VOLUME 21 • NUMBERS 1—2 • 1986

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*Acta Phytopathologica* publishes original papers on phytopathology mainly in English.

*Acta Phytopathologica* is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
H-1054 Budapest, Alkotmány u. 21.

Manuscripts and editorial correspondence should be sent to the Editorial Office

H-1525 Budapest, P.O. Box 102

*Subscription information*

Orders should be addressed to

KULTURA Foreign Trading Company
H-1389 Budapest P.O. Box 149

or to its representatives abroad

*Acta Phytopathologica* is indexed in Current Contents

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Volume 21

Akadémiai Kiadó, Budapest

1986

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 3–3 (1986)*

# Fifty Years' Anniversary of the Death of Prof. Károly Schilberszky

Károly Schilberszky (1863–1935) was the founder of the Hungarian horticultural plant pathology. As a professor of plant pathology at the Horticultural Academy, he served 32 years. He also delivered lectures at the Technical University of Budapest and the University of Economics. He was a superb teacher who introduced plant pathology as a university subject and founded a school of scientific research for plant diseases.

His research trend involved fungus diseases of potatoes, cereals and fruit trees. He was interested in the apoplexy disease of apricots, as well as in crown gall disease of trees. His most important discovery (1896) was the identification of the causative agent of potato wart disease, *Synchytrium endobioticum* (Schilb.) Perc. (Syn.: *Chrysophlyctis endobiotica* – Schilb.) from a potato sample found in Northern-Hungary in 1888.

He published a series of scientific and popular articles in different journals. Professor Schilberszky was elected as an ordinary member of the St. Stephan Academy in 1923. Dr. Schilberszky passed away unexpectedly in September 10, 1935.

Our journal published a memorial article on his scientific activity twenty years ago: G. Ubrizsy: Károly Schilberszky (1863–1935), the founder of horticultural phytopathology in Hungary. Acta Phytopathologica Hung. 1, 5–10, 1966.

*The Editoral Board*



*Acta Phytopathologica et Entomologica Hungarica 21, 1986*
*Akadémiai Kiadó, Budapest*

*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 5—14 (1986)*

# Phytoalexins and Questions that Remain Unresolved Forty-five Years after their Discovery

T. Érsek and Z. Király

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

Phytoalexins have received great attention and enthusiasm of researchers since their discovery. The antimicrobial activity and the association of these compounds with the expression of resistance implies that they have the characteristics of molecules to determine disease resistance of plants. Although phytoalexin production seems to be a general process among higher plants, there are still species where phytoalexins have not been detected. This and the finding that phytoalexin accumulation is a consequence of earlier events that may also limit the growth of the pathogen, suggest that phytoalexins are not the sole and primary factors of resistance. The resistance process seems to be a sequence of events. Phytoalexins could be one of the participants in these events. But the questions such as whether phytoalexin accumulation is a direct manifestation of the primary recognition response and, whether phytoalexins can express their *in vitro* antimicrobial activity to inhibit the pathogen *in planta*, still cannot be unequivocally answered—forty-five years after the phytoalexin theory came to light. Further research is required for the resolution of causal associations, since a potential for function is not obviously a function in effect. The present knowledge of phytoalexins and the advanced techniques in molecular biology offer a great opportunity to study the molecular basis of disease resistance.

Plants continuously confront a number of plant pathogenic microorganisms. In spite of this onslaught, a given plant is susceptible to only a relatively few microorganisms and resistant to the vast majority of them. In plant with inherited resistance the microorganism is inhibited shortly after infection. Concomitantly, the plant tissue around the infection site responds, in many cases, by undergoing the hypersensitive reaction, which results in localized death (necrosis) of plant cells (hypersensitive type resistance). In addition, such infected resistant plants may become resistant also to their regular fungal or bacterial pathogens (acquired resistance). Both types of resistance have been suggested to be due to "warding-off" compounds, the phytoalexins (Müller, 1956, Müller and Börger, 1941).

The first experiments were carried out by Müller and Börger (1941), forty-five years ago. They inoculated cut surfaces of potato tubers with an avirulent race of the potato late blight fungus, *Phytophthora infestans* and then, after some time, with a virulent race. As a result of the inoculation with the avirulent race, the tuber tissue reacted "hypersensitively" and became resistant to the virulent race. It was

shown that an antifungal substance, a "phytoalexin" is produced in and diffuses from the tissue undergoing the hypersensitive reaction. The phytoalexin has been thought to be capable of preventing the development of both the virulent race of *P. infestans* and several other potato-pathogenic fungal species.

Müller (1956) hypothesized that phytoalexins may also be active in inhibiting the growth and development of a primary infecting microorganism in plants resistant to the microorganism. Ongoing research has revealed that phytoalexins accumulate, in most cases, more rapidly and/or in larger quantities in incompatible than in compatible plant–pathogen interactions, and they possess antifungal and antibacterial activity. Accordingly, phytoalexins have the characteristics of molecules that make plant cultivars resistant to fungal and bacterial races, as well as plant species resistant to otherwise pathogenic species. In other words, phytoalexins would be responsible for the race-specific and non-host resistance, in addition to the above mentioned acquired resistance.

Ever since their discovery, phytoalexins have attracted special interest. Research has proliferated, especially during the past two decades. (For most recent reviews see: Bailey and Mansfield, 1982; Darvil and Albersheim, 1984; Keen, 1982; Kuć and Rush, 1985).

Major questions about the causal association between phytoalexin accumulation and disease resistance remain. The aim of the present paper is to reflect the main controversies concerning the causal association in a survey of different hypotheses and experimental data, as well as to introduce several area where further studies may evoke great progress in phytoalexin research.

## *Is phytoalexin production a general or a specific process?*

More than 100 compounds classified as phytoalexins have been isolated from a large number of plant species. Most of these plants are dicotyledons. Structural similarities are obvious among phytoalexins from plants within one family, but minor differences in structure and/or level of accumulation of various phytoalexins may occur. In terms of the association of phytoalexins with resistance, the most important plants belong to the *Solanaceae* (e.g. potato) and the *Leguminosae* (e.g. soybean, French bean, peas), where the predominant phytoalexins are sesquiterpenoids and isoflavonoids, respectively. Phytoalexin biosynthesis involves three basic metabolic routes, the acetate-mevalonate, acetate-malonate, and shikimate pathways. Through all the structural diversity of phytoalexins, there runs a common thread: these compounds usually are not detected in plant tissues prior to infection or other stress. That is to say, stress stimulates their *de novo* synthesis. (Phytoalexins are termed stress compounds, too.) Dead necrotic cells may serve as a sink to accumulate phytoalexins or their intermediates, which are produced by the adjacent living cells. Plants not only synthesize but also catabolize phytoalexins. The level of phytoalexins that accumulate is determined by the rate of catabolism as well as the rate of synthesis.

Table 1

The possible ways of the occurrence of resistance and susceptibility to microorganisms in plants when the role of phytoalexins is assumed

| Plant-microbe interaction | | Resistance | Susceptibility |
|---|---|---|---|
| species—species, or species—forma specialis | Cultivar-race | *Rapid phytoalexin accumulation:* | *Late phytoalexin accumulation:* |
| | | Specific elicitation | Non-specific elicitation |
| | | Non-specific elicitation and no suppression | Non-specific elicitation *versus* specific suppression |
| | | *Rapid phytoalexin accumulation:* | |
| | | Phytoalexin sensitive microbe | Phytoalexin tolerant microbe |

The molecules that signal the plant to begin the process of phytoalexin synthesis are called elicitors. All cultivars within one species carry the genetic potential for phytoalexin production, because non-specific effects (physical: injury, heat, UV-light; abiotic elicitors: various compounds; biotic elicitors: microbial products) also cause phytoalexins to accumulate. The key difference between resistance and susceptibility, therefore, must be the speed and magnitude with which this potential is expressed. This could be regulated in several ways (Table 1). In some instances low molecular weight water-soluble compounds (suppressors) are released by the virulent pathogen, which specifically counteracts the effect of non-specific elicitors. Thus susceptibility would be the specific response to infection by means of the specific suppression of a non-specific, general resistance response. A balance between elicitation and suppression would specify the amounts of phytoalexins that accumulate.

In contrast, certain cell wall substances (glucans, glycoproteins) from pathogenic fungi and bacteria have been claimed to specifically elicit phytoalexins in plants resistant to the pathogen that carries the elicitor. Accordingly, these specific biotic elicitors turn on the signal for phytoalexin synthesis, which the non-specific elicitors later enhance. A compatible interaction then would be the result of the lack of specificity.

Regardless the way in which specificity is expressed, the microorganism is capable of liberating fragments from the wall of dead or injured plant cells. These oligosaccharide fragments, termed "endogenous" elicitors, are thought to stimulate phytoalexin production initiated by the attacking microorganism.

In conclusion, phytoalexin accumulation seems to be quite a general response of higher plants to infection or other stresses. The rapid response in incompatible *versus* compatible interactions, however, is rather specific.

*The suppression of phytoalexin production leads to susceptibility*

A large body of evidence supports the theory that phytoalexins function in disease resistance. Studies indicate that the lack of phytoalexin accumulation or the suppression of their synthesis converts the plant's reaction to an avirulent pathogen from resitance to susceptibility. The classical work of Chamberlain and Paxton (1968) shows that the hypocotyls of a soybean cultivar resistant to a race of the soybean pathogenic fungus, *Phytophthora megasperma* f. sp. *glycinea* become susceptible if the phytoalexins (glyceollin and others) are eluted by a string wick. When the phytoalexins are transported in such a way from a genetically resistant cultivar to a genetically susceptible one, the latter becomes resistant.

By the use of metabolic inhibitors that influence the synthesis of phytoalexins, phytoalexin accumulation can be inhibited and, at the same time, resistance suppressed. The inhibition by L-2-amino-3-phenylpropionic acid of phenylalanine-ammonia lyase (PAL), a key enzyme in the isoflavonoid pathway, results in 90% drop in glyceollin content of soybean hypocotyls and in an increase in the growth of soybean-*Phytophthora* in a genetically incompatible interaction (Moesta and Grisebach, 1982). Blockage of isoflavonoid production at the shikimate–chorismate conversion by the herbicide glyphosate also eliminates glyceollin production and concomitantly decreases the expression of resistance of soybean leaves to the bacterium, *Pseudomonas syringae* pv. *glycinea* (Holliday and Keen, 1982). These inhibitors are quite specific, but they may influence protein biosynthesis by enhancing or lowering metabolite pools. Thus side effects unrelated to phytoalexin synthesis cannot be ruled out. This problem could be overcome by the use of specific inhibitors of enzymes that take part in the terminal synthesis of phytoalexins. Such an enzyme (a prenyl-transferase) has recently been identified from cell-free extract of elicitor-treated soybean cotyledons (Zähringer et al., 1979). There is no published information on the specific inhibition of phytoalexins synthesized by other pathways.

*Susceptibility resulting from tolerance of fungi to phytoalexins*

In several interactions of plant species and fungi non-pathogenic to them (non-host resistance) phytoalexin accumulation was not higher and/or faster than in compatible interaction, in which the plant had been inoculated with a pathogenic species. In other words, the pathogenic fungus induced the accumulation of as much phytoalexin in its host as the fungus non-pathogenic to this plant at the same time. To explain this situation investigators stressed the fact that virulent microorganisms are tolerant of phytoalexins that accumulate in the host tissue. A comparative study on phytoalexin sensitivity of numerous pathogenic fungi revealed

that only the pea-pathogenic species were tolerant to pisatin (the major phytoalexin in peas), the ones non-pathogenic to peas were sensitive (cf. Cruickshank, 1963). Van Etten and his co-workers found that tolerance to pisatin by certain fungi is due to the ability of these fungi to demethylate, and thereby detoxify, the phytoalexin. Genetic studies were carried out on the root- and stem-rot pathogen of peas, *Nectria haematococca* (imp. *Fusarium solani* f. sp. *pisi*) : a field isolate that was highly virulent and tolerant to pisatin (could demethylate pisatin) was crossed with a field isolate that lacked virulance and could not demethylate pisatin. In these crosses, all the progenies that were highly virulent on peas proved to be insensitive to pisatin and able to demethylate it (Tegtmeyer and Van Etten, 1982).

An outstanding exception to this general pattern of tolerance were given by Pueppke and Van Etten (1976) who found the pea-pathogenic *Aphanomyces euteiches* to be much more sensitive to pisatin than any other pea pathogens. Despite this extreme sensitivity *in vitro* and a very high accumulation of pisatin in the infected tissue, the fungus, and accordingly the lesions caused by the fungus continued to spread as long as for five days.

Tolerance is not always attributable to the ability of microorganisms to detoxify phytoalexins. Furthermore, tolerance has not been reported to occur in plant cultivar–pathogenic race interactions.

*Inhibition of fungal growth in susceptible plants results in resistance response*

As early as 1972, Király et al. reported that the inhibition of the hyphal growth of *Phytophthora infestans* in tubers of a susceptible potato cultivar by antibiotics results in a hypersensitive-like reaction, i.e. tissue necrosis and the accumulation of the sesquiterpenoid phytoalexin, rishitin. This pattern was similar to that in an incompatible interaction. Notwithstanding, phytoalexin accumulation was found to be as a result of attacked fungal growth. On this basis it was hypothesized that fungi are able to release substances (elicitors) in a genetically incompatible interaction only following an early growth inhibition. On the other hand, phytoalexin induction is absent or proceeds slowly in a compatible interaction under normal conditions, because the fungus can freely grow for longer time, without being inhibited or damaged in its host, and thus it is unable to release elicitors (Érsek et al., 1973; Király et al., 1972).

A possible candidate for the substance that causes the release of elicitors from fungi has been identified. For the first time it has been demonstrated that a short incubation (2 min) of isolated cell walls or live hyphae of *P. megasperma* f. sp. *glycinea* with either wounded soybean cotyledons or the endoglucanase enzyme preparation from such cotyledons results in the rapid release of water-soluble carbohydrates (Yoshikawa et al., 1981; Keen and Yoshikawa 1983). These polysaccharides are glucomannans with race-specific characteristics (Keen et al. 1983).

In bacterium–plant interactions the early inhibition of bacteria by antibiotics in a susceptible host did not result in hypersensitive resistance (Keen et al.,

1981). It is concievable, since only live bacteria capable of multiplying can elicit the hypersensitive reaction, and thus the accumulation of phytoalexins.

At present, there can be no doubt that the expression of resistance (i.e. phytoalexin accumulation) is preceded by early events that are the primary determinants of incompatibility between plant and pathogen (e.g. Keen, 1982). This phenomenon, called recognition or the 'determinative' phase of resistance, is possibly due to the interplay of surface molecules on plant and microbial cells, but it is poorly understood. Tissue necrosis occurs and phytoalexins are then produced in the subsequent 'expressive' phase.

*Phytoalexin level does not obviously correlate to the rate of race-specific resistance*

The current phytoalexin theory seems to really cope with the race-specific resistance. That is to say, the higher and/or faster phytoalexin accumulation in incompatible vs. compatible cultivar–race interactions may contribute to the resistance process. The correlation, however, is not always obvious. Both phytoalexin production and the rate of resistance are influenced, sometimes conversely, by the physiological state of plant tissue.

It has been demonstrated that soybean cultivars susceptible to *P. megasperma* f. sp. *glycinea* remained susceptible after heat-treatment of the host tissue, though the amount of glyceollin increased above an *in vitro* inhibitory level. Another experiment indicated that increased resistance may not parallel increase in phytoalexins (Lazarovits et al., 1981). The resistance of soybean to the fungus increases with the age of tissues. In contrast, the glyceollin level decreases with age.

The production of sesquiterpenoid phytoalexins in potato tubers also depends on age. Interestingly enough, this situation is just the opposite like in soybean. The freshly harvested resistant tubers accumulate very low amount of rishitin after inoculation with *P. infestans*, as compared with aged resistant tubers. Phytoalexin accumulation does not differ and is very low in unstored young tubers inoculated with either virulent or avirulent races of the fungus (Bostock et al., 1983). On the other hand, phytoalexin accumulation is rapidly expressed in older stored or aged tubers only in the incompatible interactions (Nozue et al., 1978, Sato et al., 1971). Thus, it would seem for infections by *Phytophthora* spp. that resistance and phytoalexin accumulation may correlate, the two processes, however, are independent of one another.

*Phytoalexins in acquired resistance?*

The original phytoalexin theory is based upon the phenomenon of acquired resistance, concerning its local type (local cross-protection). In many cases, there is a close association between phytoalexin accumulation induced by an avirulent bacterial or fungal strain and the local protection against a virulent pathogen, as challenge, applied to the same site as the initial inoculum. In terms of phytoalexins, reports on aquired resistance, however, are considerably less than on the

inherited resistance (e.g. Kuć and Caruso, 1977). This may be attributable to the failure in finding significance of phytoalexins in the systemic cross-protection, which seems to be economically more important than the local one.

In contrast, Vanderplank (1975) argues that phytoalexins may be of economical importance as "preformed" resistance factors against secondary infections under natural conditions that accumulate as a consequence of the primary infections, to which plants in the field are continuously exposed. Several recent papers (Ayers et al., 1976, Ebrahim-Nesbat et al., 1982, Hoppe et al., 1980) dealing with elicitors refer to the protective role of phytoalexins against fungi that attack the host only after the elicitation of sufficient amounts of these compounds. The above results lend to support Vanderplank's hypothesis which, however, needs more direct proof.

*Sequence of events in resistance process needs to be determined*

Failures in precisely determining the sequence of events in the resistance response or in finding correlation between phytoalexin concentration and resistance may be due to imperfect techniques. Most researchers extract phytoalexins from relatively large pieces of tissue that contain both infected and uninfected cells. Phytoalexin concentrations then are expressed on a unit area or a unit weight basis. Such an unprecise approach cannot give information about micro-sites where phytoalexins might contact the pathogen.

The rishitin concentration in a relatively thick (1 to 2 mm) layer of potato tuber slices inoculated with an avirulent race of *P. infestans*, immediately after slicing, was far below the level that completely inhibits the fungal growth in vitro (Sato et al., 1971). In contrast, the concentrations of phaseollin to which hyphae of an avirulent *Colletotrichum lindemuthianum* race are exposed within French bean hypocotyl cells undergoing the hypersensitive reaction are much higher than those recorded in excised pieces of tissue, which mostly contain unresponding cells. It was calculated that local concentrations approached 300 times the minimum concentration completely restricting *in vitro* growth of the fungus (Bailey and Deverall, 1971).

Glyceollin extracted from 1 cm pieces of soybean hypocotyl tissue containing the inoculation site at the center was found to exceed the $ED_{90}$ level 6 to 7 hours after the growth of the avirulent race of *P. megasperma* f.sp. *glycinea* had stopped. Dissection experiments revealed, however, that the uppermost longitudinal 500-$\mu$m layer contained glyceollin at an $ED_{90}$ level as early as 8 hours after inoculation, a time that coincides with the onset of cessation of hyphal growth. Such a high phytoalexin concentration in the compatible interaction occurs only after 24 hours, at which time the fungus has already moved into other tissue layers. Hahn et al. (1985) used radioimmunoassay for quantitation of the major soybean phytoalexin, glyceollin I, and an immunofluorescent stain for the localization of fungal hyphae in soybean roots inoculated with *P. megasperma* f.sp. *glycinea*. Most of the phytoalexin accumulated in the epidermis in both incompatible and compatible inter-

actions. The avirulent race colonized the entire cortex, despite the inhibitory in vitro concentration of glyceollin. However, it did not grow into the stele, which contained a very low glyceollin level. The virulent race also grew through the epidermal cells where the phytoalexin concentration was high and then colonized the stele. It remains to be explained why the virulent race is able and the avirulent race is unable to grow into the stele, which accumulates very small amounts of glyceollin I. Studies, similar to the one above, should be pursued on phytoalexins other than this major one, because their distribution might be different.

The effort to localize phytoalexin accumulation revealed that phytoalexins are produced even when resistance is not expressed by macroscopic hypersensitive response ("immune" type of resistance). Though no extractable quantity of isoflavonoid phytoalexins was found in soybean leaves 'immune' to a race of *Peronospora manshurica*, autofluorescence revealed a small accumulation site that probably contained glyceollin and related compounds. This site was localized just around the penetrating hyphae. Conversely, very faint and delayed autofluorescence was detected in the compatible interaction (Érsek et al., 1982).

In addition, it would also be of importance to know how the activities of phytoalexins in plant tissues mimic the activities in bioassays. Our data (Beczner and Érsek, unpublished) indicate that the non-purified organic extract from potato tubers has weaker antifungal activity on the hyphal growth of *P. infestans* than any of the separated sesquiterpenoids in their proportional concentration in the same extract. This suggests that plant tissue even may contain substances that counteract phytoalexins. In addition, most phytoalexins are lipophilic, therefore it should be proven that they accumulate in an appropriate form to be effective *in vivo*.

Because of the difficulties involved in the direct approach to proving the role of phytoalexins in resistance, future progress is likely to come from further improvement of experimental methods.

*Future progress at molecular level*

In those incompatible plant-parasite interactions in which the phytoalexin accumulation is associated with the expression of resistance, phytoalexins may serve as excellent tool in studies on the molecular basis of resistance. Most recent findings indicate that the activities of key enzymes in isoflavonoid biosynthesis increase in incompatible interaction of bean hypocotyls and races of *C. lindemuthianum* or upon elicitor treatment, just prior to the accumulation of isoflavonoid phytoalexins (Bell et al., 1984, Ryder et al., 1984, Chappell and Hahlbrock, 1984). Coincident with the increase in activity of chalcone synthase, 4-coumarate : CoA ligase and phenylalanine ammonia-lyase there is an early but localized increase in active mRNAs for these enzymes. In the compatible interaction, there is no induction of mRNA activity in the early stages of infection. This suggests that the control of resistance gene expression lies at the level of transcription or earlier. We are ignorant about what happens in plant cells during the period between the application of the microorganism or elicitor and the appearance of new mRNAs, and about

how the microorganism turns on the expression of certain plant genes and turns off that of others. The possible identification of substances of microbial origin that regulate the signals for this expression holds great promise.

Staskavicz et al. (1984) successfully mobilized gene(s) encoding avirulence of *P. syringae* pv. *glycinea* into other races, and changed race specificity from virulent to avirulent on the appropriate soybean cultivars. Whereas the wild type of the recipient race caused disease symptoms with a low level of glyceollin, the transposed one elicited hypersensitive reaction and high phytoalexin accumulation in leaves. With site-directed (Tn 5) mutagenesis two DNA fragments were found to be unique and important in determining race specificity. It is not known whether the product of the bacterium race-specificity genes interact directly with the product of the plant resistance gene or it is indirectly involved in plant recognition of the avirulent pathogen.

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*Note added in proof:* A less detailed review on the same subject has been published in Physiologia Plantarum (Érsek, T. and Király, Z. 1986: Phytoalexins: Warding-off compounds in plants? Physiol. Plantarum 68, 343—346).

*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 15—22 (1986)*

# Necessities, Problems and Results of the Development and Application of Antiphytoviral Substances against Tobacco Viruses*

G. SCHUSTER

Department of Life Sciences, Division of Plant Physiology and Microbiology, Karl-Marx-University, Leipzig, GDR

Virus diseases cause considerable losses in tobacco crop. In this situation antiphytoviral substances for chemical control would be of great importance. A survey is given about promising antiphytoviral compounds and measures which have been developed during the last years. Results of the application of antiphytoviral agents, such as Ribavirin, 2,4-dioxohexahydro-1,3,5-triazine (= DHT), compounds having the azine structure and a membrane lipid analogous compound against tobacco viruses are reported. An inerease in effectiveness was obtained by granulating DHT. This granulated preparation provides protection from some viruses all over the year after one application during planting. The most important progress in efficiency was obtained by combining two or three antiphytoviral substances in one antiphytoviral preparation, one of them being DHT. Some of the preparations were tested in field trials. The previous results obtained and reported with potato justify forthcoming field trials with tobacco.

In nearly all tobacco-cultivating countries virus diseases still cause considerable economic losses in tobacco crop. For instance, in some European regions the yields of a number of tobacco varieties, above all modern American tobacco varieties, are depressed seriously by a lot of dangerous virus diseases. Repeatedly, the losses in yield have been so high that the lucrativeness of the cultivation of these modern flavour satisfying varieties is endangered. Breeding of resistant varieties with the same flavour and other desirable properties need a lot of years, and the breeders and cultivators must hope that the taste of the cigarette smokers will not change during this time. Doubtlessly, in this situation effective antiviral compounds interfering with steps of the replication cycle of the viruses but not or only in part with the nucleic acid and protein replication of the host or other host activities would bring about a major technical advance. As remarkable progress has been

* Revised version of a report to the 8th International Scientific Tobacco Congress of CORESTA, Vienna, 7–12 October 1984.

Abbreviation of viruses: BMV = brome grass mosaic virus; CMV = cucumber musaic virus; PLRV = potato leaf roll virus; PVX = potato virus X; PVY = potato virus Y; TMV = tobacco mosaic virus; ToMV = tomato strain of TMV

achieved in recent years in developing antiphytoviral substances, it seems advantageous to take use of it also in tobacco cultivation.

A survey about the main trends and results of the search for agents capable of inhibiting plant virus multiplication is given, among others, by Schuster (1983a). There is explained that high attention has been paid world-wide to analogues of purine and pyrimidine bases or the corresponding nucleosides and nucleotides. But the most promising progress in inhibiting plant virus replication has been obtained by using analogues of anabolites and catabolites of the mentioned substances. One of the most important analogues of an anabolite of purine nucleosides is 1-$\beta$-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, developed by Sidwell et al. (e.g. 1972). This substance is known under the common name Ribavirin and the trade names Virazole (USA) and Ribamidin (SU). A promising analogue of a catabolite of pyrimidine bases is 5-azadihydro-uracil (= 2,4-dioxohexahydro-1,3,5-triazine, abbreviated as DHT, Schuster et al., 1979a). It is evident that this substance is processed by the plant in relatively slow bioconversion steps to an antiphytoviral active base analogue (Byhan and Schuster, 1980; Schuster and Arenhövel, 1984). This may be the reason for relatively high doses of DHT being well tolerated by plants. The bioconversion of 5-azadihydrouracil to an active base analogue is apparently impossible by animal and human beings. Thus, this substance is not toxic for vertebrates, and the antiphytoviral chemotherapy using DHT can be accomplished under excellent toxical and phytotoxical premises (Schuster, 1982a). Ribarivin as well as DHT inhibit PVX in tobacco excellently. But the inhibition of TMV by both substances is much less effective (Schuster, 1976; Byhan et al., 1978). We should learn from these examples that the different species of viruses are differently to be influenced by antiphytoviral substances, quite similar to the different species of fungi, which are also differently influenced by fungicides (antifungal substances).

As an effective antiphytoviral chemotherapy, for instance against tobacco viruses, is only possible, if the most important viruses of the corresponding host plant are inhibited in a satisfying manner, the chemotherapeutical measures had to be further improved before starting field trials and other efforts to ascertain the practical use of an antiphytoviral chemotherapy. With respect to this task, we started experiments in three different ways. The results of them brought about some encouraging progress, as well with regard to potato as to tobacco viruses.

The first remarkable progress was obtained by developing granulated DHT, which releases therapeutically sufficient to optimum quantities of DHT over prolonged periods of time, namely for more than three months (Schuster and Kramer, 1982). Thus, the four to five treatments with DHT, which are, because of the relatively rapid breakdown of this substance by the host plant, necessary to provide antiphytoviral protection during the whole vegetation period, can be reduced to one during planting. Moreover, PVX is reduced better by granulated DHT even in inoculated leaves of younger plants relatively short after planting. The same is true for TMV but the inhibition of this virus is smaller.

Table 1

Increase of the inhibition of different viruses by combined treatment with Ribavirin and DHT

| Virus | Host | Tested leaf time of testing (d.p.i.) test procedure | Concentration | | Reduction of virus concentration, % (control = 100%) | | |
|---|---|---|---|---|---|---|---|
| | | | Ribavirin, % | DHT, % | Ribavirin | DHT | Ribavirin +DHT |
| PVX | *Nicotiana tabacum* 'Samsun' | i, 5 (ser.) | 0.005 | 0.005 | 68* | 66** | 45*** |
| | | s, 13 (ser.) | 0.0025 | 0.0025 | 32*** | 35*** | 0*** |
| CMV | *Nicotiana glutinosa* | s, 16 (biol.) | 0.01 | 0.1 | 40*** | 38*** | 2*** |
| TMV | *Nicotiana tabacum* 'Samsun' | i, 3 (ser.) | 0.05 | 0.1 | 38** | 48** | 12*** |
| BMV | *Hordeum vulgare* | s, 3 (ser.) | 0.05 | 0.25 | 89* | 72* | 37** |

Ser., biol. = estimation of the virus concentration serologically or biologically, respectively. i = inoculated leaves; s = secondarily infected leaves, d.p.i. = days after infection

A further essential improvement of the antiphytoviral chemotherapy was obtained by combined treatment with two or more antiphytoviral substances. The remarkable antiphytoviral activities of Ribavirin and DHT were augmented synergistically by combined application of these substances. For instance, the concentration of PVX could be reduced by very small quantities, namely 15 g per hectare of each active agent, to a level, at which it was no longer detectable serologically in secondarily infected leaves (Table 1). Moreover, the concentration of some very often tobacco damaging viruses which are more difficult to be influenced by antiphytoviral substances than PVX, such as CMV and TMV, was reduced much better by combinations of Ribavirin with DHT than by treatments with the single agent (Schuster, 1982b).

Finally, new substances from different chemical groups were developed or discovered, respectively, which show a satisfactory activity, also against TMV. Interesting results have been obtained with cyclic and non-cyclic compounds owning the azine structure, such as thiadiazoles (Nos 1, 2 in Table 2, Schuster et al., 1984a), oxazoles (Nos 3, 4; Schuster and Ulbricht, 1985), and isothiosemicarbazones (No. 5; Schuster et al., 1984b). It is remarkable that more than one antiphytoviral principles are united in most of the mentioned compounds. Besides the azine structure compound 1 possesses the adamantyl group, compound 2 the nitrophenyl group, compound 3 the guanyl-guanidino and acetophenon groups and compound 4 the acetophenon group.

The most important reduction of TMV was obtained following a new concept of antiphytoviral chemotherapy. We tried to disturb membrane-bound steps of virus replication using substances with structures resembling the membrane lipids. These substances, which should be comprehended as membrane lipid analogues, have at least one long aliphatic chain which should enable the corresponding substances to be included into the lipid layer of membranes, above all in districts where the membrane layer is not covered by proteins. Beside this chain, a hydrophilic anionic, cationic or non-ionic structure is essential which is thought to influence membrane bound enzymes, membrane receptors for virus structures and so on. One of the most promising anionic substances of this group is L 333. Table 3 shows that the number of local lesions of TMV on *Nicotiana glutinosa* is reduced, by application of L 333, by 96% to 4%. But also the virus concentrations in inoculated leaves of systemically infectible host plants were reduced considerably, namely that of TMV to 25% in *N. tabacum* 'Samsun' and that of ToMV in tomato to 3%. Never before it was possible for us to reduce the TMV concentration in inoculated leaves of systemically infectible intact plants to such an extent. Also the reduction of TMV and ToMV in secondarily infected leaves is remarkable. DHT, which is less effective against TMV, augments the inhibition of TMV by L 333 scarcely, when applied in combination with L 333. On the other hand, there is a good synergistical augmentation of the activities of L 333 and DHT against PVX. Summarizing, combinations of DHT with L 333 augment the effct of DHT against some viruses, e.g. (not demonstrated in Table 3) against PVY and PVA, and besides, L 333 inhibits quite well TMV and similar viruses. A further augmentation of the antiphytoviral activities of the combination of DHT with L 333 is possible by adding a third antiphytoviral substance, as is to be seen, for instance, from Tables 5 and 6.

Although most basic work in developing or detecting antiphytoviral substances has been done with tobacco as an experimental plant, most field trials and other efforts to ascertain the practical use of an antiphytoviral chemotherapy have been done hitherto with regard to a prospective application in potato breeding and cultivation. Those experiments demonstrated, among other ones, that treatments with DHT enabled the yield of tubers of 57 virus-infected potato stocks to be significantly increased by 6.5%. On the other hand, 49 healthy stocks showed no changes in yield, whatsoever (Table 4). Thus, the yield increase of virus-infected potato stocks may be regarded as being due to antiviral effects of DHT. The results of 19 large-scale field trials with ware potatoes, the treated area being 20 ha in each trial, showed an average yield increase by 12.6% (Schuster et al., 1979b). However, it is important to note that DHT did not completely suppress virus infections in these experiments. Likewise, DHT, applied alone, reduced the natural infection of potatoes with PLRV only from 35.1% to 26%. But in combination with L 333 the reduction was much greater. Finally the combination of DHT with L 333 and cyanoguanidine (Schuster, 1983b) reduced the infections with PLRV from 35.1% to 11.7% (Table 5, Schuster, 1984). In other experiments a completely healthy potato stock was cultivated for two years in a region with high natural infection

Table 2

Reduction of the numbers of local lesions of TMV on leaves of *Nicotiana glutinosa* by substituted cyclic and non cyclic compounds owning the azine structure (>C = N–N = C<) (SCHUSTER et al. 1984a, b, c)

| Compound | Concentration and solvent | Local lesions (% of the control) |
|---|---|---|
| 1. 2-Anilino-5-adamantyl-1,3,4-thiadiazole | 0.1 %<br>Acetone 5 % | 66* |
| 2. 2-Ethylamino-5-p-nitrophenylthiadiazole | 0.1 %<br>Acetone 5 % | 29** |
| 3. m-Guanylguanidino-acetophenon-(4,5-diphenyl-oxazole-2-yl-hydrazone)-hydrochloride | $5\times 70^{-3}$ mol<br>Acetone 5 % | 34*** |
| 4. 4,5-Diphenyl-oxazole-2-yl-hydrazinium salt of 2,4--dihydroacetophenone-(4,5-diphenyloxazole-2-yl-hydrazone) | $5\times 10^{-3}$ mol<br>Acetone 5 % | 12*** |
| 5. Pyridin-3-aldehyd-S-ethylisothiosemicarbazone | $5\times 10^{-3}$ mol<br>Acetone 5 % | 10** |

Table 3

Effect of the membrane lipid analogue L 333 on TMV, ToMV and PVX, when applied alone and in combination with DHT

| Virus, host, test | Conc. of L 333, % | Conc. of DHT, % | Leaf | Reduction of local lesions or virus concentrations (control = 100 %) | | |
|---|---|---|---|---|---|---|
| | | | | L 333 | DHT | L 333 + DHT |
| TMV | | | | | | |
| *Nicotiana glutinosa* hyp. (local lesions) | 0.5 | 0.125 | i | 4*** | 82* | 3*** |
| TMV | | | | | | |
| *Nicotiana tabacum* 'Samsun', syst. serological test | 0.5 | 0.125 | i | 25** | 95* | 18*** |
| | | | s | 62* | 92* | 62** |
| ToMV | | | | | | |
| *Lycopersicon esculentum*, syst. immunoelectrophoretical test | 0.5 | 0.125 | i | 3*** | — | — |
| | | | s | 32** | — | — |
| PVX | | | | | | |
| *Nicotiana tabacum* 'Samsun', syst. serological test | 0.6 | 0.005 | i | 47** | 41** | 11*** |
| | | | s | 27*** | 19*** | 7*** |

hyp. = hypersensible host, syst. = systemically infectible host; for further declarations see Table 1

Table 4

Effect of DHT treatments on yields of healthy and virus-infected potato stocks (derived by SCHUSTER et al. 1979b)

| Type of experiment | Number of field trials | Yield (t/ha) | | Yield increase | |
|---|---|---|---|---|---|
| | | untreated | DHT | t/ha | % |
| 1. Several years' plot experiments | | | | | |
| (a) seed from virus-infected sources | 57 | 17.0 | 18.1 | 1.1 | 6.5 |
| (b) seed from healthy sources | 49 | 27.0 | 27.0 | 0 | 0 |
| 2. Large scale field experiments (20 ha each) 1977 | 19 | 18.3 | 20.6 | 2.3 | 12.6 |

Table 5

Reduction of the percentage of natural infection of potato cv. Adretta with PLRV by treatment with antiphytoviral substances

| Treatment | PLRV% Elisa- test | Reduction % |
|---|---|---|
| 1. Untreated control | 35.1 | 0 |
| 2. Granulated DHT (125 mg active substance per plant during planting) | 25.9 | 26* |
| 3. Granulated DHT (125 mg a. s.) + L 333; spray treatment 0.6% (4 ×) | 21.4 | 39* |
| 4. DHT; spray treatment 0.125% (4 x) + L 333; spray treatment 0.6% (4 ×) | 15.2 | 57*** |
| 5. DHT; spray treatment 0.125% (4 x) + L 333; spray treatment 0.6% (4 ×) + cyanoguanidine; spray treatment 0.1% (4 ×) | 11.7 | 67*** |

Table 6

Reduction of the percentage of natural virus infection of a healthy potato stock cv. Xenia by treatments with antiphytoviral substances within two years

| Treatment | Virus infections, % | | Total |
|---|---|---|---|
| | Mosaic viruses (serological) | Leaf roll virus (Elisa-test) | |
| Control | 28 | 20 | 48 |
| DHT (0.125% active substance) + cyano-guanidine 0.1% + L 333 0.5% | 0*** | 5.6*** | 5.6*** |
| DHT (0.125% active substance) + anilino-adamantyl-thiadiazole 0.1% + L 333 0.5% | 6*** | 2.4*** | 8.4*** |

pressure. Thereafter, from 120 plants one tuber was tested using potato eye cuttings and serological tests including Elisa-test for PLRV. Table 6 shows that the abovementioned combination of three substances reduced the potato leaf roll infections from 20% to 5.6%. Treatments with a combination of DHT, L 333 and anilinoadamantyl-thiadiazol (Schuster et al., 1984a) led to reductions from 20% to 2.4%, respectively (Kleinhempel et al., 1984). Thus the development of new antiphytoviral substances together with the establishement of a combination chemotherapy have enabled promising results in bringing potato stocks into a good state of health. The same should be true for tobacco.

According to the experiences from the efforts for establishing an antiphytoviral chemotherapy of potato plants, the activity of the different antiphytoviral preparations against different tobacco viruses under different economical conditions should at first be examined in a lot of small experiments. Larger-sized experiments should not begin before having elaborated a convenient mode of treatments. Each progress demands efforts, and it may be a question of the development of the virus situation in the different countries, whether it shall be possible to live with the viruses hitherto or whether it is necessary to make new efforts in the battle against viruses with the aim to establish a modern chemotherapy. As the possibilities for a successful chemotherapy have enlarged by the mentioned results, we plan to start some trials for establishing an antiviral chemotherapy of tobacco in cooperation with tobacco breeders and invite all interested tobacco research workers to join these experiments.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 23–28 (1986)*

# Resistance of Corn Hybrids and Inbred Lines to MDMV and Inheritance of Resistance

I. MILINKÓ[1], O. GYULAVÁRI[2], GY. KOVÁCS[2] and J. TÁTRAI[2]

[1]University of Agricultural Sciences, Keszthely H-8361 Keszthely, P. O. Box 71, Hungary
[2]Research Station of the Cereal Research Institute, H-9761 Táplánszentkereszt, Hungary

In Hungary modern MDMV resistant corn hybrids were found in each maturity group (e.g. in FAO 200: Pioneer 3839 SC and P. 3925 SC; in FAO 300: P. 3901 SC; in FAO 400: P. 3747 SC and in FAO 500: P. 3707 SC) in 1983 and especially in 1984 under severe natural infection pressure. Under the same circumstances inbred lines were much more susceptible to MDMV than their hybrids. For this reason MDMV is the greatest phytopathological problem in hybrid corn seed production in the areas where *Sorghum halepense* (L.) Pers. is widely extended.

The inheritance of MDMV resistance is completely or partially dominant depending on the different genotypes. Therefore, new virus resistant hybrids can be developed on the basis of a systematic breeding program for resistance.

The fact that maize dwarf mosaic virus (MDMV) spread over the Southern part of Hungary simultaneously with its appearance in the USA (Janson and Ellett, 1963) was observed more than 20 years ago (Szirmai and Paizs, 1963). The investigations (Szirmai, 1968) following the recognition proved the possibility of breeding for MDMV resistance. Later, when Johnsongrass (*Sorghum halepense* (L.) Pers.) extended from the South to the North the area damaged by MDMV increased parallelly. In the course of a country-wide observation in 1974 Milinkó found considerable MDMV-infection even in the northern counties of Hungary (Fejér and Komárom). According to the evaluations of CPS corn hybrid trials Milinkó (1974) proved resistance breeding as the most effective method of MDMV control.

Afterwards, Sum et al. (1979) found 14.3–42.4%, and Peti (1983) 11.7–40.0% kernel mass decrease caused by MDMV.

In Hungary a systematic and continuous research program started in 1981 for studying virus resistance on the basis of previous investigations (Szirmai, 1968; Milinkó, 1974, 1977, 1978a and b; Milinkó et al., 1979, 1981) in collaboration between Cereal Research Institute (Szeged-Táplánszentkereszt) and University of Agricultural Sciences (Keszthely). Some results of these examinations have already been published (Milinkó et al., 1982, 1983a, b, c and d, 1984a and b, 1985).

In the present paper we analyse only:
- MDMV resistance of current corn hybrids and inbred lines.
- Role of MDMV in hybrid seed production.
- Inheritance of MDMV resistance.

## Materials and Methods

Field experiments were carried out in 1983–1984 in the area where the Johnsongrass (*Sorghum halepense* (L.) Pers.) covering is the widest in our country (southern part of Bács county near Baja). Each year and at each trial (Bátmonostor) 2 m wide stripes of Johnsongrass were left among the replications and around the trial to guarantee the uniform natural infection pressure. The area of the corn trial plots was specially treated with herbicides. Trial plots were intentionally sown somewhat later, around the 10th of May. In case of hybrids we used a randomized complete block design with four replications (40 hills per plot). A plot consisted of two rows. Plant density was 71 500 plants/ha (0.7 m × 0.2 m). Lines were planted in two replications, 20 hills/plot each. We evaluated the plants at least twice a year, first, at the end of June or at the beginning of July, second, during the first days of August. The tables contain the results of the second survey. In the evaluations, the following scale was applied using also the decimal 5 value: 0 = 0%, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%. The date of the individual plots (hybrids and lines) were averaged. The MDMV infection of *S. halepense* was calculated to be about 30–40% by percentual estimation.

To control the visual evaluations leaf samples were collected in cooling bags for a subsequent serological, indicator plant tests and electron microscope investigations. In our trials commercial corn hybrids grown in Hungary, their parental lines, a special diallel cross and various lines as possible resistance sources and others were examined.

## Results

Especially in 1984 we found the natural infection more serious than in the previous years (Table 1) on the basis of MDMV resistance ratings of the corn hybrids. The most susceptible hybrids to MDMV were Sanora TC, Pioneer 3965 A MTC, Pioneer 3950 MSC, and Sze 378 MSC R.

At the same time, in each maturity group up-to-date and highly productive corn hybrids were found with excellent resistance to MDMV as well, e.g. in FAO 200 group Pioneer 3839 SC and Pioneer 3925 SC, in FAO 300 Pioneer 3901 SC, in FAO 400 Pioneer 3747 SC, and in FAO 500 Pioneer 3707 SC (Table 1).

In both years the MDMV resistance of inbred lines was evaluated. Inbreds were much more susceptible than their hybrids (Table 2). In 1983 two resistant and three moderately susceptible lines were found, while in 1984 four lines proved to be resistant and two lines moderately susceptible. As against it, the majority of

Table 1

*MDMV ratings of corn hybrids.* 0–5 severity scale, Bátmonostor, 1983 and 1984

| FAO groups | Hybrids | Mean scale values | | | |
|---|---|---|---|---|---|
| | | 1983 | | 1984 | |
| | Sanora TC | — | | 3.75 | VS |
| | P. 3965 A MTC | 4.6 | VS | — | |
| | P. 3839 SC | 0.9 | R | 1.5 | R |
| 200 | P. 3950 MSC | 4.5 | VS | 4.4 | VS |
| | 3288 SC | 1.3 | R | — | |
| | 3310 SC | 0.75 | R | 2.3 | MS |
| | P. 3925 SC | — | | 1.4 | R |
| | P. 3906 SC | — | | 2.5 | MS |
| | Sze SC 390 | 0.6 | R | 1.3 | R |
| | Sze MSC 378 R | 3 | VS | 4 | VS |
| 300 | Sze SC 369 | 3 | VS | — | |
| | 3368 SC | 1.9 | MS | — | |
| | P. 3901 SC | 0.5 | R | 1.3 | R |
| | P. 3747 SC | 0.3 | R | 0.5 | R |
| 400 | P. 3732 SC | 2.4 | MS | 2.5 | MS |
| | 3479 SC | 0.1 | R | — | |
| 500 | P. 3707 SC | — | | 1.5 | R |

R: resistant, MS: moderately susceptible,
VS: very susceptible

Table 2

Distribution of corn inbred lines for MDMV resistance 0–5 severity scale, Bátmonostor, 1983 and 1984

| Scale values | 1983 | 1984 |
|---|---|---|
| 0–1 | 2 | 4 |
| 1.1–2 | 1 | — |
| 2.1–3 | 2 | 2 |
| 3.1–4 | 1 | 1 |
| 4.1–5 | 14 | 14 |
| Total | 20 | 21 |

Table 3

MDMV infection of corn hybrids in diallel test 0–5 severity scale, Bátmonostor, 1983 and 1984

| | Pa 405 | A 632 | A 654 | W 153 R | W 64 A | W 401 |
|---|---|---|---|---|---|---|
| Pa 405 | 0.1<br>0.5 | | | | | |
| A 632 | 0<br>0.5 | 3<br>2.5 | | | | |
| A 654 | 0<br>0.5 | 0.5<br>1.3 | 1.8<br>2 | | | |
| W 153 R | 0.1<br>0 | 1.4<br>3.5 | 2.1<br>2.5 | 4.8<br>4.5 | | |
| W 64 A | 0<br>0.3 | 1.8<br>2.8 | 2.1<br>3 | 4<br>5 | 5<br>4.5 | |
| W 401 | 0<br>0 | 1.6<br>2.1 | 0.5<br>2.1 | 4.6<br>4.9 | 5<br>5 | 5<br>5 |

Upper numbers: 1983, lower numbers: 1984

lines, that is, 15 of them were very susceptible in both years. The lines became rather early infected. Their infection was as high as 50–100% by the middle of July.

In case of a single cross hybrid the following four combinations are theoretically possible in seed production:

susceptible female × susceptible male = very bad,
susceptible female × resistant male = somewhat better,
resistant female × susceptible male = better combination,
resistant female × resistant male = the best combination.

The most serious damage occurs in case of susceptible female lines. There is a significant yield loss because of virus effect. Moreover, the ratio of insufficiently developed kernels will be high during seed processing as a consequence of poor seed setting. This could be observed in the fields of a South-Hungarian state farm where Pioneer 3732 SC was produced. The female plants were totally infected as early as midsummer in both years of our examinations. In case of a very susceptible male line the appropriate pollen shedding fertility and nick are not guaranteed.

Two effective conclusions can be drawn from these investigations:

– In the regions where Johnsongrass is widely spread MDMV means the main phytopathological problem especially in seed production.

Table 4

MDMV infection of corn hybrids and their inbred lines, Bátmonostor, 1983 and 1984

| Hybrids | Sclae values (0—5 severity scale) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1983 | | | | 1984 | | | |
| | A | B | C | A × B(—C) | A | B | C | A × B(—C) |
| Sanora TC | — | — | — | — | 0 | 5 | 5 | 3.75 |
| P. 3965 A MTC | 4.1 | 2.6 | 5× 5 | 4.6 | — | — | — | — |
| P. 3839 SC | 0 | 5 | | 0.9 | 0 | 5 | | 1.5 |
| P. 3950 MSC | 2.6 | 5 | 5× 5 | 4.4 | 0.5 | 5 | 5× 5 | 4.4 |
| 3288 SC | 5 | 1.8 | | 1.3 | — | — | — | — |
| 3310 SC | 1.75 | 4.5 | | 0.75 | 2.5 | 5 | | 2.3 |
| P. 3925 SC | — | — | | — | 0 | 5 | | 1.4 |
| P. 3906 SC | — | — | | — | 5 | 0 | | 2.5 |
| Sze SC 390 | 1.8 | 3 | | 0.6 | 2 | 2.5 | | 1.3 |
| Sze MSC 378 R | 3 | 4.9 | 4,8× 5 | 3 | 2.5 | 2 | 5× 4.5 | 4 |
| Sze SC 369 | 4.8 | 3.3 | | 3 | — | — | | — |
| 3368 SC | 1.8 | 4.5 | | 1.9 | — | — | | — |
| P. 3901 SC | — | — | | — | 0 | 5 | | 1.3 |
| P. 3747 SC | 4.9 | 0 | | 0.3 | 3.8 | 0 | | 0.5 |
| P. 3732 SC | 5 | 0 | | 2.4 | 5 | 0 | | 2.5 |
| P. 3707 SC | — | — | | — | 0 | 5 | | 1.5 |

A, B and C: inbred lines, A× B(–C): hybrids

— If the female line is susceptible to MDMV, hybrid seed production should not be carried out in such areas, where *S. halepense* is widely distributed.

We examined the inheritance of MDMV resistance in diallel experiments (Table 3). The data and Table 3 show clearly that resistance to MDMV was generally inherited dominantly. For example the line Pa 405 was resistant in all cases, even if it was crossed with very susceptible lines, like W 153 R, W 64 A or W 401. This fact is very important because a single cross hybrid can be resistant, even if one of its parental lines is rather susceptible, but the other one has dominant resistance to MDMV.

In certain cases a hybrid with good resistance could be obtained also by crossing two moderately susceptible lines, e.g. A 632 × A 654 (Sze SC 390). However, the crossing of two susceptible lines always results in susceptible hybrids.

In case of some commercial hybrids the inheritance of MDMV resistance was only partially dominant (Table 4). Pioneer 3906 SC and Pioneer 3732 SC belong to this group. They are moderately susceptible hybrids despite of the fact that one of their parental lines is resistant. Though in Table 4 there are several resistant inbreds, none of the lines in these combinations impart resistance to the degree as the line Pa 405 does.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 29—33 (1986)*

# Relationship between Activity of Virus Vector Aphids and Virus Incidence in Seed Cucumber Fields

ZSUZSA BASKY

Vegetable Crops Research Institute 6001 Kecskemét, P. O. Box. 116, Hungary

Over a period of four years the activity of virus vector aphids was estimated by trap plants placed in two seed cucumber fields. The virus incidence in seed cucumber fields was determined from randomly selected leaf samples collected at two week intervals. The incidence of virus in the trap plants and in the field samples was determined by mechanically-inoculating herbaceous indicator plants. Aphids were trapped in yellow water pans.

Correlations were obtained between the average trap plant infection percent at intensive vector activity period and the virus incidence in fields as well as the number of trapped alate aphids.

Correlation existed between the average trap plant infection percent at intensive vector activity period and the virus incidence observed in the fields at time of virus appearance, but this correlation was not very great.

There was a good correlation between the average trap plant infection percent at intensive vector activity period and the virus incidence observed in the fields at the end of the vegetation period. There was no connection between the number of aphids caught by yellow pans and either the trap plant infection or the virus incidence in fields.

Aphids play a prominent role in spreading phytopathogenic viruses. Up to now 300 aphid species have been tested as virus vectors in connection with 300 viruses on 300 host plants; of the 300 aphid species tested approximately 193 proved to be virus vectors. These aphid species are each able to transmit at least one virus (Harris, 1980).

Since field cucumbers in Hungary are infected by the non-circulative aphid-transmitted cucumber mosaic virus and watermelon mosaic virus (Basky, 1984a, c), it is necessary to correlate aphid number, intensity of vector activity and virus incidence in the fields.

## Materials and Methods

Over a period of four years virus vector aphid activity was determined by trap plants placed in the seed production fields of the cultivars "Kecskeméti bőtermő" and "Kecskeméti keseredésmentes konzerv". Each week 25 cucumbers

at the cotyledon stage were placed in the fields and replaced by new ones from the emergence of the cucumber crop until the ripening of the fruit. After exposure the trap plants were placed under isolators to exclude aphids and after a two week incubation period leaf samples were taken from them.

Leaf samples were collected randomly at 2-weeks intervals, ten samples from each of four replicates in both varieties. The virus infection percentage was determined by mechanically-inoculating herbaceous indicator plants: *Cucumis sativus* L. "Szenzáció", *Chenopodium amaranticolor* Coste et Reyn., *Nicotiana tabacum* L. "Xanthi-nc", and *Ammi majus* L. with the sap from individual leaf samples. The infection percentage of the trap plants was determined in the same way. Aphids were collected by yellow pan traps placed on the border and in the middle of the fields. A bivariate linear regression analysis was used to establish correlations between the average trap plant infection percent at the intensive vector activity period and the virus incidence observed in the fields at the time of virus appearance and at the end of the vegetation period. Correlations were also obtained between the aphid numbers caught in traps, trap plant infection and the virus incidence in the fields.

## Results

Correlation was found between the average trap plant infection percent at the intensive vector activity period and the virus incidence observed in fields at the time of virus appearance, $r = 0.7184$; $a = -4.2006$; $b = 2.4006$; $y = = -4.2006 + 2.4006x$. In this case the correlation is valid, but it is not definitely close. The $a = -4.2006$ value indicates that trap plants became infected in the cucumber fields when field cucumbers were still healthy.

Of course, a part of field cucumbers was also infected with the trap plants together but it is impossible to reveal virus infection of field cucumbers at the time of infection. There was a close correlation between the average trap plant infection at the intensive vector activity period and the virus incidence observed in the fields at the end of the vegetation period, $r = 0.9306$; $a = 5.0436$; $b = 4.4664$.

In this case the correlation was definitively close $y = 5.0436 + 4.4664x$, $a = 5.0436$ indicates that even if trap plants are not infected virus infection does exist in the fields. Moreover, new infected plants appear after the incubation period even if the activity of virus vector aphids decreases to zero by the end of the vegetation period.

Data pairs of the bivariate linear regression analysis are presented in Table 1, a graphic representation is in Fig. 1. Data of the bivariate linear regression analysis prove the close correlation between the intensity of vector activity expressed with trap plant infection percent and the virus incidence in the seed cucumber field at the end of the vegetation period. The virus infection pressure of the field can be followed on the basis of trap plant infection as it was established by Marrou et al. (1979).

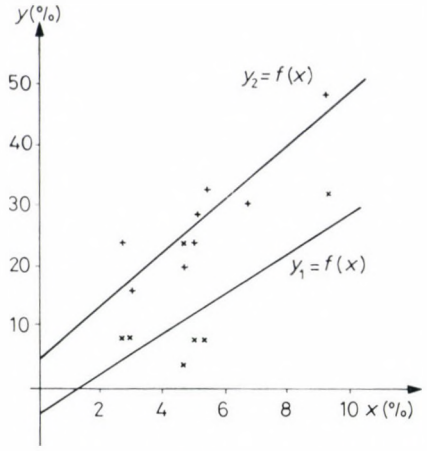



Fig. 1. Infection rate of field samples ($y$) in function of the general infection of trap plants ($x$). Value pairs of 4 years and 2 varieties are represented in Table 1. × Infection rate in the field at the appearance of virus infection. + Infection rate in the field at the end of the vegetation period

There was no correlation between the number of caught aphids, the average trap plant infection percent at the intensive aphid vector activity period or the virus incidence in the fields. There was no correlation between the number of caught alate aphids and the above-mentioned factors even if a number of alate aphids proved to be vectors of cucumber mosaic virus and watermelon mosaic virus.

Table 1

Average trap plant infection percent at intensive vector activity period, virus incidence in seed cucumber fields at the time of virus appearance and at the end of vegetation period

| Average trap plant infection, % $x$ | Virus incidence in the fields $y$ | |
|---|---|---|
| | at the time of virus appearance | at the end of vegetation period |
| 2.67 | 8.0 | 24,0 |
| 3,00 | 8.0 | 16,0 |
| 5.00 | 8,0 | 28.0 |
| 4.67 | 24.0 | 24.0 |
| 9.20 | 32.0 | 48.0 |
| 4.67 | 4.0 | 20.0 |
| 5.33 | 7.5 | 32.5 |
| 6.66 | 15.0 | 30.0 |

During the trials it was established that the high activity of virus vector aphids never coincided with the peak of aphid flights. When trap plants were infected at the end of June and during July the number of aphids was extremely low (Basky, 1984b).

## Discussion

Faan and Johnson (1951), Havránek and Láska (1972) as well as Marrou et al. (1979) found correlations between the number of caught aphids and the virus incidence in the cultivated plants. Toba and co-workers (1977) observed that the peak of aphid flights was two months earlier than the appearance of virus symptoms. As to the correlation between the number of caught aphids and the virus infection of field plants Leclant (1978) suggested that the number of alate aphids did not reflect the extent of virus transmission but rather the flight behaviour of aphids.

Harrison (1981) suggested that with air-borne vectors the activity of vectors was more important then their number. According to Quiot et al. (1982) if many vector species are able to transmit a virus then their vector efficiency may differ considerably in the field. Moreover, trapping possible vectors does not provide information as to whether each is viruliferous at the moment of catching. This information can be obtained by trapping live alatae, identifying them, and then placing them individually or in groups on indicator test plants to determine viruses being carried (Ashby et al., 1979).

Virus infection requires the presence of the virus, the vector and the susceptible host plant at the same time.

In temperate zones host plants are necessary in which the virus is able to overwinter e.g. perennial plants, or else spread by seed transmission is required.

Therefore, the study of correlations requires the complex examination of the above-mentioned factors. There was no real correlation between the number of caught aphids and the activity of virus vector aphids, because the other two important factors – the virus and the host as acceptor and donor – were not taken into consoderation.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 35—49 (1986)*

# Compatible and Incompatible Relations between *Capsicum* Species and Viruses

## I. Review

J. HORVÁTH

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

In the first part of the publication series brief survey is given of susceptibility and resistance to viruses in *Capsicum* species and varieties.

According to our present knowledge 13 species and 9 varieties of *Capsicum* are known for susceptibility to some 43 viruses. The number of viruses occurring spontaneously on *Capsicum* plants is about 18, of which tobacco mosaic virus, tomato mosaic virus, cucumber mosaic virus, potato virus Y and alfalfa mosaic virus are the most important in Hungary. We also reviewed the virus resistance of various *Capsicum* species and varieties. As far as we know 15 *Capsicum* species and varieties are resistant to 45 viruses. In the incompatible host-virus relations very important are the plants resistant to alfalfa mosaic virus, cucumber mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, tomato mosaic virus. The main tasks of the research are to analyse the resistance genes in *Capsicum* plants, the infectivity of the different virus strains and the inheritance of the resistance genes.

## Classification of the Genus *Capsicum*

In connection with the classification of the genus *Capsicum*, that is a member of the family *Solanaceae*, many publications have appeared. Among them remarkable is the work of Heiser and Smith (1953) who tried to determine the taxonomic place of the different species by interspecific crossing tests. On this basis four species *(Capsicum annuum, C. frutescens, C. pendulum, C. pubescens)* were separated. On the basis of the morphological characters Lippert et al. (1965) differentiated twelve-thirteen species. They are: *Capsicum annuum, C. cardenasii, C. chacoense, C. chinense, C. eximium, C. frutescens, C. galapagoensis, C. microcarpum* or *C. pendulum, C. praetermissum, C. pubescens, C. scolnikianum, C. schottianum.* In a recent work Heiser and Smith (1969) acknowledge five cultivated varieties of the genus *Capsicum*, four of which also grown wild, ten wild forms and about seven not closely identified species. According to our present knowledge the genus *Capsicum* includes 22 wild species, three varieties, five cultivated species and four varieties (Table 1). We think that the work related to the classification of the genus *Capsicum* is far from being completed, as suggested by the recent studies of which we only mention here a few (cf. Terpó, 1966; Pickersgill et al., 1979; Quagliotti, 1979; Somos, 1981).

Table 1

Classification of the genus *Capsicum*[1]

| Wild species | Domesticated species |
|---|---|
| *Capsicum buforum* | *Capsicum annuum* var. *aviculare* |
| *C. campylopodum* | *C. baccatum* var. *pendulum* |
| *C. chacoense* | *C. baccatum* var. *praetermissum* |
| *C.* var. *tomentosum* | *C. baccatum* var. *tomentosum* |
| *C. ciliatum* | *C. cardenasii* |
| *C. coccineum* | *C. chinense* |
| *C. cornutum* | *C. eximium* var. *tomentosum* |
| *C. dimorphum* | *C. frutescens* |
| *C. dusenii* | *C. pubescens* |
| *C. galapagoensis* | |
| *C. geminifolium* | |
| *C. hookerianum* | |
| *C. lanceolatum* | |
| *C. leptopodum* | |
| *C. minutifolium* | |
| *C. mirabile* | |
| *C. parvifolium* | |
| *C. scolnikianum* | |
| *C. schottianum* var. *flexuosum* | |
| *C. tovari* | |
| *C. cillosum* | |

[1] Reviewed by Hunziker (1958), Eshbaugh (1977) and Somos (1981).

The pepper, a native of Central- and South America, is supposed to have been introduced into Europe in the 16th century. At present Europe of all continents stands first in pepper production. In Hungary the sowing area of pepper has considerably decreased for some years; it is now 10–12 thousand hectare or so. In spite of the reduction of area pepper is a very important crop in Hungary, still occupying 7–8% of the total vegetable area of the country (Zatykó, 1979).

## Virus Susceptibility of *Capsicum* Species

The affinity of *Capsicum* species to plant viruses with different biological characteristics is very strong. The work started in the 1940s in Hungary with the study and descriptions of viruses pathogenic to pepper has recently become more intensive. Due to these four decades of research work we have now through knowledge of the virus pathogens of pepper, and very promising is the work of breeding for virus resistance, which for some years has become particularly intensive (cf. Szirmai, 1941; Petróczi, 1956; Solymosy, 1958; Solymosy and Szalay-Marzsó, 1962; Horváth, 1966, 1967, 1969, 1983, 1984a, b, c; Kapeller, 1967;

Table 2

Virus susceptibility of *Capsicum* species[1]

| *Capsicum* species | Viruses |
|---|---|
| *Capsicum annuum* | alfalfa mosaic virus* |
| | *Asparagus* virus 2 |
| | belladonna mottle virus |
| | broad bean wilt virus* |
| | carnation ringspot virus |
| | carrot mottley dwarf virus |
| | cucmber mosaic virus* |
| | henbane mosaic virus |
| | pea early browning virus |
| | pepper mild mosaic virus* |
| | pepper mottle virus* |
| | pepper veinal mottle virus* |
| | pepper yellow veinal mosaic virus* |
| | potato aucuba mosaic virus |
| | potato leaf roll virus |
| | potato mop-top virus |
| | potato spindle tuber viroid |
| | potato stunt virus |
| | potato virus X* |
| | potato virus Y* |
| | *Rumex* virus 2 (syn.: dock mosaic virus) |
| | strawberry vein clearing virus |
| | tobacco etch virus* |
| | tobacco leaf vurl virus* |
| | tobacco mosaic virus* |
| | tobacco necrosis virus |
| | tobacco rattle virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | tomato aspermy virus* |
| | tomato bushy stunt virus |
| | tomato curly top virus* |
| | tomato mosaic virus* |
| | tomato spotted wilt virus* |
| | turnip mosaic virus (syn.: cabbage ringspot virus) |
| *C. annuum* var. *cerasiforme* | potato virus Y |
| *C. annuum* var. *longum* | potato virus Y |
| *C. baccatum* | pepper veinal mottle virus |
| | tobacco mosaic virus |
| *C. cerasiforme* | tobacco mosaic virus |
| *C. chacoense* | cucumber mosaic virus |
| | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. chinense* | belladonna mottle virus |
| | cucumber mosaic virus |
| | pepper mild mottle virus |
| | tobacco mosaic virus |
| | tomato mosaic virus |

Table 2 *(continued)*

| *Capsicum* species | Viruses |
|---|---|
| *C. frutescens* | alfalfa mosaic virus* |
| | beet curly top virus |
| | belladonna mottle virus |
| | broad bean wilt virus |
| | carnation ringspot virus |
| | carrot mottley dwarf virus |
| | cucumber mosaic virus* |
| | *Datura* distortion mosaic virus |
| | eggplant mosaic virus |
| | pepper mild mottle virus |
| | pepper mottle virus* |
| | pepper veinal mottle virus* |
| | potato aucuba mosaic virus |
| | potato leaf roll virus |
| | potato stunt virus |
| | potato virus X |
| | potato virus Y |
| | sweet potato mosaic virus |
| | tobacco etch virus |
| | tobacco mosaic virus |
| | tobacco necrosis virus |
| | tobacco rattle virus* |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | tomato aspermy virus |
| | tomato bushy stunt virus |
| | tomato mosaic virus |
| | tomato ringspot virus |
| | tomato spotted wilt virus |
| *C. frutescens* var. *cerasiforme* | cucumber mosaic virus |
| *C. frutescens* var. *conoides* | cucumber mosaic virus |
| *C. frutescens* var. *Golden Dawn*× var. *Golden Dawn*× var. *minimum* | tobacco mosaic virus |
| *C. frutescens* var. *Golden Dawn*× var. *minimum* | tobacco mosaic virus |
| *C. frutescens* var. *grossum* | beet curly top virus |
| | cucumber mosaic virus |
| | tomato spotted wilt virus |
| *C. frutescens* var. *minimum* | potato virus Y |
| *C. frutescens* var. *Tobasco*× var. *Ruby King* | tobacco mosaic virus |
| *C. grandiflora* | turnip mosaic virus* |
| *C. microcarpum* | tobacco etch virus |
| | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. minimum* | alfalfa mosaic virus |
| | balladonna mottle virus |
| | cucumber mosaic virus |
| | potato virus Y |
| | tobacco mosaic virus |

Table 2 *(continued)*

| *Capsicum* species | Viruses |
|---|---|
| *C. pendulum* | potato virus Y |
| | tobacco etch virus |
| | tobacco mosaic virus |
| | tobacco rattle virus |
| | tomato mosaic virus |
| *C. praetermissum* | alfalfa mosaic virus |
| | belladonna mottle virus |
| | cucumber mosaic virus |
| | potato virus Y |
| | tobacco mosaic virus |
| *C. pubescens* | tobacco mosaic virus |
| | tobacco rattle virus |
| *C. testiculatum* | alfalfa mosaic virus |
| | belladonna mottle virus |
| | cucumber mosaic virus |
| | potato virus Y |
| | tobacco mosaic virus |

[1] Viruses designated with asterisks are naturally or spontaneously occurring pathogens on the *Capsicum* plants mentioned.

Beczner, 1969; Kovács and Horváth, 1973; Andrásfalvy, 1974; Tóbiás et al. 1978; Csilléry and Ruskó, 1980; Salamon et al., 1980a, b; Tóbiás and Molnár, 1981; Csilléry, 1982; Csilléry and Ruskó, 1983; Tóbiás and Maat, 1982; Tóbiás et al., 1982a, b; Zatykó and Moór, 1982; and others). According to the results of investigations in Hungary the following viruses of pepper are of the greatest importance: tobacco mosaic virus, tomato mosaic virus, cucumber mosaic virus, potato virus Y and alfalfa mosaic virus. In the course of earlier investigations we found various pepper varieties to be susceptible to potato virus X and potato aucuba mosaic virus (Horváth, 1969; Beczner and Horváth, 1969). Some years ago broad bean wilt virus and tomato aspermy virus were reported to occur in Hungary (cf. Salamon et al., 1980a, b; Horváth and Beczner, 1983). Here we wish to point out that the *Capsicum* species through their hypersensitive reactions (after 2 or 3 days of incubation) are highly suitable indicator plants of the potato aucuba mosaic virus. In the diagnosis of the potato aucuba mosaic virus and in its reisolation from host plants highly important, indispensable role is played by the *Capsicum* spp. (Horváth, 1964, 1976, 1977). In recent years we isolated three cucumber mosaic virus strains from *Asclepias syriaca* and *Phaseolus vulgaris* plants, which proved highly patogenic for pepper too (Schmidt and Horváth, 1984; Horváth, 1983; Horváth et al., 1983). It was again due to recent investigations that a virus disease of pepper earlier unknown in Hungary became known by the name pepper yellow veinal (Salamon et al., 1982). Its importance is increased by the fact that it has been detected in various parts of the country. At present 13

*Capsicum* species and 9 varieties are known for virus susceptibility (cf. Horváth, 1966, 1967; Thornberry, 1966; Schmelzer and Wolf, 1977; Horváth 1981; Rast, 1982; and others), if our own experiments performed in the last several years, of which account will be given following this publication are left out of consideration. The *Capsicum*-virus relations cover some 43 viruses with different characteristics (Table 2). It is remarkable that about 18 viruses have so far been found to occur spontaneously on pepper plants. Among the viruses occurring in *Capsicum* species under natural conditions very important are the tobacco mosaic virus and tomato mosaic virus which are transmitted or spread mechanically and by seed, as well as the potato virus Y, alfalfa mosaic virus and cucumber mosaic virus which spread mechanically and through aphids in a non-persistant manner. The virus infection of pepper ranges between 10 and 50 per cent in Hungary, but field stands with 100 per cent virus infection at the end of vegetation are not rare either.

## Virus Resistance of *Capsicum* Species

The host-virus research has lately been paying ever increasing attention to seeking out plants resistant to viruses and detecting the occurrence of virus resistance genes. The importance lies in the fact that the chemotherapic and physicotherapic procedures used in vivo against viruses are inapplicable in practice, the conventional plant protection methods are of poor efficiency, undesirable from the point of view of environment protection, toxicologically dangerous and ecologically limited. These facts suggest that the control of the virus pathogens of pepper is restricted almost exclusively to breeding for virus resistance and to virus resistant varieties, respectively.

Prodromal investigations related to the virus resistance of pepper can supposedly be traced back to the resistance of *Capsicum frutescens* var. *Tobasco* and *C. frutescens* var. *minimum* to tobacco mosaic virus. Responses of *Capsicum* accessions to infection by tobacco mosaic virus were found to be controlled by a series of multiple alleles: L (localisation of tobacco mosaic virus), $L^1$ (imperfect localisation of tobacco mosaic virus) and $L^+$ (mottling) (Holmes, 1937), with $L > L^1 > L^+$ (reviewed by Boukema, 1980).

The so-called "imperfect" type of resistance is characteristic of the variety *Yolo Wonder* (cf. Porter and Younkin, 1952). Here we mention a work by Cook (1966) in which he gives account of a derivative resistant to potato virus Y isolated from the variety *Yolo Wonder* as showing resistance to tobacco mosaic virus as well.

In the course of studying the resistance of five *Capsicum chinense* accessions to the pepper strain of tobacco mosaic virus found the resistance to be inherited monogenically and to be particularly dominant (Boukema, 1980). The resistance genes in these accessions appeared to be allelic and were also found to be allelic with the known alleles $L^1$ and L. It is proposed to use the symbol $L^3$ for these resistance genes.

Resistance to tobacco mosaic virus was detected among others in the following species: *Capsicum annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C. frutescens*, *C. microcarpum*, *C. pendulum*, *C. praetermissum* and *C. pubescens* (cf. Boukema, 1977, 1980; Rast, 1977, 1982 and others). Resistance based on hypersensitivity to tobacco mosaic virus is probably the easiest attainable type of virus resistance, as varified by the resistant Hungarian varieties (e.g. *Fehérözön*, *D. Cecei*, *Hárosi zöld*, *Rezisztens Keszthelyi*, *Csipke;* cf. Zatykó, 1979; Csilléry, 1982; Horváth and Kovács 1984 unpublished). Thanks are due to Szirmai (1970) for the pepper variety which shows hypersensitivity to tobacco mosaic virus and high resistance to cucumber mosaic virus (Szirmai et al., 1984).

The resistance of potato virus Y, tobacco etch virus and cucumber mosaic virus are inherited by polygenes. Resistance to potato virus Y has been demonstrated so far e.g. in the varieties *California Wonder* (Easton et al., 1958), *Long Red Cayenne* (Kahn and Monroe, 1963) and *Markgärtner* (Horváth, 1967). Cook and Anderson (1959) gave account of experiments in which two pepper accessions *(P. I. 264281, S. C. 46252)* were found to be resistant to potato virus Y. The authors could not locate the recessive gene that brought about the resistance. According to the data of Simons (1966) some clones of the variety *Italian El* while resistant to potato virus Y are extremely susceptible to cucumber mosaic virus. In the course of genetic studies on resistance to potato virus Y Cook and Anderson (1960) pointed out that the course of inheritance of resistance was recessive, while the susceptibility of the pepper trains was a dominant hereditary feature. The plant that Cook (1966) derived from the variety *Yolo Wonder* and described by the name *Yolo Y* proved resistant to potato virus Y. The progeny obtained from *Yolo Y* through self-pollination was also resistant to the virus; however, the resistance depends on a single recessive gene, therefore crossing with any commercial varieties of the variety *Yolo Y* results in a virus susceptible progeny. The recessive course of inheritance represents essential breeding problems in developing resistance to the virus.

Resistance to tobacco etch virus postulates the presence of more than one genes (Greenleaf, 1953, 1956; and others). In the experiments of Smith (1970) and Cook (1974) the pepper varieties resistant to tobacco mosaic virus were found to be also resistant to the strains of potato virus Y.

Resistance to cucumber mosaic virus shows again polygenic inheritance (Pochard, 1977). Remarkable is a work by Szirmai (1970) in which red pepper strains resistant to cucumber mosaic virus are reported to show hypersensitive resistance to tobacco mosaic virus. The complex resistance to cucumber mosaic virus and tobacco mosaic virus in red pepper plants is an inportant research result.

Breeding for virus resistance—in spite of the difficulties—is not hopeless, as testified by the results of experiments after the 1960s, in the course of which e.g. the variety *Florida VR–2* (Cook et al., 1976) resistant to three viruses (tobacco mosaic virus, tobacco etch virus and potato virus Y) and a variety *Delray Bell* tolerant to tobacco etch virus and potato virus Y were produced (Cook et al., 1977). A source of resistance to pepper mottle virus was reported in *Capsicum*

*chinense, P. I. 159236* (cf. Zitter, 1972). Subramanya (1982) studied the progenies of a cross between *Delray Bell (Capsicum annuum)* pepper cultivar tolerant to pepper mottle virus and a *Capsicum chinense* accession *(P. I. 159236)* resistant to pepper mottle virus. In the experiments the $F_1$ generation was susceptible to the virus, whereas the generations of backcross to both parents and the $F_2$ segregated in a ratio of 1 : 1. This indicates that the pepper mottle virus resistance is controlled at the same locus in both parents, or at two closely linked loci. Each parent is homozygous for one locus and the homozygous condition of either allele results in failure to support virus. In heterozygous condition as observed in the $F_1$ generation the effect of a single allele is insufficient even when one allele of each type is present, resulting in systemic infection.

