showed an antifeedant activity in the species *Locusta migratoria migratoroides* R. and F., *Leptinotarsa decemlineata* Say, *Pieris brassicae* L. and a repellent activity in *Messor structor* Latr. There was no effect observed in the *Megoura viciae* Buct. aphids and in *Tetranychus urticae* Koch spider mites. The compounds apparently did not affect the foraging activity of pollinating bees on flowering rape plants sprayed under field conditions.

The study of antifeedant materials, which disturb the trophic relations established between insect pests and their host plants, is carried out at present in many countries of the world, as these compounds offer possibilities to work out selective materials to be used in plant protection. These compounds would be advantageous also from point of view of environmental protection, compared to the insecticides still used. Antifeedant activities of many secondary plant materials have been demonstrated so far (JERMY, 1972).

The authors have selected for this purpose the bastard indigo, *Amorpha fruticosa* L. The insecticidal properties of this plant's seeds have been already reported (FUKAMI and TOMIZOCA, 1960; FUKAMI, 1962). As indicated in the literature, the latter authors have used not only the rotenoids isolated, but also emulsions made from the leaves and bark of this plant. In steppe areas aqueous extracts of whole plant homogenates were used not only as insecticides but also as a herbicide (KISPATIC and BOEHM, 1960; TARASOV, 1967). The toxicology of rotenones was dealt with by FUKAMI and co-workers and it was established that the selective toxicity depended on the differences of oxydative metabolism. By studying the materials isolated in course of the processing of *Amorpha* seeds, KONDRATENKO and ABUBAKIROV (1962) established the structure of the active material amorphin.

The amorphin itself, belonging to the biologically active rotenone group has been studied by FUKAMI and TOMIZOCA (1956) and FUKAMI (1962), who established the mode of action of the compound, among others also in the insect organism.

Materials and Methods

The preparation of plant materials

The fruits of *A. fruticosa* were collected by the end of September in the surroundings of Veszprém, then spread out on the floor of a dry room free of dust and dried by repeated turning. The dried fruits were then ground in a blendor mill and a quantity of 4 kg was extracted with 3×8 liter benzene for 3 days. By evaporating the solvent in vacuum, a thick, creamy, brown substance remained; 110 g of this material was solved in benzene, adsorbed in 150 g silicagel and fractionated on a column prepared from 700 g silicagel. The elution was commenced with pure light petrol, then continued with a mixture of light petrol and benzene. With the 8 : 2 light petrol–benzene mixture a light yellow, viscous, scented substance was eluted from the column. Simultaneously with the study of biological activity the cleaning of the substance and establishing of the molecular structure was commenced, by using UV, IR and NMR spectra.

Laboratory experiments

In course of the laboratory studies the material was used in a 1 % concentration. The host plants were sprayed to achieve complete cover; in this work a spraying apparatus producing a solid spray cone was used. The test animals were placed on the host plants after the analytically pure solvent completely evaporated. The experiments were made with four repetitions, so the data given in the following, refer always to the mean values of 4 repetitions. In the preliminary investigation of antifeedant action *Locusta migratoria migratoroides* adults were used and, following the positive results, the studies were extended to insects belonging to systematically distant orders and to mites.

Field experiments

The material was applied on a flowering *Brassica napus* stand in small plots, by using a spray liquid containing 0.8 ml active material per sq.m. The spray liquid contained also emulsifiers and surfactants (Triton X–100 and TWEEN–80 additives). The spraying was carried out by using a plot-sprayer type "Oxford", mounted with a nozzle producing a solid spray cone and operating with a pressure of 4 atm. The behaviour of insects visiting the sprayed plants was observed for 4 hours after the drying-up of the spray.

Results and Discussion

The UV, IR and NMR spectra are shown in Figs 1–3 The formulae of the three compounds studied are shown in Fig. 4.

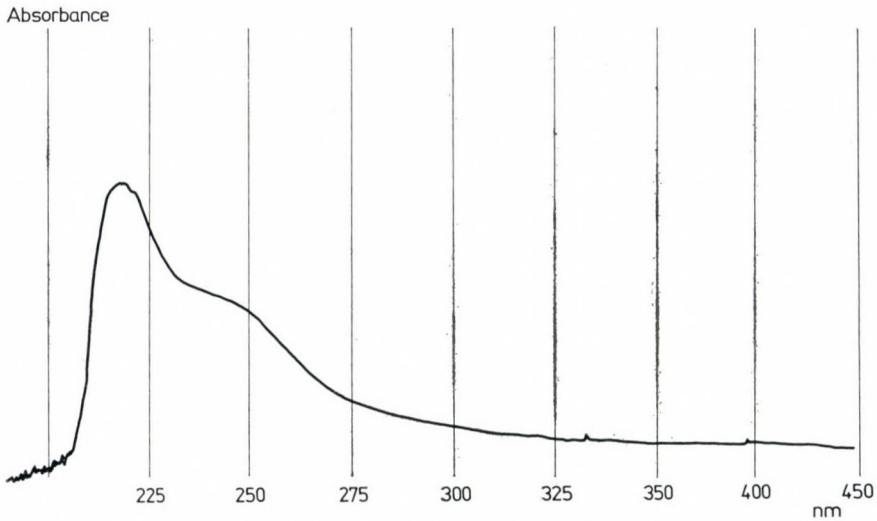

Fig. 1. UV spectrum of the fraction

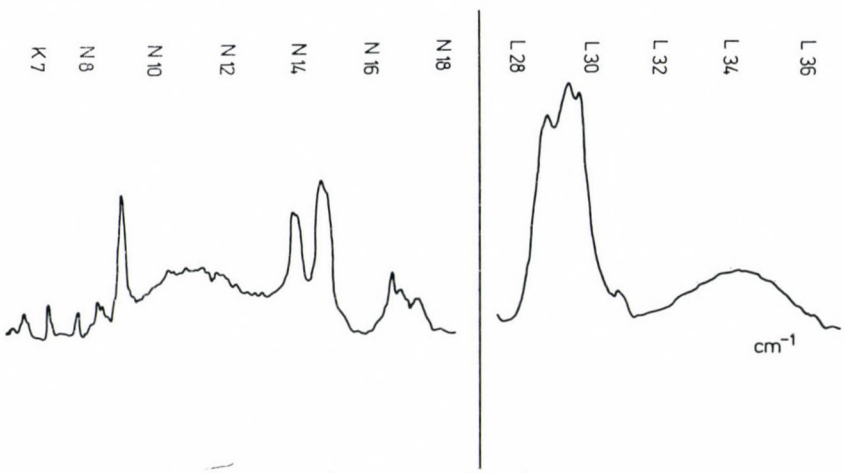

Fig. 2. IR spectrum of the fraction

In course of studies carried out for some consecutive years it was established that the three compounds contained in the extract were present in different ratios depending on year and locality. The spectra of extracts made from the fruits in 1974 did not exhibit the peak or extinction band, respectively, characteristic for the OH groups, the effectivity of the extracts however was similar to the one isolated in the preceding year. It was concluded therefore that the group responsible for the antifeedant action was contained in the basic molecular structure

