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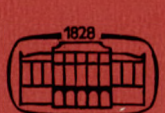

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Systemic Resistance Induced by a Strain of Alfalfa Mosaic Virus in *Phaseolus vulgaris* L.

By

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The induction of systemic resistance by alfalfa mosaic virus in *Phaseolus vulgaris* L. cv. "Borlotto nano di Vigevano" was investigated. Acquired resistance, expressed as a reduced number of local lesions, was measured at various intervals following the inducing inoculation. In half leaves and in primary leaves opposite previously inoculated ones, resistance was first detected 48 hrs later, and both its level and its successive behaviour were dependent on the severity of the primary infection. In the first trifoliate leaves the degree of acquired resistance was on the contrary independent of the concentration of the first inoculum and it appeared mainly related to the time between the two inoculations. In each case the lesion appearance in resistant tissues was delayed at least 24 hrs in comparison with the nonresistant ones. The mechanical injury induced by rubbing the leaf surface with carborundum 600-mesh did not induce any resistance. The effect of induced systemic resistance on the establishment of the new infection and on the virus multiplication and cell-to-cell movement, as well as the hypotheses concerning the development of systemic resistance are discussed.

Since the work of GILPATRICK and WEINTRAUB (1952), it is well-known that in several virus—host combinations the effect of a localized infection may extend to other parts of the plant, by inducing the development of systemic resistance against a following challenge inoculation with the same and — in some cases — with any other virus that induces localized infection. This systemic effect consists in 1. fewer lesions and 2. smaller lesions in test plants than in previously uninoculated controls.

The induction of systemic resistance has been studied in host plants which react with local necrotic lesions, for tobacco mosaic and some other viruses like tobacco necrosis, potato virus X, southern bean mosaic, cucumber mosaic, tobacco ringspot and turnip mosaic virus (ROSS and BOZARTH, 1960; ROSS, 1961, 1964 and 1966; LOEBENSTEIN, 1963; BOZARTH and ROSS, 1962 and 1964). On the contrary, the phenomenon has not yet been thoroughly investigated for alfalfa mosaic virus (AMV), although ROSS (1964 and 1966) demonstrated that this virus induces systemic resistance in Pinto bean and in Blackeye cowpea. The resistance was expressed by a decrease of both the size and the number of the lesions induced by the challenge inoculum, and was lacking in virus speci-

ficity. Ross (1961, 1964 and 1966) also reported that neither simulated inoculation nor abrasion nor hard mechanical injury induced systemic resistance.

In this paper we report the results of some experiments carried out in the attempt to better define the induction and the rate of development of systemic resistance induced by alfalfa mosaic virus in a hypersensitive bean cultivar. The effect of mechanical injury to the leaves caused by a simulated inoculation was contemporarily controlled.*

Materials and Methods

All the experiments were carried out in an insect-screened greenhouse, where the temperature ranged from 20 °C to 25 °C, with relative humidity 70–90% depending on the season. Plants of *Phaseolus vulgaris* L. cv. "Borlotto nano di Vigevano", which is a hypersensitive host for AMV, were grown in steam-sterilized soil, in large pots each containing 12–15 plants. Ten–twelve days after sowing the plants were selected for uniformity and the terminal bud was removed, except in the experiments concerning trifoliate leaves. The axillary shoots were pinched out as soon as they developed.

Purified suspensions of AMV-Mi, a strain of alfalfa mosaic virus isolated by Belli (1961), were used for all the inoculations. The virus was diluted with phosphate buffer 0.01 M pH 7.0. Plants were inoculated by the usual technique of mechanical transmission, using 600-mesh carborundum as an abrasive. Immediately after inoculation the leaves were rinsed with tap water. Controls consisted of comparable plants of the same age and similarly treated, except that the first inoculation was omitted or substituted by simple rubbing of the leaf surface with carborundum and buffer.

All the ineffectivity assays were conducted according to one of the following three standard procedures:

Half-leaf tests. Plants were inoculated on the right or left half of each primary leaf. Inocula of different concentrations were used in the various experiments, in order to induce increasing numbers of lesions, avoiding, however, tissue collapse. Control plants were either left uninoculated or were subjected to a simulated inoculation. At different times after the first inoculation, the opposite half leaf was inoculated on both test and control plants with a suspension of the same virus at a concentration expected to induce a countable number of lesions per half leaf in the controls. This second inoculation will be subsequently referred to as "challenge inoculation". In time-course experiments, groups of at least 5 plants for each of the times considered were used. The lesions induced by the challenge inoculation were always counted – in both test and control plants – 5–7 days later. The lesions evoked by the prior inoculation

* A preliminary report on this work was presented at the 2nd International Congress of Plant Pathology, September 5–12, 1973, Minneapolis, USA.

were also counted in order to establish a corrrelation between the infectivity of the first inoculum and the number of lesions induced by the challenge inoculation, *i.e.* the degree of induced resistance.

Opposite primary leaf tests. The experiments were planned as described above, the only difference being that test plants were inoculated in one of the two primary leaves. In the controls the first inoculation was omitted or substituted by the rubbing of one primary leaf. The challenge inoculation was done some days later on the opposite primary leaf of each plant. Time-course experiments were also carried out, and the lesions were always counted 5–7 days after each challenge inoculation.

Trifoliate leaf tests. In these experiments the plants were only deprived of the axillary shoots, but not of the terminal bud. After the formation of the first trifoliate leaf, all new buds were pinched out as soon as they appeared. Ten–twelve days after sowing, the two primary leaves were inoculated with a very concentrated suspension of AMV-Mi. At this time the first trifoliate leaf was already present but had not yet started to expand. Some days later, when the local lesions induced by the first inoculation were completely developed, the first trifoliate leaf was inoculated with a less or similarly concentrated suspension of the same virus. The lesions induced by the challenge inoculation were counted 7–10 days later. The control plants were not rubbed. No time-course experiments were carried out in this case, because of the progressive senescence of the primary leaves.

In each case the induced systemic resistance was determined on the basis of the reduced lesion number and not in terms of lesion size, because of the great size variability of local lesions that AMV-Mi induces in "Borlotto nano" bean.

All the results of the infectivity assays were subjected to statistical analysis (analysis of variance by comparison of the means). In the time-course experiments, the data were analyzed according to the factorial design.

Results

Resistance induction and development in the opposite half leaf. Two preliminary experiments were carried out (Table 1) in which the first inoculation of half primary leaves induced an uncountable number of lesions; in the control plants, half primary leaves were rubbed. In the first experiment the opposite half leaves were challenge inoculated (50 μg/ml) two days later, when only a few still immature primary lesions were visible. In the second experiment, the interval between the two inoculations was of 6 days, and the primary lesions were fully mature at the moment of the challenge inoculation (50 μg/ml). The results show that in both experiments the challenge inoculum induced fewer lesions in test than in control half leaves. The prior inoculation with a highly infectious suspension induced a certain level of systemic resistance (34%) even 2 days later, although the primary infection was not yet fully developed at this moment.

Table 1

Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in half primary leaves of "Borlotto nano" bean challenge inoculated 2 and 6 days following inoculation of the opposite half leaves with the same virus, and in challenged half leaves opposite previously rubbed ones (controls)

Days after the first inoculation (1)	Lesions induced by challenge inoculum (2) in half leaves opposite		Relative infectivity (3)	Percentage of inhibition (4)
	previously rubbed half leaves	previously inoculated half leaves (controls)		
2	52 (0)	68 (0)	76	34
6	30 (00)	53 (00)	57	43

(1) In both experiments the first inoculum was an AMV suspension that induced an uncountable number of lesions.
(2) The challenge inoculum had a concentration of 50 μg/ml in the two experiments. In both experiments the lesions were counted 6 days after the challenge inoculation.
(3) Number of lesions in test half leaves as a percentage of that in control half leaves.
(4) Measured in each case as: 100 — relative infectivity.
(0) Each figure is the average of 20 half leaves.
(00) Each figure is the average of 24 half leaves.
Note. The analysis of variance showed that in the two experiments the difference between tests and controls is significant at the 5% level

A little higher resistance (43%) was induced in half leaves challenged 6 days after the prior inoculation, but the difference between tests and controls was statistically significant at the same level (5%) in both cases.

The resistance-inducing effect of the prior inoculation was also compared with that of mechanical injury caused by a simulated inoculation, using challenged half leaves of previously uninoculated and unrubbed plants as controls. Results are reported in Table 2, which shows that the first inoculation with a very infectious inoculum induced a high (61% level) and significant degree (1% level) of resistance in half leaves challenged 3 days later. Also rubbing seemed to reduce the number of lesions induced by the challenge inoculum, but it did not induce a significant amount of resistance. As the difference between the effect of the first inoculation and the rubbing was statistically significant (1% level), it therefore appears that the development of systemic resistance is mainly due to viral infection and that mechanical injury is not important. Moreover, the level of induced systemic resistance seems to be mainly due to the severity of the primary infection, independently of its progress.

Two time-course experiments were then carried out in order to better define this relationship and resistance with time. In both experiments controls were uninoculated and unrubbed plants. Figure 1 shows that when the prior inoculation evoked a relatively low number of lesions per half leaf, no

Table 2

Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in half primary leaves of "Borlotto nano" bean challenge inoculated 3 days following inoculation of opposite half leaves with the same virus, in challenged half primary leaves opposite previously rubbed ones, and in challenged control half leaves opposite uninoculated and unrubbed

Lesions induced by challenge inoculum (1)		Relative infectivity (2)	Percentage of inhibition (3)
(a) Half leaves opposite previously (3 days) inoculated ones	122 (0)	39	61
(b) Half leaves opposite previously (3 days) rubbed ones	256 (0)	81	19
(c) Half leaves opposite previously uninoculated and unrubbed (controls)	314 (0)	100	—

(1) The first inoculum induced about 700 lesions per half leaf. The challenge inoculum had a concentration of 100 μg/ml. The local lesions were counted 6 days after the challenge inoculation.
(2) Number of lesions in (a) and in (b) as a percentage of that of lesions in (c).
(3) Measured in each case as: 100 — relative infectivity.
(0) Each figure is the average of 34 half leaves.
Note. The analysis of variance showed that the difference between (a) and (c) is significant at the 1 % level, the difference between (b) and (c) is not significant, and the difference between (a) and (b) is significant at the 1 % level.

difference in lesion number between tests and controls appeared in half leaves challenged 2 and 4 days later; a very high level of resistance appeared in half leaves challenged 6 days after the prior inoculation, but it decreased very rapidly during the two following days. The analysis of variance according to the factorial design showed a significant (5% level) difference between tests and controls in the experiment as a whole, but this difference appeared to be mainly related to a well-defined time (6 days) because the interaction between intervals and treatments did not appear to be significant for the experiment altogether. On the contrary, when the first inoculum was very infectious, the resistance in challenged half leaves developed much sooner (Fig. 2): it was already very high on the 2nd day, although at this moment the primary lesions were not yet fully developed. It reached its maximum on the 3rd day; later, the decrease was slower than in the previous experiment. The difference between tests and controls was significant (1% level) in the experiment as a whole, and the effect of time and the interaction time/treatment were also significant at the same level. These results confirm that the moment in which systemic resistance appears is directly correlated to the severity of the primary infection, independently of the maturation degree reached by the lesions. Moreover, once maximum resistance is

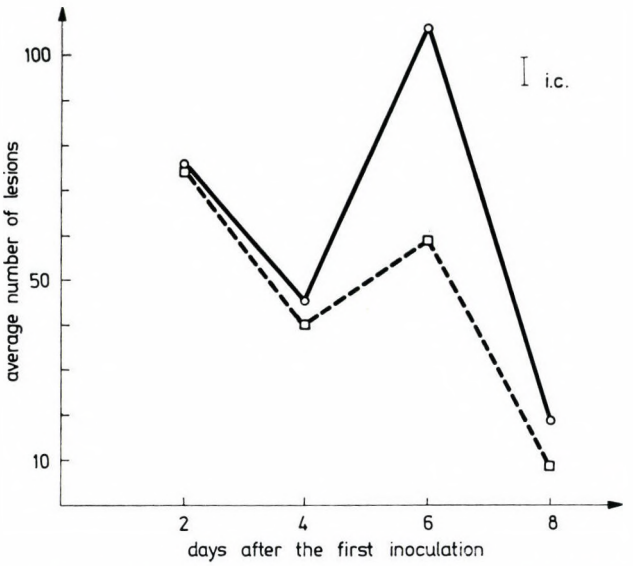

Fig. 1. Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in half primary leaves of "Borlotto nano" bean challenge inoculated at various intervals following inoculation of the opposite half leaves with the same virus, and in challenged half primary leaves opposite previously uninoculated and unrubbed ones (controls).

The first inoculum induced an average of 200 lesions per half leaf. The challenge inoculum had a concentration of 50 μg/ml. Each point is the average of 10 replicates. The lesions were counted 6 days after the challenge inoculation.

Note. The analysis of variance according to the factorial design showed a significant difference (5 % level) between test and control in the experiment as a whole, and this difference depends on the time passed since the first inoculation.

□ - - - - - - □ - - - - - - □ Half primary leaves opposite previously inoculated

○ ——— ○ ——— ○ Half primary leaves opposite previously uninoculated and unrubbed ones (controls)

I. C. Interval of confidence

reached, the resistance itself decreases rapidly if the first infection is slight whereas, in the opposite case, it remains more or less stable during the following days.

The resistance-inducing effect of both primary infection and rubbing was also measured at different intervals after the first or simulated inoculation. The results of this experiment (Fig. 3) confirm the importance of the severity of the primary infection: as in the case of the similar previous experiment (see Fig. 2), the maximum level of resistance – in comparison with the untreated control – appeared on about the 3rd day (67 hrs), in relation to the very high number of local lesions already present even if not yet fully mature in the half leaves inoculated first. Moreover, also in this case the resistance decreased slowly during the following days.

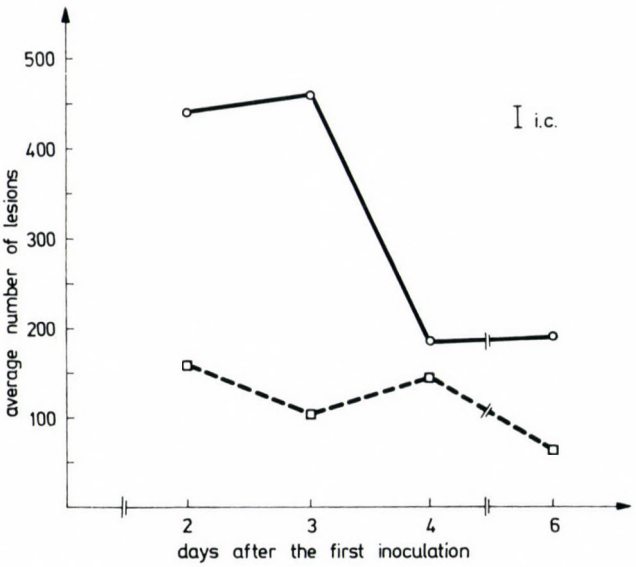

Fig. 2. Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in half primary leaves of "Borlotto nano" bean challenge inoculated at various intervals following inoculation of the opposite half leaves with the same virus, and in challenged half primary leaves opposite previously uninoculated and unrubbed ones (controls).

The first inoculum induced an average of 600 lesions per half leaf. The challenge inoculum had a concentration of 100 μg/ml. Each point is the average of 10 replicates. The lesions were counted 6 days after the challenge inoculation.

Note. The analysis of variance according to the factorial design showed a significant difference (1% level) between test and control in the experiment as a whole. The effect of the intervals and the time/treatment interaction were also found to be significant at the same level.

□ - - - - - - □ - - - - - - □ Half primary leaves opposite previously inoculated

○ ——— ○ ——— ○ Half primary leaves opposite previously uninoculated and unrubbed ones (controls)

I. C. Interval of confidence

From Fig. 3 it also appears that no systemic resistance developed in half leaves opposite those in which the primary infection had begun only 24 hrs before. This result shows that although the severity of the primary infection greatly influences the resistance development and rate, it is also indispensable that this infection has overcome its initial stage in order that the resistance itself appears. Furthermore, in comparison with the previous experiment (see Fig. 2), it would appear that in this case there was an opposite behaviour in the half leaves challenged at 43 hrs. In fact, it seemed that at this time they were much more sensitive to the infection than the untreated controls. If one considers the effect of rubbing *per se*, in comparison with the unrubbed controls, this seemed to induce a certain level of resistance already 24 hrs after the treatment. This effect disappeared during the following 24 hrs but reappeared more markedly

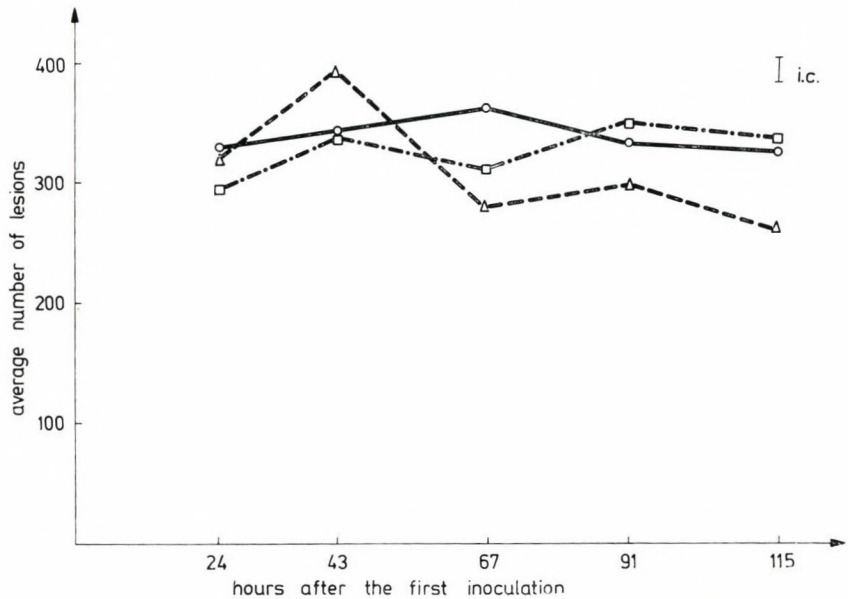

Fig. 3. Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in half primary leaves of "Borlotto nano" bean challenge inoculated at various intervals following inoculation of the opposite half leaves with the same virus, in challenged half primary leaves opposite previously rubbed ones, and in challenged half primary leaves opposite previously uninoculated and unrubbed ones (controls).

The first inoculum induced an average of 400 lesions per half leaf. The challenge inoculum had a concentration of 50 μg/ml. Each point is the average of 10 replicates. The lesions were counted 6 days after each assay.

Note. The analysis of variance according to the factorial design did not show significant differences between tests and control in the experiment as a whole. Significant differences (5% level) were found among each interval; the treatment/time interaction was found to be highly significant (1% level).

△ – – – – – – △ – – – – – – △ Half primary leaves opposite previously inoculated

□ ·–·–·–·– □ ·–·–·–·– □ Half primary leaves opposite previously rubbed ones

○ ——————— ○ ——————— ○ Half primary leaves opposite previously uninoculated and unrubbed ones (controls)

I. C. Interval of confidence

at 67 hrs; later, only negligible differences were found. The analysis of variance according to the factorial design pointed out that there are no significant differences between the two tests together compared with the control in the experiment as a whole, but significant differences exist both in the various intervals considered (5% level) and in the interactions between treatments and intervals (1% level). This indicates that, at least for some of the intervals examined, there were significant differences between the treatments. The partitioning of this inter-

action shows that only the difference between half leaves opposite previously inoculated and half leaves opposite previously rubbed is significant, while there is no significance between the untreated control and the two treatments together. This clearly shows that the induction of systemic resistance is due to the viral infection and that mechanical injury is of no importance here.

Resistance induction and development in the opposite primary leaf. A preliminary experiment showed (Table 3) that the inoculation of one primary leaf with a very infectious AMV preparation induced 4 days later – when the lesions were almost completely mature – a high degree (66% level) of resistance in the opposite one. This effect appeared highly significant (1% level) when compared with rubbed controls.

The resistance-inducing effect of the prior inoculation was also compared with that of mechanical injury (Table 4): the infectivity of the challenge inoculum was reduced by both treatments, but this decrease appeared significant (1% level) only in the leaves opposite previously (76 hrs) inoculated with a very infectious viral suspension. Moreover, the difference between the effect of the prior inoculation and of rubbing is statistically significant (5% level): this confirms that the development of systemic resistance in the opposite primary leaf is due to the infectious process and not to the mechanical injury.

In both experiments described above, the resistance developed following a very severe primary infection and reached very high levels 3–4 days later.

Table 3

Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in primary leaves of "Borlotto nano" bean challenge inoculated 4 days following inoculation of the opposite primary leaves with the same virus, and in challenged primary leaves opposite previously rubbed ones (controls)

Days after the first inoculation (1)	Lesions induced by challenge inoculum (2) in primary leaves opposite		Relative infectivity (3)	Percentage of inhibition (4)
	previously inoculated primary leaves	previously rubbed primary leaves (controls)		
4	40 (0)	116 (0)	34	66

(1) The first inoculum induced an average of 560 lesions per leaf.
(2) The challenge inoculum had a concentration of 50 μg/ml. The lesions were counted 6 days after the challenge inoculation.
(3) Number of lesions in test primary leaves (opposite previously inoculated ones) as a percentage of that in control primary leaves (opposite previously rubbed ones).
(4) Measured as: 100 – relative infectivity.
(0) Each figure is the average of 14 leaves.
Note. The analysis of variance showed that the difference between tests and controls is significant at the 1% level

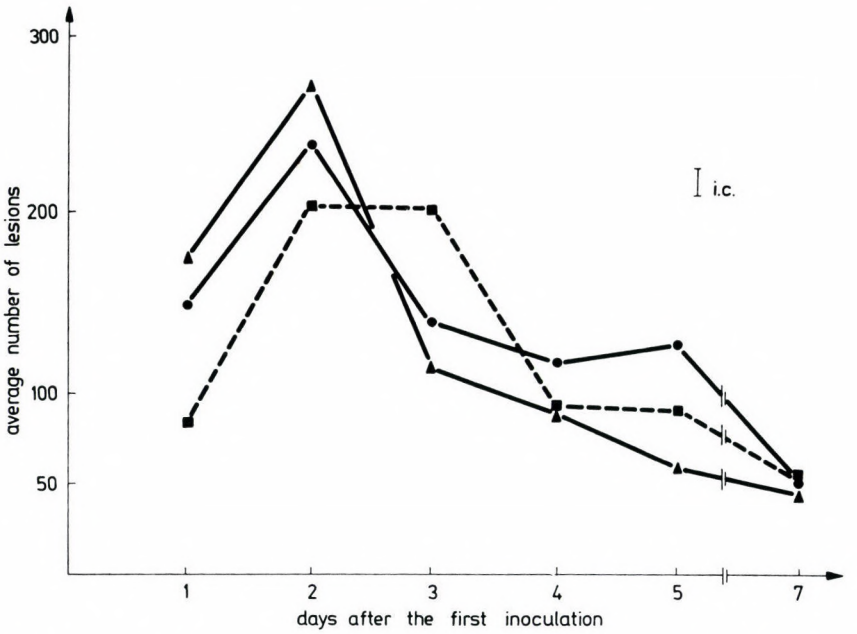

Fig. 4. Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in primary leaves of "Borlotto nano" bean challenge inoculated at various intervals following inoculation of the opposite primary leaves with the same virus, in challenged primary leaves opposite previously rubbed ones, and in challenged primary leaves opposite previously uninoculated and unrubbed ones (controls).

The first inoculum induced an average of 180 lesions per leaf. The challenge inoculum had a concentration of 50 μg/ml. Each point is the average of 6 replicates. The lesions were counted 6 days after each challenge inoculation.

Note. The analysis of variance according to the factorial design did not show significant differences between tests and controls in the experiment as a whole. Both the deviation of intervals and the time/treatment interaction were found to be highly significant (1 % level).

▲——▲——▲ Primary leaves opposite previously inoculated

■- - - -■- - - -■ Primary leaves opposite previously rubbed ones

●——●——● Primary leaves opposite previously uninoculated and unrubbed ones (controls)

I. C. Interval of confidence

A time-course experiment was then carried out in order to control 1) if systemic resistance is induced in the opposite leaf by a primary infection of low severity; 2) the behaviour of induced resistance with time; 3) if the rubbing has some effect on the susceptibility of the opposite leaf to the challenge inoculation. The results (Fig. 4) show that a primary infection of low severity delayed the resistance development in the opposite one: in fact it began to develop on the 3rd day and later increased progressively, reaching a peak on the 5th day. More-

Table 4

Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in primary leaves of "Borlotto nano" bean challenge inoculated 76 hours following inoculation of the opposite primary leaves with the same virus, in challenged primary leaves opposite previously rubbed ones, and in challenged primary leaves opposite previously uninoculated and unrubbed (controls)

Lesions induced by challenge inoculum (1)		Relative infectivity (2)	Percentage of inhibition (3)
(a) Primary leaves opposite previously inoculated (76 hrs) ones	46 (0)	24	76
(b) Primary leaves opposite previously rubbed (76 hrs) ones	139 (0)	74	26
(c) Primary leaves opposite previously uninoculated and unrubbed (controls)	188 (0)	100	—

(1) The first inoculum induced an average of 410 lesions per leaf. The challenge inoculum had a concentration of 50 μg/ml. The local lesions were counted 6 days after the challenge inoculation.
(2) Number of lesions in (a) and in (b) as a percentage of that of lesions in (c).
(3) Measured in each case as: 100 — relative infectivity.
(0) Each figure is the average of 9 primary leaves.
Note. The analysis of variance showed that the difference between (a) and (c) is significant at 1% level, the difference between (b) and (c) is not significant, and the difference between (a) and (b) is significant at the 5% level

over, the inducing mechanism seemed to be rapidly less active: on the 7th day there was no more resistance. As in the case of half leaves (see Fig. 3), resistance is therefore induced only when the primary infection has overcome its initial stage: on the contrary, it seems that during the first 48 hrs the leaves opposite previously inoculated were a little more susceptible to the challenge inoculation than untreated controls. If one considers the effect of rubbing *per se* compared with untreated controls, this seemed to induce a noticeable level of resistance 24 hrs after the treatment; this effect was reduced at 48 hrs. At the next interval considered (72 hrs) the leaf opposite previously rubbed appeared much more susceptible to the challenge inoculum, while later a certain level of resistance was again present. The analysis of variance according to the factorial design again showed no significant differences between the two tests together and the control in the experiment as a whole. Moreover, significant differences (1% level) were found both for the various intervals considered and the treatment/time interactions. The partitioning of this interaction also shows in this case that only the difference between opposite previously inoculated and opposite previously rubbed leaves is significant, while there is no significance between untreated controls and the two treatments together. These results confirm that

Table 5

Average number of lesions induced by alfalfa mosaic virus (AMV-Mi) in the first trifoliate leaf of "Borlotto nano" bean challenge inoculated 6, 7 and 13 days following inoculation of the two primary leaves with the same virus, and in challenged first trifoliate leaf of control plants with uninoculated and unrubbed primary leaves

Days after the first inoculation (1)	Lesions induced by challenge inoculum (2) in one single leaflet		Relative infectivity (3)	Percentage of inhibition (4)
	(a) first trifoliate leaf of previously inoculated plant	(b) first trifoliate leaf of control plants		
6	116 (0)	160 (0)	72	28
7	8 (00)	17 (00)	47	53
13	4 (000)	14 (000)	28	72

(1) The first inoculum had different concentrations in the three experiments; it induced an average of 340, 81 and 95 lesions per primary leaf, respectively.
(2) The challenge inoculum had a concentration of 50 μg/ml in all the experiments. The lesions were counted 6 days after each challenge inoculation.
(3) Number of lesions in (a) as a percentage of that of lesions in (b).
(4) Measured in each case as: 100 — relative infectivity.
(0) Each figure is the average of 18 leaflets (6 plants).
(00) Each figure is the average of 15 leaflets (5 plants).
(000) Each figure is the average of 12 leaflets (4 plants).
Note. The analysis of variance showed that the difference between tests and controls is significant at the 1 % level, in the three experiments

the induction of systemic resistance is, also in the case of opposite primary leaves, due to the viral infection and that mechanical injury has no effect.

Induction of resistance in the first trifoliate leaf. Three different experiments were carried out, in which challenge inoculation was done 6, 7 and 13 days respectively, after the first one. Controls were untreated plants. Different concentrations of the prior inoculum were used in the three experiments, in order to control if the severity of the primary infection induced a different level of resistance with time. The results are shown in Table 5. In each case the first inoculation induced a significant level (1 %) of resistance in the first trifoliate leaf, independently from the severity of the primary infection. Therefore, the inducing effect also acts at a long distance and it seems to be mainly connected to the period of time following the initial infection, the percentage of inhibition being 28 % on the 6th day, 53 % on the 7th and 72 % on the 13th day. Moreover, the susceptibility of the first trifoliate leaf to the challenge inoculum decreased with time. Since this was found both in already infected and in healthy plants, this phenomenon could be due to both increased concentration of the antiviral factor induced by the first inoculation, and to the natural senescence of the trifoliate leaves.

In this case, no time-course experiments were carried out, because of the progressive ageing of the primary leaves.

In all the above described experiments it was observed that the first inoculation also had the effect of delaying the appearance of the lesions in challenged tissues. This effect was never observed in the case of simulated inoculation, *i.e.* mechanical injury. In the host–virus combination tested here, the size of the local lesions is extremely variable, therefore it is impossible to say if the induced resistance is expressed also as reduced lesion size.

When the challenged resistant tissues showing almost mature lesions were examined under ultraviolet light, only a very few fluorescent "pinpoints" were visible.

The presence of the virus could never be detected in resistant tissues prior to the challenge inoculation: biological assays on *P. vulgaris* and *Vigna sinensis* always gave negative results.

Discussion

The development of systemic resistance induced by the localized infection with AMV in "Borlotto nano" bean appears to be quite similar to that reported for several other hypersensitive host–virus combinations. Acquired resistance was first detected 48 hrs after primary inoculation and was induced both in the half leaf opposite previously inoculated and at considerable distance from the site of the inducing infection, *i.e.* in the opposite primary leaf and also in the first trifoliate leaf. The rate and level of induced resistance seem – at least in the case of half leaf and opposite leaf – to be mainly related to the severity of the hypersensitive reaction evoked by the primary inoculation, and the resistance-inducing mechanism seems to be inactivated more rapidly with primary infections of low severity. The maturation reached by the primary lesions at the moment of the challenge inoculation seems to have very little influence on the resistance behaviour; however, it only appears when the primary infection has passed the initial phase.

In the case of the first trifoliate leaf, the resistance-inducing effect seems on the contrary to be related mainly to the interval between primary and challenge inoculation.

The mechanical injury provoked by rubbing the leaf surface with carborundum 600-mesh had no effect on the induction of resistance, neither near the injured tissue.

The systemic resistance-inducing effect of localized viral infection has been described and expressed in terms of lesion size and/or in terms of lesion number (Gilpatrick and Weintraub, 1952; Ross and Bozarth, 1960; Ross, 1961, 1964 and 1966; Bozarth and Ross, 1962 and 1964; Loebenstein, 1963; Davis and Ross, 1968; Kimmins, 1969; Nagaich and Singh, 1970; Simons and Ross, 1971; Van Loon and Geelen, 1971; Fritig *et al.*, 1973; Conti and Vegetti, 1973;

BATRA and KUHN, 1973 and 1975; PRITCHARD and ROSS, 1975; VAN LOON, 1976). The general basis of the two expressions of the phenomenon is thought to be the same in each case, as a consequence of the enhanced defence mechanism of the plant (ROSS, 1966; LOEBENSTEIN, 1972). Therefore, a localized infection should act with a long-distance effect, by preventing both the initial attachment of the virus to the resistant cells and the lesion enlargement, that is the replication and the movement of the challenge virus from the original site of infection to adjacent cells. If this assumption is true, it would be very interesting to ultimately assess which one of the two effects prevails. This would permit to establish at which level the resistance mechanism acts, and therefore to better characterize its biochemical and physiological bases. In fact, if the induced resistance takes place with a reduction in lesion size, it means that the inducing local necrotic infection stimulates the hypersensitivity of the host plant up to a certain level; if on the contrary resistance takes place with a consistent and significant reduction of lesion number, it means that the host hypersensitivity is stimulated at a much higher level, such as to prevent the establishment of the new infection.

ROSS (1961 and 1966) states that above all in tobacco but also in Pinto bean infected with different viruses the primary effect of induced systemic resistance appears to be on lesion size, by reducing the viral multiplication and cell-to-cell movement, rather than on the establishment of infection. In bean, the correlation between lesion size and number is fairly good, but in any case the effect on size appears before the effect on number: only when the interval between the two inoculations was long enough to induce a maximum or near-maximum effect on lesion size, a significant reduction in number also occurred. This fact seems to confirm that the defence mechanism of the plant enacts at two successive levels, the strongest of which should act secondly avoiding the attachment of the virus to the host cell. From available data, it seems reasonable to assume that the prevailing of one or other level of the plant defence mechanism can also depend on the host–virus combination examined. In fact, ROSS (1961) showed a host-specificity in relation to systemic resistance; LOEBENSTEIN (1963) found that in *Datura stramonium* and in *Gomphrena globosa* respectively infected with tobacco mosaic and potato virus X, systemic resistance was expressed by a significant reduction in lesion number, and CONTI *et al.* (1974) showed that the level and the appearance of resistance can also vary in the same host for different strains of the same virus.

The evidence presented here supports this hypothesis; in fact the AMV-induced systemic resistance in "Borlotto nano" bean mainly acts by preventing the establishment of the new infection. Our results – supported by intense statistical analyses – show in fact that the primary effect is on the lesion number and that a mechanism is activated which prevents the establishment of the new infection by blocking the initial attachment of the challenge virus to the resistant cells. This is also confirmed by the fact that when resistant tissues bearing few necrotic lesions were examined under ultraviolet light, only very few "pinpoints" – due to the accumulation of fluorescent materials around the virus-induced

lesions (BEST, 1936) – were visible. These pinpoints represent immature lesions which do not progress to enough cells to become visible necroses; adding the number of these invisible lesions with the mature ones, the total was, however, significantly lower than that found in nonresistant tissues.

Due to the extreme variability of the lesion size that AMV induces in the bean cultivar studied here, it was impossible to evaluate exactly if resistance would also act by blocking the replication and the cell-to-cell movement of the challenge virus. Nevertheless, also in this host–virus combination a certain effect of this type is showed by the fact that in resistant tissues necrotic lesions became visible with considerable delay – usually 24 hrs, but even 36 hrs when resistance was at its maximum – compared with controls.

It is well known that both lesion size and lesion number are a function of the genetic characters of both the virus and the host, and can be modified by a number of factors, e.g. temperature, light and leaf age (RAPPAPORT and WILDMAN, 1957; HELMS and MCINTIRE, 1962; BOZARTH and ROSS, 1964; TAKAHASHI, 1972 and 1974; WU, 1973).

Independently of biochemical, physiological and morphological factors that influence such variability, if it is true that the two resistance levels have a common basis, it seems to us to be less correct and less feasible to express it in terms of lesion size than on the basis of lesion number. Firstly because of the irregular shape of the lesions, mainly in dense populations (SIMONS and ROSS, 1971), it is very difficult to measure the diameters correctly. In order to render this datum significant, it would be necessary to measure a very high number of lesions, much higher than that normally measured by various authors. Moreover, it is very difficult to establish when the lesions have stopped their enlargement, especially if they have been chosen at random on the whole leaf surface, because it is well known that the lesion maturation is not simultaneous at the apex and at the base of the leaf blade; on the other hand it is not correct to measure lesions chosen only on one part of the blade, because this does not represent an overall situation. In Pinto bean infected with two different strains of TMV, HELMS and MCINTIRE (1962) found that differences in lesion size between basal and central leaf areas can be due to differences in the stage of development within the leaf. The natural occurrence of very localized physiological and morphological differences between groups of cells must also be taken into consideration, so that the cells are not uniformly susceptible to infection, and some are more favourable to lesion enlargement than others (BALÁZS *et al.*, 1976). Moreover, the operator's technique during inoculation is also important, since variation in the pressure applied to different regions of the leaf during rubbing might induce wounds of different degree or kind, which might result in lesions of different sizes. It is possible that different levels of abrasive tend to predispose differently the areas around the point of entry of the virus, therefore giving the same result. In fact, it is well known that non-specific wound-healing mechanisms may influence the process for limiting lesion size (WU, 1973). The hypothesis should also be considered of the presence of heterogeneous infective

particles within the inoculum, differing in their ability to produce lesions of different sizes: in the case of a multicomponent virus such as AMV, this assumption seems to be likely.

The production of resistance-inducing substance(s) or antiviral factors both in infected and uninfected resistant tissues, as well as the presence of inhibitors of viral infectivity in healthy plants have been described by many authors (RAGETLI and WEINTRAUB, 1962a, b; SELA and APPLEBAUM, 1962; LOEBENSTEIN, 1963; LOEBENSTEIN and ROSS, 1963; SELA *et al.*, 1964, 1965 and 1966; CHADA and MACNEILL, 1969; KIMMINS, 1969; WYATT and SHEPHERD, 1969; NAGAICH and SINGH, 1970; SMOOKLER, 1971; ANTIGNUS *et al.*, 1971 and 1975; OWENS *et al.*, 1973). The chemical nature of these inhibitors, as well as the mechanism of their activity and their involvement in localization, induced resistance and recovery are still an open question; it, was, however, demonstrated that systemic resistance is associated with the movement of substance(s) from or to resistant tissues (LOEBENSTEIN, 1972). On the basis of the depletion theory (SILBERSCHMIDT and CANER, 1967), the increased metabolic activities in the initially infected cells act as a metabolic sink and cause a depletion of metabolites in the uninfected parts of the plant, which are therefore less able to support virus synthesis and become resistant. However, from our experiments the increased sensitivity to the challenge inoculum showed by opposite half leaves and opposite leaves soon after the primary inoculation, seems to contradict the metabolite depletion theory. This initially increased sensitivity could on the contrary be explained by supposing that in the primarily infected tissues a defence mechanism is rapidly activated, which recalls naturally occurring inhibitors from uninfected tissues that therefore become more sensitive to the challenge inoculum. When the first infection is well established, this recall could cease and be substituted by the production of new (?) inhibitor(s) that could spread to uninfected tissues and induce resistance. Moreover, the hypothesis that systemic resistance is caused by drainage of metabolites is negated by other experimental results (LOEBENSTEIN *et al.*, 1968 and 1969), and ROSS (1961) postulated that a diffusible by-product of the necrotic process – specific only to virus-induced necroses – could be the actual material responsible for development of systemic resistance.

Therefore, a virus multiplying in a hypersensitive host, or the resulting necrotic process, should induce the production of a material capable of systemic movement from infected to uninfected plant parts and of altering the reaction of invdaed tissues to subsequent inoculation by stimulating the host's defence mechanism (BOZARTH and ROSS, 1964; ROSS, 1966). However, the hypothesis that primary infection could activate the *de novo* synthesis of a resistance-inducing substance at a distance in uninfected tissues should also be considered. Our experiments with the first trifoliate leaf confirm that the induction of resistance is not due to a drainage of metabolites, because resistance developed and reached a high level in leaflets that did not begin to expand until after active virus multiplication and lesion growth had stopped in the initially inoculated

primary leaves. It also appears that the resistance-inducing substance moves up the stem or that the newly induced synthesis of the inhibitor takes place at a considerable distance from the site of inducing inoculation. As the susceptibility of the first trifoliate leaf to the challenge inoculation decreases with time both in already infected and in healthy plants, the possibility that systemic resistance may also be influenced by some change in plant physiology, such as changes involved in the leaf senescence, should be considered. This hypothesis seems to be supported by the fact that in trifoliate leaves the resistance increases with time following induced inoculation, independently of its severity. However, as a higher level of resistance is present in trifoliate leaves from infected than from healthy plants, the viral infection could induce a premature ageing of resistant tissues.

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Peroxidases of Red Clovers Resistant and Susceptible to Bean Yellow Mosaic Virus[1]

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Peroxidase activity and isozymes in leaf and petiole extracts were analyzed in four red clover clones, KyC36, KyC71-8, KyC40-1, and KyC101, which exhibit the respective phenotypes of mottling, systemic necrosis, hypersensitive necrosis and symptomlessness when infected with an isolate (204-1) of bean yellow mosaic virus. Leaves of KyC36 had the lowest peroxidase level, whereas, KyC101 contained a high concentration of peroxidase in the petioles. Leaf necrosis enhanced the peroxidase activity much more than did mottling. The level of peroxidase in the petiole was not affected by symptom development in the leaf except with systemic necrosis where the peroxidase activity increased accordingly. Six peroxidase isozymes, three cathodic (C_1, C_2, C_3) and three anodic (A_1, A_2, A_3) were detected in leaves, however, the C_2 isozyme is unique for KyC40-1. Extra peroxidase bands were found in petioles. KyC40-1 and KyC101 shared a common anodic petiole isozyme. The C_2 isozyme is inherited as a single dominant factor and is linked to the hypersensitive necrosis factor with 11% crossing-over. Analysis of testcross progenies from KyC40-1 as a parent indicates that the C_2 isozyme possibly acts as a modifier contributing to virus localization and tissue necrogenesis.

The involvement of peroxidase in various aspects of pathogenesis has been investigated for many years. This enzyme consists of multiple forms (isozymes) which can be separated by electrophoresis and is present in cytoplasm and associated with cell wall (KEY *et al.*, 1967; RIDGE and OSBORNE, 1970). Past studies dealing with peroxidase in pathogenesis were limited to the change of anodic enzymes (LOEBENSTEIN, 1972; NOVACKY and HAMPTON, 1968; SOLYMOSY *et al.*, 1967). The alteration of anodic isozymes during symptom development was usually quantitative. In a few instances, new isozymes were observed in connection with necrobiosis. Since peroxidase in plants are tissue specific (SHEEN and REBAGAY, 1970), any quantitative or qualitative change ought to be related to the infected tissue rather than to the organ as a whole. Within a tissue, the distribution of peroxidases with respect to cytoplasm and cell wall also needs

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to be distinguished as they may function in pathogen localization and tissue necrogenesis.

Enhanced peroxidase activity was correlated with systemic acquired resistance in TMV infected Samsun NN tobacco which carries a dominant factor for hypersensitive necrosis (SIMONS and ROSS, 1970, 1971; VAN LOON, 1976). A single dominant factor governing hypersensitive resistance with localized necrotic lesions against bean yellow mosaic virus (BYMV) was identified in red clover *(Trifolium pratenses)* (DIACHUN and HENSON, 1965). A different dominant factor confers similar necrotic lesions at the infection sites but with subsequent lethal systemic necrosis (DIACHUN and HENSON, 1974). How the two red clover genotypes differ in peroxidases in relation to the localization of BYMV and necrogenesis was the objective of the present investigation. In addition to quantitative measurement, both anodic and cathodic isozymes were analyzed in regard to tissue specificity and cellular distribution. Two additional red clover genotypes, one conferring mottling and the other showing symptomless resistance to BYMV, were included for comparison. An abstract of this investigation has been published (SHEEN *et al.*, 1975).

Materials and Methods

Red clover clones KyC40-1, KyC71-8, KyC36 and KyC101 produce hypersensitive necrosis, systemic necrosis, veinal chlorosis (mottling) and symptomlessness, respectively, when infected with BYMV isolate 204-1. Three cuttings from each clone were grown in 8″ pots in a growth chamber where the temperature was maintained at 22°C during the daytime and 18°C at night with a photoperiod of 10 hours. Mature leaves and petiole were sampled from each pot prior to inoculation with BYMV. The same clones were inoculated with the isolate extracted from young mottled leaves of "Dwarf Gray Sugar" pea seedlings according to the procedures previously described (DIACHUN and HENSON, 1974). Leaves showing primary necrotic lesions were collected 6 days after inoculation, whereas mottled and systemically necrotic leaves were sampled 15 days later. All samples were freeze-dried, ground to pass through a 40-mesh screen and stored at −20°C.

For peroxidase analysis, pulverized tissues were homogenized in a cold mortar and pestle with 0.1 M Tris-HCl buffer, pH 8, containing 0.1% each of ascorbate and cysteine-HCl. After centrifugation at 20,000 × *g* for one hour, the extracts were spectrophotometrically assayed for peroxidase activity (LUCK, 1963) and were subjected to polyacrylamide gel slab electrophoresis. The electrophoretic procedures using 7.5% gel and Tris-glycine buffer, pH 8.5, and the detection of peroxidase isozyme by the benzidine–H_2O_2 method have been reported elsewhere (SHEEN and REBAGAY, 1970).

Cellular distribution of peroxidases was studied by differential extraction according to the method of RIDGE and OSBORNE (1970). Fresh leaves were

extracted with ice-cold 0.05 M phosphate buffer, pH 6, to yield cytoplasmic peroxidases. The residues were repeatedly washed with the same buffer until free of peroxidase activity and then were homogenized with 1 M NaCl solution to release peroxidases ionically bound to the cell wall and membrane. Peroxidases covalently bound to the cell wall and membrane were isolated by cellulose and pectinase treatment.

The finding of a cathodic peroxidase isozyme unique for KyC40-1 led to investigation of the mode of inheritance and its possible association with the hypersensitive necrosis factor. Segregating progenies of KyC40-1 crossed with KyC36 and KyC71-8 were grown in a greenhouse. In addition to the examination of BYMV symptoms, leaves were taken from each plant for peroxidase isozyme analysis. Enzyme preparation and gel slab electrophoresis were as above.

Results

Peroxidase activity. On a dry weight basis, leaf peroxidase activity was significantly lower in KyC36 than in the remaining three genotypes, among which the difference was not statistically significant (Table 1). The level of leaf peroxidase was not correlated with that of the respective petiole. The petiole of KyC101 contained higher peroxidase activity than the other clones. Leaves with primarily local necrosis increased peroxidase activity nearly 50% in KyC71-8 and more than doubled in KyC40-1. However, the enhanced level of peroxidase in the leaf did not affect the peroxidase content in the petiole. Similarly, an enhancement of peroxidase activity in the leaf but not in the petiole was observed in KyC36 showing mottling symptom. Both leaf and petiole peroxidases increased several-fold in KyC71-8 when systemic necrosis became evident.

Table 1

Peroxidase activity in the leaf and petiole of red clover clones with or without bean yellow mosaic virus infection

Clones	Virus infected			Non-infected	
	Symptom	Leaf	Petiole	Leaf	Petiole
		ΔA/mg dry wt/min			
KyC40-1	Hypersensitive necrosis	3.30	2.15	1.60	2.29
KyC101	Symptomless	—	—	1.53	3.16
KyC36	Mottling	2.68	2.39	1.24	2.29
KyC71-8	Local necrosis	2.53	2.27	1.72	1.91
	Systemic necrosis	8.32	7.07	—	—
	LSD	0.05		0.20	0.25
	LSD	0.01		0.27	0.40

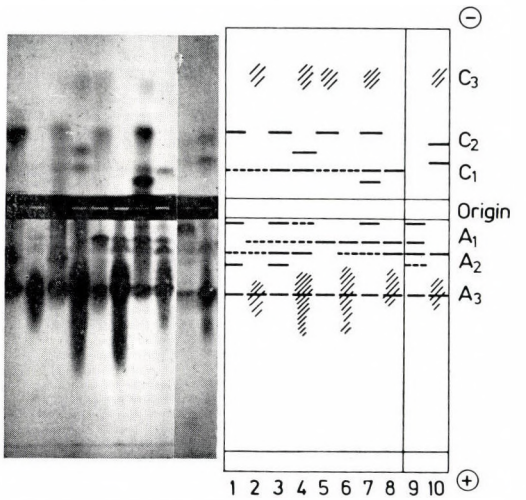

Fig. 1. Peroxidase isozymes of the petiole and leaf of red clover clones on a polyacrylamide gel slab. The A and C symbols are denoted respectively to the anodic and cathodic isozymes of the leaf. The samples under comparison are (1) KyC101 petiole; (2) KyC101 leaf; (3) KyC40-1 petiole; (4) KyC40-1 leaf; (5) KyC71-8 petiole; (6) KyC71-8 leaf; (7) KyC36 petiole; (8) KyC36 leaf; (9) wall-bound isozymes of KyC40-1 leaf; and (10) cytoplasmic isozymes of KyC40-1

Peroxidase isozymes and cellular distribution. Figure 1 shows a peroxidase zymogram of four red clover genotypes on a polyacrylamide gel slab. For the sake of illustration, the leaf peroxidase isozymes are assigned symbols C_1, C_2, C_3, and A_1, A_2, A_2 for those migrating toward the cathode and anode, respectively. The subscript numerals correspond to the relative mobility from the origin. All clones possess isozymes C_1, C_3, A_1, A_2, A_3. The A_1 and A_2 bands showed stronger intensity in the susceptible clones than in the resistant ones, whereas the C_1 and C_3 bands varied in intensity depending on the physiological stage of the leaf. KyC40-1 differed from other clones by having the C_2 band with increased intensity in necrotic leaves. Additional, but weak peroxidase bands migrating fast toward the anode were present in necrotic leaves of KyC40-1 and KyC71-8. They may represent the degradation products of peroxidase that still retain some enzymatic activity. Banding modification was not observed in mottled leaves of KyC36.

Peroxidase isozymes in the petiole shared the same bands with leaf peroxidases C_1, C_2, A_1 and A_2 but they differed from them in intensity. In addition, petioles of all four genotypes had an intense cathodic band with a relative mobility between C_2 and C_3. KyC36 had an extra cathodic band migrating slower than C_1. An intense anodic band which appeared between A_2 and A_3 isozymes was common for the two resistant genotypes.

Table 2

Chi-square tests for goodness of fit for the 1 : 1 segregation of peroxidase isozyme C_2 factor in the progenies derived from crosses of red clover clone KyC40-1

Progeny	Number of plants		P value
	C_2	c_2	
KyC40-1 × 15 × KyC71-8 × 14	42	0	—
KyC71-8 × 14 × KyC71-8 × 10	0	21	—
KyC40-1 × KyC36, H5	23	14	.10—.25
KyC40-1 × KyC71-8, H13	23	20	.50—.75
KyC71-8 × KyC40-1, H7	17	24	.25—.50
KyC71-8 × KyC40-1, H8	17	24	.25—.50
KyC40-1 (open pollination)	20	12	.10—.25

With differential extraction, isozymes C_1, C_2, C_3, and A_3 appeared to be the major peroxidases in cytoplasm, whereas bands A_1 and A_2 were mainly bound to the cell wall and/or membrane. In addition, an extremely slow-moving anodic band in the wall-bound fraction was possibly associated with vascular tissues. The latter three isozymes were also detected in the extract after cellulase and pectinase treatment although their concentrations were very low.

Inheritance of peroxidase isozyme C_2. KyC40-1 was heterozygous for the hypersensitive necrosis factor (N). Plants homozygous for N factor were obtained from selfed progenies. When the homozygous NN KyC40-1 was crossed with KyC71-8, all F_1 plants showed the C_2 band (Table 2). On the other hand, a sib-mating of KyC71-8 produced no C_2 band in the offspring. This indicates that a plant homozygous for the N locus is also homozygous for C_2 locus and that the C_2 factor is inherited in dominance. Testcrosses of KyC40-1 (NnC_2c_2) with KyC71-8 (nnc_2c_2) in a reciprocal manner confirmed the C_2 as a dominant factor and inherited in a monogenic pattern. This is further substantiated by a 1 : 1 segregation for C_2 and c_2 in the testcross progeny of KyC40-1 × KyC36. An open-pollination progeny of KyC40-1 also gave a 1 : 1 segregation, suggesting that the C_2 factor is not common in the present cultivators of red clover in Kentucky.

Genetic linkage of C_2 *factor with hypersensitive necrosis factor.* The appearance of mottled plants with or without the C_2 isozyme in the testcross progenies of KyC40-1 with KyC36 and KyC71-8 indicates that the parental KyC40-1 was heterozygous at both C_2 and N loci (Table 3). At the corresponding loci, the susceptible clones KyC36 and KyC71-8 are homozygous recessive, c_2c_2nn. By inspecting the segregation of $C_2 : c_2$ within N and n groups or N : n within C_2 and c_2 groups in the KyC40-1 × KyC36 progeny, the nonrandom assortment is evident. On the basis of this backcross data, the linkage intensity in the coupling phase was calculated as 0.11. This means that the N and C_2 loci are separated by 11 crossing-over units.

Table 3

Genetic association of hypersensitive necrosis factor and peroxidase isozyme C_2 factor in the segregating progenies of red clover infected with bean yellow mosaic virus

Progeny	Number of plants				Calculated segregation[a]				P value
	NC_2	Nc_2	nC_2	nc_2	N (or Ns) C_2	N (or Ns) c_2	nC_2	nc_2	
KyC40-1 × 15 × KyC71-8 × 14	42(7)[b]	0	0	0					
KyC71-8 × 14 × KyC71-8 × 10	0	21(21)	0	0					
KyC40-1 × KyC36, H5	22	3	1	11	16.47	2.03	2.03	16.47	.10—.20
KyC40-1 × KyC71-8, H13	22(1)	13(5)	1	7	20.32	11.93	1.18	9.67	.75—.90
KyC71-8 × KyC40-1, H7	16(3)	17(12)	1	7	19.37	11.38	1.13	9.12	.25—.50
KyC71-8 × KyC40-1, H8	16(2)	(168)	1	8	19.37	11.38	1.13	9.12	.25—.50

[a] Segregation ratios were calculated on the basis of 1% crossing over between N and C_2 factors linked in coupling phase and independent segregation of Ns and ns factors in KyC71-8. The calculated segregation ratio for KyC40-1 × KyC71-8 and its reciprocals is 47.25 : 27.75 : 2.75 : 22.25.

[b] The numeral in parentheses is the number of plants that became systemically infected with necrotic lesions.

The detection of linkage in the progenies of KyC40-1 crossed with KyC71-8 becomes difficult because local necrotic lesions can be attributed either to the N factor in KyC40-1 or to the systemic necrosis factor (Ns) in Ky71-8. However, on the basis of 11% crossing-over in the coupling phase between N and C_2 factors in KyC40-1 and the segregation of Ns and ns KyC71-8, the expected segregation ratio of N (or Ns)C_2 : N(or Ns)c_2 : n (and ns)C_2 : n (and ns)c_2 is calculated as 47.25 : 27.75 : 2.75 : 22.25. The observed data fit to the expected ratio with high probabilities for all three populations of KyC40-1 crossed with KyC71-8 (Table 3). A chi-square test for heterogeneity of the three populations gave a goodness of fit with a P value between 0.90—0.95, suggesting that they can be considered homogeneous.

By pooling the data of three populations, 6 plants out of 54 in the NC_2 group developed systemic necrosis. The expected ratio for N and Ns in the NC_2 group would be 44.50 : 2.75 which fits the observed result (48 hypersensitive necrosis : 6 systemic necrosis) with a P value of 0.05—0.10. In contrast, 25 plants became systemically infected in a population of 46 identified as Nc_2 genotype. The N : Ns segregation ratio in the c_2 group would be 5.50—22.25. The observed result showed a great excess of plants with hypersensitive necrosis. Whether this is attributed to misclassification or the presence of modifiers in favor of hypersensitive localization of BYMV is yet to be determined.

Discussion

Leaf peroxidase isozyme C_2 in KyC40-1 is of interest in several aspects. First, it is a cathodic peroxidase. Cathodic peroxidases are capable of catalyzing both oxidative and peroxidative reactions (KEY *et al.*, 1967). This renders a greater efficiency in polyphenol oxidation than the anodic counterparts. Second, the C_2 isozyme is inherited as a single dominant factor and is closely linked with the hypersensitive necrosis factor. It seems to modify the hypersensitive resistance as evidenced by a higher proportion of NC_2 plants than nc_2 ones in the segregating population of KyC40-1 × KyC36. Third, the cellular localization of the C_2 peroxidase appeared in the cytoplasm, and it is likely associated with leaf laminae. The exclusion of veinal tissues as the site of this isozyme is deduced from the fact that the vascular bundles of leaf and petiole are interconnected and the C_2 isozyme was not found in the petiole of KyC40-1. Since KyC40-1 differs from KyC71-8 only by the C_2 isozyme, the possible implication of this peroxidase in BYMV localization and tissue necrogenesis deserves evaluation.

Virus localization and necrogenesis in infected cells are regarded as separate processes. In tobacco, the induction of TMV localization was correlated with enhanced peroxidase activity (SIMONS and ROSS, 1970; 1971; VAN LOON, 1976). The present results showed a similar trend in that the clones resistant or producing local lesions to BYMV infection contain higher levels of peroxidase than the systemically mottled KyC36. However, the distinction between KyC40-1 and KyC71-8 in BYMV localization cannot be explained by the peroxidase activity alone. In addition to the isozyme C_2, one may point out that KyC40-1 shares an intense anodic peroxidase in common with KyC101 in petiole extracts. This suggests a possible close pedigree relationship between these resistant genotypes. Although KyC101 is symptomless with BYMV 204-1, this does not rule out hypersensitive response with the death of a few cells and/or hindrance of virus movement in the petiole. The possible role of this isozyme in virus localization is yet to be determined.

It has been demonstrated that virus particles can initiate the infection and subsequent spread in the leaf tissue of both hypersensitive and systemically susceptible varieties with the same rate at the early stage of infection (LOEBENSTEIN, 1972). In certain host–pathogen combinations, an increase of peroxidase takes place only during the necrotic phase. This is in agreement with the present results that necrotic leaves exhibited an enhancement of peroxidase activity; in contrast, the increase in mottled leaves was less quantitative. That an increase in banding intensity of the C_2 isozyme coincided with the expansion of necrotic lesions substantiates its possible role in necrogenesis. A time-course analysis of symptom development as well as peroxidase accumulation may delineate the C_2 isozyme from the other peroxidases. Such experiments have been undertaken and the results will be presented elsewhere.

All experimental materials used in the present investigation allow us to

examine only the interactions among N, C_2, and Ns loci at heterozygous conditions. How homozygosity at one or more loci affects symptom development is beyond the scope of this analysis. In evaluation of the available data, 96% of the C_2 plants in the KyC40-1 × KyC36 progeny are also carriers of the N factor, whereas 84% is the case for the KyC40-1 and KyC71-8 progenies. It seems to be apparent that the C_2 isozyme can be used as a genetic marker in red clover breeding programs in selection of the hypersensitive necrosis factor for BYMV resistance on the one hand and as a resistance modifier on the other.

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Increased Free Formaldehyde Level in Crude Extract of Virus Infected Hypersensitive Tobaccos

By

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The presence of free formaldehyde in pressed juice and extracts was detected by mass spectrometric measurement and by TLC in form of dimedone complex. An increased level of formaldehyde was found in the extract of TMV infected hypersensitive tobaccos as compared to the healthy control.

The presence of free formaldehyde in homogenates of different animal tissues was detected (KNECHT, 1966; PAIK and KIM, 1974) as a product of demethylation of N-methylated substances, e.g. dimethylnitrosamine (TURBERVILLE and CRADDOCK, 1971). The present paper reports on preliminary experiments carried out to demonstrate the occurrence of free formaldehyde in extracts of healthy and tobacco mosaic virus (TMV) infected hypersensitive tobacco leaves.

Nicotiana tabacum cv. Xanthi-*nc* was used in 6–8 leaf stage and was inoculated with U_1 strain of TMV. All inoculated leaves produced local necrotic lesions (hypersensitive necrosis) on the sites of virus infection. For direct detection of formaldehyde the leaf was directly applied to the double focusing mass spectrometer (JEOL JMS-01 SG-2). The samples of healthy and virus infected leaves were inserted into glass capsules and were taken into the electron impact ion source of the instrument. By slowly raising the temperature (5°C per min) from 20°C to 80–100°C, the spectrum of the evaporating components was taken in 8 or 16 sec interval. Fifty mass spectra were recorded from each sample to be able to check the mass fragmentation. (The intensity of ionizing current was 200 μA, ionizing voltage was 75 V, accelerating voltage was 10 kV, detector voltage: 1.8 kV, resolution power: 2000 and 5000.) During the mass spectrometric experiments the appearance of CHO^+ and $CH_2{=}\overset{+}{O}H$ (m/e = 29 and 31) ions and the alteration of their intensity were checked. The N_2^+ ion (m/e = 28) served as comparative basis (100%). By the investigation of healthy and virus infected leaves the intensity of ions with mass numbers 29 and 31 increased from the 4th–5th scans, but in all cases of virus infected samples we got higher values of the ion intensities. The relative intensities of the peaks at masses 29 and 31 were the following:

	m/e = 29	m/e = 31
Healthy	34.0%	22.1%
TMV infected	138.0%	62.2%

These results demonstrated the occurrence of free formaldehyde both in healthy and virus infected tobacco tissues inserted into the glass capsules, but its amount significantly increased in TMV infected necrotic tissues.

Furthermore we homogenized leaf tissue in water, or acetic acid (5%) + trichloroacetic acid (5%) (30 g in 100 ml) and then, after centrifugation (5000 rpm for 10 min), 50 mg dimedone (5,5-dimethyl-1,3-cyclohexanedione) was added to the about 100 ml clear supernatant, and was preserved overnight. Then the precipitate was collected by centrifugation and was resolved in 2 ml chloroform. Samples of 0.05 ml were taken into silica gel G layers. The location of dimedone-formaldehyde adduct (bis-condensation product) on layer was identical with authentic substance (co-TLC) in two solvent systems (benzene – ethylacetate, 95 : 5 v/v; and chloroform – ethylacetate, 90 : 10 v/v. R_f values: 0.6 – 0.65 and 0.75 – 0.80, respectively). The spots were detected by the $FeCl_3$ – $K_3Fe(CN)_6$ reagent; Folin – Ciocalteau reagent; vanillin–ethanol reagentor 2,6-dichlorchinone-chlorimide (Fig. 1). The later reagent gave very specific color

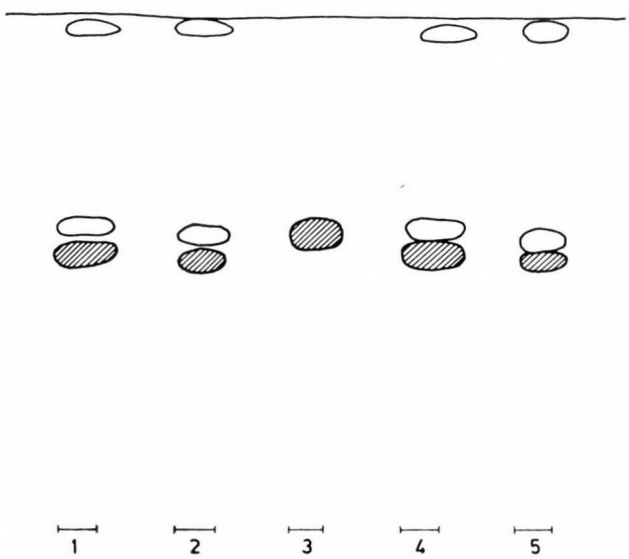

Fig. 1. TLC chromatography of formaldehyde in formaldemethone form in water extract of healthy and tobacco mosaic virus infected hypersensitive tobacco leaves. Layer: silica gel G (Merck); solvent: benzene – ethylacetate 95 : 5 v/v. Reagents: 0.2 g 2,6-dichlorchinone-chlorimide solved in 100 ml methanol, then 20 g of Na_2CO_3 solved in 100 ml water. Patterns: 1 virus infected; 2 healthy; 3 formaldemethone (10 μg); 4 virus infected + formaldemethone (5 μg); 5 healthy tobacco (15 g samples). Hatched spots = formaldemethone; empty spots = unidentified material

reaction with this dimedone-formaldehyde adduct (green in light and red in UV_{365} light). The results of TLC experiments were strengthened by the mass spectrometrical investigations. After thin-layer chromatographic investigations the amount of free formaldehyde in healthy tobacco tissues is between 15–20 ng per g tissue. The presence of formaldehyde was detected both in water and in acetic acid (5%) extracts. But it was absent in the homogenate in trichloroacetic acid (5%). These results demonstrated that the observed formaldehyde was a product of enzymatic reaction, eventually of N-demethylase. The presence of trichloroacetic acid inhibited this enzymatic activity.

In the preliminary investigations of systemically infected tobaccos *(Nicotiana tabacum* var. *Samsun)* in compatible host–parasite relation we did not find any changes in free formaldehyde levels as compared to the control.

By these experiments we firstly verified the presence of free formaldehyde in extracts of tobacco leaf tissues and its increase in the virus induced hypersensitive necrosis (incompatible host–parasite relation). The role of increased formaldehyde production during the necrogenesis remains to be determined.

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Cytokinins in White Clover Plants Infected by Clover Phyllody Mycoplasma

By

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The effect of clover phyllody infection on cytokinin levels was investigated in the leaves and flowers of white clover plants. An increase in cytokinin level of phyllodoid flowers was detected, as compared to healthy controls.

Results from chromatographic studies suggest that the active materials are the different forms of zeatine and N^6-(Δ^2 isopentenyl)-adenine. These different forms of cytokinins also were detected in the leaf extracts. However, the infected leaves contained a lower amount of cytokinins than did the healthy ones. The altered cytokinin levels in the different parts of mycoplasma-infected plants may be associated with the development of symptoms.

From the virescence of white clover (*Trifolium repens* L.) flowers to the complete phyllody, there is a large variation in the syndromes caused by the pathogen depending upon the stage of development of the host plant at the time of infection. (Cf: BOS, 1963; BOS and GRANCINI, 1965; MARAMOROSCH *et al.*, 1970). In extreme cases all flowers express the trifolia-forming vegetative stage. These morphological abnormalities suggest an altered hormonal balance in the infected plants (Cf: SEQUIERA, 1973) and in this regard it is well-known that cytokinins can potentially influence the trend of differentiation and tissue development (SKOOG and SCHMITZ, 1972). The present paper reports on research focused on changes in cytokinin levels in diseased plants.

Materials and Methods

Plant material

Field-growing white clover plants (*Trifolium repens* L.) which had been naturally infected by a clover phyllody mycoplasma agent (BECZNER and GÁBORJÁNYI, 1968) and adjacent uninfected control plants were harvested at the end of September (1973–1975) from the same clover field. The symptoms varied from virescence to complete phyllody of flowers. The diseased plants (Fig. 1) were generally stunted, and the size of their trifolia was less than one-half (47.6%) that of the healthy plants ($P = 0.01$).

Fig. 1. White clovers naturally infected by clover phyllody mycoplasma

Cytokinin extraction and determination

Samples of both flowers and leaves, from both healthy and infected plants, were extracted as described earlier (SZIRÁKI *et al.*, 1975). Separation of extracted cytokinins was carried out by paper chromatography on Whatman No. 1 paper: ethyl acetate : formic acid : water (65 : 5 : 35 v/v) upper phase (solvent A), followed by 0.03 M borate buffer, pH 8.4 (solvent B). Cytokinin standards, i.e. zeatin (Z), zeatin riboside (ZR), N^6-(Δ^2 isopentenyl)-adenine (2iP), and N^6-(Δ^2 isopentenyl)-adenosine (IPA), were used as markers for characterization of substances in chromatograms. Following chromatography with each of solvent A and solvent B, the chromatograms were divided into ten equal strips, and each strip was tested for cytokinin activity in the soybean callus assay (MILLER, 1965; KRASNUK *et al.*, 1971).

Results and Discussion

Table 1 presents the data of the soybean callus assay of the extracts prepared from healthy and phyllodoid flowers of white clover plants. The total cytokinin activity of the extract of phyllodoid flowers was much higher than

Table 1

Soybean callus bioassay of chromatograms of purified extracts from healthy and clover phyllody mycoplasma infected white clover flowers

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	111	243	271	223	147	145	192	150	134	146
Infected	150	255	284	269	184	180	134	170	228	129

Control 129
(without extract)

The extracts were chromatographed on Whatman No. 1 paper in ethyl acetate—formic acid—water, 60 : 5 : 35 v/v, upper phase. Cultures were grown on basal medium supplemented with portions of chromatograms corresponding to R_F regions. Average values of three experiments in five replicate samples. The extracts were obtained from 30 g fresh weight of flowers/1000 ml of medium

Table 2

Soybean callus assay of the samples at R_F 0.0 to 0.5 in Table 1 after rechromatography

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	83	132	136	103	80	133	112	98	106	105
Infected	88	143	112	83	93	153	93	93	187	173

Control 103
(without extract)

The materials from the chromatogram represented in Table 1 were eluted with ethanol, and rechromatographed in borate buffer (0.03 M, pH 8.4)

that of the extract purified from flowers of healthy plants. The extracts of flowers from healthy plants showed high cell division activity at R_F 0.1 – 0.4 and 0.6 – 0.8. The extract of phyllodoid flowers showed high cell division activity at R_F 0.1 – 0.4 and 0.7 – 0.9. In solvent system A the migrations of Z, TR, 2iP, and IPA corresponded to R_F regions 0.19 – 0.32, 0.18 – 0.31, 0.64 – 0.81 and 0.62 – 0.78.

Further chromatographic studies were required, because cytokinin activity had been found in other regions as well as those to which the cytokinin standards had migrated. In addition the solvent A did not separate the free base form of cytokinins from the nucleoside form of cytokinins. For this reason we used solvent B, which clearly separated the two above-mentioned groups. The chromatograms developed in solvent A were divided into two parts according to the R_F values 0.0 – 0.5, 0.5 – 1.0, and the eluted materials were chromatographed in solvent B. Table 2 presents the bioassays of the compounds eluted from the first parts of the solvent-A chromatograms R_F 0.0 – 0.5. Extracts of both healthy and diseased flowers contained active material at R_F 0.1 – 0.3 (solvent B). This activity cannot be due to any of the standards used. The region of R_F 0.5 – 0.6 (solvent B) also showed cytokinin activity, which can be attributed to the zeatin R_F 0.48 – 0.53. There was high cell division activity in the extract of diseased flowers at R_F 0.8 – 1.0 in which R_F region synthetic ZR is located (R_F 0.84 – 0.95). However, the extract of control flowers contained no cytokinin activity at these R_F values. It can be seen from Table 2 that the extract of diseased flowers contained the free base, the nucleoside forms of zeatin (and an unknown active substance) while the extract of healthy flowers contained no nucleoside form of zeatin.

The cytokinin activity of the 0.5 – 1.0 R_F region found using solvent A, after rechromatography in solvent B, was as shown in Table 3. Both the extracts of healthy and mycoplasma-infected flowers contained cytokinin activity at R_F 0.1 – 0.3. This active substance did not correspond to the migration of any synthetic cytokinin used in this work which may be due to the nucleotide form of 2iP. We also found high cytokinin activity at R_F 0.4 – 0.6. This activity corresponded to the 2iP, which migrated to R_F 0.45 – 0.57 in solvent B. At both R_F regions the extract of infected flowers showed higher cytokinin activity than did the healthy flowers. At R_F 0.8 – 1.0 there was also detectable cytokinin activity in the extract of infected flowers, which corresponds with the riboside form of 2iP (R_F 0.82 – 0.93).

The data from soybean callus assays, using leaf extract from healthy and mycoplasma-infected plants, are summarized in Table 4. Cell division activity was detectable, but variable, in all fractions as compared to the water control. The cytokinin activity was higher in the extract of healthy leaves at R_F regions 0.0 – 0.2, 0.3 – 0.4, 0.5 – 0.8. After rechromatography of the extracts in solvent B, the free base forms of cytokinins were separated from the nucleoside forms. Tables 5 and 6 present the results of bioassays of these rechromatograms (developed in solvent B) of substances which were initially eluted from the R_F regions 0.0 – 0.5 and 0.5 – 1.0 (solvent A).

Table 3

Soybean callus assay of the samples at R_F 0.5 to 1.0 in Table 1 after rechromatography

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	107	126	125	99	138	156	115	110	123	104
Infected	105	146	120	97	102	174	110	119	232	170

Control 110
(without extract)

Elution and rechromatography are as in Table 2

Table 4

Soybean callus bioassay of chromatograms of purified extracts from leaves of healthy and mycoplasma infected white clover plants

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	100	311	195	151	80	251	212	233	239	118
Infected	141	261	196	74	130	143	225	183	96	93

Control 76
(without extract)

The extracts were chromatographed on Whatman No. 1 paper in ethyl acetate — formic acid — water, 60 : 5 : 35 v/v, upper phase. Cultures were grown on basal medium supplemented with portions of chromatograms corresponding to R_F regions. The extracts were obtained from 15 g fresh weight of leaves/1000 ml of medium. Average values of three experiments in five replicate samples

Table 5

Soybean callus assay of the samples at R_F 0.0 to 0.5 in Table 4 after rechromatography

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	60	220	132	98	135	186	108	60	89	62
Infected	65	112	108	95	67	194	73	156	72	75

Control 76
(without extract)

The materials from the chromatogram represented in Table 4 were eluted with ethanol, and rechromatographed in borate buffer (0.03 M, pH 8.4)

Table 6

Soybean callus assay of the samples at R_F 0.5 to 1.0 in Table 4 after rechromatography

R_F	Fresh weight, mg/piece									
	0.0—0.1	0.1—0.2	0.2—0.3	0.3—0.4	0.4—0.5	0.5—0.6	0.6—0.7	0.7—0.8	0.8—0.9	0.9—1.0
Healthy	147	170	111	108	210	242	207	150	193	184
Infected	105	143	122	97	121	170	153	117	198	150

Control 84
(without extract)

The healthy leaves contained more cytokinins at R_F 0.1—0.3, and 0.4—0.6, than did the extracts of diseased leaves. It is noteworthy that there was higher activity in the extracts of infected leaves at R_F 0.7—0.8, corresponding with the synthetic nucleoside form of zeatin. The synthetic Z and ZR migrated in this solvent to R_F 0.45—0.6 and 0.84—0.95, respectively.

Table 6 shows similar results as does Table 5, in that there were found more cytokinins in the extracts of healthy leaves than in the extract of infected ones. The riboside form of the 2iP also was detectable in the chromatograms of extract prepared from healthy leaves.

It is very probable that the higher cytokinin content found in the infected flowers can be correlated with the morphological abnormalities resulting from mycoplasma infection. Further tRNAs isolated from different mycoplasma strains contained cytokinin-active minor nucleotides (HAYASHI *et al.*, 1969), thus this may play a role in increasing the cytokinin level in the infected flowers.

In addition to the conception of BOS (1970), we suggest that mycoplasma infection causes a hormonal imbalance in the whole plant, as is demonstrated by the lower cytokinin levels in the infected leaves as compared to the healthy controls. It also has been reported many times that the cytokinins are able to increase RNA and protein synthesis (WOLLGIEHN, 1961; OSBORNE, 1962; POZSÁR *et al.*, 1967) and to cause enlargement of leaf surface (KIRÁLY *et al.*, 1968; KURAISHI, 1959); and thus the lower cytokinin content of mycoplasma infected leaves also may correlate with the decreased leaf size. It is also noteworthy that, in general, the infected tissues differed in their free-base content relative to the healthy controls, and the increased cytokinin activity in the mycoplasma infected flowers.

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Changes in Chemical Composition of Pigeon Pea Fruits Due to Arhar (Pigeon Pea) Mosaic Virus Infection

By

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Studies on the chemical composition of pigeon pea fruits indicate that the total nitrogen and protein were higher in fruit-coat but lower in other fruit parts of the diseased plants than in healthy ones. The percentages of nitrite, nitrate and total free amino acids were higher in all parts of virus infected pigeon pea fruits than in healthy ones. Total and organic phosphorus fractions were lower in AMM and ASM infected fruit but inorganic phosphorus was higher in the same. Different carbohydrate fractions, viz. water soluble total, reducing and non-reducing sugar and starch, were higher in healthy pigeon pea fruits than in their virus-infected counterparts.

Cajanus cajan (L.) Millsp. ranks high among the pulse crops of tropica countries and entered into the daily food of a considerable number of people. It is largely eaten in the form of split pulse as *dal*, while its tender green pod constitutes a very favourite vegetable in some parts. The outer integument of its seed together with part of the kernel provide a valuable feed for the milk cattle. In recent years it has been recorded that the crop of pigeon pea suffers heavily due to infection of arhar (pigeon pea) mosaic virus (SINGH and MALL, 1976) in District Gorakhpur of eastern U.P. (India). Pods produced on diseased pigeon pea were usually fewer in number and smaller in size. Seeing the importance of pigeon pea in food and feed for human and cattle it was planned in the present study to investigate the effect of arhar (pigeon pea) mosaic virus infection on the chemical composition of pigeon pea fruits.

Materials and Methods

All the experiments in the present study were carried out in an insect proof chamber. Arhar (pigeon pea) mosaic virus–mild (AMM) and severe (ASM) strains (SINGH and MALL, 1976) were maintained on pigeon pea cv. Sarda as host plant.

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Virus inoculations were made by gently rubbing the upper surface of the primary leaves of 7 days old pigeon pea seedlings with fore-finger dipped in infected sap.

Three lots containing 120 pigeon pea cv. Sarda plants per lot were taken. The 1st and 2nd lots were inoculated with AMM and ASM strains respectively and the 3rd lot was inoculated with 0.05 M phosphate buffer (pH 7.0) to serve as the healthy control. Fully matured fruits were collected at 180 days after inoculation. Just after the harvest, fruits were hand plucked and kept in an oven at 80°C for 24 hours. Different parts of fruits, viz. fruit coat, seed coat and cotyledon (dal), were separated before chemical analysis. The total nitrogen (DONEEN, 1932), nitrate, nitrite nitrogen (HUMPHRIES, 1956), total free amino acid (WIGGINS and WILLIAMS, 1955), ammonia-col nitrogen (STROGANOV, 1964), total phosphorus (HUMPHRIES, 1956), inorganic phosphorus, total, reducing sugars and starch (SNELL and SNELL, 1954) were estimated as described. Non-reducing sugars were converted into reducing sugar by the method of SOMOGYI (1952). For total protein, the samples were ground with 10% TCA, centrifuged and the residue was placed in an oven at 70°C for drying. The nitrogen content was estimated as above and was multiplied by 6.25 to get the value of protein. The amount of organic phosphorus was calculated from the total phosphorus after deducting the amount of inorganic phosphorus.

Results and Discussion

The results (Table 1) indicate that the total nitrogen and protein were higher in fruit coat but lower in other fruit parts of the diseased plants than in healthy ones. However, the percentages of nitrite, nitrate nitrogen and total free amino acids were higher in all parts of virus infected pigeon pea fruits than in healthy ones. The increased percentage of total nitrogen and protein in virus infected cotyledons (dal) seems to be due to low availability of storable normal plant protein during the period of their growth. The accumulation of other nitrogenous forms (except ammoniacal nitrogen), is probably due to their higher rate of translocation to the fruit parts, where they were stored.

The virus infection increased the inorganic phosphorus content in the fruit but decreased the total and organic phosphorus. Some workers (REDDI, 1966; REDDI and ANJANEYALU, 1963) are of the opinion that the nucleosides from ribosomal RNA are used for viral RNA synthesis. This suggests that virus infected seeds receive low amount of total and organic phosphorus for storage than the healthy plant seeds.

Under the condition of virus infection the leaves show decreased rate of photosynthesis but have enhanced rate of respiration (GOODMAN *et al.*, 1967). Carbohydrates formed in the leaves were translocated to seeds for storage. The decrease in sugars and starch in such conditions is because of their lesser translocation to the seeds.

Table 1

Carbohydrate, nitrogen and phosphorus contents (mg/100 mg dry wt. except nitrite nitrogen) of pigeon pea fruit parts infected with virus strains

Contents	Fruit parts	Healthy	AMM	ASM
Total sugar	Fruit coat	2.40	2.20	2.00
	Seed coat	3.60	3.40	3.10
	Cotyledon	4.00	3.86	3.75
Reducing sugar	Fruit coat	1.70	1.60	1.52
	Seed coat	2.10	1.95	1.90
	Cotyledon	1.20	1.10	1.05
Non-reducing	Fruit coat	0.70	0.60	0.48
	Seed coat	1.50	1.45	1.20
	Cotyledon	2.80	2.76	2.70
Starch	Fruit coat	16.80	14.00	12.50
	Seed coat	11.00	10.50	9.80
	Cotyledon	20.00	16.70	15.00
Total nitrogen	Fruit coat	1.06	1.35	1.41
	Seed coat	1.63	1.50	1.40
	Cotyledon	4.66	4.45	4.24
Total protein	Fruit coat	4.89	5.60	5.81
	Seed coat	6.00	5.31	4.75
	Cotyledon	23.31	21.75	20.38
Nitrite nitrogen (μg/100 mg dry wt.)	Fruit coat	7.50	8.50	10.00
	Seed coat	4.80	5.00	5.50
	Cotyledon	4.80	5.00	7.00
Nitrate nitrogen	Fruit coat	0.11	0.13	0.14
	Seed coat	0.11	0.12	0.13
	Cotyledon	0.21	0.28	0.28
Ammoniacal nitrogen	Fruit coat	0.014	0.012	0.011
	Seed coat	0.020	0.017	0.015
	Cotyledon	0.025	0.020	0.018
Total free amino acid	Fruit coat	0.60	0.62	0.68
	Seed coat	0.40	0.56	0.60
	Cotyledon	1.00	1.40	1.75
Total phosphorus	Fruit coat	0.121	0.098	0.084
	Seed coat	0.054	0.054	0.048
	Cotyledon	0.421	0.241	0.177
Inorganic phosphorus	Fruit coat	0.045	0.055	0.060
	Seed coat	0.010	0.012	0.015
	Cotyledon	0.010	0.020	0.030
Organic phosphorus	Fruit coat	0.076	0.043	0.024
	Seed coat	0.044	0.042	0.033
	Cotyledon	0.411	0.221	0.147

The difference in the concentration of different nitrogenous fractions in pigeon pea fruits infected with AMM and ASM strains is probably due to the fact that both strains withdraw different amounts of the nitrogenous and phosphorus fraction from the host, for their multiplication and alteration caused to the host metabolism.

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

II. Susceptibility of Varieties of Yellow Lupin to Bean Yellow Mosaic Virus

By

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Previous investigations have shown that the chief causative agent of virus diseases of yellow lupin cultivated in Poland is bean yellow mosaic virus.

Using artificial inoculation screening tests have been performed of yellow lupin within varieties (cultivars, strains, populations and hybrids) in a total number of about 5000 plants in respect to the resistance of this plant to an isolate of the typical strain of bean yellow mosaic virus.

Under conditions of an experimental infection genotypes of a high resistance reaction have not been found.

Previous investigations (POSPIESZNY and FRENCEL, 1977) have shown that in Poland in natural virus infections of yellow lupin the bean yellow mosaic virus (BYMV). occurs in dominant frequency over some other viruses

On the ground of field observations or experimental works under greenhouse conditions a high susceptibility of this plant to BYMV has often been noticed (CORBETT, 1958; BŁASZCZAK, 1963; KSIĄŻEK, 1970, 1971). But till now detailed investigations on an extensive range of this plant material have not been performed, and this was the cause for the undertaking of screening investigations in the so far available collection of cultivars, strains, acclimatized populations and some hybrids *(Lupinus luteus* × *L. Rothmalerii)* from the point of view of plant resistance to BYMV.

Materials and Methods

The plant material has originated mostly from the Poznań Plant Breeders Research Station in Wiatrowo and partly from the Institute of Plant Genetics of the Polish Academy of Sciences in Poznań.

Greenhouse experiments were performed in two equivalent repetitions but within the next vegetative periods, chiefly during the spring–summer months (it otherwise, it will be noted). Plants in the growth phase of 2–3 pairs of leaves were inoculated mechanically with an isolate of the typical strain of BYMV, multiplied on bean. Sap from an infected bean plant was diluted with water (1 : 3).

Table 1

Susceptibility of cultivars and populations of yellow lupin to bean yellow mosaic virus (BY under conditions of artificial infection in greenhouse experiments

No.	Cultivar, strain, hybrid population	Experiment I		Experiment II	
		Ratio of the number of attacked to inoculated plants	Percentage of attacked plants	Ratio of the number of attacked to inoculated plants	Percentage of attacked plants
1	Paulaus Gelbe	9/15	60*	18/20	90
2	Neko Nev	11/18	61*	21/21	100
3	Schwako	2/18	11*	12/19	65
4	Bas	17/20	85*	19/19	100
5	Mazowiecki	6/10	60*	15/15	100
6	Sam	7/14	50*	13/13	100
7	Gorzowski	8/14	57*	20/20	100
8	Bałtyk I	12/16	75*	17/17	100
9	Bałtyk II	9/12	75*	12/12	100
10	Lila	14/20	65*	18/18	100
11	Popularny	15/20	75*	20/20	100
12	AFS	15/21	71*	21/21	100
13	Palvo	16/16	100*	21/21	100
14	Tedin	6/12	50*	15/17	100
15	Lima	12/15	80*	18/18	100
16	Karat	14/17	82*	21/21	100
17	Afus	10/12	83*	20/20	100
18	As	10/13	77*	21/21	100
19	Kaszub	10/14	71*	18/18	100
20	Pal	17/19	90*	21/21	100
21	Pałucki	12/14	85*	19/19	100
22	Refusa Nowa	10/10	100	20/20	100
23	M-pop	10/10	1 0	19/19	100
24	Reto	21/21	100	20/20	100
25	Wista	20/20	100	15/15	100
26	Sulfa	28/28	100	27/27	100
27	Neven	27/27	100	22/22	100
28	Podw. Mieszaniec	14/14	100	19/19	100
29	Super Szybkopędny	16/16	100	17/17	100
30	Martini	28/28	100	27/27	100
31	Batavo	18/18	100	21/21	100
32	Schwefelgelbe	27/27	100	21/21	100
33	Rufus	24/24	100	19/19	100
34	Sweet Yellow	20/22	91	24/24	100
35	Bianca	15/15	100	14/14	100
36	Pama	25/27	91	18/18	100
37	Gardenjaj	21/21	100	18/18	100
38	Noskowskij	28/28	100	19/19	100
39	Tarlo-essellgfürt	31/31	100	21/21	100
40	Zołtyj Karmawoj	17/17	100	19/19	100
41	Maj_2	19/20	95	22/22	100
42	Drobnonasienny Szl.	15/15	100	19/19	100
43	Raddatz Frühe	31/31	100	23/23	100

Table 1 (cont.)

No.	Cultivar, strain, hybrid population	Experiment I		Experiment II	
		Ratio of the number of attacked to inoculated plants	Percentage of attacked plants	Ratio of the number of attacked to inoculated plants	Percentage of attacked plants
44	Züneburger Gelbe	21/21	100	24/24	100
45	Fuz. Rez. Lamb.	18/18	100	21/21	100
46	Ród C	15/15	100	16/16	100
47	W—4	29/29	100	24/24	100
48	V × Alteria	27/27	100	31/31	100
49	778	8/19	42*	13/13	100
50	780	8/21	21*	17/19	90
51	846	12/21	57*	17/17	100
52	814	5/18	28*	7/12	58
53	1543/46/72N	5/10	50*	11/14	78
54	1541/46/72N	14/19	73*	16/19	86
55	331/6/74	36/36	100	23/23	100
56	331/18/74	29/29	100	22/22	100
57	331/14/74	27/27	100	21/21	100
58	331/11/74	33/33	100	24/24	100
59	331/4/74	31/34	91	27/28	93
60	268/12/73	28/34	82	23/26	88
61	268/116/73	25/34	74	22/26	85
62	268/112/73	23/29	79	23/27	85
63	268/102/73	23/27	85	21/24	88
64	331/17/74	32/32	100	27/27	100
65	Trebatsch 1908	21/21	100	20/20	100
66	Trebatsch 2901/69	21/21	100	19/19	100
67	Trebatsch 1033/70	13/13	100	14/14	100
68	Trebatsch 1442/69	19/19	100	17/17	100
69	Trebatsch 2875/69	21/21	100	20/20	100
70	Trebatsch 13309/64	19/19	100	12/12	100
71	Trebatsch 3	18/19	95	21/21	100
72	Trebatsch 10	19/19	100	20/20	100
73	Bornhoff 6328/70	18/19	95	21/21	100
74	Bornhoff 6329/70	19/19	100	20/20	100
75	Bornhoff 5855/70	19/19	100	18/18	100
76	Bornhoff 25016/70	18/18	100	17/17	100
77	Bornhoff 9633/68	21/21	100	16/16	100
78	Bornhoff 12880/68	18/18	100	16/16	100
79	Bornhoff 18394/73	15/15	100	19/19	100
80	Bornhoff 23486/70	17/17	100	19/19	100
81	Bornhoff 18395/70	17/17	100	16/16	100
82	Bornhoff 12876/70	20/20	100	19/19	100
83	Bornhoff 25820/71	18/18	100	18/18	100
84	St. 80	14/14	100	20/20	100
85	St. 77	23/23	100	25/25	100
86	St. 1332/64	25/25	100	24/24	100
87	St. 332/55	20/20	100	19/19	100
88	St. 372/55	23/24	96	25/25	100

Table 1 (cont.)

No.	Cultivar, strain, hybrid, population	Experiment I		Experiment II	
		Ratio of the number of attacked to inoculated plants	Percentage of attacked plants	Ratio of the number of attacked to inoculated plants	Percentage of attacked plants
89	St. Orange	26/26	100	27/27	100
90	T-701	10/10	100	20/20	100
91	Nr. 9633	9/17	53*	19/19	100
92	Nr. 9352	9/15	60*	18/18	100
93	Nr. 63	14/20	65*	21/21	100
94	Nr. 1/56	13/16	81*	21/21	100
95	Portugalia 1	19/19	100	20/20	100
96	Portugalia 2	23/23	100	20/20	100
97	Portugalia 3	17/17	100	23/23	100
98	Portugalia Lamb. 4	15/15	100	19/19	100
99	Portugalia Nowacki 5	14/14	100	17/17	100
100	Hiszpania kl. 1	21/21	100	20/20	100
101	Hiszpania kl. 2	26/26	100	19/19	100
102	Hiszpania kl. 5	13/13	100	17/17	100
103	Holandia	20/20	100	21/21	100
104	Anatolia kl. 1	20/20	100	21/21	100
105	Anatolia kl. 3	19/19	100	17/17	100
106	Turcja	20/20	100	20/20	100
107	Sycylia kl. 1	18/18	100	20/20	100
108	Sycylia kl. 3	14/14	100	18/18	100
109	woj. Paulseus	17/17	100	16/16	100
110	woj. Niveus	10/10	100	16/16	100
111	Popul. Kozienice	13/15	87	22/22	100
112	2546/435/72R	25/25	100	15/15	100
113	2521/422/72R	21/21	100	17/17	100
114	2520/422/72R	29/29	100	18/18	100
115	1285/429/72R	34/34	100	18/18	100
116	*Lupinus rothmalerii*	17/17	100	15/15	100

Explanations: positions 1 to 54 — cultivars;
55 to 94 — strains;
95 to 111 — populations;
112 to 115 — hybrids *(L. luteus × L. rothmalerii)*
* Experiment carried out during the autumn–winter months

In the case of an established infection plants showed typical symptoms of the Lupin Narrow Leaf disease. But in cases where doubts arose (symptoms not typical or poorly visible) an additional reisolation on test plants has been performed.

Results

Reaction of plants on virus infection is presented in Table 1.

Discussion

In the investigated plant material, despite its differentiation, in no case has been determined a total or high resistance reaction to BYMV. On the contrary, plants in general showed a high susceptibility (100% of diseased plants). In individuals of some cultivars, among them one commercial variety – Schwako – as well as some strains (814; 1543/46/72 N; 1541/46/72 N; 268/116/73; 268/112/73; 268/102/73) comparatively mild symptoms of infection were observed. This could be interpreted as an evidence of a smaller susceptibility (or a greater tolerance?) to a virus infection; disease symptoms in these plants have appeared later, they often were atypical and distinctly less visible. Sometimes, in these plants the presence of a virus has been stated only by means of reisolation on tests plants. These forms have been selected for further investigation.

In the light of up-to-date investigations and results obtained, it seems that the chances of finding resistant genotypes directly, i.e. a check and selection of genotypes in collections of cultivars, are not very promising. Rather we can still seek sources of resistance to BYMV among natural populations of yellow lupin from primary regions of their origin. On the other hand, we can hope that newer methods of remote hybridization, including embryology and tissue culture *in vitro*, will perform in this respect necessary conditions of the synthesis of desirable genotypes using wild species.

We intend to continue supplemental screening investigations on the new plant material currently available.

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

III. Tobravirus Group: Tobacco Rattle Virus

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In the course of our experiments we have pointed out further thirty herbaceous and woody, annual and perennial plants to be susceptible to tobacco rattle virus, of which 11 have been found to be local, and 19 to be local and systemic hosts. Among the host plants *Tinantia erecta* may be important as a prognostic virus host. Many of the new virus susceptible plants are also important as diagnostic plants. The five new virus resistant plants belong to the families of *Commelinaceae* and *Cucurbitaceae*.

The 30 new plants susceptible to tobacco rattle virus and the five new resistant ones play an important role in separating the different viruses. The tobacco rattle virus can be separated from 10 viruses with *Ocimum sanctum*, from 9 with each of *O. canum* and *Paulownia fargesii*, from about six with *Ammi visnaga*, and from some 14 viruses with any of the mentioned four dichotomous separators. The semiseparators resistant to tobacco rattle virus (*Commelina clandestina*, *C. communis*, *C. graminifolia*, *C. tuberosa*, *Cucurbita pepo* convar. *patissonina* f. *radiata*) render it possible to separate seven viruses from the tobacco rattle virus.

On the basis of reviewing the most detailed study written on the host range of tobacco rattle virus (R/1 : 2.3/5 + 0.9/5 : E/E : S/Ne) it can be established that the virus is able to infect more than 400 species belonging to some 50 dicotyledonous and monocotyledonous plant families (SCHMELZER, 1957). The virus which is readily transmitted mechanically, by nematode vectors (*Trichodorus* spp.) and with the seeds of some weeds (e.g. *Capsella bursa-pastoris*, *Myosotis arvensis*, *Viola arvensis*) alike belongs to those causing the most severe economic losses. The stem mottle and corky ringspot diseases of potato e.g. are among the most serious potato virus diseases in Europe and the United States of America (ROZENDAAL, 1947; WALKINSHAW and LARSON, 1959; TODD, 1965; HARRISON, 1968; ALONSO and PREECE, 1970; DAVIS and ALLEN, 1975). In the course of our experiments carried out in the past years (see HORVÁTH, 1972, 1973, 1975a, b, c, 1976) further virus susceptible herbaceous and woody, annual and perennial plants were found, some of which are of prognostic importance.

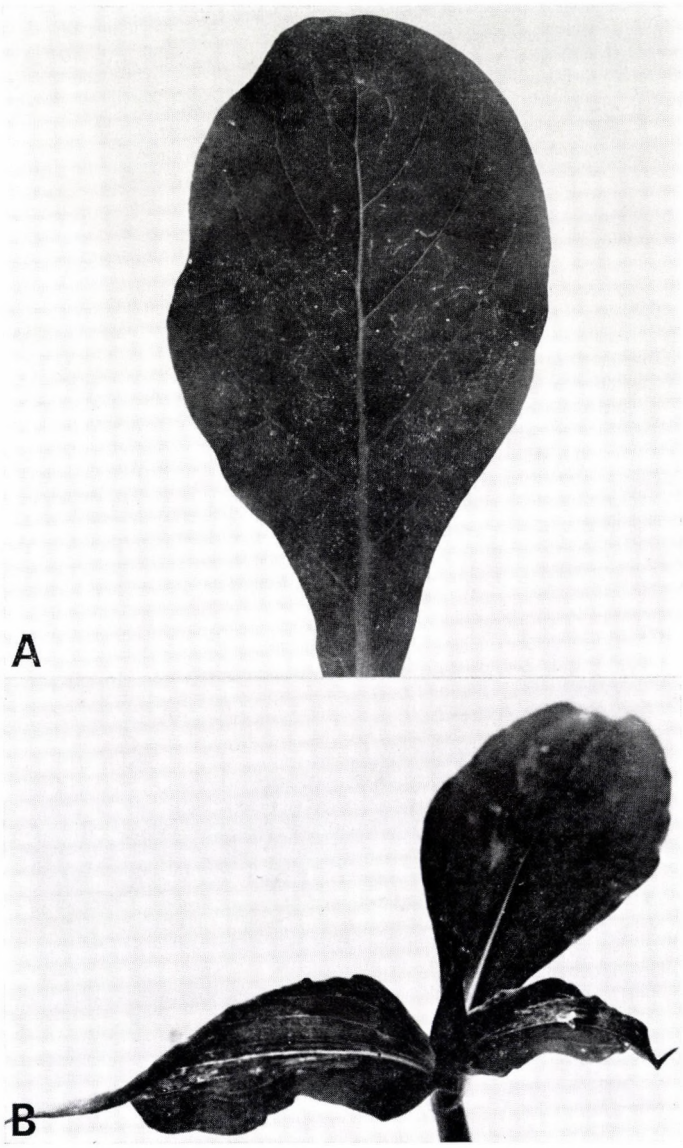

Fig. 1. Tobacco rattle virus symptoms on *Nicotiana quadrivalvis* (A) and *Tinantia erecta* (B) plants. A: local symptoms, B: systemic symptoms

Materials, Methods and Results

As regards the test plants, the methods of artificial inoculation and reinoculation, the virus(es), the host–virus relations, the possibilities of virus separation, the symbols of host–virus relation and the host–virus cryptograms, detailed information is given in the second paper of the series (cf. HORVÁTH, 1977). Any information on the materials and methods will therefore be omitted in the present and the subsequent papers, and only those results concerning the subject will be presented.

New hosts and non-hosts of tobacco rattle virus and their role in the separation of viruses

Local susceptible hosts

Ammi visnaga°*:* Umb=Api, Th // M // /+// ⊟ BCMV, BMV, PVY, WMV/, RMV, TuMV
Beta macrocarpa (B. vulgaris ssp. *macrocarpa):* Chen, Th // M // /+ // ⊟ BCMV, BYMV, PVY, WMV, TuMV, TYMV
Lycium chinense: Sol, Ph // M // /+ // ⊟ –
L. europaeum: Sol, Ph // M // /+ // ⊟ –
Ocimum canum: Lab=Lami, Th // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TuMV, TYMV
O. sanctum°*:* Lab=Lami, Th // M // /+ // ⊟ PVM, PVS, PVY, CLRV, WMV, AMV/, RMV, TYMV, BBWV/, CeMV
Paulownia fargesii°*:* Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, AMV, RMV, TYMV
Physalis aequata: Sol, ? // M // /+ // ⊟ BCMV, PVM, PVS, RMV, TYMV
P. pubescens: Sol, Th // M // /+ // ⊟ BCMV, RMV, TYMV
P. viscosa: Sol, ? // M // /+ // ⊟ BCMV, PVS, RMV, TYMV
Vigna catjang (V. cylindrica)°*:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVY, ToMV

Local and systemic susceptible hosts

Datura chlorantha (D. humilis): Sol, Th // M // +/+ // ⊟ –
D. fastuosa cv. *Alba*°*:* Sol, Th // M // +/+ // ⊟ PVY
D. gigantea (D. tatula): Sol, Th // M // +/+ // ⊟ –
D. godronii cv. *Minka*°*:* Sol, ? // M // +/+ // ⊟ BCMV, BYMV, PVY, CLRV, WMV, RMV, TYMV, CeMV
D. inermis (D. stramonium f. *inermis):* Sol, Th // M // +/+ //⊟ –
D. leichardtii: Sol, ? // M // +/+ // ⊟ –

D. meteloides: Sol, Th // M // +/+ // ⊟ −
D. quercifolia: Sol, ? // M // +/+ // ⊟ −
D. rosei°*:* Sol, ? // M // +/+ // ⊟ WMV
Nicotiana chinensis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
N. knightiana: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
N. occidentalis: Sol, Th // M // +/+ // ⊟ TYMV, CeMV
N. quadrivalvis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, TYMV, CeMV (Fig. 1A)
N. tabacum cv. *Xanthi-nc:* Sol, Th // M // +/+ // ⊟ PVM, PVS, CeMV
Solanum ochroleucum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV
S. pseudocapsicum: Sol, ? // M // +/+ // ⊟ −
Tetragonia crystallina°*:* Aiz, Th // M // +/+ // ⊟ BCMV, BMV, PVM, PVS, PVY, AMV, TYMV, CeMV
T. echinata: Aiz, Th // M // +/+ // ⊟ BCMV, BMV, PVM, PVS, TYMV, CeMV
Tinantia erecta (T. fugax)°*:* Com, H // M // +/+ // ⊟ BCMV, BYMV, PAMV, PVM, PVS, RMV, TYMV, CMV (Fig. 1B)

Resistant plants

Commelina clandestina°*:* Com, ? // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. communis (C. coelestis): Com, Th, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/CMV
C. graminifolia: Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. tuberosa°*:* Com, Th, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
Cucurbita pepo convar. *patissonina* f. *radiata*°*:* Cuc, Th // M // −/− // ⊟ TRSV/TRSV, /TMV, TNV/TNV, WMV/, ToMV/, CMV/

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

IV. Tobamovirus Group: Tobacco Mosaic Virus and Tomato Mosaic Virus

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In the course of investigations made on the range of host plants we have found 205 new tobacco mosaic virus susceptible plants belonging to 11 plant families, of which 121 have proved to be local, and 84 local and systemic hosts. Of the susceptible plant families the *Commelinaceae* and *Cucurbitaceae* deserve special attention. Three new *Commelina* species (*C. clandestina*, *C. graminifolia*, *C. tuberosa*) so far unknown in plant virology are e.g. latently susceptible, local and systemic hosts to tobacco mosaic virus. *Cucumis myriocarpus*, a plant of the family *Cucurbitaceae*, and *Cucurbita pepo* convar. *patissonina* f. *radiata*, an economically important vegetable crop have been found to be local susceptible hosts to tobacco mosaic virus. Among the plants recently found to be susceptible to tobacco mosaic virus there are many perennial *Amaranthus*, *Lycium*, *Paulownia*, *Crambe*, *Geranium* and *Physalis* species. Eighteen plants belonging to the families *Fabaceae (Leguminosae, Papilionaceae)* and *Solanaceae* have proved resistant, while three *Solanum* species (*S. acaule*, *S. cardiophyllum*, *S. vernei)* have been pointed out by hypersensitive reaction to be sources of virus resistance.