In works by Thornberry (1966), Barrios et al. (1971), Boukema (1980), Rast (1982), Wetter et al. (1984) and others important data are found concerning the incompatible (hypersensitive) host-virus relations between *Capsicum* species and viruses (Table 3). The genetic problems connected with the virus resistance of pepper are increased by the fact that the affinity between pepper and viruses is very strong, and more and more new host-virus relations are being detected which throw up at the same time genetic and breeding problems. Here we refer by way of example to pepper mottle virus and pepper mild mosaic virus recently appearing in Florida and Venezuela (Debrot et al., 1980; Nelson and Wheeler, 1972), or to pepper veinal mottle virus described in Nigeria (Lana et al., 1975), further to pepper mild mottle virus from the tobamovirus group (Wetter et al., 1984) and to the occurrence of new strains of tobacco mosaic and tomato mosaic viruses (Ramakrishnan, 1961; Horváth and Beczner, 1973; Mamula et al., 1974; Rast, 1975, 1979; Zaitlin and Israel, 1975; Hollings and Huttinga, 1976; Van den Berkmortel, 1977; Boukema et al., 1980; Csilléry and Ruskó, 1980; Nagai et al., 1981; Tošić and Videnov, 1981; Tošić, 1982; Paludan, 1982; Tóbiás et al., 1982a, b; Conti and Marte, 1983; Goto et al., 1984; Wetter, 1984), and last but not least to the so far not closely identified pepper yellow veinal mosaic virus occurring in Hungary (Salamon et al., 1982).

The work of breeding for virus resistance is made difficult by the fact that among the varieties grown earlier virus resistant ones are hardly found. In the course of inoculation experiments with eight viruses (potato aucuba mosaic virus, potato virus X, potato virus Y, tobacco mosaic virus, broad bean wilt virus, alfalfa mosaic virus, tomato aspermy virus and cucumber mosaic virus) none of the varieties *California Wonder, Bogyiszlói, Budai csípős, Cecei édes konzerv, Csokros csüngő, Csokros felálló, D. Cecei, Dokomlási 2710, Gépi konzerv, Hatvani hajtatási, Ipari vörös, Javított cecei, Kalocsai felálló, Kalocsai E–15, Keszthelyi fehér, Korai halványzöld, Kovácsházi hajtatási, Kalocsai, Magyar kincs, Maritza, Markgärtner, Paradicsomalakú zöld, Perfection, Sonnenpreis, Soroksári, Szarvas II., Tétényi hajtatási zöld* showed resistance except for Markgärtner to potato virus Y (cf. Horváth, 1966, 1967; Beczner, 1969; Salamon et al., 1980a, b).

In the course of breeding pepper for virus resistance it is therefore a basic task to seek out the resistant initial forms (species, varieties) determine the types

Table 3

Insusceptible or resistant relationships between *Capsicum* species and viruses

| *Capsicum* species | Viruses |
|---|---|
| *C. annuum* | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. annuum* cvs. (e.g. *Bruinsma's Wonder*, *Pll*, *Rama*, *Tisana*, *Turrialba* etc.) | tobacco mosaic virus |
| | tomato mosaic virus |
| | cucumber mosaic virus |
| *C. baccatum* | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. baccatum* cvs. (e.g. *Chilefen*, *Christmas Bell* etc.) | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. cardenasii* | potato virus Y |
| *C. cerasiforme* | *Nicotiana glutinosa* mosaic virus |
| *C. chacoense* | tobacco mosaic virus |
| | tomato mosaic virus |
| | cucumber mosaic virus |
| *C. chinense* | pepper mottle virus |
| | tobacco etch virus |
| | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. frutescens* | alfalfa mosaic virus |
| | *Anemone* mosaic virus |
| | bean pod mottle virus |
| | bean southern mosaic virus |
| | beet curly top virus |
| | beet yellows virus |
| | cacao swollen shoot virus |
| | clauliflower mosaic virus |
| | *Chrysanthemum* B (mild) mosaic virus |
| | cowpea mosaic virus |
| | cucumber green mottle mosaic virus |
| | cucumber mosaic virus |
| | *Hibiscus* yellow vein mosaic virus |
| | *Hydrangea* ringspot virus |
| | muskmelon mosaic virus |
| | *Nicotiana glutinosa* root necrosis virus |
| | orchid mosaic virus |
| | papaw mosaic virus |
| | papaw ringspot virus |
| | pea streak virus |
| | *Petunia* mosaic virus |
| | potato leaf roll virus |
| | potato paracrincle virus |
| | potato virus A |
| | potato virus M |
| | potato virus X |
| | potato virus Y |

Table 3 *(continued)*

| *Capsicum* species | Viruses |
|---|---|
| | spinach yellow dwarf virus |
| | squash mosaic virus |
| | sweet potato mosaic virus |
| | tobacco coarse etch virus |
| | tobacco etch virus |
| | tobacco leaf curl virus |
| | tobacco mosaic virus |
| | tobacco ringspot virus |
| | tomato mosaic virus |
| | tomato spotted wilt virus |
| | *Tropaeolum* mosaic virus |
| | watermelon mosaic virus |
| *C. frutescens* var. *cerasiforme* | beet mosaic virus |
| | cauliflower mosaic virus |
| | cucumber mosaic virus |
| | turnip mosaic virus |
| *C. frutescens* var. *grossum* | beet curly top virus |
| | beet mosaic virus |
| | cauliflower mosaic virus |
| | celery mosaic virus |
| | *Primula* mosaic virus |
| | squash mosaic virus |
| | tobacco ringspot virus |
| *C. frutescens* var. *minimum* | tobacco mosaic virus |
| *C. frutescens* var. *Tobasco* | tobacco mosaic virus |
| | cucumber mosaic virus |
| *C. microcarpum* | cucumber mosaic virus |
| | tobacco mosaic virus |
| | tomato mosaic virus |
| | potato virus |
| *C. pendulum* | cucumber mosaic virus |
| | potato virus x |
| | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. praetermissum* | tobacco mosaic virus |
| | tomato mosaic virus |
| *C. pubescens* | tobacco mosaic virus |
| | tomato mosaic virus |

of resistance, clear up the transmissibility of the resistance genes and improve the possibility of interspecific crossing. The work of breeding for resistance requires internationally identified and characterized viruses (virus strains); in this respect research is faced with a highly important task. Owing the chemical and biological characteristics of the plant viruses the control of viruses pathogenic for pepper can be achieved almost exclusively by producing new, virus resistant varieties. For this very reason highly important are the investigations aimed at locating virus resistance genes present in various *Capsicum* species and finding the way of crossing certain *Capsicum* species with each other.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 51–58 (1986)*

# Compatible and Incompatible Relations between *Capsicum* Species and Viruses

## II. New Compatible Host-Virus Relations (Susceptible Plants)

J. HORVÁTH

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

The present paper gives an account of studies on the responses of 21 *Capsicum* species and varieties (8 of which are new in the virological literature) to 14 viruses, as a part of investigations on the host-virus relations. In the course of experiments we detected 144 compatible relations.

The classification for virus infection was *(1)* chlorotic/necrotic local lesions and systemic mosaic (group I), *(2)* necrotic local lesions and systemic necrosis (group II), and *(3)* local and systemic necrotic lesions, leaf drop, death of the plants (group III).

In the literature of plant virology the interpretation of virus susceptibility and virus resistance (of different types) in plants is not always uniform. Differences in the interpretation and denomination of the same phenomenon have given many occasions for misunderstanding. In our papers published lately we described the host-virus relations according to the part of the virus-infected plant becoming disordere and the part (parts) of the plant from which the virus could be reisolated (cf. Horváth, 1976, 1977, 1983, 1985a, b). When the infection only affected inoculated leaves, we regarded the relationship between plant and virus as local host-virus relation. When the infection extended to the non-inoculated or subsequently developed leaves, we considered the relationship between plant and virus to be a systemic host-virus relation. When the infection affected both the inoculated and non-inoculated leaves, the relationship between plant and virus was regarded as a local and systemic relation. To these three kinds of relationship we gave the comprehensive name of compatible host virus relation. In our recent works for the local host-virus relations (hypersensitive reaction) we used the term "transitional compatibility" too; this manifested itself in the initial susceptibility and "subsequent" or "late" resistance of a plant to a certain virus (cf. Horváth, 1984, 1985b). Those plants which after the inoculation remained virus-free both locally and systemically, and the virus could not be reisolated from them to indicator plants, were regarded as resistant or immune. To the latter relationships were given the comprehensive name incompatible host-virus relation.

Some years ago Cooper and Jones (1983) made a proposal on a uniform terminology related to the response of plants to virus inoculation. On the basis

of this proposal the relationships between the *Capsicum* plants and the viruses discussed in this paper can be placed in two groups. One of the groups includes those plants as can be infected with the different viruses (infectible hosts), while to the other group those plants belong which cannot be infected with them (immune or nonhosts). In a part of the infectible plants the reproduction and invasion of the viruses could be pointed out (susceptible plants), while in the other plants the infection was, though, successful, but the reproduction of the virus in the plant slowed down or stopped after some time, with the consequence that the course of the disease was also checked and the infection became localized (resistant plants). The relationship between susceptible plants and viruses is called compatible host-virus relation, while the one between resistant or immune plants and viruses is an incompatible host-virus relation.

By the above terms, in the present, second part of the publication series those new compatible host-virus relations – and infectible plants, respectively – are dealt with in which the reproduction and invasion of viruses could be demonstrated.

Table 1

*Capsicum* species and varieties investigated in the experiments

| *Capsicum* species and varieties[1] | Source[2] |
|---|---|
| *Capsicum annuum* var. *cerasiforme* | C |
| *C. annuum* var. *conoides** | C, N |
| *C. annuum* var. *longum* | C, N |
| *C. annuum* var. *minimum** | N |
| *C. annuum* var. *paramidale** | N |
| *C. baccatum* | N |
| *C. barbatum* var. *pendulum** | N |
| *C. cardenasii* | B |
| *C. chacoense* | G, C, N, B |
| *C. chinense* | N |
| *C. eximium** | B, N |
| *C. flexuosum* | N |
| *C. frutescens* | C, G, N |
| *C. grossum** | C |
| *C. luteum** | N |
| *C. minimum* | N, K |
| *C. nigrum** | C |
| *C. pendulum* | G |
| *C. praetermissum* | B, N |
| *C. pubescens* | G |
| *C. testiculatum* | N |

[1] Plants marked with asterisks are species new to experimental virology

[2] Abbreviations used for sources of seed: *B* = University of Birmingham, Department of Plant Pathology, Birmingham (England), C = Botanical Garden, University of Copenhagen, Copenhagen (Danmark), *G* = Central Institute of Genetics and Cultivated Plants, Gatersleben (German Democratic Republic), *K* = Botanical Garden, Botanical Institute, Kaunas (Soviet Union), *N* = Botanical Garden of the University of Nijmegen (the Netherlands)

In the third part of the publication series we are discussing those incompatible *Capsicum*-virus relations for which we are able to prove the ability of plants to localize the virus or viruses on the one hand, and their immunity of the virus or viruses, on the other.

## Materials and Methods

In the course of experiments performed with *Capsicum* species and varieties we studied the responses of 15 species and 6 varieties of *Capsicum* to various strains of 14 viruses (Tables 1 and 2). In the case of the potato virus Y we used the mixed inoculum of the C-, normal-, veinal necrosis- and anomale strains for inoculation. Present paper discusses the compatible relations between 20 *Capsicum* species and varieties and 13 viruses. Young seedlings were inoculated with the different viruses at the 6–8-leaf stage. The viruses were obtained from the gene bank of our laboratory (see Table 2). The 600 mesh carborundum used in the course of the inoculation was applied onto the leaves with a special duster before the infection. Inoculation was carried out with virus-containing tissue sap diluted with distilled water at a ratio of 1 : 1. After the inoculation the infected plants

Table 2

Viruses inoculated in the experiments

| Viruses | Strains or isolates | Literature |
|---|---|---|
| Alfalfa mosaic virus (AMV) | $K_2$, MvS70 | Beczner (1972) |
| Belladonna mottle virus (BMV) | H | Horváth et al. (1976) |
| Broad bean wilt virus (BBWV) | Tm | Horváth and Szirmai (1975) |
| Cucumber mosaic virus (CMV) | U/246, W, U/Ascl | Horváth (1976, 1977), Schmidt and Horváth (1982) |
| *Melandrium* yellow fleck virus (MYFV) | LB | Hollings and Horváth (1981) |
| Potato aucuba mosaic virus (PAMV) | SS | Horváth (1976, 1977) |
| Potato virus X (PVX) | G | Horváth (1976, 1977) |
| Potato virus Y (PVY) | C, N, R, An | Horváth (1976, 1977) |
| Tobacco mosaic virus (TMV) | $U_1$ | Siegel and Wildman (1954) |
| Tobacco necrosis virus (TNV) | f | Szirmai (1964) |
| Tobacco rattle virus (TRV) | H | Horváth (1976, 1977) |
| Tobacco rinsgpot virus (TRSV) | D | Horváth (1976, 1977) |
| Tomato aspermy virus (TAV) | Tm12 | Horváth et al. (1980) |
| Tomato mosaic virus (ToMV) | D/H, Ob | Horváth (1976, 1977) Mamula et al. (1974), Juretić et al. (1977), Csilléry et al. (1983) |

were sprayed with tap water. The experiments were performed in vector-free virological glasshouse. Symptoms were recorded after 10 and 21 days. Plants responding with local and systemic necrosis were regarded as susceptible.

## Results and Discussion

Between the 20 *Capsicum* species and varieties included in the experiment described in present paper and 13 viruses 144 compatible host-virus relations affecting both the inoculated and non-inoculated leaves were found. On the basis of the symptoms the local and systemic susceptibility of the plants was placed in three groups.

The 67 relations established between 18 *Capsicum* species and varieties and 12 viruses belonging to group I were characterized by cholorotic/necrotic lesions appearing on the inoculated leaves, as well as by a mosaic symptom displayed by the non-inoculated leaves (Table 3). Remarkable was the susceptibility to broad bean wilt virus in the species examined. The increasing frequency of the aphid-borne virus in pepper cultures (cf. Lovisolo and Conti, 1976; Conti and Masenga, 1977; Salamon et al., 1980a, b) is of an ever greater importance. The legume or bean strain (U/246) of cucumber mosaic virus isolated from *Phaseolus vulgaris* in Hungary (cf. Schmidt and Horváth, 1982; Horváth and Beczner, 1983) also proved highly pathogenic for the individual *Capsicum* species. The so-called pepper strain (Ob) of tomato mosaic virus isolated from *Capsicum annuum* cv. *Soroksári* (cf. Csilléry and Ruskó, 1980; Csilléry et al., 1983) while only inducing systemic symptoms in some *Capsicum annuum* species *(D-Cecei, Westlandia)* caused both local and systemic ones in others (Tóbiás et al., 1982). Among the *Capsicum chinense* varieties there were, likewise, ones only showing systemic susceptibility (e.g. *D7)* while some of them (e.g. *D8, Miscucho, P. I. 159236)* exhibited local susceptibility (Tóbiás et al., 1982; Csilléry et al., 1983). In the experiments performed by Tóbiás et al. (1982) *Capsicum frutescens* var. *Tobasco* was found to be locally and systemically susceptible. According to the results of our own experiments the *Capsicum frutescens* plants responded with chlorotic/necrotic local lesions and systemic mosaic to infection by the pepper strain of tomato mosaic virus. Out of the new *Capsicum* species susceptible to the belladonna mottle virus (see Table 3) *Capsicum frutescens* is of special importance, because – as it is known – in the United States of America the virus even occurs spontaneously in it (Lee et al., 1975). In Europe the *Capsicum* spp. has not been known so far as a natural host to the virus (Horváth, 1979), although *Capsicum annuum* is a known indicator of the virus.

The resistance of *Capsicum chinense (P. I. 159237)* to the pepper strain of tomato mosaic virus (Tóbiás et al., 1982; Csilléry et al., 1983) is important if only because the accession in question is known to be resistant to the pepper mottle virus as well (Zitter, 1972). *Capsicum barbatum* var. *pendulum* showed the recovery phenomenon when infected by the systemic strain (MvS70) of the alfalfa

Table 3

Reaction of *Capsicum* species and varieties to different viruses (Group I: Chlorotic/necrotic local lesions and systemic mosaic)

| *Capsicum* species and varieties[1] | Viruses[2] |
|---|---|
| *C. annuum* var. *cerasiforme* | BBWV, CMV-U/246, PVX, ToMV-Ob |
| *C. annuum* var. *conoides** | BBWV, CMV-U/246, PVY, ToMV-Ob, TRSV |
| *C. annuum* var. *longum* | BBWV |
| *C. annuum* var. *minimum* | BBWV |
| *C. annuum* var. *paramidale** | CMV-U/246, PVX, PVY |
| *C. baccatum* | BBWV, CMV-U/246, CMV-W, BMV, MYFV, PVX, PVY, TRV, ToMV-D/H |
| *C. barbatum* var. *pendulum* | AMV-MvS70, BBWV, PVX, ToMV-D/H |
| *C. cardenasii* | AMV-$K_2$, BBWV |
| *C. chinense* | AMV-MvS70, BBWV, BMV, MYFV, TMV-$U_1$, TRV, ToMV-D/H |
| *C. eximium** | AMV-MvS70, BBWV |
| *C. flexuosum* | BBWV, BMV, MYFV, PVX, TRV |
| *C. frutescens* | CMV-U/Ascl, BMV, MYFV, ToMV-Ob |
| *C. grossum** | CMV-U/246, PVX, PVY |
| *C. luteum** | BBWV, BMV, MYFV, PVX, PVY, TRV |
| *C. minimum* | BMV |
| *C. nigrum** | CMV-U/246 |
| *C. praetermissum* | BMV |
| *C. testiculatum* | CMV-U/246, BBWV, BMV, PVX, PVY, TRSV, TAV, ToMV-D/H |

[1] Plants marked with asterisks are species new to experimental virology
[2] The names of viruses are listed in Table 2

mosaic virus. The local and systemic susceptibility to 9 viruses (alfalfa mosaic virus, belladonna mottle virus, broad bean wilt virus, cucumber mosaic virus, *Melandrium* yellow fleck virus, potato virus X, potato virus Y, tomato mosaic virus, tobacco ringspot virus) of the six *Capsicum* spp. and varieties (Table 3) which to our best knowledge have not been known so far in the literature on plant virology, is remarkable. The economic importance of the *Melandrium* yellow fleck virus, a plant virus recently isolated in Hungary (Hollings and Horváth, 1981), is suggested by the fact that some *Capsicum* species and varieties (e.g. *Capsicum baccatum*, *C. chinense*, *C. flexuosum*, *C. frutescens*, *C. luteum*) are susceptible to it.

In group II the 13 cases of relationship between 10 *Capsicum* species and varieties and 5 viruses were characterized by necrotic lesions on the inoculated leaves of plants and various degrees of necrotization on the non-inoculated leaves; the plants belonging to this group were relatively tolerant compared to those in group III, as they were not destroyed by the disease (Table 4). Particularly remarkable is the susceptibility to tobacco ringspot virus in the new species and

Table 4

Reaction of *Capsicum* species and varieties to different viruses (Group II: Necrotic local lesions and systemic necrosis)

| *Capsicum* species and varieties[1] | Viruses[2] |
|---|---|
| *C. annuum* var. *cerasiforme* | TRSV |
| *C. annuum* var. *longum* | AMV-$K_2$ |
| *C. baccatum* | TRSV |
| *C. barbatum* var. *pendulum* | TRSV |
| *C. chacoense* | AMV-$K_2$ |
| *C. eximium** | TRSV |
| *C. flexuosum* | TRSV |
| *C. grossum** | TRSV |
| *C. luteum** | TRSV |
| *C. nigrum** | BBWV, PVX, PVY, TRSV |

[1] Plants marked with asterisks are species new to experimental virology
[2] The names of viruses are listed in Table 2

Table 5

Reaction of *Capsicum* species and varieties to different viruses (Group III: Local and systemic necrotic lesions, leaf drop, death of the plants)

| *Capsicum* species and varieties[1] | Viruses[2] |
|---|---|
| *C. annuum* var. *cerasiforme* | AMV-$K_2$, AMV-MvS70, PAMV, TMV-$U_1$, ToMV-D/H |
| *C. annuum* var. *conoides** | AMV-$K_2$, PAMV, PVX, TMV-$U_1$, ToMV-D/H |
| *C. annuum* var. *longum* | PAMV, PVX, TMV-$U_1$, TRSV, ToMV-D/H |
| *C. annuum* var. *minimum** | AMV-$K_2$, PVY |
| *C. annum* var. *paramidale** | AMV-$K_2$, BBWV, PAMV |
| *C. baccatum* | AMV-$K_2$, AMV-MvS70, CMV-U/246, PAMV, TMV-$U_1$, ToMV-Ob |
| *C. barbatum* var. *pendulum** | CMV-U/Ascl, CMV-U/246, PAMV |
| *C. chinense* | PAMV, PVX |
| *C. eximium** | CMV-U/246, PVX, ToMV-D/H |
| *C. flexuosum* | CMV-U/Ascl, TMV-$U_1$, ToMV-D/H |
| *C. grossum** | AMV-$K_2$, BBWV, PAMV, TMV-$U_1$, ToMV-Ob |
| *C. luteum** | AMV-$K_2$, CMV-U/246, CMV-U/Ascl, CMV-W, PAMV |
| *C. minimum* | AMV-$K_2$, CMV-U/246, PVY |
| *C. nigrum** | AMV-$K_2$, PAMV, TMV-$U_1$, ToMV-D/H, ToMV-Ob |
| *C. pendulum* | ToMV-Ob |
| *C. praetermissum* | AMV-$K_2$, BBWV, CMV-U/246, PAMV, PVX, PVY |
| *C. testiculatum* | AMV-$K_2$, PAMV |

[1] Plants marked with asterisks are species new to experimental virology
[2] The names of viruses are listed in Table 2

varieties (*Capsicum barbatum* var. *pendulum*, *C. eximium*, *C. grossum*, *C. luteum*, *C. nigrum*), which – as far as we know – have not been studied so far in plant virology; but no little importance is attached to the susceptibility of *Capsicum nigrum* to broad bean wilt virus, potato virus X and potato virus Y either.

In the 64 relations of group III between 17 *Capsicum* species and varieties and 9 viruses the plants exhibited very severe local and systemic necrotic symptoms. As a consequence of the disease the plants died (Table 5). These plants are highly responsive to infection by the viruses concerned. The 31 host-virus relations described in the case of species see (Table 5) known to us as new in plant virology are important from a diagnostical point of view as well.

## Acknowledgements

I am indebted to the following botanical gardens and institutes: University of Birmingham, Department of Plant Pathology, Birmingham (England), Botanical Garden, University of Copenhagen, Copenhagen (Danmark), Central Institute of Genetics and Cultivated Plants, Gatersleben (German Democratic Republic), Botanical Garden, Botanical Institute, Kaunas (Soviet Union) and Botanical Garden of the University of Nijmegen (the Netherlands) for putting the seeds of the experimental *Capsicum* species and varieties at our disposal.

Thanks are due to Miss K. Molnár and Mrs É. Bősze for their technical assistance in the experiments.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 59–62 (1986)*

# Compatible and Incompatible Relations between *Capsicum* Species and Viruses

## III. New Incompatible Host-Virus Relations (Resistant and Immune Plants)

J. HORVÁTH

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

Between 11 *Capsicum* species and varieties and 6 viruses we pointed out 19 resistant host-virus relations in which the inoculated plants responded with local necrotic lesions and leaf drop to infection. After the infection restricted to the inoculated leaves the viruses could not be reisolated to indicator plants from the resistant plants.

Between *Capsicum annuum* var. *minimum* and *C. annuum* var. *paramidale* on the one hand, and tobacco necrosis virus on the other, we found incompatible relation in which neither the inoculated nor the non-inoculated leaves of the plants could be infected (immune plants), and from which the virus could not be reisolated to indicator plants.

In the course of virological experiments carried out with various *Capsicum* species and varieties a part of the plants though got infected by the different viruses, but the reproduction of the viruses did not cause systemic disease to the plants. As pointed out in a previous publication we considered these plants – similarly to other authors (e.g. Rast, 1982) – to be resistant (cf. Horváth, 1986b). Another part of the plants could not be infected with the different viruses. We regarded them as immune plants. From the point of view of breeding the resistant and immune plants are very important. In the course of experiments aimed at locating the resistance genes some new sources of virus resistance became known of which we give account in the present paper.

## Materials and Methods

In the course of studying *Capsicum* species and varieties we succeeded in finding 13 *Capsicum* species and varieties, respectively, which were resistant to or immune of certain viruses. According to our knowledge 7 of the species and varieties in question are new to virology. We inoculated the *Capsicum* plants with viruses from our virus gene bank using carborundum-spatula technique (see Horváth, 1986b). Following the inoculation both the inoculated and non-inoculated leaves were surface disinfected with 2% NaOH solution, then washed with a strong jet

of water. After that with the water diluted tissue sap of inoculated and non-inoculated leaves virus reisolation was carried out to specifically responsive indicator plants (e.g. *Chenopodium amaranticolor*, *Nicotiana glutinosa*, *N. tabacum* cv. *Xanthi-nc*, *Solanum demissum A6-hybrid;* cf. Horváth, 1976, 1977, 1984a, b, c; Zschüttig and Horváth, 1968). Symptoms were recorded after 10 and 21 days. Plants reacting only with chlorotic/necrotic local lesions followed by abscission of the inoculated leaves (hypersensitive reaction) were considered resistant, while those which showed no symptoms and the viruses could not be reisolated from them were regarded as immune.

## Results and Discussion

1. *Resistant plants*

In the course of our inoculation experiments 11 *Capsicum* species and varieties proved infectible hosts for 6 viruses; the reproduction of the virus and the appearance of symptoms were confined to the inoculated leaves of them. Appearance of local necrotic lesions and leaf drop of inoculated leaves (hypersensitive reaction)were found in 19 relations between 11 plants and 6 viruses (Table 1). Attempts to reisolate the virus(es) from non-inoculated leaves were successful in no case, while from inoculated leaves exhibiting disease symptoms the virus(es) could be reisolated to indicator plants. Thus, the infection only affected inoculated leaves, the viruses did not ever cause systemic disease to the plants. Of the new sources of resistance special attention is worth being paid to *Capsicum barbatum* var. *pendulum* and *C. pubescens* which proved resistant to five viruses (see Table 1). Resistance to cucumber mosaic virus was pointed out in *Capsicum annuum* var. *longum* and *C. pubescens* plants. Also remarkable is the resistance to potato virus Y in *Capsicum barbatum* var. *pendulum*, *C. chinense*, *C. eximium*, *C. flexuosum* and *C. pubescens*. The resistance of *Capsicum chinense* to potato virus Y (see Table 1) is important only because some accessions of the plant in question are known to be resistant to the tobacco- and tomato strains of tobacco mosaic virus (cf. Rast, 1982). The reason why we consider the resistance of the wild *Capsicum eximium* to potato virus Y important is that this wild species is readily crossed with the species *Capsicum cardenasii* (Esbaugh, 1975) which has recently been found also resistant to potato virus Y (reviewed by Horváth, 1986a).

2. *Immune plants*

In our experiments *Capsicum annuum* var. *minimum* and *C. annuum* var. *paramidale* plants equally showed immunity to tobacco necrosis virus (Table 2). Neither the inoculated nor the non-inoculated leaves showed symptoms of inoculation by the virus, and the virus could not be reisolated from them to indicator plants. Of the two *Capsicum* plants, *C. annuum* var. *minimum* was found to be susceptible to alfalfa mosaic virus, broad bean wilt virus, potato virus Y, while

Table 1

Resistant *Capsicum* species and varieties to different viruses
(Local necrotic lesions with leaf drop)

| *Capsicum* species and varieties[1] | Viruses[2] |
|---|---|
| *C. annuum* var. *conoides** | TNV |
| *C. annuum* var. *longum* | CMV-U/246, TNV |
| *C. barbatum* var. *pendulum** | PVY, TAV, TMV-$U_1$, ToMV-Ob |
| *C. chinense* | PVY |
| *C. eximium** | PVY |
| *C. flexuosum* | PVY |
| *C. grossum** | TNV |
| *C. luteum** | TNV |
| *C. praetermissum* | ToMV-D/H |
| *C. pubescens* | CMV-U/246, PVY, ToMV-D/H, ToMV-O |
| *C. testiculatum* | ToMV-Ob, TNV |

[1] Plants marked with asterisks are species new to experimental virology

[2] Abbreviations used for viruses: CMV = cucumber mosaic virus (bean strain), PVY = potato virus Y, TAV = tomato aspermy virus, TMV = tobacco mosaic virus (common strain), TNV = tobacco necrosis virus, ToMV-D/H = tomato mosaic virus (tomato strain), ToMV-Ob = tomato mosaic virus (pepper strain)

Table 2

Immune *Capsicum* varieties to tobacco necrosis virus
(Plants cannot be infected)

| *Capsicum* varieties[1] | Virus |
|---|---|
| *C. annuum* var. *minimum* | Tobacco necrosis virus |
| *C. annuum* var. *paramidale* | Tobacco necrosis virus |

[1] The investigated two *Capsicum* varieties are new to experimental virology

*C. annuum* var. *paramidale* to alfalfa virus, broad bean wilt virus, cucumber mosaic virus, potato aucuba mosaic virus, potato virus X and potato virus Y, as we pointed it out in a previous publication (Horváth, 1986b). To our best knowledge the incompatible relationship between *Capsicum annuum* and tobacco necrosis virus is only important from a general virological–first of all virus separation–point of view, therefore the immunity of the above species is of minor consequence for the work of resistance breeding.

## Acknowledgements

I am indebted to the following botanical gardens and institutes: University of Birmingham, Department of Plant Pathology, Birmingham (England), Botanical Garden, University of Copenhagen, Copenhagen (Denmark), Central Institute of Genetics and Cultivated Plants, Gatersleben (German Democratic Republic), Botanical Garden, Botanical Institute, Kaunas (Soviet Union) and Botanical Garden of the University of Nijmegen (the Netherlands) for putting the seeds of the experimental *Capsicum* species and varieties at our disposal.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 63—72 (1986)*

# Physiological and Biochemical Studies on Soybean, Bean and Lettuce Plants Infected with Seed-Borne Viruses

R. A. OMAR, F. F. MEHIAR, E. A. ZAYED and A. A. DIEF

Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt

The soybean mosaic virus (SBMV), bean common mosaic virus (BCMV) and lettuce mosaic virus (LMV) caused a significant reduction in chlorophyll a, b and carotenoid contents in leaves.

Reducing and non-reducing sugars were decreased significantly in leaves and seeds of infected hosts. Glucose was not affected as a result of viral infection, while sucrose and fractose contents in leaves were decreased in most cases. Only fractose and sucrose were found in the seeds produced by infected hosts, though they were decreased as a result of the infection. Only raffinose was found in the seeds of infected lettuce plants, and was also decreased as a result of the infection.

The concentration of total amino acids and free amino acids in infected leaves and seeds differed according to the virus, the host plant and the duration of sampling.

The soybean mosaic virus (SBMV), bean common mosaic virus (BCMV) and lettuce mosaic virus (LMV) are economic seed-borne viruses. Like other plant viruses, they are known to induce several changes in the physiological and biochemical features of the host plant. The present work aimed to investigate the effect of these viruses on leaf pigments, reducing and non-reducing sugars, total amino acids and free amino acids in the respective host plants. It is an attempt to find any common features which make the transmission of these viruses through seeds possible. The study may also reveal any abnormalities in the host plants resulting from virus infection, and which may prove useful in detecting infected seeds.

## Materials and Methods

The plants of soybean var. Calland, bean var. Suisse Blanc and lettuce var. Balady grown in 15 cm diameter pots, filled with sandy loam soil under insect-proof greenhouse conditions were used in these studies. Seedlings of the soybean, bean and lettuce hosts were mechanically inoculated with SBMV, BCMV and LMV, respectively. Uninoculated seedlings were left for comparison. The studied viruses were isolated and identified from the naturally infected plants according

to host range, stibility modes of transmission and electron microscopy. Leaf pigments, sugars, total amino acids and free amino acid contents were determined as follows:

*Leaf pigments*

Samples were taken at an early stage of infection (10 days after inoculation) and following disease establishment (35 days after inoculation). Chlorophyll a, b and carotenoids were determined according to the method of Wettstein (1975) using spectrophotometer model spectronic 20.

*Sugar contents*

Leaf samples were collected 10 and 45 days after inoculation, while seed samples were collected after maturity. Total soluble sugars and reducing sugars were determined according to the method of Shaffer and Somogyi (1945). Thin layer chromatographic technique was used for the qualitative and quantitative analyses of soluble sugar content fractions of leaf and seed samples (Gauch et al., 1979). For quantitative determination of sugar fractions, the fractionated sugars were eluted from the chromatogram and colorimetrically measured using the phenol-sulphuric acid method as described by Smith et al. (1956).

*Amino acid contents*

Leaf samples were collected 10 and 45 days after inoculation, while seed samples were collected after maturity. Total amino acids composition of leaves and seeds were determined using the method of Block et al. (1958). One dimensional descending paper chromatographic technique was used for separation and determination of free amino acids according to the method of Ronald and Gross (1954). Two replicates were used for each sample.

Statistical analysis was performed using the multiple range test (Duncan, 1955).

## Results

*Leaf pigment contents*

Data presented in Table 1 indicate that the amounts of chlorophyll a, b and carotenoids and their total amount were significantly reduced in infected host leaves at all sampling durations.

*Sugar contents*

Data presented in Tables 2 and 3 show that reducing sugars and non-reducing sugars and their total in leaves and seeds were all significantly decreased as a result of viral infection.

Table 1

Effects of SBMV, BCMV and LMV on the content of pigments (mg/g dry weight) in soybean, bean and lettuce leaves, respectively

| Pigments content | Content of pigments in leaves after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | | | BCMV | |
| | 10 days | | 35 days | | 10 days | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Chlorophyll a | 3.71[a] | 3.08[b] | 5.83[a] | 4.27[b] | 6.63[a] | 4.74[b] |
| Chlorophyll b | 1.27[a] | 1.08[b] | 2.82[a] | 2.09[b] | 1.61[a] | 1.54[b] |
| Carotenoids | 1.49[a] | 1.09[b] | 1.91[a] | 1.81[b] | 0.92[a] | 0.79[b] |
| Total amount | 6.47[a] | 5.25[b] | 10.56[a] | 8.17[b] | 9.16[a] | 7.07[b] |

| Pigments content | Content of pigments in leaves after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | BCMV | | LMV | | | |
| | 35 days | | 10 days | | 35 days | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Chlorophyll a | 6.50[a] | 5.28[b] | 7.04[a] | 5.86[b] | 9.90[a] | 7.60[b] |
| Chlorophyll b | 2.60[a] | 2.02[b] | 1.86[a] | 1.72[b] | 2.65[a] | 2.02[b] |
| Carotenoids | 10.10[a] | 1.01[b] | 1.34[a] | 1.12[b] | 2.77[a] | 2.67[b] |
| Total amount | 19.20[a] | 8.31[b] | 10.24[a] | 8.70[b] | 15.32[a] | 12.29[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test.

Table 2

Effects of SBMV, BCMV and LMV on soluble sugar contents (mg glucose/g dry weight) in soybean, bean and lettuce leaves, respectively

| Content of soluble sugars in leaves | Period after inoculation (days) | Soybean infected with SBMV | | Bean infected with BCMV | | Lettuce infected with LMV | |
|---|---|---|---|---|---|---|---|
| | | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Reducing | 10 | 22.25[a] | 14.41[b] | 23.20[a] | 15.45[b] | 21.40[a] | 15.29[b] |
| | 45 | 24.98[a] | 18.91[b] | 27.19[a] | 20.98[b] | 25.37[a] | 19.42[b] |
| Non-reducing | 10 | 1.13[a] | 0.91[b] | 2.15[a] | 1.66[b] | 1.85[a] | 1.03[b] |
| | 45 | 1.33[a] | 1.08[b] | 3.29[a] | 2.19[b] | 2.09[a] | 1.54[b] |
| Total amount | 10 | 23.38[a] | 15.32[b] | 25.35[a] | 17.11[b] | 23.25[a] | 16.32[b] |
| | 45 | 26.31[a] | 19.99[b] | 30.48[a] | 23.17[b] | 27.46[a] | 20.96[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test.

Table 3

Effects of SBMV, BCMV and LMV on soluble sugar contents (mg glucose/g dry weight) in soybean, bean and lettuce seeds, respectively

| Soluble sugars | Content of soluble sugars in seeds of | | | | | |
|---|---|---|---|---|---|---|
| | Soybean obtained from | | Bean obtained from | | Lettuce obtained from | |
| | Healthy | SBMV infected plants | Healthy | BCMV infected plants | Healthy | LMV infected plants |
| Reducing | 15.61[a] | 12.10[b] | 18.49[a] | 14.22[b] | 12.84[a] | 10.63[b] |
| Non-reducing | 2.11[a] | 1.48[b] | 2.33[a] | 1.65[b] | 1.06[a] | 0.81[b] |
| Total amount | 17.72[a] | 13.58[b] | 20.82[a] | 15.87[b] | 13.90[a] | 11.44[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test.

Table 4

Effects of SBMV, BCMV and LMV on soluble sugar fractions (mg/g dry weight) in soybean, bean and lettuce leaves, respectively

| Sugars | Samples of leaves collected at 10 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Glucose | 2.80[a] | 3.30[a] | 4.30[a] | 4.30[a] | 3.60[a] | 4.20[a] |
| Fractose | 7.50[a] | 4.60[b] | 6.20[a] | 4.90[b] | 7.00[a] | 5.00[b] |
| Sucrose | 8.50[a] | 7.30[b] | 7.00[a] | 6.00[b] | 4.20[a] | 3.10[b] |

| Sugars | Samples of leaves collected at 45 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Glucose | 5.10[a] | 4.00[b] | 4.00[a] | 3.50[a] | 8.00[a] | 5.50[b] |
| Fractose | 10.50[a] | 7.60[b] | 9.70[a] | 8.00[a] | 9.40[a] | 7.50[b] |
| Sucrose | 70.00[a] | 7.00[b] | 10.00[a] | 6.00[b] | 8.20[a] | 5.10[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test.

Data in Table 4 show that glucose was not significantly affected in soybean leaves 10 days after inoculation with SBMV, whereas it was significantly decreased at 45 days. The same virus caused a highly significant reduction in fractose and sucrose contents in soybean leaves at both sampling dates, i.e. 10 and 45 days after inoculation. No significant effect was observed in glucose content in bean

Table 5

Effects of SBMV, BCMV and LMV on soluble sugar fractions (mg/g dry weight) in soybean, bean and lettuce seeds, respectively

| Sugars | Seeds produced from plants infected with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Glucose | — | — | — | — | — | — |
| Fractose | 5.50[a] | 4.50[b] | 6.90[a] | 5.30[b] | 4.00[a] | 2.70[b] |
| Sucrose | 10.50[a] | 7.50[b] | 12.20[a] | 8.50[b] | 5.30[a] | 4.20[b] |
| Raffinose | — | — | — | — | 3.00[a] | 2.10[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test.
— = Absent.

leaves 10 and 45 days after inoculation with BCMV. Fractose was decreased 10 days after inoculation, whereas it was not significantly affected at 45 days. BCMV caused significant reduction in sucrose at 10 and 45 days. LMV had no significant effect on glucose at 10 days after inoculation, but reduced it significantly at 45 days. Fractose and sucrose were both significantly decreased 10 and 45 days after inoculation with LMV.

Data in Table 5 show that only fractose and sucrose were found in the seeds produced by infected soybean, bean and lettuce plants, though they were decreased as a result of the infection. Only raffinose was found in the seeds of infected lettuce plants and was also decreased as a result of the infection.

*Amino acid contents*

Table 6 presents data on contents of total amino acid in leaves. Some amino acids were significantly affected (increased or decreased), whereas others were not significantly affected as a result of viral infection. Leucine, proline, glutamic acid, aspartic acid, arginine and threonine were significantly increased at 10 days of inoculation, whereas methionine and serine were decreased at 45 days of virus inoculation. The other amino acids in the infected plants were varied according to the virus, the host plant and the duration of sampling.

Table 6

Effects of SBMV, BCMV and LMV on total amino acid contents (mg/100 g dried leaves in soybean, bean and lettuce leaves, respectively

| Amino acids | 10 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Leucine | 41.6[b] | 47.6[a] | 41.7[b] | 46.5[a] | 30.1[b] | 33.6[a] |
| Isoleucine | 19.9[b] | 22.1[a] | 30.3[a] | 27.5[b] | 17.7[a] | 17.6[a] |
| Phenylalanine | 105.6[a] | 89.9[b] | 85.7[a] | 76.6[b] | 90.5[b] | 108.6[a] |
| Cysteine | T | T | — | — | — | — |
| Methionine | 41.2[a] | 37.3[b] | 36.0[a] | 22.1[b] | 28.4[a] | 28.5[a] |
| Proline | 224.7[b] | 237.5[a] | 181.2[b] | 201.9[a] | 162.9[b] | 166.7[a] |
| Alanine | 71.4[b] | 77.6[a] | 54.1[b] | 63.2[a] | 50.4[a] | 50.6[a] |
| Valine | T | T | T | T | 20.6[a] | 17.4[b] |
| Glycine | 76.4[a] | 78.2[b] | 63.1[a] | 54.1[b] | 47.1[a] | 44.5[b] |
| Glutamic acid | 82.0[b] | 87.5[a] | 45.9[b] | 53.2[a] | 44.4[b] | 52.5[a] |
| Aspartic acid | 140.3[b] | 147.4[a] | 88.2[b] | 130.7[a] | 74.8[b] | 86.1[a] |
| Serine | 31.4[a] | 28.2[b] | 21.3[b] | 30.5[a] | 25.3[b] | 25.7[a] |
| Arginine | 152.4[b] | 171.4[a] | 120.4[b] | 163.2[a] | 84.6[b] | 140.4[a] |
| Threonine | 52.5[b] | 54.4[a] | 22.7[b] | 25.5[a] | 21.4[b] | 22.9[a] |
| Tyrosine | — | — | T | T | T | T |
| Hydroxyproline | T | T | T | T | T | T |
| Total | 1039[b] | 1079[a] | 795[b] | 895[a] | 698[b] | 795[a] |

| Amino acids | 45 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Leucine | 50.8[b] | 55.1[a] | 43.4[b] | 54.4[a] | 43.3[a] | 42.1[b] |
| Isoleucine | 19.9[b] | 23.8[a] | 31.7[b] | 35.2[a] | 20.3[a] | 19.4[b] |
| Phenylalanine | 150.9[b] | 162.5[a] | 124.5[b] | 144.2[a] | 116.8[b] | 136.0[a] |
| Cysteine | T | T | — | — | — | — |
| Methionine | 50.5[a] | 40.9[b] | 53.9[a] | 42.9[b] | 35.8[a] | 32.2[b] |
| Proline | 270.8[a] | 260.3[b] | 208.0[b] | 235.8[a] | 210.1[b] | 216.5[a] |
| Alanine | 83.0[b] | 90.4[a] | 74.7[a] | 74.9[a] | 66.1[a] | 66.4[a] |
| Valine | T | T | T | T | 25.5[a] | 25.5[a] |
| Glycine | 75.0[b] | 85.9[a] | 63.2[a] | 50.5[b] | 37.7[b] | 57.2[a] |
| Glutamic acid | 80.7[b] | 94.3[a] | 60.9[b] | 67.3[a] | 52.0[a] | 50.6[b] |
| Aspartic acid | 164.5[b] | 173.0[a] | 107.3[b] | 135.4[a] | 86.7[b] | 100.9[a] |
| Serine | 44.5[a] | 40.0[b] | 37.5[a] | 35.7[b] | 36.1[a] | 30.3[b] |
| Arginine | 204.4[a] | 203.8[b] | 196.9[a] | 185.8[b] | 121.8[b] | 136.5[a] |
| Threonine | 63.6[a] | 55.4[b] | 29.6[a] | 24.9[b] | 22.3[b] | 25.7[a] |
| Tyrosine | — | — | T | T | T | T |
| Hydroxyproline | T | T | T | T | T | T |
| Total | 1259[b] | 1288[a] | 1032[b] | 1087[a] | 877[b] | 939[a] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test. T = Trace; — = Absent.

Table 7

Effects of SBMV, BCMV and LMV on total amino acid contents (mg/100 g dried seed) in seeds of soybean, bean and lettuce, respectively

| Amino acids | Total amino acid contents in seeds produced by plants infected with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Leucine | 75.85[a] | 61.12[b] | 54.75[a] | 54.75[a] | 38.00[a] | 32.75[b] |
| Isoleucine | 40.10[b] | 45.00[a] | 29.25[b] | 35.65[a] | 28.10[a] | 25.85[b] |
| Phenylalanine | 116.35[a] | 82.22[b] | — | — | — | — |
| Cysteine | T | T | — | — | — | — |
| Methionine | 52.04[a] | 43.12[b] | 55.90[a] | 55.54[a] | 42.10[a] | 36.25[b] |
| Proline | 248.30[b] | 253.15[a] | 228.90[a] | 203.72[b] | 235.25[b] | 242.10[a] |
| Alanine | 65.67[b] | 70.30[a] | 63.15[a] | 56.87[b] | 60.00[b] | 65.50[a] |
| Valine | T | T | T | T | T | T |
| Glycine | 82.95[a] | 72.06[b] | 53.40[b] | 57.92[a] | 53.70[b] | 58.60[a] |
| Glutamic acid | 73.72[a] | 65.50[b] | 65.72[a] | 51.12[b] | 56.75[b] | 67.12[a] |
| Aspartic acid | 140.10[b] | 147.30[a] | 111.50[b] | 114.00[a] | 118.70[a] | 103.10[b] |
| Serine | 66.95[a] | 49.45[b] | 54.72[a] | 41.70[b] | 26.72[b] | 29.37[a] |
| Argenine | 140.65[b] | 169.60[a] | 140.50[b] | 156.40[a] | 104.40[b] | 115.60[a] |
| Threonine | 27.97[b] | 37.25[a] | 21.12[b] | 22.05[a] | 19.58[b] | 22.70[a] |
| Tyrosine | — | — | — | — | — | — |
| Hydroxyproline | — | — | — | — | — | — |
| Total | 1129.7[a] | 1096.1[b] | 878.9[a] | 851.3[b] | 783.3[a] | 778.9[b] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test. T = Trace; — = Absent.

Data in Table (7) show total amino acid contents in seeds. The three studied viruses caused an increase of threonine and arginine, but caused an increase or decrease of other amino acids depending on the virus isolate and the host species.

Data on free amino acids in leaves are presented in Table 8. The viral infection resulted in a marked effect (increase or decrease) on the amounts of the different free amoni acids at different sampling durations. Proline, alanine and aspartic acids were significantly increased 10 days after inoculation. Only proline was increased, whereas methionine and serine were decreased 45 days after viral inoculation. The other free amino acids were varied according to the virus, the host and the duration of sampling.

Table 8

Effects of SBMV and LMV on free amino acid contents (mg/100 g fresh leaves) in soybean, bean and lettuce, respectively

| Amino acids | 10 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Leucine | 26.5[a] | 26.5[a] | 23.5[b] | 26.3[a] | 22.5[a] | 22.5[a] |
| Isoleucine | 13.5[b] | 15.2[a] | 20.0[a] | 20.0[a] | 13.7[b] | 15.2[a] |
| Phenylalanine | 40.04[a] | 36.2[b] | 54.3[a] | 50.2[b] | 73.6[b] | 90.4[a] |
| Cysteine | 10.3[b] | 14.3[a] | — | — | — | — |
| Methionine | 22.6[a] | 18.6[b] | 24.4[a] | 19.3[b] | 18.7[a] | 18.7[a] |
| Proline | 120.7[b] | 125.3[a] | 166.8[b] | 171.3[a] | 93.8[b] | 115.5[a] |
| Alanine | 38.3[b] | 48.2[a] | 33.3[b] | 39.8[a] | 34.6[b] | 39.1[a] |
| Valine | 16.3[a] | 16.3[a] | — | — | T | T |
| Glycine | 56.3[b] | 69.1[a] | 36.3[b] | 44.3[a] | 36.2[a] | 30.2[b] |
| Glutamic acid | 51.8[a] | 50.2[b] | 27.3[a] | 22.4[b] | 40.2[b] | 45.3[a] |
| Aspartic acid | 76.3[b] | 96.8[a] | 77.4[b] | 84.3[a] | 60.6[b] | 73.2[a] |
| Serine | 16.9[b] | 27.1[a] | 17.6[a] | 17.6[a] | 17.5[a] | 17.5[a] |
| Argenine | 74.1[a] | 70.0[b] | 64.8[b] | 100.3[a] | 56.3[b] | 64.3[a] |
| Threonine | 37.2[a] | 35.6[b] | 16.5[a] | 16.5[a] | 14.4[a] | 14.6[a] |
| Tyrosine | — | — | T | T | — | — |
| Hydroxyproline | — | — | — | — | — | — |
| Total | 601[b] | 650[a] | 561[b] | 612[a] | 481[b] | 594[a] |

| Amino acids | 45 days after inoculation with | | | | | |
|---|---|---|---|---|---|---|
| | SBMV | | BCMV | | LMV | |
| | Healthy | Infected | Healthy | Infected | Healthy | Infected |
| Leucine | 32.9[b] | 34.2[a] | 33.2[a] | 30.2[b] | 28.6[a] | 22.6[b] |
| Isoleucine | 26.7[a] | 20.0[b] | 25.0[b] | 27.2[a] | 15.6[a] | 15.6[a] |
| Phenylalanine | 93.6[a] | 86.2[b] | 103.5[b] | 96.2[b] | 92.3[b] | 110.7[a] |
| Cysteine | 17.2[b] | 18.7[a] | — | — | — | — |
| Methionine | 30.8[a] | 26.2[b] | 37.2[a] | 24.3[b] | 26.2[a] | 21.3[b] |
| Proline | 160.7[b] | 180.2[a] | 152.4[b] | 177.6[a] | 180.3[b] | 186.2[a] |
| Alanine | 70.7[b] | 75.5[a] | 44.1[a] | 44.1[a] | 40.7[a] | 36.6[b] |
| Valine | 44.7[a] | 34.0[b] | — | — | T | T |
| Glycine | 60.8[b] | 64.5[a] | 41.3[a] | 34.3[b] | 34.2[a] | 30.7[b] |
| Glutamic acid | 71.5[a] | 71.5[a] | 44.0[a] | 34.7[b] | 39.5[a] | 34.6[b] |
| Aspartic acid | 117.7[b] | 139.2[a] | 96.2[a] | 96.2[a] | 64.3[b] | 76.5[a] |
| Serine | 35.8[a] | 30.2[b] | 26.3[a] | 20.6[b] | 26.3[a] | 22.3[b] |
| Argenine | 143.6[a] | 113.3[b] | 98.2[b] | 110.6[a] | 100.2[b] | 107.3[a] |
| Threonine | 50.8[a] | 36.3[b] | 18.2[a] | 18.2[a] | 19.0[b] | 21.4[a] |
| Tyrosine | — | — | T | T | — | — |
| Hydroxyproline | — | — | — | — | — | — |
| Total | 958[a] | 930[b] | 720[a] | 714[b] | 665[b] | 686[a] |

Means followed by the different letter are significantly different (P = 0.05) according to Duncan's new multiple range test. T = Trace; — = Absent.

## Discussion

The obtained results revealed that the amounts of chlorophyll a, b and carotenoids in infected hosts were significantly decreased. These results agree with those of Diener (1963), Platt et al. (1979) and Abo El-Ghar (1981). Diener (1963) explained that the colour changes typical of most virus diseases make it obvious that chlorophyll is either not synthesized at the rate as in healthy plants or that some chlorophyll is destroyed as a consequence of virus infection.

The present study revealed that reducing and non-reducing sugars in both leaves and seeds were significantly decreased as a result of viral infection. Similar results were obtained by Singh and Mall (1978) and Abo El-Ghar (1981). Glucose was not affected as a result of viral infection, with the exception of two cases, i.e. at 45 days of infection in both sampling dates in all infected host plants, with the exception of fractose at 45 days of infection in bean. These results agree with those of Platt et al. (1979). Further, the viral infection led to a decrease in fractose and sucrose contents of seeds of the three studied hosts, a similar decrease in raffinose content was obtained in lettuce seeds only. Generally, the obtained results are in harmony with those of Diener (1963) who reported that the decreased photosynthetic activity, coupled with the increased respiratory rate generally observed in virus-infected leaves, should lead to decreased concentrations of assimilate, i. e., carbohydrates.

The current results indicate that the total amino acid contents differed in infected leaves according to the time of sampling after inoculation and the type of virus. Leucine, proline, glutamic acid, aspartic acid, arginine and threonine were significantly increased at 10 days after inoculation, whereas methionine and serine were decreased at 45 days after inoculation. These results agree with those obtained by Tu and Ford (1970), Sheir and Fegla (1975) and Hovis et al. (1970). Regarding seeds produced by virus infected plants, some amino acid contents increased (especially threonine and arginine) as a result of viral infection, whereas the others were increased or decreased depending on the virus and the host. These results agree with those obtained by Hovis et al. (1980). Similarly, free amino acid contents in leaves were increased or decreased as a result of viral infection. These results corroborate those of Tu and Ford (1970) and El-Halwagy (1977). Proline, alanine and aspartic acids were increased at the early stage of infection, whereas only proline was increased following disease establishment. The other free amino acids in infected plants were varied according to the virus species, the host plant and sampling duration. These results are in agreement with those obtained by Bozarth and Diener (1963) and Tu and Ford (1970.)

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 73—85 (1986)*

# Physiology of Rice Tungro Virus Disease: Possible Cause of Carbohydrate and Amino Acid Accumulation due to Infection

S. K. MOHANTY and R. SRIDHAR*

Department of Plant Pathology, Central Rice Research Institute, Cuttack 753006, India

Tungro infection resulted in significant accumulation of reducing sugars and soluble amino acids and decrease in total protein and an increase in protease activity in the leaf blades attached to plants. Similar changes were also characteristic of detached and dark-incubated healthy senescing leaf blade segments. The rise in the levels of reducing sugars and amino acids and proteolysis were retarded by kinetin and accentuated by abscisic acid in senescing healthy leaf blade segments. Tungro infection caused a reduction in the electrolyte leakage from the leaf blades, in contrast to an increase of the same in senescing leaf blade segments. The disease-induced metabolic alterations were the greatest in the leaf blades of a sensitive cultivar, and were less pronounced in those of an intermediate cultivar and a tolerant line. The significance of reduced cell permeability as a possible cause for the accumulation of low molecular weight compounds in the leaf blades, and for the inhibition or delay in flowering of tungro-diseased rice plants is discussed.

Tungro is a virus complex caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) transmitted in a semipersistent manner by green leafhoppers (Hibino et al., 1978; Saito et al., 1981). Tungro-diseased rice leaf blades accumulate low molecular weight compounds like sugars and amino acids (Sridhar et al., 1976). This phenomenon is a common feature in many virus-diseased plants (Matthews, 1981). Doubts have been expressed (Bawden, 1959) on the proposal that the disturbance in translocation due to phloem necrosis might be responsible for this. On the other hand, some effect of virus infection on mesophyll cells not yet understood has been thought of a possible cause which may interfere with the translocation of these plant constituents out of the leaves (Watson and Watson, 1951).

Many of the metabolic disturbances occurring in the virus-infected plants appear to be nonspecific. Similar alterations occur not only in infected plants, but also can appear during ageing under normal conditions or can be induced by environmental factors (Farkas and Solymosy, 1965; Matthews, 1981). In this

* Author to whom correspondences should be addressed.

report we compare certain metabolic changes induced by disease and senescence, suggesting a possible cause for the accumulation of low molecular weight compounds in tungro-affected rice leaf blades.

## Materials and Methods

*Plant materials and inoculation*

Rice (*Oryza sativa* L.) cultivars, Taichung Native 1 (sensitive) and IR 20 (intermediate), and the line CR 44–955 (tolerant) differing in their reaction to tungro by producing severe, intermediate and mild symptoms in response to inoculation were used. The method of plant culture is essentially the same as described in a previous communication (Mohanty and Sridhar, 1982). Twenty-five-day-old plants at the 3 to 4 leaf stage were caged individually in galvanized iron wire-net cages and were inoculated by confining two viruliferous green leaf hoppers [*Nephotettix virescens* (Distant)] in each cage for 8 h (Sridhar et al., 1976). Control plants were similarly treated with non-viruliferous insects.

*Tissue analyses*

The symptoms of the disease appeared in the form of interveinal chlorosis on the fifth leaf blades of the plants, which had emerged fully six days after inoculation. The fifth leaf blades were collected separately from 10 healthy and 10 virus-infected plants at 3 day intervals from 6 to 21 days after inoculation (zero to 15 days of leaf emergence). For the estimation of reducing sugars and soluble amino acids, leaf blade samples were extracted in boiling ethanol (Mahadevan and Sridhar, 1982). The concentrations of reducing sugars (Nelson, 1944) and total soluble amino acids (Moore and Stein, 1948) present in the alcohol extract were determined. Proteins present in the alcohol-insoluble residue were precipitated with 5 per cent trichloroacetic acid (Mahadevan and Sridhar, 1982), and their concentration was determined (Lowry et al., 1951). Protease activity was assayed (Naito et al., 1979) following the release of aromatic amino acids from casein solution using the aqueous extract of the enzyme (Mahadevan and Sridhar, 1982) obtained from another set of leaf blade samples. Protein content of the enzyme extract was determined (Lowry et al., 1951) after they were precipitated with 10 per cent trichloroacetic acid (Mahadevan and Sridhar, 1982). The enzyme activities are expressed in units. One unit is the amount of enzyme which caused a change of increase of one $A_{750}$ unit per milligram protein per hour.

*Permeability of cells*

Leaf blade samples (500 mg) placed in bathing solution contained in 100-ml Erlenmeyer flasks were incubated for 6 h at room temperature (28 $\pm$ 2 °C) with reciprocal shaking at 200 strokes per minute. The electrolytic conductance of the

ambient solution was measured in a conductivity meter (Systronics, Type 302) using a conductivity cell with 0.6 constant (Mahadevan and Sridhar, 1982). The cell permeability of leaf blades is expressed as microohms per gram fresh weight of the tissue.

*Senescence studies*

Changes in senescing healthy leaf blade segments were investigated following the floatation method (Tetley and Thimann, 1974). Five-cm apical segments of fully expanded second leaf blades from 20-day-old healthy seedlings of sensitive cultivar were floated with their adaxial side up in groups of five on 50 ml of either distilled water or $2 \times 10^{-5}$ M kinetin (Loba Chemie, Austria) of abscisic acid (Sigma Chemical Co., U.S.A.), in 10-cm Petri dishes. The Petri dishes were incubated in the dark at $28 \pm 2\,^{\circ}C$ for 3 days. The leaf segments remained floating throughout the experiment. The senescing leaf blade segments were collected at day zero and at 24 h intervals for three days, and were used for the determination of reducing sugars, amino acids and protein contents, protease activity and electrolyte leakage as described above. Although this study was performed with all the three rices differing in their sensitivity to the disease, results obtained only with the leaf blades of sensitive cultivar are presented as the trend of senescence-induced changes did not differ between cultivars/line.

*Number of determinations*

Data represent the mean of four determinations performed in duplicate with each of two identical extracts in case of tissue analysis and four replications in case of permeability measurements. The experiments were repeated once, and the data presented are from a typical experiment.

## Results

*Reducing sugars*

The concentration of reducing sugars gradually increased with the age of the leaf blades attached to plants in both healthy and tungro-infected plants (Fig. 1A–C). Significant sugar accumulation due to infection was noticed in the leaf blades of sensitive cultivar as early as 6 days after inoculation, while in those of intermediate cultivar and tolerant line, it occurred only after 9 days of inoculation. The rate of disease-induced sugar accumulation gradually increased with the disease development in the leaf blades of both sensitive and intermediate cultivars. However, in the leaf blades of tolerant line, the degree of sugar accumulation due to infection declined 12 days after inoculation, and subsequently showed a slow increase. The rate of disease-induced accumulation of sugars was the greatest in the leaf blades of sensitive cultivar when compared with those of intermediate cultivar and the tolerant line.

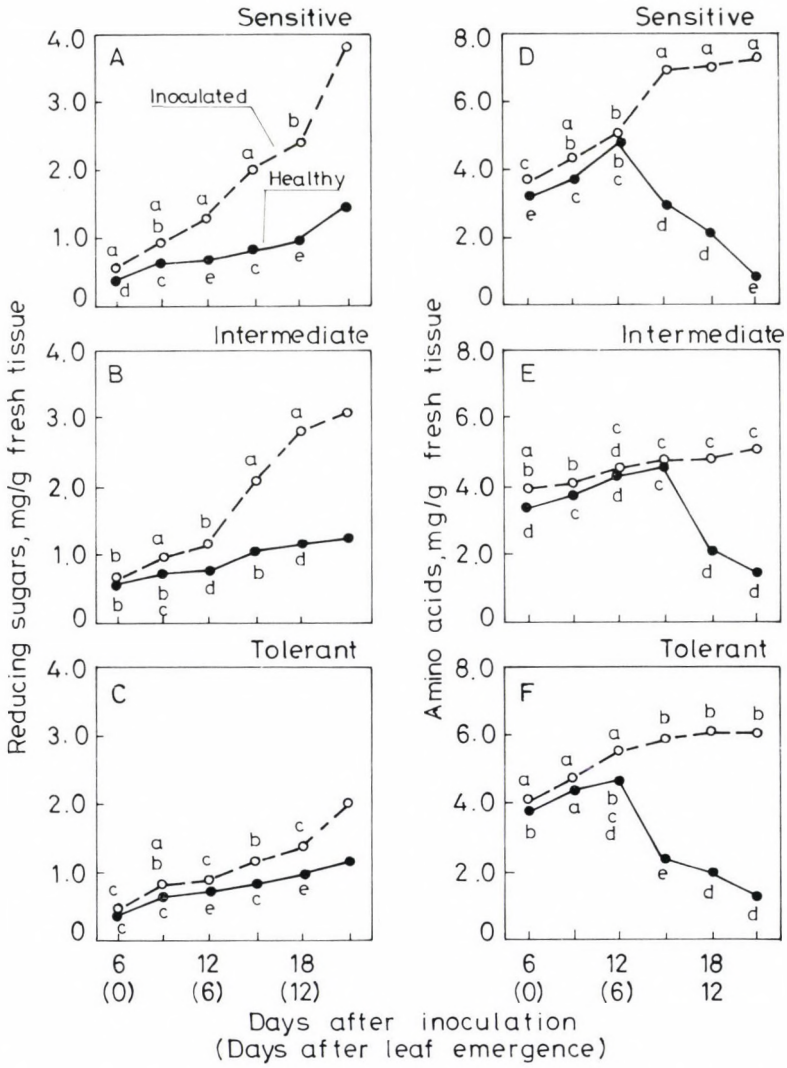



Fig. 1. Reducing sugar (A—C, glucose equivalents) and total soluble amino acid (D—F, glutamic acid equivalents) contents in the leaf blades of healthy (solid line) and tungro-infected (broken line) plants differing in their reaction to the disease. Rice cultivars/line sensitive (Taichung Native 1), intermediate (IR 20) and tolerant (CR 44–955) to tungro were inoculated at 3 to 4 leaf stage, and the fifth leaf blades were used for the determinations. Data points of a specific day of determination marked by different letters represent significant differences ($P = 0.05$) according to Fisher's least significant difference test

Reducing sugars invariably accumulated to a high level in the healthy leaf blade segments during three days of senescence in the dark (Fig. 2A). Kinetin retarded the rise in reducing sugar concentration during senescence. On the other hand, abscisic acid (ABA) markedly accelerated the increase in the quantity of reducing sugars in the leaf blade segments until 2 days of incubation. However, on the third day of incubation, the concentration of reducing sugars in the ABA-treated leaf blade segments was lower than that of untreated segments.

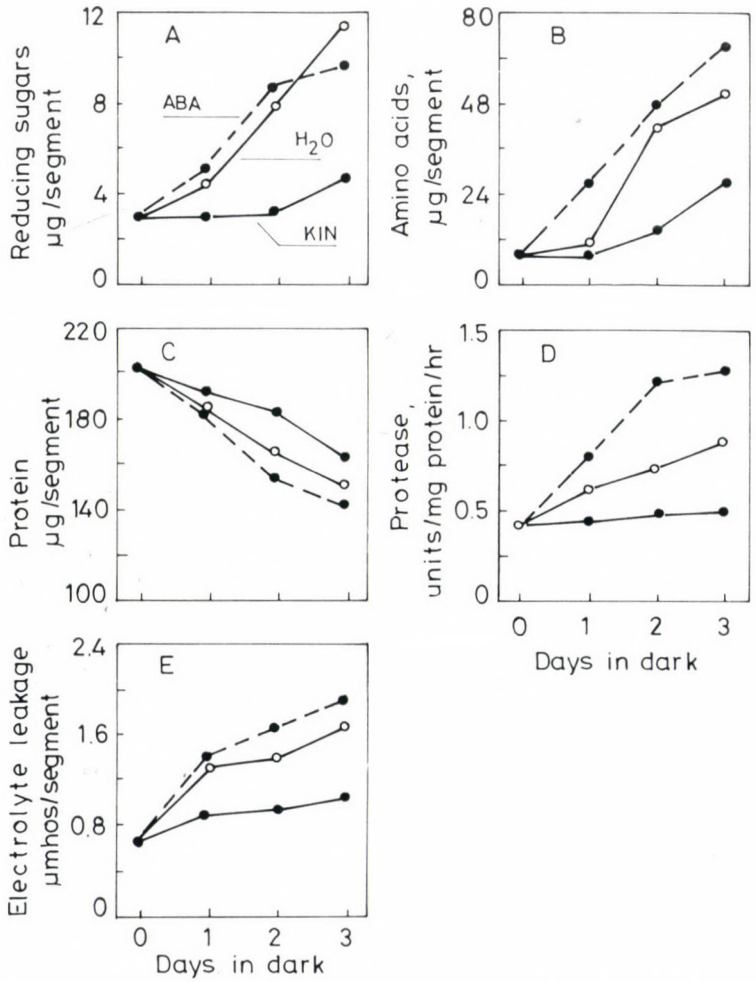



Fig. 2. Time course of reducing sugar (A), amino acid (B) and protein (C) concentrations, proteolytic enzyme activity (D) and of electrolyte leakage (E) in dark-incubated healthy senescing leaf blade segments of sensitive cultivar, Taichung Native 1 floated on $H_2O$ (open circle solid line) and on $2 \times 10^{-5}$ M kinetin (KIN; closed circle, solid line) and abscisic acid (ABA; closed circle, broken line) solutions

*Soluble amino acids*

The concentration of total soluble amino acids increased in the fifth leaf blades of healthy plants from day zero to 6 days of their emergence in sensitive cultivar and tolerant line, and until 9 days in those of intermediate cultivar, and declined thereafter (Fig. 1D–F). In contrast, the amount of soluble amino acids steadily increased in the leaf blades of inoculated plants until 15 days of leaf emergence in sensitive and intermediate cultivar and in those of tolerant line. Tungro infection resulted in significant accumulation of soluble amino acids in the leaf blades of sensitive and intermediate cultivars and in those of tolerant line even as early as 6 days after inoculation. However, the difference in the total concentration of soluble amino acids between healthy and infected leaf blades were not statistically significant 12 days after inoculation in sensitive cultivar, 12 and 15 days after inoculation in intermediate cultivar and 9 days after inoculation in tolerant line. Nevertheless, in general, the disease-induced increase in soluble amino acids was maximum in the leaf blades of sensitive cultivar.

The concentration of total soluble amino acids increased strikingly in senescing healthy leaf blade segments (Fig. 2B). Kinetin retarded the senescence-induced accumulation of soluble amino acids, while ABA stimulated it markedly.

*Total protein*

The fifth leaf blades of healthy plants contained the highest amounts of total proteins on the day of their complete emergence (Fig. 3A–C). Thereafter, protein content declined with leaf maturation in all the test plants. Tungro infection markedly reduced the concentration of total proteins in the leaf blades of sensitive cultivar from 6 to 21 days after inoculation. The rate of disease-induced decrease in protein content increased with the period after inoculation. The reduction in total protein content due to infection was significant in the leaf blades of intermediate cultivar only after 18 days of inoculation, while in those of tolerant line, significant decrease in the level of total protein was observed only 6 and 15 days after inoculation.

Total protein content markedly declined in detached healthy leaf blade segments during 3 days of senescence in the dark (Fig. 2C). Kinetin markedly retarded the reduction in protein content in the leaf blade segments, while ABA accelerated the loss of protein.

*Protease*

Protease activity gradually increased in the fifth leaf blades of healthy plants with the leaf maturation. In general, tungro infection significantly augmented the protease activity in the leaf blades of sensitive and of intermediate cultivars (Fig. 3D–E). The increase in protease activity due to infection was not significant in intermediate cultivar only in samples collected 9 and 12 days after

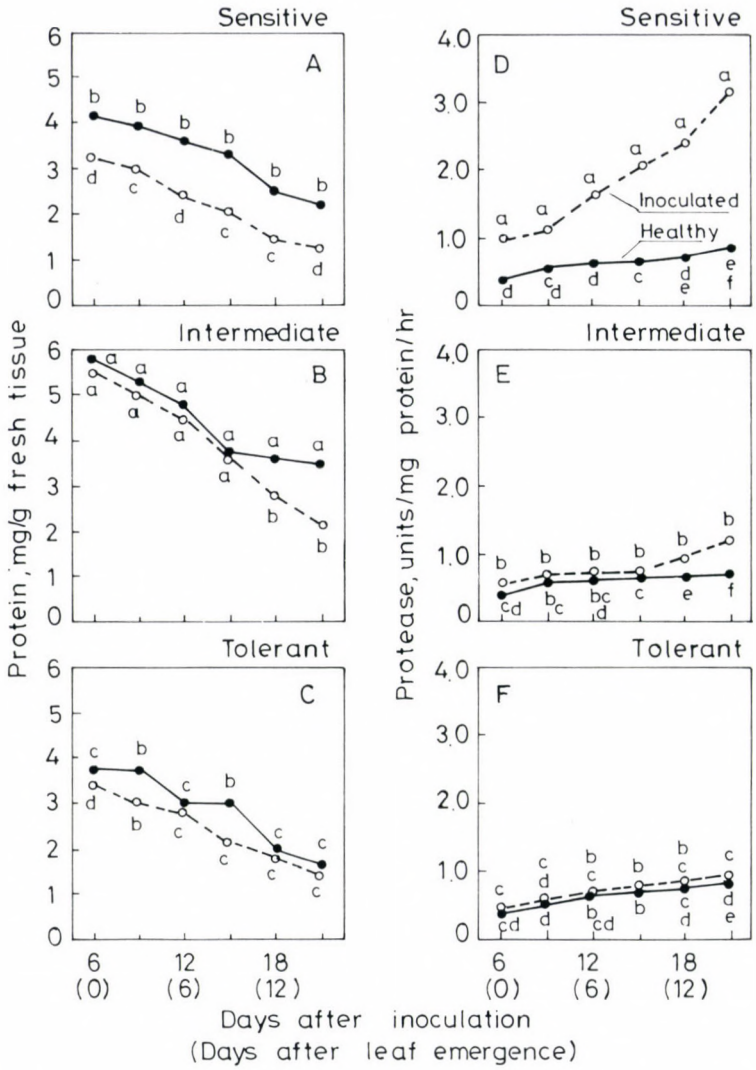



Fig. 3. Protein (bovine serum albumin equivalents) content (A—C) and protease activity (D—F) of the leaf blades of healthy (solid line) and tungro-infected (broken line) plants differing in their reaction to the disease. Rice cultivars/line sensitive, Taichung Native 1; intermediate, IR 20; and tolerant, CR 44–955 to tungro were inoculated at 3 to 4 leaf stage, and the fifth leaf blades were used for the determinations. Data points of a specific day of determination marked by different letters represent significant differences ($P = 0.05$) according to Fisher's least significant difference test

inoculation. Though there was slight increase in protease activity due to infection in tolerant cultivar, the changes were not significant excepting in samples collected 21 days after inoculation. The rate of disease-induced increase in protease activity was maximum in the sensitive cultivar, and it rose with the symptom development.

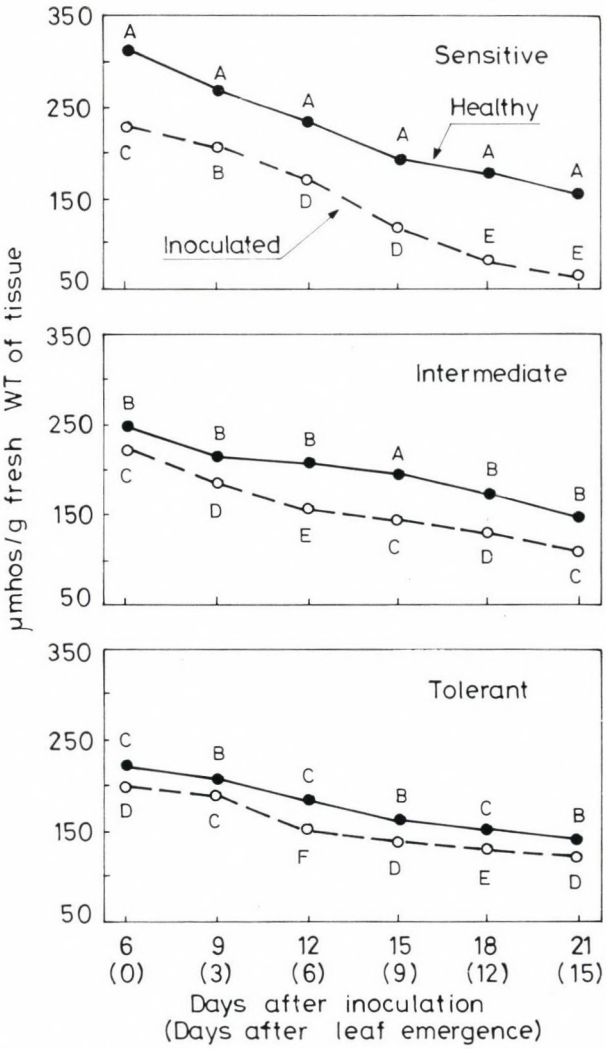



Fig. 4. Electrolyte leakage from leaf blades of healthy (solid line) and tungro-infected (broken line) plants differing in their reaction to the disease. Rice cultivars/line sensitive, Taichung Native 1; intermediate, IR 20; and tolerant, CR 44–955 to tungro were inoculated at 3 to 4 leaf stage, and the fifth leaf blades were used for the determinations. Data points of a specific day of determination marked by different letters represent significant differences ($P = 0.05$) according to Fisher's least significant difference test

Data on protease activity in senescing leaf blade segments (Fig. 2D) reveal that senescence induced marked increases in the protease activity of the leaf blade segments. Kinetin slowed down the increase in protease activity, while ABA greatly augmented the enzyme activity in senescing leaf blade segments.