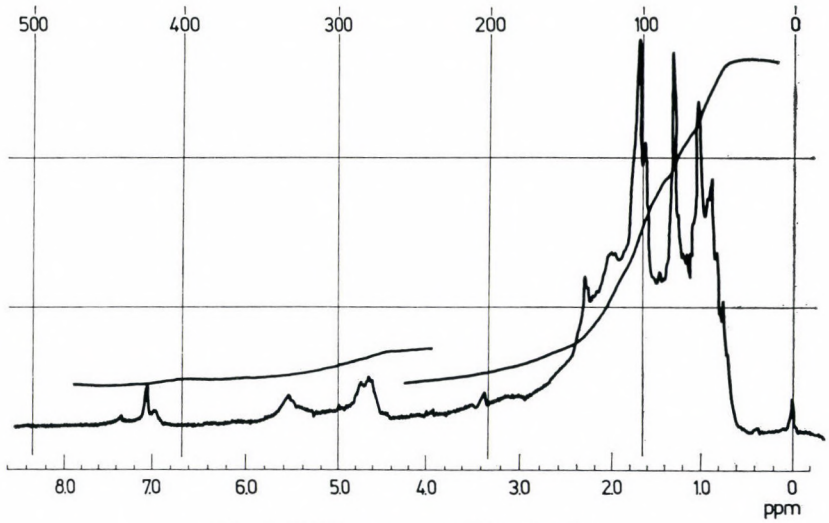

Fig. 3. NMR spectrum of the fraction

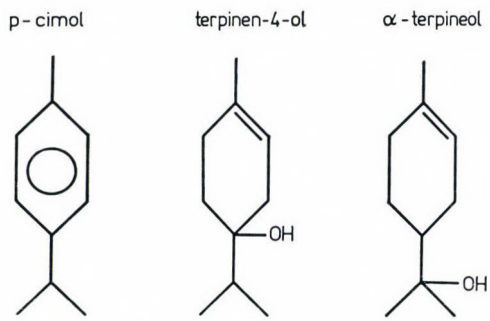

Fig. 4. Formulae of the three compounds

itself. To corroborate this assumption, fruits were collected from different locations and the antifeedant fractions of these samples were further investigated.

Results got with the different test animals:

a) *Locusta migratoria migratoroides* R. et F. (*Saltatoria*)

In the migratory locust adults the material caused a 100 per cent, permanent feeding inhibition, the treated host plant was not accepted at all. During the 72 hours of the experiment some of the animals starved to death and among the survivors a high grade of cannibalism was observed. If a possibility of food selection was given by presenting simultaneously treated and untreated food to the locusts a similar observation could be made (Fig. 5). Following the total consumption

of the untreated bundle of wheat leaves, the treated leaves were left intact after a brief nibbling; later the same symptoms of starvation and cannibalism appeared, although somewhat delayed.

Fig. 5. In case of possibility of food selection the *L. migratorius migratoroides* adults accepted the untreated food only after 24 hours

b) *Leptinotarsa decemlineata* Say (*Coleoptera*)

The effectivity of the extract was separately studied on third and fourth instar larvae and adults.

The young, third instar larvae left the treated potato leaves after a nibbling and did not resume their feeding during the experiment (Fig. 6A), whereas in the control the larvae completely consumed the potato leaves placed into the petri dish (Fig. 6B).

The older, fourth instar larvae also left the treated leaves after the first nibbling, then returned repeatedly to the leaves and tried to feed on them. By the end of the 4-hour period about 15 per cent, after 24 hours, 55 per cent of the treated leaf surface was consumed; at the same time a 100 per cent leaf consumption could be observed in the untreated control.

Similar results were got with young (freshly emerged) Colorado beetle adults, which were starved for 24 hours prior to the experiment; both the behaviour and rate of food consumption corresponded to those of the fourth instar larvae.

Fig. 6. The third instar larvae of *Leptinotarsa decemlineata* did not feed on the treated leaves (A) while in the control (B) a 100 per cent consumption was observed

In a food selection experiment third instar larvae, which had been previously exposed to treated leaves, showed for a shorter or longer period (max. for 2 hours) difficulties in finding the untreated leaves. The adults tasted and immediately left the treated leaves, but – contrary to the larvae – did not exhibit afterwards signs of disturbed orientation i.e. difficulties in finding the untreated food.

c) *Pieris brassicae* L. (*Lepidoptera*)

The experiments were carried out with fifth instar larvae. The larvae did not feed in the first 4 hours on the treated cabbage leaves and even after 24 hours only 30 per cent of the leaf surface was consumed. During the same period in the control the untreated leaves were consumed to 100 per cent.

If the possibility of food selection was presented, the larvae began to feed within 1 hour on the untreated cabbage leaves and only after these were consumed (by the beginning of the second 24 hours), were the treated leaves accepted. 48 hours after the beginning of the experiment 45 per cent of the treated area was consumed. In the untreated control – in which the amount of food was identical with the total amount presented in the food selection – already at the end of the first 24 hours a 100 per cent food consumption was noted (Fig. 7).

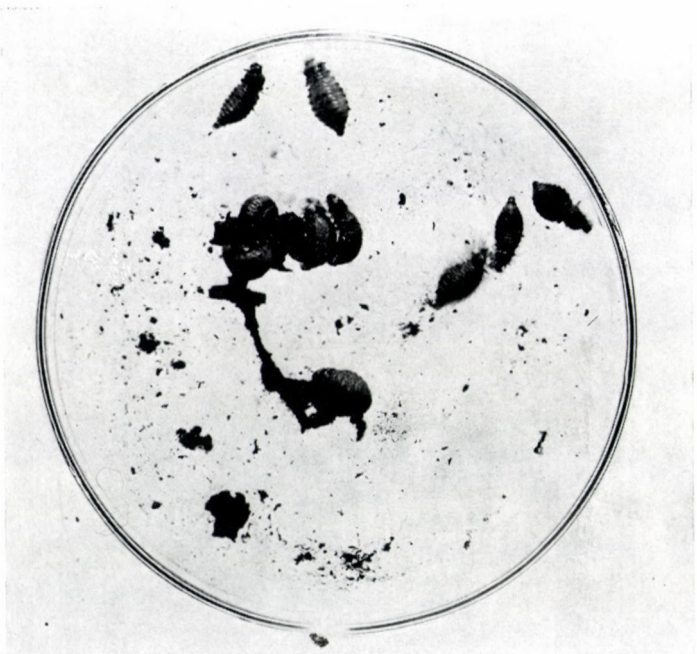

Fig. 7. In case of possibility of food choice the fifth instar larvae of *P. brassicae* consumed first the untreated leaves

d) *Messor structor* Latr. (*Hymenoptera*)

The material was applied in 10 cm wide and 0.5 m long bands, diagonally to the trail of ants. After the treatment the foraging ants changed their direction and went around the treated surface in an U-shaped line, then continued their run in the original direction. The treated area was avoided even after 72 hours; the observations were discontinued after this time.

In case of the *Megoura viciae* Buct. (Homoptera) aphids and *Tetranychus urticae* Koch (Acarina) spider mites no effect was observed.

Field experiments

In the field tests no effectivity was noted in connection with the species *Meligethes aeneus* F., *Ceutorrhynchus assimilis* Pay and *C. quadridens* Panz. (Coleoptera). The pollinating bees continued to visit the flowers following the treatment and did not interrupt their foraging activity. Besides various wild bees – which were difficult to determine to species without catching them –

Fig. 8

honey bees and bumblebees (*Bombus terrestris* L., *B. lapidarius* L.) were observed in the treated area.

At the time of the investigations (29. IV – 5. V. 1975) in the experimental area (Veszprém-Szabadságpuszta) a considerable swarming of cockchafers (*Melolontha melolontha* L.) was noted. The beetles avoided the treated area while heavy damage was observed on the foliage of untreated rape plots.

Conclusions

It can be concluded from the experimental results that the antifeedant material extracted from *Amorpha fruticosa* fruits exerted its effectivity by contact action. The antifeedant (less frequently repellent) action observed in the different insect orders can be different within the orders, even families. The effect may depend also in some cases on the developmental instar of the given species. To get closer insight into the mode of action and spectrum of effectivity, the authors intend to continue their investigations both in the laboratory and under field conditions.