In the course of experiments related with the host range of tomato mosaic virus 75 new hosts of ten plant families have been found of which 44 have proved locally, two systemically and 29 locally and systemically susceptible. Among the susceptible plants several perennial and woody species are found (e.g. *Atropa belladonna*, *Bryonia alba*, *B. dioica*, *Lycium europaeum*, *L. ruthenicum*, *Paulownia fargesii*, *P. tomentosa*, *Rorippa islandica*).

We have described about 600 combinations (possibilities) for separating the tobacco mosaic virus and tomato mosaic virus from other viruses.

The host range of tobacco mosaic virus (R/1 : 2/5 : E/E : S/O) and tomato mosaic virus (R/1 : (2)/5 : E/E : $S/_*$), two members of the tobamovirus group occurring all over the world, is — according to the data known so far — probably the second largest one outnumbered only by the hosts of the cucumber mosaic virus (cucumovirus group, R/1 : 1/18 : S/S : S/Ap). According to a detailed though relatively early paper published on the host range of tobacco mosaic virus (cf. HOLMES, 1946) among 310 species from 49 families 202 species have proved susceptible and 108 resistant to the virus. Two decades after the publication of HOLMES' (ibid.) excellent paper THORNBERRY (1966) listed about 350 virus susceptible plants in his book. During the twenty years' period information concerning the susceptible plants grew substantially wider, as shown by a recent

Table 1

Reaction of some differential and non-differential test plants for tobacco mosaic virus and tomato mosaic virus[1]

Host plants	Host reaction[2]	
	Tobacco mosaic virus	Tomato mosaic virus
Differential (after MAMULA *et al.*, 1974)		
Chenopodium amaranticolor	L	L+S
Ch. quinoa	L	L+S
Nicotiana sylvestris	L+S	L
Plantago major	L	L+S
Differential (after RAST, 1975)		
Nicotiana rustica	S	L
N. sylvestris	S	L
N. tabacum cv. *White Burley*[3]	S	L
Petunia hybrida	S	L
P. nyctaginiflora[4]	S	L
Physalis ixocarpa	S	L
Solanum giganteum	L	S
Non-differential (after RAST, 1975)		
Nicotiana glutinosa	L	L
N. tabacum cv. *Xanthi nc*	L	L
N. tabacum cv. *Samsun NN*	L	L
N. tabacum cv. *Samsun*	S	S
N. tabacum cv. *White Burley*	S	S
Petunia nyctaginiflora[4]	S	S

[1] MAMULA *et al.* (1974) and RAST (1975)
[2] L = local lesions, S = systemic symptoms
[3] "Necrotic" line or "Dutch A"
[4] Lines originating from seeds kindly supplied by DR. SCHADE, Martin Luther Universität, Halle, DDR

work of SCHMELZER and SCHMIDT (1977) according to which the host range of tobacco mosaic virus includes about 583 plants. The virus susceptibility of some economically important plant families (e.g. *Cucurbitaceae*) has but recently been established. Owing to the exact virus reisolation and inoculation experiments a latent systemic susceptibility has been pointed out for many plants earlier considered as only locally susceptible. For example, while HOLMES (1946) found only a local susceptibility in 12 species of seven plant families, WEIL (1961) demonstrated the latent systemic susceptibility in them. In the course of his

experiments WEIL (ibid.) pointed out that e.g. outdoor ornamentals as latent hosts of the tobacco mosaic virus were highly important virus reservoirs.

As for the host range of tomato mosaic virus comprehensive data are – to our best knowledge – not available apart from the works by MAMULA *et al.* (1974) and HOLLINGS and HUTTINGA (1976). The reason is perhaps that it was only after the investigations of KNIGHT (1963), WITTMANN (1965), WANG and KNIGHT (1967) and WETTER (1968) that owing to chemical and serological differences between the tobacco mosaic virus and tomato mosaic virus the two viruses of the tobamovirus group had to be distinguished and given separate names (cf. HARRISON *et al.*, 1971; ZAITLIN and ISRAEL, 1975; HOLLINGS and HUTTINGA, 1976). On the basis of the results of investigations made so far it can be established that the host ranges of the tomato mosaic virus and tobacco mosaic virus (type or common strain) though very similar show some differences (MAMULA *et al.*, 1974; RAST, 1975, see Table 1).

In the course of experiments performed in the past years (cf. HORVÁTH, 1968a, b, 1969a, b, c, d, e, 1970, 1972a, b, c, 1973a, b, 1974a, b, c, 1975a, b, c, d, HORVÁTH and BECZNER, 1968, 1973; BECZNER and HORVÁTH, 1974; MAMULA *et al.*, 1974) a number of new hosts to tobacco mosaic virus and tomato mosaic virus were found, which are of great importance not only for the diagnosis of the two viruses but also in separating them from other viruses.

Materials, Methods and Results

As regards the materials and methods of experiments detailed information is given in the second publication of the series (cf. HORVÁTH, 1977). The results of experiments related with tobacco mosaic virus and tomato mosaic virus are summed up as follows.

1. New hosts and non-hosts of tobacco mosaic virus and their role in the separation of viruses

Local susceptible hosts

Amaranthus angustifolius (A. graecizans)°: Ama, Th // M // /+ // ⊟ –
A. ascendens (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ –
A. atropurpureus (A. hybridus)°: Ama, Th // M // /+ // ◫ –
A. aureus (A. paniculatus var. *flavus):* Ama, Th // M // /+ // ◫ –
A. bouchoni°: Ama, Th // M // /+ // ⊟ –
A. caracu (A. hypochondriacus): Ama, Th // M // /+ // ⊟ –
A. caudatus: Ama, Th // M // /+ // ⊟ –
A. caudatus cv. *Atripurpureus (A. caudatus* var. *sanguineus)°:* Ama, Th // M // /+ // ⊟ –
A. chlorostachys (A. hybridus)°: Ama, Th // M // /+ // ⊟ –

A. chlorostachys f. *strictus (A. chlorostachys* f. *leucocarpus)°:* Ama, Th // M // /+ // ⊟ –
A. chlorotachys var. *powelli°:* Ama, Th // M // /+ // ⊟ –
A. cruentus (A. paniculatus): Ama, Th // M // /+ // ⊟ –
A. deflexus: Ama, H // M // /+ // ⊟ –
A. deflexus var. *rufescens°:* Ama, H // M // /+ // ⊟ –
A. dubius: Ama, Th // M // /+ // ⊟ –
A. emarginatus (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ PVS
A. gangeticus var. *multicolor (A. tricolor)°:* Ama, Th // M // /+ // ⊟ –
A. hypochondriacus°: Ama, Th // M // /+ // ⊟ PVS
A. hypochondriacus var. *Monstrosus°:* Ama, Th // M // /+ // ⊟ –
A. mantegazzianus°: Ama, Th // M // /+ // ⊟ –
A. oleraceus (A. lividus var. *oleraceus)°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus (A. cruentus): Ama, Th // M // /+ // ⊟ PVS
A. paniculatus cv. *Roter Dom°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus cv. *Roter Paris°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus cv. *Sanguineus nanus°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus var. *flavus (A. aureus)°:* Ama, Th // M // /+ // ⊟ –
A. patulus (A. hybridus ssp. *cruentus* var. *patulus):* Ama, Th // M // /+ // ⊟ –
A. retroflexus: Ama, Th // M // /+ // ⊟ BMV, PVS
A. speciosus (A. paniculatus f. *speciosus)°:* Ama, Th // M // /+ // ⊟ –
A. spinosus: Ama, Th // M // /+ // ⊟ –
A. sylvestris (A. graecizans var. *sylvestris)°:* Ama, Th // M // /+ // ⊟ –
A. tricolor cv. *Malten Fire°:* Ama, Th // M // /+ // ⊟ –
A. viridis (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ –
Aptenia cordifolia: Aiz, Th, H // M // /+ // ⊟ BCMV, PAMV, PVY, RMV, TYMV, CMV
Chenopodium capitatum: Chen, Th // M // /+ // ⊟ PVS, AMV/
Ch. murale: Chen, Th // M // /+ // ⊟ PVY
Ch. quinoa: Chen, Th // M // /+ // ⊟ BMV41
Ch. quinoa f. *rubescens:* Chen, Th // M // /+ // ⊟ BMV41
Ch. quinoa f. *viridescens°:* Chen, Th // M // /+ // ⊟ –
Cucumis myriocarpus: Cuc, Th // M // /+ // ⊟ BCMV, PAMV, PVM, PVS, PVY, CLRV, RMV, TYMV, CMV/
Cucurbita pepo convar. *patissonina* f. *radiata°:* Cuc, Th // M // /– //⊟ BCMV, BYMV, BMV, PAMV, PVM, PVS, PVX, PVY, CLRV, TRSV/, TNV/, TRV, WMV/, AMV, TuMV, CMV/
Datura aegyptiaca (D. fastuosa): Sol, Th // M // /+ // ⊟ PAMV37/, PVY
D. arborea: Sol, Ph // M // /+ // ⊟ PAMV, PVY, TRV43
D. carolinianum°: Sol, ? // M // /+ // ⊟ BMV/, PAMV/, AMV/
D. ceratocaula°: Sol, Th // M // /+ // ⊟ BMV41/, PAMV/, PVX/, TRSV/, AMV/, CMV/
D. chlorantha (D. humilis): Sol, Th // M // /+ // ⊟ BMV/, PAMV37/, TRSV/, TRV/, AMV/, CMV/

D. fastuosa cv. *Alba*°: Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVX/, PVY, TRSV/, TRV/, AMV/, CMV/
D. gigantea (D. tatula): Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVX/, TRSV5/, TRV/, AMV/, CMV/
D. godronii cv. *Minka*°: Sol, ? // M // /+ // ⊟ BCMV, BYMV, BMV/, PAMV/, PVX/, PVY, CLRV, TRSV/, TRV/, WMV, AMV/, RMV, TYMV, CMV/, CeMV
D. inermis (D. stramonium f. *inermis)*: Sol, Th // M // /+ // ⊟ BMV41/, PAMV45/, TRSV/, TRV/, AMV/, CMV/
D. innoxia: Sol, Th // M // /+ // ⊟ BMV41/, PAMV45/, TRSV/, TRV43/, AMV1/, CMV45/
D. leichardtii: Sol, Th // M // /+ // ⊟ BMV/, PAMV37/, TRSV/, TRV/, AMV/, CMV/
D. metel var. *inermis*°: Sol, ? // M // /+ // ⊟ BMV/, PAMV/, PVX/
D. metel var. *muricata*°: Sol, ? // M // /+ // ⊟ BMV/, PAMV/, PVX/, PVY/, AMV/
D. quercifolia: Sol, ? // M // /+ // ⊟ BMV/, PAMV37/, TRSV/, TRV/, ToMV/
D. rosei°: Sol, ? // M // /+ // ⊟ BMV/, PAMV/, PVX/, TRSV/, TRV/, WMV, AMV/, CMV/
D. stramonium var. *chalybea (D. tatula)*: Sol, Th // M // /+ // ⊟ BCMV, BYMV, BMV/, PAMV/, PVX/, PVY
D. stramonium f. *inermis*°: Sol, Th // M // /+ // ⊟ BCMV, BYMV, BMV/, PAMV/, PVX/, PVY
D. stramonium var. *tatula (D. stramonium* var. *chalybea)*°: Sol, Th // M // /+ // ⊟ BCMV, BYMV, BMV/, PAMV37/, PVX/, PVY, TRSV5/, AMV34/
Erodium ciconium°: Ger, Th // M // /− // ⊟ BCMV, BMV, PVX/, PVY, TRSV, WMV, TYMV, CMV/
E. cicutarium (E. cicutifolium): Ger, Th // M // /− // ⊟ BCMV, BMV41, PVY, CLRV/, TRSV, TRV45/, WMV, TuMV48/, TYMV, CMV45/
E. gruinum: Ger, Th // M // /− // ⊟ BCMV, BMV, PVX, PVY, TRSV, TYMV, CMV/
E. manescavi°: Ger, H // M // /− // ⊟ BCMV, BMV, PVX, PVY, TRSV, TYMV, CMV/
E. moschatum: Ger, Th // M // /− // ⊟ BCMV, BMV, PAMV/, PVX, PVY, CLRV/, TRSV, TYMV, CMV/
Lycium australe°: Sol, Ph // M // /+ // ⊟ −
L. europaeum: Sol, Ph // M // /+ // ⊟ AMV34
L. flexicaule°: Sol, Ph // M // /+ // ⊟ AMV
L. horridum°: Sol, Ph // M // /+ // ⊟ AMV
L. ruthenicum: Sol, Ph // M // /+ // ⊟ AMV
L. turconamicum°: Sol, H // M // /+ // ⊟ AMV
Nicotiana tabacum cv. *Bel 61–10*: Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVM, PVS, PVX/, PVY/, TRSV/, WMV, RMV, TYMV, CeMV

Obione sibirica (Atriplex sibirica)°: Chen, Th // M // /+ // ⊟ BCMV, CLRV/, TRSV/, TYMV, CeMV
Paulownia fargesii°: Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, AMV, RMV, TYMV, CMV/
P. tomentosa (P. imperialis): Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, RMV, TYMV
Pentstemon alpinus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, TRSV/, AMV/, CMV
P. attenuatus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, TRSV/, CMV
P. calycosus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
P. cardinalis°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CLRV/, TRSV/, CMV
P. glaucus°: Scrop, H // M // /+ // ⊟ TRSV/
P. hirsutus°: Scrop, H // M // /+ // ⊟ TRSV/, CMV
P. laevigatus°: Scrop, H // M // /+ //⊟ PAMV/, PVY, CLRV/, CMV
P. murrayanus°: Scrop, H // M // /+ // ⊟ –
P. ovatus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
P. pubescens°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. stenopetalus°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. utahensis°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. venustus°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. whippleanus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
Phaseolus vulgaris cv. *Aladin:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Andrásbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Cardinal:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Cukorbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fehér gyöngy°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fertődi 5.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Fertődi 23.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Harkovszkaja:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Icar Fundulea 51.:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Japán gyöngybab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Kompolti gyöngybab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Kőbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Középbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Májbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Michelite:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Moldovszkaja:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Őrségi cseresznyebab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Perlicska:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Princess:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Robust:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Saxa:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Soproni lapos°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tápiószelei barna°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS

Ph. vulgaris cv. *Tendergreen:* Fab = Legu = Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Tétényi cukorbab°:* Fab = Legu = Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tétényi gyöngybab°:* Fab = Legu = Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tétényi fehér középbab°:* Fab = Legu = Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Tétényi középbab°:* Fab = Legu = Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tétényi nagyszemű fehér°:* Fab = Legu = Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Wade:* Fab = Legu = Pap, Th // M // /+ // ⊟ PVS
Tetragonia crystallina°: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, PVY, CLRV/, TRSV/, TRV/, AMV, TuMV/, TYMV, CMV/, CeMV
T. echinata: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, CLRV/, TRSV/, TRV/, TuMV/, TYMV, CMV/, CeMV
Vigna catjang (*V. cylindrica*)°: Fab = Legu = Pap, Th // M // /+ // ⊟ BCMV, PVY, TRSV/, ToMV, BBWV35/

Local and systemic susceptible hosts

Browallia cordata°: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
B. grandiflora: Sol, Th // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
B. roezli°: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, TYMV/, AMV
B. viscosa: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, AMV, TYMV
Capsicum annuum cv. *Bogyiszlói vastaghúsú°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Cecei édes°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros csüngő°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros felálló I°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros felálló II°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Hatvani hajtatási°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Korai halványzöld°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Kovácsházi hajtatási°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Magyar kincs°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Maritza°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Sonnenpreis°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Tétényi hajtatási zöld°:* Sol, Th // M // +/+ // ⊟ –
Commelina clandestina°: Com, ? // M // –/– // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, RMV, TuMV
C. communis (C. coelestis): Com, Th, H // M // –/– // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV, TYMV
C. graminifolia°: Com, ? // M // –/– // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV, TYMV
C. tuberosa°: Com, Th, H // M // –/– // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV

Crambe armena°: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
C. cordifolia°: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
C. hispanica°: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
C. maritima: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
C. orientalis°: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
C. tatarica°: Cru = Bras, H // M // −/− // ⊟ PAMV, PVX, PVY, RMV, TYMV, CMV
Geranium cristatum°: Ger, ? // M // −/− // ⊟ PAMV, PVY, CMV
G. columbianum°: Ger, Th // M // −/− // ⊟ PAMV, PVY, CMV
G. dissectum: Ger, Th // M // −/− // ⊟ PAMV, PVX/, PVY, CMV/
G. lucidum°: Ger, Th, TH // M // −/− // ⊟ PAMV, PVY, CMV
G. molle: Ger, Th // M // −/− // ⊟ PAMV, PVY, CMV
G. pratense: Ger, H // M // −/− // ⊟ PAMV, PVY, CMV
G. pusillum°: Ger, Th // M // −/− // ⊟ BMV41, PAMV, PVY, CMV
G. pyreanicum°: Ger, H // M // −/− // ⊟ BMV, PAMV, PVY, CMV
G. robertianum: Ger, Th // M // −/− // ⊟ BMV, PAMV, PVY, BBWV44, CMV
G. rotundifolium: Ger, Th // M // −/− // ⊟ BMV, PAMV, PVY, CMV; ⊞ CMV45
G. sibiricum°: Ger, H // M // −/− // ⊟ BMV, PAMV, PVY
Gomphrena decumbens°: Ama, Th // M // +/+ // ⊟ BCMV, BMV, PAMV, PVY, CLRV, WMV, RMV, TYMV, CeMV
Lycopersicon esculentum cv. *Kecskeméti 363*°: Sol, Th // M // +/− // ⊟ −
L. esculentum cv. *Kecskeméti konzerv*°: Sol, Th // M // +/− // ⊟ −
L. esculentum cv. *Pécs gyöngye*°: Sol, Th // M // +/+ // ⊟ −
Nicotiana tabacum cv. *Debreceni*°: Sol, Th // M // +/− // ⊟ −
N. tabacum cv. *Érdi*°: Sol, Th // M // +/+/ // ⊟ −
N. tabacum cv. *Hevesi*°: Sol, Th // M // +/+ // ⊟ −
N. tabacum cv. *Hicks Fixed A2−426*°: Sol, Th // M // +/+ // ⊟ −
N. tabacum cv. *Kerti*°: Sol, Th // M // +/− // ⊟ −
Ocimum canum: Lab = Lami, Th // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
O. sanctum°: Lab = Lami, Th // M // +/+ // ⊟ PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
Petunia atkinsiana°: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
P. axillaris: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV
P. violacea: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV
Physalis aequata: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, WMV, RMV, TYMV
P. ixocarpa: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, WMV, RMV, TYMV

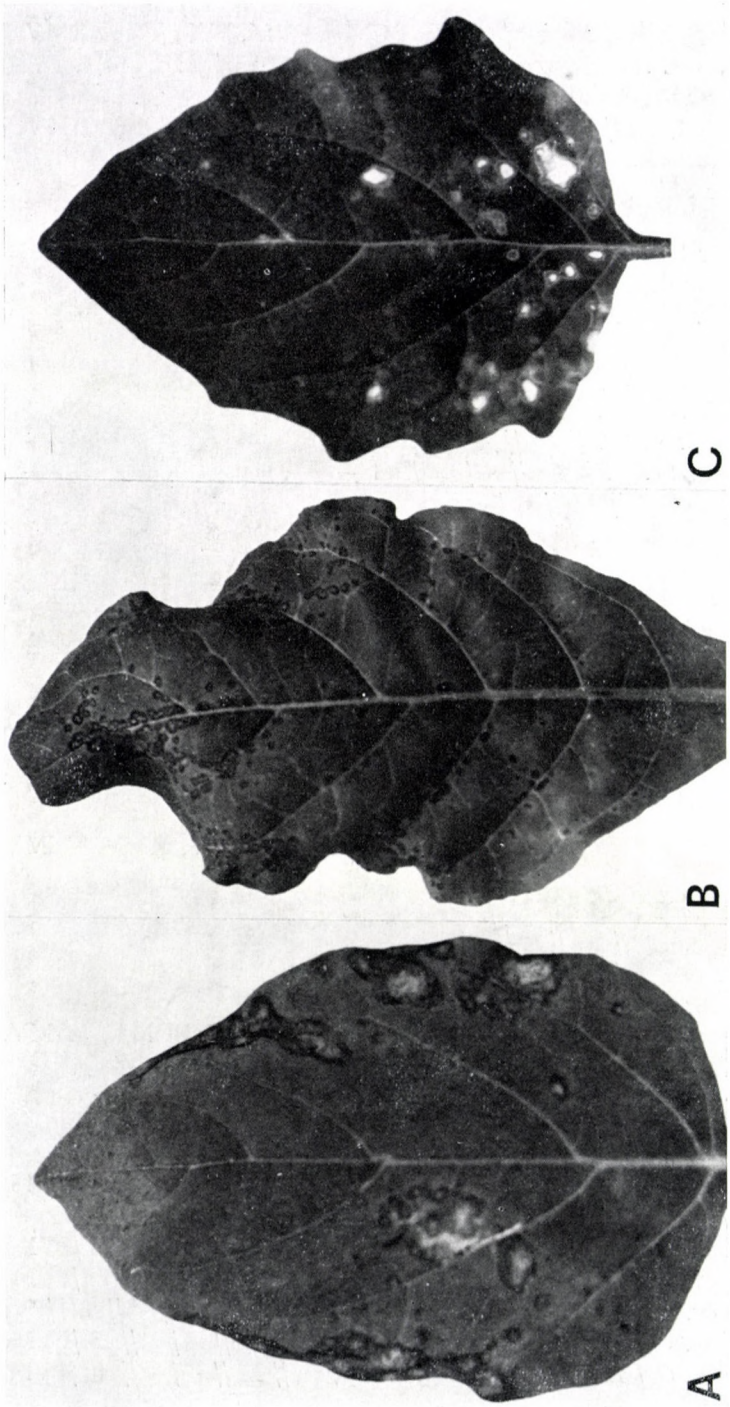

Fig. 1. Local symptoms of tobacco mosaic virus on *Solanum ochroleucum* (A). Local (B) and systemic (C) symptoms of tomato mosaic virus on *Nicotiana quadrivalvis* (B) and *Solanum capsicastrum* (C) plants

P. peruviana: Sol, H // M // +/+ // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
P. peruviana var. *macrocarpa*°: Sol, H // M // +/+ // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
P. pruinosa: Sol, Th // M // +/+ // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Solanum acroscopicum: Sol, ? // M // +/+ // ⊟ −
S. ajanhuiri°: Sol, ? // M // +/+ // ⊟ −
S. berthaultii: Sol, ? // M // +/+ // ⊟ −
S. boliviense°: Sol, ? // M // +/+ // ⊟ −
S. brachycarpum°: Sol, ? // M // +/+ // ⊟ −
S. brevicaule°: Sol, ? // M // +/+ // ⊟ −
S. canasense°: Sol, ? // M // +/+ // ⊟ −
S. chacoense: Sol, ? // M // +/+ // ⊟ −
S. ehrenbergii°: Sol, ? // M // +/+ // ⊟ −
S. etuberosum°: Sol, ? // M // +/+ // ⊟ −
S. famatianae: Sol, ? // M // +/+ // ⊟ −
S. gourlayi: Sol, ? // M // +/+ // ⊟ −
S. guerreroense: Sol, ? // M // +/+ // ⊟ −
S. hjertingii°: Sol, ? // M // +/+ // ⊟ −
S. hougasii°: Sol, ? // M // +/+ // ⊟ −
S. jamesii: Sol, ? // M // +/+ // ⊟ −
S. kurtzianum: Sol, ? // M // +/+ // ⊟ −
S. leptophyes°: Sol, ? // M // +/+ // ⊟ −
S. medians°: Sol, ? // M // +/+ // ⊟ −
S. megistacrolobum: Sol, ? // M // +/+ // ⊟ −
S. ochroleucum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV (Fig. 1A)
S. rostratum: Sol, Th // M // −/− // ⊟ BCMV, CLRV, RMV, TYMV
S. sanctae-rosae: Sol, ? // M // +/+ // ⊟ −
S. simplicifolium: Sol, ? // M // +/+ // ⊟ −
S. sucrense: Sol, ? // M // +/+ // ⊟ −
S. tarijense: Sol, ? // M // +/+ // ⊟ −
S. verrucosum: Sol, ? // M // +/+ // ⊟ −
Tinantia erecta (T. fugax)°: Aiz, H // M // −/− // ⊟ BCMV, PAMV, PVM, PVS, RMV, TYMV, CMV

Resistant plants

Phaseolus vulgaris cv. *Annelise:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM' /AMV
Ph. vulgaris cv. *Barnabab*°: Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Caroline:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Fullcrop:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM
Ph. vulgaris cv. *GN 59.:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *GN 123.:* Fab=Legu=Pap, Th // M // −/− // ◫ /PVM, /AMV

Ph. vulgaris cv. *Icar Fundulea 416.*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Kanizsai csikosbab°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Kereskedelmi hosszú fürjbab°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Kinghorn Wax*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Korai fürj°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Olomucka Zelenoluska*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Processor*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Red Kidney*: Fab = Legu = Pap, Th // M // −/−// ⊟ /PVM, CLRV/CLRV, /TNV, /AMV
Ph. vulgaris cv. *Refugee*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Tápiószelei fürj°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Tápláni fekete "cirádás" fürjbab°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Tápláni nagyszemű cseresznyebab°*: Fab = Legu = Pap, Th // M // −/− // ⊟ /PMV, /AMV

Virus resistant sources

Solanum acaule P. I. 208856: Sol, Th // M // HR // ⊟ PVS45/, PVX45/, PVY47/
S. cardiophyllum P. I. 283063: Sol, Th // M // HR // ⊟ PVX45/, PVY47/
S. vernei P. I. 230468: Sol, Th // M // HR // ⊟ −

2. New hosts and non-hosts of tomato mosaic virus and their role in the separation of viruses

Local susceptible hosts

Aptenia cordifolia: Aiz, Th, H // M // /+ // ⊟ PAMV, PVY, RMV, TYMV, CMV
Beta lomatogona: Chen, Th // M // /+ // ⊟ BMV, PVY, CLRV/, CMV/
B. macrocarpa (*B. vulgaris* ssp. *macrocarpa*): Chen, Th // M // /+ // ⊟ BCMV, BYMV, PVY, CLRV/, WMV, AMV/, TuMV, TYMV, CMV/
Bryonia alba: Cuc, H // M // /+ // ⊟ BCMV, PAMV, PVX, PVY, CLRV, TRSV, AMV, RMV, TuMV, TYMV, CMV47/
B. dioica (*B. cretica* ssp. *dioica*): Cuc, H // M // /+ // ⊟ BCMV, PAMV, PVX, PVY, CLRV, TRSV, AMV, RMV, TuMV, TYMV; ⊞ TuMV45, ⊞ TYMV48

Chenopodium amaranticolor (Ch. giganteum): Chen, Th // M // /+ // ⊟ BMV, TRSV5/ AMV34/

Cucumis myriocarpus: Cuc, Th // M // /+ // ⊟ BCMV, PAMV, PVM, PVS, PVY, CLRV, WMV40/, RMV, TYMV, CMV

Cucurbita pepo convar. *patissonina* f. *radiata*°*:* Cuc, Th // M // /+ // ⊟ BCMV, BYMV, BMV, PAMV, PVM, PVS, PVX, PVY, CLRV, TRSV/, TNV/, TRV, AMV, TuMV, CMV/

Datura chlorantha (*D. humilis*): Sol, Th // M // /+ // ⊟ BMV/, PAMV37/, TRSV/, TRV/, AMV/, CMV/

D. fastuosa cv. *Alba*°*:* Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVX/, PVY, TRSV/, TRV/, AMV/, CMV/

D. gigantea (*D. tatula*): Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVX/, TRV/, AMV/, CMV/

D. godronii cv. *Minka*°*:* Sol, ? // M // /+ // ⊟ BCMV, BYMV, BMV/, PAMV/, PVX/, PVY, CLRV, TRSV/, TRV/, WMV, AMV/, RMV, TYMV, CMV/, CeMV

D. inermis (*D. stramonium* f. *inermis*): Sol, Th // M // /+ // ⊟ BMV41/, PAMV45/, TRSV/, TRV/, AMV/, CMV/

D. innoxia: Sol, Th // M // /+ // ⊟ BMV41/, PAMV45/, TRSV/, TRV43/, AMV1/, CMV45/

D. leichardtii: Sol, ? // M // /+ // ⊟ BMV/, PAMV37/, TRSV/, TRV/, AMV/, CMV/

D. rosei°*:* Sol, ? // M // /+ // ⊟ BMV/, PAMV/, PVX/, TRSV/, TRV/, WMV, AMV/, CMV/

Erodium ciconium°*:* Ger, Th // M // /− // ⊟ BCMV, BMV, PVX/, PVY, TRSV, WMV, TYMV, CMV/

E. cicutarium: Ger, Th // M // /− // ⊟ BCMV, BMV3, PVY, TRSV, WMV, TYMV

E. gruinum: Ger, Th // M // /− // ⊟ −

E. moschatum: Ger, Th // M // /− // ⊟ BCMV, BMV, PAMV/, PVX, PVY, TRSV, TYMV, CMV/

Lycium europaeum: Sol, Ph // M // /+ // ⊟ −

L. ruthenicum: Sol, Ph // M // /+ // ⊟ −

Nicotiana glutinosa: Sol, Th // M // /+ // ⊟ BCMV47, BYMV47, PVM47, CLRV, WMV40

N. tabacum cv. *Bel 61–10:* Sol, Th // M // /+ // ⊟ BMV/, PAMV/, PVM, PVS, PVX/, PVY/, TRSV/, WMV, RMV, TYMV, CeMV

N. tabacum cv. *Xanthi-nc:* Sol, Th // M // /+ // ⊟ BMV41/, PAMV/, PVM, PVS, PVX/, PVY/, TRSV/, TRV/, CeMV

Obione sibirica (*Atriplex sibirica*)°: Chen, Th // M // /+ // ⊟ BCMV, CLRV/, TRSV/, TYMV, CeMV

Paulownia fargesii°*:* Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, AMV, RMV, TYMV, CMV/

P. tomentosa (*P. imperialis*): Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, RMV, TYMV, CMV23/
Pentstemon alpinus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, TRSV/, AMV/, CMV
P. attenuatus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, TRSV/, CMV
P. calycosus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
P. cardinalis°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CLRV/, TRSV/, CMV
P. grandiflorus: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. hirsutus°: Scrop, H // M // /+ // ⊟ TRSV/, CMV
P. laevigatus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CLRV/, CMV
P. murrayanus°: Scrop, H // M // /+ // ⊟ −
P. ovatus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
P. pubescens°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. utahensis°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. venustus°: Scrop, H // M // /+ // ⊟ CLRV/, TRSV/
P. whippleanus°: Scrop, H // M // /+ // ⊟ PAMV/, PVY, CMV
Tetragonia crystallina°: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, PVY, CLRV/, TRSV/, TRV/, AMV, TuMV/, TYMV, CMV/, CeMV
T. echinata: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, CLRV/, TRSV/, TRV/, TuMV/, TYMV, CMV/, CeMV
T. tetragonoides (*T. expansa*): Aiz, Th // M // /+ // ⊟ BCMV, BMV41, PVS4, CLRV/, TRV43/

Systemic susceptible hosts

Atropa bella-donna: Sol, H // M // +/ // ⊟ BCMV, BYMV, PVY47, CLRV, TRSV, WMV, RMV, TuMV, TYMV
Nicotiana knightiana: Sol, Th // M // +/ // ⊟ PVM, PVS, /TNV, WMV, RMV, TYMV, CeMV

Local and systemic susceptible hosts

Browallia cordata°: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
B. demissa (*B. americana*): Sol, Th // M // +/+ // ⊟ BCMV, PVM, PVS, TYMV
B. grandiflora: Sol, Th // M // +/+ // ⊟ BCMV, PVM, PVS, AMV, TYMV
B. roezli°: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
B. viscosa: Sol, ? // M // +/+ // ⊟ BCMV, PVM, PVS, AMV, TYMV
Capsicum annuum: Sol, Th // M // +/+ // ⊟ WMV, TuMV6
Commelina clandestina°: Com, ? // M // −/− // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, RMV, TuMV
C. communis (*C. coelestis*): Com, Th, H // M // −/− // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV, TYMV

C. graminifolia°: Com, ? // M // −/− // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV, TYMV
C. tuberosa°: Com, Th, H // M // −/− // ⊟ BCMV, BYMV, PAMV, PVM, PVS, PVY, TRV, WMV, RMV, TuMV
Datura metel: Sol, Th // M // +/+ // ⊟ −
D. meteloides: Sol, Th // M // +/+ // ⊟ PVY47
D. quercifolia: Sol, ? // M // +/+ // ⊟ −
Gomphrena decumbens°: Ama, Th // M // +/+ // ⊟ BCMV, BMV, PAMV, PVY, CLRV, WMV, RMV, TYMV, CeMV
Lycopersicon esculentum cv. *Kecskeméti 363.*°: Sol, Th // M // +/+ // ⊟ −
L. esculentum cv. *Kecskeméti konzerv*°: Sol, Th // M // +/+ // ⊟ −
L. esculentum cv. *Pécs gyöngye*°: Sol, Th // M // +/+ // ⊟ −
Malva pusilla: Mal, Th // M // +/+ // ⊟ −
Nicotiana chinensis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
N. quadrivalvis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, TYMV, CeMV (Fig. 1B)
Ocimum basilicum: Lab=Lami, Th // M // +/+ // ⊟ PVM, PVS4, PVY38, CLRV, WMV40, TuMV6
O. canum: Lab=Lami, Th // M // +/+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
O. sanctum°: Lab=Lami, Th // M // +/+ // ⊟ PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
Rorippa islandica: Cru=Bras, H // M // −/− // ⊟ BMV
Solanum capsicastrum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV (Fig. 1C)
S. ochroleucum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV
S. pseudocapsicum: Sol, ? // M // +/+ // ⊟ −
S. rostratum: Sol, Th // M // −/− // ⊟ BCMV, CLRV, RMV, TYMV
Tinantia erecta (*T. fugax*)°: Com, H // M // −/− // ⊟ BCMV, PAMV, PVM, PVS, RMV, TYMV, CMV

Resistant plants

Cheiranthus cheiri: Cru=Bras, Th, H // M // −/− // ⊟ /TRSV, TMV45/, /TRV43, AMV34/, TuMV47/, TYMV47/, CMV47/
Phaseolus vulgaris cv. *Pinto:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TNV
Ph. vulgaris cv. *Red Kidney:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, CLRV/CLRV, /TNV, /AMV
Vigna catjang (*V. cylindrica*)°: Fab=Legu=Pap, Th // M // −/− // ⊟ BCMV/, BCMV, /PVM, /PVS, TRSV/TRSV, /TMV, /TNV, /TRV, /AMV, BBWV35/
V. sinensis var. *sesquipedalis*°: Fab=Legu=Pap, Th // M // −/− // ⊟ −

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Relationship Among Some Tobamoviruses I. A Symptomatological and Serological Comparison

By

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Some tipical tobamovirus isolates originated from tobacco, tomato, pepper and *Solanum dulcamara* (N/Tb-3, C/Sz, L/DH, C13 and Sd) were compared with authentic strains. On the basis of the symptoms on *Nicotiana sylvestris* we separated tobacco mosaic virus and tomato mosaic virus (TMV and ToMV) groups. The differentiation of these isolates on *Chenopodium quinoa* or on *Phaseolus vulgaris* seemed to be inconvenient. The isolates of TMV group (U1, N/Tb-3, C/Sz) showed serological identity. The members of ToMV group were separated into distinct serotypes: the isolates L/DH, M II-16 and S7 were serologically closely related, while the other members of this group represented independent serotypes (U2, C13, and Sd). On the basis of symptomatology and serological cross reactivity the Sd isolate was considered as the most divergent type.

In the past decades several attempts have been made to prevent glasshouse tomato crops from severe virus infections by preinoculation of seedlings with selected or artificially induced mild virus strains (OSHIMA *et al.*, 1965; MARROU and MIGLIORI, 1971; RAST, 1972, 1973; VLASOV, 1976).

One of the most important virus diseases of tomato crop is tomato mosaic or in another term, single virus streak disease. The causal agent is tobacco mosaic virus (TMV) or tomato mosaic virus (ToMV). Many authors presume that two different viruses can cause mosaic disease on tomato, namely TMV or ToMV. Others consider that the tomato mosaic virus is only a strain of TMV (cf. RAST, 1975; ZAITLIN and ISRAEL, 1975; VAN REGENMORTEL, 1975; BROADBENT, 1976; HOLLINGS and HUTTINGA, 1976; GIBBS, 1977). In relation to the classification of the causal agent the main problem is that there are controversial results as regards the cross-protection between TMV and ToMV (cf. MARROU and MIGLIORI, 1971; BROADBENT, 1976).

In the present paper we compared the properties of some tobamoviruses isolated in Hungary from pepper (SZIRMAI, 1960; SALAMON, 1978), tomato (HORVÁTH and BECZNER, 1973; BECZNER and HORVÁTH, 1974; MAMULA *et al.*, 1974) and from *Solanum dulcamara* (BECZNER *et al.*, 1976) with some authentic virus strains, isolated from tobacco and tomato.

Materials and Methods

Virus strains. The isolates or strains of TMV and ToMV used in the present paper are listed in Table 1. All the nine isolates were reisolated from a single local lesion on *Nicotiana tabacum* cv. Xanthi-*nc* before being propagated on *Nicotiana tabacum* cv. Samsun. Maximum virus yield was obtained in inoculated leaves about 20 days after the inoculation. In this study the common mechanical inoculation method was used, applying carborundum as abrasive. The crude tobacco sap, diluted with Sörensen buffer (pH 7.0), served as inoculum.

Virus purification. The method of GOODING and HEBERT (1967) was followed with little modification. The harvested leaves of Samsun plants were frozen overnight and homogenized in 0.5 *M* phosphate buffer (pH 7.2) containing 1% mercaptoethanol. After the filtration through double chese-cloth 8 ml *n*-butanol/100 ml was added by gentle stirring, and put in the refrigerator for 1 hr at 4°C. Then the crude sap was centrifuged (30 min, 10,000 *g*). Four grams polyethylene glycol/100 ml (PEG mol wt 6000) was added to the supernatant to precipitate the virus. After 1 hr the solution was centrifuged (30 min, 10,000 *g*). The sedimented virus was resuspended in 0.01 *M* phosphate buffer (20/100 ml v/v of initial volume). The supernatant was clarified again by adding PEG (4 grams PEG plus 4 grams NaCl/100 ml) and after 1 hr the second precipitate was centrifuged again (15 min, 10,000 *g*). The pellet of the second precipitation was resuspended in 0.01 *M* phosphate buffer (20/100 ml v/v). Sucrose gradient centrifugation (10–40% linear gradient; 2 hrs; SW 27 rotor Beckman Model; 24,000 rev/min) was also used for virus purification.

Antisera production. One ml purified virus (10 mg/ml mixed with Freund's complete adjuvant 1 : 1 v/v) was injected intramuscularly into the rabbits. If the titre remained below 512 in a two weeks period, the injection was repeat-

Table 1

List and origin of tobamovirus isolates or strains

Isolate or strain	Original host	Reference
U1	tobacco	SIEGEL and WILDMAN, 1954
C/Sz	pepper	SZIRMAI, 1960. Unpublished
N/Tb-3	tobacco	GÁBORJÁNYI, 1975. Unpubl.
L/DH	tomato	HORVÁTH and BECZNER, 1973; MAMULA *et al.*, 1974.
M II-16	tomato	RAST, 1975.
S7	tomato	VLASOV, 1976. Unpublished.
U2	tobacco	SIEGEL and WILDMAN, 1954.
C/13	pepper	SALAMON 1978. Unpublished
Sd	*Solanum dulcamara*	BECZNER *et al.*, 1976.

ed. The first blood samples were taken after two weeks, then were repeated five times in one week intervals. The sera were separated by centrifugation (10,000 *g*, 20 min) and were stored at −18°C.

Microprecipitin serological test. The titers were estimated by microprecipitin reaction under paraffin oil (VAN SLOGTEREN, 1954). The heterologous and homologous titers were measured in the same way. Fresh purified antigens were always used for serological reactions. One mg/ml standardized antigen was diluted to 1 : 10, 1 : 20 and 1 : 100. The purified antigens were stored at −18°C by adding glycerine (1 : 1 v/v). Sörensen buffer (0.067 *M*) was used for antigen dilutions. The antisera samples were diluted by normal saline solution containing 0.05% sodium azide. The samples of antisera were checked by samples of healthy tobacco sap.

Gel diffusion. The double diffusion tests were carried out by the method of OUCHTERLONY (1962) in 0.8% Difco Agar Noble, containing 0.02% sodium azide in physiological saline solution. The antisera were diluted to 1 : 2, 1 : 4, . . . 1 : 16 with saline solution. The intragel adsorption test was used according to VAN REGENMORTEL (1967).

Results

Symptoms. The host reaction to the nine tobamo virus isolates are summarized in the Table 2. It is concluded that all of the isolates are similar as regards the disease symptoms. On the basis of symptoms on *Nicotiana sylvestris* we can divide the isolates into two groups. The first group (U1, C/Sz and N/Tb-3) induced systemic infection and the other (L/DH, S7, M II-16, U_2, C13, Sd) induced only local infection on *N. sylvestris*. The local lesions of isolate Sd were small in size, white and completely different from the others.

The symptomatological differentiation of the isolates on the basis of symptoms on *N. sylvestris* corresponded to the grouping within tobamoviruses (cf. Descriptions of Plant Viruses, see the Literature). In the following we also used the terms: TMV and ToMV groups to differentiate the U1, C/Sz and N/Tb-3 isolates from the L/DH, S7, M II-16, U2, C13 and Sd isolates. The validity of this grouping was also strengthened by the reactions of *Petunia hybrida*. Isolates of the first group did not cause visible symptoms on inoculated *Petunia* leaves, only local necroses occurred sometimes on the veins. They never induced typical local necrotic lesions, but severe systemic reactions. The isolates of the second group (ToMV type) induced many local lesions, and in some cases (S7, L/DH, Sd) we observed a symptomless systemic infection. By testing on *Nicotiana glutinosa* we got very few local lesions.

Some authors (MAMULA *et al.*, 1974; JURETIĆ, 1971) considered the *Chenopodium quinoa* as a differential host plant for tobacco mosaic and tomato mosaic virus groups. Under our experimental conditions only isolate Sd produced systemic infection regularly, the other ToMV isolates were not able to infect

Table 2

Host reactions of some tobamo virus isolates

Host plants	Isolates								
	U1	C/Sz	N/Tb-3	L/DH	M II-16	S7	U2	C13	Sd
Chenopodium amaranticolor	$LL_{c,n}$ / —	LL_n / —	LL_n / —	$LL_{c,n}$ / s	LL_n / —	$LL_{c,n}$ / —	LL_n / —	LL_n / —	LL_c / s
Chenopodium quinoa	LL_c / —	LL_c / —	LL_c / —	$LL_{c,n}$ / —	LL_c / s	LL_c / —	LL_n / —	$LL_{c,n}$ / —	$LL_{c,n}$ / s
Datura stramonium	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —
Gomphrena globosa	LL_c / St, s	LL_c / St, s	LL_c / St, s	LL_c / St, s	LL_c / Mo	LL_c / St, s	LL_c / St, s	LL_c / St, s	LL_c / St, s
Nicotiana glutinosa	LL_n / —	LL_n / —	LL_n / —	LL_n / LL_n	LL_n / LL_n	LL_n / LL_n	LL_n / LL_n	LL_n / LL_n	LL_n / LL_n
Nicotiana sylvestris	Mo, Ma	Mo, Ma	Mo, Ma	—	—	—	—	—	—
N. tabacum cv. Samsun	— / Mo, Ma	— / Mo, Ma	— / Mo, Ma	— / Mo	— / Mo	— / Mo	— / Mo	— / Mo, Ma	— / Mo
N. tabacum cv. Xanthi-*nc*	LL_n /	LL_n /	LL_n /	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —	LL_n / —
Petunia hybrida	(LL_n) / VC, Mo, Ma	(LL_c) / VC, Mo	— / VC, VN, Mo	LL_n / s	LL_n / —	LL_n / s	LL_n / —	LL_n / —	LL_n / CNS
Phaseolus vulgaris cv. Pinto	LL_n / —	LL_n / —	LL_n / —	— / —	— / —	— / —	— / —	(LL_n) / —	— / —

Key: LL = local lesion; c = chlorotic; n = necrotic; Mo = mosaic; Ma = malformation; St = stunting; s = latent systemic infection; VC = vein cleaning; VN = vein necrosis; CNS = chlorotic necrotic spot; — = no symptoms, negative recovery test; ( ) = occasionally; numerator = inoculated leaf; denominator = systemic reaction

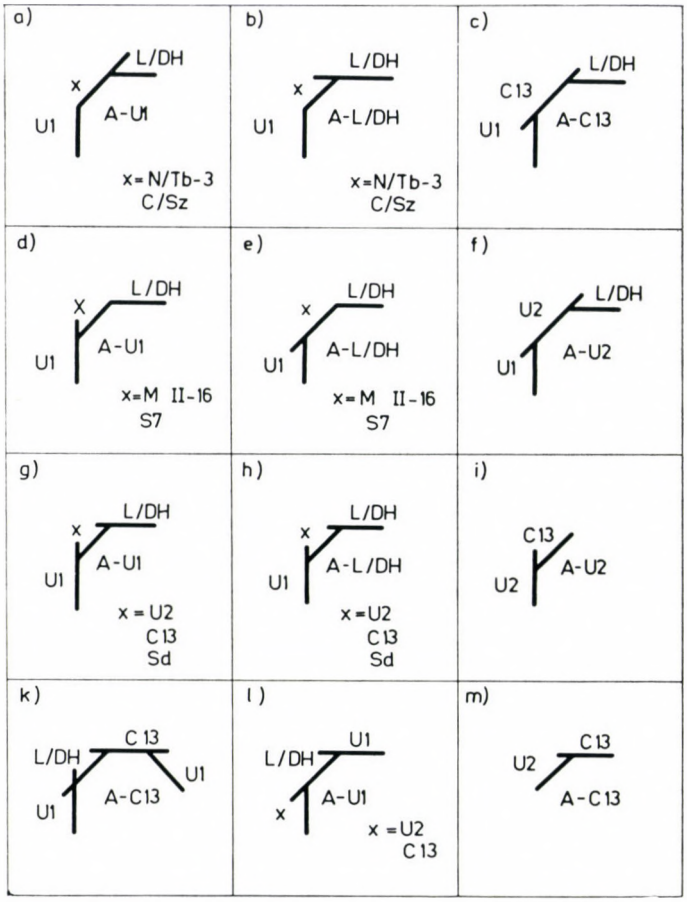

Fig. 1. Results of agar gel double diffusion test among some tobamoviruses. Abbreviations see in text

systemically this host plant. The reaction is noteworthy, because the isolate L/DH in previous experiments of MAMULA *et al.* (1974) induced systemic infection. We suppose that these little differences might be due to small alterations in greenhouse conditions, and we are not considering this plant to be meaningful for differentiation of our isolates. This is true also for Pinto bean, because the C13 isolate caused sometimes little necrotic pinprick-like lesions.

Isolate Sd caused divergent symptoms as compared to other isolates. For instance in Samsun tobacco a very slight mosaic pattern with characteristic white spots appeared after inoculation (Fig. 1).

Microprecipitin serological test. The results of homologous and heterologous serological reactions are summarized in Table 2. It does not contain the serological reactions of S7 isolate. These will be published elsewhere (VLASOV *et al.*, 1978).

Table 3

Serological relationship among the tobamovirus isolates

Antiserum	Test antigen	SDI	SDI in reciprocal test	Average SDI
Anti-U1	N/Tb-3	0	1	0.5
	C/Sz	0.6	0.33	0.5
Anti-N/Tb-3	C/Sz	1	0	0.5
Anti-U1	L/DH	3.3	1	2.2
	M II-16	3.3	2.5	2.9
	U2	5.6	2	3.8
	C13	5	1.3	3.2
	Sd	6	5.3	5.65
Anti-N/Tb-3	L/DH	3.3	2	2.7
	M II-16	3.3	3.5	3.4
	U2	3.6	1.6	2.6
	C13	4.6	1	2.8
	Sd	4.6	6	5.3
Anti-C/Sz	L/DH	2	1	1.5
	M II-16	3	1	2
	U2	3	2	2.5
	C13	3.3	2.3	2.8
	Sd	3	6	4.5
Anti-L/DH	M II-16	0.6	2	1.3
	U2	2.3	2	2.2
	C13	2	1.3	1.7
	Sd	3	7	5
Anti-U2	C13	3.3	0.6	2
	Sd	1.6	4	2.8
Anti-C13	Sd	0.3	4.6	2.5

SDI values represent the difference between homologous and heterologous titers expressed as Neg. Log_2.

After two injections to rabbits we got very similar homologous titers (1024–2048) except with the M II-16 and U2 isolates, where similar titers were observed after one injection.

The first group of the isolates (TMV group) which was separated on a symptomatological basis behaved more or less identically both in the homologous and heterologous reactions. The serological differentiation index (SDI) varied between 0 and 1. This conclusion was valid in the reciprocal titers too, demonstrating that these 3 isolates formed a closely identical group.

In the case of the second group there was a large variability in the heterologous titers, especially in the reciprocal titers (Tables 3 and 4). The statistical insignificance, originated from the small number of rabbits and the blood

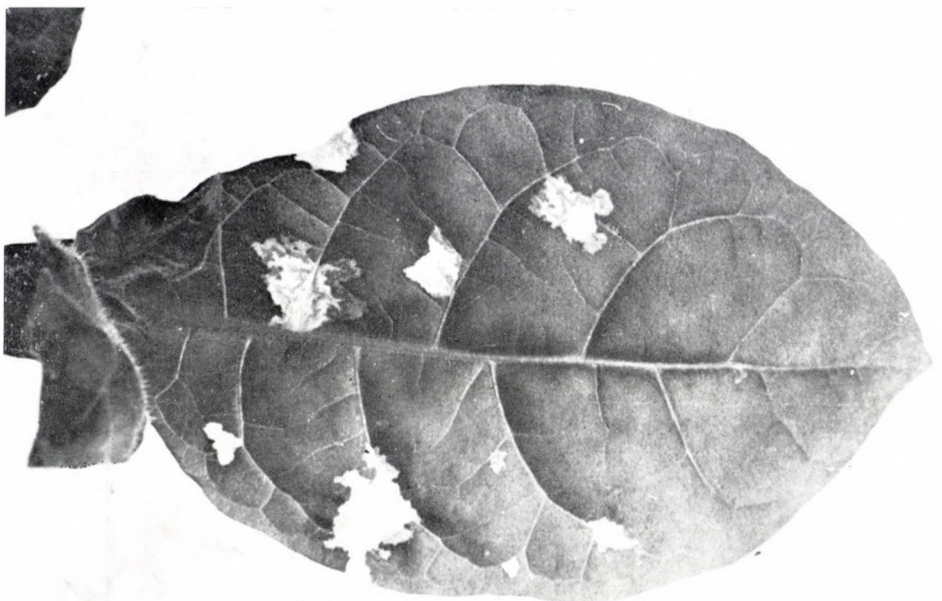

Fig. 2. Symptoms caused by isolate Sd on *Nicotiana tabacum* cv. Samsun

samples, did not allow the exact quantitative characterization of the serological relationship among the isolates, but in extreme cases gave some information about the similarity or heterogeneity.

The variability of ToMV group was larger in the serological cross reactivity expressed with reciprocal SDI, indicating that there are different isolate types in this group. For example we could conclude that Sd differed from both isolate groups (TMV and ToMV).

Agar gel diffusion test. There were no qualitative differences between TMV isolates (U1, C/Sz, N/Tb-3) either in homologous or in heterologous reactions. The precipitation lines gave fusion in all cases, demonstrating the serological identity between these isolates. Among the tomato mosaic virus isolates L/DH, S7, and M II-16 there was no difference, they showed serological uniformity, forming one, distinct serotype.

The U2, C13 and Sd isolates gave spur formation in the gel with both the TMV type and the L/DH group. They were serologically different from each other too, representing new, distinct, independent serotypes. All of these results are summarized in Fig. 2. Sometimes double precipitation lines occurred, and made the decisions difficult. In the case, where the SDI value was high (e.g. between Sd and U1 isolates) even the 1 : 2 dilution of the antisera

Table 4

Homologous and heterologous titers

Antisera	Days after the last injection	Antigens	U1		N/Tb-3		C/Sz	
			T	SDI	T	SDI	T	SDI
U1	14		1024	0	1024	0	512	1
	25		1024	0	1024	0	512	1
	42		1024	0	1024	0	1024	0
	mean:			0		0		0.66
N/Tb-3	14		1024	0	1024	0	512	1
	25		512	2	2048	0	1024	1
	42		1024	1	2048	0	1024	1
	mean:			1		0		1
C/Sz	14		1024	1	2048	0	248	0
	25		4096	0	4096	0	4096	0
	42		1024	0	1024	0	1024	0
	mean:			0.33		0		0
L/DH	21		512	0	256	1	256	1
	38		512	2	512	2	1024	1
	49		512	1	128	3	512	1
	mean:			1		2		1
M/II-16	28		512	2	512	2	1024	1
	44		—	—	—	—	—	—
	55		512	3	128	5	1024	1
	mean:			2.5		3.5		1
U2	14		512	1	512	1	1024	0
	29		256	2	512	2	512	2
	57		512	3	1024	2	256	4
	mean:			2		1.66		2
C13	23		1024	1	1024	1	256	3
	35		512	2	1024	1	512	2
	49		1024	1	1024	1	512	2
	mean:			1.3		1		2.33
Sd	14		64	3	32	4	16	5
	21		16	7	64	5	32	6
	28		32	6	16	7	16	7
	mean:			5.33		6		6

of some tobamovirus isolates

L/DH		M II-16		U2		C13		Sd	
T	SDI	T	SDI	T	SDI	T	SDI	T	SDI
128	3	64	4	16	6	16	6	16	6
128	3	128	3	16	6	128	3	16	6
64	4	128	3	32	5	16	6	16	6
	3.33		3.33		5.66		5		6
128	3	128	3	64	4	256	2	128	3
128	4	128	4	256	3	64	5	64	5
256	3	256	3	128	4	16	7	32	6
	3.33		3.33		3.66		4.66		4.66
256	3	512	2	256	3	128	4	256	3
1024	1	256	4	512	3	512	3	256	4
256	2	128	3	128	3	128	3	256	2
	2		3		3		3.33		3
512	0	256	1	256	1	256	1	128	2
2048	0	2048	0	256	3	128	4	128	4
1024	0	512	1	128	3	512	1	128	3
	0		0.66		2.33		2		3
1024	1	2048	0	512	2	64	5	128	4
—	—	—	—	—	—	—	—	—	—
512	3	2048	0	128	5	512	3	256	4
	2		0		3.5		4		4
1024	0	128	3	1024	0	128	3	512	1
512	2	1024	1	2048	0	256	3	1024	1
256	4	256	4	4096	0	256	4	512	3
	2		2.66		0		3.33		1.66
512	2	256	3	1024	1	2048	0	1024	1
1024	1	1024	1	1024	1	2048	0	2048	0
1024	1	512	2	2048	0	2048	0	2048	0
	1.33		2		0.66		0		0.33
16	5	4	7	64	3	128	2	512	0
16	7	4	9	128	4	16	7	2048	0
4	9	4	9	64	5	64	5	2048	0
	7		8.33		4		4.63		0

gave slight, diffuse reaction. If cross spur was observed, the estimation was far from being settled. To be sure in these cases too, we had to make some intragel adsorption tests.

Intragel adsorption test. These experiments were carried out only in the above mentioned dubious cases, where the characterization of the double diffusion test was difficult. The result of these experiments helped us to construe and verify the data of Fig. 2.

Conclusions

In the experiments outlined above we collected different types of the isolates originating from Hungary. First of all from tomato, where the L/DH isolate was the first tomato mosaic virus described in Hungary (MAMULA *et al.* 1974). On the other hand the C13 (SALAMON and MOLNÁR, 1978) from pepper in preliminary experiments differed from our other tobamovirus isolates. The characterization of Sd was important for us because this isolate was different in its properties and originated from an exceptional source (BECZNER *et al.*, 1976).

From the symptomatological and serological experiments we can conclude that these isolates differed from each other. According to the symptoms the isolates N/Tb-3 and C/Sz are identical with the common tobacco mosaic virus (U1).

It is very important that the Hungarian tomato mosaic virus isolate L/DH did not differ from the authentic M II-16 and S7 attenuated or mild strains. In the intragel adsorption test they were identical, only in the micro-precipitin test we got some variability between the homologous and heterologous titers. In our experiments the U2 isolate differed from the common TMV and the typical ToMV too (GIBBS, 1977).

The C13 was similar in its host range reactions both to TMV and ToMV group, but serologically differed from them. It seems that among the virus isolates originating from pepper there occurred sometimes unusual strains too (cf. FELDMAN and OREMIANER, 1972).

The most problematic question is the characterization of the isolate Sd because it is extremely deviating from others in the quality of symptoms and it represents an independent serotype. To decide exactly whether the isolate Sd is a strain of one tobamovirus or– an individual one, we should investigate more details of its properties.

Acknowledgement

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Comparison of the Hungarian and the Type Isolates of Dulcamara Mottle Virus

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The Hungarian isolate of dulcamara mottle virus was further characterized and compared with DMV—G, BMV and EMV. DMV—H and DMV—G were slightly different in their electrophoretic migration. The molecular weight of protein subunits of DMV—H is 20,500—21,000 dalton. The molar base ratio of its RNA is identical with that of DMV—G.

The antibody compositions of antisera were determined by intragel adsorption and Ouchterlony-test. The relationships between viruses and isolates were confirmed by immunoelectrophoretic investigations. According to the results the DMV—H is considered as the serotype of DMV—G, and the Hungarian isolate is more closely related to BMV and EMV than the DMV—G.

In an earlier paper the presence of dulcamara mottle virus (DMV) in Hungary and its characterization were described (BECZNER *et al.*, 1976). The aim of this study is to confirm the previous results by other investigations and to compare the properties of the Hungarian isolate (DMV—H) with those of the authentic isolate (DMV—G) described by GIBBS and co-workers (1966). The two isolates are similar in their host-range and symptoms but they are not identical in the serological behaviour, therefore some physicochemical properties were chosen for comparative studies: i.e. the determination of molecular weight of the viral protein subunits and the molar base ratio. To determine the serological connection of the two DMV isolates and their relation to other viruses, intragel adsorption test and immunoelectrophoresis were used.

Materials and Methods

The one lesion cultures of DMV—H, DMV—G, belladonna mottle virus (BMV) and eggplant mosaic virus (EMV) were maintained on *Nicotiana glutinosa* and the viruses were propagated in *N. glutinosa* or *N. megalosiphon*. The viruses and isolates used in these experiments were identical with those described previously (BECZNER *et al.*, 1976). The purification was carried out according to GIBBS *et al.* (1966) and DUNN and HITCHBORN (1965).

The electrophoretic migration of viruses was investigated in 1% agarose in 0.05 *M* phosphate buffer, pH 7.0, at 4.5 V/cm for 150 minutes (KOENIG, 1970)

using purified virion samples and virus components separated by sucrose density gradient centrifugation. WEEKE's (1973) dying method was used. The preparation of viral protein subunits was carried out on the basis of PAUL's (1974) method (C modification of basic treatment).

For determining the molecular weight the digested samples were used diluted with 0.1 *M* phosphate buffer pH 7.0. The determination of the molecular weight of the viral protein subunits was carried out with polyacrylamide disc-electrophoresis after KOENIG *et al.* (1970) and PAUL (1974). The 7.5% polyacrylamide gel contained urea of 0.8 *M*. The electrophoresis procedure lasted for 3 hours at 5 mA/gel, at 4°C, using LMIM OE-107 (Hungary) instrument. To the calibration curve the following marker proteins were used: ribonuclease, 4 times crystallized (Reanal) (13700), trypsin inhibitor from soybean (Reanal) (21000), trypsin (Merck) (23800), bovine serum albumin (Merck) (66000), tobacco mosaic virus U_1 strain dimer (35600) and its monomer subunits (17800), and BMV (20700) (PAUL and BUCHTA, 1971), EMV (20600) BERCKS *et al.*, 1971), DMV–G (20900) (BERCKS *et al.*, 1971). The preparation of TMV-U_1 dimer and monomer subunits, and BMV and EMV protein subunits A was carried out as described above. The gels were dyed with amido black 10 B, then washed with 7% acetic acid.

The base composition of the nucleic acid prepared by the method of POULSON (1973) was estimated by a chromatographic method described by MARKHAM (1955) and the fluorescent screen described by KATZ (1962) was used to detect the spots. For the identification of the spots their R_f values were calculated and to determine the recovery chromatographic and spectrophotometric estimations with authentic samples were carried out.

In the serological investigation antisera prepared by BECZNER and co-workers (1976) were used. The composition of the applied gel: 0.8% Difco Agar Noble containing 0.02% sodium azid, in Sörensen buffer pH 7.0. In the intragel adsorption test 24 hours prior to filling in the antisera, adsorbing antigens were filled in the place of antisera, according to the optimum antigen-antibody ratio determined beforehand (BALL, 1974).

Each antiserum was adsorbed by all the four antigens, separately. The circumstances and the evaluation of the experiment were corresponding to OUCHTERLONY's method (1962). The immunoelectrophoresis was carried out at 3 V/cm for 120 min in 0.025 *M* phosphate buffer pH 8.0 containing 1% agarose (KOENIG, 1976) on LMIM OE 206 (Hungary) semimicro-electrophoretic instrument.

Results and Discussion

The electrophoretic migrations of the two DMV isolates and those of their related viruses (EMV and BMV) are shown in Fig. 1. The results are in good agreement with KOENIG's data (1976), since all the four virions migrated into negative direction.

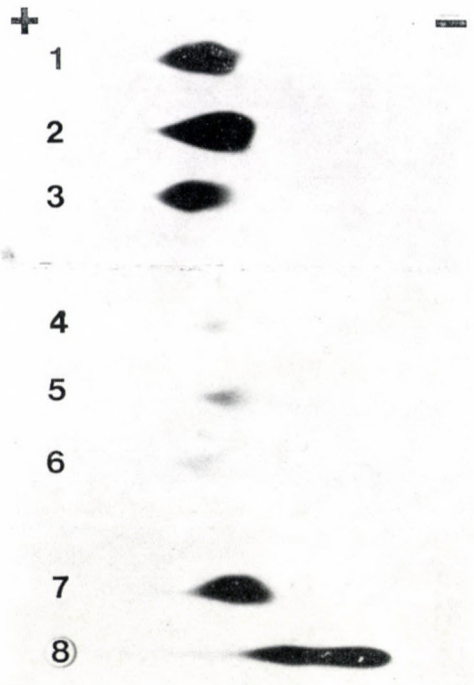

Fig. 1. Electrophoretic migration of DMV—H (1), DMV—H "empty" (2), DMV—H "full" (3), DMV—G (4), DMV—G "empty" (5), DMV—G "full" (6), BMV (7), EMV (8)

Their electrophoretic mobilities are close to each other, except that of EMV, which definitely differs from the others. The EMV forms two not entirely separated spots that refer to the "empty" and "full" particles. The mobilities of DMV—H and DMV—G are different but only slightly. After separation the "empty" particles migrated faster than the "full" ones.

In the experiments the molar base ratios of DMV—H and DMV—G were determined. The experimental data and the results from the literature (GIBBS *et al.*, 1966) concerning the DMV—G were compared mathematically, and accordingly, they can be regarded as identical results. Twenty-eight analyses were done from five RNA preparations of DMV—H and six investigations from three RNA samples of DMV—G. The molar base ratios of DMV—H and DMV—G RNA-preparations were compared by means of Student's *t*-probe (Table 1). On the basis of the results and the statistical analysis, the molar base ratios of the two DMV-isolates are not significantly different.

The results of determination of molecular weight of the viral protein subunits with SDS-polyacrylamide gel electrophoresis are summed up in Fig. 2. The molecular weight of DMV—H protein subunit is 20,500—21,000 dalton in our estimation and this value is identical with the molecular weight of sub-

Table 1

Comparison of the molar base ratios of DMV—H and DMV—G

Bases	Molar base ratio				t	p
	DMV—H mean	$s_{\bar{x}}$	DMV—G mean	$s_{\bar{x}}$		
Guanine	18.65	0.51	17.49	0.91	0.98	N.S.
Adenine	22.40	0.47	22.41	0.72	0.09	N.S.
Cytidine	31.97	0.43	31.39	0.49	0.58	N.S.
Uridine	26.99	0.31	28.7	0.56	1.71	N.S.

units of the related viruses. Fifteen analyses were carried out from five subunit preparations and five electrophoretic runnings were done for the calibration.

The results of the intragel adsorption investigations are shown in Figs 3 and 4. After the intragel adsorption of DMV—H antiserum with DMV—G the precipitations against DMV—G and EMV disappeared and became weaker against BMV. When it was adsorbed with BMV, the reactions with BMV and

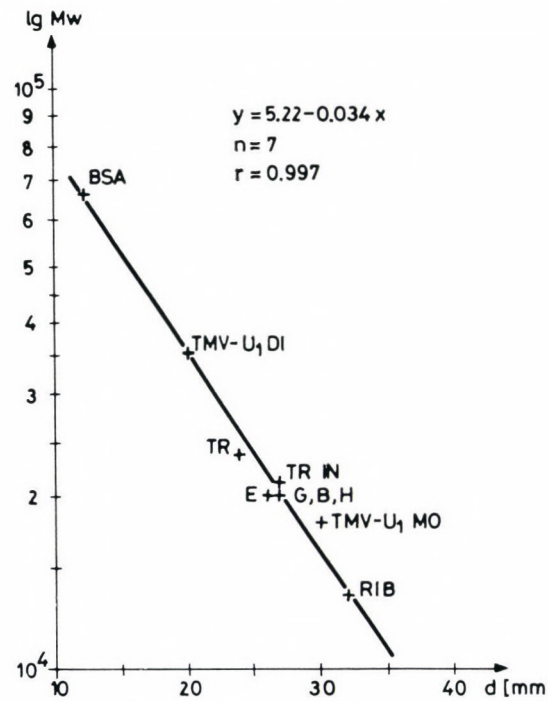

Fig. 2. Determination of the molecular weight of DMV—H protein subunits. (BSA = bovine serum albumin, TR = trypsin, TR IN = trypsin inhibitor, RIB = ribonuclease, E = EMV, B = BMV, G = DMV—G, H = DMV—H protein subunits, respectively)

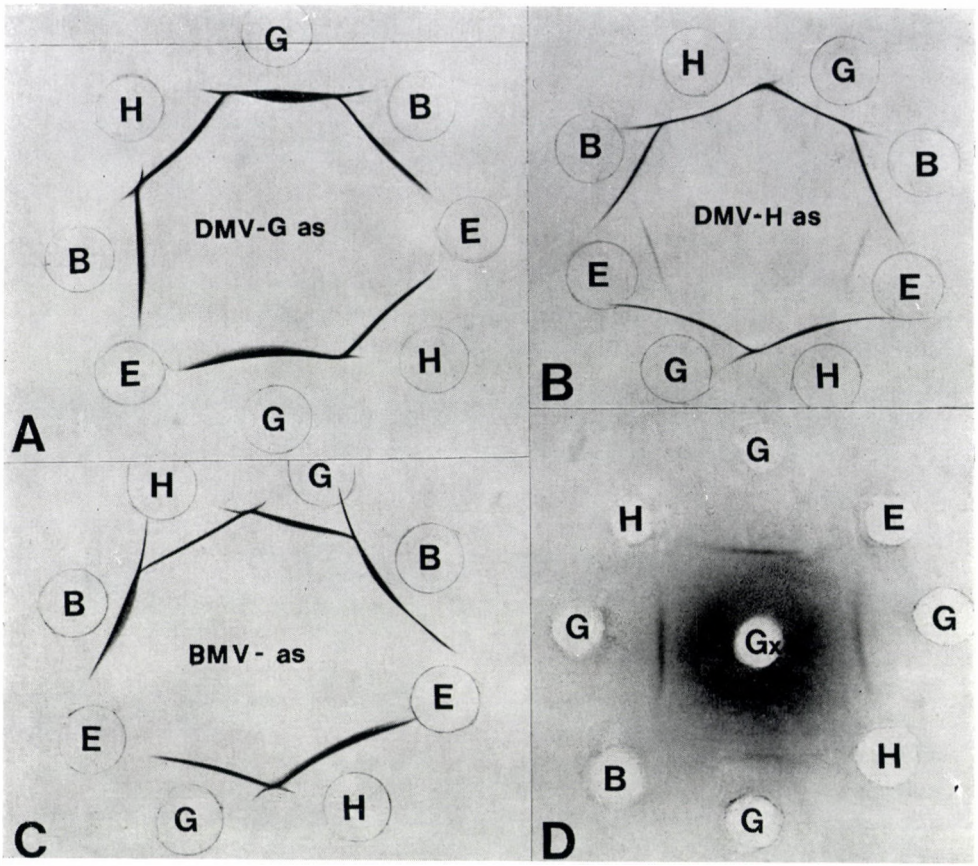

Fig. 3. Immunodiffusion tests with DMV—H (H), DMV—G (G), BMV (B) and EMV (E) antigen. Central well is filled with antisera of DMV—G (A), DMV—H (B), BMV (C) and surrounding wells with 1 mg/ml homologous and heterologous antigens. Gx indicates the antiserum of DMV—G adsorbed by DMV—H, BMV and EMV (D)

EMV disappeared. Using the EMV as adsorbing antigen, only the reactivity of DMV—H antiserum with EMV was lost. Taking the control reactions also into consideration the possible antibody composition of DMV—H antiserum is as follows: h, hg, hb, hbg, hbge, hx, where the small letters indicate the types of antigen determinants in the virion.

Adsorbing the DMV—G antiserum with DMV—H the precipitations characteristic of DMV—H and EMV disappeared. A similar result was obtained after adsorption with BMV: the reaction of BMV and EMV also disappeared. Therefore, the possible antibody composition of DMV—G antiserum is the following: g, gh, ghb, hbge, gx.

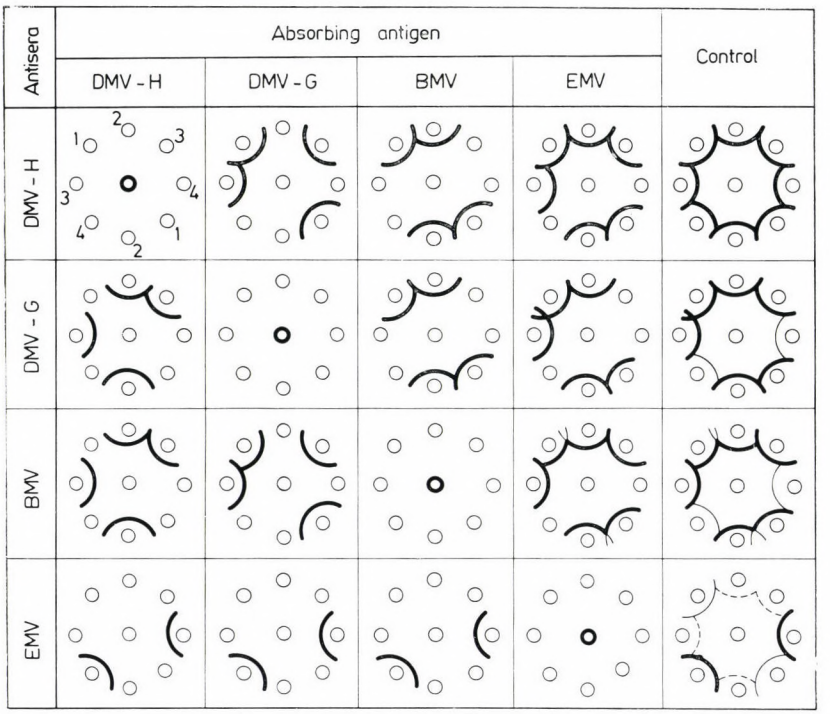

Fig. 4. Intragel adsorption test with DMV—H (1), DMV—G (2), BMV (3) and EMV (4) antigens

Adsorbing the BMV antiserum, comparing to the control reactions, only the precipitation lines specific to the adsorbing antigens and the EMV were lost. The antibody composition of BMV antiserum: b, bg, bh, bhg, hbge, bx.

When adsorbing the EMV antiserum with any of the antigens, all the heterologous reactions disappeared, independently from the heterologous antigen used. The possible antibody composition of EMV antiserum: e, hbge, ex_1.

The immunoelectrophoretic investigations confirmed the results described above and gave further information for the antibody composition of EMV antiserum: e, eh, ehg, hbge, ex_2 (Figs 5 and 6).

We managed to demonstrate the specific antibody(ies) of the English isolate characteristic only for it (Fig. 3D), if we have exhausted the DMV—G antiserum in succession with all heterologous antigens. After one hour 37 °C incubation we removed the precipitate built and the virion particles not participating in the reaction with ultracentrifugation. The completeness of the exhaustion was controlled by means of determining the titres of the antiserum concerning the exhausting antigen.

The investigations proved that a serological difference existed between the English and Hungarian isolates of dulcamara mottle virus. However, the relation-

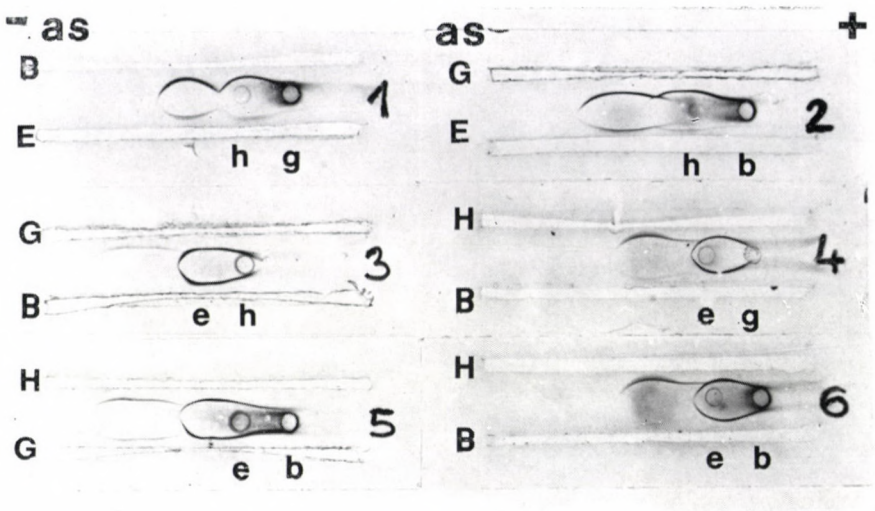

Fig. 5. Immunoelectrophoresis of DMV—H (h), DMV—G (g), BMV (b) and EMV (e) antigens. The channels contained antisera to DMV—H (H), DMV—G (G), BMV (B) and EMV (E)

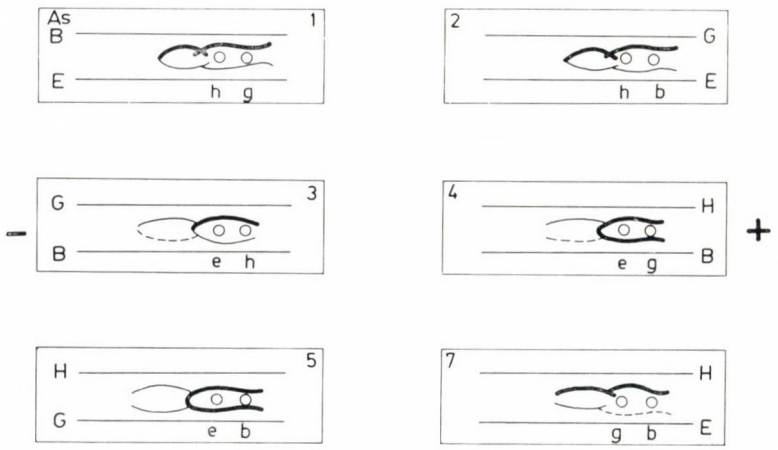

Fig. 6. Results of immunoelectrophoresis of DMV—H, DMV—G, BMV and EMV. Abbreviations see in Fig. 5

ship is of great extent because the BMV antiserum can differentiate the two isolates with cross spur being always weaker than the precipitation lines before crossing, while in case of DMV—G antiserum the cross spur between DMV—H and BMV is always stronger. In the serological classification system of tymoviruses (KOENIG, 1976) the DMV—H can be placed as a serotype of DMV.

Since the molecular weight of viral protein subunits and the molar base ratios of the RNA-s of the two isolates are identical, the DMV—H is considered as a serotype of DMV—G (GIBBS *et al.*, 1966) as it was presumed earlier (BECZNER *et al.*, 1976).

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Interactions in Plants Infected with Viruses and Fungi

I. Interactions between Each of Cucumber Mosaic Virus and Watermelon Mosaic Virus against *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Pollacci, in some Cucurbitaceous Hosts*

By

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The present work reports an antagonistic interaction in three cucurbitaceous hosts, between each of the viruses cucumber mosaic (CMV; cryptogram R/1 : 1/18 : : S/S : S/Ap) and watermelon mosaic (WMV; cryptogram */* : */* : E/E : S/Ap) from one side and *Sphaerotheca fuliginea* (Schlecht ex Fr.) Pollacci from the other side. Host plants were *Cucumis sativus* L. (cucumber), *Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois. (patisson) and *Cucurbita pepo* L. (squash).

Virus-infected plants were investigated for their reaction to challenge-inoculations with the fungal pathogen. Reactions of fungus-infected plants to challenge-inoculations with the involved viruses were not carried out.

Suspending conidia of *Sphaerotheca fuliginea* in extracts of virus-infected hosts revealed that those extracts were free from any substance that might be of an effect on the germinability of conidia of the mentioned fungal pathogen.

The development of a disease on its host can, sometimes, be greatly affected by the presence of another. Such interactions may increase the severity of either or both diseases, a phenomenon known as synergism. Severity may be decreased and in such a case the term antagonism is applied to describe the interaction. Antagonism may be due to direct interaction between the pathogens on or in their host, e.g. one fungal pathogen could prevent others from infecting its host by forming an impenetrable mycelial cover on a leaf surface or by producing antibiotic substances which interfere with spore germination or growth in other pathogens. Other interactions, however, are caused by the effects of one pathogen on the metabolism of its host so that its inherent resistance or susceptibility to other pathogens is altered. When studying the interactions between pathogens related to different systematic categories (fungus–bacterium, bacterium–virus or fungus-virus) it is most advisable to speculate the possible way or ways through which each pathogen can affect the other.

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In the present work, where interactions between plant pathogenic viruses and fungi are involved, the author finds it suitable to summarize the possible ways through which a plant pathogenic fungus can affect a viral infection of its host and *vice versa*.

As far as the possible ways through which a phytopathogenic fungus can affect the orientation of a virus in its host, the following possibilities should be taken into consideration:

1. The presence of virus inhibitors, as an ingredient in the bioproducts of many fungi were reported by several authors (JOHNSON and HOGGAN, 1937; JOHNSON, 1938; GOLDIN, 1938; FULTON, 1943; TAKAHASI, 1942, 46; GUPTA and PRICE, 1950; UTECH and JOHNSON, 1950; YARWOOD, 1951, 56; BLUMER *et al.*, 1955; SINGH *et al.*, 1970 and YAGER, 1973).

2. Infection with certain fungi remarkably affects the level of cytokinin production of the infected host. Cytokinins are known to affect multiplication and symptom visualization of viruses. In this respect, works of KIRÁLY *et al.* (1967) MILO and SRIVASTAVA, 1969; GÁBORJÁNYI *et al.* (1972) ALDWINCKLE, (1975) BALÁZS *et al.* (1976) SZIRÁKI *et al.* (1976) and GÁBORJÁNYI (1977) are worth mentioning.

On the other hand, and as far as the possible ways through which virus infection may affect a secondary fungal infection of the same host is concerned, the following facts should be regarded:

1. In case of virus diseases metabolism of the host is no longer entirely governed by the host's genetic system alone. Genetic informations encoded in the nucleic acid of the virus interfere, leading to physiological and more specifically biochemical disturbances resulting in the multiplication of the virus and visible symptoms on the host organs (BOS, 1970). As a matter of fact, when dealing with virus diseases, pathogenesis has to be considered a genetic process. Accordingly, the inherent susceptibility or resistance of a given host to a certain pathogen can be altered due to viral infection.

2. Literature is very rich in works dealing with the production of phytoalexins and similar antifungal compounds by plants infected with viruses, especially those causing local lesions (PURKAYASTHA and DEVERALL, 1964, 65a, b; DEVERALL, 1967; CRUICKSHANK and PERRIN, 1971; INGHAM, 1972; KLARMAN and HAMMERSCHLAG, 1972; BAILY, 1973 and KUĆ, 1976).

3. Viral infection affects the osmotic value of sap of host cells which in turn affects its reaction to certain fungal diseases (SCHNATHROST, 1959).

4. Viral infection may result in an increase of the exudation of nutrients from roots of infected hosts which may enhance the potentiality of root fungal pathogens of those hosts (BEUTE and LOCKWOOD, 1968).

The present work is undertaken to investigate possible interactions between each of the viruses cucumber mosaic and watermelon mosaic against the powdery mildew *Sphaerotheca fuliginea*, in three cucurbitaceous host plants (cucumber, patisson and squash).

The reaction of mildewed cucurbits to infection with viruses was investi-

gated in earlier works of several authors (e.g. BLUMER *et al.*, 1955; and EL-HAMMADY *et al.*, 1977). That is why attention, in the present work, was directed towards studying the reaction of virus-infected cucurbits to infection with powdery mildew.

Materials and Methods

Interactions between the ascomycete *Sphaerotheca fuliginea* (Schlecht. ex. Fr.) Pollacci and two isolates of cucumber mosaic virus (CMV; cryptogram R/1 : 1/18 : S/S : S/Ap) in addition to an isolate of the general strain of watermelon virus (WMV; cryptogram*/* : */* : E/E : S/Ap) were investigated on three cucurbit host plants. These were *Cucumis sativus* L. (cucumber), *Cucurbita pepo* L. var. *patissonina* Greb. f. *radiata* Nois. (patisson) and *Cucurbita pepo* L. (squash).

The mildew was of common occurrence in the greenhouse and it was identified according to the system given by SZ. NAGY (1970). *S. fuliginea* was propagated on patisson on which it sporulated very heavily under conditions of the greenhouse. No difficulty was faced in preparing large volumes of conidial suspensions. Conidia were collected by gentle tapping of mildewed patisson leaves. Fungal inoculum was prepared by suspending 1.0 g conidia in 1000 ml distilled water. Application of the conidial suspension onto leaves of test plants was accomplished by the aid of a camel's-hair paint brush.

One of the two examined isolates of cucumber mosaic virus (CMV-G/Sc) was isolated from naturally diseased *Scopolia carniolica* Jacq. in Yugoslavia (HORVÁTH *et al.*, 1975). The other isolate (CMV-G/ET*) was recovered from naturally virus-infected musk-melon (*Cucumis melo* L. cv. *cantalaupensis*) which was grown in the experimental fields of the Faculty of Agriculture (of the University of Alexandria) in El-Sabahhia near Alexandria, Egypt. It was identified on the basis of its host range, aphid (*Myzus persicae* Sulz.) transmissibility and physical properties. In sap of patisson, the thermal inactivation point (TIP) of the isolate was between 58 °C and 60 °C, its dilution end point (DEP) between 10^{-4} and 2×10^{-5} and *in vitro* it survived for four days.

The examined isolate of the general strain of watermelon mosaic virus (WMV-G/PW) was obtained from naturally virus-infected patisson plants which were grown in the experimental fields of the Horticultural Department of the Faculty of Agronomy (one of the Faculties of the University of Agricultural Sciences, Keszthely) at Mosonmagyaróvár, Hungary (HORVÁTH *et al.*, 1975a).

To avoid the diminishing of infectivity of CMV to cucurbitaceous hosts, resulting from prolonged propagation of the virus in tobacco plants, CMV-G/Sc was propagated in cucumber (Fig. 1A) while both CMV-G/ET and WMV-G/PW in patisson (Fig 1B and C, respectively).

* Unpublished work.

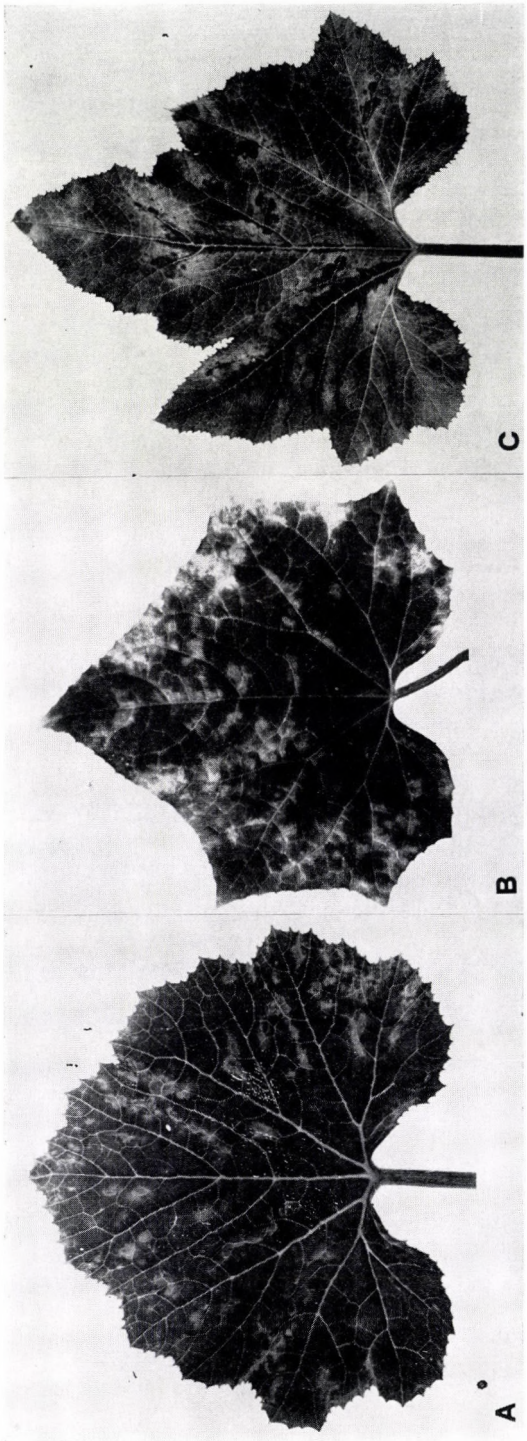

Fig. 1. Systemic symptoms of CMV—G/ET on patisson (A), CMV—G/Sc on cucumber (B), WMV—G/PW on patisson (C)

Test plants were inoculated, with the involved viruses, by practicing the carborundum-spatula technique.

Examined host plants were grown in 16-cm clay pots containing manured soil (compost) and sand in the ratio 3 : 1. Four seeds were germinated per pot. A set of 20 plants was involved in each treatment while a set of an equal number served as control.

The cotyledonary leaves of 8–10 days old cucumber, patisson and squash were mechanically inoculated with each of the before-mentioned viruses. About 15 days later, when test plants attained the four-foliage leaf stage and by that time systemic virus symptoms used to be well expressed, all virus-infected as well as control plants were inoculated with the conidial suspension of *S. fuliginea*. Seven to ten days after inoculations with the fungal pathogen and when powdery mildew symptoms were well pronounced on controls (only inoculated with the mentioned fungus), leaves of treated (first inoculated with virus and then challenged with fungus) as well as those of control plants were harvested.

The number of powdery mildew colonies on each leaf of the treated test plants and also the surface area of the leaf was determined. This number was compared with that developed on leaves of control plants. Comparison was made on basis of the number of mildew colonies developed per 100 cm^2 surface area of leaf.

To determine if there was any direct effect of the virus-bearing sap of the examined hosts on the germinability of conidia of the powdery mildew under investigation, healthy test plants were inoculated with conidia suspended for different periods of time (simultaneous, 6, 12, and 24 hours) in saps of each of the involved hosts which were infected with each of the mentioned viruses. Conidia suspended for the same intervals of time in saps of healthy plants served as controls. Conidial suspensions were kept in a refrigerator maintained at 5 °C. After their development, mildew colonies were counted on leaves of hosts inoculated with each of the two types of suspensions. Obtained numbers were compared with each other. Again comparison was made on basis of the number of mildew colonies recorded on 100 cm^2 surface area of leaf.

During the whole period of the experiments, test plants were kept in the greenhouse where temperature was maintained around 25 °C.

All experiments were repeated twice. Obtained data were statistically analysed to determine significant differences between their means. Statistical analyses were carried out according to the methods described by SVÁB (1973).

Results and Discussion

In comparison to controls, cucumber, patisson and squash plants pre-infected with any of the examined viruses showed very remarkable resistance against infection with *S. fuliginea*. Fig. 2 illustrates, on leaves of patisson, the recorded antagonistic interactions between each of CMV-G/ET, CMV-G/Sc

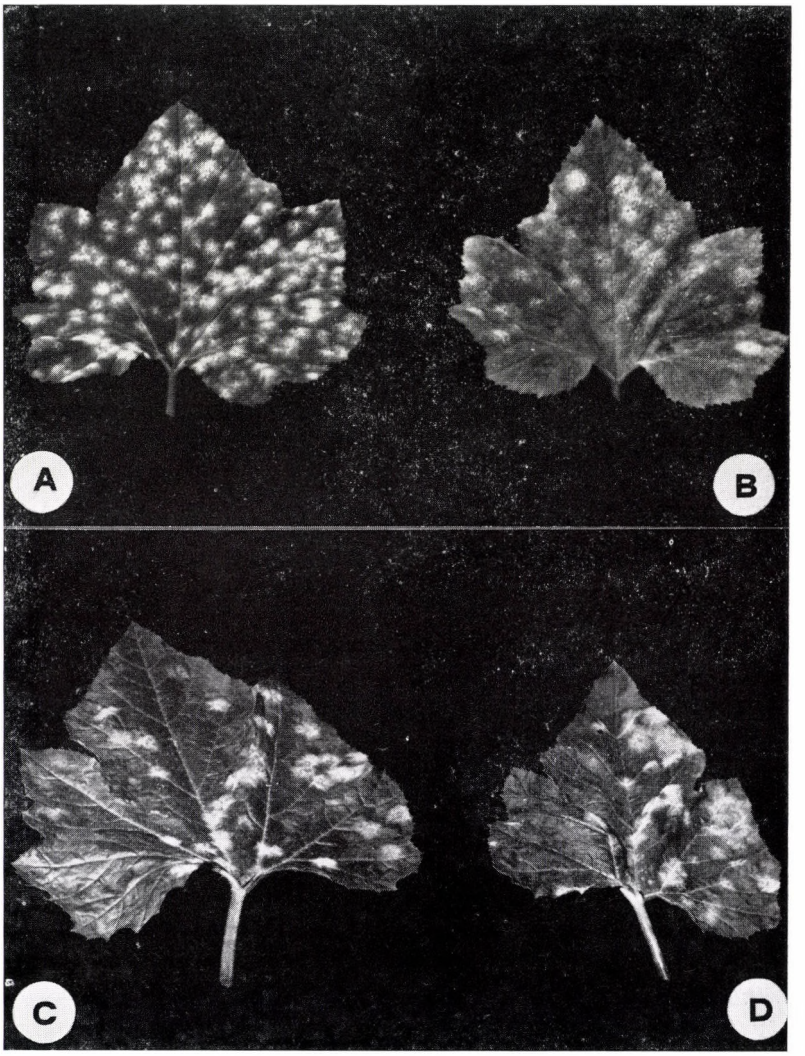

Fig. 2. Antagonistic interaction between *S. fuliginea* and each of CMV—G/ET, CMV—G/Sc and WMV—G/PW on leaves of patisson. A: Control (only infected with *S. fuliginea*). B: First infected with CMV—G/ET and then challenged with *S. fuliginea*. C: First infected with CMV—G/Sc and then challenged with *S. fuliginea*. D: First infected with WMV—G/PW and then challenged with *S. fuliginea*

and WMV-G/PW on one side and *S. fuliginea* on the other side. In agreement with the observations of BLUMER *et al.* (1955), CHADHA and RAYCHAUDHURI (1968) and EL-HAMMADY *et al.* (1977), mildew growth in virus-infected leaves was observed to be mainly restricted to the green areas leaving mosaic spots

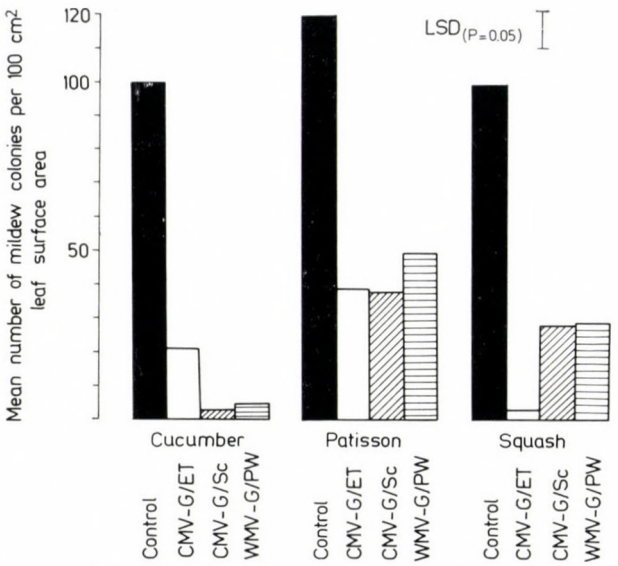

Fig. 3. Effect of viral infection on the response of tested host plants to challenge-inoculation with the fungal pathogen (n = 20)

free. As far as the susceptibility to infection with the mentioned powdery mildew (expressed in terms of number of mildew colonies developed per 100 cm^2 surface area of leaf) is concerned, all treated hosts significantly differed (at the statistical level of P = 0.05) from their controls (Table 1, Fig. 3). Data of Table 1 also show that the recorded virus–fungus antagonistic interaction was the highest in cucumber when preinfected with CMV-G/Sc, in patisson when preinfected with the same above mentioned virus and in squash when preinfected with CMV-G/ET.

Obtained data also show that there was no significant difference between the treatment involving CMV-G/ET and that involving CMV-G/Sc. On the other hand, significant difference was recorded between the treatment involving CMV-G/ET and that involving WMV-G/PW (Table 1). In other words, in this particular interaction, no significant differences were recorded between treatments involving isolates of one and the same virus. Meanwhile, treatments involving different viruses significantly deviated from each other. Tested host plants significantly deviated from each other in their response to the mentioned virus–fungus antagonistic interaction (Table 1).

In general, virus infection caused a significant reduction in the surface area of leaves of tested cucurbit hosts. However, such significant reduction was neither detected in case of cucumber infected with WMV-G/PW nor in case of patisson when infected with CMV-G/Sc (Table 2). This fact indicates that in these two particular cases and without any drastic effects, virus infection

Table 1

Effect of viral infection on the response of examined hosts to challenge-infection with the fungal pathogens
(n = 20)

Treatments	Mean number of mildew colonies per 100 cm^2 surface area of leaf			Mean of treatments
	Cucumber	Patisson	Squash	
Control	99.25	118.73	97.21	105.06
CMV-G/ET	20.38	38.07	2.35	20.26
CMV-G/Sc	2.71	37.60	27.73	22.28
WMV-G/PW	4.65	48.85	28.42	27.30
Mean of hosts	31.75	60.81	38.92	

L.S.D. (P = 0.05) between means of hosts 5.16
L.S.D. (P = 0.05) between means of treatments 5.96
L.S.D. (P = 0.05) between means of any host and any treatment 10.33

Table 2

Effect of viral infection on the surface area of leaf
(n = 20)

Treatments	Surface area of leaf in cm^2		
	Cucumber	Patisson	Squash
Control	82.56	72.05	130.05
CMV-G/ET	56.68	52.15	52.31
CMV-G/Sc	51.86	*62.18*	79.31
WMV-G/PW	*65.53*	48.95	105.39

L.S.D. (P = 0.05) 17.33

had protected the mentioned hosts against a serious secondary infection with the powdery mildew in question.

When compared with their controls, the number of mildew colonies developed on leaves of tested hosts inoculated with conidia suspended for different intervals of time in virus-bearing saps did not show any significant deviation. This means that virus-bearing saps did not contain any substance that might have an effect on the germinability of mildew conidia.

Resistance of the virus-infected hosts against infection with the mentioned fungal pathogen may be attributed to the absence, in saps of virus-infected hosts, of certain metabolites important for the powdery mildew to be established. Resistance can be also due to a change in the osmotic concentration caused by

virus infection of saps of examined plants. SCHNATHORST (1959) stated that resistance of lettuce plants against infection with powdery mildew *(Erysiphe cichoracearum)* was related to the osmotic value of cell sap.

In this respect GOHEEN and SCHNATHORST (1961) reported the resistance of leafroll-diseased grapevines against infection with powdery mildew *(Uncinula necator)*. They found that leafroll-affected vines were characterized by an abnormal high level of carbohydrate accumulation. The mentioned authors concluded that this high level of carbohydrate content had increased the osmotic value of cell sap of virus-infected grapevines rendering them resistant against infection with the mentioned mildew.

On the other hand, EL-HAMMADY *et al.* (1977) reported a lower level of carbohydrate content in virus-infected cucumber plants than in healthy ones. They attributed the resistance of virus-infected plants against powdery mildew *(Erysiphe cichoracearum)* to their poorer content of carbohydrates. At any rate, in cases of systemic virus infections, where no phytoalexins are known to be produced, the osmotic value of host cell sap seems to play of an important role in the resistance of virus-infected plants against infection with powdery mildews.

The author finds it worthy to mention that, as far as he is aware, patisson plant has not been involved before in any study investigating interactions between plant pathogenic viruses and fungi.

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Transmission de Germes de Type Rickettsoïde par la Plante Parasite: *Cuscuta Subinclusa* Durr. et Hilg

par

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Des études en microscopie électronique ont révélé la présence de germes rickettsoïdes en relation avec une maladie à dépérissement des trèfles blancs (*Trifolium repens* L.) et des trèfles incarnats (*T. incarnatum*), detectée dans le sud de la France.

Cette maladie a, pour la première fois été transmise à des trèfles ainsi qu'à des pervenches (*Vinca rosea* L.) sains, par l'intermédiaire de la plante parasite *Cuscuta subinclusa* Durr. et Hilg. Les plantes infectées expérimentalement et les plantes malades naturellement présentent exactement les mêmes symptômes. Chez *T. repens* ils sont caractérisés par un dépérissement général de la plante, accompagné d'une réduction de la taille des folioles. Les jeunes feuilles à l'émergence ont une taille encore plus réduite, sont rougissantes puis marquées de distorsions du limbe avec gaufrage.

Chez *T. incarnatum*, on retrouve les mêmes symptômes, encore plus prononcés avec en plus, la naissance de bouquets grêles de feuilles à la base de pieds, donnant à la plante un aspect proliférant.

Le jaunissement, la chute des feuilles basales et la réduction de taille des feuilles accompagnée de leur distorsion sont les symptômes typiques se manifestant chez *V. rosea* après transmission.

Des études comparatives ont été réalisées sur la multiplication et l'effet cytopathogène des germes au niveau cellulaire chez la Cuscute et les autres plantes hôtes. Au niveau des haustoriums et des tiges de Cuscute, plus de 80% des cellules phloemiques sont parasitées par un grand nombre de germes de type rickettsoïde entourés d'un double système membranaire, mesurant 1 à 2 μm de long et 0.4 à 0.5 μm de large. Ces germes sont responsables de la dégradation progressive du cytoplasme avec libération de particules granulaires denses dans les cellules phloemiques de toutes les plantes.

La morphologie ultrastructurale des germes, ainsi que leurs effets cytopathogènes se retrouvent identiques dans toutes les autres plantes hôtes. Cependant, la Cuscute se révèle particulièrement favorable à la multiplication des germes. Cette propriété pourrait permettre en premieu lieu, de révéler des infections de bas niveau chez les plantes hôtes primaires et en second lieu d'étudier certaines caractéristiques des germes extraits des tissus.

Nous avons ainsi réalisé la première transmission de germes de type rickettsoïde par la plante ventrice *C. subinclusa*. Elle ouvre la voie à l'étude comparative sur les plans de l'ultrastructure et du mode d'action au sein d'une même plante hôte, de rickettsoïdes du trèfle et d'autres procaryotes phytopathogènes biologiquement transmis.

Les germes phytopathogènes qui, dans la nature, dépendent d'un cycle de transmission biologique par des arthropodes, peuvent aussi être transmis expérimentalement par greffes ou par cuscutes, plantes parasites susceptibles de jouer le rôle de vecteur. Ces dernières constituent dans bien des cas, le seul moyen efficace de transmission lorsque les vecteurs naturels ne sont pas disponibles et que le greffage difficile ou même impossible ne permet pas l'entretien du matériel sur une plante hôte convenable.

L'utilisation de la cuscute en tant que vecteur est connue depuis de nombreuses années puisque BENNET (1940) et JOHNSON (1941) établissent déjà la transmission de maladies présumées de nature virale par *Cuscuta campestris* Yunck. VALENTA (1961) obtient la transmission du stolbur par ce type de végétal parasite et ouvre ainsi la voie à la transmission par ce moyen de nombreuses maladies de type «jaunisse» à différentes plantes, notamment *Vinca rosea* L., facilitant ainsi la conservation de ces affections et leur étude symptomatologique comparée. Ces travaux se sont encore étendus avec la découverte de l'étiologie mycoplasmique de beaucoup de jaunisses végétales.

Cependant, la transmission par les plantes parasites, des jaunisses à rickettsoïdes qui présentent à beaucoup d'égards les mêmes difficultés d'étude que les mycoplasmoses végétales ne semble pas jusqu'à présent avoir été explorée. C'est cependant sur une plante appartenant à ce genre *C. subinclusa* DURR et HILG., que GIANNOTTI et al. (1970) ont pour la première fois observé des procaryotes intracellulaires à paroi pouvant rappeler les rickettsies d'animaux.