*Permeability of cells*

In rice, permeability of the cells was influenced both by age of the leaf blades and virus infection (Fig. 4). With the maturation of leaf blades, the electrolyte leakage decreased denoting a decline in permeability of the cells. Tungro infection significantly reduced the electrolyte leakage from the leaf blade segments as early as 6 days after inoculation, and the decrease in electrolyte leakage increased with the severity of the disease. The reduction in electrolyte leakage due to the disease was greater in the leaf blades of sensitive cultivar than in those of intermediate cultivar, and was the least in the leaf blades of tolerant line.

Senescence of detached leaf blade segments caused an increase in the electrolyte leakage (Fig. 2E). Kinetin reduced the electrolyte leakage from the senescing leaf blade tissues, while ABA enhanced it.

## Discussion

Accumulation of reducing sugars and free amino acids in the leaf blades of tungro-affected plants was correlated with symptom severity. It is of interest to note that despite reduction in chlorophyll content (Sridhar et al., 1976), sugars accumulated in tungro-diseased rice plants, suggesting that the chlorophyll content may not be a rate-limiting factor for photosynthesis (Jensen, 1972). The increase in concentration of free amino acids and amides in virus-infected tissues might result from metabolic alterations including enhanced synthesis (Tu and Ford, 1970) and release from host proteins or from inhibition of normal protein synthesis (Matthews, 1981). Our data on protein-proteolytic activity of diseased tissue lend support for the possible breakdown of host proteins in tungro-diseased plants. For, the protein content of the sensitive cultivar showed marked reductions during the period of active virus multiplication which is related to increase in protease activity. Presumably, virus synthesis and hydrolysis of normal proteins take place simultaneously (Takahashi, 1941).

Changes in the concentrations of reducing sugars and amino acids, and proteolysis in tungro-diseased leaf blades were similar to those occurring in detached senescing leaf blade segments. The concentrations of reducing sugars and amino acids rise in senescing leaf blade tissues incubated in the dark (Martin and Thimann, 1972, Tetley and Thimann, 1974), while protein level declines (Osborne, 1962) with concomitant increase in proteolytic enzyme activity (Martin and Thimann, 1972). If these changes are characteristic of senescing tissues, then, kinetin known to retard (Osborne, 1962) and ABA known to accelerate senescence (Gep-

stein and Thimann, 1980) might further slow down or accentuate them, respectively. A striking exception to the effect of ABA, however, is the lower level of reducing sugars in the leaf blade segments floated on ABA solution than in those floated on water. The increased leakiness of the cell membrane caused by ABA in senescing tissues might explain this. These are supported by the data on senescence presented in this paper. A previous study from this laboratory (Mohanty et al., 1979) has shown that tungro infection increases markedly ABA levels in a sensitive rice cultivar. This indicates that part of the increase in the concentrations of reducing sugars and amino acids in leaf blades of tungro-diseased plants might be due to enhanced senescence triggered by the disease induced alterations in the endogenous hormone levels.

However, the concentration of amino acids in healthy leaf blades attached to plants declined with age after an initial rise in contrast to a steady accumulation in senescing healthy, detached leaf blades. Some modest differences have been reported between the senescence process in attached and detached leaves. The decline in the amino acids after an initial rise in its level in the attached leaves has been attributed to the transport of amino acids from the ageing leaves to the roots (Thimann, et al., 1974). The disease altered the normal phenomenon, for the amino acids in the virus-infected attached leaves showed steady increase until 15 days of leaf emergence which is similar to that occurring in senescing detached healthy leaf blade segments. These two effects together show that transport of low molecular weight compounds out of the leaf blades is hampered in diseased plants.

As both the viruses associated with the tungro complex are typically phloem-borne in rice plants (Saito et al., 1976, Hibino et al., 1978), it can be argued that disruption in pholem translocation might be the cause for the accumulation of low molecular substances in the infected plants. Although it is true that tungro infection causes phloem necrosis in rice plants (Saito et al., 1975), the extent or degree of phloem necrosis caused by tungro in rice has not been critically examined. In addition, accumulation of low molecular weight compounds especially carbohydrates, in sugar beet plants infected with yellows becomes evident before phloem necrosis is apparent (Klinkengerg, 1945). Similar situation might exist in tungro-diseased leaf blades, since high levels of reducing sugars and amino acids occurred as soon as the complete emergence of the leaf blades. Virus-induced changes in the permeability of cells in the infected tissues have been thought to be a possible cause for carbohydrate accumulation (Wynd, 1943, Matthews, 1981). However, it has so far not been supported with experimental evidence. Evidence for decreased permeability of cell membrane induced by tungro infection in rice has been provided in this paper. Presumably, the decreased permeability of the cells may well have obstructed the movement of sugars and amino acids from the cells, into the phloem, thus resulting in their accumulation in the cells where they are synthesized.

Despite a close correlation between the major criteria of senescence (accumulation of sugars and amino acids, and proteolysis) and tungro syndrome, differential response in changes in cellular permeability between these two was

observed. The permeability of cells was enhanced greatly in senescing healthy leaf blade segments incubated in the dark. Kinetin retarded and ABA accelerated the electrolyte leakage in these senescing leaf blade segments, as they do the senescence (Trippi and Thimann, 1983). Thus, the relation between disease and senescence may not be a simple one. It is perhaps more surprising that tungro infection caused a decrease in electrolyte leakage from the leaf blades, despite increase in ABA level (Mohanty et al., 1979). The evidence for the reduction in electrolyte leakage which is increased by chilling in cucumber (Rikin and Richmond, 1976, 1979), bean (Wilson, 1976) and in cotton (Rikin et al., 1979) cotyledonary tissues due to treatment with ABA might support the observation with tungro-diseased leaf blades. Plant hormones may have an important role in the regulation of membrane permeability (Van Steveninck, 1976). Nevertheless, the significance of ABA in the present connection is unclear.

Tungro infection delays flowering in less-sensitive rice plants, while tungro-infected highly sensitive plants may not flower at all (Ling, 1972). It is possible that decreased permeability of cell membrane may be responsible for the delay or absence of flowering of diseased plants either by preventing the specific flowering hormone from reaching the floral appex (Chailakhyan, 1968) or by reducing the influx of phosphate ion required in higher amounts for cell differentiation into the cytoplasm and/or into organelles (Baxter, 1972) or by both, besides causing the accumulation of low molecular weight compounds in the diseased leaf blades.

It can be concluded that the reduced permeability of cells may prowide new explanation for the accumulation of low molecular weight compounds and delay or inhibition of flowering in tungro-diseased rice plants.

We thank Dr. H. K. Pande, Director for his interest in the study and for permission to publish this work, and Mr. A. Surya Rao for the statistical analysis of the adata.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 87–91 (1986)*

# Virulence-specific Antigens in *Pseudomonas syringae* pv. *phaseolicola*

S. El-Kady, G. Somlyai, Dóra Simon, Mária Hevesi and Z. Klement

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

Four *Pseudomonas syringae* pv. *phaseolicola* Tn5 transposon containing derivatives, which lost their ability to elicit hypersensitive reaction (HR) and two spontaneous "rough" (R) mutants from which one was avirulent, were compared serologically by using crossed-immunoelectrophoresis (CIE) technique. The results revealed that the mutants lost some antigens compared to the wild-type (S21). Mutants S21–3 and S21–35 lost five antigens (A, C, D, G, H and A, D, G, H, K), while S21–8 and S21–39 lost the same antigen (A). Similarly missing antigens in the avirulent rough mutant were also detected. These results support the hypothesis that the detected antigens play an important role in pathogenesis.

The Tn5 transposon recently is a widely applied tool to generate mutation in plant pathogenic bacteria (Anderson et al., 1985; Staskawicz et al., 1983). To isolate mutants exhibiting variability in their ability to incite disease symptoms or to induce hypersensitive reaction (HR) we performed Tn5 mutagenesis on *Pseudomonas syringae* pv. *phaseolicola* (hereafter *P. s. phaseolicola)*. First 6 mutants from 2200 independent Tn5 containing colonies were isolated which were not able to elicit HR in the incompatible tobacco leaf ($Hyp^-$). Four of them also did not cause disease symptoms in the susceptible bean cultivar, while two induced mild symptoms (Somlyai et al., unpublished).

The serological differences between the virulent *Agrobacterium tumefaciens* and its avirulent derivatives have been established recently (El-Kady et al., 1982). We could also detect differences in antigenic structure between the wild-type *P. s. phaseolica* and its six $Hyp^-$ derivatives (El-Kady et al., unpublished).

We report here the serological differences between the pathogen wild-type and recently isolated four $Hyp^-$ strains (from 1500 Tn5 containing colonies) and two spontaneous rough mutants studied by crossed-immunoelectrophoresis (CIE).

## Materials and Methods

*Strains and mutants* used were recorded in Table 1.

*Preparation of antigens and immunization.* Bacterial cells were harvested from 3-day-old shaked broth culture by centrifugation. The precipitate was washed three times with 0.85% NaCl then the bacterial cells were sonicated by

Table 1
Bacterial strains

| Strains | Characteristics | Source references |
|---|---|---|
| S2 | wild-type, $Hyp^+$, $Path^+$ | K. Rudolph |
| S21 | $Rif^r$ derivatives of S2 $Hyp^+$, $Path^+$ | Somlyai et al. |
| S21–3 | Tn5 induced $Hyp^-Path^-$ | this work |
| S21–8 | Tn5 induced $Hyp^-Vir^-$ | this work |
| S21–35 | Tn5 induced $Hyp^-Path^-$ | this work |
| S21–39 | Tn5 induced $Hyp^-Vir^-$ | this work |
| R2 | rough derivatives of S2 $Path^+Hyp^+$ | this work |
| S1 | wild-type, $Hyp^+Path^+$ | this work |
| R3 | rough derivatives of S1 $Path^-Hyp^-$ | this work |

Rif, rifampicin; r, resistant; $Hyp^+$, induced hypersensitive reaction (HR); $Hyp^-$, unable to elicit hypersensitive reaction; $Path^+$, typical water-soaking symptom (WS); $Path^-$, unable to elicit water-soaking symptom; $Vir^-$, induce mild symptom

MSE unltrasonic disintegrator for 10 min. and the protein contents were determined according to Lowry et al. (1951) and adjusted to 20 mg/ml.

Antigens of *P. s. phaseolicola* strain S21 were injected in the rabbit after mixing with the complete adjuvant in 1 : 1 ration. Each rabbit received 8 injections (subcutaneously then intramuscularly) starting with one ml until five ml and increasing by 0.5 ml for the next injection. The rabbit was bled after one week from the last injection.

Antiserum was prepared, and immunoglobulins were isolated according to Axelsen et al. (1973).

The antibodies absorption experiment was carried out according to Király et al. (1970).

*Crossed-immunoelectrophoresis (CIE) technique.* CIE technique was used according to Axelsen et al. (1973) using 1.5 mm layer of 1% agarose (REANAL, Budapest) and barbital buffer, pH 8.6, ionic strength 0.02. Application wells had a diameter of 4.0 mm. Ten $\mu$l antigen samples were applied in the wells. First-dimension electrophoresis was carried out at 10 V/cm for 1 h, second-dimension run was made applying 1 V/cm for 16 h at 15 °C. Second-dimensional gels contained 15 $\mu$l purified, concentrated antibody per 1 $cm^2$. Immunoprecipitates were, stained with Coomassie brilliant blue R–250.

## Results

The bacterial transposon Tn5 was used to generate mutation in *P. s. phaseolicola*. Isolating and screening 3700 independent mutants in to experimental series, altogether 10 mutants were found which lost their ability to elicit hypersensitive reaction (HR) in tobacco leaf ($Hyp^-$ mutants). In the first experiments the missing antigens in six $Hyp^-$ strain were verified. Then investigated whether the recently

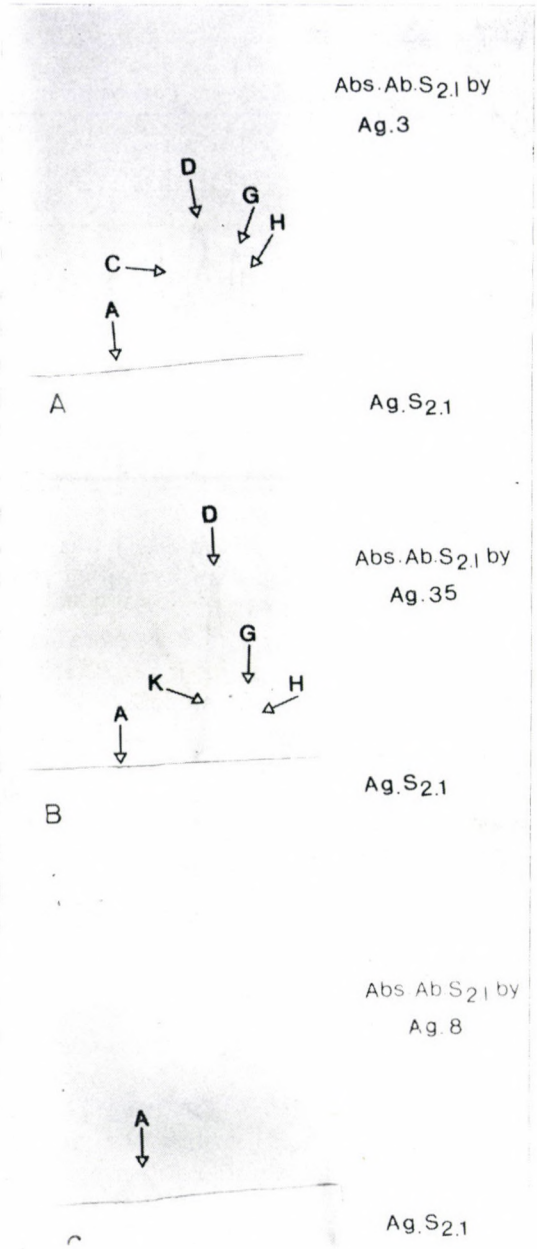



Fig. 1. Crossed-immunoelectrophoresis (CIE) of *Pseudomonas syringae* pv. *phaseolicola* (S21) strain. Antigen of S21 (AgS21) was electrophoresed, the upper gel contained the absorbed antibody of S21 (Abs.Ab.S21) by the mutant S21–3 (Ag. 3) Fig. 1A; S21–35 (Ag. 35) Fig. 1B; S21–8 (Ag. 8) Fig. 1C

Table 2

Number of precipitin peaks detected in the crossed immuno-electrophoresis technique between antigens of *Pseudomonas syringae* pv. *phaseolicola* strains and its derivatives

| Antibody | Antigens used for absorbtion | Number of precipitin peaks detected with antigens of Tn5 induced Hyp⁻ strains | | | | |
|---|---|---|---|---|---|---|
| | | S21 | 3 | 8 | 35 | 39 |
| S21 | S21 | — | — | — | — | — |
| | 3 | 5 | — | 4 | 1 | 4 |
| | 8 | 1 | — | — | — | — |
| | 35 | 5 | 1 | 4 | — | 4 |
| | 39 | 1 | — | — | — | — |
| | antibody S21 was not absorbed | 34 | 29 | 33 | 29 | 33 |

isolated four Hyp⁻ strains also lost the same six antigens which had been proved earlier (A, B, C, D, E, F).

The antiserum against *P. s. phaseolicola* (S21) was obtained with a titre 1 : 2560. To determine the missing antigens in the mutants, the antibodies of S21 were absorbed by the antigens of each mutant respectively. The mutant antigens separated in the first dimension were electrophoresed against the non-absorbed antibodies in the second dimension. The number of precipitin peaks detected by reciprocal analysis procedure is recorded in Table 2.

The results proved that the mutant S21–3 and S21–35 lost five antigens (A, C, D, G, H; A, D, G, H, K), (Fig. 1A, B), while the S21–8 (Fig. 1 C) and S21–39 lost only one antigen (A). We found that the antigens A, C and D are common with those antigens which were not present even in the first six Hyp⁻ mutants. The new missing antigens G, H and K were detected in the recently isolated Hyp⁻ strains (S21–8; S21–39).

Similar experiments were applied to investigate the antigen pattern of two spontaneous mutants (R2, R3). We found that the R2, which was able to elicit mild disease symptoms, did not show any difference comparing to its wilt-type strain S2. On the other hand, the mutant R3 lost three antigens which were detected when it was electrophoresed against the absorbed antiserum of S21 absorbed by R3.

To investigate the thermosensitivity of these antigens sonicated cells were boiled at 100 °C for 1 hour. Using these heat-treated antigens no precipitin peaks appeared, indicating that these antigens were not thermostable.

## Discussion

The serological differences between the virulent wild-type strain of *P. s. phaseolicola* and its Tn5 containing derivatives were detected. We found six different missing antigens in the four Hyp⁻ strains. Two mutants lost five antigens

from which four were common (A, D, G, H) and one antigen was different (C in S21–3 while K in S21–35). We found only one missing antigen (A) in two mutants (S21–8, S21–39). The antigens G, H and K were present in the isolated first six mutants. That some other antigens were lost from the new four Hyp$^-$ strains suggest that the Tn5 integrated into different parts of the chromosome. These speculations, however, require further experimental support by Southern technique.

We concluded that the CIE is an acceptable method to detect missing antigens in the Tn5 induced Hyp$^-$ mutants. Because the wild-type and Hyp$^-$ mutants were cultivated in broth medium for the preparation of antigens, we could compare only those gene products which genes were expressed *in vitro*. It is also known that not all, but only 30–40 gene products are detectable by the CIE technique as antigens. We proved that 1–5 out of 34 detectable antigens were absent in the Hyp$^-$ mutants. This fact leads us to believe that mutagenised genes by Tn5 were expressed *in vitro* in the Hyp$^-$ mutants. Furthermore because this antigens were detectable by this technique, it is very probable that these antigens are present in a relatively high amount in the bacteria.

In our earlier work no missing antigens were detected in Tn5 mutants which kept HR-inducing capacity (Hyp$^+$). Therefore, it seems that the detected missing antigens somehow correlate with the HR-inducing ability of the mutants. A similar result was obtained with the two spontaneous rough mutants R2 and R3, because we could detect missing antigens using CIE only in the mutant R3 which was avirulent and HR negative.

The serological differences between pathogenic *Agrobacterium tumefaciens* and its non-pathogenic derivatives were also proved by El-Kady and Süle (1982). They also found that the specific antigens for virulence were thermosensitive.

We also showed that the investigated antigens were thermosensitive, indicating that these may be proteins.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 93—98 (1986)*

# Immunoelectrophoretic Studies of three *Pseudomonas syringae* Pathovars

EL-KADY S., MÁRIA HEVESI, G. SOMLYAI and Z. KLEMENT

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

Crossed-immunoelectrophoresis (CIE) techniques were used for studying the differences in the antigenic structure of *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Pseudomonas syringae* pv. *tabaci*. The results revealed that 29, 34 and 32 precipitin peaks (antigens) were detected in homologous reactions of these three species. Comparing the three pathovars to each other 2, 3 and 6 strain specific antigens were found, respectively. Serological differences among strains of the same pathovar were also detected. One antigen, which was present in *P. s. tabaci* strain ATCC No11528, was absent in the strains H3G and H46 of *P. s. tabaci*. *P. s. syringae* strain $P_2$ differed from the other *P. s. syringae* strain H13 in one antigen while two antigens of *P. s. phaseolicola* strain S21 were not found in the strain Sl.

Species specific antigens have been studied by using gel diffusion test for several plant pathogenic *Pseudomonas* nomen species. (Lucas and Grogan, 1969a; Lovrekovich and Klement, 1961). The properties of these specific antigens in *Pseudomonas syringae* pv. *lachrymans* and *Pseudomonas syringae* pv. *phaseolicola* (hereafter *P. s. phaseolicola)* were investigated by Lucas and Grogan (1969b). They found that the chemical differences in the specific antigens of different *Pseudomonas* isolates may account for their serological specificities.

In our earlier work we investigated and found serological differences between the wild type *P. s. phaseolicola* and its Tn5 transposon induced mutant derivatives (El-Kady et al., unpublished). This study, using crossed-immunoelectrophoresis technique (CIE) for detecting specific antigens in *P. s. phaseolicola* *Pseudomonas syringae* pv. *syringae* (hereafter *P. s. syringae*) and *Pseudomonas syringae* pv. *tabaci* (hereafter *P. s. tabaci)* will be the basis of this work, namely the investigation of *P. s. tabaci* and its Tn5 induced derivatives.

## Materials and Methods

The investigated strains of *Pseudomonas* are listed in Table 1.

*Immunization of rabbits and preparation of antibodies:* Antigens of the tested strains were prepared according to El-Kady and Süle (1982). Protein contents

Table 1

Pseudomonas strains used

| *Pseudomonas* species | | Host plant | Source/reference |
|---|---|---|---|
| *Pseudomonas syringae* pv. *tabaci* | ATCC No 11528 | tobacco | B. Goodmen |
| | H3G | tobacco | Mária Hevesi |
| | H46 | tobacco | Mária Hevesi |
| *Pseudomonas syringae* pv. *syringae* | $P_2$ | pepper | Mária Hevesi |
| | P60 | pepper | Mária Hevesi |
| | H13 | cherry | Mária Hevesi |
| *Pseudomonas syringae* pv. *phaseolicola* | S21 | bean | G. Somlyai |
| | S1 | bean | K. Rudolph |

were determined and adjusted to 20 mg/ml according to Lowry et al (1951). Antigens of *P. s. tabaci* strain ATCC No 11 528, *P. s. phaseolicola* strain S21 and *P. s. syringae* strain P2 were used for injection of rabbits. The complete Freund adjuvant was mixed with the sonicated baterial cell suspension at ratio 1 : 1. Each rabbit received 8 injections (subcutaneously followed by intramuscularly) with doses 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml.

Antisera were obtained, and immunoglobulins were isolated by means of ammonium sulfate precipitation according to Axelsen, Kroll and Weeke (1973) and Harboe and Ingild (1973).

*Cossed-immunoelectrophoresis (CIE) techniques:* CIE, CIE with intermediate gel and CIE with antibodies absorption *in situ* were used according to Axelsen, Kroll and Weeke (1973) with some modification. 1% agarose (Reanal, Budapest) and barbital buffer, pH 8.6 ionic strength 0.02 were used. Ten $\mu$l antigen samples were electrophoreised in the first dimension at 10 V/cm for 1 h at 15 °C, and at 2 V/cm for 16 h in the second dimension. Immunoprecipitates were stained with Coomassie brilliant blue R–250 according to Axelsen, Kroll and Weeke (1973).

## Results

Antisera against *P. s. tabaci* (ATCC No 11528), *P. s. syringae* (P2) and *P. s. phaseolicola* (S21) with a titre 1 : 2560 were obtained. Antibodies against *P. s. tabaci* indicated 32, 31 and 31 precipitin peaks when it was electrophoresed with its homologous ATCC No 11528, H3G and H46 antigens respectively (Table 2). The *P. s. syringae* strains (P2, P60, H13) gave 29, 29 and 28 precipitin peaks, while the *P. s. phaseolicola* strains (S21 and S1) gave 34 and 32 peaks when the homologous antiserum was used.

Table 2

Number of precipitin peaks detected among *Pseudomonas* strains by using crossed immunoelectrophoresis techniques in the reciprocal analysis procedure

| Antibodies against | Antigens used for absorption | Number of precipitin peaks detected with antigens of | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *P. s. tabaci* strains | | | *P. s. syringae* strains | | | *P. s. phaseolicola* strains | |
| | | ATCC | H3G | H46 | P | P60 | H13 | S21 | S1 |
| | ATCC No 11528 | — | — | — | — | — | — | — | — |
| *P. s. tabaci* | H3G | 1 | — | — | — | — | — | — | — |
| strain | H46 | 1 | — | — | — | — | — | — | — |
| ATCC No11528 | $P_2$ | 6 | 5 | 5 | — | — | — | — | — |
| | P60 | 6 | 5 | 5 | — | — | — | — | — |
| | H13 | 6 | 5 | 5 | — | — | — | — | — |
| | S21 | 6 | 5 | 5 | — | — | — | — | — |
| | S1 | 6 | 5 | 5 | — | — | — | — | — |
| | — | 32 | 31 | 31 | 26 | 26 | 26 | 26 | 26 |
| | ATCC No 11528 | — | — | — | 3 | 3 | 2 | 1 | 1 |
| *P. s.* | H3G | — | — | — | — | — | — | 1 | 1 |
| *syringae* | H46 | — | — | — | — | — | — | 1 | 1 |
| strain $P_2$ | $P_2$ | — | — | — | — | — | — | — | — |
| | P60 | — | — | — | — | — | — | — | — |
| | H13 | — | — | — | 1 | 1 | — | — | — |
| | S21 | — | — | — | 2 | 2 | 1 | — | — |
| | S1 | — | — | — | 2 | 2 | 1 | — | — |
| | — | 26 | 26 | 26 | 29 | 29 | 28 | 27 | 27 |
| | ATCC No 11528 | — | — | — | 3 | 3 | 3 | 8 | 6 |
| *P. s.* | H3G | — | — | — | 3 | 3 | 3 | 8 | 6 |
| *phaseolicola* | H46 | — | — | — | 3 | 3 | 3 | 8 | 6 |
| strain S21 | $P_2$ | — | — | — | — | — | — | 5 | 3 |
| | P60 | — | — | — | — | — | — | 5 | 3 |
| | H13 | — | — | — | — | — | — | 5 | 3 |
| | S21 | — | — | — | — | — | — | — | — |
| | S1 | — | — | — | | — | — | 2 | — |
| | — | 26 | 26 | 26 | 29 | 29 | 29 | 34 | 32 |

To determine differences in the antigen pattern of the three *Pseudomonas syringae* pathovars CIE with intermediate gel and CIE with antibody absorption *in situ* were applied.

The results revealed that *P. s. tabaci* (ATCC No 11528) had 6 antigens more than *P. s. syringae* or *P. s. phaseolicola* (Fig. 1). Comparing the homologous strain of *P. s. tabaci* ATCC No 11528 with the other two *P. s. tabaci* strains, (H3G, H46) one plus peak was found in the former.

When the *P. s. syringae* ($P_2$) was compared with *P. s. phaseolicola* (S21) and *P. s. tabaci* (ATCC No 11528) two (Fig. 2) and three peaks appeared indicating the differences between the pathovars. Investigating the antigens of the three *P. s.*

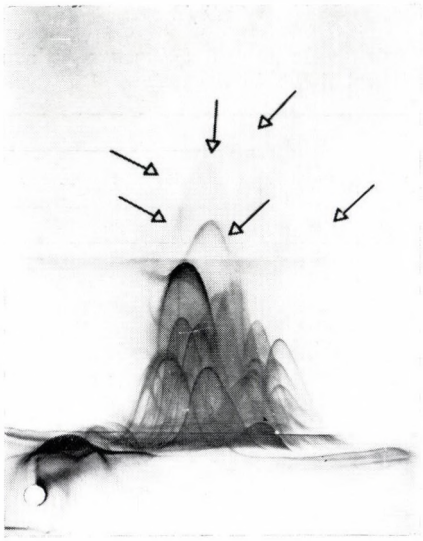


Fig. 1. CIE with intermediate gel. Antigens of *P. s. tabaci* strain ATCC No 11528, the intermediate gel contained antibodies against *P. s. phaseolicola* strain S21, the reference gel contained antibodies against *P. s. tabaci*. Arrows indicate the species specific antigens of *P. s. tabaci*

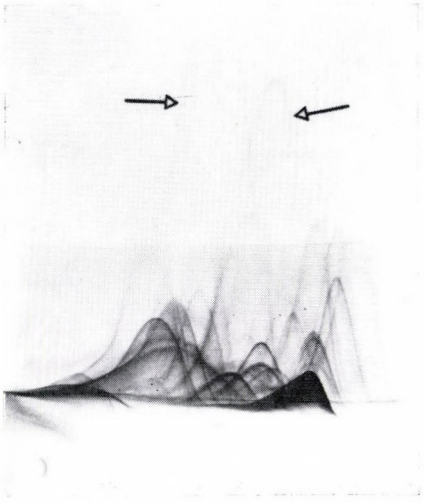


Fig. 2. CIE with intermediate gel. Antigens of *P. s. syringae* strain $P_2$ were electrophoresed, the intermediate gel contained antibodies against *P. s. phaseolicola* strain S21, the reference gel contained antibodies against $P_2$. Arrows indicate the precipitin peaks of the species specific antigens of *P. s. syringae*

*syringae* strains we found that the strains $P_2$ and P60 had 29 common antigens while the H13 differed in one antigen from the former two strains.

Finally the *P. s. phaseolicola* strain (S21) was compared with *P. s. syringae* and *P. s. tabaci* strains. It was found that the *P. s. phaseolicola* contained five and

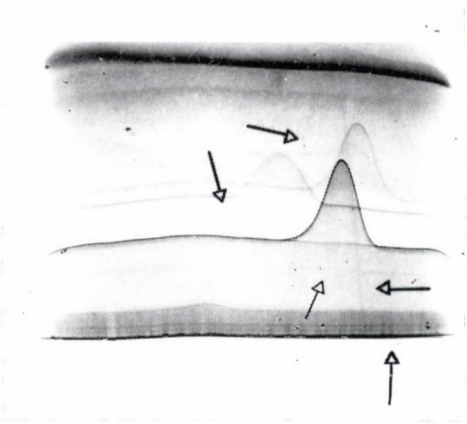


Fig. 3. CIE with antibodies absorption *in situ*. Antigens of S21 was electrophoresed, the intermediate gel contained concentrated antigens of *P. s. syringae* H13, the reference gel contained S21 antibody. The five arrows indicate that these antigens were absent in *P. s. syringae*

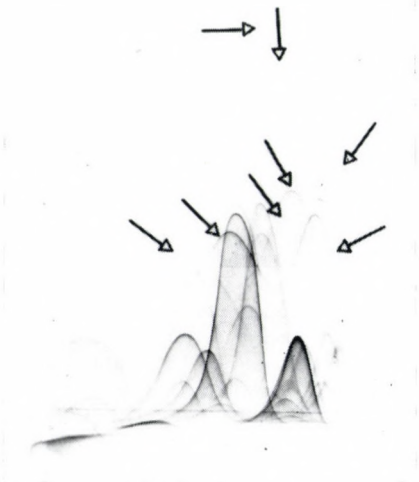


Fig. 4. CIE with intermediate gel. Antigens of *P. s. phaseolicola* S21 were electrophoresed, the intermediate gel contained *P. s. tabaci* antibody, the reference gel contained S21 antibody The eight arrows indicate that these antigens were absent in *P. s. tabaci*

eight antigens which were absent in the strains of *P. s. syringae* (P60) (Fig. 3) and *P. s. tabaci* (ATCC No 11528) (Fig. 4) respectively.

The strain specific antigens for each species of three *Pseudomonas* pathovars are listed in Table 2 by means of reciprocal analysis procedure.

## Discussion

Three *Pseudomonas* species were distinguished serologically by using the CIE technique. Two, 3 and 6 antigens were found, which were present only in the *P. s. syringae*, *P. s. phaseolicola* and *P. s. tabaci* respectively. Similarly, strain specific antigens were found; *P. s. tabaci* ATCC No 11528 contained one antigen which was absent in *P. s. tabaci* strain H3G and H46. These results are in agreement with Lovrekovich and Klement (1961) that *P. s. tabaci* has strain specific antigens. Also *P. s. syringae* strain $P_2$ and P60 contained one antigen which was absent in *P. s. syringae* H13; and *P. s. phaseolicola* S21 contained two antigens which were absent in *P. s. phaseolicola* S1.

We could prove in our earlier work (El-Kady et al. unpublished) that the Tn5 transposon induced mutants of *P. s. phaseolicola* (S21) which lost their ability to elicit HR, also lost some antigens. In our future work we are interested in determining whether the Tn5 mutants of *P. s. tabaci*, which also lost their ability to induce HR, will lose some specific antigens, or antigens which are common with *P. s. phaseolicola*.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 99—108 (1986)*

# Highlights on Pear Disorder in Egypt

NABIL S. FARAG, FAIZA G. FAWZI, FAYEZ BISHAY, TAHA A. EL-SHARKAWY and KAMEL Y. MICKAIL

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

Symptoms similar to fire blight have been observed on pear trees. Media selective for the fire blight pathogen failed to give rise *E. amylovora* (Burrill) Winslow et al. The bacteriological characteristics of the isolated pathogen are different from those known for *P. syringae* van Hall but conform with those of *P. syringae* pv. *phaseolicola* (Burkholder) Young et al.

Leaf as well as pod inoculation of *Phaseolus vulgaris* resulted in typical halo blight symptoms after 14 days incubation at 19 °C. The inability of these bacteria to infect green lemon fruits, ability to produce ooze from pear fruits larger than 1.5 cm in diameter and versatile pigment production are among the major sources of confusion with the fire blight organism.

Pear orchards in certain districts of Egypt have been ravaged by several disorders. The prevalence of symptoms similar to those known for fire blight were reported by El-Goorani (1964) and El-Helaly et al. (1964). Nine years later, El-Goorani (1973) reported failure to isolate any pathogenic bacteria during a monitoring study extending over a six-years period.

In april 1982 and 1983, many cases of blosom and twig blast symptoms occurred at certain localities in Egypt. Paulus (1982) found no evidence of fire blight on pears, and later concluded (1983) that many causes could contribute to the decline and the possibility of fire blight must be seriously investigated. Zwet (1983) attributed the decline to fire blight, blast, and severe insect damage. He identified the pathogen isolated in Egypt as *Erwinia amylovora* based on the following differential tests: (a) presence of ooze from fruits and twigs on inoculation (b) absence of reaction with UV light (c) growth on Miller Schroth's (MS) medium (d) fatty acids biochemistry. He also reported few interesting cases of shepherd's crook due to borers infestation.

This work was undertaken to define the cause of the problem in the affected orchards in Egypt.

## Materials and Methods

*Affected plant material*

Reasonable samples of blossoms, leaves and twigs, as well as cankerous branches were collected from pear and quince trees. The samples were subjected to macro-, and microscopic examinations.

*Isolation procedures*

Selected samples of the affected tissues were surface terilized with 0.5% sodium hypochlorite for 3 minutes. After rinsing with sterile water, the tissues were comminuted in droplets of sterile water and kept for 15 minutes before streaking. Plating was made on 5% sucrose nutrient agar (Ark, 1937); $D_3$ and high sucrose media selective for *E. amylovora* (Kado and Heskett, 1970; Crosse and Goodman, 1973). The $D_4$ medium selective to *Pseudomonas* spp. was also tried. Incubation was made at 28 °C for 4–7 days. In case of blosoms and cankerous branches, plating was made without surface sterilization on the afore-mentioned selective media.

*Pathogenicity*

Green pear fruits (1.5 and 3 cm in diam.) were surface sterilized with 0.5% sodium hypochlorite and inoculated by gentle stabbing with a needle laden with the bacteria. Incubation was made under humid conditions at 25–28 °C for 6 days. Inoculation of potted pear, apple, and quince seedlings was made with a turbid suspension of the bacteria by hypodermic injection. Inoculated plants were kept under humid conditions for the development of symptoms. Re-isolation was made on $D_3$ and $D_4$ media.

Leaves of *Phaseolus vulgaris* variety Giza 3 were inoculated with a turbid suspension of the bacteria. Incubation was made under humid conditions for 2 days, followed by 12 days under normal conditions. Pods and green lemon fruits were inoculated by pricking and kept under humidity, at 19–20 °C, for 14 days. Re-isolation was made on $D_4$ medium.

*Identification of the isolated bacteria*

Representative isolates that showed pathogenicity to the hosts under investigation were subjected to complete identification:

(a) *Morphological characters:* Air dried films from 48 hrs cultures were Gram stained as modified by Kopeloff and Beerman (Cruickshank et al., 1975) using acetone-alcohol as a decolourizer. Demonstration of capsules was made in wet India ink films. Motility was detected according to Dowson (1957). Flagella staining was made by method of Rhodes (1958). Colony morphology and other growth characteristics were described on glucose nutrient agar.

(b) *Physiological characters:* Detection of flourescent pigments was made on King's B medium (King et al., 1954) and Paton's medium (1959). Fluorescence was observed with UV light at 2537 Å. Other characters were determined for the isolated bacteria employing methods as indicated along each test as follows: Oxygen requirements and reducing substances from sucrose (Dye, 1968). Utilization of glucose (Hugh and Leifson, 1953). Production of acids from carbohydrates in peptone-free basal medium (Dowson, 1957) and in peptone water base (Dye and Kemp, 1977). Production of levan, 2-ketogluconate, egg-yolk reaction, and rot of potato slices (Lelliott et al., 1966). Oxidase (Kovacs, 1956). Arginine dihydrolase (Thornley, 1960). Amino acids decarboxylases (Møller, 1955). Catalase, VP, and MR tests (Cruickshank et al., 1975). Reduction of nitrate, production of ammonia, indole, and hydrolysis of strach (Dowson, 1957). Gelatin liquefaction was tested in nutrient gelatin (Lelliott et al., 1966), and on nutrient gelatin agar (Cruickshank et al., 1975). Lithmus milk reaction (Dowson, 1957) and milk digestion (Cruickshank et al., 1975). Hypersensitive reaction on tobacco leaves (Klement, 1963).

## Results and Discussion

*Disease syndrome and field observations*

Early in April 1982 and 1983 many cases of blossom and twig blast of pear occurred at certain localities in Egypt. Withering of flowers and blackening of twigs were among the attractive symptoms on affected trees. The severity of attack ranged from almost no disease in some orchards to severe in others. The presence of unaffected flowers in the same truss of blasted ones was a common feature under many circumstances. Affected blossoms kept in polyethylene bags showed a minute viscid exudates. Their consistency was stick enough to produce threads, approximately 2 cm length, on being touched. The affected buds did not show any sticky exudates on keeping in polyethylene bags. The leaves showed marginal necrosis and turned black with time.

Loosened periderm below spurs and new twigs, in some instances, assumed a papery appearance. Small fruits blackened and mummified without dropping. However, many green unaffected fruits beside seriously affected ones were frequently observed.

Longitudinal sections made in twigs, below affected flowers and leaves showed inconsistent black discolouration. Several types of bark cankers on the main trunk and old branches, without any milky oozes, were observed. Terminal branches, on limited occasions, gave the appearance of shepherd's crook, however, micro-tunnels supposedly produced by some insect(s), were observed below the affected tips.

It is worthy to note that sporadic cankerous pear trees have been observed near a young apple orchard free from any disorder. Similar observations were

reported by Paulus (1983) and Zwet (1983) who found one acre of affected quince surrounded by twenty acres of symptomless pear trees. The absence of ooze on cankerous branches and trunks was also reported by El-Goorani (1973) and Paulus (1982 and 1983).

Confusion between fire blight and blossom blast is most likely to occur when blossom infection is considered. In literature, the bacterial ooze on blasted pear buds is associated only with *E. amylovora*, and absence of ooze has been one of the feature differentiating *P. s. syringae* infection. However, the production of ooze by an unidentified species of *Pseudomonas*, on Magness pear blossom and buds, was reported by Zwet and Keil (1979). Another species was described in Russia as *P. s. pyri*, causing a disease which differs from fire blight and blast in that the bark cracks and peels off, with no infection on unripe fruits (D'yakova, 1955). Such reports render the proper identification of the pathogen, isolated in Egypt, of paramount and decisive importance (Zwet and Keil, 1979).

*Isolation of bacteria*

Isolation made on $D_4$ medium, from unsterilized samples, gave rise to uniform growth of bacteria. Trials made from sterilized plant materials, on the same medium as well as sucrose nutrient agar, resulted in a very low frequency of bacterial colonies. Plating on $D_3$ and high sucrose media resulted in no colonies after 7 days incubation. Development of bacteria on $D_4$ medium suggested that *Pseudomonas* spp. might be contributing to the current problem of pear. The low frequency of growth after sterilization may be attributed to the presence of bacteria in the outer cortical layers and/or symptoms may be considerably ahead of the invasive bacteria, as was suggested by Rosen and Bleecker (1933).

*Pathogenicity*

The isolated bacteria proved to be pathogenic on pear, apple, and quince. Blackening of twigs and leaf petioles was observed 3–4 days after inoculation. Bark of the inoculated twigs cracked at the point of inoculation and the vessels showed black discolouration. This may indicate to the semi-systemic spread of bacteria due to the method of inoculation. The systemic infection with *P. s. syringae* on other host plants was reported by Cameron (1962).

Leaf inoculation of *Phaseolus vulgaris* resulted in typical systemic infection and mosaic like symptoms after 7–14 days incubation at 19–20 °C. Inoculation of green pods resulted in water soaking with the production of ooze at the site of inoculation.

Inoculation of lemon fruits did not lead to any pathological reaction. The noninfectivity of these bacteria to lemon fruits eliminates the possibility of *P. s. syringae* or may indicate a very special form of this species (Sabet and Dowson, 1952). McKeen (1955) in a similar situation in Canada identified a bacteria as *P. s. syringae* despite its inability to infect green lemon fruits and its ability to infect kidney beans.

Inoculation of pear fruits, 1.5 cm in diam., produced a blackened area without milky ooze about needle prick. However, fruits larger than 3 cm in diam., gave the same sign together with sticky tan exudates at the point of inoculation. Such controversy relating to ooze production and the results reported herein may indicate that the bacteria in consideration are not *E. amylovora.*

Susceptibility of immature pear fruits to the fire blight pathogen is known to correlate with high asparagin content (Ark, 1937). The use of small sized fruits, about 2 cm in diam., was stressed as essential for proper diagnosis (Crosse et al., 1960; Schroth and Hildebrand, 1980).

*Identification of the isolated bacteria*

(a) *Cultural and morphological characters:* On nutrient glucose agar the colonies are smooth, convex, and opaque; circular, 1–2 mm in diam., with entire margins. Dirty white centers occasionally occur. The growth is filiform, moderate with bluish tinge at periphery. Fluorescent pigment is slowly produced in cultures, 3–4 days after incubation; being intensified in Paton's medium. Such an enhancement of pigment secretion is necessary in case of weak fluorescence (Paton, 1959; Palleroni and Doudoroff, 1972). Growth in nutrient broth is feeble without pellicle, however, in glucose nutrient broth pellicle and sediment are formed.

The cells occur singly or in pairs. Rod shaped, the average size is $1.4 \times 0.6$ $\mu$, motile with 2–3 wavy polar flagella. The cells are encapsulated and nonsporulating. No differences could be observed between isolates either for morphological or cultural characters (Table 1).

(b) *Physiological characters:* The bacteria metabolized glucose oxidatively, and they are catalase positive. Levan produced, oxidase negative, and arginine dihydrolase not produced. The bacteria produced hypersensitive reaction on tobacco leaves and did not rot potato slices. Based on these characteristics the pathogen could be placed in group Ia fluorescent Pseudomonas (Lelliot et al, 1966).

All isolates did not produce 2-ketogluconic acid according to the method of Lelliot et al., (1966). Reducing substances were produced from sucrose as well as decarboxylases of arginine and lysine, but not glutamic decarboxylase. Nitrate reduction and lipase were negative. Gelatin liquefaction and starch hydrolysis were negative. Slight alkaline reaction was produced in litmus milk without curdling. Casein was not hydrolyzed by all cultures. Other tests are shown in Table 1.

(c) *Production of acids from carbohydrates:* Acid was produced by all cultures in peptone–free basal medium and peptone water base from L-arabinose, D-glucose, and D-galactose. Slow acid was produced from sucrose. No acid from D-arabinose, D-xylose, fructose, lactose, raffinose, glycerol, mannitol, salicin, and aesculin. Peptone free basal medium was more appropriate than peptone water base for acid detection. The latter medium showed an alkaline reaction with sucrose after prolonged incubation. Sush reaction may be attributed to the action of decarboxylases in media containing peptone. These enzymes are favoured by low oxygen tension and low pH (Thornley, 1960; Cruickshank et al., 1975).

Table 1

Basic characteristics of bacteria isolated from pear and quince

| Tests | Isolates | | | | | |
|---|---|---|---|---|---|---|
| | 412 | 415 | 510 | 112 | 113 | Q |
| Gram stain | (−) | (−) | (−) | (−) | (−) | (−) |
| Spores | − | − | − | − | − | − |
| Capsules | + | + | + | + | + | + |
| Flagella | Polar | Polar | Polar | Polar | Polar | Polar |
| Fluorescence | green | green | green | green | green | green |
| Aerobiosis | + | + | + | + | + | + |
| O/F | O | O | O | O | O | O |
| Levan* | + | + | + | + | + | + |
| Oxidase* | − | − | − | − | − | − |
| Potato rot* | − | − | − | − | − | − |
| Arginine dihydrolase* | − | − | − | − | − | − |
| Tobacco hypersensitivity* | + | + | + | + | + | + |
| Gelatin liquefaction | − | − | − | − | − | − |
| Casein hydrolysis | − | − | − | − | − | − |
| Litmus milk: | | | | | | |
| Curdling | − | − | − | − | − | − |
| reaction | alk. | alk. | alk. | alk. | alk. | alk. |
| Egg-yolk: | | | | | | |
| Lecithinase | − | − | − | − | − | − |
| lipase | − | − | − | − | − | − |
| Decarboxylases: | | | | | | |
| arginine | + | + | + | + | + | + |
| lysine | + | + | + | + | + | + |
| Glutamic | − | − | − | − | − | − |
| Catalase | + | + | + | + | + | + |
| Red. Sugars from sucrose | + | + | + | + | + | + |
| Oxidation of gluconate | − | − | − | − | − | − |
| Starch hydrolysis | − | − | − | − | − | − |
| Production of ammonia | − | − | − | − | − | − |
| Indole | − | − | − | − | − | − |
| $H_2S$ | − | − | − | − | − | − |
| Acetoin | ± | ± | ± | ± | ± | ± |
| MR | − | − | − | − | − | − |
| Nitrate reduction | − | − | − | − | − | − |
| Growth in: | | | | | | |
| nutrient broth | w | w | w | w | w | w |
| Glucose nutrient broth | Ps | Ps | Ps | Ps | Ps | Ps |

Alk = alkaline, w = weak, Ps = pellicle and sediment.
* LOPAT test.

The inability of these bacteria to produce acid from fructose, xylose, raffinose, and mannitol is different from those reported for *Ps. syringae* (Dye, 1953; Breed et al, 1957; Sands et al., 1970), as well as *E. amylovora* (Dye, 1968).

*Identity of the isolated bacteria*

Detection of pale green fluorescent pigment, polar flagellation and growth on $D_4$ medium suggested that the bacteria in concern might be a species of the genus *Pseudomonas*. Other characters such as oxidative metabolism of glucose, production of arginine and lysine decarboxylases, inability to ferment salicin or to liquefy gelatin are sufficient to eliminate the possibility of *E. amylovora* (Breed et al., 1957; Dye, 1968; Buchanan and Gibbons, 1974).

According to LOPAT tests (Table 1) these bacteria could be placed in the group Ia of the scheme of Lelliot et al. (1966) that comprises nine *Pseudomonas* species including *P. s. phaseolicola* and *P. s. syringae*. The physiological characters of the bacteria under investigation, on the other hand, correspond with those of sub-group IB, *P. s. phaseolicola*, in the scheme of Sands et al (1970). The latter species is considered as a pathovar of syringae and given the name *P. s. syringae* pv. *phaseolicola* (Dye et al., 1980).

From the pathological point of view, the ability of these bacteria to produce halo blight symptoms on pods and seedlings of kidney beans, as well as its inability to infect green lemon fruits are reasonable enough to eliminate the possibility of *P. s. syringae* pv. *syringae*. The ability of these bacteria to produce ooze from pear fruits larger than 1.5 cm in diameter may be the cause of confusion in many parts of the world including Egypt (Mckeen, 1955; El-Helaly et al., 1964; Abo El-Dahab et al., 1983).

*Sources of confusion in previous reports*

Although one must not underestimate the value of diagnosis based on disease expression, it is important to differentiate between conditions necessary for disease development and those necessary for outbreaks. In Egypt, predisposing conditions at the time of blooming are favourable for infection and disease expression. However, neither internal symptoms nor milky ooze could be observed on twigs and trunks (El-Goorani, 1973; Paulus, 1982 and 1983). Zwet (1983) reported one 'Alexandrine' pear tree showing remnant streaks along the bark surface that were attributed to bacterial ooze early in spring. In the same orchard he found few younger apple trees free from either blast or blight symptoms. Under these circumstances the remnant streaks may be attributed to the limb borer, *Zeuzera pyrina*, a major problem of pome trees in Egypt. However, the presence of young apple trees in that orchard, free from blight and blast symptoms, could not be explained. Meanwhile, he identified a non-fluorescent bacteria isolated from quince in Egypt as *P.s. syringae*, that has not been listed before on *Cydonia oblonga* (Elliott, 1951; Cameron, 1962). Furthermore, he identified two isolates as *E. amylovora*,

despite the inability to grow on MS medium or to produce ooze from fruits and shoots. This indicates that neither symptoms nor identification, as indicated by Zwet (1983), could justify the presence of fire blight in Egypt. Interpretation of results show that his conclusion was based on the fatty acids biochemistry being used for differentiation between *E. amylovora* and *P.s. syringae*.

The first record on the occurrence of fire blight in Egypt was made by El-Helaly et al. (1964). Although the record came free from description of diagnostic symptoms, many contradictions have been observed in the identification of the pathogen. In their trial to verify the identity of the isolated bacterium, they followed the methods of Mckeen (1955) and Billing et al (1960). They found that the pathogen neither infects kidney bean seedlings nor lemon and lime fruits. The noninfectivity to these test hosts strongly suggested that the isolated bacterium was not *P.s. syringae*. However, it was not justifiable to consider it *E. amylovora*. In the literature, few species of *Pseudomonas* were reported on pear and apple; without indication to their infectivity to such differential hosts (Elliot, 1951; Breed et al., 1957).

From the bacteriological point of view, El-Helaly et al. (1964) found that the Egyptian isolates of *E. amylovora* could utilize glucose with acid production, however, with dextrose no acid was produced. The same finding was reported by El-Goorani (1964). Such specificity for different synonyms of one and the same substance is not known for *E. amylovora* (Ark, 1937; Breed et al., 1957; Buchanan and Gibbons, 1974). Meanwhile, they found that the bacteria did not produce acid from lactose and curdled litmus milk; did not digest casein and cosidered the bacteria to be proteolytic. Moreover, they found that 5% sucrose with gelatin medium decreased the time of liquefaction from 15 to 4 days. Such case is contrary to what is known about protein sparing action of carbohydrates (Salle, 1961).

Such discrepancies in the identification of the so called "Egyptian isolates of *E. amylovora*" and the absence of diagnostic symptoms of fire blight are looked upon with great concern in Egypt.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 109—114 (1986)*

# Host-Pathogen Interaction in Bacterial Blight Pathosystem in Rice

P. NAYAK

Department of Plant Pathology, Central Rice Research Institute, Cuttack 753006, India

Significant differential interaction between 10 rice cultivars and 18 isolates o *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye indicated a vertical relationship between resistance of the host and virulence of the pathogen. Specificity of resistance/virulence was observed in specific cultivars—isolate combinations. Among the rice cultivars, IR 20, DV 85 and CB II. possessed differential ability. The most virulent isolates like Kota and CR 3 possessed all the matching virulence genes against the resistance genes Xa4, xa5, Xa7 and xa11, while none of the cultivars possessed resistance against all the isolates tested. The host cultivars IR 20, DV 85, Indira and CB II appeared to possess unidentified genes for resistance against certain virulent Indian isolates while IR 8 possessed resistance against certain weak isolates.

Bacterial blight disease, incited by *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, is one of the most serious diseases of rice crop. Several attempts to control this disease through chemicals have not met with any success. Hence host resistance is given priority in the disease control strategy. Variation in virulence among the isolates of the pathogen has been a major limiting factor in breeding for resistance. Although pathogenic variability has been reported (Devadath and Padmanabhan 1969, Rao et al., 1971, Ezuka and Horino 1974, Horino et al., 1981, Mew et al., 1981, 1982), limited attempt has been made to recognize distinct differential interaction *sensu* Vanderplank (1968) between pathogen-isolates and host cultivars (Reddy and Ou 1976, Addy 1979, Mew and Veracruz 1979). The present experiment was aimed at recognition of significant differential interaction and host specificity of the pathogen isolates in the bacterial blight pathosystem in rice.

## Materials and Methods

Five IRRI differentials (IR 20, IR 8, IR 1545, DV 85, Cemposelak) with known genes for resistance to bacterial blight disease and five Indian cultivars (BJ 1, CB II, Ramakrishna, Indira, TKM 6) with varying degrees of resistance to the disease, were used in this experiment. Fifteen day old seedlings raised in Petridishes were planted in 25 cm diameter earthen pots filled with 5 kg of soil.

Fertilisier was applied at the rate equivalent to 80 kg N/ha in the form of ammonium sulphate in two split doses (basal and 5 days before inoculation).

Eighteen isolates of the pathogen from leaf samples collected from different states of varied geographical regions of India (Orissa, West Bengal, Bihar, Andhra Pradesh, Rajsthan, Manipur) were selected using the Central Rice Research Institute collection of isolates of *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye. The stored bacterial isolates were revived on Wakimoto's medium and incubated at 28 °C for 72 h. Forty-day-old plants were inoculated by clipping the top one centimeter of each leaf with a pair of scissors every time dipped into a bacterial cell suspension, prepared from the revived 48 h old culture of each isolate, containing ca. $10^9$ cells/ml in sterile distilled water. A separate pair of scissors was used to inoculate each isolate. The experiment was conducted in a completely randomised design with two replications. The length of the lesion developed below the point of inoculation was measured with a centimeter scale on the 15th day of inoculation. The presence of a singificant differential interaction was tested by F test through analysis of variance. Qualitative evaluation of the disease reaction of each variety-isolate combination was also made by 1–9 scale of SES (Anonymous, 1980).

## Results

The analysis of variance revealed highly significant differences among the cultivars, the isolates of the pathogen and in the interaction between the two (Table 1). The isolates, as a group, exhibited a wide range of virulence on the host cultivars tested (Fig. 1). Based on the mean virulence of each isolate on all the cultivars, Pusa and Kota were most virulent while M-1, M-3 and M-4 were least compatible with all the host cultivars. The rest of the isolates exhibited a differential virulence on different host cultivars. Despite such wide variation in virulence of these isolates on the host cultivar tested, isolates with similar magnitude of virulence appeared to belong to distinct groups of high and low virulence. Accordingly, the isolates of Pusa, Kota, Hoogly, CR-e, CR-2, CR-3, Faizabad and Chinsurah could be grouped as highly virulent while rest of the isolates as low virulent types.

The virulence pattern of 18 isolates on 10 host genotypes (Table 2) indicated that none of the known genes present in these cultivars provide resistance against all isolates. It is only xa5, Xa7 and xa11 present in DV 85, were effective against all except the most virulent isolates Pusa, Kota, CR-3 and Faizabad. Similarly, the Xa4 gene present in IR 20 was effective against all except the most virulent isolates Kota, CR-1, CR-2 and CR-3. Each of the cultivars IR 20, CB II, DV 85, Indira and IR 8 appeared to carry unidentified genes against certain Indian isolates.

The number of virulence factors carried by each isolate with the number of resistance genes in relation to which virulence could be evaluated is given in the last row of Table 2. The spectrum of virulence factors in the pathogen-isolates

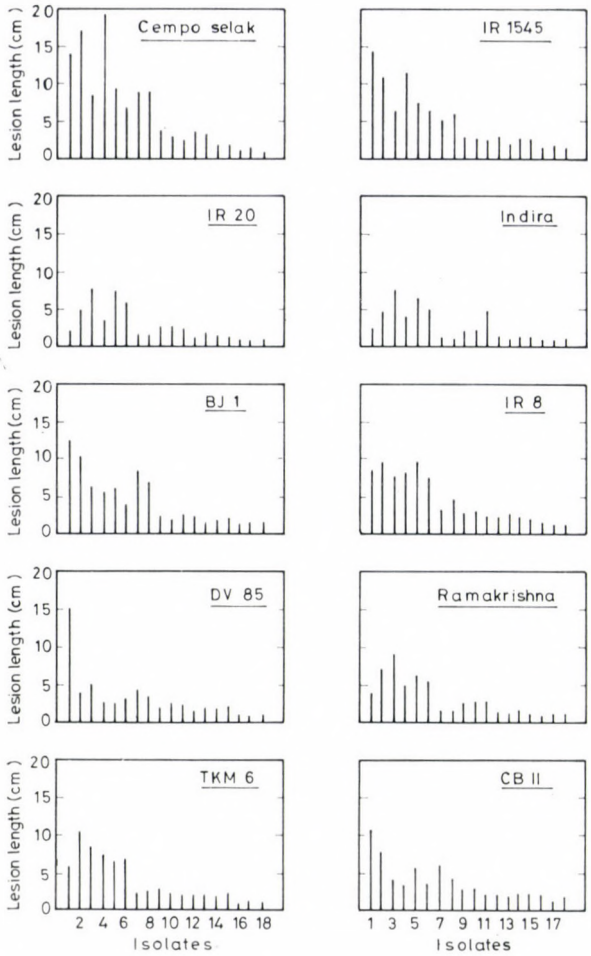



Fig. 1. Level of pathogenicity of each isolate of the pathogen on different cultivars. The isolates are indicated in the figure by corresponding numbers noted in Table 2

ranged from 0 to 4. The isolates of Manipur, M-1, M-2, M-3, M-4, Patna, CR-4, Hyderabad and Athanga did not possess any virulence factors to overcome the resistance genes present in these host cultivars. The isolates Pusa, Kota and CR-3 on the other hand possessed a maximum of 4 virulence factors, besides those acting against the unknown resistance genes present in the host cultivars Indira, IR 8 and Cemposelak. The isolated Pusa possessed all the virulence factors in addition to the unknown factors present against the unidentified genes present in Indira and IR 20.

Table 1

Analysis of variance of cultivar x isolate interactions

| Source | Df | SS | MS | F |
|---|---|---|---|---|
| Cultivar (C) | 9 | 453.2998 | 50.3666 | 99.49** |
| Isolate (I) | 17 | 2525.5108 | 148.5595 | 293.45** |
| Interaction (C × I) | 153 | 1217.0469 | 7.9546 | 15.71** |
| Error | 180 | 91.1243 | 0.5062 | |

** Significant at P = 0.01

Table 2

Reaction of 18 isolates on 10 host cultivars

| Cultivars | Resistance genes | 1. Pusa | 2. Kota | 3. CR-3 | 4. Hoogly | 5. CR-1 | 6. CR-2 | 7. Faiza-bad | 8. Chins-urah | 9. Cutt-ack |
|---|---|---|---|---|---|---|---|---|---|---|
| IR 20 | Xa4 | R | S | S | R | S | S | R | R | R |
| TKM 6 | Xa4 | S | S | S | S | S | R | R | R | R |
| Ramakrishna | Xa4 | S | S | S | S | S | S | R | R | R |
| CB II | Xa4, xa5 | S | S | S | R | S | R | S | S | R |
| IR 1545 | xa5 | S | S | S | S | S | S | S | S | R |
| BJ 1 | xa5, xa11 | S | S | S | S | S | S | S | S | R |
| DV 85 | xa5, Xa7, xa11 | S | S | S | R | R | R | S | R | R |
| Cemposelak | ? | S | S | S | S | S | S | S | S | S |
| IR 8 | ? | S | S | S | S | S | S | R | S | R |
| Indira | ? | R | S | S | S | S | S | R | R | R |
| Number of virulence factors/Number of resistance factors | | 4/4 | 4/4 | 4/4 | 3/4 | 3/4 | 3/4 | 3/4 | 2/4 | 0/4 |

| Cultivars | Resistance genes | 10. CR-4 | 11. M-2 | 12. Patna | 13. Hyder-abad | 14. Athan-ga | 15. Mani-pur | 16. M-3 | 17. M-1 | 18. M-4 |
|---|---|---|---|---|---|---|---|---|---|---|
| IR 20 | Xa4 | R | R | R | R | R | R | R | R | R |
| TKM 6 | Xa4 | R | R | R | R | R | R | R | R | R |
| Ramakrishna | Xa4 | R | R | R | R | R | R | R | R | R |
| CB II | Xa4, xa5 | R | R | R | R | R | R | R | R | R |
| IR 1545 | xa5 | R | R | R | R | R | R | R | R | R |
| BJ 1 | xa5, xa11 | R | R | R | R | R | R | R | R | R |
| DV 85 | xa5, Xa7, xa11 | R | R | R | R | R | R | R | R | R |
| Cemposelak | ? | R | R | R | R | R | R | R | R | R |
| IR 8 | ? | R | R | R | R | R | R | R | R | R |
| Indira | ? | R | R | R | R | R | R | R | R | R |
| Number of virulence factors/Number of resistance factors | | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |

R = Resistant
S = Susceptible,

## Discussion

Interaction in a pathosystem is the mathematical expression of the biological phenomenon of the response between strains of the pathogen and genotypes of the host (Zadoks and Schein, 1979). This could be studied either by means of analysis of variance or by ranking of the isolates. The significant differential interaction observed in the present study indicated a vertical relationship between the resistance of the host-cultivars and virulence of the pathogen-isolates.

The specificity of resistance in the host virulence in the pathogen is also characterised by the presence or absence of differential interactions. The existence of significant differential interactions also indicated such specificity of response in specific genotype-isolate combinations. While a group of 9 isolates did not possess any of the virulent factors matching the resistant genes present in this set of host cultivars, the three most virulent isolates Pusa, Kota and CR-3 possessed all the matching virulent factors against the available resistant host genes.

Amongst the IRRI differentials IR 20 (Xa4) and DV 85 (xa5, Xa7, and xa11) showed distinct differential reactions. IR 1545 (xa5) and Cemposelak (?) although differentiated the isolates of high and low virulence, they could not further differentiate amongst isolates of high virulence and both exhibited reaction patterns similar to that in BJ 1 (xa5, xa11) and IR 8 (?). The response of TKM 6 (Xa4) and Ramakrishna was also similar indicating that Ramakrishna carried the Xa4 gene from its parent TKM 6. IR 20 and Indira also showed a similar reaction pattern. Only CB II possessed the differential ability to differentiate some of the virulent isolates.

Based on the reaction-pattern, the cultivar IR 20 and Indira appear to possess additional genes governing reistance against the most virulent isolate Pusa; DV 85 against Hoogly, CR-1 and CR-2; IR 20 against Hoogly; CB II against Hoogly and CR-2 which are yet to be identified.

It is interesting to point out here that the IRRI differentials, except IR 20 and DV 85 did not possess resistance genes to overcome the virulence factors present in the virulent Indian isolates. In other words, Indian isolates like Kota and CR 3 possess all the matching virulence factors against all the resistance genes present in this set of cultivars tested (Xa4, xa5, Xa7 and xa11). Mew et al. (1981) reported the availability of matching virulence genes for xa5 in majority or the Bangladesh isolates and those for Xa4 in the Indonesian isolates. From the results of the present experiment and those reported by Mew and Veracruz (1971) and Mew et al. (1981) on the specificity of resistance/virulence in specific isolate-genotype combinations, it is reasonable to assume that the host pathogen genetic system for bacterial blight disease is dynamic and genes in one part of the system interact with corresponding genes in the other part in a manner similar to the gene-for-gene relationship.

## Acknowledgements

The author is thankful to Dr. H. K. Pande, Director, CRRI, for providing necessary facilities and Dr. P. R. Reddy, Scientist S-2 (Plant Pathology) for his generosity in providing the isolates used in this experiment.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 115—122 (1986)*

# Streptomycin in Relation to Potato Brown Rot Control

NABIL S. FARAG[1], FAIZA G. FAWZI[1], SAID I. A. EL-SAID[2] and MAURICE S. MIKHAIL[3]

[1] Plant Pathology Research Institute, Giza, Egypt, [2]Plant Protection Lab., National Research Center, Dokki, Egypt, [3]Plant Pathology Dept., Fac. of Agric., Cairo Univ., Giza, Egypt

*Pseudomonas solanacearum* E. F. Smith, the causative agent of potato brown rot, is not sensitive to chloramphenicol or penicillin. Both virulent and avirulent forms of the pathogen are sensitive to streptomycin and dihydrostreptomycin.

Control trials with streptomycin were made on an early maturing, disease-susceptible cv. King Edward and late maturing disease-tolerant cv. Alpha. Application of the antibiotic at the rates 150 as well as 400 ppm, increased wilt incidence. Root exudates of the first cultivar were rich enough in amino acids as compared with those of the latter, however, approximately equal amounts were exuded after antibiotic application.

King Edward showed high microbial size in the rhizosphere as compared with Alpha. Although streptomycin application increased amino acids exudation by both cultivars, inoculated plants of the first showed sharp decline in microbial densities in the rhizosphere, and the second showed only a considerable increase.

The increase in disease incidence after streptomycin application was correlated with increased root exudation at early stages of growth.

Bacterial wilt, i.e. brown rot, caused by *P. solanacearum* E. F. Smith is a serious disease of a variety of economical food crops including potato (Kelman, 1953). The disease has been reported in Egypt (Sabet, 1961; Lelliott, 1964) being serious in the Nili plantation (Mickail et al., 1974).

The use of antibiotics in controlling a variety of bacterial diseases has long been tried. Although screening tests of a number of antibiotics *in vitro* demonstrated effectiveness against many pathogens, trials *in vivo* frequently failed to corroborate them (Zaumeyer, 1958). The effective use of antibiotics similar to erythromycin at rates of 500 and 1000 ppm was reported by Shetty and Rangaswami (1969) in the control of brown rot.

This work was made to study the possible use of antibiotics in brown rot control.

## Materials and Methods

*Sensitivity of the pathogen to antibiotics*

Virulent and avirulent isolates of the pathogen were obtained from the Dept. of Bacterial Diseases, Plant Pathology Institute. The isolates were tested for sensitivity to streptomycin, dihydro-streptomycin, penicillin, and chloramphenicol. The disk-diffusion method was used according to Gould and Bowie (1952). Filter paper disk impregnated with the desired concentration(s) of the antibiotics were prepared according to Cruickshank et al. (1975). The concentrations tested were 100, 250, 500 and 1000 ppm.

Mixed inocula of the isolates under investigation were used, to avoid differences in sensitivity to a single antibiotic (Seif-Elyazal, 1980). Surface of nutrient agar plates was seeded with 0.1 ml of five, mixed virulent, isolates propagated on nutrient broth for 48 hrs at 35 °C. The same method was used in case of avirulent isolates of the pathogen. Twelve replicates (4 disks/plate) were used for each concentration and the mean diameter of inhibition zones was measured after 3 days incubation at 35 °C.

*Effect of antibiotics on disease control*

Streptomycin as well as dihydrostreptomycin at the rates of 150 and 400 ppm were used in control trials. The early maturing Arran Banner potato cultivar was used, in a preliminary trial in this work, due to the reported high susceptibility to brown rot (Farag, 1970). Standard eye-pieces were removed from tubers by using semi-spherical knife. The cut surface of the pieces was infested with a bacterial slime of naturally infected tubers and dipped in antibiotic solution(s) for two minutes. Eye-pieces of control treatment were infested in the same manner and dipped in tap water before planting. Pots (25 cm in diam.) filled with Nile silt were used for planting. Each treatment was represented by twenty pots. The number of developed stems were counted and the percentage of wilt was determined.

In a second trial the same method was followed for testing the effect of streptomycin on wilt control of tolerant late maturing-Alpha cultivar, as compared with the susceptible early maturing-King Edward one (Farag, 1970; Mahmoud et al., 1974).

*Densities of total microbial flora in the thizosphere of treated plants*

Standard eye-pieces of cvs. Alpha and King Edward were prepared and inoculated as mentioned before. Streptomycin application was made by dipping either inoculated or un-inoculated pieces in solution containing 150 and 400 ppm for 2 minutes. Control treatments were prepared without either inoculation or antibiotic application.

The total microbial flora was determined by the dilution plate count technique. The soil extract agar medium ($gl^{-1}$: glucose, 1, $K_2HPO_4$ 2, Soil extract 100 ml, agar pH 6.8–7) was used in all determinations (Farag, 1976). Each treatment was represented by three plants at each interval.

*Effect of streptomycin on root exudation*

Alpha and King Edward potato cultivars were used in this experiment. Sterilization of tubers and preparation of eye-pieces for planting were made according to Farag (1976). Plastic non-perforated pots, filled with sterilized acid washed sand, were used for planting. Streptomycin application was made by dipping sterilized eye-pieces in 150 and 400 ppm solutions for 2 minutes before planting. Control treatments were prepared without antibiotic application. The pots were kept under aseptic condition for four weeks. The plants were up-rooted with their root system intact as possible elution and concentration of exudates were made according to Farag (1976).

Quantitative determination of amino acids was made by spotting of 0.1 ml, of the concentrated exudates, on Whatman No. 1 paper chromatorgaphy. The spots, without running, were made visible with 0.1% ninhydrin and extracted with 50% ethanol (5 ml/tube). The intensity of colour was determined at 510 nm Spectronic colorimeter. The amount of total amino acids was calculated from a standard curve constructed for leucine. Data are presented as mg leucine/10 g dry roots.

## Results

*Sensitivity of the pathogen to antibiotics*

Table 1 shows that both virulent and avirulent isolates of *P. solanacearum* are sensitive to streptomycin and dihydrostreptomycin. Penicillin and chloramphenicol, on the other hand, did not show any antimicrobial effect against the brown rot pathogen. The mixed isolates showed higher sensitivity to dihydro-

Table 1

Sensitivity of *P. solanacearum* isolates to antibiotics

| Antibiotics ppm | Mean diameter of inhibition zones in mm | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Virulent | | | | Avirulent | | | |
| | 1000 | 500 | 250 | 100 | 1000 | 500 | 250 | 100 |
| Dihydrostreptomycin | 23 | 18 | 16 | 14 | 22 | 19 | 17 | 16 |
| Streptomycin | 16 | 14 | 13 | 11 | 18 | 16 | 15 | 13 |
| Penicillin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2

Effect of antibiotics on disease incidence (first trila, Arran Banner*)

| Treatments | % infected plants/days after planting | | | Mean |
|---|---|---|---|---|
| | 43 | 50 | 56 | |
| Control | 5.0 | 25.0 | 40.0 | 23.33 |
| Streptomycin 150 ppm | 26.3 | 52.6 | 63.2 | 47.37 |
| Streptomycin 400 ppm | 25.0 | 65.0 | 70.0 | 53.33 |
| Dihydrostreptomycin, 150 ppm | 45.0 | 60.0 | 75.0 | 60.00 |
| Dihydrostreptomycin; 400 ppm | 38.8 | 77.8 | 83.3 | 66.00 |

* Early maturing, LSD 5%, 8.74

streptomycin as compared with streptomycin. It seems probable that the avirulent form of the pathogen is more sensitive to the tested antibiotics than the virulent ones.

*Effect of antibiotics on wilt incidence*

Data in Table 2 show that application of streptomycin and dihydrostreptomycin increased the percentage of wilt incidence on Arran Banner potato cultivar. It is interesting to note that application of dihydrostreptomycin caused a significant increase in wilt incidence as compared with that of streptomycin application. Meanwhile, no significant increase in wilt could be observed between the high and the low rate of application for the same antibiotic.

Table 3 shows the effect of streptomycin on wilt incidence on the early maturing-susceptible Kind Edward cultivar as compared with the late maturing-tolerant Alpha. Application of the high rate of the antibiotic to the first cultivar caused significant increase in wilt incidence. The same trend could be observed for the second cultivar at either high or low rates of application.

*Densities of total microbial flora in the rhizosphere*

The densities of microbial flora in the rhizosphere of King Edward and Alpha potato plants are shown in Table 4. The un-inoculated plants of cv. King Edward were characterized by higher densities of microbial flora in the rhizosphere as compared with cv. Alpha. Application of streptomycin to both cultivars increased the densities of total microbial flora.

Table 3

Effect of streptomycin on disease incidence (2nd trial, King Edward and Alpha)

| Treatments | % infected plants/days after planting | | | Mean |
|---|---|---|---|---|
| | 32 | 42 | 60 | |
| *King Edward* | | | | |
| Control | 6.7 | 46.7 | 76.6 | 43.32 |
| Streptomycin, 150 ppm | 12.5 | 50.0 | 75.0 | 45.83 |
| Streptomycin, 400 ppm | 35.71 | 78.6 | 85.7 | 66.67 |
| *Alpha* | | | | |
| Control | 00.0 | 30.3 | 67.5 | 32.61 |
| Streptomycin, 150 ppm | 20.0 | 50.0 | 75.0 | 48.30 |
| Streptomycin, 400 ppm | 12.5 | 55.6 | 88.9 | 52.31 |

LSD 5%, 11.58

Table 4

Effect of streptomycin on densities of microbial flora in the rhizosphere of King Edward and Alpha potato plants

| Treatments | *Microbial flora ($\times 10^6$)/days after planting | | | |
|---|---|---|---|---|
| | 40 | | 60 | |
| | Inoculated | Uninoculated | Inoculated | Uninoculated |
| *King Edward* | | | | |
| Control | 3117.6 | 761.4 | 5970.1 | 1747.4 |
| Streptomycin, 150 ppm | 1345.4 | 2252.2 | 2549.0 | 2976.2 |
| Streptomycin, 400 ppm | 480.7 | 1509.9 | 1538.5 | 3190.5 |
| *Alpha* | | | | |
| Control | 351.5 | 34.8 | 923.1 | 609.8 |
| Streptomycin, 150 ppm | 372.1 | 243.9 | 1200.0 | 685.9 |
| Streptomycin, 400 ppm | 918.6 | 362.3 | 2600.0 | 1378.0 |

* Counts per g dry soil

Infected plants of cv. Alpha showed considerable increase in densities, due to antibiotic application, as compared with respective controls. Under the same conditions, however, King Edward showed a sharp decrease in microbial densities after 40 and 60 days from planting.