Acknowledgements

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Data to the Knowledge of Moth Communities Occurring on Fruit Trees and Shrubs in Hungary

By

G. REICHART

Research Institute for Plant Protection, 1525 Budapest, P. O. Box 102, Hungary

Regarding the moth communities living on fruit-bearing plants in Hungary it was established that their seasonal grouping reflects better their composition than their differentiation according to host plants alone. So in our studies spring, summer and autumn aspects of these moth communities were distinguished. In regular studies carried out in the spring period for 32 years, collections were made in 216 localities with 300 surveys and a total number of 35,085 individuals was reared and determined. These belonged to 11 families and 68 species (Tables 1 and 2). In present paper also the dominance conditions and numbers of host plants were summarized for the most common 6 species (Tables 3—6). The summer and autumn aspects could not be delimited so sharply as the spring one. In less extensive surveys in 138 localities and 227 surveys 13,700 individuals were reared and determined. These belonged to 7 families and 26 species (Table 7).

With the intensification of the Hungarian fruit production an increasing demand has presented itself that the faunal elements occurring in the orchards be thoroughly investigated. The earlier literarure data (SAJÓ, 1895; 1902a; 1902c; JABLONOWSKI, 1902; KADOCSA, 1938, 1942, 1944) indicated the presence of many moth species in the orchards, their number however seemed to be quite limited.

In our regular studies commenced in 1945 after the end of World War II, the composition of moth communities occurring on the most important fruit trees and shrubs has been investigated for 32 consecutive years. The detailed data of this study are summarized in Table 1. After the initial studies (REICHART, 1950) the diversity of moth communities of orchards showed that only a long-range survey can furnish reliable data. So as a first step suitable field survey methods and laboratory rearing methods had to be worked out; the investigations were commenced in the commercially most important culture, in apple orchards (REICHART, 1953). Later the surveys were successively extended to other pomaceous fruits (REICHART, 1972a) and stone fruits (REICHART, 1972b; 1973a) and for comparison studies were made also on some wild growing fruits (REICHART, 1973b).

In the last period the surveys were further broadened to include berry fruits (REICHART, 1973c) in the spring aspect. The investigations however did not include the following plants: *Cornus mas* L., *Corylus avellana* L., *Castanea sativa* Mill., *Juglans regia* (L.).

During the 32 years mentioned, the surveys were less methodical in the second halves of the vegetation period, but orienting studies were made on fruits

listed in Table 2, by comparing also their moth communities with the ones of some wild growing fruits. In the summer and autumn surveys only those species could be considered which influenced the yield as pests, so economically less significant leafminers and other foliage-eating Microlepidoptera could not be included. It has been established that the common names used in the plant protection as "leafrollers" or "fruit moths" are more or less suitable for the practice, as these refer to well visible changes and distortions caused on the host plants. From scientific point of view however, it proved to be more exact to differentiate between the spring, summer and autumn aspects of moth communities. In present work the data referring to the spring aspect are given in more details; the observations on the summer and autumn aspects (although these cause mostly the actual injuries on fruits) were less regular and can be considered only as informative.

Material and Methods

In the surveys the "100 shoot method" (REICHART, 1952) was used. This consisted of collecting 100 – 100 infested (distorted or wilted) shoots from two crown levels, from 4 sides of the trees according to the cardinal points. The survey was carried out in stone fruits after the appearance of leaf buds and on pomaceous trees between the budding stage and the flowering. In collecting the actually damaged shoots also the average percentage of infested versus intact shoots could be noted. In course of the surveys also the environment of the investigated fruit trees was considered and ranged according to the following biotope types: "culture area" (= urban), "agricultural area" and "brush", "forest" surroundings. The material was partly collected by co-workers using the uniform survey method mentioned above, throughout the country, in different locations. These localities were selected in the most representative parts of Hungary, but within these territories they were scattered at random. From 1971 in constant locations the specialists of the Plant Protection Service made collections and sent the larval materials to our laboratory. The author wants to acknowledge the valuable help of the entomologists of Plant Protection Stations of Baranya, Csongrád, Szabolcs-Szatmár and Heves departments. In the department Pest the materials were collected by the author himself and his co-workers (REICHART *et al.*, 1972, 1973, 1975, 1976). The infested shoots were investigated in the laboratory and the larvae taken out from the dissected shoots were determined based on their macroscopical characteristics; the larvae were then grouped (according to species, locality and host plant) and reared to adults. So the mistakes made eventually in the first determination could be corrected later based on adult morphology and, besides, the rearing of parasites was also possible. The larvae which died during the rearing were identified by their chaetotaxy, so the moth communities and their dominance conditions were established by many-sided methods.

Table 1

Data of larval materials collected to study the spring aspects of moth communities living in Hungarian orchards

Host plant	Time period	No. of years	No. of collection localities	No. of samplings	Individual numbers studied	Plant types
Cydonia oblonga Mill.	1952—1977	8	10	14	1469	
Pyrus communis L.	1945—1974	8	16	18	1339	
Pyrus achras Gärtn.	1960	1	1	1	150	
Malus silvestris Mill.	1974—1977	4	1	4	335	Total 17,243 individuals on pomaceous fruits
Malus domestica Borkh.	1945—1977	16	57	67	9741	
Sorbus aucuparia L.	1975—1977	3	1	3	306	
Mespilus germanica L.	1952—1974	4	6	7	1251	
Crataegus oxyacantha L.	1945—1972	10	16	27	2652	
Rubus idaeus, R. caesius L.	1957—1967	11	8	11	2154	Berry fruits
Fragaria spp.	1957—1967	10	9	10	1947	
Cerasus mahaleb Mill.	1960—1962	2	4	4	427	
Cerasus avium Mönch.	1945—1976	9	13	17	2814	
Cerasus vulgaris Mill.	1957—1976	5	10	14	604	
Amygdalus communis L.	1959—1966	7	4	16	1869	Total 12,657 individuals on stone fruits
Armenica vulgaris Lam.	1945—1977	10	9	20	2162	
Persica vulgaris Mill.	1945—1977	14	17	25	2014	
Prunus spinosa L.	1955—1960	3	4	6	620	
Prunus domestica L.	1945—1977	11	15	21	2147	Total 5186 individuals on berry fruits
Ribes uva-crispa L.	1957—1964	7	7	7	396	
Ribes spp.	1957—1966	8	8	8	688	
Total	between 1945 and 1977	during 32 years	216 localities	300 surveys	35 085 individuals	

Table 2

List of moth species reared in the period 1945—1976 from larval materials collected during

Moth species reared*	Host plants studied							
	1. Cydonia oblonga Mill.	2. Pyrus communis L.	3. Pyrus achras Gärtn.	4. Malus silvestris Mill.	5. Malus domestica Borkh.	6. Sorbus aucuparia L.	7. Mespilus germanica L.	8. Crataegus oxyacantha L.
I. INCURVARIIDAE:								
1. Lampronia rubiella Bjerk.								
II. PLUTELIDAE:								
2. Ypsolophus parenthesellus L.								
3. Ypsolophus persicellus F.								
4. Ypsolophus asperellus F.					×	×		
5. Ypsolophus scabrellus Z.								×
6. Argyresthia pruniella Cl.					×			×
III. COLEOPHORIDAE:								
7. Coleophora serratella L.		×			×			×
8. Coleophora paripenella Z.					×			
9. Coleophora albicastella Dup.								
10. Coleophora anatipenella Hbn.		×			×			
11. Coleophora hemerobiella Sc.				×				×
IV. HYPONOMEUTIDAE:								
12. Scythropia crataegella L.								×
13. Hyponomeuta padellus L.								
14. Hyponomeuta malinellus L.					×		×	
15. Swammerdamia pyrella Vill.		×			×			
V. GLYPHIPTERYGIDAE:								
16. Anthophila pariana Cl.	×	×	×	×	×			×
VI. COSMOPTERYGIDAE:								
17. Blastodacna atra Haw.		×	×		×			
VII. OECOPHORIDAE:								
18. Diurnea fagella F.					×			
19. Cheimophila salicella Hbn.		×			×			×
VIII. GELECHIIDAE:								
20. Anarsia lineatella Zell.								
21. Compsolechia subsequella Hbn.								×