Au cours de nos recherches sur l'étiologie de maladies affectant différentes plantes, nous avons remarqué chez certaines d'entre elles et plus particulièrement chez les trèfles *Trifolium repens* L. et *T. incarnatum* L., des syndromes accompagnés d'évolutions pathologiques peu communes dans nos régions. La recherche en cytologie ultrastructurale nous ayant permis de mettre en évidence la présence de germes de type rickettsoïde dans certaines cellules libériennes criblées de *T. repens* et *T. incarnatum*, nous avons cherché à reproduire la maladie sur des plantes hôtes secondaires telles *V. rosea*. Comme les transmissions mécaniques se sont révélées inefficaces, et que les vecteurs ne sont pas connus, nous avons effectué la transmission des rickettsoïdes par la plante parasite *C. subinclusa*. Nous avons alors étudié au niveau cellulaire les modalités d'infection des rickettsoïdes sur ce vecteur végétal comparativement aux différentes plantes hôtes et notamment *V. rosea*.

Matériel et Méthodes

Les trèfles malades se rencontrent le long de la bordure sud des Cévennes. Ils sont prélevés puis repiqués en pots de 13 cm de diamètre dans un substrat composé de terreau additionné de sable. Maintenus en serre à une température comprise entre 20 °C et 28 °C, ils subissent la photopériode journalière normale. Après leur reprise, les pieds reçoivent des filaments de *C. subinclusa* provenant d'une souche mère saine issue de semis. Trois à quatre semaines plus tard alors

que la cuscute parasite effectivement les trèfles, et qu'elle manifeste une croissance plus ou moins marquée, un pont est établi avec une plante hôte saine sur laquelle la cuscute sera maintenue plusieurs mois, en limitant sa croissance afin d'éviter un affaiblissement trop marqué de l'hôte.

Les plantes hôtes saines sont représentées par des pieds de trèfles sains: *T. repens* et *T. incarnatum* d'une part et par des pieds de *V. rosea* d'autre part. Une fois la maladie transmise par la plante vectrice *C. subinclusa*, les pervenches sont secondairement multipliées par greffage.

Les examens en microscopie électronique sont réalisés sur les plantes malades prélevées dans la nature, sur les cuscutes qui les parasitent, les trèfles et les pervenches utilisés pour les transmissions ainsi que sur des plantes indemnes de toute infection.

Les fragments de tissus prélevés sur chacune de ces plantes sont fixés dans une solution de glutaraldéhyde à 2.5% dans un tampon cacodylate de sodium 0.1 M à pH = 7.4, puis postfixés au tétroxyde d'osmium à 1% dans le même tampon à 4 °C. Les pièces sont deshydratées dans un gradient d'acétone puis incluses à l'«Epon». Les coupes ultra-fines sont contrastées à l'acétate d'uranyle et au citrate de plomb selon REYNOLDS et examinées au microscope électronique.

Résultats

A) *Symptomatologie et transmission expérimentale*

1 – *Aspect des plantes infectées naturellement*

Les pieds de *T. repens* naturellement malades se caractérisent par une tendance très nette au rabougrissement accompagné dans la plupart des cas d'un rougissement des folioles affectant surtout la bordure marginale, mais pouvant s'étendre à de larges secteurs de la surface foliaire. L'état de ces plantes repiquées et maintenues en serre évolue vers un nanisme et un rachitisme de plus en plus marqués avec notamment l'apparition de jeunes feuilles plus ou moins jaunissantes recourbées passagèrement en virgule. Le déclin de ces végétaux malades se poursuit pendant plusieurs mois à 25 °C–30 °C et aboutit lentement à la mort.

Chez *T. incarnatum* récolté dans la nature, les mêmes symptômes se retrouvent à un degré plus élevé encore. Le jaunissement des nouvelles feuilles est fortement accusé et s'accompagne d'une torsion en virgule. A la base des pieds, on note la présence de bouquets grêles de feuilles d'aspect chlorotique donnant à la plante un aspect proliférant. L'état des plantes évolue habituellement vers un nanisme de plus en plus intense qui débouche souvent sur une mort brutale.

2 – *Transmission expérimentale par C. subinclusa*

Chez *T. repens* et *T. incarnatum* sains, reliés aux pieds malades de *T. repens* par un «pont de cuscute», on note deux mois et demi à trois mois après l'accrochage des suçoirs l'apparition d'un rougissement foliaire. Chez les feuilles adultes il

évolue vers le desséchement tandis qu'il s'estompe progressivement chez les jeunes feuilles au niveau desquelles apparaissent ensuite des distorsions du limbe accompagnées de gaufrage. Par la suite, naissent de nouvelles feuilles chlorotiques et de taille réduite, pouvant présenter une forme en virgule, portées par un pétiole grêle, ce qui donne à la plante un aspect rachitique et éclairci de plus en plus prononcé.

Chez *V. rosea* sain relié à *T. repens* malade, on observe, six à sept mois après l'établissement des haustoriums de la cuscute, un jaunissement prématuré des feuilles basales allant jusqu'à leur desséchement et leur chute ce qui donne à la plante atteinte un port «squelettique». Seules les feuilles de l'extrémité des tiges subsistent. Elles sont de taille réduite, d'aspect lancéolé et marquées aussi par des distorsions plus ou moins accusées du limbe. La floraison n'est pas complètement inhibée, mais les fleurs sont d'une taille nettement inférieure à celle des plantes saines et présentent en outre une certaine décoloration. La maladie peut aussi être retransmise par greffage chez cette plante test. Dans ces conditions, les premiers symptômes apparaissent au bout de 6 à 7 semaines.

3 — *Mise en évidence de manifestations pathologiques chez la cuscute*

Peu après son installation sur la plante hôte malade, la cuscute manifeste une croissance ralentie et développe des filaments grêles dont le diamètre est environ de moitié inférieur à celui des plantes parasites saines. De plus, elle présente une tendance marquée à l'apparition précoce des fleurs qui se groupent en multiples inflorescences peu fournies. Son installation secondaire sur une plante hôte saine ne modifie pas ce comportement alors que les cuscutes saines conduites dans les mêmes conditions sur des hôtes sains poussent vigoureusement.

B) *Étude ultrastructurale des tissus des plantes infectées*

1 — *Recherche des germes au niveau de C. subinclusa*

Les observations en microscopie électronique permettent de constater que de nombreuses cellules libériennes criblées de la plante vectrice *C. subinclusa* contiennent un grand nombre de microorganismes qui n'apparaissent jamais chez les sujets sains (Fig. 1). Ces éléments sont peu polymorphes. Leur longueur varie de 1 à 2 μm et leur diamètre de 0.2 à 0.3 μm. Ils sont tous limités par deux enveloppes trilamellaires. La membrane cytoplasmique est régulièrement tendue et son épaisseur est d'environ 8 nm. La membrane externe (9 nm) est plus ondulée. Les deux enveloppes sont séparées par un espace clair aux électrons (6 nm). L'appareil nucléaire, libre dans le cytoplasme, est formé de fibrilles d'ADN groupées au centre du germe sous forme de réseau, et de granules ribosomiens disposés en général à la périphérie (Fig. 2). La morphologie et le caractère intracellulaire de ces micro-organismes, montrent que ce sont des procaryotes de type

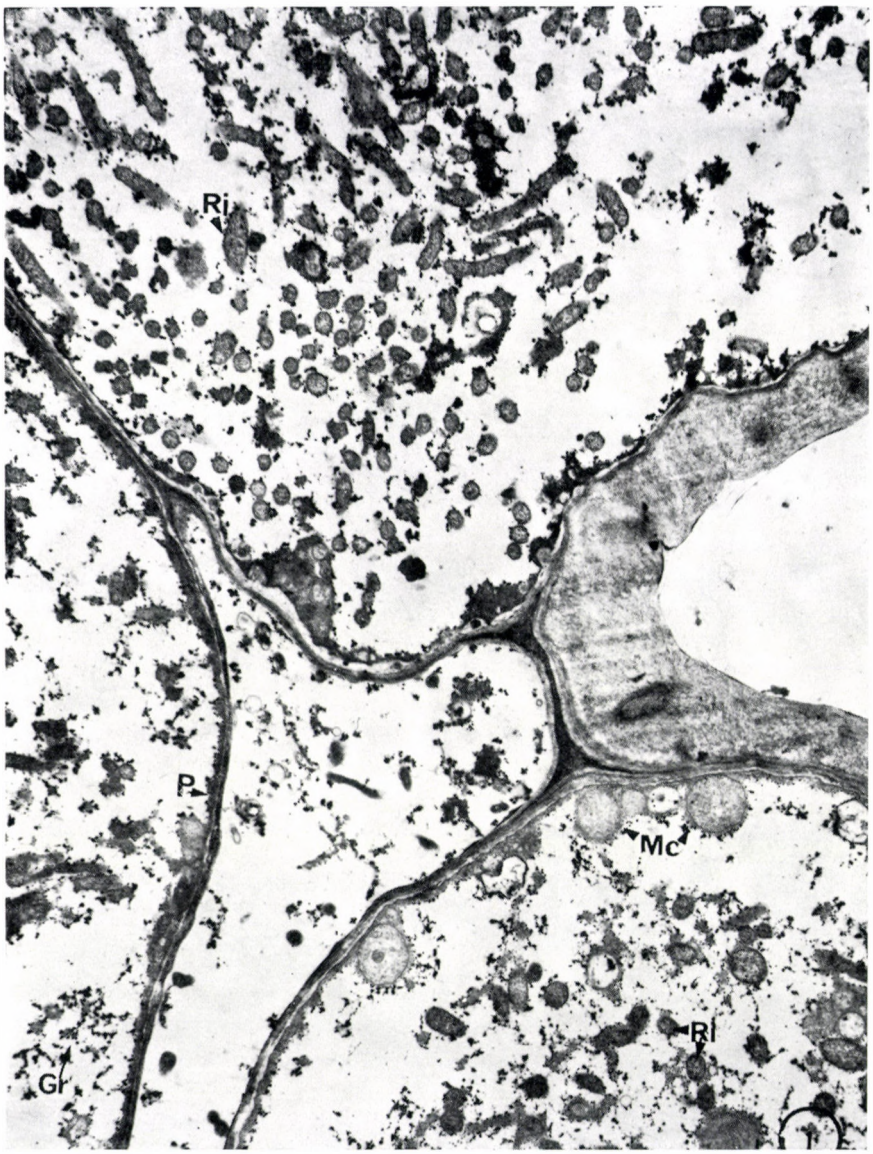

Fig. 1. Germes rickettsoïdes infectant des cellules criblées de *C. subinclusa.* ×10500. Ri: Rickettsoïde, Mc: Mitochondrie, P: Paroi végétale, Gr: Granulations denses aux électrons

rickettsien, désignés en pathologie végétale sous le terme de «rickettsoïdes» (GIANNOTTI *et al.* 1974 b). Ils peuvent présenter différents aspects. C'est ainsi que dans certaines cellules criblées encore jeunes et en tout début d'infection, leurs membranes interne et externe sont accollées (Fig. 3). Par contre, dans d'autres cellules plus

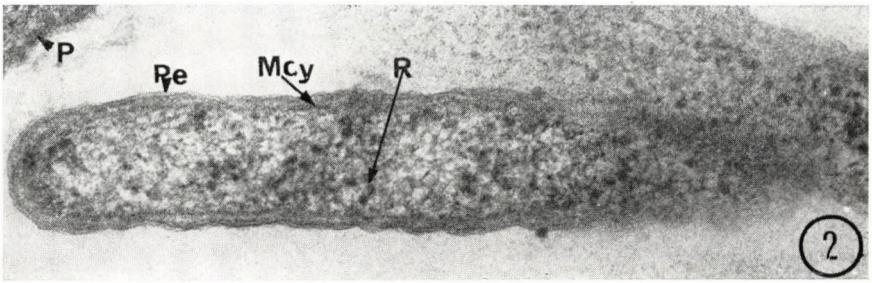

Fig. 2. Détail d'un germe rickettsoïde. × 59000. Pe: Paroi externe, Mcy: Membrane cytoplasmique (double système membranaire), R: Ribosome, P: Paroi végétale

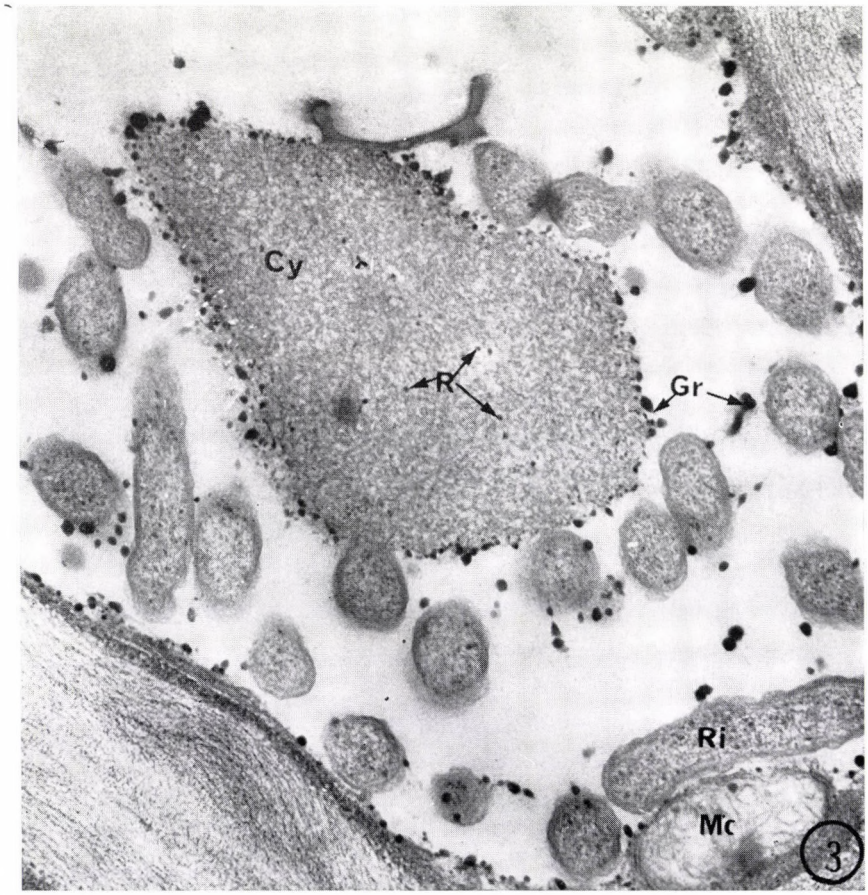

Fig. 3. Cellule libérienne criblée de *T. incarnatum* en début d'infection. × 37.000. Mc: Mitochondrie, Ri: Rickettsoïde, Cy: Cytoplasme résiduel, R: Ribosome, Gr: Granulations denses aux électrons

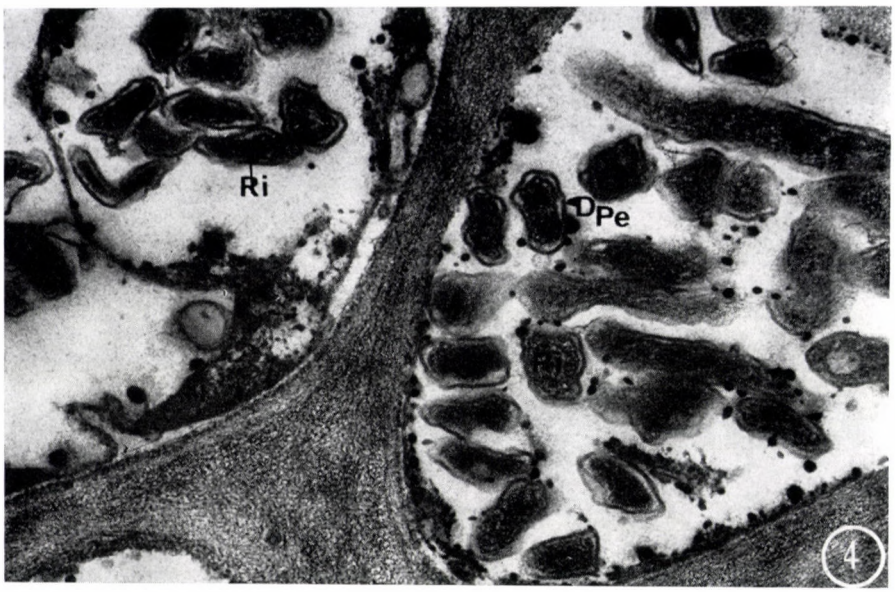

Fig. 4. Aspect des germes rickettsoïdes dans des cellules criblées de *T. repens* anciennement parasitées. ×27600. Dpe: Décollement de la paroi externe, Ri: Rickettsoïde

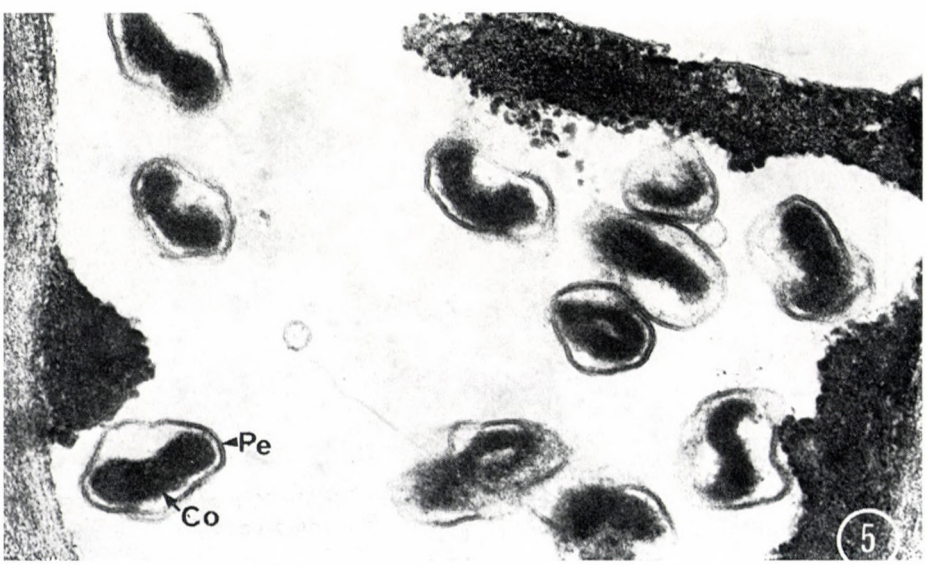

Fig. 5. Stade ultime de la dégénérescence des germes rickettsoïdes. ×55.000. Co: Coagulum retracté entraînant la membrane cytoplasmique, Pe: Paroi externe des germes

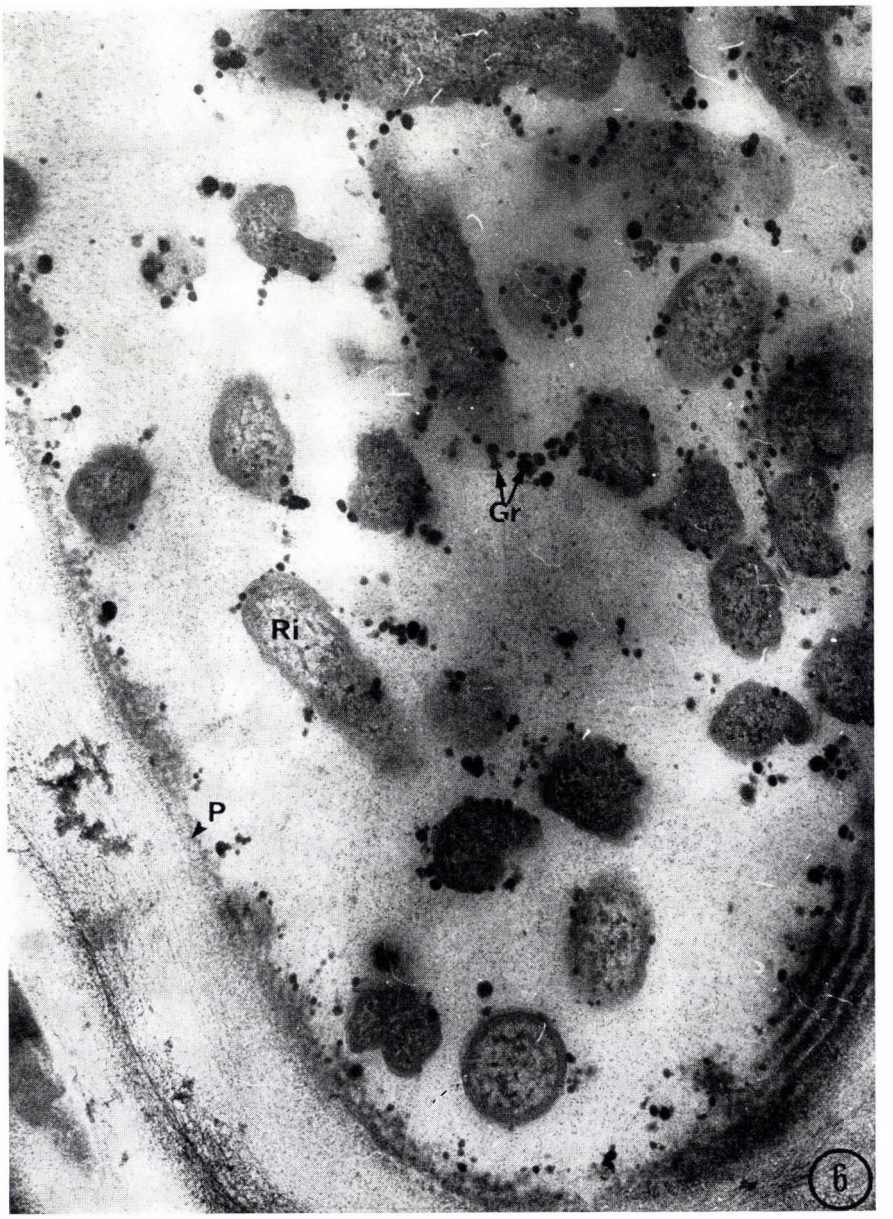

Fig. 6. Germes rickettsoïdes parasitant une cellule criblée de *V. rosea* après transmission expérimentale par *C. subinclusa*. × 45.000. Gr: Granulations denses aux électrons, Ri: Rickettsoïde, P: Paroi végétale

mûres où le parasitisme est sans doute plus ancien, on note un décollement de la membrane externe qui finit par ne plus adhérer à la membrane interne (Fig. 4). La membrane externe devenant de plus en plus festonnée, peut parfois se dilater alors que le corps central des germes a tendance à se rétracter, en entraînant la membrane interne (Fig. 5). Dans les stades ultimes de cette dégénérescence, seule la membrane externe reste nette et elle renferme un coagulat rétracté, dense aux électrons, et de forme peu définie. Les germes que nous venons de décrire chez *C. subinclusa* présentent les mêmes caractères morphologiques, ultrastructuraux et évolutifs que ceux que l'on rencontre chez *T. repens* et *T. incarnatum* malades, plantes qui sont à l'origine de l'infection des cuscutes, ainsi que chez les plantes hôtes soumises aux essais de transmission.

2 – *Évolution des structures cytologiques des cellules criblées infectées*

Nous avons analysé l'impact de ces rickettsoïdes sur les cellules libériennes criblées de *C. subinclusa*, en suivant l'évolution de l'infection à partir des stades précoces. De tels stades se reconnaissent à la présence simultanée dans certains tubes criblés d'un nombre réduit de germes co-existant avec des organites cellulaires tel que: noyau, mitochondries ou plastes (Fig. 3).

Dans les cellules libériennes saines, la dégradation cytoplasmique normale se manifeste progressivement avec apparition de fibrilles de protéine P. Par contre, dans les cellules criblées parasitées, le cytoplasme se scinde en plages qui hébergent encore quelques ribosomes (Fig. 3). Les plages se disloquent en îlots de plus ou moins grande taille à la périphérie desquels se forment des granulations inégales, opaques aux électrons, pouvant croître par agrégation.

Les ribosomes disparaissent progressivement de ces plages cytoplasmiques, tandis que le processus de formation de ces granulations denses se généralise à toute la masse cytoplasmique et semble précéder sa dislocation complète. Ce sont les reliquats de cytoplasme pariétal qui participent les derniers à cette évolution. La cellule criblée renferme alors des germes de plus en plus nombreux, une multitude de granulations éparses, le plasmalemne et quelques mitochondries résiduelles (Fig. 1). Puis les particules granulaires se dissolvent progressivement alors que les premiers signes de senescence des rickettsoïdes se manifestent (Fig. 4). Dans quelques cas, on observe des germes dégénerés emprisonnés dans un réseau de matériel fibrillaire. Malgré cette évolution des tubes criblés, et le degré souvent prononcé de la dégénerescence des procaryotes, les cellules libériennes conservent leur turgescence et on ne note aucun phénomène de nécrose par laminement des cellules infectées.

C) *Répartition des germes selon la plante hôte*

La répartition des germes dans les cellules a été appréciée à partir des examens effectués sur huit pieds de *T. repens*, deux pieds de *T. incarnatum*, de nombreuses tiges et crampons de *C. subinclusa*, ainsi que sur deux pieds de *V. rosea*.

L'abondance des germes varie considérablement d'une espèce à l'autre. C'est au niveau de la cuscute qu'ils sont incontestablement les plus nombreux. Leur densité est très importante dans 80% des cellules criblées, quel que soit le lieu du prélèvement. C'est ainsi, qu'ils sont présents en aussi grande quantité dans l'haustorium qu'à l'extrémité des tiges et même dans les pédoncules et les réceptacles floraux.

Par contre, chez les autres plantes hôtes, le niveau d'infection est plus faible. On note que:

- chez *T. incarnatum*, chaque coupe transversale de pétiole ou de nervure comporte en général 25% de cellules parasitées par un grand nombre de germes dans chaque faisceau libérien.
- *chez V. rosea*, les coupes transversales faites au niveau des pétioles, des nervures, ou de la partie apicale, ne révèlent que 10% de cellules criblées infectées par de nombreux microorganismes dans chacun des faisceaux libériens.
- enfin, chez *T. repens*, le degré de parasitisme est encore plus faible et surtout les cellules parasitées ne recèlent qu'un nombre réduit de germes.

Discussion et Conclusion

Les résultats et les observations que nous venons d'exposer peuvent être évalués à différents points de vue.

Le premier, concerne la transmission de germes de type rickettsoïde par le végétal parasite *C. subinclusa* infecté primitivement à partir de ces mêmes procaryotes issus des trèfles malades récoltés dans la nature.

A l'issue de la transmission, les symptômes apparaissant chez les trèfles soumis aux expérimentations sont identiques à ceux primitivement notés chez les plantes malades naturellement. D'autre part, les études cytologiques réalisées montrent les mêmes effets cytopathogènes des germes. Cel permet d'établir que les rickettsoïdes sont vraisemblablement les agents de la maladie.

Il se dégage ainsi, que le spectre des potentialités vectrices de la Cuscute, déjà fortement ouvert pour les virus et les mycoplasmes, s'élargit à travers ces résultats aux germes de type rickettsoïde qui, jusqu'à présent, n'ont semble-t-il pu être transmis par cette méthode.

Ce premier exemple de transmission pourrait ouvrir la voie à des essais semblables avec d'autres affections où sont impliqués des germes rappelant les rickettsies, affections dont certaines ont de sérieuses incidences agronomiques: Greening des agrumes (Lafleche et Bove, 1970; Saglio et al. 1971; Moll et Martin, 1974); Prolifération de la carotte (Giannotti et al. 1974), Maladie «Phony» du pêcher (French, 1974).

Le second a trait au passage des rickettsoïdes à la plante test *V. rosea*, couramment utilisée dans l'étude des mycoplasmoses végétales. Il se traduit par des manifestations pathologiques nettement caractérisées. Cette sensibilité aux

rickettsoïdes est à rapprocher de celle déjà rapportée pour cette même plante par WINDSOR et BLACK (1973) pour le «clover club leaf» transmis dans ce cas par la cicadelle vectrice: *Agalliopsis novella* Say.

Ces deux exemples nous permettent de dire que des études comparées de symptomatologie, d'ultrastructure et du mode d'action dans une même plante hôte sont désormais possibles pour les rickettsoïdes phytopathogènes biologiquement transmis.

Le troisième concerne l'action pathogène de ces rickettsoïdes qui s'exerce non seulement sur les plantes hôtes mais aussi sur la plante parasite *C. subinclusa*. Malgré le nombre considérable de transmissions de maladies de plantes effectuées jusqu'à présent dans le monde à l'aide des cuscutes, les observations rapportant l'action pathogène d'agents transmis sur le vecteur végétal lui-même se ramènent aux quelques cas signalés par VALENTA (1958), CAUDWELL (1965); GIANNOTTI (1974). Les caractéristiques propres des cuscutes (dépigmentation; absence de feuilles), ainsi que leur capacité à tolérer l'infection des germes transmis expliquent certainement les difficultés de mise en évidence de symptômes nets. Avec les rickettsoïdes du trèfle, la mise en défaut de cette tolérance chez *C. subinclusa* se manifeste, non seulement au niveau de son potentiel de développement mais aussi au niveau cellulaire avec la formation de matériel granulaire libéré par le cytoplasme dégénérescent. Une telle évolution n'a été signalée jusqu'ici ni avec les procaryotes mycoplasmiques ni avec les procaryotes de type rickettsoïde décrits, exception faite des cas signalés par BEHNCKEN (1974) en Australie et MARKHAM (1975) en Grande-Bretagne. Elle semble donc représenter un processus original, commun seulement à quelques germes.

Enfin, nous pouvons constater que la cuscute favorise remarquablement la multiplication des rickettsoïdes. Cette propriété offre un double intérêt: elle permet tout d'abord de mieux révéler des infections de faible niveau chez les plantes hôtes primaires comme ici chez *T. repens*. Cette aptitude à multiplier les germes est également utilisée dans le cas du greening des agrumes (GHOSH *et al.* sous presse). En second lieu elle pourrait ouvrir la voie à l'étude de certaines propriétés des germes extraits des tissus.

Summary

Electron microscopy studies revealed the presence of Rickettsia-like organisms associated with a degrading disease in white (*Trifolium repens* L.) and Crimson (*T. incarnatum* L.) clover plants in the south of France.

For the first time, the disease was transmitted to healthy clovers as well as to healthy *Vinca rosea* L. plants through the parasitic plant *Cuscuta subinclusa* DURR. et HILG. Symptoms on the experimentally transmitted and naturally infected clovers are exactly the same. In *T. repens*, symptoms are characterized by a general stunting of the plant, and a reduction of the leaflets. Young emerging leaves are much reduced in size, reddish in colour then club shaped and curled.

In *T. incarnatum*, symptoms are the same, but yet more pronounced and severe, with also production of several thin bunches of leaves at the foot of the plants giving a proliferation appearance.

Yellowing, falling of lower leaves, reduction in the size of the upper leaves and curling are the typical symptoms on *V. rosea* infected after transmission.

Comparative studies were made on the multiplication and cytopathogenic effects of the germ in cellular level in the dodder and in the other host plants.

In dodder haustoria and stems, more than 80% of the phloem cells are infected by a greater number of double membraned Rickettsia-like organisms measuring 1 to 2 μm in length and 0.4 to 0.5 μm in diameter. These germs are responsible for the progressive cytoplasmic degradation with release of dense granular particles in the phloem cells.

The ultrastructural morphology of the germs and their cytopathogenic effects described in the dodder are the same in all the other host plants.

However, the dodder is remarkably favourable for multiplication of Rickettsia-like organisms. This property would permit first to reveal low infections in primary host plants as we noticed in *T. repens*, secondly to study some characteristics of the germs extracted from the tissues.

This first successful transmission of Rickettsia-like organism by the plant vector *C. subinclusa* could permit the comparative study of the structure and cytopathogenic effects of Rickettsia of clovers with other biologically transmitted phytopathogenic procaryots, in a same host plant.

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The Effect of Bacterial Wilt on the Uptake of Manganese and Zinc in Alfalfa

By

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Alfalfa plants (*Medicago sativa* L.) of the relatively resistant (R) variety Hodonínka and very susceptible (S) variety Přerovská were, in the stage of cotyledons, infected with *Corynebacterium insidiosum* (McCulloch) Jensen. After 30 days from inoculation, labelled compounds of $^{54}MnCl_2$ and $^{65}ZnSO_4$ were added to the nutrient medium. Radioactivity per plant and specific activity (concentration of the labelled element) in the roots and above-ground parts were determined after 25 days from the application of the labelled compounds.

The uptake of ^{54}Mn by the infected plants, compared with the control, was lower by almost 93% in the S-variety and insignificantly lower, by only 11%, in the R-variety. The transport of ^{54}Mn from roots to the above-ground parts of the plants was not reduced in the infected plants of the R-variety since the concentration of ^{54}Mn in the above-ground parts was even higher than in the controls. The above-ground parts of the infected plants of the R-variety had a ^{54}Mn concentration almost eight times as high as those of the S-variety.

The uptake of ^{65}Zn by the infected plants of the S-variety was statistically insignificantly higher (by 17%); in the R-variety it was significantly lower (by 53%).

The increased amount of ^{65}Zn, taken up by the infected plants, accumulated mainly in the roots. The concentration of ^{65}Zn in the roots of infected plants of the S-variety was about three times higher than in the control and four times than in the R-variety.

The metabolism of minerals is a field of phytopathological physiology which has been, so far, on the margin of the interest of researchers (SADASIVAN, 1965). In a previous study the authors of this paper examined the uptake and distribution of phosphorus, sulphur, and, just for brief information, manganese in alfalfa plants after infection with bacterial wilt (TAIMR *et al.*, 1975).

The present study deals with the effect of bacterial wilt on the uptake and distribution of zinc and manganese in lucerne plants possessing different levels of resistance.

Materials and Methods

Plant material

The plants of alfalfa (*Medicago sativa* L.), used in the experiment, belonged to two varieties: 'Přerovská', which is very susceptible to bacterial wilt, and 'Hodonínka', characterized by a certain degree of resistance (KŮDELA, 1971;

KŮDELA and REZÁČ, 1972). In further text the 'Přerovská' variety will be referred to as a susceptible variety (S) and the 'Hodonínka' as a resistant variety (R).

The plants were grown in pots filled with silicon sand to which HELLRIEGEL's nutrient solution had been added. The pots with the plants were placed in a glasshouse where the daily temperatures ranged between 22 and 25 °C and nightly temperatures between 15 and 19 °C.

Artificial infection of the plants with bacterial wilt

At the age of 8 days the plants were subjected to inoculation on the cotyledons (KREITLOW, 1963). Three isolates of *Corynebacterium insidiosum* (McCulloch) Jensen were used for the inoculation. The density of the inoculum corresponded to the concentration of about 3.7×10^6 bacterial cells per 1 ml sterile distilled water. Twice 25 plants of each variety were inoculated for the study of the uptake and distribution of one labelled element. The same number of plants was in the control.

The application of labelled compounds

Aqueous solution of the labelled compounds $^{65}ZnSO_4$ and $^{54}MnCl_2$ were applied to the infected and control plants after 30 days from inoculation. The $^{65}ZnSO_4$ solution having the activity of 40.095 μC ml^{-1} (1483.52 kBq) and $^{54}MnCl_2$ solution with the activity 8496 μC ml^{-1} (314.35 kBq) was pipetted to each plant in the proximity of the root neck, the application rate being 0.5 ml, i.e. 20.048 μC (741.76 kBq) in $^{65}ZnSO_4$ and 4.248 μC (157.18 kBq) in $^{54}MnCl_2$. Hence the activity supplied to one pot with 50 plants was 212.400 μC (7859 kBq) from the application of $^{54}MnCl_2$ and 1002.375 μC (37.088 kBq) from the application of $^{65}ZnSO_4$.

Determination of $^{65}ZnSO_4$ and $^{54}MnCl_2$ in the plants

The lucerne plants were harvested and processed 25 days after the application of labelled compounds. The plants were carefully drawn from sand and their roots were thoroughly washed in water stream. The roots were separated from the above-ground parts and the plants were dried at 60–70°C for 48 hours. Then the material was weighed.

After desiccation the above-ground parts and roots were mineralized on sand bath in an adequate volume of HNO_3, corresponding to the weight of the plants, and in half the volume of $HClO_4$. After cooling the very acid mineralization product was neutralized with concentrated NaOH to the level of pH 6. The translucent liquid was poured into measuring cells and distilled water was added to complement the volume to 25 ml. The samples of 0.1 ml each were measured by the scintillation method in SLE 31 (Spolana Neratovice) on the Mark I apparatus (Nuclear Chicago Liquid Scintillation Computer).

Radioactivity (imp. per sec per plant) and specific activity (concentration) in the above-ground parts and roots (imp. per sec/1 g dry weight) were calculated from the measured values.

At the same time, along with the analytic processing of the plants, random-selected control and infected plants were subjected to autoradiography.

Method of the evaluation of results

The results of measurement were processed by the method of the analysis of dispersion in two-way classification (infected and control plants, Hodonínka' and 'Přerovská' varieties). The significance of the differences and interactions was evaluated by means of Student's *t*-test, as referred to in detail in our previous paper (TAIMR *et al.*, 1975).

Results

The effect of bacterial wilt on plant weight

Bacterial wilt considerably reduced the weight of the plants (Figs 1, 2). The weight of the roots of the infected plants was reduced by a half in both varieties. The weight of the above-ground parts was decreased by the infection by about a half in the S-variety, whereas no such reduction in the weight of the above-ground parts, or just a slight reduction, was observed in the R-variety.

The uptake and distribution of microelements in the infected and control plants

The parameter indicating the effect of bacterial wilt on the uptake of the studied microelements is the difference in the radioactivity of the given element in conversion to the value per 1 infected and control plant (Tables 1, 3).

The transport and distribution of the elements in the plant are estimated according to the results of the measurement of the radioactivity of the roots and above-ground organs in conversion per 1 g of dry matter weight (Tables 2, 4) and according to the autoradiograms of the infected and control plants (Figs 3–6).

Manganese

In comparison with the control, the uptake of ^{54}Mn by the infected plants was decreased in the S-variety by almost 93% (significant at $\alpha = 0.005$), and in the R-variety by only 11% (statistically insignificant).

The transport of ^{54}Mn from the roots to the above-ground parts was not reduced at all in the infected plants, since in conversion per 1 plant the above-ground organs showed even an increased uptake of this element (significant

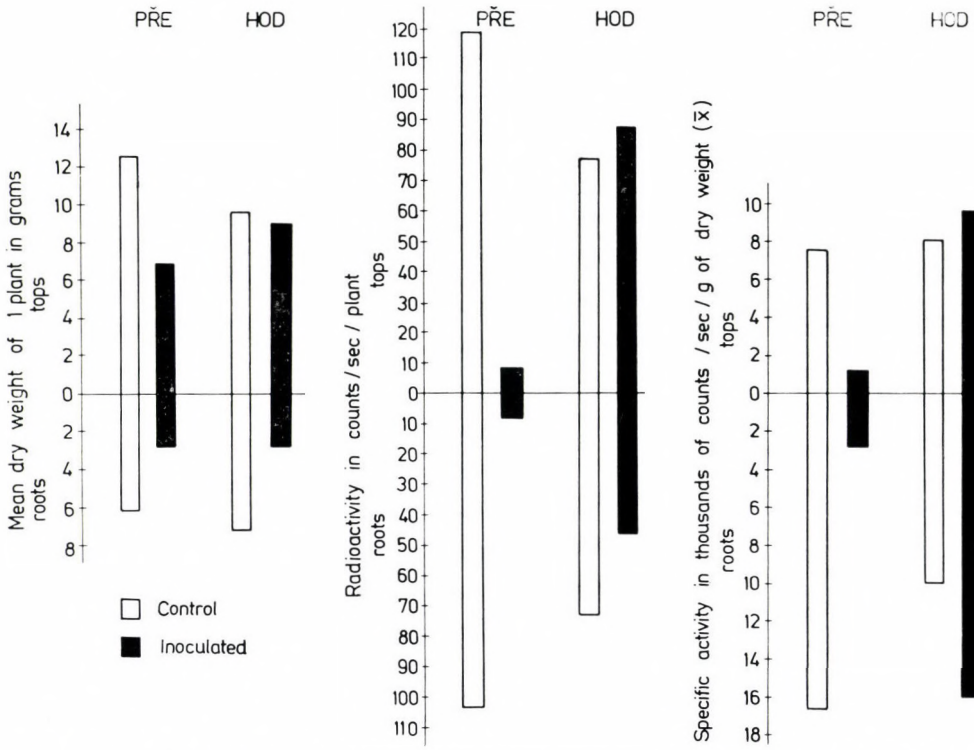

Fig. 1. The effect of bacterial wilt on plant weight and uptake of manganese in alfalfa. (PŘE = variety Přerovská; HOD = variety Hodonínka)

at $\alpha = 0.05$) in the infected plants, unlike in the control. The concentration of ^{54}Mn in the above-ground parts was higher by almost 20% than in the control plants (significant at $\alpha = 0.025$).

The reduction of the transport of ^{54}Mn to the above-ground parts of the infected plants of the S-variety was proportionate to the abrupt decrease of the uptake of this element owing to infection. The concentration of ^{54}Mn in the above-ground parts of the infected plants was significantly lower (at $\alpha = 0.005$) than in the control plants.

Zinc

The uptake of ^{65}Zn by the infected plants of the S-variety, compared with that in the controls, was statistically insignificantly higher (by 17%), whereas in the R-variety it was lower by 47% (significant at $\alpha = 0.025$).

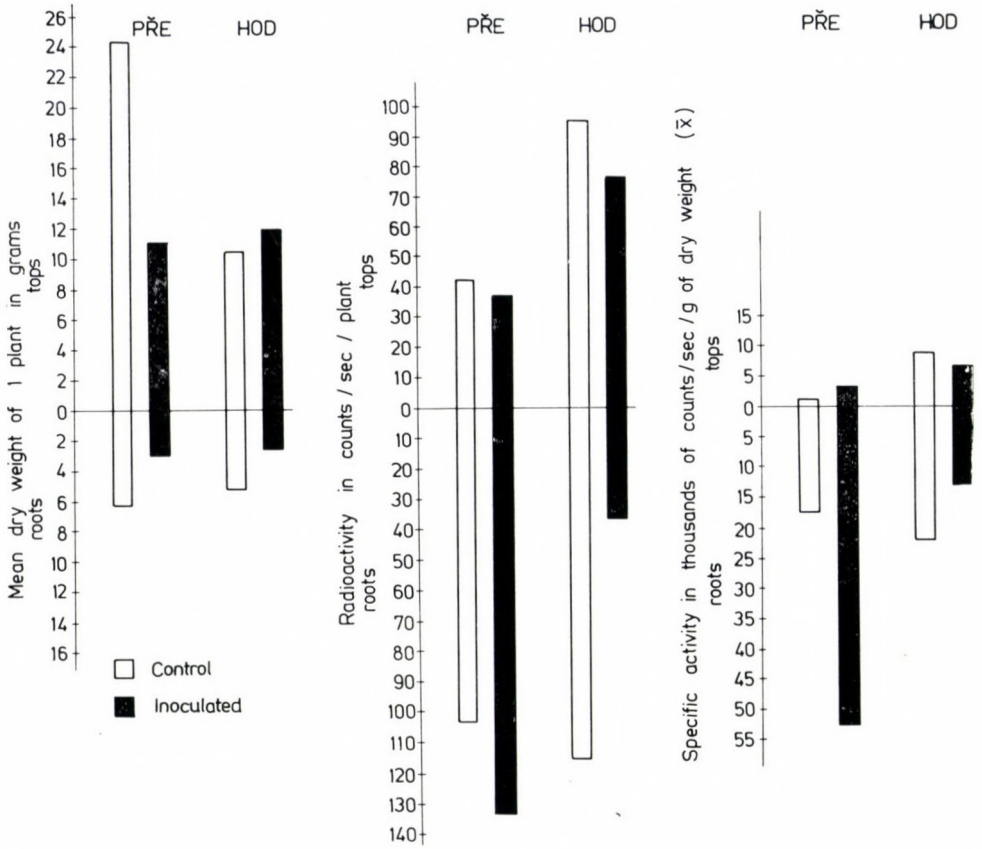

Fig. 2. The effect of bacterial wilt on plant weight and uptake of zinc in alfalfa. (PŘE = variety Přerovská; HOD = variety Hodonínka)

^{65}Zn, taken up by the infected plants of the S-variety, accumulated mainly in the roots where the concentration of this element was approximately three times higher than in the roots of the control plants (significant at $\alpha = 0.005$).

The concentration of ^{65}Zn in the infected plants of the R-variety was lower by about a third than in the control plants, this drop being greater in the above-ground parts (significant at $\alpha = 0.025$) than in the roots (significant at $\alpha = 0.25$).

Varietal differences in the uptake of ^{54}Mn and ^{65}Zn

The healthy plants of the S-variety took up more ^{54}Mn (by about a third, on an average per plant) than the plants of the R-variety (significant at $\alpha = 0.01$); this corresponds with the fact that the weight of the control plants of the S-variety was higher than that of the R-variety.

Fig. 3. Alfalfa plant of variety Hodonínka (at left) and its autoradiogram (at right) after inoculation with *Corynebacterium insidiosum* and application of ^{54}Mn to the nutrient medium

Fig. 4. Alfalfa plant of variety Přerovská (at left) and its autoradiogram (at right) after inoculation with *Corynebacterium insidiosum* and application of ^{54}Mn to the nutrient medium

Fig. 5. Alfalfa plant of variety Hodonínka (at left) and its autoradiogram (at right) after inoculation with *Corynebacterium insidiosum* and application of ^{65}Zn to the nutrient medium

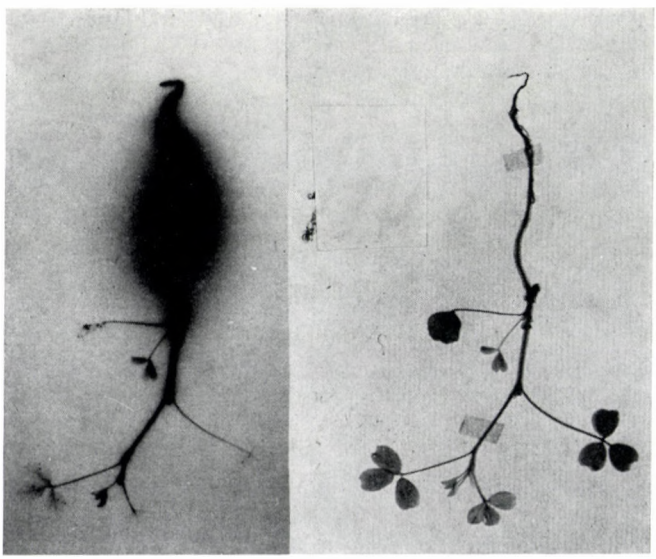

Fig. 6. Alfalfa plant of variety Přerovská (at left) and its autoradiogram (at right) after inoculation with *Corynebacterium insidiosum* and application of ^{65}Zn to the nutrient medium

Table 1

Radioactivity of infected and control plants of alfalfa in counts/sec/plant. Differences expressed as per cents of infected plants as compared to controls

Labelled compound	Cultivar	Part of the plant	Control plants $\bar{x}$ (=100)	Infected plants		
				$\bar{x}$	%	% ± of control
$^{54}MnCl_2$	Přerovská	Roots	104	8	7.60	−92.31******
		Overground part	119	8	6.72	−93.28******
		Whole plant	223	16	7.17	−92.83******
	Hodonínka	Roots	73	46	63.01	−36.99*
		Overground part	77	87	112.99	+12.99***
		Whole plant	150	133	88.66	−11.34
	Both cultivars ($\bar{x}$)	Roots	89	27	30.34	−69.66******
		Overground part	98	48	48.98	−51.02******
		Whole plant	187	75	40.11	−59.89******
$^{65}ZnSo_4$	Přerovská	Roots	104	136	130.77	+30.77*
		Overground part	43	36	83.72	−16.28
		Whole plant	147	172	117.01	+17.01
	Hodonínka	Roots	116	36	31.03	−68.97****
		Overground part	95	76	80.00	−20.00*
		Whole plant	211	111	52.61	−47.39****
	Both cultivars ($\bar{x}$)	Roots	110	86	78.18	−21.82*
		Overground part	69	56	81.16	−18.84*
		Whole plant	179	142	79.33	−20.67*

* significance at α = 0.25
** 0.10
*** 0.05
**** 0.025
***** 0.01
****** 0.005

Owing to infection, the uptake of ^{54}Mn in the S-variety was reduced to such an extent that the concentration of this element in the roots was 5.7 times lower (significant at $\alpha = 0.005$), and in the above-ground parts even 7.8 times lower (significant at $\alpha = 0.005$), than in the R-variety.

On an average per one plant, the healthy plants of the R-variety showed an uptake of ^{65}Zn higher by about 44% than the plants of the S-variety (significant at $\alpha = 0.10$), although the weight of the plants of the R-variety was smaller than in the S-variety.

Even despite the considerable decrease of weight, infection increased the uptake of ^{65}Zn by the plants of the S-variety, whereas the plants of the R-variety

Table 2

Specific activity of roots and overground parts of infected and control plants of alfalfa in counts/sec/g of dry weight ($\bar{x}$). Differences expressed as per cents of infected plants as compared to controls

Labelled compound	Cultivar	Part of the plant	Control plants $\bar{x}$ (=100)	Infected plants		
				$\bar{x}$	%	% ± of control
$^{54}MnCl_2$	Přerovská	Roots	16.759	2.824	16.85	−83.15******
		Overground part	9.466	1.224	12.93	−87.07******
	Hodonínka	Roots	10.009	16.103	159.45	+59.45****
		Overground part	8.062	9.643	119.61	+19.61****
	Both cultivars	Roots	13.429	9.463	70.47	−29.53***
		Overground part	8.764	5.434	62.00	−38.00******
$^{65}ZnSO_4$	Přerovská	Roots	16.629	52.433	315.31	+215.31******
		Overground part	1.747	3.291	188.38	+88.38**
	Hodonínka	Roots	22.096	13.343	60.39	−40.28*
		Overground part	9.052	6.439	71.13	−28.87****
	Both cultivars	Roots	19.363	32.888	169.85	+69.85****
		Overground part	5.399	4.864	90.09	−9.91

* significance at α = 0.25
** 0.10
*** 0.05
**** 0.025
***** 0.01
****** 0.005

reduced their ^{65}Zn uptake. Nevertheless, the concentration of ^{65}Zn in the above-ground parts of the infected plants of the R-variety was higher than in the same parts of the plants of the S-variety (significant at $\alpha = 0.01$). On the other hand, the roots of the infected plants of the S-variety had about four times higher concentration of ^{65}Zn than the roots of the infected plants of the R-variety (significant at $\alpha = 0.005$).

Discussion

The changes in the metabolism of manganese and zinc, induced by infection, were much greater in the susceptible plants than in those which were resistant. This can be inferred from the great reduction of the uptake of manganese and from the excessive accumulation of zinc in the roots of the infected plants of the susceptible variety.

Table 3

Radioactivity of control and infected plants of alfalfa in counts/sec/plant ($\bar{x}$). Differences expressed as per cents of the cultivar Hodonínka as compared to the cultivar Přerovská

Labelled compound	Part of the plant	State of plants (C = control plants, I = infected plants)	Cultivar Přerovská $\bar{x}$ (=100)	Cultivar Hodonínka		
				$\bar{x}$	%	% ± of Přerovská
$^{54}MnCl_2$	Whole plant	C	223	150	66.96	−33.04*****
		I	16	153	831.25	+831.25******
	Roots	C	104	73	70.19	−29.81**
		I	8	46	575.00	+475.00**
	Overground part	C	119	77	64.70	−35.30******
		I	8	87	1087.50	+987.50******
$^{65}ZnSO_4$	Whole plant	C	147	211	143.54	+43.54**
		I	172	111	64.53	−35.47**
	Roots	C	104	116	111.54	+11.54
		I	136	36	26.47	−73.53*****
	Overground part	C	43	95	220.93	+120.93*****
		I	36	76	211.11	+111.11******

* significance at α = 0.25
** 0.10
*** 0.05
**** 0.025
***** 0.01
****** 0.005

The metabolism of manganese seems to be connected with the defence mechanisms of the host and that this bond is much closer than in the metabolism of zinc. It can be believed that resistant varieties are characterized by the ability of taking up and distributing manganese from the roots to the above-ground organs in an unchanged or even increased amount even after infection. The susceptible plants, once infected, take up manganese just in a reduced extent. On the other hand, the excessive concentration of zinc in the root tissues is, apparently, an accompanying, subsequent, or conditioning phenomenon in the increased susceptibility of lucerne to bacterial wilt. The conspicuously high accumulation of zinc in the roots of the infected plants suggests that the toxic action of zinc on the host plants may have played its role in this case.

A similar behaviour of manganese, like in our experiments, i.e. a decrease of the concentration of the element owing to infection in the tissues of susceptible plants and a smaller or only slight decrease in the resistant plants, was observed in *Cajanus cajan* after infection with *Fusarium udum* (SUBRAMANIAN, 1963a).

Table 4

Specific activity of roots and overground parts of control and infected plants of alfalfa in counts/sec/g of dry weight ($\bar{x}$). Differences expressed as per cents of the cultivar Hodonínka as compared to the cultivar Přerovská

Labelled compound	Part of the plant	State of plants (C = control plants, I = infected plants)	Cultivar Přerovská $\bar{x}$ (= 100)	Cultivar Hodonínka		
				$\bar{x}$	%	% ± of Přerovská
$^{54}MnCl_2$	Roots	C	16.759	10.009	60.26	−39.74****
		I	2.824	16.103	570.22	+470.22******
	Overground part	C	9.466	8.062	85.16	−14.84****
		I	1.224	9.643	787.83	+687.83******
$^{65}ZnSO_4$	Roots	C	16.629	22.096	132.88	+32.88
		I	52.433	13.343	25.45	−74.55******
	Overground part	C	1.747	9.052	518.15	+418.20******
		I	3.293	6.437	195.48	+95.48*****

* significance at α = 0.25
** 0.10
*** 0.05
**** 0.025
***** 0.01
****** 0.005

In other tests (SUBRAMANIAN, 1963b), manganese was added to the soil, or sprayed as top-dresser, or the seeds were soaked with solutions of manganese sulphate, and these treatments reduced, or entirely stopped, the manifestations of the disease in artificially infected plants. The addition of manganese adjusted the physiological processes of the infected susceptible plants to the level of the control healthy plants, or resistant plants after artificial infection. The surprisingly effective therapeutic action of manganese is ascribed to the inactivation of the toxins or enzymes produced by the pathogen.

The results obtained by FORSTER and ECHANDI (1975) can be mentioned here to support the hypothesis that the susceptibility of the plants is accompanied by an increased uptake of zinc by the infected individuals. The tomato plants sufficiently supplied with the calcium showed the highest degree of attack by *Corynebacterium michiganense* and, among other things, their zinc content was almost twice as high as in the plants having enough calcium and attacked by the disease to a lesser degree.

JONES and WOLZ (1967) studied the effect of liming and of the application of manganese and zinc on *Fusarium* wilt in tomatoes. The addition of manganese and zinc in one experiment increased the occurrence rate of the disease (having eliminated the favourable effect of liming), but in another experiment the course of the disease remained unchanged.

It is a well-established fact that zinc and manganese either activate or inhibit a number of enzymes, thus interfering with the processes of photosynthesis, formation of growth substances, sugars, amino acids, as well as secondary products (alkaloids, terpenes, etc.). The knowledge of the metabolism of microelements is important for understanding the principles of the resistance of plants to vascular diseases, since a blockade of the conductive tissues, resulting in a limited uptake of nutrients, may be responsible for the initiation of complex enzymic changes, characteristic of the physiology of a diseased plant. The successes that have been achieved, though scarcely for the time being, in the improvement of the resistance of plants to attack by diseases through the application of manganese (SUBRAMANIAN, 1963b) should encourage efforts in this field of pathological physiology, thus contributing to the enrichment of the knowledge of the possibilities of the use of chemical immunization in the protection of plants against pathogens affecting the conductive tissues.

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Histopathology of Vascular Tissue System in Grapevine Affected by Legno Riccio (Rugose Wood, Stem Pitting)

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The legno riccio (rugose wood, stem pitting) symptoms in the woody cylinder of scion of the weakly growing, 6-year-old, fruiting vines cv. *Pinot gris* did not developed, but in their rootstock (*V. berlandieri* X *V. riparia* cv. *Teleki 5C*) symptoms were intensive. In the rootstock seven annual rings formed totally, the eighth was incomplete. In the second annual ring commenced formation of such xylem segment which consisted of parenchymatic cells typical for rays but did not contain xylem vessels. Formation of this kind of abnormal tissue continued also in the younger annual rings and its ratio in the sixth ring was 51.3—82.0 per cent, while in the seventh one reached already 69.6—95.0 per cent. The total area of the fourth and fifth annual rings increased strongly while the following rings decreased. Thus in the sixth one it decreased by 24.6%, in the seventh one by 49.3% as compared to the area of the fifth ring. This fact indicates the atrophy of the stock. The phloem thickened abnormally, the fibers were missing or developed only poorly herein. All these are followed by a transporting insufficiency as a consequence which may be associated with the delay of bud burst and the decay of the vine, particularly if the weather prior to burst, in winter and in spring, is permanently dry. Comparative analysis of annual rings gave some proofs to the explanation of untimely decay of the legno riccio sensitive vines from European and rootstock varieties, within 10 years after planting.

Some virus and mycoplasma diseases of grapevine were studied histopathologically, to clear up what kind of alterations are caused by the disease process in the host tissue system. The investigations showed that slight changes or severe disorders can develop in the tissues.

On the effect of *fanleaf* virus infection many intracellular cordons are formed in the vascular elements, fibers, parenchyma rays and in the secondary phloem parenchyma. They are always oriented radially and occur frequently in apparent continuity one to another contiguous adjacent cell (PETRI, 1910, cit.: GRANITI and RUSSO, 1965; GIFFORD *et al.*, 1956; GRANITI and RUSSO, 1965). The primary anatomical effect of the *leafroll* virus is phloem degeneration in vascular bundles of leaves, stems and fruit pedicles with crushing and obliteration of sieve elements, companion cells and phloem parenchyma cells, while secondary symptoms are hypertrophy and hyperplasy of phloem parenchyma, furthermore production of gum and formation of tyloses in the xylem elements (HOEFERT and GIFFORD, 1967). *Pierce's disease* induces gum development and plugging

in the xylem vessels that paralyses its function. These symptoms are usually accompanied by the excessive development of tyloses in the wood (ESAU, 1948). *Flavescence dorée* influences the normal development of tissue system and excess phloem is formed at the expense of the xylem that is associated with phloem necrosis. The phloem fiber bands are irregular, if present at all (CAUDWELL, 1964). Some time after the appearance of phloem necrosis black pustules develop in an abnormal number on the bark by cell division below the stomata (GÄRTEL, 1959; MENDGEN, 1971). The *corky bark* virus infection influences drastically the function of vascular and cork-cambium and causes more severe alterations in the secondary tissue of *LN–33*, than those mentioned above. The differentiation of cells in the vascular cambial zone is disturbed and the production of cells toward the xylem side is drastically reduced, while an abnormally large amount of secondary phloem is produced. The phloem contains sieve-tube-like cells, but these cells occur in narrow bands between the abnormally wide rays. No normal cork is formed at all, but there is a stimulation of cork-like cells in the region of the phloem where the cork-cambium usually arises (BEUKMAN and GIFFORD, 1969).

Histological changes associated with the legno riccio were investigated first by GRANITI and MARTELLI (1965). They established the following characteristics: wavy cambial zone, abnormally thick bark, phloem necrosis, reduction of the xylem vessels, hypertrophy and parenchymatosis which develops both in xylem and phloem.

The purpose of this investigation was to secure histological proofs which explain and verify the gradual decline of the legno riccio sensitive vines in a few years after planting.

Materials and Methods

For the present anatomical studies the diseased six-year-old fruiting *Pinot gris* vines grafted on "*Teleki 5C*" stocks were collected at Balatonarács (Veszprém County, at the northern shore of Lake Balaton) in a cooperative vineyard on 4th August 1975. Two vines were grubbed with total root system, from weakly growing affected ones (Fig. 1). These vines showed no symptoms in the xylem of scion part but developed characteristic symptoms on the rootstock xylem (Fig. 2), therefore merely from the upper, middle and lower part of the stock 20 mm large portions were cut out. The hard lignified material was softened by soaking it in distilled water for some days, then sectioned transversally without embedding by microtome knife in an oblique angled position. Upper parts of the rootstocks were too large to to cut, therefore we prepared no entire transverse sections of them, while sections cut from the lower part of the second sample were unfitted for further microscopic investigations, because the tissue was seriously injured mechanically. Sections 25–30 microns thick were stained by safranin, or phloroglucinol in 20 per cent HCl.

Fig. 1. Legno riccio diseased, weakly growing, 6-year-old cv. *Pinot gris* vine grafted on *Teleki 5C* rootstock (Vine of the first sample)

The sections were investigated microscopically and photomicrographs were also taken from the entire transverse sections as well from their details. For comparing the annual rings, 18.8×, 15.0× and 15.7× linearly enlarged pictures of the entire transverse section on Dokumbrom photopaper were used (Figs 3, 4 and 5). On the pictures the lines between the annual rings and within the rings between functioning and trachea-free parts of the xylem were drawn precisely with white China water-proof ink, under strict microscopic control of adequate sections (Fig. 6). The annual rings were cut out and each ring was divided by scissors along the lines on two, namely functioning and trachea-free, parts and both were weighed on analytical balance, with 0.1 mg accuracy. Sum of both gave the total weight of the annual rings and with these we calculated the extension of surface of the rings and finally the surface of the entire transverse sections on pictures. For calculation 1 g = 10472, 2510 mm^2 basic value was used, obtained from a half part lighted and developed Dokumbrom photopaper, from which 15 discs of 50 mm diameter were cut out, weighed and from their weight the above mean value of weight: surface ratio was counted. Finally,

Fig. 2. Typical symptom of the legno riccio on the woody cylinder of the rootstock

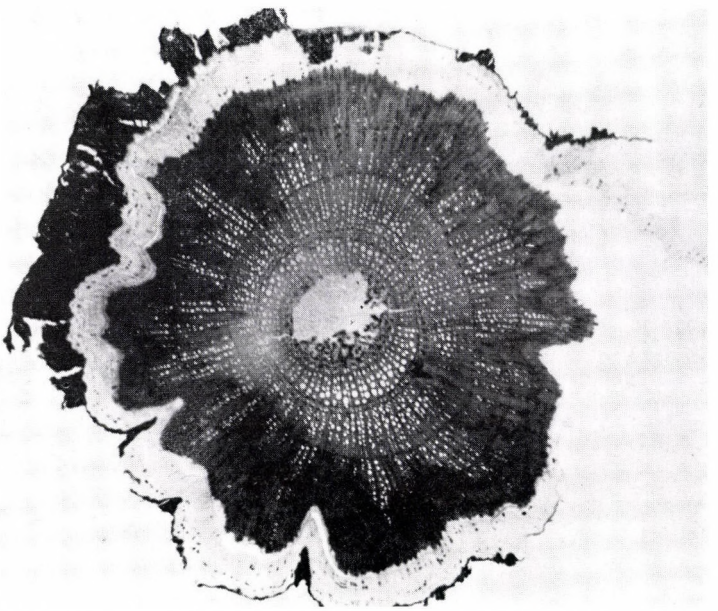

Fig. 3. Total transverse section in middle of the rootstock, 4 : 1 (First sample)

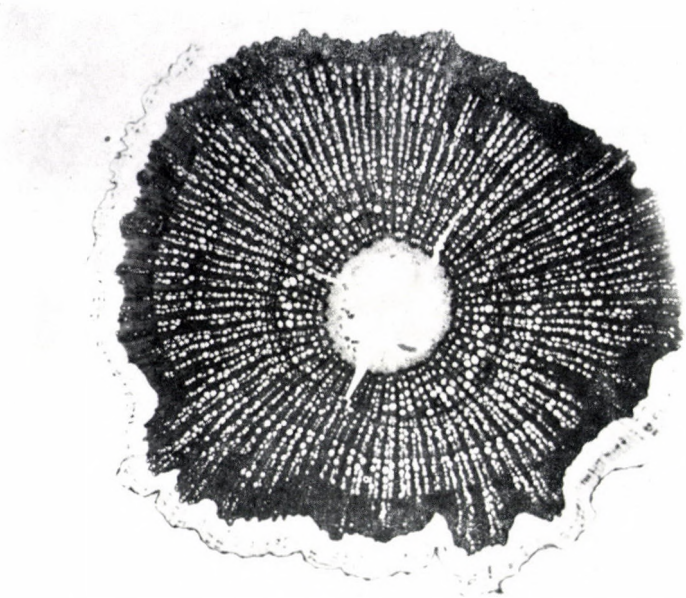

Fig. 4. Total transverse section at the bottom of the rootstock, 5 : 1 (First sample)

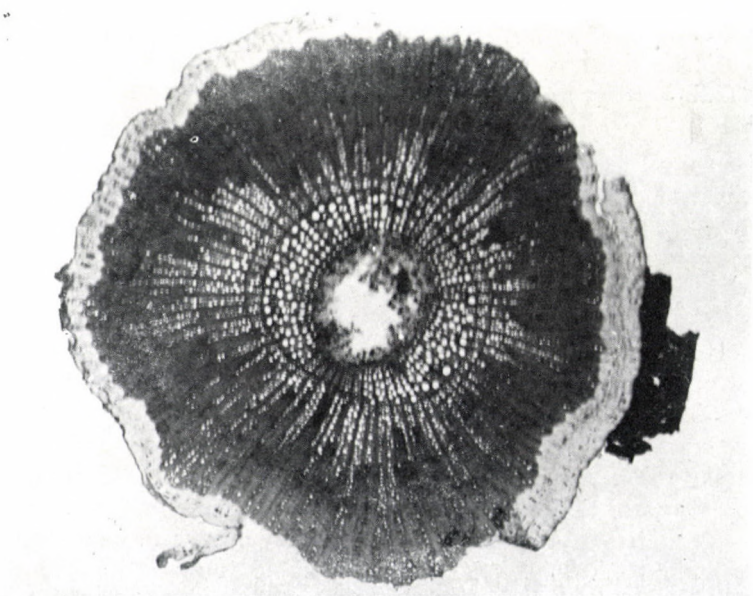

Fig. 5. Total transverse section in middle of the rootstock, 5 : 1 (Second sample)

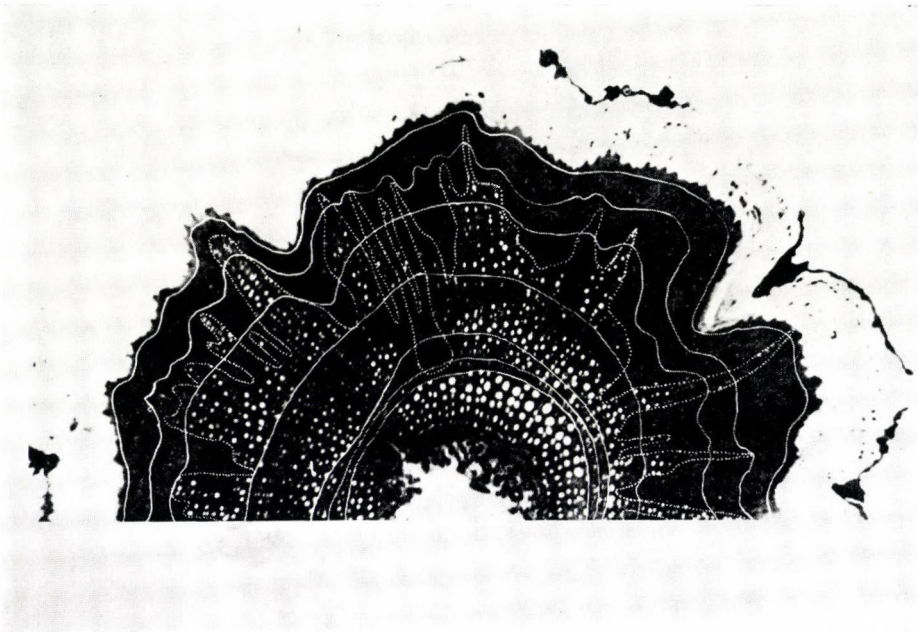

Fig. 6. Detail of prepared picture for comparative analysis with the boundary of annual ring, and with the dividing line (broken line) between functioning and trachea-free xylem parts

from data of pictures and taking linear enlargement of pictures into consideration we calculated the data of tissue structure of original transverse sections shown in Table 1.

Results

Samples of diseased vines were investigated visually and it was established that typical symptoms of legno riccio developed only on the rootstocks. Symptoms were most seriously expressed on the upper part of rootstocks and downward they became gradually milder.

In the tissue system of rootstock eight annual rings were discernible, but the eighth, the youngest, had not yet developed totally. Among these, the innermost one, namely the first, was the original ring of the rootstock cane, the second one developed in the nursery, and the third one formed after planting in the first year. Accordingly, the grubbed fruiting vines were 6-year-old in the vineyard.

Comparing the area of the annual rings shows that the tissue area of the second and the third rings decreased in proportion to the first one. The amount of the fourth and the fifth annual rings markedly increased, however, followed by the beginning of reduction in the sixth annual ring and its area decreased in average by 24.6%; the seventh ring area was, in average 49.3% smaller in compared to the fifth one (Table 1).

Table 1

Comparative analysis of rootstock tissue of the legno diseased cv. *Pinot gris* vine grafted on *Teleki 5C*[1]

Annual rings	1. Rootstock of the first sample					
	In the middle					
	Xylem area		Total area of annual ring	Ratio of the trachea-free tissue	Increase or decrease of the ratio of	
	with vascular elements	without vascular elements			total area of the annual ring	trachea-free tissue
					as compared to the previous annual ring	
	mm²	mm²	mm²	%	%	
1.	10.50	0.00	10.50	0.0	—	—
2.	2.70	0.03	2.73	1.2	−74.0	∞
3.	3.33	0.32	3.65	8.7	+33.7	+625.0
4.	18.69	1.20	19.89	6.1	+444.9	−29.9
5.	22.67	11.30	33.97	33.3	+70.8	+445.9
6.	8.45	16.56	25.01	66.2	−26.5	+98.8
7.	1.17	22.01	23.18	95.0	−7.3	+43.5
8.[3]	0.05	8.23	8.28	99.3	−64.3	+4.6

Annual rings	At the bottom					
	Xylem area		Total area of the annual ring	Ratio of the trachea-free tissue	Increase or decrease of the ratio of	
	with vascular elements	without vascular elements			total area of the annual ring	trachea-free tissue
					as compared to the previous annual ring	
	mm²	mm²	mm²	%	%	
1.	15.08	0.00	15.08	—	—	—
2.	5.24	0.00	5.24	0.0	−65.3	—
3.	5.13	0.00	5.13	0.0	−2.1	—
4.	28.60	0.07	28.67	0.2	+458.9	∞
5.	35.44	2.92	38.36	7.6	+33.8	+3700.0
6.	10.61	11.19	21.80	51.3	−43.2	+575.0
7.	4.23	9.70	13.93	69.6	−36.1	+35.7
8.[3]	2.17	3.71	5.88	63.1	−57.8	−9.9

[1] The upper part of both rootstock samples could not be cut for their thickness

[2] The lower part of the second rootstock sample could not be investigated for mechanical injury

[3] The eighth annual ring did not develop totally (time of collection: August 4), therefore its data were not taken into consideration

Table I (cnnt.)

Annual rings	2. Rootstock of the second sample[2]					
	In the middle					
	Xylem area		Total area of the annual ring	Ratio of the trachea-free tissue	Increase or decrease of the ratio of	
	with vascular elements	without vascular elements			total area of the annual ring	trachea-free tissue
					as compared to the previous annual ring	
	mm^2	mm^2	mm^2	%	%	
1.	11.75	0.00	11.75	0.0	—	—
2.	4.76	0.00	4.76	0.0	−59.5	—
3.	2.95	0.15	3.10	4.9	−34.9	∞
4.	13.03	6.34	19.37	32.7	+524.8	+567.3
5.	8.50	13.46	21.96	61.3	+13.4	+87.5
6.	3.80	17.25	21.05	82.0	−4.1	+33.8
7.	2.66	9.09	11.75	77.3	−44.2	−5.7
8.[3]	4.13	8.94	13.07	68.4	+11.2	−11.5

Comparing the total transverse sections of the middle and lower parts of the stock of the first sample shows clearly that symptoms at the middle are more serious than at the bottom (Figs. 3 and 4). The symptoms in transverse section of the middl epart of the second sample are similar to those of the first sample (Fig. 5).

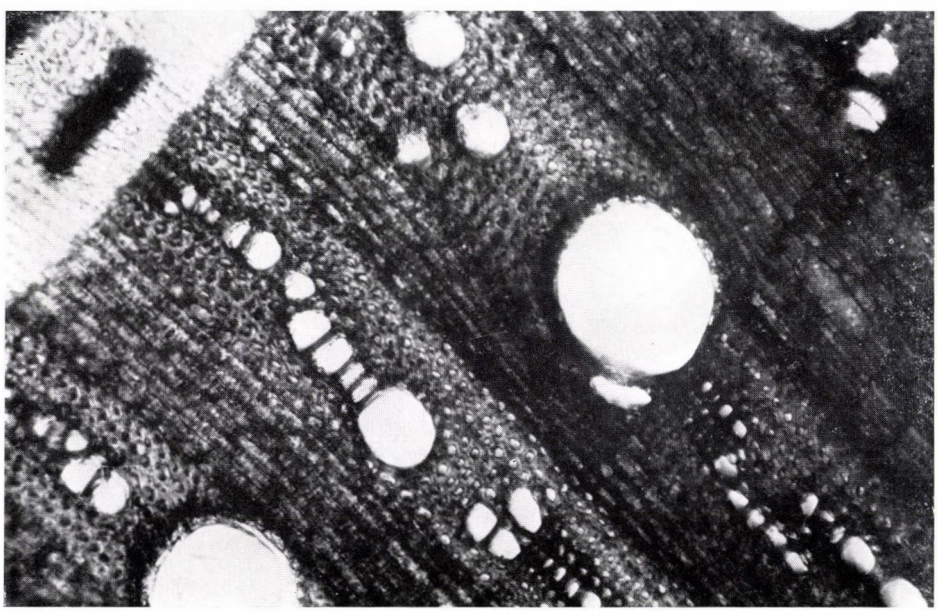

Fig. 7. Phloem and xylem part of the healthy vine, 100 : 1

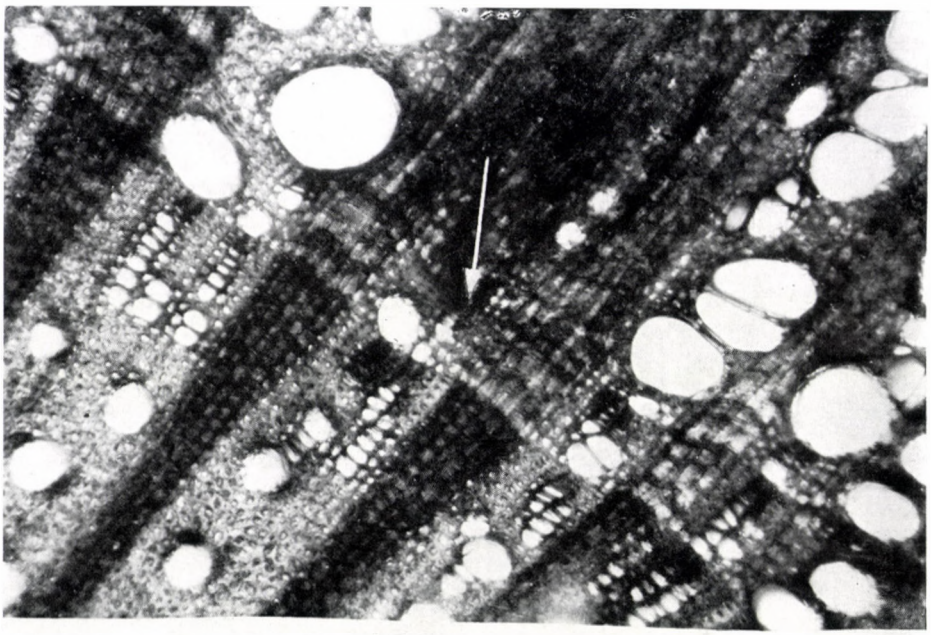

Fig. 8. Region of boundary of the first, second and third annual rings, with the appearance of trachea-free tissue in the third ring (vessels are of narrow cavity because the section was prepared from that sector of the rootstock where there was formerly the hollow side of the original cane), 100 : 1

The normally active cambial zone of a healthy vine forms inwards narrow rays and broad xylem segments which latter contain vessels in a great number (Fig. 7), whereas in the diseased one the cambial zone activity is abnormal and lays down also parenchyma in place of xylem segment; it forms a parenchymatosis without vessels, or occasionally within some radially arranged small xylem vessels. Numerical data of sections show (Table 1) that the first trachea-free segments may appear in the second annual ring (the year of nursery) in the middle part of the stock (Figs 3 and 6) but until the fourth year, though increasing the rate of the trachea-free tissue part, symptoms are not excessively serious. From the fourth and fifth years, nevertheless, 50% of xylem does not contain tracheae. In the seventh and eighth annual rings of the first sample at middle there are scarcely tracheae. Symptoms at the bottom appear merely in the fourth annual ring and become serious in the sixth one. It should be noted that in those abnormal tissue regions where xylem vessels form at all, their number is fewer and they are narrower than in the xylem of the healthy vines.

Symptoms of the trachea-free stage appear suddenly in certain segments at the boundary of the annual ring or farther in (Fig 8 and 9) but a narrow xylem fiber line with one or two tracheae may continue towards the periphery. In the sub-

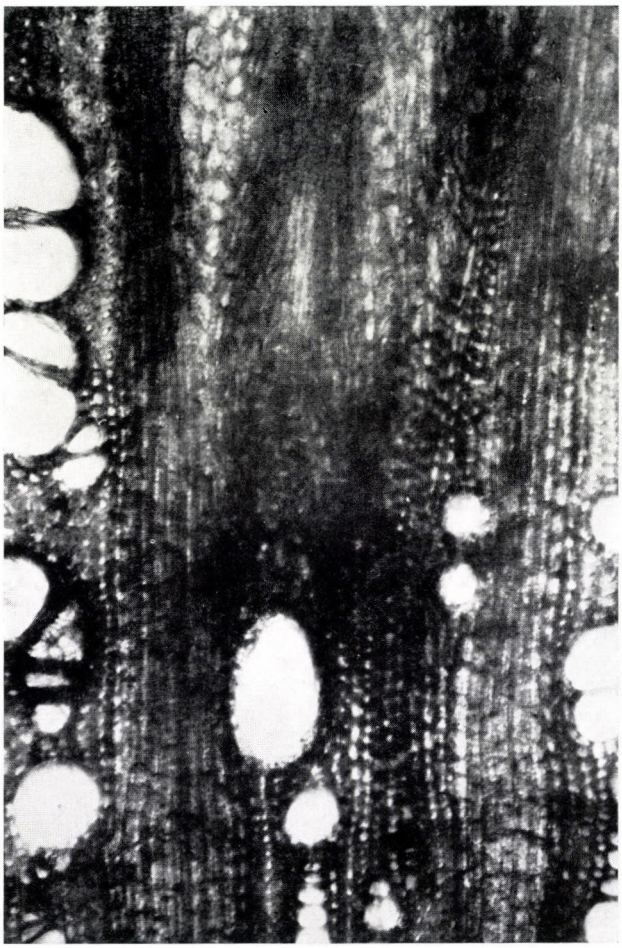

Fig. 9. Sudden disappearance of the tracheae and the xylem fibers in the fifth annual ring, 100 : 1

sequent years new segments become trachea-free and form broad strips which are broken by radially oriented narrow trachea rows (Figs 10 and 11). The ratio of the trachea-free abnormal tissue increases quickly (445.9–625.0% sometimes 3700.0%); after beginning of its formation increase becomes slower (33.8–98.8%) (Table 1). In some places where the cambial activity is still normal, the amount of the formed xylem is greater while in the sections where the cambial activity is abnormal a lesser amount of ray parenchyma is formed, hereby the cambial region and contour of the xylem become wavy. The cog-wheel-like wavy contour of the xylem is shown by Figs 12 and 13. The cork-cambium also adapts itself to this waviness. Peeling of periderm and cortex of

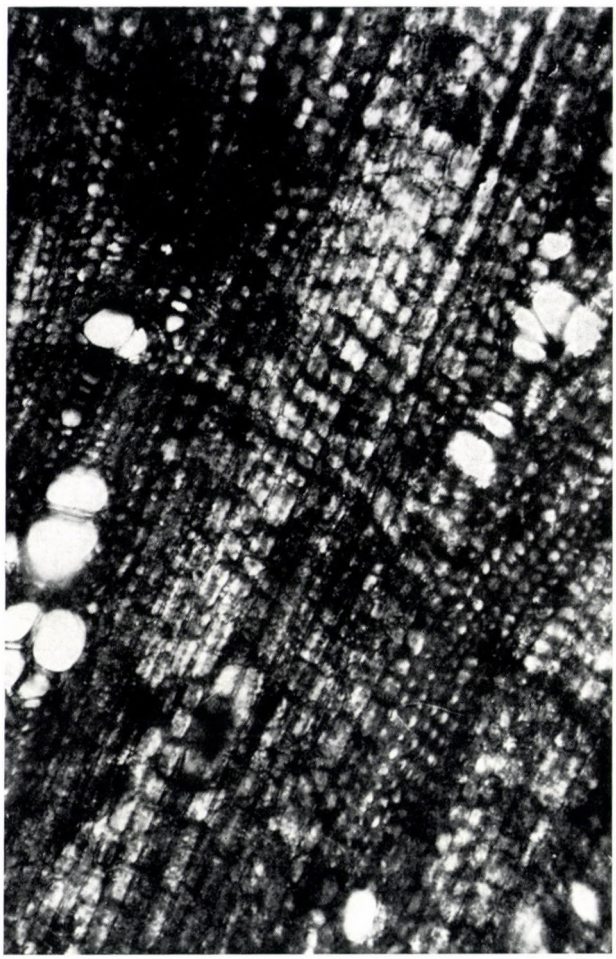

Fig. 10. Excess of parenchyma cells (parenchymatosis) in the xylem, 100 : 1

the rootstock reveals the typical symptoms of legni riccio in the xylem (Fig. 2). The proportion of the abnormal trachea-free tissue in the consecutive annual rings increases mostly constantly and in the seventh annual ring is already very high and reaches 69.6–95.0% (Table 1). It is interesting to mention that the ratio of the trachea-free tissue in the total area of all annual rings is in the middle of the first sample 43.23%, at the bottom of the first sample 18.62%, while in the middle of the second sample 49.54% (Table 1).

The phloem becomes thick and the fibers are missing or develop only weakly in it (Fig. 14). Formation of parenchymatosis can also be observed here though sligther than in the xylem. Proliferation also appears at the outer line of the phloem.

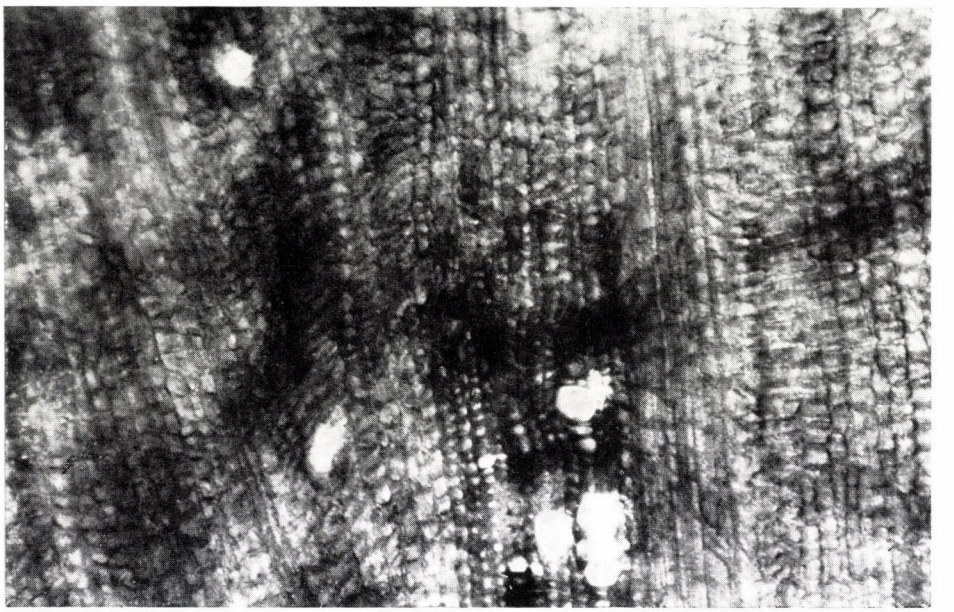

Fig. 11. There are vascular elements in a slight number in the sixth annual ring, 100 : 1

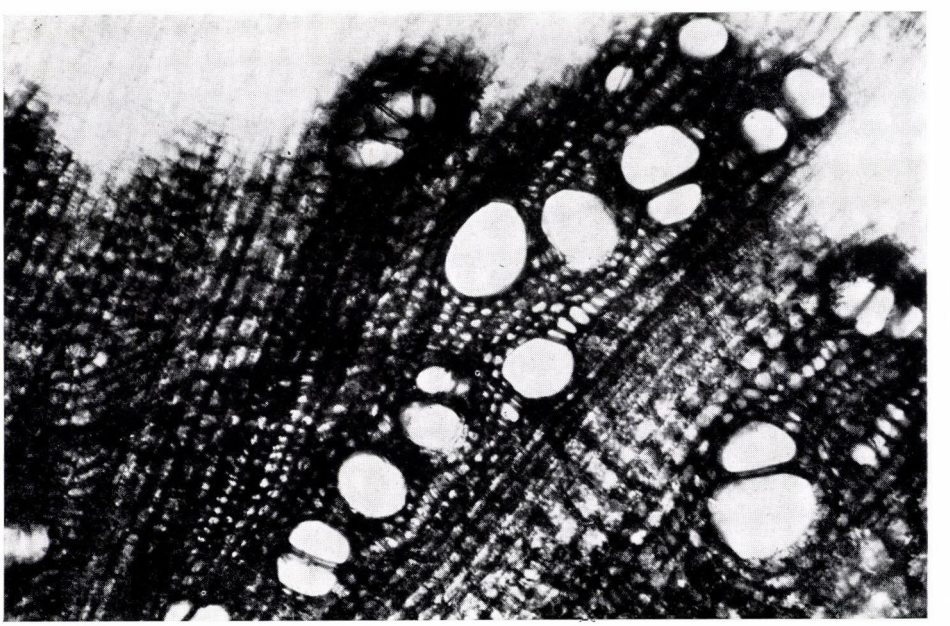

Fig. 12. Wavy boundary line of xylem and phloem, 100 : 1

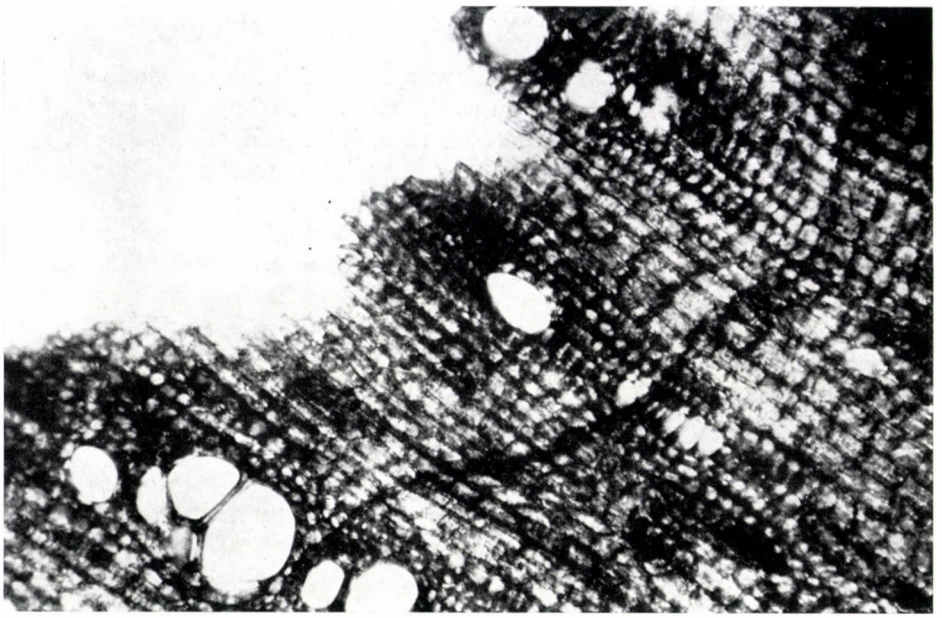

Fig. 13. Xylem of the eighth annual ring with slight vascular elements and wavy line, 100 : 1

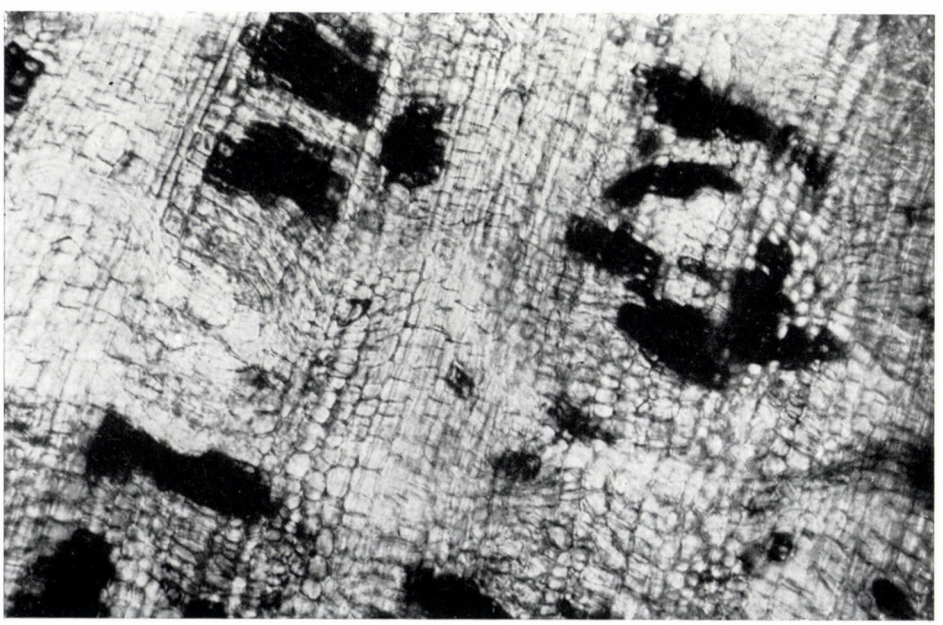

Fig. 14. Fibers in the phloem are dispersed irregularly, 100 : 1

Discussion

Main changes of the vascular tissue system of the legno riccio diseased vine are reported in this paper, such as the reduction of xylem vessels, hypertrophy in phloem, formation of parenchymatosis in phloem and xylem, wavy cambial line and contour of woody cylinder which agree with the results of former investigations (GRANITI and MARTELLI, 1965, 1970; GARAU *et al.*, 1974).

In our samples the beginning of the formation of abnormal tissue in the xylem tissue of the *Teleki 5C* rootstock was observed in the second annual ring whereas GARAU *et al.* (1974) found similar changes in the third ring, though in other rootstock species.

Results of histological comparison of annual rings show that by the effect of virus infection at first a small cell group changes its activity in the cambial region in spring or later during the vegetation period and the cells, due to disturbed cell division, are unable to form normal differentiation. Later the local functional trouble in the cambial zone, presumably after spreading of virus, extends periclinally and this continues in the subsequent years too, while the rate of the abnormal tissue increases permanently. It is supposed that the diseased vine suffers already seriously when the rate of the trachea-free tissue reaches 50%. In the case of our samples this took place in the fifth and sixth rings. If the ratio increases further, menaces more and more with the danger of transporting insufficiency. In our samples the ratio of the abnormal tissue in the seventh ring reaches 69.6–95.0%. On the basis of our results, it seems to be certain that with the profound pathological changes of the xylem structure, this vascular tissue becomes unable to function.

From the results it is concluded that the functional trouble of cambial zone is reversible to some extent because it could be observed that after a permanent growth, the ratio of the trachea-free tissue part decreased again and some tracheae reappeared with narrow cavity in a formerly trachea-free sector. In spite of this fact the healthy tissue structure was not re-established by this process.

It was experienced that the total area of certain annual rings decreases some years after planting, e.g. in the sixth ring by an average 24.6% and in the seventh one by an average 49.3% as compared to the fifth ring. This indicates the atrophy of the rootstock and we consider it as a consequence of the disease process, which was reported previously (LEHOCZKY, 1972). The reduction of the second and third annual ring area is explained by the influence of bench-graft making, nursery and planting, namely by exogenous factors.

It is supposed that the profound changes in the vascular tissue system of the rootstock impede storage of metabolites herein and this can also be the reason of gradual worsening of the condition of the diseased vine.

It is considered that the results of this paper supply proofs enough to explain why the condition of diseased vine declines inevitably some years after planting. The reduced growth of the affected vines (Fig. 1), decrease of bleeding

Fig. 15. Late bud burst of the legno riccio diseased vine for dry weather prior to burst

in spring on the pruned vine parts, strong bleeding sometimes externally and locally at the basal part of the trunk where the legno riccio symptoms developed intensively, delay of bud burst by one, two (or three) weeks (Fig. 15), particularly when the weather conditions are dry in winter and in spring, are all characteristic concomitant symptoms of the disease. Frequently just the dry weather period causes decay of the diseased vines two to four years after planting when the ratio of the abnormal trachea-free tissue reached 50% in the youngest annual ring. The decay of the young diseased vines ensues usually within 10 years after planting as it was already shown previously (LEHOCZKY, 1972). This catastrophic state could not be attributed merely to the consequence of

partial dysfunction of the youngest annual ring which contains already very slight vascular element, but presumably it could come from the summarized effect of functional troubles of all older annual rings. Some papers also report about stunting of the legno riccio diseased vines (ENGELBRECHT, 1971; HEWITT and NEJA, 1971; BOVEY and BRUGGER, 1973), while AGRIOS (1971) found that some years after appearance of the symptoms, the diseased vines decline and die. According to present knowledge, among the virus diseases of grapevine the legno riccio and the corky bark exhibit the most serious changes in the secondary vascular tissues.

Acknowledgements

We wish to thank Mrs. ANNE LADÁNYI and Mrs. CATHLEEN GYURJÁCS for their technical assistance.

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Types of Mycoparasitic Die-back Diseases in Apple Trees

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Five principal types of mycoparasitic diseases of the apple die-back are distinguished: 1. armillariosis (superseding often peripremnosis in young trees), 2. phytophthorosis, 3. tubercullariosis, 4. sphaeropsidosis, and 5. cytosporosis (main pathogens: *Cytospora schulzeri* and *C. sacculus*). The occurrence of *Cytospora nivea* = *C. leucostoma* on apple tree is reported from two Czechoslovak localities.

While surveying (about the middle of September) South Slovak orchards, some apple trees can always be observed the crown of which shows typical symptoms of a nearly total moniliosis. In contrast to apparently healthy and broad-leaved non-flowering shoots, most flower- or fruit-bearing shoots exhibit dry flowers or undeveloped fruit, the crop being largely lost. This disease seems to be confined to trees of 10 or more years of age (according to our experience made chiefly at Vinosady near Pezinok, South Slovakia).

For the time being, there is only little indication that moniliosis may be the cause of an apoplectic die-back of apple trees. Let us mention, however, what the late Czech botanist J. KLIKA (1924) observed during and after a severe attack, by *Sclerotinia fructigena*, in the wet summer of 1917 (translated from Czech): There were not only apples (fruit of the rennets) destroyed, but twigs themselves were severely damaged. The injured trees died back next year.

Apple trees planted too deep suffer from a physiological disease, the peripremnosis, and are known to perish at the age of 4 years or so (PACLT, 1969). Roots of the trees dying back which were set free, may often show an attack by specific fungus species *(Dematophora necatrix, Armillaria mellea)*. On the other hand, the infection by the honey agaric, *Armillaria mellea* (Vahl ex Fr.) Kummer, is on no account limited to young deep-planted trees. This species of root-invading fungi has long been known to be one of the causes of a sudden death in apple trees.

Type One: Armillariosis

The infection affects living trees and sometimes those considered to be healthy from every point of view. It occurs primarily by root-like rhizomorphs *(Rhizomorpha subterranea)* which are known to grow freely through soil. These

Fig. 1. Apple tree dying back due to *Armillaria mellea* (19 June 1972)

subterranean rhizomorphs can penetrate unwounded bark by mechanical pressure and enzymatic activity. The disease is easily recognized by white mycelial fans occurring in bark layers and the cambial region. Flattened rhizomorphs (*Rhizomorpha subcorticalis*) may replace fans between bark and wood in advanced stages of disease. When infection is heavy, the white mycelial fans spread in more distant areas of the host plant and may be found also near "shot" or exit holes produced by the apple bark beetle, *Scolytus mali* (Bechstein), a vector of the disease.

About in the middle of June, 1972, a typical case of the sudden death due to *Armillaria* has been observed at Ivanka p. D. (SW Slovakia). General appearance of the wilting apple tree (15 years old; 6.5 m high; 14 cm in the diameter of the breast-height [1.3 m above the ground]) on June 19th, 1972, is shown in Fig. 1. The foliage withered invariably and the tree was allowed to stand dead all the year round (trunk cut down on July 3rd, 1973). Two additional apple trees – one dead while the other wilting – were felled at the same time. At the

Table 1

No. 001	. . .	+ middle of June, 1972 (Fig. 1); numerous fruiting bodies (1974)
No. 002	. . .	+ (1972: already dead); fruiting bodies in masses (1974)
No. 003	. . .	+ middle of June, 1973; fruiting bodies pretty often (1974)
No. 004	. . .	+ June, 1974; rhizomorphs present
No. 005	. . .	+ end of June/early in July, 1974 (Fig. 2); rhizomorphs present
No. 006	. . .	living; premature fall of the leaf (Oct., 1974); rhizomorphs present

end of October, 1974, six records of the sudden death of apple trees attacked by *Armillaria* were already available at Ivanka p. D. (orchard of a poultry farm). The trees referred to were all 15 to 20 years old. Notes on the development of the pathogen and decay dates for the individual trees may be found in Table 1.

Apple trees injured by the honey agaric become usually subject to infestation with bark beetles as secondary invaders. Sometimes one finds pycnidia of *Cytospora* on single twigs or stem of the injured trees. On the other hand, the occurrence of *Cytospora nivea* (Hoffm.) Sacc. = *C. leucostoma* Fr. on injured apple trees has only recently been reported from an orchard of the Agricultural Technical

Fig. 2. Crown of a dead apple tree initially infected by *Armillaria mellea* (30 July 1974)

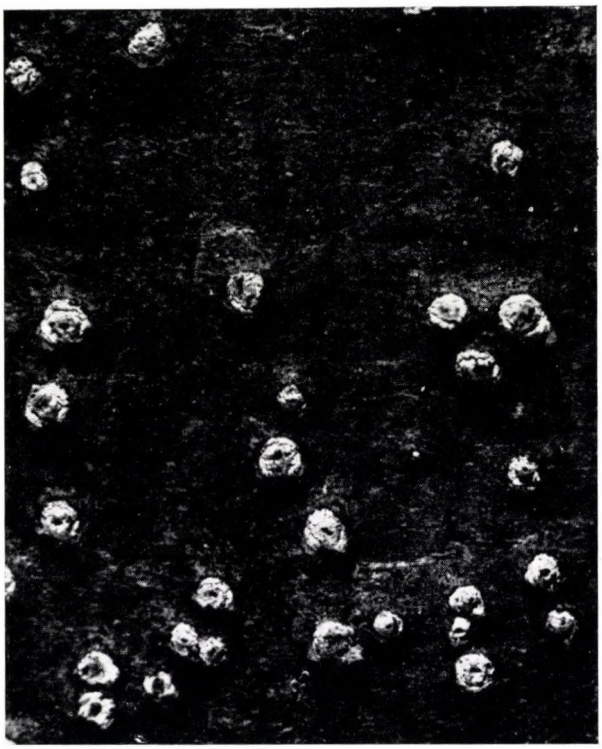

Fig. 3. White pycnidia of *Cytospora leucostoma* on stem of a dead apple tree (locality Šurany, Slovakia)

School (SPTŠ) at Šurany (distr. Nové Zámky). The fungus, which is normally a perthophyte damaging on poplar and willow, has been collected there on *Malus domestica* (Fig. 3) on September 12th, 1974 (PACLT, 1975).

Type Two: Phytophthorosis

Rotting known as "collar rot", which follows infection by several *Phytophthora* species, is not uncommon among kernel fruits as well as stone fruits. The pathogen most frequently responsible for the collar rot proved to be *P. citricola* Sawada sensu Waterhouse (synonyms: *P. cactorum*, *P. omnivora*). According to repeated records (SAREJANNI, 1935; KOUYEAS, 1971), apricots suffer from a phytophthorosis in South European culture. Apple trees were affected by the infection in a number of cases: Rhineland and the Netherlands (KOTTE, 1958), Great Britain (SEWELL *et al.*, 1974), Switzerland, U. S. A., and Canada (cf. KOUYEAS, 1971).

Type Three: Tuberculariosis

Although the fungus *Tubercularia vulgaris* Tode ex Fr. cannot infect healthy tissues immediately, it may enter wounds and pass thence as a pertophyte into living branches. The "wither-tip" form of the disease has been observed on an old apple tree at Banská Belá, Slovakia (sample collected on Oct. 14th, 1974). On the other hand, a case of sudden death in a middle-aged apple tree has been seen at Ždánice, Moravia, Oct. 10th, 1974, with simultaneous occurrence of *Tubercularia vulgaris* and (!) *Cytospora leucostoma* Fr.

Type Four: Sphaeropsidosis

According to KLINGNER and PONTIS (1974) the fungus *Sphaeropsis malorum* Peck is responsible for the die-back of severely attacked apple trees in Argentina.