*Effect of streptomycin on exudation*

Table 5 shows that root exudates of Alpha plants are normally poor in free amino acids as compared with those of King Edward. The low rate of strepto-

Table 5

Effect of streptomycin on exudation of free amino acids from roots of Alpha and King Edward potato cultivars

| Treatments | Free amino acids*/cultivars | |
|---|---|---|
| | Alpha | King Edward |
| Control | 0.130 | 0.380 |
| Streptomycin (150 ppm) | 0.626 | 0.570 |
| Streptomycin (400 ppm) | 0.800 | 0.665 |

* mg/10 g dry weight of roots

mycin application to both cultivars increased the amounts of amino acids exuded. No quantitative difference could be observed in exudates of both cultivars after antibiotic application. The high rate of application gave the same trend.

## Discussion

Bacterial wilt disease, brown rot of potato, has been reported as a serious problem in Egypt (Sabet, 1961; Lelliott, 1964). Control measures based on changes in certain agricultural practices have been suggested, however, no practical control could be achieved (Kelman, 1953; Farag, 1970; Mahmoud et al., 1974, and 1978).

Mixed isolates of virulent as well as avirulent forms of *P. solanacearum* showed pronounced sensitivity to either dihydrostreptomycin or streptomycin. The avirulent form showed a slightly higher sensitivity to these antibiotics and dihydrostreptomycin revealed higher deterimental effect as compared with streptomycin. This is in accordance with Seif-Elyazal (1980).

In a preliminary experiment, application of the aforementioned antibiotics, at the rates 150 and 400 ppm to artificially inoculated eye-pieces of cv. Arran Banner increased wilt incidence. Such inverse relation between *in vivo* and *in vitro* results led to another trial with physiologically different potato cultivars.

King Edward, an early maturing wilt-susceptible cultivar and Alpha a late maturing wilt-tolerant one (Mahmoud et al., 1974) were used in this work. These cultivars showed a significant increase in wilt following antibiotic application at the used rates. This indicates that neither differences in maturity nor differences in susceptibility could be contributed to the significant increase in disease incidences.

Inoculated plants of the cultivars under investigation showed higher densities of microbial flora in the rhizosphere as compared with the respective controls. Such increase may be attributed to possible changes in permeability of root membrane following infection. The change in membrane permeability of plant cells after inoculation was reported by many investigators. (Lai et al., 1968;

Cook and Stall 1968; Burkowicz and Goodman, 1969). Moreover, broth cultures of *P. solanacearum* were found to contain two toxic fractions, one of which was considered to be a plasma poison causing wilting by changing permeability in leaves (Kunz, 1952).

Application of streptomycin before planting to cv. Alpha increased the microbial densities in the rhizosphere of either inoculated or uninoculated plants. However, inoculated King Edward showed sharp decline in densities after antibiotic application, as compared with the respective controls.

Streptomycin application to both cultivars, increased the amino acids in root exudates. No pronounced quantitative differences in the determined amounts could be observed between King Edward or Alpha after this treatment. Such increase in amino acids exuded may indicate a considerable change(s) in permeability of root membranes.

It is well known that most substances involved in cellular metabolism of higher plants are released by exudation (Rovira, 1965). Therefore, the increased exudation of amino acids for antibiotic treated plants may coincide with a proportional increase in exudation of other nutrients necessary for growth of microorganisms, hence, high microbial size(s) was established in the rhizosphere of uninoculated plants. Such quantitative attributes, however, did not explain the reported decline in densities in case of inoculated antibiotic-treated King Edward. Under these circumstances the reported decline might be attributed to a greater increase of the pathogen population that inhibited other microorganisms, in the rhizosphere. The inhibitive ability of *P. solanacearum* against soil microorganisms was reported by Farag et al. (1980) and efficiency of potato root exudates in supporting growth of the pathogen was indicated by Mahmoud et al. (1978).

Generally, it could be concluded that the increase in wilt incidence after streptomycin application might be due to the increase in exudation by treated plants that changed the microbial equilibrium in favour of the pathogen. The correlation between the composition of root exudates of different potato varieties and disease susceptibility was studied by Farag (1976). Meanwhile, the increase in exudation as a result of antibiotic application, as well as following the pathogenesis with *P. solanacearum* may indicate to a possible alteration in plant water relation(s) that results in increased wilt incidence.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 123–126 (1986)*

# Integrated Control of *Fusarium* Wilt of Pepper

A. R. T. SARHAN[1] and F. M. SHARIF[2]

[1] Department of Biology, University of Salahaddin, Erbil, Iraq, [2] Agricultural and Water Resources Research Center, Scientific Research Council, Baghdad, Iraq

Incidence and severity of pepper wilt incited by *Fusarium oxysporum* f.sp. *redolens* were reduced after application of nitrogen and lime. Treatment with nutrient solution in which the nitrogen was high, plants were less diseased than plants that received low nitrate nitrogen. Similarly, plants growing in soil with an initial pH of 7.7.–8.3 showed lower wilt symptoms than plants growing at an initial pH of 6.4–7.0. By applying lime (2 g or 4 g $Ca(OH)_2$/kg soil) in conjunction with 420 and 630 ppm nitrogen in the nitrate form, greater disease reduction was obtained than by using the components individually. The two factors which individually reduced the wilt, although they did not control it, were additive when they were combined. Inoculated plants, in which wilt symptoms were controlled, were equal in quality and as regards flowering to uninoculated plants.

*Fusarium* wilt of pepper caused by *Fusarium oxysporum* f. sp. *redolens* is among the most serious diseases which limits production in different areas of Iraq. Based on the principles of integrated control and using different methods of control, the use of growth stimulators is recommended for the recovery of host plants. Effective and economical control of *Fusarium* wilt diseases in different hosts is depending on chemicals (Corden, 1965; Gould and Miller, 1970). Nitrate nitrogen supply has reduced the development of *Fusarium* wilt in tomato (Huber, 1980; Sarhan, et al., 1982) and in chrysanthenum (Woltz and Engelhard, 1973).

The liming of certain soils to a pH of about 7.0 to 8.0 reduced the severity of *Fusarium* wilt tomato of (Jones and Woltz, 1970; Sarhan, 1982). It was found that two mutually dependent factors, pH and nitrate nitrogen, significantly increased *Fusarium* wilt resistance and decreased wilt injury of cotton (Albert, 1946) as well as of tomato (Woltz and Jones, 1973; Sarhan and Király, 1981).

This study evaluates the individual and interrelated effects of nitrate nitrogen and lime upon the severity of *Fusarium* wilt of pepper caused by *F. oxysporum* f. sp. *redolens*.

## Materials and Methods

The experiments were carried out in the green-house under conditions of 12 hours day length and 25 ± 2 °C minima and 35 ± 2 maxima. A wilt susceptible cultivar of pepper, California Wonders, was used. Seeds were sown into soil, two weeksl ater seedlings were to be transplanted to a limed soil : sand mixture (2 : 2) in 12 cm pots. Four-week-old plants were inoculated by up-rooting them dipping the roots in inoculum, and repotting the plants in the original pots. The inoculum was a spore suspension ($2 \times 10^5$ spore/ml) of a pathogenic strain of *Fusarium oxysporum* f. sp. *redolens*. The pathogen grown on PDA medium at 28 °C (7-day-old) was used to infest the pepper plants. In each experiment 10 plants per treatment were used. Dry $Ca(OH)_2$ was thoroughly incorporated in the soil and allowed to equilibrate for one week prior to potting. Potted pepper plants were supplied with nutrients based on Hogland's solution but containing different levels of nitrogen most of it as nitrate, according to our previous experiences (Sarhan et al., 1982) all pots received 40 ml of appropriate solution twice a week for 7–8 weeks and the remainder of the water requirement being supplied as water.

Soil pH was determined for each treatment because of the significance of this parameter of the soil environment in the development of *Fusarium* wilt. The soil samples for determination of pH were prepared with 50–50 (v/v) soil–deionized water which had equilibrated 4 weeks after inoculation. The plant stems were cut and examined for vascular browning. Disease was evaluated for each plant by the method of Edgington and Walker (1958), 0, plant healthy; 20, one leaf wilted; 40, two to several leaves wilted; 60, all leaves wilted except growing tip; 80, all leaves wilted; 100, plant dead. Plants were harvested at the end of the period and the fresh weight of shoots was determined.

## Results and Discussion

The results are presented in Table 1. Disease was most severe as measured by vascular discoloration and wilting of leaves of plants grown in soil (as control) and in the sandy soil when the nitrogen concentrations were at 70 and 280 ppm. Plants grown at 420 and 630 ppm $NO_3$ nitrogen level were significantly more resistant to wilt than plants grown at other concentrations. Soil pH was raised by lime (Table 2) and the elevated pH values resulted in less disease symptoms and the growth of plants (fresh weight) increased. The soil pH was also raised by nitrate nitrogen as well as by the greater amount of liming material, and disease was less. Vascular browning was inversely related to soil pH. The evidence has been presented (Sarhan and Király, 1981) that high soil pH produced by lime application may increase the resistance of plants so *Fusarium* infection by interfering with the nitrate nitrogen nutrition. It is suggested by Woltz and Jones (1973) that *Fusarium* wilt of tomato is affected by inorganic nutrients in the soil environment and by other factors affecting availability of some elements to the host

Table 1

Effect of different levels of nitrate nitrogen and liming on the severity of *Fusarium* wilt of pepper

| Concentrations of nitrogen (ppm) | Wilt disease index** | | | | Vascular discoloration in per cent* | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount of lime g $Ca(OH)_2$/kg soil | | | | Amount of lime g $Ca(OH)_2$/kg soil | | | |
| | 0 | 1 | 2 | 4 | 0 | 1 | 2 | 4 |
| 0 | 84.3 | 80.8 | 69.5 | 60.1 | 79.4 | 71.4 | 62.2 | 53.3 |
| 70 | 77.9 | 72.3 | 55.9 | 56.1 | 72.1 | 69. 1 | 60.0 | 51.6 |
| 280 | 79.8 | 70.8 | 50.2 | 51.8 | 72. 8 | 66.3 | 58.9 | 48.7 |
| 420 | 40.5 | 38.8 | 29.7 | 22.4 | 53.3 | 48.5 | 41.2 | 32.1 |
| 630 | 29.7 | 31.4 | 20.4 | 18.5 | 38.6 | 35.1 | 22.5 | 20.5 |
| $LSD_{0.05\%}$ | 8.4 | 6.6 | 11.3 | 10.2 | 9.6 | 8.1 | 5.8 | 6.5 |

* Length of stem with vascular discoloration as percentage of whole stem length
** Each figure represents the average index of 3 replicates of 10 plants each

Table 2

Effect of different levels of nitrate nitrogen and liming on soil pH and on the growth of pepper

| Concentrations of nitrogen (ppm) | pH values | | | | Mean fresh weight (g/plant)* | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount of lime g $Ca(OH)_2$/kg soil | | | | Amount of lime g $Ca(OH)_2$/kg soil | | | |
| | 0 | 1 | 2 | 4 | 0 | 1 | 2 | 4 |
| 0 | 6.4 | 6.8 | 7.3 | 8.0 | 9.2 | 16.5 | 20.0 | 21.3 |
| 70 | 6.4 | 6.7 | 7.4 | 7.9 | 14.1 | 18.5 | 22.9 | 20.4 |
| 280 | 6.5 | 6.8 | 7.4 | 8.0 | 21.0 | 25.6 | 29.7 | 28.0 |
| 420 | 6.6 | 6.8 | 7.6 | 8.1 | 34.2 | 34.8 | 41.2 | 43.2 |
| 630 | 6.6 | 7.0 | 7.7 | 8.3 | 37.4 | 39.5 | 45.5 | 44.0 |
| $LSD_{0.05\%}$ | | | | | 4.8 | 7.3 | 5.9 | 8.6 |

* Each number is an average of three replicates of 10 plants each

and the pathogen. Previously it was hypothesized (Sarhan, 1982) that the control of *Fusarium* wilt of tomato by lime resulted from high soil pH which affected the macronutrients in the soil. Macronutrients may influence the growth of the pathogen. Our findings agree in general with those of Albert (1946) and Woltz and Engelhard (1973). However, the results for one hostparasite relationship do not necessarily apply to other *Fusarium* wilt disease. It is apparent from the data presented, together with other previously published information about *Fusarium* wilt of tomato (Sarhan et al., 1982), that the increased resistance of host under high nitrogen supply is related to a juvenility effect.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 127—133 (1986)*

# *Fusarium* Post-emergence Damping-off of *Eucalyptus* and its Control Measures in Egypt

S. H. MICHAIL[1], A. B. EL-SAYED[1] and M. A. SALEM[2]

[1]Faculty of Agriculture, Alexandria University, Alexandria, Egypt,
[2]Faculty of Agriculture Menoufeia University, Shebin El-Kom, Egypt

*Fusarium solani* (Mart.) Sacc. was found to be seed-borne in *Eucalyptus*. The fungus caused post-emergence damping-off to 10 *Eucalyptus* spp. tested. The disease was effectively controlled by drenching the seedlings by Benlate or TBZ at the rate of 2 ml (0.1 g/l l water) / 35 g soil, or biologically controlled by adding hyphal spore suspension or filtrate of *Trichoderma longibrachiatum* Rifai or *Aspergillus sydowii* (Bainer & Sartory) Thom & Church at the rate of 2 ml/35 g soil in which the seedlings were grown. Each ml contained 115 200 conidia in case of *A. sydowii* and 64 000 conidia in case of *T. longibrachiatum*. *T. longibrachiatum* and *A. sydowii* decreased mycelial dry weight, sporulation and spore germination of *F. solani*.

Eucalypt (*Eucalyptus camaldulensis* L.) is a common forest tree in Egypt.

Mahmoud (1971) in Pakistan isolated *Fusarium solani* from the roots of damped-off seedlings of *Eucalyptus camaldulensis*. Arya and Jain (1962) in India, isolated *Fusarium oxysporum* Schlecht. f. sp. *eucalyptii* from roots of wilted *Eucalyptus gomphocephala* DC. Seedlings.

As there is no work written on damping-off of *Eucalyptus* in Egypt, except that by Salem et al. (under publication), the present work was undertaken to investigate the causal organism(s) of damping-off of several species of *Eucalyptus*, along with chemical and biological control measures.

## Materials and Methods

Seeds of *Eucalyptus camaldulensis* placed in a muslin bag were pretreated in 1% sodium hypochlorite solution for 10 minutes, then plated on PDA medium at the rate of 10 seeds/dish. Dishes were incubated at 20 °C under alternating cycles of NUV light and darkness for 7 days. Two hundred seeds were used (Ista, 1976).

The pathogenic potentiality of the isolated fungus was tested on 15-day-old seedlings of the local cultivar, *E. camaldulensis* along with other recently introduced species, viz. *E. astringes* Cunn. *E. camaldulensis* (Morocco), *E. camaldulensis* (Tunisia), *E. camaldulensis lakalbacotia*, *E. cladocalyx* F. Muell, *E. maideni* Smith, *E. occidentalis* Endl., *E. salubris* F. Muell and *E. tereticornis* Smith.

Soil inoculation was carried out using plastic multi-pot plates. Each plate had 140 small pots of 35 g soil/pot. Autoclaved potted soil was inoculated with hyphal/spore suspension of 7-day-old culture of the isolated fungus, grown on potato dextrose liquid medium at room temperature (24–29 °C), at the rate of 2 ml/pot. Pots served as check were kept uninoculated. Ten seedlings were raised per pot. Four pots were used per treatment.

Along with the pathogenicity tests, chemical and biological control measures were carried out at the same time in the multipot plates. Certain systemic fungicides, namely, Benlate, TBZ, Vitavax 200 and Vitavax 300 (Vitavax + Captan) were used as soil drench at concentration of 0.1 g/l. Fifteen-day-old seedlings of 10 *Eucalyptus* species were planted in *F. solani*-inoculated soil in the plastic multipot plates, were immediately drenched with the fungicides at the rate of 2 ml/pot. Pots served as checks were similarly treated but without drenching with fungicides. Four pots were used/treatment. Percentages of post-emergence damping-off were recorded periodically for 60 days.

For studying the effect of the filtrate concentration of *A. sydowii* and *T. longibrachiatum* on sporulation of *F. solani*, the latter fungus was grown on filtrate concentration incorporated in PDA medium in 9-cm Petri dishes and kept at room temperature for 7 days. Four discs (4 mm diameter), half way between colony centre and the edge were agitated in 1 ml water. Few drops were transferred to a haemocytometer and the number of spores/ml was computed.

*Aspergillus sydowii* and *Trichoderma longibrachiatum* were used as biological control measure. Seven-day-old blended cultures previously grown on potato dextrose liquid medium at room temperature (24–29 °C) and their filtrates were used. Hyphal/spore suspension and filtrate of each fungus were immediately added to 15-day-old seedlings of *Eucalyptus* spp. after planting in *F. solani*-inoculated soil in the plastic multipot plates, at the rate of 2 ml/pot. Each ml contained 32 000 conidia. Pots served as check were similarly treated but without adding *A. sydowii* or *T. longibrachiatum*. Four pots were used/treatment. Percentages of post-emergence damping-off were recorded periodically for 60 days.

## Results

### *Seed health testing*

Seed health testing of *Eucalyptus camaldulensis* showed the presence of *Fusarium solani** at the rate of 3%.

### *Pathogenicity tests*

Seedlings of the ten *Eucalyptus* spp. tested varied in their susceptibility to *F. solani* (Table 1). *E. astringens*, *E. terticornis* and *E. occidentalis* were the most

* Identified by Dr. C. Booth of the CMI and kept under IMI No. 279 787.

Table 1

Effect of fungicidal drenching of *Eucalyptus* spp. with certain systemic fungicides on postemergence damping-off caused by *F. solani*

| *Eucalyptus* spp. | % of post-emergence damping-off in *F. solani*—inoculated soil | | | | |
|---|---|---|---|---|---|
| | Check | Benlate | TBZ | Vitavax 200 | Vitavax 300 |
| *E. astringens* | 88.54[a] | 4.16[e] | 9.82[a] | 69.79[a] | 32.29[g] |
| *E. camaldulensis* (Egypt) | 80.00[bc] | 9.02[cd] | 18.68[a] | 52.49[c] | 37.50[f] |
| *E. camaldulensis* (Marocco) | 70.06[d] | 9.02[cd] | 19.39[a] | 66.38[a] | 46.87[de] |
| *E. camaldulensis* (Tunisia) | 83.88[abc] | 11.62[cd] | 10.55[a] | 17.77 | 28.33[g] |
| *E. camaldulensis lakalbacotia* | 82.50[abc] | 8.33[d] | 10.27[a] | 45.00[d] | 81.10[a] |
| *E. cladocalyx* | 72.08[d] | 45.83[a] | 19.84[a] | 54.16[c] | 48.05[d] |
| *E. maideni* | 32.08[e] | 5.62[e] | 12.59[a] | 26.66[e] | 28.33[g] |
| *E. occidentalis* | 86.38[ab] | 11.25[cd] | 10.55[a] | 46.51[d] | 38.75[ef] |
| *E. Salubris* | 77.50[cd] | 20.62[b] | 9.16[a] | 54.16[c] | 55.48[cf] |
| *E. tereticornis* | 86.66[ab] | 19.12[b] | 7.29[a] | 58.67[b] | 67.02[b] |
| Average | | 61.41 | 63.15 | 26.81 | 29.59 |

Means followed by the same letter within the same treatment are not significantly different at 0.05 level according to LSD.

susceptible as the percentage of post-emergence damping-off reached up to 88.54, 86.66 and 86.38% respectively. *E. maideni* was moderately susceptible as the percentage of post-emergence damping-off was 32.08%.

*Chemical control*

Data on the effect of the four systemic fungicides on controlling the post-emergence damping-off of *Eucalyptus* spp. are presented in Table 1. Data in Table 1 show that Benlate and TBZ had the best effect in combatting post-emergence damping-off of *Eucalyptus* seedlings. Benlate had the best effect on *E. camaldulensis* (Egypt), *E. astringens*, *E. camaldulensis* (Morocco), *E. camaldulensis lakalbacotia* and *E. maideni*. However, TBZ had the best effect on *E. cladocalyx*, *E. tereticornis*, *E. occidentalis*, *E. salubris*, *E. camaldulensis* (Tunisia) and significant differences between the different fungicides were detected. The fungicide TBZ and Benlate were the most effective.

Table 2

Effect of *A. sydowii* and *T. longibrachiatum* or their filtrates in controlling post-emergence damping-off of *Eucalyptus* spp. caused by *F. solani*

| *Eucalyptus* spp. | % of post-emergence damping-off in *F. solani* — inoculated soil | | | | |
|---|---|---|---|---|---|
| | Check | *A. sydowii* | | *T. longibrachiatum* | |
| | | Fungus | Filtrate | Fungus | Filtrate |
| *E. astringens* | 88.54[a] | 17.50[d] | 7.50[a] | 12.50[a] | 7.50[cd] |
| *E. camaldulensis* (Egypt) | 80.00[bc] | 17.36[d] | 16.52[a] | 5.00[a] | 8.12[cd] |
| *E. camaldulensis* (Morocco) | 70.06[d] | 17.50[d] | 18.12[a] | 0.00[a] | 5.00[d] |
| *E. camaldulensis* (Tunisia) | 83.88[abc] | 38.05[a] | 15.62[a] | 8.57[a] | 0.00[e] |
| *E. camaldulensis lakalbacotia* | 82.50[abc] | 10.62[e] | 10.83[a] | 2.77[a] | 0.00[e] |
| *E. cladocalyx* | 72.08[d] | 35.00[b] | 20.55[a] | 5.00[a] | 30.00[b] |
| *E. maideni* | 32.08[e] | 0.00[g] | 5.00[a] | 10.83[a] | 0.00[e] |
| *E. occidentalis* | 56.38[ab] | 7.14[f] | 12.50[a] | 43.33[a] | 5.00[d] |
| *E. salubris* | 77.50[cd] | 10.00[e] | 18.33[a] | 10.00[a] | 35.00[a] |
| *E. tereticornis* | 86.66[ab] | 30.83[c] | 15.26[a] | 17.50[a] | 10.00[c] |
| Average | | 54.56 | 58.94 | 61.41 | 62.96 |

Means followed by the same letter are not significantly different at 0.05 level according to LSD.

Table 3

Effect of filtrate concentrations of *A. sydowii* and *T. longibrachiatum* on the dry weight of *F. solani* grown on potato dextrose liquid medium at room temperature (24—29 °C)

| Filtrate conc. (filtrate + PD liquid medium | Dry weight (mg)* | |
|---|---|---|
| | *A. sydowii* | *T. longibrachiatum* |
| 0+10 | 0.19[a]** | 0.19[a] |
| 1+9 | 0.19[a] | 0.19[a] |
| 2+8 | 0.08[b] | 0.11[b] |
| 3+7 | 0.05[b] | 0.06[c] |
| 4+6 | 0.03[c] | 0.02[d] |

* Mean of four replicates

** Means followed by the same letter are not significantly different at 0,05 level according to LSD

*Biological control*

*In vitro* studies of the effect of *Aspergillus sydowii* (Bainer & Sartory) Thom & Chirch* and *Trichoderma longibrachiatum* Rifai** on PDA medium show a marked antagonistic effect against *F. solani*. This led to study the effect of several concentrations of *A. sydowii* and *T. longibrachiatum* filtrates on the dry weight (Table 3) and sporulation of *F. solani* (Table 4). Data (Table 3) show that the filtrate of each of *A. sydowii* and *T. longibrachiatum* affected the dry weight of *F. solani*. The decrease in the dry weight was proportional with the increase in the filtrate concentration. Filtrate of *A. sydowii* seemed to be more effective in diminishing the dry weight of *F. solani*.

Filtrates of *A. sydowii* and *T. longibrachiatum* (Table 4) caused marked decrease in the sporulation of *F. solani*. The decrease in the number of spores formed was proportional with the increase in the filtrate concentration of *A. sydowii* or *T. longibrachiatum*.

The effect of filtrates of *A. sydowii* and *T. longibrachiatum* on spore germination of *F. solani* was studied in Van Tieghem cells for 18 hours at room temperature (24–29 °C). Data (Table 5) show that the filtrate of *A. sydowii* and *T. longibrachiatum* restricted the percentage of spore germination of *F. solani*.

*In vivo* studies (Table 2) show that in general, the filtrates of *A. sydowii* or *T. longibrachiatum* were more effective in checking the post-emergence damping-off

Table 4

Effect of filtrate concentraions of *A. sydowii* and *T. longibrachiatum* incorporated in PDA medium on sporulation of *F. solani* after 7 days

| Filtrate conc. (filtrate + PDA) | Number of *F. solani* spores/ml* | |
|---|---|---|
| | *A. sydowii* | *T. longibrachiatum* |
| Check (water) | 84000[a]** | 84000[a] |
| 0.5+9.5 | 66400[b] | 65000[b] |
| 1.0+9.0 | 38400[c] | 34800[c] |
| 1.5+8.5 | 16000[d] | 13000[d] |
| 2.0+8.0 | 6800[e] | 3400[e] |
| 2.5+7.5 | 1400[e] | 1800[f] |

* Mean of 10 readings

** Means followed by the same letter are not significantly different at 0.05 level according to LSD

* Identified by Dr. B. L. Laurence of CMI, and kept under IMI No. 285 710.

** Identified by Dr. Brady of CMI, and kept under IMI No. 281796.

Table 5

Effect of filtrates of *A. sydowii* and *T. longibrachiatum* on spore germination of *F. solani* after different periods at room temperature (24—29 °C)

| Period (h) | % of germination of *F. solani* spores | | |
|---|---|---|---|
| | Control (water) | *A. sydowii* | *T. longibrachiatum* |
| 1 | $0.0^{f}$ | $0.0^{d}$* | $0.0^{f}$ |
| 2 | $6.6^{e}$ | $2.1^{c}$ | $1.8^{e}$ |
| 4 | $34.2^{d}$ | $5.0^{b}$ | $4.2^{d}$ |
| 6 | $68.6^{c}$ | $5.5^{b}$ | $7.6^{c}$ |
| 8 | $88.2^{b}$ | $7.8^{b}$ | $8.3^{b}$ |
| 10 | $100.0^{a}$ | $10.9^{a}$ | $8.5^{b}$ |
| 18 | $100.0^{a}$ | $11.7^{a}$ | $11.2^{a}$ |

* Means followed by the same letter are not significantly different at 0.05 level according to LSD

of the ten *Eucalyptus* spp. tested with the exception of *E. camaldulensis* (Egypt), *E. camaldulensis* (Morocco), *E. salubris* and *E. cladocalyx*. The filtrate of *T. longibrachiatum* was more effective than that of *A. sydowii*. The effectiveness of each fungus and its filtrate varied in checking the postemergence damping-off of *E. maideni*. Also, significant difference were found between the average means of the fungi and their filtrates. The fungus *T. longibrachiatum* was more effective than the *A. sydowii* in their fungal suspension and filtrate.

## Discussion

The present work showed that *F. solani* is seed-borne in *Eucalyptus*. The isolated fungus could cause damping-off to ten species of *Eucalyptus* in soil inoculation tests. Benlate and TBZ could check the post-emergence damping-off in most of the tested *Eucalyptus* spp. tested. The filtrate of *T. longibrachiatum* effectively controlled the *F. solani* post-emergence damping-off of *E. camaldulensis* (Tunisia), *E. camaldulensis lakalbacotia*, *E. maideni* and *E. occidentalis*. The fungal growth of *T. longibrachiatum* substantially decreased the postemergence damping-off of *E. camaldulensis* (Morocco), *E. camaldulensis* (Egypt) and *E. cladocalyx*. Such results agree with the findings of Raicu and Stan (1975) who found that *Trichoderma* sp. was highly antagonistic to *Fusarium oxysporum*. The mycelial/spore suspension of *A. sydowii* or its filtrate profoundly decreased the post-emergence damping-off *E. maideni*, *E. camaldulensis lakalbacotia*, *E. astringens*, *E. occidentalis*. Such results agree with Khanna et al. (1979) who found that the filtrate of *Aspergillus niger* completely inhibited spore germination of *Fusarium roseum*.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 135—140 (1986)*

# Variability in *Pyricularia oryzae* Cav. in Successive Generations

B. PADHI and N. K. CHAKRABARTI*

Central Rice Research Institute, Cuttack 753006, India

The increase and decrease in virulence of the blast pathogen, *P. oryzae* could be observed when different isolates were passed through successive generations on the host differentials. The variability was maximum in the 3rd generation and was minimum in the first. The re-appearance of mother race IC-30 was 8.58% while it was 7.36 in the case of ID-2. The appearance of other new races in different generations from both mother races was variable. New race groups viz. IE (from mother isolate IC-30) IC and IE (from mother isolate ID-2) appeared in different generations. Interestingly, the new races from the race groups IC, ID and IE were only restricted and the appearance of other races (from race groups like IA, IB, IF and IH etc.) could not be observed.

The monoconidial subcultures of two physiologic races of *P. oryzae* were studied on a set of international differentials through successive generations by Giatgong and Frederiksen (1967, 1969) who reported that in each generation the subcultures differed in their pathogenicity to the differentials. Bidaux and Notteghem (1973) observed that several strains of the blast fungus exhibited different virulence spectra when tested on a set of rice cultivars. However, Veeraraghavan (1975) claimed that *P. oryzae* isolates reach a stability in their pathogenicity and do not show any variation. These observations prompted us to study the variations in the pathogenicity of virulent and avirulent isolates of *P. oryzae* as influenced by subculturing.

## Materials and Methods

The international set of blast differentials viz., Raminad Str. 3, Zenith, NP 125, Usen, Dular, Kanto-51, CI 8970(S) and Caloro along with a highly blast susceptible check CO. 13 were sown in GI nursery trays of $50 \times 20 \times 10$ cm size. Fertilizers were added in the form of ammonium sulphate in two split doses (once at sowing and the other a week before inoculation i.e. 15 day old seedlings) 80 kg/ha. The 21 days old seedlings with vigorous growth and dark green in colour

* Director, Jute Agricultural Research Institute, Barrackpore 743101, West Bengal, India

were ready for artificial inoculation. Physiologic race IC-30 (isolated from cultivar Np 125 and treated as avirulent strain) infected cultivars NP 125 and CI 8970(S) while, race ID-2 (isolated from Usen and treated as virulent strain) infected cultivars Usen, Dular, Kanto-51 and CI 8970(S) of the international set of blast differentials. Both the races produced susceptible reaction on the check cultivar CO. 13.

Spore suspension was prepared in sterilized water from both the cultures (mother race IC-30 and ID-2) grown in Oat Meal Agar (OMA) medium. Artificial inoculation was carried out from the spore suspension in the evening hours when the atmospheric temperature touched 20 °C (Padmanabhan et al., 1967) separately on two different nursery trays.

The infected leaf samples were collected from infected cultivars of the differentials separately 10 days after inoculation and the pathogen was isolated separately. The monosporic cultures of the isolates thus obtained were subcultured on OMA medium artificially and kept 10 days for sporulation. Again, the spore suspensions were prepared and inoculated to fresh sets of differentials (21 days old seedlings) for race identification. The inoculation of the differentials and identification of races were carried out three times consecutively covering three generations. The isolation of the pathogen was done from the infected leaf samples of all the varieties of the differentials wherever there was infection. The identification of races also was done with the help of artificial inoculation on the international set of blast differentials (Ling and Ou, 1969).

## Results

After a period of three successive generations, it was observed from both mother races (IC-30 and ID-2) that the variation in appearance of different races was more in $G_3$ compared to other two generations (Table 1).

It was noticed (Fig. 1) that the mother race IC-30 gave rise to IC-17, IC-29, IC-30 and IE-5 in $G_1$, IC-17, IC-22, IC-29, IC-30, IE-2, IE-5, IE-7 and IE-8 in $G_2$ and IC-17, IC-18, IC-22, IC-23, IC-29, IC-30, IC-31, IE-2, IE-3, 'E-5, IE-7 and IE-8 in $G_3$. The percentages of appearance (Table 1) of races belonging to 'IC' were 1.83 in $G_1$, 5.52 in $G_2$ and 14.71 in $G_3$, while the other group 'IE' was 0.61 in $G_1$, 3.06 in $G_2$ and 10.42 in $G_3$. It could be marked that the percentage of reappearance of race group 'IC' was maximum (14.71) followed by 'IE' (10.42%) in $G_3$ and was minimum in $G_1$, being 1.83% and 0.61% respectively while the appearance of other race groups were of intermediate type in $G_2$. However in all the three generations, the appearance of 'IC' group was more.

It was observed (Fig. 1) that the mother race ID-2 gave rise to more number of races (IC-17, ID-2, ID-6 and IE-2 in $G_1$, IC-17, IC-18, IC-22, IC-29, ID-2, ID-6, ID-10, ID-13, IE-1, IE-2, IE-4, IE-5, IE-8 in $G_2$ and IC-17, IC-18, IC-22, IC-29, IC-30, ID-2, ID-6, ID-7, ID-9, ID-10, ID-11, ID-13, ID-16, IE-1, IE-2, IE-5, IE-6, IE-7 and IE-8 in $G_3$) than IC-30 after three successive generations. In

Table 1

Variation in the occurrence (in percentage) of different race groups of *P. oryzae* in different generations

| Generations (G) | Race groups | Mother race IC-30 (in percentage) | Mother race ID-2 (in percentage) |
|---|---|---|---|
| $G_1$ | IC | 1.83 | 0.61 |
| | ID | — | 1.83 |
| | IE | 0.61 | 0.61 |
| $G_2$ | IC | 5.52 | 3.67 |
| | ID | — | 5.52 |
| | IE | 3.06 | 4.29 |
| $G_3$ | IC | 14.71 | 12.26 |
| | ID | — | 14.71 |
| | IE | 10.42 | 20.23 |

this case also, the number of races appeared were more in $G_3$ followed by $G_2$ and $G_1$. From Table 1 it can be noticed that in $G_1$ percentage of IC group was 0.61, ID as 1.83 and IE as 0.61 while in $G_2$ they were 3.67%, 5.52% and 4.29% and in $G_3$ 12.26%, 14.71% and 20.23% respectively. Among these, 'ID' group was more prevalent in $G_1$ and $G_2$, while in $G_3$ 'IE' group was maximum.

When compared, race group 'IC' (mother race IC-30) was observed to be more in all generations while group 'IE' (from mother race ID-2) was observed to be more in $G_3$ only. The appearance of IC race group was more (22.06% in total) from mother race IC-30 while, it was IE group which was having 25.13% from mother race ID-2.

It was noticed (Fig. 2) that the mother race IC-30 gave rise to more number of races from IC group than IE. Races IC-18, IC-23, IC-31 and IE-3 appeared in least percentages compared to IC-30 (more than 24%) and IE-5, IE-8, IC-22 and IC-29 while the other races were of intermediate in their appearance.

Considering mother race ID-2, it was noticed that more number of races reappeared than mother race IC-30. The maximum percentages of reappearance was IE-5, ID-2, IE-8, IC-17, intermediate types were IC-29, ID-6, ID-10, ID-13, IE-2 and IE-7 while the rest were of least in their percentages of appearance.

The overall frequency of variation using the original isolate IC-30 in all the three generations gave rise to distinctly 12 different races, while, the mother race ID-2 gave rise to 20 races. The variation was more in the later case when it was passed through the host plant for a period of continuous three successive generations. Interestingly when both mother races IC-30 and ID-2 were subjected to pass through three successive generations, the appearance of races from only three race groups viz., IC, ID and IE were restricted.

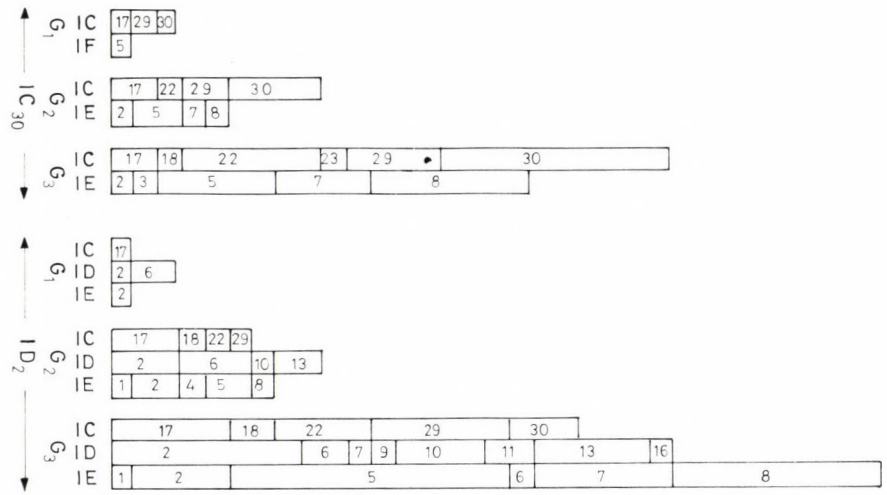



Fig. 1. Proportions of races in *P. oryzae* in successive generations

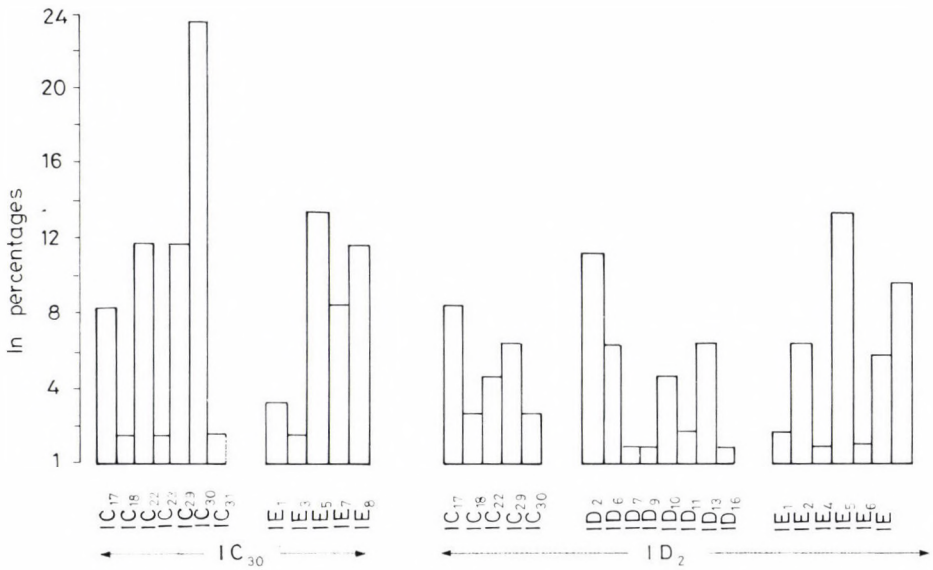



Fig. 2. Appearance of race of *P. oryzae* after three generations

## Discussion

In the present investigation, it was observed that when physiologic races IC-30 and ID-2 were passed through rice cultivars of international set of blast differentials and a susceptible cultivar, CO. 13, variation in the occurrence of pathogenic types was observed and several races from different race groups were detected which might be due to the genetic changes within the pathogen as observed by Giatgong and Fredericksen (1967, 1969). The variation was found to be more frequent with mother race ID-2. The virulence of the pathogen was found to decrease in most of the cases except a few, where it was increased as was observed by Bidaux and Notteghem (1973).

The variation of the fungus due to passing through successive generations might be cause for the genetic changes resulted from heterokaryosis within the pathogen as was suggested by Suzuki (1963), Giatgong and Fredericksen (1967, 1969), Genovesi (1975) and Fatemi and Nelson (1977). In the present study unlike the findings of Bidaux and Notteghem (1973) both increase and decrease in virulence of the pathogen was observed and this might explain the variation in pathogenicity noticed. This observation was similar to the findings of Hooker (1957) and Cherwick (1958) as discussed above who also observed considerable variation on smut races even in the 10th generation of selection.

## Acknowledgements

The authors are grateful to Dr. H. K. Pande, Director for allowing this manuscript for publication.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 141–146 (1986)*

# Growth and Nutrition of *Alternaria alternata* Pathogenic to Peas

L. Susuri and D. J. Hagedorn

Agricultural Faculty, University of Kosova, 38000 Prishtine, Yugoslavia, and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA

Two isolates of *Alternaria alternata* the causal organism of leaf spot and blight of peas (*Pisum sativum*) grew well on potato dextrose agar between 20 and 32 °C: with optimum at 28 °C. Mannose, dextrin, cellobiose and fructose were the best carbon sources. As nitrogen sources peptone, calcium nitrate and urea supported maximum mycelial growth. Casein hydrolysate, proline and arginine were the best amino acid sources for mycelial growth. Both isolates grew well on media at 3.7–7.1 with optimum at 6.5 pH.

A highly visible leaf spot and blight of Wisconsin processing peas (*Pisum sativum* L.), incited by *Alternaria alternata* (Fries) Keissler, has been described by Susuri and Hagedorn (1979, 1982) and studied in laboratory and greenhouse. These studies indicated that high and prolonged relative humidity (RH) is the most important environmental condition for severe disease development. In view of the importance of the pea crop in Wisconsin and the need to study the disease involved, it seemed desirable to learn more of the physiology of the fungus which cause *Alternaria* leaf spot. Such studies would be especially useful for efficient growing of inoculum for disease resistance studies. This paper will report the results of these investigations.

## Materials and Methods

Two isolates of *A. alternata* were used: isolate P from a diseased pea plant collected in a commercial field of processing peas near Fairwater, WI in 1976; and for comparative purposes isolate S from soybean (*Glycine max.*/L./Murr.) growing in the University of Wisconsin Linden Drive greenhouses in the fall of 1976.

The effect of temperature on colony growth was tested at 4 °C intervals from 4–36 °C. Petri dishes with potato dextrose agar medium were used for this experiment. They were inoculated with a 5 mm disc of mycelium from a 5 day. old culture and incubated at the test temperatures for ten days. The radial growth was determined after 1, 2, 3, 6 and 10 days of incubation.

The effect of various carbon sources on the growth of *A. alternata* was compared in 50 ml of a synthetic liquid medium in 250 ml pyrex Erlenmeyer flasks. Individual carbon sources were added to the basal medium before sterilization at the rate equivalent to 10 g dextrose per litre. The basal synthetic medium consisted of $NaNO_3$, 2.0 g; $MgSo_4 \cdot 7H_2O$, 0.5 g; $KH_2PO_4$, 0.5 g, $Na_2HPO_4$ 0.65 g; KCl, 0.5 g; distilled water, 1000 ml.

The various media were sterilized by autoclaving for 15 min at 121 °C. The pH of each of the media was adjusted to 6.5–6.8. The flasks were inoculated with a single 5 mm disc of mycelium cut from the advancing margin of a five-day-old culture of isolate P or S *(A. alternata)* growing on PDA at 28 °C and inocubated for ten days at 28 °C. Four flasks from each treatment were harvested, the mycelium was filted and washed with distilled water using a Buchner Funnel with Whatman 202 filter paper, then dried for 24 h at 80 °C and weighed.

The effect of various nitrogen sources on the growth of *A. alternata* was studied in a synthetic liquid basal medium with dextrose as the carbon source. The N compounds were added similarly (equivalent) to the amount of N present in 3.5 g of $KNO_3$. The basal liquid medium consisted of $KH_2PO_4$, 5.0 g; $MgSO_4 \cdot \cdot 7H_2O$, 2.5 g; $FeCl_3$, 0.02 g; dextrose, 20.0 g; $Na_2HPO_4$, 12.5 g; citric acid, 5.2 g; distilled water, 1000 ml. Peptone was used at the same rate, i.e. 3.5g/l. The urea was added aseptically to a sterilized basal liquid medium through sterile filters. Before sterilization the pH of the medium was adjusted to 6.8. After sterilization, flasks were inoculated as above. The cultures were incubated for ten days at 28 °C, then mycelium was harvested, dried and weighed as above. The effect of different amino acids as nitrogen sources was investigated in a synthetic liquid medium at the rate of 0.4 g N/l. The experiment was the same as with N compounds.

The influence of pH on growth of *A. alternata* was tested from pH 2.6 to pH 7.5, using Wolpert's (10) liquid basal synthetic medium modified by adding 10 g dextrose and 0.005 g $FeSO_4$. Pyrex Erlenmeyer flasks with capacity of 125 ml were used, and each flask contained 35 ml of adjusted medium to various pH levels. Sterilization and incubation was done as above. The cultures were incubated for ten days at 28 °C, then the mycelium was harvested, washed, dried and weighed.

## Results

### *Carbon nutrition*

Isolates differed in their responses to the carbon sources. Mannose supported maximum growth of isolate P, while isolate S produced the greatest amount of mycelium on cellobiose (Table 1). Isolate P also had excellent mycelium growth on dextrin and cellobiose, while isolate S responded similarly on dextrin. The poorest growth of both isolates was shown by inulin.

*Nitrogen nutrition*

Both isolates (P and S) produced the maximum dry weight of mycelium on peptone and considerable growth on calcium nitrate (Table 1). The least growth was in a medium with sodium nitrite.

Among the amino acides tested, casein hydrolysate and proline gave the best growth. Other amino acids tested supported good growth except tyrosine and tryptophane which showed the poorest mycelium growth.

*Hydrogen-ion concentration*

*Alternaria alternata* showed moderate growth from pH 3.7 to pH 7.1 with maximum at pH 6.5. Poorest mycelium growth was at or below pH 3.0 and at pH 7.5.

*Temperature*

Both isolates grew well over a wide range of temperatures with the optimum at 28 °C (Fig. 1). Growth at 36 °C was as good as at 20 °C. *A. alternata* sporulates well under continuous white light intensity 10.2 cd.

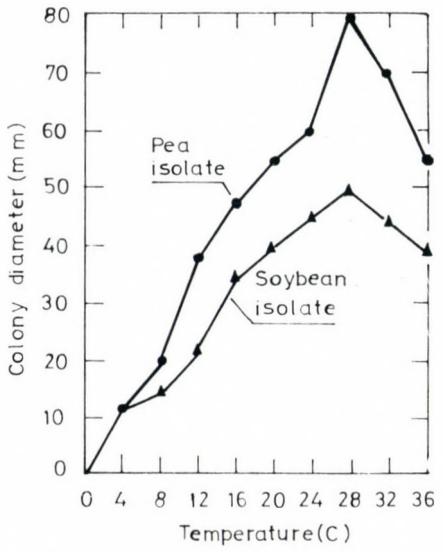



Fig. 1. Growth of two isolates of *Alternaria alternata* on potato-dextrose agar after 10 days at different temperatures

Table 1

Effect of various carbon and nitrogen sources on two isolates of *Alternaria alternata*

| Isolate P | | Isolate S | |
|---|---|---|---|
| Carbon source | Dry wt of mycelium/mg/[a] | Carbon source | Dry wt of mycelium (mg) |
| Mannose | 64[b] | Cellobiose | 93 |
| Dextrin | 50[b] | Dextrin | 42[b] |
| Cellobiose | 42[bc] | Mannose | 37[bc] |
| Fructose | 33[cd] | Fructose | 34[bcd] |
| Dextrose | 31[de] | Lactose | 29[cde] |
| Maltose | 28[def] | Xylose | 25[def] |
| Raffinose | 28[def] | Maltose | 19[efg] |
| Xylose | 28[def] | Raffinose | 19[efg] |
| Sorbose | 27[defg] | Sorbose | 18[fg] |
| Galactose | 22[efgh] | Galactose | 16[fgh] |
| Sucrose | 22[efgh] | Mannitol | 15[fgh] |
| Arabinose | 19[fgh] | Dextrose | 14[gh] |
| Lactose | 18[gh] | Sucrose | 14[gh] |
| Mannitol | 16[h] | Arabinose | 12[gh] |
| Ribose | 16[h] | Ribose | 11[gh] |
| Inulin | 3[i] | Inulin | 6[hi] |
| Control | 1[i] | Control | 1[i] |
| N source | | | |
| Peptone | 233[a] | Peptone | 205[a] |
| Calcium nitrate | 122[b] | Calcium nitrate | 114[b] |
| Urea | 107[bc] | Potassium nitrate | 88[bc] |
| Sodium nitrate | 80[cd] | Sodium nitrate | 70[cd] |
| Potassium nitrate | 78[cde] | Ammonium sulfate | 55[cd] |
| Ammonium nitrate | 62[def] | Urea | 53[cd] |
| Ammonium sulfate | 58[ef] | Ammonium nitrate | 48[cde] |
| Sodium nitrite | 35[f] | Sodium nitrite | 35[de] |
| Control | 12[g] | Control | 13[e] |
| Amino acid N source | | | |
| Casein hydrolysate | 292[a] | Glutamic acid | 73[cde] |
| Tyrosine[c] | 205[b] | Alanine | 65[def] |
| Proline | 200[bc] | | |
| Arginine | 88[c] | | |
| Asparagine | 82[c] | | |
| Glycine | 80[cd] | | |
| Aspartic acid | 65[def] | | |
| Leucine | 61[ef] | | |
| Methionine | 53[ef] | | |
| Tryptophane | 27[g] | | |
| Control | 22[g] | | |

[a] Growth was measured in mg of dry mycelium 10 days after incubation at 28 °C, data are average of three trials of four replicates each.

[b] Means followed by the same letter are not significantly different as determined by Duncan's Multiple Range Test at the $P = 0.05$ level.

[c] Tyrosine flasks contained large amounts of precipitate at harvest time, and mycelium grew slowly.

## Discussion

Carbohydrates are usually the best sources of carbon for fungal growth (Hawker, 1968). *A. alternata* grew well on a number of different carbon sources. The good growth of both isolates on oligosaccharides and polysaccharides is probably due to conversion into reducing sugar by appropriate extracellular enzymes, or to breakdown during autoclaving. Saad and Hagedorn (1970) found that *A. alternata* grew well on several tested carbon sources. Taber et al. (1968) reported that *A. raphani* utilized all sugar carbon sources tested and that rates of growth were less affected by differences in carbon sources than by differences in nitrogen sources.

Both isolates of *A. alternata* grew well on most tested N sources. The poorest growth was shown by urea and sodium nitrite. Hawker (1968) reported that the amount of nitrogen required is less than the amount of carbohydrates, but it is still high in comparison with other elements required.

The influence of pH on fungal growth is a very important factor for nutritional studies. Cochrane (1958) reported that many factors may influence pH, such as temperature, time of harvest, nitrogen sources, calcium and magnesium levels. Although Saad and Hagedorn found a considerably better growth of *A. alternata* in their pH test, their optimum was pH 6.5 which coincides with the optimum for *A. alternata* in the present work.

The influence of temperature on development of *A. alternata* is also in general agreement with the work of other authors. Saad and Hagedorn (1970) found that the best temperature for growth of *A. alternata* was 28 °C, although above the optimum growth decreased much more rapidly than with *A. alternata*. Pearson and Hall (1975) found that optimal temperature for sporulation of *A. alternata* was 27 °C; below 15 °C, and above 33 °C sporulation was inhibited. Stavely an Main (1970) reported that the optimum growth of *A. alternata* occurred at 27 °C. Likewise, Pound (1951) found that the best temperature of *A. solani* Sorauer was 28 °C, and for disease development, the optimum temperature was 16 °C.

## Acknowledgements

Published with the approval of the Director, Wisconsin Agricultural Experiment Station as a contribution from Project 120. Technical assistance by R. E. Rand and S. A. Vicen is gratefully acknowledged.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 147–150 (1986)*

# An Ascochytosis of the Chickpea (*Cicer arietinum L.*) Caused by *Didymella rabiei* (Kov.) v. Arx: Imperfect *Ascochyta rabiei* (*Pass.*) Lab. in Hungary

## (Short Communication)

GY. KÖVICS*, L. HOLLY** and E. I. SIMAY

NIAVT Research Centre for Agrobotany; H-2766 Tápiószele, Hungary

*Ascochyta rabiei* was isolated from lesions of diseased stems and leaves of chickpea plants. The teleomorph of the fungus (*Didymella rabiei*) was observable only on the overwintered parts of diseased plants, especially on the pods.

This paper is the first report on the occurrence of *Didymella rabiei* in Hungary.

Ascochytosis of chickpea, caused by *Ascochyta rabiei* (Pass.) Lab. (syn.: *Phyllosticta rabiei* Trot.; *Phoma rabiei* Khune et Kapoor) was previously reported in 26 countries (*viz.* Nene, 1980). However, to the best of our knowledge, the perfect state of the fungus was not observed in countries other than Bulgaria (Kovachewski, 1936), USSR (Gorlenko and Bushkova, 1958) and Greece (Zachos et al., 1963).

The severe ascochytosis of chickpea was observed first in 1978 in Hungary by Bea (1979), but the causal fungus was not isolated. In June 1984 a severe disease was also observed in Hungary (village Tápiószele). The ascochytosis caused decay on numerous experimental plots in that year.

The symptoms were lesions on the stems, petioles, leaves, pods and seeds. The lesions had a light-brown or brown area. The most serious diseases were on the pods and the stems (Fig. 1), because the mycelia could grow into the seeds from the pod, and the parts of the diseased plants above the stem-lesions frequently dried. The pycnidia (black points) were in concentric rounds in the lesions.

Isolations were made from lesions of stems and leaves. After surface sterilization (0.5% NaOCl for 2 min) the prepared pycnidia were macerated in 0.9% NaCl solution with 0.05% Tween 80. The conidialsuspension was incubated on oat meal agar at 20 °C in the dark.

The single-conidial cultures were grown on oat meal agar. We tested 9 other media for growing too, at 10, 15, 20, 25, and 30 °C. We made a comparison between the effects of continuous light and dark at 20°C on growing and maturation.

* Present address: BIOGAL, Debrecen, Hungary
** Present address: ICARDA, Aleppo, Syria

The mycelium was septate and grown on the surface of the media. The culture was light-grey to brownish-black with a white growing zone (Fig. 2). The pycnidia formed under the surface of the media. The fungus grew and sporulated well on chickpea seed meal agar, oat meal agar and Richard's media, similar to the data of other workers (Bedi and Aujla, 1970; Kaiser, 1973; Reddy and Nene, 1979; Reddy et al., 1984). We observed intensive growing on bean meal agar, pea meal agar, corn meal agar and potato dextrose agar. The best temperature for growing and maturation was 20–25 °C. The fungus abundantly formed pycnidia both in light and dark. The perithecial state was not observable in any culture.

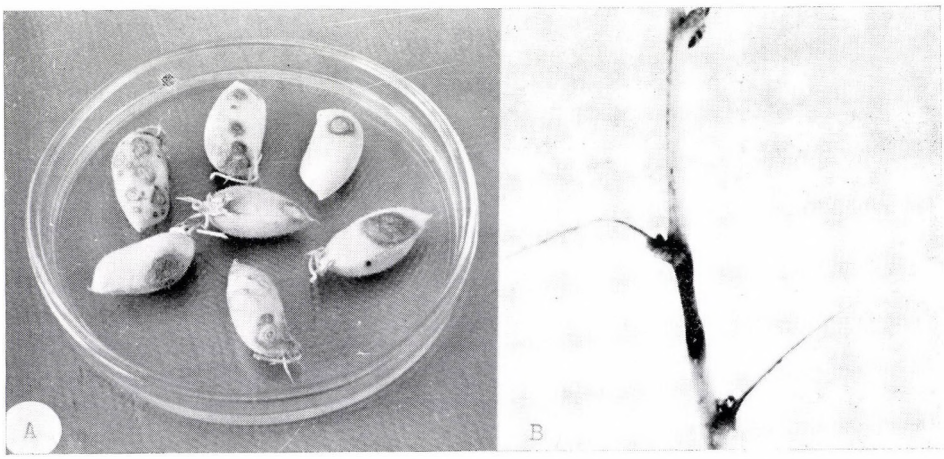



Fig. 1. Symptoms of *Ascochyta rabiei;* (A) Lesions on pods. (B) Lesion and leaf abscision on stem

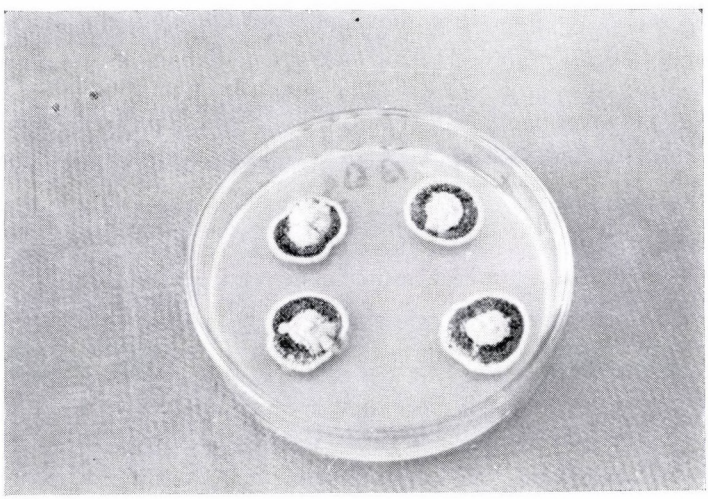


Fig. 2. Two week old culture of *Ascochyta rabiei* on potato dextrose agar at 20 °C

We tested the pathogenicity on two-week-old chickpea plants inoculating with washed conidia-suspension ($4 \times 10^5$ conidia $\times$ $ml^{-1}$) from 14 day old culture growing on potato dextrose agar. The suspension was atomized onto the plants (0.5 ml per plant). The inoculated plants were covered with a polyethylene tent for 4 days.

The isolated fungus was strongly pathogenic for all the investigated lines (coded; II-12/22, 121, 134, 155 and 307). The young plants frequently showed top necrosis.

For the investigation of overwintering the diseased whole plants were placed onto the surface of soil. The first perithecia occured in March. The perithecia formed on all parts of the overwintered plants, especially on the pods. We could infect healthy chickpea plants with ascospore suspension.

The microscopic characterisation of fungus was carried out measuring 50 of pycnidia and peritecia, and 100 of conidia and ascospores.

Pycnidia are subepidermal, rather globose, dark-brown with an ostiole or a pore. Their diameter is 80–(170)–260 micrometers. The ostiole open to the surface of epidermis and their diameter is 48–(51)–56 mikrometers. (Fig. 3.)

Conidia are commonly unicellular, hyaline. Bicellular conidia occur only in the older cultures in 5–8%. The measure is 5.2–14.5 × 3.2–5.4 micrometers.

Perithecia are globose or subglobose measuring 82–(133)–196 × 110–(165)–240 micrometers. Their colour is dark-brown to brownish-black. They formed in the dead epidermis.

Asci are hyaline, double-walled, cylindrical and containing 8 spores (Fig. 4).

Ascospores are hyaline, 1 septate, 12–(15)–18 × 5.2–(7.2)–8.4 micrometers. The two cells are not equal.

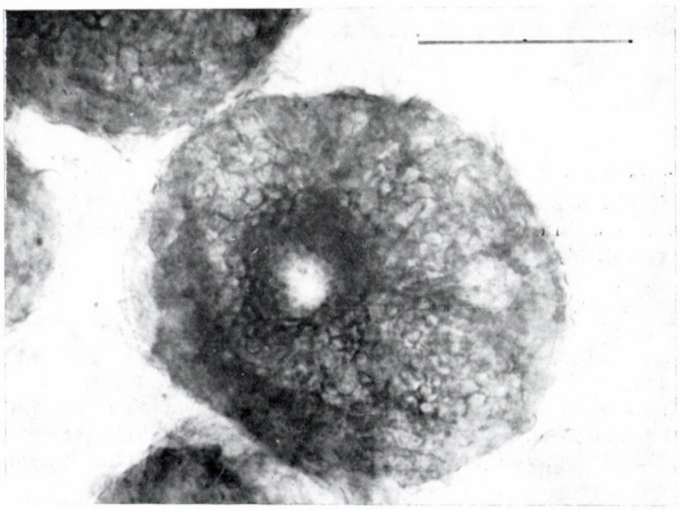


Fig. 3. Pycnidia of *Ascochyta rabiei* from leaf. Bar represents 100 $\mu$m

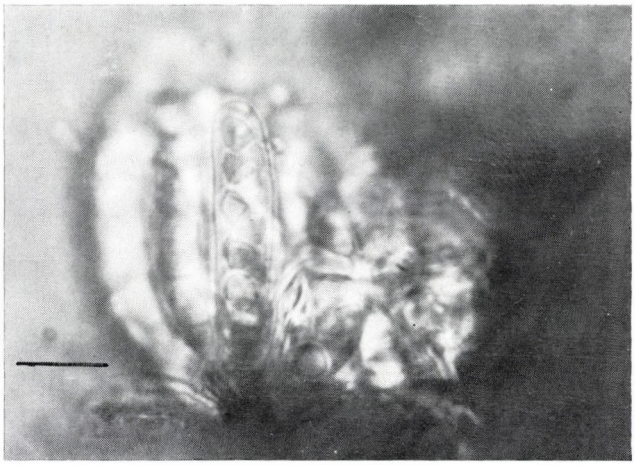


Fig. 4. Ascus with ascospores of *Didymella rabiei*. Bar represents 20 $\mu$m

On basis of field symptoms and artificial symptoms and morphology we could identify the fungus as *Didymella rabiei* (Kov.) v. Arx; imperfect *Ascochyta rabiei* (Pass.) Lab.

Interaction with other fungi on the chickpea, as well as comparative studies on Hungarian cultivars are in progress.

## Acknowledgements

The authors wish to thank Mrs. J. Molnár and Mrs. A. Boros for excellent technical assistance.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 151—156 (1986)*

# Adsorption of Some Nonionic Tenzides on Different Carriers

## I. Adsorption Strength

T. CSERHÁTI

Plant Protection Institute of Hungarian Academy of Sciences, 1022 Budapest, Herman O. u. 15, Hungary

Relative adsorption strength of 19 nonionic tenzides belonging to two homologous series on kaolin, diatomaceous earth, Sillilin N8 and Ultrasil was determined by thin-layer chromatography. Adsorption strength depended both on the type of hydrophobic part of the tenzides and on the number of ethyleneoxide groups per molecule, however their influence on the adsorption strength varied on each carrier.

The biological efficiency of a pesticide molecule depends considerably on the mode of formulation that is on the quantity and quality of tenzides, solvent, inert carriers and other materials present in the commercial products. The tenzides applied in pesticide formulations can modify the effect of the active ingredient (Valkenburg, 1973; Müller and Burth, 1982), they themselves display marked microbicidal effect too (Cserháti and Kecskés, 1978; Cserháti and János, 1979; Lundahl et al., 1973; Clifford and Hislop, 1975; Baicu et al., 1980). The inert carriers in the formulation can adsorb the active ingredients and tenzides decreasing in this way the performance of the formulation. The degree of activity loss depends on the adsorbed quantity and on the strength of adsorption.

As the classical methods to measure adsorption energy are time consuming and they require complicated instrumentation they were in some cases successfully replaced by gas chromatography. However the gas chromatographic method has serious limitations: both compounds have to be stable at the temperature of the measurement, one of them has to be volatile and the other highly non volatile. In the thin-layer chromatography these requirements are irrevelant.

## Theoretical Considerations

In the case of adsorption from solution of solids an exponential correlation exists between the surface tension of the solution and the energy of adsorption (Kipling, 1965). From the combination of Gibbs equation with Szyszkowski

relationship follows:

$$\frac{B_1\gamma_0}{RT} \cdot \frac{1}{A} = K\, e^{\lambda/RT} \qquad (I)$$

where $\gamma_0$ is the surface tension of the solvent, the $\lambda$ is the work required to remove one mole of solute from the surface layer into the bulk of solvent.

When the adsoprtion takes place from a solvent system of water and a water miscible organic solvent (in our case acetone) the adsorption energy is determined also by the surface tension of the solvent mixture. As the surface tension of water: acetone mixtures decreases monotonously at growing acetone concentrations also the adsorption energy decreases at growing concentration of acetone.

In the thin-layer chromatography the $R_f$ value increases at growing rate of desorption of the molecule from the sorbent. The rate of desorption $\left(-\frac{dn}{dt}\right)$ and the adsorption energy ($\lambda$) are exponentially correlated:

$$-\frac{dn}{dt} = B_2 \cdot n \cdot e^{-\lambda/RT} \qquad (II)$$

consequently in the same eluent a higher $R_f$ value indicates a lower energy of adsorption.

According to Eq. II the same $R_f$ value for a molecule on a sorbent of different adsorptivity can be obtained only by the modification of the adsorption energy which is influenced by the surface tension of the eluent (water: acetone mixtures) that is by the change of the acetone conecentration From Eqs I and II follows:

$$\lambda = a + b \cdot \log R_f + b \cdot \log C_{acetone}. \qquad (III)$$

In the case of constant acetone concentration the adsorption energies are related to the logarithm of the $R_f$ values. In a very narrow range of $R_f$ values this correlation can be replaced by a linear one.

We assume that Eq. III can be applied to the comparison of adsorption energies without the knowledge of the exact numerical values of the intercept and slopes and after calibration it is suitable for the rapid determination of adsorption energy.

## Experimental

*Materials*

We investigated the adsorption of 11 nonylphenyl-ethyleneoxide polymers (containing 4, 5, 6, 8, 9, 10, 11, 15, 23 and 30 ethyleneoxide groups per molecule in average) and that of 8 tributylphenyl-ethyleneoxide polymers (containing 4, 6, 8, 10, 13, 18, 30 and 50 ethyleneoxide groups per molecule in average). These

Table 1
Eluent systems to study the adsorption of nonionic tenzides on different carriers

| Number | Eluent | | Carrier |
|---|---|---|---|
| | composition | vol. ratio | |
| I | water : acetone | 1:1 | kaolin and |
| II | 2M NaCl : acetone | 1:1 | diatomaceous earth |
| III | 1M $MgCl_2$ : acetone | 1:1 | |
| IV | 1M NaCl : acetone | 1:1 | |
| V | 1M CsCl : acetone | 1:1 | |
| VI | water : acetone | 4:1 | |
| VII | 2M NaCl : acetone | 4:1 | Sillilin N8 |
| VIII | 1M $MgCl_2$ : acetone | 4:1 | |
| IX | 1M NaCl : acetone | 4:1 | |
| X | 1M CsCl : acetone | 4:1 | |
| XI | water : acetone | 9:1 | Ultrasil |

compounds belong to the nonionic tenzides applied extensively in pesticide formulations. The adsorbents under investigation were kaolin, diatomaceous earth and two synthetic products, Sillilin N8 and Ultrasil.

*Preparation and development of thin-layer chromatographic plates*

A cellulose layer of 0.25 mm thickness (Cellulosepulver MN 300) was applied to the lower part of a chromatographic (glass) plate in a height of 25 mm. The aqueous suspension of the carrier (one part carrier to two parts of water) was layered on the cellulose free surface of the plate in the same thickness and dried at 105–110 °C temperature. The cellulose layer prevents the detachment of the carrier from the plate immersed in the eluent and it assures a steady flow of eluent to the carrier layer. The tenzides were dissolved in methanol at a concentration of 5 mg/cm$^3$; 4 mm$^3$ of solution was spotted on the carrier at 1 cm above the cellulose layer. The plates were developed by the common TLC technique dipping only the cellulose layer into the eluent. The different eluents are summarized in Table 1. After development the plates were dried at 105–110 °C and the tenzides were detected by the modified Burger reagent (Longman, 1977). Due to the inherent low reproducibility of TLC only the $R_f$ values measured on the same plate were compared. All determinations were carried out in quadruplicate.

## Results and Discussion

The strength of adsorption of tenzides on kaolin and on diatomaceous earth are highly similar, it is lower on Sillilin N8 and very low on Ultrasil (compare eluent compositions in Table 1). Due to its very low adsorptivity Ultrasil was not

Table 2

Parameters of linear correlations between the $R_f$ values of tenzides ($y$) and the number of ethyleneoxide groups per molecule ($x$). Eluent water: acetone mixtures

$y = a + bx$

| Hydrophobic moiety | Carrier | a | b | r |
|---|---|---|---|---|
| A | diatomaceous | 0.63 | −0.013 | 0.9257 |
| B | earth | 0.79 | −0.010 | 0.9588 |
| A | kaolin | 0.84 | −0.007 | 0.9001 |
| B | | 0.80 | −0.005 | 0.8540 |
| A | Sillilin N8 | 0.23 | 0.011 | 0.9476 |
| B | | 0.32 | 0.003 | 0.8662 |
| A | Ultrasil | 0.68 | 0.005 | 0.7386 |
| B | | 0.73 | 0.003 | 0.7667 |

$r_{95\%} = 0.6021$ for nonylphenyl derivatives (A)
$r_{95\%} = 0.7067$ for tributylphenyl derivatives (B)

included in other investigations. The adsorptivity of tenzides increases significantly at growing number of ethyleneoxide groups per molecule on kaolin and on diatomaceous earth, however these effects are very low. An inverse correlation was observed on Sillilin N8 and on Ultrasil (Table 2).

It can be concluded from our data that the character of adsorption on kaolin and on diatomaceous earth differs from that on Sillilin N8 and on Ultrasil. The ethyleneoxide groups lower the strength of adsorption on Sillilin N8 and on Ultrasil but they enhance it on kaolin and on diatomaceous earth.

The differences between the $R_f$ values of tenzides containing the same number of ethyleneoxide groups but various hydrophobic parts show the same behaviour pattern on kaolin and on Ultrasil (nonylphenyl group results always in higher $R_f$ value than the tributylphenyl group). This finding proves that hydrophobic interactions also play a considerable role in the adsorption of tenzides on these two carriers (Table 3). An inverse relation was found on Sillilin N8 and on diatomaceous earth; they adsorbed less strongly the compounds with the more lipophilic tributylphenyl substructure.

The ionic environment decreases significantly the $R_f$ values that is they enhance the adsorptive strength between tenzides and carriers (Table 4).

In the most cases we were able to prove that the differences in ion radii ($Na^+ - Cs^+$) and in the ion charge ($Na^+ - Mg^{2+}$) influence significantly the adsorption. This observation can be explained by the fact that the molecules in solution are surrounded by a solvate shell (Lewin, 1974). The solvate shell is not the same around the various substructures of the molecule, its character depends

Table 3

Differences in the $R_f$ values of nonylphenyl- and tributylphenyl ethyleneoxide polymers containing the same number of ethyleneoxide groups per molecule (water: acetone eluents)

| No. of ethyleneoxide groups | Carrier | | | |
|---|---|---|---|---|
| | kaolin | diatomaceous earth | Sillilin N8 | Ultrasil |
| 4 | 0.06 | −0.15 | −0.04 | 0.04 |
| 6 | 0.06 | −0.15 | −0.04 | 0.03 |
| 8 | 0.05 | −0.16 | −0.03 | 0.03 |
| 10 | 0.03 | −0.14 | −0.03 | 0.02 |
| 13 | 0.03 | −0.11 | −0.02 | 0.03 |
| 30 | 0.02 | −0.10 | −0.02 | 0.02 |

Table 4

Effect of ions on the adsorption of nonylphenyl ethyleneoxide polymer containing 8 ethyleneoxide groups per molecule ($t_{93\%} = 3{,}71$)

| Eluent numbers | Carrier | $R_f$ values | $t_{calculated}$ |
|---|---|---|---|
| I–II | kaolin | 0.91–0.80 | 19.32 |
| II–III | | 0.80–0.71 | 6.06 |
| I–III | | 0.91–0.71 | 11.36 |
| V–IV | | 0.88–0.83 | 1.20 |
| I–II | diatomaceous | 0.43–0.06 | 27.05 |
| II–III | earth | 0.06–0.15 | 10.16 |
| I–III | | 0.43–0.15 | 61.58 |
| V–IV | | 0.20–0.19 | 1.38 |
| VI–VII | Sillilin N8 | 0.32–0.06 | 37.06 |
| VII–VIII | | 0.06–0.11 | 4.17 |
| VI–VIII | | 0.32–0.11 | 13.98 |
| X–IX | | 0.12–0.08 | 10.19 |

considerably on the hydrophobic properties of the substituents. The ions (according to their charge and radii) modify the structure of solvate shell decreasing in this way its neutralizing effect.

The competition between the tenzides and ions for the adsorptive sites of carriers also has to be taken into consideration but our data suggest that under our experimental conditions this effect is of secondary importance.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 157–164 (1986)*

# Tridemorph Acts on the Cell Membrane of *Plasmopara halstedii*

G. Oros and F. Virányi

Plant Protection Institute of the Hungarian Academy of Sciences, H-1525 Budapest 114, P.O. Box 102, Hungary

CALIXIN 75 EC (tridemorph) had no killing effect on the mycelia and did not stop the formation of zoosporangia of *Plasmopara halstedii* but it inhibited the systemic development of the fungus in sunflower plants. Although the differentiation of zoospores within sporangia seemed not to be inhibited by tridemorph or other morpholine derivatives, the zoospores became sensitive just before their liberation. A total inhibition was observed at 30–60 $mgl^{-1}$ and the minimal effective concentration of the active ingredient ranged between 0.1–0.5 $mgl^{-1}$.

The damage on semi-permeability of cell membrane of the zoospores has lead to electrolyte leakage even in case of sublethal doses. At higher concentrations, however, a complete destruction of zoospores was detected.

The fungicide CALIXIN 75 EC had no persistent effect on downy mildew as foliar spray and, when applied through the soil, it retarded the growth of sunflower stems.

## Introduction

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni, Peronosporaceae) is one of the most important diseases of sunflower. The pathogen survives by soil-borne oospores from which zoosporangia arise. The zoospores liberating from such sporangia can infect all parts of young sunflower plants in the spring (primary infection), initiating the systemic development of the fungus. As a result, the plants show retarded growth of stem, appearance of chlorotic spots on the leaves and, under favourable conditions, formation of zoosporangia on invaded tissue surfaces (Nishimura, 1922; Novotel'nova, 1966). Such sporangia may result in a range of secondary infections (Cohen and Sackston, 1973). Because of the incomplete resistance of sunflower cultivars to downy mildew (Vear, 1978), chemical control is of importance in reducing lossess caused by this disease. However, to be truly effective, a fungicide should control both primary and secondary infections and should have preventive as well as eradicant properties (Virányi and Oros, 1986). The contact fungicides, like Bordeaux mixture, may control the aerial spread of downy mildew to some extent, but they are of little practical value

in our case because of the continuous development of sunflower during the season and the endobiotic nature of *P. halstedii*.