the spring aspect of moth communities living in Hungarian orchards

Host plants studied													
9. Rubus idaeus L. Rubus caesius L.	10. Fragaria moschata D. Fragaria vesca L.	11. Cerasus mahaleb Mill.	12. Cerasus avium Mönch.	13. Cerasus vulgaris Mill.	14. Amygdalus communis L.	15. Armenica vulgaris Lam.	16. Persica vulgaris Mill.	17. Prunus spinosa L.	18. Prunus domestica L.	19. Ribes uva-crispa L.	20. Ribes nigrum L. Ribes rubrum L.	No. of host plants
×												1
×					×	×	×					1 3
			×	×		×	×		×			2 1 7
×		×	×	×		×		×	×			9 2
	×											1
			×	×		×						5 2
								×	×			3
								×	×			2 2
						×						3
			×				×	×	×			10
												3
×	×					×				×		5
×					×	×	×		×	×		9
			×		×	×	×		×			5
			×	×				×	×			5

Table 2

Moth species reared*	Host plants studied							
	1. Cydonia oblonga Mill.	2. Pyrus communis L.	3. Pyrus achras Gärtn.	4. Malus silvestris Mill.	5. Malus domestica Borkh.	6. Sorbus aucuparia L.	7. Mespilus germanica L.	8. Crataegus oxyacantha L.
22. Gelechia scotinella HS.								
23. Recurvaria leucatella Cl.	×	×	×		×	×	×	×
24. Recurvaria nanella Hbn.	×	×	×	×	×	×	×	×
25. Argolamprotes micella Schiff.								
IX. TORTRICIDAE:								
26. Sparganothis pilleriana Schiff.					×			
27. Pandemis dumetana Tr.				×	×			
28. Pandemis heparana Schiff.	×	×	×	×	×	×	×	×
29. Pandemis corylana F.								
30. Pandemis ribeana Hbn.	×	×	×		×		×	×
31. Argyrotaenia pulchellana Haw.	×				×			×
32. Choristoneura sorbiana Hbn.					×			
33. Archips rosanus L.	×				×			×
34. Archips crataeganus Hbn.				×	×	×		×
35. Archips xylosteanus L.					×			
36. Archips podanus Scop.	×			×	×			
37. Syndemis musculana Hbn.								
38. Clepsis strigana Hbn.								
39. Clepsis semialbana Gn.								
40. Clepsis spectrana Tr.		×						
41. Adoxophyes reticulana Hbn.					×		×	×
42. Ptycholoma lecheanum L.	×				×			
43. Lozotaenia forsterana L.								
44. Neosphaleroptera nubilana Steph.					×			×
45. Tortrix viridana L.								×
46. Aleimma loefflingiana L.								
47. Croesia holmiana L.	×				×			
48. Croesia forskaleana L.						×		
49. Acleris rhombana Schiff.	×				×			×
50. Acleris tripunctana Hbn.								
51. Acleris ferrugana Schiff.	×	×	×	×	×			×
52. Acleris variegana Schiff.					×			
53. Acleris apiciana Hbn.								
54. Paracelypha rivulana SC.								
55. Orthotaenia undulana Schiff.								

continued

Host plants studies												
9. Rubus idaeus L. Rubus caesius L.	10. Fragaria moschata D. Fragaria vesca L.	11. Cerasus mahaleb Mill.	12. Cerasus avium Mönch.	13. Cerasus vulgaris Mill.	14. Amygdalus communis L.	15. Armenica vulgaris Lam.	16. Persica vulgaris Mill.	17. Prunus spinosa L.	18. Prunus domestica L.	19. Ribes uva-crispa L.	20. Ribes nigrum L. Ribes rubrum L.	No. of host plants
			×			×						2
×		×	×	×	×	×	×	×	×	×	×	18
×		×	×	×	×	×	×	×	×	×	×	19
×												1
×	×						×					4
×	×		×	×	×		×		×		×	10
×	×	×	×	×	×	×	×	×	×		×	19
×												1
×	×	×	×	×	×	×	×	×	×	×	×	18
×	×	×	×	×		×		×	×			11
									×			2
×			×	×		×			×	×	×	10
		×	×	×		×						8
×			×	×		×		×				6
×			×	×		×	×	×	×	×	×	12
×												1
	×											1
	×											1
	×									×	×	4
×	×	×	×	×	×	×	×	×	×	×	×	15
×			×	×	×	×			×			8
											×	1
		×	×	×		×		×	×			8
												1
			×			×						2
			×	×								4
												1
			×	×				×	×			7
×												1
		×	×	×		×	×	×	×			13
	×		×	×				×	×			6
×												1
											×	1
×												1

Table 2

Moth species reared	Host plants studied							
	1. Cydonia oblonga Mill.	2. Pyrus communis L.	3. Pyrus achras Gärtn.	4. Malus silvestris Mill.	5. Malus domestica Borkh.	6. Sorbus aucupaira L.	7. Mespilus germanica L.	8. Crataegus oxyacantha L.
56. Hedya pruniana Hbn.								×
57. Hedya nubiferana Haw.	×	×	×	×	×	×	×	×
58. Ancylis achatana Schiff.	×				×			×
59. Ancylis tineana Hbn.								×
60. Ancylis comptana Fröl.								
61. Notocelia roborana Tr.								
62. Notocelia uddmanniana Schiff.								
63. Eucosma expallidana Haw.								
64. Spilonota ocellana F.	×	×	×		×	×	×	×
65. Grapholitha molesta Busck	×	×			×			
X. PHYCITIDAE:								
66. Rhodophaea advenella Zck.					×		×	×
67. Catacrobasis obtusella Hbn.		×	×					
XI. PYRAUSTIDAE:								
68. Eurrhypara hortulata L.								
Total number of species per host plant	16	16	10	9	35	8	9	26

Results and Discussion

In the period 1945–1976, during 32 years, in 216 localities and in 300 surveys 35,085 individuals were collected, reared and identified. This work yielded 68 species belonging to 11 families as shown in Table 2, in which also the number of host plants per moth species is indicated. The most commonly occurring species were ranged into dominance groups and the standard deviations were calculated. In Table 3 the most common and therefore most significant 6 species were presented, which were found constantly in the spring aspect of moth communities living on pomaceous fruits. Similarly, the most important species and their dominance conditions were presented for stone fruits in Table 4 and for berry fruits in Table 5. The most polyphagous 7 species (occurring on the majority of fruit

continued

Host plants studied												
9. Rubus idBeus L. Rubus caesius L.	10. Fragaria moschata D. Fragaria vesca L.	11. Cerasus mahaleb Mill.	12. Cerasus avium Mönch.	13. Cerasus vulgaris Mill.	14. Amygdalus communis L.	15. Armenica vulgaris Lom.	16. Persica vulgaris Mill.	17. Prumus spinosa L.	18. Prunus domestica L.	19. Ribes uva-crispa L.	20. Ribes nigrum L. Ribes rubrum L.	No. of host plants
			×	×				×	×			5
×	×	×	×	×		×	×	×	×			17
		×				×		×	×			7
								×	×			3
×	×											2
×												1
×												1
×						×	×					2
		×	×	×	×	×	×	×	×			15
									×			4
												3
												2
											×	1
28	14	13	28	24	11	26	17	22	29	9	12	

* The species are ranged into systematical order according to the Fauna Hungariae editions of Gozmány *et .al* (1955—68), Fauna Catalog of Gozmány (1952) and list of Nomenclature (Gozmány, 1968)

tree species in Hungary) were listed in Table 6. The latter moth species can live both on hosts belonging to the families Rosaceae, Grossulariaceae, but also on various forest trees and shrubs belonging to different plant families. The summer and autumn aspects were not separated yet as the referring surveys were more limited. In course of the 32 years mentioned, in 138 localities 227 surveys were carried out, yielding 13,700 individuals. The adults reared from the larval materials belonged to 26 moth species (7 families) as shown in Table 7.