Type Five: Cytosporosis

The disease is widely distributed in Czechoslovakia and many other countries. It may be common even in mountain localities (e.g., Švermovo, Slovakia, altitude 890 m above sea level). The identity of the pathogen has been established with the following result: *Cytospora schulzeri* Sacc. et Syd. (synonyms: *C. capitata* Sacc. et Schulzer, *C. carphosperma* auct. nec Fr.), related as imperfact state to *Valsa malicola* Urban (synonym: *Valsa ambiens* auct.). Another species, *Cytospora sacculus* (Schw.) Gvrit., causes canker and die-back of apple trees in Japan, and is related as imperfect state to *Valsa ceratosperma* (Fr.) Maire (synonym: *Valsa mali* Miyabe et Hemmi). The severity of the disease varies in different districts. In Slovakia, a case of heavy cytosporosis occurred in the neighbourhood of Ilava (locality Savčina – Podvažie, middle-aged apple tree, May 1975, pathogen: *Cytospora schulzeri*). The disease, caused by *C. schulzeri*, may do substantial damage even in the interior of Asia (Kirgizia, Kazachstan) where it may destroy from 56.5% up to 90% of mature apple trees.

In Victoria, New South Wales, and South Australia, the cytosporosis of apple trees (Figs 4 and 5) seems to be due exclusively to *Cytospora leucostoma* Fr. Curiously enough, larvae of a Curculionid beetle, the so-called fruit tree root weevil (root borer), are often implicated in the disorder. For details on this species – *Leptopius squalidus* (Boh.) – see FROGGATT (1920), HARRIS (1922), FRENCH (1934), ANONYMUS (1936), O'LOUGHLIN (1950), FENNER (1962), ANONYMUS 1970; BAXTER and HUTCHINSON, 1975. Severe and chronic decline of single limbs and eventually the whole tree occurs, although the roots attacked by the larvae remain functional. Decline of the tree continues even when infestations of *L. squalidus* have been greatly reduced by traps and insecticides (BAXTER and HUTCHINSON, 1975).

Fig. 4. Injury by *Cytospora leucostoma* to a young apple tree in Victoria. Root system undamaged. Orig. P. BAXTER, Ferntree Gully, Victoria

Apple trees under the die-back due to *Cytospora* spp. show sometimes the symptom of a microphylly (Fig. 6; July 25th, 1973).

Appendix

In addition to the five principal types, the following fungi are considered to cause occasionally die-back in apple trees: *Dermatea polygonia* (Fuckel) Rehm (cf. PŘÍHODA, 1974); *Dematophora necatrix* Hartig (cf. AGARWALA and SHARMA, 1972); *Botryosphaeria dothidea* (Moug ex Fr.) Ces. et de Not. and *Sclerotium rolfsii* Sacc. (cf. TAYLOR and MCGLOHON, 1975); *Haplosporella malorum* Sacc. (cf. SHANDILYA, 1974).

Fig. 5. Die-back of apple tree in Victoria due to *Cytospora leucostoma*. Orig. P. BAXTER, Ferntree Gully, Victoria

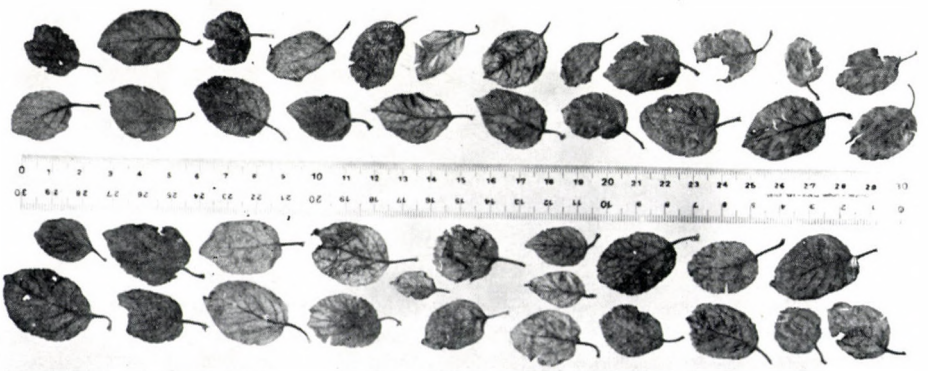

Fig. 6. Microphylly of an apple tree dying back due to *Cytospora schulzeri* (locality Skýcov, Slovakia)

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Changes in Peroxidase Isoenzymes in Blast Diseased Rice Leaves

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Gel electrophoretic analysis of the peroxidase isoenzymes of the healthy and blast infected rice leaves revealed the presence of three new isoenzymes in the blast diseased rice leaves and the quantitative intensification of the other three isoenzymes present in the healthy leaves. Inhibition of these parasitically induced peroxidase isoenzymes by actinomycin-D suggests that the enhanced peroxidase activity of blast diseased leaves is due to *de novo* synthesis in response to pathogenic infection.

Increased peroxidase activities have been observed to occur in a number of host plants infected with fungal pathogens (FARKAS and KIRÁLY, 1958; URITANI and AKAZAWA, 1959). The peroxidase activity of rice (*Oryza sativa* L.) leaves is significantly enhanced in response to infection with *Pyricularia oryzae* Cav. (TOYODA and SUZUKI, 1960; SRIDHAR, 1972). In order to understand the physiological significance of the activation of peroxidase in rice leaves in response to blast infection, it is necessary to ascertain whether the enhanced activity results from *de novo* synthesis of the enzyme or from activation of the previously inactive form of the enzymes.

Materials and Methods

Rice seedlings of cultivar Khao-teh-haeng 17 were grown in a soil seed bed in the greenhouse and 20-day–old seedlings were inoculated by spraying with a spore suspension of *P. oryzae* (ca. 50,000 spores per ml) and kept under humid conditions for 36 hr at 24 ± 1 °C and then removed to the greenhouse. Portions of heavily infected 4th leaves from the bottom of the plant, 7 cm in length, were cut out 3 days after inoculation and floated for 24 hr on the surface of a solution containing 25 μg of actinomycin-D per ml, or water in Petri dishes under laboratory conditions. Before the enzyme extraction the edges of the leaf bits were removed and washed in distilled water. In view of the localization of enhanced peroxidase activity in the lesion area and to avoid the dilution of the sample with uninfected tissue, single lesions with a ring of apparently healthy

tissue were cut out and used for the enzyme extraction. Healthy leaves from uninoculated plants served as controls. Lesions from intact plants were also collected similarly 4 days after inoculation and were used.

Tissues were extracted by crushing in 0.1 *M* tris (hydroxymethyl) aminomethane (Tris) buffer at pH 8.0 containing 17 per cent sucrose, 0.1 per cent ascorbic acid and 0.1 per cent cysteine hydrochloride as described by FARKAS and STAHMANN (1966). Total proteins in the extract were estimated by the method of LOWRY *et al.* (1951). Enzyme assays were performed in a Beckman DU spectrophotometer following the method described by HAMPTON (1963). Enzyme activity was expressed as the change in optical density (ΔO.D.) under these conditions during 1 min/mg of protein. Control cuvettes contained the same reaction mixture without hydrogen peroxide. For the quantitative peroxidase assay, ascorbic acid and cysteine were omitted from the extraction medium.

Alterations in peroxidase isozyme spectrum of the samples were analyzed using polyacrylamide gel electrophoresis as described by BREWBAKER *et al.* (1968). The gels were stained with ethanolic solution of *o*-dianisidine, consisting of 100 mg of *o*-dianisidine in 70 ml of 95 per cent ethanol and adding 10 ml of 1.5 *M* acetate buffer (pH 4.7), 2 ml of 3 per cent hydrogen peroxidase and 18 ml of water. The experiments were repeated twice and the data on the enzyme activities represent the average of triplicates.

Results and Discussion

Rice leaves exhibited increased peroxidase activity in response to infection with *P. oryzae* (Table 1). The peroxidase isozyme patterns (Fig. 1) from the blast diseased rice leaves obtained from intact plants showed three new peroxidase isozymes and intensification of the other three isozymes present in

Table 1

Influence of actinomycin-D on the peroxidase activity of healthy and blast infected rice leaves 4 days after inoculation

Treatment	Peroxidase activity ΔO.D.(min/mg of protein) at 470 nm
Healthy	1.09
Inoculated	1.62
Healthy, floated on water	1.18
Inoculated, floated on water	1.75
Inoculated, floated on actinomycin-D solution	1.24

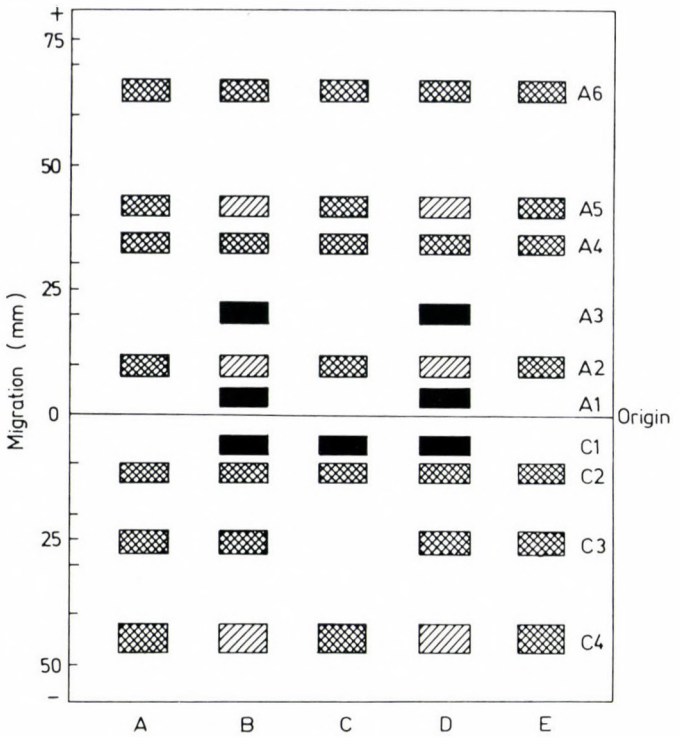

Fig. 1. Electrophoretic pattern of peroxidase isozymes. Leaf extracts from healthy (A), inoculated (B) intact plants and healthy (C), inoculated (D) water treated, and inoculated actinomycin-D treated (E) leaves of Khao-teh-haeng 17

healthy rice leaf tissues of intact plants indicating a quantitative increase of them (variants A and B). When the healthy leaves were cut and floated on water (variant C) a new cathodic isoenzyme (band C1) was found which was also present in the intact diseased leaves (variant B) suggesting that this particular isoenzyme is produced both in response to infection and cutting injury. Actinomycin-D, which is an inhibitor of DNA-dependent RNA synthesis, inhibited the parasitically induced peroxidase isoenzymes (variant E), suggesting that the increase of peroxidase is due to the *de novo* synthesis of the enzyme in response to fungal infection and indicates the participation of DNA-dependent RNA synthesis in the appearance of symptoms in the inoculated plants (MAHADEVAN, 1970). The rate and time at which rice plants exhibit the enhancement in peroxidase activity are greatly influenced by the race of the pathogen to which the host is exposed, which would also influence the peroxidase isoenzyme pattern of the blast infected tissues.

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Changes in the Activities of Some Enzymes in Relation to Pathogenesis of *Fusarium* Root Rot of Pea

By

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Fusarium solani f. *pisi* (Jones) Snyder and Hansen, the incitant of root rot disease in pea, causes browning and rotting of the primary and the secondary root and the foot region resulting in the wilting of plants. Both polyphenol oxidase and peroxidase activities were found to be more at the infection site than in healthy tissue. IAA oxidase activity was found to decrease where polyphenol oxidase activity increased. RNase activity decreased while DNase activity increased in all the parts of the diseased plant. The possible role of these enzymes in the symptom development has been discussed.

Fusarium solani f. *pisi* causes a serious root rot disease of pea, attacking plants of all ages. Characteristic aerial symptom is a dark brown lesion at the collar region. The primary and the secondary roots become brown in colour and then get rotted as a result of which the whole plant wilts. As any infection is associated with characteristic changes in enzymic activities peculiar to that disease (FARKAS and KIRÁLY, 1958), attempts were made to find out the change in some of the enzymic activities in healthy and diseased tissue during disease development with a view to find out the possible role of enzymes in the symptom development.

Materials and Methods

Culture

Pure culture of *Fusarium solani* f. *pisi* isolated from an infected pea plant from I.A.R.I. field was used.

Plant material

A commonly grown susceptible pea variety Bonneville was taken for the experiments.

Inoculation was done by keeping the seeds in standard spore suspension (4×10^5 spores/ml) for 5 days and then transplanting in vermiculite. Seeds soaked in sterile water and then transplanted to vermiculite served as controls.

Enzyme preparation

Sampling was done at four different periods of infection; (i) after 5 days of inoculation when both the primary root and the shoot get little stunted and the root a little bit discoloured, (ii) after 10 days when the primary root becomes dark brown and some brown streaks, or sometimes a wedge shaped lesion develops at the foot (region above ground level), (iii) after 15 days when the secondary roots also become discoloured and the lesion at the foot encircles the stem base, the plants become partially flaccid, and (iv) after 18 days when the secondary roots are reduced, become brown; the plants wilt. For both healthy and infected lot the plant parts were sampled in five groups, *viz.*, primary root (PR), secondary root (SR), foot (F) (0.5 cm above collar region for 5 days lot and 1.5 to 2.0 cm for other periods), stem (S) and leaves (L). Healthy and diseased plants have been designated as H and D, respectively.

Samples were collected in the morning between 7 and 8. a. m. They were washed with distilled water, blotted and then weighed and crushed with a pinch of acid washed quarter sand with suitable buffer solution. The homogenized pulp was squeezed through three layers of cheese cloth and then centrifuged at 2000 r. p. m. for 10 minutes. Clear supernatant was used as enzyme preparation. The experiments were done at 25°C.

Enzyme assays
Polyphenol oxidase

Polyphenol oxidase (PPO) was assessed following MATTA and DIMOND's (1963) method with some modification. The reaction mixture consisted of 1 ml distilled water, 1 ml 0.2 *M* phosphate buffer (pH 7.0), 0.5 ml of 0.5 *M* freshly prepared catechol and 0.5 ml of enzyme. The optical density was measured by a Hitachi 124 spectrophotometer at 410 mμ at minute intervals up to 5 minutes.

Peroxidase

Peroxidase (PO) activity was spectrophotometrically measured following FARKAS and STAHMANN (1966). The reaction mixture consisted of 2.7 ml of Tris HCl buffer (pH 7.5), 0.2 ml of 2 μ moles freshly prepared orthodinisidine, 0.1 ml enzyme and 0.02 ml of 20 μ moles H_2O_2. Absorbance changes were recorded at 420 mμ at 30 second intervals up to 2 minutes and 30 seconds.

IAA oxidase

The method followed was of MUMFORD *et al.* (1961). The assay solution contained 1 ml enzyme, 2 ml of IAA stock solution (10^{-3} *M* IAA and 5×10^{-4} *M* $MnCl_2$ in 0.01 *M* phosphate buffer), 0.5 ml phenol-indo-2,6-dichlorophenol (10^{-3} *M*) and 0.01 *M* phosphate buffer (pH 6.1), added to make up the total volume to 10 ml. After incubation for 1 hr the residual IAA was determined with Salkawski ferric chloride reagent.

Ribonuclease

RNase was assessed following UDVARDY *et al.* (1969) with some modification. The enzyme activity was assayed by measuring the increase in absorption at 260 mμ of the uranyl acetate–trichloroacetic acid (TCA) soluble digestion product of commercial, purified RNA incubated with the enzyme for 30 min. The reaction mixture consisted of 0.5 ml of 0.1 *M* acetate buffer (pH 5.5), 1 ml substrate (2 mg RNA/ml of 0.1 *M* acetate buffer at pH 5.0), and 0.5 ml enzyme. MacFadyen's reagent, prepared by dissolving 0.25% uranyl acetate in 2.5% trichloroacetic acid was added to slop the reaction. Precipitation was removed by centrifugation and absorbance of the supernatant was measured at 260 mμ against a zero-time blank.

Deoxyribonuclease

Method was the same as that of RNAse with slight modification. Reaction mixture consisted of 0.3 ml acetate buffer, 1.0 ml substrate (0.5 mg DNA/ml buffer), 0.5 ml enzyme and 0.2 ml $MnCl_2$ (0.1 *M*). Incubation period was 2 hours.

Results

An increase in polyphenol oxidase and peroxidase activity at the infection site, that is, the primary and secondary roots and the foot region, is evident from Tables 1 and 2, respectively. Though the percentage increase varied with the period of noculation and plant parts it attained a maximum value and then declined again, in both cases. For PPO activity maximum increase was observed at 15 days of infection for primary root and the foot region while for PO it was at 10 days of inoculation for primary and secondary root and 15 days for the foot. At the initial stages of infection the PPO activity of stem and leaves was observed to increase a little and then decreased. In this case also percentage decrease attained a maximum value and then declined again. In stem and leaves PO activity did not follow the same rule as PPO; though some decrease was noted in the stem the leaves showed more PO activity in the infected plants.

Table 3 indicates that IAA oxidase reacted in a reverse way as PPO in the infected plants. At all intervals of inoculation, infection caused considerable reduction in IAA oxidase activity in primary and secondary roots and in the foot region. Percentage decrease was maximum at 10 days interval for primary root and the foot and 15 days for secondary root. Stem and leaves showed increased activity in the infected plants. For both of them maximum increase was observed at 10 days interval.

Tables 4 and 5 show that the activity of both RNase and DNase was affected due to infection. In all parts the activity of RNase decreased while that of DNase increased. Percentage decrease in RNase activity in primary root and foot was

Table 1

Polyphenol oxidase activity in different parts of healthy (H) and *F. solani* f. *pisi* infected (D) pea (var. Bonneville) plants at various intervals of inoculation

Days after inoculation	Polyphenol oxidase activity (absorbance at 410 mμ/min/ 0.05 g fresh weight)					Percentage increase (+)/decrease (−) over healthy in				
	Primary root (PR) D/H	Secondary root (SR) D/H	Foot (F) D/H	Stem (S) D/H	Leaves (L) D/H	PR	SR	F	S	L
5	0.031/0.034	—	0.026/0.026	0.026/0.024	0.06/0.054	−5.9	—	0.0	+8.33	+11.11
10	0.054/0.033	0.033/0.029	0.033/0.025	0.030/0.031	0.052/0.065	+63.63	+13.45	+32.0	−3.22	−20.0
15	0.08/0.032	0.035/0.031	0.039/0.021	0.015/0.030	0.02/0.071	+150	+12.0	+85.71	−50.0	−71.83
18	0.056/0.032	0.04/0.036	0.042/0.026	0.026/0.030	0.023/0.027	+75.0	+11.11	+61.54	−13.33	−14.81

Table 2

Peroxidase activity in different parts of healthy (H) and *F. solani* f. *pisi* infected (D) pea (var. Bonneville) plants at various intervals of inoculation

Days after inoculation	Peroxidase activity (absorption at 420 mμ/ 30 sec/ 0.002 g fresh weight)					Percentage increase (+)/decrease (−) over healthy in				
	Primary root (PR) D/H	Secondary root (SR) D/H	Foot (F) D/H	Stem (S) D/H	Leaves (L) D/H	PR	SR	F	S	L
5	0.145/0.08	—	0.055/0.054	0.04/0.042	0.120/0.120	+81.25	—	+1.85	−4.76	0.0
10	0.220/0.116	0.10/0.084	0.094/0.059	0.031/0.034	0.170/0.092	+89.65	+19.04	+59.32	−8.82	+16.3
15	0.215/0.142	0.138/0.134	0.124/0.074	0.042/0.030	0.138/0.114	+51.40	+2.98	+67.56	+40.0	+21.05
18	0.128/0.138	0.134/0.125	0.096/0.063	0.047/0.052	0.197/0.128	−7.24	+7.2	+52.38	−9.61	+53.90

Table 3

IAA oxidase activity in different parts of healthy (H) and *F. solani* f. *pisi* infected (D) pea (var. Bonneville) plants at various periods of inoculation

Days after inoculation	IAA oxidase (amount of IAA destroyed in mg by 0.1 g fresh weight) in					Percentage increase (+)/decrease (−) over healthy in				
	Primary root (PR) D/H	Secondary root (SR) D/H	Foot (F) D/H	Stem (S) D/H	Leaves (L) D/H	PR	SR	F	S	L
5	0.263/0.266	—	0.252/0.225	0.131/0.132	0.198/0.171	−1.13	—	+12.0	−0.76	+15.2
10	0.076/0.273	0.249/0.259	0.096/0.214	0.192/0.140	0.050/0.042	−72.12	−0.38	−55.14	+37.14	+19.23
15	0.122/0.286	0.144/0.278	0.144/0.195	0.186/0.162	0.146/0.132	−57.34	−48.2	−22.15	+14.81	+10.6
18	0.210/0.243	0.218/0.245	0.224/0.237	0.234/0.232	0.207/0.212	−13.58	−10.9	−5.48	+0.86	−2.36

Table 4

Ribonuclease activity in different parts of heathy (H) and *F. solani* f. *pisi* infected (D) pea (var. Bonneville) plants at various intervals of inoculation

Days after inoculation	RNase activity (optical density at 260 mμ/30 min /0.0012 g fresh weight)					Percentage decrease over healthy in				
	Primary root (PR) D/H	Secondary root (SR) D/H	Foot (F) D/H	Stem (S) D/H	Leaves (L) D/H	PR	SR	F	S	L
5	0.240/0.29	—	0.24/0.24	0.40/0.41	0.67/0.76	17.24	—	0.0	2.44	11.84
10	0.11/0.24	0.28/0.42	0.17/0.28	0.21/0.23	0.43/0.46	33.33	46.15	39.28	8.7	6.52
15	0.09/0.19	0.165/0.432	0.07/0.13	0.23/0.27	0.535/0.565	52.63	61.62	46.15	14.81	5.31
18	0.05/0.12	0.11/0.215	0.03/0.12	0.20/0.21	0.46/0.46	58.33	48.83	75.0	4.76	0.0

Table 5

Deoxyribonuclease activity in different parts of healthy (H) and *F. solani* f. *pisi* infected (D) pea (var. Bonneville) plants at various intervals of inoculation

Days after inoculation	DNase activity (optical density at 260 mμ/2 hrs/0.037 g fresh weight)					Percentage increase over healthy in				
	Primary root (PR) D/H	Secondary root (SR) D/H	Foot (F) D/H	Stem (S) D/H	Leaves (L) D/H	PR	SR	F	S	L
5	0.065/0.06	—	0.02/0.015	0.03/0.03	0.140/0.13	8.33	—	33.33	0.0	7.69
10	0.06/0.03	0.05/0.02	0.03/0.020	0.03/0.03	0.08/0.06	100.0	150.0	50.0	0.0	33.33
15	0.02/0.015	0.05/0.025	0.045/0.022	0.04/0.022	0.06/0.032	33.33	100.0	104.54	100.0	84.4
18	0.025/0.010	0.027/0.02	0.06/0.022	0.018/0.015	0.018/0.01	150.0	35.0	172.72	20.0	80.0

in increasing order with sampling interval, while that of leaves was in decreasing order. For secondary root and stem, maximum percentage decrease was observed at 15 days of inoculation.

Percentage increase in DNase activity of the infected tissue over healthy ones was observed to be in increasing order in the foot and decreasing order in secondary root. In case of stem and leaves it was in increasing order for first 15 days, followed by some decrease. In general, the infection was observed to cause more decrease in RNase and increase in DNase activity in the roots and the foot region, in comparison to the stem and leaves.

Discussion

Increased activities of polyphenol oxidizing enzymes in the diseased tissue are the most responsive reaction on the part of the host (KIRÁLY and FARKAS, 1959). These enzymes are important from the point of symptom development as well as defense mechanism (FARKAS and KIRÁLY, 1958). In the absence of reducing agents the oxidation of phenols proceeded to form quinones, which react with other cell constituents and form the brownish black melanin pigments. These quinones are toxic for both the host tissue and the pathogen (GEIGER, 1946).

In the case of this disease a positive correlation has been found between the symptom development and the activity of PPO and PO.

The activity of PPO and PO and so also the brown pigment was observed to increase with time in the primary root, the secondary root and the foot region. Again that brown colour was found to be more intense in the primary root and the foot than in the secondary root and so also the activity of PPO and PO. Necrosis of the brown tissue occurred gradually, showing a positive correlation between the activity of phenol-oxidizing enzymes and tissue necrosis. The incitant being a soil borne pathogen events connected with defense mechanism took place in the roots, which confirms the findings of RETIG (1974). Reduction of the polyphenol oxidizing enzymes in the later period of infection may be due to inhibition of these enzymes by high concentration of phenolics (VAN KAMEN and BROWER, 1964; KOSUGE, 1969). The question arises whether decreased PPO and PO activity in the healthy portions is due to translocation and accumulation of these enzymes at the infection site or to spatial separation between polyphenols and these enzymes in the healthy cells (GOODMAN *et al.*, 1967).

The activity of PPO inhibits the IAA oxidase activity, thereby regulating the auxin level in tissues (RAY, 1958). On analysing the activities of PPO and IAA oxidase in both healthy and infected plants it was found that the activity of IAA oxidase decreased where PPO activity increased. Some variations were observed which may be considered to be due to some other physiological and biochemical processes involved in the mechanism of pathogenesis. As the activity of IAA oxidase is inversely proportional to IAA concentration (PILET, 1957),

decreased IAA oxidase activity may cause accumulation of IAA. On the other hand oxidative products of phenol, the quinones, are reported to bind auxin thus decreasing its concentration (LEOPOLD and PLUMMER, 1961; ZENK and MULLER, 1963). Therefore, it may be suggested that the altered activity of IAA oxidase and PPO in the infected plants over healthy ones, ultimately lead to some change in IAA concentration.

So far as the activities of RNase and DNase are concerned, the former was found to decrease and the latter increase in all parts of the infected plants over the corresponding healthy ones. As there is an inverse relationship between the RNase activity and RNA concentration (KESSLER and ENGELBERG, 1962), in the present studies decreased RNase activity may be suggested to lead to accumulation of RNA. Accumulation of RNA at the infection site has been reported by many workers (PERSON, 1960; ROHRINGER and HEITEFUSS (1961); QUICK and SHAW, 1964). Some decrease in DNA at the infection site was observed by HEITEFUSS (1965) and MILLIKAN *et al.* (1965). Ribonuclease activity is related to growth and RNA content (KESSLER and ENGELBERG, 1962). Therefore, it may be suggested that the abnormal activity of RNase and DNase as well as the altered concentration of IAA in the infected plants affect the growth of the plant while increased PPO and PO activities are responsible for browning of the infected tissue.

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Occurrence of *Corynespora cassiicola* (Berk. & Curt.) Wei on Soybean in Hungary

Short communication

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Target spot disease caused by *Corynespora cassiicola* (Berk. & Curt.). Wei on soybean was first reported in the U.S.A. (OLIVE *et al.*, 1945), and it was later described in Cambodia China, Japan, Nicaragua (cf.: SINCLAIR and SHURTLEFF, 1975), Canada (SEAMAN *et al.*, 1965) and more recently in Brasil (ALMEIDA *et al.*, 1976). The fungus affects leaves, petioles, stems, pods seeds, hypocotyls and roots and can result in yield losses of 18 to 32 per cent.

The symptoms characteristic of target spot (Fig. 1) were observed on primary roots, hypocotyls as well as on stems of plants with surprisingly reduced growth in a plot of Hungarian soybean cultivar ISZ-10 in Iregszemcse (Western Hungary) at the beginning of June, 1977.

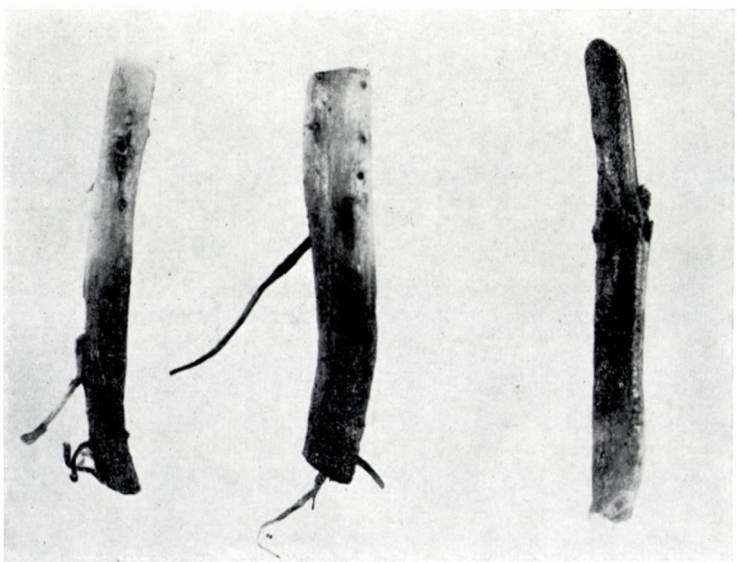

Fig. 1. Symptoms of *Corynespora cassiicola* infection on root, hypocotyl and stem of ISZ-10 soybean from a field plot

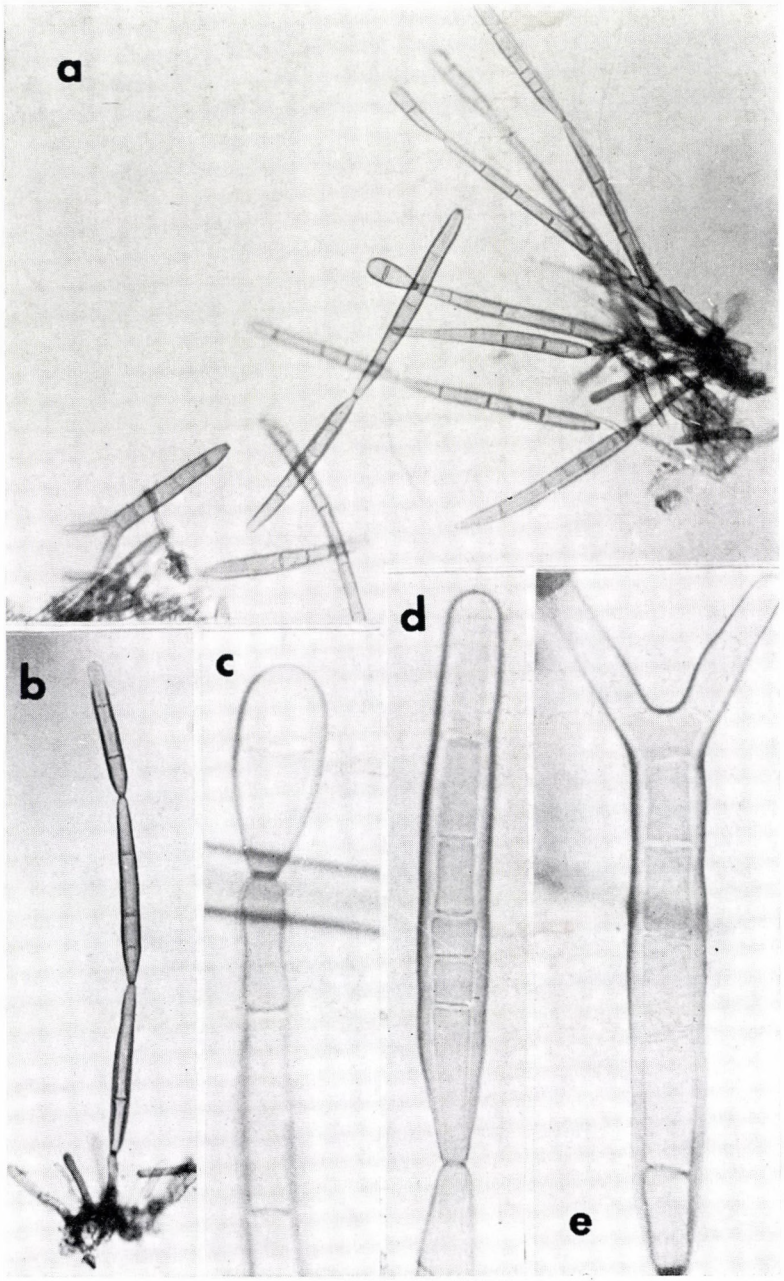

Fig. 2. Conidia and conidiophores of *Corynespora cassiicola* (a, b), × 130; developing conidium (c), most common form of conidia (d), Y-shaped conidium (e), × 360

Fig. 3. One-month-old colony of *Corynespora cassiicola* grown on potato dextrose agar at 25°C

Fig. 4. Symptom of target spot on ISZ-10 soybean leaflet 21 days after inoculation with *Corynespora cassiicola*

The fungus isolated from the affected parts of ISZ-10 soybean plants, after they were surface-sterilized in 0.2% NaOCl and 70% ethanol for 2 minutes then incubated at 18°C for a week, proved to be *C. cassiicola*. On the basis of microscopic measurements it was established that average size of conidiophores and that of conidia is 164 μm × 11 μm and 183 μm × 16 μm, respectively. Conidiophores are averagely 4-septate and conidia 5-pseudoseptate (Fig. 2). These data corresponded to that of the literature cited (ELLIS and HOLLIDAY, 1971).

Optimum growth of the Hungarian isolate on potato dextrose agar occurred at 25°C (Fig. 3). This temperature was higher than that reported on Canadian isolates (20°C) and lower than it was published in the U.S.A. and Japan (28°C) (cf.: SEAMAN *et al.*, 1965).

Inoculation of 3- to 5-week-old ISZ-10 and Merit soybean seedlings resulted in the development of numerous reddish-brown pinpoint lesions on leaves in an incubation period of 14–18 days (Fig. 4). Inoculation was carried out with the homogenate of one-month-old culture on potato dextrose agar. The homogenate consisted of mycelial fragments and conidia in 100 ml distilled water containing 0.02% Tween 80. Inoculated plants were kept in moist chamber at 15±2°C for 3 days then under greenhouse conditions at 20±3°C. Pinpoint lesions on leaves enlarged if plants were replaced into moist chamber, lesions, however, were exclusively confined to the foliage. Reisolation of the fungus from inoculated leaves was successful.

C. cassiicola has not been reported on any of its host plants in Hungary, moreover, this paper is apparently the first account of the occurrence of the fungus on soybean all over Europe.

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Some Questions on the Population Dynamics of the San José Scale, *Quadraspidiotus perniciosus* Comstock *(Homoptera: Coccoidea)*

By

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The present paper discusses the changes in significance of different (abiotic, biotic, anthropogenic) factors, governing the population dynamics of *Quadraspidiotus perniciosus* on continental, zonal, regional and host plant population levels. There is an organic, essential connection between the factors modifying and controlling population density and their significance varies in space and time. For the construction of a reliable model on population dynamics a comprehensive survey work is needed, carried out for some decades and covering a large geographical area.

In the judgement of the role of different factors governing the changes in densities of insect populations, in the last decades many authors took the view that it is incorrect to exaggerate the significance of one or the other, taken out from its context (MILNE, 1957; VIKTOROV, 1971). So complex and synthetic theories were created. The author tried to construct his working hypothesis on the factors influencing the fluctuations of *Q. perniciosus* individual numbers and on the possibilities of their study, based on the interpretations of HUFFAKER and MESSENGER (1964) and VIKTOROV (1976). The complexity of these theories and their dynamic approach have been acknowledged and it is not the aim of this paper to consider the differences between the viewpoints of various authors.

According to the opinion of YAKHONTOV (1964) there are no sufficient data available at present to construct reliable systems of population dynamics. In case of the San José scale, however, we considered to have enough concrete data to present a starting point to future research work. It is beyond question that the complicated interactions and significances of the factors can be cleared only in studies carried out for many decades in different geographical localities, based on uniform principles. In surveys made in restricted geographical units and in some detail problem the work should be planned so as the data gained could be inserted later into the lattice of data on *Q. perniciosus*. It was therefore considered as important to draw the outlines of a system of population dynamics, which may be a guiding principle in the future research on *Q. perniciosus*.

In the present paper we tried to summarize the data of the literature and our own observations into a uniform system, by taking into account also the

theories on population dynamics mentioned and the important points of other studies.

In models on population dynamics and in life-table studies (GEIGER and HILMANN, 1971) it occurs frequently that the authors calculate with end numbers of certain processes or developmental instars, omitting at the same time the study of causes of intermediary changes. Thus considerable factors of uncertainty are built in into the models. However, even these models are very important in the research until more reliable ones can be worked out. These seemingly "perfect" models, however, create the feeling that the problem is solved and therefore do not stimulate further work.

The factors influencing the population dynamics of *Q. perniciosus* have been studied by many authors both in Hungary and abroad. The investigations were extended to the role of abiotic factors in the winter mortality (GYŐRFFY, 1936; KOZÁR, 1972a; POPOVA, 1962), to the influence of parasites and predators (GYŐRFFY, 1934; HUBA, 1969; POPOVA, 1962; SZELÉNYI, 1934, 1952), to the role of host plants in the changes of individual density (KOZÁR, 1972b; THIEM and SCHETTERS, 1958; ZAGAYNÜJ, 1964) and to the role of agrotechnical factors (KALABEKOV, 1973). The authors mentioned studied, however, the different factors separately, so their results were unsuitable for preparing a synthesis summarizing the informations gained so far, and indicating the directions of further research.

I. Establishment of populations in new areas

Before discussing the role of different factors which play a role in population dynamics, we have to mention also the question of populations invading new areas. We do not want to touch upon the territorial increase and speed of invasion (ELTON, 1963), but only the changes in population density, occurring in the already populated new area. This phenomenon is by no means restricted only to newly introduced or colonized species, as also in case of endemic ones marked pulsations can be observed increasing and decreasing continuously the area occupied (MAYR, 1968; VARGA, 1971), where the problems of repeated colonization are fairly similar.

The San José scale *Q. perniciosus* occupied hundred years ago only a very small area. Its subsequent worldwide expansion occurred as a result of some ecological changes, and – undoubtedly – as a consequence of transport of goods between countries. In course of its expansion the San José scale got among different ecological circumstances; it was around 1920 that its presence was stated also in Hungary (SZELÉNYI, 1953). As a newly introduced insect species it presents a good example to follow its establishment into the Hungarian fauna. A spectacular series of observations is presented on a similar phenomenon by VARLEY and GRADWELL (1960) in case of the winter moth, *Operophtera brumata* L. It is a pity that only few reviews of this type have been published.

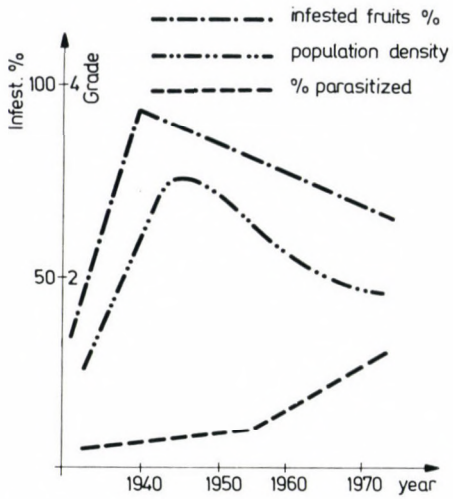


Fig. 1. The formation of *Q. perniciosus* infestation in Hungary after its establishment

FLANDERS (1960) reported on the process of establishment of *Q. perniciosus* in North America, on its outbreak and subsequently on the decrease of its significance; the author mentioned that the causes of the declining individual density are not known. According to the data of KLETT (1963) the outbreak of the scale decreased after 1910 and this could be attributed to the activity of its parasite, *Prospaltella perniciosi* Tower, causing mortalities of 80–90 per cent.

Q. perniciosus appeared within a period of 10 years in whole Hungary (ANON., 1940, Fig. 1), mostly as a result of faulty horticultural practice. It reached in a short time a high density in the orchards, promoted by the lack of adequate control and natural enemies and by the presence of suitable host plant varieties. As a result of the damage caused the fruit trees began to die off. As within the same varieties the less susceptible trees could survive, the population densities of the pest had decreased to some extent and later also the methods of control were improved. During the second World War the infested area increased again. In the 1950s the most important means of its spread could be prevented, by producing infestation-free propagating material. Later, by the improvement of control methods also the spread of the pest could be prevented and its individual density decreased even in the already infested orchards (KOZÁR, 1972c).

Soon after its establishment in Hungary, the appearance of animal communities, basing their existence on San José scale populations could be observed. Already in the 30s the presence of the polyphagous parasite *Aphytis proclia* WALKER was noted in the scale colonies (SZELÉNYI, 1934); GYŐRFFY (1934) reported practically in the same period on the activity of polyphagous scale predators. In the early 50s the introduction of the monophagous parasite

Prospaltella perniciosi Tower was commenced (JERMY, 1967) which became established and distributed in Hungary (KOZÁR, 1975). In the meantime also the presence of other, endemic parasites could be demonstrated in the colonies. So the parasitization of the females increased from the 1% level of 1934 to 30% by 1975 (KOZÁR, 1975). Similar data were reported by RUBTZOV (1961) in the Soviet Union.

These antecedents led up to the present situation, in which about 50% of the orchards has been found infested (Fig. 1). The measure of the infestation, however, reaches only on the susceptible varieties of the suitable host plant species a high level (red and black currant, apple, pear, etc.), i.e. grades rated 3 to 4 on a scale of 0–5.

It can be considered that the species is at present in the transition from the "instable" phase following the introduction to the more "stable" phase of an established pest.

In Hungary only sporadic data have been collected during the important period of establishment; from the point of view of population dynamics it would be of utmost importance to study the period following the introduction into a given area. This could be made in many countries, in the first place in the Soviet Union where the species invades every year newer and newer territories (SMOLYANNIKOV, 1973). It would be especially important to study the process of establishment both in areas with strong human influence and in nearly natural environments.

II. Changes in the importance of different factors influencing population densities, according to geographical categories

The individual densities of populations are influenced within the area of distribution of the species to a different degree, by different factors. According to RUBTZOV (1953) the role of abiotic factors becomes prominent especially in the parts of the distribution area with unfavourable climatic conditions. As MILNE (1957) presented in his tables, the increasing role of density-independent environmental conditions leads to the formation of zones with very low densities. According to the theory of HUFFAKER and MESSENGER (1964) the role of "forming" forces is more pronounced in places with unfavourable physical conditions, whereas in the favourable areas the importance of density-dependent factors increases.

As regards these questions, also observations on *Q. perniciosus* are available, and we tried to apply them for that species framing a base for further research. In Fig. 2 the system of already known factors exerting the utmost influence on the reproduction of *Q. perniciosus* is presented. Most data are available so far concerning the abiotic and anthropogenic factors as well as several biotic factors (parasites, predators, common host plant fertility and sexual ratio). Our knowledge is, however, very scanty in the fields of entomopathogens, ac-

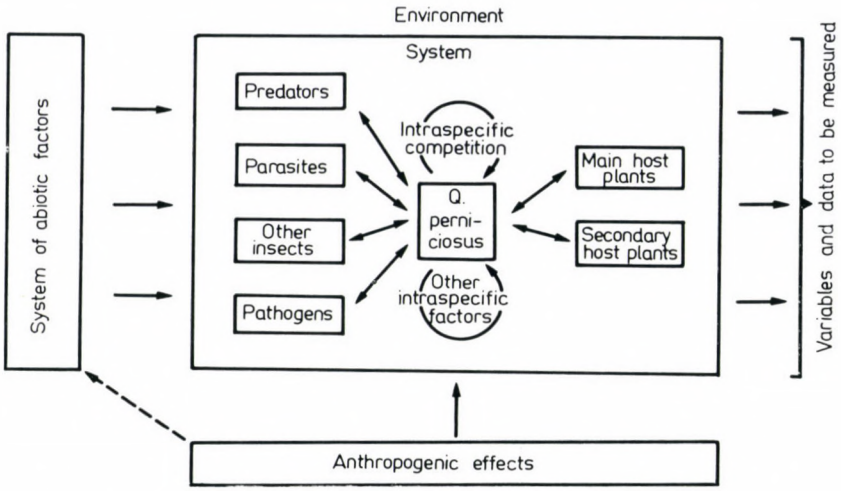


Fig. 2. System of factors determining the population dynamics of *Q. perniciosus*

cidental host plants and regarding the influence of inter- and intraspecific competition.

The relations shown in Fig. 2 manifest themselves to different degrees under different circumstances and these will be discussed in the following, with special regard to the abiotic, anthropogenic and other immediate effects. Our knowledge regarding the indirect effects is rather scanty, as mentioned before.

A) Factors acting in continental dimensions

The potential possibilities of establishment and spread of *Q. perniciosus* are given and determined in continental dimensions basically by abiotic factors.

1. Abiotic factors

The establishment of *Q. perniciosus* has been demonstrated in regions where the average temperature of the coldest month reached −24.2°C with an absolute minimum of −42.7°C,the hottest summer month, however, averaged 31.5°C temperature. The precipitation values changed in these territories between 348 and 2465 mm (SHUTOVA, 1973). The temperature and humidity requirements determine the distribution of *Q. perniciosus* on a given continent.

According for the data of HUBA (1963) a temperature sum of 770°C is required for the development of a whole generation (measured over the temperature threshold of 7.3°C). Therefore, in most parts of its area of distribution, the possibility is given for the development of more than one generation, which

Table 1

Intensity of effects influencing the population dynamics of *Q. perniciosus*, at different geographical-ecological dimensions

Factors	Continent	Zone	Region	Host plant population	Isolated plant population
	A	B	C	D	E
Temperature	+++	++	+	+	++
Other abiotic factors	+++	+++	++	+	+
Host plant	+	+	++	+++	+++
Natural enemies	+	+	++	+++	+++
Interspecific effects	+	+	+	++	++
Intraspecific factors	+	+	+	++	+++
Anthropogenic effects	+	+	++	+++	+++

\+ Low effect ++ Medium effect +++ Strong effect

is very important from the point of view of establishment. So the group of abiotic factors can be regarded as most important in determining the individual density (Table 1).

2. Biotic factors

a. Host plants. Being a polyphagous species, the distribution area of possible host plants surpasses considerably the one of *Q. perniciosus*, not mentioning the frequency of the host plants within its area, so this factor is clearly less significant than the above mentioned.

b. Natural enemies. d. Intraspecific factors. Based on data available, their role is not significant in continental dimensions.

c. Interspecific competition. Its role can be neglected in the formation of population in continental dimensions. Although there are examples known in which animals obstructed the distribution of others as biotic factors by their presence (cf. the expression "niche already occupied" by DOUTT and DEBACH, 1964; MAYR, 1968), regarding *Q. perniciosus* no such data are available, and the appearance of such factors seems very unlikely.

3. Anthropogenic factors

Man has a considerable influence in promoting or retarding the distribution of a given species within the limits of an area determined by the abiotic factors mentioned. With human intervention the species is able even to surpass these limits (possibilities created by glasshouses, irrigation, etc.). In spite of this, in continental dimensions the anthropogenic factors seem to be of low importance on a long run in controlling the reproduction of an already established species.

B. Factors acting in zonal dimensions

In studying the population dynamics of *Q. perniciosus* it is practical to regard those areas as zones independent of region where the species is able to develop in certain number of generations. So zones can be distinguished which permit the development of a single or more (two, three or more) generations; accordingly, these will be called "cold" (northern), "temperate", "subtropical" or "tropical" (southern) zones. The effectivity levels of individual factors are shown in column B of Table 1.

1. Abiotic factors

Their role is in zonal dimensions less significant than in continental dimensions, but they can be still regarded as the most important ones. The climatic conditions of the zone determine the number of generations per year as mentioned earlier and their influence in controlling the individual number of populations in the cold, subtropical and tropical zones. This effect manifests itself in an increased mortality both in northern and southern zones (DEFREITAS, 1964, 1975). The climatic conditions exert their influence in the warmer regions also via the higher number of parasite generations (HUBA, 1969).

2. Biotic factors

a. Host plants. Their role is in some zones somewhat more pronounced, as the cold, northern zone contains besides deciduous forests also many coniferous species, so the number of host plants suitable for mass reproduction is limited. The same is valid also for the subtropical and tropical regions where evergreen plants inhibit the spread of *Q. perniciosus*. The role of host plants is less important from this point of view in the temperate zone as plants suitable for mass colonization are practically everywhere present.

b. Natural enemies. Their role becomes more significant in the cold, tropical and subtropical zones according to the general rules (HUFFAKER and MESSENGER, 1964; MAYR, 1968; MILNE, 1957), but still does not become decisive. POPOVA (1962) reported from the subtropical regions predator activities of 70–98 per cent or parasitization of 90 per cent in some years.

c. Interspecific competition. No concrete studies have been carried out in this direction according to our knowledge; in zonal dimensions no other insect species is known which would exhibit ecological requirements identical with the ones of *Q. perniciosus*.

d. Intraspecific factors. In zonal dimensions the role of intraspecific factors is not significant to our knowledge and is also unlikely.

3. Anthropogenic factors

Their role is not significant in zonal dimensions except in the cold zone where in limited areas (e.g. Poland) the species could be eradicated (HUBA, 1963).

C. Factors acting in regional dimensions

By a region we mean a territory more or less uniform from climatic, pedological or botanical points of view; so e.g. the lowlands of the Carpathian Basin which enable the development of two San José scale generations. The effectivity levels of individual factors are shown in column C of Table 1.

1. Abiotic factors

The climatic conditions of Hungary can be regarded suitable for outbreaks of *Q. perniciosus*. The average temperature of the hottest summer month varies between 16.1 and 22.7°C, while the one of the coldest month between −10.7 and −15.3°C, with an absolute minimum (average) of −32.2°C. The mean precipitation per year varies in the different parts of the country from 496 mm to 843 mm (averages of 50 years, BACSÓ, 1958). These data show that the conditions for the development are considerably more suitable than in the marginal regions of the distribution area (SHUTOVA, 1973).

In Hungary, where according to BACSÓ (1958) the temperature sum of the summer period lies between 2900 and 3500°C (or, by calculating only with temperatures above the 7.3°C developmental threshold: 1700−2300°C) the possibilities for the development of two complete generations and − in some places − of a third incomplete generation are given. Generally, the region suitable for the development of two generations is considered as optimal for *Q. perniciosus* (HUBA, 1963). So it is by no means accidental that the first data on the occurrence are from this type of climate (Austria, Yugoslavia, Hungary, Romania, Spain, Soviet Union, etc.). In the northern areas, where only one generation develops, the distribution slows down because of the prolongation of the 1st larval instar. As this instar is the most sensitive to the adverse abiotic factors, an increased mortality can result from the prolongation. The survival of the species is endangered also by low winter temperature minima.

Although the different climatic factors are able to influence considerably the population densities in special developmental instars (e.g. high summer and winter larval mortality), if the milieu is otherwise suitable, the species is able to recover its initial density in a short time, as mentioned also by HUBA (1969).

The indirect influence of abiotic factors is advantageous also via the host plants and natural enemies.

It may be concluded that in regional dimensions, and especially in the regions of the temperate zone, the abiotic factors play only a restricted role in the fluctuations of density.

2. Biotic factors

a. Host plants. The host plants occurring in Hungary present favourable conditions for the outbreaks of *Q. perniciosus*, so their role in the short-range individual density fluctuations (periods of 1 – 10 years) cannot be significant.

b. Natural enemies. Their role can be important only in biotopes with reduced human influence and also here mostly on not quite suitable host plants. The significance of natural enemies increased in course of the 50 years following the introduction of *Q. perniciosus* (Fig. 1). From this group mainly the parasites and predators have to be mentioned, although in case of high individual densities also high mortalities caused by entomopathogenic fungi can be observed (KOZÁR, unpublished).

c. Interspecific competition, d. Intraspecific factors. Their role seems to be insignificant in regional dimensions.

3. Anthropogenic factors

Their role may be important in the regional dimensions, especially in the long run, via the host plants, e.g. planting or destruction of orchards, afforestation or the expansion of field crops in a given area, introduction of less susceptible fruit varieties, improvement in plant protection, etc. In smaller geographical areas the significance of anthropogenic factors increases in the formation of population density.

D. Factors acting on the host plant level

Host plant populations are defined as relatively isolated areas in which the individual plants are in immediate contact (securing also connections and interactions of faunal elements living on them) and in which the biotic, climatic, etc. conditions are relatively uniform as e.g. in a farm orchard, backyards of a village, neighbouring vineyards.

Each zoocenosis is based on a given plant population (SZELÉNYI, 1957) to which several populations of different ecological roles are linked; this unit, held together by functional relations, is regarded as the base unit of population dynamics. These units make possible the most detailed investigations and form the "corner-stones" of factor analyses on regional, zonal and continental levels. On the host plant level all connections appear in an extremely complicated form. Nearly each factor may become decisive in a given situation or may act with identical effectivity. The connections and roles of different factors are shown in column D of Table 1.

1. Abiotic factors

On the host plant population level their role is insignificant. They may exert an important effect on the population density level in years with extreme weather conditions, but in the long run the population dynamics of the species are determined by other factors.

2. Biotic factors

a. Host plants. On this level their role may be decisive, especially in case of resistant varieties (Table 2). On varieties with medium resistance the population density is determined by a combined effect of many factors. On susceptible species and varieties the density may show big variations caused by other factors, in spite of the possibility of outbreaks. The structure of the host plant population may be also very important in the final outcome of density fluctuations.

b. Natural enemies. In natural biotopes their role may be significant, but cannot be neglected even in biotopes with human influence, if on the given plants the use of insecticides is restricted or if the number of scales is usually low, e.g. in case of sweet or sour cherry. According to the studies of YASNOSH (1976) some Aphelinid species follow the increase in the host population density only to a certain level, then level off their own density (e.g. by decreasing the percentage of females in their population). This observation is supported also by our experiences gained in numerous studies on the parasitization; even in scale populations with a high individual density, parasitization rarely exceeds 30% (KOZÁR, unpublished).

c. Interspecific competition. Within the same orchard or on the same fruit tree the competition between different species may be important. Such interactions are possible between *Q. perniciosus* and the following other scales: *Quadraspidiotus ostreaeformis* Curtis and *Epidiaspis leperii* Signoret. On the other hand, the number of plant species in which this situation may occur is rather restricted,

Table 2

Factors acting on the host plant population level, at different infestations

Factors	Infestation	
	Low	High
Host plant	+++	++
Abiotic factors	++	+
Natural enemies	++	+
Structure of the environment	++	+
Interspecific effect	+	++
Intraspecific effect	+	++
Antropogenic effect	+	+++

\+ Low effect ++ Medium effect +++ Strong effect

primarily apple, pear and plum. Competition with other insect pests rarely occurs as they occupy other "niches".

d. Intraspecific factors. Their role may be significant on the host plant population level. The most data known refer to the intraspecific competition. POPOVA (1962) observed that the increase in the density of *Q. perniciosus* from 1 per sq. cm to 30 (the latter corresponding to infestation level IV) resulted in a decrease of females to 4 per cent. Our preliminary data showed that mortality of crawlers seeking colonization sites increased with increasing population density. In this field our knowledge is rather scanty. In JERMY's opinion (1956) the "psychic" interactions of individuals and populations merit further investigations.

Although at present no genetical differences are known among different *Q. perniciosus* populations, concluded from the examples of other insects, their existence is not impossible and merits also studies in the future.

e. Structure of the environment. In the first place here the frequency of host plants, the presence and the degree of isolation towards the neighbouring *Q. perniciosus* populations have to be considered. The effect of these factors may act in two directions: in case of low densities the increase of the species' density can be accelerated, on the other hand, it can also secure the survival and immigration of natural enemies in places where their presence is possible. The polyphagous parasites need intermediate hosts, the adults of parasitic insects need flowering plants for their maturation feeding. Our knowledge on the environmental effects is rather poor in connection with *Q. perniciosus* populations, in the population dynamics of small areas they become important factors.

3. Anthropogenic factors

On host plant population level the role of anthropogenic factors may be decisive and this can manifest itself in different ways. The density of the species can be directly influenced by chemical or biological control methods (Fig. 3). Anthropogenic factors may act in an indirect way through the planting of host plants or through the agrotechnics applied. These factors show themselves the most conspicuously through planting susceptible host plant varieties, on which the population densities can be hold, nevertheless, on low levels by applying insecticides. Here the controlling effect of man is decisive (Fig. 3).

If, however, other circumstances modify the situation (e.g. hosts of low susceptibility) in case of low population densities the anthropogenic factors are negligible. In spite of chemical plant protection measures the farming-scale cherry orchards in Hungary are infested to 77%. Here, however, the role of natural enemies and of hidden infestation foci increases, the latter especially in neighbouring plant stands (Table 2).

Areas with continuous low or high infestation levels may exist in nature. From highly infested areas, in which the role of natural enemies and environmental factors is negligible, areas of low infestation can be made by human intervention. Although the latter areas are not quite identical with the ones with

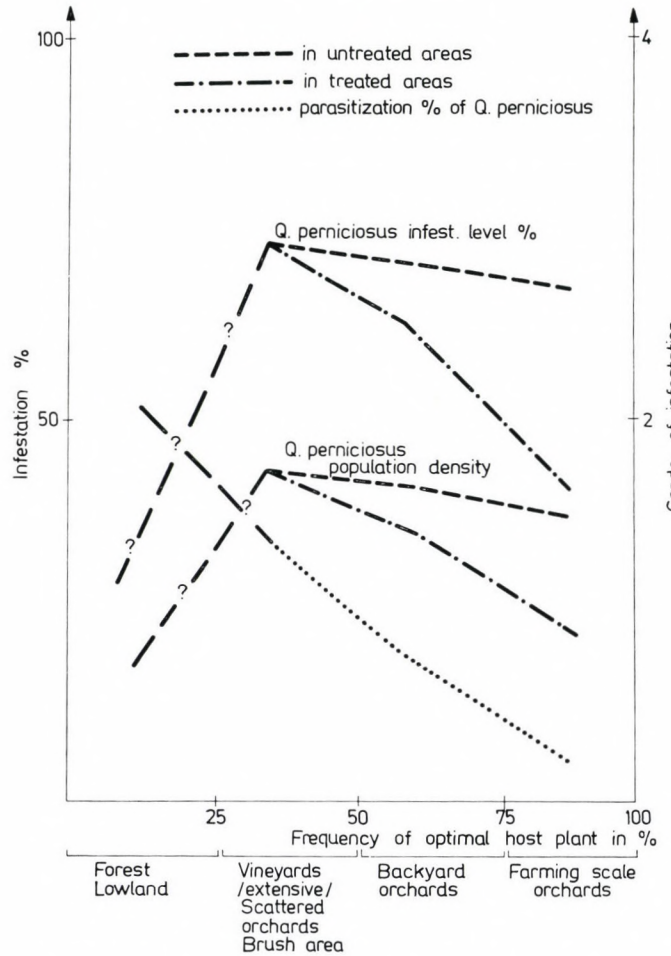


Fig. 3. Frequency of *Q. perniciosus* in different biotope types

"originally" low infestation, the role of natural enemies and infestation foci increases also in their case, according to the law of "change in the control mechanism" of VIKTOROV (1976).

The category "host plant population" covers also populations situated in different biotopes where both the frequency of host plants and of human influence vary. Accordingly, also the frequencies of *Q. perniciosus* and parasite populations connected to the former show variations, as shown in Fig. 3 in which we presented the data of our surveys carried out in 1971. In course of these studies it has been established that the infestation of orchards (in %) and the level of infestation (population density) are the highest in neglected, scattered orchards.

On the other hand, both values were significantly lower in backyard orchards receiving chemical treatments and in farming-scale large orchards.

The parasitization data (%) showed that with the increase of human influence their role diminishes. To a real estimation of their importance the knowledge of their absolute population density would be necessary, such data on the parasites are, however, still lacking.

As shown in Fig. 3 the studies on population dynamics have to be extended to different biotopes because otherwise no clear picture can be formed on the role of the individual factors.

E. Extremely isolated populations

Within the host plant populations very often also extremely isolated populations occur, in which the effect of some factors very appears markedly.

This case occurs not too often with the polyphagous *Q. perniciosus*, especially under Hungarian conditions, although it may happen as in case of a population living on a single *Crataegus* tree in the middle of a sandy plain. The restricted movement of the scale isolates considerably the trees standing apart to a longer distance, this, however, does not apply to the more mobile predators and parasites, belonging to higher trophic levels.

The significance of population dynamic factors is identical in many respects with the ones dealt with under paragraph D, however, there are also substantial differences, because the extreme isolation intensifies many effects. Mortalities up to 100 per cent are not uncommon, as mentioned also by SOUTHWOOD and WAY (1970). The possible factors are presented in column E of Table 1.

1. Abiotic factors

On isolated plant individuals their role again increases even in the temperate zone suitable for the outbreaks of *Q. perniciosus*; while in larger areas (host plant population, region) the eventual high winter mortalities are compensated readily by the surviving populations, these mortalities on single plants can be fatal. Under the conditions of Hungary the possibility of the latter is rather low.

2. Biotic factors

a. Host plant. Its role may be decisive in the fate of the population. On one hand the age of the host plant influences the *Q. perniciosus* population living on it, on the other, the physiological alterations caused by the feeding of the scale may determine the fate of the scale population itself. It is well proven that the physiological status of the host plant influences fertility (POPOVA, 1962) and body size of the females (MAKSIMOVA, 1973).

b. Natural enemies. Their role has not been studied yet from this point of view; it can be assumed that their role is rather restricted.

c. Interspecific competition. The possibility of this phenomenon is rather small in case of *Q. perniciosus.*

d. Intraspecific factors. Their role can be very important on isolated plants. Their effect shows primarily in the fluctuations of mortality, fertility and sex ratio. These effects may prolong for years the low individual densities and expose thus the population to total extinction. (See item a.)

e. Structure of the environment. Its role under extreme isolation is negligible.

3. Anthropogenic factors

These factors can influence decisively the individual densities both by chemical means and by agrotechnical measures (e.g. by cutting off the infested plant parts).

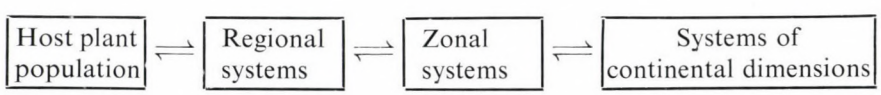
Conclusions

The changes in the individual density of *Q. perniciosus* populations are results of complicated connections between numerous factors.

As the enumeration of the most important factors showed, nearly all of them can be decisive under given circumstances. In other words, the circumstances favour rarely the formation of a numerically determined system of factors but one or the other factor deviates largely from its usual range, becomes decisive and determines finally the fate of the population; this can hardly happen in a large area (MILNE, 1957). Consequently, the factors can exert in this case very seldom a regulatory effect, their function remains merely modificatory. This, however, is in contrast with the opinion of GRUYS (1970): "as separate populations, the density effect must have some regulatory effect, at least theoretically".

The incomplete picture outlined in the present paper confirms our opinion that a reliable model on the population dynamics of the San José scale can be prepared only with a very detailed work for many decades, based on international co-operation in large geographical areas. However, neither the studies of partial connections, nor the partial models constructed from the former can dispense with a uniform, basic point of departure. The connections and their values vary considerably both in time and space; larger spatial connections can be recognized only by decoding the network of local effects.

The system of effects can be constructed from the following interacting units, ranged according to their geographical dimensions:



There is an organic connection between the *regulatory* and *modificatory* factors mentioned and discussed as causes of changes in the population densities within the different spatial units. Each factor has a place and role in the given system, only their importance and effectivity vary according to the different levels, time periods and geographical places.

The horizontal changes of the factors affecting *Q. perniciosus* individual densities can be observed also in vertical directions but while the horizontal effects develop in a large geographical area and are well pronounced, these effects show rapid changes vertically and can be separated often only with difficulties.

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Feeding of Larvae of the Silkworm *Philosamia ricini* Boisd. under the Influence of Different Photoperiods

By

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The effect of photoperiods: 0/24, 10/14, 11/13, 12/12, 13/11 and 14/10 hr light/dark per day on the food uptake, digestibility and growth of *Philosamia ricini* Boisd. was studied. Results showed that the experimental photoperiods were found to produce specific responses on food consumption, and consequently on digestion and growth of larval instars. First and fifth instar larvae consumed more food under short photophase (10 hr), while the fourth instar consumed more food under long photophase (14 hr). A concept of critical photoperiod was displayed by the larvae in all instars in food consumption, digestion and growth by using the 12/12 hr light/dark regime. Photophases more than 12 hr obviously increased the growth of the first four larval instars, while this tendency was altered in the fifth instar.

Introduction

Photoperiods have been known to play a central role in ecological adaptations related to insect growth, activity, determination of both estival and hibernal diapause, host sequence and voltinism. The role of photoperiod in synchronizing food consumption was verified, as the authors are aware, only in *Colaphellus sophiae* Schall. (SARINGER, 1960) and in *Subcoccinella vigintiquattuorpunctata* L. (ALI, 1973). Food consumption in *Ph. ricini* Boisd. is considered to be one of the biotic factors affecting development and silk production (SALEM, 1974), and in turn is influenced by other abiotic factors, therefore, it is worthy to study the role of these factors.

The experimental photoperiods were shown to produce specific responses on food consumption and digestibility of *Ph. ricini* Boisd, although the locomotion and feeding rhythms were found being influenced as stated by many others (HARKER, 1956; ROBERT, 1956; EDWARDS, 1964 and 1965; and MCCLUSKEY 1965).

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Caterpillars of most lepidopterous species are differing markedly in their response to photoperiodic regimes. Growth and diapause in these species are mostly regulated by various environmental factors, particularly by photoperiod (GEISPITZ, 1953; DANILEVSKII, 1961; TOSHIMA *et al.*, 1961; and ZARANKINA and GEISPITZ, 1963).

Materials and Methods

The food consumed, excreta produced and the weight gained by individual larvae per day from hatching till pupation were determined when larvae were exposed to photoperiods of 0/24, 10/14, 11/13, 12/12, 13/11, and 14/10 hour light/dark per day and at a constant temperature of 25°C. Larvae under treatment were reared in boxes (15 × 10 × 5 cm) with a glass cover and two open spaces covered with muslin for ventilation. Food was made available to larvae continuously during the larval period. Dry weights of ingested and digested food were assessed according to the procedure described by WALDBAUER (1964). Briefly the method involved was cutting the leaves of castor oil plant *(Ricinus communis)* fed to the larvae into two symmetrical portions along the midrib. One half was weighed fresh and fed to the larvae, the other half was dried to a constant weight in order to determine the dry weight of introduced food. The difference between the amount of food consumed and faeces produced during the instar was considered as the weight of food digested.

In the expression of results, the indices used by WALDBAUER (1964) were used with some modifications in order to facilitate the discussion of results. The approximate digestibility was calculated as follows:

$$\text{A. D.} = \frac{\text{Approximate dry weight of food digested}}{\text{Dry weight of food consumed}} \times 100$$

Results

1. *Effect of photoperiod on food consumption*

The rhythms of general activity, presumed to be mainly feeding in larvae of *Ph. ricini* Boisd. which is sessile form, appeared to show a quite complex pattern with stage dependent changes. The predilection for feeding throughout every instar, as shown in Fig. 1, might be partially controlled by the experimental photoperiods. The highest amounts of consumed food were conducted in first, second, third and fifth instars by 10 hours photoperiod per day. In the fourth instar the highest amount of consumed food was performed under 14 hours photoperiod. The least amounts of consumed food were performed by 14, 0, 12, 0 and 0 photoperiods in the first, second, third, fourth and fifth instars, respectively (Fig. 1).

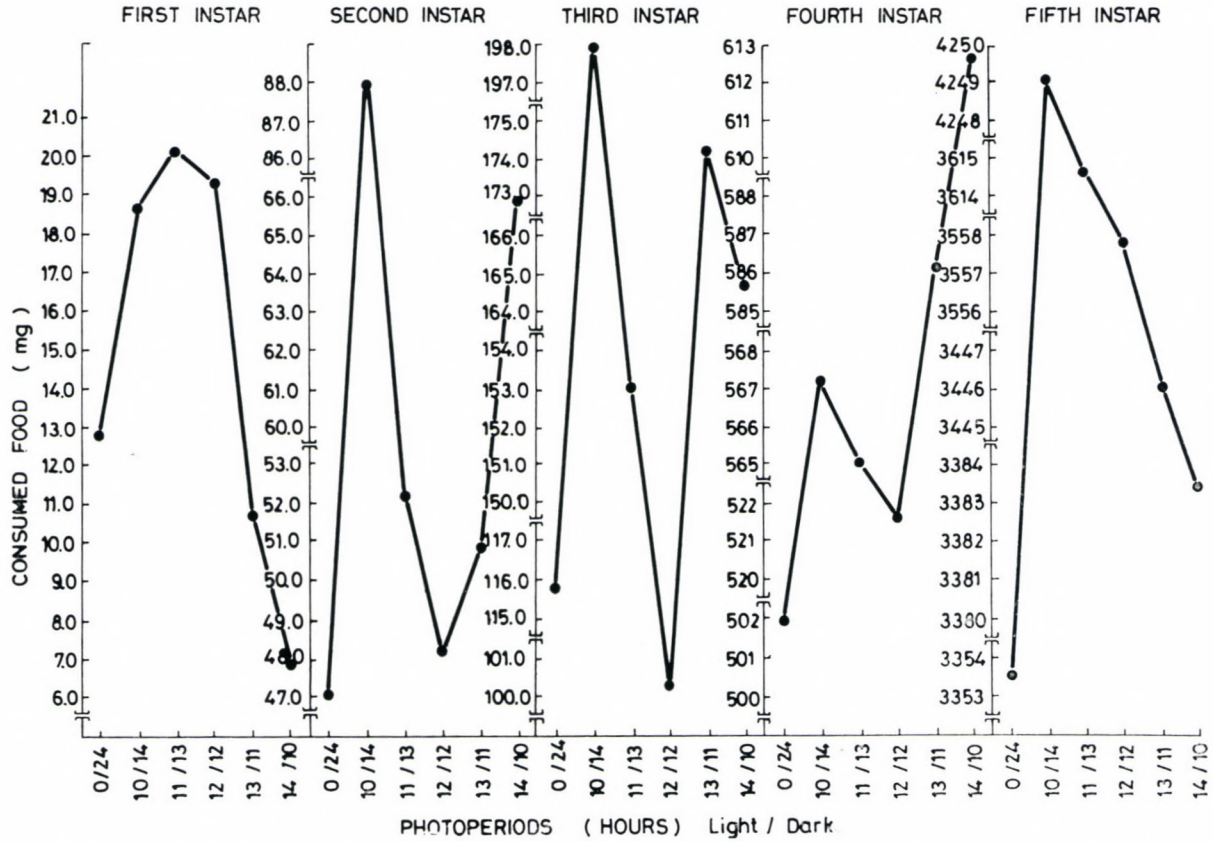

Fig. 1. Effect of photoperiods on the consumed food by larvae of the eriworm *Philosamia ricini* Boisd.

It is worth noting that a concept of critical photoperiod was displayed by the larvae of *Ph. ricini* in all instars. This critical photoperiod, as shown in Fig. 1, was the point in the photoperiodic response curves at which the sign response changed. This critical photoperiod was encountered by using 12 hr photoperiods.

However, the total consumed food (mg dry weight) throughout the larval duration was gradually decreased in the following order from the viewpoint of experimental photoperiods (hrs): 10 (5120.7), 11 (4403.9), 13 (4267.9), 12 (4246.9), 14 (4233.6) and 0 (4020.8). The statistical analysis of the data showed that significant differences prevailed among the averages obtained.

2. *Effect of photoperiod on digested food*

The digestion of food in *Ph. ricini* larvae, as a physiological function which is endogenously controlled, showed an interaction with the exogenous stimuli brought by using different light regimes. As shown in Fig. 2, the photoperiodic response curves of the different instars reflected generally the tendencies depicted in the preceding topic, except in the fourth instar response. It may be of concern to note that values of digested food were not just reflection for consumed food. This result may be illustrated by inspection of the outwards of the digestibility and food consumption curves (Figs 1 and 2). In the first instar, digestibility in larvae under 12/12 regime was extremely downrated than their food consumption. This trend was performed by larvae under constant complete darkness conditions in the second instar. The curve of digestibility was properly upset in the fourth instar.

However, the concept of critical photoperiod, with respect to digestion, was displayed by the larvae of *Ph. ricini* in the different instars and thus reflecting properly the same tendency of 12/12 hr regime effect on food consumption.

The total digested food (mg dry weight) throughout the larval period gradually decreased in the following order: 2538.1 (10/14), 2164.7 (11/13), 1970 (13/11), 1860.2 (0/24) and 1852.9 (14/10). The statistical analysis of the data showed that significant differences prevailed among averages obtained.

3. *Effect of photoperiod on growth*

Weight of larvae could be considered the overall efficiency of conversion of ingested food to body material. This physiological process showed an interaction with the experimental photoperiod regimes.

From the curves of weight responses (Fig. 3), it is evident that weights of larvae in the different instars were correlated with the consumed food (Fig. 1) rather than with the digested food. However, in the first four successive instars the performed weights (mg fresh weight) decreased, in response to photoperiod regimes, as follows: 1174.9 (14/10), 1165.3 (11/13), 1145.4 (10/14), 1111.9 (0/24), 1096.3 (13/11) and 971.0 (12/12). However, in mature larvae the gained growth

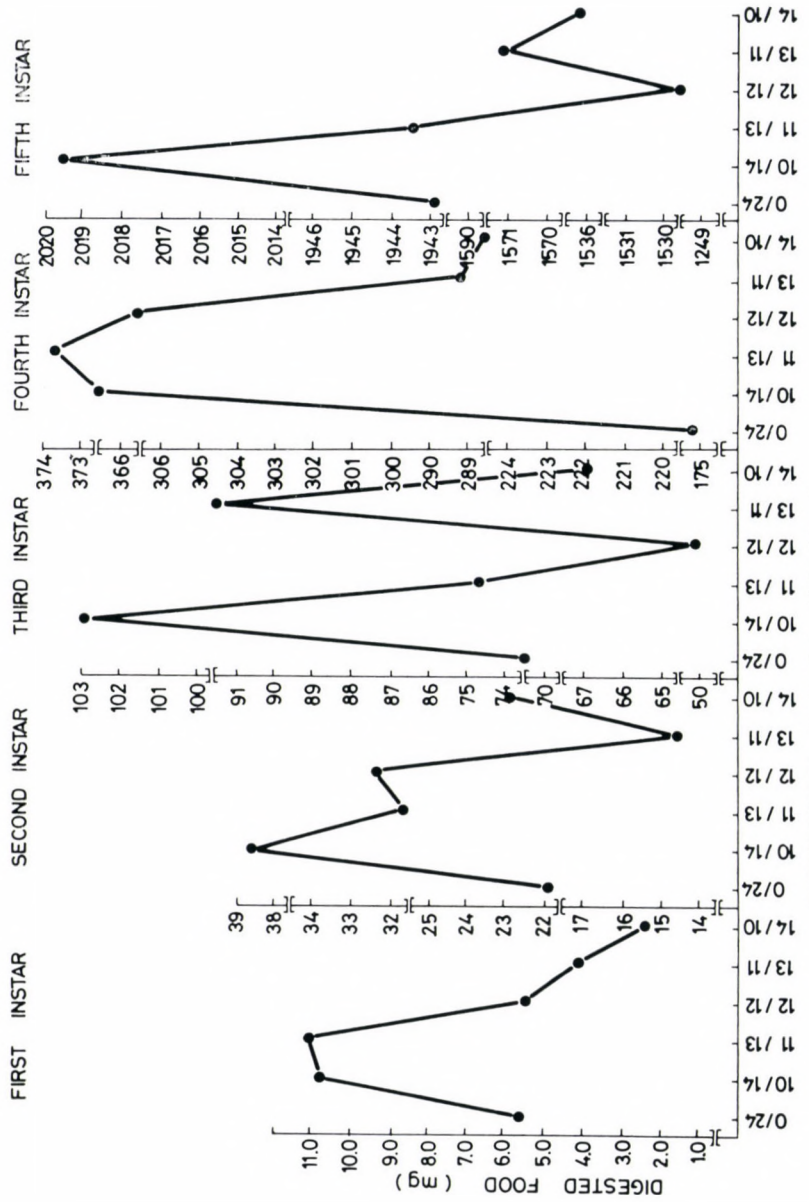

Fig. 2. Effect of photoperiods on the digested food by larvae of the eriworm *Philosamia ricini* Boisd.

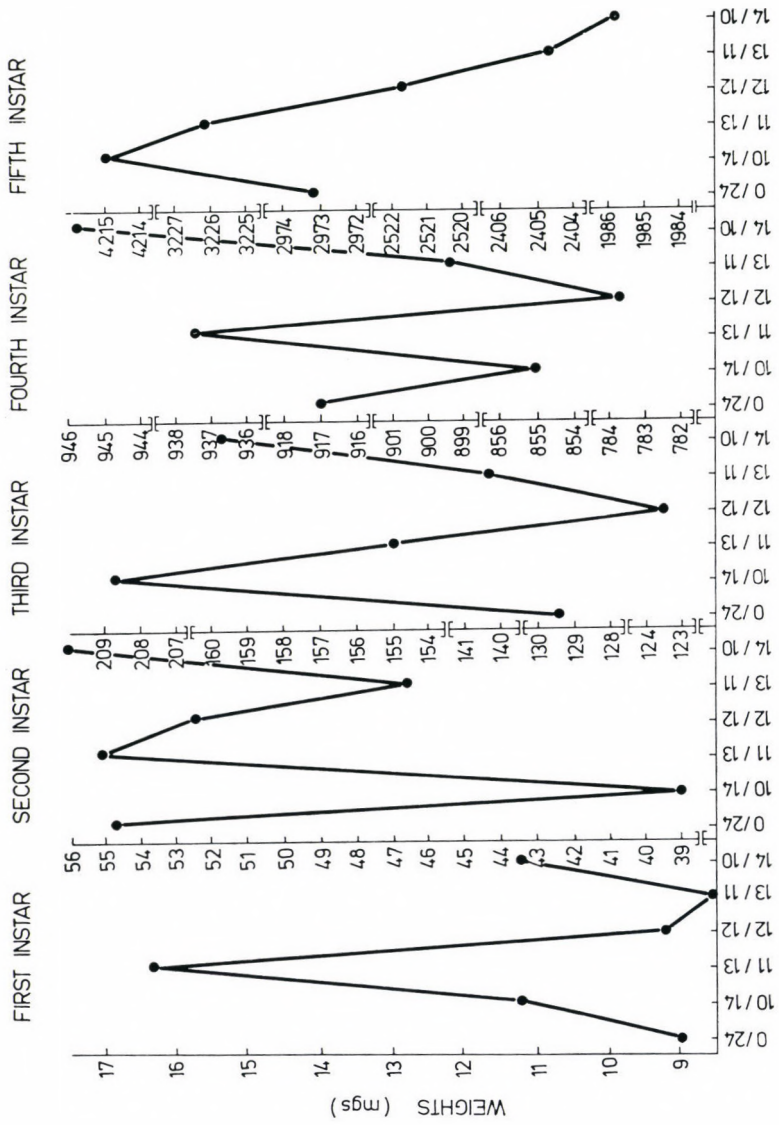

Fig. 3. Growth gained of *Philosamia ricini* Boisd. larvae as influenced by different photoperiods

through the fifth instar altered the previous order and the weights increased as follows: 5360.3 (10/14), 4391.4 (11/13), 4085.0 (0/24), 3500.9 (13/11), 3492.7 (12/12) and 3160.7 (14/10). These averages were significantly different.

Discussion

Behaviour may be regarded as the outward expression of the ability of an organism to respond to its internal and external environment. The external factors can act not only to release behaviour but also to influence motivation. Little is currently known about the specific physiological mechanism by which photoperiod stimuli influence the growth. There is disagreement, however, as to whether or not the photoperiodic effects are always produced because of phase-regulating influences on endogenous rhythmic functions, rather than on reaction systems.

In this work the experimental photoperiods were shown to produce specific responses on food consumption and consequently on digestion and growth of *Ph. ricini* larvae with instar dependent variations.

Although the locomotion and feeding rhythms were found by HARKER (1956) as being influenced by a physiological hunger cycle, yet the possibility does exist that feeding rhythm was affected by photoperiods. Activity patterns were found to be quite complex, with developmental stage dependent changes and also with interaction between temperature and photoperiod, on several species of defoliating caterpillars (EDWARDS, 1964 and 1965). However, the responses obtained under constant darkness could be considered as activities which are endogenously controled and display rhythmicity (HARKER, 1956; MCCLUSKEY, 1965; ROBERTS, 1956).

From the findings of GEISPITZ (1953), ZARANKINA and GEISPITZ (1963) and DANILEVSKII (1961), the caterpillar species differ markedly in the effect of day-length on growth, with some being stimulated by long days and others are inhibited by similar photoperiods. It is likely to conclude that photoperiods affected the food consumption of *Ph. ricini* larvae as reported by SARINGER (1960) in *Colaphellus sophiae* and by ALI (1973) in *Subcoccinella vigintiquattuorpunctata* L. These effects, however, were varied in the different instars, as larvae of first and fifth instars consumed more food under short photophase (10 hrs) while those of the fourth instar consumed more food under long photophase (14 hrs).

The food consumption was properly reflected on growth and the experimental photoperiodic regimes showed variation in growth approximating those reported by SALEM (1974) in his studies on the effect of variation of food regimes on growth of *Ph. ricini* larvae. It could be generally concluded that the first four instars showed a peculiarity, i. e. growth increase by using photophases more than the critical (12 hrs) was higher than growth increase by using photophases less than the critical one, while this tendency was altered in the fifth instar.

The concept of a critical day length displayed by the larvae of *Ph. ricini* in all instars may be considered as the estimate of the response threshold (TOSHIMA *et al.*, 1961; DANILEVSKII, 1961).

Acknowledgement

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Specificity of Sexual Attractant Traps for Signalization of Oriental Fruit Moth (*Grapholitha molesta* Busck)

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Studies were carried out on the specificity of synthetic sexual attractant pheromone for luring of *G. molesta*, and it was also investigated how specificity was, influenced by some factors.

The investigations were carried out first of all in peach plantations, near Budapest. The placing out of the traps was partly on May 2, 1976, partly in the first days of June, and they worked until the middle of October. In course of this time males of 27 *Tortricidae* species — belonging to 11 genera — were captured.

Among the three research areas there were considerable differences, regarding the number of *Tortricidae* species collected by the traps, and consequently, regarding their specificity as well. In agricultural surroundings the specificity was stronger than in the vicinity of forests, but the percentage of the oriental fruit moth increased over 60% only after the middle of September, even in the large-scale peach orchards. In addition to *G. molesta*, the most frequently attracted species were: *G. funebrana*, *Pammene gallicolana*, *P. albuginana*, *Epiblema* (scutulana) and *Cnephasia alternella*.

The population dynamics of this pest can be followed by sexual attractant traps, even at a so little population density at which there is no detectable fruit-damage. In another respect — because of the unstableness of the specificity — the investigation of genitalia is essential to obtain reliable results.

The observation of development of population density by sexual attractant traps is a new, spreading all over the world, for signalization of certain insect method pests. An excellent peculiarity of this method is that it is usable even at a very little abundance, consequently, serviceable even in such orchards in which — at certain periods, because of the regular and intensive chemical control — the presence of the pest is not detectable by other means. It is disadvantageous, on the other hand, that the specificity of most of the synthetic sexual attractanst is unsatisfactory (COMEAU and ROELOFS, 1973; CHAMBON and d'AGUILAR, 1974; ZIVANOVIC, 1974).

In the year 1976, in the Research Institute for Fruit-growing and Ornamentals, Budapest — linked with the researches on dispersion and population dynamics — we studied the degree of specificity of synthetic sexual attractant pheromone for luring of male oriental fruit moths (*Grapholitha molesta* Busck), and investigated how the specificity is changing depending on place and period.

Materials and Methods

The investigations were carried out at Érd–Elvira-major, in the Research Station of the Research Institute for Fruit-growing and Ornamentals, Budapest; in the state farm at the village Törökbálint; and at Nagykovácsi–Julia-major (in the research orchard of the Research Institute for Plant Protection, Budapest). All of the three orchards are located near to Budapest.

At Érd–Elvira-major 4, at Törökbálint 18, at Nagykovácsi–Julia-major 5 traps functioned. The traps were placed out partly (and first of all) into peach, partly into other fruit (apple, apricot, quince) plantations. One of the traps was in the forest close to the orchard at Julia-major. (At a distance of 200 m from the orchard.)

The inside surface of the used cylindric traps (20 cm in diameter, hanged at 1.2 m height) was coated with a non-siccative stick. In the case of the traps at Julia-major the used pheromone was Orfamone (product of Zoecon Corporation, USA), while on the other two places we used pheromone preparation designated as "Atramol" (produced in Romania). The pheromone caps were changed every fifth week. The captured moths were taken out weekly. The placing out of the traps was on May 2, 1976 at Julia-major, on June 1 at Elvira-major, and on June 2 at Törökbálint. They worked until the middle of October (Oct. 15, Oct. 12 and Oct. 14, respectively) in all of the three orchards. The collected material was determined on the basis of genitalia.

Results and Discussion

In 1976 in the sexual attractant traps on the three research areas we found altogether 27 species of *Tortricidae*, belonging to 11 genera (Table 1). From these 27 species only two, *Grapholitha molesta* and *G. funebrana*, may be regarded as pests in orchards. The latter two species occurred in a large amount in all of the three research areas, and further five were found in all of the three orchards (*Grapholitha janthiana*, *Pammene gallicolana*, *P. albuginana*,* *Epiblema scutulana* and *Cnephasia alternella*). (The last four in a large amount.)

Most species (24) occurred at Julia-major, and here was the proportion of *G. molesta* the lowest. At Törökbálint 15, and at Elvira-major 7 *Tortricidae* species flew into the traps.

For the differences among the three research areas ground is given by the disparity of their surroundings and localization: The orchard at Julia-major is a little one, and it is surrounded by hornbeam-oak forests (it is the reason of the large-scale occurrence of *Pammene* species). The orchards at Törökbálint, and

* Most of the authors regard *P. gallicolana* as a synonym of *P. albuginana*, but N. L. WOLFF (1968) cleared that they are different species. We dwell on the systematic and taxonomic problems, arisen in course of investigations, in another paper.

Table 1

Tortricidae species captured by sexual attractant traps for *Grapholitha molesta*, and their number on the three research territories in 1976

Research stations		Törökbálint	Elvira-major	Julia-major	Total
Number of the traps		18	4	5	27
Working period of the traps		VI. 2—X. 14	VI. 1—X. 12	V. 2—X. 15	
Captured *Tortricidae* species	Grapholitha molesta Busck	3890	182	133	4205
	G. funebrana Tr.	3728	578	445	4751
	G. tenebrosana Dup.	8	—	40	48
	G. janthiana Dup.	5	1	5	11
	Pamenne gallicolana Lienig-Z.	212	4	807	1023
	P. albuginana Gn.	156	4	642	802
	P. querceti Gozm.	1	—	50	51
	P. argyrana Hb.	—	—	38	38
	P. fasciana L.	14	—	5	19
	P. inquilina Fletch.	—	—	19	19
	P. gallicana Gn.	10	—	1	11
	P. suspectana Lienig-Z.	—	—	5	5
	P. spiniana Dup.	—	—	5	5
	P. aurantiana Stgr.	3	—	2	5
	P. insulana Gn.	—	—	4	4
	P. aurana Hb.	—	—	2	2
	P. splendidulana Gn.	—	—	2	2
	P. regiana Z.	2	—	—	2
	Epiblema (scutulana* Schiff.)	263	86	16	365
	Cnephasia alternella Steph.	30	122	3	155
	Celypha rurestrana Dup.	3	—	—	3
	Isotrias hybridana Hbn.	—	—	2	2
	Pandemis dumetana Tr.	—	—	1	1
	Paramesia gnomana Cl.	—	—	1	1
	Argyroploce lacunana Schiff.	—	—	1	1
	Apotomis lineata Schiff.	1	—	—	1
	Hedya nubiferana Haw.	—	—	1	1

* The species of the genus Epiblema are not separable surely from one another on the basis of the male genitalia, but the specimens, the wings of which were not examinable because of the stick, proved to be E. scutulana

at Elvira-major are big farms (several thousands, and several hundreds ha, respectively), but while the peach plantations at Törökbálint are bordered by oak forests from one side, the research station at Elvira-major is surrounded only by plough lands.

Apart from the *Tortricidae* species from which only one was captured, or two specimens at Julia-major, and at Törökbálint, moths belonging to the same four genera flew into the traps on all of the three territories. Regarding the number of species, the difference between the two territories investigated by

Atramol (Törökbálint and Elvira-major) is the same as the difference between Julia-major (investigated by Orfamone) and Törökbálint. These facts prove that the differences among the investigated territories do not arise from a difference between the pheromone preparations.

It is a further evidence that in addition to the traps having been worked throughout the examined period, we placed out some recurrent Orfamone-traps too at Törökbálint, and regarding to the captured species, the collected material was the same as in the Atramol-traps in this plantation.

The species-composition of the material, collected by the sexual attractant traps, is not accidental. It is an evidence of this that the majority of the 22 *Tortricidae* species collected in French peach orchards by Orfamone-traps (CHAMBON and d'AGUILAR, 1974) (most of the *Grapholitha* and *Pammene* spp., *Epiblema scutulana*, *Cnephasia alternella*) agree with species collected by our traps (Orfamone and Atramol) in Hungary.

Regarding the weekly development of percentage of most frequent species, and of the genus *Pammene*, we can state that this greatly changed during the examined period (Table 2). Simultaneously, the specificity of the traps, concerning *G. molesta*, changed as well.

G. molesta flew at more than 60% at Törökbálint only after the middle of September, while at Julia-major only at middle of October (when the signalization had no remarkable importance). Earlier this percentage fluctuated between 7.86% and 59.85% at Törökbálint, between zero and 43.90% at Elvira-major, and between zero and 30.00% at Julia-major.

At Törökbálint and at Elvira-major the main factor reducing the specificity is the large-scale flying of *G. funebrana* males into the traps (max. values were 82.01% and 93.67%, respectively). It can cause a problem if only because of it, that segregation of *G. funebrana* and *G. molesta* is not possible reliably on the basis of external morphological characteristics.

On either above mentioned territories *Epiblema scutulana* flew almost constantly, while in June and in the first part of July, *Cnephasia alternella* became dominant at Elvira-major (VI. 22: 79.17%; VI. 29: 61.90%). These two *Tortricidae* species – achording to recent knowledge living only on herbaceous plants – are well separable from *G. molesta* and *G. funebrana* on the basis of the size and the wingpatterns, even if they are more or less permeated by stick of the trap.

G. funebrana flew regularly, and in a large number into the traps at Julia-major too, but on this territory *Pammene* species predominated in the remarkable part of the investigated period. These species sprang from the near forests. Their percentage rose here above 50% in the case of 11 examined weeks, and above 80% in four weeks.

Of course, in the case of the unique definite traps, in definite periods, the percentage of non-oriental fruit moths was even higher. This is demonstrated in Table 3, in which we showed the maximal weekly percentages on average of all the traps, worked on the definite territory, and the maximums in the case of a definite trap.

Table 2

Percentage of the most frequent species, and the genus *Pammene* that flew into the traps on the three research areas

Date of taking out of moths	*G. molesta*			*G. funebrana*			*Pammene* spp.			*Epiblema (scutulana)*			*Cnephasia alternella*		
	Török-bálint	Érd–Elvira	Julia-major	Török-bálint	Érd–Elvira	Julia-major	Török-bálint	Érd–Elvira	Julia-major	Török-bálint	Érd–Elvira	Julia-major	Török-bálint	Érd–Elvira	Julia-major
V. 6.	—	—	0.09	—	—	8.49	—	—	87.74	—	—	1.89	—	—	0.00
V. 14.	—	—	3.95	—	—	4.50	—	—	91.28	—	—	0.27	—	—	0.00
V. 21.	—	—	2.01	—	—	6.78	—	—	90.70	—	—	0.50	—	—	0.00
V. 28.	—	—	7.69	—	—	7.69	—	—	84.62	—	—	0.00	—	—	0.00
VI. 3.	—	—	0.00	—	—	13.13	—	—	66.67	—	—	0.00	—	—	0.00
VI. 8—11.	7.86	0.00	11.11	62.86	45.83	38.89	9.28	0.00	44.44	19.29	29.14	5.56	0.71	20.83	0.00
VI. 15—18.	22.22	2.13	7.14	41.03	44.68	57.14	13.68	2.12	7.14	15.38	40.43	0.00	3.42	10.64	0.00
VI. 22—25.	22.78	3.13	10.38	48.10	10.42	13.79	6.33	0.00	13.79	11.39	7.29	3.45	8.86	79.17	0.00
VI. 29—VII. 2.	59.85	19.05	4.54	32.58	19.05	22.73	3.49	0.00	4.55	1.52	0.00	0.00	2.27	61.90	0.00
VII. 6—9.	37.93	2.86	17.39	46.55	71.43	52.17	2.59	0.00	8.70	4.31	2.86	0.00	8.62	12.90	8.70
VII. 13—16.	29.16	10.42	18.97	53.33	77.08	68.97	10.83	0.00	8.62	5.00	4.17	0.00	1.67	8.33	0.00
VII. 21—23.	27.01	2.70	4.23	33.05	89.19	32.39	35.34	0.00	60.56	3.74	2.70	0.00	0.86	5.41	2.81
VII. 27—30.	50.88	33.33	10.26	27.19	46.67	64.10	18.86	6.67	25.64	3.07	13.33	0.00	0.00	0.00	0.00
VIII. 3—6.	46.41	43.90	5.97	39.06	31.71	55.22	6.88	4.88	35.82	7.66	19.51	2.99	0.00	0.00	0.00
VIII. 10—13.	49.49	16.18	2.07	42.54	64.71	40.69	3.98	0.00	53.79	3.98	19.12	1.38	0.00	0.00	0.00
VIII. 17—19	44.51	1.38	4.69	46.96	91.67	19.59	5.84	1.39	73.44	2.80	5.56	1.56	0.00	0.00	0.00
VIII. 24—27.	29.31	13.02	9.91	65.86	78.70	40.54	1.69	0.59	49.55	3.14	7.69	0.00	0.00	0.00	0.00
VIII. 31—IX. 3.	14.26	1.26	8.79	82.01	93.67	49.45	1.97	1.27	40.66	1.77	3.80	1.10	0.00	0.00	0.00
IX. 7—10.	41.12	0.00	13.16	55.72	85.71	34.21	1.45	0.00	50.00	1.70	14.29	0.00	0.00	0.00	0.00
IX. 14—17.	60.66	17.11	20.63	38.24	78.95	31.75	0.35	1.32	46.03	0.55	2.63	1.59	0.00	0.00	0.00
IX. 21—24.	84.76	68.75	15.15	14.33	31.25	9.09	0.30	0.00	12.12	0.61	0.00	0.00	0.00	0.00	0.00
IX. 28.—X. 1.	90.10	76.47	25.00	9.38	23.53	12.50	0.00	0.00	62.50	0.52	0.00	0.00	0.00	0.00	0.00
X. 5—8.	97.38	91.18	30.00	2.33	8.82	0.00	0.00	0.00	70.00	0.29	0.00	0.00	0.00	0.00	0.00
X. 12—15.	99.60	100.0	66.69	0.20	0.00	0.00	0.19	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00

Table 3

Maximal weekly percentage of the most frequent species, and the genus *Pammene*, that flew into the traps average of all the traps; (1), and the maximal percentage in the case of a single trap (2) (Date of observations in brackets)

		G. molesta	*G. funebrana*	*Pammene* spp.	*Epiblema (scutulana)*	*Cnephasia alternella*
Törökbálint	(1)	99.60% (X. 13.)	82.01% (IX. 1.)	35.34% (VII. 22.)	19.29% (VI. 9.)	8.86% (VI. 23.)
	(2)	100% (X. 13.)	91.96% (IX. 1.)	77.36% (VII. 22.)	70.00% (VI. 9.)	25.00% (VI. 16.)
Elvira-major	(1)	100% (X. 12.)	93.67% (VIII. 31.)	6.67% (VII. 27.)	40.43% (VI. 15.)	79.17% (VI. 22.)
	(2)	100% (X. 12.)	97.06% (VIII. 31.)	16.67% (VII. 27.)	46.16% (VI. 15.)	89.19% (VI. 22.)
Julia-major	(1)	66.67% (X. 15.)	68.97% (VII. 16.)	91.28% (V. 14.)	5.56% (VI. 11.)	8.70% (VII. 9.)
	(2)	100% (X. 15.)	72.22% (VII. 16.)	94.12% (V. 14.)	25.00% (VI. 11.)	50.00% (VII. 9.)

It is to be emphasized from these data that in a definite trap, on September 1, at Törökbálint, the percentage of *G. funebrana* was 91.96% and on August 31 at Elvira-major it was 97.06%. Moreover, at Törökbálint (although here the amount of *Pammene* spp. was not a large one regarding the whole examined period) on July 22 there was such a trap in which the percentage of *Pammene* species was more than 75%.

Comparing the development of the average number/week of *G. molesta* males (that is the development of swarming dynamics) to the development of the average number/week of all *Tortricidae*, collected by traps (Table 4), their maximums coincided only in a part of the cases (at the swarm of the first summer generation at Elvira-major, and at the swarm of the third summer generation at Elvira-major and at Törökbálint).

Corresponding to the above mentioned factors, this fact shows also the snags of the application of synthetic sexual attractant traps.

Conclusions

The specificity of synthetic sexual attractant preparations for luring of *G. molesta* males in general is not satisfactory, and it depends on the surroundings of the orchard, and on the point of time. In agricultural surroundings the specificity is stronger than in the vicinity of forests, but in our investigations the per-

Table 4

Number (trap) week of *G. molesta* and of all *Tortricidae* on the three research areas (The outstanding values underlined)

Date of taking out of moths	*G. molesta*			All of the *Tortricidae*		
	Törökbálint	Elvira-major	Julia-major	Törökbálint	Elvira-major	Julia-major
V. 6.	—	—	0.20	—	—	21.21
V. 14.	—	—	*5.80*	—	—	*146.80*
V. 21.	—	—	1.60	—	—	79.60
V. 28.	—	—	0.40	—	—	5.20
VI. 3.	—	—	0.00	—	—	3.00
VI. 8.—11.	0.61	0.00	0.40	*7.78*	6.00	3.40
VI. 15.—18.	1.44	0.25	0.20	6.50	11.75	2.80
VI. 22.—25.	1.00	0.75	0.60	4.39	*24.00*	5.80
VI. 29—VII. 2.	*4.39*	*2.00*	0.20	*7.33*	10.50	4.40
VII. 6.—9.	2.44	0.25	0.80	6.44	11.67	4.60
VII. 13.—16.	2.06	1.25	*2.20*	7.06	*12.00*	14.05
VII. 21—23.	5.33	0.25	0.60	*20.47*	9.25	*17.28*
VII. 27—30.	6.82	1.25	0.80	13.41	3.75	9.75
VIII. 3—6.	16.50	*4.50*	0.80	35.56	10.25	13.40
VIII. 10—13.	*21.39*	2.75	0.60	43.22	17.00	*29.00*
VIII. 17—19.	21.17	0.25	1.20	47.56	18.00	25.66
VIII. 24—27.	20.22	*5.50*	*2.20*	*69.00*	*42.25*	22.20
VIII. 31—IX. 3.	8.06	0.25	1.60	56.50	19.75	18.20
IX. 7—10.	9.94	0.00	1.00	24.18	7.00	7.60
IX. 14—17.	*24.50*	3.25	*2.60*	40.39	19.00	12.60
IX. 21—24.	15.40	2.75	1.00	18.22	4.00	6.60
IX. 28—X. 1.	9.61	3.25	0.40	10.67	4.25	1.60
X. 5—8.	19.65	7.75	0.60	20.18	8.50	2.00
X. 12—15.	*27.78*	*9.25*	0.80	*27.89*	*9.25*	1.20

centage of male oriental fruit moths increased over 60% only after the middle of September, even in the traps in the large-scale peach orchards.

On the other hand, the sensitivity of the traps, working with synthetic sexual pheromones, is very large. Using this method, the population dynamics of the oriental fruit moth can be followed even at so little population density, at which there is no wed detectable fruit damage. In another respect – because of the unstableness of the specificity – the investigation of genitalia is essential to obtain reliable results.

Acknowledgements

One part of the examinations was carried out in the course of a study tour in the Research Institute for Plant Protection, Budapest. I wish to thank Dr. T. JERMY, Dr. B. NAGY, and Dr. Z. MÉSZÁROS providing the opportunity and conditions for this.

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Evidence and Extraction of a Female Sex Pheromone from the Winter Moth *Operophtera brumata* (L.)

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On the basis of the analysis of mating behaviour and field trapping with females evidence was gained for the existence of a female-secreted sex pheromone in *O. brumata*. The pheromone could be extracted from virgin females and the activity of the extract was verified by laboratory bioassay.

The winter moth is known in Hungary as a common forest pest. Occasionally it also attacks orchards situated near forests. Reichart *et al.* (1974) have found that in 1973 it was the dominant pest in 7 of the 16 plots studied. According to Szontágh (1976) 65 per cent of the captured Geometrids by light traps during 1971 appeared to be *O. brumata*. In the year 1972 its damage-area covered some 25306 ha.

In the present study we provide evidence for the existence of a female-secreted sex pheromone which could be extracted.

Behavioural observations

For elucidating the successive behavioural steps in the mating behaviour we studied the course of about 20 copulations both under laboratory and field conditions. As a result the following stages could be recognized:

(1) Females show a calling position extruding their ovipositors while sitting or slowly creeping upwards on the trunks (Fig. 1).

(2) Males exhibit spontaneous activity running in zigzag patterns on the surface of the trunks and fluttering their wings.

(3) Coming close to a calling female the male nudges her by his antennae and "slaps" her with his wings.

(4) The female remains stationary if receptive (possible tactile stimuli from the male), the male continuously "slapping" her with his wings (this could last up to 5–10 sec).

(5) The male extends his cluspers curving his abdomen toward the female and copulation occurs.

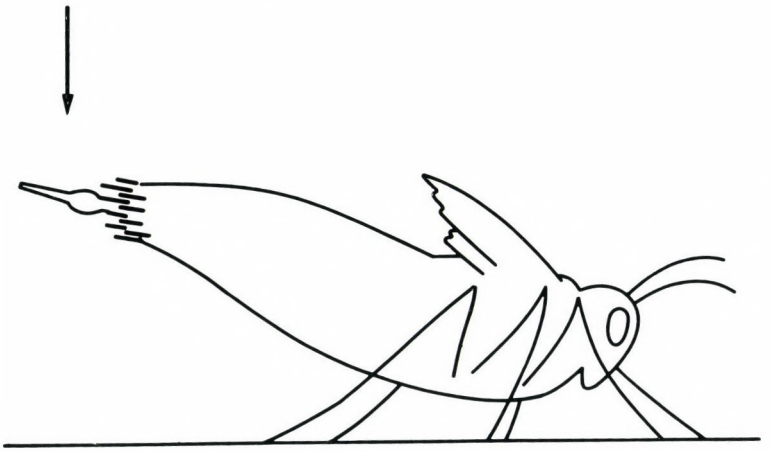

Fig. 1. Typical calling posture of female *O. brumata*. The arrow points to the extruded ovipositor

(6) After some seconds the pair assumes a tail to tail position always with the female upwards. The copulation can last for hours; very often the female drags with herself the male while climbing up to the tree.