Among the fungicides reported by Kis et al. (1979), Ridomil 25 WP (metalaxyl) and Previcur N (propamocarb) showed effectivity against sunflower downy mildew. Ridomil has curative action with concomittant host tissue regeneration (Garcia-Baudin et al., 1980; Virányi and Oros, 1985) but in effective concentrations it is slightly phytotoxic (Garcia-Baudin et al, 1980; Iliescu, 1980; Steiner, 1980).

Calixin 75 EC (tridemorph) has widely been used as a foliar spray against powdery mildews and other fungal diseases (Otto et al., 1971; Phillips and Frost, 1975). It has both contact and systemic eradicant effects. In laboratory experiments it was found to be active against representatives of nearly all groups of Ascomycetes and Basidiomycetes, however, it showed less effectivity on different species of Oomycetes *(Pythium, Phytophthora, Albugo)* and Zygomycetes *(Mucor, Rhizopus)* (Bergman et al. 1975; Buchenauer, 1975; Kerkenaar et al. 1979, Kerkenaar and Sijpesteijn, 1979; Kradel and Pommer, 1971; Verma and Petrie, 1979). Tridemorph is also effective against Grampositive bacteria (Kerkenaar and Sijpesteijn, 1979; Sijpesteijn, 1972) as well as a few species of phytopathogenic bacteria belonging to *Corynebacterium* and *Xanthomonas* but its practical use is limited because of phytotoxicity (Süle and Oros, 1979).

The morpholine fungicides influence different metabolic pathways in various organisms: e.g. respiration chains, protein biosynthesis, lipid metabolism or steroid biosynthesis. Sijpesteijn (1972) suggested that tridemorph interferes with cell membrane functions. According to Fisher (1974), the first moments of meeting with cell membrane have decisive importance for biological action of this compound. Buchenauer (1975) reported the membrane action to be important in the respect of selectivity among different species of fungi. Moreau (1976) supposed that tridemorph acts as a cationic detergent. The membrane action also seems to be important for a selective antibacterial activity (Oros and Süle, 1980).

In this paper the mode of action of tridemorph on *P. halstedii*, in particular the fungal membranes in vitro and in vivo, are studied and some practical implications of its use are provided.

## Materials and Methods

*In vitro experiments*

The effect of various concentrations of morpholine derivatives was determined in a watery suspension of zoosporangia of *P. halstedii* ($2 \times 10^5$ sporangia per ml), the fungicidal concentrations varying between 0.00005–0.2% of active substance. The minimal inhibitory and effective concentrations (MIC and MEC, respectively) as well as $ED_{50}$ values were calculated according to Hewitt (1977).

The electrolyte leakage of zoospores induced by Calixin 75 EC was measured by means of a Radelkis OK-102/1 conductivity-meter as follows: a suspension of zoosporangia ($5 \times 10^5$ sporangia per ml) was incubated at 16 °C for 2 hours prior to

being centrifugated at 500 rpm and 10 °C for 5 minutes, and the supernatant containing the zoospores was used for the experiment. Calixin 75 EC with final concentrations of 1.5, 15 and 150 $mgl^{-1}$ of tridemorph was added to 15 ml of the zoospore suspension under continuous stirring, and the conductivity was determined in every 10 second. The results were calculated in percent of differences between the starting and final values of conductivity.

*In vivo experiments*

The apical buds of two-leaf-stage sunflower plants (*Helianthus annuus* L. cv. *VNIIMK 6540*) were inoculated with a watery suspension of zoospores of *P. halstedii*, isolate *H*1 ($2 \times 10^5$ sporangia per ml), by using the method of Cohen and Sackston (1973).

CALIXIN 75 EC treatments were made 1 or 2 days before, and 1, 2 or 5 days after inoculation. The fungicide was applied in form of watery emulsion (0.0003 %–0.2 % a.i.) either as soil drench or as foliar spray, 3.0 or 0.5 ml per plant respectively. The plant height as well as infection rate (i.e. the appearance of chlorotic leaf spots, fungal sporulation and retarded stem growth) were assessed at the end of the experiments. In addition, the internal development of the pathogen was also determined microscopically (Virányi, 1977). Of the chemicals CALIXIN 75 EC(tridemoph; BASF), CORBEL 75 EC (fenpropimorpf; BASF), MELTATOX 40 EC (dodemorph; BASF) commercial grades were used. The aldimorph was supplied by FALIMA.

## Results and Discussion

The results concerning the effectivity of tridemorph on the germination of zoosporangia of *P. halstedii* and on the movement of zoospores are demonstrated in Fig. 1. The movement of liberated zoospores became slower soon after the treatment with tridemorph (2–10 s) and it stopped within 2 to 30 seconds.The sensitivity of zoosporic movement to fungicide depended exponentially on the concentrations used. The zoospores swam very slowly at concentrations around $ED_{10}$ value and they stopped in a few time after their liberation. Degeneration of plasma of the non-germinated zoosporangia characterized by appearance of dark and roughly granulated structure was observed at concentrations higher than $ED_{10}$, and higher concentrations of tridemorph (and other morpholines) lead to increasing degeneration. All sporangia seemed to be similarly died at MIC value (Table 1). This type of degeneration differed from that of died zoosporangia of natural origine the latter showing fine granulated structure.

These results suggest that the zoosporangia of *P. halstedii* become sensitive to tridemorph and other morpholines in the final stage of zoosporogenesis and that the velocity of zoospore destruction correlates to fungicide concentration only after opening of zoosporangia (Table 1 and Fig. 1). The inhibitory effect of

Table 1

Inhibitory effect of morpholine derivative fungicides on the zoospore release of *P. halstedii*

| Compound | MIC | $ED_{50}$ | MEC |
|---|---|---|---|
| | | $mgl^{-1}$ | |
| Aldimorph | 62–125 | 15–30 | 1–2 |
| Dodemorph | 250–500 | 62–125 | 31–62 |
| Fenpropimorph | >1000 | 15–31 | 2–4 |
| Tridemorph | 31–62 | 4–8 | 0.2–0.5 |

morpholine derivatives manifests itself very rapidly, i.e. after the zoospore discharge. One of the possible causes of such decrease in zoosporic movement could be the inhibition of the flagellar apparatus. An other possibility could come from the destruction of membrane due to morpholines (Fisher, 1974; Kerkenaar et al., 1979).

Changes in the conductivity of the zoospore suspension induced by the treatment with tridemorph (15 $mgl^{-1}$ final cc.) are shown in Fig. 2. After a short latent period of 2 minutes a well defined electrolyte leakage took place over a period of 18–25, min., then, as the zoospores had been destroyed, the conductivity of the suspension became stabile.

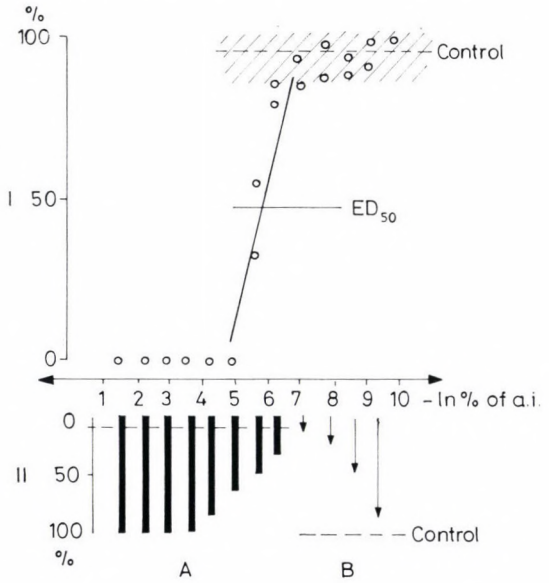



Fig. 1. Effect of CALIXIN on the liberation and movement of *P. halstedii* zoospores (I) Germination of zoosporangia, (II) A — ratio of zoosporangia with degenerated plasma, B — relative intensity of motion of the zoospores

Table 2

Efficacy of CALIXIN 75 EC applied at 100 $mgl^{-1}$ in the glasshouse against sunflower downy mildew

| Treatment | Dose ml/plant | Time of application (days) | | | | |
|---|---|---|---|---|---|---|
| | | before inoculation | | after inoculation | | |
| | | 1 | 2 | 1 | 2 | 5 |
| Soil drench | 3.0 | + | − | + | + | + |
| Foliar spray | 0.5 | − | + | + | + | + |

+ — leaf chlorosis
− — no symptom
} recorded 10 days after inoculation

When the zoospores were incubated together with tridemorph at concentrations of 1.5, 15 and 150 $mgl^{-1}$ for 10 min, respectively, they were unable to infect sunflower plants. However, despite of treatment with CALIXIN 75 EC, *P. halstedii* could sporulate freely on the affected host tissues. All these data mentioned above indicate that tridemorph destroys the membrane functions of zoospores but has no effect on the membranes of zoosporangia and mycelia, probably because of the cell-wall barrier. These findings correspond well with those obtained in vitro for *Phytophthora infestans* (Érsek and Oros, 1981).

The effect of CALIXIN 75 EC on the development of downy mildew was nvestigated under glasshouse conditions. When applied through the soil, CALI-

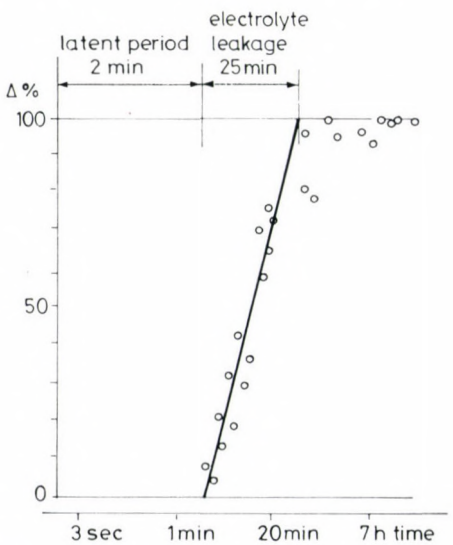



Fig. 2. Electrolyte leakage from the zoospores of *P. halstedii* induced by CALIXIN

XIN could protect the plants only if the treatment was made 2 days before inoculation, while treatments 1 day before, and 1, 2 or 5 days after inoculation had no result (Fig. 3 and Table 2). Although the systemic development of *P. halstedii* was inhibited by preventive applications (at 0.025 and 0.2% of a.i.), fungal sporulation occurred on the invaded host tissues indicating that CALIXIN has no lethal effect on the mycelia of the fungus.

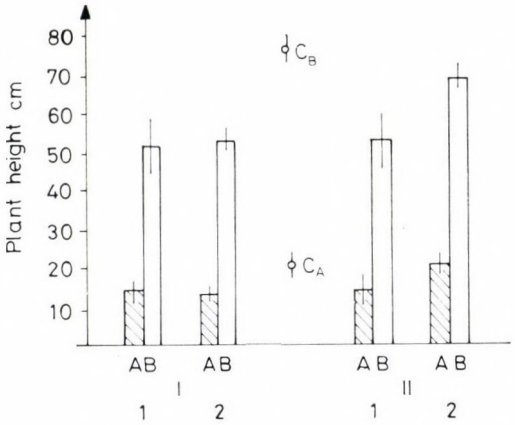



Fig. 3. Effect of CALIXIN applied as soil drench on the development of downy mildewed sunflower. A — assessed 10 days after treatment, B — assessed 40 days after treatment, 1 — treatment made 1 day before inoculation, 2 — treatment made 2 days before inoculation, I — 0.2% tridemorph applied, II — 0.025% tridemorph applied, $C_A$, $C_B$ — non-treated and non-infected control assessed 10 and 40 days after the inoculation, respectively

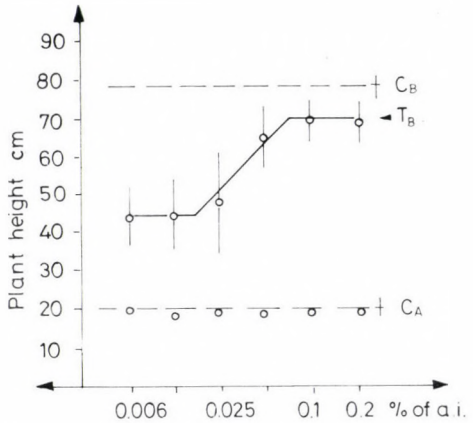



Fig. 4. Effect of CALIXIN applied as foliar spray on the retardation of sunflower growth due to mildew infection. $C_A$, $C_B$ — non-treated and non-infected control plants assessed 10 and 40 days after the inoculation, respectively, $T_B$ — treated and infected plants assessed 40 days after the inoculation

CALIXIN 75 EC (at concentrations of 0.05–0.2% of a.i.) as a foliar spray was effective only preventively (Fig. 4 and Table 2) and resulted in the localization of the pathogen in treated plants. When applied 5 days after inoculation, this fungicide did not inhibit the systemic downy mildew development and was unable to prevent symptom appearance.

A remarkable retardation of sunflower stems due to CALIXIN 75 EC treatment was evident in case of soil drench (Fig. 3).

## Conclusions

1. Tridemorph has lethal effect on the zoospores of *P. halstedii* but does not affect the formation of zoosporangia and the differentiation of zoospores in them.

2. The destruction of zoosporic membrane and its functions could be the possible cause of this lethal effect of tridemorph.

3. CALIXIN 75 EC is unable to eliminate *P. halstedii* at mycelial stage but inhibits its systemic development in sunflower.

4. The phytotoxicity and growth retardant effect to sunflower as well as a short persistance of the fungicide effect limit the practical use of CALIXIN 75 EC against *P. halstedii*.

## Acknowledgement

The authors wish to thank Mrs. and Dr. Kőmives for their helpful discussion.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1–2), pp. 165–177 (1986)*

# The Effect of Dicamba on the Petiole Tissues of the Sunflower (*Helianthus annuus* L.)

F. HERDI

Plant Protection Institute, Budapest, P.O.B. 102, H-1525, Hungary

The effect of three concentrations of Dicamba (2-methoxy3,6-dichlorobenzoic acid) on petioles of sunflower was studied. I measured cell wall thickness of vascular bundles, of bundle sheaths and of tracheae as reference parameters. Cell wall thinning in vascular bundles and in their sheaths was registered which was related to Dicamba-concentration. Tracheal cell walls were more thin in all treatments than in the control. No large difference in these effects with time was found. I observed vascular bundle multiplication, especially at the concentration of 1000 ppm, 7 weeks after treatment. Tracheal arrangement, radial in the control, became irregular. Non-differentiated xylem was found within the vascular bundles.

## Introduction

Hormone-based herbicides are mostly applied in cereals, with sprayings often from the air. Drifting herbicide can damage the neighbouring dicotyledonous crops. It is of interest therefore, what damage can be caused by different concentrations of herbicides in such crops.

Different distortions and malformations were reported in different plant organs like shoots, leaves, fruits and roots (Buhl, 1958; Ubrizsy, 1962; Way, 1962, 1963a, b, c, 1964a, b; Kiermayer cit. Audus, 1964; Szatala, 1967; Ubrizsy and Gimesi, 1969; Terpó-Pomogyi and Terpó, 1971). The sunflower is especially sensitive to hormone-based herbicides (Ubrizsy, 1962). These herbicides cause disturbances in cell differentation (Watson, 1948). Intensive cell divisions were observed by Eames (1951) in the meristematic tissues and shoot phloem. The cell wall structure of different parts like phloem, endodermis, cambium, embryonal pericycles (Swanson cit. Klingman, 1963; Beal cit. Audus, 1964, Pate et. al., 1965), xylem and phloem (Meyer, 1970) is affected by different herbicides as 2,4-D, dichlobenil, dicamba, 2, 4, 5-T. Herdi (1979, 1981, 1982) reported on the morphological changes in the beetroot and sunflower in response to a treatment with 2,4-D, MCPA and dicamba. Here similar data are presented on the changes in the structure of petiole tissues of sunflower following a treatment of different dosages of dicamba.

## Materials and Methods

Sunflower seedlings (Variety VNIIMK 6541) sown 12 May were treated 12 June at two-leaf stage. Technical grade dicamba (2-methoxy-3,6-dichlorobenzoic acid) was sprayed at 100, 1000, and 5000 ppm concentrations; control was untreated. Samples were taken 14 June, 21 June, 11 July. Leaf samples were stored in 40% ethylene. Sections, staining and evaluation was made as reported earlier (Herdi, 1983).

## Results

Tissue deformations after dicamba treatments are shown on Figs 1–7. The numbers of cell layers in the laminar collenchyma and in the cambium are shown on Tables 1 and 2.

*Untreated sample, 14 June*

The petiole was triangular, bifacial, slightly undulated on convex surface. Both petiole surfaces contained laminar collenchyma (Table 1), with single, scattered distributed multi-cell bristles. There were 12–15 open, collateral vascular bundles (VB), distant from each other; three of them larger than the others (Figs 4a, 6a). Bundle sheath (BS) surrounded the VB with amylaceous sheath at the phloem. Cambium cell layer numbers are on Table 2. Tracheae and tracheids (TT) are radially arranged. VBs are not ramified.

Table 1

Cell layer numbers in the laminar collenchyma in relation to different concentrations of dicamba

| Treatment | Sampling date (1978) | Cell layer numbers in laminar collenchyma | | |
|---|---|---|---|---|
| | | concave surface | convex surface | edge |
| Control | June 14th | 2–3 | 2–3 | 4–5 |
| | June 21st | 3–4 | 3 | 4–7 |
| | July 11th | 4–6 | 5–8 | 6–9 |
| 100 ppm | June 14th | 2–4 | 3–4 | 4–5 |
| | June 21st | 3–5 | 4–5 | 5–8 |
| | July 11th | 3–4 | 3–5 | 6–8 |
| 1000 ppm | June 14th | 3–4 | 3–5 | 4–7 |
| | June 21st | 4–5 | 4–6 | 5–7 |
| | July 11th | 5–8 | 6–8 | 7–10 |
| 5000 ppm | June 14th | 2–4 | 4–5 | 4–6 |
| | June 21st | 2–3 | 2–4 | 3–6 |
| | July 11th | 2–4 | 2–3 | 3–4 |

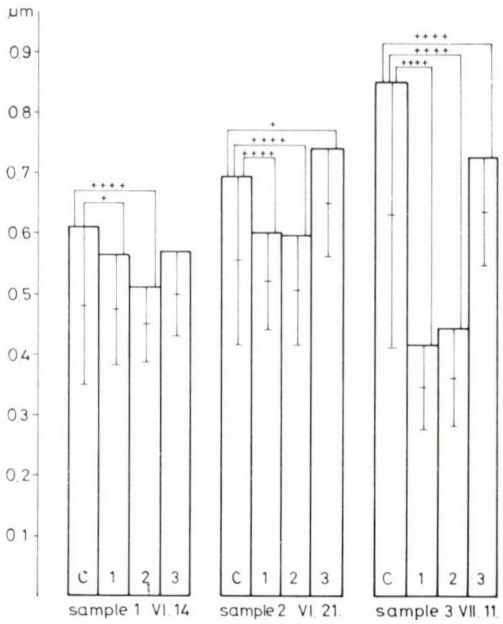



Fig. 1. Changes in the cell wall thickness of bundle sheath coll in relation to different concentrations of dicamba. C = control, 1 = 100 ppm, 2 = 1000 ppm, 3 = 5000 ppm, + = $p < 5\%$, ++++ = $p < 0.1\%$

Table 2

Cell layer numbers in the bundle sheath and cambium of the vascular bundles in sunflower petioles, in relation to different concentrations of dicamba

| Treatment | Sampling date (1978) | Bundle sheath cell layer numbers | | Cambium cell layer numbers |
|---|---|---|---|---|
| | | above phloem | near xylem | |
| Control | June 14th | 4–7 | 2–3 | 3–4 |
| | June 21st | 4–7 | 2–3 | 4–6 |
| | July 11th | 6–10 | 2–4 | 4–6 |
| 100 ppm | June 14th | 4–9 | 2–3 | 4–6 |
| | June 21st | 5–15 | 2–3 | 5–7 |
| | July 11th | 9–16 | 2–4 | 3–6 |
| 1000 ppm | June 14th | 4–8 | 1–2 | 4–7 |
| | June 21st | 6–9 | 2–3 | 5–7 |
| | July 11th | 7–10 | 2–3 | 3–7 |
| 5000 ppm | June 14th | 7–10 | 2–3 | 5–7 |
| | June 21st | 6–14 | 3–4 | 6–10 |
| | July 11th | 4–12 | 2–3 | 4–8 |

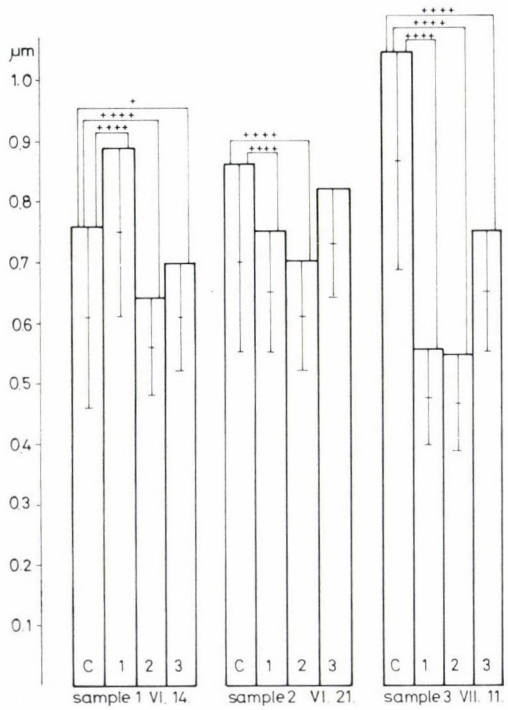



Fig. 2. Changes in the cell wall thickness in phloem cell in relation to different concentrations of dicamba. C = control, 1 = 100 ppm, 2 = 1000 ppm, 3 = 5000 ppm. + = $p < 5\%$, ++++ = $p < 0.1\%$

*Untreated sample, 21 June*

Petiole structure was similar to the one found earlier, with more layers of laminar collenchyma (Table 1, Figs. 4b, 6b). BS structure and cambium thickness was also similar (Table 2).

*Untreated sample, 11 July*

The petiole grew more thick which is reflected in the cell layer numbers of laminar collenchyma (Table 1) and of BS and cambium (Table 2). VBs were 16–18; their distribution was similar to earlier samples (Figs. 4c, 6c), as well as that of tracheae and tracheids.

*The effect of 100 ppm dicamba, 14 June*

Petiole shape showed no difference from the control. Laminar collenchyma was found on both surfaces (Table 1); angular collenchyma was locally seen. The multi-cell bristles were distributed as in the control. Some of them was wider than one cell and they were on the convex surface.

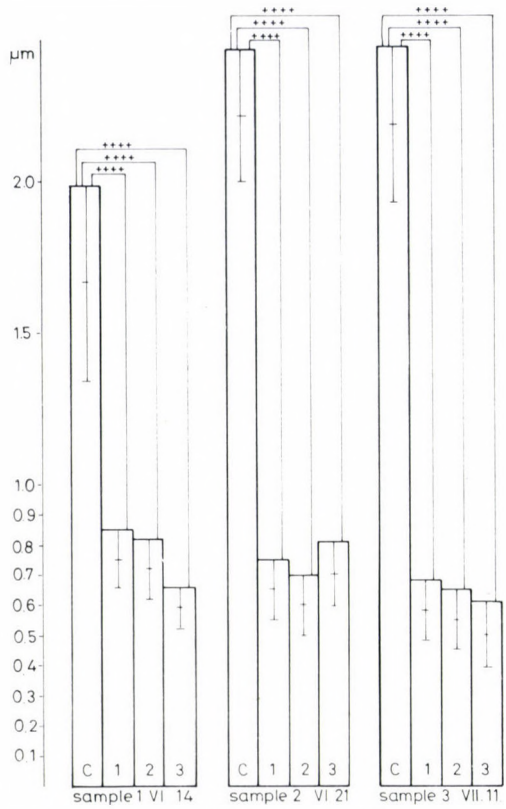



Fig. 3. Changes in tracheal wall thickness in relation to different concentrations of dicamba. C = control, 1 = 100 ppm, 2 = 1000 ppm, 3 = 5000 ppm. ++++ = $p < 0.1\%$

VB numbers and distribution was similar to that of in the control (Figs 4d, 6d). BS and cambium cell layer thickness is shown on Table 2. TT were radially arranged with some of them scattered.

VB cell walls were thinner than in the control (Fig. 1). BS cell walls were, on the other hand, thicker than in the control or later in the same treatment (Fig. 2). Similar change was observed in the tracheae (Fig. 3) with respect to the control. Tracheae from other treatments showed similar cell wall thickness.

VB multiplication was slight, by division and by differentiation from procambium foci. TT were not ramified.

*The effect of 100 ppm dicamba, 21 June*

Petiole was like in the control with the concave surface slightly undulated. There was an increase in the amount of angular collenchyma (Table 1) on the convex side. Multi-cell bristles were like before. VB numbers were 11–15, similarly

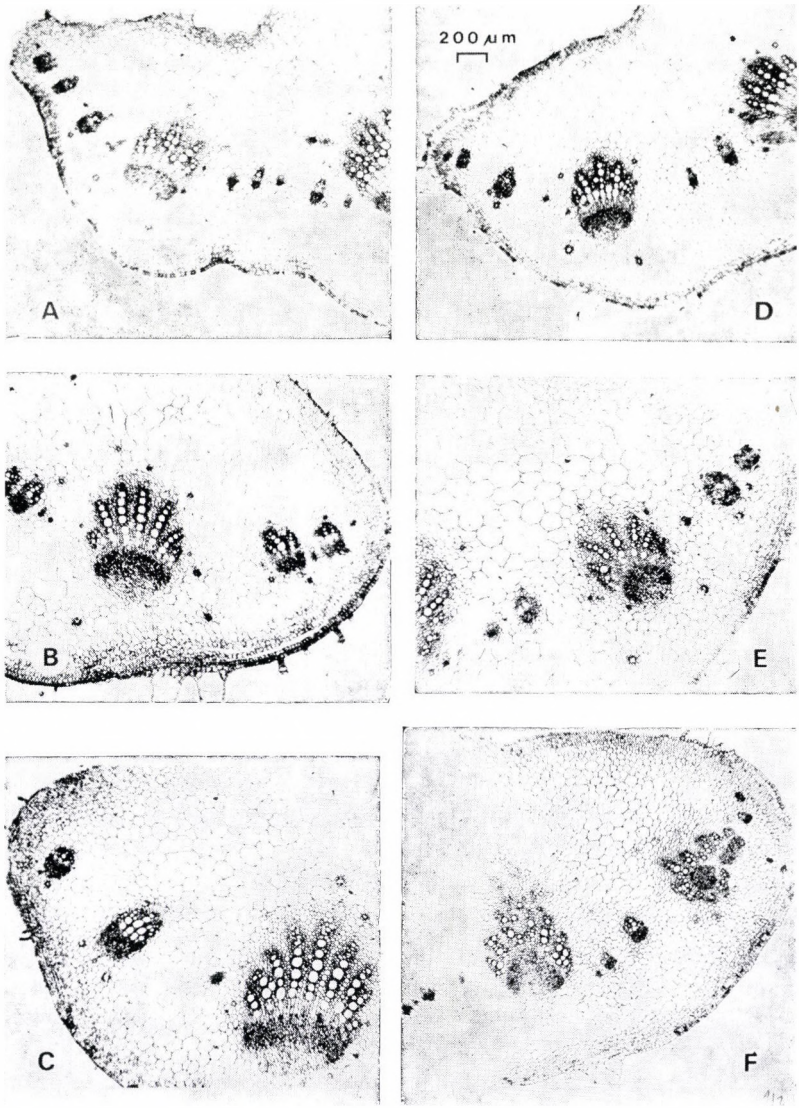



Fig. 4. Arrangement of the vascular bundles in the petiole of the sunflower. A—C = control, D—F = 100 ppm, A, D = 1st sample, 14 June, B, E = 2nd sample, 21 June, C, F = 3rd sample, 11 July. All are to the same scale

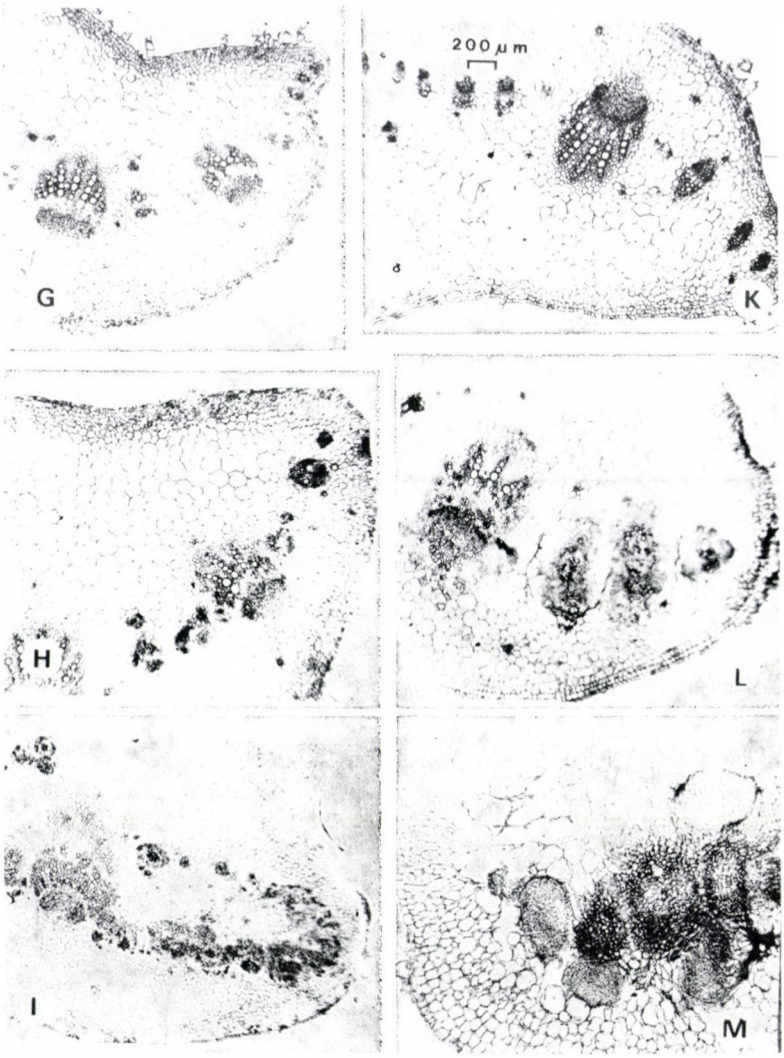



Fig. 5. Arrangement of vascular bundles in the petiole of the sunflower. G—I = 1000 ppm, K—M = 5000 ppm, G, K = 1st sample, 14 June, H, L = 2nd sample, 21 June, I, M = 3rd sample, 11 July. All are to the same scale

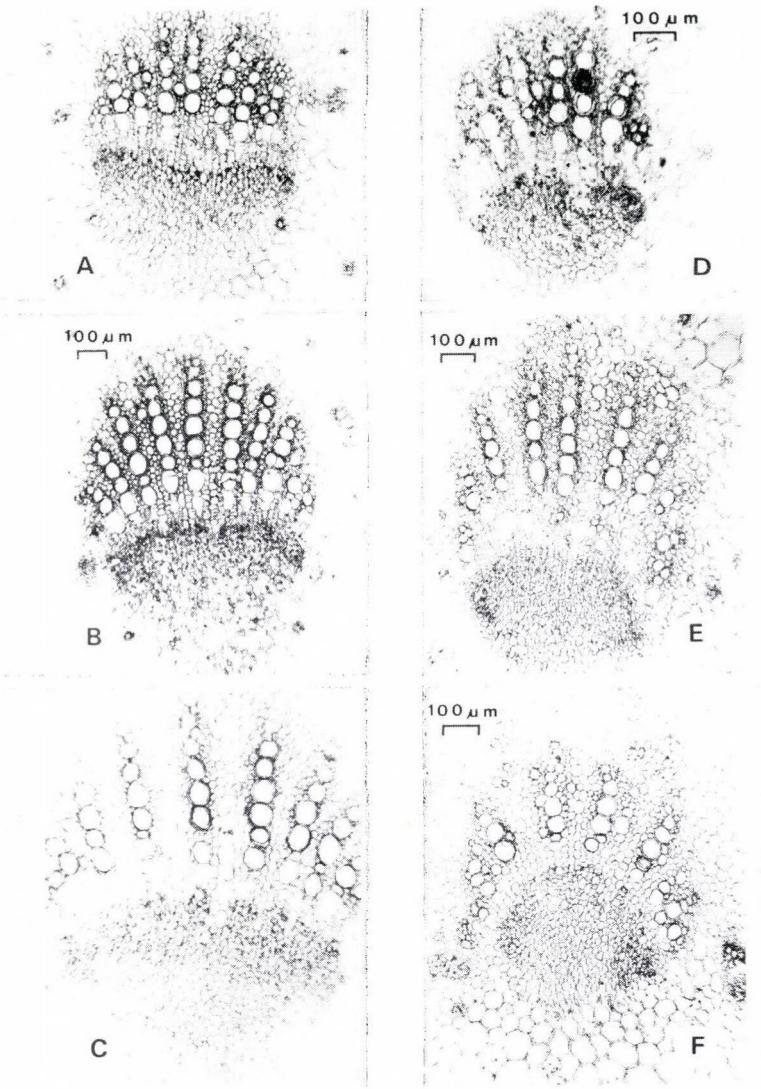



Fig. 6. Changes in the structure of vascular bundles in the petiole of sunflower. A—C = = control, D—F = 100 ppm. A, D = 1st sample, 14 June B, E = 2nd sample, 21 June. C, F = 3rd sample, 11 July. The scale of figures A, B and C are the same

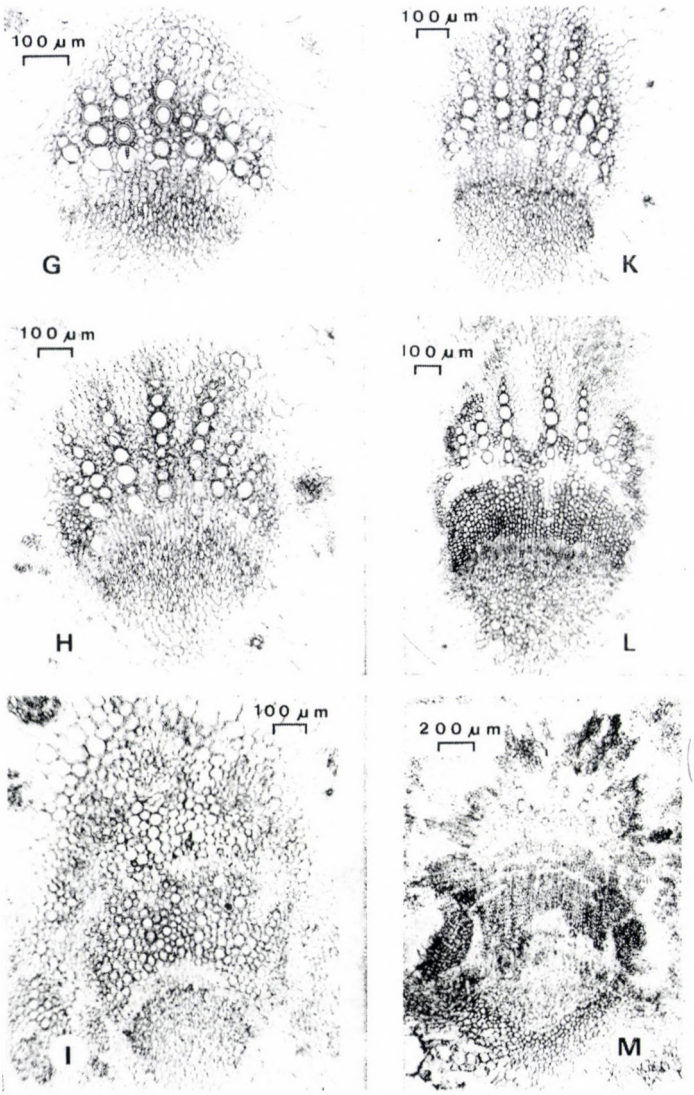



Fig. 7. Changes in the structure of vascular bundles in the petiole of sunflower. G—I = 1000 ppm, K—M = 5000 ppm, G, K = 1st sample, 14 June, H, L = 2nd sample, 21 June, I, M = 3rd sample, 11 July

structured as in the control (Fig. 4e). BS and cambium grew thicker (Table 2). TT arrangement was radial with scattered ones.

BS cell walls were thinner than the ones in the control (Fig. 1). Phloem parenchyma cells showed similar reaction in comparison to both the control and the 14 June sample (Fig. 2). TT reacted with cell wall thinning, too (Fig. 3). VB multiplication was limited, mainly via differentitation from procambium foci; ramification was not observed.

*The effect of 100 ppm dicamba, 11 July*

Petiole shape was not deformed; both surfaces were slightly undulated. Elements of angular collenchyma within the laminar collenchyma layer were observed. Bristle distribution was like that in the control.

VB numbers were 23–25, with size distribution and arrangement similar to the control. (Figs 4f, 6f). BS had more cell layers than found in earlier sample (Table 2); cambium became thinner. BS and phloem parenchyma cell walls were also thinner with respect to the control (Figs 1, 2). Tracheal cell walls were also thinner (Fig. 3) but this change was not time-related. TT multiplication proceeded as in the control; no ramification was found.

*The effect of 1000 ppm dicamba, 14 June*

Petiole shape was like in the control; slightly undulated on concave surface. Angular collenchyma was observed on the convex side and locally only. Bristle distribution and arrangement was normal.

VB numbers were 14–16, their distribution was normal (Figs 5g, 7g). BS and cambium was only slightly thicker than in the control (Table 2). BS cell walls were thinner than in the other treatments or in the control (Fig. 1). Cell wall thinning was observed in the phloem parenchyma (Fig. 2) and tracheae (Fig. 3). TT arrangement was radial, with some of them scattered. VB multiplication proceeded mainly via differentiation from procambium foci. Ramification was not found.

*The effect of 1000 ppm dicamba, 21 June*

Petiole surfaces were slightly undulated. Angular collenchyma was present. Bristle distribution was normal but the number of multi-cell bristles increased on the convex side.

There were 15–18 VBs with the normal size distribution and arrangement (Figs 5h, 7h). BS and cambium grew slightly thicker (Table 2). TT arrangement was as in the previous sample. BS cell wall was even thinner (Fig. 1), similar to that in the phloem parenchyma and tracheae (Figs 2, 3). VB division was observed as a way of VB multiplication. Ramification was not seen.

*The effect of 1000 ppm dicamba, 11 July*

Both petiole surfaces were slightly undulated. The presence of angular collenchyma was observed as in earlier samples. Bristle density increased; number of multi-cell bristles was fairly high.

VBs are so numerous that they form a continuous layer (Fig. 5i) in two rows; the three larger ones are still conspicious (Fig. 7i). BS grew somewhat thicker but not the cambium (Table 2). TT were radially distributed and scattered. BS cell wall thickness was less compared to the control and 5000 ppm, third sample (Fig. 1). Cell wall thickness in phloem parenchyma was less than in the control only (Fig. 2). Similar phenomenon was observed for tracheal cell wall thickness. (Fig. 3).

VB multiplication by differentiation from procmabium foci was observed. No ramification was found.

*The effect of 5000 ppm dicamba, 14 June*

Petiole shape, laminar collenchyma and bristle distribution was as in the control. VB number was 19–23, three of them larger; their arrangement was as in the control, only the central was surrounded by smaller VBs (Figs 5k, 7k).

BS cell layer was slightly thicker than in the control (Table 2), TT were radially arranged, BS and phloem parenchyma cell wall thickness did not differ from that of the control (Figs 1, 2), or in other treatments. The walls of the tracheae, however, were thinner than in the control (Fig. 3).

VBs multiplied by procambium foci differentiation. No ramification was observed.

*The effect of 5000 ppm dicamba, 21 June*

Petiole shape was similar to that of the control; angular collenchyma within the laminar collenchyma layer was not found. Multi-cell bristles were more on the convex side.

VBs formed a continuous layer, they cannot be counted (Fig. 5L). The three larger ones were still distinguishable (Fig. 7L). BS and cambium cell layers were more numerous than in the control or the previous sample (Table 2). TT were radially arranged.

BS cell walls were thicker than in the control and in other samples (Fig. 1). Phloem parenchyma cell walls were as thick as in the control but thicker than in other treatments (Fig. 2). Tracheal walls were thinner than in the control while the opposite came true when compared to other treatments (Fig. 3).

VB multiplication was of small extent and proceeded by procambium foci differentiation. In the large VBs an undifferentiated layer of xylem was observed next to cambium, below which the radially arranged TT were found. The larger VBs were surrounded by elongated parenchyma cells. No VB ramification was observed.

*The effect of 5000 ppm dicamba, 11 July*

Petiole shape was strongly distorted, VBs occupied ca. 75% of the petiole cross-section. Multi-cell bristles were single and distant from each other on both surfaces.

VBs were scattered distributed but formed a continuous layer; the three larger VBs could be observed (Figs 5m, 7m). BS and cambium was thick but not more than in the previous sample. TT were radially arranged. BS and phloem parenchyma cell walls were conspiciously thinner than in the control but thicker than the third samples from the 100 and 1000 ppm treatments (Figs 1, 2).

Tracheal walls were thinner than in the control but similar to the situation found in other treatments (Fig. 3). VBs did not multiply. In the larger VBs a large amount of undifferentiated, thick-walled xylem layer next to cambium was seen. TT are located below this and radially arranged. The larger VBs were surrounded with elongated parenchyma cells. VB ramified seriously.

## Conclusion

I conclude that the dicamba, in all concentrations applied, damaged the VBs by influencing cell wall thickness of BS, phloem parenchyma and tracheae. BS cell walls were affected to the largest extent by 100 ppm; for phloem parenchyma, this concentration was 100 and 1000 ppm. Tracheal walls were most sensitive to the dicamba concentrations applied. The effect of 5000 ppm was the appearence of undifferentiated xylem formation in the second and third samples. This concentration damaged tracheal walls to the greatest extent.

Petiole and VB structure was most affected by the concentrations of 1000 and 5000 ppm. 5000 ppm caused strong distortion of the petiole.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 179—186 (1986)*

# Effect of Precocene 2 on Second Instar Larvae of the Leaf-Curling Peach Aphid, *Brachycaudus schwartzi* CB. (Hom., *Aphididae*)

E. T. E. DARWISH[1], A. I. FARAG[1] and B. DARVAS[2]

[1] Department of Plant Protection, Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt, [2] Research Institute for Plant Protection, Hungarian Academy of Sciences, P. O. Box 102, Budapest, H-1525, Hungary

Exposure of 2nd instar larvae of the leaf-curling peach aphid, *Brachycaudus schwartzi*, to precocene-treated (P2) peach shoots resulted in an increased level of larval mortality and increased proportion of alate females. Moreover, we observed a greater incidence of malformation in the tibia, tarsal and antennal joints in those adults which developed from the survivors, together with a reproduction in fecundity.

The leaf-curling peach aphid, *Brachycaudus schwartzi* is one of the most important economic pests occurring in all of the peach growing areas of Europe and Asia as well as in the Mediterranean countries and in South America (Mostafawy, 1967). The morphology of different larval and adult stages and the ecology of *B. schwartzi* have been studied by Darwish (1983a, b).

Precocenes induce precocious metamorphosis in larvae of certain sensitive Hemiptera (Bowers, 1976; Bowers et al., 1976; Bowers and Martinez-Pardo, 1977; Bowers, 1977; Farag and Varjas, 1981; Farag, 1982) and locusts (Pener and Orshaw, 1977; Ĉhenevert et al., 1978). These ageratochromenes (Alertsen, 1961) and their derivates are necrogenic to the corpus allatum (CA) because of their in situ massive bio-oxidation (Pratt et al., 1980) to highly reactive epoxides (Brooks et al., 1979). Accordingly, the term "pro-allatocidin" has been adopted (Hamnett et al., 1981; Pratt, 1983) for the precocenes in the light of this mode of action. Precocene treatment has many different effects, most of which are a consequence of reduced juvenile hormone (JH) production. These responses include precocious metamorphosis, distruption of morph determination in the case of polymorphism, anatomical and morphological disturbances, sterilization, inhibition of sex attractant production, disturbances of embriogenesis, antifeedant and toxic effects (Bowers, 1985; Darvas et al, 1987).

There is some evidence which favours a hormonal basis for the control of polymorphism in aphids (Hardie, 1984). For example Mackauer et al. (1979) found that treatment of young apterous viviparous females of *Acyrthosiphon pisum* with P2 induced allate production in isolated apterae and that the effect could be reversed with JH treatment. The same phenomenon was observed to occur in *Macrosiphum euphorbiae* (Delisle et al., 1983) whilst Hales and Mittler

(1981) showed that P3 had a classical precocene effect in that it caused precocious metamorphosis in *Myzus persicae*, and resulted in the complete degeneration of the CA in precociously metamorphosing individuals. Hales and Mittler (1983) were the first authors to demonstrate that P3 caused male determination in the aphid *M. persicae*. This observation provided the first example of a specific endocrine influence on chromosome behaviour in sex determination. Maternal treatment with P3 using apterous viviparous *M. persicae* (Hales and Mittler, 1981) and *A. pisum* (Kambhampati et al., 1984) resulted in a significant increase in the proportion of alates and precocious adultoids. The precocious adultoids were sterile.

The present investigations, were designed to study the effect of P2 on second instar larvae of *B. schwartzi*. We describe effects on fecundity, and on the external morphology of malformed adults.

## Materials and Methods

Leaf-curling peach aphids were collected during the second half of July from a field at the Experimental Station, Research Institute for Plant Protection, Nagykovácsi, Hungary.

In order to obtain aphids of known age, apterous viviparous females were transfered from the stock colony onto cut peach shoots standing in tap water. The offspring produced over a 2 hour period were transferred to 10–15 cm long peach shoots.

P2 (6,7dimethoxy-2,2dimethyl-chromene) was dissolved in acetone at concentrations of 1.0, 0.5, 0.1, 0.05 and 0.01 %. Single peach shoots were sprayed with 0.5 ml of a spray solution using a "Rofra" atomizer. The control shoots were sprayed with acetone.

Sixty young second instar larvae were isolated in small plastic cages enclosing one treated or one control peach shoot. The cages were held at 25 °C in a growth chamber, under a photophase of 16 hours (short-night condition) and were checked daily for adult survival and offspring production. Observations were restricted to a 9 day period after parturity. The percentage of alate progeny was calculated in the offspring produced by 5 treated adults. The morphogenetic action was investigated in Plant Protection Department, Faculty of Agriculture, Shibin El-Kom.

## Results and Discussion

Investigation of survival, the external morphology, and the rate of development, after P2 treatments revealed 5 main types of responses: (1) Increased larval mortality, (2) A higher proportion of alate females in the treated generation, (3) Malformation in adults, (4) Reduced rate of reproduction.

*Larval mortality*

One of the most striking effects of P2 upon *B. schwartzi* was the mortality rate. The results summarised in Table 1 indicated that the mortality of precocene-treated aphids, within the treated generation ranged from 55 to 80% whereas mortality of the control aphids varied from 5 to 10%. In general, larvae succumbed within the first 24 hr after treatment. Similar results were published by Anastasis and Brown (1982) for *Nilaparvata lugens* (Hom., Delphacidae) and Darvas et al. (1985) for *Quadraspidiotus perniciosus* (Hom., Diaspididae).

*Percentage of alate females in the P2 treated generation*

The percentage of apterous viviparous females was 91–93% in the surviving adults of the control group, but the ratio of these forms was decreased to less than 10% by precocene treatment (Table 1). On the contrary, the ratio of alate females was considerably higher in the precocene treated generation. The proportion of alate females ranged from 33% to 99% at P2 concentrations of 0.01% to 1.0% (Table 1).

Lees (1977) and Hardie (1980, 1981) found that alate/apterae morph determination was mainly dependent upon scotophase until the middle of the second instar. A short night favoured development of apterous adults and a long night favoured the alate form. However, the control of developmental processes may change in third instar alates because short night conditions at this stage of development might bring about an increased incidence of juvenilized forms (Lees,

Table 1

Effect of P2 on larval mortality and production of alate females in the treated generation*

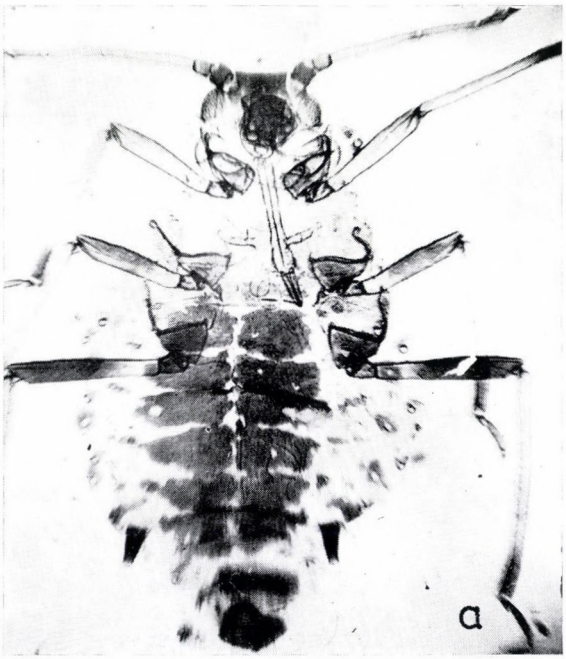
| Compound | Conc. % | Total mort. % | % of survivors as viviparous females | | |
|---|---|---|---|---|---|
| | | | Alate | | Apterous |
| | | | Normal | Malformed | |
| Precocene 2 | 1.00 | 80.0 | 50.0 | 41.7 | 8.3 |
| | 0.50 | 80.0 | 33.3 | 33.3 | 33.3 |
| | 0.10 | 63.3 | 18.2 | 27.3 | 54.5** |
| | 0.05 | 58.0 | 28.0 | 12.0 | 60.0 |
| | 0.01 | 55.0 | 29.6 | 3.7 | 66.7 |
| Control-1 | | 10.0 | 9.3 | 0.0 | 90.7 |
| Control-2 | | 5.0 | 7.0 | 0.0 | 93.0 |

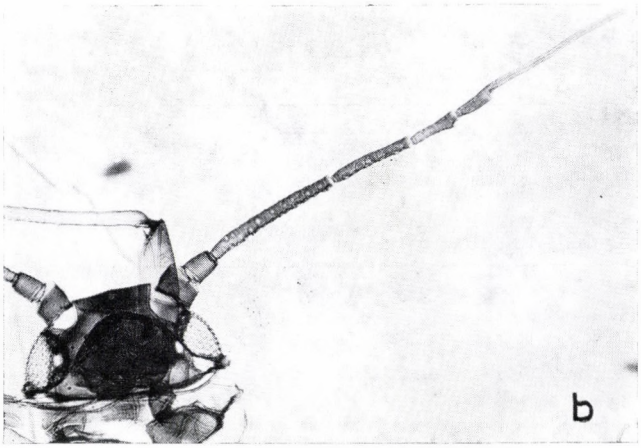
* Second instar larvae of *B. schwartzi* were treated, 60 larvae in each group.

** About 50% of apterous females were malformed adults.

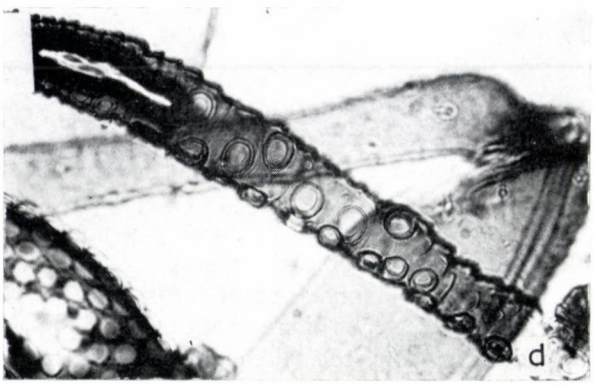
1980). This is substantiated by Hardie et al. (unpubl. data, cit: Hardie, 1984) by finding higher levels of JH in the case of short-night reared aphids.

The experiments reported here show that treatment of 2nd instar larvae of *B. schwartzi* with P2 under short–night conditions favour development of the alate morph, although the wings were damaged in many cases. This leads us to suppose that the allatocidal activity of P2 in this species might be a consequence of reduced JH levels in the larval stages and that lower JH levels are responsible for alate development.





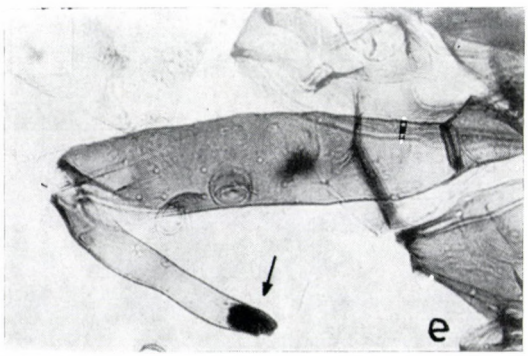




Fig. 1. *B. schwartzi* following application of P2 (a) Normal apterous viviparous female; (b) Normal antennae of alate viviparous female; (c) Antennae of malformed apterous viviparous female; (d) Antennae of malformed alate viviparous female; (e) Tibia of malformed apterous viviparous female

*Morphology of the malformed adults*

The morphological studies on the control adult females clearly show that the legs are usually covered with numerous fine hairs, that the hind tarsal joint II is about 1.3 mm long and that the antennae are normally 6-jointed and about 0.45–0.74 times as long as the body (Fig. 1a, b). Following P2 treatment however, the antennal joints of both apterous and alate viviparous forms were abnormal. The damage was especially obvious at the fourth and fifth antennal joints and at the processus terminalis (Fig. 1c, d). Moreover, the tibia and tarsal joints were destroyed (Fig. 1e). In addition, the third antennal joint of the malformed alate vivipara had more secondary rhinaria than it was obvious on a normal insect (Fig. 1d), whilst the wings were similar to those apparent in the 50% juvenilized forms produced in Lees' (1977) experiments.

*Inhibition of reproduction*

The reproductive potential of surviving adults was significantly decreased. For example, in apterous viviparous females, the number of larvae deposited during their reproductive life was 58 for five untreated aphids, decreasing to 30 and 10 for those treated with 0.01% and 1.0% of P2, respectively (Table 2).

Hales and Mittler (1981) treated *M. persicae* young adults with P3 and found that the offspring underwent precocious metamorphosis and these adultoids could not deposit their mature embryos because of the incomplete differentiation of the external reproductive tract. We did not notice any incomplete differentiation of the external reproductive ducts of females in the case of treatment of the second instar larvae of *B. schwartzi*. However, offspring mortality was the same in both groups. Although from the middle of fourth instar onwards there are not only daughter embryos present in the maternal ovaries but also the next generation

Table 2

Number of progeny following application of P2 on 2nd instar larvae

| Compound | Conc. % | No. of progeny produced* |
|---|---|---|
| Precocene 2 | 1.00 | 10 |
| | 0.50 | 14 |
| | 0.10 | 20 |
| | 0.05 | 22 |
| | 0.01 | 30 |
| Control-1 | | 42 |
| Control-2 | | 58 |

* Produced by five apterous viviparous females during 9-days period

which is already developing in the ovaries of older daughter embryos (Hardie, 1984), nevertheless we failed to observe any precocious metamorphosis among the offspring of the treated aphids. Most probably the daughter embryos in the second instar larvae are not as sensitive to precocene as the mature embryos in the young adults (Hales and Mittler, 1981; Kambhampati et al., 1984).

## Acknowledgements

The authors want to express their sincere thanks to L. Varjas (Res. Inst. Plant Prot., Budapest), H. S. Radwan (Plant Prot. Dept., Fac. Agr., Shibin El-Kom) and K. M. Stott (AFRC Inst. Phys. Bioch. Group, Brighton) for critical reading of manuscript.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (1—2), pp. 187—188 (1986)*

# Book Reviews

HILL, S. A.: *Methods in Plant Virology. Methods in Plant Pathology.* Blackwell Scientific Publ., Oxford, 1984. Vol. 1, pp. 167

"Methods in Plant Virology", the first volume of the series "Methods in Plant Pathology" deals with the fundamental technical-methodological questions of virological studies. The methods discussed are not based on the most up-to-date laboratory techniques—possessed by very few institutes—,they postulate instead the existence of a minimum laboratory equipment. The book is therefore a stop-gap work that offers help mainly to those who are compelled to work in less modern virological laboratories. Considering the rapid progress of virology and the ever widening circle of the new virologist generation the book may command great interest.

After a brief introduction Dr. Hill discusses the individual virus groups (carnation latent virus, closteroviruses, hordeiviruses, potexviruses, potyviruses, tobamoviruses, tobraviruses, bromoviruses, caulimoviruses, comoviruses, cucumoviruses, geminiviruses, ilarviruses, luteoviruses, nepoviruses, monotypic viruses, phytoreoviruses, Fijiviruses, tobacco necrosis virus group, tomato spotted wilt virus group, tombus-viruses, tymoviruses, alfalfa mosaic virus group, plant rhabdoviruses). In 5 subsequent chapters (Histological and other basic methods, Basic virus characterization and storage, Transmission tests, Serological techniques, Electron microscopy) questions of virus methodology are dealt with in detail. After each chapter fundamental works of the relevant literature are listed for the sake of orientation. The book gives many illustrative drawings, figures and tables. The index at the end of the book of 167 pages helps the reader to find his way among the subjects.

Dr. Hill's book is an indispensable guide for those who wish to be familiar with the methodological questions of plant virology.

J. HORVÁTH

J. BÁNHEGYI, S. TÓTH, G. UBRIZSY and J. VÖRÖS: *A Manual for the Identification of the Microscopic Fungi of Hungary.* Akadémiai Kiadó, Budapest, 1985. Vols I—II, pp. 1152

This book is a comprehensive elaboration of the microscopic fungi present in Hungary, the first successful attempt to give a monographic survey of the *Micromycetes* flora occurring in the country and in the surrounding parts of the Carpathian-basin. The work is, in fact a taxonomical handbook, presents an illustrated key for the identification of the fungi found in the above-mentioned geographical region. The phylogenetic system proposed by the authors depends on the conceptions of Gäumann (1951, 1964), Bessey (1950), Martin (1954, 1961), Alexopoulos (1962, 1966), Greis (1943), Sparrow (1958), and Chadefaud (1960)

and shows remarkable similarities to Ainsworth's (1966) classification, although the two systems were independently developed. Regarding nomenclatural rules, the "International Code of Botanical Nomenclature" (1956, 1961) was accepted as guiding principle; the synonyms and homonyms are mentioned in that case only, if these are widely used in applied mycology. Over its scientific value this handbook serves practical purposes too, since detailed descriptions sufficient to identify fungal specimens collected in the country are given. The book is well illustrated, presents nearly 600 photographs and drawings. A selected bibliography is given at the end of the book, where basic works, comprehensive studies, monographs and Hungarian records are cited.

This book is very useful for all of us, who are working in the field of applied mycology. Teachers and students interested in fungi will also find this monograph as an extremely valuable work.

L. HORNOK

Wolfgang BRANDENBURGER: *Parasitische Pilze an Gefäßpflanzen in Europa (Parasitic fungi of Vascular plants in Europe)*
Gustav Fischer Verlag, Stuttgart—New York, 1985. With 403 figures on 150 pages.

The author gives of parasitic fungi known on indigenous, introduced and cultivated vascular plants in Europe. Fungal organisms causing disease symptoms and/or producing fruiting bodies (e.g. spores) on living plants or parts of plants are taken into account and registered as parasites.

The first compilation of plant parasitic fungi, 'Rabenhorst's Kryptogamenflora von Deutschland, Österreich und Schweiz (1884—1910)', was followed by similar works in a number of other countries. However, a European review of these parasites have not yet been published. Thus, the present book provides the first general view of plant parasitic fungi in Europe.

After a short information on handling and explanation of the used symbols and abbreviations, the book presents the host plants and their parasites on 887 pages. The host plants are followed in taxonomic order, according to works of Melchior and Werdermann (1954) and Melchior (1964). Within the plant families the genera are listed alphabetically, and are divided into plant parts (root, stem, leaf, flower, etc.). The parasites follow under these entries systematically. A brief description of fungi are given at their first occurrence.

In the next chapter (p. 888—1195) the parasitic fungi are ordered taxonomically. The description of genera and type-species are completed with the concerning literature and well illustrated with drawings.

The description of fungi is based on the up-to-date monographies. However, in some cases there is a deviation from the valid fungus names for practical reason. A lot of synonyms are listed, e.g. in the class Fungi imperfecti (*Deuteromycetes*), since a recent work of Sutton on *Coelomycetes* (1980) does not involve the decription of type-species of a lot of important genera (e.g. *Septoria*). Furthermore, in the case of pleomorph fungi the anamorphs are often used.

Finally, the terminology, the author's names, the literature cited, as well as a list of hosts and parasites (families and genera) are given.

This excellent book is a great advantage for all who are interested in plant pathology and mycology.

Gyöngyvér Sz. NAGY

# HEAVY METALS IN WATER ORGANISMS

Edited by *J. Salánki*

In English. 1985. XVI + 440 pages.
17×25 cm. Hardcover $45.00/DM 126.—/£32.50
ISBN 963 05 4195 5
Symposia Biologica Hungarica Vol. 29

Chemical substances of anthropogenic origin polluting surface waters are dangerous to aquatic organisms and have varying effects on microbes, plants and animals. Living organisms frequently accumulate pollutants, signalling in this way their presence in the environment. Accumulation is particularly characteristic of heavy metals entering surface waters as industrial, agricultural and communal wastes. Heavy metals have come into the focus of environmental biological research all over the world. Beyond the monitoring of pollution, special attention is being paid to the various organisms which make early detection possible.

The papers in this volume were presented at a symposium held in Hungary at which scientists from 12 countries provided information on the latest results in this field. The most important topics discussed were accumulation of heavy metals, ecological monitoring of heavy metal pollution, organisms as indicators and the effect of heavy metal pollution on vital functions. The Hungarian participants gave an account of the state of the art of research into the heavy metal pollution of Lake Balaton for the first time.

The importance of the problem of heavy metal pollution is reflected by the fact that it has assumed prominence in one of the main projects of IUBS.

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# The Fauna of the Kiskunság National Park Vol. I.

Edited by

S. MAHUNKA

In English. Forthcoming 1986. 512 pages, 48 figures, 8 tables. 17×25 cm.
Hardcover approx. $48.00 ISBN 963 05 3875 X

(Series: Natural History of the National Parks of Hungary 4.)

The Kiskunság National Park situated in the centre of the Great Hungarian Plain, as well as in that of the Carpathian Basin, is one of the most interesting national parks of Hungary, since within its boundaries there are highly varied biotopes like the saline puszta, the sand dunes, Juniper woodlands, inundation groves and a series of marshes.
These biotopes harbour a very special fauna which, elsewhere in Central Europe, has partly become extinct. This is why it is of paramount importance to explore and study this characteristic animal community. A multilateral investigation was proposed and subsequently carried out by the staff of the Hungarian Natural History Museum.
The field work lasted for four years (1977—1981). The collected material has been worked up by an international team of scientists. The taxonomic, faunistical, zoogeographical and ecological results brought to light many new features of interdependence. Basides the documentation of the natural gene bank "protected" here, the obtained data may be a source of inspiration for further comparative zoogeographical and ecological studies. The first volume includes contributions on the following groups of animals: worms, crustaceans, insects, mites, amphibians, reptiles and birds.
The book will be a useful handbook not only for zoologists or other specialists engaged in the study of animals but also for teachers and the layman interested in nature.

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# *Results of the "Síkfőkút" Project*

## *Vol. 1. Structure, Primary Production and Mineral Cycling*

## Series: Ecology of an Oak Forest in Hungary

In English. 1985. 545 pages. 16 photoplates,125 figures 205 tables, 17×25 cm. Hardcover
$49.00/DM 124,—/£34,25 ISBN 963 05 3371 5

The ecological investigations of climax oak-forests in Hungary are conducted as a part of the temperate-forest studies of the "Man and Biosphere" programme organized by UNESCO. Ecologists of 22 institutes have been cooperating since 1972 in studying a fairly undisturbed forest community (Quercetum petraeae-cerris) composed by Quercus petraea and Quercus cerris treespecies in the northern mountain region of Hungary. Similar forests, being characteristic of the Pannonicum region, once covered about a quarter of the country's territory. The synthetic ecological studies, carried out in a forest of 16 hectares equipped for permanent field-studies, are planned to continue until 1990. The first results of these investigations are included in this book.

Chapters I—III outline the main objectives and organization of the whole ecological research, and also the topics of investigations that have been carried out. Geomorphological, pedological and climatic data on the wider geographical surroundings are also included, followed by a phytosociological and pedological description of the sample area, together with the meteorological trends in the sample period.

The following part include only the results of studies on autotrophic levels until 1977. The material from investigations since 1977 and from the studies on heterotrophic levels will be published in the volume of "*Results of the "Síkfőkút" Project II.*"

Chapter IV gives the structural parameters of the forest, Chapter V deals with phytomass and primary production of organic matter, Chapter VI includes the results of growth- and pigment-studies on assimilatory organs, Chapter VII deals with energy-relationship of the autotrophic level and efficiency, Chapter VIII with the element content of higher plants and mosses and Chapter IX contains the results of element-circulation and litter-decomposition studies. The above mentioned main chapters are divided into numerous smaller units. The text and tables always contain results of investigations repeated at last over three years.

The book is the first basic work of its kind in Hungary in a field of terrestrial ecology, whose literature is also rather scarce world-wide. Its final chapter calls attention to trends of global environmental changes (atmospheric $CO_2$ accumulation, soil-acidification, break-down of element-cycles) based on the data given in the book.

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Index: 26.032

OLUME 21 • NUMBERS 3—4 • 1986

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 189 (1986)*

# Gábor Farkas
# 1925—1986

Professor Gábor Farkas, pioneer in plant pathophysiology died after a long illness in Budapest on April, 1986. As a brilliant student, he studied at the Budapest University and was the member of the Eötvös College. Originally he wanted to be trained in mathematics, but under the influence of a meeting with Professor Gimesi, the head of the Plant Physiology Department of the University, he became a biologist mainly interested in the biochemistry and physiology of plants.

Professor Farkas's interests covered many areas of plant biochemistry, including stress and disease physiology. As a young scientist and teacher, he influenced many students because his enthusiasm was infectious. In the first years of the fiftieth he joined the Agricultural Research Center of the Hungarian Academy of Sciences, Martonvásár. He worked on the physiology of water stress and later, under the influence of plant pathologists, on the biochemistry of plant disease and particularly on disease resistance. In 1956 he joined the Plant Protection Research Institute in Budapest and became one of the founding of the plant "pathophysiology school". With his colleagues he covered many diverse topics: the enzymology of respiration in diseased plants, the phenol metabolism in relation to disease resistance, the relation of senescence to disease symptoms etc. In 1974 he became the director of the Plant Physiology Institute of the Hungarian Academy of Sciences and later moved to Szeged. Here he was engaged to work mostly on the biosynthesis of plant viruses in protoplasts and on the biochemistry of virus-infected plants. At the Szeged University he was also engaged in teaching a course on plant physiology. As a member of the Hungarian Academy of Sciences he actively influenced the scientific policy of Hungary.

Professor Farkas was a talented writer and an excellent speaker. He is the author of a book on Plant Biochemistry (in Hungarian). He started to translate this book into English in his hospital room, but a few weeks later he gave up the idea because the progress in his disease. His papers appeared in Nature, Science, Naturwissenschaften, Virology, Experientia, Phytopathology, Physiological Plant Pathology, FEBS Letters, Plant Sci. Letters, etc. In addition to the scientific papers and reviews, he contributed essays to journals and newspapers describing the problems of education and research in his homeland.

His character was an original one. He always supported the talented, enthusiastic young people, and always admired the great and genious persons. He particularly liked to travel and discuss philosophical and political problems in friendly circles. He enjoyed sports and music. He was a generous and caring friend and a man of culture. Gábor Farkas will be remembered with affection and admiration as a distinguished scientist and a very influential person.

*Z. Király*



*Acta Phytopathologica et Entomologica Hungarica 21, 1986*
*Akadémiai Kiadó, Budapest*

*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 191–192 (1986)*

# Kristiu K. Kristev
# (1912–1986)

Death come to Professor Kristev after a brief illness on June 9, 1986, at the age of 74. Being one of the most outstanding Bulgarian plant pathologists, his name is closely associated with the development, advancement and worthy representation of Bulgarian plant pathology in his homeland and abroad.

Professor Kristev was born into a family of civil servants in 1912, in the town Khaskovo. He attended primary and secondary school in his hometown and then was educated at the Sofia State Agro-Forestry Institute (College). He had a difficult life. He lost his mother at the tender age of three, and after he graduated from high school, his father also died. He was obliged to work during his College years to support himself. He was deeply influenced by Professor Atanasov (founder Bulgarian plant pathology) at the Department of Plant Pathology. It was that time that his love and passion for the science of plant pathology were aroused, which were to give him inspiration for more than fifty years till the end of his life. He was trained as a young agronomist in 1935 at the newly established Institute of Plant Protection. Later he worked as a teacher (lecturer) in the Agricultural School in Sapareva Banya. He was appointed for one year as a quarantine officer at the port of Varna. In 1940 he became an assistant of Professor Atanasov. Dr. Kristev served in the Department of Plant Pathology at the Sofia Agro-Forestry Faculty for 11 years, during which time he earned a doctor's degree (his Dissertation was completed in 1943 on smut and bunt diseases of wheat). In 1954 he became a fulltime associate professor and subsequently in 1962 full professor and head of the Plant Pathology Department at the Vasil Kolarov College of Agriculture in Plovdiv till 1976. Thereafter he was appointed as head of the International Wheat Immunity Laboratory at the Dobroudja Wheat and Sunflower Institute near General Toshevo. He served here for three years (1976–1979).

Dr. Kristev was actively involved in his country's scientific activities. He was a member of several scientifix boards, e. g. of the Editorial Boards of Plant Science (in Bulgarian) and Cereal Research Communications. Professor Kristev had been teaching for 36 years during which he wrote five textbooks and published numerous papers in the fields of plant pathology and patho-physiology. His influence on the students activities was basically important. He directed the students association of the College for many years. He educated thousands of agricultural experts and under his guidance 12 postgraduate scientists received their Ph. D. degrees.

He became closely associated with Hungarian scientists in 1956 till the end of

his life. He enthusiastically joined to the Budapest school of patho-physiology and became a close friend of Dr. Farkas and Dr. Király. He worked in the laboratory of the Plant Protection Institute of the Hungarian Academy of Sciences, Budapest several times as a visiting professor.

Professor Kristev was an outstanding experts who was able to integrate basic research and applied practical plant pathology in his activity. On the one side, the goal of his scientific research was to explain plant disease resistance with biochemical terms, on the other hand he was engaged in disease control and as a wheat breeder in practical disease resistance. He published widely cited articles on the enzymology of respiration of rust and virus infected plants. He created the first soft wheat cultivar that was resistant to bunt, a rust resistant wheat cultivar (Balkan) etc., and also participated in the innovation of new Bulgarian fungicides.

Colleagues, friends and students remember him as an extremely enthusiastic scientist, a devoted friend and a really kind man. They always keep his memory with respect and deeply-felt gratitude.

*Professor Nicola Mitov*
*Professor Z. Király*

*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 193—201 (1986)*

# Antibacterial Activity of a Protein(s) from Tomato

TIBOR ÉRSEK,[1] ARTHUR L. KARR and ROBERT N. GOODMAN

University of Missouri, Columbia, Missouri 65211, USA

Extracts from tomato seeds contain both agglutinating and antibacterial activities. Both activities elute from a gel filtration column coincidently with a single peak of protein. The activities are expressed only between pH 3 and 4 and are present in a heat-stable, basic, SDS-insoluble, protease-sensitive component. In drop-agglutination tests, the tomato protein (TP) is capable of differentially agglutinating various bacterial species. The agglutination titer obtained with a species is unrelated to the capacity to cause disease in tomato. TP possesses antibacterial activity against *Pseudomonas syringae* pv. *tomato* and *P. syringae* pv. *glycinea* but not to the saprophyte *P. fluorescens*. This antibacterial activity of TP is inhibited by thiol reagents.

When nonhost plants are inoculated with phytopathogenic bacteria, the bacteria are quickly localized at or near the site of introduction (Huang et al., 1975; Sequeira et al., 1977). It has been suggested that this localization phenomenon results from the action of plant-derived proteins that possess agglutinating activity in *in vitro* assays (Romeiro et al., 1981a; Sequeira and Graham 1977). One such protein has been isolated from apple seeds and shoots and partially characterized (Meyer-Bothling and Karr 1984, Romeiro et al., 1981*a*, *b*). Although preparations of this protein agglutinate both virulent and avirulent strains of *Erwinia amylovora*, they possess a much higher titer against avirulent, acapsular strains. This activity correlates with the selective localization observed when avirulent, acapsular strains of *E. amylovora* are introduced into apple tissue (Huang et al., 1975). The apple protein possessing this agglutinating activity is chemically and physically similar to the purothionins derived from wheat (Meyer-Bothling and Karr, 1984) and, indeed, can be replaced by $\beta$-purothionin in the agglutination assay. The similarity of this protein to purothionin is of interest because the purothionins are reported to exhibit antimicrobial activity against, among others, phytopathogenic bacteria (Fernandez de Caleya et al., 1972). The purpose of the work described in this paper was to determine whether tomato (*Lycopersicum esculentum* Mill.) produces a similar agglutinating protein and to determine whether the agglutinating activity is related to the antimicrobial activity, which might influence plant-parasite specificity.

[1] On leave from the Plant Protection Institute, Hungarian Academy of Sciences, Budapest 1525, P.O. Box 102, Hungary

Contribution from the Missouri Agricultural Experiment Station Journal. Series Number 9962

## Materials and Methods

*Bacteria and medium*

Nutrient broth, supplemented with yeast extract and glucose prepared as described previously (Ayers et al., 1979), was the basic liquid (NYGB) and solid (NYGA) medium used. All bacterial species used in this study were maintained on slants of NYGA at 25 °C. Bacterial suspensions were prepared by washing the cells from slants with either deionized water or phosphate-buffered saline (PBS; 0.01 M sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl).