By considering the species found most common in the spring aspect, both from point of view of polyphagy and frequency of occurrence, the same 6—7 species were found the most significant (Tables 3—6).

There were no significant differences between the frequencies of the first 5 species mentioned in Table 3 as the most common 6 species on pomaceous fruits. In the first half of present century KADOCSA (1938, 1944) already reported the first 3 species as most common on fruit trees in Hungary and even concluded that these 3 species were responsible for the shoot rolling symptoms in the spring. Our investigations demonstrated the presence of considerably more species in the orchards in the given period (Table 2). The species composition of moth communities living on pomaceous and stone fruit trees is basically the same only with differences in the frequency of some species; it has to be mentioned however that on stone fruits among the most common species as new element *Pandemis heparana* Schiff. appeared (Table 4). The most common species of moth communities on berry fruits did not differ considerably of the ones living on the two host plant groups mentioned above (Table 5) as *Adoxophyes reticulana* Hbn. and *Recurvaria nanella* Hbn. are concerned, the species *Pandemis heparana* Schiff. however appears as "connecting" element, "linking" so to say the communities to the ones of stone fruits. Finally, the 3 further most common species: *Archips rosanus* L., *Archips podanus* Sc. and *Clepsis spectrana* Tr. seem to be elements characteristic for berry fruits.

The summer and autumn aspects are not sharply delimited. In this period the communities consist mostly of species which develop inside the fruits and from the summer and autumn larval populations of the multivoltine species which played a role already in the spring aspect. These latter damage mostly on the surface of fruits. The communities consist further of other species living in the foliage like leaf moths, leaf miners (these are in present paper not dealt with) and the bark moth (Table 7).

Table 3

The most common 6 moth species occurring in Hungary in the spring aspect of the moth community of pomaceous trees. Based on data collected about the middle of the 20th century

Species name	Frequency %
1. Spilomota ocellana F.	83.7 ± 10
2. Recurvaria nanella Hbn.	83.3 ± 15
3. Hedya nubiferana Haw.	78.4 ± 22
4. Recurvaria leucatella Cl.	73.0 ± 23
5. Adoxophyes reticulana Hbn.	69.3 ± 25
6. Pandemis ribeana Hbn.	53.9 ± 32

Table 4

The most common 6 moth species occurring in Hungary in the spring aspect of the moth community of stone fruits. Based on data collected about the middle of the 20th century

Species name	Frequency %
1. Recurvaria nanella Hbn.	95.0 ± 5
2. Spilonota ocellana F.	72.6 ± 25
3. Adoxophyes reticulana Hbn.	68.3 ± 17
4. Pandemis heparana Schiff.	67.8 ± 18
5. Pandemis ribeana Hbn.	66.7 ± 15
6. Hedya nubiferana Haw.	62.5 ± 21

Table 5

The most common 6 moth species occurring in Hungary in the spring aspect of the moth community of berry fruits. Based on data collected about the middle of the 20th century

Species name	Frequency %
1. Adoxophyes reticulana Hbn.	85.4 ± 15
2. Archips rosanus L.	71.4 ± 25
3. Recurvaria nanella Hbn.	68.2 ± 26
4. Archips podanus Sc.	59.8 ± 21
5. Clepsis spectrana Tr.	58.2 ± 21
6. Pandemis heparana Schiff.	55.4 ± 28

Table 6

The most polyphagous species according to the moth communities studied in the spring aspect, on 20 host plant species

Species	Number of host plants
1. Recurvaria leucatella Cl.	18
2. Recurvaria nanella Hbn.	19
3. Pandemis heparana Schiff.	19
4. Pandemis ribeana Hbn.	18
5. Adoxophyes reticulana Hbn.	15
6. Hedya nubiferana Haw.	17
7. Spilonota ocellana F.	15

Table 7

List of moth species reared in the period 1945—76 from larval materials collected (only the ones actually damaging

Moth species reared*	Host plants studied								
	1. Cydonia oblonga M.	2. Pyrus communis L.	3. Pyrus achras Gärthn.	4. Malus silv. Mill.	5. Malus dom. Borkh.	6. Sorbus aucuparia L.	7. Mespilus g. L.	8. Crataegus ox. L.	9. Rosa canina L.
I. PLUTELIDAE:									
1. Argyresthia conjugella Z.					×	×			
II. GLYPHIPTERYGIDAE:									
2. Anthophila pariana Cl.	×	×			×	×	×	×	
III. COSMOPTERYGIDAE:									
3. Blastodacna hellerella Dup.								×	
IV. GELECHIIDAE:									
4. Anarsia lineatella Z.	×	×			×				
V. CARPOSINIDAE:									
5. Carposina scirrhosella HS.									×
VI. TORTRICIDAE:									
6. Pandemis heparana Schiff.	×	×			×				
7. Pandemis ribeana Hbn.	×	×			×				
8. Argyrotaenia pulchellana Haw.		×			×				
9. Choristoneura sorbiana Hbn.		×			×				
10. Archips rosanus L.	×	×			×				
11. Archips podanus Scop.		×			×				
12. Adoxophyes reticulana Hbn.		×	×	×	×				
13. Hedya nubiferana Haw.		×			×		×		
14. Ancylis achatana Schiff.		×			×				
15. Spilonota ocellana F.	×	×			×		×		
16. Enarmonia formosana Scop.			×	×	×				
17. Grapholitha molesta Busck	×	×			×		×		
18. Grapholitha lobarzewskii Nov.									
19. Grapholitha funebrana Tr.									
20. Grapholitha tenebrosana Dup.									×
21. Grapholitha janthinana Dup.						×	×	×	
22. Laspeyresia pomonella L.	×	×	×	×	×		×		
23. Laspeyresia pyrivora Danil.		×	×						
24. Laspeyresia amplana Hbn.									
25. Laspeyresia splendana Hbn.									
VII. PHYCITIDAE:									
26. Rhodophaea advenella Zck.						×	×	×	
Total number of species par host plant:	8	15	4	3	16	4	7	4	2

* The species are ranged into systematical order according to the Fauna Catalog of

during the summer and autumn aspects of moth communities the fruits were considered)

Host plants studied														
10. Cerasus avium Mönch.	11. Cerasus vulg. Mill.	12. Amygdalus c. L.	13. Armenica v. Lam.	14. Persica vulg. M.	15. Prunus spinosa L.	16. Prunus dom. L.	17. P. insititia J.	18. P. domestica spp. rotunda W.	19. Corylus av. L.	20. Fagus silv. L.	21. Castanea sat. Mill.	22. Quercus spp.	23. Juglans regia L.	No. of host plants
														2
			×		×	×	×							10
														1
			×	×		×	×							7
														1
			×	×	×	×	×	×						9
			×	×	×	×	×	×						9
			×	×										4
			×	×										2
			×	×		×	×	×						8
			×	×	×	×	×	×						8
×			×	×	×	×	×	×						11
			×											4
					×									2
														5
×	×	×	×	×	×	×	×	×						12
			×	×		×	×	×						9
					×	×	×	×						4
			×		×	×	×	×						5
					×									1
														4
			×	×									×	9
									×		×	×	×	2
									×	×	×	×	×	4
														5
														3
2	1	1	13	10	10	11	11	9	2	1	2	2	3	

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Anti-Ecdysone Activity of Compounds Related to Triarimol

By

B. TÓTH, M. TÜSKE, G. MATOLCSY and L. VARJAS

Research Institute for Plant Protection,

H—1525 Budapest, P. O. Box 102, Hungary

A series of compounds related to triarimol was prepared and tested for anti-ecdysone activity. Some of them exert a marked delay of pupation of the insects tested. Relationships between structure and activity are briefly discussed.