The behavioural events prior to copulation were described in detail in two other geometrids, *Lambdina fiscellaria lugubrosa* (Hulst.) (OSTAFF *et al.*, 1974) and *Rheumaptera hastata* (L.) (WERNER, 1977). The mating behaviour of *O. brumata* is by and large similar to that of the two above mentioned species, the main difference being the "slapping" of the female by the male.

Trapping

In order to prove the existence of the pheromone we were conducting trapping studies in a cherry orchard (part of the experimental orchard of the Institute for Plant Protection, Budapest). In a distance of about 100 m there was a forest abundant with oak trees.

The traps used were of cylindrical type, 35 cm long and 20 cm wide with the two ends open. Three females in a wire screen cage joined to a watering bottle for maintaining humidity in the cage were used in the traps as a lure. The control traps contained no females. The females used were field collected ones which had copulated in the preceding night. Earlier laboratory observations revealed that paired females call also during the ensuing nights. The inner bottom surface of all traps was supplied by sticky material. The traps were placed near to the trunks at the heights of 0.5; 1.5; 2.5; and 4 m, respectively. At every elevation three female-baited and three control traps were placed. The captured males were counted after six days.

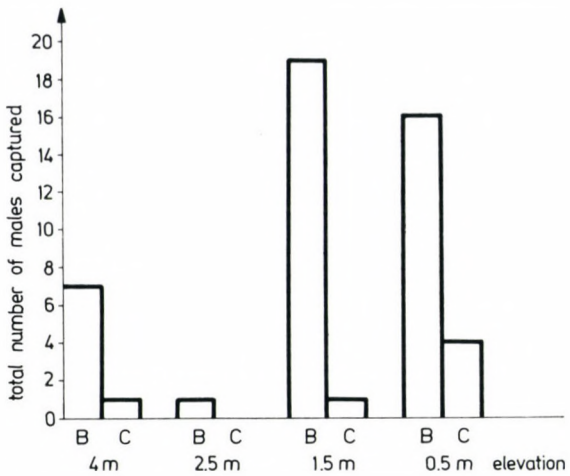

Fig. 2. Number of *O. brumata* males caught by traps baited with females and placed at differen elevations. B = baited, C = unbaited control

The total number of males caught, especially by the traps placed at 1.5 and 0.5 m, clearly indicate the presence of an attractive female sex pheromone (Fig. 2).

This finding seems to be consistent with other results obtained with Geometridae. Successful trapping with females was also reported in *Erannis defoliaria* (Cl.), *Erannis aurantiaria* (Hb.) (TVERMYR, 1969), *Ennomos subsignarius* (Hübner) (GRANETT, 1973), *L. fiscellaria lugubrosa* (OSTAFF *et al.*, 1974) and *R. hastata* (WERNER, 1977).

Extraction

For the extraction of the sex pheromone the abdomen tips of 100 virgin females (originating from a culture maintained on oak leaves and under natural conditions) were excised and extracted in n-hexane. The extract was held in a refrigerator until use.

Biotest

In order to monitor the activity of the extract, a laboratory bioassay technique was developed. Four field collected males were placed in a glass container (200 ml) and the container was covered by a glass lid several hours prior to the beginning of the test. The containers were held at $+7\pm1°C$ and the photoperiod was regulated according to the outdoor conditions (investigations were made during November, 1977). At the time when in the nature most of the copulations occur, a filter paper of 10 × 10 mm with 5 μl of n-hexane was put into the container. The number of males exhibiting locomotor activity and wing

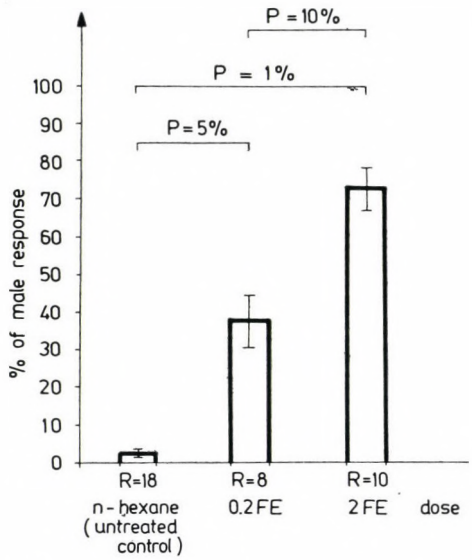

Fig. 3. Response of male *O. brumata* to the sex pheromone extract. Concentrations in female equivalents. Bars = standard deviation, R = repetitions

fanning was registered during the ensuing 60 seconds in order to survey the general spontaneous activity of males which was used as a control. Thereafter a filter paper of the same size with n-hexane extract of the pheromone was added after having allowed the hexane to evaporate. The activity of the males was also observed during the following 60 seconds.

The percentage of male response was 72.5 at a dose of 2 FE which differed significantly from the spontaneous activity at the 1% probability level (P). Significant difference could be found also between the spontaneous activity and the male response obtained with 0.2 FE (P = 5%) and between the responses to 0.2 and 2 FE (P = 10%). According to the data the presence of the sex pheromone in the extract could be verified.

Though sex pheromones have been extracted from *E. subsignarius* (GRANETT, 1973) and *R. hastata* (WERNER, 1977) the chemical nature of the substance(s) was not reported. BIWER *et al.* (1975) report the capture of *Sterrha biselata* (Hufn.) by the synthetic sex pheromone of the grape vine moth, *Lobesia botrana* (Schiff.), E-7,Z-9-dodecadien-1-yl acetate. This is consistent with the data of ANDO *et al.* (1977), who trapped *Sterrha imbecilla* (Inoue) with Z-7-dodecen-1-yl acetate. As neither of these species is closely related to *O. brumata* the structure of the sex pheromone extracted from the latter may not have similar structure.

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Evidence for a Female Sex Pheromone in the Lucerne Seed Chalcid, *Bruchophagus roddi (Hymenoptera, Eurytomidae)*

By

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The existence of a female sex pheromone in *Bruchophagus roddi* has been proved in field experiments.

The lucerne seed chalcid (*Bruchophagus roddi* Guss.) often devastates 20–30 per cent of the seed crop, but occasionally the damage is even heavier (Manninger, 1960; Strong, 1960). The females immigrate into the field only when the first young green pods suitable for egg-laying appear. At that time pollinating wild bees are also present which makes the control even more difficult. Evening application of pesticides of short persistence provides a partial solution only, for the plant-stand suitable for egg-laying will be repopulated soon. However, frequent repetition of the treatment is also not desirable with regard to the protection of beneficial insects.

In search for new control measures experiments were carried out to demonstrate the existence of a sex pheromone in the species. Sex pheromones have already been found in several species of *Chalcidoidea* (Rao and Debach, 1969; Assem, 1970; Hermann *et al.*, 1974).

Materials and Methods

Unmated females emerged mostly in 1–4 days and held at about 5–10°C to the starting point of the experiment were used. The insects were confined into a cage made of wire (D = 3 cm, L = 7 cm, mesh = 0.4 mm). The cage was attached inside a cylindrical metal trap (D = 20 cm, L = 25 cm) supplied with sticky paper sheeting (Stikem-Special, Tanglefoot). The traps were positioned 20 cm above ground level in a 40–60 cm high lucerne stand phenologically suitable for egg-laying where the simultaneous presence of both sexes could be expected. The traps were situated 2 m apart in an approximately 2 ha lucerne field. The evaluation was conducted on the basis of the number of males captured. In 1973 unmated males, and females with males as additional variations were also used. (The number of the insects used per cage was 25 unmated females in 1972 and 1977. In 1973 there were 29 unmated females and males, resp., and

18 pairs per cage.) Significant mortality in the cages was not noted, and the dead insects were not removed. Chalcids were fed honey water solution. Data were recorded at the termination of the experiment.

Results

In two subsequent experiments in Aug. 1972 some 79.9 and 90.9 per cent of the males was found in the traps baited with unmated females, resp. [total number of males captured (n) = 902 ♂♂ and 512 ♂♂, 4 traps per treatment, $p < 0.05$ and $p < 0.01$, resp.]. The results of the experiment in 1973 are presented in Table 1.

Table 1

Results of field trapping on the female sex pheromone of *Bruchophagus roddi* at Kompolt (Hungary), from 17 to 22 Aug. 1973 (4 traps per treatment)

Traps baited with	Mean number (±S.D.) of males per trap
Unmated females	209.0±47.7a
Unmated males	7.8±1.4b
Females+males	12.0±3.2b
Check (with empty cage)	8.3±1.9b

Means (±S.D.) followed by the same letters are not significantly different at the 0.1% probability level. (One way analysis of variance. Random block design.)

There were almost no females captured (0–3 per trap).

No other significant difference was shown out between treatments.

In Sept. 1977 similar highly significant difference was noted in the number of captured males in favour of traps baited with unmated females (n = 67 ♂♂, 3 traps per treatment, $p < 0.001$).

Discussion and Conclusions

The results clearly indicate the existence of a sex pheromone in the unmated females. Females confined together with males did not attract males probably because they lost attractiveness soon after mating. This result is in contradiction with the view of MATTHEWS (1975) that recently mated females of *Pteromalidae* are just as attractive as are virgins.

The lower level of significance found in 1972 may be attributed to experimental error.

Acknowledgements

We thank Dr. Á. SZENTESI and M. TÓTH for their useful suggestions in our work.

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Synthesis of Some Highly Lipophilic N-Cycloalkyl-imidazoles and Triazoles

By

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The synthesis of N-menthyl, N-1-adamantyl and N-cholesteryl derivatives of imidazole and of 1,2,4-triazole is described.

N-alkyl-imidazoles and -1,2,4-triazoles are known to exert a great variety of biologic actions, such as inhibition of cholesterol biosynthesis in humans, inhibition of drug oxidation, insecticide-synergistic effect based on suppression of oxidative detoxication, antifungal action resulting by inhibited synthesis of vital fungal steroles, and others (ATKINS *et al.*, 1972; BUCHENAUER, 1976; BUCHENAUER and GROSSMANN, 1976; WILKINSON *et al.*, 1974a; 1974b). The majority of these divergent effects can be traced back to binding of the imidazole or triazole ring, respectively, to the functional heme of hemoproteins, thus preventing the redox change of the iron atom.

In our previous papers (MATOLCSY *et al.*, 1976; 1977) we reported on our studies on the mechanism of antifungal action of several N-cycloalkyl-imidazoles and -1,2,4-triazoles prepared by us in order to investigate structure–activity relationship within this group. Due to the general biological interest of this type of compounds, we now wish to report on their synthesis.

The compounds synthesized by us represent imidazoles and 1,2,4-triazoles linked with highly lipophilic cycloaliphatic moieties, such as menthyl, 1-adamantyl and cholesteryl, respectively. Structural formulae are shown in Fig. 1.

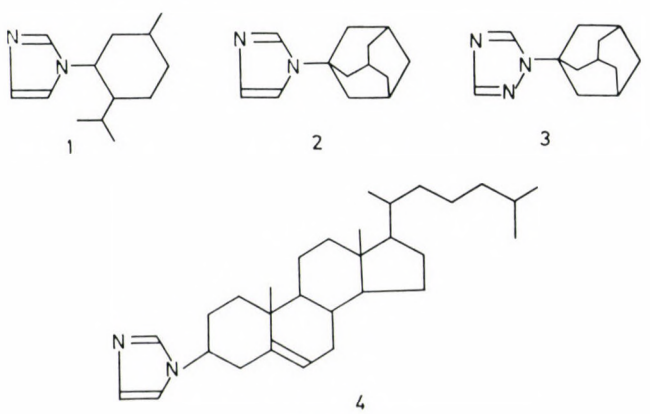

Fig. 1

The synthesis of these compounds was performed by reacting the corresponding cycloalkyl-halide with imidazole or 1,2,4-triazole, respectively, according to the general synthesis principle of N-substituted imidazoles and triazoles. No reaction occurred, however, under standard conditions, therefore drastic conditions were needed, such as heating at 190–200°C of the corresponding halide in excess of the heterocycle, serving simultaneously as solvent and as hydrogen-halide acceptor.

The method applied by us seems to be generally applicable for the preparation of N-cycloalkyl heterocycles from cycloalkyl-halides of low reactivity.

Experimental

N-menthyl-imidazole (1). 8.7 g (0.05 mole) of menthyl-chloride and 17.0 g (0.25 mole) of imidazole were stirred for 3 hours at 180–190°C. After addition of water the product was extracted with ether, the extract dried, the ether was distilled off and the remainder distilled in vacuo. Yield 3.8 g (36.8%). Colorless liquid, B. p. 122–127°C/14 mm.

$C_{13}H_{22}N_2$ (206.3): Calcd. C, 75.67%; H, 10.75%; N, 13.58%. Found C, 75.32%; H, 10.97%; N, 13.33%.

N-1-adamantyl-imidazole (2). A mixture of 6.42 g (0.03 mole) 1-bromoadamantane and 13.62 g (0.2 mole) imidazole was stirred for 2 hours at 190–200°C temperature. The product was treated with water, extracted with chloroform, the extract was dried, the chloroform was distilled off and the product distilled in vacuo. Yield 2.45 g (40.4%). Viscous, colorless oil, B. p. 147–149°C/2 mm.

$C_{13}H_{18}N_2$ (202.3): Calcd. C, 77.18%; H, 8.97%; N, 13.85%. Found C, 76.92%; H, 8.67%; N, 14.03%.

IR (KBr): νCH bands (adamantane): 2920 and 2860 cm^{-1}; $\nu C=N$ and $\nu C=C$ type skeleton vibration bands (imidazole): 1490 and 1455 cm^{-1}.

PMR ($CDCl_3$): δCH: ~1.75 and ~2.05 ppm, 2 × *m* (15 H) (adamantane); δH–5: 6.95 ppm, *d* (1 H), δH–4: 7.00 ppm, 2 × *d* (1 H), δH–2: 7.55 ppm *d* (1 H) (imidazole ring).

N_1-1'-adamantyl-1,2,4-triazole (3). A mixture of 2.14 g (0.01 mole) 1-bromoadamantane and 2.76 g (0.04 mole) 1,2,4-triazole was stirred for 2 hours at 190–200°C. After treatment with water, the product was extracted with chloroform, the extract was repeatedly washed with water and dried. The solution was evaporated to afford a crystalline mass. The crude product (2.2 g) was dissolved in 150 ml boiling ether, the solution was filtrated from the insoluble product, the filtrate evaporated to dryness, the product dissolved in 120 ml abs. ethanol, treated with activated carbon, filtrated and evaporated to dryness at 60–80°C in order to remove the rest of unreacted 1-bromoadamantane by sublimation. Yield 1.48 g (72.8%), M. p. 85–87°C.

$C_{12}H_{17}N_3$ (203.3): Calcd. C, 70.90%; H, 8.43%; N, 20.67%. Found C, 71.03%; H, 8.49%; N, 20.48%.

IR (KBr): νCH bands (adamantane): 2910 and 2840 cm^{-1}; $\nu C=N$ type skeleton vibration bands (triazole): 1490 and 1470 cm^{-1}.

PMR ($CDCl_3$): δCH: 1.80 and 2.20 ppm, $2 \times m$ (15 H) (adamantane); $\delta H-5$: 7.85 ppm, s (1 H), $\delta H-3$: 8.10 ppm, s (1 H) (triazole ring).

Cholesteryl-imidazole (4). A mixture of 4.5 g (0.01 mole) cholesteryl-bromide and 4.1 g (0.06 mole) imidazole was stirred for 2 hours at 190–200°C. The product was treated with ether, the solution washed repeatedly with water, dried, treated with activated carbon and the ether removed by distillation. The crude product was dissolved in 10 ml boiling abs. ethanol and the solution was kept at −10°C for 24 hours. The crystals were separated by suction and discharged. The filtrate was treated with water until complete precipitation and the product was extracted with ether. The ether extract was then dried and the solvent removed by distillation. Viscous, colorless product, which solidifies while prolonged standing. 1.4 g (32.1%).

$C_{30}H_{48}N_2$ (436.7): Calcd. C, 82.51%; H, 11.08%; N, 6.42%. Found C, 82.77%; H, 11.29%; N, 6.58%.

IR (KBr): $\nu(=CH)$: 3110 cm^{-1}; $\nu C=N$ and $\nu C=C$ type skeleton vibration bands (imidazole): 1490 and 1460 cm^{-1}; νCH (sterane skeleton): 2960–2840 cm^{-1} bright, very intensive band.

PMR ($CDCl_3$): δCH_3: 0.70, 0.80, 0.90 and 1.07 ppm, $4 \times s$ ($\sim$ 15 H); δCH(OH): 3.75 ppm, bright (1 H): sterane skeleton H–3; $\delta(=CH)$: 5.40 ppm, bright (1 H): sterane skeleton H–6; $\delta(=CH)$: 6.95 ppm, bright (2 H): imidazole ring H–4.5; $\delta(=CH)$: 7.55 ppm, d (1 H): imidazole ring H–2.

Acknowledgement

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Quantitative Relationship between Structure and Anti-Ecdysone Activity of Triarimol Analogues

By

I. LUKOVITS,* B. TÓTH,** L. VARJAS** and G. MATOLCSY**

The quantitative relationship between structure and anti-ecdysone activity was investigated by the Free–Wilson analysis. For triarimol analogues no common "parent" structure could be found. The calculated group contributions are in good agreement with the experimental data of the earlier study. The method described can be considered as a special type of substructure analysis.

The anti-ecdysone activity of a series of compounds related to triarimol was described in a previous paper (TÓTH *et al.*, 1977), where some relationships between structure and activity were also discussed briefly. The purpose of the present paper is to establish some quantitative structure–activity relationship in this type of compounds enabling us to make predictions on the expectable biological activity of new derivatives too.

Materials and Methods

Calculations

The method proposed originally for molecules of the same "parent" structure (FREE and WILSON, 1964) was applied. According to this approach, the biological activity can be expressed as the sum of the contributions of the substituents and the average activity value of the set of molecules under consideration. Assuming that the activities of N different derivatives are known, the activity of the j-th molecule ($j = 1, \ldots, N$) A_j can be expressed as

$$A_j = \sum_i^n a_i X_{ij} + \mu \qquad (j = 1, \ldots, N), \tag{1}$$

where a_i denotes the group contributions to be determined, μ is the mean activity of the series of compounds considered and n is the number of substituents. The

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Table 1

Compounds	No.	X	R	R_1	R_2	R_3
	1.	OH	—	5-pyrimidinyl	phenyl	2,4-dichlorophenyl
	2.	Cl	—	5-pyrimidinyl	phenyl	2,4-dichlorophenyl
	3.	OOC—CH_2Cl	—	5-pyrimidinyl	phenyl	2,4-dichlorophenyl
	4.	OH	—	5-pyrimidinyl	4-chlorophenyl	2-chlorophenyl
	5.	OH	—	3-pyridyl	phenyl	phenyl
	6.	OH	—	3-pyridyl	phenyl	4-methoxyphenyl
	7.	OH	—	3-pyridyl	4-methoxyphenyl	4-methoxyphenyl
	8.	OH	—	3-pyridyl	phenyl	4-hydroxyphenyl
	9.	OH	—	3-pyridyl	phenyl	4-chlorophenyl
	10.	OH	—	2-pyridyl	phenyl	phenyl
	11.	OH	—	2-pyridyl	phenyl	4-chlorophenyl
	12.	OH	—	phenyl	phenyl	phenyl
	13.	Cl	—	phenyl	phenyl	phenyl
	14.	1-imidazolyl	—	phenyl	phenyl	phenyl
	15.	1,2,4-triazol-1-yl	—	phenyl	phenyl	phenyl
	16.	OH	—	phenyl	phenyl	4-chlorophenyl
	17.	1-imidazolyl	—	phenyl	phenyl	4-chlorophenyl
	18.	OH	—	phenyl	phenyl	2,4-dichlorophenyl
	19.	OH	—	phenyl	phenyl	2-chlorophenyl
	20.	1-imidazolyl	—	phenyl	phenyl	2-chlorophenyl
	21.	OH	—	phenyl	phenyl	3-sulphonylphenyl-phenyl
	22.	1-imidazolyl	—	phenyl	phenyl	3-sulphonylphenyl-phenyl

1. Triarimol
4. Fenarimol
20. Clotrimazol

	23.	OH	—	4-chlorophenyl	4-chlorophenyl	4-chlorophenyl
	24.	OH	—	cyclohexyl	phenyl	phenyl
	25.	OH	—	methyl	phenyl	phenyl
	26.	OH	—	methyl	methyl	phenyl
	27.	OH	—	methyl	methyl	methyl
	28.	OH	—	4-chlorophenyl	$COO-CH(CH_3)_2$	4-chlorophenyl
	29.	OH	—	phenyl	phenyl	4-geranyloxy-phenyl
	30.	OH	—	phenyl	phenyl	4-isopentenyloxy-phenyl
	31.	OH	—	—	—	—
	32.	$OOC-CH_3$	—	—	—	—
	33.	—	H	—	—	—
	34.	—	4-chloro	—	—	—
	35.	—	H	—	—	—
	36.	—	4-chloro	—	—	—
	37.	—	4-methoxy	—	—	—
	38.	—	H	—	—	—
	39.	—	4-chloro	—	—	—
	40.	—	4-methoxy	—	—	—

28. Rospin
31. Du-Ter
32. Brestan

matrix elements X_{ij} are indicating how many times molecule j contains substituent i. In our case X_{ij} equals to 0 or 1, or 2 or 3 ($i = 1, \ldots, n; j = 1, \ldots, N$). Thus matrix X has n rows and N columns ($N = n$).

The interesting feature of these investigations is that the molecules considered do not have any common "parent" structure as defined by FREE and WILSON (1964) but even the central atom (C) can be replaced by another one (Sn).

The procedure described briefly above may be considered as a special case of substructure analysis (CRAMER *et al.*, 1974). In order to determine the unknown values of the group contributions a_i ($i = 1, \ldots, n$) further restrictions have also to be considered:

$$\sum_{i}^{n_k} a_i v_i = 0, \tag{2}$$

where v_i denotes the number of times substituent i occurs at site k in the series of N molecules, n_k is the number of substituents considered at site k. In order to include Eq. (2) into Eq. (1), matrix X must be changed. The new matrix X' has $n-k$ rows and N columns, details are given in the literature (PURCELL et al., 1973). The reduced matrix X' cannot be inverted if all substituents connected to the central atom are considered equal. To avoid singularity of matrix X' the substituents Nos 1, 2, 4, 5, 6, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and substituents Nos 3, 7, 8, 9, 25 as well the substituents Nos 10, 24 were grouped together. Then the group contributions a_i can be determined by using standard regression techniques. With these values of a_i the expected activities of other derivatives which have not been synthetized, can be estimated. The calculations were performed on a CDC 3300 computer by a program written by us.

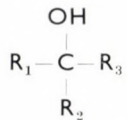
Chemistry

Most of the compounds listed in Table 1 are known and have been described either in literature or in our previous paper (TÓTH *et al.*, 1977). The exceptions are compounds Nos 29, 30 prepared on the analogy of known synthetic methods:

Table 2

		Compound No.	
		29	30
M.p., °C		132–133	110–111
Formula		$C_{29}H_{32}O_2$	$C_{24}H_{24}O_2$
Yield, %		30.6	36.0
Carbon	Calcd.	84.46	83.72
	Found	84.10	83.70
Hydrogen	Calcd.	7.76	6.97
	Found	7.90	7.38

Table 3

Compounds	No.	R_1	R_2	R_3	Retardation ratio (RR)
	24.	cyclohexyl	phenyl	phenyl	1.04
	25.	methyl	phenyl	phenyl	1.07
	26.	methyl	methyl	phenyl	1.00
	27.	methyl	methyl	methyl	1.00
	28.	4-chlorophenyl	$COO-CH(CH_3)_2$	4-chlorophenyl	1.19
	29.	phenyl	phenyl	4-geranyloxy-phenyl	1.31
	30.	phenyl	phenyl	4-isopentenyl-oxyphenyl	1.26

from the appropriate ketones (4-geranyloxy-benzophenone and 4-isopentenyl-oxy-benzophenone) with Grignard reaction (GOMBERG and CONE, 1905; STERLING and PAWLOSKI, 1967).

Structures were identified by methods of elementary, IR and NMR analyses. Physical and analytical data are given in Table 2.

Biology

The biological tests were carried out in the same way as described in previous papers (MATOLCSY *et al.*, 1975; TÓTH *et al.*, 1977). Some of the compounds have not been tested for anti-ecdysone activity and their RR (retardation ratio) values are given in Table 3.

Results and Discussion

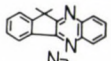
The experimental and recalculated activities are contained in Table 4. The experimental data are in fairly good agreement with the calculated group contributions, with a calculated correlation coefficient between them $R = 0.78$ which is satisfactory regarding the uncertainties of the experimental data and the heterogeneity of the molecules. The standard error of the estimated values is $s = 0.23$. From Table 4 it can be seen that the Free and Wilson model also applies for this rather heterogeneous type of compounds. The calculated group contributions (a_i) are listed in decreasing order of activity in Table 5. The more often the substituents occur, the more reliable is the calculated contribution of the given group. Table 5 shows that groups containing two nitrogen atoms (1, 2) are much more active than the others. Most of the investigated substituents (3–24) do not improve or decrease the anti-ecdysone activity of the molecules considerably. Replacement of the OH group by Cl or $OOC-CH_2Cl$ reduces the biological activity markedly. The differences in calculated group contribution are low. It was not possible to find any correlation between these values and the

Table 4

Compound No.	Retardation ratio	
	experimental	calculated
1.	1.70	1.61
2.	1.10	1.29
3.	1.25	1.25
4.	1.72	1.61
5.	1.25	1.15
6.	1.10	1.15
7.	1.00	1.15
8.	1.00	1.00
9.	1.28	1.18
10.	1.10	1.29
11.	1.50	1.32
12.	1.00	1.23
13.	1.10	0.91
14.	1.21	1.28
15.	1.26	1.26
16.	1.50	1.26
17.	1.21	1.32
18.	1.40	1.29
19.	1.10	1.07
20.	1.09	1.12
21.	1.00	1.21
22.	1.48	1.27
23.	1.25	1.32
24.	1.04	1.04
25.	1.07	1.14
26.	1.00	1.04
27.	1.00	0.95
28.	1.19	1.19
29.	1.31	1.31
30.	1.26	1.26
31.	1.00	1.00
32.	1.00	1.00
33.	1.91	1.68
34.	1.50	1.72
35.	1.25	1.11
36.	0.82	1.11
37.	1.25	1.11
38.	0.95	1.10
39.	0.93	1.13
40.	1.44	1.10

substituent constants defined by HAMMETT (1947). Our earlier investigations show, however, that the anti-ecdysone activity may be related with the lipophilic character of the molecules as it was to be expected on the basis of experimental data.

Table 5

Substituents	No.	Number of occurrences	Group contributions
	1.	2	0.45
	2.	4	0.34
	3.	4	0.07
	4.	1	0.06
	5.	2	0.05
	6.	4	0.04
	7.	1	0.03
−OH	8.	31	0.02
−COO-CH_3	9.	1	0.02
	10.	38	0.01
	11.	14	0.01
	12.	1	0.01
	13.	55	0.00
3SO_2Ph	14.	2	−0.03
	15.	5	−0.05
	16.	5	−0.06
-COO-CH(CH_3)	17.	1	−0.08
-CH_3	18.	6	−0.10
	19.	3	−0.12
	20.	3	−0.14
	21.	2	−0.18
	22.	1	−0.20
	23.	1	−0.21
	24.	2	−0.22
−Cl	25.	2	−0.32
−COO-CH_2Cl	26.	1	−0.35

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Book Reviews

Current Topics in Plant Pathology. Edited by Z. KIRÁLY, Akadémiai Kiadó, 1977. 443 pages. Distributor: Kultura, Budapest, H-1389 POB. 149.

This volume contains the proceedings of the International Symposium held at the Hungarian Academy of Sciences, Budapest, 24—27 June 1975.

Following the opening address of R. K. S. WOOD on the specificity in plant diseases, the majority of presented papers deals with the resistance of plants to pathogens and its physiological background. In the first chapter: Resistance to Fungus Diseases, the problem of "induced resistance and susceptibility" is discussed in connection with the protein synthesis. TANI *et al.* dealt with proteins synthesized in incompatible host that are associated with resistance. BARNA *et al.* reported on inhibitors of host protein metabolism that can reverse the amino acid induced resistance. According to OUCHI *et al.*, the protein synthesis is closely related to accessibility induction, especially in the early stage of infection. The altered DNA metabolism was the subject of the work of YAMAMOTO *et al.* in connection with the hypersensitive reactions. The important role of phytoalexins against infections or against secondary infections is emphasized by STOESSL, ÉRSEK and SCHLÖSSER.

In the second part of the book which deals with the metabolic aspects of fungus diseases the altered photosynthesis of infected plants by obligate parasites (MAGYAROSY *et al.*), the respiration of rust infected wheats (KRISTEV), the modified carbohydrate translocation (FARRELL) are discussed. Increased activity of mitochondrial cytochrome oxidase and peroxidase was detected in *Fusarium* infected wheats by SÁGI and MESTERHÁZY. In the miscellaneous aspects of fungus diseases mainly the effects of environmental conditions on the artificial inoculation are discussed. EL HAMMADY *et al.* describe an antagonism between the cucumber mosaic virus and *Erysiphe cichoracearum*.

In the chapter: Resistance to Bacterial Diseases the mechanism of bacterial HR is discussed. GOODMAN *et al.* reported on the mechanism of immobilization of the incompatible bacterium as a new plant defense mechanism. DURBIN and KLEMENT discussed the different phases of hypersensitive reaction. Ultrastructural differences between resistant and susceptible plants are presented by SIGEE and EPTON.

In the following chapter: Biochemistry and Physiology of Virus Infection, the authors give a review on the protein and RNA biochemistry of the host in relation to host resistance (GIANNINAZZI and MARTIN), viral RNA synthesis (FÖGLEIN *et al.*), cytokinins (WU *et al.*), histones and cytokinins isolated from TMV (BALÁZS *et al.*, SZIRÁKI and BALÁZS). Some papers dealt with the biochemistry of symptom expression especially with virus induced stunting (BAILISS) and the development of necrosis (PAYNOT, SANTILLI, KALPAGAM).

In the last chapter works on mycoplasma diseases are collected. At the end of the book Z. KIRÁLY, the organizer of the Symposium, summarizes the results of the conference and gives a general review of this developing branch of plant pathology.

The book is greatly recommended for theestablished research workers and for advan- students.

R. G.

LUCAS, G. B.: *Diseases of Tobacco*. Third Edition. Harold E. Parker and Sons, Printers Fuguay-Varina, N. C., Raleigh, North Carolina 1975. 621 pages. U.S. $ 30.-

GEORGE B. LUCAS' work "Diseases of Tobacco" ran into three editions between 1958 and 1975. The reviews unanimously had the highest opinion of the book. Since its first edition in 1958 the "Diseases of Tobacco" has been ranked with the standard works. Its importance is underlined by the fact that it deals with the diseases of an important industrial crop which occurs on every continent, is grown in some 87 countries, and its total world production in 1973 was estimated at 10.4 thousand million pounds.

The completely revised and enlarged third edition which appeared 10 years after the second summarizes the status of knowledge of tobacco diseases as of 1975, and presents a broad overview of the latest developments in tobacco disease research. Compared to the second edition this new one is essentially richer and discusses such important recent problems as e.g. *(1)* use of mathematics in epidemiology and disease assessment, *(2)* improved concepts of integrated pest management systems, *(3)* mycoplasmas as disease agents, *(4)* mycotoxins and chemical residues, *(5)* parasexual techniques, *(6)* virus genetics and chemistry, *(7)* role of the hypersensitive reaction and phytoalexins in disease resistance, *(8)* new diseases of tobacco.

The book has 621 pages including six tables, 8 coloured and 270 black-and-white figures, and more than 3000 literary data, and consists of nine parts and 59 chapters, with the following contents:

Part I. *General* (Introduction: Tobacco, The sovereign weed, Seed beds in relation to disease control, Cropping systems in relation to tobacco diseases, Breeding tobacco for disease resistance).

Part II. *Nematode diseases* (Root knot, Tobacco cyst nematode, Brown root rot, Stem-break, Ectoparasitic nematodes).

Part III. *Fungus diseases* (Black shank, Black root rot, Damping-off, Sore shin, Southern stem and root rot, Fusarium wilt, Verticillium wilt, Olpidium seedling blight, Charcoal rot, Miscellaneous root diseases, Blue mold, Brown spot, Powdery mildew, Frogeye, Anthracnose, Miscellaneous leaf diseases, Gray mold, Barn rots, Storage molds).

Part IV. *Bacterial diseases* (Granville wilt, Hollow stalk and black leg, Frenching, Crown gall, Leafy gall or fasciation, Wildfire and angular leaf spot, Philippine bacterial leaf spot).

Part V. *Mycoplasma diseases* (Aster yellows, Stolbur, Big bud and yellow dwarf).

Part VI. *Virus diseases* (Mosaic, Vein banding, Tomato spotted wilt, Cucumber mosaic, Etch, Tobacco vein mottle, Rosette and bushy top, Peanut stunt, Alfalfa mosaic, Leaf curl, Beet curly top, Rattle, Ringspot, Streak, Necrosis, Tobacco stunt, Miscellaneous virus diseases).

Part VII. *Diseases caused by flowering plants* (Broomrape, Witchweed, Dodder).

Part VIII. *Malnutritional diseases* (Deficiencies and toxicities).

Part IX. *Miscellaneous diseases* (Non-parasitic leaf spots, Weather fleck, Bassara, Poisonous fumes, Sulfur dioxide, Diseases caused by weather extremes or other abiotic factors, False broomrape, Genetic abnormalities).

The "Diseases of Tobacco", this fundamental work, is a most useful reference book for plant pathologists, virologists, mycologists, bacteriologists, entomologists, research specialists, teachers, students, producers, — in short, for anyone interested in being informed on the tobacco diseases of the world.

J. HORVÁTH

DIENER, T. O.: *Virology in Agriculture.* Beltsville Symposia in Agricultural Research (BARC Symposia I). Allanheld, Osmun et Co. Publishers, Inc., New Jersey 1977. 293 pages.

The reader will certainly welcome the excellent initiation and excellent execution when taking in hand and reading the book "Virology in Agriculture" which is the first in a series of books that report proceedings of the annual Beltsville Symposium in Agricultural Research. The serious virus diseases occurring in agriculture, e.g. foot-and-mouth disease, Marek's disease, as well as many plant viruses, attracted thousands of researchers to this interesting field of science where highly important results have recently been obtained. The economic importance of virus diseases in agriculture is inestimable, and for this very reason the acknowledgement of the results attained in this field and the importance of the current researches are equally documented by this work which was chosen as the topic for the first Beltsville Symposium in Agricultural Research. In May 1976 more than 200 virologists and other biologists from eight countries met for three days at the Beltsville Agricultural Research Centre (BARC) to discuss the latest information and ideas on virology as it relates to agriculture. This excellent book contains the contributions of 18 speakers invited to the Symposium. Besides 64 figures and 31 tables the book is completed with some 949 references listed after the respective papers.

The texts of the lectures delivered at the Symposium are grouped as follows:

I. *Molecular biology of animal and plant viruses*
   1. Foot-and-mouth disease virus: properties, molecular biology, and immunogenicity by HOWARD L. BACHRACH
   2. Replication of plant viruses — an overview by MILTON ZAITLIN
   3. Plant viruses with multipartie genomes by LOUS VAN VLOTEN-DOTING, J. F. BOL and E. M. JASPARS

II. *Virology in the service of agriculture*
   4. The etiology and control of Marek's disease of chickens and the economic impact of a successful research program by H. GRAHAM PURCHASE
   5. Bovine reproductive diseases by D. G. MCKERCHER
   6. Practical applications of insect viruses by A. M. HEIMPEL
   7. Large-scale culture of insect cells for virus production by J. L. VAUGHN and R. H. GOODWIN

III. *Molecular biology of insect and microbial viruses*
   8. Properties and replication of insect baculoviruses by T. W. TINSLEY
   9. Microbial viruses: physical-chemical properties and modes of replication by E. L. CIVEROLO
   10. Fungal viruses and agriculture by PAUL A. LEMKE

IV. *Approaches to disease problems*
   11. Immunity against porcine enteric viral infections by EDWARD H. BOHL
   12. Recent advances in bovine leukemia research by M. J. VAN DER MAATEN and J. M. MILLER
   13. Defective plant viruses by I. R. SCHNEIDER
   14. Mycoplasmaviruses: virus-cell interactions by JACK MANILOFF, JYOTIRMOY DAS and JAN A. NOWAK

V. *Nucleic acids in disease processes*

15. Deoxyribonucleic acids of baculoviruses by MAX D. SUMMERS
16. Nature of plasmids in phytopathogenic bacteria with special reference to *Agrobacterium tumefaciens* plasmids by CLARENCE I. KADO
17. Translation of the RNAs of brome mosaic virus by PAUL KAESBERG
18. Viroids in agriculture by T. O. DIENER

On the lectures and of the papers and results found in the book special attention is worth being paid to those on the first effective cancer vaccine developed against a destructive neoplasm of chickens, the Marek's disease, the multipartie nature of certain viral genomes first established with a number of plant viruses, the replication of plant viruses, the subviral plant pathogens, the viroids and with the translation of the RNAs of the brome mosaic virus.

The highly valuable work which discusses the fundamental questions of agricultural virology and presents its recent results is indispensable for those who try to solve the theoretical and practical problems of agricultural virology and reduce the losses caused by the viruses.

Thanks are due to all those who took part in the work aimed at making these excellent lectures available for those interested in agricultural virology all over the world.

J. HORVÁTH

GRUNEWALDT-STÖCKER, G. and NIENHAUS, F.: *Mycoplasma-ähnliche Organismen als Krankheitserreger in Pflanzen (Mycoplasma-like organisms as plant pathogens)*. Acta Phytomedica (Beihefte zur Phytopathologischen Zeitschrift; Suppl. J. Phytopathology). Verlag Paul Parey, Berlin und Hamburg 1977. 115 pages DM 44.-

In the last twenty years since the discovery of mycoplasma-like agents in plants a number of comprehensive studies have been published. A recently published German review "Mycoplasma-ähnliche Organismen als Krankheitserreger in Pflanzen" (Mycoplasma-like organisms as plant pathogens) written by GISELA GRUNEWALDT-STÖCKER and FRANZ NIENHAUS, German researchers known the world over, besides giving an excellent survey of the results obtained in the past twenty years in the course of studying the mycoplasma of plants call attention to the necessity of further investigations.

The book contains 8 tables, 36 figures and more than 300 references; the subject is discussed in the following seven chapters: *1.* Introduction, *2.* Nomenclature and properties of Mycoplasmatales, *3.* Properties of mycoplasma-like organisms (MLO), *4.* Host plants of MLO, *5.* Methods of identification, *6.* Control, *7.* Relationships between MLO and other microorganisms as well as viruses.

The book is completed by English and German summaries, a list of references and a subject index.

Of the excellent review compiled by the authors we have to underline the tables which list the natural and artificial vectors (some 76 species) taking part in the transmission of mycoplasmas, as well as the host plants, and give information on the incubation time of mycoplasma in the different vectors and host plants, on the occurrence of mycoplasmas in various plants (about 200 plants), further on the symptoms caused by them, and finally, on the joint occurrence of mycoplasma and virus infection in plants. The English and German descriptions of the symptoms of diseases as well as the proposals on short forms for them are particularly welcomed by those who deal with the symptomatology of mycoplasmas.

The excellent work which presents the most recent information and research results concerning the mycoplasmas is indispensable for those who try to acquire a thorough knowledge of mycoplasmas causing serious damages and losses all over the world, and elaborate methods to control them.

J. HORVÁTH

Plant Disease: An Advanced Treatise (Vol. I: How Disease Is Managed). Edited by JAMES G. HORSFALL and ELLIS B. COWLING. Academic Press, New York—San Francisco—London, 1977. 465 pages.

Since the publication of the first edition of this book 20 years have gone. This new multivolume publication — as it is mentioned in the title — really is a new advanced treatise of plant pathology, which represents a new synthesis of scientific achievements throughout the world. The Advisory Board including distinguished scientists from different countries and the long list of authors, well known specialists of different topics of plant pathology, make this publication a comprehensive account of what is known. At the same time these books may be considered as a challenge of phytopathologists to penetrate even more deeply and to resolve many existing unknown problems of plant diseases.

The first volume includes 21 chapters. Each of them deals with different aspects of disease management; with the sociology and theory of plant pathology, the disease control through environmental management, the management through host genes, chemicals. The book also deals with diagnosis, the damage induced by disease, with use of chemotherapy and heat treatment, with problems of antiviral agents and weed management by pathogens. It covers societal aspects of disease control and education for the practitioners.

This volume, I think, will be a greet contribution to the further development of plant pathology, stimulating the thinking of a new generation of plant pathologists in the last two decades of this century.

L. VAJNA

CO_2 Metabolism and Plant Productivity Ed. by R. H. BURRIS and C. C. BLACK. Univ. Park Press, Baltimore—London—Tokyo, 1976. 431 pages.

This book contains the proceedings of the Fifth Annual Harry Steenbock Symposium, held in Madison (Wisconsin, U. S. A.) in 1975. The aim of the symposium was to integrate the informations on photosynthetic CO_2 fixation, photorespiration and crop productivity. A number of well-known specialists of these fields helped to fulfill this task. Various aspects of CO_2 assimilation (C_3 and C_4 pathways, photosynthetic enzymes and their regulation, photosystems, etc.) and photorespiration were the subjects of fifteen papers. Characteristics and mechanisms of CO_2 assimilation in CAM plants were presented by five contributions, and relationships between photosynthesis, crop growth and yield were discussed in six papers. Although it gives a good survey on present status of knowledge and potential in plant breeding, the paper on the anther-derived haploids is relatively out of the scope of the symposium. On the other hand, such important topics like photosynthetic and energetic problems of N_2-fixation, effect of various chemicals widely used in plant management on photosynthetic CO_2 assimilation, metabolism and plant productivity are dealt with very sparsely, and impact of leaf diseases is not mentioned at all. Studying the proceedings, breeders of both cereal and leguminous plants, crop physiologists and plant biochemists equally can get a deep insight into the complexity of basic problems and processes acting and interacting behind the crop productivity. The obtained results and progress of the research demonstrated by this volume are certainly stimulating, even if the integration of all available and important informations connected with plant productivity could not be completely achieved.

F. SÁGI

MURIEL J. O'BRIEN and AVERY E. RICH: *Potato Diseases*. Agriculture Handbook No. 474. U. S. Department of Agriculture, Washington, D. C., 1976. 79 pages. Price: $ 2.40.

The potato, *Solanum tuberosum* L., is widely grown in the world and it is one of the most important food crops. A number of biotic and abiotic injuries often causes large losses in its yield. A well-founded knowledge of disease symptoms and causal agents is required of everyone being somehow in connection with potato production.

This handbook containing a descriptive compilation of major diseases and related problems of potato, meets the claim and provides useful hints for agricultural experts, scientists, plant protection officials, growers and students as well. The contents cover diseases caused by fungi, bacteria, viruses, mycoplasmas, aviroid, nematode and insect injuries, abiotic factors as well as control measures. The "Modified key to potato diseases and disorders" listing the symptoms of different organs of potato plant and referring to the page where the actual disease is discussed in detail seems to be a useful tool in the identification of the causal agent. This part is followed by a ready-reference table of 8 pages outlining the disease name or disorder in alphabetic order, the causal agent, contributing factor, plant part affected, mode of transmission, seasonal carry-over and control. The detailed part is illustrated by 44 very good colour figures (photos) of disease symptoms. There is also a glossary of scientific terms and there are tables of measurements and dilutions, too.

The Handbook is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D. C. 20402.

T. ÉRSEK

Photoperiodism in Animals and Plants. Proceedings of a Symposium held November 26–29 1974 in Leningrad. Editor-in-Chief O. A. SCARLATO, Editor V. A. ZASLAVSKY. USSR Academy of Sciences, Zoological Institute, Leningrad, 1976. 211 pages.

The Proceedings gives the full text of lectures presented at the Symposium held in Leningrad, in 1974.

The best experts in ornithology, botany and entomology being concerned in photoperiodism had participated in the Symposium.

Among earthly conditions, the alteration of length of the day and night periods and the existence of photoperiodical phenomena suggest that such reactions are regulated by the same or at least similar way in living organisms. The more thorough knowledge of this mechanism regulated by the photoperiodism may give a possibility for the management of the reproduction of harmful or beneficial species, to monitor their developmental processes and for directional selection.

The role of photoperiodism in plants

In the field of botany, the number of articles concerning photoperiodism has exceeded ten thousand. The role of photoperiodism with plants varies greatly. It influences the whole development: flowering, hibernation, optimal circumstances for infection by certain pathogens, etc. A single leaf of a plant may react to a change of photoperiod, or only even one third of a leaf may absorb enough information of this kind (MOSHKOV, B. S.: Actinorhytmism as a general phenomenon in biology). AKSENOVA, N. P. et al. (Photoperiodic reaction of flowering in plants) have found four distinct reaction types. They also studied the influences of photoflashes during dark period. According to V. V. SKRIPTINSKY it might be more successful to study the mode of action of photoperiodism using unicellular organisms (e.g. green algae)

The role of photoperiodism in insects

There was a "two-step universal photoperiodic model" accomplished by V. A. ZASLAVSKY (A model of the photoperiodic control based on the interaction of the activating and inhibiting endocrine centers) on the basis of transitional photoperiodic reactions discovered in recent years. According to ZASLAVSKY's opinion, the new modelcould explain a great number of reaction types that had not been known to exist before. However, the model was strongly criticized by some participants and some of its substantial insufficiencies were pointed out.

Another important event of the Symposium happened to be V. P. TYSHCHENKO's effort to narrow the validity of his earlier, so called "two-oscillatory model" to the sensitive stadium and to quantitative photoperiodic reactions. This model was formerly considered

to be universal. It was established that the quantity of photoperiodic information is not "heat sensitive", i.e. it does not degrade by high temperatures in insects. There was a diapause-inducing substance found in the diapausing eggs of *Calliphora vicina* which, subsequently fed by *Barathra brassicae* larvae, could increase the incidence of short-day diapause.

Numerous data of K. F. GEISPITZ and her co-workers (Ecological principles of the regulation of the active and diapausing stages and seasonal turnover in arthropods) showed the photoperiodic reactions to be extremely diverse according to geographic circumstances, therefore, our recent knowledge of them is rather limited. The physiological mechanism of photoperiodic reactions was also discussed by the contributors. The diapause seems to be controlled by the cerebral and suboesophageal ganglia acting by means of a two-factor hormonal system. It appears that the ratio of hormones is the most important.

The role of photoperiodism in birds

This field is more advanced. One of the reasons for this progress can be that while in insects the photoperiodical reactions are usually studied at the population level, in birds the same processes could be investigated at the level of a single organism. Also more numerous are the physiological data. Attention was drawn to the fact that the measurement of hormone titers in birds could be misleading as there exist a diurnal and a seasonal rhythm.

The Symposium was closed after a fruitful debate. A number of participants has pointed out that it is not feasible to seek similar photoperiodic mechanisms with different organisms. It was also argued whether only the qualitative or also the quantitative reactions would belong to the phenomenon of photoperiodism.

The recent state of research in the field of photoperiodism is well summarised by the Proceedings. As the results of the school founded by A. S. DANILEVSKY in the USSR are recognized throughout the world this collection of the newest results might be of great use for the experts working on both theoretical and applied fields of science. It is regretful that the publication of the Proceedings of the Symposium was somewhat delayed.

F. KOZÁR

USHATINSKAYA, R. S. and YIRKOVSKIY, G. G.: Экология и физиология жука. [Ecology and physiology of the Colorado potato beetle.] "Nauka" Editing House, Moscow, 1976, 130 pp., 32 figs, 387 refs. (In Russian)

This is an excellent concise critical treatise of the present knowledge on the species specific adaptedness of the Colorado potato beetle (*Leptinotarsa decemlineata* Say, Col., Chrysomelidae) to the most important environmental factors. Main emphasis is laid on the ecological and physiological aspects of the ontogenesis especially concerning the following points: Effects of temperature and humidity on the speed of the ontogenesis and on the activity of larvae and adults. Characteristics of the metabolism of different ontogenetic stages. Daily rhythm of oxygen consumption and CO_2 production in active, diapausing, hibernating and reactivating adults. Variations in the sensitivity of different ontogenetic stages to noxious environmental effects, as high and low temperatures, and sublethal doses of toxic substances.

The different types of dormancy, which were thoroughly studied by the first author and her co-workers, are dealt with in details. The following types were found: (1) consecutive (physical) dormancy being the immediate consequence of the influence of environmental factors at values outside the range enabling the beetles' activity, and (2) prospective (physiological) dormancy having two types: aestivation and hibernation, both showing four degrees of dormancy, i.e. sleep, oligopause, diapause and superpause. The role of the photoperiod in diapause induction, the physiological and biochemical characteristics as well as the ecological factors affecting the formation and maintenance of each type of dormancy, and the lability and reversibility of the latter are discussed.

Finally, conclusions are drawn concerning the use of ecological and physiological characteristics of the beetle in the praxis of its control. The necessity of further investigations on the control of the pest with hormones, pheromones, trophic attractants, or substances of non hormonal nature disrupting diapause is emphasized.

This booklet is an extremely valuable source of data not only for insect ecologists and physiologists but also for specialists developing new insect pest control methods.

T. JERMY

BÁNKI, L.: *Bioassay of Pesticides in the Laboratory*. (Research and quality control.) Akadémiai Kiadó, Budapest, 1978. 489 pp., 73 figs, 400 refs.

The increasing importance of pesticides in the world's food production, the overall demand for a steady control of environmental pollution by pesticides and especially of their effect on non-target organisms, augment the need for measuring the toxic effect of pesticides by biological methods besides chemical and physical ones. Although such methods have been widely used for several decades all over the world, a synthesis of logical, metrological, biological and biometrical questions arising in connection with the bioassay of pesticides was still lacking. Basing on extended experimental and theoretical work carried out in that field for thirty years the author succeeded to produce that synthesis in an excellent way.

As a general theoretical approach to the problems of bioassay, the similarities and dissimilarities in the measurements carried out on living and non-living systems, respectively, are discussed. The clear-cut answer to all relevant questions is warranted by the exact definition of the notions used. The laws determining the principles of measurement are traced back to their biological, statistical and logical relations always underlining the interdependence of theory and measurement of pesticide action. By this means the author makes the process of measurements and calculations shown in the examples (Part III) understandable also for experts who are not familiar with all relevant biological, toxicological or statistical theories.

The toxic effect is thoroughly discussed as a dynamic process. The author has clearly proved by many examples that the "biological total effect", i.e. the measurement of the toxic effect as a process in time, gives a much more differentiated picture of it than the usual consideration of the end point (mortality) only. Basing on this principle a new model and new methods of the measurement of additive, synergistic and antagonistic phenomena, equally important in theory and practice, are also described. It is shown that the two kinds of approaches can give quite different results.

It has to be emphasized that the concrete examples taken from experiments with all three main categories of pesticides showing in details the most important methods of computation, make the book a valuable guide for pesticide experts of the third world who often have to work under difficult circumstances. This is the more so since the appendix contains tables with all necessary data for the calculations, thus the use of other manuals is superfluous.

This book is a comprehensive source of knowledge not only for scientists, teachers and factory experts directly dealing with pesticides but also for specialists working in adjacent domains. As a matter of fact the book is useful for all kind of theoretical and applied scientific activities carried out in connection with studying the effect of chemicals on biological systems. E.g. plant protection (research, production and use of pesticides, teaching); human and animal hygiene (all kind of effects of chemicals on man, including labour safety, human genetics, etc.); firms and institutions dealing with research, production or use of chemicals (especially pesticides, drugs and intermediary compounds); organisations of environmental protection (measuring pollution of soil, water and food).

Probably due to the relatively long time gap between the submission of the manuscript and the appearance of the book, a chapter on the use of modern computer methods is lacking. A second edition should be completed accordingly.

The manuscript has been supervised by the distinguished expert in theoretical and practical aspects of medicinal biometry, Dr. I. JUVANCZ.

T. JERMY

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Relationship of Peroxidase and Polyphenoloxidase Activity to Virus Symptoms in Red Clover

By

S. J. SHEEN and STEPHEN DIACHUN

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In red clover-bean yellow mosaic virus combinations, the hypersensitive necrosis clone KyC13 doubled peroxidase level one day after inoculation, whereas the increase of this oxidase in the systemic necrosis clone KyC71-8 was delayed. The mottling genotype KyC36 showed an enhanced peroxidase activity after inoculation, but had no further increase during symptom development. The enhanced peroxidase activity was associated with increase in the banding intensity of cathodic isozymes in the necrosis genotypes but with anodic isozymes in the mottling clone. Viral infection resulted in a slight decrease in polyphenoloxidase activity in KyC36 but increased activity in the necrosis genotypes. Two anodic polyphenoloxidase isozymes appeared in all three clones, and the development of viral symptoms altered binding intensity only. Ethrel treatment and environmental stress activated cathodic peroxidase and in most cases caused a reduction in number and size of necrotic lesions. Our results support other reports that peroxidase activity in plants increases during hypersensitive reaction to viruses and also add evidence that cathodic peroxidase isozymes may play a major role in this reaction, especially necrogenesis.

In a previous paper (SHEEN and DIACHUN, 1978) we reported a correlation of peroxidase (PRO) activity in a number of red clover (*Trifolium pratense* L.) genotypes with resistant and susceptible reactions to an isolate of bean yellow mosaic virus (BYMV). One cathodic PRO isozyme was found to be unique to the resistant clone which exhibited hypersensitive necrosis to the isolate. This isozyme is inherited as a dominant trait and is closely linked with the hypersensitive necrosis factor. Its possible role as a modifier for necrogenesis in hypersensitive reaction was suggested.

The mechanism of hypersensitive reaction to virus infection in plants has been extensively investigated with Samsun-NN tobacco and tobacco mosaic virus (TMV) system (SIMONS and ROSS, 1971; VAN LOON, 1976). It was suggested that necrogenesis in the TMV-infected leaf of hypersensitive tobacco is due to PRO-mediated accumulation of toxic quinones at infection sites as well as in the cells just in advance of virus infection. WESTSTEIJN (1976) recently postulated that PRO mediates the formation of a product, an "inducing" agent which elicits resistance mechanism in the cells neighboring to the infection sites. The importance of PRO in hypersensitive reaction has also been documented in many other host-virus com-

binations (LOEBENSTEIN, 1972; SOLYMOSY *et al.*, 1967; VEGETTI *et al.*, 1975). Most of these studies, however, were limited to comparisons between a local necrosis genotype and a non-necrosis counterpart or manipulated the size of necrotic lesions by alteration of physiological state of the host as it may be related to PRO activity. In red clover, primary local necrosis may or may not cause the localization of BYMV. Local necrosis followed by systemic necrosis is conditioned by a pair of dominant factors which differ from the hypersensitive necrosis factor (DIACHUN and HENSON, 1965; 1974). Therefore, the red clover-BYMV combination provides a unique system to examine the mechanism of virus localization after delineating it from necrogenesis. Furthermore, differences between necrosis and chlorosis genotypes may enlighten the physiological processes of necrogenesis in response to viral infection.

Cell necrosis has been associated with the accumulation of phenolic compounds which are oxidized by PRO and polyphenoloxidase (PPO) to form brown substances. Increase of PPO activity in parallel with necrogenesis in plants hypersensitive to virus has been reported (LOEBENSTEIN, 1972). To delineate the effect of PPO from that of PRO and vice versa on hypersensitive reaction, it is necessary to determine the activity of both oxidases during viral symptom development. The present study attempted to elucidate the association of PRO and PPO with BYMV symptom development in three red clover genotypes of which the host-virus hypersensitive reaction has not been studied. In addition, alterations in activity of both oxidases by Ethrel treatment or environmental variables were measured as they may modify the physiological state of the host and consequently the symptoms of BYMV on red clover leaves.

Materials and Methods

Red clover clones KyC36, KyC71-8, and KyC13 representing systemic chlorosis and systemic and hypersensitive necrosis genotypes in response to the isolate 204-1 of BYMV, respectively, were grown in 8" pots in an environmental chamber under conditions previously reported (SHEEN and DIACHUN, 1978). Clone KyC13 was derived from a cross of KyC40-1 × KyC36. It is heterozygous at both hypersensitive necrosis and cathodic PRO isozyme C_2 loci and differs from KyC40-1 by producing numerous local lesions on BYMV-inoculated leaf. Three plants of each genotype were selected for growth uniformity to constitute a replication and three replications were used. One leaflet of each trifoliate leaf was inoculated with BYMV according to a conventional procedure (DIACHUN and HENSON, 1974), whereas a second leaflet was rubbed with water as control. Eight leaflets, either inoculated or control, were collected from 1/6 sector of the plant at 0, 1, 2, 4, 6, and 8 days after inoculation and stored at −70 °C until analysis.

For preparation of crude oxidases, frozen tissues were transferred into liquid nitrogen prior to extraction. This facilitates tissue pulverization and extraction which were carried out with cold mortar and pestle in 0.1 M Tris-HCl buffer, pH 8,

containing 0.1% each of ascorbate and cysteine-HCl. The homogenized slurry was centrifuged at 20,000 *g* for one hour. The supernatant fluid was subsequently passed through a Sephadex G-25 column (150 × 20 mm) equilibrated with 25 mM solution of the same buffer. The greenish solvent front containing large molecular weight proteins was collected as the crude oxidase preparation.

Peroxidase and PPO were assayed with spectrophotometric methods (SHEEN and CALVERT, 1969). Oxidase activity is expressed on the basis of protein which was quantified by LOWRY's method (1951) using bovine serum albumin as standard. The same oxidase preparation in the amount of 100 μg and 200 μg protein was applied to polyacrylamide gel slab electrophoresis to determine PRO and PPO isozymes, respectively. Electrophoretic techniques and the detection of PRO and PPO have been detailed previously (SHEEN and CALVERT, 1969).

The effect of altered levels of endogenous PRO on viral symptom in KyC13 was studied by spraying 1,000 ppm Ethrel, an ethylene-releasing agent, in 0.1% Tween-20 solution at 1, 2 and 4 days before inoculation. Plants which received 0.1% Tween-20 only served as control. Leaf samples from three plants for each treatment were taken at the time of inoculation for oxidase determination, and six days later the number of local necrotic lesions was counted. Clones KyC13, KyC36 and KyC71-8 grown in a greenhouse in early summer showed various degrees of weather fleck due to air pollution. Leaf samples taken from these plants for oxidase assay were sorted into classes of severe, mild, and no visible injury. Meantime, the plants were inoculated with BYMV for observation of symptom development.

In other experiments, leaflets of trifoliate leaves were treated with Kelthane, a miticide, by a camel-hair brush, whereas the neighboring leaflets were rubbed with water as control. Two days later leaves were collected for oxidase determination. Effect of moisture levels on oxidase activity was conducted with nine plants in 4" × 4" pots of each genotype which were divided into three groups subjected to drought stress, excessive water and normal moisture conditions for one week prior to leaf sampling. Both miticide and moisture experiments were conducted in environmental chambers with conditions similar to aforementioned experiments.

Results

Changes of PRO and PPO activity during symptom development. Clone KyC13 maintained a near constant level of PPO activity up to six days after inoculation; thereafter its activity increased in parallel with the development of necrotic lesions (Fig. 1). Activity of PRO doubled within 24 hours after inoculation, and further increase coincided with necrogenesis which became evident at the fourth day. The death of cells neighboring to the primary necrotic lesions was recognizable one week after inoculation. The same pattern of symptom development was observed in KyC71-8 (Fig. 2). The PPO activity was stimulated in KyC71-8 during the initial 48 hours of viral infection. During the same period the PRO level was unchanged; however, it became elevated during necrogenesis. The control leaf of

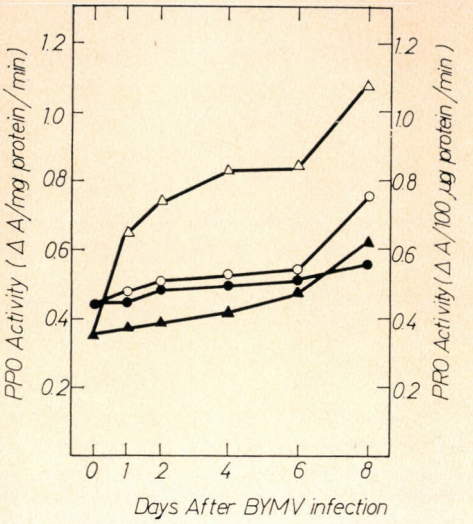

Fig. 1. Activity of PRO (△—△) and PPO (○—○) during the development of hypersensitive necrotic lesions in KyC13 leaves infected with bean yellow mosaic virus. Solid symbols represent experimental controls

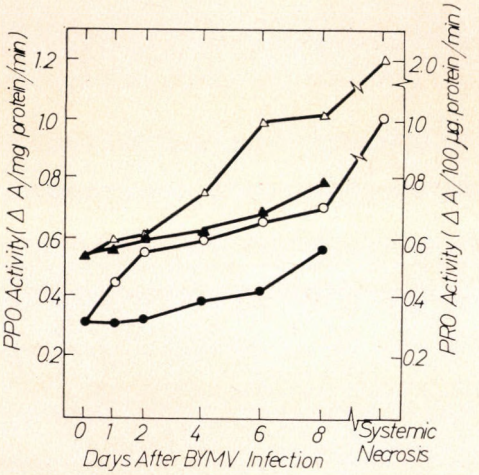

Fig. 2. Activity of PRO and PPO during the development of primary and systemic necrotic lesions in KyC71-8 leaves infected with bean yellow mosaic virus. Leaves showing systemic necrosis were samples three weeks after inoculation. Symbol designation as in Fig. 1

KyC36 contained an extremely low level of PPO (Fig.3). Viral infection caused a decrease in its activity. Peroxidase activity in KyC36 was stimulated soon after infection; however, further increase that occurred in the other two genotypes during necrogenesis was not observed. The mottled leaves of KyC36 maintained the same level of PRO but slightly increased PPO activity. Both oxidases were greatly increased in the KyC71-8 leaves showing systemic necrosis.

Isozyme pattern of PRO and PPO during symptom development: Comparisons between control leaves and those exhibiting mottling or necrosis revealed that viral infection changes PRO isozymes only in a quantitative manner. Anodic isozymes

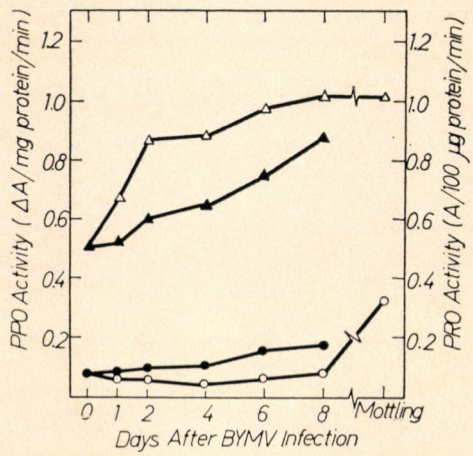

Fig. 3. Activity of PRO and PPO after bean yellow mosaic virus infection and the appearance of mottling symptom three weeks later in KyC36 leaves. Symbol designation as in Fig. 1

intensified in mottled leaves, while cathodic ones appeared in the senescent and virus-free leaves of KyC36 (Fig. 4). Leaf necrotization in either primary lesion or systemic infection was associated with enhanced banding intensity of cathodic isozymes. Systemic necrosis also resulted in an intensified A_3 band. Under the identical electrophoretic conditions, the oxidase preparation resolved two PPO isozymes migrating toward the anode irrespective of viral symptoms. Nevertheless, the hypersensitive necrosis genotype differs from the susceptible ones by having a slower mobility of the slow-moving band.

During the symptom development, the change of PPO isozyme pattern was quantitative for all three genotypes. The effect of viral infection on PRO zymogram is illustrated by densitometer tracing of the representative stages as shown in Fig. 5. The necrosis genotypes shared a common feature in that the development of primary necrotic lesions had little effect on the intensity of anodic isozymes but enhanced the intensity of cathodic bands, especially during necrogenesis. A slight

increase of cathodic banding intensity also occurred in control leaves, possibly as a result of leaf maturation and senescence. The initial increase of PRO activity in BYMV-infected KyC36 (Fig. 3) can be correlated with the intensification of A_2 band.

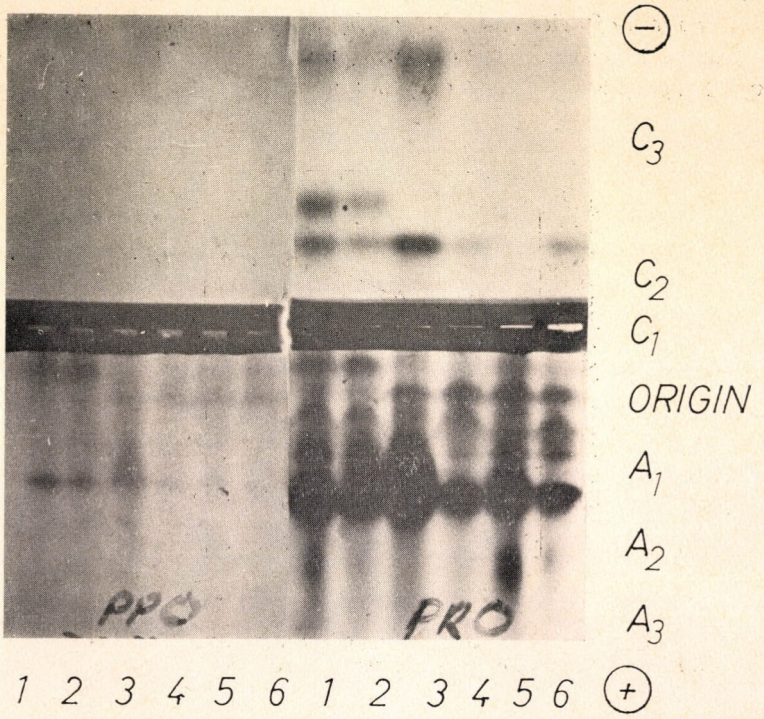

Fig. 4. Zymograms of PPO (left-half gel) and PRO (right-half gel) isozymes in bean yellow mosaic virus-infected and healthy leaves of three red clover clones. The samples are (1) KyC13 showing hypersensitive necrotic lesions; (2) healthy KyC13 leaves; (3) KyC71-8 leaves showing systemic necrosis; (4) healthy KyC71-8 leaves; (5) mottled KyC36 leaves; and (6) healthy KyC36 leaves. The designation of anodic (A_1 to A_3) and cathodic (C_1 to C_3) PRO isozymes is the same system described in a previous paper (SHEEN and DIACHUN, 1978)

Effect of Ethrel on symptom development. Ethrel induced increases in activity of PRO and PPO in KyC13 (Fig. 6). This coincided with an increase in isozyme banding intensity; however, in the case of PRO the cathodic bands intensified to a much greater extent than the anodic ones. Endogenous levels of PRO at the time of inoculation can modify lesion number and size. Ethrel treatment resulted in fewer necrotic lesions when inoculation was performed within two days after treatment. When treatment was applied four days prior to inoculation, the number of necrotic lesions was greater than the control leaf. Lesion number appeared to be positively

correlated with lesion size, although no attempt was made to measure lesion size. In a separate experiment where KyC13 plants were treated with Ethrel 12 hours before inoculation, the number and size of necrotic lesions showed greatest reduction.

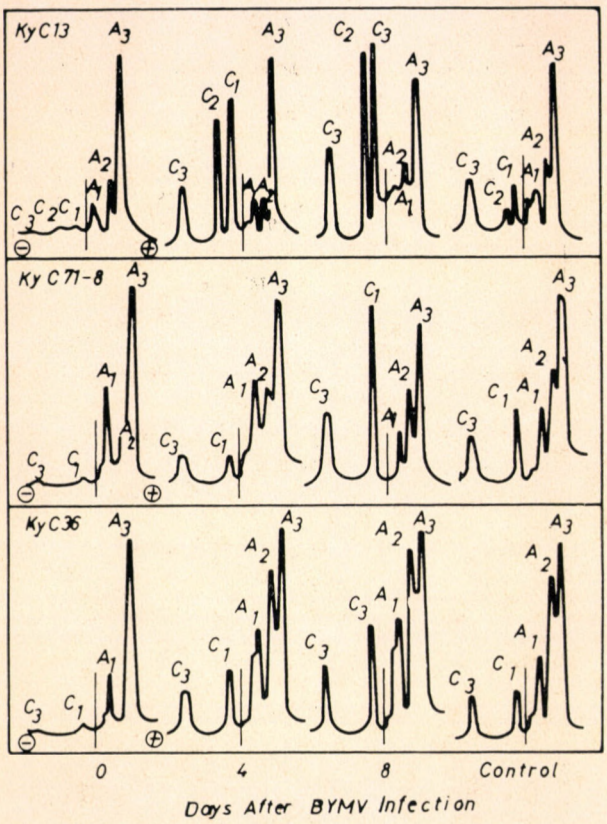

Fig. 5. Densitometric tracings of PRO zymograms with the Photovolt densitometer model 530 and the Varicord 43 recorder. Control represents the non-inoculated leaflets of the 8th day sample

Alteration of oxidase activity due to environmental stresses. Table 1 summarizes the oxidase activity in red clover clones subjected to environmental stresses. The degree of injury due to air pollutants was parallel with increase in PRO level in the leaves of all three genotypes. Air pollutants caused a decrease of PPO activity in KyC13 but a slight increase in the susceptible clones. Upon BYMV infection the non-injured leaves developed primary necrotic lesions, whereas symptoms were not detectable against the background of necrotic weather fleck in the pollutant-injured tissues. However, the necrotic lesions attributable to viral infection became

Table 1

Effects of environmental factors on oxidase activity in red clover

Clone	Activity of PPO (ΔA/mg protein/min) and PRO (ΔA/100 μg protein/min)					
	Air pollutants					
	No injury		Moderate injury		Severe injury	
	PPO	PRO	PPO	PRO	PPO	PRO
KyC13	0.52	0.42	0.20	0.94	0.17	1.03
KyC71-8	0.35	0.72	0.36	0.78	0.49	0.96
KyC36	0.08	0.71	0.09	0.76	0.10	1.52
	Miticide					
	Control		Treatment			
	PPO	PRO	PPO	PRO		
KyC13	0.44	0.39	0.30	0.61		
KyC71-8	0.41	0.54	0.33	0.71		
KyC36	0.09	0.59	0.07	0.84		
	Moisture conditions					
	Normal		Water stress		Excess water	
	PPO	PRO	PPO	PRO	PPO	PRO
KyC13	0.57	0.52	0.12	0.69	0.05	1.32
KyC71-8	0.32	0.61	0.28	0.68	0.21	0.82
KyC36	0.10	0.89	0.08	0.88	0.07	1.01

enlarged and distinguishable from weather flecks when the inoculated plants were kept in darkness several days after inoculation.

Both miticide and abnormal moisture conditions enhanced PRO content in red clover clones. On the contrary, PPO activity decreased in all cases. Miticide-treated leaves of KyC13 produced few lesions or failed to show visible viral symptoms. Moisture stress resulted in growth abnormality so that inoculation tests were abandoned. All the above environmental stresses failed to modify the isozyme patterns of both oxidases. The increased PRO activity again caused a great enhancement in banding intensity of cathodic isozymes.

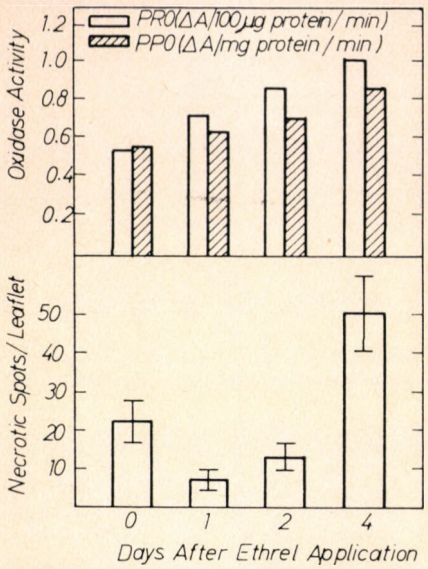

Fig. 6. Effects of Ethrel treatment on the oxidase activity and the number of necrotic lesions per leaflet in KyC13. Experimental procedures are described in Materials and Methods

Discussion

The pattern of change in PRO and PPO activity during the development of BYMV symptoms exhibited a distinctive contrast among the three red clover genotypes. That the PPO activity was either stimulated or suppressed by viral infection in the susceptible clones or increased slightly in the hypersensitive genotype suggests a minor role of this oxidase in hypersensitive reaction, if any. This supports other's findings (JOCKUSCH, 1966; LOEBENSTEIN, 1972). Necrotization of infection sites with or without virus localization, as is the case in KyC13 and KyC71-8, respectively, differed from each other in the timing of PRO induction. This is especially evident during the initial 24–48 hours after inoculation, at which time active virus multiplication and cell to cell movement would be expected. The rapidity of increasing PRO activity at infection sites and, subsequently, in neighboring cells seems to be associated with virus localization. This is in agreement with the conclusion drawn from investigations on hypersensitive reaction in the tobacco-TMV combination (SIMONS and ROSS, 1971; VAN LOON, 1976).

Hypersensitive reaction to virus infection is usually associated with necrosis at or near infection sites. Necrotic substances were found to be a complex of protein and quinone as reported in tobacco leaves infected with tobacco streak virus (HAMPTON, 1970). Quinones are the products of polyphenol oxidation which is catalyzed by PRO and PPO. These oxidases may become the protein moiety of the

necrotic substances as a result of product inhibition (SHEEN, 1974; WEYBREW and LONG, 1970). On the basis of total leaf proteins, the relative concentration of PRO in healthy and diseased tissues is much greater than that of PPO. This renders PRO as the major oxidase for oxidation of polyphenols. This is substantiated by its quantitative increment preceding the appearance and enlargement of necrotic lesions in the virus-infected leaves of KyC13 and KyC71-8.

Quantitative changes of PRO activity can be correlated with increase in banding intensity of cathodic isozymes in the necrosis genotypes. Isozymes migrating toward the cathode under the present electrophoretic conditions at pH 8.6 must be rich in basic amino acids in their composition. It has been suggested that primary and secondary amines can be readily substituted at the 6'- position of the quinones derived from polyphenol oxidation (PIERPOINT, 1966). If so, the more basic amino acids, the greater the chance will be for the protein to complex with quinones. Polymerization of quinone-protein complexes is believed to be the cause of cell necrosis. On the basis of these chemical reactions, the increased activity of cathodic PRO isozymes would be favorable for necrogenesis. The presence of an extra cathodic isozyme C_2 in KyC13 could enhance the process of necrobiosis.

Questions may be asked as to the possible role of cathodic PRO isozymes in virus localization. Enhanced activity of cathodic PRO isozymes was reported in the systemic acquired resistance to TMV of tobacco plants carrying the hypersensitive necrosis factor (VAN LOON, 1976). This acquired resistance was characterized by reduced lesion number and size, suggesting that virus localization takes place sooner after challenging inoculation than in primary infection (SIMONS and ROSS, 1971). The same explanation may be applied to the present results that the activated level of PRO, most of which are cathodic isozymes, by environmental factors reduced the number and size of local lesions in KyC13. However, an exception is that in the plants treated with Ethrel four days before inoculation, lesion number and size increased. A similar phenomenon was observed in tobacco where the effect of ethylene on the development of necrotic lesions can be either stimulative or inhibitory, depending on the time of application and the physiological state of the plant (PRITCHARD and ROSS, 1975). The possible association of cathodic PRO with virus localization and necrogenesis is also substantiated by the absence of these isozymes in KyC36 showing a mottling symptom. The increased activity of anodic PRO in virus-infected leaves of KyC36 obviously contributes nothing to hypersensitivity. This points to the necessity of examining both cathodic and anodic isozymes as they are related to the increased PRO activity for elucidation of hypersensitive reaction in plants.

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Acid Soluble Free Nucleotides in Chlorotic Spot Virus (GCSV) Infected Groundnut Leaves (*Arachis hypogaea* L.)

By

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The total acid soluble free nucleotides were less in GCSV infected groundnut leaves when compared to healthy leaves. In virus infected leaves total adenosine, uridine, cytosine, and guanosine phosphates and individual cytosine and uridine phosphates and ATP were less, ADP more, and AMP, GMP, GDP, GTP levels showed no specific pattern of change. The total adenosine and guanosine phosphates decreased while total cytosine and uridine phosphates increased as the leaves aged in both healthy and infected plants. The sum of the adenine and uracil phosphates was lower than the sum of cytosine and guanine phosphates in virus infected leaves when compared to healthy leaves.

Nucleotide composition changes according to the physiological status of host. The changes that occur in different metabolic systems as a result of virus infection would be reflected in the acid soluble nucleotides of the leaf since they play a central role in the mediation and utilization of respiratory energy and in the synthesis of proteins, lipids, carbohydrates and nucleic acids. BROWN (1965) noted an apparently close correlation between ATP production and utilization, and pointed out that the quantity of free nucleotides might be an indication of the amount of metabolic activity. Therefore, the changes in the soluble nucleotides during symptom development, symptom severity and their masking at a later stage in groundnut chlorotic spot virus (GCSV) infected groundnut leaves were investigated.

Materials and Methods

Freshly harvested seeds of groundnut (var. TMV-2) were sown in earthen pots containing garden soil and kept inside the insect-proof wire mesh house. The plants at 5 leaf stages were inoculated mechanically with GCSV (1 g/20 ml 0.1 M NaCl). Comparable healthy plants were inoculated only with 0.1 M NaCl solution. Inoculated leaves never showed the symptoms. Sampling of the leaf material at 5 different stages was the same as reported earlier (SREENIVASULU *et al.*, 1977).

The method of STEWART and GUINN (1971) was employed for extraction of acid soluble free nucleotides and they were separated by ion-exchange column

chromatography as described by CHERRY and HAGEMAN (1960). Throughout the operation 5 ml fractions were collected automatically. The absorbance of the fractions was read in Beckman DU-2 Spectrophotometer at 260 nm. The nucleotides were identified by comparing the elution pattern with standards and previous reports. For quantitative estimation optical density of the pooled fractions in each peak area was taken at 260 and 290 nm. The difference in optical density between 260 and 290 nm was used to calculate the individual nucleotides quantitatively from a standard curve prepared with adenosine-5-monophosphate in a similar way and expressed as μg/20 g fresh weight of the material.

Results

The amount of total nucleotides in virus inoculated and systemically infected leaves was less by 4.69%, 18.07%, 13.9% and 13.56% at stages 1, 3 to 5 and more by 5.8% at stage 2, respectively, when compared to corresponding healthy leaves (Table 1). The young leaves (stage 3) of both the samples had higher amount of nucleotides, and it decreased as the leaves aged (stages 3 to 5).

Table 1

Total acid soluble nucleotide content of healthy (H) and virus infected (VI) leaves at various stages of infection [Values given are average of triplicate determinations; variation was expressed as per cent increase (+) or decrease (−) over healthy]

Stage	H	VI	Variation
	mg/20 g fresh weight	mg/20 g fresh weight	
1	4.63	4.56	− 4.69
2	4.26	4.51	+ 5.80
3	7.15	5.86	−18.07
4	5.58	4.80	−13.90
5	4.97	4.30	−13.56

The elution pattern of nucleotides of both types of samples at stage 4 is given in Fig. 1. The virus inoculated and systemically infected leaves had less ATP and more ADP at all stages when compared to corresponding healthy leaves (Fig. 2). The difference in the content of AMP of virus inoculated and healthy leaves at stages 1 and 2 was negligible, and it decreased from stage 3 onwards in virus infected leaves. The total adenosine phosphate content was less in virus infected leaves at all stages when compared to corresponding healthy leaves. They were more in young leaves of both samples and decreased with leaf aging. CMP was less in virus infected leaves at stages 2 to 5 when compared to healthy leaves (Fig. 3). The virus infected leaves had less CDP at all stages except at stage 3 where it was

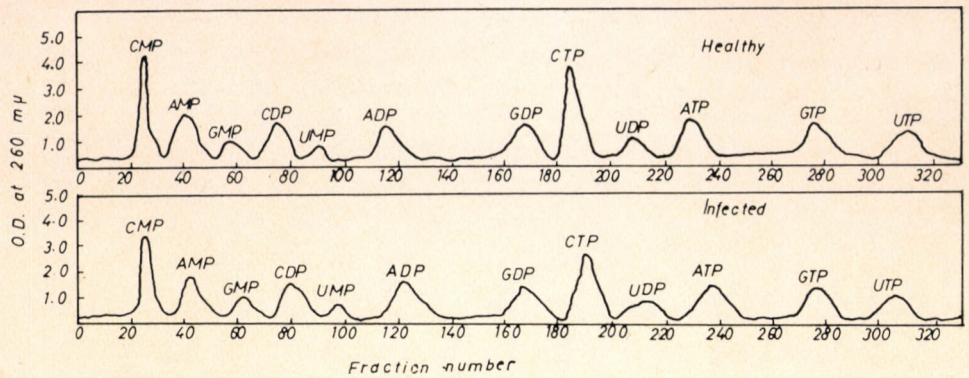

Fig. 1. Elution chromatogram of acid soluble free nucleotides from healthy and virus infected leaves at stage 4

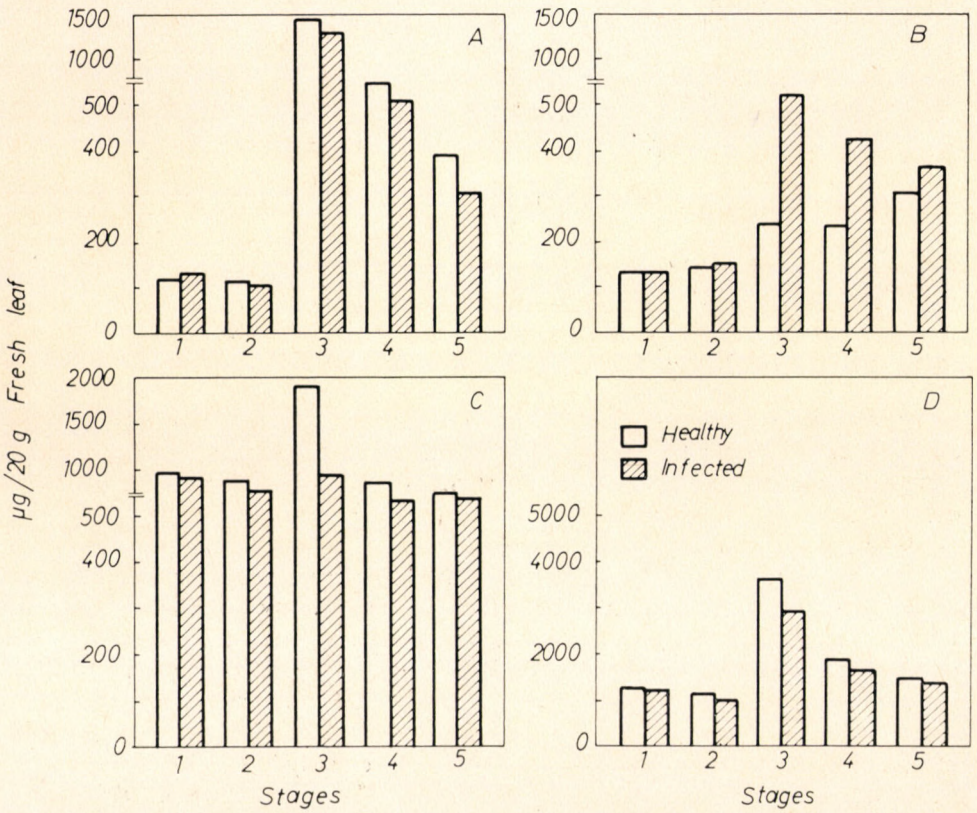

Fig. 2. Composition of adenosine phosphates in healthy and virus infected leaves at various stages of virus infection. A, adenosine monophosphate (AMP); B, adenosine diphosphate (ADP); C, adenosine triphosphate (ATP); D, total adenosine phosphates

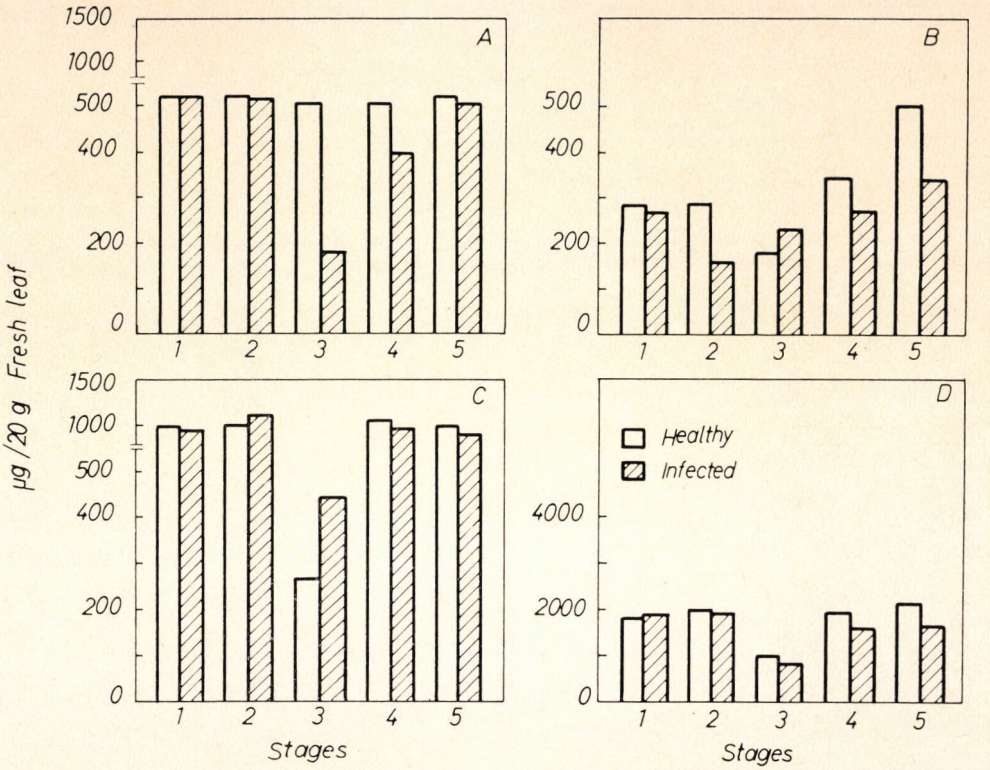

Fig. 3. Composition of cytosine phosphates in healthy and virus infected leaves at various stages of virus infection. A, cytosine monophosphate (CMP); B, cytosine diphosphate (CDP); C, cytosine triphosphate (CTP); D, total cytosine phosphates

more when compared to healthy leaves. CTP in virus infected leaves was less at stages 4 and 5 and more at stages 2 and 3 when compared to healthy leaves. With age all the 3 increased in both types of samples but the total cytosine phosphate content in virus infected leaves was less at stages 2 to 5 with negligible difference at stage 1 when compared to healthy leaves.

In virus infected leaves GMP was more at stages 1 and 4, and less at the remaining 3 stages when compared to corresponding healthy leaves (Fig. 4). GDP was less in virus infected leaves at stages 3 to 5 and there was no notable change at stages 1 and 2 when compared to healthy leaves. The GTP content in virus infected leaves was less at stages 1, 2, 4 and 5 and more at stage 3 when compared to corresponding healthy leaves. With age GMP and GDP decreased in both samples; on the other hand GTP level decreased with age only in virus infected leaves and showed no specific pattern in healthy leaves. The total guanosine phosphate content in virus infected leaves was less when compared to healthy leaves. GDP content was more when compared to GTP and GMP, and GMP was less when compared to GTP and GDP in both samples at all stages.

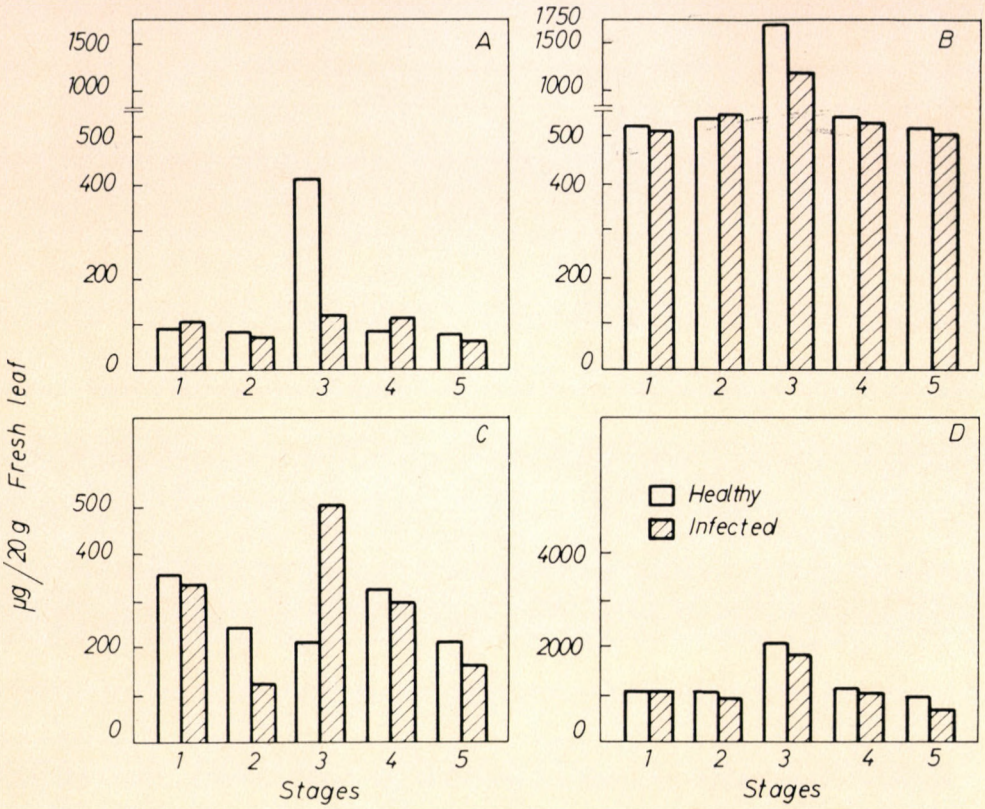

Fig. 4. Composition of guanosine phosphates in healthy and virus infected leaves at various stages of virus infection. A, guanosine monophosphate (GMP); B, guanosine diphosphate (GDP); C, guanosine triphosphate (TP); D, total guanosine phosphates

The amount of UMP in virus infected leaves was less at all stages except at stage 1 where it was more when compared to corresponding healthy leaves (Fig. 5). The virus infected leaves had more UDP at stages 1 to 3 and less at stages 4 and 5 when compared to healthy leaves. With age UMP and UDP showed no specific pattern of change in both samples. In virus infected leaves UTP was more at stages 1 and 3 and less at the remaining 3 stages when compared to corresponding healthy leaves. With age it increased in both samples. The total uridine phosphates were less in virus infected leaves at stages 3 to 5 and showed no notable change at stages 1 and 2 when compared to healthy leaves. UDP was more in both samples at all stages except in healthy sample at stage 3 when compared to UMP and UTP. Further, UMP in both samples was less at all stages except in healthy sample at stage 3 (where it was more than UDP and UTP).

There was no change in pyrimidine and purine phosphates of virus infected leaves when compared to healthy leaves at stage 1. But both were less in virus infected leaves at stages 2 to 5 when compared to healthy leaves. With age the pyrimidine

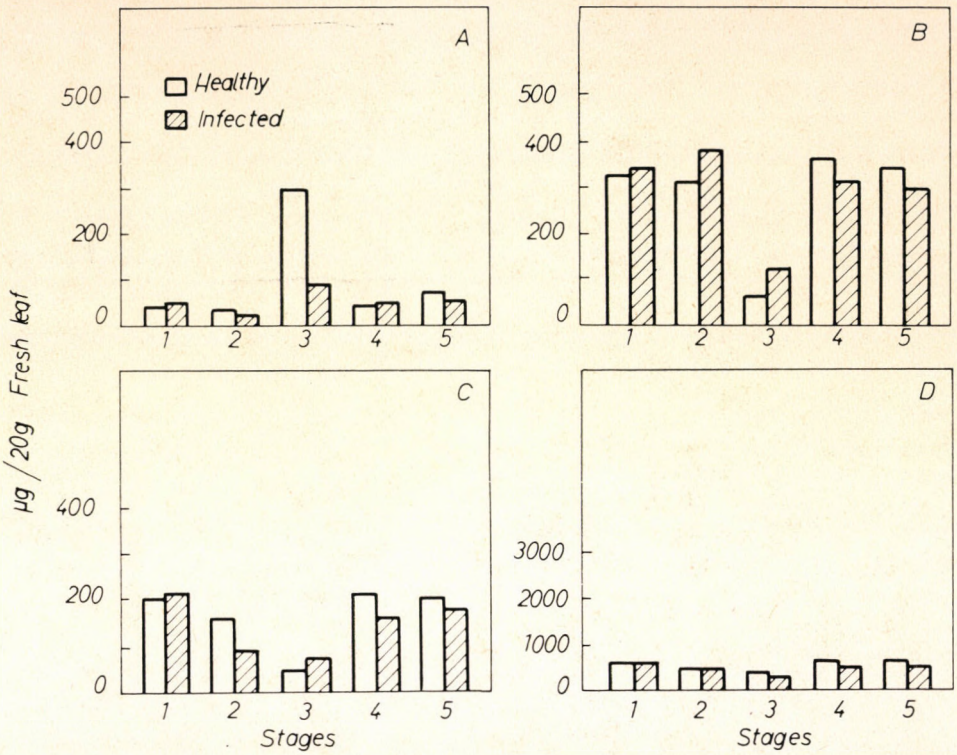

Fig. 5. Composition of uridine phosphates in healthy and virus infected leaves at various stages of virus infection. A, uridine monophosphate (UMP); B, uridine diphosphate (UDP); C, uridine triphosphate (UTP); D, total uridine phosphates

phosphates increased and purine phosphates decreased in both samples. The highest content of purine phosphates was noted at stage 3 (young leaves) in both samples but pyrimidine phosphates were less at stage 3 and they were more at stage 5 (mature leaves).

Discussion

In the present host-virus system the amount of acid soluble nucleotides was less in virus infected leaves (Table 1) when compared to healthy leaves, confirming the results of SINGH (1971) in tristeza infected key 'lime'. He attributed the decrease in total nucleotides to their utilization in the synthesis of viral particles. This decrease in total acid soluble free nucleotides may be correlated with high amount of buffer soluble RNA in GCSV infected groundnut leaves (SREENIVASULU and NAYUDU, 1978).

In virus infected leaves the content of ATP was less when compared to healthy leaves. This may be due to its greater utilization or lesser rate of synthesis both by

photo- and oxidative-phosphorylations as evident from the increased leaves of ADP (Fig. 2). The reduced level of CTP at stages 4 and 5 in virus infected leaves (Fig. 3) may affect the lipid metabolism as less phosphatidyl choline and phosphatidyl glycerol are synthesized in such leaves (SREENIVASULU *et al.*, 1977). The decreased rate of synthesis of phosphatidyl choline may be a reason for lower level of CMP in virus infected leaves as this happened to be a product in the formation of phosphatidyl choline. While in bean leaves, the GMP, GDP, GTP, UMP, UDP, UTP, CDP and CTP were unaffected and AMP, ADP and ATP decreased due to southern bean mosaic virus infection (BOZARTH and BROWING, 1970) the various nucleotide phosphates, except of guanine, specifically decreased in GCSV infected groundnut leaves. The total adenosine and guanosine phosphates decreased while total cytosine and uracil phosphates increased as the leaves aged in both samples. The increase in the 4 nucleotides from the total RNA, adenylic, cytidylic, guanylic and uridylic acids in the GCSV infected groundnut leaves (SREENIVASULU and NAYUDU, 1978) seems to be correlated with decreased levels of total acid soluble free nucleotides. The sum of the adenine and uracil phosphates was less than the sum of cytosine and guanine phosphates in virus infected leaves. This suggests that the bases adenine and uracil were depleted during the period of virus infection to a much greater extent than guanine and cytosine, contrary to the suggestion of SINGH (1971) and SINGH and MISRA (1974). Further studies of the enzymes involved in the synthesis of nucleotides may, therefore, be useful.

*

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Detection of Potato Virus X in Tubers and Foliage of Potatoes

By

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Of the four different types of serological tests compared here, viz. microprecipitin (MP), double diffusion (DD), single radial diffusion (RD), and agglutination tests, MP and RD were found to be much more sensitive than the agglutination test which is currently used for routine screening of PVX in potatoes in Hungary. It was found that the MP test was by far the most sensitive, being able to detect purified virus at a concentration of 0.5 μg/ml and virus in the plant dilution of 1/256 to 1/352. RD on the other hand, could detect purified virus (D-proteins) at 1 μg/ml and virus in the plant dilution of 1/8 to 1/176.

Large scale virus-free seed certification programme of potato in Hungary is normally undertaken relying on serodiagnosis of the various viruses infecting potato by the agglutination test. In view of the reported sensitivity of immunodiffusion tests in agar gel, in detecting low concentrations of potato virus X in infected plant tissue (SHEPARD, 1969), a study was undertaken to compare the various serodiagnostic tests in vogue in Europe, in terms of sensitivity and ease of handling so as to suit rapid laboratory screening of seed potatoes.

The different methods tested were the bentonite flocculation test of KAHN *et al.* (1967), the microprecipitin test of VAN SLOGTEREN (1955), the Ouchterlony double diffusion test, the slide agglutination test and the single radial diffusion test of MANCINI *et al.* (1963).

Materials and Methods

Virus isolate and hosts: The isolate of potato virus X (PVX) used in these studies was the PVX-G strain of the Research Institute for Plant Protection, Budapest. This was multiplied in *Nicotiana tabacum* cv. *Bel 61-10* plants grown in the glass house at an average temperature of 20°C and lighted for 16 hours, harvested after three weeks and used as source material for purification of the virus.

Virus purification: Two methods of purification were tried using polyethylene glycol 6000 (PEG). One was that of SHEPARD and SECOR (1969) where borate buffer was used as suspension medium. A second method using phosphate buffer instead of borate buffer was tried where PEG precipitation was carried out in the butanol clarified extract before high speed centrifugation. Moreover, all operations were carried out at pH 7.1 to 7.4. Since roughly six times the quantity of virus was isolated by the first method, it was followed throughout these studies. This preparation was free of proteins as determined spectrophotometrically. Also no antibodies specific to host proteins were detected when these preparations were used for immunization.

PVX degraded protein: Purified PVX was degraded by treatment with 2 M guanidine hydrochloride according to RALPH and BERGQUIST (1967). Instead of sodium citrate buffer, 0.05 *M* borate buffer, pH 7.2 was used as the suspension medium. The fragmented virus was then dialysed for 24 hours against the same buffer containing 0.37% formaldehyde. Formaldehyde stabilization of PVX D-protein was used in view of the results reported by SHEPARD and SHALLA (1970).

PVX and PVXD-protein preparations were assayed for concentration spectrophotometrically with a UNICAM SP 800 spectrophotometer using an extinction coefficient of 2.7 at 260 mμ for a 1% solution (SHEPARD and SECOR, 1969).

Antiserum production: PVX antiserum was produced by the same schedule as described by SHEPARD and SECOR (1969). Antiserum to degraded PVX protein was produced by 4 weekly intramuscular injections of antigen emulsified 1 : 1 with 2 ml Freund's incomplete adjuvant followed by a booster intramuscular injection 2 weeks after the last injection. In view of the reported immunochemical cross reactivity between the dissociated capsid proteins of PVY group (SHEPARD *et al.*, 1974) in long term (12 weeks) antisera, only primary (4 to 5 weeks) antisera of the PVXD-proteins were tested.

Serological tests: Ouchterlony, double diffusion (DD) and radial immunodiffusion (RD) tests using antisera against PVX and PVXD-protein were tested in 90 mm Pyrex glass Petri dishes. In the double diffusion tests, 10 ml of 1.5 per cent (Difco) agar dissolved in the appropriate buffer was poured into each plate and 3 mm wells 8 mm away from each other was made. In RD tests, various dilutions of antiserum were mixed with equal volumes of buffered 3 percent agar at 50°C, 10 ml of the medium were used per plate. 4 mm diam. wells 10 mm away from each other and 2 mm diam. wells 7 mm away from each other were tested on these plates. The plates were incubated at room temperature.

Microprecipitin test: In addition to the gel diffusion tests, the bentonite flocculation test (KAHN *et al.*, 1967), the microprecipitin test (MP) (VAN SLOGTEREN, 1954) and the agglutination test were compared for sensitivity and adaptability with the PVX antiserum.

Results

Initial experiments were conducted with antiserum prepared against intact PVX and with the "Keszthely" antiserum which is used for routine tests. Comparative studies with microprecipitin tests showed that the present antiserum (designated Juliamajor-JM) had a higher titre (2048 to 4096) than the Keszthely (256) antiserum.

Comparison of MP, DD and RD tests using whole PVX antiserum and D-protein antiserum

Tests were conducted with freshly purified preparations of PVX and D-protein as well as with host extracts treated with 30 percent pyridine, against PVX antiserum. MP, DD and RD tests were compared to determine the titre of the serum and the nature of bands formed in gel diffusion plates.