*Extraction of agglutinating protein (TP) from tomato seeds*

The extraction procedure is based on the method developed for isolation of the agglutinating protein from apple (Meyer-Bothling and Karr, 1984). Tomato seeds of cultivar VF-198 were ground and the resulting meal was packed in a chromatography column and exhaustively washed with acetone to remove seed lipids. The defatted meal was dried overnight and extracted with 95% (v/v) ethanol (1 g meal per 10 ml) by stirring the suspension for 2 h at room temperature. The suspension was centrifuged at 8 000 g for 20 min and the pellet was extracted with eight volumes of 50 mN sulfuric acid for 1 h at room temperature with stirring. The resulting suspension was centrifuged and the supernatant liquid was retained. Trichloroacetic acid was added to a final concentration of 12% (w/v) and the solution was stirred for one h and kept at 4 °C overnight. The resulting precipitate was collected by centrifugation at 8.000 g for 20 min. The pellet was extracted with 0.1 M sodium acetate buffer (SAB; pH 3.7) by stirring for 2 h at room temperature, insoluble material was removed by centrifugation at 12,000 g for 30 min and the supernatant liquid was passed through a Millipore filter (0.45 $\mu$m). The filtrate was dialyzed against 400 volumes of half-strength SAB, dialyzed against deionized water and lyophilized.

*Fractionation of TP*

Routinely, a 50-mg sample of lyophilized seed extract was suspended in 5 ml of SAB (pH 3.7) and placed in a boiling water bath for 10 min. Any undissolved material was removed by passing the suspension through a 0.45 $\mu$m Millipore filter. The sample was placed on a Bio Gel P-100 column (2.5 × 100 cm) at room temperature, eluted with SAB, and 5-ml fractions were collected. Protein was located by measuring $A_{280}$ or by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

*Assay for agglutinating activity*

Bacterial cells were washed from slants with water and adjusted, turbidmetrically, to a concentration of $5 \times 10^9$ cells $ml^{-1}$. For the drop-agglutination assay

(Romeiro et al., 1981*a*), TP was dissolved in the appropriate buffer (2 mg $ml^{-1}$) and a two-fold dilution series was prepared. Twenty microliter aliquots of TP solution and bacterial cell suspensions were mixed on polystyrene plates. Agglutination reactions were scored under a dissecting microscope after incubation periods up to 3 h at 28 °C.

*Assay for antibacterial activity*

A 100 µl aliquot of bacterial suspension ($10^5$ to $10^9$ cells $ml^{-1}$ of water) was mixed with an equal volume of TP dissolved in the appropriate buffer. The mixture was incubated on a rotary shaker at room temperature. Subsamples were collected at regular intervals by vigorously shaking the mixture for 10 sec and removing a 50 µl aliquot. The subsample was transferred to the center of a 9 cm culture plate, 10 ml of NYGA maintained at 45 °C was added and the sample was throughly mixed with the agar. The agar plate was incubated for 24 – 48 h at 26 °C and the number of colonies determined. Results are expressed as colony forming units (cfu) remaining after the treatment. In the case of assays in liquid medium, subsamples (100 µl) were transferred to 25 ml of NYGB in 125 ml flasks and incubated on a rotary shaker at 23 °C.

*Treatments that destroy the agglutinating and/or antibacterial activities of TP*

*Aspergillus*-protease, type XIII (Sigma Chemical Company St. Louis, MO) was dissolved in half-strength SAB at a concentration of 8.2 mg $ml^{-1}$. Equal volumes of protease solution and TP solution, in the same buffer, were mixed and incubated at 28 °C. Subsamples (100 µl) of the mixture were removed after 3-, 6-, and 24-h incubation periods and mixed with 100 µl aliquots of bacterial suspension ($10^5$ to $10^9$ cells $ml^{-1}$). The agglutinating and antibacterial activities were measured as described above. Controls consisted of incubation mixtures containing protease which had been inactivated by boiling.

To test the effect of thiols on agglutinating and antibacterial activity, solutions of dithiothreitol (DTT) or glutathione (GSH) were prepared in half-strength SAB and mixed with an equal volume of TP dissolved in the same buffer. The solution containing the thiol and TP was mixed with an equal volume of bacterial suspension and the agglutinating and antibacterial activities were measured as described above.

## Results

*Extraction and fractionation of TP*

TP was prepared by a modification of a procedure used to extract a similar protein from apple (Meyer-Bothling and Karr 1984). TP prepared by this procedure possessed both agglutinating and antibacterial activities (Tables 1 and 2).

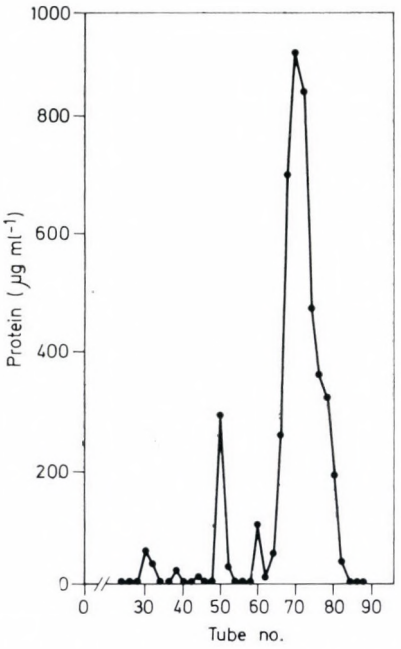



Fig. 1. Elution profile of TP from Bio Gel P-100 column. Protein values are relative to a standard curve prepared with BSA and determined by the method of Lowry et al. [1951]. Column: 2.5× 100 cm; fraction volume: 5 ml

Inclusion of the ethanol extraction step increased the solubility of TP after lyophilization.

The antibacterial activity of TP was sensitive to freezing and thawing. Three to four cycles of freezing at −20 °C and thawing at room temperature led to total loss of activity. Agglutinating activity was not affected. Even with inclusion of the ethanol extraction step, lyophilized preparations of TP were only moderately soluble in water or SAB at room temperature but were readly dissolved when placed in a boiling water bath for 10 min. Neither the antibacterial nor agglutinating activities of the unfractionated TP were diminished by boiling.

Most of the protein (90%) applied to the Bio Gel P-100 column was recovered in a single peak (fractions 63 through 84; Fig. 1). These fractions also contained all of the applied agglutinating and antibacterial activities.

*Agglutinating activity of TP*

The optimal pH for agglutination of bacterial cells was between pH 3 and 4. Therefore, agglutination assays were carried out in SAB.

The agglutination titer (Romeiro et al., 1981) for a typical preparation of TP was measured with a number of bacterial species (Table 1). The ability of TP to

Table 1

Agglutinating activity of fractionated TP present in tomato seeds

| Bacterium spp. | Agglutination titer |
|---|---|
| *Pseudomonas syringae* pv. *tomato** | 128 |
| *P. syringae* pv. *glycinea* | 16 |
| *P. fluorescens* | 256 |
| *P. syringae* pv. *tabaci* | 0 |
| *Erwinia amylovora*, $E^8$ (acapsular) | 4 |
| *E. amylovora*, $E^9$ (capsular) | 0 |
| *E. carotovora* pv. *carotovora* | 0 |
| *Corynebacterium michiganense** | 0 |
| *C. insidiosum* | 0 |
| *Xanthomonas campestris* pv. *vesicatoria** | 128 |
| *X. campestris* pv. *malvacearum* | 128 |

* Indicates species pathogenic to tomato.
Drop agglutination assay was carried out as described in Materials and Methods.

agglutinate a bacterial species was unrelated to that species ability to cause disease symptoms in tomato. Of the three species studied further, the agglutinating titer was highest for the saprophyte *P. fluorescens*, intermediate for the tomato pathogen *P. syringae* pv. *tomato*, and lowest for the soybean pathogen *P. syringae* pv. *glycinea*.

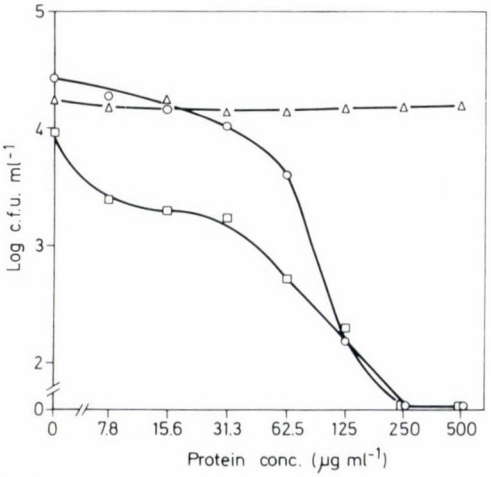



Fig. 2. Effect of TP obtained from Bio Gel P-100 column (combined fractions 63 to 84) on cfu of *P. syringae* pv. *tomato* (○), *P. syringae* pv. *glycinea* (□) and *P. fluorescens* (△) in NYGA. Bacterial suspension ($2 \times 10^5$ $ml^{-1}$) was exposed to equal volumes of TP at concentrations two times those indicated for 15 min before incorporating subsamples (50 μl) into the medium

*Antibacterial activity of TP*

TP possessed antibacterial activity only in the pH range where it caused agglutination (between pH 3 and 4). The data in Table 2 were obtained by treating cells of *P. syringae* pv. *tomato* with unfractionated TP at pH 3.7 for 30 min. Treatments were terminated at 5, 15, 25 and 29 min by addition of enough NaOH to increase the pH to 4.8. Only a 5-min treatment with TP is required to cause a

Table 2

Effect of unfractionated TP on the survival of P. syringae *pv.* tomato

| TP ($\mu$g ml$^{-1}$) | Treatment time (min) | | | |
|---|---|---|---|---|
| | 5 | 15 | 25 | 29 |
| 0 | 100 | 100 | 100 | 100 |
| 3.9 | 60±40 | 13±3 | 10±3 | 22±9 |
| 7.8 | 10±1 | 6±3 | 6±1 | 15±2 |
| 15.6 | 32±5 | 12±1 | 7±1 | 15±3 |
| 31.3 | 39±15 | 19±1 | 16±3 | 22±6 |
| 62.5 | 78±3 | 64±32 | 26±2 | 42±9 |

Values are the mean percent survival of cfu obtained from three separate experiments. Treatment was terminated at the above intervals by addition of 0.1 N NaOH to increase the solution pH to 4.8.

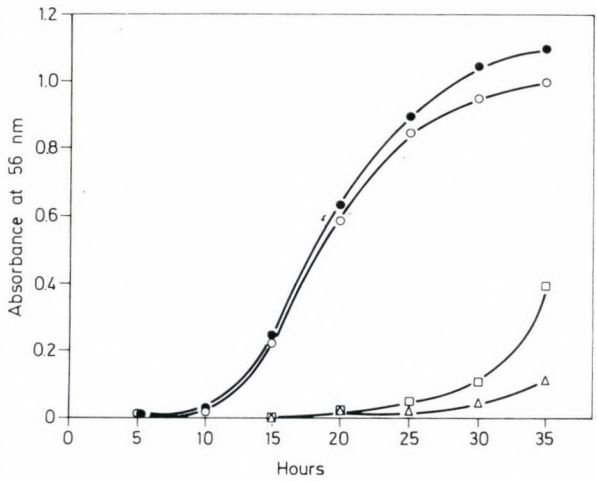



Fig. 3. Effect of fractionated TP on *P. syringae* pv. *tomato* in NYGB at actual concentrations of 500 (△), 250 (□) and 125 (○) $\mu$g ml$^{-1}$ after mixing with the bacterial suspension. After 30 min incubation 100-$\mu$l subsamples were added to the medium. The initial bacterial concentration in the medium (0 time) was $5\times10^5$ ml$^{-1}$. The control (●) consisted of bacterial cells treated with SAB

significant decrease in the survival of bacterial cells. Treatment with TP for periods longer than 25 min did not further decrease bacterial cell survival. Termination of the treatments by dilution in NYGA gave similar results.

The antibacterial activity of combined fractions 63 to 84 from the gel filtration column (Fig. 1) increased with increasing protein concentration (Fig. 2) for both *P. syringae* pv. *tomato* and *P. syringae* pv. *glycinea* and with *P. syringae* pv. *tomato* in liquid medium (Fig. 3). This is in contrast to the unfractionated TP (Table 2) which exhibited decreased activity at higher protein concentrations. The survival of *P. fluorescens* was not affected by any concentration of the TP preparation tested.

*Protein characteristics of TP*

Treatment of TP with protease resulted in a time-dependent decrease in both the agglutination and antibacterial activities of TP. After a 24-h treatment both activities were eliminated. TP, like the purothionins and thionin-like proteins, was insoluble in the presence of SDS (Jones and Cooper, 1980). TP in SAB had absorption maxima at 280 nm and 237 nm, the latter suggesting the possible presence of sulfur-containing amino acids.

The protein(s) in combined fractions 63 through 84 possessed a large net positive charge and moved rapidly when subjected to agarose gel electrophoresis (Romeiro and Karr 1981*a*). In addition, the protein(s) was capable of precipitating bacterial LPS (isolated from *E. amylovora)* when subjected to rocket electrophoresis.

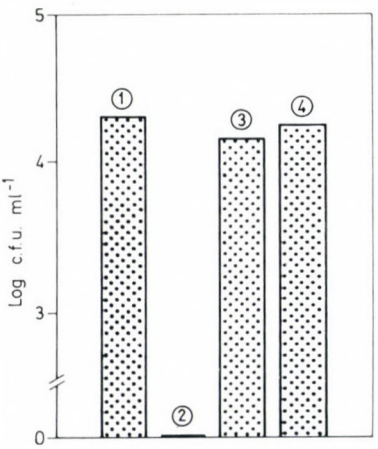



Fig. 4. Inhibition of bactericidal activity of fractionated TP on *P. syringae* pv. *tomato* by GSH Equal volumes (100 $\mu$l) of the solutions and bacterial suspension ($2\times 10^5$ ml$^{-1}$) were mixed and incubated for 30 min before incorporation into NYGA. 1, control: SAB; 2, TP: 500 $\mu$g ml$^{-1}$ SAB; 3, TP (1000 $\mu$g ml$^{-1}$ SAB) plus GSH (1000 $\mu$g ml$^{-1}$ SAB) in equal volumes (50 $\mu$l each); 4, GSH 500 $\mu$g ml$^{-1}$ SAB

Thiols, such as GSH (and DTT, data not presented), prevented the antibacterial effect of TP on *P. syringae* pv. *tomato* (Fig. 4). This is similar to the protective effect of thiols reported for purothionin (Fernandez de Caleya et al., 1972). Neither GSH nor DTT affected the agglutinating activity of the TP preparation.

## Discussion

Reports from this laboratory (Meyer-Bothling and Karr, 1984, Romeiro et al., 1981*a*, *b)* have suggested that purothionin-like protein(s) from apple may be responsible for the microscopically observed localization of avirulent, acapsular strains of *E. amylovora* (Huang et al., 1975). In this paper, we report the isolation of chemically similar protein(s) from tomato.

TP possessed both agglutinating and antibacterial activities. The antibacterial activity is sensitive to the presence of thiol reagents. It is similar to the apple protein(s) in that it is a low molecular-weight, very basic, protease-sensitive component. Some differences do exist between the tomato- and apple-derived proteins. First the two proteins elute in different fractions from the same Bio Gel P-100 column. The tomato-derived protein(s) elutes in earlier fractions. Second, although the tomato-derived protein(s) only exhibits antibacterial activity between pH 3 and 4, the apple protein is antibacterial at pH 7.0 (unpublished results).

TP decreases cfu (Table 2 and Fig. 2) and turbidimetrically measured bacterial cell concentrations (Fig. 3). We believe that these effects are due to decreased cell survival and are not due to aggregate formation resulting from the agglutinating activity of the preparation for two reasons. First, GSH and DTT prevent the TP-caused decrease in measured survival of *P. syringae* pv. *tomato* (Fig. 4) without affecting agglutinating activity. Second, *P. fluorescens* is the most strongly agglutinated of the bacterial species tested, but its survival is not affected by TP. If the apparent decrease in survival was due to aggregate formation caused by the agglutinating activity of TP, then *P. florescens* should also show decreased survival. Thus, agglutination *per se* is not a prerequisite for bacterial cell death.

The reason for the decreased antibacterial activity of unfractionated, but not fractionated TP, at higher protein concentrations (Table 2) is not clear. It is possible that unfractionated preparations contain a molecule which mitigates TP activity by aggregating TP, or by protecting the bacterial cells from TP.

The mechanism by which the purothionin-like protein(s) decreases survival of bacterial cells is not known. The fact that thiol reagents protect the bacterial cells suggests that the proteins interact with important cell thiol groups or interfere with cell sulfur metabolism, possibly on the cell surface. It is unlikely that the thiol reagents merely act to reduce important disulfide bonds in the protein. With the apple protein, disulfide bonds were only broken by reaction with 4-vinylpyridine at reflux (Meyer-Bothling and Karr, 1984).

Although this class of proteins may be involved in the observed localization of acapsular strains of phytopathogenic bacteria, present evidence is insufficient

to determine whether they are exclusive or participate as one of several factors involved in localization. It is also possible that these proteins play a role in preventing growth of incompatible phytopathogenic bacteria.

Research was supported by the National Science Foundation (PCM 8216395).

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 203–206 (1986*

# Survival of *Agrobacterium tumefaciens* in Berlandieri × Riparia Grapevine Rootstock

S. SÜLE

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

Two hundred and eighty bacteria were isolated from symptomless 5-year-old Berlandieri × Riparia T5C grapevine rootstocks. Separation of the dead bark, phloem and xylem has shown that the pathogenic *Agrobacterium tumefaciens* strains were in detectable number only in phloem tissue. Enrichment of the population was achieved by incubating the roots for 3–4 weeks. The newly formed callus tissue contained high number of pathogenic bacteria. Comparison of serological and bacterilogical identifications revealed that the results of the two methods were in agreement, however none of them can substitute the pathogenicity test. The use of selective medium (3DG) for biovar 3 facilitated the isolation.

Crown gall of grapevine (*Vitis vinifera* L.), caused by *Agrobacterium tumefaciens*, is a disease of economic concern in many parts of the world (Chamberlain, 1962; Lehoczky, 1968; Panagopoulos et al., 1978; Burr and Katz, 1983; Dhanvantari, 1983). The pathogen has three biovars and according to recent works (Kerr and Panagopoulos, 1977; Süle, 1978; Burr and Katz, 1983) it can be distinguished by biochemical tests. Grapevine is infected mainly by biovar 3 strains, although all of the three described biovars can be isolated from it (Süle, 1978).

Host range of biovar 3 is primarily a property of the pTi (tumor inducing plasmid), but the chromosomal background also plays a role (Knauf et al., 1982). The pathogen can induce tumorlike galls on nearly all parts of the plant, but trunk infections are the most serious as the vegetative vigor, fruiting and long life of the entire vine depends on its continued healthy state. The bacterium is usually present in high numbers in the tumor tissue and can infect systemically the vascular and root system (Lehoczky, 1968, 1971, 1978; Burr and Katz, 1983, 1984) of the cultivated grapevine (*Vitis vinifera* L.). Grapevine cultivars differ in susceptibility (Szegedi, 1982) and the ability of the pathogen to survive in different *Vitis sp.* may vary also.

The purpose of this investigation was to understand more fully the nature of root infestation of the most videly used *Vitis berlandieri* × *Vitis riparia* T5C rootstock.

## Materials and Methods

Material was obtained in December 1983 from a five-year-old vineyard of cv. Merlot on the *Vitis berlandieri* × *Vitis riparia* T5C rootstock. The scion was heavily infected showing typical trunk canker and galls, but the rootstock was apparently

healthy without any galls. Five roots (5 cm long and 6–7 mm diameter) were excised from each plant and were washed with shaking in 50 ml sterile water. The dead bark of the roots were removed with a sterile scalpel and suspended in the same washing water. Bacteria living on the root surfaces were isolated from this suspension. The washed roots were surface sterilized for 5 minutes in 1% sodium hypochlorite and rinsed 4 times in sterile water. The phloem was separated from the xylem with the help of pincers and both of them were macerated in sterile water (1 g/ml). The whole suspension was left in refrigerator (4 °C) for overnight and in the following day, aliquots of 0.1 ml from dilutions were spread over the surface of media.

Parallel with this direct isolation, surface sterilized roots were incubated in moist perlit at 20 °C for 3–4 weeks, until the new callus tissue appeared at the ends of roots. The callus tissue was macerated in sterile water and plated onto media. Two media were used all over the experiments: nutrient agar (NA) supplemented with 0.1% yeast extract, 0.1% glucose, 30 mg/l actidione and 10 mg/l hexachlorophene and the selective medium (3DG) prepared according to Brisbane and Kerr (1983). At the time of plating, previously identified strains were streaked onto both media for comparison of the colonies. Plates were incubated for 5 days at 25 °C before suspect colonies were isolated.

Isolates that produced typical colonies on culture media were tested for pathogenicity and characterized to biovar (Süle, 1978). Agglutination tests, using rabbit antiserum (Süle and El-Kady, 1983) produced against a biovar 3 strain, were performed in microtiter plates. Colonies were removed from the medium and suspended in 0.1 ml sterile saline (0.85% NaCl). This bacterial suspension was mixed with 0.1 ml diluted antiserum (1: 50) and the plates were incubated for 3 hours at 25 °C.

## Results

From the surface of the unsterilized roots, only non-pathogenic isolates were obtained all over the investigations. None of the 95 isolates (Table 1) induced gall either on sunflover or tomato seedlings, however 19 of them were serologically positive. Of those, eleven were identified as biovar 3 and eight as biovar 1. A few pathogenic strains were obtained from the phloem demonstrating that the pathogen can survive in this tissue. Of 41 isolates 5 were pathogenic, 21 were agglutinated with antiserum and all the 21 belonged to biovar 3. None of the strains isolated from the xylem were pathogenic. Of 46 strains 20 were serologically positive, 16 were characterized as biovar 3 and 4 as biovar 1. Thirty root segments (5 cm long and 6–7 mm diameter) were incubated at 20 °C for 3–4 weeks in moisted sterile perlit. To prevent fungal contaminations, the roots were submerged in 0.1% Benlate suspension during overnight. Inspite of this treatment eight root segments had to be discarded because of fungal contaminations. By the end of incubation, all of the roots developed at their ends a white callus tissue. Isolations from these tissues

Table 1

Characters of isolates from *Vitis berlandieri* × *Vitis riparia* T5C rootstock roots

| Isolated from | Number of isolates | | | | |
|---|---|---|---|---|---|
| | Total | Pathogen | Agglutinated | Biovar 1 | Biovar 3 |
| Rhizosphere | | | | | |
| NA | 85 | 0 | 12 | 3 | 9 |
| 3DG | 10 | 0 | 7 | 5 | 2 |
| Phloem | | | | | |
| NA | 18 | 1 | 4 | 0 | 4 |
| 3DG | 23 | 4 | 17 | 0 | 17 |
| Xylem | | | | | |
| NA | 21 | 0 | 8 | 4 | 4 |
| 3DG | 25 | 0 | 12 | 0 | 12 |
| Callus | | | | | |
| NA | 38 | 14 | 21 | 3 | 18 |
| 3DG | 60 | 42 | 48 | 0 | 48 |

NA = nutrient agar as specified in text
3DG = selective medium of Brisbane and Kerr (1983)

were always successful. Of 98 strains 56 were pathogenic, 69 reacted positively in serological tests and 66 were characterized as biovar 3 and 3 as biovar 1.

We ran serological tests on a total of 280 isolates, 129 from those were agglutinated with antiserum and all of the 129 were characterized later as *Agrobacterium sp.* There was no serological difference between pathogenic and nonpathogenic strains.

Isolations from 3DG medium were more successful than from NA. Of 118 colonies isolated from 3DG 84 *Agrobacterium sp.* and 46 were pathogenic. Beside agrobacteria, 3–4 other types of bacteria grew also on this medium, but pearly like colonies of agrobacteria were easily distinguished. NA supported as well, growth of biovar 3 strains but beside agrobacteria 6–8 other bacteria also grew on this medium which could sometimes overgrow agrobacteria. Of 162 isolates from NA 45 were *Agrobacterium sp.* and only 15 were pathogenic. The use of reference strain on both media made easier to recognize the developing colonies.

## Discussion

Results from this study clearly demonstrate that *Agrobacterium tumefaciens* can survive in *Vitis berlandieri* × *Vitis riparia* T5C rootstock. This phenomenon may be extremely important as the pathogen can from the roots reinfect the scion and insure the inoculum for new infections. Pathogenic agrobacteria were not

isolated from root surfaces and the rhizosphere seems to be not a common place of biovar 3 strains. Both 3DG medium and the antiserum were useful in isolation and identification of agrobacteria. Unfortunately none of them can replace pathogenicity tests and to distinguish pathogenic and non-pathogenic forms it is necessary to conduct pathogenicity tests. Attempts to use absorbed antisera (Süle and El-Kady, 1983) were promising but they failed in routine works because their titers became very low after absorption. The efficiency of direct isolation of the pathogen from root tissue is very low. Probably number of bacteria is not sufficient to detect with the available methods. Enrichment of the pathogenic population can be achieved by incubating the roots for 3–4 weeks (Lehoczky, 1971). The newly formed callus tissue contained always high number of pathogenic bacteria.

*

This work was supported by a Research grant (3–0015–44 MTA KKA) of the Hungarian Academy of Sciences.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 207—213 (1986)*

# Common Virulence Specific Antigens in Five *Pseudomonas* Pathovars

S. EL-KADY, G. SOMLYAI, MÁRIA HEVESI and Z. KLEMENT

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

It has been proved that the Tn5 induced *P. s.* pv. *phaseolicola* mutants, which lost their ability to induce hypersensitive reaction (HR), also lost some antigens (A, B, C, D, E, F, G, H, K). It was suggested that these antigens might have a specific role in the HR induction pathogenesis. Comparing four *Pseudomonas* pathovars it was found that in the *P. s.* pv. *tabaci* A, D, E, G, K antigens exist; in the *P. s.* pv. *syringae* C, D, E; in the *P. s.* pv. *pisi* and *P. S.* pv. *lachrymans* C and E antigens were present from the nine antigens mentioned above. None of these antigens were detected in the non-pathogenic strain (*P. s. syringae* DV2) and in the two saprophytic strains (*P. fluorescens*, *P. aeruginosa*).

*Pseudomonas syringae* pv. *phaseolicola*, (*P. s.* pv. *phaseolicola*), causal agent of halo blight of bean has been used to generate mutants for use in the molecular analysis of pathogenesis and in the recognition process of the plant by bacterium. Three thousand and seven hundred independent mutants were tested for the ability to induce hypersensitive reaction (HR) in the tobacco leaf to cause watersoaking symptoms in bean trifoliates and pods. At least ten mutants were found which failed to elicit HR ($HRi^-$) (Somlyai et al., 1986; El-Kady et al., 1986*b*) and six out of ten mutants were non-pathogenic ($Path^-$) on bean while four had reduced virulence ($Vir^-$). Crossed-immunoelectrophoresis was used to compare serologically the wild type *P. s.* pv. *phaseolicola* with its mutant derivatives. Altogether nine different missing antigens (A, B, C, D, E, F, G, H, K) were proved in the ten mutants (El-Kady et al., 1986*a*; El-Kady et al., 1986*b*) from the detected 34 antigens. We concluded that these antigens play an important role in the induction of HR.

In an independent experimental series we compared the antigen pattern of three *Pseudomonas* pathovars (El-Kady et al., 1986*c*) showing that the *P. s.* pv. *tabaci* had 26 common antigens with *P. s.* pv. *syringae* and *P. s.* pv. *phaseolicola* and 6 strain-specific antigens. The *P. s.* pv. *syringae* had 2, while *P. s.* pv. *phasolicola* has 3 strain-specific antigens while more than 20 were common.

In this study we aimed to investigate that the 9 antigens, which were missing in the 10 $HRi^-$ *P. s.* pv. *phaseolicola* mutants, are also detectable in the other wild strains of *Pseudomonas* pathovars supporting the assumption that these are common antigens.

## Materials and Methods

The bacterial strains and mutants used are listed in Table 1.

Table 1

Bacterial strains used

| Strains | Characteristics | Source/reference |
|---|---|---|
| P. s. pv. phaseolicola (S21) | $Path^+$ | G. Somlyai (1986) |
| P. s. pv. tabaci | | |
| ATCC No 11528 | $Path^+$ | R. Goodman |
| 466 | $Path^+$ | M. Hevesi |
| P. s. pv. syringae P14 | $Path^+$ | M. Hevesi |
| P60 | $Path^+$ | M. Hevesi |
| P. s. pv. pisi | $Path^+$ | M. Hevesi |
| P. s. pv. lachrymans | $Path^+$ | M. Hevesi |
| P. s. pv. syringae DV2 | $Path^-$ | De Vay |
| P. fluorescens | $Path^-$ | |
| P. aeruginosa | $Path^-$ | |
| 21—35 | $HRi^-$, $Path^-$ derivatives of S21 | El-Kady 1986b |
| 21—989 | $HRi^-$, $Path^-$ derivatives of S21 | G. Somlyai 1986 |

Preparation of antigens, procedures for immunization of rabbits, preparation of antibody, absorption of antisera and crossed-immunoelectrophoresis (CIE) procedures were performed as described in El-Kady et al. (1986*a*).

## Results

In order to investigate the common antigens of different *Pseudomonas* pathovars we tested two strains of *P. s.* pv. *syringae*, P14, P60 and one of *P. s.* pv. *lachrymans* using antibody of *P. s.* pv. *phaseolicola* (S21). It was found that among the common antigens of the S21 and *P. s.* pv. *syringae* three antigens (C, D, E) belonged to the group of antigens (Fig. 1) which may play an important role in the HR induction (El-Kady et al., 1986*a*). Similarly two of their antigens (C, E; Fig. 2) were also present in *P. s.* pv. *lachrymans*.

Comparing the antigenic pattern of S21 with *P. s.* pv. *tabaci* we used antibody of S21 absorbed by the antigens of an $HRi^-$ mutant (S21—35) of S21. It was proved earlier (El-Kady et al., 1986*b*) that this mutant lost A, D, G, H and K

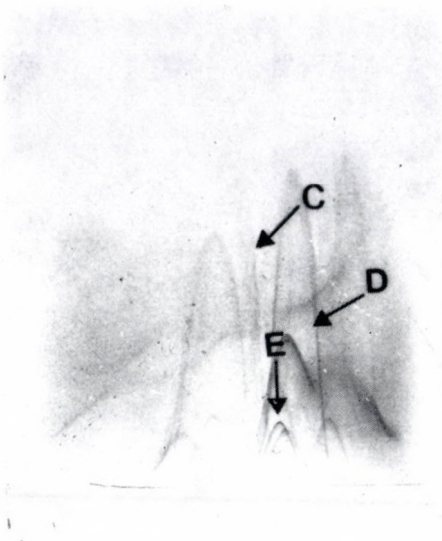



Fig. 1. Crossed-immunoelectrophoresis of the antigens of *P. s.* pv. *syringae* and antibodies of *P. s.* pv. *phaseolicola*. The antigens of *P. s.* pv. *syringae* were electrophoresed, the upper gel contained the antibodies of *P. s.* pv. *phaseolicola*. The arrows indicate three precipitin antigens (C, D, E) which are present in *P. s.* pv. *syringae* as well in *P. s.* pv. *phaseolicola*

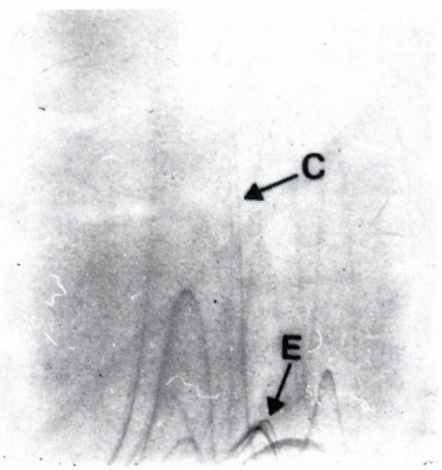



Fig. 2. Crossed-immunoelectrophoresis between antigens of *P. s.* pv. *lachrymans* and antibodies of *P. s.* pv. *phaseolicola*. The antigens of *P. s.* pv. *lachrymans* were electrophoresed, the upper gel contained the antibodies of *P. s.* pv. *phaseolicola*. The arrows indicate two precipitin antigens (C, E) which are present in *P. s.* pv. *lachrymans* as well in *P. s.* pv. *phaseolicola*

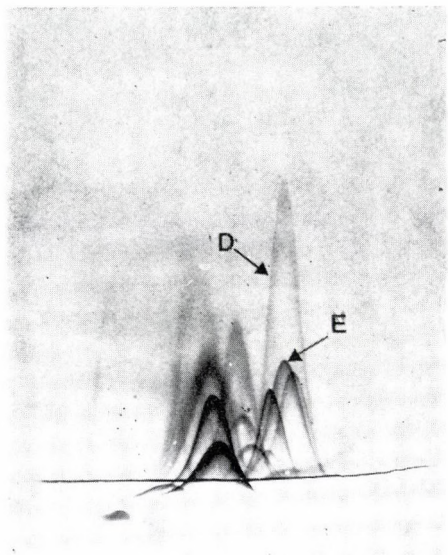



Fig. 3. Crossed-immunoelectrophoresis between antigens of *P. s.* pv. *tabaci* and antibodies of *P. s.* pv. *tabaci* absorbed by antigens of the mutant S21–989. The antigens of *P. s.* pv. *tabaci* were electrophoresed, the upper gel contained the absorbed antibodies of *P. s.* pv. *tabaci*. The arrows indicate the common antigens (D, E) between the two species

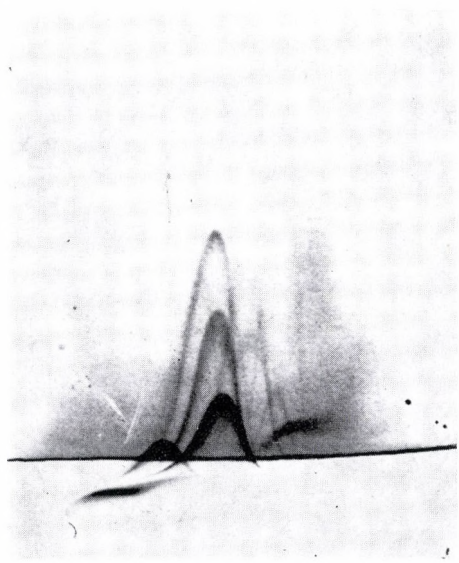


Fig. 4. Crossed-immunoelectrophoresis between antigens of *P. s.* pv. *tabaci* and antibodies of *P. s.* pv. *tabaci* absorbed by antigens of *P. s.* pv. *phaseolicola* (S21). The antigens of *P. s.* pv. *tabaci* were electrophoresed, the upper gel contained the absorbed antibodies of *P. s.* pv. *tabaci*. The precipitin peaks show the serological differences between the two species

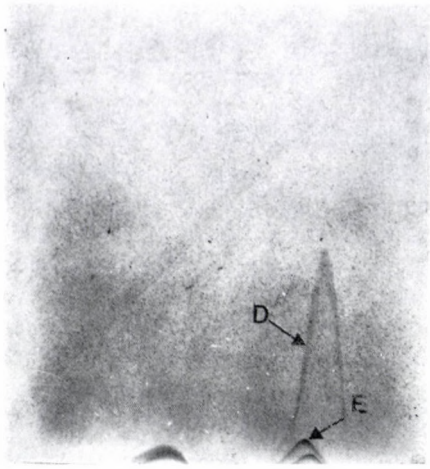



Fig. 5. Crossed-immunoelectrophoresis between antigens of *P. s.* pv. *phaseolicola* and antibodies of *P. s.* pv. *tabaci* absorbed by antigens of the mutant S21–989. The antigens of *P. s.* pv. *phaseolicola* were electrophoresed, the upper gel contained the absorbed antibodies of *P. s.* pv. *tabaci*. The arrows indicate two antigens (D, E) which are absent in mutant S21–989 but exist in *P. s.* pv. *phaseolicola* and *P. s.* pv. *tabaci*

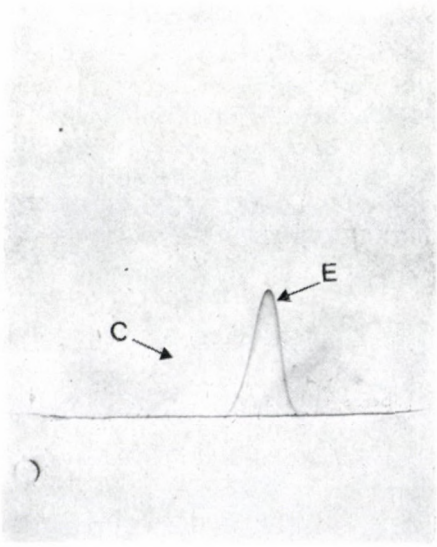



Fig. 6. Crossed-immunoelectrophoresis between the antigens of *P. s.* pv. *pisi* and antibodies of *P. s.* pv. *phaseolicola* absorbed by antigens of the mutant S21–35. The antigens of *P. s.* pv. *pisi* were electrophoresed; the upper gel contained the absorbed antibodies of *P. s.* pv. *phaseolicola*. The arrows indicate the common antigens (C. E) between the two species

antigens. In this case the peaks represent the common antigens, which might play a role in the HR induction of *P. s.* pv. *phaseolicola* (Fig. 4). Another HRi$^-$ mutant (S21 – 989) was also used for absorption of *P. s.* pv. *tabaci* antibodies for electrophoresis of *P. s.* pv. *tabaci* antigens. We found that two antigens (D, E; Fig. 3) were present in *P. s.* pv. *tabaci*, but when the *P. s.* pv. *tabaci* antiserum was absorbed with the wild type antigens of *P. s.* pv. *phaseolicola* (containing all the antigens) we did not detect these two peaks in homologous reaction of *P. s.* pv. *tabaci* (Fig. 4). These two specific antigens, however, also were detected when S21 antigens were electrophoresed against the absorbed antibodies of *P. s.* pv. *tabaci* by S21 – 989 mutant derivatives of S21 (Fig. 5).

Similarly, the S21 antiserum was absorbed by the antigens of the HRi$^-$ mutant S21 – 35 and electrophoresed against the antigens of *P. s.* pv. *pisi* (Fig. 6). In this case two common antigens (C, E) were detected from the five which were missing in the mutant.

In the control experiment we compared the two virulent *P. s.* pv. *phaseolicola* (S21) and *P. s. tabaci* strains, respectively with a non-virulent strain of *P. s.* pv. *syringae* (DV2) and with two saprophytes *(P. fluorescens, P. aeruginosa)*, however, none of the nine antigens (A, B, C, D, E, F, G, H, K) were detected in the non-virulent and saprophytic bacteria.

## Discussion

In the last few years several articles have reported on isolation of non-pathogenic mutants of different plant pathogenic bacteria, (Anderson and Mills, 1985; Daniels et al., 1984; Lindgren et al., 1985; Somlyai et al., 1986) which usually were also unable to induce HR.

The serological differences between the wild type *P. s.* pv. *phaseolicola* and its HRi$^-$ derivatives were demonstrated in our previous papers (El-Kady et al., 1986*a*; El-Kady et al., 1986*b*). We suggested that those nine antigens which were missing in the ten different HRi$^-$ mutants, may be necessary for both the induction of HR and pathogenesis.

We aimed to investigate whether these non-virulent related antigens could be present in other *Pseudomonas* pathovars or whether they exist only in the *P. s.* pv. *phasolicola*. The results show that some of the nine non-virulent antigens are present in the *P. s. tabaci*, *P. s.* pv. *syringae*, *P. s.* pv. *lachrymans* and *P. s.* pv. *pisi* (Table 2.) but none of them were detected in the non-virulent strain of *P. s.* pv. *syringae* and in the two saprophytic *Pseudomonas* species. We found that only one common antigen (E), which exists in all tested phytopathogenic pseudomonads, may play a specific role in the pathogenesis. However, this conclusion requires more evidence. We suppose that some antigens of *Pseudomonas* pathovers exist as common virulent related antigens, but it is most likely that they have also virulence-related pathovar-specific antigens.

Table 2

The common virulence-specific antigens among Pseudomonas species, detected by using CIE technique

| Species | Common virulence-specific antigens |
|---|---|
| P. s. pv. phaseolicola (S21) | A, B, C, D, E, F, G, H, K, |
| P. s. pv. tabaci ATCC No 11528 | A, D, E, G, K, |
| 466 | A, D, E, G, K, |
| P. s. pv. syringae P14 | C, D, E, |
| P60 | C, D, E, |
| P. s. pv. pisi | C, E, |
| P. s. pv. lachrymans | C, E, |
| P. s. pv. syringae DV2 | — |
| P. fluorescens | — |
| P. aeruginosa | — |

## Literature

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 215–230 (1986)*

# Genetic Distance in Fungus Genus *Fusarium* Measured by Comparative Computer Analysis of Isoelectrofocusing Esterase Profiles

Á. Szécsi and L. Hornok

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

Soluble protein extracts from 39 species of the fungus genus *Fusarium* were subjected to isoelectric focusing in polyacrylamide gel rods. The pI values of the esterase isoenzymes were determined and the data were used for calculation of genetic distance matrices and the construction of genetic distance trees by computerized classificatory techniques.

Evidence presented indicates that chemotaxonomic studies based on IEF esterase isoenzyme comparison could be a valuable additional tool in the identification of species within the genus *Fusarium* and a suitable method for the determination of genetic distance between fungi at the species level.

The genus *Fusarium* Link ex Fr. contains anamorphs of the *Ascomycetes* genera *Calonectria* de Not, *Giberella* Sacc., *Micronectriella* Höhnel, and *Nectria* Fr. *Fusarium* is therefore a heterogeneous and taxonomically difficult fungal genus. Classification of *Fusarium* is exclusively based on morphological characters, and although excellent taxonomic monographs have been published in the last 15 years (Booth, 1971; Joffe, 1974; Bilay, 1977; Gerlach and Nirenberg, 1982; Nelson et al., 1984) there is much debate over the status of several taxa and their correct placement in sections (sections contain related species).

In recent years important chemotaxonomic investigations have been made to determine interspecific differences between a wide range of Fusaria. Pioneer serological comparisons of *F. moniliforme*, *F. oxysporum* and *F. solani* (ova-shaped microconidia) by Madhosingh (1964) and a study of serological relationships of the species belonging to the sections "Gibbosum" and "Discolor" by means of crossed immunoelectrophoresis (Hornok, 1980) are worthy of note. Ianelli et al. (1982) reported distinctive antigenic characteristics between *F. oxysporum*, *F. moniliforme* and *F. xylarioides*. Several enzyme patterns of 11 species of *Fusarium* were compared by Saito et al. (1980, 1983) to estimate possibilities of a taxonomic identification on this basis. In an extensive comparative study the structural ribosomal proteins of 172 isolates of several species were analysed by polyacrylamide gradient gel electrophoresis in SDS (Partridge et al., 1984). Szécsi and Dobrovolszky (1985*a*, 1985*b*) established phylogenetic relationships among 11 *Fusarium* species by means of DNA reassociation studies and by comparing DNA thermal denaturation profiles.

Table 1

Isolates of *Fusarium* species and varieties used in IEF studies

| Species | Culture collection No. | Place of origin | Habitat/Host | Collected and identified by | Year of isolation |
|---|---|---|---|---|---|
| *acuminatum* Ellis and Everhart | 22—8 | Hungary | wheat root | L. Hornok | 1970 |
| *aqueductum* Lagh | IMI 86 977 | No record | — | C. Booth | — |
| *arthrosporioides* Sherb. | IMI 163 537 | Australia | wheat root | S. Chambers | 1971 |
| *avenaceum* (Chorda ex Fr.) Sacc. | 22—2 | Hungary | alfalfa root | L. Hornok | 1972 |
| *buharicum* Jaczewski | DSM 62 125 | Iran | Hibiscus sp. | W. Gerlach | — |
| *camptoceras* Wollenw. and Reink. | IMI 162 622 | Australia | wheat root | S. Chambers | 1971 |
| *coccophilum* (Desm.) Wollenw. and Reink. | — | Japan | pine shall | J. Aoki | — |
| *concolor* Reinking | IMI 152 479 | Australia | wheat root | S. Chambers | 1970 |
| *culmorum* (W. G. Smith) Sacc. | 22—13 | Hungary | wheat seedling | L. Hornok | 1972 |
| *decemcellulare* Brick | Rd 25 | No record | pecan | P. E. Nelson | — |
| *dimerum* Penzig in Saccardo | M 61 | No record | banana | A. Wallbridge | — |
| *equiseti* (Corda) Sacc. | 22—59 | Hungary | barley root | L. Hornok | 1970 |
| *flocciferum* Corda | IMI 131 516 | No record | — | C. Booth | — |
| *fusarioides* (Frag. and Cif.) Booth | IMI 153 430 | Australia | wheat root | S. Chambers | 1970 |
| *graminearum* Schwabe | 22—39 | Hungary | wheat seed | L. Hornok | 1972 |
| *heterosporum* Nees ex Fr. | 22—115 | Hungary | wheat leaf | L. Hornok | 1973 |
| *lateritium* Nees | — | Australia | apricot | M. V. Carter | — |
| *merismoides* Corda | E-22 | No record | soil | P. E. Nelson | — |

| | | | | | |
|---|---|---|---|---|---|
| *moniliforme* Sheldon | 22—161 | Hungary | onion bulb | L. Hornok | 1972 |
| *moniliforme* var. *subglutinans* Wr. and Reink | 22—82 | Hungary | wheat seed | L. Hornok | 1970 |
| *nivale* (Fr.) Ces. | 74—131 | Finland | rye | A. Ylimaki | 1972 |
| *oxysporum* Schlecht | 22—181 | Hungary | carnation root | L. Hornok | 1973 |
| *oxysporum* var. *redolens* (Wollenw.) Gordon | IMI 181 129 | India | potato tuber | S. K. Bhattacharyya | — |
| *poae* (Peck.) Wollenw. | 22—189 | Hungary | wheat ear | L. Hornok | 1970 |
| *sambucinum* Fuckel | 22—125 | Hungary | wheat seedling | L. Hornok | 1972 |
| *sambucinum* var. *coeruleum* Wollenw. | IMI 162 621 | Australia | wheat root | S. Chambers | 1970 |
| *semitectum* Berk abd Rav.. | 22—22 | Hungary | alfalfa root | L. Hornok | 1972 |
| *semitectum* var. *majus* Wollenweber | 22—209 | Hungary | Cucurbita sp. | L. Hornok | 1975 |
| *solani* (Mart.) Sacc. | 22—50 | Hungary | wheat stalk | L. Hornok | 1970 |
| *solani* var. *coerileum* (Sacc.) Booth | W 48/1 | GDR | potato | B. Pett | — |
| *stilboides* Wollenweber | FS Tanz. | Tanzania | coffe berry | C. J. Mappes | 1975 |
| *sporotrichioides* Sherb. | 22—26 | Hungary | alfalfa root | L. Hornok | 1972 |
| *sulphureum* Schlechtendall | — | Scotland | potato tuber | W. Boyd | — |
| *tabacinum* (Beyma) W. Gams | IPO 1114 | South Flevoland | soil | J. van der Spek | 1971 |
| *trichothecioides* Wollenweber | IMI 150 555 | Australia | wheat seed | S. Chambers | 1970 |
| *tricinctum* (Corda) Sacc. | 22—27 | Hungary | alfalfa root | L. Hornok | 1972 |
| *tumidum* Sherb. | IMI 102 084 | No record | — | C. Booth | — |
| *udum* Butler | IMI 205 514 | No record | — | — | — |
| *xylarioides* Steyaert | 11 781 | Ethiopia | Coffea excelsa | M. Mogk | — |

Isoelectric focusing (IEF) in polyacrylamide gels provides a method of high resolving power, isoenzymes occurring in very low amounts concentrate at a specific point in the gel, their pH values corresponding to the isoelectric point (pI) of the respective isoenzymes. In this way the isoenzymes are better isolated in comparison with electrophoresis in polyacrylamide gel and their diffusion is lower. Besides the characterization of the individual taxon by its isoenzyme patterns, we also get an important physico-chemical constant of each isoenzyme: its pI value. This technique has been little used in fungal taxonomy (Erselius and DeVallavieille, 1984; Jeffries et al., 1984). Esterases have been among the enzymes systems most frequently studied. These enzymes provide discrimination and give reliable information regarding microbial classification and identification (Goodfellow and Board, 1980).

The aim of the present study was to examine relationships among 39 *Fusarium* species and varieties on the basis of their esterase isoenzyme patterns resolved by IEF. The data obtained with this technique were subjected to computerized classificatory techniques and affinities established between species were expressed as genetic distance trees.

## Materials and Methods

*Organisms and growth conditions.* The origins of the 39 *Fusarium* species and varieties examined are given in Table 1. The fungi were maintained on potato-sucrose agar at 15 °C in the dark. For electrophoresis fungi were cultured on a liquid medium containing salts of the standard Czapek-Dox solution plus glucose (20.0 g), yeast extract (Difco, 1.0 g), peptone (Difco, 5.0 g) per litre of distilled water. One plung (10 mm diameter) each from the peripheral region of a seven days old colony was transferred to 50 ml of the above mentioned liquid medium in 300 ml Erlenmayer flasks and incubated for 7 days in the dark at 25 °C. The fungal material was harvested by filtration, washed three times with distilled water, blotted dry between paper towels. The fungal material was lyophilized and powdered. The fungal powder was stored at −15 °C until used.

*Preparation of protein extracts and samples for IEF.* Aqueous enzyme extract was made from the powder of lyophylized fungal material. 100 mg of fungal powder was ground in a chilled mortar containing 1.0 ml of bidistilled water. The homogenate is centrifuged in a Microcentrifuge (Type 320a, Poland) 15 min at 4 °C and 12 000 g. The protein concentrate was estimated spectrophotometrically on the basis of the 260 nm/280 nm ratio (Layne, 1957) and adjusted to 50 mg protein $ml^{-1}$ for IEF. The supernatant was mixed with an equal volume of a Sephadex solution composed as follows: Sephadex G-200 superfine (Pharmacia) swollen in an aqueous sucrose solution (20%) and 1% Pharmalyte (pH range 5 to 8, Pharmacia). Sephadex prevents proteins, which may precipitate or aggregate at the beginning of IEF, from plugging up the gel surface (Klose, 1983).

*IEF in polyacrylamide gel rods and esterase staining.* IEF in polyacrylamide gel rods was performed according to Laas and Fast–Johansson (1979), where T 5%, C 3% is used with Pharmalyte diluted 1 to 15. (DL) glutamic acid (0.01 M) was used as the anode electrode solution, ethanolamine (0.01 M) as the cathode electrode solution. The sample was focused at 1 mA gel rod $^{-1}$ (10 cm long) for 1 hour and 2 mA gel rod$^{-1}$ for 3 hours setting a maximum voltage of 500 volts (Pharmacia Electrophoresis Constant Power Supply ECPS 2000/300) at 4 °C. After IEF is completed the gels were removed from the glass tubes by rimming with a water-filled syringe with a narrow gauge, long needle. Esterase bands were stained as follows: the gel columns were placed in 0.1 M phosphate buffer (pH 6.5) for 15 min at 4 °C and then transferred to a solution containing 30 mg alpha naphtyl acetate (Sigma) dissolved in 2 ml 50% acetone, 100 mg Fast Blue RR 12630 (Gurr Ltd.) and 90 ml of 0.1 M phosphate buffer (pH 6.5). The gel rods were incubated in the staining solution for 1 hour at room temperature (Reddy and Stahmann, 1972)

*Determination of pI values of esterase bands and construction of genetic distance tree by computerized classificatory technique.* The pI value of each esterase band was estimated according to a calibration curve obtained by a "Pharmacia pI Calibration Kit" (High pI Calibration Kit, pH 5 to 10.5). The resulting patterns (the gel rods were stained for esterase) were automatically analyzed with a Simadzu Scanner CS-930 (Japan). The pI values were determined from the densitometric graph paper profile by use of a pI calibration curve. A clustering program that produced a genetic distance tree was obtained by a modified UPG methods (Li, 1981). Programs based on pI values – averages of three individual determination – of *Fusarium* esterase bands were run on HP 9845B desktop computer (Hewlet–Packard).

## Results and Discussion

The densitometric esterase profile of *F. culmorum* is demonstrated in Figure 1.

Esterase isoenzymes of the investigated *Fusarium* species and varieties produce 18 bands between pI 4.9 and 9.0 (Table 2). Bands at the pI value of pH 8.6 and 8.8 are present only in *F. concolor*, *F. equiseti* and *F. moniliforme* esterase profile. *F. nivale* has only two esterase bands at the pI value of pH 4.9 and 5.1, while *F. concolor* has 17 esterase isoenzymes (Table 2).

The classification based on IEF esterase patterns is shown as genetic distance trees (Figs 2–10) constructed according to the modified Li method (Li, 1981). Genetic distances between *Fusarium* species and varieties within sections and between sections should be expressed numerically on the basis of branch lengths in the tree (Figs 2–10).

The potential value of IEF in pilyacrylamide gel rods as an adjacent to the taxonomic methods for differentiating and identifying Fusaria has been demonstrated by this study. According to our preliminary studies (Szécsi et al., 1976) only

Table 2

pI values of *Fusarium*

| Species | pI values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4.9 | 5.1 | 5.4 | 5.6 | 5.8 | 6.0 | 6.2 | 6.4 |
| *acuminatum* | + | + | + | + | + | + | 0 | + |
| *aqueductum* | + | + | + | 0 | + | 0 | 0 | 0 |
| *arthrosporioides* | + | + | + | 0 | 0 | 0 | 0 | + |
| *avenaceum* | + | + | + | + | + | + | 0 | + |
| *buharicum* | + | + | 0 | + | 0 | + | 0 | 0 |
| *camptoceras* | + | + | + | + | + | + | 0 | + |
| *coccophilum* | + | + | + | 0 | + | + | 0 | 0 |
| *concolor* | + | + | + | + | + | + | 0 | + |
| *culmorum* | + | + | 0 | + | + | + | 0 | + |
| *decemcellulare* | 0 | + | + | 0 | + | 0 | 0 | 0 |
| *dimerum* | + | + | + | + | + | 0 | 0 | 0 |
| *equiseti* | + | + | + | + | + | + | 0 | + |
| *flocciferum* | + | + | + | + | + | 0 | + | + |
| *fusarioides* | + | + | + | 0 | + | + | + | + |
| *graminearum* | + | + | 0 | + | + | + | 0 | 0 |
| *heterosporum* | + | + | 0 | + | + | + | 0 | + |
| *lateritium* | 0 | 0 | 0 | 0 | + | + | 0 | + |
| *merismoides* | + | + | + | + | + | 0 | 0 | 0 |
| *moniliforme* | + | + | + | + | + | 0 | + | 0 |
| *m. var. subglutinans* | + | + | + | + | + | 0 | + | 0 |
| *nivale* | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| *oxysporum* | + | + | + | + | + | 0 | + | + |
| *o. var. redolens* | + | + | + | + | + | + | + | + |
| *poae* | + | + | + | + | + | 0 | + | 0 |
| *sambucinum* | + | + | 0 | + | + | + | 0 | + |
| *s. var. coeruleum* | + | + | 0 | + | + | + | 0 | + |
| *semitectum* | + | + | + | + | + | + | + | 0 |
| *s. var. majus* | + | + | + | + | + | 0 | + | 0 |
| *solani* | 0 | 0 | + | + | + | + | 0 | + |
| *s. var. coeruleum* | 0 | 0 | + | + | + | + | 0 | + |
| *sporotrichioides* | + | + | + | 0 | + | + | + | + |
| *stilboides* | + | + | + | + | 0 | + | 0 | + |
| *sulphureum* | + | + | 0 | + | 0 | 0 | 0 | 0 |
| *tabacinum* | + | + | + | + | 0 | 0 | + | 0 |
| *trichothecioides* | + | + | 0 | + | + | + | 0 | 0 |
| *tricinctum* | + | + | + | + | + | 0 | + | 0 |
| *tumidum* | 0 | + | 0 | 0 | + | + | 0 | 0 |
| *udum* | + | + | + | + | + | + | + | + |
| *xylarioides* | + | + | + | + | + | + | + | + |

the presence or absence of the esterase band in the investigated profile is suitable for differentiation of fungus species. IEF analysis is a suitable method for taxonomic investigation of fungi, as substantial differences occur only between species and not within species (Szécsi and Tóth, 1987).

esterase isoenzymes

| 6.6 | 6.8 | 7.0 | 7.4 | 7.6 | 7.8 | 8.0 | 8.6 | 8.8 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|
| + | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | 0 | + | + | + | 0 | 0 | 0 | 0 |
| + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | 0 | + | 0 | 0 | 0 |
| + | + | + | + | + | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | + | + | 0 | 0 | + | + | 0 |
| + | + | + | + | 0 | 0 | + | + | + | + |
| + | + | + | + | + | 0 | 0 | 0 | 0 | 0 |
| + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | + | + | + | + | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | + | + | + | + | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | 0 | + | + | + | + | + | + | + | 0 |
| + | 0 | 0 | + | 0 | + | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | + | + | 0 | 0 | 0 | 0 | 0 |
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| + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | + | + | + | + | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Most taxonomic problems are posed by that group of Fusaria which was formerly named as "Roseum" and was divided by certain taxonomists into different sections. This was the reason why four sections (Arthrosporiella, Discolor, Gibbosum, Lateritium), which – according to Booth's system (Booth, 1971) – contain

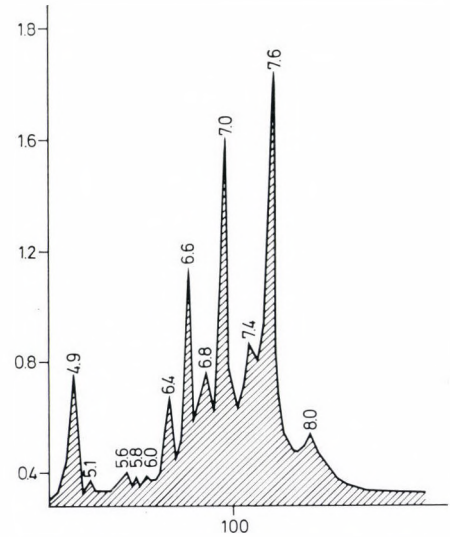



Fig. 1. Densitometric esterase profile of *Fusarium culmorum* with pI values of the isoenzymes

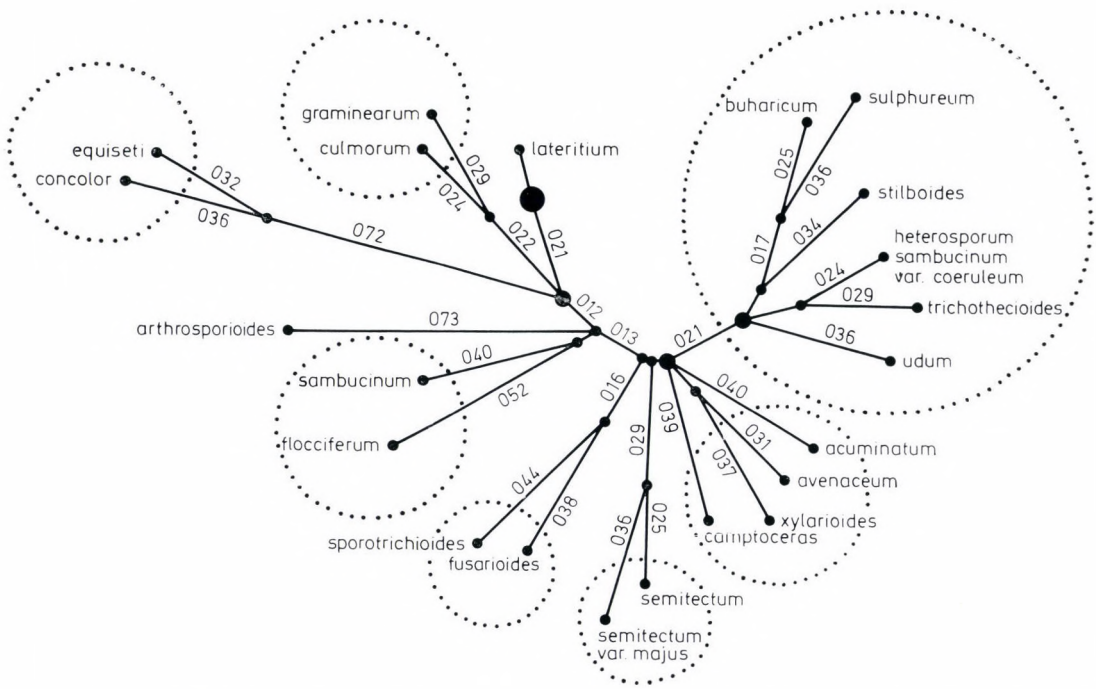



Fig. 2. Genetic distance tree for species grouped in the sections: Arthrosporiella, Discolor, Gibbosum, Lateritium

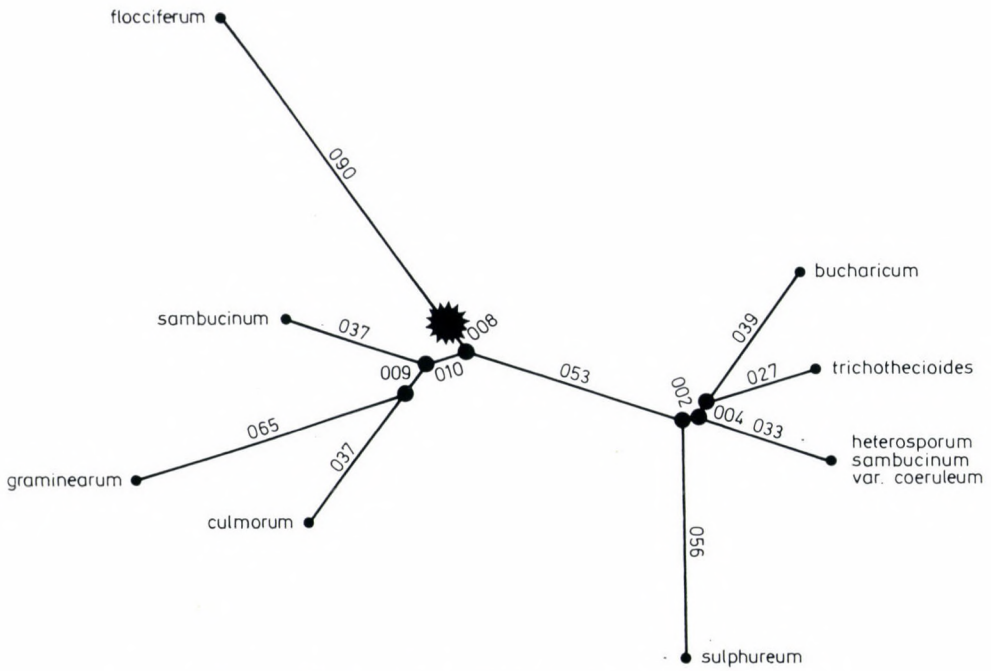



Fig. 3. Genetic distance tree for species being in the section Discolor

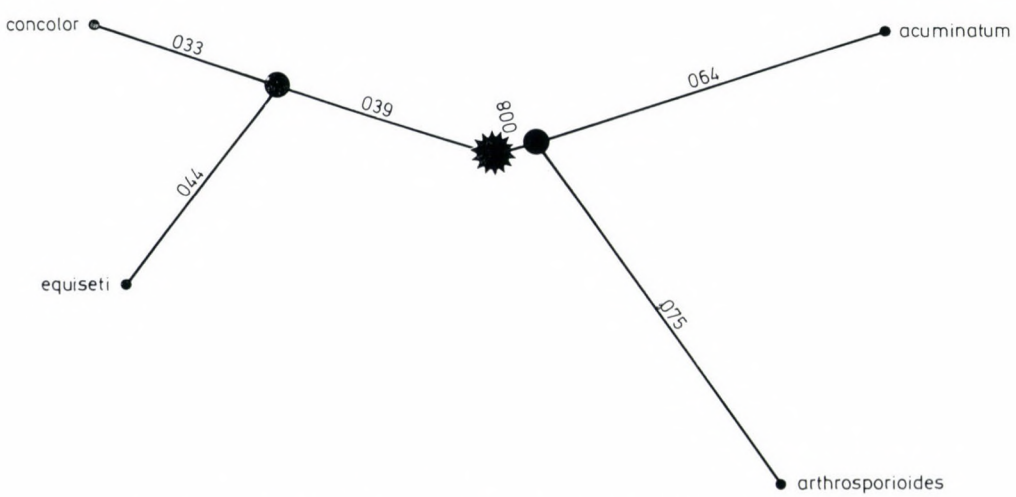



Fig. 4. Genetic distance tree for species being in the section Gibbosum

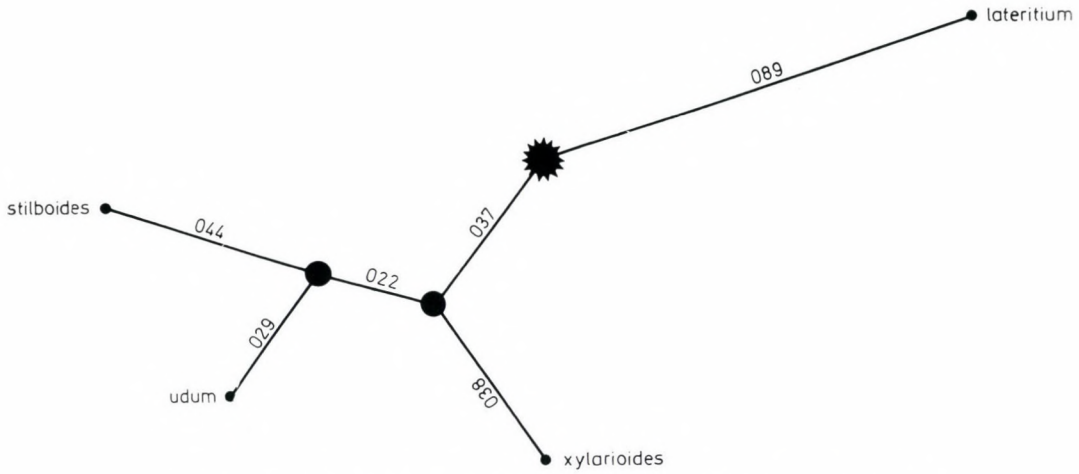



Fig. 5. Genetic distance tree for species being in the section Lateritium

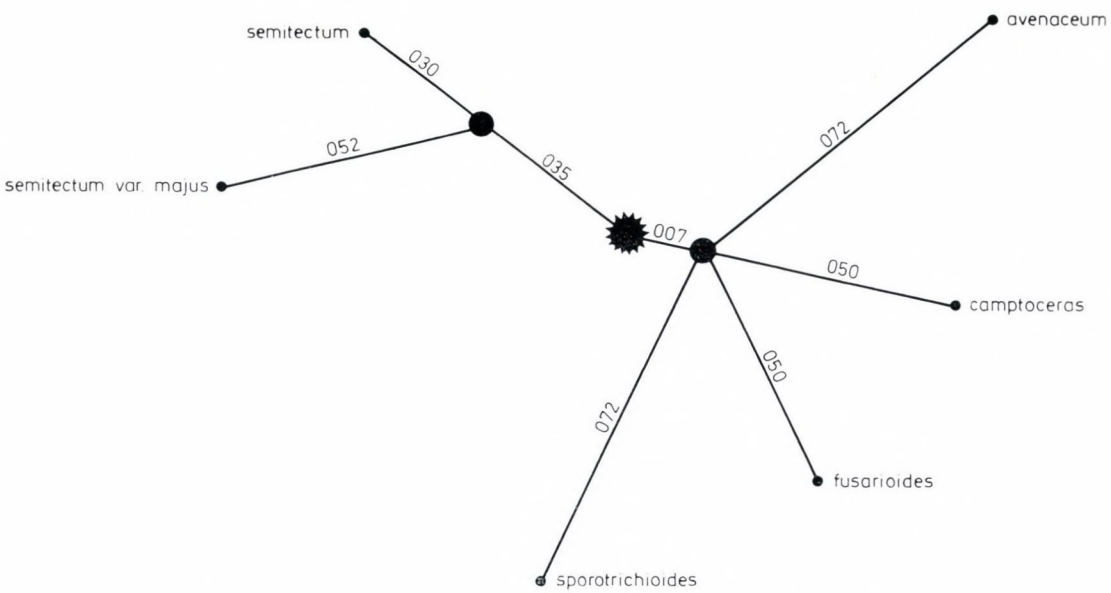



Fig. 6. Genetic distance tree for species being in the section Arthrosporiella

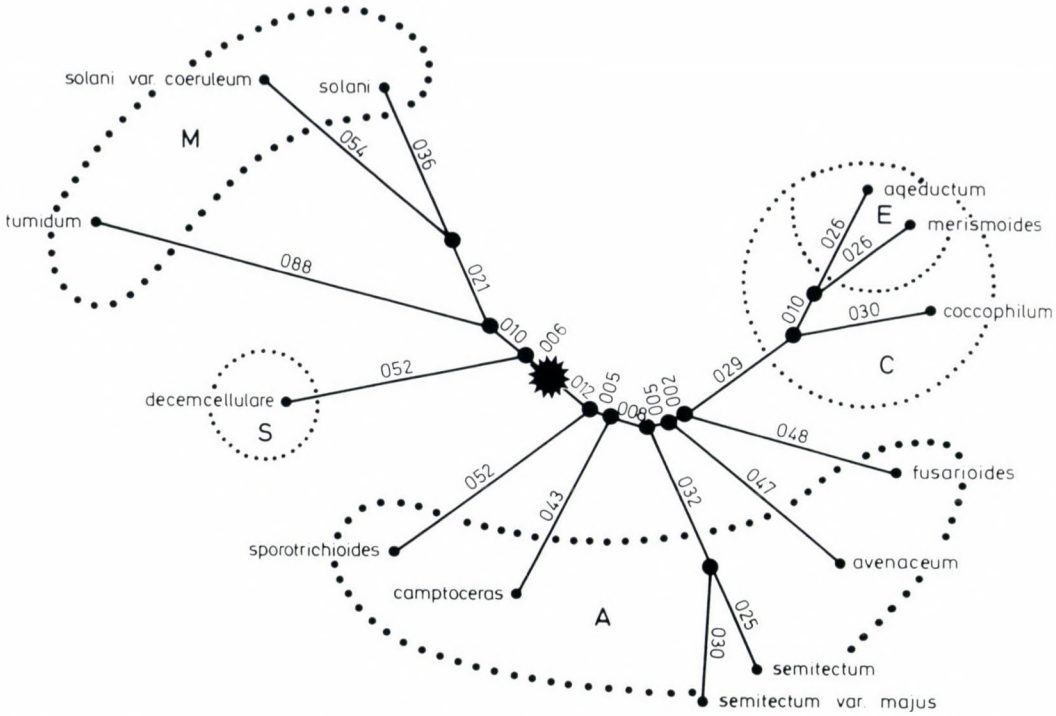



Fig. 7. Genetic distance tree for species ranged in the sections of Arthrosporiella, Coccophilum, Episphaeria, Martiella and Spicarioides

the majority of the so-called "Roseum" species, were first grouped in a genetic distance tree (Fig. 2). As can be seen the 23 species and varieties did not form distinct groups, which would coincide with the sectional arrangements of the morphological systems. In our diagram there are remarkable overlaps between the sections; the phylogenetic relationships established among these species on the basis of their IEF esterase patterns are thus different from those proposed by the morphological systems.