The anti-ecdysone activity of triarimol, α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol was described in a previous paper (MATOLCSY *et al.*, 1975). This finding prompted us to prepare a series of compounds related to triarimol and to test them for anti-ecdysone activity. The purpose of the present study is to determine the connection between chemical structure and biological effect and to investigate the structural requirements of this action as well as to search for further active derivatives of the same type.

Materials and Methods

Chemical

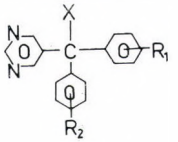
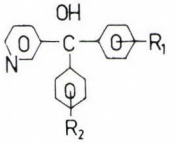
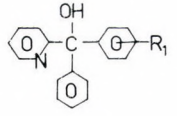
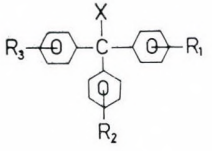
The majority of the compounds tested has been described in literature. Most of them are known to act as fungicides, some others possess an antibacterial effect (Lilly, ELI and CO., 1968; VAN HEYNINGEN and TAYLOR, 1969; WHALEY and TAYLOR, 1970; DAVENPORT *et al.*, 1971).

The preparation of compounds listed in Table 1 was carried out by known synthetic methods described in literature (GOMBERG and DAVIS, 1903; GOMBERG and CONE, 1905; PROOST and WIBAUT, 1940; PEARSON *et al.*, 1962; VAN HEYNINGEN, 1968; DAVENPORT *et al.*, 1971; BÜCHEL *et al.*, 1971, 1972). Compounds Nos 4, 24, 25 were obtained by extraction of commercially available pesticides.

Compounds Nos 3, 8, 21, 22, 27, 28, 29, 30, 34 have not been described as yet and were prepared on analogy of known methods. α-(4-Hydroxyphenyl)-α-phenyl-3-pyridinemethanol (8) was prepared by ether cleavage of the corresponding 4-methoxyphenyl derivative.

Structures were identified by methods of elementary, IR and NMR analyses. Physical and analytical data are listed in Table 2.

Table 1

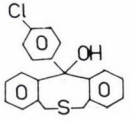
Compounds	No.	X	R_1	R_2	R_3	Retardation ratio (RR)
	1.	OH	2,4−Cl_2	H	—	1.70*
	2.	Cl	2,4−Cl_2	H	—	1.10
	3.	OOC−CH_2Cl	2,4−Cl_2	H	—	1.25
	4.	OH	4−Cl	2−Cl	—	1.72*
	5.	—	H	H	—	1.25
	6.	—	4−OCH_3	H	—	1.10
	7.	—	4−OCH_3	4−OCH_3	—	1.00
	8.	—	4−OH	H	—	1.00
	9.	—	4−Cl	H	—	1.28
	10.	—	H	—	—	1.10
	11.	—	4−Cl	—	—	1.50
	12.	OH	H	H	H	1.00
	13.	Cl	H	H	H	1.10
	14.	1-imidazolyl	H	H	H	1.21*
	15.	1,2,4-triazol-1-yl	H	H	H	1.26*
	16.	OH	4−Cl	H	H	1.50
	17.	1-imidazolyl	4−Cl	H	H	1.21*
	18.	OH	2.4−Cl_2	H	H	1.40
	19.	OH	2−Cl	H	H	1.10
	20.	1-imidazolyl	2−Cl	H	H	1.09*
	21.	OH	3-phenylsulfonyl	H	H	1.00
	22.	1-imidazolyl	3-phenylsulfonyl	H	H	1.48*
	23.	OH	4−Cl	4−Cl	4−Cl	1.25*

	24.	OH	—	—	—	1.00
	25.	$OOC-CH_3$	—	—	—	1.00
	26.	—	H	—	—	1.91*
	27.	—	4—Cl	—	—	1.50*
	28.	—	H	—	—	1.25*
	29.	—	4—Cl	—	—	0.82*
	30.	—	$4-OCH_3$	—	—	1.25*
	31.	—	H	—	—	0.95*
	32.	—	4—Cl	—	—	0.93*
	33.	—	$4-OCH_3$	—	—	1.44*

* dissolved in DMSO-water (5 : 5)

1. Triarimol
4. Fenarimol
20. Clotrimazol
24. Du-Ter
25. Brestan

Table 1 (continued)

Compounds	No.	X	R_1	R_2	R_3	Retardation ratio (RR)
	34.	—	—	—	—	0.93*
4-(4-chlorophenyl)-imidazol		—	—	—	—	1.10
Imidazol		—	—	—	—	0.77*
1,2,4-Triazol		—	—	—	—	0.97*

* dissolved in DMSO-water (5 : 5)

Table 2

Compounds No.	M. p. C	Yield %	Formula	Carbon		Hydrogen		Nitrogen		Chlorine	
				Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
3.	113—119	68.8	$C_{19}H_{13}Cl_3N_2O_2$	55.88	55.70	3.18	3.35	6.88	6.72	26.19	26.08
8.	82—85	21.6	$C_{18}H_{15}NO_2$	78.00	77.40	5.41	5.41	5.50	5.52	—	—
21.	123—125	58.0	$C_{25}H_{20}O_3S$	75.00	74.98	5.00	5.31	—	—	—	—
22.	192—193	51.5	$C_{28}H_{22}N_2O_2S$	74.66	74.79	4.88	5.20	6.22	6.40	—	—
27.	277—279	49.7	$C_{21}H_{13}ClN_2O$	73.15	73.37	3.77	4.20	8.12	7.96	10.30	10.22
28.	145—146	93.8	$C_{20}H_{16}O_2$	83.33	83.25	5.55	5.78	—	—	—	—
29.	121—124	62.5	$C_{20}H_{15}ClO_2$	74.41	74.40	4.65	4.78	—	—	11.00	11.00
30.	105—107	63.0	$C_{21}H_{18}O_3$	79.24	79.24	5.66	5.93	—	—	—	—
34.	187—189	60.3	$C_{21}H_{17}ClOS$	71.49	71.18	4.82	5.01	—	—	10.07	9.75

Biological

The biological tests were carried out on the same way as described in a previous paper (MATOLCSY *et* al., 1975).

Full grown larvae of *Neobellieria (Sarcophaga) bullata* were used as test organisms. Ten μg of the compounds dissolved or finely disperged in 2 μl ethanol-water (2 : 8) or DMSO-water (5 : 5) were injected into "water treated" insects.

The activity of compounds manifested in the retardation of pupation (pupariation) can be expressed as the retardation ratio (RR) calculated according to the formula:

$$RR = \frac{t_{50i}}{t_{50c}}$$

where t_{50i} is half time of pupation of the insects treated with the compounds and t_{50c} is half time of pupation of the insects treated with solvent only.

The half time of pupation means the interval (in hours) between treatment and pupation of 50 per cent of larvae calculated by graphical method (MILLER and TAINTER, 1944).

RR values higher than 1.00 indicate retardation effect.

Results and Discussion

Our results in Table 1 indicate that some of the compounds related to triarimol exert a marked delay of pupation of the insects tested.