In the MP tests, no precipitates were seen in the D-protein (purified) – PVX antiserum reaction and in the pyridine treated plant sap, PVX antiserum gave non-specific precipitation. The results of DD systems were essentially similar to that reported by SHEPARD and SECOR (1969) in that a curved line close to the antiserum depot was obtained with PVX and its homologous antiserum whereas no line was perceptible with the D-protein antiserum. Straight precipitin lines midway between the two wells were obtained in the D-protein and its homologous antiserum if the antigen well was charged 1 hour before the antiserum well.

In the RD test using pyridine treated plant sap and PVX antiserum a titre of 1 : 16 was obtained as against 1 : 8 in DD test. Similar results were obtained with D-protein antiserum and pyridine treated plant sap. Pyridine and pyrrolidine were tested as protein degrading agents. Pyridine gave cleaner and stronger precipitate in treated tobacco extracts. Tris-HCl buffer gave clearer precipitate than phosphate buffer.

Sensitivity of the MP test: Experiments were conducted to see the minimum amount of purified PVX detectable by the MP test using homologous antiserum (titre 1 : 2048), as well as PVX present in infected plant sap. Clear precipitates were seen at a concentration of 0.5 μg/ml of purified PVX. In infected tobacco Bel plants PVX was detected in a sap dilution of 1 : 256 and in 4-week-old potato (var. Desire) up to a dilution of 1 : 352. No reaction was noticed with any dilutions of uninfected plant sap.

Sensitivity of RD test: Experiments were conducted on similar lines as above to determine the sensitivity of single radial diffusion test using PVX as D-protein antiserum and purified PVX D-protein or pyridine treated plant sap. Antiserum dilution of 1 : 4 (homologous titre 1/16) was used. Faint precipitin lines were seen at a concentration of 1 μg/ml D-protein antigen when 2 mm diameter wells were used. When 4 mm diameter wells were used a concentration of 10 μg/ml D-protein antigen was detectable. Virus in infected tobacco plants was detected up to a dilution of 1/8 and in potato up to a dilution of 1/176.

Comparison of sensitivity of MP and RD tests with infectivity tests on *Gomphrena globosa* showed that infectivity test was most sensitive, detecting a virus concentration of 0.1 μg/ml followed by MP at 0.5 μg/ml and RD at 1 μg/ml (D-protein).

Detection of PVX in potato sprouts and foliage: MP and RD tests were conducted on natural 0.5 to 1 cm long sprouts of potato var. Gülbaba. It was found that in the MP test there was some nonspecific precipitation when normal serum and grindates of potato sprouts were mixed, in spite of filtration. There was difference in the type of virus specific precipitate formed, yet it was found difficult to differentiate between these two types of precipitates. However, 91 out of 119 positives were obtained in sprouts of Gülbaba by MP test; the foliage of these 119 tubers in later tests proved to be infectious. On the other hand, in single radial diffusion tests, it was found that PVX could be easily detected in germinating sprouts about 1 cm long.

Foliage of 342 samples of potato var. Gülbaba was tested by MP, RD and agglutination tests and compared with infectivity assay on *Gromphrena globosa*. While agglutination test detected the virus in 248 out of 342 samples, all 342 samples were found to have PVX by the other three tests. For one lot of potato var. Desire, 13 out of 17 samples were found to have PVX by MP and RD tests, whereas only 4 out of 17 were detected to be positive by the agglutination test.

Discussion

Of the four different types of serological tests compared here, viz. microprecipitin, double diffusion, single radial diffusion and agglutination tests, MP and RD were found to be much more sensitive than the agglutination test which is currently used for routine screening of PVX in potatoes in Hungary. It was found that the MP test was by far the most sensitive, being able to detect purified virus at a concentration of 0.5 μg/ml and virus in the plant dilution of 1/256 to 1/352. RD on the other hand, could detect purified virus (D-proteins) at 1 μg/ml and virus in the plant dilution of 1/8 to 1/176. However, MP test has a disadvantage in that the plant sap has to be centrifuged at low speed to remove fast sedimenting particles whereas in the RD test the tissue samples including sprouts could be pressed treated with a drop of pyridine and directly placed in the wells concerned. This ease of handling makes it definitely more advantageous when large number of samples have to be handled.

The bentonite flocculation test of KHAN *et al.* (1967), although reported to be more sensitive, did not give reproducible results under the present experimental conditions.

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

V. Potexvirus Group: Potato Virus X and Potato Aucuba Mosaic Virus

By

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In the course of studying the range of artificial host plants to potato virus X we carried out experiments with 114 new plants belonging to nine families. The families *Aizoaceae* and *Geraniaceae* can be regarded as new experimental plant families. Of the 114 new experimental plants 98 proved to be susceptible to potato virus X, 57 of them locally, 21 systemically and 20 locally and systemically. Four new virus susceptible species *(Erodium ciconium, Geranium dissectum, Tetragonia crystallina, T. echinata)* of the two new experimental plant families *(Aizoaceae, Geraniaceae)* are particularly remarkable from a diagnostical point of view. Of the examined plants 16 species and varieties proved to be resistant.

When carrying out investigations into the range of host plants to potato aucuba mosaic virus 72 new virus susceptible plants belonging to the families *Geraniaceae, Labiatae (Lamiaceae), Scrophulariaceae* and *Solanaceae* were found of which 6 proved locally, 16 systemically and 50 locally and systemically susceptible. The families *Geraniaceae* and *Labiatae (Lamiaceae)* can be regarded as new potato aucuba mosaic virus susceptible families. Of the virus susceptible plants 43 species and varieties proved to be new diagnostical plants for the science. Some perennial, epidemiologically important plants are also found among them (e.g. *Paulownia, Physalis* spp.). In the course of our experiments 33 virus resistant plants have been detected.

While studying the ranges of host plants to potato virus X and potato aucuba mosaic virus we found many new dichotomous separators and semiseparators (e.g. *Nicotiana tabacum* cv. *Bel 61-10, Phaseolus aureus, Gomphrena decumbens, Ocimum canum, O. sanctum, Paulownia tomentosa*), and for 22 viruses described about 600 new virus separation possibilities.

Introduction

On the basis of major comprehensive works published so far on the host range of potato virus X [R/1 : 2.1/6 : E/E : S/(Fu)], a mechanically readily transmitted, economically important virus known all over the world, it can be established that the about 120 susceptible plant species known at present belong to nine families (*Amaranthaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Labiatae* [*Lamiaceae*], *Polygonaceae, Rutaceae, Scrophulariaceae, Solanaceae*), while the about

twenty plants resistant to the virus are members of the families *Cruciferae (Brassicaceae)*, *Fabaceae (Leguminosae, Papilionaceae)* and *Solanaceae* (LADEBURG *et al.*, 1950; MACLEOD, 1962; THORNBERRY, 1966; BODE, 1968; SPAAR and HAMANN, 1977).

The potato aucuba mosaic virus, ($R/_{*}$: */5 : E/E : S/Ap) though known the world over, is uncommon and economically less important. Apart from works by HOLLINGS (1959), KOLLMER and LARSON (1960), HORVÁTH (1964) and DE BOKX (1975) very few data on its host range are available. On the basis of the above cited works it can be established that the host plants of the virus are found first of all in the families *Solanaceae*, *Amaranthaceae* and *Fabaceae (Leguminosae, Papilionaceae)*, though some plants in the families *Aizoaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Polygonaceae*, *Portulaceae* and *Scrophulariaceae* also proved susceptible. According to the most recent study of DE BOKX (ibid.) *Gomphrena globosa*, *Phaseolus vulgaris* cv. *Bruine stam* and *Pisum sativum* – known to be important in virus diagnosis – were found to be reactive to inoculation with the virus. Special attention is worth being paid to *Browallia*, *Cyphomandra*, *Melilotus*, *Lycopersicon* and *Nicotiana* species recently pointed out to be symptomless hosts and thereby important prognostical plants.

In the course of experiments carried out for several years some new host plants of potato virus X and potato aucuba mosaic virus have been identified, which besides their diagnostical value are important from the point of view of virus separation (see the works 10 to 38 in Literature).

Materials, Methods and Results

As to the materials and methods of experiments information was given in the second publication of the series (cf. HORVÁTH, 1977). The results of experiments carried out with potato virus X and potato aucuba mosaic virus are summed up below.

1. New Hosts and Non-Hosts of Potato Virus X and their Role in the Separation of Viruses

Local susceptible hosts

Amaranthus angustifolius (A. graecizans)°: Ama, Th // M // / + // ⊟ –
A. ascendens (A. lividus var. *ascendens)°:* Ama, Th // M // / + // ⊟ –
A. atropurpureus (A. hybridus)°: Ama, Th // M // / + // ⊟ –
A. aureus (A. paniculatus var. *flavus):* Ama, Th // M // / + // ⊟ –
A. bouchoni°: Ama, Th // M // / + // ⊟ –
A. caracu (A. hypochondriacus): Ama, Th // M // / + // ⊟ –
A. caudatus: Ama, Th // M // / + // ⊟ –

A. caudatus cv. *Atripurpureus (A. caudatus* var. *sanguineus)°:* Ama, Th // M // /+ // ⊟ –
A. chlorostachys (A. hybridus)°: Ama, Th // M // /+ // ⊟ –
A. chlorostachys f. *strictus (A. chlorostachys* f. *leucocarpus)°:* Ama, Th // M // /+ // ⊟ –
A. chlorostachys var. *powelli°:* Ama, Th // M // /+ // ⊟ –
A. cruentus (A. paniculatus): Ama, Th // M // /+ // ⊟ –
A. deflexus: Ama, H // M // /+ // ⊟ –
A. deflexus var. *rufescens°:* Ama, H // M // /+ // ⊟ –
A. dubius: Ama, Th // M // /+ // ⊟ –
A. emarginatus (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ PVS4
A. gangeticus var. *multicolor (A. tricolor)°:* Ama, Th // M // /+ // ⊟ –
A. graecizans (A. graecizans var. *sylvestris, A. sylvestris):* Ama, Th // M // /+ // ⊟ –
A. hypochondriacus°: Ama, Th // M // /+ // ⊟ PVS4
A. hypochondriacus cv. *Monstrosus°:* Ama, Th // M // /+ // ⊟ –
A. leucocarpus (A. chlorostachys var. *leucocarpus)°:* Ama, Th // M // /+ // ⊟ –
A. lividus (A. lividus var. *ascendens):* Ama, Th // M // /+ // ⊟ –
A. mantegazzianus°: Ama, Th // M // /+ // ⊟ –
A. oleraceus (A. lividus var. *oleraceus)°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus (A. cruentus): Ama, Th // M // /+ // ⊟ PVS4
A. paniculatus cv. *Roter Dom°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus cv. *Roter Paris°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus cv. *Sanguineus nanus°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus var. *flavus (A. aureus)°:* Ama, Th // M // /+ // ⊟ –
A. patulus (A. hybridus ssp. *cruentus* var. *patulus):* Ama, Th // M // /+ // ⊟ –
A. speciosus (A. paniculatus f. *speciosus)°:* Ama, Th // M // /+ // ⊟ –
A. spinosus: Ama, Th // M // /+ // ⊟ –
A. sylvestris (A. graecizans var. *sylvestris)°:* Ama, Th // M // /+ // ⊟ –
A. tricolor cv. *Malten Fire°:* Ama, Th // M // /+ // ⊟ –
A. viridis (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ –
Beta macrocarpa (B. vulgaris ssp. *macrocarpa):* Chen, Th // M // /– // ⊟ BCMV, BYMV, PVY, CLRV/, WMV, AMV/, TuMV, TYMV, CMV/
Chenopodium botrys: Chen, Th // M // /+ // ⊟ PAMV, PVS4, AMV/
Ch. capitatum: Chen, Th // M // /+ // ⊟ PVS4, TRV46/, AMV
Ch. foetidum (Ch. schraderianum): Chen, Th // M // /+ // ⊟ PVM, PVS4, TRV46/, AMV/, BBWV47/
Ch. giganteum (Ch. amaranticolor): Chen, Th // M // /+ // ⊟ PVS4
Ch. murale: Chen, Th // M // /+ // ⊟ PVY, CLRV48/, AMV1/
Ch. quinoa f. *viridescens°:* Chen, Th // M // /+ // ⊟ –
Cucumis myriocarpus: Cuc, Th // M // /– // ⊟ BCMV, PAMV, PVM, PVS, PVY, CLRV, WMV43/, RMV, TYMV, CMV
Gomphrena decumbens°: Ama, Th // M // /+ // ⊟ BCMV, BMV, PAMV, PVY, CLRV, TRSV/, TMV/, WMV, ToMV/, RMV, TYMV, BBWV/, CeMV

Lycium australe°: Sol, Ph // M // /+ // ⊟ −
L. carolinianum°: Sol, ? // M // /+ // ⊟ AMV/
L. chinense: Sol, Ph // M // /+ // ⊟ AMV9/
L. europaeum: Sol, Ph // M // /+ // ⊟ AMV9/
L. flexicaule°: Sol, Ph // M // /+ // ⊟ AMV/
L. horridum°: Sol, Ph // M // /+ // ⊟ AMV/
L. ruthenicum: Sol, Ph // M // /+ // ⊟ AMV/
L. turcomanicum°: Sol, Ph // M // /+ // ⊟ AMV/
Paulownia fargesii°: Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, AMV, RMV, TYMV, CMV/
Solanum demissum Redd. 530-hybrid°: Sol, Th // M // /+ // ⊟ BMV
S. demissum Stamm S-hybrid°: Sol, Th // M // /+ // ⊟ BMV
Tetragonia crystallina°: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, PVY, CLRV/, TRSV/, TRV/, AMV, TuMV/, TYMV, CMV/, CeMV
T. echinata: Aiz, Th // M // /+ // ⊟ BCMV, BMV, PVM, PVS, CLRV/, TRSV/, TRV/, TuMV/, TYMV, CMV/, CeMV

Systemic susceptible hosts

Datura ceratocaula°: Sol, Th // M // +/ // ⊟ /BMV44, /TRSV, /TMV, /CMV
D. fastuosa cv. *Alba*°: Sol, Th // M // +/ // ⊟ /BMV, PVY, /TRSV, /TMV, /TRV, /ToMV, /CMV
D. gigantea (D. tatula): Sol, Th // M // +/ // ⊟ /BMV, /TRSV6, /TMV, /TRV, /ToMV, /CMV
D. godronii cv. *Minka*°: Sol, ? // M // +/ // ⊟ BCMV, BYMV, /BMV, PVY, CLRV, /TRSV, /TMV, /TNV, /TRV, WMV, /ToMV, RMV, TYMV, /CMV, CeMV
D. metel var. *inermis*°: Sol, ? // M // +/ // ⊟ /BMV, /TMV
D. metel var. *muricata*°: Sol, ? // M // +/ // ⊟ /BMV, /TMV /TNV
D. rosei°: Sol, ? // M // +/ // ⊟ /BMV, /TRSV, /TMV, /TNV, /TRV, WMV, /ToMV, /CMV
D. stramonium var. *chalybea (D. tatula)*: Sol, Th // M // +/ // ⊟ BCMV, BYMV, /BMV, PVY53, /TMV, /TNV
D. stramonium f. *inermis*°: Sol, Th // M // +/ // ⊟ BCMV, BYMV, /BMV, PVY, /TMV, /TNV
D. tatula (D. stramonium var. *chalybea)*°: Sol, Th // M // +/ // ⊟ BCMV, BYMV, /BMV, PVY, /TMV, /TNV
Geranium dissectum: Ger, Th // M // −/ // ⊟ PVM, PVY, /TMV
Nicotiana attenuata: Sol, Th // M // +/ // ⊟ /BMV, /AMV
N. auriculata°: Sol, Th // M // +/ // ⊟ /BMV, /AMV
N. langsdorfii: Sol, Th // M // +/ // ⊟ PVM, PVS, /TRV, /AMV
N. plumbaginifolia: Sol, Th // M // +/ // ⊟ PVM, PVS
N. solanifolia: Sol, Th // M // +/ // ⊟ PVM, PVS, /CLRV48
N. tabacum cv. *Bel 61-10*: Sol, Th // M // +/ // ⊟ /BMV, PVM, PVS, /PVY, /TRSV, /TMV, WMV, /ToMV, RMV, TYMV, CeMV

N. tabacum cv. *Hicks Fixed A2-426°:* Sol, Th // M // +/ // ⊟ /TMV
N. tabacum cv. *Szuloki°:* Sol, Th // M // +/ // ⊟ –
N. tabacum cv. *Xanthi-nc:* Sol, Th // M // +/ // ⊟ /BMV44, PVM, PVS, /TRSV, /TMV52, /TNV, /ToMV, CeMV
N. texana: Sol, Th // M // +/ // ⊟ –

Local and systemic susceptible hosts

Browallia cordata°: Sol, ? // M // +/– // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
Br. demissa (Br. americana): Sol, Th // M // +/– // ⊟ BCMV, PVM, PVS, TYMV
Br. grandiflora: Sol, Th // M // –/+ // ⊟ BCMV, PVM, PVS, AMV, TYMV
Br. roezli°: Sol, ? // M // +/– // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
Capsicum annuum cv. *Bogyiszlói vastaghúsú°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Cecei édes°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros csüngő°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros felálló I°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Csokros felálló II°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Hatvani hajtatási°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Korai halványzöld°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Kovácsházi hajtatási°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Magyar kincs°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Maritza°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Sonnenpreis°:* Sol, Th // M // +/+ // ⊟ –
C. annuum cv. *Tétényi hajtatási zöld°:* Sol, Th // M // +/+ // ⊟ –
Erodium ciconium°: Ger, Th // M // –/– // ⊟ BCMV, BMV, PVY, TRSV, WMV, TYMV
Ocimum canum: Lab=Lami, Th // M // –/+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
O. sanctum°: Lab=Lami, Th // M // –/+ // ⊟ PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
Physalis viscosa: Sol, ? // M // –/– // ⊟ BCMV, PVS, RMV, TYMV

Resistant plants

Bryonia alba: Cuc, H // M // –/– // ⊟ /TNV, /TRV46, /ToMV, CMV53/
Br. dioica (Br. cretica ssp. *dioica):* Cuc, H // M // –/– // ⊟ /TNV, /TRV46, /ToMV, CMV49/
Cestrum parqui°: Sol, ? // M // –/– // ⊟ /BMV
Cheiranthus cheiri: Cru=Bras, Th, H // M // –/– // ⊟ /TRSV, /TRV46, AMV9/ AMV9, TuMV53/TuMV53, TYMV53/TYMV53, CMV53/
Crambe abyssinica: Cru=Bras, H // M // –/– // ⊟ /TNV, RMV/RMV, TYMV/ TYMV
C. armena°: Cru=Bras, H // M // –/– // ⊟ TMV/TMV

C. cordifolia°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. hispanica°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. maritima: Cru=Bras, H // M // −/− // TMV/TMV
C. orientalis°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. tataria°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
Cucurbita pepo convar. *patissonina* f. *radiata*°: Cuc, Th // M // −/− // ⊟ TRSV/TRSV, /TMV, TNV/TNV, WMV/, /ToMV, CMV/
Diplotaxis viminea°: Cru=Bras, ? // M // −/− // ⊟ RMV/RMV, TYMV/TYMV, CMV/CMV
Erodium gruinum: Ger, Th // M // −/− // ⊟ /TMV, /TNV, /TRV46, /ToMV, /RMV, TYMV, CMV/CMV
E. manescavi°: Ger, H // M // −/− // ⊟ /TMV, /TNV, /RMV, TYMV, CMV/CMV
E. moschatum: Ger, Th // M // −/− // ⊟ PAMV/PAMV, CLRV/, /TMV, /TNV, /ToMV, /RMV, TYMV, CMV/CMV

2. New Hosts and Non-Hosts of Potato Aucuba Mosaic Virus and their Role in the Separation of Viruses

Local susceptible hosts

Lycium europaeum: Sol, Ph // M // /+ // ⊟ AMV9/
L. horridum°: Sol, Ph // M // /+ // ⊟ AMV/
L. ruthenicum: Sol, Ph // M // /+ // ⊟ AMV/
L. turcomanicum°: Sol, H // M // /+ // ⊟ AMV/
Paulownia fargesii°: Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, AMV, RMV, TYMV, CMV/
P. tomentosa (P. imperialis): Scrop, Ph // M // /+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, TRSV/, RMV, TYMV, CMV27/

Systemic susceptible hosts

Datura carolinianum°: Sol, ? // M // −/ // ⊟ /AMV, /TMV
D. ceratocaula°: Sol, Th // M // −/ // ⊟ /TMV, /CMV
D. fastuosa cv. *Alba*°: Sol, Th // M // −/ // ⊟ /BMV, PVY, /TRSV, /TMV, /TRV, /ToMV, /CMV
D. gigantea (D. tatula): Sol, Th // M // −/ // ⊟ /BMV, /TMV, /TRV, /ToMV, /CMV
D. godronii cv. *Minka*°: Sol, ? // M // −/ // ⊟ BCMV, BYMV, /BMV, PVY, CLRV, /TRSV, /TNV, /TRV, WMV, /ToMV, /CMV
D. metel: Sol, Th // M // +/ // ⊟ /BMV44, /PVM55, /TNV, /ToMV
D. metel var. *inermis*°: Sol, ? // M // +/ // ⊟ /BMV, /TMV, /TNV
D. metel var. *muricata*°: Sol, ? // M // +/ // ⊟ /BMV, /TMV, /TNV
D. rosei°: Sol, ? // M // −/ // ⊟ /BMV, /TRSV, /TMV, /TNV, /TRV, WMV, /ToMV, /CMV

D. stramonium var. *chalybea (D. tatula):* Sol, Th // M // −/ // ⊟ BCMV, BYMV, /BMV53, /TMV, /TNV
D. stramonium f. *inermis*°*:* Sol, Th // M // −/ // ⊟ BCMV, BYMV, /BMV, PVY, /TMV, /TNV
Nicotiana tabacum cv. *Ambalema:* Sol, Th // M // −/ // ⊟ −
N. tabacum cv. *Bel 61-10:* Sol, Th // M // −/ // ⊟ /BMV, PVM, PVS, /PVY, /TRSV, /TMV, WMV, /ToMV, RMV, TYMV, CeMV
N. tabacum cv. *Hicks Fixed A2-426*°*:* Sol, Th // M // −/ // ⊟ /TMV
N. tabacum cv. *Xanthi-nc:* Sol, Th // M // −/ // ⊟ PVM, PVS, /TRSV, /TMV52, /TNV, /ToMV, CeMV
N. texana: Sol, Th // M // −/ // ⊟ −

Local and systemic susceptible hosts

Browallia cordata°*:* Sol, ? // M // −/− // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
Br. demissa (Br. americana): Sol, Th // M // +/− // ⊟ BCMV, PVM, PVS, TYMV
Br. grandiflora: Sol, Th // M // +/− // ⊟ BCMV, PVM, PVS, AMV, TYMV
Br. roezli°*:* Sol, ? // M // +/− // ⊟ BCMV, PVM, PVS, PVY, AMV, TYMV
Br. viscosa: Sol, ? // M // +/− // ⊟ BCMV, PVM, PVS, AMV, TYMV
Capsicum annuum cv. *Bogyiszlói vastaghúsú*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Cecei édes*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum var. *cerasifera*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Csokros csüngő*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Csokros felálló I*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Csokros felálló II*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Dokomlási 2710*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Hatvani hajtatási*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Kalocsai E-15*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Kalocsai felálló*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Korai halványzöld*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Kovácsházi hajtatási*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Magyar kincs*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Markgärtner*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Maritza*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Sonnenpreis*°*:* Sol, Th // M // +/+ // ⊟ −
C. annuum cv. *Tétényi hajtatási zöld*°*:* Sol, Th // M // +/+ // ⊟ −
Erodium moschatum: Ger, Th // M // −/− // ⊟ BCMV, BMV, PVX, PVY, TRSV, TYMV
Nicotiana chinensis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
N. knightiana: Sol, Th // M // −/− // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
N. quadrivalvis: Sol, Th // M // +/+ // ⊟ PVM, PVS, WMV, TYMV, CeMV
Ocimum canum: Lab=Lami, Th // M // −/+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV

O. sanctum°: Lab=Lami, Th // M // −/+ // ⊟ BCMV, PVM, PVS, PVY, CLRV, WMV, RMV, TYMV, CeMV
Pentstemon alpinus°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. attenuatus°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. calycosus°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. cardinalis°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. laevigatus°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. ovatus°: Scrop, H // M // −/− // ⊟ PVY, CMV
P. whippleanus°: Scrop, H // M // −/− // ⊟ PVY, CMV
Petunia atkinsiana°: Sol, Th // M // −/− // ⊟ PVM, PVS, WMV, RMV, TYMV, CeMV
P. axillaris: Sol, Th // M // −/− // ⊟ PVM, PVS, WMV, RMV, TYMV
P. hybrida cv. *Rose de Haven amélioré*°: Sol, Th // M // −/− // ⊟ PVM, PVS, CLRV
P. parviflora°: Sol, Th // M // −/− // ⊟ PVM, PVS, CLRV, WMV
P. violacea: Sol, Th // M // −/− // ⊟ PVM, PVS, WMV, RMV, TYMV
Physalis aequata: Sol, ? // M // +/− // ⊟ BCMV, PVM, PVS, RMV, TYMV
Ph. alkekengi (Ph. franchetti): Sol, H // M // +/− // ⊟ BCMV, PVM, PVS, RMV, TYMV, BBWV50
Ph. ixocarpa: Sol, ? // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Ph. peruviana: Sol, H // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Ph. peruviana var. *macrocarpa*°: Sol, H // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Ph. philadelphica: Sol, ? // M // +/− // ⊟ BCMV, RMV, TYMV
Ph. pruinosa: Sol, Th // M // +/− // ⊟ BCMV, PVM, PVS, TNV, RMV, TYMV
Ph. pubescens: Sol, Th // M // +/− // ⊟ BCMV, RMV, TYMV
Solanum capsicastrum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV
S. ochroleucum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV

Resistant plants

Aptenia cordifolia: Aiz, Th, H // M // −/− // ⊟ /CLRV, TRSV/, TMV/, TNV/, TRV46/, AMV9/AMV9, ToMV/
Bryonia alba: Cuc, H // M // −/− // ⊟ /TNV, /TRV46, /ToMV, CMV53/CMV53
Br. dioica (Br. cretica ssp. *dioica)*: Cuc, H // M // −/− // ⊟ /TNV, /TRV46, /ToMV, TuMV49/TuMV49, TYMV54/TYMV54, CMV49/CMV49
Cheiranthus cheiri: Cru=Bras, Th, H // M // −/− // ⊟ /TRSV, TMV8/TMV8, /TRV8, AMV9/AMV9, TuMV53/TuMV53, TYMV53/TYMV53
Chenopodium botrys: Chen, Th // M // −/− // ⊟ /BYMV41, /PVY, AMV/ AMV
Commelina clandestina°: Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. coelestis (C. communis): Com, Th, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV53/

C. graminifolia°: Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. tuberosa°: Com, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
Crambe abyssinica: Cru=Bras, H // M // −/− // ⊟ /TNV, RMV/RMV, TYMV/TYMV
C. armena°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. cordifolia°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. hispanica°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. maritima: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. orientalis°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
C. tatarica°: Cru=Bras, H // M // −/− // ⊟ TMV/TMV
Cucumis myriocarpus: Cuc, Th // M // −/− // ⊟ /TRSV, /TMV, /TNV, WMV43/, /ToMV
Cucurbita pepo convar. *patissonina* f. *radiata*°: Cuc, Th // M // −/− // ⊟ TRSV/TRSV, /TMV, TNV/TNV, WMV2, /ToMV, CMV/
Geranium cristatum°: Ger, ? // M // −/− // ⊟ TMV/TMV
G. colombianum°: Ger, Th // M // −/− // ⊟ TMV/TMV
G. dissectum: Ger, Th // M // −/− // ⊟ TMV/TMV, CMV/
G. lucidum°: Ger, Th, TH // M // −/− // ⊟ TMV/TMV
G. molle°: Ger, Th // M // −/− // ⊟ TMV/TMV
G. pratense: Ger, H // M // −/− // ⊟ TMV/TMV
G. pusillum°: Ger, Th // M // −/− // ⊟ TMV/TMV
G. pyrenaicum°: Ger, H // M // −/− // ⊟ TMV/TMV
G. robertianum: Ger, Th // M // −/− // ⊟ TMV/TMV
G. rotundifolium: Ger, Th // M // −/− // ⊟ TMV/TMV, CMV49/
G. sibiricum°: Ger, H // M // −/− // ⊟ TMV/TMV, CMV
Gomphrena decumbens°: Ama, Th // M // −/− // ⊟ /BYMV, /PVM, /PVS, TRSV/TRSV, TMV/TMV, /TNV, ToMV/ToMV, BBWV/BBWV, CMV/CMV
Phaseolus aureus (Ph. mungo): Fab=Legu=Pap, Th // M // −/− // ⊟ BCMV53/, BYMV53/; ⊞ BYMV53, ⊟ TRSV6/, /TMV8, /TNV45, TRV46/TRV46, AMV9/, BBWV39/BBWV39, CMV53/
Ph. vulgaris cv. *Red Kidney:* Fab=Legu=Pap, Th // M // −/− // ⊟ BCMV/BCMV, BYMV/BYMV, /PVM, CLRV/CLRV, /TNV, /AMV
Tinantia erecta (T. fugax)°: Com, H // M // −/− // ⊟ BYMV/, PVY/, CLRV/, TMV/, TRV/, AMV/, ToMV/, TuMV/

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

VI. Carlavirus Group: Potato Virus M and Potato Virus S

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In the course of experiments carried out to get a better knowledge of the range of artificial hosts to potato virus M we studied 157 species, varieties and cultivars of ten plant families. Of 103 susceptible plants belonging to the families *Amaranthaceae*, *Chenopodiaceae*, *Fabaceae (Leguminosae, Papilionaceae)* and *Solanaceae* 90 showed local, 10 systemic and 3 local and systemic susceptibility in artificial inoculation experiments. On the other hand, 54 species of the families *Aizoaceae*, *Chenopodiaceae*, *Commelinaceae*, *Fabaceae (Leguminosae, Papilionaceae)*, *Labiatae (Lamiaceae)*, *Scrophulariaceae* and *Solanaceae* proved resistant to inoculation with the virus. Of the susceptible and resistant plants 64 can be regarded as new experimental plants in plant virology. Of the new local i.e. theoretically important hosts the species *Amaranthus* and *Chenopodium* and the cultivars *Phaseolus vulgaris*, while among the new systemically susceptible hosts the *Lycopersicon* species have diagnostical importance in the first place; the *Lycopersicon* species are also significant as virus production hosts.

When studying the host range of potato virus S we found that among 101 plants belonging to 8 families (*Commelinaceae* is a new experimental plant family) 8 *Chenopodium*, *Gomphrena*, *Obione*, and *Vigna* species were locally, 4 *Atropa* and *Lycopersicon* species systemically and 3 *Solanum* species locally and systemically susceptible, while 86 plants showed resistance. Of the virus susceptible plants the perennial *Atropa bella-donna* is the most important from an epidemiological point of view. *Lycopersicon glandulosum*, *L. hirsutum* and *L. peruvianum* are of importance as new virus production hosts.

During our studies on the ranges of host plants to potato virus M and potato virus S we have made new discoveries of a number of plants important from the point of view of both virus diagnosis and separation. These plants make it possible to apply some 800 variations of virus separation for 22 plant viruses.

Introduction

The carlavirus group is named after carnation latent virus and includes other well known viruses such as potato virus M and potato virus S. Investigations into the range of hosts to potato virus M ($_*$/* : $_*$/* : E/E : S/Ap) – a mechanically and (some strains) aphid transmitted stylet-borne virus discovered hardly more than two decades ago and since spread all over the world – have contributed little to the science of virology, and even the results are in many cases contradictory in the world literature. Apart from our own recently written papers five major comprehensive

studies have been published on the host range of potato virus M (BAGNALL *et al.*, 1956, 1959; HORVÁTH, 1964; VULIĆ and HUNNIUS, 1967; KOWALSKA and WAŚ, 1976).

On the basis of the mentioned works it can be established that the host plants of the virus are found first of all in the families *Amaranthaceae*, *Chenopodiaceae*, *Fabaceae (Leguminosae, Papilionaceae)* and *Solanaceae*. It is remarkable that the virus has relatively few systemic hosts. As virus production hosts *Lycopersicon esculentum*, *Solanum demissum* and *S. tuberosum* are of importance in the first place. Of the recently described indicator plants special attention is worth being paid to various *Chenopodium* species (HORVÁTH and DE BOKX, 1972) as well as to *Cucumis sativus* whose cotyledons – according to the experiments of KOWALSKA (1973) and KOWALSKA and WAŚ (1976) – react after 10–16 days of incubation with chlorotic local lesions of 3 mm diameter to inoculation with potato virus M in autumn, winter and spring, while in the hot summer period no symptom can generally be observed.

Investigations into the range of host plants to potato virus S ($_*$/* : $_*$/* : E/E : : S/Ap) – a mechanically and (some strains) aphid transmitted stylet-borne virus serologically related to and similarly cosmopolitan in potato as the potato virus M – have not made much progress, and even the available data are in many cases contradictory. On the basis of major studies published so far on the host range of potato virus S (BAGNALL *et al.*, 1956, 1959; HORVÁTH, 1964; VULIĆ and HUNNIUS, 1967; DE BOKX, 1970; KOWALSKA and WAŚ, 1976) it can be established that its host plants are found first of all in the families *Amaranthaceae*, *Chenopodiaceae*, *Fabaceae (Leguminosae, Papilionaceae)* and *Solanaceae*. In a prominent paper published by DE BOKX (1970) not long ago 81 species of some 15 families are pointed out to be resistant, and only 16 species to be susceptible to inoculation with potato virus S. The host-virus relations are mostly of local nature. A relatively small proportion of the host plants (e. g. *Nicotiana debneyi*, *Solanum rostratum*, *Lycopersicon chilense)* are systemically susceptible to the virus inoculation. Of the recent studies those calling attention to a possible host–virus relation between the species *Lycopersicon* (ROSS, 1968; HORVÁTH, 1972a) and *Chenopodium* (DE BOKX, 1970) and the potato virus S bear special importance.

In the course of experiments carried out in connection with studies on the host plants of potato virus M and potato virus S we have recently identified further plants important from the point of view of both virus diagnosis and virus separation (see works 10 to 34 in Literature). The results of these experiments are presented in this paper.

Materials, Methods and Results

As for potato virus M and potato virus S strains included in our experiments in the course of studying the host–virus relation, as well as of the test plants, host–virus cryptogram, etc. detailed account was already given in the second publication of the series (cf. HORVÁTH, 1977), so we do not repeat them here. The results of our experiments are summed up below.

1. New Hosts and Non-Hosts of Potato Virus M and their Role in the Separation of Viruses

Local susceptible hosts

Amaranthus ascendens (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ –
A. atropurpureus (A. hybridus)°: Ama, Th // M // /+ // ⊟ –
A. bouchoni°: Ama, Th // M // /+ // ⊟ –
A. caracu (A. hypochondriacus): Ama, Th // M // /+ // ⊟ –
A. caudatus cv. *Atripurpureus°:* Ama, Th // M // /+ // ⊟ –
A. chlorostachys f. *strictus (A. chlorostachys* f. *leucocarpus)°:* Ama, Th // M // /+ // ⊟ –
A. cruentus (A. paniculatus): Ama, Th // M // /+ // ⊟ –
A. dubius: Ama, Th // M // /+ // ⊟ –
A. gangeticus var. *multicolor (A. tricolor)°:* Ama, Th // M // /+ // ⊟ –
A. hypochondriacus°: Ama, Th // M // /+ // ⊟ –
A. hypochondriacus cv. *Monstrosus°:* Ama, Th // M // /+ // ⊟ –
A. leucocarpus (A. chlorostachys var. *leucocarpus)°:* Ama, Th // M // /+ // ⊟ –
A. mantegazzianus°: Ama, Th // M // /+ // ⊟ –
A. oleraceus (A. lividus var. *oleraceus)°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus (A. cruentus): Ama, Th // M // /+ // ⊟ –
A. paniculatus cv. *Roter Dom°:* Ama, Th // M // /+ // ⊟ –
A. paniculatus var. *flavus (A. aureus)°:* Ama, Th // M // /+ // ⊟ –
A. retroflexus: Ama, Th // M // /+ // ⊟ BMV46, PVS6, BBWV52/
A. speciosus (A. paniculatus f. *speciosus)°:* Ama, Th // M // /+ // ⊟ –
A. spinosus: Ama, Th // M // /+ // ⊟ –
A. sylvestris (A. graecizans var. *sylvestris)°:* Ama, Th // M // /+ // ⊟ –
A. tricolor: Ama, Th // M // /+ // ⊟ PVS6
A. viridis (A. lividus var. *ascendens)°:* Ama, Th // M // /+ // ⊟ –
Chenopodium ambrosioides var. *ambrosioides°:* Chen, Th, TH // M // /+ // ⊟ PVS6
Ch. ambrosioides var. *anthelminticum:* Chen, Th // M // /+ // ⊟ PVS6
Ch. aristatum (Teloxis aristata)°: Chen, Th // M // /+ // ⊟ –
Ch. ficifolium (Ch. album var. *ficifolium):* Chen, Th // M // /+ // ⊟ PVS6, AMV4/
Ch. foliosum (Blitum virgatum): Chen, Th // M // /+ // ⊟ PVS6, TRV51
Ch. giganteum (Ch. amaranticolor): Chen, Th // M // /+ // ⊟ PVS6
Ch. glaucum: Chen, Th // M // /+ // ⊟ PVS6
Ch. hybridum: Chen, Th // M // /+ // ⊟ –
Ch. opulifolium: Chen, Th // M // /+ // ⊟ BMV46
Ch. polyspermum: Chen, Th // M // /+ // ⊟ –
Ch. quinoa f. *viridescens°:* Chen, Th // M // /+ // ⊟ –
Ch. rubrum (Blitum rubrum): Chen, Th // M // /+ // ⊟ –
Ch. vulvaria: Chen, Th // M // /+ // ⊟ PVS6
Gomphrena decumbens°: Ama, Th // M // /+ // ⊟ BCMV, BMV, PAMV, PVY, CLRV, TRSV, TMV/, WMV, ToMV/, RMV, TYMV, BBWV/, CMV/, CeMV

Obione sibirica (Atriplex sibirica)°: Chen, Th // M // /+ // ⊟ BCMV, CLRV/, TRSV/, TYMV, CeMV
Phaseolus vulgaris cv. *Aladin:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Andrásbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Annelise:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Barnabab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Cardinal:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Caroline:* Fab=Legu=Pap, Th // M // /+ // ⊟ TMV
Ph. vulgaris cv. *Cukorbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fehér gyöngy°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fehér középbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fertődi 5.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Fertődi 23.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Fullcrop:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *GN 59.:* Fab=Legu=Pap, Th // M // /+ // ⊟ TMV
Ph. vulgaris cv. *GN 123.:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Harkovszkaja:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Icar Fundulea 51.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Icar Fundulea 416.°:* Fab=Legu=Pap, Th // M // /+ // ⊟ TMV
Ph. vulgaris cv. *Japan gyöngybab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Kanizsai csíkosbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Kentucky Wonder:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Kereskedelmi hosszú fürjbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Kinghorn Wax:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Kompolti gyöngybab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Korai fürj°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Kőbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Középbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Májbab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Michelite:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Michigan:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Moldovszkaja:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Olomucka Zelenoluska:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Őrségi cseresznyebab°:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Perlicska:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Pinto:* Fab=Legu=Pap, Th // M // /+ // ⊟ BMV, PVS, ToMV
Ph. vulgaris cv. *Prinzess:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Processor:* Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Red Kidney:* Fab=Legu=Pap, Th //M// /+// ⊟ BCMV/, BYMV49/, BMV, PAMV, PVS; ⊞ PVS41; ⊟ CLRV/, TMV, ToMV, RMV
Ph. vulgaris cv. *Refugee:* Fab=Legu=Pap, Th // M // /+ // ⊟ TMV
Ph. vulgaris cv. *Robust:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS

Ph. vulgaris cv. *Soproni lapos*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Szegedi fehér*°: Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Sztepnaja:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tápiószelei barna*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tápiószelei fürj*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Tápláni fekete „cirádás" fürjbab*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS, TMV
Ph. vulgaris cv. *Tápláni nagyszemű cseresznyebab*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Tétényi cukorbab*°: Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Tétényi gyöngybab*°: Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Tétényi fehér középbab*°: Fab=Legu=Pap, Th // M // /+ // ⊟ –
Ph. vulgaris cv. *Tétényi nagyszemű fehér*°: Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Ph. vulgaris cv. *Wade:* Fab=Legu=Pap, Th // M // /+ // ⊟ PVS
Vigna catjang (V. cylindrica)°: Fab=Legu=Pap, Th // M // /+ // ⊟ BCMV/, PVY, TRSV/, ToMV, BBWV36/

Systemic susceptible hosts

Atropa bella-donna: Sol, H // M // –/ // ⊟ BCMV, BYMV, /BMV46, /PVX43, PVY, CLRV, TRSV, WMV, RMV, TuMV, TYMV
Lycopersicon esculentum cv. *Red Cherry:* Sol, Th // M // +/ // ⊟ PVS
L. glandulosum: Sol, ? // M // +/ // ⊟ –
L. hirsutum: Sol, ? // M // +/ // ⊟ BBWV52
L. humboldtii: Sol, ? // M // +/ // ⊟ PVS
L. peruvianum: Sol, ? // M // +/ // ⊟ –
L. pimpinellifolium: Sol, ? // M // +/ // ⊟ PVS, TuMV59; ⊞ TuMV58
L. pyriforme: Sol, ? // M // +/ // ⊟ PVS
L. racemiflorum°: Sol, ? // M // +/ // ⊟ PVS
L. racemigerum: Sol, ? // M // +/ // ⊟ PVS

Local and systemic susceptible hosts

Solanum capsicastrum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV
S. demissum A6-hybrid: Sol, Th // M // +/+ // ⊟ BMV
S. ochroleucum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV

Resistant plants

Browallia cordata°: Sol, ? // M // –/– // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
Br. demissa (Br. americana): Sol, Th // M // –/– // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, PVY/, TRSV/TRSV, ToMV/ToMV, RMV/RMV, CMV/CMV

Br. grandiflora: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, PVY/PVY, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV

Br. roezli°*:* Sol, ? // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, CLRV/, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV

Br. viscosa: Sol, ? // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV

Cheiranthus cheiri: Cru=Bras, Th // M // −/− // ⊟ /TRSV, /TRV51, TuMV58/, TYMV58/, CMV58/

Chenopodium anthelminticum (Ch. ambrosioides): Chen, Th // M // −/− // ⊟ −

Ch. bonus-henricus: Chen, H // M // −/− // ⊟ −

Ch. foetidum (Ch. schraderianum): Chen, Th // M // −/− // ⊟ /PVX, /PVY, CLRV53/CLRV53, TRV51/TRV51, AMV/AMV, BBWV52/BBWV52

Commelina clandestina°*:* Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/

C. communis (C. coelestis): Com, Th // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV58/

C. graminifolia°*:* Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/

C. tuberosa°*:* Com, Th // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/

Cucumis myriocarpus: Cuc, Th // M // −/− // ⊟ /PVX, /TRSV, /TNV, WMV45/, /ToMV

Cucurbita pepo convar. *patissonina* f. *radiata*°*:* Cuc, Th // M // −/− // ⊟ TRSV/TRSV, /TMV, TNV/TNV, WMV/, /ToMV, CMV/

Galega hartlandii°*:* Fab=Legu=Pap, H // M // −/− // ⊟ BYMV/, AMV/

G. officinalis: Fab=Legu=Pap, H // M // −/− // ⊟ BYMV39/, /TRV51, AMV4/

Nicotiana acuminata: Sol, Th // M // −/− // ⊟ PVX43/PVX43, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV51/, AMV35/, /TuMV58

N. alata: Sol, Th // M // −/−// ⊟ PVX58/PVX58, PVY58/, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56

N. chinensis: Sol, Th // M // −/−// ⊟ BMV/BMV, PAMV/PAMV, PVX43/PVX43, PVY55/, CLRV/CLRV, TRSV/TRSV, TMV58/TMV58, /TNV, TRV/TRV, ToMV/ToMV, /TuMV, CMV/CMV

N. fragrans: Sol, Th // M // −/− // ⊟ PVX55/PVX55, PVY55/PVY55, TMV58/TMV58, TRV51/

N. knightiana: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, CLRV/CLRV, TRSV/TRSV, TMV58/TMV58, /TNV, TRV/TRV, ToMV/, CMV/CMV

N. langsdorffii: Sol, Th // M // −/− // ⊟ PVY58/PVY58, TRSV7/TRSV7, TMV9/TMV9, /TNV37, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/, CMV58/

N. longiflora: Sol, Th // M // −/− // ⊟ PVX55/, PVY55/PVY55, TRSV7/TRSV7, TMV9/TMV9, TRV51/, AMV35/, /TuMV58, BBWV56/

N. paniculata: Sol, Th // M // −/− // ⊟ BMV/BMV, PVY, CLRV7/CLRV7,

TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56, CMV58/

N. plumbaginifolia: Sol, Th // M // −/− // ⊟ PVX/, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV55/TRV55

N. quadrivalvis: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/PVY, CLRV/CLRV, TRSV58/TRSV58, TMV58/TMV58, TRV/TRV, AMV35/, ToMV/ToMV, /TuMV58, BBWV/, CMV/CMV

N. rustica: Sol, Th // M // −/− // ⊟ BCMV58/BCMV58, PAMV55/, PVX43/PVX43, PVY58/, TRSV7/TRSV7, TMV9/TMV9, /TNV58, TRV51/TRV51, AMV58/, /TuMV58, BBWV52/

N. sanderae (N. alata x *N. forgetiana):* Sol, Th // M // −/− // ⊟ PVX58/, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56, CMV/CMV

N. solanifolia: Sol, Th // M // −/− // ⊟ PVX/, PVY/, CLRV53/CLRV53, TRSV55/TRSV55, TMV9/TMV9

N. tabacum cv. *Bel 61-10:* Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/, PVX/, PVY/PVY, TRSV/TRSV, /TMV, /ToMV

N. tabacum cv. *Xanthi-nc:* Sol, Th // M // −/− // ⊟ BMV46/BMV46, PAMV/, PVX/, PVY/, TRSV/TRSV, /TMV57, /TNV, TRV/TRV, /ToMV

Ocimum basilicum: Lab=Lami, Th // M // −/− // ⊟ PVX55/PVX55, TRSV/TRSV, TMV44/TMV44, /TNV44, /TRV51, AMV35/, ToMV/ToMV, BBWV52/, CMV55/

O. canum: Lab=Lami, Th // M // −/− // ⊟ PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, /TRV, AMV/, ToMV/ToMV

O. sanctum°: Lab=Lami, Th // M // −/− // ⊟ PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, /TRV, AMV/, ToMV/ToMV, BBWV/, CMV/CMV

Paulownia fargesii°: Scrop, Ph // M // −/− // ⊟ /PAMV, /PVX, TRSV/TRSV, /TMV, /TRV

P. tomentosa (P. imperialis): Scrop, Ph // M // −/− // ⊟ /PAMV, TRSV/TRSV, /TMV, /TRV, /ToMV, CMV18/CMV18

Petunia atkinsiana°: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, TRSV/TRSV, TMV/TMV, /TNV, AMV/AMV, TuMV/, CMV/CMV

P. axillaris: Sol, Th // M // −/− // ⊟ PAMV/PAMV, PVY/, TRSV/TRSV, TMV/, TMV, /TNV, AMV/AMV

P. hybrida: Sol, Th // M // −/− // ⊟ BYMV5/; ⊞ BYMV58; ⊟ BMV46/, PAMV 38/PAMV38, PVX58/, PVY58/, TRSV7/TRSV7, TMV9/TMV9, TNV55, TRV51/, AMV35/, TuMV58/, BBWV52/, CMV58/

P. hybrida cv. *Rose de Haven amélioré*°: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, AMV/AMV, CMV/CMV

P. parviflora°: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, AMV/AMV, CMV/CMV

P. violacea: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX43/PVX43, PVY58/, TRSV7/TRSV7, TMV/TMV, /TNV, AMV/AMV, TuMV/, CMV58/CMV58

Physalis aequata: Sol, ? // M // −/− // ⊟ PAMV/PAMV, PVX14/PVX14, PVY/PVY, TRSV/TRSV, TMV/TMV, /TNV, /TRV
Ph. alkekengi (Ph. franchetti): Sol, H // M // −/− // ⊟ PAMV/PAMV, TNV9/TMV9, /TNV55, CMV14/CMV14
Ph. angulata: Sol, ? // M //−/− // ⊟ PAMV14/PAMV14, PVX55/PVX55, PVY1/PVY1, TRSV7/TRSV7, TMV7/TMV7, TRV51/, AMV35/, /TuMV8, 25, CMV14/ CMV14
Ph. floridana: Sol, ? // M // −/− // ⊟ BYMV5/, PAMV55/PAMV55, PVX14/PVX14, PVY58/PVY58, TRSV7/TRSV7, TMV14/TMV14, /TNV14, TRV51/, AMV35/, BBWV52/, CMV14/CMV14
Ph. ixocarpa: Sol, ? // M // −/− // ⊟ PAMV/PAMV, PVX14/PVX14, PVY55/PVY55, TRSV/TRSV, TMV/TMV, TNV/, TRV51/TRV51, AMV35/, CMV/CMV
Ph. peruviana: Sol, H // M // −/− // ⊟ PAMV/PAMV, PVY58/PVY58, TRSV/TRSV, TMV/TMV, TRV51/TRV51
Ph. peruviana var. *macrocarpa*°: Sol, H // M // −/− // ⊟ PAMV/PAMV, PVY/, TRSV/TRSV, TMV/TMV, AMV/AMV, CMV/CMV
Ph. pruinosa: Sol, Th // M // −/− // ⊟ PAMV/PAMV, PVY/PVY, TRSV/TRSV, TMV/TMV, TRV51/, AMV/AMV, CMV/CMV
Tetragonia crystallina°: Aiz, Th // M // −/− // ⊟ /BYMV, /PVX, CLRV/CLRV, TRSV/TRSV, /TMV, /TNV, TRV/TRV, /WMV, /ToMV, /RMV, TuMV/TuMV, CMV/CMV
T. echinata: Aiz, Th // M // −/− // ⊟ /BYMV, /PVX, CLRV/CLRV, TRSV/TRSV, /TMV, /TNV, TRV/TRV, /WMV, /ToMV, /RMV, TuMV/TuMV, /BBWV, CMV/CMV
Tinantia erecta (T. fugax)°: Com, H // M // −/− // ⊟ BYMV/, PVY/, CLRV/, TRSV/, TMV/TMV, /TNV, TRV/TRV, AMV/AMV, ToMV/ToMV, TuMV/

2. New Hosts and Non-Hosts of Potato Virus S and their Role in the Separation of Viruses

Local susceptible hosts

Chenopodium hybridum: Chen, Th // M // /+ // ⊟ BBWV56
Ch. opulifolium: Chen, Th // M // /+ // ⊟ BMV46
Ch. polyspermum: Chen, Th // M // /+ // ⊟ −
Ch. quinoa f. *viridescens*°: Chen, Th // M // /+ // ⊟ −
Ch. rubrum (Blitum rubrum): Chen, Th // M // /+ // ⊟ −
Gomphrena decumbens°: Ama, Th // M // /+ // ⊟ BCMV, BMV, PAMV, PVY, TRSV/, TMV/, WMV, ToMV/, RMV, TYMV, BBWV/, CMV/, CeMV
Obione sibirica (Atriplex sibirica)°: Chen, Th // M // /+ // ⊟ BCMV, CLRV/, TRSV/, TYMV, CeMV
Vigna catjang (V. cylindrica)°: Fab=Legu=Pap, Th // M // /+ // ⊟ BCMV/, PVY, TRSV/, ToMV, BBWV36/

Systemic susceptible hosts

Atropa bella-donna: Sol, H // M // −/ // ⊟ BCMV, BYMV, PVY58, CLRV, TRSV, /TNV, WMV, RMV, TuMV, TYMV
Lycopersicon glandulosum: Sol, ? // M // −/ // ⊟ −
L. hirsutum: Sol, ? // M // −/ // ⊟ BBWV52
L. peruvianum: Sol, ? // M // −/ // ⊟ −

Local and systemic susceptible hosts

Solanum capsicastrum: Sol, ? // M // +/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV
S. demissum A6-hybrid: Sol, Th // M // +/+ // ⊟ BMV
S. ochroleucum: Sol, ? // M // −/+ // ⊟ BCMV, BYMV, RMV, TuMV, TYMV

Resistant plants

Browallia cordata°: Sol, ? // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
Br. demissa (Br. americana): Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, TRSV/TRSV, ToMV/ToMV, RMV/RMV, CMV/CMV
Br. grandiflora: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, PVY/PVY, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, BBWV54/, CMV/CMV
Br. roezli°: Sol, ? // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
Br. viscosa: Sol, ? // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, TRSV/TRSV, TMV/TMV, ToMV/ToMV, RMV/RMV, CMV/CMV
Cheiranthus cheiri: Cru=Bras, Th, H // M // −/− // ⊟ /TRSV, TMV9/, /TRV51, AMV35/, TuMV58/, TYMV58, CMV58/
Commelina clandestina°: Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. communis (C. coelestis): Com, Th, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV58/
C. graminifolia°: Com, ? // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
C. tuberosa°: Com, Th, H // M // −/− // ⊟ CLRV/, TRSV/, TMV/TMV, /TNV, ToMV/ToMV, CMV/
Cucumis myriocarpus: Cuc, Th // M // −/− // ⊟ /PVX, /TRSV, /TMV, /TNV, WMV45/, /ToMV
Cucurbita pepo convar. *patissonina* f. *radiata*°: Cuc, Th // M // −/− // ⊟ TRSV/TRSV, /TMV, TNV/TNV, TYMV/, /ToMV, CMV/
Galega hartlandii°: Fab=Legu=Pap, H // M // −/− // ⊟ BYMV/, AMV/
G. officinalis: Fab=Legu=Pap, H // M // −/− // ⊟ BYMV39/, /TRV51, AMV4/
Lycopersicon esculentum cv. *Red Cherry*°: Sol, Th // M // −/− // ⊟ PVM/
L. humboldtii: Sol, ? // M // −/− // ⊟ PVM/

L. pimpinellifolium: Sol, ? // M // −/− // ⊟ PVM/, TMV9/, TRV51/, TuMV58/; ⊞ TuMV59; ⊟ BBWV52/

L. pyriforme: Sol, ? // M // −/− // ⊟ PVM/

L. racemiflorum°: Sol, ? // M // −/− // ⊟ PVM/

L. racemigerum: Sol, ? // M // −/− // ⊟ PVM/, PVX58/,

Nicandra physaloides: Sol, Th // M // −/− // ⊟ BMV46/, PAMV38/PAMV38, PVX43/PVX43, PVY58/, TRSV7/TRSV7, TMV9/TMV9, /TNV58, TRV5/TRV5, AMV35/, /TuMV58, BBWV52/, CMV58/

Nicotiana acuminata: Sol, Th // M // −/− // ⊟ PVX43/PVX43, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58

N. alata: Sol, Th // M // −/− // ⊟ PVX58/PVX58, PVY58/, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56

N. chinensis: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX43/PVX43, PVY55/, CLRV/CLRV, TMV58/TMV58, /TNV, TRV/TRV, ToMV/ToMV, /TuMV, CMV/CMV

N. fragrans: Sol, Th // M // −/− // ⊟ PVX55/PVX55, PVY55/, TMV58/TMV58, TRV51/TRV51

N. knightiana: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, CLRV/CLRV, TRSV/TRSV, TMV58/TMV58, /TNV, TRV/TRV, ToMV/, CMV/CMV

N. langsdorffii: Sol, Th // M // −/− // ⊟ PVX/, PVY58/, TRSV7/TRSV7, TMV9/TMV9, /TNV58, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/, CMV58/CMV58

N. longiflora: Sol, Th // M // −/− // ⊟ PVX55/, PVY55/, TRSV7/TRSV7, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/

N. paniculata: Sol, Th // M // −/− // ⊟ BMV/BMV, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56, CMV58/

N. plumbaginifolia: Sol, Th // M // −/− // ⊟ PVX/, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV55/TRV55

N. quadrivalvis: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/PYV, CLRV/CLRV, TRSV58/TRSV58, TMV58/TMV58, TRV/TRV, AMV35/, ToMV/ToMV, /TuMV58, BBWV/, CMV/CMV

N. sanderae (N. alata x *N. forgetiana):* Sol, Th // M // −/− // ⊟ PVX58/, PVY/, TRSV7/TRSV7, TMV9/TMV9, TRV51/TRV51, AMV35/, /TuMV58, BBWV56/BBWV56, CMV58/CMV58

N. solanifolia: Sol, Th // M // −/− // ⊟ PVX/, PVY/, CLRV53/CLRV53, TRSV55/TRSV55, TMV9/TMV9

N. sylvestris: Sol, Th // M // −/− // ⊟ BCMV58/, BYMV58/, BMV/BMV, PAMV 38/PAMV38, PVX58/, PVY58/, TRSV7/TRSV7, TMV9/TMV9, /TNV48, TRV51/TRV51, AMV35/, /TuMV58, BBWV52/BBWV52, CMV58/CMV58

N. tabacum cv. *Bel 61-10:* Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/, PVX/, PVY/PVY, TRSV/TRSV, /TMV, /ToMV

N. tabacum cv. *Xanthi-nc:* Sol, Th // M // −/− // ⊟ BMV46/BMV46, PAMV/, PVX/, PVY/, TRSV/TRSV, /TMV57, /TNV, TRV/TRV, /ToMV

Ocimum canum: Lab=Lami, Th // M // −/− // ⊟ PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, /TRV, AMV/, ToMV/ToMV, BBWV/, CMV/CMV
O. sanctum°*:* Lab=Lami, Th // M // −/− // ⊟ PAMV/PAMV, PVX/PVX, TRSV/TRSV, TMV/TMV, /TRV, AMV/, ToMV/ToMV, CMV/CMV
Paulownia fargesii°*:* Scrop, Ph // M // −/− // ⊟ /PAMV, /PVX, TRSV/TRSV, /TMV, /TRV, /ToMV, CMV/CMV
P. tomentosa (P. imperialis): Scrop, Ph // M // −/− // ⊟ /PAMV, TRSV/TRSV, /TMV, /TNV, /ToMV, CMV18/CMV18
Petunia atkinsiana°*:* Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, TRSV/TRSV, TMV/TMV, /TNV, AMV/AMV, TuMV/, CMV/CMV
P. axillaris: Sol, Th // M // −/− // ⊟ PAMV/PAMV, PVY/, TRSV/TRSV, TMV/TMV, /TNV, AMV/AMV
P. hybrida cv. *Rose de Haven amélioré*°*:* Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, AMV/AMV, CMV/CMV
P. parviflora°*:* Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVY/, AMV/AMV, CMV/CMV
P. violacea: Sol, Th // M // −/− // ⊟ BMV/BMV, PAMV/PAMV, PVX43/PVX43, PVY58/, TRSV7/TRSV7, TMV/TMV, /TNV, AMV/AMV, TuMV/, CMV58/CMV58
Phaseolus vulgaris cv. *Annelise:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Barnabab*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Cordon:* Fab=Legu=Pap, Th // M // −/− // ⊟ −
Ph. vulgaris cv. *Falomiteana:* Fab=Legu=Pap, Th // M // −/− // ⊟ −
Ph. vulgaris cv. *Fertődi 5.*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Fullcrop:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM
Ph. vulgaris cv. *GN 123.:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Japan gyöngybab*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Kanizsai csíkosbab*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Kentucky Wonder:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Kereskedelmi hosszú fürjbab*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Korai fürj*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Michelite:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Harkovszkaja:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Őrségi cseresznyebab*°*:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Perlicska:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV

Ph. vulgaris cv. *Pinto:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TNV
Ph. vulgaris cv. *Prinzess:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Red Kidney:* Fab=Legu=Pap, Th // M // −/− // ⊟ BCMV/BCMV, BYMV49/BYMV49, /PVM; ⊞ /PVM41; ⊟ CLRV/CLRV, /TNV, /AMV
Ph. vulgaris cv. *Robust:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Soproni lapos°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Sztepnaja:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM
Ph. vulgaris cv. *Tápiószelei barna°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Tápiószelei fürj°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Tápláni fekete "cirádás" fürjbab°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /AMV
Ph. vulgaris cv. *Tétényi cukorbab°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Tétényi gyöngybab°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Tétényi középbab°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Tétényi nagyszemű fehér°:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Ph. vulgaris cv. *Wade:* Fab=Legu=Pap, Th // M // −/− // ⊟ /PVM, /TMV, /AMV
Physalis aequata: Sol, ? // M // −/− // ⊟ PAMV/PAMV, PVX14/, PVY/PVY, TRSV/TRSV, TMV/TMV, /TNV, /TRV
Ph. alkekengi (Ph. franchetti): Sol, H // M // −/− // ⊟ PAMV/PAMV, TMV9/TMV9, /TNV55, CMV14/
Ph. angulata: Sol, ? // M // −/− // ⊟ PAMV14/PAMV14, PVX55/, PVY1/, TRSV7/TRSV7, TMV9/TMV9, TRV51/, AMV35/AMV35, /TuMV25,8, CMV14/
Ph. ixocarpa: Sol, ? // M // −/− // ⊟ PAMV/PAMV, PVX14/, PVY55/, TRSV/TRSV, TMV/TMV, /TNV, TRV51/TRV51, AMV35/AMV35, CMV/CMV
Ph. peruviana: Sol, H // M // −/− // ⊟ PAMV/PAMV, PVY58/, TRSV/TRSV, TMV/TMV, TRV51/TRV51, AMV/AMV, /TuMV14, BBWV52/, CMV/CMV
Ph. peruviana var. *macrocarpa°:* Sol, H // M // −/− // ⊟ PAMV/PAMV, PVY/, TRSV/TRSV, TMV/TMV, AMV/AMV, CMV/CMV
Ph. pruinosa: Sol, Th // M // −/− // ⊟ PAMV/PAMV, PVY/PVY, TRSV/TRSV, TMV/TMV, TRV51/TRV51, AMV/AMV, CMV/CMV
Ph. viscosa:* Sol, ? // M // −/− // ⊟ PAMV14/PAMV14, PVX/PVX, PVY47/PVY47, TRSV7/TRSV7, TMV14/TMV14, /TNV, /TRV, CMV/CMV

* Newly, FELDMAN and GRACIA (1977) demonstrated in their paper entitled "Studies of weed plants as source of viruses. V. Occurrence of alfalfa mosaic virus on Origanum crops and on some weeds in Argentina" (*Phytopath. Z. 90*, 87—90) that *Physalis viscosa* is a natural host of alfalfa mosaic virus. The infected plants reacted with bright yellow or a whitish

Tetragonia crystallina°: Aiz, Th // M // −/− // ⊟ /BYMV, /PVX, CLRV/CLRV, /TMV, /TNV, TRV/TRV, /WMV, /ToMV, /RMV, TuMV/TuMV, CMV/CMV

T. echinata: Aiz, Th // M // −/− // ⊟ /BYMV, /PVX, CLRV/CLRV, TRSV/TRSV, /TMV, /TNV, TRV/TRV, /WMV, /ToMV, /RMV, TuMV/TuMV, /BBWV, CMV/CMV

Tinantia erecta (T. fugax)°: Com, H // M // −/− // ⊟ BYMV/, PVY/, CLRV/, /TRSV, TMV/TMV, /TNV, TRV/TRV, AMV/AMV, ToMV/ToMV, TuMV/

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The Occurrence of Tobacco Rattle Virus and its Vectors in Hungary

(Preliminary report)

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In course of surveys on the distribution of NEPO viruses in Hungary, in the northern part of the country (Ipolyvece) also a NETU virus: tobacco rattle virus (TRV) has been isolated partly from roots of weed plants and partly from the soil. In soil samples taken in the same locality also the presence of two nematode species, *Trichodorus viruliferus* Hooper and *T. sparsus* Szczygiel, known as vectors of TRV was demonstrated.

At the site of the virus isolation (humid sandy soil) previously strawberries had been grown for many years; then subsequently from 1970 cabbage and potato were grown on the plot. In the year of the isolation (1974) rye was grown in the same field.

The virus infection of weeds was established by testing the roots of plants collected in April of 1974. The roots were washed to remove soil particles, triturated and with the sap leaves of *Nicotiana tabacum* L. var. White Burley plants were inoculated. From the soil sample — taken in October of 1974 — the virus was isolated with the bait plant method described by CADMAN and HARRISON (1960). For attractants cucumber plants (*Cucumis sativus* L. cv. *Delicatess*) were used. The bait plants were grown in soil samples for 5—6 weeks, then their roots were washed, triturated and with the plant sap *Nicotiana langsdorffii* plants were inoculated. From some of the soil samples taken in the infested area the nematodes were washed out by using the Oestenbrink funnel method.

The virus was isolated also from the roots of many weed plants: *Capsella bursa-pastoris* (L.) Medik., *Plantago media* L. and *Senecio vulgaris* L. From these *C. bursa-pastoris* and *S. vulgaris* were known as natural hosts of TRV, according to the data of LISTER and MURANT (1967) and HARRISON (1973), respectively.

The characteristic symptoms of TRV appeared partly on the leaves and partly on the root necks of the 10 *Nicotiana langsdorffii* plants inoculated in the bait plant isolation.

No publications are known to us so far as regards the occurrence of tobacco rattle virus in Hungary. We had, however, a personal communication of J. HORVÁTH (Research Institute for Plant Protection, Budapest, present address: Agricultural

University, Keszthely) who isolated TRV in one case in Southwest Hungary (Lábod-Nagyatád) from tobacco (*Nicotiana tabacum* cv. *Szuloki*) plants.

From both soil samples taken in the locality the individuals of *Trichodorus viruliferus* and *T. sparsus* were isolated. The occurrence of the latter was mentioned earlier from Hungary (ANDRÁSSY 1973), but *T. viruliferus* is new for the Hungarian fauna.

T. viruliferus is a well known transmitter of TRV (HARRISON 1963, VAN HOOF 1964). At the same time *Trichodorus sparsus* has not been mentioned yet in the literature as a TRV vector, or at least not under this name. VAN HOOF *et al.* (1966) collected among other Trichodorus species also a not identified form in North Italy in studying the occurrence of various TRV strains and their vectors. This type, named temporarily as "Trichodorus X" has been included also into further studies (VAN HOOF 1968) when the author investigated the TRV vectors occurring in Holland. It has been proved that the individuals of "Trichodorus X" were able to transmit TRV. The actual description of the species as *Trichodorus sparsus* by SZCZYGIEL (1968) followed only later and this turned out to be identical with the form called temporarily "Trichodorus X" (personal communication of Dr. H. A. VAN HOOF, Instituut voor Plantenziektenkundig Onderzoek, Wageningen, Holland). So, the two species, *Trichodorus viruliferus* and *T. sparsus*, found in the site of TRV infection can be well considered as having played a role as vectors of TRV in Hungary.

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Natural Occurrence of a Strain of Tomato Mosaic Virus on Potato in Hungary

By

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Tomato mosaic virus (ToMV, tobamovirus group) was isolated from potato plants *(Solanum tuberosum* L. cv. *Astilla)* in Jánoshalma, near Szeged, Hungary. Diseased potato plants showed strong stunting, deformation with leaf roll and stem necrosis. No necrotic symptoms were observed on leaves. The virus was identified on the basis of host range, symptomatology, serology, cytology and particle morphology. By mechanical inoculation *Chenopodium amaranticolor* Coste et Reyn., *Datura stramonium* L., *Nicotiana glutinosa* L., *N. sylvestris* Speg. et Comes and *N. tabacum* L. cv. *Xanthi-nc* reacted only with local lesions, *Gomphrena globosa* L. and *N. tabacum* cv. *Samsun* with local and systemic symptoms. The *Cucumis sativus* L., *Cucurbita pepo* L. convar. *patissonina* Greb. f. *radiata* Nois. and *Phaseolus vulgaris* L. plants proved to be resistant against inoculation.

In slide precipitin tests, the virus gave a positive reaction against sera of both ToMV and tobacco mosaic virus (TMV, tobamovirus group). It is worth mentioning that *Nolana prostrata* L. *(Nolanaceae)* which is a new host in plant virology reacted both locally and systemically when inoculated with our isolate of ToMV.

Well developed crystalline inclusions in the form of more or less regularly shaped hexagonal prisms similar to those of the members of tobamovirus group were detected in leaf hair cells of *Gomphrena globosa* and *Nicotiana tabacum* cv. *Samsun.*

In electron microscopy using dip method, numerous, straight, rigid particles of about 300 × 18 nm were found. The virus *in vitro* withstood 95°C for 10 minutes, dilution 10^{-5} and more than 21 days at room temperature.

This is apparently the first report of the natural occurrence of ToMV in potato.

Introduction

Since more than six decades the plant virologists have been specially interested in the virophil potato plant (*Solanum tuberosum* L.) and its pathogenic viruses. Among the new researches carried out in this respect it is worth mentioning those which revealed the host-virus relationship between the potato plant and each of the following viruses: cucumber mosaic virus (CMV, cryptogram: R/1 : 1/18 : S/S : : S/Ap; cucumovirus group), alfalfa mosaic virus (AMV, cryptogram: R/1 : 1.3 + +1.1 + 0.9/18 : U/U : S/Ap; monotypic group), as well as tobacco mosaic virus (TMV, cryptogram: R/1 : 2/5 : E/E : S/O; tobamovirus group) (reviewed by

HANSEN, 1960; PHATAK and VERMA, 1967; HORVÁTH, 1968; SCHMELZER and SPAAR, 1975; BODE, 1975; HORVÁTH, 1976; SPAAR and HAMANN, 1977).

In the few last years during the isolations and identifications of viruses from potato plants we noticed the occurrence of a virus isolate which gave a host-virus range which sharply deviated from those given by the known potato pathogenic viruses (cf. HORVÁTH, 1976). It was of a great interest for us to continue carrying on tests necessary for the identification of this isolate.

The present paper reports a strain of tomato mosaic virus (ToMV, cryptogram: R/1 : (2)/5 : E/E : S/O; tobamovirus group) naturally infecting a potato variety *(Astilla)* in Jánoshalma, near Szeged, Hungary. The diseased *Astilla* plants showed very strong stunting, deformation, leaf rolling and stem necrosis. Leaves were free from any necrotic symptoms.

Materials and Methods

Using the carborundum-spatula technique, test plants were inoculated by sap extracted from *Astilla* diseased leaves after mortaring them with an equal volume of a phosphate buffer (0.1 *M*, pH 7.0). Test plants *(Chenopodium amaranticolor* Coste et Reyn., *Cucumis sativus* L., *Cucurbita pepo* L. convar. *patissonina* Greb. f. *radiata* Nois., *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. sylvestris* Speg. et Comes, *N. tabacum* L. cv. *Samsun*, *N. tabacum* cv. *Xanthi-nc*, *Phaseolus vulgaris* L. cv. *Pinto*, *Ph. vulgaris* cv. *Red Kidney)* used for studying the host range of the virus isolates were inoculated by sap extracted from leaves of artificially inoculated *Gomphrena globosa* showing severe systemic symptoms.

Light microscope observations were performed on living cells in several plants, but especially on systemically infected leaves of *Gomphrena globosa* and *Nicotiana tabacum* cv. *Samsun* plants.

The examinations of the virus particles in infected tissues were carried out with an ELMI D-2 Zeiss electron microscope. The leaf samples of *Gomphrena globosa* were examined by the leaf dip method (cf. BRANDES and WETTER, 1959; BRANDES, 1964).

Serological reactions were performed by means of slide precipitin test using the procedure previously described (HORVÁTH, 1971).

Physical properties of the virus were determined using extracted *Nicotiana tabacum* cv. *Samsun* sap. *Datura stramonium* was used as assay host. The thermal inactivation point was determined in ultrathermostat (E. Mot., Type FF. 100/45) where 1 ml aliquots of infected sap were exposed to each degree of temperature from 60 to 98°C for 10 minutes. Other properties examined were the dilution end-point of infectious sap and longevity *in vitro* of the virus in sap at room temperature (see HORVÁTH, 1969).

In the insect transmission studies the green peach aphid (*Myzus persicae* Sulz.) was used. The aphids were starved for 3 hours, then permitted to feed on

infected *Samsun* tobacco leaves for 8–10 minutes. Ten aphids were then transferred to healthy test plants *(Nicotiana tabacum* cv. *Samsun)* for 24 hours and finally destroyed with Phosdrin insecticide. The experiments were carried out in an insect-proof greenhouse.

Results and Discussion

Two to four days after inoculation only local lesions were observed on leaves of the following test plants: *Chenopodium amaranticolor* (Fig. 1A), *Datura stramonium* (Fig. 1C), *Nicotiana glutinosa*, *N. sylvestris* (Fig. 1B) and *N. tabacum* cv. *Xanthi-nc*. *Gomphrena globosa* and *Nicotiana tabacum* cv. *Samsun* reacted with both local and systemic symptoms (Fig. 2A and B), while only systemic symptoms were observed on *Lycopersicon esculentum*. *Cucumis*, *Cucurbita* and *Phaseolus* plants proved to be resistant. In the course of host range studies our attention was attracted to the new experimental plant, *Nolana prostrata* L. *(Nolanaceae)*, which gave both local and systemic symptoms (Fig. 3A and B).

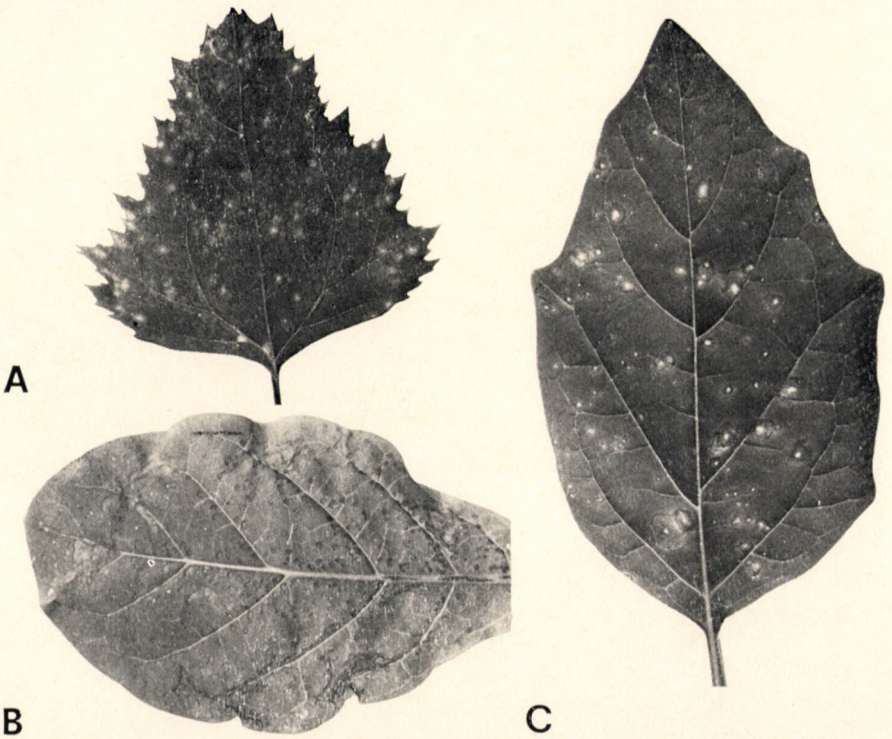

Fig. 1. Cholorotic and necrotic local lesions on *Chenopodium amaranticolor* Coste et Reyn. (A), *Nicotiana sylvestris* Speg. et Comes (B) and *Datura stramonium* L. (C)

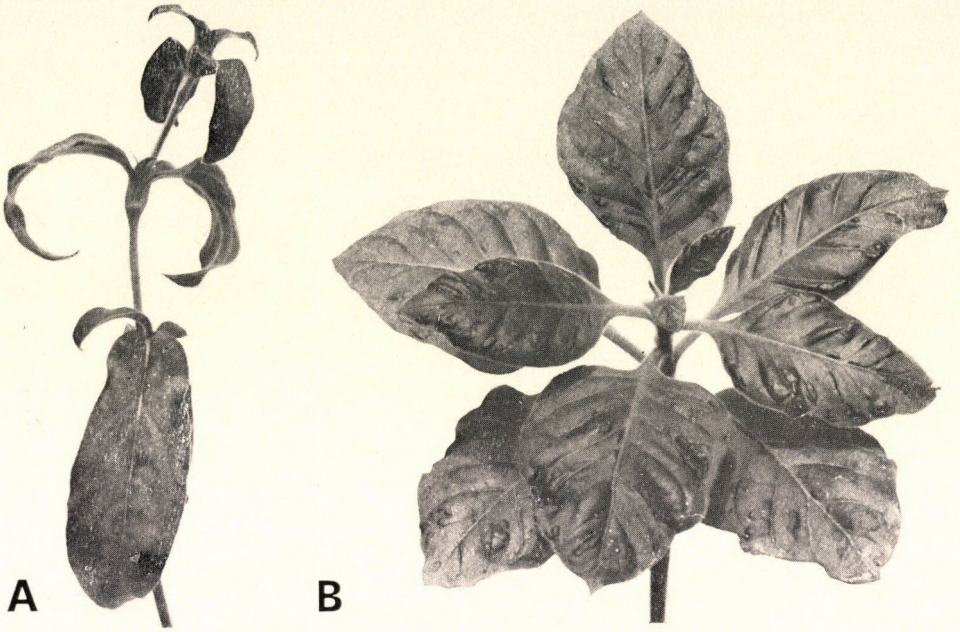

Fig. 2. Systemic symptoms on *Gomphrena globosa* L. (A) and *Nicotiana tabacum* L. cv. *Samsun* (B) plants

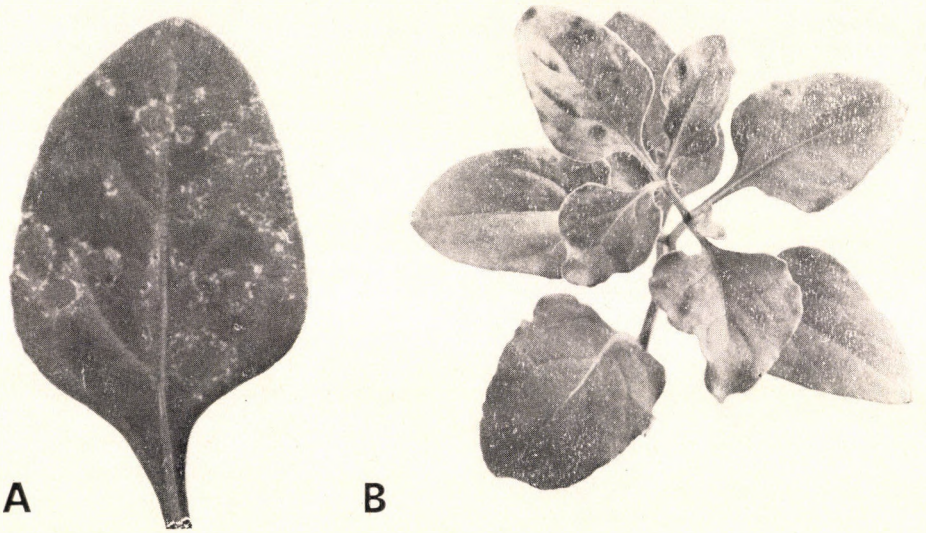

Fig. 3. Local (A) and systemic symptoms (B) on *Nolana prostrata* L.

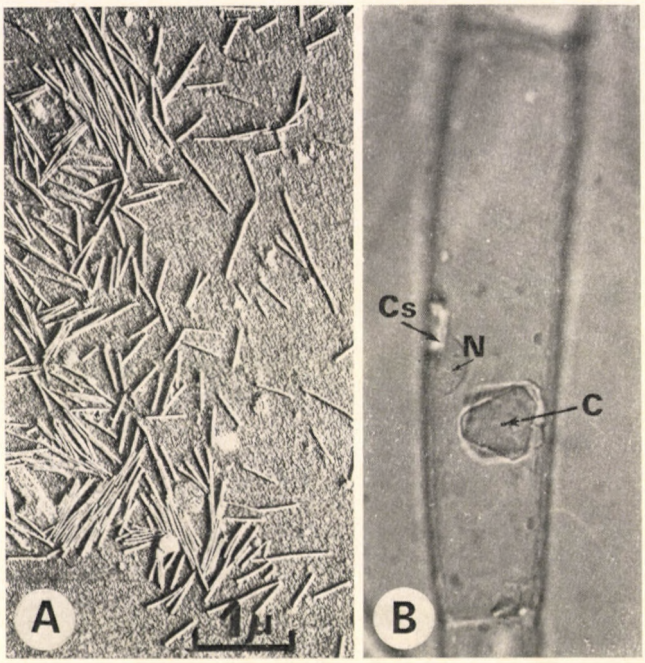

Fig. 4. A: Virus particles from artificially inoculated *Gomphrena globosa* L. plants (leaves showing systemic symptoms). B: A cell of an epidermal hair of a *Nicotiana tabacum* L. cv. *Samsun*, showing inclusion bodies of tomato mosaic virus (C, crystalline prisms in polar view; Cs, crystalline prisms in side view; N, nucleus). Magn.: × 840

On the basis of the symptomatological results obtained it seems probable that the infection of potato plants had been caused by a strain of ToMV which was earlier isolated from tomato plants *(Lycopersicon esculentum* cv. *Moneymaker)* in Hungary (cf. HORVÁTH and BECZNER, 1973; MAMULA *et al.*, 1974; HORVÁTH, 1976). Generally the reactions obtained on the used test plants corresponded to those provoked by some viruses of the tobamovirus group. In this respect the reaction of *Datura stramonium*, *Nicotiana glutinosa* and *N. tabacum* cv. *Xanthi-nc* which all repeatedly exhibited symptoms on inoculated leaves only is worth mentioning. However, the lack of systemic symptoms in *Nicotiana sylvestris* indicated that the isolate under examination differs from common TMV, and is probably identical with the ToMV (see HOLLINGS and HUTTINGA, 1976).

In India PHATAK and VERMA (1967) isolated a tobamovirus from potato. This isolate in contradiction to ours produced local lesions on *Phaseolus vulgaris* and systemic symptoms on *Nicotiana sylvestris*. Accordingly the Indian virus isolate is identical with the common TMV (cf. ZAITLIN and ISRAEL, 1975).

Preparations from systemically infected *Gomphrena* plants contained numerous, straight, rigid tubules, about 300 × 18 nm (Fig. 4A). These diameters are very close to those of ToMV (cf. HOLLINGS and HUTTINGA, 1976).

Light microscope cytological observations were performed on inoculated plants in order to establish the type of cytoplasmic inclusion bodies, if present. In *Gomphrena globosa* and *Nicotiana tabacum* cv. *Samsun* leaf hair-cells well developed crystalline inclusions in the form of more or less regularly shaped hexagonal prisms similar to those of the members of tobamovirus group were found (Fig. 4B). The investigated virus was tested with antisera against ToMV and TMV, using slide precipitin test. The reaction was positive, followed by the occurrence of pronounced precipitation which showed that the virus belonged to the tobamovirus group. The reaction against ToMV antiserum was much more stronger than that against TMV antiserum. Thermal inactivation point of the virus ranged between 95° and 97°C. The dilution end-point was in the range from 10^{-5} to 2×10^{-6}, the longevity *in vitro* was more than 21 days.

Transmission experiments with *Myzus persicae* aphids were unsuccessful.

On the basis of host range, symptomatology as well as serology, virus morphology and particle length, inclusion bodies and physical properties, the virus isolated from potato and studied here has been concluded to be a virus of the tobamovirus group, and identical with ToMV.

This is the first report about the natural occurrence of ToMV in potato.

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Studies of the Soil Transmission of the Nigerian Okra Mosaic Virus*

By

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Disease free seedlings of cowpea, green gram and okra developed okra mosaic virus (OMV) symptoms when grown in a field in which OMV infected plants had been grown or when grown in soil from areas of prior disease incidence. Successive soil transmission can be attributed to either root contact in the soil or virus containing debris or virus contaminated soil particles. Control plants did not become infected and no insect eggs were found on the roots of both infected and disease free plants.

In the course of studies on a Nigerian isolate of okra mosaic virus (OMV), leachate of the soil in which infected okra plants were growing was found to be highly infective and volunteer okra seedlings developed foliar mosaic symptoms similar to those induced by OMV (LANA and BOZARTH, 1975). Further experiments were therefore conducted to determine the following: (1) Whether the observed foliar mosaic symptoms in the volunteer seedlings were induced by OMV, (2) whether OMV is soil-borne and (3) the possible vectors of soil-borne OMV. This paper reports the results of investigations.

Materials and Methods

In the experiments, soil taken from the top 20 cm of the field plot where OMV occurred is referred to as "infested soil". Sterilized soil or soil from a different site where OMV disease had neither been observed nor recorded is referred to as "non-infested soil". All observations lasted for at least 6 weeks. Series of experiments were carried out to determine if transmission was through the soil and by what means.

In one of the experiments, OMV indicator hosts cowpea (*Vigna unguiculata* L. Walp cv. New Era), green gram (*Vigna radiata*), and okra (*Abelmoschus esculentus* L. Moench) were grown and observed for symptom development in a plot where volunteer okra seedlings from a previous experiment had developed mosaic symptoms. When symptoms appeared on the indicator plants, both the

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pattern of disease distribution and the presence of any insect vectors were recorded weekly for 5 weeks. Okra grown in adjacent plot about 18 meters away and where okra plants had not been grown for two previous seasons served as control.

In another experiment, field-soil was collected in 120 black plastic pots from 3 inches around OMV infected plants. Seeds of cowpea, green gram and okra were grown respectively in 40 pots each and the seedlings were observed for symptom development in an insect-proof greenhouse. Seedlings of the same indicator hosts grown in a similar way in non-infested soil served as control.

In a third experiment, 250 ml of crude sap obtained from leaves of OMV-infected plants, ground in sterilized mortar and pestle was added to pots containing sterilized soil in which the indicator hosts mentioned above were growing. These were also observed for disease syndrome while similarly treated soil but with crude sap of healthy okra plants served as control.

Soil suspensions were diluted to 10^{-2}, 10^{-3} and 10^{-4} with sterile distilled water plated on potato dextrose agar, cassava dextrose agar and malt extract agar. Pure cultures of the fungi obtained were communited and the mycelia suspension of each fungus was poured into sterilized soil in which OMV indicator hosts were growing.

For possible nematode transmission, soil was collected every 3 weeks for 3 months from the infested plot for nematode recovery, identification and population assessment (HIBBEN and WALKER, 1971). Attempts were also made to induce infection on indicators of OMV by using two nematodes (*Xiphinema halocum* and an unidentified *Helicotylenchus* ssp.) frequently extracted from okra growing in infested soil. *Xiphinema halocum* was cultured on *Begonia* plants growing in sterilized soil while large numbers of *Helicotylenchus cavenessi* were constantly extracted from cocoa growing soils at the Cocoa Research Institute of Nigeria, Ibadan, Nigeria. To ascertain that these nematodes were virus-free, they were first fed on healthy indicator hosts of OMV in 32 pots containing sterilized soil for 6 weeks. Another set of healthy indicator plants replaced the former and these new sets were mechanically inoculated with OMV, allowed to grow in the same pot containing the nematodes for another six weeks. These virus infected plants were removed again and replaced with healthy indicators of OMV and observed for 6 weeks for disease syndrome.

Identification of the virus involved in transmission: In all experiments, roots, stem and leaves of the test plants were assayed for virus by mechanical inoculation, and by purification of infected plants for electron microscopy and serology.

Results

When seeds of indicator hosts were grown in the field where heavily OMV-infected okra plants had been grown the previous season, the first true leaves of the okra seedlings showed mosaic symptoms. Infection was distributed in patches on okra growing in the plot but later appeared on cowpeas and green gram grow-

ing in the same plot. When some of these infected plants were assayed for virus, the virus was the same as that originally isolated from okra (LANA *et al.*, 1974).

The presence of the virus in the soil is suspected from the fact that virus free plants became infected when grown in soil collected from infested sites and when grown in the same flats with infected plants in sterilized soil. Plants grown in soil from non-infested plots did not become infected.

Soil from tests in which high levels of infection occurred were examined for the presence of nematodes known to be vectors of plant viruses but only *Xiphinema halocum* species was found and this has not yet been reported as a virus vector. The following other plant parasitic nematodes were also found in twenty samples of soil collected over a period of three months: *Helicotylenchus cavenessi* (frequently isolated in great numbers), *Pratylenchus* spp. and *Hoplolaimus* spp. However, Table 1 shows that *Xiphinema halocum* (2.8 and 1.7%) and *Helicotylenchus cavenessi* (1.2% and 2.1%) transmitted the virus into cowpea and greengram. No nematode transmission to okra test plant was recorded on test plants.

Conidia of *Aspergillus flavus* Link, *A. niger* van Tiegh, *Fusarium moniliforme* Sheld, *Penicillium digitatum*, Sacc, and zoospores of an unidentified species of *Pythium* were isolated from 20 infested soil samples examined, but no virus transmission occurred when mycelial suspensions of each of these fungi was added to sterilized soil in which indicator hosts were growing.

Virus from infected leaves was purified and the purified virus was tested against the OMV antiserum and analyzed by electron microscopy. The results indicated that the virus being transmitted in these experiments was the Nigerian OMV.

Discussion

The results presented in Table 1 suggest that OMV may be soil-borne. The levels of soil transmission which occurred in the test reported here are quite low and suggest that the virus may be seedborne. However, in an earlier study (LANA and BOZARTH, 1975) no seed transmission was detected when different OMV infected hosts were analyzed.

The pattern of distribution of this virus disease in the field during the experiments is in agreement with HARRISON's view on distribution of soilborne virus infection in the field (HARRISON, 1960). Although infection appeared rapidly, after six weeks initial infection and distribution were slow and occurred in patches.

OMV was transmitted to both green gram and cowpeas by *Xiphinema halocum* and *Helicotylenchus cavenessi* using the bait plant method (HIBBEN and WALKER, 1971). These nematodes, however, could not transmit OMV to its natural host. The transmission by these nematodes is, however, regarded as contamination.

Based on HARRISON's concept of a soilborne virus (HARRISON, 1960), the fact that OMV is transmitted from infected plants to test plants in the same container in the absence or presence of root contact indicates that the mode of transmission reported here belongs to the category of "soil-borne viruses".

Table 1

Evidence of soil transmission of the Nigerian OMV by different experimental methods

Method of transmission	Indicator host used					
	Green gram (*Vigna radiata*)		Cowpea (*Vigna unguiculata*)		Okra (*Abelmoschus esculentus*)	
Soil from infected plot	1* / 40	2.5%**	0 / 40	0	7 / 40	17.5%
Sterile soil in which OMV sap was added	0 / 80	0	0 / 80	0	0 / 80	0
Healthy plants grown in same flats with infected plants	5 / 120	4.1	0 / 80	0	16 / 120	13.3
Helicotylenchus spp.	1 / 85	1.2	1 / 47	2.1	0 / 40	0
Xiphinema spp.	2 / 73	2.8	2 / 120	1.7	0 / 120	0
Fungi	0* / 20	0	0 / 42	0	0 / 20	0

* Plant infected over plants tested (average of four replicates).
** Percentage infection.
None of the control plants used in these experiments became infected.

Results from these experiments suggest that even if no organisms were found to be involved in transmission, it seems certain that something other than free virus in the soil is required for infection to occur, for seedlings did not become infected when watered with highly infective virus crude sap. It is hereby stated that this occasional transmission of OMV could have occurred as a result of abrasion of healthy roots or shoots in the soil with virus-containing debris or virus-contaminating soil particles. This necessarily may not involve soil inhabiting nematodes but might provide a source of virus-infected plants from insect vectors that could be the means of secondary spread.

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Disease Resistance in Cereals*

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The role of antifungal and antibacterial substances in disease resistance in cereals is reviewed. Prohibitins, phytoalexins and other post-infectionally accumulated antimicrobial substances play a crucial role in defense. The possible use of phytoalexins in the chemical control of plant diseases is envisaged as an indirect method of disease control.

It is suggested that phytoalexins are not specific to a particular disease but to the diseased plant. Various phenolics and flavonoids reported from rice may be considered as prohibitins. Consideration of momilactones A and B as phytoalexins in rice is questioned as they are present in healthy plants. MBOA [6-methoxyl-2(3)-benzoxazolinone] must also be considered as a prohibitin in wheat, rather than as phytoalexin. The concepts of induced resistance and induced accessibility in barley are discussed in detail. Phytoalexins induced by an incompatible pathogen confer resistance in barley against the compatible race of powdery mildew organism. Phytoalexins in maize appear to influence its resistance to *Helminthosporium turcicum*. Avenacin is a very active prohibitin in oats. 2(3)-Benzoxazolinone is the major prohibitin in rye.

Phytoalexin production in cereals is largely unexplored. Search for the possible use of chemicals which can induce phytoalexin production in plants is suggested.

There is enough evidence to believe that disease resistance in plants is an active biochemical process. Studies on the chemical basis of disease resistance have clearly shown that prohibitins and phytoalexins play a crucial role in the hosts' innate defence. The concept of prohibitins formulated by MAHADEVAN (1970) is widely accepted and these preformed antifungal substances have recently been reviewed by OVEREEM (1976) and SCHÖNBECK and SCHLÖSSER (1976). Phytoalexins, the post-infectionally formed antimicrobial substances, have been reviewed by many workers (CRUICKSHANK, 1963, 1974; CRUICKSHANK and PERRIN, 1964; CRUICKSHANK *et al.*, 1971; DEVERALL, 1972a, b, 1976; INGHAM, 1972, 1973; KUĆ, 1968, 1972, 1976; KUĆ *et al.*, 1976; MAHADEVAN, 1973; PURKAYASTHA, 1971, 1973, 1976; STOESSL *et al.*, 1976; VANETTEN and PUEPPKE, 1976).

Indeed phytoalexins have attracted increasing attention of researchers during the recent years. This may be attributed to their possible role as natural defensive agents (CRUICKSHANK and PERRIN, 1964; INGHAM, 1972; KUĆ, 1966;

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ROHRINGER and SAMBORSKI, 1967; STOESSL, 1970). Besides, the induction of disease resistance in plants through phytoalexin induction can also be envisaged as an indirect method of plant disease control (SADASIVAN, 1965).

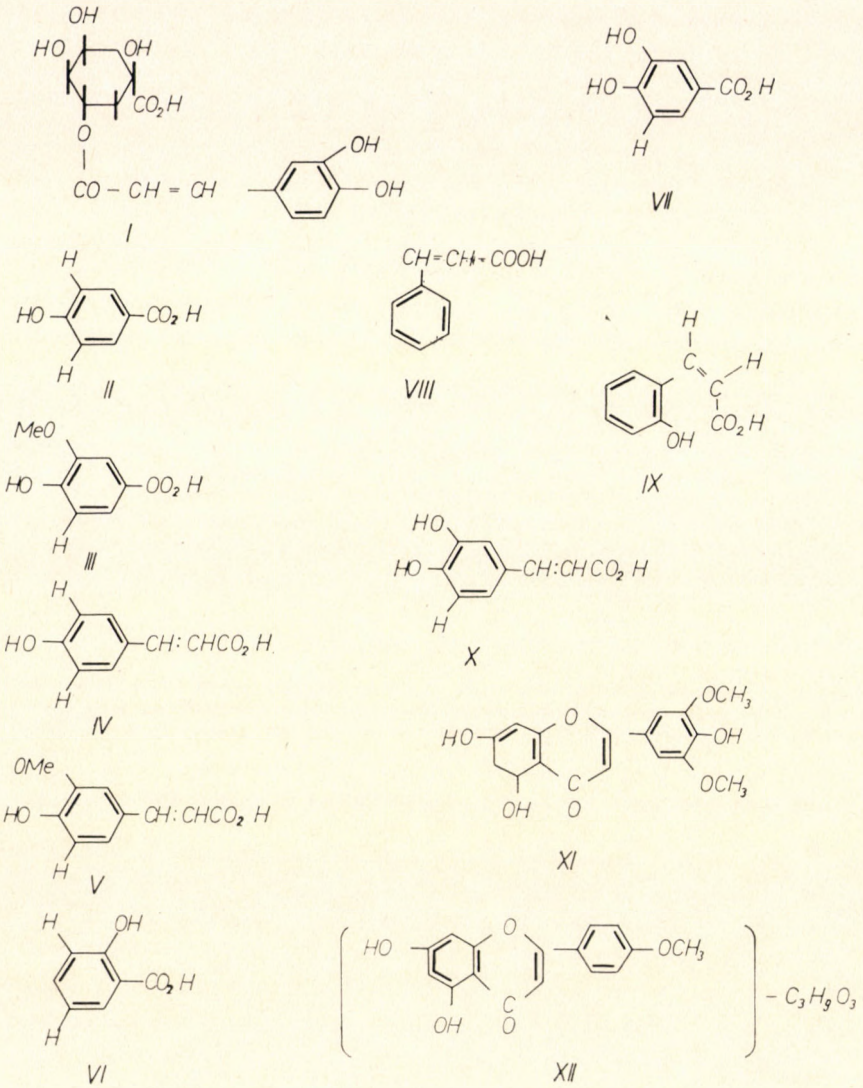
It is quite inappropriate for KUĆ (1976) to observe that phytoalexins are "most thoroughly studied". However, we suggest that in the field of Plant Pathology and disease resistance in particular, phytoalexins form the subjectmuch talked about and less worked out. It is quite surprising and disheartening to note that little work has been done on the biochemistry of disease resistance in the cereals which form the important staple food all over the world. This review is mainly intended to present a comprehensive account of the role of antifungal and antibacterial substances in disease resistance of some cereals.

Rice (*Oryza sativa* L.)

Earlier work on disease resistance in rice by AYYAMPERUMAL *et al.* (1968) and VEERRAJU and PRASAD (1971) revealed the presence of a phenolic pre-infectional antifungal substance in rice. This inhibitor was antifungal against *Helminthosporium oryzae* causing malformations on the spores. VEERRAJU and PRASAD (1972) screened a number of rice varieties for this inhibitor and observed that CO 4 and CO 13 possess highest and lowest concentration of the inhibitor respectively. Surprisingly enough, these varieties are highly resistant and susceptible, respectively, to 'blast' disease caused by *Pyricularia oryzae*. Phenolic compounds have been associated with host defense because of their rapid accumulation in the immediate vicinity of the infected tissues and that phenols and their oxidation products are highly fungitoxic (FARKAS and KIRÁLY, 1962; KUĆ, 1966; ROHRINGER and SAMBORSKI, 1967; RUBIN and ARTSIKHOVSKAYA, 1964; TOMIYAMA, 1963). A number of phenolic compounds, viz. chlorogenic (I) acid (SUZUKI *et al.*, 1953); p-hydroxybenzoic (II), vanillic (III), p-coumaric (IV) and ferulic (V) acids (KUWATSUKA and OSHIMA, 1961a, b); salicylic (VI) acid (ISHII *et al.*, 1962); and protocatechuic (VII), cinnamic (VIII), o-coumaric (IX) and caffeic (X) acids (VARGA, 1970) and flavonoids such as tricin (XI) (KUWATSUKA and OSHIMA, 1961b), and oryzagenin (XII), inetin (XIII), homoinetin, homooryzatin (XIV), inabanin (XV) and homoinabatin (XVI) (KUWATSUKA, 1962) reported from rice leaf tissues may probably serve as prohibitins.

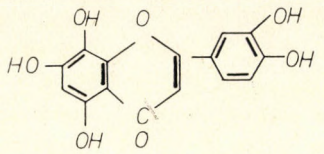
Blast resistant rice varieties were reported to contain more polyphenols than susceptible cultivars (SUZUKI, 1965; WAKIMOTO and YOSHII, 1958) whereas a reverse relationship was observed by RAMAKRISHNAN (1966). Similarly, SRIDHAR (1972a, b) could not find any relationship between the resistance of rice varieties and their phenolic level.

Recently, SRIDHAR and OU (1974a, b) studied the changes in phenolic levels in blast infected rice. They observed increased levels of total and ortho-dihydroxy phenolics, peroxidase and ascorbic oxidase in resistant rice cultivars and only a slower rate of increase in susceptible cultivars after inoculation with *P. oryzae*. They detected salicylic (VI), p-coumaric (IV) and ferulic (V) acids from the rice blast le-



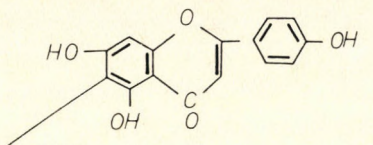
sions and suggested the formation of a protein-quinone complex after the oxidation of these phenolic acids *in vivo*.

Phenolics upon oxidation become highly toxic to pathogens and pathogenic enzymes, thus inhibit the development of pathogen in the tissues (FARKAS and KIRÁLY, 1962; MAHADEVAN, 1966). The oxidation of phenolics is by polyphenol oxidase and peroxidase. In the absence of O-diphenol oxidase in healthy and blast infected rice plants (SRIDHAR, 1972a; TOYODA and SUZUKI, 1960), peroxidase



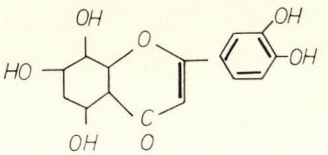
$C_{12}(OH)_9\ H_{12-16}\ O.H_2O$

XIII

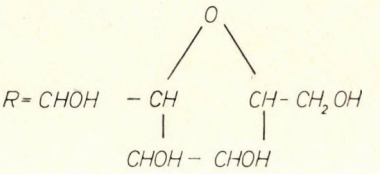


$C_{12}(OH)_9\ H_{12-16}\ .O$

XIV

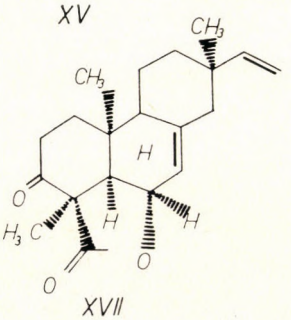


$R = (CHOH)_5\ CH_2OH\ (?)$

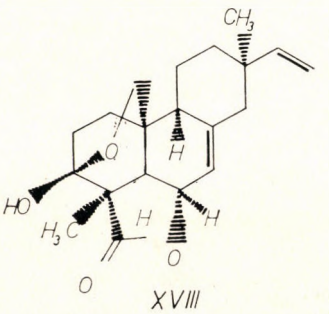


XVI

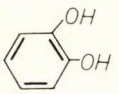
XV



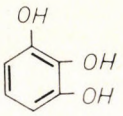
XVII



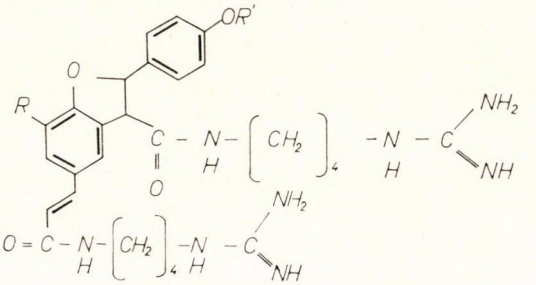
XVIII



XIX



XX



XXI: $R = R' = H$

XXII: $R = OMe,\ R' = H$

indirectly mediates oxidation of phenols in rice—*P. oryzae* interaction (SRIDHAR, 1972a; THOMSON, 1964; TOYODA and SUZUKI, 1960).