When species of a given section were compared, some noteworthy affinities were established. In the section Discolor (Fig. 3) *F. culmorum* and *F. graminearum* were found to be closely related; these species, together with *F. sambucinum*, formed one distinct subgroup, while the remaining five taxa *(F. buharicum, F. heterosporum, F. sambucinum* var. *coeruleum, F. sulphureum, F. trichothecioides)* formed the other. *F. flocciferum* was only remotely related to the other members of the section. Surprisingly, *F. heterosporum* and *F. sambucinum* var. *coeruleum* produced esterase patterns. In the section Gibbosum (Fig. 4), there were two subgroups: *F. concolor* and *F. equiseti* were very closely related to one another, but more distant relationship was found between *F. acuminatum* and *F. arthro-*

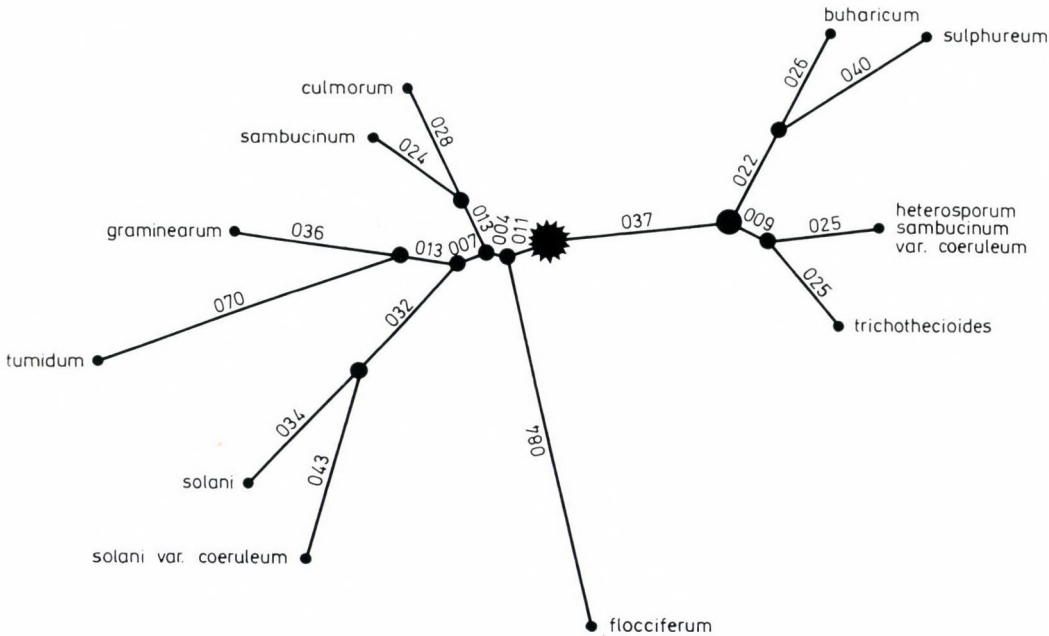



Fig. 8. Genetic distance tree for determination of sectional status of *F. tumidum* (comparison of Discolor and Martiella sections)

*sporioides*. These findings seem to confirm our former serotaxonomical results (Hornok, 1980). The section Lateritium (Fig. 5) contains three more or less related species *(F. stilboides. F. udum, F. xylarioides)* and a completely distinct one *(F. lateritium)*. In the section Arthrosporiella (Fig. 6) *F. semitectum* and its var. *majus* were – as could be expected – closely related, while the other four species formed another subgroup. *F. camptoceras* did not show particular affinity to *F. semitectum.*

In our experiments Arthrosporiella proved to be the most heterogeneous "Roseum"-type section. The only common character of the species in this section is the polyblastic conidiogeneous cell, in other morphological properties there are remarkable differences among them; microconidia, if present at all, may be oval to obovate; macroconidia vary from spindleshaped apedicellate to curved, dorsiventral, pedicellate formation; in certain species no clear distinction between micro- and macroconidia could be made. This heterogeneous nature of the section was reflected in the esterase patterns, too. Because of its seemingly transitional position Arthrosporiella was compared with other sections (Coccophilum, Episphaeria, Martiella, Spicarioides) of the genus (Fig. 7). In this comparison a very clear sectional arrangement, that coincided with the sectional classification proposed by morphological systems, was established. All these sections formed distinct groups on the basis of their IEF esterase patterns. This finding suggests, that

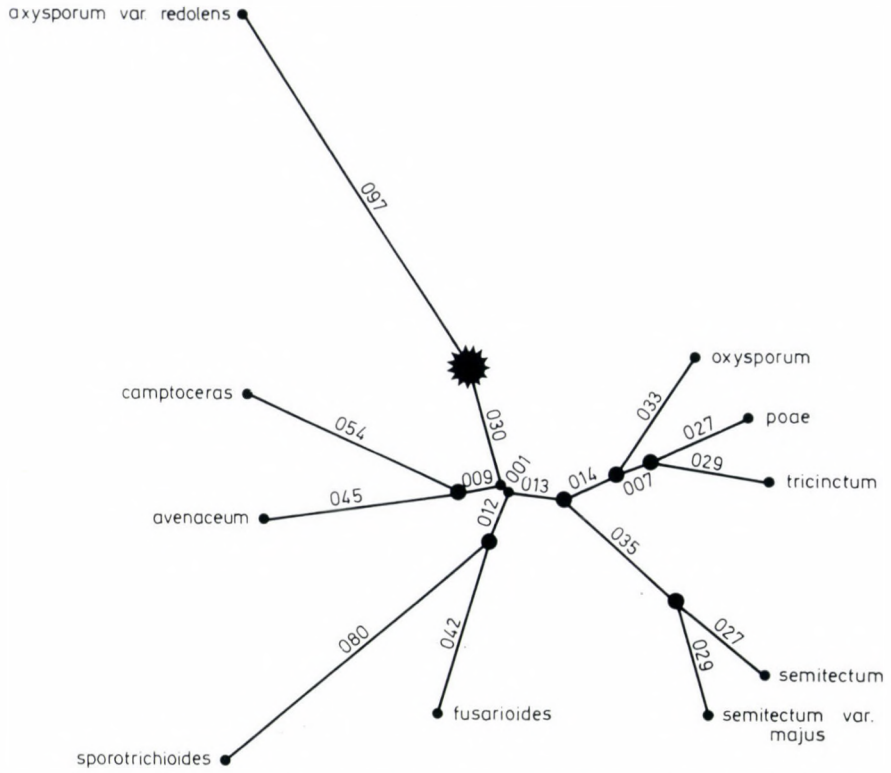



Fig. 9. Genetic distance tree for species grouped in the sections Arthrosporiella, Elegans and Sporotrichiella

chemotaxonomic studies based on IEF esterase isoenzyme comparison could be valuable additional tool in the classification of fungi.

There are controversies over the sectional status of several species. *F. tumidum*, for example, is regarded to be relative to Martiella species in one proposal (Booth, 1971), but considered as a member of the Discolor section in other conceptions (Joffe, 1974; Gerlach and Nirenberg, 1982; Nelson et al., 1984). When IEF esterase patterns of the Discolor- and Martiella-species were compared (Fig. 8), *F. tumidum* was found to be most closely related to *F. graminearum* (a Discolor species), but the second and third close relatives of the species were two typical members of the section Martiella *(F. solani, F. solani* var. *coeruleum)*. We consider *F. tumidum* as a transitional species, and since this fungus has no teleomorph, the indefiniteness as to its sectional arrangement seems to be reasonable.

Another interseting question is the taxonomic position of the species that produce distinct microconidia and have or supposed to have teleomorphs in the genus *Gibberella*. The sections Arthrosporiella, Elegans, Liseola and Sporotrichiella, contain these species. Oval shaped microconidia are produced by *F. oxysporum*,

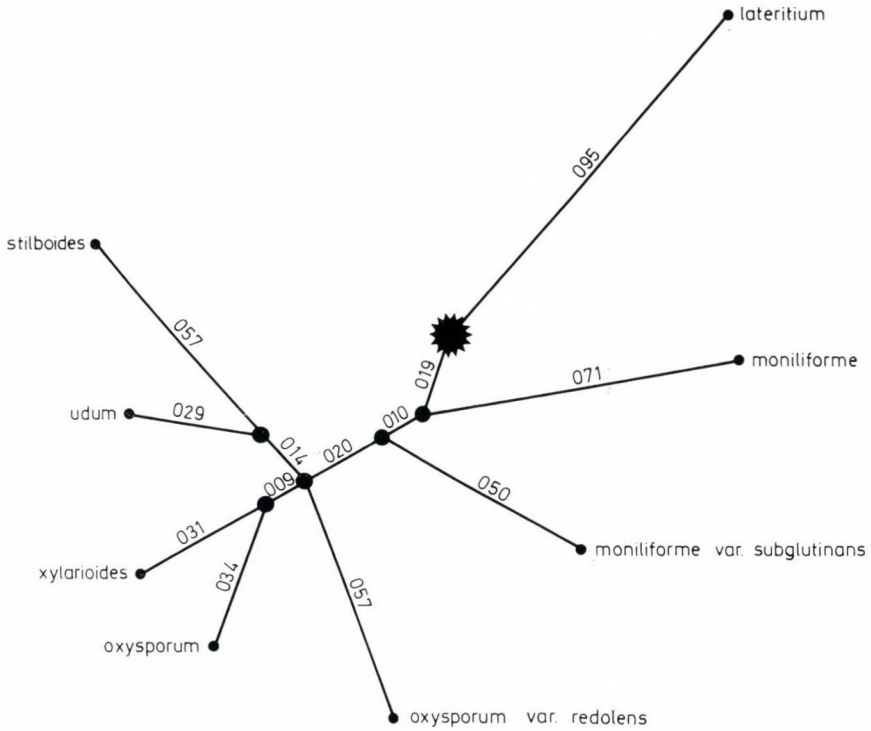



Fig. 10. Genetic distance tree for determination of sectional status of *F. udum* and *F. oxysporum* var. *redolens* (comparison of sections Elegans Lateritium, Liseola)

*F. oxysporum* var. *redolens* (Elegans), *F. monoliforme*, *F. monoliforme* var. *subglutinans* (Liseola) and *F. udum* (Lateritium). *F. poae*, *F. tricinctum* (Sporotrichiella), and *F. fusarioides*, *F. sporotrichioides* (Arthrosporiella) produces pyriform to obovate microconidia. The genetic distance tree showing the relationships of these fungi are presented in Figs 9 and 10. As can be seen *F. fusarioides* and *F. sporotrichioides* relate to other species, that have polyblastic conidiogenous cells, while *F. poae* and *F. tricinctum* show affiinity to *F. oxysporum*, a species which produces simple phialides as sporogenous cells. These relationships support Booth's conception, who transferred *F. fusarioides* and *F. sporotrichioides* to Arthrosporiella because of their polyblastic conidiogenous cells, and regarded the shape of microconidia as a character of secondary importance in this case (Booth, 1971). There is a disagreement in placing *F. udum* to the correct section. Booth (1971) proposed to arrange this species into Lateritium, while Gerlach and Nirenberg (1982) assigned it to Elegans, and Nelson et al. (1984) vacillated between Elegans and Lateritium. Other workers did not accept *F. udum* as a distinct species and reduced it to a forma specialis of *F. oxysporum*. Serotaxonomic and electrophoretic investigations seemed to support this latter opinion (Kaiser and Gupta, 1976). In our studies there was a

close interrelationship between Elegans and certain members of the section Lateritium as suspected by earlier workers *(cf.* Booth, 1971). We found *F. udum* to be more closely related to *F. stilboides* and *F. xylariodes*, than to *F. oxysporum*. Further interesting results were obtained in these comparisons: 1) *F. lateritium* was not closely related to other members of the section Lateritium; 2) *F. oxysporum* and its var. *redolens* showed greater affinity to Lateritium, than Liseola *(F. moniliforme* and its var. *subglutinans)*; 3) *F. oxysporum* var. *redolens* was quite different from its basic species in one comparison (Fig. 9), but the two taxa showed closer relationship in the other (Fig. 10); from this we conclude, that *F. oxysporum* var. *redolens* is worthy to maintain at least at a variety level, but even its restoration as a distinct species as proposed by Gerlach and Nirenberg (1982) seems to be justifiable. Cytological sudies made by Punithalingam (1975) may be recalled here; he proposed the distinction between *F. oxysporum* and *F. oxysporum* var. *redolens* at a species level on the basis of difierent chromosome numbers of the two fungi.

One more remark should be made on the section Arachnites. Three species *(F. dimerum, F. nivale, F. tabacinum)* were investigated from this group. Their IEF esterase patterns were found to be completely different from the other Fusaria, i.e. these fungi gave esterase bands at the start of the pH gradient. The unique esterase pattern of *F. nivale* is not surprising, since this fungus – because of its annelate condiogenous cells – was recently transferred to a new genus, *Gerlachia*, under the name *Gerlachia nivalis* (Ces. ex Sacc.) W. Gams et E. Müller (Gams and Müller, 1980).

The results reported in this paper indicated that the comparative analysis of IEF esterase patterns in polyacrylamide gels was a suitable method for the determination at the species level of genetic distance between fungi.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 231—249 (1986)*

# Breeding Corn Against Fusarial Stalk Rot, Ear Rot and Seedling Blight

Á. MESTERHÁZY[1] and KATALIN KOVÁCS[2]

[1]Cereal Research Institute, Szeged, Hungary, [2]Research Station of Seed Trade Company, Dept. Field Crops, Szentes, Hungary

After several forgotten reports the importance of working with more isolates in inoculation tests came into prominence again.

As stalk rot heavily influences ear rot response, genetically valid ear rot data can be obtained only on healthy stalks.

In corn there exist several mechanims for resistance to *Fusarium* spp., therefore no general agreement was found between seedling, stalk and ear rot responses. There is, however, a type of resistance which gives protection to the seedlings, stalk and ear rot, respectively. Inbreds and hybrids with these selected resistances were created in a breeding program. In this case the seedling resistance can be used as a preselection method making possible a more effective screening for stalk and ear rot. We also found a correlation between the reaction of corn ears and seedlings to *F. graminearum* and *F. culmorum*. We consider therefore as corner stone the selection for general *Fusarium* resistance giving protection against the major species.

The diseases caused by *Fusarium spp.* are among the most destructive to corn in Hungary and elsewhere. Important species are *F. graminearum*, *F. culmorum*, and *F. moniliforme* var. *subglutinans* (Bíróné 1975, Békésy and Hinfner 1970, Mesterházy and Vojtovics 1977).

*Fusarium* species in Hungary cause root rot, stalk rot, seedling blight, and ear rot (Manninger 1967, 1977, 1978, Petróczi 1956). The susceptibility of seedlings often results in severe losses, but seed dressing gives a reasonably good protection. Solid correlations of the seedling tests with stalk and ear rot responses have not been produced until now (Christensen and Wilcoxson 1966, Hooker 1956, Marič 1981, Smiljakovič 1972, Thompson et al. 1971). Therefore breeding programs everywhere are separated to stalk and ear rot resistance. The efficacy of screening is low, because the symptoms develop after pollination and no indirect way is given to predict resistance (Christensen and Wicoxson 1966). The problem of ear infection is aggravated also by the toxin infection of feedstuffs which occurs nearly every year on a smaller or larger acreage of corn (Barócsai, 1983).

However, Barnes (1957) and Bausch et al. (1982) found a relationship between seedling and stalk rot response, Young (1949) was not successful in presenting this in every case, so he concluded that the same resistance factor cannot generally control the two forms of resistance. Positive correlation between seedling and ear reaction was reported only by Mesterházy (1982). It is important that Barnes (1957), Mesterházy (1982) and Young (1949) used more parallel isolates. This need was stressed also by Christiansen and Wilcoxson (1966). As stalk rot acts a pseudo-resistance factor for ear rot (Mesterházy 1983*a*), this inhibits finding a positive

relationship between ear rot and stalk rot response if such exists genetically at all; only Holbert et al. (1923) reported on a complex reaction in this respect. Recently only Mesterházy (1983*a*) could demonstrate such a relationship based on field data.

These considerations suggest the reinvestigation of the possible role of the seedling reaction in a selection program and clarify relationships between different forms of resistance to *Fusarium* spp.

## Materials and Methods

The experiments were concluded (1980–1982) with commercial hybrids and our experimental ones, as well as inbred lines from our breeding supplemented by those used internationally (153R, Ma4, W64A/Z, WF9, C22, WI53R, 337, GK-3, GK-15 and A632). The selection of new inbreds begun in 1973–1974 within lines Ia153, A90 and continued 1979 from the local variety Kz lila, and the hybrid A632 × WF9.

### *Creating inbreds*

The mass selection by seed inoculation was first reported for wheat by Mesterházy (1977, 1983*b*). After careful washing seeds were submerged at room temperature for 24h in a suspension of *F. graminearum* previously tested for pathogenicity to wheat germs by the Petri dish method (Mesterházy 1978). The inoculated seeds (15–18 000 for each genotype in 1973–1974 and 2000 in 1979 were then sown in sterile perlite in a glasshouse in plastic pots (30–50 cm in diameter) at a depth of 30 mm. After 14–18 days the healthy plants were selected, where none were found, the most healthy were taken, and in early May these were planted in the field for each genotype 20–50. For about two weeks they were irrigated every second day. About 90% of these could be selfed. About 1 month after 50% silking for individuals plant height, rind resistance was measured, and in autumn (end of September – beginning of October), after splitting stalks, root rot, basal stalk rot, and stalk rot (each on a 0–5 scale) were recorded. All ears were collected and treated individually.

Seedling tests were made with seeds of each plant after harvest until December. Where all characteristics were positive and the weight of seed/plant was normal, the progenies were sown next spring. In later years, the ear-to-row method was used and in every row at least 3–5 pollinations were made. Up to 1979 we selected only for stalk rot. After this time the material was tested also for ear rot. At there was a continuous selection, the material changed from year to year.

### *Field tests in 1980–82*

The number of tested inbred lines in the three year of testing (1980–1982) was 35, 26 and 27, that of the hybrids 31, 35 and 28. The plant density for 1980–1981 was 40 000/ha and 60 000/ha in 1982. Three locations were used, Szeged-Alsótelep, Szeged-Öthalom and Szarvas, the last only in 1982.

*Ear rot tests*

In wheat there was a significant, but not stable isolate-genotype interaction (Mesterházy 1981*a*) as found in corn ear rot tests (Mesterházy 1982). Earlier Barnes (1957) and Young (1949) had come to a similar conclusion in corn seedling and stalk rot tests, and recently Atlin et al. (1983) published data supporting the presence of this interaction. Therefore, as Christensen and Wilcoxson (1966) suggested, more isolates were used for inoculation to neutralize these interaction effects. 15–20 plants in a row were inoculated with one isolate, altogether 4–6 isolates were used (tooth pick method, Young 1943). The control rows were treated with sterile toothpicks and two rows per genotype received no ear treatment. The isolates, isolated and identified by the author, with the help of the Booth (1971) manual, used for ear tests were as follows: 1980: *F. graminearum* Nos 12270, 12282, 12377, and *F. culmorum* No. 12375: 1981: *F. graminearum* Nos 12208, 12270, 12295, 12377 used for inbreds, but for hybrids these were supplemented by Nos 12216 and 12465: 1982: *F. graminearum* Nos 12216, 12377 and 12454, *F. culmorum* Nos 12375, and 12465.

The toothpicks were inserted through the husk into the middle of the ear (DeVay et al. 1957, Sutton and Baliko 1981) 18 days after silking in 1980, 1981, but 1982 this was reduced to 10 days because the poor water economy of the soil in Öthalom hastened stalk rot development and shortened the vegetation period. Ear rot was evaluated on a scale 0–10, their mean multiplied by ten gave the *Fusarium* covered surface of the ear. From the kernel moisture data we claculated ear rot spreading rate, where this value was divided by the number of days from inoculation to reaching 28% kernel moisture, given as % of spreading/day.

*Evaluation of stalk rot*

For this the stalk splitting method was used (Mesterházy 1979). Only natural infection was assessed, artificial inoculation was not applied. In 1980 we evaluated root, stalk and lower stalk rot, each on a scale 0–5. As relationships between the three values were very close (Mesterházy 1979), in 1981 and 1982 only basal stalk rot was assessed from the health condition of pith tissue in the stalk cross section 5 cm above ground (0–5). Evaluation was made at the end of the vegetation period, usually 2nd week of October.

According to Koehler (1959) the best indirect way to estimate stalk rot is the amount of *premature death*. He insisted, however, that data must be comparable. It was recorded from the death of the first plant until all plants had died or no more changes occurred. A plant was considered dead when it had dried green from the top and had no more living green leaves left. In the most intensive drying period 4 days intervals between assessments were used, otherwise these were one week.

To make data comparable we used the *kernel moisture* data. These were evaluated in 1981 once (7th October), in 1981 twice (9th and 27th September), in 1982 twice (31st August and 13th September), 2–2 ears per genotype. With

these data we calculated the date of reaching 28% kernel moisture. From the premature death data we could identify the amount of PD at 28% kernel moisture (KM). This was supplemented by the average PD up to 28% and 22% KM. Using the date of reaching 28% KM, we could count the number of days between inoculation and physiological ripening (reaching 28% KM) which correlates closely with earlyness or lateness.

Beside the pathological factor another component of the stalk strength is the rind thickness (RT) its improvement played a major role over the past two decades (Jugenheimer 1976). There is no general agreement between this and stalk rot resistance (Mesterházy 1981*b*, Christensen and Wilcoxson 1966), but many genotypes have both. The RT was determined by the method of the rind puncture (Colbert and Zuber 1978) using a spring balance (Mesterházy 1981*b*). The determinations were made on green plants at the end of August in the middle of the third internode, on the opposite side to the pistillate branch.

*Glasshouse experiments*

As a result of the selfings and crosses we had from each genotype 3–5 or more ears. The seed samples of every ear were tested separately and their mean reaction was considered to be characteristic for the genotype. To raise inoculum, the bubble breeding method was used (Mesterházy 1977, 1981*a*) and resulted in a suspension of conidia and mycelium. On the basis of pathogenicity tests, the grade of dilution was determined (Mesterházy 1982). In 1980 one *F. graminearum* isolate No. 12377, $0.55 \times 10^6$ conidia/ml after dilution 1 : 1 with distilled water. In 1981 *F. graminearum* isolates were used (Nos 12270, 12295 and 12377) 1–1, with three replicates and two uninoculated controls (conidium concentrations 0.25, 0.65, and $0.93 \times 10^6$). In 1982 *F. graminearum* No. 12216 undiluted and 1 : 2 diluted (1.05 and $0.35 \times 10^6$ conidia/ml), No. 12377 1 : 0.5 dilution, $0.39 \times 10^6$/ml, and *F. culmorum* No. 12375, 1 : 2, $1.60 \times 10^6$/ml; with four replicates for each genotype. The seed inoculation was made according to the method of Mesterházy (1974, 1982). In the first two years plastic pots (15 cm in diameter) were used, they were filled 2/3rd full with perlite. Its surface was made smooth, carefully irrigated with distilled water and after that uniformly impregnated with 50 ml inoculum. Ten seeds were sown per pot at a depth of 25–30 mm. In 1982 the same method was applied, but pots of 7 cm diameter were used with five seeds and 20 ml inoculum per pot. As cootrols two replicates were sown. The experiment was evaluated three weeks after sowing. Up to this date the number of seeds germinated and later the number of dead seedlings were recorded every second day. The plants were washed out of the pots and root infection (0–10) was evaluated. Also the height of each plant was measured before washing out. From the data rate of germination, the death rate of the last reading, the average death rate of the last four readings were counted. Here also the non-germinated seeds were taken into account and the performance of the control gave the limit values.

As biostatistical methods correlation and variance analyses were used.

## Results

*Interaction between genotype and isolate*

As the literature quoted earlier demonstrated very well the interaction between genotype and *Fusarium* isolate, we present (Table 1) to demonstrate its effect and point out its significance in testing resistance. As shown, the highly resistant and highly susceptible genotypes react similarly to any of the isolates used, but the genotypes with intermediate forms of resistance gave very different disease severities with various isolates.

Table 1

Fusarium ear rot reaction of corn hybrids to different strains and species of the genus

| Genotype | 12377[1] | 12375[2] | 12465[2] | 12216[1] | 12452[1] | $\bar{x}$ |
|---|---|---|---|---|---|---|
| | isolates | | | | | |
| 39/1a/3–Pi 3965 | 46.36 | 82.00 | 19.20 | 54.29 | 2.86 | 40.94 |
| 39/1a/3/3–GK15 | 14.09 | 36.67 | 14.55 | 30.77 | 0.50 | 19.30 |
| 39/1a/3/3–GK3 | 20.00 | 47.27 | 10.83 | 40.00 | 0.00 | 23.60 |
| A632–W64A/Z | 17.50 | 36.50 | 11.87 | 60.00 | 3.24 | 25.80 |
| Ma4–C22 | 100.00 | 100.00 | 97.85 | 100.00 | 46.88 | 88.90 |
| Ia153× C22 | 70.00 | 81.33 | 32.72 | 82.50 | 8.13 | 54.90 |
| Ia153× 51/4a/1 | 20.83 | 98.66 | 30.00 | 76.86 | 5.26 | 46.30 |
| Ia153 4/1–C22 | 43.57 | 79.28 | 38.50 | 79.37 | 4.00 | 48.90 |
| Ia153 4/51/4a/1 | 25.00 | 80.52 | 38.50 | 50.76 | 2.06 | 39.40 |
| Ia153 4–39/3/3 | 27.50 | 95.71 | 52.27 | 55.38 | 3.50 | 46.90 |
| Ia153–39/3/3 | 20.00 | 68.66 | 22.75 | 44.54 | 4.06 | 32.20 |
| Ia153 4/1–A632 | 26.36 | 11.76 | 7.89 | 33.52 | 1.67 | 18.20 |
| 39/1a/3/3–A632 | 6.76 | 19.41 | 15.00 | 29.41 | 1.05 | 14.30 |
| 39/1c/1b–A632 | 23.07 | 88.66 | 18.75 | 71.33 | 0.28 | 40.40 |
| 39/1a/3/2–Ma4 | 34.16 | 52.00 | 36.36 | 73.63 | 2.14 | 39.70 |
| 39/1a/3/1× A632 | 20.00 | 32.63 | 24.00 | 60.00 | 0.50 | 27.40 |
| 39/1a/3/2–GK15 | 17.33 | 48.23 | 23.12 | 69.37 | 1.67 | 31.90 |
| 39/1a/3/2–GK3 | 23.84 | 45.38 | 19.16 | 42.50 | 2.08 | 26.60 |
| A90 1979–A632 | 3.84 | 77.05 | 12.30 | 54.66 | 0.29 | 29.60 |
| A90 1979–153R | 13.12 | 61.66 | 20.66 | 52.66 | 10.83 | 31.80 |
| 51/4a/1–GK15 | 7.35 | 38.57 | 25.45 | 63.33 | 2.50 | 27.40 |
| 51/4a/1× A632 | 15.00 | 28.42 | 11.25 | 65.71 | 0.67 | 24.20 |
| 51/4a/1–39/1a/3 | 54.54 | 95.00 | 42.50 | 56.25 | 2.14 | 50.10 |
| A90 1979–GK15 | 15.50 | 51.66 | 15.83 | 78.33 | 2.69 | 32.80 |
| A90–153R | 12.69 | 60.71 | 26.42 | 16.66 | 4.58 | 24.20 |
| 51/4a/1–153R | 13.75 | 55.00 | 20.90 | 54.44 | 4.67 | 29.00 |
| SzeSC 444 | 8.75 | 18.67 | 100.00 | 22.85 | 0.63 | 12.20 |
| PiSC 3732 | 11.00 | 40.67 | 15.00 | 31.00 | 0.67 | 19.70 |
| 51/4a/1–Ia153 4/ | 2.83 | 67.85 | 25.00 | 27.27 | 2.08 | 25.00 |
| Ia153 4/1–Pi3950 | 37.27 | 85.00 | 25.45 | 77.69 | 7.27 | 46.50 |
| Mean | 25.40 | 59.50 | 25.50 | 55.17 | 4.29 | 33.20 |
| LSD 5% | | | | | | 16.87 |

Table 1. continued

| Isolates | Correlations | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 1 | — | 0.51*** | 0.75**** | 0.60**** | 0.72**** |
| 2 | — | — | 0.63**** | 0.52*** | 0.40* |
| 3 | — | — | — | 0.53*** | 0.81**** |
| 4 | — | — | — | — | 0.48*** |

**** P = 0,1 % *** P = 1 % * P = 5 %
[1] *F. graminearum* [2] *F. culmorum*

*Relationship between stalk, ear and seedling rot reaction*

Comparing the performance of the same hybrids in 1980 and 1981, as well as the data of the two locations in 1982 (Table 2), we see that stalk rot has an enormous effect on ear rot. Similar ear rot values were presented with a similar amount of stalk rot. Much higher ear rot values were recorded for stalk rot susceptible hybrids on heatlhy stalks than on severely stalk rot affected plants. A high infection on stalks greatly reduced ear rot even by 1/3rd or more compared to the

Table 2

Stalk rot and ear rot reactions of several hybrids in 1980 and 1981, as well as in Szeged and Szarvas in 1982

| Hybrid A | Stalk rot scale 0—5 | Ear rot % | Stalk rot scale 0—5 | Ear rot % |
|---|---|---|---|---|
| | 1980 | | 1981 | |
| *Hybrids with similar stalk rot response* | | | | |
| PiMSC 3709 | 0.07 | 33.60 | 0.90 | 33.70 |
| SzeSc 444 | 0.19 | 18.60 | 0.70 | 15.90 |
| PiMSC 3709 | 0.00 | 31.37 | 0.90 | 33.70 |
| PiSC 3732 | 0.05 | 46.17 | 1.00 | 52.70 |
| $\bar{X}$ | 0.08 | 32.44 | 0.88 | 34.01 |
| *Hybrids with different stalk rot response* | | | | |
| 39/1c× C22 | 0.24 | 79.47 | 2.50 | 29.24 |
| 39/1a× C22 | 0.04 | 59.15 | 2.20 | 34.50 |
| Ia153× A90 51/3a | 1.63 | 31.90 | 3.10 | 21.40 |
| PiMSC 3901 | 0.05 | 46.44 | 1.30 | 24.30 |
| PiTC 3950 | 0.22 | 36.60 | 2.20 | 17.40 |
| $\bar{X}$ | 0.43 | 50.71 | 2.26 | 25.37 |

Table 2. continued

| Hybrid B | | Szarvas 1982 | | Szeged 1982 | |
|---|---|---|---|---|---|
| *Hybrids with similar stalk rot response* | | | | | |
| Ma4× C22 | | 2.40 | 76.70 | 1.60 | 88.90 |
| 39/1a/3× A632 | | 1.40 | 14.90 | 0.70 | 14.30 |
| 39/1a/3/1× A632 | | 0.30 | 36.80 | 0.10 | 27.40 |
| 39/1a/3/2× GK 15 | | 0.80 | 25.60 | 0.56 | 31.90 |
| 39/1a/3/2× GK 3 | | 0.90 | 21.30 | 1.20 | 26.60 |
| 39/1a/3/3× GK 3 | | 0.90 | 21.30 | 1.00 | 23.60 |
| | $\bar{X}$ | 1.12 | 32.77 | 0.86 | 35.45 |
| *Hybrids with different stalk rot response* | | | | | |
| A632× W64A/Z | | 0.89 | 15.70 | 0.05 | 25.80 |
| Ia153× C22 | | 2.40 | 20.50 | 1.00 | 54.90 |
| Ia153× A90 51/4a/1 | | 4.20 | 22.00 | 2.00 | 46.30 |
| A90178/7× A632 | | 1.50 | 14.80 | 0.60 | 29.60 |
| A90 51/4a× A632 | | 2.20 | 9.70 | 0.60 | 24.20 |
| | $\bar{X}$ | 2.24 | 16.54 | 0.85 | 36.16 |

performance on healthy stalks. As shown, this effect is important first of all in the stalk rot susceptible genotypes, where environment has a dominant effect. This effect is much less expressed in stalk rot resistant genotypes.

The correlations sepecially refer to this. In the case of the inbreds (Tables 3, 4 and 5) the correlations between stalk rot and ear rot are negative in 1980 and 1981, showing the negative effect of stalk rot on ear rot, but in 1982 the tendency is turned; due to selection most of the stalk rot susceptible materials were discarded and replaced by inbreds, where the stalk rot and ear rot resistance were closer. So the positive significant correlations show the success of the selection work for combined resistance to both parameters.

There is a similar tendency also for the hybrids (Tables 6, 7 and 8). In 1981 we do not have significant relationship between the two parameters, in 1981 we have significant negative relationships, but for 1982 with a large change of the hybrids for combined stalk and ear rot resistance the negative tendency turned out to be positive, showing that selection for combined resistance is possible.

Considering the way evaluating stalk rot we see that the premature death values are more exact than the stalk rot values. The explanation is that the PD values are calculated for 28% kernel moisture, so the genotypes with different ripening groups are comparable. As stalk rot was evaluated at the same time, the earlier genotypes were in 1–2 weeks overripening, e.g. they had more stalk rot than they would have at the optimal time of evaluation. As the exact timing of the stalk

Table 3

Reaction of inbreds to *Fusarium* in the field and the greenhouse
Correlations

| Parameters | Field parameters | | | | Greenhouse parameters | | |
|---|---|---|---|---|---|---|---|
| | Ear rot | Stalk rot | Rind puncture | Premature death | Plant height | Germination | Killed plants, mean of 4 readings |
| Ear rot | — | 0.0720 | −0.1062 | −0.0472 | −0.2730 | −0.2649 | $0.3890^{++}$ |
| Stalk rot | — | — | $-0.6002^{++++}$ | $0.7048^{++++}$ | $0.5305^{++++}$ | $0.3990^{+++}$ | $-0.4900^{++++}$ |
| Rind puncture | — | — | — | $-0.5820^{++++}$ | −0.0748 | 0.0391 | 0.0830 |
| Premature death | — | — | — | — | 0.2504 | 0 0924 | −0 2550 |
| Plant height | — | — | — | — | — | $0.7620^{++++}$ | $-0.9095^{++++}$ |
| Germination | — | — | — | — | — | — | $-0\ 7319^{++++}$ |

$^{++++}P = 0.1\%$ $^{+++}P = 1\%$ $^{++}P = 2\%$

Table 4

Reaction of inbreds to *Fusarium* in the field and the greenhouse in 1981, experiment Szeged-Öthalom
Correlations

| Parameters | Field parameters | | | | | Greenhouse parameters | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ear rot | Stalk rot | Rind puncture | Premature death 9 September | Seed humidity 9 September | Plant height | Germination | Killed plants last reading | Killed plants mean |
| Ear rot | — | −0.31 | 0.18 | −0.15 | −0.18 | −0.18 | $-0.34^{b}$ | 0.19 | 0.24 |
| Stalk rot | — | — | $-0.67^{a}$ | $0.77^{a}$ | $-0.63^{a}$ | 0.00 | −0.14 | 0.09 | 0.04 |
| Rind puncture | — | — | — | $0.70^{a}$ | $0.68^{a}$ | 0.02 | 0.16 | −0.14 | −0.10 |
| Premature death | — | — | — | — | $-0.75^{a}$ | 0.08 | 0.07 | 0.05 | 0.01 |
| Kernel moisture | — | — | — | — | — | 0.05 | 0.16 | −0.23 | −0.17 |
| Plant height | — | — | — | — | — | — | $0.76^{a}$ | $-0.90^{a}$ | $-0.93^{a}$ |
| Germination | — | — | — | — | — | — | — | $-0.77^{a}$ | $-0.85^{a}$ |
| Killed plants, last reading | — | — | — | — | — | — | — | — | $0.97^{a}$ |

$^{a}P = 0.1\%$ $^{b}P = 10\%$

Table 5

Evaluation of corn inbreds for stalk and ear rot in the field and seedling blight. 1982
Correlation

| Parameters | Field parameters | | | | | | | | Greenhouse parameters | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stalk rot 0—5 scale | Ear rot | | Rind puncture, kg | Kernel moisture 31.8, % | (KM) 13,9, % | Premature death | | Days from inoculation to 28% KM | Plant height as a % | Germination of chech | Ratio of killed | |
| | | spreading rate, %/day | % | | | | at 28% RM % | until 28% RM % | | | | plants last reading, % | mean of the last four readings, % |
| Stalk rot | — | 0.29 | 0.14 | −0.68[a] | −0.58[b] | −0.87[a] | 0.50[b] | 0.67[a] | −0.77[a] | 0.18 | −0.24 | −0.20 | −0.13 |
| Ear rot %/day | — | — | 0,97[a] | −0.54[b] | −0.37[e] | −0.49[c] | 0.47[c] | 0.42[d] | −0.16 | −0.13 | −0.22 | 0.04 | 0.12 |
| Ear rot % | — | — | — | −0.47[e] | −0.28 | −0.33[e] | 0.48[c] | 0,40[d] | 0.03 | −0.20 | −0.24 | 0.12 | 0.19 |
| Rind puncture | — | — | — | — | 0.39[d] | 0.68[a] | −0.46[c] | −0.47[c] | 0.44[d] | 0.02 | 0.29 | 0.05 | −0.03 |
| Kernel moisture 1 | — | — | — | — | — | 0.71[a] | −0.53[b] | −0.57[b] | −0.57[b] | 0.07 | 0.24 | −0.01 | −0.09 |
| Kernel moisture 2 | — | — | — | — | — | — | 0.42[d] | −0.59[b] | 0.86[a] | 0.18 | 0.20 | 0.18 | 0.12 |
| Premature death 1 | — | — | — | — | — | — | — | 0.88[a] | −0.07 | −0.05 | −0.27 | 0.00 | 0.08 |
| Premature death 2 | — | — | — | — | — | — | — | — | 0.32 | 0.10 | −0.14 | −0.10 | −0.06 |
| Days from inoculation to 28% KM | — | — | — | — | — | — | — | — | — | 0.27 | 0.02 | 0.28 | 0.26 |
| Plant height | — | — | — | — | — | — | — | — | — | — | 0.74[a] | −0.89[a] | −0.87[a] |
| Germination | — | — | — | — | — | — | — | — | — | — | — | −0.78[a] | −0.83[a] |
| Killed plants 1 | — | — | — | — | — | — | — | — | — | — | — | — | 0.98[a] |

[a] P = 0,1% [b] P = 1% [c] P + 2% [d] P = 5% [e] P = 10%

Table 6

Performance of the hybrids for ear rot, stalk rot, rind resistance, premature death and water content of the ears in 1980

Correlations

| Parameters | Ear rot | Stalk rot | Rind resistance | Premature death | Cob water content |
|---|---|---|---|---|---|
| Ear rot | — | 0.1884 | −0.4344* | −0.0443 | −0.3810 |
| Stalk rot | — | — | −0.4353* | 0.4274* | −0.3195 |
| Rind resistance | — | — | — | 0.4329* | 0.2443 |
| Premature death | — | — | — | — | −0.3402 |

* P = 5%

evaluation by splitting is not easy, we suggest the application of the PD value for evaluation of pathological stalk resistance. When we compare the PD values, we see that the average PD up to reaching 20% kernel moisture is more suitable than the PD value measured on the day of the physiological ripening. By this way the area below the curve can be calculated and this is more secure.

In the case of the ear rot we see that the ear rot spreading rate gives more close relationships with other parameter than the original value in percentage. The explanation is similar like in the case of premature death, e.g. by this way we can compare the genotypes of different ripening groups more exact. Therefore we suggest the application of this parameter for the ear rot susceptibility.

The rind thickness has a negative correlation with stalk rot, this is significant in every test. This means that a higher rind thickness tends to secure more resistant stalks, but this is not valid for every genotype. The tendency is, however, clear: the lower the rind thickness values, the higher is the susceptibility of stalks to rot and vice versa. The rind thickness, however, influences also the ear rot values. Its way of acting is the influencing stalk rot and through this the water content of the ears. This explains the significant positive correlation between rind thickness and water content of the ears.

The days between inoculation and reaching 28% kernel moisture are closely related with the earliness or lateness. As this value does not correlate significantly with ear rot values (Tables 5, 8) we can state genetically fixed ear rot resistance is not more evident in any of the ripening groups. With premature death is the situation similar or we have only weak significant relationships, which show the possibility of a high resistance to stalk rot in early materials.

As a result of these studies we see highly complicated interactions between the parameters investigated and, of course, this has several consequences also to test these parameters under field conditions.

The correlation tables give also an insight into the seedling and adult stage resistance relationships. The correlations are moderately close if significant at all and in several cases no relationship was detected. Knowing the complicated inter-

Table 7

Evaluation of corn hybrids for *Fusarium* reaction and other characteristics in 1981
Correlations

| Parameters | Ear rot | | Stalk rot 0—5 | Rind puncture, kg | Kernel moisure (KM) | | Premature death (PD) | | Days from inoculation to 28% KM |
|---|---|---|---|---|---|---|---|---|---|
| | Spreading rate, %/day | % | | | 9.9 | 27.9 | 28% at KM 22% | | |
| | | | | | % | | | | |
| Ear rot, %/day | — | 0,91[a] | −0.35[d] | −0.12 | −0.37[d] | −0.43[c] | −0.37[d] | −0.34[d] | −0.32 |
| Ear rot as a % | — | — | −0.31 | −0.17 | −0.07 | 0.13 | −0.14 | −0.13 | 0.08 |
| Stalk rot | — | — | — | −0.60[a] | −0.20 | 0.03 | 0.71[a] | 0.67[a] | 0.13 |
| Rind puncture | — | — | — | — | 0.36[d] | 0.21 | −0.52[b] | −0.55[a] | −0.14 |
| KM at 9th Sept. | — | — | — | — | — | 0.64[a] | 0.19 | 0.06 | 0.71[a] |
| KM at 27th Sept. | — | — | — | — | — | — | 0.46[c] | 0.43[d] | 0.77[a] |
| PD at 28% | — | — | — | — | — | — | — | 0.90[a] | 0.62[a] |
| PD at 22% | — | — | — | — | — | — | — | — | 0.55[a] |

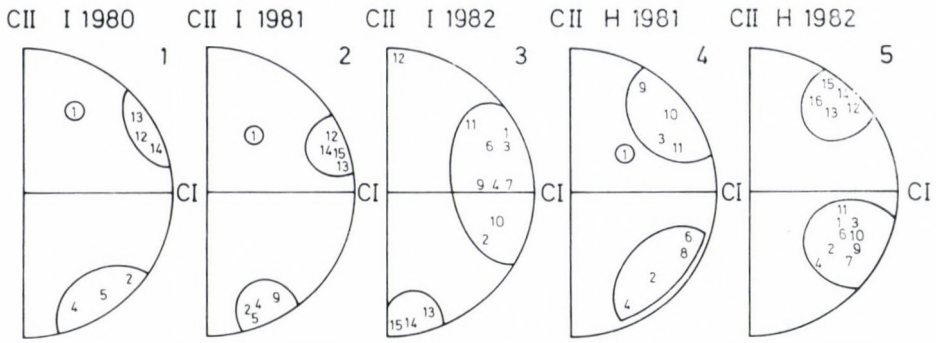
[a] P = 0.1% [b] P = 1% [c] P = 2% [d] P = 5%

Table 8

Correlations between the characteristics investigated in hybrid experiment in 1982, Experimental Station Szeged-Öthalom

| Parameters | Field parameters | | | | | | | Greenhouse paramaters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ear rot, %/day | % | Rind puncture, kg | Kernel moisture (KM) | | Premature death (PD) | | Days from inoculation to 28% KM | Plant height | Killed plants last reading | Mean value | Germination | Root infection |
| | | | | 31.8 | 13.9 | at | until | 38% KM | | | | | |
| | | | | % | | % | | | % | | | | |
| Stalk rot | 0.29 | 0.16 | $-0.60^{b}$ | $-0.70^{a}$ | $-0.54^{b}$ | $0.61^{a}$ | $0.56^{b}$ | $-0,67^{a}$ | $-0.18$ | 0.11 | 0.16 | $-0.27$ | 0.11 |
| Ear rot, %/day | — | $0.98^{a}$ | $-0.43^{d}$ | $-0.57^{b}$ | $-0.59^{a}$ | $0.60^{a}$ | $0.70^{a}$ | $-0.28$ | $-0.43^{d}$ | $0,32^{e}$ | 0.27 | $-0.25$ | 0.16 |
| Ear rot, % | — | — | $-0.38^{d}$ | $-0.45^{c}$ | $-0.48^{b}$ | $0.56^{b}$ | $0.66^{a}$ | $-0.11$ | $-0.37^{e}$ | 0.28 | 0.21 | $-0.19$ | 0.14 |
| Rind puncture | — | — | — | $0.71^{a}$ | $0.51^{b}$ | $-0.43^{d}$ | $-0.40^{d}$ | 0.32 | 0.08 | $-0.05$ | 0.00 | 0.09 | 0.01 |
| KM 1 | — | — | — | — | $0.67^{a}$ | $-0.44^{c}$ | $-0.55^{b}$ | $0.67^{a}$ | 0.23 | $-0.19$ | $-0.17$ | 0.25 | $-0.22$ |
| KM 2 | — | — | — | — | — | $0.52^{b}$ | $-0.65^{a}$ | $0.74^{a}$ | $0.37^{e}$ | $-0,22$ | $-0.23$ | 0.13 | $-0.11$ |
| PD 1 | — | — | — | — | — | — | $0.91^{a}$ | $-0.32^{e}$ | $-0.45^{c}$ | $-0.24$ | 0.24 | $-0.22$ | 0.16 |
| PD 2 | — | — | — | — | — | — | — | $-0.37^{e}$ | $-0.33^{d}$ | 0.15 | 0.13 | $-0.12$ | 0.09 |
| Days from inoculation to 28% KM | — | — | — | — | — | — | — | — | 0.45*s* | $-0.28$ | $-0.35^{e}$ | 0.31 | $-0.22^{e}$ |
| Plant height | — | — | — | — | — | — | — | — | — | $-0.77^{a}$ | $-0.79^{a}$ | $0.60^{a}$ | $-0.70$ |
| Killed plants 1 | — | — | — | — | — | — | — | — | — | — | $-0.96$ | $-0.74^{a}$ | 0.71 |
| Killed plants 2 | — | — | — | — | — | — | — | — | — | — | — | $-0.83^{a}$ | 0.67 |
| Gemination | — | — | — | — | — | — | — | — | — | — | — | — | 0.41 |

[a] P = 0.1% [b] P = 1% [c] P = 2% [d] P = 5% [e] P = 10%




Figures 1–5

Principal component analyse for the inbred (I) and hybrid (H) *Fusarium* experiments in 1980–1982

*Field parameters*

1. Stalk rot.
2. Ear rot, covered surface of ear as a percentage.
3. Ear rot spreading as % per day.
4. Rind puncture.
5. Premature death at harverst.
6. Premature death at 28% kernel moisture.
7. Premature death, mean up to reaching 28% kernel moisture.
8. Premature death at 22% kernel moisture.
9. Kernel moisture, 1st record.
10. Kernel moisture, 2nd record.
11. No. of days from inoculation to 28% kernel moisture.

*Greenhouse parameters*

12. Plant height.
13. Germination.
14. Killed plant number, last reading.
15. Killed plant number, mean of the last four readings.
16. Root infection rate.

relations of stalk and ear rot parameters we are aware that ear rot or stalk rot values might be far from the genetically fixed reaction. Therefore to have a more detailed information, we made principal component analyses for the three years of inbred and two years of hybrid (Figs 1 – 5).

In all cases the seedling parameters were in separate groups from the field parameters, where the selection brought all field parameter into one group. This group is somewhat looser for the inbreds and more compact for the hybrids as here the selection pressure was greater. This shows the possibilities of selecting materials with stalk and ear rot resistance. Analysing the correlations of field and seedling data, we find that correlations are below r – 0.50, especially with plant height, were not strong enough to bring this parameter into the stalk rot–ear rot group.

We think, however, that with a continuous selection we might produce a number of inbreds where the three characteristics are closely coppeled. There are several now, but among the materials tested there were many materials with different resistance structure (e.g. SRS, SRR, etc.) which break correlation. Behind this phenomenon the specific resistance sources should be identified (rind thickness, rapid physiological dry down of kernels, specific seedling resistance, etc.).

*The structure of the resistance*

Investigating the resistance reaction of different genotypes for seedling, stalk and ear resistance, we see that in many cases considerable differences occur. For example the original Ia153 line had very good seedling resistance, but poor stalk rot and ear rot performance. Its improved version become worse for seedling resistance, but significantly improved for stalk and ear reaction. B37 has excellent stalk rot resistance, but its ear and seedling performances are very poor, and the examples can be continued. So the resistance has a distribution in the plant and has different amount in the development. Therefore we may name the resistance of the plant as structured. If we consider (for convenience) only two types of resistance, resistant (R) and susceptible (S), the following combinations can be formed (Table 9). For us that group is the most important, where every parameter can be classified as resistant. The examples listed give a picture about the resistance structure of several inbreds and hybrids.

*Results at selecting inbreds and hybrids*

In Table 10 we present the inbreds selected in the ten year period according to the 1982 results. In Ia153 we were successful in selecting for field (stalk and ear) resistance, but lost seedling resistance being present in the original material. In A90 the line 178/7a was improved for all three parameters, in 51/4a/1a no improvement for stalk rot was combined with better resistance to ear rot and seedling blight. Among the lines selected from the hybrid KSC 360 the line 39/1c/1a is interesting with its good stalk reaction, excellent seedling response and moderate stalk rot severity. More interesting are, however, the lines selected from the A632 × × WF9 hybrid which have an excellent stalk and ear rot resistance and its seedling reaction is also better than average. We may list the inbred from Kz lila in this category, too. These latter inbreds are young, morphologically rather uniform, and further segregations may modify the present picture. Among the control inbreds, A632 is important with its considerable resistance to stalk and ear rot, as well as a somewhat less seedling response. The absolute negative example is the inbred Ma4 which represents the minimum for ear rot and seedling blight, but is somewhat better in stalk response.

The hybrid performances are more uniform, as the majority of hybrids with extreme susceptibility to some forms of resistance were discarded (Table 7). The hybrids with A632 are generally good in complex resistance (there are exceptions),

Table 9

Structure of resistance of corn to fusarial diseases. Simplified model, only susceptible (S) and resistant (R) variants are considered

| Form of resistance against | Possible combinations of resistance or susceptibility | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Seedling blight | S | S | S | S | R | R | R | R |
| Stalk rot | S | S | R | R | S | S | R | R |
| Ear rot | S | R | R | S | S | R | S | R |
| Example: Inbreds | A 90 Ma4 | | Ia154 4/1 | C22 B37 | Ia153 39/1c/1a/1 | GK3 | A632 A90 178/7 W64A/Z A632× WF9 $S_3$ Kz lila | |
| Hybrids | Ma4× C22 | | A90178/7× A632 | PiSC 3732 | | SzeSC 444 | A632× W64A/Z 39/1a/3× GK3 39/1a/3× GK15 | |

Table 10

Results of selection for *Fusarium* resistance
Creating inbreds, based on 1982 data

| Inbred | Field test | | Greenhouse test | |
|---|---|---|---|---|
| | Ear rot spreading | Rind puncture, kg | Premature death until 28% kernel moisture (%) | Germination, % |
| A632 | 0.26 | 7.02 | 28.0 | 71.7 |
| C22 | 1.80 | 4.79 | 76.1 | 65.0 |
| Ma4 | 2.23 | 4.51 | 40.7 | 17.5 |
| GK3 | 0.96 | 4.26 | 67.4 | 86.2 |
| GK15 | 0.50 | 5.33 | 7.2 | 65.0 |
| Ia153 | 1.60 | 4.48 | 65.8 | 77.5 |
| 4/1a* | 0.74 | 5.71 | 44.2 | 55.0 |
| 4/1b* | 0.40 | 5.82 | 51.5 | 48.6 |
| A90 | 1.01 | 4.05 | 53.3 | 28.0 |
| 178/7* | 0.78 | 5.79 | 24.8 | 70.0 |
| 51/4a* | 0.54 | 4.10 | 60.2 | 73.7 |
| A632× WF9aS* | 0.06 | 9.15 | 6.0 | 80.0 |
| A632× WF9b* | 0.04 | 6.52 | 6.0 | 69.6 |
| Kz lila* | 0.25 | 5.50 | 25.4 | 77.5 |
| $\bar{X}$ | 0.73 | 5.20 | 42.1 | 63.1 |
| LSD 5% | 0.59 | — | — | 27.5 |

* New inbreds

Table 11

Creating hybrids with superior resistance to *Fusarium* diseases. Results of 1982

| Hybrid | Field test | | | Greenhouse test | |
|---|---|---|---|---|---|
| | Ear rot spreading rate, %/day | Rind resistance, kg | Kernel moisture on 13.9, % | Premature death until 28% kernel moisture, % | Plant height |
| A632× W64A/2 | 0.51 | 13.08 | 30.10 | 0.80 | 66.0 |
| Ma4× C22 (susceptible control) | 2.02 | 4.09 | 14.90 | 45.30 | 32.0 |
| Ia153 4/1× A632 | 0.38 | 8.01 | 23.70 | 19.00 | 49.5 |
| 39/1a/3/3× A632 | 0.34 | 6.99 | 14.60 | 10.50 | 53.5 |
| 39/1a/3/1× A632 | 0.52 | 7.95 | 29.50 | 7.30 | 58.0 |
| 39/1a/3/2× GK15 | 0.56 | 8.58 | 32.30 | 15.10 | 67.0 |
| A90 178/7× A632 | 0.68 | 8.78 | 24.50 | 13.10 | 34.0 |
| A90 51/4a/1× A632 | 0.51 | 8.75 | 23.80 | 19.20 | 41.0 |
| 39/1a/3/3× GK15 | 0.35 | 6.18 | 31.20 | 0.00 | 65.6 |
| SzeSC 444 | 0.24 | 5.85 | 28.50 | 12.60 | 65.0 |
| Pi 3732 | 0.41 | 8.37 | 28.60 | 21.80 | 36.0 |
| $\bar{X}$ (n = 28) | 0.72 | 6.81 | 21.81 | 22.53 | 50.0 |
| LSD 5% | 0.36 | — | — | — | 17.0 |

and we have the same experience with GK-15 and GK-3. In these cases we could combine the high seedling, stalk and ear rot resistances and also the rind resistance seems to be sufficient.

*Relation between F. graminearum* and *F. culmorum reaction*

In the ear tests we worked with these two species in 1982. We produce means for the 2–2 isolates used. Their relationships for inbreds are r = 0.8209 P = 0.1% and for hybrids r = 0.6984, P = 0.1%, respectively.

## Discussion

*Role of using more isolates and the stalk rot–ear rot interactions in resistance tests*

The findings underline again the advantageous effect of using more isolates in resistance testing. This result is not brand new, and earlier authors have pointed out its significance (Christensen and Wilcoxson 1966, Mesterházy 1981*a*, 1982). We mention it, because the testing procedure in breeding programs uses only one single isolate and this may often lead to misinformation regarding the resistance level. As reaction to different isolates correlate fairly well, in our tests generally above

$r = 0.50$, for screening one pathogenic isolate seems to be enough. This agrees well with the way of thinking of Atlin et al. (1983), and recently we also came to the same conclusion (Mesterházy 1982). But in tests where possible exact differences should be produced and also the amount of the resistance is crucial, the use of one isolate or a mixture of isolates giving only one pathogenicity level is not acceptable, at least 4 isolates with different pathogenicity are necessary. By this way we can model better the natural conditions, where the plants are infected by a number of isolates, species and not by the progenies of a single isolate.

It is very important that stalk rot influences ear rot response. Its significance and the methodical consequences were discussed in detail by Mesterházy (1983). They prove that ignorance of these two factors (variability of the pathogen, influence of stalk rot on ear rot) is responsible for the majority of experimental failures in determining ear rot resistance. The other side of the stalk-ear relationship when ear rot inflences stalk rot is well documented (Michaelson 1957, Christensen and Wilcoxson 1966), our results support the earlier findings. The conclusion is that stalk and ear rot should be tested separately.

*Why we might speak about a new possibility in selection?*

It is true that selecting parallel for more agronomic traits is not a new finding. The consideration of the variability of the pathogen has its history, but found no practical application in this special field. To suppose more resistance mechanism is also not a new idea but was not developed further. However, there was no system created which could work in practical breeding. The development in these fields presented here, however, helped us to create a system which works not only in theory, but also in practice. For this the basic information required is the more exact determination of resistance and the presence of resistance which gives protection in seedlings, stalk and ear.

This allows the use of seedling resistance and a significant decrease in amount of field work, even thousands of seeds from a population can be screened. The result is a highly increased efficacy of selection. Of course, this system works when the desirable resistances for seedling, stalk and ear in the population to be screened in other cases the failure is guaranted.

This supposes the careful creation of the outgoing material, where the resistance structure of the parents is known. We have to mention resistance that in stalks, etc. may be determined by more sources of resistance. For example for stalk quality rind thickness is as important as pathological resistance.

The knowledge of the resistance structure is important in planning hybrids with the desirable resistances. At least one of the parents should contain the necessary seedling, stalk or ear resistance. This information is important also for the grower to estimate the risks of growing this or that hybrid, and also for the seed producer, because inbreds are generally more sensitive than hybris and even in good hybrids one parent might be deficient of one or more forms of resistance to *Fusarium*.

The results explain many of the contradictions between the field and greenhouse results, as well as between the stalk and ear rot response. As we know now, there are genetical (presence of different resistance mechanisms and ignorance of the variability of the pathogen), physiological (the stalk and ear rot relations) and methodological (how we can receive data reflecting the genetically determined resistance) problems, explaining these contradictions and they help us to have a more reliable view about resistance to Fusarium diseases in corn.

The authors wish to express their thanks to research assistants Dr. Irma Mózsik, and Ilona Pusztai, and the field staff in Szarvas.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 251–254 (1986)*

# Effect of Nitrogen, Phosphorus and Potassium on Sensitivity of Pepper Plants to *Fusarium* Wilt Toxin

A. R. T. SARHAN and A. M. A. HEGAZI

Department of Biology, College of Science, University of Salahaddin, Erbil, Iraq

The role of nitrogen, phosphorus, and potassium nutrition in relation to plant growth and development of pepper wilt symtoms caused by culture filtrate of *Fusarium oxysporum* f. sp. *redolens*, and by fusaric acid were studied. The high levels of nitrogen and potassium reduced the susceptibility of pepper plants to culture filtrate and to fusaric acid. Phosphorus in soil could not show such effect. The culture filtrate and fusaric acid were showing similar effects on the plants and on the symptoms (chlorosis, necrosis and epinasty).

Resistance of certain plants to various pathogenic soil organisms may be affected by subjecting the host plants to nutritional conditions which inflence the physiological responses of the plant (Király, 1976; Huber, 1980).

In previous studies with *Fusarium* wilt of tomato it was shown that the extent of disease development decreased with increasing the nitrogen concentration in the nutrient solutions (Sarhan et al., 1982). The fungal metabolite, fusaric acid (5-n-butyl picolinic acid) is produced by several *Fusarium* species which cause wilt disease in many important crops (Davis, 1969; David et al., 1978; Chakrabati and Basuchaudhary, 1980; and Barna et al., 1983). However, there is very little information about the effects of nutrition on the resistance of host plants to fungal toxins and especially to those produced by *Fusarium* spp.

The aim of this work was to investigate the effects of nitrogen, phosphorus and potassium nutritions in soil on resistance of pepper plants to culture filtrate of *Fusarium oxysporum* f. sp. *redolens* and to fusaric acid.

## Materials and Methods

Pepper *(capsicum frutescens*, var. California Wonder) was used for the experiments. Plants were grown in soil in the green house and 2–3 weaks later seedlings were transplanted to a sandy soil in pots of 12 cm in diameter. The plants were supplied with nutrients based on Hogland's solution which was containing different levels of nitrogen, phosphorus and potassium. The chemical composition of the nutrient solutions containing different levels of elements is given in Table 1. All pots received 40 ml of appropriate solutions twice a week for 6–8 weeks, the remainder of the water requirement was being supplied as tap water. For each treatment 25 plants were used. *Fusarium oxysporum* f. sp. *redolens* was used for

Table 1

Chemical composition of the nutrient solution based on Hogland's solution

| Stock solutions (1M) | ml of stock solution per liter of nutrient solution* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Basal | High N | Low N | High P | Low P | High K | Low K |
| $Ca(NO_3)_2 \cdot H_2O$ | 5 | 5 | — | 5 | 5 | 5 | 5 |
| $KNO_3$ | 5 | 5 | 2 | 5 | 5 | 5 | — |
| $KH_2PO_4$ | 1 | 1 | 1 | 1 | 1 | 1 | — |
| $MgSO_4 \cdot 7H_2O$ | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| $CaCl_2 \cdot 2H_2O$ | — | — | 5 | — | — | — | — |
| $NaNO_3$ | — | 30 | — | — | — | — | 5 |
| KCl | — | — | 3.5 | — | 9 | 12 | 1 |
| $NaH_2PO_4 \cdot 4H_2O$ | — | — | — | 2 | — | — | — |
| NaCl | 30 | — | 30 | 27 | 30 | 18 | 25 |

* One ml of minor elements stock solution ($H_3Bo_3$, 0.28 g; $CuCl_2 \cdot 2H_2O$, 0.004 g; $ZnCl_2$, 0.003 g; $MnCl_2 \cdot 4H_2O$, 0.18 g; $FeCl_3 \cdot 6H_2O$, 0.05 g; distilled $H_2O$ 100 ml) added to each nutrient solution.

production of toxic material. The fungus was grown in modified Richard's medium (Kuo and Scheffer, 1964). Medium was inoculated with small pieces of mycelial mat and fungus was grown in stillculture at 28 °C. After incubation for 14 days, the culture was filtered through cheese cloth, and then through membrane filter to obtain a crude stock toxin solution.

To test the effect of fusaric acid, a solution of this toxin ($0.5 \times 10^{-3}$ M) was used. To assess the effects of test solutions on detached leaves of similar positions, pepper leaves were put into small tubes and allowed to take up the toxin solutions or the filtrate or the medium for control experiments. The test tubes were incubated under laboratory conditions. Visual observations were made daily up to 4–6 days

## Results and Discussion

Pepper leaves exposed to the normal atmosphere and treated with culture filtrate obtained from the virulent strain of *Fusarium oxysporum* f. sp. *redolens*, and with $0.5 \times 10^{-3}$ M fusaric acid produced similar symptoms of grey-green necrotic spots on the leaves besides epinasty as described by Barna et al. (1983). Both culture filtrate and toxin solution exerted similar effects on the treated plants. The most severe symptoms occured on controlplants and on plants treated with the basal solution. The filtrate and toxin solutions caused chlorosis in 50–70 percent leaves. The high nitrogen and high potassium regimes produced plants which were the most resistant to the culture filtrate as well as to the fusaric acid solutions. However, phosphorus had no significant effect against toxins (Table 2).

Table 2

Effect of nitrogen, phosphorus, and potassium nutrition on the development of symptoms caused by culture filtrated of *F. oxysporum* f. sp. *redolens* and by fusaric acid on pepper

| Treatments | Mean height of plants (cm) | Leaves showing chlorosis (%) | | Disease intensity* | |
|---|---|---|---|---|---|
| | | (C.f) | (F. a) | (C. f) | (F. a.) |
| Control ($H_2O$) | 22.3 | 53.1 | 68.3 | +++ | +++++ |
| Basal | 28.1 | 25.5 | 32.8 | +++ | +++ |
| Low N | 25.4 | 17.2 | 20.0 | +++ | ++ |
| High N | 34.9 | 2.8 | 5.6 | − | + |
| Low K | 26.6 | 15.1 | 13.5 | +++ | +++++ |
| High K | 29.6 | 4.2 | 3.3 | − | ++ |
| Low P | 24.2 | 18.3 | 15.4 | +++ | ++++ |
| High P | 25.3 | 8.2 | 10.0 | ++ | ++ |

* Disease intensity; +++++ = severe symptom; + = very mild symptoms; − = no symptoms.

Pepper plants grew better with basal, high nitrogen and high potassium containing soils. The data obtained in the present investigation strengthen the hypothesis that toxins produced by the pathogen could be involved in pathogenesis and it was primarily responsible for the disease symptoms in wilt of pepper.

Chakrabarti and Chaudhary (1980) have also observed a similar effect in case of *Fusarium oxysporum* f. sp. *carthami* on safflower plants.

Although in all treatments, leaves of the same age were used, their physiological states due to the various treatments was different. Thus leaves of plants grown with high levels of nitrogen and potassium were physiologically more juvenile than those in other treatments. In previous work (Sarhan et al., 1980) we considered that factors associated with juvenility are involved in the decreased sensitivity of the plants to necrotizing factors (toxins, enzymes) secreted by the pathogen.

These results are consistent with those of Sarhan et al. (1980; 1982) and Barna et al. (1982; 1983) who concluded from experiments with *Fusarium* wilt of tomato that factors which maintained juvenility in plants could increase plant resistance to the pathogen and to its toxins.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 255—259 (1986)*

# A Modified Technique for Inoculation in Leaf Stripe of Barley

A. M. HAMMOUDA*

Plant Pathology Institute, Agricultural Reaearch Centre, Giza, Egypt

Seven techniques for inoculating seeds of a susceptible barley cultivar with isolates of *Drechslera graminea* were compared. One of the techniques consistently achieved higher infection values and relatively high percentage of seed germination. This technique, therefore, was considered as the most effective method for inducing leaf stripe infection in barley. Healthy, surface-sterilized seeds of barley were placed on an actively growing 8-day-old mycelial culture and incubated at 25 °C for 10 days. Afterwards, culture and germinated seed were carefully removed and planted.

Barley leaf stripe caused by *Drechslera graminea* (Rabh. ex Shlecht.) Shoem. is common wherever the crop is grown and sometimes causes considerable damage (Mathur et al., 1964).

Spraying barley plants with conidial and mycelial suspension failed to induce typical symptoms of stripe. In this case, tiny brown flecks may appear, but never expand further (Smedegard—Petersen, 1972). Typical symptoms of leaf stripe was obtained when dehulled barley grains of susceptible cultivars were germinated in contact with mycelium, and then planted (Ravn, 1900). It was possible to obtain a high percentage of infection by removing the hull of seeds and inoculating with spores and mycelium during germination (Johnson, 1925). Inoculation of flowers and germinating kernels gave relatively high rates of infection (Isenbeck, 1930). Infection rate was 5.4% when milky and green mature stages of barley were inoculated with spore suspension (Yu and Hwang, 1935).

Surface-sterilized kernels of barley were mixed with mycelium of *D. graminea* growing on autoclaved wheat grains-media in 125 ml Erlenmeyer flask. When the inoculated kernels were planted, only 50% of them germinated with high rate of stripe infection (Arny and Shands, 1942).

Percentage of stripe infection was apparently high when barley kernels were submerged in water for one hour, then germinated on 8-day-old culture of *D. graminea* for eight days before planting in pots (Smedegard—Petersen, 1972).

Four techniques of inoculating dehulled and undehulled (normal) kernels of susceptible barley varieties with *D. graminea* were compared (Mohammad and Mahmood, 1974). The most effective technique consisted of placing dehulled seeds in between two layers of an actively growing 7-day-old mycelial culture, incubating them at 25 °C for 60hr (until sprouting) and then carefully removing and sowing them.

The present investigation was conducted to compare seven techniques for inducing artificial infection of leaf stripe of barley.

* Present address: Agricultural Research Department, P.O. Box 18475, Salalah, Sultanate of Oman.

## Materials and Methods

*Isolation of the causal fungus*

Small pieces of striped leaves of barley were surface-sterilized in 0.2% mercuric chloride for two minutes, washed several times with sterile distilled water, carefully dried in between two Watman No. 1 papers, transferred to potato dextrose agar (PDA) plates and incubated at 25 °C. Pure cultures were obtained by cutting hyphal tip (Brown, 1924).

*Inoculation techniques*

Six techniques, previously used, as well as a new modified one, were compared during the present study. These techniques were carried out using kernels of susceptible barley cultivar, Giza 119, as follows:

*1. Flask technique* The kernels were surface-sterilized, placed in 125 ml Erlenmeyer flasks containing *D. graminea* growth on wheat media, shaken immediately to mix kernels with inoculum and incubated for 4 days at 25 °C. The entire contents of the flasks were planted in the soil (Arny and Shands, 1942).

*2 Submerging of kernel* Surface-disinfected kernels were submerged in sterile water for one hr, then germinated on 8-day-old culture of *D. graminea* for 8 days before planting in soil (Smedegard–Petersen, 1972).

*3. Dip of normal kernel* Surface-disinfected kernels were dipped in conidial and mycelial suspensions, kept at 25 °C for 60 hr, until the seeds sprouted, and then sown in soil.

*4. Dip of dehulled seed* Dehulled seeds were surface-sterilized, immersed in conidial and mycelial suspensions, kept and sown as in (3).

*3 Embedding of normal kernel* Surface-disinfected kernels were placed on an activity growing 7-day-old mycelial culture of *D. graminea* on PDA in Petri-plates. A similar mycelial mat of the fungus culture was aseptically removed and inverted over kernels; embedding them in between the two layers of inocula, and incubated at 25 °C for 60 hr. Then the sprouted seeds were carefully removed and sown.

*6. Embedding of dehulled seed* Dehulled seeds were surface-sterilized, sprouted in between two layers of fungus culture as in (5) and then sown (Mohammad and Mahmood, 1974).

*7. The modified technique* Surface-disinfected kernels were placed on an activity growing 8-day-old mycelial culture on PDA in Petri-plates, and then incubated at 25 °C for 10 days. The entire contents of Petri-plates were removed carefully and planted in pots.

*Seedling inoculation*

Mycelial and conidial suspension of *D. graminea* were also used for inoculating plants of the susceptibe variety Giza 119 at ages of 2.4 and 8 weeks. The inoculated plants were placed under 90% relative humidity at 25 °C for 48 hr.

Final observations were taken after 45 days.

## Results

Tiny brown flecks were found to occur on leaves of seedlings of susceptible barley cultivar inoculated with conidial and mycelial suspension of *D. graminea* at different ages. These flecks never expanded further.

Barley seeds, soaked in mycelial suspension or sprouted on cultures of *D. graminea*, developed seedlings with typical leaf stripe.

The various techniques of inoculation, which produced typical symptoms, were found to result different values of infection (Table 1).

Table 1

Effect of seven inoculation techniques on percentage of seed germination and stripe infection in barley

| No. | Inoculation technique | % seed germination | % infection |
|---|---|---|---|
| 1 | Flask technique | 49.3 | 74.0 |
| 2 | Submerging of kernel | 92.3 | 72.3 |
| 3 | Dip of normal kernel | 92.5 | 11.8 |
| 4 | Dip of dehulled seed | 80.5 | 20.5 |
| 5 | Embedding of normal kernel | 90.5 | 34.0 |
| 6 | Embedding of dehulled seed | 82.0 | 72.8 |
| 7 | The modified technique | 92.3 | 90.0 |
| | LSD (P = 0.05) | 5.3 | 15.7 |

Low rates of infection (11.8% and 20.5%) were obtained when normal and dehulled seed, respectively, were dipped in conidial and mycelial suspension for 60 hr before planting.

The infection value increased to be 34% when normal kernels were placed in between two layers of mycelial mat for 60 hr.

A high rate of infection (about 72%) was obtained by placing dehulled seeds in between two layers of mycelial mat for 60 hr, or submerging kernels in water for one hr and germinating them on cultures for 8 days, before planting.

The infection value was 74% when normal kernels were mixed with fungus growth in flasks for 4 days then planted.

The highest percentage of infection (90%) was achieved by a new modified technique. In this technique, disinfected kernels were placed on actively growing mycelial cultures in Petri dishes for 10 days, then the entire contents were carefully removed and planted.

Rate of seed germination was affected also by the inoculation techniques. It was apparently low in the flask technique (No. 1). On the contrary, dip of normal kernel (No. 3) achieved the highest percentage of seed germination (92.5%).

The dip of dehulled seed technique (No. 4) gave a high percentage of germination (80.5%).

Rates of seed germination were 82 to 92.3% in the other techniques, i.e.; embedding of dehulled seed (No. 6), embedding of kernel (No. 5), submerging of kernel (No. 2) and the new technique (No. 7).

## Discussion

Inoculation technique is an essential procedure in the study of host-pathogen relationship especially that of disease control such as varietal resistance and chemical control. More accurate records can be made when successful methods of inoculation are used.

Mycelilal and conidial suspensions of *Drechslera graminea* were sprayed on barley leaves for inducing stripe infection. Tiny brown flecks were obtained, but never expended further (Smedegard-Petersen, 1972).

Several other methods of artificial inoculation were adopte to obtain typical symptoms of stripe. Normal or dehulled barley grains were germinated in contact with mycelium, or dipped in conidial and mycelial suspension of *D. graminea* (Ravn, 1900; Johnson, 1925; Isenbeck, 1930; Arny and Shands, 1942; Smedegard – Petersen, 1972; Mohammad and Mahmood, 1974).

In the present study, a modified technique was used and compared with other six methods adapted before. This new technique has achieved the highest percentage of stripe infection. Also, high rate of seed germination has been obtained.

The concept of the new technique is related to that of submerging kernel technique (Smedegard – Petersen, 1972) and embedding-kernel technique (Mohammad and Mahmood, 1974), with two modifications:

1. The kernels were placed on fungus culture in Petri plates for *ten days*.
2. The entire contents, consisting of *germinated seeds and cultures*, were transferred and planted.

## Acknowledgements

Thanks are due to Professor E. Ghobrial, Head of Cereal Diseases Division, Plant Pathology Institute, Egypt, for providing facilities, and to Mamdouha M. Hussein for technical assistance.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 261–266 (1986)*

# Sclerotium Production of *Sclerotinia sclerotiorum* (Lib.) *de Bary* in Sunflower

J. ENISZ

NEVIKI, Department of Biology, H-8201, Veszprém, P.O. Box 160

In regard to the epidemiological prediction the knowledge of starting disease potential is an important fact. In the case of *Sclerotinia sclerotiorum* this disease potential depends on the number of sclerotia per hectare. Regarding sunflower we do not have enough data to estimate this number.

During our work we evaluated a field in centre of West-Hungary using eight different cultivars/hybrids to determine the number of new sclerotia that were produced by an infected plant in a normal crop-rotation year and we compared this with an experimental sunflower monoculture. In the first year only the evidently infected plants were collected and on the average 108.45 sclerotia — with a mean weight of 27.5 mg per sclerotia — were found in one infected plant. It appears that there was a significant difference among the cultivars/hybrids. Therefore in the second year a statistically planned experiment with four replications was set up. Each plant was examined indiscriminately and it was found, that in crop-rotation on the average 59.57 sclerotia per infected plant, while in monoculture 59.04 sclerotia per infected plant were produced. Significant differences do appear among the cultivars/hybrids.

On the basis of our data we can predict 50–100 new sclerotia per infected sunflower plant at natural conditions like it in Hungary. Accordingly, relative low, 5% frequency of *S. sclerotiorum* infestation can result 125–250 000 new sclerotia per hectare at a mean rate — 50 000 plant per hectare — of sunflower.

Grogan (1979) summarized the status of the biology and epidemiology of *Sclerotinia* species at a "Symposium on Sclerotinia" in 1978. Athough much information on the species is available, Grogan's rewiew is still valid. This work was initiated to determine the *in vivo* facts, rather than in vitro measure qualifications of this fungus. For example, sclerotia produced in vitro or sclerotia germination etc. on artifical media are not similar to those of natural Sclerotinia species. The biology of *Sclerotinia sp.* and the prediction of their disease potential are also difficult to assess. In this regard the work of Huang and Hoes (1980) concerning the infection of sunflower plants by *S. sclerotiorum* found in the soil, should be mentioned.

At present we have an incomplete picture concerning the quantity of the emerging number of sclerotia in vivo. This is an important link in the disease potential since the level of inoculum density of this shows what kind of sclerotia determines the degree of infestation after a known disease incidence.

In the past two years we have determined the number of sclerotia following a Sclerotinia rot and wilt disease incidence in sunflowers. Information on sclerotial density can contribute to better forecasting of disease potential under climatic

conditions similar to those found in Hungary. Our tests were made in the years of 1981 and 1982. The examined field is located in the centre of West-Hungary at the 18 degree of east-longitude and at the 47 degree of north-latitude. The average annual rainfall is about 580 mm.