Most of the compounds were soluble in ethanol–water mixture but some others in DMSO-water only. Both solvents themselves are inactive and they do not influence the activity of the tested compounds as indicated by equal RR values of triarimol in both cases ($RR_{ethanol} = 1.63 \pm 0.24$, $RR_{DMSO} = 1.70 \pm 0.27$).

Compounds Nos 4, 11, 16, 18, 22, 26, 27, 33 proved to be the most active, but only α-(4-chlorophenyl)-α-(2-chlorophenyl)-5-pyrimidinemethanol (4) and 11-H-11-hydroxy-11-phenyl-indeno[1,2-b]quinoxaline (26) reached the activity of triarimol.

Although unambiguous conclusion cannot be drawn regarding structural requirements of anti-ecdysone activity within this series, but some trends have been revealed by our experimental results. Thus the presence of one heterocyclic nucleus seems to be favouring activity, but triphenylmethanols may also possess an activity similar to that of heterocyclic derivatives in case of a suitable substitution.

Our former finding on the reversal of triarimol action by ecdysteron (MATOLCSY *et al.*, 1975) and structural similarities of the compounds as well as the fact that the antifungal activity of these compounds is due to the inhibition of fungal sterols (RAGSDALE and SISLER, 1972, 1973; MATOLCSY *et al.*, 1976) suggest that the effect of the tested compounds as well as that of triarimol is a result of the inhibition of ecdysone biosynthesis.

Further studies on this line are in progress.

Experimental

Preparation of the α-(4-hydroxyphenyl)-α-phenyl-3-pyridinemethanol (8)

0.6 g (0.002 moles) of α-(4-methoxyphenyl)-α-phenyl-3-pyridinemethanol and 9 ml 48% hydrobromic acid were refluxed for 3 hours. After cooling, the solid product was separated, extracted with diluted sodium carbonate solution, filtrated, the filtrate acidified with acetic acid, the pinkish product separated by suction and dried.

Preparation of the α-chloroacetoxy-α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinylmethane

To a mixture of 3.31 (0.01 mol) α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol, 1.01 g (0.01 mol) triethylamine and 30 ml ether 1.13 g (0.01 mol) chloroacetylchloride dissolved in 5 ml ether was added and the mixture was refluxed for 30 minutes. The solvents were evaporated and the solid product washed with water, dried and recrystallized from methanol-water 2 : 1.

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Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 12 (3—4), pp 383—385 (1977)

Study on the Factors Influencing the Decomposition of Linuron

II. The chemical and microbiological decomposition of linuron in soil

By

T. CSERHÁTI and A. VÉGH

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

The decomposition of linuron was investigated in sterilized and intact soil at 10, 18, 26 and 37°C. The rate of decomposition was not considerably influenced by the physico-chemical characteristics of the soil. The microbiological breakdown between 10—26°C increases nearly linearly with temperature, its energy of activation was found 13.3 kcal/mol. In sterilized soil its value is 19.4 kcal/mol. At higher temperature the rate of the microbiological decomposition is decreasing. The proportion of the chemical decomposition in the all-over breakdown of linuron is notable at low (10°C) and high (37°C) temperatures.

Introduction

Our investigations concerning the pH and temperature dependence of the linuron decomposition in model solutions gave us the possibility to resolve into chemical and microbiological components the breakdown of linuron in soil. In this study the applicability of the method is demonstrated with a Hungarian soil type.

Materials and Methods

To 10 g sterilized and intact samples of the chernozem soil of Martonvásár 100 μg linuron was added and incubated for 5 weeks at 10, 18, 26 and 37°C. Samples of 1 g weight were taken weekly, 1 ml hexan added, shaked thoroughly for a minute, separated and investigated by electron capture gas chromatography. Other method of purification of the hexan extract was not applied because the hexan did not solved any soil components influencing the gaschromatographic determination of linuron. During the experiment the samples were held at the 60% of their water binding capacity. From the data at 10, 18, 26 and 37°C the linear regressions characterizing the first order kinetics

$$\text{Time of incubation} = b \times \log \text{concentration}$$

were calculated for sterilized and intact soils.

The difference of the rate constants of a sterilized and intact soil gave the rate constant of the microbiological decomposition at the given temperature. On the base of the Arrhenius equation from the rate constants measured at different temperatures the energy of activation of the chemical and microbiological decomposition can be determined. The proportions of the chemical and microbiological breakdown too were calculated at the temperatures of incubation.

Results and Discussion

The temperature dependence of the rate constants of the linuron decomposition in sterilized soils is nearly logarithmic (Fig. 1), their values do not differ significantly from the values measured in model solutions. From the facts above mentioned we draw the conclusion that the physico-chemical characteristics of the soil do not influence considerably the decomposition of linuron. The microbiological breakdown is insignificant at 10°C, from 10°C up to 26°C is increasing nearly linearly, at 37°C decreases (Fig. 2).

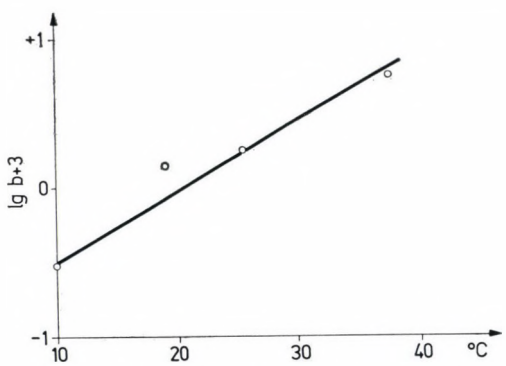

Fig. 1. The rate constants of the breakdown measured in sterilized soils

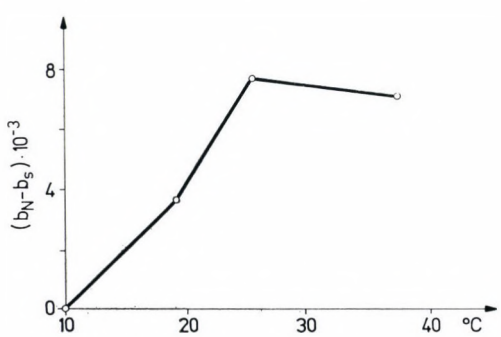

Fig. 2. The rate constants of the microbiological breakdown

The energy of activation calculated from the rate constants measured in sterilized soils is

$$\Delta H_a = 19.4 \text{ kcal/mol},$$

this value is in good agreement with the value measured in model solutions.

The energy of activation of the microbiological decomposition in the temperature interval 18–26°C was found

$$\Delta H_a = 13.3 \text{ kcal/mol}.$$

This significantly lower energy of activation indicates that in this case the decomposition of linuron presumably proceeds on an other biochemical pathway as the chemical decomposition.

At 10°C the chemical decomposition predominates, its proportion is decreasing with temperature, but newly increases at 37°C (Fig. 3).

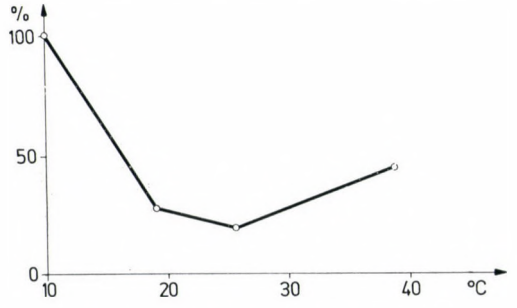

Fig. 3. The proportion of the chemical breakdown at different temperatures

Book Review

ADRIAN GIBBS and BRYAN HARRISON: *Plant Virology*. The Principles. Edward Arnold (Publishers) Ltd., London 1976, 292 pp.