As for postinfectional antifungal substances, UEHARA (1958) was the first to show their production in rice. A drop diffusate technique with detached rice leaves using a conidial suspension of *P. oryzae* indicated the antifungal activity of the

supernatant identified as a phenol. Both *Xanthomonas oryzae* and the blast pathogen *P. oryzae* are sensitive to the supernatants from the diffusates. Similar phytoalexin production occurred as a result of interaction between the rice plant and the leaf blight bacterium, *X. oryzae*. Phytoalexin from this host-parasite combination was effective against both *X. oryzae* and *P. oryzae* (UEHARA, 1960). UEHARA concluded that phytoalexins of rice did not display any specificity in their antibiotic action.

The effect of mixed inoculation on lesion formation and the production of antifungal substances was studied by OHATA and KOZAKA (1967). Mixtures of spores of avirulent and virulent races of *P. oryzae* produced smaller lesions than when the virulent race alone was used. Lesion size was inversely related to the concentration of avirulent spores in the mixed inoculum. However, mixed inocula containing *P. oryzae* and some nonpathogens of rice, *Alternaria brassicola*, *Botrytis cinerea*, *Colletotrichum glycines*, *Erwinia aroideae*, *Fusarium oxysporum* f. *lycopersici*, and *Septoria lactucae* did not show this effect. Two ultraviolet fluorescing phytoalexin-like substances were detected in and around the lesion area. Neither of them was present in healthy rice plants, in culture filtrates or in the mycelium of *P. oryzae*. The one with high Rf value (0.89) was more inhibitory to spore germination of *P. oryzae* and also to nonpathogens *Alternaria brassicola*, *Botrytis cinerea*, *Cochliobolus miyabeanus*, *Fusarium oxysporum* f. *lycopersici* and *Pestalotia* spp. than the one with 0.69 Rf value in rice-blast system. These substances are also produced in rice plants infected with *C. miyabeanus*, *X. oryzae* and stripe virus respectively and also in plants injured with copper sulfate. These two substances were not identified with any phenolics or coumarins already reported from diseased rice. They appear to be specific to the diseased rice plant but not to the blast disease alone.

Momilactones A (XVII) and B (XVIII) were claimed as the first to be characterized from a member of the Gramineae and were considered as phytoalexins (CARTWRIGHT *et al.*, 1977). These phytoalexins are significantly different from all the previously described phytoalexins in that the former are diterpene lactones whereas the latter are phenols or sesquiterpenes. This report also substantiated the idea that fungicide treatment may enhance the ability of a plant to synthesize phytoalexins in response to infection (DAY, 1974; SISLER, 1977). CARTWRIGHT *et al.* (1977) also demonstrated phytoalexin induction by ultraviolet irradiation of leaves or dark-grown coleoptiles of rice. We, however, believe that momilactones A and B cannot be considered as phytoalexins as they are present in healthy plants. Instead, we prefer to call them prohibitins. Though momilactones A and B have been reported in rice – *P. oryzae* system, search can be made for these in rice infected by other microorganisms.

Histochemical evidence for disease resistance in rice against *H. oryzae* infection was provided by OKU (1962) in Japan. He clearly showed the rapid browning of cells in resistant but not in the susceptible varieties. Phenolic substances and related quinones accumulated in the invaded cells and in a few cell layers in their immediate vicinity in resistant rice varieties. Therefore OKU related the formation of quinones to disease resistance factors in rice against brown leaf spot disease.

Breakdown of disease resistance of rice plants was reported by the addition of reducing agents like ascorbic acid or glutathione to *H. oryzae* spore inoculum (OKU, 1960). Phytoalexin production occurs in the host-parasite interaction of brown spot disease of rice (OKU and NAKANISHI, 1962). Furthermore, these authors found that the more resistant the variety, the higher its antifungal activities.

SINHA and TRIVEDI (1969) attempted to induce resistance in rice cultivars susceptible to *H. oryzae*, by prior inoculation with an avirulent race. Germinating fungal fluids or heated and spore free inoculum of the avirulent race was equally effective in inducing resistance to virulent races. Antifungal substances that reduce germination and germ tube growth accumulated in the tissues inoculated with the protecting avirulent race. Further work by SINHA and DAS (1972) revealed that induced resistance in rice against *H. oryzae* infection depends upon a number of factors. One of these is the concentration of spores in the inoculum. Induced resistance was effective at concentrations up to 5×10^5 spores/ml, but rapidly declined at high concentrations. Age of the plant was also important in that maximum resistance was displayed by 21-day-old plants, which effect gradually disappeared in older plants. Resistance was maximum with a 2-day interval between inoculation with protecting race and the pathogenic race. Besides, germination fluids conferred better protection at higher concentrations than the comparable avirulent spore suspensions. SINHA and TRIVEDI (1972), however, demonstrated that the spore suspension was more effective in inducing resistance in rice.

PURKAYASTHA and MUKHOPADHYAY (1974) studied the factors affecting the colonization of rice leaves by *H. oryzae*. PURKAYASTHA and CHATTOPADHYAY (1975) and PURKAYASTHA and MUKHOPADHYAY (1976) detected greater antifungal activity in rice resistance to *H. oryzae* than in susceptible rice. They further confirmed that there was a close correlation between low antifungal activity of diffusates and high incidence of disease.

There is little work on disease resistance in rice against bacterial pathogens. PURUSHOTHAMAN and PRASAD (1971) indicated the possible involvement of prohibitins of heat-labile nature in resistance to *X. oryzae*. PURUSHOTHAMAN (1974) further pointed out that resistant varieties showed increased levels of total and orthodihydric phenols than the susceptible ones after infection with *X. oryzae*. Besides, two new diazotized sulphanilic acid reacting spots were present in the resistant varieties but absent in susceptible varieties.

NAKANISHI and WATANABE (1977a) recently studied the role of ethyl acetate-extractable antibacterial substances in the resistance of rice plants against *X. oryzae* infection. These substances started accumulating within 24 h after inoculation and increased rapidly to a maximum level within 3–5 days in incompatible reaction. They were not detected, however, till the lesion formation in compatible combinations. Minute quantities of these antibacterial substances were also detected in uninfected rice leaves. These authors (1977b) further partially purified these substances by silica gel column chromatography following thin-layer chromatography.

Healthy leaves of rice cultivar resistant to the bacterial leaf streak, *X. translucens* f. sp. *oryzicola*, contained higher quantities of total phenols than the susceptible

varieties. In the resistant host-parasite combinations phenols decreased initially but increased subsequently (RANGA REDDY and SRIDHAR, 1975).

Detoxification of the pathogen produced toxins by plant constituents is also an important defense mechanism. Piricularin, for example, was detoxified by chlorogenic and ferulic acids by forming a nondiffusible complex in rice plant tissues (TAMARI *et al.*, 1965).

Wheat (*Triticum vulgare* Vill.)

NEWTON and ANDERSON, as early as 1929, correlated the phenolic content in wheat varieties to rust resistance and developed "The Phenol Hypothesis" which reads: "resistance to stem rust in wheat is due to phenolic compounds set free in the cell on the entrance of the fungus; these kill the cell and inhibit the growth of the parasite". Besides, protocatechuic acid (VII), catechol (XIX) and pyrogallol (XX) which might be considered as prohibitins were detected in wheat by KARGOPOLOVA (1935). No definite correlation, however, existed between the phenol content and stem rust resistance of wheat varieties (KIRÁLY and FARKAS, 1962), even though rapid accumulation of phenolics was associated with resistant reactions.

Water and methyl alcohol extractable components from wheat seeds inhibited fungi and Gram-positive bacteria (ARK and THOMPSON, 1958). VIRTANEN *et al.* (1957) identified the antifungal 6-methoxy-2(3)-benzoxazolinone (MBOA) (XXIV) from young wheat plants, active against *Fusarium nivale* and *Sclerotinia trifoliorum*. It appeared as a hydrolytic product of the glycoside present in healthy wheat. WAHLROOS and VIRTANEN (1961) also detected an antifungal 4-o-glucosyl-1,2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one from healthy wheat. Recently an active substance effective against *Puccinia braminis* var. *tritici* and *Helminthosporium sativum* was isolated from an incompatible interaction between wheat leaves and *P. graminis tritici* (DEVERALL, 1976). The active component was identified as methoxy-benzoxazolinone (MBOA). Further, DEVERALL suggested a possible release of MBOA from an inactive glycoside present in healthy cells after post-infectional hydrolysis. DEVERALL (1976) was cautious in calling MBOA a phytoalexin since its role in wheat resistance requires evaluation, but we feel it should be regarded as a prohibitin. Recently, BAKER and SMITH (1977) showed that the antifungal benzoxazine gradually decreased in wheat cultivars resistant and susceptible to *Septoria nodorum* 3–4 weeks after germination. However, these could not be detected in field-grown plants. Healthy and diseased field-grown wheat cultivars revealed the presence of additional antifungal substances but their role in disease resistance is yet to be ascertained.

Studies by ELNAGHY and LINKO (1962) on the resistance of wheat to stem rust caused by *P. graminis* var. *tritici* showed higher concentrations of the glycoside 4-o-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistant varieties and the concentrations varied with the degree of resistance in wheat. Enzymatic release of this glycoside occurred after rust infection.

Naturally occurring quinones in wheat with fungicidal rather than fungistatic effect on the spores and mycelium of loose smut fungi *Ustilago nuda* and *U. tritici* were observed by MACE and HEBERT (1963).

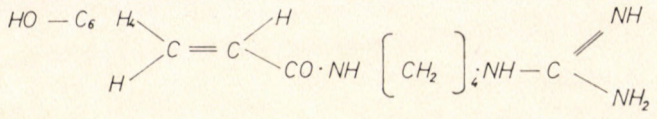
The causal role for peroxidase in stem rust resistance in wheat was ruled out by SEEVERS and DALY (1970a, b) and DALY *et al.* (1970). In later studies, DALY *et al.* (1971) concluded that peroxidase activity was non-specific and appears even after mechanical wounding. MAYAMA *et al.* (1975) recently interpreted the development of *P. graminis tritici* in resistant and susceptible wheat varieties to glucosamine content, using ligand exchange chromatography. Significant increase in glucosamine content indicated rapid fungal growth in compatible hosts before symptom development. Persistent growth of the pathogen in compatible hosts was attributed to the smaller glucosamine increase.

Barley (*Hordeum vulgare* L.)

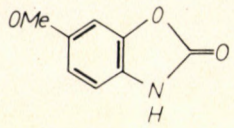
Antimicrobial substances effective against fungi and Gram-positive bacteria have been reported from the barley seeds (ARK and THOMPSON, 1958). Naturally occurring quinones active against loose smut fungi were also found in barley (MACE and HEBERT, 1963).

Our knowledge of the antifungal substances in barley mainly stems from the work of STOESSL and his group in Canada. LUDWIG *et al.* (1960) reported the ability of barley seedlings to resist invasion by *Helminthosporium sativum*. Antifungal activity was recovered from extracts of 5-day-old barley coleoptiles, but not in 6-day-old ones. These authors suggested that an inhibitor of antifungal activity appears to reduce activity after 5 days. Ca ions present in older coleoptiles inhibited the antifungal activity of the extracts. KOSHIMIZU *et al.* (1963) described the preparation and properties of some highly active antifungal factors in barley. STOESSL (1965) isolated p-coumaroylagmatine (XXIII) from young barley shoots, showing weak antifungal activity. From his exhaustive studies on the isolation, structure and synthesis of antifungal factors in barley, STOESSL (1966a, b; 1967) finally attributed the antifungal activity to hordatines A (XXI) and B (XXII) and their glycosides. This inhibitory effect was clearly demonstrated by STOESSL and UNWIN (1970) on the spore germination of *Colletotrichum coccodes*, *Fusarium solani*, *Glomerella cingulata*, *Helminthosporium sativum* and *Monilia fructicola*. Complete spore germination inhibition in these fungi occurred at 10 ppm, whereas *H. sativum* was less sensitive at this concentration.

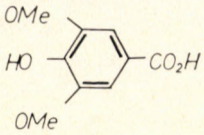
OUCHI *et al.* (1974a, b) induced disease resistance in barley by preliminary inoculation with an incompatible race of *Erysiphe graminis*. They demonstrated induced susceptibility or induced accessibility in barley – a process where prior inoculation with a compatible race rendered the barley leaves accessible to the originally incompatible races. Histological evidence was presented for the localized nature of the induced susceptibility or induced accessibility. OUCHI *et al.* (1976) further reported the localization of induced resistance in barley leaves elicited by a nonpathogenic race of *E. graminis*. Barley leaves first inoculated with a compatible



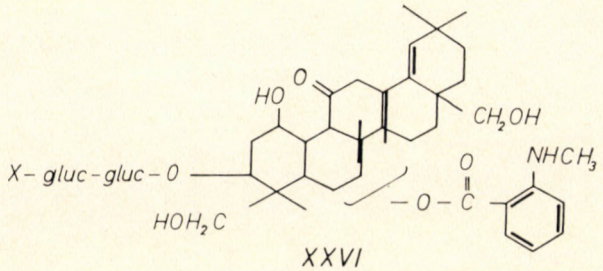
XIII



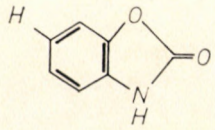
XXIV



XXV



XXVI



XXVII

race of *Erysiphe graminis* became locally susceptible to *Sphaerotheca fuliginea*, a bean pathogen.

Phytoalexin production in obligate host-parasite combinations is a fertile field for research. OKU *et al.* (1975a) started work on these lines, although indications of phytoalexin production were reported earlier (LEATH and ROWELL, 1970; SCOTT *et al.* 1957). Further, OKU *et al.* (1975b) detected two phases of phytoalexin production in the barley – *E. graminis* system. The phytoalexin activity detected 12 h after inoculation, the first phase, was more pronounced in incompatible combinations than in compatible ones, but this phytoalexin accumulation was lost by treatment at 50°C for 5 min. In the second phase, the phytoalexin production was restricted to the fungal colonies formed on the leaves of compatible cultivar-race interaction and in this case phytoalexin was equally effective against all the races. Breakdown of resistance and simultaneous breakdown of phytoalexin production in incompatible combinations by heat treatment was reported (OKU *et al.* 1975c). Phytoalexin production during the first phase of an incompatible interaction was suppressed when the barley leaves were first inoculated with a compatible race. This could be interpreted as induction of accessibility by suppression of phytoalexin production. Thus "Once host cells recognize a microbe as compatible, phytoalexin production cannot be initiated by the subsequent infection with an incompatible race. Conversely, once host cells were conditioned towards rejection phytoalexin was induced even if a compatible race were later introduced. These results also show that the

phytoalexin production is a result of host cell recognition of the parasite, and phytoalexin could possibly be involved in the mechanism of host-parasite, specificity in powdery mildew disease of barley" (OKU and OUCHI, 1976). They further suggested that "The phytoalexin production of barley was parallel to the degree of incomeatibility in the predisposed leaves. Thus, phytoalexin may be a product of the plant's defense reaction".

The possible involvement of phytoalexins in the resistance of barley to net blotch caused by *H. teres* was suggested by KEELING and BANTTARI (1975). But details are yet to be worked out.

Maize (*Zea mays* L.)

VIRTANEN *et al.* (1956, 1957) and SMISSMAN *et al.* (1957) isolated 6-methoxy-2(3)-benzoxazolinone (MBOA) which is antifungal. MBOA is formed by the degradation of 1,4-benzoxazine which exists in bound form as a glucoside (HIETALA and VIRTANEN, 1960; WAHLROOS and VIRTANEN, 1959). Attempts to search for the free aglucones in the uninjured resistant corn lines were first made by WAHLROOS and VIRTANEN (1964), who suggested that "MBOA is not present in fresh plant tissue even of the resistant maize inbred W 22, but that the free aglucone is present in appreciable concentration in this maize variety and accordingly, may vary widely in different maize strains. The aglucone which has antifungal properties may thus be the resistance factor in maize". Further studies by VIRTANEN and WAHLROOS (1963) and WAHLROOS and VIRTANEN (1964) led them to presume the occurrence of free DIMBOA aglucone in the plant. HOFMAN and HOFMANOVA (1971) also presumed that there was a correlation between the amount of free DIMBOA in the corn plant and its resistance. Their experiments showed that free 2,4-dihydroxyl-7-methoxyl-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) was absent in uninjured corn plants. They also failed to detect the possible aglucones of the known glucosides reported earlier (HOFMAN and HOFMANOVA, 1969; HOFMAN *et al.*, 1969, 1970). These authors concluded that "The resistance of plants is not determined by the presence of free aglucone in the uninjured plant, but corresponds to a high content of glucosides of 1,4-benzoxazine in the resistant lines and by the capacity to release from them antifungally active aglucones".

COUTURE *et al.* (1971) presented evidence for the production of cyclic hydroxamates such as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one and suggested that they were responsible for resistance to *H. turcicum* in maize. Spore germination was inhibited at concentrations of 1 to 10 ppm.

The role of phenolics in resistance to *Helminthosporium* and *Fusarium* was demonstrated by MOLOT (1969). The phenolics implicated were p-hydroxybenzoic (II), vanillic (III) and syringic (XXV), p-coumaric (IV) and ferulic acids (V). MACE and VEECH (1973) observed inhibitory activity of the diffusates to *H. turcicum* spore germination from susceptible or chlorotic-lesion-type resistant corn leaves 1–3 days after inoculation with *H. turcicum*. The pH of the leaf diffusates had a marked effect on the inhibition of spore germination. Changes in host phenols and pigments

associated with the resistance of maize to anthracnose caused by *Colletotrichum graminicola* were recently studied by HAMMERSCHMIDT and NICHOLSON (1977). These authors observed a rapid increase in total phenol in the resistant and hypersensitive resistant inbreds compared with the susceptible inbreds which showed no change. Three phenolics, M_1, M_2 and M_3, were detected, two of which (M_1 and M_2) were identified as flavonoids of the flavone group. These two compounds showed marked inhibitory activity to *C. graminicola* spore germination. The third compound (M_3) was not inhibitory. Accumulation of anthocyanin pigments was also shown in the resistant and hypersensitive resistant plants, but not in the susceptible varieties (HAMMERSCHMIDT and NICHOLSON, 1977).

Oats (*Avena sativa* L.)

TURNER (1956) reported the presence of an antifungal inhibitor in leaf sap of oat seedlings that is effective against *Ophiobolus graminis*. The oat roots contained a factor that inhibited the invasion by *O. graminis*, a non pathogen of oats, and which caused the take-all disease of wheat (TURNER, 1960, 1961). This prompted TURNER to suggest that the oat pathogen *O. graminis* var. *avenae* produced an enzyme which detoxified the resistance factor enabling the pathogen to invade its host. Further work by MAIZEL *et al.* (1964) led to the isolation of avenacin from oat roots. Avenacin inhibited the growth of *O. graminis* and some other microorganisms at 3–50 ppm concentrations. Its structure was elucidated by BURKHARDT *et al.* (1964) and evidence for the mechanism of action of the avenacinase enzyme to detoxify the avenacin (XXIV) was also presented by these authors.

An inhibitory factor isolated from the young oat leaves grown in dark (OLSEN, 1971a, b) is present in the plant as methoxyhydroquinone glucoside. The inhibitory activity of this compound, or its oxidation product methoxybenzoquinone, was demonstrated against *O. graminis* var. *avenae* and *O. graminis* var. *graminis*, but not to *Fusarium oxysporum* f. *lycopersici*. OLSEN (1972, 1973a, b) has suggested that avenacin acts like saponin by binding to sterols in the cytoplasmic membrane which disrupts membrane permeability.

TANI *et al.* (1975a) from their studies on induced resistance in *Avena sativa* by an incompatible race of the crown rust fungus *Puccinia coronata* var. *avenae* observed that the protecting effect of the preinoculant diminishes as the time interval between the two inoculations increased. TANI *et al.* (1975b) further showed that the first events determining host resistance appeared between 8 and 12 hr after inoculation and that they are not necessarily associated with host cell collapse.

Rye (*Secale cereale* L.)

The earliest known antifungal substance from a member of Gramineae was isolated from the pressed juice of green rye seedlings (VIRTANEN *et al.*, 1957). This compound, 2(3)-benzoxazolinone (XXVII), inhibitory to *F. nivale* and *Sclerotinia trifoliorum*, was first thought to be the disease resistance factor in rye. It was

later found to be an artifact. The substance naturally occurring in plants is 2-glucosyloxy-4-hydroxy-1,4-benzoxazin-3-one. When the plants were crushed the aglucone was released by the action of β-glucosidase. Upon heating this aglucone loses the 2-carbon atom leading to the formation of 2-benzoxazolinone and formic acid (HIETALA and VIRTANEN, 1960; HONKANEN and VIRTANEN, 1961).

Ragi (*Eleusine coracana* [L.] Derf.)

Phytoalexin production in finger millets is largely unexplored. The paper by PURUSHOTHAMAN and MARIMUTHU (1974) appears to be the first to indicate phytoalexin production in ragi.

VIDHYASEKARAN (1976) found higher phenolic content and polyphen oloxidase activity in the leaves of finger millets resistant to *H. nodulosum* and *H. tetramera.* Besides, he demonstrated that oxidized phenols markedly inhibited spore germination, mycelial growth and pectic and cellulolytic activity of the pathogen.

Conclusions

Most of the researchers have compared the physiological and biochemical changes in the inoculated resistant and susceptible plants. In fact, the literature is sprinkled with such factual data (Tables 1 – 3). However, such studies will only help in identifying certain biochemical markers for resistance and susceptibility. Despite the work done to understand the mechanism(s) of resistance of cereals against pathogenic microorganisms our present knowledge is almost in the beginning of this interesting aspect of study. Admittedly, for years to come the lacuna will continue to exist.

Nevertheless, resistance is the fundamental attribute of plants. By studying the mechanisms by which plants resist parasites, it is possible to manipulate them to our advantage. Either by fixing the defending power genetically into the plants or by inducing resistance by certain treatments, can one successfully protect plants from debilitating and devastating parasites.

That phytoalexins can be successfully utilized to control plant diseases is quite attracting. Indeed, DUCZEK and HIGGINS (1976) provided experimental evidence in support of this view. They showed that phytoalexins from red clover when treated to *Helminthosporium carbonum* spores prevented from infecting their own host plant corn. The possible use of substances apart from bactericides and fungicides in inducing disease resistance in plants is quite challenging. In fact, VAN LOON (1977) showed that ethephon, an ethylene releasing compound, induced systemic resistance in tobacco against tobacco mosaic virus (TMV). This may open a new approach in chemical control of plant diseases.

Substances besides prohibitins and phytoalexins may also serve as disease resistance factors in plants. Indeed, the recent report of participation of some basic proteins in the disease resistance of peas (HADWIGER *et al.*, 1977) is quite stimulating and it necessitates the search for similar substances in other host–parasite systems.

Table 1

Occurrence of prohibitins in cereals

Common name	Latin name	Prohibitin	Reference
Rice	*Oryza sativa* L.	Chlorogenic acid	SUZUKI *et al.* (1953)
		p-Hydroxybenzoic acid	KUWATSUKA and OSHIMA (1961a, b)
		Vanillic acid	KUWATSUKA and OSHIMA (1961a, b)
		p-Coumaric acid	KUWATSUKA and OSHIMA (1961a, b)
		Ferulic acid	KUWATSUKA and OSHIMA (1961a, b)
		Salicylic acid	ISHII *et al.* (1962)
		Protocatechuic acid	VARGA (1970)
		Cinnamic acid	VARGA (1970)
		O-coumaric acid	VARGA (1970)
		Caffeic acid	VARGA (1970)
		Tricin	KUWATSUKA and OSHIMA (1961b)
		Oryzagenin	KUWATSUKA (1962)
		Inetin	KUWATSUKA (1962)
		Homoinetin	KUWATSUKA (1962)
		Homooryzatin	KUWATSUKA (1962)
		Inabanin and homoinabatin	KUWATSUKA (1962)
Wheat	*Triticum vulgare* Vill.	Protocatechuic acid	KARGOPOLOVA (1935)
		Catechol	KARGOPOLOVA (1935)
		Pyrogallol	KARGOPOLOVA (1935)
		6-Methoxy-2(3)-benzoxazoline (MBOA)	VIRTANEN *et al.* (1957)
		4-O-Glucosyl-1,2,4-dihydroxy-7-methoxy-1,4,-benzoxazine-3-one	VIRTANEN (1961) ELNAGHY and LINKO (1962)
Barley	*Hordeum vulgare* L.	Hordatine A	STOESSL (1966a, b; 1967)
		Hordatine B	
		p-Coumaroylagmatine	STOESSL (1965)
Maize	*Zea mays* L.	6-Methoxy-2(3)-benzoxazolinone (MBOA)	VIRTANEN *et al.* (1956, 1957) SMISSMAN *et al.* (1957)
		1,4-Benzoxazine	HOFMAN and HOFMANOVA (1969) HOFMAN *et al.* (1969, 1970)
		p-Hydroxybenzoic acid	MOLOT (1969)
		Vanillic acid	MOLOT (1969)
		Syringic acid	MOTOT (1969)
Oats	*Avena sativa* L.	Avenacin	MAIZEL *et al.* (1964) BURKHARDT *et al.* (1964)
		Methoxyhydroquinone	OLSEN (1971a, b)
Rye	*Secale cereale* L.	2(3)-Benzoxazolinone	VIRTANEN *et al.* (1957)

Table 2

Relationship between the sensitivity of plant parasites to prohibitins and pathogenicity

Common name	Latin name	Prohibitin	Microorganism	Sensitivity	Pathogenicity	Reference
Rice	*Oryza sativa* L.	Unidentified	*Helminthosporium oryzae*	+	+	AYYAMPERUMAL *et al.* (1968)
		Unidentified	*Xanthomonas oryzae*	+	+	PURUSHOTHAMAN and PRASAD (1971)
		Flavonoids	*Pyricularia oryzae*	−	+	WAKIMOTO *et al.* (1961)
			Helminthosporium oryzae	−	+	
Wheat	*Triticum vulgare* Vill.	6-Methoxy-2(3)-benzoxazolinone (MBOA)	*Fusarium nivale*	+	−	VIRTANEN *et al.* (1957)
			Sclerotinia trifoliorum	+	−	ELNAGHY and LINKO (1962)
		4-O-Glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one	*Puccinia graminis* var. *tritici*	+	+	
		Naturally occurring quinones	*Ustilago nuda*	+	+	MACE and HEBERT (1963)
			U. triciti	+	+	
Barley	*Hordeum vulgare* L.	Hordatines A and B	*Monilinia fructicola*	+	−	
			Helminthosporium sativum	+	−	STOESSL and UNWIN (1970)
			Glomerella cingulata	+	−	
			Fusarium solani	+	−	
			Colletotrichum coccodes	+	−	

Maize	*Zea mays* L.	2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one	*Helminthosporium turcicum*	+	+	COUTURE *et al.* (1971)
		p-Hydroxybenzoic, vanilic, syringic, p-coumaric and ferulic acids	*Helminthosporium*	+	−	MOLOT (1969)
			Fusarium	+	−	
Oats	*Avena sativa* L.	Avenacin	*Ophiobolus graminis*	+	−	TURNER (1961)
			O. graminis avenae	+	+	
		Methoxyhydroquinone	*O. graminis* var. *avenae*	+	+	OLSEN (1971a, b)
			O. graminis var. *graminis*	+	−	
			Fusarium oxysporum f. *lycopersici*	−	−	
Rye	*Secale cereale* L.	2(3)-Benzoxazolinone	*Fusarium nivale*	+	+	VIRTANEN *et al.* (1957)
			Sclerotinia trifoliorum	+	+	
			Penicillium roquefortii	+	−	
			Aspergillus niger	+	−	
			Mucor sp.	+	−	

Table 3

Sensitivity of plant parasites to phytoalexins and their capacity to cause disease

Common name	Latin name	Phytoalexin	Parasite	Sensitivity	Pathogenicity	Reference
Rice	*Oryza sativa* L.	Unidentified	*Pyricularia oryzae*	+	+	UEHARA (1958)
			Xanthomonas oryzae	+	+	UEHARA (1960)
		Two ultraviolet fluorescing and unidentified compounds	*P. oryzae*	+	+	OHATA and KOZAKA (1967)
			Cochliobolus miyabeanus	+	−	
			Fusarium oxysporum f. *lycopersici*	+	−	
			Alternaria brassicola	+	−	
			Botrytis cinerea	+	−	
			Pestalotia spp.	+	−	
		Momilactones A and B (?)	*P. oryzae*	+	+	CARTWRIGHT *et al.* (1977)
			Cladosporium cucumerinum	+	−	
		unidentified	*X. oryzae*	+	+	NAKANISHI and WATANABE (1977a)
Wheat	*Triticum vulgare* Vill.	−	−	−−	−−	−
Barley	*Hordeum vulgare* L.	Unidentified	*Helminthosporium teres*	+	+	KEELING and BANTTARI (1975)
		Unidentified	*Erysiphe graminis hordei*	+	+	OKU *et al.* (1975a)
			Cochliobolus miyabeanus	+	−	−
Maize	*Zea mays* L.	Unidentified	*Helminthosporium turcicum*	+	+	MACE and VEECH (1973)
Oats	*Avena sativa* L.	−	−	−−	−−	−
Rye	*Secale cereale* L.	−	−	−−	−−	−
Ragi	*Eleusine coracana* (L.) Derf.	Unidentified	*P. setariae*	+	+	PURUSHOTHAMAN and MARIMUTHU (1974)
			H. oryzae	+	−	

In this connection a few antimicrobial substances isolated from a marine red alga, *Marginisporum aberrans* (OHTA and TAKAGI, 1977), are quite challenging for the algologists.

Further, the mechanism(s) of action to confer resistance by the antimicrobial substances is much less studied and proper answers for the following questions are difficult to get:

Do the plants produce specific phytoalexins in response to infection?

Why are certain physiological races of the pathogens specialized to certain cultivars?

Does the phytoalexin play a major role in determining the plant resistance?

All these compelling questions require careful investigation – a fertile field for imaginative researchers.

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Phenolic Changes in Mung (*Phaseolus aureus*) Infected by *Rhizoctonia solani*

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Mungbean *(Phaseolus aureus)* hypocotyls infected by *Rhizoctonia solani* were analysed for qualitative and quantitative changes in phenols. The healthy tissues contained larger quantities of total phenols than the inoculated ones. In the inoculated tissues there appeared two new compounds. Tentative identification of the phenolic compounds has also been reported.

Mungbean *(Phaseolus aureus)* is an important seed legume grown in India, and forms one of the main sources of protein in cereal based diet of the people. Several viral, fungal and bacterial diseases have been described which affect this crop (PATEL and JINDAL, 1972). Damping-off of mung caused by *Rhizoctonia solani* is one of the serious diseases of this protein rich crop. Since in the recent years phenols have gained much attention (FARKAS and KIRÁLY, 1962; KOSUGE, 1969), in the present studies the changes in phenolic constituents of the mungbean were investigated and reported in this communication.

Materials and Methods

Mungbean *(Phaseolus aureus)* variety ML-1 seeds were germinated in garden soil in iron trays (18" × 12") and plants 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 (BOOHT, 1971). The mycelial mats were fragmented in a waring blender for one minute and diluted to 20 ml/mat (VAN ETTEN *et al.*, 1967). Plants were infected by dribbling the mycelial suspension over the hypocotyls at the rate of 100 ml/tray. Plants were removed immediately after inoculation (0 hr) and at suitable intervals for analysis.

Extraction of plant material. Hypocotyl portions of plants were analysed for phenols. 15 gms of hypocotyls were refluxed with 100 ml absolute alcohol for three hours on steam bath. The alcohol extract was filtered through Whatman No. 1 filter paper and concentrated under vacuum.

Estimation of total phenols. The total phenols were estimated by employing Folin-Denis reagent (SWAIN and HILLIS, 1959). The intensity of the blue colour was read in Spekol spectrocolorimeter (Germany) at 725 mμ.

Qualitative analysis of phenols. 15 mgs of hypocotyls were refluxed with 100 ml absolute alcohol for three hours on steam bath. The extract was filtered through Whatman No. 1 and concentrated under vacuum. Plant samples collected at different stages were analysed.

Chromatographic separation of phenols: 100 μl of the extract was spotted on Whatman No.1 chromatographic paper (28 × 23 cm) and developed descendingly in a solvent system of n-butanol : acetic acid : water 4 : 1 : 5 (v/v) for the first direction and 2 per cent acetic acid for the second run. The papers were air dried and sprayed with different chromogenic reagents, such as ferric chloride, potassium ferrocyanide for phenolic compounds (KIRBY, *et al.*, 1953), bromocresol green for phenolic acid (LUGG and OVERELL, 1947), vanillin reagent for catechins and leucoanthocyanins (CARTWRIGHT and ROBERT, 1954), bisdiazotized benzidine for flavonoids (HASLAM, 1966) and sodium metaperiodate benzidine for glycosides (CIFONELLI and SMITH, 1954).

Results

Total phenols. Table 1 presents the changes in total phenols of mungbean inoculated with *Rhizoctonia solani.*

Chromatographic separation of phenols. Table 2 presents the qualitative changes in phenols in mungbean. Tentative identification of individual phenols has been made by using different chromogenic reagents.

Five phenolic compounds were found to be present in all the samples at 0 hr. Inoculation clearly favoured the formation of new phenols and two new substances appeared while one substance disappeared after 24 hrs.

Table 1

Changes in total phenols* in *Phaseolus aureus* as influenced by *Rhizoctonia solani* inoculation

Sampling time after inoculation	Healthy	Inoculated	Per cent change
0 hr	2.52	2.11	− 16.2
24 hr	2.44	2.15	− 11.4
2nd day	2.31	1.82	− 21.2
3rd day	1.88	1.75	− 6.9
4th day	1.94	1.84	− 5.1

* mg of total phenols/g of oven dry tissue in tannic acid equivalents;
− : decrease

Table 2

Tentative identification of phenolic compounds present in mung *(Phaseolus aureus)* hypocotyls

Spot No.	Rf values		Colour in			Colour with chromogenic reagents*	
	BAW	2% ACOH	Visible light	UV light	UV + NH_4 vapour	1	2
1	0.70	0	NC	LY	NC	NC	NC
2	0.63	0.27	NC	V	NC	NC	NC
3	0.62	0.62	NC	LB	NC	NC	NC
4	0.67	0.61	NC	NC	B	NC	NC
5	0.65	0.80	NC	NC	B	NC	NC
6	0.37	0.72	NC	YB	NC	B	Y
7	0.29	0.90	NC	YB	NC	B	Y

Colour with chromogenic reagents*			Sampling time after inoculation					Tentative identification
3	4	5	0 hr	24 hr	2nd day	3rd day	4th day	
NC	NC	NC	+	+	+	+	+	Flavonol
NC	NC	NC	+	+	−	−	−	Flavonoid glycoside
NC	CM	NC	−	+	+	+	+	Flavonoid aglycon
NC	NC	NC	+	+	+	+	+	Flavonoid aglycon
NC	NC	W	−	+	+	+	+	Flavonoid glycoside
NC	NC	NC	+	+	+	+	+	Phenolic acid
NC	NC	NC	+	+	+	+	+	Phenolic acid

* Chromogenic reagents:
1. Ferric chloride + potassium ferrocyanide
2. Bromocresol green
3. Vanillin reagent
4. Bisdiazotized benzidine
5. Sodium metaperiodate benzidine

Discussion

In the healthy plants total phenols were more than in the inoculated plants. There is a decrease in the total phenolic constituents after inoculation at different stages of growth. This relationship confirms the general view that plants resistant to diseases contain higher amounts of phenols than the susceptible ones (FARKAS and KIRÁLY, 1962; CHOPRA *et al.*, 1974). The rate of decrease of phenols in the inoculated tissues was not uniform. In 0 hr sample, decrease in phenolic contents was 16.2 per cent while in 24 hr sample it was 11.4 per cent. The decrease in con-

tents was the least, 5.1 per cent in 4th day sample. The fall in phenolic contents after infection which is related to increase in susceptibility is in agreement with the observations in chillies infected with *Colletotrichum capsici* (BHULLAR *et al.*, 1972) and in potato roots infected with *Verticillium alboatrum* (PATIL *et al.*, 1962).

Apart from changes in total phenols in the *Rhizoctonia solani* inoculated mungbean, two new, bisdiazotized benzidine and sodium metaperiodate benzidine positive, compounds appeared after 24 hr of inoculation and these have tentatively been identified as flavonoid aglycon and flavonoid glycoside, respectively. The compound with Rf 0.70 having no mobility in 2% acetic acid appears to be a flavonol and the compound with Rf 0.63/0.27 which disappeared after 24 hr appears to be a flavonoid glycoside. Another compound Rf 0.67/0.61 gave blue colour under UV light + ammonia vapour and is identified as flavonoid aglycon. Two compounds (Rf 0.37/0.72, 0.27/0.90) giving yellow colour against blue background with bromocresol green are identified as phenolic acids. The leucoanthocyanins and catechins were found to be absent as shown by negative reaction with modified vanillin reagent.

Acknowledgements

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Abbreviations used

ACOH	—	Acetic acid
B	—	Blue
BAW	—	Butanol : acetic acid : water
CM	—	Claret maroon
LB	—	Light blue
LY	—	Light yellow
NC	—	No colour
U.V.	—	Ultra violet
W	—	White
Y	—	Yellow
+	—	Present
—	—	Absent

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Effect of *Ceratocystis paradoxa* (Moreau) Dade Infection on Sugar and Protein Content of Banana Fruits

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Ceratocystis paradoxa was isolated from diseased banana fruits and peduncles. Pathogenicity test was carried out. Different sugars were used as a carbon source in the medium, maximum growth of the fungus was achieved on sucrose followed by starch.

Determination of sugar content showed that reducing and non reducing sugar contents were lower in infected fruits with the exception of reducing sugar content in the infected skin.

Protein and total nitrogen determination showed a slighter increase in protein and nitrogen content of infected fruits than of healthy ones.

Banana is an important fruit crop in tropical and subtropical countries. The banana fruits are harvested while still green and then riped artificially. During marketing and storage, banana fruits are invaded with different fungi; one of the most important is *Ceratocystis paradoxa* (Moreau) Dade, which causes black peduncle and finger rot (BECZE, 1932a; HEIM, 1946; ANDRADE *et al.*, 1956; JOLY, 1961; MEREDITH, 1961; GREEN and GOOS, 1963; ROTH and LOEST, 1965; WARDLAW, 1972). In Egypt ELAROSI and WASFY (1972) recorded *C. paradoxa* as a causal organism of black rot to peduncles of *Musa cavendishii* Lamb.

The object of the current investigation was to study the black finger (fruit) rot caused by *C. paradoxa* and its effect on the sugar and protein content of the banana fruits.

Materials and Methods

Isolation and pathogenicity. Diseased banana fruits (*Musa cavendishii* Lamb. cv. Hendi) showing black discoloration of the skin, and a soft dark-brown pulp, as well as black peduncles were collected from Alexandria markets for isolation purpose.

In isolation trials small pieces of diseased fruits and peduncles were sterilized by 0.1% mercuric chloride for 2 min, washed thoroughly in sterile water and then transferred onto potato dextrose agar (PDA) in Petri dishes and incubated at room temperature. The isolated fungus was purified by using single spore isolation.

Pathogenicity test was carried out by applying discs of 7 days old PDA culture of the isolated fungus to healthy surface sterilized wounded banana fruits cv. Hendi. Check treatment was the same but with PDA discs only. Reisolation was made from the infected banana fruits, and the developed fungus was reidentified to insure the validity of the results.

Effect of different carbon sources on fungal growth

The ability of *C. paradoxa* to utilize carbon from different sources was studied. Five different carbon sources of mono-, di- and polysaccharides were chosen for this purpose. Czapeck Dox liquid medium was used and amended by 8 g/l of carbon as glucose, galactose, maltose, sucrose or starch. Three flasks were used for each treatment, and were then inoculated with equal discs of *C. paradoxa* and incubated at 20°C for 7 days, and the dry weight of the fungus was determined.

Determination of reducing and non reducing sugars in diseased and healthy fruits

The effect of *C. paradoxa* on the reducing and non reducing sugar content of banana fruits was estimated.

Extraction of sugars was carried out by using 80% ethyl alcohol for 3 hours, followed by 50% ethyl alcohol for the same time. One g of the dried banana fruits was used.

The alcoholic extracts were dried at 50°C and then dissolved in 100 ml 70% ethyl alcohol and filtered through Whatman No. 1 filter paper.

Reducing sugars extracted from the banana fruits were determined colorimetrically following Nelson's method (NELSON, 1944). For this purpose, one ml of the alcoholic extracts of each treatment was added to one ml of Nelson's alkaline copper reagent (Nelson's reagent I). The mixture was agitated and heated for exactly 20 minutes in a boiling water bath. After cooling, 1 ml of arsenomolybdate reagent (Nelson's reagent II) was added to the mixture and reagitated and diluted by adding 7 ml of distilled water. The optical density of the tube was read by colorimeter at 680 nm (SPECOL-Spectrocolorimeter, Carl Zeiss, Jena, Germany) and then the reducing sugars were calculated as micrograms per 1 ml of the extracts.

Total sugars were determined by the same method after acid hydrolysis. 25 ml of the extracts were put in 100 ml volumetric flasks and 5 ml of HCl (specific gravity = 1.0294) was added, the mixture was put in water bath at 60–65°C for 15 minutes, then NaOH (12 N) was added till neutrality, and the volume was completed to 100 ml by distilled water (JACOBS, 1958). One ml of the mixture was used for total sugar determination. Non reducing sugars were calculated by the following equation: Non reducing sugars=Total sugars–Reducing sugars.

A glucose standard curve was prepared by using Nelson's method which served for determining the total and reducing sugars in the banana extracts.

Determination of protein and total nitrogen

Protein and nitrogen contents of healthy and diseased fruits were estimated by using the Macro Kjeldahl method (A.O.A.C., 1970).

Results

Isolation and pathogenicity

Isolation trials from diseased fruits and peduncles revealed the presence of *Ceratocystis paradoxa* (Moreau) Dade. Verification of this fungus was kindly made by the Commonwealth mycological Institute, England.*

Artificial infection of banana fruits cv. Hendi showed black discoloration of the skin, followed by outgrowing masses of fungal mycelium. On the other hand infection reduced the pulp to a dark-brown, soft wet consistency (Fig. 1).

Fig. 1. Healthy banana fruit (cont.) and artificially infected fruit with *C. paradoxa* showing black discoloration of skin

Effect of different carbon sources on fungal growth

Five different sugars were used as a carbon source in Czapeck Dox medium. Table 1 shows the dry weight of the fungus after 7 days of inoculation. From the presented data, the maximum growth of the *C. paradoxa* was on the medium amended by the sucrose followed by starch, glucose, maltose, and then the minimum growth was obtained on the medium amended with galactose.

* Report from Dr. SUTTON (IMI 205043).

Table 1

Effect of different carbon sources on the growth of *C. paradoxa* 7 days after of inoculation

Carbon sources		Dry weight of the fungus in mg*
Monosaccharides	glucose	568
	galactose	471
Disaccharides	maltose	520
	sucrose	643
Polysaccharide	starch	596

* Mean of three replicates

Determination of reducing and non reducing sugars

Reducing and non reducing sugar contents were determined using Nelson's method. From the data presented in Table 2 it is clear that banana fruit infection with *C. paradoxa* resulted in reduction of sugar content compared with the healthy ones with the exception of infected skin, in which reducing sugar content was increased by 4%.

Table 2

Effect of infection by *C. paradoxa* in banana fruits on reducing and non reducing sugar content 7 days after of infection

Treatment		Micrograms/1 ml of the extracted juice*			
		Reducing	% change	Non reducing sugars	% change
Skin	control	1500		900	
	infected	1560	+ 4	200	−55.5
Pulp	control	3048		5272	
	infected	2700	−11.41	1220	−76.85

* Mean of three replicates

The maximum reduction in non reducing sugars was in case of infected pulp, followed by infected skin.

Reducing sugar content of the infected pulp was decreased by 11.41%.

Determination of protein and total nitrogen

Protein and nitrogen contents of pulp and skin of banana fruits, either healthy or infected with *C. paradoxa*, were estimated. The obtained data (Table 3) showed that the protein and nitrogen contents are 11.15% and 1.78% in the healthy pulp and 12.34% and 1.97% in the healthy skin. The protein and nitrogen contents in the infected fruits were increased. The infected pulp contains 12.12% protein and 1.94% nitrogen, while the infected skin contains 13.42% protein and 2.15% nitrogen.

Table 3

Protein and nitrogen contents of healthy and infected banana fruits with *C. paradoxa*, as well as the fungal mycelium*

Treatment		Protein, %	Nitrogen, %
Skin	control	12,34	1.97
	infected	13.42	2.15
Pulp	control	11.15	1.78
	infected	12.12	1.94
C. paradoxa		17.21	2.75

* All figures are means of three replicates

Discussion

Ceratocystis paradoxa was able to incite soft rot symptoms on banana fruits (BECZE, 1932; HEIM, 1946; ANDRADE *et al.*, 1956; JOLY, 1961; MEREDITH, 1961; GREEN and GOOS, 1963; ROTH and LOEST, 1966). ELAROSI and WASFY (1972) stated that *Ceratocystis paradoxa* isolated from black peduncle of banana failed to incite disease symptoms on banana fruits in all stages of formation and maturity. Our obtained results showed that *C. paradoxa* isolated even from diseased fruits or from black peduncles of banana succeeded in inciting the disease symptoms on mature wounded banana fruits. The isolated fungus differed from the isolate obtained by ELAROSI and WASFY (1972). The *in vitro* studies on the effect of carbon sources on the growth of *C. paradoxa* showed that sucrose and starch were the most suitable sources of carbon to growth. These results agree well with what is known about the banana fruit composition. Fresh banana fruits contain starch and about 10.37% sucrose (WINTON and WINTON, 1945), and this may explain the rapid spread of the fungus on the banana fruits during storage and marketing.

Determination of sugar content of the healthy and infected fruits showed that non reducing sugar content was decreased both in pulp or skin of infected fruits. Sucrose and starch as non reducing sugars form a great portion of fruit.

The great reduction in the non reducing sugar content may be due to the sugar consumption during fungal growth and disease spread.

Determination of reducing sugar content in the infected skin showed a slight increase, that may be due to enzymatic activities of the pathogen, which resulted in releasing reducing sugars from pecteolytic or cellulolytic substances in the fruit skin. The slight degradation of the skin component may explain the light soft rot of banana fruits due to infection with *C. paradoxa.*

Determination of protein and nitrogen content showed a slight increase in protein and nitrogen content in infected fruits compared with the healthy ones. The slight increase in protein and nitrogen content in infected skin and pulp may be attributed to the difference in consumption rates of the carbohydrates and proteins in infected fruits by the fungus, and this resulted in the increase of protein and nitrogen in infected fruits.

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Alternaria Fruit Rot of Pepper

By

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Fruit rot of pepper was observed in Hammam El-Alil, Mosul, Iraq, causing substantial losses in the yield. *Alternaria alternata* proved to be the causal organism. The pathogen is seed-borne. Vitavax at 0.04% a.i. was effective against damping-off losses.

Alternaria fruit rot of pepper (*Capsicum annuum* L.) was observed for the first time in the Experimental Station of the College of Agriculture and Forestry, Hammam El-Alil, Mosul, Iraq, in October and November, 1977. The disease caused almost 70% loss in the fruit crop. It was characterized by black lesions that ranged in size from small flecks, 2–3 mm in diameter, to large lesions involving one half or more of the fruit surface. The fungus was sporulating profusely on the large lesions. In large lesions, blackening usually extended into the carpel wall and in the locules.

Alternaria rot of pepper fruit was reported to affect up to 80% of the crop in Illinois, USA (QUEBRAL and SHERTLEFF, 1965). WARD *et al.* (1973) reported *Alternaria alternata* as an incitant of rot to pepper fruits in Canada. A virulent strain of *A. alternata* causing leaf and fruit spot of pepper was reported from India (SPEEKANTIAH *et al.* 1973). Also, *A. alternata* was recorded as a causal organism of rot of tomato fruits (BUTLER, 1959; MITCHELL *et al.*, 1968; LOCKHART *et al.*, 1969; KAUSHIK *et al.*, 1969; BARKAI-GOLAN, 1973; ABDEL-REHIM *et al.*, 1974; MEHTA *et al.*, 1974, PEARSON and HALL, 1975).

The present work was undertaken (i) to elucidate the effect of an isolate of *Alternaria* obtained from rotted pepper fruits on pepper, tomato and eggplant fruits, (ii) to test the presence or absence of *A. alternata* on seeds of pepper, tomato and eggplant, (iii) to determine the effect of certain fungicides on the *Alternaria* isolate *in vitro* and *in vivo*.

Materials and Methods

Isolations were carried out from pepper fruits showing black rot symptoms. Such fruits were collected from the Experimental Station of the College of Agriculture and Forestry, Hammam El-Alil.

The infectivity of the isolated fungus was tested on green and red fruits of pepper, green mature tomato fruits, and mature eggplant fruits, all of local cultivars. Fruits were surface sterilized with about 1% sodium hypochlorite solution (commercially known as Fast) for five minutes. Inoculation was done either by inserting inocula through needle-prick wounds at two opposite sites in the middle, or by spraying the spore suspension on apparently healthy fruits. Fruits serving as checks were similarly treated but were not inoculated with the fungus. Four replicates were used in each treatment. Each treatment consisted of four fruits of pepper, tomato or eggplant, placed in a perforated polyethylene bag and kept at room temperature (17–25°C) for 15 days.

One hundred seeds, each of pepper, tomato and eggplant, of local cultivars, were plated on moist blotters in pyrex petri dishes (9 cm diameter) at the rate of 25 seeds/dish. The dishes were incubated at 22–26°C under alternating cycles of 12 hours near ultraviolet (NUV) light and darkness for seven days. The NUV tubes were of Philips TL 40W/08 RS 40 BLB. The fungus was examined using the stereobinocular microscope.

The fungicides used were:

1 – Benomyl [50% methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate].
2 – Captan 50 (50% N-trichlorethyl thioltetrahydprophthalimide).
3 – Dithane M-22 (80% manganese ethylene-bis-dithio-carbamate, 16.5% metallic manganese).
4 – Dithane M-45 (80% manganese ethylene-bis-dithio-carbamate, 16% metallic manganese + 2% zinc).
5 – Dithane Z-78 (65% zinc ethylene-bis-dithiocarbamate).
6 – Plantvax (5,6-dihydro-2-ethyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide).
7 – Vitavax (75% 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide).

The effect of different concentrations of the tested fungicides on the isolated fungus was studied on potato dextrose agar (PDA). The concentrations used of each fungicides were: 0.04, 0.08, 0.15, 0.22 and 0.3% a.i. Check treatment was carried out on PDA without fungicide. Inocula, 54 mm in diameter, were taken from the growing margin of 5-day-old PDA culture of the fungus. Three replicates in each treatment were used, and incubated at 25°C.

Fungicides listed earlier were used for treating seeds of pepper, tomato and eggplant, at the rate of 3 g a.i./kg seeds. The treated seeds were sown in asbestos trays with compartments (12 cm in diameter × 16 cm deep) filled with sterilized soil previously inoculated with the tested fungus. Twenty-five treated seeds were sown in each compartment (replicate). Each treatment comprised three replicates. Untreated seeds sown in each of inoculated and non-inoculated soil were used as checks.

Results

Isolation and pathogenicity tests

Isolations carried out from pepper fruits showing black rot symptoms always yielded *Alternaria alternata* (Fr.) Keissl. (= *A. tenuis* Nees) (SIMMONS, 1967). Pure cultures of the fungus were maintained on PDA slants.

The pepper, tomato and eggplant fruits which were inoculated with the *A. alternata* isolate were examined after 15 days of incubation. Wound-inoculated fruits showed longitudinal cracks (Figs 1 and 2), whereas spray-inoculated fruits

Fig. 1. Pepper fruits, healthy (left) and wound-inoculated with *A. alternata* (middle and right)

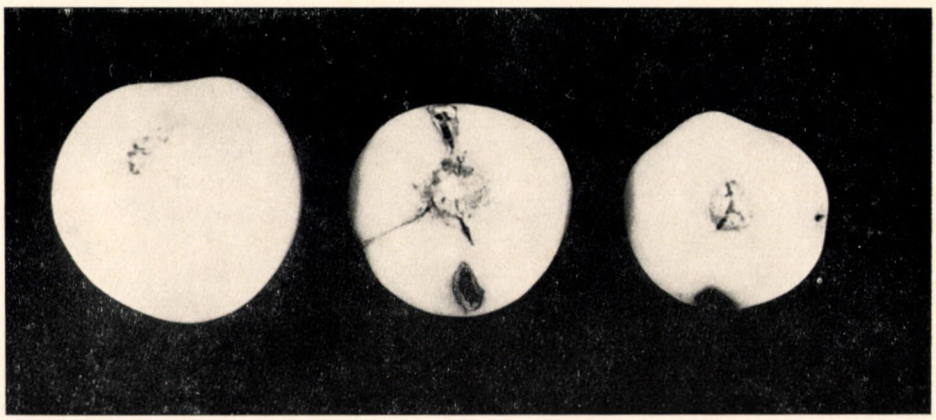

Fig. 2. Tomato fruits, healthy (left) and wound-inoculated with *A. alternata* (middle and right)

Fig. 3. Tomato fruits, healthy (left) and spray-inoculated with *A. alternata* (middle and right)

showed only surface greenish brown lesions which often coalesced and covered appreciable area (Fig. 3).

Effect of temperature on the fungal growth

The effect of temperature on mycelial growth of the pathogen grown on PDA medium at temperatures ranging from 5 to 35°C at 5°C intervals showed that the fungus grew from less than 10°C to above 35°C with an optimum at 25°C (Table 1).

Table 1

Effect of temperature on linear growth of *A. alternata* grown on PDA

Temp., °C	Mean daily increase in diameter (mm)
5	0.0
10	1.8
17	9.0
25	11.4
30	7.5
35	0.0

Seed health testing for A. alternata

Seeds of pepper, tomato and eggplant plated on moist blotters under NUV and examined after seven days showed the presence of *A. alternata* at the rates of 39, 1, and 9% respectively. Most of the radicles of the germinating seeds on which the fungus was grown showed browning and poor development.

Effect of the tested fungicides on the pathogen

In vitro studies of the tested systemic and non-systemic fungicides on *A. alternata* at 25°C showed that Captan 50 (at 0.04% a.i.) and Vitavax (at 0.04% a.i.) were the most effective in checking the mycelial growth of the pathogen (Table 2).

Table 2

Lowest inhibitory concentration of the tested fungicides on the growth of *A. alternata in vitro*

Lowest inhibitory concentration, % a.i. of fungicides						
Benomyl	Captan 50	Dithane M-22	Dithane M-45	Dithane Z-78	Plantvax	Vitavax
0.08	0.04	0.15	0.15	0.30	0.15	0.04

In vivo studies showed that Vitavax at 0.3% a.i. was the best fungicide in protecting pepper, tomato and eggplant seeds/seedlings against *A. alternata* isolate. Captan 50 (0.3% a.i.) gave also good protection to tomato seeds. Benomyl, on the other hand, did not support sufficient protection.

Table 3

Effect of seed dressing with certain fungicides on damping-off incidence of pepper, tomato and eggplant caused by *A. alternata*

Plant species	Average survivals of seeds after 30 days of sowing*					
	untreated seeds in non-inoculated soil	untreated seeds in inoculated soil	Benomyl	Captan 50	Vitavax	L.S.D. at 5%
Pepper	22.3	12.3	20.0	18.0	21.7	1.8
Tomato	20.0	7.0	12.7	19.0	18.7	2.4
Eggpant	19.0	13.0	15.0	16.0	18.3	1.6

* 25 seeds represented a replicate, 3 replicates were used per treatment

Discussion

Fruit rot of pepper incited by *Alternaria alternata* resulted in substantial losses in the yield at Hammam El-Alil, during October and November, 1977. In inoculation tests, the fungus showed to be pathogenic to pepper, tomato and eggplant fruits when apparently healthy fruits were wound-inoculated. Insects might play an important role in invasion of the fungus through ripe pepper fruits during that period of the year. The magnitude of infection was more pronounced in mature green and ripe pepper fruits than in tomato or eggplant fruits. Host

range studies carried out by QUEBRAL and SHERTLEFF (1965) indicated that tomato and eggplant fruits were also susceptible to *A. alternata*. Spray-inoculation resulted only in surface blemishing of the fruit skin. This result supports that reached by QUEBRAL and SHERTLEFF (1965) who found that no infection occurred unless pepper fruits were injured.

Mosul is a semiarid region in the north of Iraq. Under the prevailing environmental conditions in Mosul during October and November, 1977, there was little rainfall, however, the pathogen might be stimulated to germinate in the dew occurring on the surface of pepper fruits and then could invade and cause the disease. Dew was shown to be important in the development of blackmold of tomato fruits incited by *A. alternata* in California (PEARSON and HALL, 1975). ROTEM and REICHERT (1964) emphasized the importance of dew in the development of early blight epidemics in tomato caused by *Alternaria solani* in the semiarid regions.

The present work showed that the fungus was seed-borne in pepper, tomato and eggplant. The fungus demonstrated itself up to 39% on pepper seeds. The result supports the findings of other investigators (NOBLE and RICHARDSON, 1968) concerning the presence of *A. tenuis* on seeds of those hosts. Vitavax at 0.3% a.i. showed to be the best fungicide in protecting the seeds/seedlings against *A. alternata*, that coincided with the *in vitro* tests concerning the effect of certain fungicides on the fungal growth. This is in line with the findings of CRISAN (1976) who found that Vitavax at 0.05–0.02% was effective against *A. alternata* isolates from pepper, tomato and eggplant.

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On Inadequancy of the Double Diffusion Test in *Fusarium* Serotaxonomy

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Two distantly relative species of the genus *Fusarium*, *F. graminearum* and *F. equiseti*, were compared in double diffusion test using anti-F graminearum serum in order to evaluate the applicability of this method in *Fusarium* serology. Four precipin lines were observed both in homologous and in heterologous reactions and the heterologous titre was two steps higher than the homologous one indicating inadequacy of the double diffusion test. By means of tandem-crossed immunoelectrophoresis 13 antigens specific to the homologous relationship were detected suggesting superiority of this technique. It was also verified that the high homologous titre is due to a single common antigen present in an extreme amount in the heterologous fungus. On basis of the qualitative differences determined by tandem-crossed immunoelectrophoresis the two species could be distinguished well.

Although there were several attempts to apply serological methods in classification of the genus *Fusarium* (e.g. TEMPEL, 1959; MADHOSINGH, 1964; KALYANASUNDARAM *et al.*, 1967; KALYANASUNDARAM and CHARUDATTAN, 1969; SPAAR and VESPER, 1970; KAISER and GUPTA, 1976; VESPER *et al.*, 1976) no general success was won in this field mainly because of the limited resolution power of the double diffusion test used widely in such studies. As shown by AXELSEN (1971, 1973), in fungal serology the antigen-antibody reactions take place in highly complex systems thus the serological similarity can be determined only in that case, if these reactions are investigated by more polyvalent techniques.

The aim of the present communication is to demonstrate superiority of the tandem-crossed immunoelectrophoresis on such a selected example where the serological similarity determined by double diffusion test is quite inconsistent with the taxonomical relationship. This work is a by-product of the comprehensive serotaxonomical study on *Fusarium* species being in progress at this laboratory.

Materials and Methods

Cultures. F. graminearum Schwabe strain 22—1 and *F. equiseti* (Corda) Sacc. strain 22—37 were obtained from our own culture collection. Potato-sucrose broth (200 g peeled, sliced potatoes and 20 g sucrose per 1 litre distilled water)

enriched with 1 g/l NH_4NO_3 was used as culture medium. Stationary cultures were grown by inoculating 60 ml medium in 300 ml Erlenmeyer flasks with 10^5 conidia and incubating at 25°C for five days in the dark.

Preparation of antigens. These procedures were carried out as described earlier (HORNOK and SZÉCSI, 1977) except that protein content of the extracts was adjusted to 30 mg/ml.

Antiserum production. Hyperimmune mouse ascitic fluid against *F. graminearum* 22–1 antigens was prepared according to HORNOK and SZÉCSI (1977). Mice received five immunizing injections at two weeks' intervals. Immunoglobulins from the immune ascites pool were purified by means of $(NH_4)_2SO_4$ precipitation and chromatography on DEAE-Sephadex A 50 column (HARBOE and INGILD, 1973). Purified immunoglobulin preparation was concentrated to one-fourth of the original fluid volume.

Double diffusion test. This was made in a 2 mm thick layer of 1% agarose (Reanal, Budapest) dissolved in barbital buffer (pH 8.6, I=0.02) using a micro-modification of the Ouchterlony technique (OUCHTERLONY and NILSSON, 1973). Reservoirs had a diameter of 6 mm with an intercentre distance of 10 mm. After 24 h incubation at 25°C, precipitin lines were stained with Coomassie brilliant blue G-250 (Serva, Heidelberg) as described by WEEKE (1973).

Immunoelectrophoresis. Tandem-crossed immunoelectrophoresis was carried out according to KRØLL (1973) using a 1.5 mm layer of 1% (w/v) agarose and barbital buffer, pH 8.6, ionic strength 0.02. Application wells had a diameter of 3.0 mm. Five μl antigen samples were applied in the wells. First-dimension electrophoresis was carried out at 5 V/cm for 1 h, second-dimension run was made applying 1 V/cm for 18 h at 15°C. Second-dimensional gels contained 10 μl purified, concentrated anti-*F. graminearum* serum per 1 cm^2. Dimensions of the antibody containing gel were 4.5×6.5 cm. Single-crossed immunoelectrophoresis for the homologous reaction only was carried out at the same parameters. Every immunoelectrophoretic run was made in duplicate. Immunoprecipitates were stained with Coomassie brilliant blue G-250.

Results and Discussion

Although the test species *F. graminearum* and *F. equiseti* used in this study are taxonomically quite different ones and they are easily distinguishable on the basis of their morphological characters, the well-known general concordance between taxonomical difference and serological behaviour could not be demonstrated in the gel diffusion experiments. When undiluted samples were reacted in double diffusion test anti-*F. graminearum* serum formed four precipitin lines both in the homologous and in the heterologous relations (Fig. 1). The antigen identity as marked by arrows could be determined only at two precipitates.

Twofold serial dilutions were then prepared from both fungal extracts and each dilution steps were reacted with purified, concentrated antiserum in order to

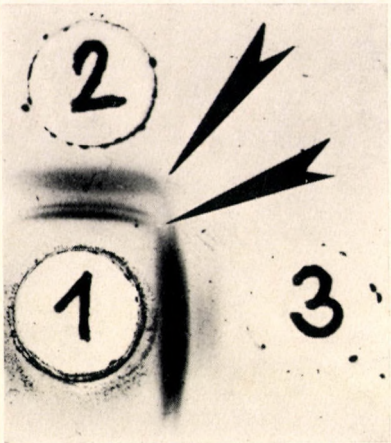

Fig. 1. Double diffusion test: (1) 20 µl anti-*F. graminearum* serum; (2) 20 µl antigenic preparation of *F. graminearum* 22—1; (3) 20 µl antigenic preparation of *F. equiseti* 22—37

demonstrate qualitative antigenic differences between the two fungi. As can be seen in Table 1 the only antigen surplus for homologous fungus was found at the dilution 1 : 4 and the final heterologous titre (1 : 64) was two steps higher than the homologous one (1 : 16). The results show great uncertainties of the double diffusion test; i.e. the precipitate pattern obtained by this method is strongly dependent on the antigen-antibody ratio and the high heterologous titre cannot logically be explained.

In order to achieve more exact antigenic analysis *F. graminearum* 22—1 antigens were subjected to crossed immunoelectrophoresis with the homologous antibody preparation. By this method 24 anodic precipitates were detected indicating the presence of 24 water-soluble antigens in *F. graminearum* (Fig. 2).

Table 1

Results of the double diffusion test using anti-*F. graminearum* serum

Dilutions	Number of precipitin lines	
	F. graminearum	*F. equiseti*
undiluted	4	4
1 : 2	4	4
1 : 4	3	2
1 : 8	2	2
1 : 16	2	2
1 : 32	—	1
1 : 64	—	1
1 : 128	—	—

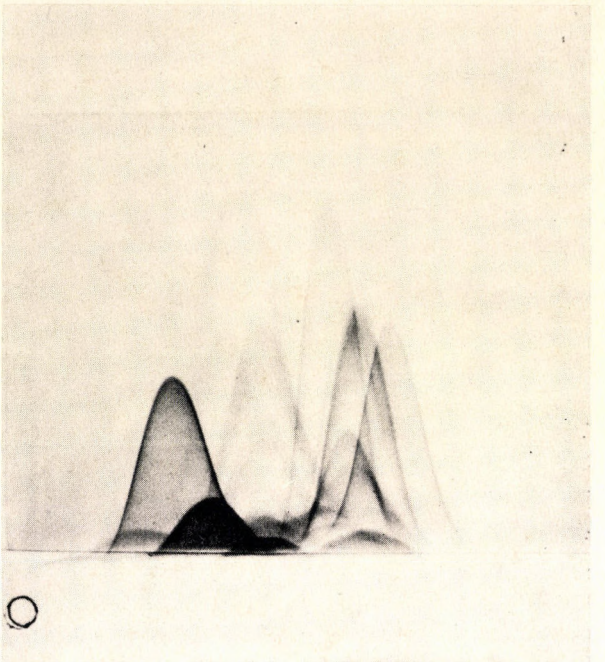

Fig. 2. Crossed immunoelectrophoresis of *F. graminearum* 22—1. The second-dimension gel contained 10 μl/cm^2 purified, concentrated anti-*F. graminearum* serum. (Anode to the right during the first-dimensional run and at the top during the second-dimensional run)

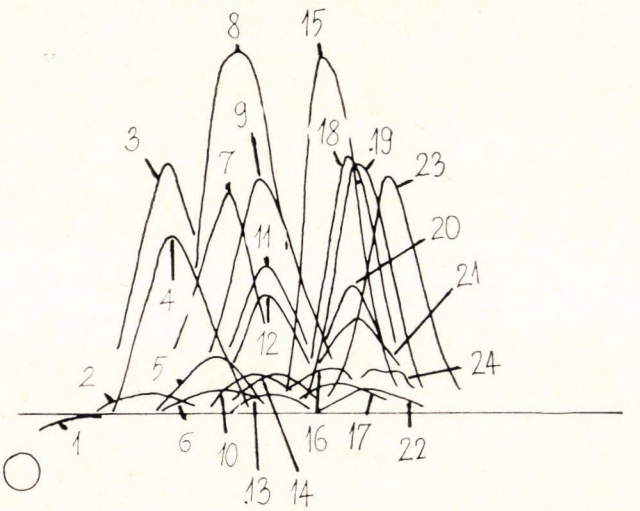

Fig. 2*a*. Tracing of Fig. 1 with enumeration of the antigens

Fig. 2*a* is a tracing of the original slide with enumeration of the antigens. Due to the differences in quantity and immunogenicity among the individual antigens the precipitates differed in their staining intensity. Some antigens, like nos. 2, 4, 5, 13, 15, 19, 22, and 23 seem to be dominant ones.

The antigenic differences between *F. graminearum* 22–1 and *F. equiseti* 22–37 were determined by means of tandem-crossed immunoelectrophoresis. As shown in Figs 3 and 3*a* four main types of the antigen correspondence were present in our system: 1. identity indicated by fused wavy patterns (nos. 2 and 2*a*, 9 and 9*a*, 15 and 15*a*, 22 and 22*a*); 2. identity indicated by complete

Fig. 3. Tandem-crossed immunoelectrophoresis comparing antigens of *F. graminearum* 22–1 and *F. equiseti* 22–37 using anti-*F. graminearum* serum. (Anode like in Fig. 2)

fusion of the two antigens (nos. 19+19*a*, 23+23*a*); 3. partial identity indicated by presence of a spur (nos. 5 and 5*a*); 4. probable identity where the antigen in question is present in the heterologous fungus, however, fusion between the two precipitin lines cannot be detected (nos. 3 and 3*a*, 16 and 16*a*, 18 and 18*a*, 24 and 24*a*). To sum up these only 11 common antigens are present between *F. graminearum* and *F. equiseti*. This means that as much as 13 antigens specific to *F. graminearum* 22–1 can be detected by means of tandem-crossed immunoelectrophoresis in that system where no graminearum-specific antigen could be demonstrated by the double diffusion test. Some of these graminearum-specific antigens seem to be dominant ones (like Nos 4 and 13).

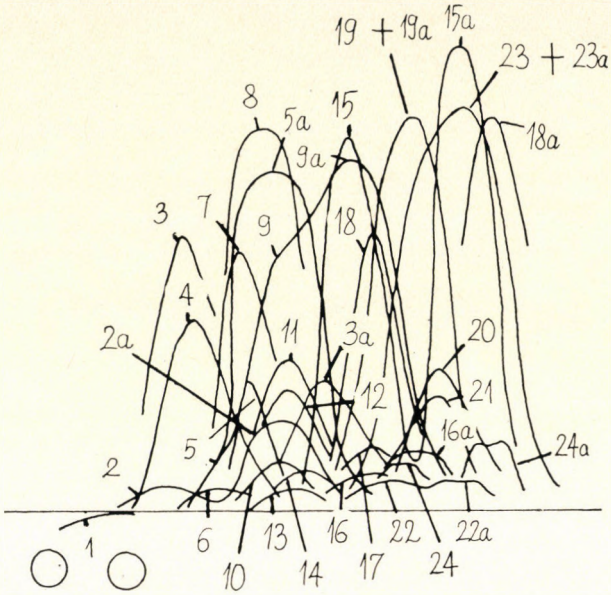

Fig. 3*a*. Tracing of Fig. 3 with enumeration of the antigens. (Heterologous antigens are footnoted with *a*)

On the basis of the semiquantitative nature of crossed immunoelectrophoresis the high heterologous titre can also be explained. As is shown in Figs 3 3*a* precipitates No. 5 in *F. graminearum* and No. 5*a* in *F. equiseti* indicate a partial identity. Since the height (or area) of a precipitate is proportional to the antigen amount the remarkable difference found between nos. 5 and 5*a* in this respect suggests the existence of some sixfold excess in quantity for the antigen no. 5*a*. In the case of a dominant antigen, such an excess for the heterologous fungus may cause even a two-step increase in the titre, however, the taxonomical importance of this quantitative difference found at a single pair of antigens is negligible in comparison with the qualitative differences demonstrated above.

In conclusion, the double diffusion test is not a suitable method for the serological comparison of crude *Fusarium* antigens since on the basis of some dominant antigens misleading serological similarity may be observed between distant species. By means of tandem-crossed immunoelectrophoresis species- or strain-specific antigens and remarkable qualitative differences can be demonstrated.

Acknowledgement

Mrs. M. SZABÓ is thanked for expert technical assistance.

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Pod and Stem Blight of Soybeans in Hungary

(Short Commucation)

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Pod and stem blight caused by *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* Wehm. (Syn.: *D. sojae* Leh.; imperfect stage: *Phomopsis sojae* Leh.) was first reported in the USA in 1923. The occurrence of this disease has since been published from some other countries such as Brazil, Canada, India, Japan, China, USSR *etc.* (*cf.:* SINCLAIR and SHURTLEFF, 1975). Infections occur on stems, petioles, pods and sometimes on leaves during the periods of warm, wet weather. Large numbers of pycnidia develop in a linear arrangement perpendicular on the stems and pods late in the growing season.

The symptoms characteristic of pod and stem blight were observed on stems and pods of Merit and ISZ-10 soybean cultivars in Iregszemcse (Western Hungary) in late July, 1977 (Fig. 1). Earlier observations of this disease are also available (SZILI, 1975; SZALAY, 1976), but neither formal mycological description nor artificial inoculations were carried out by the authors.

On the basis of microscopic observations, the pycnidia developing beneath the epidermis in compact groups of black, pulvinate stromata averaged in size 168×110 μm. The pycnidia had one cavity and contained unicellular, hyaline, elliptical, biguttulate conidia with average size of 8.6×2.1 μm (Fig. 2).

The pycnidia of the fungus isolated from the stem of Merit cultivar produced dense, white, closely septate hyphae. Yellow areas frequently occurred in the culture on potato-dextrose agar at 26°C, and darkening of the media was also visible. Pycnidia rarely occurred in the culture even in continuous light.

The pathogenicity of the culture was studied by inoculating soybean plants (cultivars Merit and ISZ-10) in the greenhouse. Inoculations were made by inserting tooth-pick with agar-block of 3-week-old mycelium grown on potato-dextrose agar into a wound in the stem. After inoculations made in the leaf scar at the first, second and third node (ATHOW and CALDWELL, 1954), the 6-week-old plants were incubated in moist chamber for 4 days, then replaced into normal greenhouse conditions. Around the inoculation site brown discoloration occurred 2—3 days after the inoculation. The lesions were larger on cv. ISZ-10 than on cv. Merit. Three to four weeks later reddish, immature pycnidia in linear rows were visible both on stems below and on petioles immediately above the inoculation site

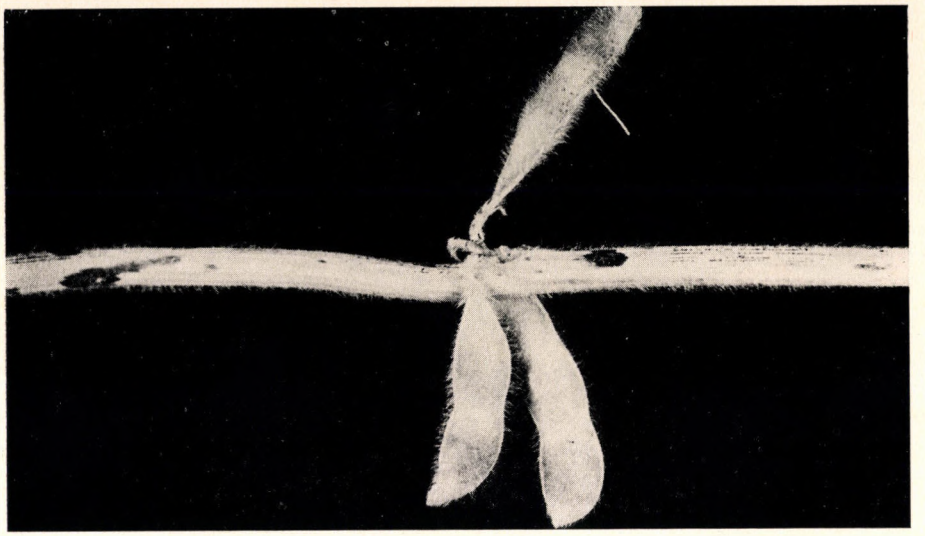

Fig. 1. Symptoms of pod and stem blight disease

(Fig. 3). The colour of pycnidia gradually turned to black, much earlier on cv. ISZ-10 than on cv. Merit which latter is less susceptible to the fungus. Pycnidia reisolated from the artificially infected plant produced culture characteristic of *Phomopsis sojae*, the imperfect stage of *D. phaseolorum* var. *sojae*.

The perithecial stage of the fungus that occurs in overseasoned debris has not been found, the description of the imperfect form, however, is the first formal report from Hungary.

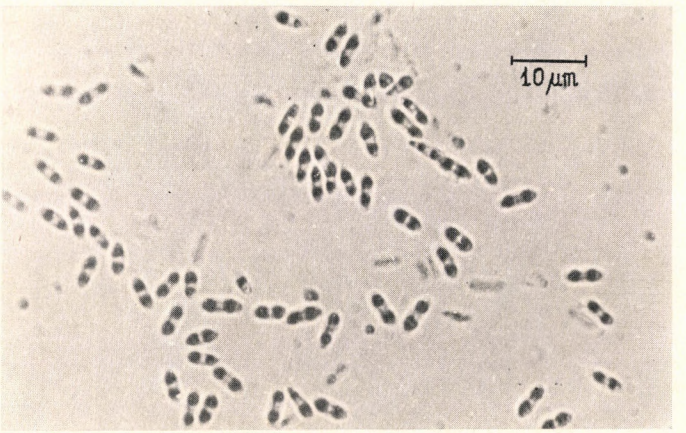

Fig. 2. Conidia of *Diaporthe phaseolorum* var. *sojae*

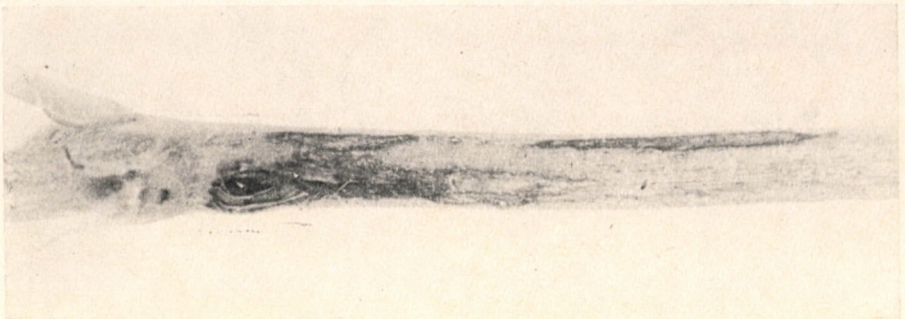

Fig. 2. Symptom caused by *Diaporthe phaseolorum* var. *sojae* on stem of ISZ-10 soybean 3 weeks after inoculation

Because of the economic importance of the disease (infected seeds fail to germinate or may be the source of systemic infection) the selection of resistant soybean varieties is of great importance. Such a work on different varieties mostly produced in Hungary is in progress.

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A New Type of Defence Reaction of Tobacco against Incompatible Bacterium, *Pseudomonas pisi*

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An early inhibition (3—6 h) of incompatible *P. pisi* was demonstrated in tobacco plant, which was not identical either with the hypersensitive reaction (HR) or with acquired resistance (premunity) of plants. The inhibition of bacterial multiplication was proved by the lack of the HR. The induction of this defence mechanism was not specific, however, the effect of the inhibition on bacteria was specific.

Since the bacterially induced hypersensitive reaction (HR) of plants was described (KLEMENT *et al.* 1964) many authors have studied this phenomenon as a disease resistance mechanism. Host specificity of a pathogenic bacterium has been explained by inability of the bacterium to induce the HR (KLEMENT *et al.* 1968). However, there were exceptions in which it was impossible to demonstrate incompatibility by the HR. For these reasons RUDOLPH (1976) concluded that the primary importance of the HR in disease resistance has not yet been established and that other mechanisms of resistance to bacteria should be sought (cf. KIRÁLY *et al.*, 1977). KIRÁLY *et al.* (1972) argued that the HR is a consequence rather than the cause of resistance in some fungal diseases.

During our investigation of the mechanism of the HR a new type of host resistance (early selective protection, ESP) described below has been found.

Materials and Methods

To assess the resistance of the host, multiplication of bacteria in infected tissues was measured. However, it is very difficult to do this by the dilution plate technique in the earliest stages of infection. For this reason, inhibition of bacterial multiplication in infected tissue was demonstrated indirectly by the absence of either hypersensitive or normosensitive necrosis because it had been shown earlier that HR and normosensitive necrosis are induced only by multiplying bacteria (DURBIN and KLEMENT, 1977; KLEMENT, HEVESI and SASSER, unpublished). Consequently, if bacterial multiplication is inhibited in host tissues, necrosis does not develop.

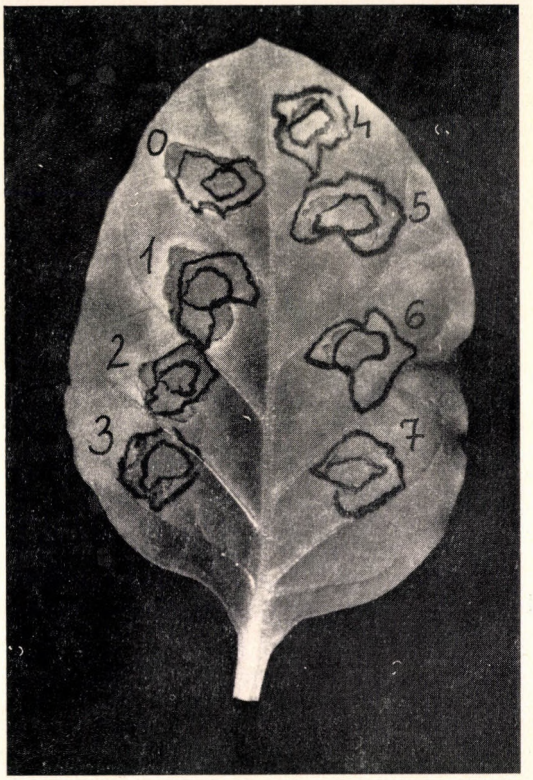

Fig. 1. Demonstration of ESP by the lack of necrosis in the outer zone 3—4 h after the inoculation of central areas with *P. pisi* 5×10^8 cells ml^{-1}. The bacterium and the inhibitor were rinsed out with sterile distilled water from the central areas into the outer zones at different intervals (numbers in hours) after the inoculation of central zones

Intercostal areas (ca. 1 cm^2) of leaves of intact tobacco plants (*Nicotiana tabacum* L. cv. Xanthi *nc.* and cv. Samsun) were injected with a suspension of 5×10^8 cells ml^{-1} *P. pisi*, Saccket (ATCC No. 11055) pathogenic to pea. These infiltrated central areas were immediately marked with nontoxic ink (Fig. 1). After 1 h intervals the bacterial cells and the assumed antibacterial materials were rinsed out from intercellular spaces of central areas into the surrounding tissue (outer zone). Flushing of the intercellular spaces was done by the reinjection of the central areas with steril distilled water so that the water soaking extended to the surrounding tissue (outer zone). If any antibacterial inhibitor was produced in the central area, then the multiplication of *P. pisi* was inhibited and so HR did not develop in the outer zone.

The same method was used for the demonstration of aspecificity of the inducer. In this case tobacco leaves were pretreated by injection of (ca. 1 cm^2) leaf areas with a suspension of streptomycin-sensitive strain of *P. tabaci* (Wolf and Foster)

Stevens (ATCC No. 11528) pathogenic to tobacco, *P. pisi* pathogenic to pea and saprophytic *P. fluorescens* Migula (ATCC No. 13525) respectively. Suspensions contained 5×10^8 cells ml^{-1} bacteria. Controls were injected with sterile water. In this experiment, the flushing of intercellular spaces of the central zones was done with 1000 $\mu g\ ml^{-1}$ streptomycin solution containing 5×10^7 cells ml^{-1} of streptomycin resistant mutant of *P. pisi* or *P. tabaci*.

The effect of light on the development of inhibitor was investigated in a growth-chamber under controlled conditions at 28°C, either without light or illuminated with a light intensity of 3000 lux. One half of leaves of tobacco plants were pretreated with either 5×10^5 cells ml^{-1} of *P. tabaci* or 10^6 cells ml^{-1} of *P. pisi* or 10^8 cells ml^{-1} of *P. fluorescens*. The other half was injected with water as a control. The pretreated plants were kept in light or in dark from 24 h before the pretreatment until 16 h after the challenge inoculation. Different concentrations of *P. pisi* as challenge were injected into about 1 cm^2 leaf areas 6 h after the pretreatment.

Results and Discussion

Inhibition of growth of *P. pisi* in tobacco leaf was demonstrated by the lack of the HR. The lack of the necrosis appeared in the outer zones, where the rinsing of central areas was done 3–4 h after the inoculation (Fig. 1). Since the produced inhibitor and many cells of *P. pisi* were rinsed out with sterile distilled water from the central area into the outer zone, further multiplication of this bacterium was inhibited in outer zones. Inhibition of *P. pisi* in central areas was not appreciable, because the HR had already been previously induced before the inhibitor developed. During the experiment, the cell number of *P. pisi* in the outer zones was measured and it had to be sufficient for the induction of the HR.

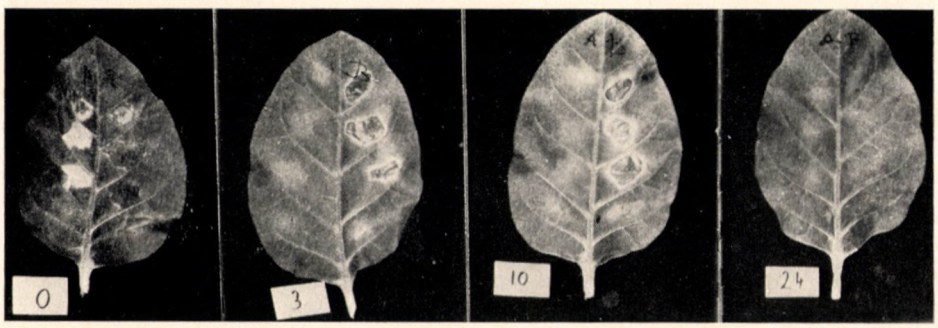

Fig. 2. Inhibition of the development of necrosis in tobacco leaf pretreated with *P. fluorescens*. The left half of leaves were challenged with *P. phaseolicola* (incompatible) and the right ones were injected with *P. tabaci* at 6 h after the pretreatment. Concentrations of challenge inocula were 5×10^6, 10^7, 10^8 and 10^9 cells ml^{-1} from the bottom

When we repeated this experiment investigating the biological characters of the inhibitor, it was established that the inhibitor can be induced not only by incompatible, but by compatible and saprophytic bacteria, too (Table 1).

Table 1

Appearance of necrosis in tobacco leaf panels pretreated with water, compatible, incompatible and saprophytic bacteria

Hours between pretreatment and challenge inoculation	Water		Pretreatment with					
			P. tabaci		*P. pisi*		*P. fluorescens*	
	C	O	C	O	C	O	C	O
1	+	+	+	+	+	+	+	+
1.5	+	+	+	+	+	+	±	+
2	+	+	+	+	+	+	−	+
2.5	+	+	$+^P$	+	$+^P$	+	−	+
3	+	+	$+^P$	−	$+^P$	±	−	+
3.5	+	+	$+^P$	−	$+^P$	−	−	±
4	+	+	$+^P$	−	$+^P$	−	−	−
5	+	+	$+^P$	−	$+^P$	−	−	−
6	+	+	$+^P$	−	$+^P$	−	−	−

Appearance of necrosis (+) in central areas (C) and in outer zones (O) of tobacco leaf panels. Only central areas were pretreated either with water (control) or with the compatible *P. tabaci* or with the incompatible *P. pisi* or with the saprophytic *P. fluorescens*. Suspensions of these bacteria contained 5×10^8 cells ml^{-1}. The effect of ESP was demonstrated with a streptomycin resistant mutant of *P. pisi* 5×10^7 cells ml^{-1}. This challenge inoculum was suspended in 1000 $\mu g\ ml^{-1}$ streptomycin solution and injected into the central areas so that the water-soaking area extended to the surrounding tissue (outer zone). The sign — indicates the absence of the HR. The sign $+^P$ indicates the necrosis induced by *P. tabaci* or *P. pisi* as preinoculum. Once the induction time is complete, development of necrosis is not prevented by the killing of primary inocula. Each tested in triplicate.

A similar experiment was carried out with the alteration that the compatible *P. tabaci* as challenge was used. In this case the normosensitive necrosis always appeared in both the central and outer zones. All these experiment prove that the ESP inhibits multiplication of incompatible bacteria but not the compatible one.

Selectivity of the inhibitory effect was further investigated. A suspension of 10^8 cells ml^{-1} of *P. fluorescens* was preinjected into whole tobacco leaves. These leaves were challenge injected in ca. 1 cm^2 areas (half leaf with *P. tabaci*, other half with *P. phaseolicola*) at different intervals after the pretreatment (Fig. 2). In compatible host-parasite combination (tobacco–*P. tabaci*) the development of normosensitive necrosis was not or just a little inhibited by ESP, until the development of acquired resistance until 14–20 h. However, in the compatible combination (tobacco–*P. phaseolicola*) the HR appeared, but only in the first 2 h following pretreatment. This result also indicates that the effect of the ESP on compatible

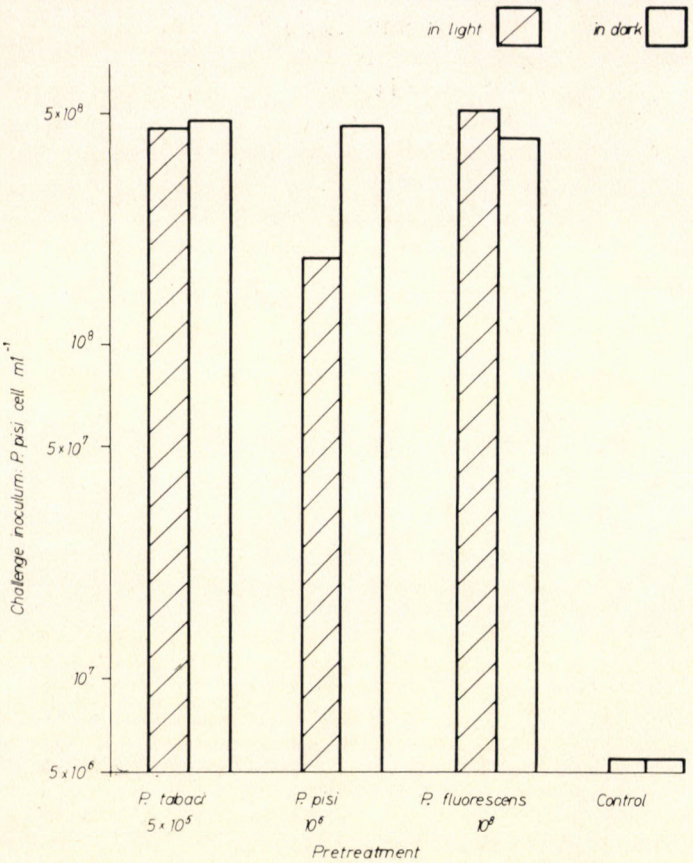

Fig. 3. Inhibition of *P. pisi* in tobacco leaf, in light and in dark. The ESP was induced by *P. tabaci* 5×10^5 cells ml^{-1}, *P. pisi* 10^6 cells ml^{-1} and *P. fluorescens* 10^8 cells ml^{-1}, respectively. The inhibition of *P. pisi* was demonstrated by the lack of the HR. The challenge bacterium was injected at 6 h after pretreatments

and on incompatible bacteria is different. As mentioned earlier, although the inhibitor is produced in the compatible host-pathogen combination too, the compatible bacterium counteracts its effect, therefore, the inhibitor has a selective specificity. The compatible bacterium (normosensitive necrosis) was also inhibited but only after the development of acquired resistance. This selective inhibition distinguishes the ESP from the acquired resistance phenomenon.

Suppression of normosensitive and hypersensitive necrosis by heat killed bacteria has been reported earlier (LOVREKOVICH and FARKAS, 1965; LOZANO and SEQUEIRA, 1970). This phenomenon (acquired resistance) was characterized as a non-specific, time- and light-dependent reaction of plant (SEQUEIRA, 1976). Since light is required for acquired resistance we investigated its effect on ESP and found that it had no effect (Fig. 3).

In summary, it seems that incompatible bacteria can induce an early defence reaction (ESP) in tobacco leaf against incompatible *P. pisi*, which reaction is not light-dependent. Saprophytic bacteria are supposedly also inhibited by ESP, however, there is no suitable method to demonstrate the inhibitory effect *in vivo*. The induction of the ESP is not specific but its inhibitory effect is selectively specific, because the multiplication of compatible *P. tabaci* was not inhibited. Our results also suggest that the ESP is not identical with the HR or acquired resistance.

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The Production of Polygalacturonase by *Xanthomonas manihotis*

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A study has been made of the activity of polygalacturonase in culture filtrates of *Xanthomonas manihotis*. The activity of the enzyme is enhanced in increasing concentrations of polygalacturonic acid. Activity was also high in media containing starch. The presence of glucose in growth medium depressed enzyme activity. It is suggested that polygalacturonase is an inducible enzyme in this bacterium.

It is known that most plant pathogens produce enzymes that degrade components of primary cell walls of plants (WOOD, 1967). The ability of a pathogen to produce pectic enzymes has been correlated with its virulence and the critical role of such enzymes in infection. But although the involvement of pectic enzymes in many host-parasite interactions is uncontested their importance in pathogenicity is often doubtful (WIESE *et al.* 1970).

The diseases caused by plant pathogenic bacteria of the genus *Xanthomonas* are quite unlike those induced by the pectolytic soft rot *Erwinia Bacillus* and *Pseudomonas* species and suggest that xanthomonads probably lack the ability to degrade pectic substances (SMITH, 1958b; NASUNO and STARR, 1967). Nevertheless there are a few reports on the pectolytic activity of xanthomonads. Thus 14 out of 25 *Xanthomonas* spp. were found to liquefy pectate gel although more slowly and less vigorously than did the soft rot erwinias (BURKHOLDER and STARR, 1948). Similar observations were presented by other workers (SABET and DEWSON, 1951; SMITH, 1958a, b; DYE, 1960). Even though most of the cultures liquefied pectate gel and produced pectinesterase (PE) none was shown to produce hydrolytic polygalacturonase or pectin *transeliminase* (NASUNO and STARR, 1967).

The aim of this work is to investigate the production of polygalacturonase by *Xanthomonas manihotis* maintained under various cultural conditions.

Materials and Methods

(a) *Organism*

Xanthomonas manihotis strain No CMI B 5530 was used.

(b) *Growth of cultures*

Cells were reconstituted from a freeze-dried ampoule with sterile distilled water and grown for 27 h on KELMAN's (1954) TTC medium after which subcultures were made from single colonies on to Kelman's medium without TTC. Inoculated plates were incubated for 48h at 30°C after which bacterial growth was washed off with sterile distilled water and standardized to an absorbance of 0.3 unit (ca. 3.2×10^7 cells/ml).

The liquid salt medium of STARR (1946) was used as a basal growth medium. Different carbon sources at appropriate concentrations were separately sterilized and then added aseptically to the basal medium. Five ml of standardized bacterial suspension was added to penicillin flasks (Jencons, Hemel Hempstead) containing 300 ml of the medium. The cultures were maintained shaking in the dark on a rotary shaker (94 rev/min) at 25°C. The cultures were harvested after various periods and then centrifuged to separate the cells from culture filtrates. The latter was used as enzyme source.

(c) *Assay for polygalacturonase activity*

The activity of polygalacturonase (PGase) was measured at 25°C by a method modified from KERTÉSZ (1955) and NASUNO and STARR (1966). To 90 ml of 0.5% polygalacturonic acid in 0.1 M NaCl previously buffered to pH 5.2 with sodium acetate buffer was added 10 ml of culture filtrate. The mixture was immediately stirred. At zero time and at 5 minute intervals thereafter 5 ml of the reaction mixture were withdrawn into a conical flask containing 0.9 ml of 1 M Na_2CO_3. Immediately 5 ml of 0.1 N iodine solution was added to each flask and left for 20 minutes at room temperature. After the 20 minute period, 2 ml of 2 M H_2SO_4 was added and the residual iodine titrated against 0.05 N sodium thiosulphate using starch as indicator.

The titre values were plotted against time. The slope of such graphs related to one microgram of the protein contents of the culture filtrate represents the activity of PGase. The protein contents of culture filtrates were determined by the folin phenol method of LOWRY *et al.* (1951).

(d) *Paper chromatography of products*

This was done essentially by the method of NASUNO and STARR (1966).

Results

Culture filtrates of *X. manihotis* contain PGase activity (Fig.1). Such activity increased with the age of culture. The major product of reaction was found by paper chromatography to be galacturonic acid (Data not shown). The production of PGase by *X. manihotis* was, however, found not to be peculiar to cells grown in

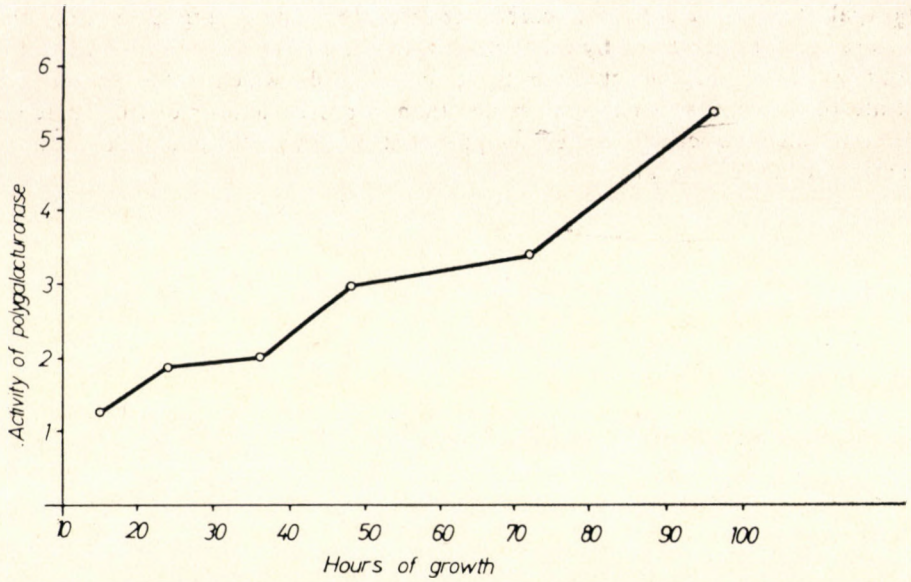

Fig. 1. Time course of polygalacturonase activity in *Xanthomonas manihotis* grown with polygalacturonic acid as sole source of carbon

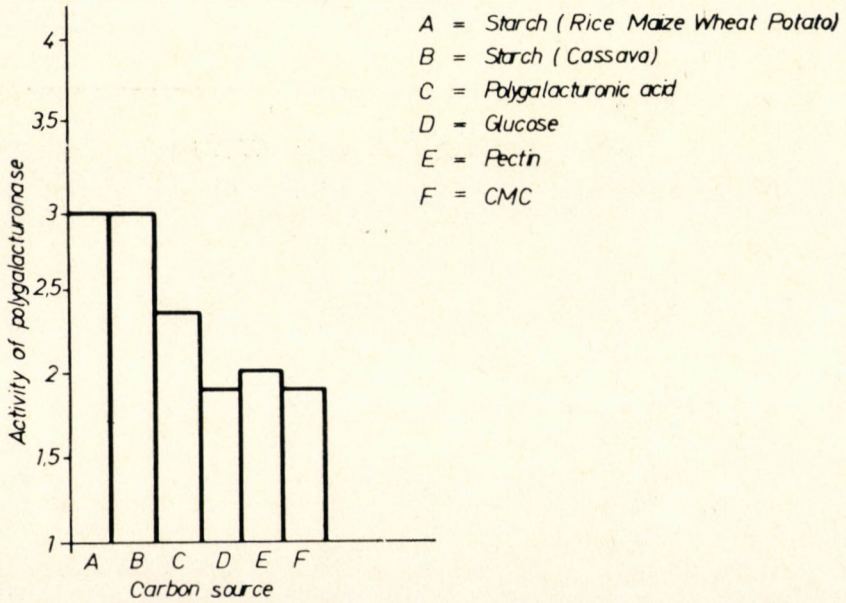

Fig. 2. Effects of various carbon sources on inducibility of polygalacturonase

polygalacturonic acid as sole carbon source. The data in Fig. 2 indicate that PGase could be produced by cells grown with cassava or maize starch as carbon sources. Indeed enzyme activity in this polysaccharide, which is the natural substrate of the enzyme *in vivo*, was higher than in polygalacturonic acid. There was also considerable activity in media supplemented with pectin, glucose or carboxymethyl cellulose (Fig. 2).

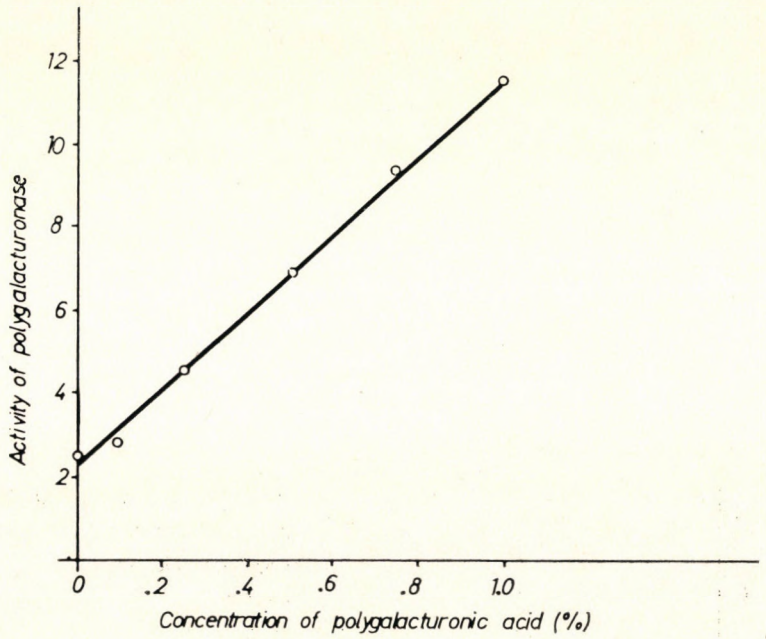

Fig. 3. Effect of polygalacturonic acid (PG) concentration on polygalacturonase activity. PG supplied as sole carbon source in growth medium

PGase was assayed in culture filtrates of *X. manihotis* grown in basal salts medium containing different concentrations of polygalacturonic acid. There was a linear increase in PGase activity with increase in concentration of polygalacturonic acid (Fig. 3). Figure 4 shows the effect of different concentrations of glucose on PGase activity in cells grown in 0.5% polygalacturonic acid as the sole carbon source. There was increasing suppression of polygalacturonase activity with increasing concentration of glucose in the medium. This suppression of enzyme activity was doubled at 2% concentration of glucose. These data suggest that PGase is an inducible enzyme in *X. manihotis*.