This up-to-date book was compiled by two of the most famous personalities among virologists. It is an admirable account of all aspects of virology and it will certainly be an indispensable volume for plant pathologists, plant virologists, plant physiologists, molecular biologists and even entomologists. The book contains chapters dealing with the history and scope of virology, the virus names, effects of viruses on plants, methods of experimental and natural transmission, the composition and structure of the virus particles, the purification, properties of the purified preparations, infectivity assay, serological and physical-chemical methods and analysis, effects of inactivators, the behaviour of viruses in plants, variation, strains and classification, ecology, ways of preventing crop losses, viruses of organisms other than higher plants, origins of viruses and plant pathogens confused with viruses (e.g. mycoplasmas, rickettsia-like organisms).

The chapters on nomenclature and classification deserve special attention. Of special interest are the chapters on the composition and structure of particles of plant viruses, and the behaviour of viruses in the plants, as well as viruses of organisms other than higher plants (origins of viruses).

The book contains about 300 outstanding illustrations, including many original diagrams, photographs and electron micrographs, and over 1000 references to material in research papers and provides a synthesis of the science and practice of plant virology. The index of virus names, at the end of the book, gives name, cryptogram and groups of each included virus. It is of an invaluable help to the readers.

This excellent book have been received in the international scientific world with an enthusiasm which they merited: this worthy appreciation may be attributed with good reasons not only to its pioneering character but also to the high standard of its contents.

The authors deserve credit for publishing this book, and full homage is rendered to the Edward Arnold Ltd. publishing house.

J. HORVÁTH

DELUCCHI, V. L.: *Studies in Biological Control. — International Biological Programme 9.* — Cambridge University Press. Cambridge—London—New York—Melbourne. 1976. pp. 304. (Price: £ 13.00)

It is known pesticides pollute environment, therefore to the overcome of pests, integrated control methods are beeing worked out. The work is done in the frame of international programme in which hundreds of entomologists and more than thirty countries have a part. Some studies of the book resume in five programmes the results of 7—8 years work. In the

first part of the book we find a list of collaborators. Following is the introduction of DELUCCHI in which he gives a summary of the five themes and that is followed by the chapter "Definition and planning of the project" by WATERHAUSE.

The first project, fruit flies, is coordinated by M. A. BATEMAN. In the next larger chapter he summarizes the results: Life-table studies and pupal mortality. Significance of fly marking of oviposition site. Response to colour stimuli. Adults movements. Sexual behaviour of pest tephritids. Population and ecological genetics.

The second project is the coordination of M. MACHAUER and M. J. WAY of *Myzus persicae* Sulz., an aphid of world importance. In the following chapters results are discussed: General biology and population dynamics of *Myzus persicae*. Biological methods of aphid control. Integrated control of *Myzus persicae*.

The third project are Rice stem-borers coordinated by K. YASUMATSU, who expounds the results: The study area. Taxonomy and distribution of rice stem-borers and their natural enemies. The rice ecosystem. Ecology of rice stem-borers. Natural control of rice stem-borers. Feasibility of integrated control. Rice production and stem-borer damage.

The fourth project, Armoured scale insects coordinated by P. DE BACH and D. ROSEN. They discuss the results in the following chapters: Background. The natural enemies of armoured scale insects. Biological control attempts and their consequences. Exchange of natural enemies. World list.

The fifth project, Spider mites coordinators were N. W. HUSSEY and C. B. HUFFAKER. They discuss the results in the following chapters: Biology of spider mites. Biology and ecology of natural enemies of spider mites. Effect of pesticides on spider mites and their natural enemies. Biological control of spider mites on various crops.

The title of the last chapter of the book is Concluding remarks written by M. J. WAY.

The book demonstrates beautifully that the preliminary new system of defence is the profound knowledge of the ecology, ethology and physiology of the species. In our days to attain results it is only possible with central international coordination.

The book closes with hundreds of literary references index of animal names and subject index.

GY. SÁRINGER

MARIAN HORZINEK: *Kompendium der allgemeinen Virologie*. Verlag Paul Parey, Berlin und Hamburg, 1975. pp. 172.

Wohl niemand unter den naturwissenschaftlich Interessierten vermag heute noch das gesemte Gebiet der Virologie bei der sprunghaften Zunahme der Kenntnisse zu überschauen. Zwei Aspekte sind es, die das Erscheinen dieses Kompendiums besonders wertvoll erscheinen lassen; erstens fehlte es wenn von dem Erscheinen der „*Arthropodenviren*" von A. KRIEG (1973) abgesehen wird, in den letzten Jahren im deutschen Sprachgebiet an einem Buch, das den zahlreichen Neuentwicklungen in der allgemeinen Virologie genühend Rechnung trägt, und zweitens wurde als Neuheit eines solchen Buches der Versuch unternommen, im Sinne der Forderungen der Virologie und der Nachbardisziplinen der Naturwissenschaften überschaubar und damit die moderne Virologie deutlich zu machen.

Das Buch enthält vier Kapitel: das Virus als Partikel, das Virus als infektiöse Einheit, das Virus als Krankheitserreger, das Virus als Seuchenerreger. Am Ende jeden Abschnittes befinden sich weiterführende Literaturangaben. Im ersten, 81 Seiten umfassenden Abschnitt wurden Vermehrung und Nachweis, Quantifizierung, Konzentrierung und Reinigung, Charakterisierung, Verhalten gegenüber physikalischen und chemischen Einflüssen, Klassifizierung und Nomenklatur, Struktur der DNS-Viren, Struktur der RNS-Viren und Viren mit unsicherer systematischer Stellung sowie Viroide kurz beschrieben.

Im zweiten Abschnitt (32 Seiten) wurden die Einschritt-Vermehrungskurve, Phasen des Infektionszyklus, Folgen der Infektion für die Zelle, Genetik und Interferenz besprochen.

Im dritten Abschnitt des Buches (23 Seiten) kann sich der Leser einen Überblick über Grundzüge der Pathogenese und Immunologie der Viruserkrankung verschaffen. Im letzten, vierten Abschnitt des Buches (21 Seiten) wurden die Mechanismen der horizontalen Übertragung, Mechanismen der vertikalen Übertragung, die Rolle des Wirts, der Ursprung der Epidemien, die wirtschaftliche Bedeutung von Viruskrankheiten und diagnostische und angewandte Virologie kurz beschrieben.

Das Kompendium behandelt in erster Linie die animalen Viren. Die Pflanzenviren und die pflanzenvirologischen Methoden wurden leider relativ kurz besprochen, obwohl diese in der allgemeinen Virologie eine verhältnismässig grössere Rolle spielen. Die Architektur des Tabakmosaik-Virus als eines Vertreters der helikalen Symmetrieklasse zeigt die Abb. 16 (Seite 37), nicht aber die Abb. 19 (vgl. Seite 77); abgesehen von diesem kleinen Druckfehler ist das Buch fehlerfrei.

Der Verfasser hult es für wünschenswert, die zum Studium von Menschen-, Tier-, Pflanzen- und Bakterienviren verwendeten wichtigsten Methoden zu sammeln. Deshalb ist dieses moderne Kompendium nicht nur für Studierende der Medizin, Veterinärmedizin, Biologie und Landwirtschaft geschrieben, deren Examensvorbereitung das Buch wesentlich zu erleichtern vermag, sondern auch für Lehrende und Lernende in Fach- und allgemeinenbildenden Schulen sowie Forschungsinstituten.

Die Fülle des behandelten Stoffes ist erstaunlich, die reiche Zahl an ausgezeichneten Abbildungen steigert den Wert des vom Verlag gut und schőn ausgestatteten Buches.

J. HORVÁTH

Current Topics in Plant Pathology

Proceedings of a Symposium held at the Hungarian Academy of Sciences, Budapest, 24—27 June, 1975.

Edited by Z. KIRÁLY

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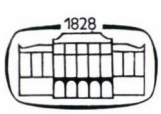

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