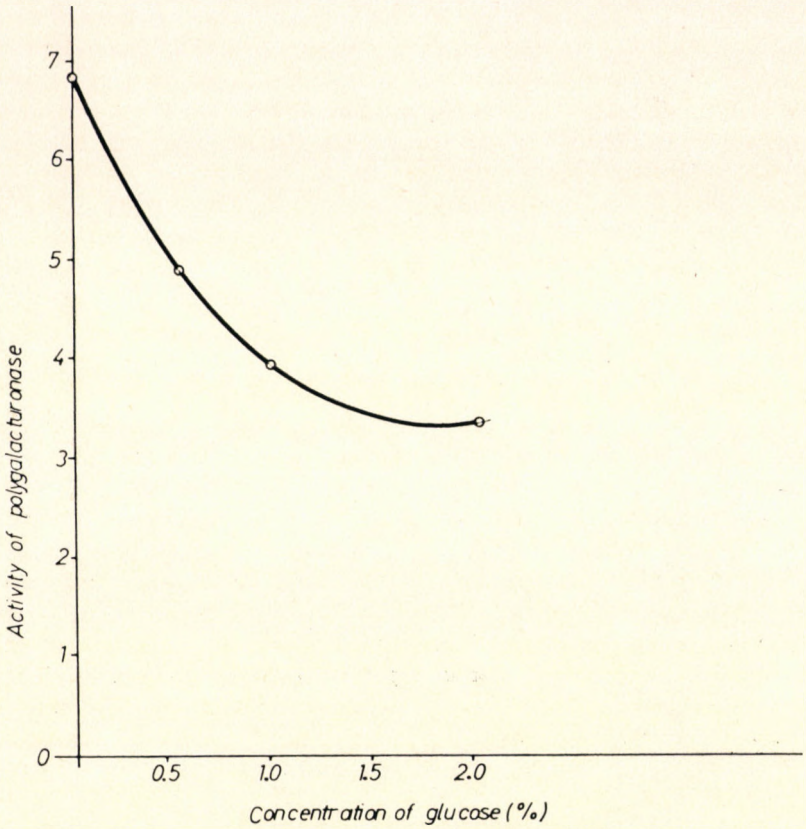

Fig. 4. Effect of different concentrations of glucose on polygalacturonase activity in cells grown in 0.5% PG as carbon source

Discussion

Results obtained in this study indicate that *Xanthomonas manihotis* can produce polygalacturonase in culture media supplemented with various sources of carbon such as starch, polygalacturonic acid and pectin (Figs 1–3). This is in line with the work of ZUCKER *et al.* (1972) who reported that soft rot bacteria produce 100–1000 times more enzymes per cell under optimal conditions than did other bacterial pathogens or saprophytes. Activity of the pectic enzymes of *Erwinia carotovora* is in the order of over 1000 (STEPHENS, 1974).

Generally, Xanthomonads are thought not to possess strong pectolytic activity because of the nature of disease they cause. However, a few species of this genus are known to produce cell wall degrading enzymes. Production of pectic enzymes has been reported for *X. campestris*, *X. vasculorum*, *X. phaseoli*, *X. paper-*

vericola (SMITH, 1958a) and other Xanthomonads including *X. manihotis* (DYE, 1960). Electron micrographs of cassava tissues infected by *X. manihotis* have confirmed the involvement of pectic enzymes in pathogenicity (IKOTUN, unpublished).

Chromatograms of the reaction mixture show that the major end product of breakdown of polygalacturonic acid by this enzyme is galacturonic acid. This is similar to the results obtained for the polygalacturonase of *Erwinia carotovora* (NASUNO and STARR, 1966) and those of various plant pathogens (SMITH, 1958b).

Polygalacturonase production continued to increase for up to 96 h of growth in culture medium. Loss of enzyme activity beyond 16 h of growth has been reported for *E. carotovora* (STARR and MORAN, 1962) and 36–48 h for *X. campestris* (NASUNO and STARR, 1967). These do not agree with the results presented here. It is possible that the growth medium used here and the assay methods employed being different accounted for differences in results.

That increased glucose concentration suppressed production and enzyme activity in this study is supported by the work of STARR and MORAN (1962). Glucose being another carbon source is a more readily available energy source and must have been utilized in preference to the polygalacturonic acid provided.

The results obtained in Figs 1–3 here have some similarity to those of ABO-EL-DAHAB (1964) in which *X. malvacearum* was shown to produce pectic enzymes only in the presence of pectic substrates. Like *X. malvacearum* it is probable that pectic enzyme production by *X. manihotis* is induced and adaptive, not constitutive. This adaptive process probably occurs inside host plant tissues where pectic substrates are present in adequate amounts in the middle lamella and elsewhere in plant cell walls.

Acknowledgements

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Histopathology of Cassava Tissues Infected by *Xanthomonas manihotis*

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The histopathology of cassava tissues (leaf and stem) infected by *Xanthomonas manihotis* was investigated by electronmicroscopy. The pathogen multiplied in the intercellular spaces of cells from where it attacked and degraded the pectic substances in the middle lamella. Undegraded cellulose microfibrils in the cell walls were exposed and dispersed and cytoplasmic contents of cells were disorganized and scattered.

The degraded cell walls were separated and the pathogen entered the cytoplasm and destroyed the cellular contents. All tissues of infected plants were invaded and disintegrated enzymatically. *X. manihotis* should be regarded as a parenchyma not a vascular pathogen, since vessels were not physically blocked by the pathogen but were degraded and disintegrated.

The symptoms caused by the cassava blight bacterium *Xanthomonas manihotis* include leaf spots and blight, wilt of leaves and of young shoots, gum exudation and stem die-back (BONDAR, 1912, 1915; AMARAL, 1942, PEREIRA and ZAGATTO, 1967; LOZANO and SEQUEIRA, 1974a). The pathogen has been regarded as a vascular pathogen which advanced systemically through the xylem (DRUMMOND and HIPOLITO, 1941; AMARAL, 1942; PEREIRA and ZAGATTO, 1967; LOZANO and SEQUEIRA, 1974a; LOZANO and BOOTH, 1974; LOZANO, 1975). Movement through phloem and pith tissues has also been reported (AMARAL, 1942; PEREIRA and ZAGATTO, 1967).

The aim of this work was to investigate how and in which tissues the pathogen advances through infected plants and the effect of the pathogen on individual plant cells.

Materials and Methods

Infected tissues were observed under the stereoscan and transmission electronmicroscopes (SEM and TEM respectively).

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SEM

Infected tissues (leaf and stem) were cut into ca. 1 mm thick sections with a razor blade and immediately fixed in 2.5% glutaraldehyde in 0.1 *M* Na-cacodylate buffer (pH 7) for 2 h, washed twice for 10 min each time in the same buffer and dehydrated in ethanol (10% steps) with two changes in absolute ethanol, then placed in increasing acetone : alcohol proportions up to absolute acetone, then critical point dried at 36°C. The dried specimens were then stuck to flat-topped nails and coated with gold.

TEM

Diseased stems and leaves were cut into pieces (ca. 0.5 mm thick) and fixed in 2.5% glutaraldehyde in 0.2 *M* Na-cacodylate buffer (pH 7) (final concentration 0.1 *M*), for 2 h at room temperature, washed twice (10 min each) in the buffer, and post fixed in 1% osmium tetroxide in 0.2 *M* Na-cacodylate buffer for 1 h at room temperature. Specimens were then dehydrated in ethanol (10% steps) up to absolute alcohol with two changes (30 min each), then transferred to a 1 : 1 mixture of absolute alcohol–epoxypropane after which specimens were left in epoxypropane–resin mixture for 15 min and the specimens were transferred to fresh resin after evaporating epoxypropane. There were three changes of resin (24 h each) and the specimens embedded in resin were left to polymerize at 60°C for 48 h. Thin sections were cut on a Cambridge-Huxley ultramicrotome and silver sections were picked up on copper grids, stained for 30 min in uranyl acetate at 60°C and in lead citrate for 15 min at room temperature.

Results

Coated and stained specimens were observed under the stereoscan and transmission electron microscopes and micrographs of suitable information were taken using an Agfa black and white negative film. Representative results are given in Figs 1–14.

Leaf infection

X. manihotis multiplied in intercellular spaces of the spongy mesophyll of infected leaves after entry through stomata and wounds (Figs 1 and 2). In intercellular spaces, bacterial cells were surrounded by extracellular polysaccharide (produced by the bacterium), the excess of which broke off in small fragments and became dispersed around them. Most bacterial cells moved in the plant polar end first. Cytoplasmic contents of the plant cells adjacent to invaded intercellular spaces became disorganized and scattered. This took place while the bacterial cells were still some distance away from affected plant cells (Fig. 2). The pathogen then invaded cells of both spongy and palisade mesophylls and degraded the plant tissues (Fig. 3). Cytoplasmic contents of invaded cells were discoloured by deposits of osmiophilic substances. The plasma membranes of cells became detached from cell walls before cytoplasmic contents were scattered (Fig. 4).

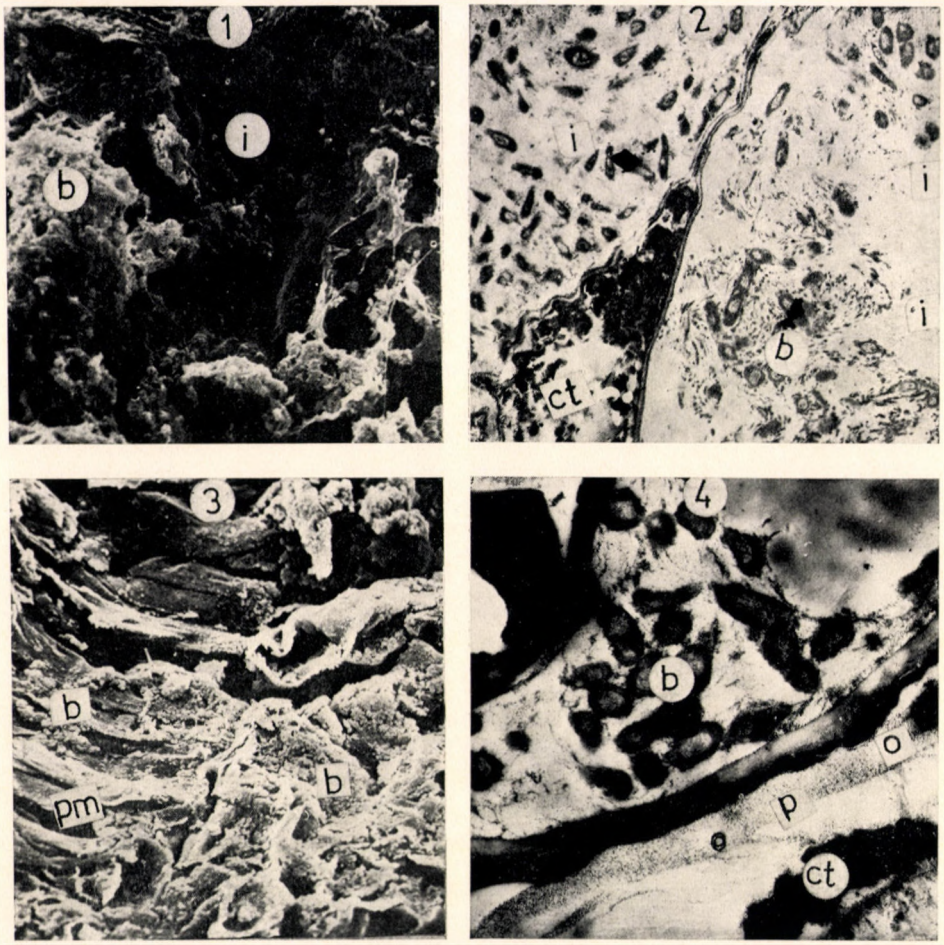

Fig. 1. Stereoscan electron micrograph (SEM) of cassava leaf tissue showing cells of *X. manihotis* multiplying in the intercellular spaces of spongy mesophyll. × 2350

Fig. 2. Transmission electron micrograph (TEM) of infected cassava leaf showing bacterial cells multiplying in intercellular spaces of spongy mesophyll. Note the dispersal of cytoplasmic contents and fragments of extracellular polysaccharide around bacterial cells. × 7400

Fig. 3. SEM of infected cassava leaf tissues showing *X. manihotis* advancing from spongy to palisade mesophyll. × 1050

Fig. 4. TEM of cassava leaf infected by *X. manihotis* showing separation of the plasma membrane from the cell wall. Note also the darkening of cellular contents due to phenolic and osmiophilic substances. × 10,200

TEM of a cell of a healthy cassava stem showed empty intercellular spaces, clear and continuous cell walls, and the plasma membranes of cells adjacent to intercellular spaces were appressed to the cell walls and cytoplasmic contents remained in place (Fig. 5).

After entry into the stem through wounds and other means, *X. manihotis* multiplied in intercellular spaces. The plasma membrane of cells adjacent to invad-

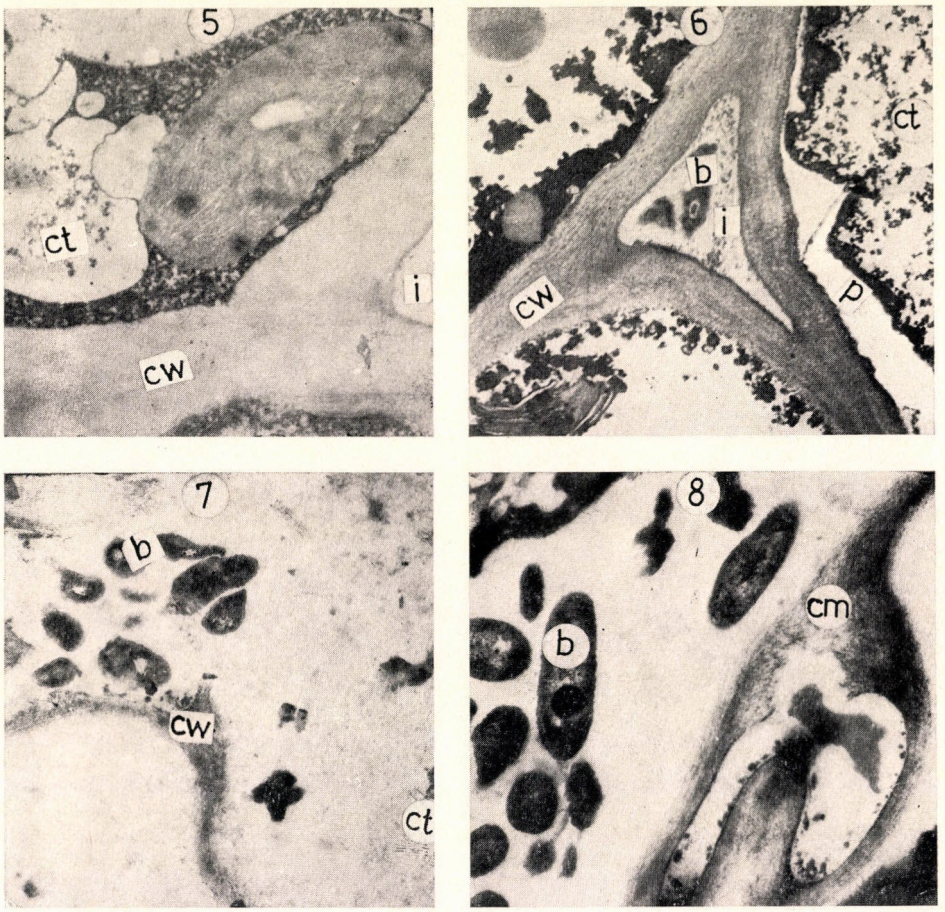

Fig. 5. TEM of healthy cassava stem showing healthy cell wall, intercellular space and healthy cytoplasm. × 10,000

Fig. 6. TEM of cassava stem infected by *X. manihotis* showing bacterium multiplying in intercellular space. Note plasma membrane separated from cell wall and dispersal of cytoplasmic contents in adjacent cells. × 7500

Fig. 7. TEM of cassava stem showing degradation of cell wall, exposure of cellulose microfibrils and bacterial cells breaking out of intercellular spaces into an adjacent cell. × 15,600

Fig. 8. TEM of infected cassava stem showing exposure of cellulose microfibrils in the cell wall after other substances had been degraded by *X. manihotis*. × 12,000

ed intercellular spaces became separated from the cell wall (right side cell) and cellular contents were disorganized and scattered. Cell walls became discoloured (Fig. 6). Middle lamella but not cellulose microfibrils were degraded and the bacterial cells moved through the degraded cell wall of undegraded cellulose microfibrils into the cell (Fig. 7).

Bacterial cells moved variously in the stem; some moved polar and first (to the plane of sectioning), whereas others moved broad side first (Fig. 8).

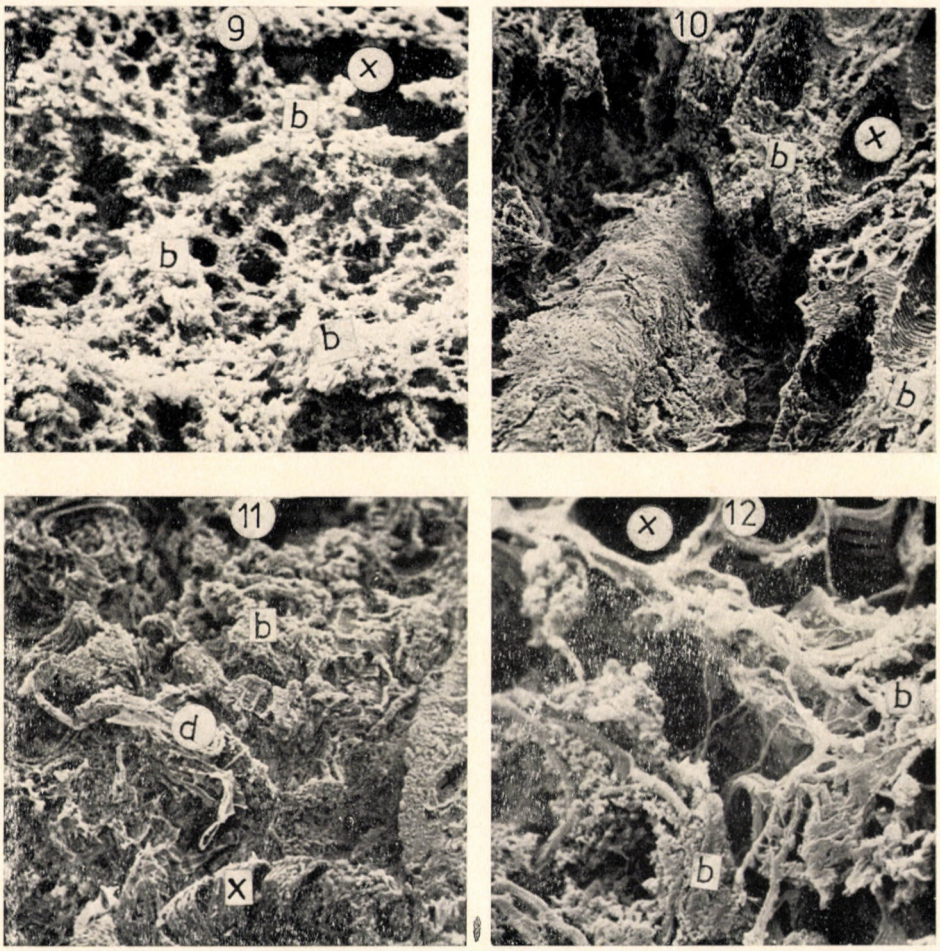

Fig. 9. SEM of cassava stem infected by *X. manihotis* showing the bacterium advancing along the cell wall and not in the lumen of xylem vessels. ×200

Fig. 10. SEM of infected cassava stem showing degradation of xylem vessels by bacterial masses whereas the lumen of xylem vessels was not blocked by the pathogen. ×1900

Fig. 11. SEM of infected cassava stem showing debris of collapsed xylem elements. ×1000

Fig. 12. SEM of infected cassava stem showing disintegration and collapse of xylem vessels whereas several vessels were still not blocked by bacterial cells. ×2500

Long after infected plants had wilted, cells of *X. manihotis* were not found in the lumen of xylem vessels. Bacterial cells were found on the cell walls of xylem parenchyma and of vessels but not in xylem vessels (Fig. 9). Cross and lateral longitudinal sections also showed that the lumen of xylem vessels was not physically blocked by bacteria (Fig. 10). Because of degradation of pectic substances in the middle lamella of cell walls, cortical and vascular elements collapsed and disintegrated (Figs 11 and 12). The collapsed and disintegrated tissues and bacterial cells cemented together by extracellular polysaccharide produced by *X. manihotis*, filled the volume formerly occupied by cortical and vascular elements to form a "bacterial pocket". In this pocket, outlines of disintegrated cells were still faintly discernible (Figs 13 and 14). Many of the intact xylem vessels were still not blocked by bacterial cells long after the formation of the bacterial pocket from which the bacterium oozed out to the epidermis of the stem as a copious exudate.

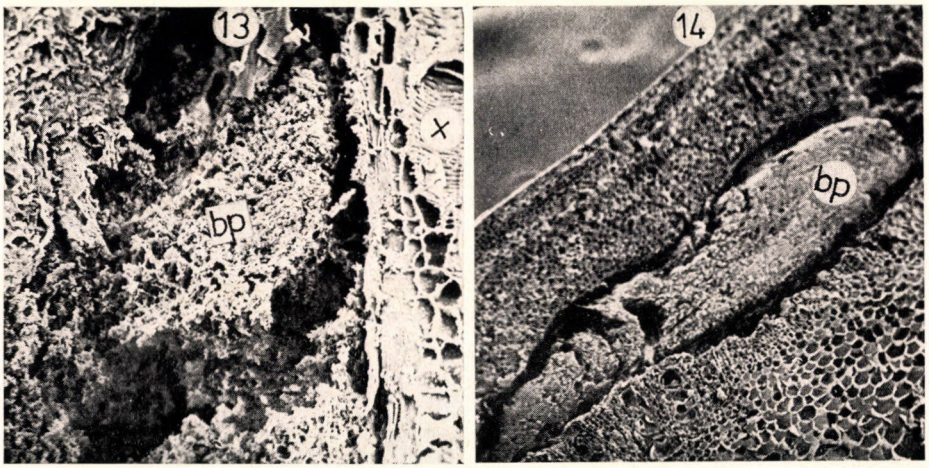

Fig. 13. SEM of infected cassava stem showing degraded and disintegrated tissues plus bacterial cells forming a "bacterial pocket" where tissues had once been. × 550
Fig. 14. SEM of cross section of infected cassava stem showing bacterial pocket in the cortical region from where bacterial cells and plant debris ooze out on to the epidermal surface. × 50

List of notations used in figures

b = bacteria
bp = bacterial pocket
cm = cellulose microfibrils
ct = cytoplasm
cw = cell wall
d = degraded tissues
i = intercellular spaces
o = osmiophilic substances
p = plasmolysed cell
pm = palisade mesophyll
x = xylem vessel

Discussion

Infection of leaves by *X. manihotis* is considered secondary since primary infections arise from infected cuttings and symptoms from these appear as gum exudates, wilt and die back of stems (LOZANO and SEQUEIRA, 1974b). Secondary symptoms (leaf infections) usually arise from rain splashes from primary infections.

Observation of and results obtained from more than 1500 sections of infected cassava stems show that the pathogen was not restricted to vascular tissues. Bacterial cells were found in all tissues from epidermis to the pith. Results obtained from this study do not agree with those of DRUMMOND and HIPOLITO (1941), AMARAL (1942), PEREIRA and ZAGATTO (1967) and LOZANO (1975), who regarded *X. manihotis* as a vascular pathogen. True vascular pathogens primarily invade the xylem tracheary elements and effect water transport in the host (BUDDENHAGEN and KELMAN, 1964).

This work agrees in part with that of DRUMMOND and HIPOLITO (1941), AMARAL (1942) and PEREIRA and ZAGATTO (1967) who reported that the bacterium occurs in the phloem and pith tissues as well as in vessels.

X. manihotis multiplied in intercellular spaces of leaves and stems and degraded the middle lamella of cell walls. The degradation of middle lamellae caused cells and the tissues to collapse.

It was the disintegration and collapse of cortical and vascular tissues and not physical blockage of xylem elements that probably caused infected plants to wilt.

Therefore, *X. manihotis* cannot be regarded primarily as a vascular wilt pathogen but rather as a parenchyma pathogen. It can be grouped among pathogens "that mainly affect parenchymatous tissue and cause wilting indirectly" (BUDDENHAGEN and KELMAN, 1964).

Indications of a possible role for enzymes in pathogenesis has been given by LEU and CHEN (1972). Such activity is more likely to be responsible for pathogenicity of *X. manihotis* rather physical blockage of conducting vessels by bacterial cells which alone cannot account for the syndrome (IKOTUN, 1975).

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Survey of Scale Insect (*Homoptera: Coccoidea*) Infestations in European Orchards

Changes in the Scale infestation Levels in Hungarian Orchards between 1971 and 1976

By

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A comparative study in Hungary revealed that infestation levels of scale insects had decreased in apple orchards, while they had increased on peach and plum in the period 1971—1976. *Quadraspidiotus perniciosus* appeared in lower individual numbers, *Parthenolecanium corni* and *Sphaerolecanium prunastri*, however, became more common. The infestations of *Q. perniciosus* were observed to decline in apple, pear, and plum orchards. The densities of *Epidiaspis leperii* decreased on apple and pear, while they increased in plum orchards. No remarkable changes were demonstrated in ratios of populations of individual scale insect species in 5 types of fruit orchards since 1971.

The last gradation of *P. corni*, well expressed in natural biotopes, was observed also in orchards, especially on plum, where no more than 3 chemical treatments were carried out. Under these conditions considerable parasitation in scales was found. Mass appearance of *S. prunastri* was observed in natural biotopes as well as in peach orchards. Infestations with this species could be detected even in orchards sprayed with insecticides 10 times a year. The rate of parasitation in *S. prunastri* proved rather variable in untreated orchards, too.

In 1976 a series of investigations, organized by the specialists of EPPO and COMECON, has been set up on the distribution and the population levels of scale insects damaging various fruits in Europe. This study will enable us to reveal the factors influencing the territorial and temporal changes in populations, moreover, to predict some possible tendencies.

Faunistical research concerning distribution of endemic species does not give information on population densities which could be very important for plant protection. It is also of considerable interest that the expansion of some species *(Pseudaulacaspis pentagona, Q. perniciosus)* in Europe is still in progress. Earlier surveys were confined either to certain plants or to individual territories, countries (KOZÁR, 1976; POVSUN, 1962; RASINYA, 1972). These considerations led the authors of the present paper, in cooperation with a specialist of the Leningrad Zoological Museum (E.S. SUGONYAEV), to organize a systematical collection of scale insects in a number of European countries.

A similar country-wide study in Hungary was conducted in 1971 (KOZÁR, 1972, 1976). Now we are able to estimate the changes in scale insect populations which had occurred since that time. This is only a five-year period and it must be

admitted that for a clear indication of changes, especially in case of certain species, at least 10 years would be necessary. We can remark, however, that even within relatively short period a considerable outbreak of plum scale *(S. prunastri)* and acacia scale *(P. corni)* could be observed.

In our investigations the following questions have been studied:

1. dominant species in individual fruit orchards;
2. role of agrotechnics (variety and age of host plant, form of tree-crown, pesticide treatments, plot-size etc.) in the formation of population dynamics;
3. rate of parasitation under different circumstances;
4. competition between scale insect species;
5. population density differences;
6. temporal changes detectable in respect to the above questions (with the use of earlier data gathered in Hungary).

Materials and Methods

For the survey in Europe standardized sample-collection methods were elaborated. In individual countries, from each type of fruit orchards (apple, pear, peach, plum, and cherry) 50 samples were collected. Twenty of these samples were taken in backyard gardens (size: below 1 hectare), another 20 in commercial orchards of large state farms (above 1 hectare), and 10 in scattered, neglected orchards. In the former two categories half of the samples derived from sprayed orchards, the other half from non-treated ones. Separate plant samples were collected from old, commercial orchards as well as from young, still non-fruit-bearing ones.

The method of sample collection was the following:

In the orchards selected for regular sampling, 20 cm long, 2-year old branches and 2 × 10 cm sized bark pieces were taken from 10 trees, positioned randomly along the diagonal axe of the plot. The time for sample collection was fixed between 15th and 30th of June to the north of the Kiev–Warsaw–Berlin line and between 1st and 15th of June to the south of it. After drying for 2 weeks, the plant samples were preserved in sealed, coarse paper bags. In some countries these materials were also fumigated.

In 'information sheets' enclosed to the samples, the following questions had to be answered: 1. Name of country; 2. place of sample collection (settlement, department); 3. plant species, variety, age, form of tree-crown; 4. number of chemical treatments per season; 5. characterization of cultivation type – grassy, cultivated, irrigated; 6. condition of the orchard – good, moderate, bad; 7. date of sampling; 8. name of sample collector; 9. remarks.

The course of evaluation was the following:

Under a dissection microscope, we determined the scale insects occurring in the sample. The population densities, the degrees of infestation of individual species

Table 1

Scoring system of infestation degree used for estimation of population densities of scale insects. After BORCHSENIUS (1950) and GOANCA *et al.* (1974) with considerable modifications

Degree (score)* of infestation	Specimens of scale insects on unit plant sample**														
	Pseudococcidae					*Coccidae*					*Diaspididae*				
	root	trunk	branch	leaves	fruits	root	trunk	branch	leaves	fruits	root	trunk	branch	leaves	fruits
	I. Females														
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	traces	1–5	traces	traces	traces	traces	1–5	1–5	traces	traces	traces	1–5	1–5	1–5	1–5
2	1	6–10	1	1	1	1	6–10	6–20	1	1	1	6–20	6–20	6–20	6–20
3	2–5	11–20	2–5	2–5	2–5	2–5	11–20	21–50	2–5	2–5	2–5	21–100	21–100	21–100	21–100
4	>5	>20	>5	>5	>5	>5	>20	>50	>5	>5	>5	>100	>100	>100	>100
	II. Larvae (uniform system for each scale species and plant samples)														
	0														
1	1–10														
2	11–50														
3	51–100														
4	>100														

* System of scores: 0 = no infestation; 1 = a few individuals; 2 = little colonies; 3 = large colonies; 4 = continuous infestation

** Unit plant samples: root = 10 cm long; trunk = 2 × 10 cm sized; branch = 10 cm long; leaves = 10 pieces; fruits = 10 pieces

were estimated with the use of a modified scoring system of BORCHSENIUS (1950). In this modification the methods of GOANCA *et al.* (1974) were also taken into consideration (Table 1).

From the samples 5 female specimens in each scale insect species were isolated into glass vials for microscopic inspection and determination. The preparation method of HOWELL and KOSZTARAB (1972) with some modifications, was used. The procedure was the following:

1. The insect material was boiled in 10% NaOH for 5–10 minutes.
2. Thereafter the specimens were put into Essig's Aphid Fluid which was mixed with some fuxine, erythrosine and lignin pink. In this fluid the insect samples were boiled for 10–30 minutes until reaching the appropriate coloration. (In case of *Coccids* and *Diaspidids* this period was shorter than with *Pseudococcids* and *Eriococcids*.)
3. From the staining solution the insects were carried over into 96% ethanol.
4. The following step was the immersion of samples in clover oil for 10 minutes.
5. Finally the scale insects were transferred to microscopic slides and mounted into Canada balsam, covered, and dried at room temperature for 2 weeks.

At the primary evaluations the presence and density of other pests or diseases (e.g. eggs of mites, bark necrosis) could be also registered.

The parasitation in scale insects was also studied in the samples. The bottom of the paper bags where the plant materials were preserved, often contained adults of hymenopterous parasitoids which had emerged after sample collection. By count-

Table 2

The distribution of sampling sites and the numbers of samples in a survey of scale insect infestations in Hungary in 1976

Department (county)	Number of samples				
	Apple	Pear	Peach	Plum	Cherry
Szabolcs-Szatmár	17				
Zala	18	16			
Borsod-Abaúj-Zemplén				20	
Baranya			12		
Somogy				20	
Veszprém		6	16		
Pest		15	9		9
Heves				9	11
Bács-Kiskun	18				
Csongrád					20
Sum total	53	37	37	49	40

ing the emergence holes of parasitoids on the scales, the rate of parasitation was accurately determined.

As seen in Table 2, in 1976 we received 216 branch and bark samples of fruit trees from various departments (counties) and fruit orchards in Hungary. This year the number of samples was less than in 1971. The statistical evaluation of the former material led us to the conclusion that in Hungary even 50 samples can provide satisfactory informations (KOZÁR, 1975). For a more detailed study of certain questions (e. g. parasitation in scale insects) the number of trees was increased from 4 to 10 within a sampling unit. The sampling sites and trees were selected in both years (1971, 1976) in the same manner, i.e. randomly.

Results and Discussion

Investigations on infestation levels of different species

In 1976 in 216 plant samples collected from 5 types of fruit orchards the occurrence of 8 scale insect species was detected in Hungary:

Coccidae
1. *Eulecanium mali* (Schrank);
2. *Parthenolecanium corni* (Bouché);
3. *Sphaerolecanium prunastri* (Fonscolombe);

Diaspididae
4. *Epidiaspis leperii* (Signoret);
5. *Lepidosaphes ulmi* (L.);
6. *Quadraspidiotus ostreaeformis* (Curtis);
7. *Quadraspidiotus perniciosus* (Comstock);
5. *Pseudaulacaspis pentagona* (Targioni-Tozzetti).

In 1971 inspection of 1327 samples derived from 6 types of fruit orchards revealed 2 additional, generally rare species, *Quadraspidiotus pyri* (Lichtenstein) and *Palaeolecanium bituberculatum* (Targioni-Tozzetti).

The changes in average infestation rates of individual species damaging various fruits, observed within these 5 years, are demonstrated in Fig. 1. The statistical analysis of data was conducted with χ^2 test. Our results equivocally showed that the infestation rates on different fruits, in case of nearly all scale insect species, had only slight changes. However, the significant decrease in the infestations with the San José scale is remarkable. At the same time the infestations with other species exhibited tendencies of some extension. It was of considerable significance to notice that the frequency of occurrence in 2 species, *P. corni* and *S. prunastri*, had increased and come close to the extent found in *E. leperii*. This question will be dealt with later in detail.

As Fig. 1 shows, similarly to 1971, in 1976 *Q. perniciosus* was the most important species infesting various fruits in Hungary. By comparing the infestation

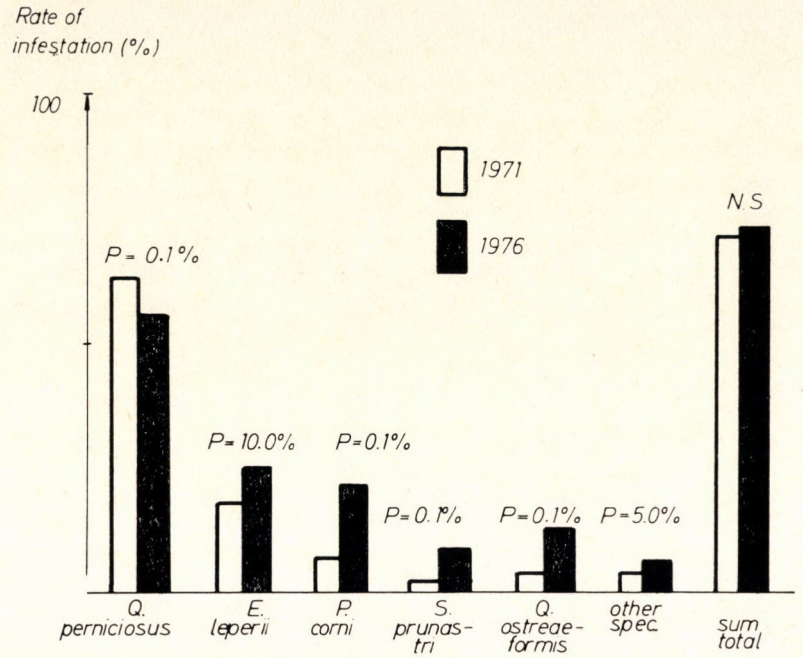

Fig. 1. Differences in average infestation rates of scale insect species damaging various fruits in Hungary between 1971 and 1976. (The levels of significance are indicated; N. S. = not significant)

degrees (scores) of different scale insect species (Table 3), it is obvious that their economical significance is in correlation with the corresponding degrees of infestation. The role of *E. leperii* seems more important on the basis of the population density than regarding its frequency of occurrence.

In contrast to the fact that the country-wide average of scale insect infestations exhibited only slight changes within 5 years, relatively significant differences

Table 3

The importance of individual scale insect species on the basis of infestation degree in 1976

Species	Average degree of infestation in 5 types of fruit orchards
Q. perniciosus	0.71
E. leperii	0.53
P. corni	0.23
S. prunastri	0.16
Q. ostreaeformis	0.15
Other species	0.11

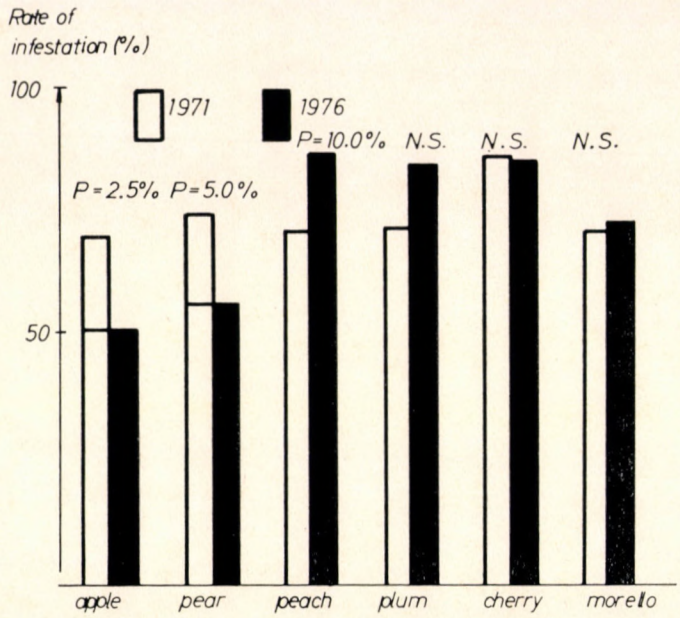

Fig. 2. Differences in average infestation rates of scale insects in various orchards between 1971 and 1976

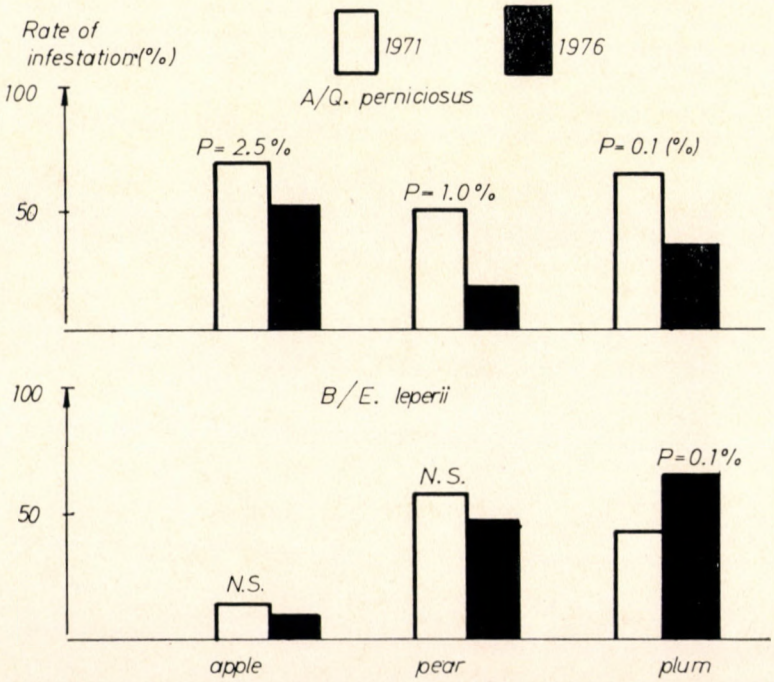

Fig. 3. Average infestation rates of *Q. perniciosus* and *E. leperii* in various orchards in 1971 and 1976

of infestation rates on individual fruit species can be observed (Fig. 2). The scale infestations in apple orchards decreased by 20% (significant at p = 2.5%) while the populations in plum and peach orchards increased. This can be attributed to the expansion of 2 species *(P. corni* and *S. prunastri)*.

A thorough investigation of the infestations on apple, pear, and plum with the most important 2 species *(Q. perniciosus* and *E. leperii)* demonstrated (Fig. 3) that the infestation rates of *Q. perniciosus* changed by 1976 to significantly lower

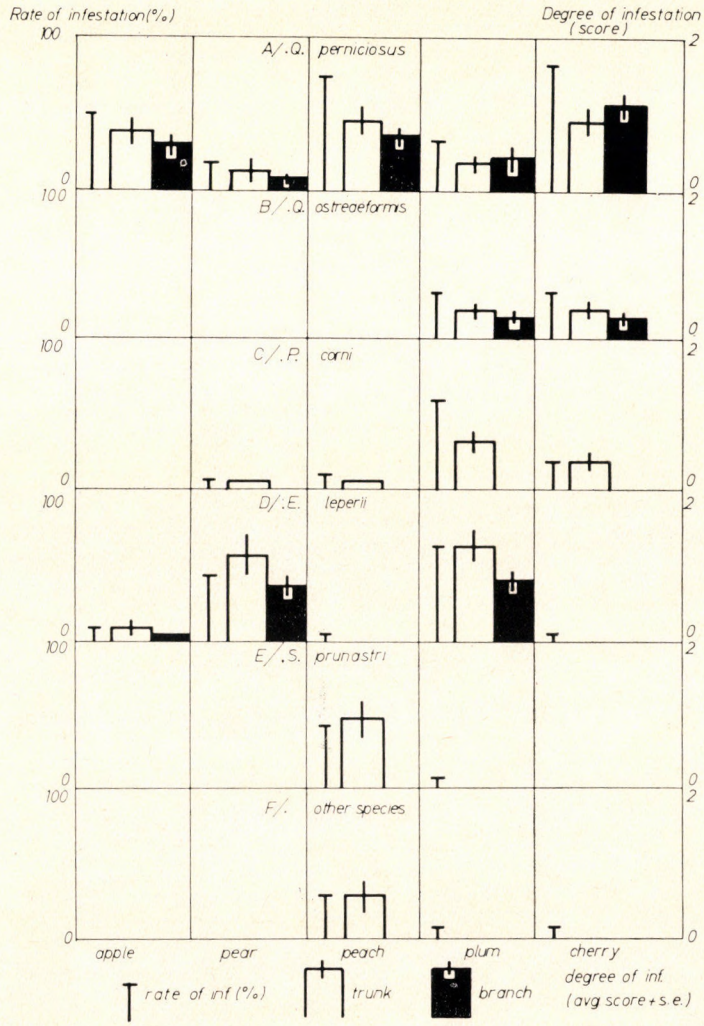

Fig. 4. Average infestation rates and infestation degrees of some scale insect species in various orchards in 1976

levels in all the 3 types of orchards, especially in plum orchards. The rates of *E. leperii* infestations on apple and pear seemed to decrease (no significant difference) while on plum an increase by 20% (significant at p = 0.1%) was found. In both species the degree of infestation showed values which could be correlated with the corresponding infestation rates in all the 3 types of fruit orchards (Table 4).

Table 4

Infestation degrees (scores) in various orchards estimated in 1971 and 1976

Orchard	Degree (score) of infestation							
	Q. perniciosus				*E. leperii*			
	1971		1976		1971		1976	
	avg.	s.d.	avg.	s.d.	avg.	s.d.	avg.	s.d.
Apple	1.55	1.35	0.81	1.23	0.20	0.60	0.15	0.56
Pear	0.81	1.02	0.29	0.86	1.17	1.20	1.14	1.56
Plum	0.94	0.88	0.41	0.89	0.64	0.87	1.24	1.36

The rates of scale insect infestations on different species, estimated in 1976, are demonstrated in Fig. 4. The occurrence of *Q. perniciosus* was in each type of fruits very considerable. Especially high infestation levels could be seen on cherry and peach where the degree of infestation proved rather high. The lowest rates were found on pear. *Q. ostreaeformis* appeared at detectable levels only on plum and cherry.

The presence of *P. corni* was shown in each type of orchards, high infestation rates and higher scores of infestation were revealed barely on plum. The occurrence of *S. prunastri* was detected only in peach and plum orchards. This species infested plum at low frequencies, at the same time, considerable infestations and population densities could be demonstrated on peach.

Other scale insect species were found only 'in traces' on cherry and plum as well as on peach where high rates (e.g. in case of *P. pentagona*) were also seen.

In *Diaspidid* species the infestations on bark and branch samples were separately evaluated. These results generally show similar values, except in case of *E. leperii* on pear and plum where the branches exhibited significantly lower infestations.

In Table 5 the per cent infestations of scale insects on various fruit species and the degrees of these infestations in 1971 and 1976 are demonstrated. The variable infestation rates in individual departments (counties) of Hungary can be also studied. In this respect, no significant changes have occurred since 1971. The results relating to one type of fruit are less demonstrative because in 1976 in average only 6–20 samples per fruit species in a department were available. So these latter results can be considered merely as of preliminary significance.

Table 5

Comparison of infestation rates and infestation degrees of *Q. perniciosus* and *E. leperii* in different orchards, departments (counties) and years

Orchard	Department (country)	Rate of infestation (%)				Degree of infestation (score)			
		Q. perniciosus		*E. leperii*		*Q. perniciosus*		*E. leperii*	
		1971	1976	1971	1976	1971	1976	1971	1976
Apple	Szabolcs	46.6	41.2	6.6	0.0	1.06	0.59	0.20	0.00
	Zala	55.5	66.7	0.0	16.7	1.33	0.72	0.00	0.38
	Bács	55.5	44.4	0.0	5.6	0.77	1.10	0.00	0.05
Pear	Zala	62.5	18.6	37.5	50.0	0.62	0.31	0.75	1.40
	Pest	27.2	33.3	63.6	16.7	0.36	0.30	1.63	0.17
	Veszprém	47.0	13.3	82.3	46.6	0.52	0.27	1.88	1.27
Peach	Baranya	40.0	83.3	0.0	0.0	0.50	1.00	0.00	0.00
	Veszprém	84.6	87.5	0.0	6.3	1.30	1.25	0.00	0.06
	Pest	80.0	44.4	0.0	11.1	1.20	0.33	0.00	0.11
Plum	Heves	28.5	66.7	42.8	88.9	0.28	0.89	0.71	2.33
	Somogy	27.2	15.0	36.3	65.0	0.27	0.10	0.72	0.85
	Borsod	77.7	40.0	16.6	45.0	1.00	0.40	0.22	1.15
Cherry	Heves	100.0	90.0	0.0	9.1	1.25	1.55	0.00	0.19
	Csongrád	100.0	85.0	0.0	0.0	2.00	1.05	0.00	0.00
	Pest	70.0	66.7	0.0	0.0	0.80	0.78	0.00	0.00

Investigations on the outbreak of P. corni

The last outbreak of *P. corni* in Hungary was observed around 1955. Since that time the gradations appearing every 11 years ceased most probably due to the high mortality caused by a parasitoid, *Blastotrix confusa* (Erdős) (SAAKYAN-BARANOVA *et al.*, 1971). In 1975 and 1976 in many regions of Hungary but also in the Soviet Union (SHELUDKO, 1976) a new gradation of this species was found indicating that in the future periodical outbreaks of *P. corni* can be taken into account. Simultaneously with these gradations in natural biotopes, the population densities increased in orchards, too, especially in chemically rarely treated plum and cherry orchards (Fig. 4).

In search of circumstances affecting these mass appearances we could determine the corresponding infestation rates that reached 56% in backyard gardens, 47% in commercial orchards of large state farms, and 83% in scattered, neglected gardens. The degree of infestation was estimated 0.6, 0.5, and 0.9, respectively, in

these orchards. The gradations were especially typical for natural territories (which was in accordance with higher infestation rates and degrees in scattered, neglected gardens), so they cannot be considered as dangerous for orchards intensively sprayed with pesticides.

Where chemical applications exceeded 3 in a season, populations of *P. corni* could not be detected. Beside the influence of pesticides, parasitoids were possibly also active (e. g. in Nagyréck in a plum orchard treated 3 times 50%, in Szücsi in a plum orchard treated twice, 80% parasitations were found).

Investigations on the gradation of S. prunastri

A gradation of *S. prunastri* was observed in natural biotopes and in orchards between 1973 and 1976. This species infested mainly peach even in chemically treated orchards (Fig. 4). VÁLYI (1976) was the first to call attention to the outbreak of *S. prunastri*.

We could determine infestation rates of 30% in backyard gardens, 47% in orchards of large state farms, and 56% in scattered, neglected gardens. The infestation degrees reached 0.6, 1.0, and 1.2, respectively. The high infestation rates in commercial orchards of state farms show that this species finds favourable conditions for a gradation not only under natural circumstances but also in intensively treated orchards. Therefore, it was not at all unlikely that even in an orchard sprayed 10 times a year we could estimate an infestation degree of score 4. Some side effects of the chemical pest control often establish favourable conditions for a gradation of this species (SZIRÁKI, 1977). It must be emphasized, however, that we are faced here with a gradation taking place under natural conditions and not with a gradation evoked by chemical control.

The influence of parasitoids in chemically treated orchards was found generally low, detectable only 'in traces'. In backyard gardens and scattered, neglected orchards parasitism proved also very variable. We often could not determine any parasitoids or only a few of them but, in other cases, the females of scale insects were parasitized up to 80% or 90%. Even in the latter instance, the high rate of parasitism did not prevent the development of a gradation of *S. prunastri*. (A few examples: Romonya: peach sprayed 7 times, parasitation in traces; Csopak: peach chemically treated 5 times, parasitation in traces; Pécs: peach, non treated, 90% parasitism; Balatonalmádi: peach, non treated, parasitation not detectable.)

Population dynamics of other scale insect species will be discussed together with data of other countries. Conditions affecting infestation levels can be then better studied and understood. The recent outbreaks of *P. corni* and *S. prunastri* emphasized their relative significance, therefore we treated them separately of the others.

Acknowledgements

We would like to express our sincere thanks to Dr. G. MATHYS, general director of EPPO and to Prof. Dr. W. WEGOREK, head of Coordination Centre for Plant Protection Research of COMECON as well as to Dr. T. JERMY, director of Research Institute for Plant Protection, Budapest, for the kind help in the organization of detailed sample and data collection in a number of European countries. For the careful work of collecting infested plant samples we are very much obliged to the specialists of Centre of Plant Protection and Agrochemistry of Ministry of Agriculture and Food Affairs, Hungary.

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Translocation and Metabolism of Triadimefon in Different Plant Species*

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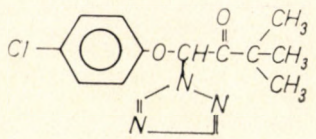
The uptake, translocation and metabolism of triadimefon have been studied in four plant species. It was established that triadimefon becomes translocated in higher plants also basipetally, in accordance with literature data. However, the rate of uptake and subsequently the measure of transport depend substantially on the mode of leaf treatment. There are also differences in the amounts of translocated triadimefon according to plants; in higher plant species triadimefon metabolizes and as a result triadimenol is formed. The transformation into triadimenol (i.e. activation) begins already in the host plant tissues. Differences were established also regarding the speed of activation in the different plant species, the selectivity of systemic fungicidal effect is thus influenced by the host itself.

1. Introduction

Triadimefon (MEB 6447), a new systemic fungicide [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanone] (GREWE and BÜCHEL, 1973, FROHBERGER, 1973, KASPERS *et al.*, 1975) has been developed by the research group of Bayer AG with the following structural formula:



Special significance is given to this compound by its property to be transported in the plants not only acropetally – as in case of most systemic fungicides known so far – but also basipetally (BUCHENAUER, 1975, 1976). Its range of effectivity is quite broad, including many pathogens of mildew and rust diseases of cereals and also some smut fungi. The biochemical mechanism of fungicidal effect seems to be founded on the inhibition of ergosterine synthesis (BUCHENAUER, 1977).

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The active material is commercialized in different formulations (seed dressing preparations, wettable powders and dusts). Promising experiments are being conducted with one of the preparations (Bayleton 25 WP) to be used in the plant protection practice in Hungary.

In the past two years the measure of acropetal and basipetal transport has been studied on bean and barley plants by using bioautographic method (BUCHENAUER, 1975, 1976). Its metabolism in barley plants and in the soil has been investigated by the radioactive tracer method (VOGELER, 1976). However, no comparative studies seem to have been conducted between different plant species, regarding both translocation and metabolism, although these are decisive for establishing the most effective range and way of application.

The importance of the host plant itself in the development of systemic fungicidal action has been studied in our earlier experiments with the active materials triforine and ethazole (GASZTONYI and JOSEPOVITS, 1975, GASZTONYI, 1976) and it was demonstrated that the amounts of active material showed considerable differences within the host plants. These differences could be attributed either to differences in inactivation and/or to differences in translocation. Both factors depend on the grade of mobilization of the active material and this, in turn, may be influenced by the adsorption to plant tissues.

2. Materials and Methods

2.1. *Materials*

Triadimefon, crystallized active material, m. p. = 80°C (m. p. = 82.3°C in the literature),

Bayleton 25 WP, wettable powder for spraying purposes, containing 25% triadimefon (both materials produced by Bayer AG).

2.2. *Incubation with leaf homogenates*

In the experiments the following plant species and varieties have been used:

Cucumber (Csemege fürtös 77), tomato (Hebros 70), bean (Cheroquee), wheat (Little club 74).

The leaf homogenates were prepared from the true leaves of cucumber seedlings with two leaves, from the leaves of tomato plants with 3 true leaves and from leaves of 10-day-old wheat seedlings and from the first trifoliate leave of bean seedlings. After a disinfection with 10% H_2O_2 solution and washing in sterile distilled water, 1 g of leaf blades (green weight) was homogenized in an MSE homogenizer and incubated in distilled water containing 100 ppm triadimefon (under sterile conditions) in a Vibroterm vibrator at 25°C for 1 and 24 hours, respectively. Simultaneously, under identical conditions homogenate-free triadimefon solution and triadimefon-free homogenates were kept for the same time periods as controls.

Each variation was repeated 3 times. Each repetition contained the components mentioned in quantities of 20 ml. The addition of triadimefon was carried out from an alcohol stock solution (0.2 ml to 20 ml) and the same amount of pure alcohol was added also to the controls.

After the incubation was finished, the mixture was filtered through filter paper and washed with 5 ml distilled water. To the filtrate 3 ml saturated NaCl solution was added and extracted with 15 ml chloroform. By evaporating the solvent in an air stream the remaining material was dissolved immediately in 5 ml acetone. The tissue debris remaining on the filter was ground with quartz sand and extracted with 10 ml acetone. Following a filtration and acetone washing the filtrate was dried and the residue immediately dissolved in 5 ml acetone.

2.3. *Triadimefon treatment and processing of plants*

The glasshouse treatments were made with the same four plant species mentioned under paragraph 2.2. Each experimental unit consisted of 2 cucumber, tomato and bean plants each, and 8 wheat seedlings, cultivated in containers holding 100 g soil. Two types of treatments were applied: a) a 10^{-2} M triadimefon acetone solution was applied on the leaves in quantities of 0.8 ml (= 2.35 mg active material) per experimental unit and in case of tomato plants in quantities of 0.2 ml (= 0.59 mg a. i.); b) the aqueous suspension of the preparation Bayleton 25 WP was applied in concentration of active material and in quantities corresponding to treatment type a). The treatments were made on the two upper leaves of cucumber seedlings with 4 true leaves, on the three upper leaves of tomato plants with 6–7 true leaves, on both trifoliate leaves of bean plants with two leaves and on the upper leaf of 2-leaved wheat seedlings. The untreated parts of the plants and soil surface were covered from eventual contamination of the treating solution. From the time of treatment until processing the plants were watered via the bottom of the containers. By treating plants with corresponding amounts of pure acetone and leaving untreated control plants as well, each variation was repeated three times and the whole experiment was repeated at another time.

Three days after the treatment the treated leaves, untreated aerial parts, roots and soil were separately processed. The plant parts were ground with quartz sand and extracted with acetone (5 ml per 1 g green weight). By filtering on filter paper and washing with acetone, chloroform was added to the eluate in a quantity to form a distinct aqueous phase originating from the water content of the tissues. The latter phase was removed and the remaining acetone–chloroform phase was dried over anhydrous sodium sulphate, evaporated in air stream and the residue immediately dissolved in 5 ml acetone. In grinding up one of the control plants 0.1 ml of 10^{-2} M triadimefon solution was added and the recovery data were used in the corrections of measured values.

The extraction of soil was made by treating air-dried soil samples with acetone (5 ml per g). The extraction was followed by evaporation of the solvent and re-dissolution of the residue in 1/5 part of solvent.

2.4. *Determination of triadimefon by gas chromatography*

The triadimefon quantities were measured by injecting the acetone solutions (or their appropriate dilutions) prepared as described above. Gas chromatography parameters:

Column packing: 3% SE-30/Chromosorb W 60/80 mesh (h = 180 cm, d = 3 mm); detector: ECD

Temperatures: column 180°C, injector 220°C, detector 190°C; carrier gas: Nitrogen (25 p. s. i.)

2.5. *Thin layer chromatography with biological detection*

The acetone extracts prepared according to the methods described in paragraphs 2.2 and 2.3 were chromatographed with a benzene–acetone (9 : 1) solvent mixture. After evaporation of the solvent the plates were detected by bioautogratphy. For that purpose a *Cladosporium cucumerinum* culture maintained in malnutrient solution was disintegrated in a homogenizer in 2% malt solution, filtered through a double-layer cheesecloth and the thick suspension (containing besides the mycelium fragments also spores of the fungus) was sprayed onto the chromatogram. Incubated in a wet chamber at 25°C for 2 days the Cladosporium develops on the layer and the inhibition zones show up clearly.

3. Results

3.1. *Study of stability of the active material*

For the quantitative study of triadimefon in biological objects it seemed necessary to investigate first the chemical, photochemical stability and volatility of the compound, to be able to correct the recovery data with the eventual decrease of active material as measured under our experimental conditions.

The results of the study of stability are summarized in Table 1.

Table 1

Decrease of triadimefon content due to physical and chemical factors

Initial concentration	Medium and treatment	Time period of exposure	Decrease in %
100 ppm	dist. water	72 hours	Ø
100 ppm	dist. water + UV irradiation	20 minutes	11
100 ppm	2% malt nutrient solution	48 hours	Ø
100 ppm	0.01 N HCl	72 hours	Ø
100 ppm	0.01 N NaOH	72 hours	Ø
20 $\mu g/cm^2$	glass surface	72 hours	44
40 $\mu g/cm^2$	glass surface	72 hours	34

The determination of the remaining triadimefon content has been carried out from aqueous solutions by chloroform extraction (with subsequent evaporation of the solvent), from glass surfaces by acetone eluation; both types of treatment followed by gas chromatography.

The chemical stability of the compound has been mentioned also in the literature (SPECHT, 1977); no data were found, however, regarding the photochemical decomposition of the active material. By the known relatively low vapour tension of triadimefon (10^{-6} m bar) the high loss due to sublimation measured on glass plates was somewhat unexpected, but nevertheless in accordance with recent data on the fungicidal effectivity experienced in the vapour phase (SCHLÜTER, 1977; SCHEINPFLUG *et al.*, 1977). (On the speed of sublimation no quantitative data were published so far).

According to these results the sublimation losses have to be taken into consideration in the recovery studies of active material following leaf treatments. In course of processing the experimental materials also the time had to be minimized when the active material was exposed to the air. As the photochemical decomposition was relatively low and did not occur in diffuse daylight, this factor was not considered in the following work.

3.2. *Incubation with leaf tissue homogenates*

The fate of active material was followed in aqueous solutions containing 100 ppm triadimefon, incubated for 1 hour and 24 hours, respectively, with leaf tissue

Table 2

Changes in triadimefon content and distribution of the active material in leaf tissue homogenates

Time	Fraction	Unit	Triadimefon in the homogenates of				$SD_{95\%}$
			wheat	bean	cucumber	tomato	
1 hour	filtrate	mg	1.53	1.48	1.41	1.46	0.22
1 hour	filter-stock	mg	0.23	0.28	0.24	0.17	0.08
1 hour	filtrate	%	77	74	71	73	
1 hour	filter-stock	%	11	14	12	9	
1 hour	total	%	88	88	83	82	
24 hours	filtrate	mg	1.40	1.36	1.22	1.45	0.12
24 hours	filter-stock	mg	0.22	0.18	0.14	0.26	0.08
24 hours	filtrate	%	70	68	61	72	
24 hours	filter-stock	%	11	9	7	13	
24 hours	total	%	81	77	68	85	
24 hours	deficiency	mg	0.38	0.46	0.64	0.29	0.15
24 hours	deficiency	%	19	23	32	15	
	loss from 1st to 24th hour	%	7	11	15	Ø	

homogenates of four plant species. The triadimefon content of the filtrate as well as the filter-stock remaining on the filter were studied by proper extraction, followed by t. l. c. analysis. The data, corrected with the recovery data of plant-free control are contained in Table 2.

The distribution of active material between the solid phase and solution came into equilibrium practically within 1 hour. The insoluble components of the plant homogenates adsorbed reversibly 9 – 14% of the triadimefon added and the plant species studied did not show significant differences in this respect, not even in such extent as shown with ethazole (GASZTONYI, 1976).

The total recoverable amount of active material was between 82 and 88% by the end of the first hour, while it decreased to 68 – 85% during the 24-hour incubation period, according to plant species. Regarding the loss of active material, significant differences were noted between the plant species by the end of the 24-hour incubation. The highest decrease was observed in the cucumber homogenate (32%, of which 15% fell to the period between the 1st and 24th hour). This had to be attributed to a considerable degree to the metabolism of triadimefon. The lowest losses in triadimefon recovery were noted in the tomato leaf homogenates (total 15%), while intermediate values were recorded in the wheat and bean homogenates. (The loss in wheat homogenate differed significantly only from the one of cucumber while between the other plants the differences were significant.)

3.3. *Translocation and metabolism in plants under glasshouse conditions*

The translocation of triadimefon was measured in four plant species by gas chromatography, 3 days following the leaf treatment. The results are summarized in Tables 3 and 4, according to the two methods applied.

In evaluating the data shown here also the volatility of triadimefon has to be considered, to which the two last lines of Table 1 give information. In our case the volatility may have caused losses of only some tenths of mg on the treated leaves, especially in case of treating with acetone solution, which presented a larger evapo-

Table 3

Triadimefon found in plants treated with acetone solution

	Wheat	Bean	Cucumber	Tomato	$SD_{95\%}$
Amount applied, mg	2.35	2.35	2.35	0.59	
Amount found in treated leaves, mg	0.39	0.79	1.37	0.21	0.08
Amount found in untreated parts, mg	0.15	0.51	1.10	0.03	0.06
µg/g in untreated parts (fresh weight)	190	130	220	22	
Translocation in % of the amount applied	6.4	22	47	5.1	

ration surface. Most recent reports (SCHLÜTER, 1977; SCHEINPFLUG *et al.*, 1977) pointed out that in the vicinity of plants treated with triadimefon also untreated plants could become protected against mildew infection, due to the distribution of active material in the vapour phase; these authors, however, did not give quantitative data. In our experiment the triadimefon quantity transmitted onto untreated surfaces via sublimation remained under the detection level of gas chromatography because no triadimefon could be found on control plants placed into the same group with treated ones. So the amounts of active ingredient determined in the untreated parts of experimental plants could be really regarded as measures of basipetal transport.

Table 4

Triadimefon found in plants treated with aqueous suspension

	Wheat	Bean	Cucumber	Tomato	$SD_{95\%}$
Amount applied, mg	2.35	2.35	2.35	0.59	
Amount found in treated leaves, mg	0.32	1.54	1.38	0.41	0.10
Amount found in untreated parts, mg	0.002	0.003	0.003	0.03	0.02
μg/g in untreated parts (fresh weight)	2.2	0.6	0.5	18	
Translocation in % of the amount applied	0.1	0.1	0.1	5.1	

In the roots of plants and in the soil in most cases no triadimefon could be demonstrated and the traces found in some cases hardly exceeded the limits of detection. So the data regarding roots and soil were not included in the Tables.

By comparing the data of Tables 3 and 4, the influence of the method of application becomes obvious. In case of acetone solutions the quantities of translocated triadimefon amounted to 130–220 μg in three plant species, related to 1 g of plant (green weight); in similar treatments with aqueous suspensions the same values fell between 0.5 and 2.2 μg/g. The differences of two orders of magnitude can be interpreted by assuming that the amounts of active ingredient passing into the untreated parts are limited not by the phloem transport but by the measure of uptake through the leaf epidermal tissue and the latter process is greatly increased by the acetone solution.

The comparatively high amounts of triadimefon found in the untreated plant parts after a longer exposure indicate that both the translocation and metabolism are continued after 3 days. So in bean plants processed 10 days after the treatment

with aqueous solution more triadimefon was found translocated (0.02 mg, or, expressed in another way 4.2 μg/g green weight) than after the first 3 days.

Another interesting phenomenon was the behaviour of tomato, different from the other plants from the point of view of translocation. After acetone treatment (Table 3, Fig. 1) in tomato plants significantly less triadimefon translocated

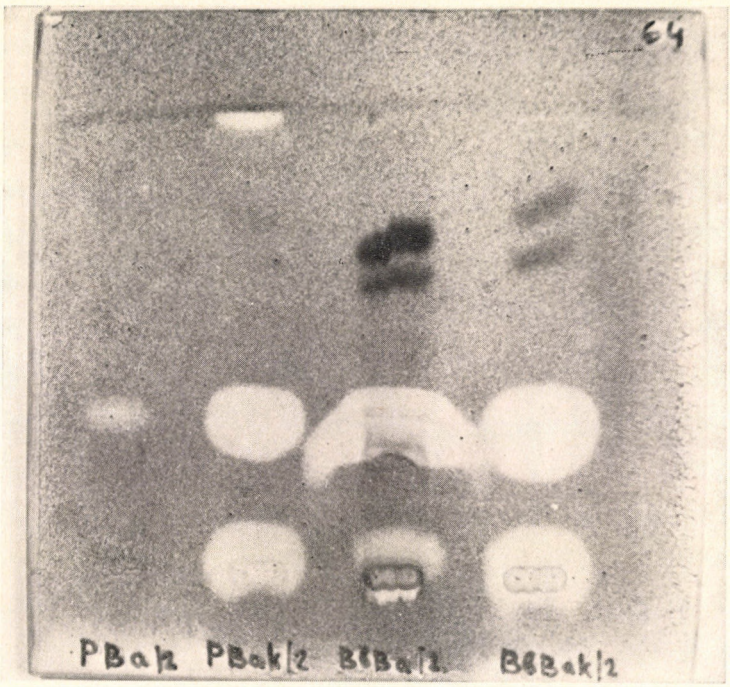

Fig. 1. Thin layer chromatograms made with extracts of tomato (P) and bean (Bb) plants treated with triadimefon acetone solution. Extracts of treated parts = PBak2 and BbBak2; extracts of untreated parts = PBa2 and BbBa2

than in the other species either expressed in weight unit or in percentage of the amount applied (although in the latter form the difference from wheat was not significant, but this could be attributed to the smaller amounts applied on tomato). After the treatment with aqueous suspension, however, it was just tomato in which more triadimefon (amounts higher by an order of magnitude) translocated than in the other three species.

As shown by the bioautographical analysis of the thin layer chromatograms made with the extracts, in course of the triadimefon metabolism a fungitoxic metabolite is formed (RF = 0.05–0.1) which has been identified (GASZTONYI and JOSEPOVITS, in press) and found identical with triadimenol [1-(4-chlorophenoxy)-

3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanol] described earlier by KRÄMER *et al.*, (1975), VOGELER (1976), FROHBERGER (1977), TRÄGNER-BORN and VAN DEN BOOM (1977). It has been also established (GASZTONYI and JOSEPOVITS, in press) that triadimenol is formed also in some fungi (mostly in ones sensitive to triadimefon) and its fungitoxic activity exceeds the one of triadimefon. This type of metabolism of triadimefon takes place both in plant homogenates and intact plants, it is, how-

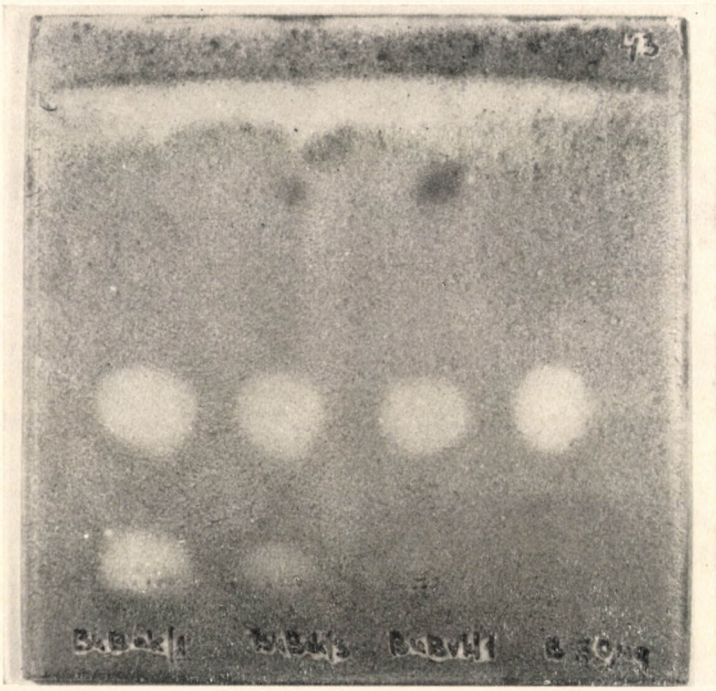

Fig. 2. Thin layer chromatogram of extracts of wheat leaves treated with triadimefon. From left to right: plants treated with acetone solution (BuBak1 and BuBak3) and with aqueous suspension (BuBvk1), and 30 μg triadimefon applied as standard (B)

ever, more intensive in the latter. Differences were noted from this point of view between the two methods of application; triadimenol formed in the leaves of the four plant species treated with the acetone solution of triadimefon, gave well visible inhibition zones on the layer chromatograms. On the other hand, in leaves treated with the aqueous suspension no, or only very small quantities of triadimenol could be demonstrated (cucumber). This difference is illustrated in Fig. 2 on the example of leaf extracts. From the plant species studied the metabolism was the highest in cucumber, in which the presence of triadimenol could be detected not only in leaves treated with acetone solution (Fig. 3) and to a smaller extent also in untreated leaves

and stalks, but even in leaves treated with aqueous suspension (Fig. 4) although in the latter near to the detection limit.

The influence of the method of application on the metabolism could be explained by the higher uptake of triadimefon from the acetone solution, so a higher amount participated in the metabolism. So the methods of treatment exerted their

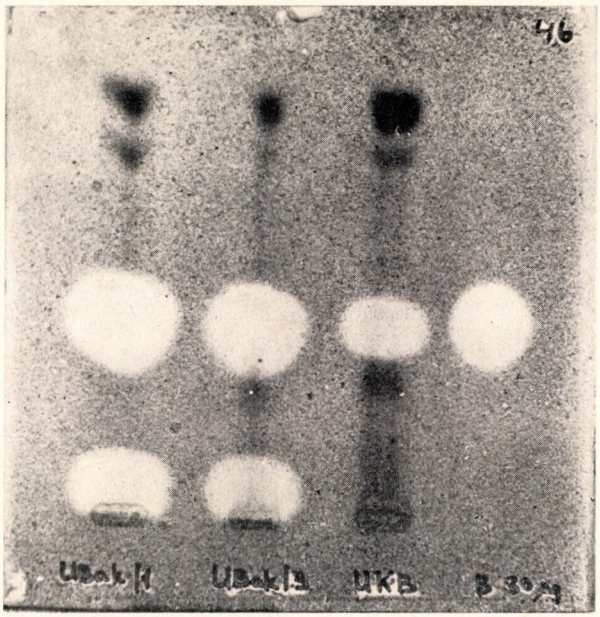

Fig. 3. Thin layer chromatograms of extracts of cucumber plants treated with triadimefon acetone solution (UBak1 and UBak3) and of untreated cucumber plants to which triadimefon has been subsequently added (UKB). Besides these 30 μg triadimefon has been used as standard

influence on the metabolism and translocation (Tables 3 and 4) through the same factor, i.e. through the difference in uptake.

The presence of the metabolite clearly demonstrated on the thin layer chromatograms showed that the treatment with acetone solution increased the uptake also into tomato leaves. The significantly lower level of triadimefon in the untreated parts, as compared to the other plants, could be interpreted with a slower phloem transport in tomato. At the same time, the uptake from aqueous suspensions and the subsequent translocation was higher in tomato than in the other plants, as shown by g. l. c. analysis (Table 4).

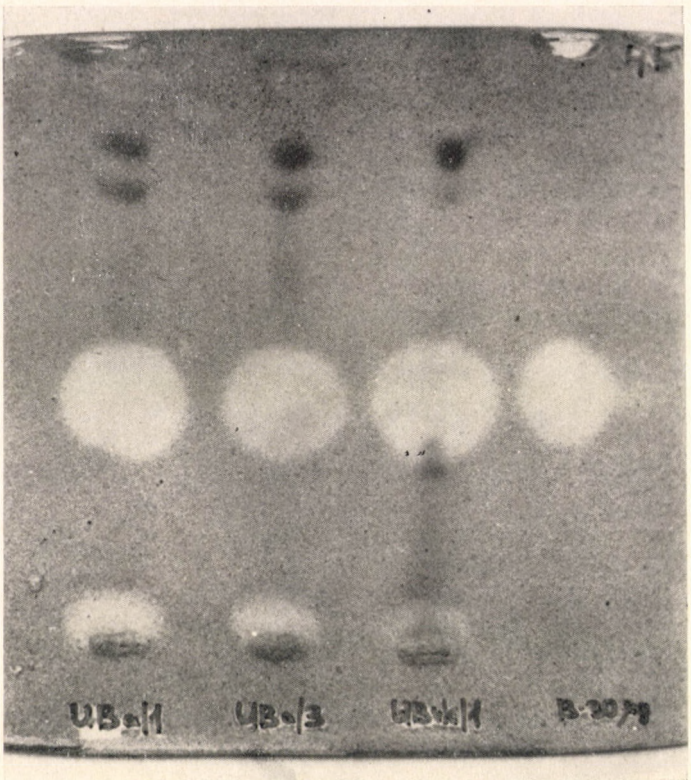

Fig. 4. Thin layer chromatograms of extracts of cucumber plants treated with triadimefon. Untreated parts of plants treated with acetone solution = UBa1 and UBa3; leaves treated with aqueous suspension = UBvk1; 30 μg triadimefon used as standard = B 30 μg

4. Conclusions

In our experiments carried out with higher plants we tried to establish the possible role of the host played in the formation of systemic fungitoxic effect of triadimefon. The reversible and irreversible bindings of the active material to the plant components did not show big differences in the plant species studied (Table 2). The uptake into intact leaves was greatly influenced by the way of application. From acetone solution significantly higher amounts passed into the treated leaves than from aqueous suspensions, which became expressed both in the measure of translocation (Tables 3 and 4) and in the metabolism (Fig. 1). From the practical point of view this calls the attention to the importance of proper formulation techniques. In earlier bioassays, BUCHENAUER (1975) found in cucumber plants, following a leaf treatment with aqueous suspension, the amount of basipetally translocated active material 18 μg/g plant material (fresh weight). In more recent studies

(SCHEINPFLUG *et al.*, 1977) in wheat plants a basipetal transport amounting to 2 – 3% of the triadimefon quantity applied was demonstrated by radioactive tracer technique. In our experiments the applications with acetone solution on wheat, bean and cucumber plants resulted in a higher translocation rate of triadimefon than the amounts reported in the literature, whereas the treatments with aqueous suspension showed lower values than the literature data. This indicates that because of the importance of application methods the various literature data are hardly comparable. In our experiments the tomato plants differed from the three other species since in the untreated leaves and stalks of plants treated with aqueous suspension, triadimefon amounts higher by an order of magnitude were found than in the corresponding parts of wheat and cucumber plants treated with the same method (Table 4). This would mean that the leaves of tomato are able to take up enough active material even from aqueous suspensions. The rate of transport remained in tomato even after acetone application on the same level (Table 3), indicating that here not the uptake (via the leaves) but the translocation in the phloem limit the amounts of triadimefon transported into untreated parts; the phloem transport seems to be slower in tomato than in other plants.

Also the metabolism of triadimefon could be established in plant tissues. This was found in homogenates to be of a much lower level than in intact plants. One product of the metabolism is identical with triadimenol found in barley plants by VOGELER (1976). The formation of this biologically active substance has been established in all the four plant species studied, but it appeared in the highest amounts in cucumber (Figs 3 and 4). In wheat and bean plants the metabolism was of lower level. In tomato leaf homogenates the triadimefon deficiency observed after 1 hour of incubation (Table 2) could be attributed either to a metabolism running along another pathway or to an irreversible binding to the structural parts of plant tissues, because at this time the appearance of triadimenol could not be demonstrated on the bioautograms and, on the other hand, this deficiency of active material did not increase during the subsequent 23 hours.

The measure of triadimenol formation was in treated, intact tomato plants similar to the ones found in wheat and bean plants, but nevertheless it fell behind the one established in cucumber plants.

As the precursor character of triadimefon has been proven in experiments made with fungus mycelia (GASZTONYI and JOSEPOVITS, in press), these data point out that the activation of triadimefon begins already in the host plant. So the fungitoxicity asserting itself via the host may be more effective against some, relatively resistant pathogens than the immediate contact effect of triadimefon. There were found also differences in the speed of activation between the plant species, so the selectivity of triadimefon depends not only on the biochemical properties of a given fungal pathogen but also on the characteristics of the host plant itself.

*

The authors have to acknowledge the valuable technical assistance of Mrs. M. VÖDRÖS in the experimental work.

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Study on the Physico-Chemical Parameters of Pesticide Formulations

I. The Investigation of the Wettability of Dispersible Powders

By

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It was proved that the determination of the slope and the intercept of the linear function between the logarithms of the radius of a spray drop on paraffin surface and of its volume are suitable for measuring and comparing the wettability of dispersible powders. The function between the log radius of the drop and the log concentration of the dispersible powder in water is of a linear character too, its slope characterizes well the wettability. The wettability is not influenced considerably by the water hardness, it increases up to fivefold hardness of the WHO standard water then does not change or decreases. The radius of a drop is changing in time: at first it grows then decreases according to the quality of the formulation, the temperature and the humidity of the air. The wettability measured on paraffin surface approaches well but is not equal to the wettability measured on vegetable wax surfaces.

The easy and economical application of biologically active molecules makes necessary the development of optimal combinations of solid or liquid surfactants, detergents, carriers or solvents and active ingredients. The physico-chemical parameters of the commercially formulated products influence considerably – increase or decrease – the performance of the active ingredient (VALKENBURG, 1973). Taking into consideration the great variety of the fields of application and the multiplicity of formulations it is understandable that the physico-chemical parameters of a pesticide formulation have to comply with different, often contradictory requirements. For example in the case of dispersible powders:

1. The good wettability that enables the formulations to cover the biggest possible surface at a given drop volume.

2. The good adhesive character that increases the resistance of the desiccated drop and the active ingredient to rain and to other mechanical effects (TELLE, 1970).

3. The good self-wettability that helps the ready and complete mixing of the formulation in waters of different degree of hardness.

4. The good suspensibility that promotes the uniform distribution of the active ingredient in the spray prepared from waters of different degree of hardness and decreases the velocity of the sedimentation (Handbook, Shell, 1959).

5. The low particle size that increases the stability of the suspension and generally the biological activity too.

6. The good flowability that promotes the moving and the distribution of the formulations (MÜLLER, 1971).

7. The low hygroscopicity. The hygroscopicity can be the cause of the caking, the decreasing of the wettability and of the flowability of the formulations in the case of inadequate, vapor permeable packaging.

Beside the requirements mentioned above the surfactants and carriers applied in the formulations

1. do not adsorb considerably the active ingredient, because it decreases the availability,

2. do not catalyze the decomposition of the active ingredient and

3. promote the penetration of the active ingredient.

The commercially available formulations without exception make a compromise with these theoretical principles, for example the decrease of the particle size and the specific weight of the carrier generally involves the decrease of the bulk density, the good adhesive character often reduces the wettability and the ability to penetrate.

The majority of the physico-chemical parameters is not easily measurable, therefore it is understandable that control methods with good reproducibility only for several parameters are presently at our disposal (e.g. suspensibility, Módszertani Gyűjtemény, 1973).

The aim of our investigations is to develop new, up-to-date, more reproducible and standardizable methods instead of the traditional ones which are not well reproducible, generally subjected to subjective errors and cannot be well quantified numerically. In this study we adopted a method easily realizable with a little standard deviation to measure the wettability of dispersible powder formulations, investigated its applicability for some formulations and determined the effect of the concentration, of the water hardness and of the humidity of the air on the wettability.

Under the products applied in the plant protection the requirements of wettability were set down only for the wettability of the solutions of wetting agents. The value of the surface tension was generally accepted to characterize the wettability, however, the correlation between the surface tension and wettability is not perfectly unequivocal (WENZEL and KAHL, 1950), moreover the determination of the surface tension in the case of suspensions is realizable only with difficulties. For these reasons we preferred the direct determination of the wettability.

Material and Methods

A wetting agent performs better when the radius of its drop of a given volume is bigger on the surface to be wetted. The correlation between the log radius and the log volume of the drop is of linear character, and the slope and the intercept are bigger in the case of the better wettability (BUZÁGH, 1954).

For our investigations we applied paraffin surfaces that model better the waxy surface of plants than the glass surface generally used. A thin layer of paraffin was layered on microscopic slides dipped in a solution in n-hexane or in molten paraffin. The drops of 1–20 μl volume were put on the slides with micropipettes, then projected on a screen, and the drop radius and the magnification were determined. Four formulations of different origin containing different types of active ingredient (further formulation 1·, 2·, 3·, 4·) were studied as follows:

a) The verification of the function

$$\log r = b \cdot \log V + a$$

(r = the radius of the drop in mm, V = the volume of the drop in μl) in the suspensions of 1% prepared from distilled and standard hard water (Specifications for Pesticides, 1961).

b) To determine the influence of the concentration of the dispersible powder on the wettability of the suspension, the dependence of the radius of 10 μl drops was measured between the concentrations 0.5–5%.

c) The extension of our investigations in *b)* to the intervals of 1–10 μl and 0.1–2%.

d) The determination of the wettability in distilled, WHO standard water and in waters 2.5, 5, 10 and 15 times harder than the standard water.

e) The change of the drop radius of the best and worst formulations was followed in time at very low and saturated humidity.

f) To determine, whether the wettability measured on paraffin surface characterizes adequately the wettability on the plant surface to be protected, the waxy layer from cabbage leaves was extracted with acetone, the solution filtered, dried at room temperature, the rest solved in n-hexane, layered on microscopic slide and the wettability determined as above.

Results and Discussion

The linear plots between the log radius of the drop and the log volume can be seen in Fig. 1, the parameters of the linear regressions are summarized in Table 1.

As it can be observed from the data in the case of dispersible powder suspensions too there is a very strong correlation between the logarithms of the two variables.

The values of the slopes and intercepts generally give the same sequence for wettability both in distilled and standard water, the order of wettability is $1 > 2 > 3 > 4$. The average coefficient of variation was found to be 1.50%, that is the method is suitable to detect relatively little differences. The wettability of all the four formulations both in distilled and standard hard water on paraffin surface was significantly better than that of the waters used. Significant differences can be observed, except between the formulations 3 and 4.

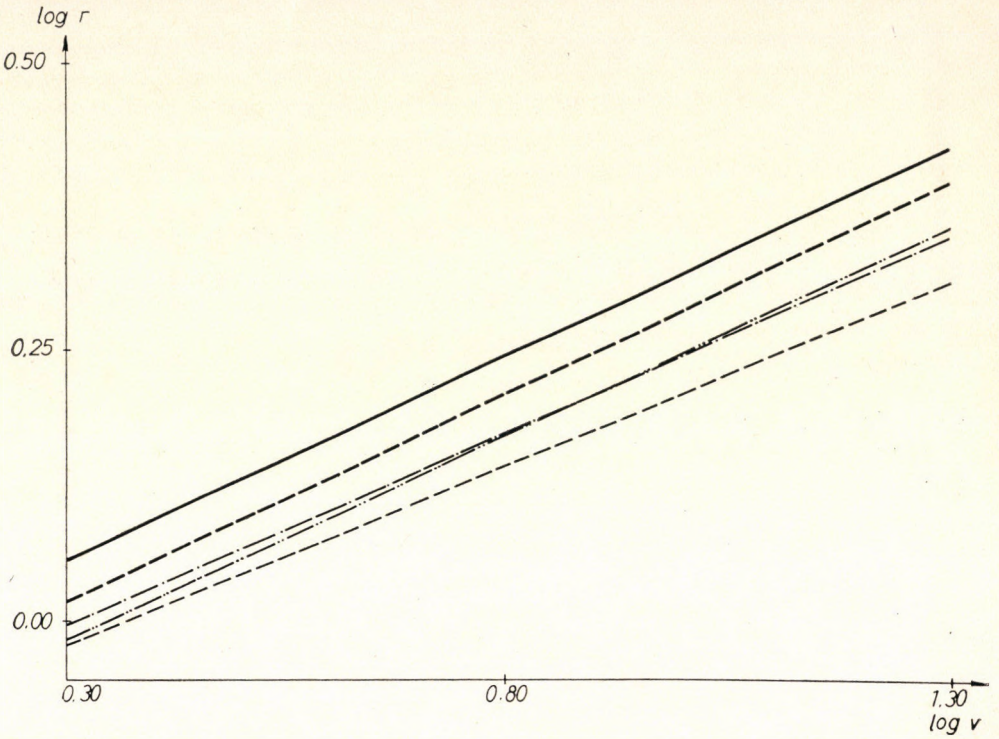

Fig. 1. The correlation between the logarithm of the drop radius and of the drop volume in distilled water. (——— Formulation 1, — — — Formulation 2, —·—·—·— Formulation 3, —··—··— Formulation 4, — — — distilled water)

Table 1

The parameters of the linear regressions between the logarithms of the drop radius and of the drop volume ($\log r = b \cdot \log V + a$)

	n	$a+1$	b	r
Distilled water	21	0.8770	0.3346	0.9973
Formulation 1 in dist. water	21	0.9385	0.3834	0.9975
Formulation 2 in dist. water	21	0.9072	0.3806	0.9993
Formulation 3 in dist. water	21	0.8776	0.3697	0.9964
Formulation 4 in dist. water	21	0.8920	0.3510	0.9981
WHO standard hard water	21	0.8869	0.3389	0.9979
Formulation 1 in stand. water	21	0.9835	0.3745	0.9989
Formulation 2 in stand. water	21	0.9254	0.3623	0.9988
Formulation 3 in stand. water	21	0.9128	0.3615	0.9984
Formulation 4 in stand. water	21	0.9035	0.3572	0.9990

The dependence of the drop radius from the concentration of the dispersible powders is shown in Fig. 2.

The data of Fig. 2 indicate that the wettability is not changing linearly with the concentration; approaching the higher concentrations the curve becomes flatter. In the theoretical interpretation of our data we supposed that the surfactants accumulate on the surface of the drop, their concentration being different on the surface and inside the drop. At low total concentrations the number of tenside molecules accumulating on the surface is growing sharply, and wettability is quickly improving. After the surface saturation with tenside molecules in mono- or bilayer

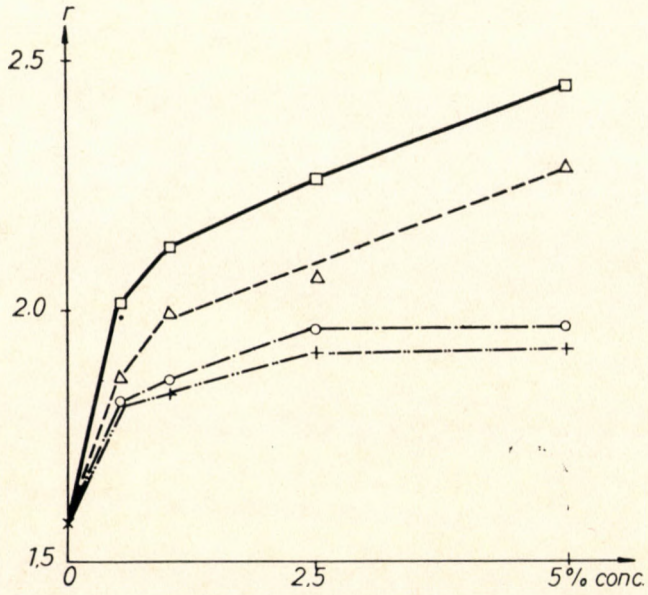

Fig. 2. The correlation between the drop radius and the concentration (symbols as in Fig. 1, the drop radius of the dist. water is plotted on the y-axis)

the further increase of the inner concentration is not accompanied by the change of the surface concentration of the surfactants, that is by the improving of the wettability. The surface-volume rate depends on the volume (in the case of little volume the relative surface increases), therefore for little drops it can be supposed that the character of the correlation between the concentration and the drop radius will be different than for big drops. Otherwise: at the same concentration the relative wettability of a little drop is worse than that of a big drop.

To prove the theory mentioned above the correlation between the radius of 1 and 10 μl drops and the concentration was investigated. From our results the data concerning the formulation 2 are presented in Fig. 3.

The correlation was in all cases of saturation character similar to the Freundlich isotherms, in the form

$$\log r = b \cdot \log c + a$$

which can be easily linearized (r = drop radius; c = concentration of the dispersible powder). The goodness of the fit was always above the significance level of 95%. In the interval investigated (1 – 10 μl) we did not succeed in proving our theory that the correlation between the drop radius and the concentration of the dispersible powder depends on the drop volume. The averaged slopes of the linearized functions are presented in Table 2.

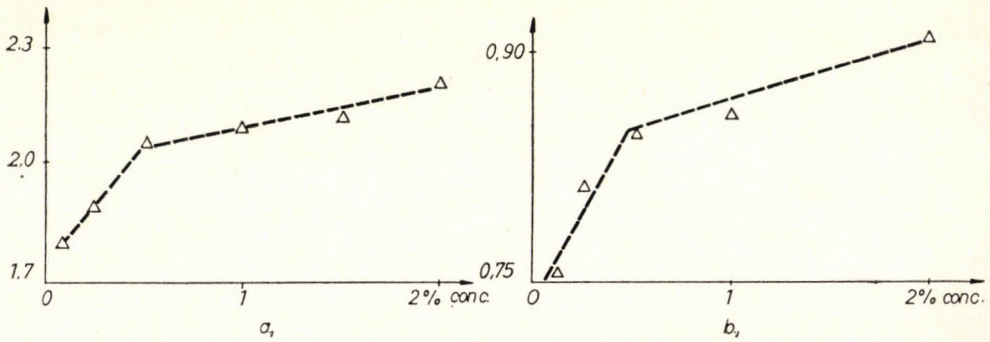

Fig. 3. The concentration dependence of the 1 and 10 μl drop radius of the formulation 2. *a*, Drop volume 10 μl; *b*, drop volume 1 μl

Table 2

The parameters of the linear regressions between the logarithms of the drop radius and of the concentration ($\log r = b \cdot \log c + a$)

Formulation	n	b	r
1	24	0.0659	0.9957
2	24	0.0636	0.9778
3	24	0.0419	0.9543
4	24	0.0401	0.9720

On the basis of our data it can be concluded that these slopes too characterize well the wettability of the formulations, as do the slopes of the function $\log r = b \cdot \log V + a$.

The effect of the water hardness on the wettability of the formulations is shown in Fig. 4.

It can be observed that the water hardness – in the case of surfactants adequately selected – does not influence considerably the wettability. Up to fivefold hardness of the WHO standard water the wettability of all the four dispersible powders is increasing which can be explained by the formation of the surfactant soaps from the anionic tensides and from the cations of the water. The further increase of

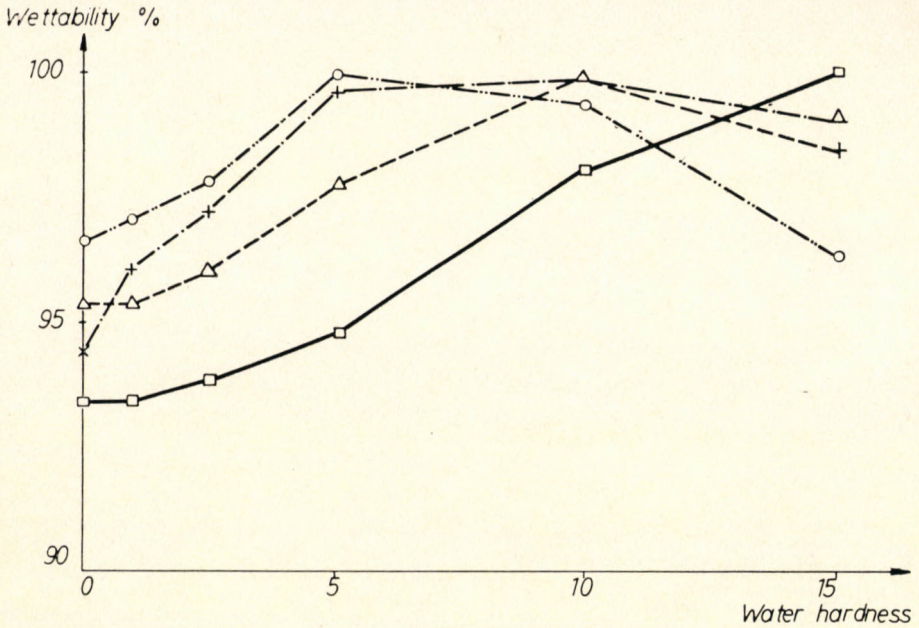

Fig. 4. The effect of the water hardness on the wettability of formulations in per cent of their maximum wettability. Concentration 1%. Symbols as in Fig. 1

the water hardness decreases the wettability in order of the relative wettability of the formulations. The effect is especially pronounced in the case of the worst formulation.

The changes in time of the drop radius of the best (1) and worst (4) dispersible powder suspensions are shown in Fig. 5. It can be seen from Fig. 5 that the drops at high vapor content reach in a short time their maximum extent: the relative extension in time even in per cent of the originally bigger drop radius at 0 time is bigger for the formulation 1. Very characteristic differences can be observed at low vapor content: in the case of the formulations investigated the rate of desiccation is considerably lower for the formulation 1 than for the formulation 4 with bad wettability. The wettability values measured on slides impregnated with cabbage wax in per cent of the values measured on paraffin surface are summarized in Table 3.

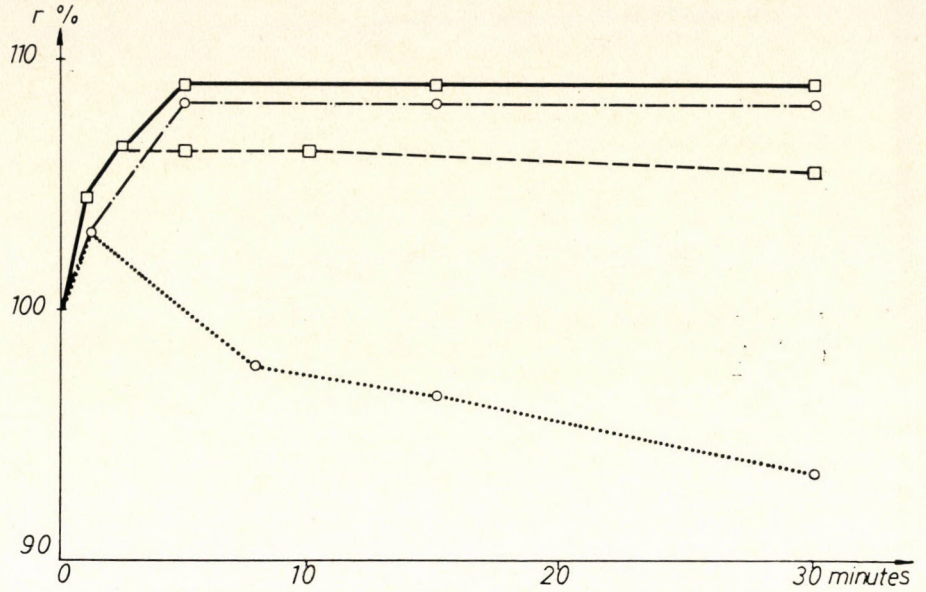

Fig. 5. The changes of 1% drop radius in time in per cent of the extent at 15 sec at very low and very high vapor content (—□— Formulation 1: high vapor content, – –□– – Formulation 1: low vapor content, – · –○– · – Formulation 2: high vapor content, · · ·○· · · Formulation 2: low vapor content)

Table 3

The wettability of dispersible powder suspensions on cabbage wax surface in percent of the wettability measured on paraffin surface (concentration 1%, drop volume 4 and 10 μl)

Formulation	Wettability, %
1	96.3
2	101.0
3	105.3
4	101.1

From the data the following conclusions can be drawn:

the wettability measured on paraffin or vegetable wax surfaces is not equal in all cases that is the use of the wax of the plant to be protected is advisable for the exact investigations,

the differences in the wettability are not great from the practical point of view, the paraffin surface approaches fairly well the waxy surface of plants,

the differences do not depend on the wettability but on the quality of the surfactants applied.

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Book Review

JÜRGEN KRANZ, HEINZ SCHMUTTERER and WERNER KOCH (eds): *Diseases, Pests and Weeds in Tropical Crops*. Verlag Paul Parey, Berlin and Hamburg, 1977. pp. 666 (Price: DM 98.—)

The book of 666 pages containing 238 figures, 6 tables and 251 illustrations in colour on 64 plates was compiled with the collaboration of some 152 internationally known scientists and published under the editorship of the three professors of the Justus Liebig University (Giessen) and University of Hohenheim (Stuttgart-Hohenheim) famous all over the world. It is the first excellent work of world literature which gives information on diseases and pests of plants found in tropical and subtropical regions (banana, barley, bean, cacao, cassava, citrus, groundnut, maize, papaya, potato, rice, sugarcane, tomato, wheat) as well as of numerous ornamentals, vegetables, certain fruit trees and plants yielding drugs and dyes, and in one volume weeds occurring on these areas are discussed.

The work consists of the following three parts:

1. Diseases in tropical crops (edited by Prof. Dr. J. KRANZ)
2. Pests in tropical crops (edited by Prof. Dr. H. SCHMUTTERER)
3. Weeds in tropical crops (edited by Prof. Dr. W. KOCH)

The part "*Diseases in tropical crops*" deals with viruses and viroids (42 pages), mycoplasma (10 pages), bacteria (22 pages) and fungi (162 pages), and describes the diseases caused by these pathogens (cryptograms, synonyms and diseases, geographical distribution, hosts and differential hosts, symptoms, epidemiology, morphology, control, physical specialization, literature) on a total of 236 pages. This part of the book — written by 64 well-known specialists — gives detailed description of 25 of the most important viruses, viroids and mycoplasmas, 10 bacteria and more than 70 fungi. Besides discussing the individual pathogens and diseases at length, it also lists the host-pathogen relations occurring in the tropics.

The part "*Pests in tropical crops*" — written with the contribution of 79 well-known specialists — discusses on 304 pages the pests of tropical areas (nematodes, mites, grasshoppers, locusts, crickets, mole crickets, thrips, termites, bugs, leafhoppers, white flies, aphids, scale insects, ants, beetles, butterflies and moths, true flies, birds, rodents) most important from an economic point of view. Several hundreds of the 17 thousand species of nematodes known at present e.g. are pests of particular economic importance. According to data of 1968 yield losses caused by nematodes amounted to US $ 310 million. Leafhoppers as plant suckers are also serious pests; especially important of them are those playing a role in transmitting viruses and mycoplasmas. Beside the economically most important pests — over 130 in number — described in detail (synonyms, common names, geographical distribution, host plants, symptoms, economic importance, morphology, life cycle, ecology, natural ene-

mies, control, literature), the names of those found on tropical areas as potential pests are also listed. The groups of pests discussed with the fullest particulars including the widest range of pests are: butterflies and moths (86 pages), leafhoppers, white flies, aphids, scale insects (69 pages), beetles (47 pages), true flies (21 pages) and nematodes (17 pages).

The last section of the book "*Weeds in tropical crops*" prepared with the participation of 10 specialists of fame supplies detailed information (common names, related species, geographical distribution, habitat, description, development, control, literature) on 73 pages on some 46 major weeds of 25 plant families, and more than 120 related species, most frequently occurring in the tropics. Considering that the life history and population dynamics of weeds occurring in tropical regions are hardly if at all known, though no efficient chemical control can be carried out without a knowledge of them, this excellently written part of the book is equally important for the theory and practice of weed control.

At the end of the book a list of books on diseases, pests and weeds in the tropics is found which by presenting bibliographic data on some 140 books enables the reader to obtain further information on the subject. The three indexes (Index of diseases by hosts, Index of pests by host plants and General Index) on the last 45 pages of the book are of considerable help to the reader's orientation.

The excellent work of "*Diseases, Pests and Weeds in Tropical Crops*" published by the Verlag Paul Parey under the editorship of JÜRGEN KRANZ, HEINZ SCHMUTTERER and WERNER KOCH with the collaboration of some 152 well-known specialists deserved with is valuable content, well constructed tables and properly chosen black-and-white and coloured illustrations the appreciation of the highest professional circles. It is indispensable for everybody taking pains to acquire a scientific knowledge with the view of a better and fuller provision for the human race. Great acknowledgement is due to the authors, editors and publisher, and we hope that the work will soon appear in German, French and Spanish languages too.

J. HORVÁTH

Contents

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PESTICIDE CHEMISTRY

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