Table 1

The number of sclerotia and their weight at harvest in sunflower plants infected by *Sclerotinia sclerotiorum* (Veszprém, Hungary 1982)

| Cultivar/Hybrid | No. sclerotia per infected plant* | | Mean weight of one sclerotia, mg* | | % Wilted plants |
|---|---|---|---|---|---|
| Crop-rotation | | | | | |
| NS-H-26 | 55.6 | 72.3 | 21.63 | 24.15 | 26 |
| | | 39.0 | | 19.11 | |
| NS-H-27 | 36.5 | 51.0 | 15.79 | 19.70 | 22 |
| | | 21.9 | | 11.89 | |
| Marianne | 75.7 | 101.7 | 18.51 | 21.23 | 24 |
| | | 49.7 | | 15.80 | |
| Luciole | 82.9 | 118.3 | 20.86 | 24.08 | 8 |
| | | 54.1 | | 17.65 | |
| Tsakinski | 12.6 | 2.2 | 56.27 | 2.75 | 36 |
| | | 47.8 | | 115.30 | |
| GK-70 | 49.6 | 68.8 | 16.94 | 18.58 | 25 |
| | | 30.3 | | 15.31 | |
| NS-H-33 | 42 3 | 53.4 | 23.41 | 27.83 | 11 |
| | | 31.2 | | 18.99 | |
| Iregi csíkos | 52.7 | 71.9 | 17.27 | 20.07 | 29 |
| | | 33.5 | | 14.48 | |
| Monoculture | | | | | |
| NS-H-26 | 61.7 | 76.0 | 19.05 | 22.09 | 45 |
| | | 47.4 | | 16.00 | |
| NS-H-27 | 76.8 | 96.1 | 38.00 | 52.91 | 42 |
| | | 57.4 | | 23.09 | |
| Marianne | 42.7 | 60.2 | 31.44 | 39.67 | 65 |
| | | 25.2 | | 23.21 | |
| Luciole | 30.4 | 38.2 | 16.14 | 20.70 | 35 |
| | | 22.6 | | 11.59 | |
| Tsakinski | 70.2 | 95.8 | 17.74 | 20.76 | 67 |
| | | 44.6 | | 14.73 | |
| GK-70 | 53.3 | 84.4 | 25.79 | 36.52 | 53 |
| | | 38.1 | | 15.06 | |
| NS-H-33 | 62.4 | 84.0 | 22.09 | 31.66 | 58 |
| | | 40.8 | | 12.53 | |
| Iregi csíkos | 66.1 | 83.4 | 18.34 | 20.90 | 59 |
| | | 48.8 | | 15.77 | |

* The means and confidence limits (P = 0.05) calculated of more than 20 infected plants

## Materials and Methods

Tests were set up on a "Ramman's brown forest soil", in 1981 in an area having an average rainfall during the growing season. Its distribution in 1981 was unusual since half of the rain fell in June. In 1982 rainfall distribution was more uniform, but its total value exceeded the average by 50%. In 1981 plants from different cultivars/hybrids, that showed white rot symptoms, were collected. In the following year (1982) the different cultivars/hybrids were sowed into plots with 40 metres long rows. Each row was limited to a different cultivar or hybrid. All sowings were replicated four times in two blocks. The tests were set up in a normal crop-rotation and a three-year-old sunflower experimental monoculture. Seed was

Table 2

Comparison of the sclerotial number per plant from 8 sunflower cultivars/hybrids with Student's tests

| Block I | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Crop-rotation | | | | | | | | |
| 1. NS-H-26 | — | — | — | — | — | — | — | — |
| 2. NS-H-27 | N | — | — | — | — | — | — | — |
| 3. Marianne | N | S | — | — | — | — | — | — |
| 4. Luciole | N | S | N | — | — | — | — | — |
| Monoculture | | | | | | | | |
| 5. NS-H-26 | N | S | N | N | — | — | — | — |
| 6. NS-H-27 | N | S | N | N | N | — | — | — |
| 7. Marianne | N | N | S | S | N | S | — | — |
| 8. Luciole | S | N | S | S | S | S | N | — |
| **Block II** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Crop-rotation | | | | | | | | |
| 1. Tsakinski | — | — | — | — | — | — | — | — |
| 2. GK-70 | N | — | — | — | — | — | — | — |
| 3. NS-H-33 | S | N | — | — | — | — | — | — |
| 4. Iregi csíkos | N | N | N | — | — | — | — | — |
| Monoculture | | | | | | | | |
| 5. Tsakinski | S | N | S | N | — | — | — | — |
| 6. GK-70 | N | N | N | N | N | — | — | — |
| 7. NS-H-33 | N | N | N | N | N | N | — | — |
| 8. Iregi csíkos | N | N | S | N | S | N | N | — |

(P = 0.05), S = significant, N = non-significant difference

sown at a rate of 66 000 per hectare. The varieties included two French sunflower hybrids "Marianne" and "Luciole". Hybrids designated with an "NS – H" were Jugoslav ones. The variety "Tsakinski" was a Soviet cultivar an the variety "GK – 70" was selected in Hungary from the Soviet cultivar "VNIIMK 6540". A Hungarian land type cultivar "Iregi csíkos" was also included.

A disease evaluation was made at the full ripening stage of "Marianne" since this variety has the shortest growing season. Whole plants were harvested, examined and those with Sclerotinia rot was delivered to laboratory for evaluation. After examination of each infested plant sclerotia were collected and counted. Sclerotial weights were measured after brushing and three-weeks of drying at room temperature. The minimal number of plants examined was 20 per replicate.

## Results

In 1981 using a mixture of various cultivars and hybrids it was found, that on the average 108.45 sclerotia were in each wilted or rotted sunflower plant. The mean weight was 27.5 mg per sclerotia. Since more sclerotia were found in certain cultivars/hybrids, it was decided to repeat test with statistically designed experiments. Table 1 contains the results of this test. The last column lists the percent of plants that were infected. From previous experiences "Luciole" showed the lowest incidence of disease, i.e.: "a typical field resistance". With crop-rotation a little higher incidence, than the average in Hungary was found (Békési, personal communication), but it was substantiated with the higher value and it may have been caused by the higher level of rainfall.

The comparison of average in each block with Student's tests can be found in Tables 2 and 3. Summarizing the data by cultivars/hybrids obtained in both the crop-rotation and monoculture tests we obtained average values shown in Table 4. There was no difference in numbers of sclerotia per plant, but the average weight of sclerotia was significantly higher from the monoculture tests.

## Discussion

An apparent contradiction in the data obtained in 1981 and 1982 appears, because the number of sclerotia per diseased plant in 1981 was nearly double than the number in the following year. The average weight of one sclerotium was slightly higher, too. This is probably due to the fact, that the data compared in 1981 were primarily from seriously infected plants but in 1982 all plants were harvested and evaluated. At present there is no adequate explanation for differences between the numbers and the weight of sclerotia per diseased plant between cultivars/hybrids, as well as between the data in monoculture and crop-rotation. There are higher differences in the data from normal crop-rotation than in monoculture. For example in the case of "Tsakinski" mostly head-infection occured with crop-rotation,

Table 3

Comparison of the mean weight of sclerotia from 8 sunflower cultivars/hybrids with Student's tests

| Block I | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Crop-rotation | | | | | | | | |
| 1. NS-H-26 | — | — | — | — | — | — | — | — |
| 2. NS-H-27 | S | — | — | — | — | — | — | — |
| 3. Marianne | N | N | — | — | — | — | — | — |
| 4. Luciole | N | N | N | — | — | — | — | — |
| Monoculture | | | | | | | | |
| 5. NS-H-26 | N | N | N | N | — | — | — | — |
| 6. NS-H-27 | S | S | S | S | S | — | — | — |
| 7. Marianne | S | S | S | S | S | N | — | — |
| 8. Luciole | S | N | N | N | N | S | S | — |

| Block II | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Crop-rotation | | | | | | | | |
| 1. Tsakinski | — | — | — | — | — | — | — | — |
| 2. GK-70 | N | — | — | — | — | — | — | — |
| 3. NS-H-33 | N | S | — | — | — | — | — | — |
| 4. Iregi csíkos | N | N | S | — | — | — | — | — |
| Monoculture | | | | | | | | |
| 5. Tsakinski | N | N | S | N | — | — | — | — |
| 6. GK-70 | N | N | N | N | N | — | — | — |
| 7. NS-H-33 | S | N | N | N | N | N | — | — |
| 8. Iregi csíkos | N | N | S | N | N | N | N | — |

(P = 0.05), S = significant, N = non-significant difference

Table 4

The number and weight of sclerotia from sunflower plants diseased by *Sclerotinia sclerotiorum* on an average of 8 cultivars/hybrids (Veszprém, Hungary 1982)

| | Mean number of sclerotia per infected plant* | | Mean weight of one sclerotia, mg* | |
|---|---|---|---|---|
| Crop-rotation | 59.57 | 66.93<br>52.20 | 20.18 | 21.72<br>18.65 |
| Monoculture<br>(3th year) | 59.04 | 64.52<br>53.56 | 24.64 | 28.07<br>21.21 |

* Mean and confidence limits (P = 0.05)

accounting for a reduced sclerotia number and a higher sclerotial weight. In the case of "Marianne" the high proportion of head-infection occurred also, but "screen-like" sclerotia didn't develop, for this reason the mean weight did not increase.

On the basis of our data we can also predict 50–100 new sclerotia per infected sunflower plant at natural condition similar to those as in Hungary. These can be considered as the starting-potential that depends on many other conditions whether an infection results directly from sclerotia or from ascospores emerging from apothecia. In another respect our observation is interesting, that in the sunflower monculture the infection-rate by *S. sclerotiorum* does not change considerable from the third year (unpublished data), therefore under nontreated conditions an equilibrium level is approached, the disease does not progress further.

## Acknowledgements

I would like to thank Prof. J. Altman (Colorado State Univ.) for critical reading and correction of the manuscript.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 267—271 (1986)*

# Production of IAA in the Sunflower Leaf-spot and Blight Disease Caused by *Alternaria helianthi*

MAHIPAL S. GHEMAWAT[1], S. C. SHARMA and J. M. AGRAWAT[2]

Department of Botany, Government College, Kota RAJ. 324002, India

*Alternaria helianthi*, the causal organism of the sunflower leaf-spot and blight disease, produces IAA in vitro. IAA is also produced in vivo when *A. helianthi* isolates are inoculated on a susceptible sunflower cultivar. Uninoculated control plants do not produce any IAA. The amount of IAA produced in vivo has a negative correlation to the degree of virulence of the fungal isolates. The least virulent isolate, which is associated with the maximum IAA in vivo, causes the maximum shot holes in sunflower leaves. The shot-hole formation, which restricts the disease, is possibly due to IAA production. Hence IAA production may be involved in disease resistance to *A. helianthi*. IAA production may also result in the accumulation of phenols which also can confer resistance.

*Alternaria helianthi* (Hanfs.) Tubaki & Nishihara causes a serious leaf-spot and blight disease of oilseed sunflower (*Helianthus annuus* L.) in India, causing heavy losses to the crop. For studying the symptomatology of the disease, seven isolates of the pathogen were collected from all over India. Out of these, three isolates (from Akola, Jalgaon and Kota) were selected on the basis of different degrees of virulence, the Akola isolate being the least, the Jalgaon isolate being moderately virulent and the Kota isolate being the most virulent.

The Akola isolate produced the least disease with small spots, accompanied frequently by shot holes and much crinkling of the leaves. Shot holes and the crinkling of leaves were less with the Jalgaon isolate and the least with the Kota isolate. These symptoms may be due to hyperauxinity. Kavanogh and Williams (1981) reported that *Plasmodiophora brassicae* causes hyperauxinity in *Brassica oleracea* by producing IAA and 3-Indoleacetonitrile. Accumulation of IAA in the leaf spots and shot holes of mango leaves incited by *Colletotrichum gloeosporioides* has also been observed (Vidhyasekaran and Durairaj, 1973). It has been further reported that higher amount of IAA leads to a greater accumulation of phenols (Daly, 1972), which play an important role in resistance in many plants against various pathogens.

Hence it was decided to study the production of IAA in vitro and in vivo by *A. helianthi* and by the diseased sunflower leaves, respectively.

[1] Present address (and address for reprints): Indra Bhawan, Mala Road, Kota RAJ. 324002, India.

[2] Agricultural Station, Sukhadia University, Borkhera, Kota RAJ., India.

## Materials and Methods

For estimating the in vitro production of IAA, flasks containing Czapek's medium with or without tryptophan were seeded separately with 2 ml of spore-suspension ($3 \times 10^3$ spores/ml) of each isolate. There were three flasks of each type (with or without tryptophan) for each isolate and the experiment had three replicates. After incubation at $27 \pm 2$ °C for 15 days, cultures were filtered through a Buchner funnel. Filtrates from three flasks containing the same medium and seeded with the same isolate in each replicate were then pooled together and IAA was extracted as per the method described by Mahadevan and Sridhar (1982).

Leaves of ten 30-day-old sunflower plants (susceptible cultivar EC 68414) were separately inoculated with 50 ml of spore-suspension ($3 \times 10^3$ spores/ml) of each isolate. This experiment also had three replicates. After incubation of the inoculated and the control plants at a very high relative humidity (> 95%) for two days, all the plants were incubated under greenhouse conditions (ca. 50–65% relative humidity). One hundred g of the diseased leaves were collected for each isolate in each replicate 15 days after inoculation and IAA was extracted as per the method described by Mahadevan and Sridhar (1982). Controls consisted of leaves from the uninoculated plants (sprayed with distilled water).

One hundred $\mu$l of each extract and 0.3% pure IAA solution were spotted on chromatographic paper (Whatman No. 1) and separated as per the method described by Mahadevan and Sridhar (1982). Colour intensities and $R_F$ values of the spots were noted and compared.

Table 1

Production of IAA in the sunflower leaf-spot and blight disease caused by *Alternaria helianthi*

| | Akola isolate | | Jalgaon isolate | | Kota isolate | | Uninoculated | |
|---|---|---|---|---|---|---|---|---|
| In vivo | +++ | | ++ | | + | | − | |
| | Czapek's | Czapek's + Trypto-phan | Czapek's | Czapek's + Trypto-phan | Czapek's | Czapek's + Trypto-phan | Czapek's | Czapek's + Trypto-phan |
| In vitro | − | ++ | − | +++ | − | + | − | − |

− No IAA
\+ Faint spot of IAA
++ Moderate spot of IAA
+++ Dark spot of IAA

## Results

All the three isolates studied, produced IAA in vitro (Table 1) when they were grown on Czapek's medium supplemented with tryptophan but its amount differed with different isolates. The most virulent Kota isolate produced the least amount of IAA whereas its amount was the maximum with the moderately virulent Jalgaon isolate. On the other hand, none of these isolates produced any IAA when cultured on Czapek's medium without tryptophan.

In vivo, the most virulent Kota isolate was associated with the least amount of IAA while the least virulent Akola isolate was associated with the maximum amount. No IAA production was observed in the control plants (Table 1).

## Discussion

In the present studies, all the three isolates of *A. helianthi* produced IAA only in the tryptophan-supplemented Czapek's medium, indicating that the pathogen may be using tryptophan as the precursor for the synthesis of IAA. Similar observations have also been recorded for *A. macrospora* (Bhaskaran, 1975) and for other fungi (Gruen, 1959; Pegg, 1976). No correlation was found between the IAA production in vitro and the degree of virulence of the pathogen isolates as also reported for *Pyricularia oryzae* from rice (Purushothaman et al., 1971). However, Bhaskaran (1975) reported a positive correlation of virulence with IAA production in vitro by two races of *A. macrospora* from cotton.

The least amount of IAA production in vivo associated with the most virulent Kota isolate and its maximum production associated with the least virulent Akola isolate shows a negative correlation of IAA production with the degree of virulence of the concerned isolate. Working with *Helminthosporium nodulosum* leaf-spot of *Eleusine coracana*, Vidhyasekaran (1975) initially recorded a negative correlation between the IAA amount produced in vivo and the degree of virulence of the pathogen isolates. But later on, this negative correlation changed into a positive one (within three days in case of a susceptible variety and within seven days in case of a resistant variety) which is just the reverse of what has been recorded in the present studies in which IAA production was studied 15 days after inoculations.

The least virulent Akola isolate, which was associated with the maximum amount of IAA in vivo, caused most shot holes in the host leaves. Shot-hole formation checks the spread of the disease as the leaf tissue is cut off which then falls down. It has been reported that IAA stimulates the rate of abscission (Bidwell, 1979). Hence, it is possible that IAA is responsible for the formation of shot holes in sunflower leaves following infection with *A. helianthi*. With the infected leaf segments falling, the pathogen is at least partly lost and hence this may be responsible for the least progress of the disease with the Akola isolate. It has been opined that shot-hole formation in *Prunus* spp. in response to *Clasterosporium carpophilum*

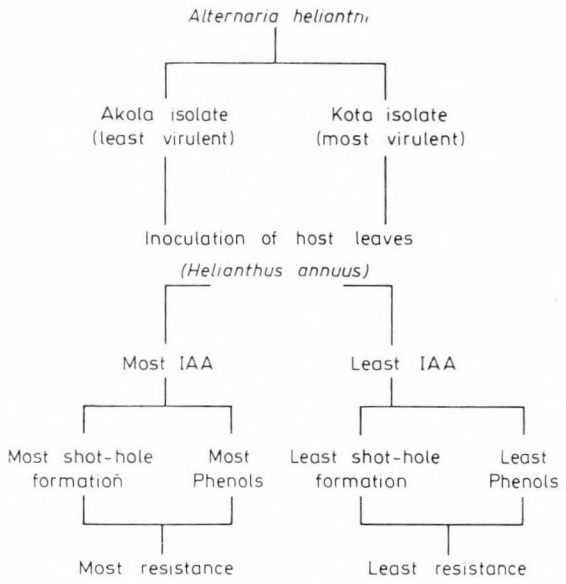



Fig. 1. Schematic representation of the possible role of IAA in the resistance of *Helianthus annuus* leaves to *Alternaria helianthi*

is basically a hypersensitivity reaction of intermediate degree in which the spread of pathogen is halted relatively late (Muller, 1959). Besides, a higher amount of IAA leads to a greater accumulation of phenols (Daly, 1972) which is said to be responsible for resistance to various pathogens. Hence, the greater production of IAA when the Akola isolate is inoculated may also be the cause for its least virulence through more production of phenols (Fig. 1).

## Acknowledgements

Grateful thanks are due to the University Grants Commission, New-Delhi for project funds and a Junior Research Fellowship to SCS.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 273—277 (1986)*

# Cercosporin Production by Both Peanut Leaf Spotting Agents, *Cercospora arachidicola* and *Cercosporidium personatum*

M. K. Abo-El-Dahab, E. H. Wasfy, M. A. El-Goorani, H. M. El-Kasheir and E. E. Wagih

Dept. of Plant Pathology, College of Agriculture, University of Alexandria, Alexandria, Egypt

The involvement of microbial toxins in the pathogensis of many plant diseases has been repeatedly indicated by several investigators. However, little work has been carried out on the production and characterization of toxins produced by *Cercospora* spp. and related genera. Frandsen (1955), Schlosser (1962) and Schlosser (1971) were the first to report on the production of a toxin from *Cercospora beticola*, the causal agent of leaf spot disease of sugar beet and called it *Cercospora beticola* toxin (CBT).

Recently, Fajola (1978) isolated two entirely different compounds from several other *Cercospora* species by thin layer chromatography using ethyl acetate: methanol (4 : 1) as developing solvents. These two compounds banded as a yellow band ($R_f = 0.73$) and a red band ($R_f = 0.68$) which he named cercosporin. The newly discovered toxin (Cercosporin) was found to be produced by 12 isolates, out of 20 isolates of 17 cercospora species tested. In contrast, eight other isolates of eight different species produced no detectable cercosporin. According to the conidiospore morphology (Sobers, 1968; Deighton, 1967, 1973; Fajola, 1978*a*, *b*), it was found that the 12 cercosporin producing isolates were true *Cercospora* sp. whereas the 8 other isolates were not and might belong to other related genera such as *Cercosporidium* and *Cercosporella* (Fajola, 1978).

It was, therefore, of interest to study the reliability of Fajola's hypothesis that Cercosporin is a *Cercospora* specific toxin. In our study, we have compared the production of Cercosporin in six different isolates belonging to two well-identified genera, *Cercospora arachidicola* and *Cercosporidium personatum*.

## Materials and Methods

*Growth conditions*

Two sets each of three isolates belonging to either *Cercospora arachidicola* or *Cercosporidium personatum* were grown in petri dishes on Peanut seed extract-oatmeal agar medium (Abo-El-Dahab et al., 1982) and kept in a growth cabinet under continuous illumination emitted from fluorescent tubes (1000 lux).

*Extraction of the toxin*

Mycotoxins were extracted by grinding 15-day-old cultures in acetone. Pieces of gel and mycelium were pelleted by centrifugation at 2250 g (4,000 rpm using a bench type IEC centrifuge). The supernatants were saved and pellets were reextracted by the same solvent and subjected to the same centrifugal force. The resultant supernatants were added to the corresponding supernatants from the first extraction step. Acetone extracts were concentrated on a water bath at 40 °C. The process was accelerated by exposing the extracts to a stream of air. The concentrates (of the acetone extracts) were fractionated by thin layer chromatography using thin layer silica gel plates (20 cm × 20 cm × 0.25 mm) and ethyl acetate: methanol (4: 1, v/v) as a developing solvent system (Schlosser, 1971).

*Isolation and physical characterization of the toxin*

Thin layer silica gel plates were overloaded with the crude concentrate obtained from the Cercosporin-producing isolates. Following chromatography, the red band of the cercosporin toxin was isolated and eluted in acetone. The acetone solution of the red band was spectrophotometrically scanned over a wide range of visible and invisible light to determine the maximum absorption.

Table 1

The *in vitro* production of cercosporin by *Cercospora arachidicola*, isolates 7.10 and 11 and *Cercosporidium personatum*, isolates 1.2 and 4

| Fungus/isolate No. | Production of the red toxin |
|---|---|
| *Cercospora arachidicola* | |
| 7 | ± |
| 10 | + |
| 11 | − |
| *Cerosporidium personatum* | |
| 1 | − |
| 2 | +++ |
| 4 | − |

+ = The red toxin was produced as evidenced from the colour of the medium; the number of pluses indicates the degree of colour.
± = Red colour was doubtful.
− = No red colour was observed.

## Results

*Pigmentation of culture medium*

The results obtained from visual inspection of 15-day-old cultures, grown on slant agar, for the production of the red coloured cercosporin can be summarized in the following table.

Results indicate that not all tested isolates were able to colourize the surrounding medium. Only two isolates, isolate 10 of *C. arachidicola* and isolate 2 of *C. personatum*, could colourize the medium with varying degrees. The latter isolate was more active than the former in producing the red pigment. In contrast, isolate 11 of *C. arachidicola* and isolate 1 and 2 of *C. personatum* could not change the colour of the medium. Isolate 7 of *C. arachidicola* was doubtful in this respect.

*Separaton of Cercosporin*

Following thin layer chromatography of the crude acetone extracts, all isolates but isolate 11 of *C. personatum* produced a yellow ($R_f = 0.87 \pm 0.01$) and/or a red ($R_f = 0.83 \pm 0.01$) bant(s). The following table presents the obtained results.

Table 2

The visible bands observed on thin layer chromatograms for the crude acetone extracts of *Cercospora arachidicola*, isolates 7, 10 and 11 and *Cercosporidium personatum*, isolates 1, 2 and 4

| Band observed / Isolate No. | *Cercospora arachidicola* | | | *Cercosporidium personatum* | | |
|---|---|---|---|---|---|---|
| | 7 | 10 | 11 | 1 | 2 | 4 |
| Yellow band Rf. | 0.86 | 0.86 | — | 0.88 | 0.88 | — |
| Red band (cercosporin) Rf. | — | 0.81* | — | — | 0.84* | t0.82 |

* The cercosporin toxin is produced in large quantity.

It appears from table 2 that the only *Cercospora* isolate capable of producing the red toxin (Cercosporin) is isolate 10. In this isolate cercosporin was associated with the yellow compound. Isolate 7 of the same fungus produced the yellow compound only. Surprisingly enough isolate 11 of the fungus produced neither the yellow nor the red (Cercosporin) band.

In contrast, two isolates (2 and 4) of *Cercosporidium* produced the red toxin but with different degrees. Isolate 2 was the most active in producing the toxin which was accompanied by the yellow compound. It is worthwhile to mention here that isolate 2 of *Cercosporidium* produced the largest quantity of cercosporin as

compared with the quantities produced by the other cercosporin producing isolates (i.e. isolate 10 of *Cercospora* and isolate 4 of *Cercosporidium)*. Comparitively, isolate 4 produced the red toxin in much smaller quantities with no detectable amount of the yellow compound. Dissimilarly, isolate 1 produced the yellow compound but no cercosporin was detected.

*Physical characterization of the red toxin*

The red toxin produced by isolate 10 of *C. arachidicola* and isolates 2 and 4 of *C. personatum* were isolated after being separated by thin layer chromatography using ethyl acetate : methanol (4 : 1; v/v) as a developing solvent. The red band obtained from each of the three different isolates was eluted in acetone and the resultant pure toxin solution was scanned for absorbancy in the visible light region. The three toxin solutions were identical in that they showed a characteristic peak at 470 nm.

The red toxin was found soluble in aceton, fairly soluble in diethyl ether and ethyl alcohol and insoluble in water and petroleum ether.

## Discussion

Thin layer chromatography of the crude cultural acetone extract of some isolates of *Cercospora arachidicola* and *Cercosporidium personatum* has revealed the presence of at least two compounds. The two compounds appeared as a yellow band ($R_f = 0.87 \pm 0.01$) and a red band ($R_f = 0.83 \pm 0.01$). Although the $R_f$ values of the two bands are different from those of Fajola's, the difference between them (0.05) is almost the same as that between the two compounds isolated by Fajola. This may suggest that the two bands isolated in the present study are similar to those reported by Fajola and the discrepancy in the $R_f$ values could be due to different experimental conditions and/or to the use of different chemical patches. The red band isolated in this study seems to be identical to the cercosporin toxin isolated by Fajola, as both compounds showed maximum absorption at 420 nm and were insoluble in water and Petrolium ether, fairly soluble in diethyl ether and ethyl alcohol and soluble in acetone. The ability of some isolates (e.g. isolate 7 of *Cercospora* and isolate 1 of *Cercosporidium)* to produce only a single (yellow) band with $R_f$ value of $0.87 \pm 0.01$, may resemble the situation faced by earlier investigators who studied CBT (Frandsen, 1955; Schlosser, 1962, 1962, 1971). Although, there is little difference between the Rf value of the yellow band of our study ($R_f = 0.87$) and that reported for CBT ($R_f = 0.82$), the two compounds could be similar if not identical.

In the study carried out by Fajola (1978) it was found that out of 20 isolates of 17 species of *Cercospora* obtained from 16 different hosts, only 12 isolates were able to produce the red cercosporin toxin. He found that the 12 cercosporin-producing isolates belonged to the genus *Cercospora* as their conidia were acircular

with a dark hilum scar (Sobers, 1968; Deighton, 1967, 1973; Fajola, 1978*a*, 1978*b*). In contrast the eight other isolates were not true Cercospora. He suggested that the eight-isolates might belong to other related genera such as *Cercosporidium* or *Cercosporella.*

The three isolates (1, 2 and 4) of *Cercosporidium* used in the present investigation are not true *Cercospora* (Abo-El-Dahab et al., 1982) based on the criteria previously outlined by Sobers (1968) and Deighton (1967, 1973). However, two of these three isolates (2 and 4) were able, though with different degrees, to produce the red toxin (Cercosporin). The amount of cercosporin produced by isolate 2 of *Cercosporidium* was much greater than that produced by isolate 4 or even isolate 10 of *Cercospora.*

Since some isolates (1 and 4) of *C. personatum* (Table 1) did not change the colour of the culture medium red but gave a cercosporin band following TLC (Table 2), visual inspection of culture media for the production of the red colour cannot be taken as a sole evidence for the ability of a certain isolate to produce cercosporin in the medium.

On the basis of these results the production of cercosporin cannot, therefore, be taken as a reliable taxonomic criterion for Cercospora spp. a fact that strongly oppose the hypothesis put forward by Fajola (1978) that cercosporin is a cercospora specific toxin.

The inability of some isolates of *C. arachidicola* (isolates 7 and 11) *C. personatum* (isolate. 1) to produce cercosporin inspite of their virulence, as leaf spotting agents, rules out the involvement of Cercosporin in pathogenesis as was previosuly suggested by Fajola (1978).

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 279–286 (1986)*

# A Contribution to the Ecology of the Cucumber Mosaic Virus

ILSE HAACK

Institut für Phytopathologie der AdL der DDR
DDR 4320 Aschersleben, Theodor-Roemer-Weg

Samples have been collected from cultivated and wild plants in the region of Aschersleben and Dresden. Out of several thousands of samples 609 cucumber mosaic virus (CMV) isolates resulted, which were checked by symptomatology and additionally by serological double diffusion tests.

It could be demonstrated that CMV-N (= B) is by far predominating and can be considered endemic in the region of Aschersleben.

However, under special conditions in several crops the share of CMV-U (=C) may increase during summer up to more than 50%, as proved by characterization of CMV isolates collected in tobacco plots in Dresden.

Besides other reasons, this fact may be due to a natural selection of CMV strains on the basis of their different thermosensitivity.

There could not be found any strains specially adapted to either cultivated plants or weeds. Furthermore, there was no evidence of any special isolates causing epidemics.

As far known, strains of the cucumber mosaic virus (CMV) can be divided into two large groups, the so-called N (=B) and U (= C) groups (Richter et al., 1972; Marrou et al., 1975), which correspond with the two serological groups ToRS and DTL (Devergne and Cardin, 1973; Richter, 1983). However, until now poor information exists about the distribution of these two groups in various geographical areas with different climate.

Comprehensive and detailed investigations in this field have been done in the Avignon area in the South-East of France (Quiot et al., 1979) which suggest the "need to undertake similar studies in other regions" (Quiot, 1980). In the present paper we should like to give an account on the spread of the two groups under the environmental conditions of the Aschersleben region, which is located in the central part of the GDR.

## Materials and Methods

During three years, samples have been collected all the year round from cultivated, ornamental, and wild plants in the region of Aschersleben–Halle–Quedlinburg and from May till September in an experimental field of the tobacco breeding station in Desden–Zschieren with special regard to spinach and tobacco which, because of their high susceptibility to CMV, play the role of natural bait

plants. 609 CMV isolates were obtained and tested by symptomatology and serology.

Test plants were grown in an insect-proof greenhouse. Inoculation was done by rubbing the inoculum with glass spatulas onto leaves dusted with carborundum using 0.02 M phosphate buffer pH 7.2. As main differential host plants *Nicotiana tabacum* L. cv. 'Samsun' and *N. glutinosa* were used to distinguish the two groups CMV – N and CMV – U. By means of *Phaseolus vulgaris* L. and *Vigna unguiculata* (L.) Walp. the so-called legume strains (CMV – U/L) were separated from those strains not systemically infecting bean (CMV – U/nL).

Serological double diffusion tests were carried out in agar plates, the medium used being 1.2 g agar in 100 ml 0.02 M phosphate buffer pH 7.2, containing 0.9% NaCl, 0.025% Chelaplex III (EDTA) and 0.3% $NaN_3$ (Tochinara, 1970). Extracts were prepared by grinding the leaves in mortars in 0.5 M sodium citrate buffer pH 6.5, containing 0.001 M EDTA, 0.1% thioglycolic acid (TGA) and 0.3% $NaN_3$.

## Results

*Spinach.* Samples from spinach were collected in breeding and commercial crops near Aschersleben and Quedlinburg. In dependence on the season and the spinach variety the degree of CMV infection ranged between 0 and 100%. Most of the plants infected during late autumn died within the wintertime.

Out of 147 CMV isolates more than 90% belonged to the "normal" CMV – N group, even those isolates obtained from totally damaged overwintering spinach crops. Five isolates only were identified as CMV – U (Table 1).

Table 1

Characterization of CMV isolates from spinach

| Month of sampling | Group/subgroup of CMV | | | | Total |
|---|---|---|---|---|---|
| | N | U/nL | U/L | unknown | |
| I–III | 2 | 0 | 0 | 0 | 2 |
| IV–VII | 79 | 0 | 1 | 2 | 82 |
| IX–XII | 56 | 2 | 2 | 3 | 63 |
| Total | 137 | 2 | 3 | 5 | 147 |
| % | 93.2 | 1.4 | 2.0 | 3.4 | 100 |

*Tobacco.* Tobacco crops may be severely attacked by CMV, and various types of symptoms are to be observed (Hidaka and Tomaru, 1960; Häni, 1971; Moldovan, 1979). Contrary to spinach, which is cultivated during the cool months of the year, the vegetation period of tobacco encloses the warmest summer months. The length of the vegetation period and the largeness of the plants enable repeated

Table 2

Progress of infection by CMV strain groups within a plot of tobacco 'Samsun' at Aschersleben

| Date of sampling | Rate of infected plants* | Group/subgroup of CMV | | |
|---|---|---|---|---|
| | | N | U/nL | U/L |
| 1. VII. | 6/30 | 6 | 0 | 0 |
| 12. VIII. | 1/30 | 1 | 0 | 0 |
| 29. IX. | 22/30 | 19 | 1 | 2 |

* Numerator = number of plants infected, denominator = number of plants tested

sampling from the same plants and following up the development of the CMV populations during the season (Table 2).

It could be established that by the middle of July 20% of the plants were infected by CMV–N. In August, when the weather was very warm, almost no CMV could be detected. But at the end of September 70% of the plants proved to be infected by CMV, three of them by CMV–U strains.

Further study was undertaken in Dresden–Zschieren where around the tobacco experimental field of the breeding station several kinds of vegetables and ornamentals susceptible to CMV were grown in gardens and plastic shelters. Thus, a great number of CMV sources can be assumed. By randomly sampling during July and September 75 CMV isolates from tobacco were obtained. In both years during September more CMV–U isolates were identified than in July (Table 3).

Revising this tendency, in the next summer samples were collected monthly from 156 designed plants of six tobacco varieties and three *Nicotiana* species. 76 CMV isolates were obtained, the characterization of which is demonstrated in Table 4. At the end of summer in several plants formerly infected by CMV–N no

Table 3

Characterization of CMV isolates from tobacco plants (Dresden–Zschieren)

| Year | July Group/subgroup of CMV | | | September Group/subgroup of/CMV | | |
|---|---|---|---|---|---|---|
| | N | U/nL | U/L | N | U/nL | U/L |
| 1981 | 5 | 3 | 1 | 9 | 12 | 3 |
| 1982 | 18 | 1 | 3 | 9 | 6 | 5 |
| Total | 23 | 4 | 4 | 18 | 18 | 8 |

CMV or CMV – U alone or CMV – N + CMV – U were detected. This led in connection with a greater number of new CMV – U infections to an absolute and relative decrease of the CMV – N population.

Table 4

Development of the two CMV populations within a tobacco experimental field in Dresden – Zschieren

| Group/subgroup of CMV | Month of sampling | | | |
|---|---|---|---|---|
| | VI. | VII. | VIII. | IX. |
| N | 1 | 4 | 6 | 3 |
| U/nL | 0 | 13 | 13 | 26 |
| U/L | 0 | 0 | 2 | 1 |
| N + U | 0 | 1 | 1 | 5 |
| Number of isolates | 1 | 18 | 22 | 35 |

By these findings the results of the previous years were confirmed.

*Other hosts.* The occurrence of the two CMV strain groups was established likewise in various other hosts. Out of the isolates sampled all around the year in the farther distance of Aschersleben more than 90% belonged to CMV – N group (Table 5). On the contrary, within the 59 isolates obtained during the summer months in Dresden–Zschieren (from wild plants in May/June, from cultivated plants in July – September) both CMV groups were approximately equally distributed (Table 6).

## Discussion

The results of this paper confirm that the CMV strain groups N (= serotype ToRS) and U (= serotype DTL, Devergne and Cardin, 1973) may occur in agricultural and horticultural plants as well as in annual and perennial weeds. Though weeds in comparison with cultivated plants only show week or no symptoms, as a rule (cp. Tomlinson et al., 1970) no CMV isolates were found specialized either for cultivated plants or weeds – comparable with Harrison's (1981) "culpad" and "wilpad" viruses. Likewise, no special epidemic isolates, for instance in spinach, were isolated.

The fact that in the Aschersleben region during the three year studies CMV – N strains always were prevailing is in conformity with the observations of other authors (Richter et al., 1972; Weber, 1981; Weber personal information). CMV – N, therefore, is considered endemic in the region.

Investigations in Dresden–Zschieren indicated that under special conditions the share of CMV – U may increase by the end of summer up to more than 50%,

Table 5

Characterization of CMV isolates from various plant species (Aschersleben region)

| Host species | Number of isolates Group/subgroup of CMV | | | | Total |
|---|---|---|---|---|---|
| | N | U/nL | U/L | unknown | |
| Cultivated plants | | | | | |
| *Apium graveolens* | 1 | 0 | 0 | 0 | 1 |
| *Beta vulgaris* | 1 | 0 | 0 | 0 | 1 |
| *Brassica spec.* | 2 | 0 | 0 | 0 | 2 |
| *Cucumis sativus* | 8 | 0 | 0 | 0 | 8 |
| *Cucurbita pepo* | 5 | 1 | 1 | 0 | 7 |
| *Lactuca sativa* | 24 | 0 | 0 | 0 | 24 |
| *Lupinus luteus* | 11 | 0 | 0 | 0 | 11 |
| *Lycopersicon esculentum* | 5 | 2 | 0 | 0 | 7 |
| *Scorzonera hispanica* | 1 | 0 | 0 | 0 | 1 |
| Ornamental and official plants | | | | | |
| *Aquilegia vulgaris* | 0 | 0 | 2 | 0 | 2 |
| *Dahlia pinnata* | 3 | 0 | 0 | 0 | 3 |
| *Delphinium spec.* | 1 | 0 | 0 | 0 | 1 |
| *Digitalis purpurea* | 1 | 0 | 0 | 0 | 1 |
| *Echinops sphaerocephalus* | 0 | 0 | 1 | 0 | 1 |
| *Hesperis matronalis* | 0 | 1 | 0 | 0 | 1 |
| *Lupinus polyphyllus* | 0 | 0 | 4 | 0 | 4 |
| *Philadelphus falconeri** | 51 | 0 | 0 | 0 | 51 |
| *Valeriana officinalis* | 6 | 0 | 0 | 1 | 7 |
| Weeds | | | | | |
| *Alliaria petiolata* | 7 | 0 | 1 | 0 | 8 |
| *Amaranthus retroflexus* | 1 | 0 | 0 | 0 | 1 |
| *Chelidonium majus* | 1 | 0 | 0 | 0 | 1 |
| *Fumaria officinalis* | 1 | 0 | 0 | 0 | 1 |
| *Lycium barbarum* | 1 | 0 | 0 | 0 | 1 |
| *Melilotus officinalis* | 2 | 0 | 0 | 0 | 2 |
| *Mentha aquatica* | 1 | 0 | 0 | 0 | 1 |
| *Mercurialis annua* | 6 | 0 | 0 | 1 | 7 |
| *Senecio vulgaris* | 5 | 0 | 0 | 0 | 5 |
| *Sisymbrium altissimum* | 1 | 0 | 0 | 0 | 1 |
| *Solanum nigrum* | 5 | 1 | 0 | 0 | 6 |
| *Stellaria media* | 31 | 0 | 0 | 0 | 31 |
| *Trifolium pratense* | 0 | 0 | 1 | 0 | 1 |
| *Trifolium ochroleucum* | 1 | 0 | 0 | 0 | 1 |
| *Vicia dumetorum* | 1 | 0 | 1 | 0 | 2 |
| Total | 184 | 5 | 11 | 2 | 202 |
| % | 91.1 | 2.5 | 5.4 | 1.0 | 100 |

* From Berlin—Baumschulenweg

Table 6

Characterization of CMV isolates from various plant species (Dresden—Zschieren)

| Host species | Number of isolates Group/subgroup of CMV | | | | Total |
|---|---|---|---|---|---|
| | N | U/nL | U/L | un-known | |
| Cultivated and ornamental plants | | | | | |
| *Capsicum annuum* | 17 | 2 | 0 | 0 | 19 |
| *Cucumis sativus* | 2 | 17 | 0 | 2 | 21 |
| *Lycopersicon esculentum* | 0 | 1 | 0 | 0 | 1 |
| *Trifolium resupinatum* | 0 | 1 | 0 | 0 | 1 |
| *Zinnia elegans* | 0 | 4 | 0 | 1 | 5 |
| Wild plants | | | | | |
| *Galinsoga parviflora* | 2 | 0 | 0 | 0 | 2 |
| *Rorippa sylvestris* | 0 | 4 | 0 | 0 | 4 |
| *Senecio vulgaris* | 2 | 0 | 0 | 0 | 2 |
| *Stellaria media* | 4 | 0 | 0 | 0 | 4 |
| Total | 27 | 29 | 0 | 3 | 59 |
| % | 45.8 | 49.1 | 0 | 5.1 | 100 |

whereas the number of isolates belonging to CMV—N group decreases. This corresponds with the situation in South-East of France described by Quiot (1980). Because of the limited number of CMV—U isolates collected within the samples from the Aschersleben region this tendency is not so clearly to ascertain. Most of these CMV—U isolates, however, were obtained during late summer and autumn or from perennial and overwintering host plants, respectively.

As the main reason for this phenomenon the different thermosensitivity of both CMV groups can be supposed (Marchoux et al., 1976). So the thermostable CMV—U strains are more capable to overcome high summer temperatures than the thermolabile CMV—N strains (Quiot, 1980). As Douine et al. (1979) stated, at 22 °C tobacco cv. 'Xanthi n c.' containing CMV—N was partially protected against CMV—U infection, at 32 °C the protection disappeared. The natural selection effect was probably supported by the extraordinary high summer temperatures during the years 1982 and 1983.

Though from most other countries until now no detailed investigations about the CMV strain spectrum are submitted, observations of other authors suggest that the spread of the two main CMV strain groups depends on the climate of the region concerned. Already Hitchborn (1956) stated strong differences in virus multiplication between CMV strains isolated in regions with different summer temperatures. Thus, N strains seem to represent the normal CMV populations in countries with a cool, temperate climate (cp. "typical English field isolates", Hollings et al., 1968).

There is some indication that the higher the summer temperature of a region the more frequently U strains are to be found (Richter et al., 1972; Quiot, 1980). For instance, out of 72 CMV isolates from Hungarian pepper plants 55 were characterized by Tobias (1984) as CMV – N and 17 as CMV – U, respectively. Horváth and Beczner (1983) stated that U strains occur more frequently in Hungarian cucurbitaceae than N strains. CMV strains isolated from *Leguminosae* in Hungary without exception belonged to CMV – U (Horváth, 1983). García-Luque et al. (1983) in Spanish crops only identified U-strains, and Migliori et al. (1978) stated the "systemic Vigna strains" (CMV – U/L) to be the main CMV strains in Guadeloupe. Unfortunately, in most publications the type of CMV strains concerned is not reported.

In the course of our studies the share of legume strains within the CMV – U group (subgroup CMV – U/L) varied probably depending on the number of sources in the vicinity. Contrary to the statements of Horváth (1983) *Leguminosae* contained not only CMV – U, but according to Schmidt (1982) also CMV – N. Therefore, the term "bean strains" for the subgroup systemically infecting bean would be more correct than the term "legume strains".

The studies described refer above all to the region of Aschersleben located in the central part of GDR and a special location in the Dresden area. It would be desirable to get further results also from other geographical regions. From a practical point of view such research is important as the different properties of the strain groups must be taken into consideration in virus control as well as in breeding for resistance (Eisbein and Haack, 1985).

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 287—290 (1986)*

# Preservation of Plant Viruses by Chemical Dehydration Method

E. Pocsai

Laboratory of Virology, Plant Protection and Agrochemistry Centre of Ministry of Agriculture and Food, Velence, Hungary

Chemical dehydration method for the preservation of plant viruses has been already studied concerning alfalfa mosaic, arabis mosaic, cucumber mosaic, grapevine Bulgarian latent, plum pox, prunus necrotic ringspot, raspberry ringspot and tomato bushy stunt viruses.

These viruses were propagated in different host plants. The virus-infected leaves were taken off in optimal time after their inoculations. Virus infected leaf tissues were dehydrated in Petri-dishes over anhydrous calcium chloride as a desiccant. Petri-dishes were sealed by plastic sticky tape. Dehydrated virus isolates in Petri-dishes were stored in a refrigerator maintained at 4 °C.

Effectivity of the preservation of virus isolates was examined by bioassay. Alfalfa mosaic virus in *Chenopodium quinoa* leaf-tissues has been maintained its infectivity for more than seven years. *Arabis* mosaic virus and cucumber mosaic virus in cucumber leaves gave good results after three year storage. Plum pox virus (necrotic strain) was preserved in leaves of *Chenopodium foetidum*. This virus remained infective after five year preservation period, but its activity was reduced. Prunus necrotic ringspot virus had the shortest survival time. Some of its isolates had lost its infectivity within a single year.

Raspberry ringspot virus, grapevine Bulgarian latent virus and tomato bushy stunt virus in leaves of *Chenopodium quinoa* gave good symptomes on the same mentioned host plant after 16 months storage.

Chemical dehydration have long been used for storage of plant viruses in their host tissues. It is a simple but effective way of preservation of plant viruses. This method was introduced by McKinney (1947) and was described by McKinney and Silber (1968). McKinney et al. (1965) used calcium chloride for the dehydration of the virus-infected tissues, which was dehydrated in aluminium baking pans. No more than 50 g of infected leaf-tissue was put into a pan. The weight of calcium chloride in the bottom of the pan was at least double the weight of the water in the leaf tissue. Pans were closed tightly and placed in a refrigerator, where the temperature was 1 °C. After dehydration, the dehydrated leaf tissue was stored over anhydrous magnesium perchlorate in glass bottles.

Since that time, this method was developed and successfully applied for quite a number of viruses (McKinney et al., 1965, Bos 1969, 1973, 1977, Barrados 1978, Horváth and Besada 1980). Preservated virus-infected plant tissues proved to be suitable not only for inoculations but they were also successfully applied in serology (Scott, 1961, Purcifull and Gooding 1970, Purcifull et. al 1975, Bos and Benetti 1979.

The presently used procedure is almost the same as that described by Bos (1969), but virus infected plant tissues were kept over anhydrous calcium chloride in Petri-dishes throughout all the preservation period. This way of perservation is more simple and suitable for the virus storage. It saves virus transmission to living plants which consumes time and requires big areas in greenhouses.

## Materials and Methods

Effectivity of chemical dehydration method for preservation of plant viruses was studied on the following viruses: alfalfa mosaic, arabis mosaic, cucumber mosaic, grapevine Bulgarian latent, plum pox, prunus necrotic ringspot, raspberry ring spot and tomato bushy stunt.

Arabis mosaic virus, cucumber mosaic virus and prunus necrotic ringspot virus were propagated in cucumber plants, plum pox virus (necrotic strain) in *Chenopodium foetidum* and the others in *Chenopodium quinoa* plants. The infected leaves were taken off in optimal time after their inoculations. Virus infected leaves were dehydrated in 100 mm diameter Petri-dishes. About 20 g anhydrous calcium chloride (which was previously heated at 150 °C for 5 hours) was poured into the lower or upper part of the Petri-dish. A filter paper disc was cut out, which separated the desiccant from the leaves of the plant. The filter paper was perforated by a needle. In generaly, four leaves were put into a Petri-dish. The cucumber leaves were cut with shears int small pieces approximately 2–3 mm stripe.

The leaves of *Chenopodium foetidum* and *Chenopodium quinoa* were not cut before dehydration. The lower part of the Petri-dish was covered with another lower one.

Then Petri-dishes were sealed with plastic sticky tape, and storage was a 4 °C in a refrigerator where it remained until the end of the experiment.

Effectivity of the preservation of virus isolates was examined by bioassay. The preserved virus preparations were opened at the time of inoculation. For investigation of the infectivity of preserved viruses, the dried leaves were taken from the Petri-dish, ground in a mortar together with two ml of 0.02 M phosphate buffer (pH 8.0). Before inoculations, test plants were dusted with 600 mesh carborundum.

Alfalfa mosaic virus was investigated on *Phaseolus vulgaris*, plum pox virus on *Chenopodium foetidum*, arabis mosaic, cucumber mosaic and prunus necrotic ring spot viruses on *Cucumis sativus*, the other tested viruses on *Chenopodium quinoa*.

Effectivity was determined in terms of obtained symptoms on the test plants.

## Results

Obtained results of longevity test on virus species preserved by chemical dehydration are shown in Table 1. The chemical dehydration method gave good results with numerous plant viruses. If at least a double weight of calcium chloride

is used, the relative humidity of the air within the Petri-dish will not be too high. This humidity is enough for the good preservation of the virus. But, the relative humidity of air within the closed Petri-dish depends on the water content of calcium chloride, that is why, the calcium chloride was heated at 150 °C for five hours before use.

Table 1

Longevity of viruses in leaf tissue dehydrated over calcium chloride in Petri-dish and stored in refrigerator at 4 °C

| Virus | Propagative host | Assay plant | Data of preservation | Preservation period (months) | Activity of virus |
|---|---|---|---|---|---|
| Alfalfa mosaic virus | Chenopodium quinoa | Phaseolus vulgaris | 1974. III. 8. | 84 | medium |
| Arabis mosaic virus | Cucumis sativus | Chenopodium quinoa | 1977. X. 28. | 42 | medium |
| Cucumber mosaic virus | Cucumis sativus | Cucumis sativus | 1978. VIII. 9. | 32 | medium |
| Grapevine Bulgarian latent virus | Chenopodium quinoa | Chenopodium quinoa | 1979. IX. 21. | 16 | medium |
| Prunus necrotic ringspot virus | Cucumis sativus | Cucumis sativus | 1977. V. 30. | 8 | very low |
| Plum pox virus (necrotic strain) | Chenopodium foetidum | Chenopodium foetidum | 1975. VII. 21. | 55 | low |
| Raspberry ringspot virus | Chenopodium quinoa | Chenopodium quinoa | 1979. IX. 14. | 16 | medium |
| Tomato bushy stunt virus | Chenopodium quinoa | Chenopodium quinoa | 1979. IX. 4. | 16 | high |

According to results, shown in Table 1, alfalfa mosaic virus has maintained its infectivity after a preservation period of 84 months. Plum pox virus was well preserved in leaves of *Chenopodium foetidum*. This virus remained infective after five year preservation period. But the activity of the virus was reduced during this period.

Prunus necrotic ringspot virus had the shortest survival time. The infectivity of this virus was lost after 12 months.

Cucumber mosaic virus proved to be highly infective when its activity was investigated after a preservation period of 32 months. Horváth and Besada (1980) had examined the activity of 2 isolates of Cucumber mosaic virus, which were preserved over calcium chloride for 13 months. They found both isolates highly active.

Bos (1969) had examined 11 isolates of cucumber mosaic virus and they were infective after 2–10 year preservation period. According to McKinney et al. (1965), cucumber mosaic virus can survive in chemically dehydrated plant tissues even for 19 years.

Raspberry ringspot virus, tomato bushy stunt virus and grapevine Bulgarian latent virus were preserved at almost the same time in Chenopodium quinoa laeves. Their infectivity were tested on leaves of *Chenopodium quinoa*. We found that all the three viruses were active after a preservation period of 16 months.

Taking into consideration these obtained results one can conclude that chemical dehydration is considered to be the most simple and effective method for the preservation of plant viruses.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 291—295 (1986)*

# Occurrence of a Tobamovirus Similar to Ribgrass Mosaic Virus in Water of the Hungarian River Zala

N. JURETIĆ,[1] J. HORVÁTH[2] and M. KRAJAČIĆ[3]

University of Zagreb, Institute of Botany, YU-41000 Zagreb, Marulićev trg 20/II, Yugoslavia[1] and [3], and University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P.O. Box 71, Hungary[2]

An isolate (W) closely related to ribgrass mosaic virus (RiMV) was isolated from water samples of the river Zala in Hungary. The virus was transmitted from resuspended sediments of ultracentrifuged water samples to *Chenopodium murale* L. and *C. quinoa* Willd. Lesions formed in these plants were used as virus sources for inoculation to other test plants. The isolate was identified on the basis of reactions in test plants, particle morphology, occurrence and type of viral cell inclusion bodies and serological reactions.

Recently it was demonstrated (Tomlinson et al., 1982, 1983*a*, *b*; Tomlinson and Faithfull, 1984) that certain plant viruses, e.g. tobacco necrosis virus (TNV), tomato bushy stunt virus (TomBSV) and tobacco mosaic virus (TMV) could be isolated from lakes and rivers in various parts of the world. Other viruses have been found in German rivers and lakes (Koenig and Lesemann, 1984, 1985; Koenig, 1986) and in Italian rivers (Piazzolla et al., 1986).

Among tobamoviruses Van Dorst (1969) isolated cucumber green mottle mosaic virus (CGMMV) from drainage water from cucumber glasshouses in The Netherlands. TMV was isolated from river water in Yugoslavia, Italy and USA (Tošić and Tošić, 1984; Piazzolla et al., 1986). In addition, Koenig and Lesemann (1985) have found tobamoviruses in the German Federal Republic other than TMV but these isolates were not identified.

In this paper we report the isolation and identification of a tobamovirus from river water samples from Hungary.

## Materials and Methods

Water samples of 250 ml were ultracentrifuged for 120 min at 100 000 g using methods similar to those used by Koenig and Lesemann (1985). The resultant pellets were resuspended in a few drops of water and used to inoculate the leaves of *Chenopodium murale* L. and *C. quinoa* Willd. plants. Infected leaves with local lesions were then used as inoculum for transmission to other test plants. The virus isolate obtained was designated isolate W. Light microscopical analysis of the viral cell inclusions was accomplished on living cells. The virus was purified from tobacco (*Nicotiana tabacum* L.) by the method of Gooding and Hebert (1967) and serological tests were made using agar gel double-diffusion methods using

purified viruses. Isolate W was serologically compared with common TMV and the cruciferous isolate of RiMV – K described by Juretić et al. (1973) using antisera to TMV and RiMV – K.

## Results and Discussion

The W isolate was mechanically transmitted to various test plants. Symptoms produced in these plants are described in Table 1. The local infection of *Datura stramonium* L. and *Nicotiana glutinosa* L. suggested that W isolate was probably a tobamovirus. However, unlike common TMV it did not infect *Phaseolus vulgaris* L.

Table 1

Symptoms in test plants caused by the isolate W

| Plant species | Symptoms |
|---|---|
| *Chenopodium amaranticolor* Coste et Reyn. | necrotic local lesions and chlorotic zones, necrotic spots on top leaves with leaf distortion |
| *C. murale* L. | necrotic local lesions |
| *C. quinoa* Willd. | chlorotic local lesions |
| *Datura stramonium* L. | necrotic local lesions |
| *Nicotiana glutinosa* L. | necrotic local lesions |
| *N. megalosiphon* Heurck et Muell. | systemic necrotic spots, and mottling |
| *N. tabacum* L. cv. *Samsun* | systemic mosaic and mottling |
| *Phaseolus vulgaris* L. | no infection |

Light microscope analysis of infected cells of *Nicotiana megalosiphon* Heurck. et Muell. and *N. tabacum* cv. *Samsun* showed that the W isolate caused typical crystalline inclusions in form of rounded plates (Fig. 1) which are characteristic of some tobamoviruses, e.g. RiMV, CGMMV, sunn-hemp mosaic virus (SHMV) and Odontoglossum ringspot virus (ORV, cf. Miličić, 1969). The investigated isolate did not produce hexagonal prisms which are produced by TMV, tomato mosaic virus (ToMV) and some other tobamoviruses

On the basis of reactions on test plants (see Table 1) the W isolate could not be differed from RiMV. However, it could be differed from other tobamoviruses which also produce crystalline inclusions in form of rounded plates: unlike CGMMV the W isolate produced local and systemic symptoms in *Chenopodium amaranticolor* (Hollings et al., 1975), it failed to infect *Phaseolus vulgaris* as does SHMV (Kassanis and Varma, 1975) and it differed from ORV which does not infect *Datura stramonium* (Paul, 1975). Reactions in test species, therefore, indicated that W isolate is more related to RiMV (Oshima and Harrison, 1975) than to some other tobamoviruses.

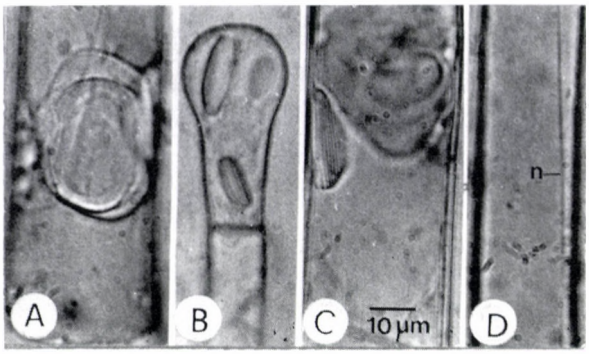



Fig. 1. Cell inclusions of the isolate W in tobacco hair cells. A: rounded plate in surface view with eccentric discs; B and C: rounded plates in side view; D: needle-shaped crystalls (n)

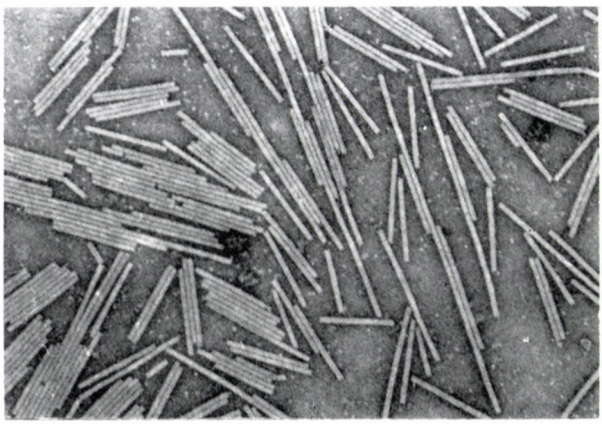


Fig. 2. Particles of the isolate W about 300 nm long in a partially purified preparation

Purified preparations of isolate W contained rod-shaped virus particles about 300 nm in length (Fig. 2). In gel diffusion tests where the W isolate was compared serologically with TMV using TMV-antiseum, a distinct precipitation spur was formed (Fig. 3). Also, in the majority of tests a spur was formed when the W isolate was tested against RiMV – K using RiMV – K antiserum. These spurs were always smaller than ones obtained during comparison of the W isolate with TMV. It was concluded, therefore, that the W isolate was serologically distinguishable from TMV as well as from RiMV – K. However, on basis of type cell inclusions and symptoms on test plants the W isolate seems to be more closely related to RiMV than to toher tobamoviruses. The extent of serological relationship between the W isolate and RiMV will be established when an antiserum to the W isolate will be at disposal.

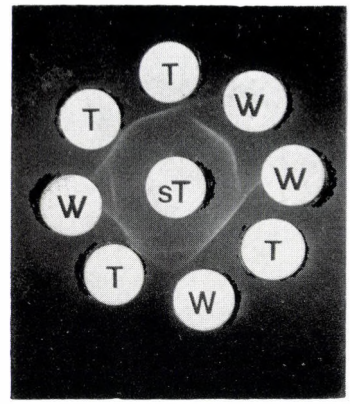



Fig. 3. Serological reactions in agar gel double-diffusion test obtained between the isolate W and TMV (T). sT: antiserum to TMV

The occurrence of RiMV in river water is not altogether surprising because RiMV naturally infects a wide range of wild plant species in various plant families (Juretić and Miličić, 1977) Since a number of viruses can be released from undamaged roots of infected plants into the soil and drainage water and persist for long periode (Koenig and Lesemann, 1985), it seems possible that one source of RiMV in river water is its release from of infected plants roots. However, sewage, waste material from various plants as well as compost may be other sources whereby RiMV can gain access to river water. The water samples tested contained great numbers of bacteria which sedimented together with virus during ultracentrifugation. The bacteria caused sharp local symptoms in some test plants making great difficulties in work with test plants. To eliminate most of the bacteria, a low-speed centrifugation of water samples is recommendable before ultracentrifugation.

## Acknowledgements

The authors wish to thank Dr. J. A. Tomlinson, National Vegetable Research Station, Wellesbourne, Warwick, England for critically reading the manuscript and correcting the English text. We are indebted to A. Škof, Zagreb and K. Molnár and É. Bősze, Keszthely for technical assistance.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 297–318 (1986)*

# Compatible and Incompatible Relations between *Phaseolus* Species and Viruses

## I. Review

J. HORVÁTH

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

In the first part of our publication series we reviewed the virus susceptibility and resistance of various *Phaseolus* plants. It was demonstrated that 137 viruses could infect 40 different *Phaseolus* plants. Of the viruses 35 are naturally occurring pathogens. The two most important viruses are bean common mosaic virus and bean yellow mosaic virus.

We also reviewed the virus resistance of various *Phaseolus* plants. As far as we know 20 different *Phaseolus* species are resistant to 93 viruses. In the incompatible host-virus relations very important are the plants resistant to bean common mosaic virus, bean yellow mosaic virus and cucumber mosaic virus.

The main tasks of research are to analyse the interactions between the resistance genes in bean plants, the pathogenicity of the different virus strains and the inheritance of the resistance genes.

Among the higher plants the family of *Leguminosae (Fabaceae)* which includes the *Phaseolus* genus is the largest family after the *Orchidaceae* and *Compositae*. Among the 17 thousand species of the about 700 genera belonging to the family of *Leguminosae (Fabaceae)* the *Phaseolus* species are very important from an economic point of view. Out of the about 200 perennial and annual *Phaseolus* species common in the tropical, subtropical and temperature zones *Phaseolus acutifolius*, *P. aureus*, *P. coccineus*, *P. lunatus*, *P. mungo* and *P. vulgaris* are of high importance. The dry seeds of these plants with their rich protein contents are important sources of food for man. According to the data of world literature the production area of dry-, green- and green shelled beans is more than 22 million hectares (Anonymous, 1975), even exceeding the area of potato world production.

### *Virus susceptibility of Phaseolus Species*

The efficiency of bean cultivation is adversely influenced by various diseases and insects. More than 250 diseases and more than 100 insect species have been identified that can cause economically significant damage to *Phaseolus vulgaris*, for instance (cf. Ruppel and Idrobo, 1962; Wellman, 1968; Mancia and Cortéz, 1975). The affinity of beans to plant viruses with different biological properties is very strong. According to our best knowledge the bean-virus relations cover 40 different *Phaseolus* plants and some 137 viruses (cf. Schmelzer and Pop, 1957; Thornberry, 1966; Gibbs et al., 1970–1984; Nene, 1972; Schmelzer et al., 1972; Edwardson, 1974; Provvidenti, 1976; Meiners et al., 1977; Schmelzer and Wolf,

1977; Schmidt, 1977; Schmidt et al., 1980; Jayasinghe, 1982; Horváth, 1982*a*, *b*, 1983*a*, *b*; Horváth and Beczner, 1982; Horváth et al., 1983;. Boswell and Gibbs, 1983; Cupertino et al., 1984; Horváth, 1985 and others; cf. Table 1). It is remarkable that so far about 35 viruses have been pointed out to occur spontaneously on bean plants. Mixed infections are not rare either; in his doctor's thesis Jayasinghe (1982) attributed the chlorotic mottle disease of bean to a complex infection by three viruses (bean mild mosaic virus, cucumber mosaic virus, Southern bean mosaic virus).

Among the viruses occurring in bean plants under natural conditions the bean common mosaic virus and bean yellow mosaic virus transmissible mechanically and by aphids in a non-persistant manner, and spread with the seeds of infected plants are highly important (Schmidt et al., 1981, 1987). The yield losses caused by these viruses considered as yet the most dangeorus virus pathogens of beans may even be 80 per cent in the case of bean common virus and 35 per cent with bean yellow mosaic virus (Taubitz, 1952; Kaiser et al., 1968;. Leon and Calot, 1973; Hampton, 1975; Schmidt and Schubert, 1978). Similarly remarkable is the cucumber mosaic virus as a recent pathogen of bean plants. The appearance of bean plants of biologically highly different cucumber mosaic virus strains and their transmission by bean seeds and aphids (cf. Marrou et al., 1969; Bird et al., 1974; Bos and Maat, 1974; Schmelzer and Schmidt, 1975; Provvidenti, 1976; Marchoux et al., 1977; Meiners et al., 1977; Babović et al., 1979; Fegla et al., 1980; Davis et al., 1981; Faccioli et al., 1982; Schmidt and Horváth, 1982; Jayasinghe, 1982) will probably lead to the spread of the virus and an increasing damage caused by it to bean cultures. According to Schmidt and Schubert (1978) the yield loss caused by cucumber mosaic virus may reach 38 per cent. Of all viruses known so far cucumber mosaic virus has the widest host range; covering more than 1000 annual, perennial and woody plants (cf. Horváth, 1976, 1979, 1980), and this makes the spontaneous infection of bean cultures possible. The problem of the cucumber mosaic virus resistance of bean varieties and the difficulties encountered in this field are of high concern for breeders both in Hungary and abroad. The difficulties are increased by the fact that resistance to cucumber mosaic virus has only been found in 5 *Phaseolus* spp. of the 195 bean lines tested so far (Provvidenti, 1976). Two of these species *(Phaseolus acutifolius* var. *latifolius* and *P. polyanthus)* were crossed with *Phaseolus vulgaris* with some success (Lorz, 1954; Honma, 1956). According to Meiners et al. (1977) *Phaseolus coccineus*, *P. lunatus* and 34 cultivars of *P. vulgaris* proved equally susceptible to the Puerto Rico strain of cucumber mosaic virus. In our own experiments various *Phaseolus* species (e.g. *P. aconitifolius*, *P. coccineus*, *P. lunatus*, *P. mungo*) and some 87 *Phaseolus vulgaris* varieties were found to be susceptible to cucumber mosaic virus (Horváth and Beczner, 1982; Horváth et al., 1983). In our opinion the alfalfa mosaic virus, a pathogen widespread in Hungary (cf. Beczner, 1973, 1974) and the peanut stunt virus that has recently appeared on *Trifolium pratense* in Hungary too (Beczner and Devergne, 1979) are highly important potential sources of infection. The bean pathogen cherry leaf roll virus has not caused so

Table 1

Virus susceptibility of *Phaseolus* species[1]

| *Phaseolus* species | Viruses |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* spp. *aborigineus)* | bean common mosaic virus* |
| | bean yellow mosaic virus |
| | cucumber mosaic virus |
| *P. aconitifolius* | alfalfa mosaic virus |
| | bean common mosaic virus* |
| | bean yellow mosaic virus* |
| | cucumber mosaic virus |
| | mung bean yellow mosaic virus |
| | plum line pattern virus |
| | rose mosaic virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | urd bean leaf crinkle virus |
| | urd bean mosaic mottle virus |
| *P. acutifolius* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean mild mosaic virus |
| | bean yellow mosaic virus |
| | cowpea mottle virus |
| | cucumber mosaic virus |
| | mung bean yellow mosaic virus |
| | pea common mosaic virus |
| | pea enation mosaic virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| *P. acutifolius* var. *acutifolius* | bean mild mosaic virus |
| | Southern bean mosaic virus |
| *P. acutifolius* var. *latifolius* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean mild mosaic virus |
| | bean yellow mosaic virus* |
| | clower (white) mosaic virus |
| | cucumber mosaic virus |
| | pea common mosaic virus |
| | Southern bean mosaic virus |
| | tobacco ringspot virus |
| *P. adenanthus* | cucumber mosaic virus |
| *P. angularis* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean yellow mosaic virus |
| | broad bean wilt virus |

[1] With asterisk designated viruses are naturally or spontan occurred pathogen on the mentioned *Phaseolus* plants.

Table 1 (continued 1)

| *Phaseolus* species | Viruses |
|---|---|
| | carrot mottle virus |
| | clover (white) mosaic virus |
| | cowpea aphid-borne mosaic virus |
| | cucumber mosaic virus |
| | plum line pattern virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | watermelon mosaic virus |
| *P. atropurpureus* | bean common mosaic virus* |
| | mung bean yellow mosaic virus* |
| | soybean mosaic virus |
| *P. aureus* (syn.: *P. radiatus*) | alfalfa mosaic virus |
| | apricot chlorotic leaf roll virus |
| | artichoke Italian latent virus |
| | bean common mosaic virus |
| | bean double mosaic virus |
| | bean yellow mosaic virus |
| | blackgram mottle virus |
| | broad bean wilt virus |
| | caraway yellow mottle virus |
| | carrot mottle virus |
| | chicory yellow mottle virus |
| | clover (white) mosaic virus* |
| | cowpea mosaic virus |
| | cowpea mottle virus |
| | cucumber mosaic virus |
| | elm mottle virus |
| | garden bean crinkle mosaic virus |
| | *Kennedya* yellow mosaic virus |
| | mung bean mosaic mottle virus |
| | mung bean yellow mosaic virus* |
| | pea enation mosaic virus |
| | plum line pattern virus |
| | Southern bean mosaic virus |
| | tobacco necrosis virus |
| | tobacco rattle virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | urd bean leaf crinkle virus |
| | urd bean mosaic mottle virus |
| *P. bracteatus* | cucumber mosaic virus |
| | plum line pattern virus |
| *P. calcaratus* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean yellow mosaic virus* |
| | broad bean wilt virus |
| | cucumber mosaic virus |
| | plum line pattern virus |
| | Southern bean mosaic virus |

Table 1 (continued 2)

| *Phaseolus* species | Viruses |
|---|---|
| | tobacco ringspot virus |
| *P. caracalla* | cucumber mosaic virus |
| *P. coccineus* (syn.: *P. multiflorus*) | alfalfa mosaic virus |
| | *Arabis* mosaic virus* |
| | bean common mosaic virus* |
| | bean yellow mosaic virus |
| | cowpea mosaic virus |
| | cucumber mosaic virus |
| | plum line pattern virus |
| | tobacco rattle virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | Southern bean mosaic virus |
| *P. coccineus* var. *albiflorus* | bean yellow mosaic virus |
| *P. coccineus* ssp. *coccineus* | bean mild mosaic virus |
| | cucumber mosaic virus |
| *P. coccineus* ssp. *polyanthus* | bean mild mosaic virus |
| | cucumber mosaic virus |
| *P. dumosus* (syn.: *P. lunatus*, *P. limensis*) | cucumber mosaic virus |
| *P. erythroloma* | cucumber mosaic virus |
| *P. filiformis* | bean common mosaic virus |
| | bean yellow mosaic virus |
| | cucumber mosaic virus |
| *P. formosus* | bean yellow mosaic virus |
| *P. lathyroides* | bean common mosaic virus |
| | bean yellow mosaic virus* |
| | cowpea aphid-borne mosaic virus |
| | cowpea mottle virus |
| | mung bean yellow mosaic virus |
| | passionfruit woodiness virus |
| | plum line pattern virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco ringspot virus |
| | watermelon mosaic virus |
| *P. lunatus* (syn.: *P. dumosus*, *P. limensis*) | *Abutilon* mosaic virus |
| | alfalfa mosaic virus |
| | apple stem growing virus |
| | bean common mosaic virus* |
| | bean double mosaic virus |
| | bean golden mosaic virus |
| | bean pod mottle virus |
| | bean yellow mosaic virus* |
| | beet curly top virus |
| | broad bean wilt virus |
| | *Canavalia* mosaic virus* |
| | clower (white) mosaic virus |
| | clower (red) mottle virus |
| | clower (red) vein mosaic virus |

Table 1 (continued 3)

| *Phaseolus* species | Viruses |
|---|---|
| | cowpea aphid-borne mosaic virus* |
| | cowpea mosaic virus |
| | cowpea mottle virus |
| | cucumber mosaic virus |
| | peanut mottle virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco mosaic virus |
| | tobacco necrosis virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | tomato ringspot virus |
| | *Wisteria* vein mosaic virus |
| *P. lunatus* var. *macrocarpus* | bean common mosaic virus |
| | clower (subterranean) mosaic virus |
| | cowpea aphid-borne mosaic virus |
| | cowpea mosaic virus |
| | cucumber mosaic virus* |
| *P. lunatus* × *P. lunatus* var. *macrocarpus* | bean pod mottle virus |
| | Southern bean mosaic virus |
| *P. multiflora* | tobacco ringspot virus |
| *P. mungo* (syn.: *P. radiatus*) | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean yellow mosaic virus* |
| | broad bean wilt virus |
| | *Canavalia* mosaic virus* |
| | carrot mottle virus |
| | clower (white) mosaic virus |
| | cowpea aphid-borne mosaic virus |
| | cowpea mottle virus |
| | raspberry bushy dwarf virus |
| | Southern bean mosaic virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| | urd bean leaf crinkle virus |
| *P. nigricans* | bean common mosaic virus |
| | soybean mosaic virus |
| *P. oblongus* | bean common mosaic virus |
| *P. panduratus* | cucumber mosaic virus |
| *P. phyllanthus* | cucumber mosaic virus |
| *P. pilosus* | cucumber mosaic virus |
| *P. polystachios* | cucumber mosaic virus |
| *P. radiatus* (syn.: *P. aureus*, *P. mungo*) | bean common mosaic virus* |
| | cucumber mosaic virus |
| *P. radiatus* var. *aureus* | bean common mosaic virus |
| *P. retusus* | alfalfa mosaic virus |
| *P. speciosus* | bean common mosaic virus |
| | soybean mosaic virus |
| *P. trilobatus* | cucumber mosaic virus |

Table 1 (continued 4)

| *Phaseolus* species | Viruses |
|---|---|
| *P. trilobus* | tobacco mosaic virus |
| *P. trinervius* | cowpea aphid-borne mosaic virus |
| *P. vulgaris* | *Abutilon* mosaic virus* |
| | alfalfa mosaic virus* |
| | apple mosaic virus |
| | apple stem growing virus |
| | apricot chlorotic leaf roll virus |
| | apricot ring pox virus |
| | *Arabis* mosaic virus |
| | artichoke Italian latent virus |
| | artichoke vein banding virus |
| | artichoke yellow ringspot virus |
| | *Aster* ringspot virus |
| | *Atropa* belladonna mosaic virus |
| | azuki bean mosaic virus |
| | bean common mosaic virus* |
| | bean golden yellow mosaic virus* |
| | bean leaf wilt virus |
| | bean mild mosaic virus* |
| | bean necrotic mosaic virus* |
| | bean pod mottle virus* |
| | bean yellow mosaic virus* |
| | beet curly top virus |
| | beet ringspot virus |
| | black raspberry latent virus |
| | blackgram mottle virus |
| | broad bean chlorosis virus |
| | broad bean mottle virus |
| | broad bean necrosis virus |
| | broad bean stain virus |
| | broad bean true mosaic virus |
| | broad bean wilt virus |
| | brome mosaic virus |
| | cabbage black ring virus |
| | cacao necrosis virus |
| | caraway yellow mottle virus |
| | carnation ringspot virus |
| | carrot mottle virus |
| | cehrry leaf roll virus |
| | chicory yellow mottle virus |
| | *Citrus* leaf rugose virus |
| | *Citrus* tatter leaf virus |
| | *Citrus* variegation virus |
| | *Clitoria* yellow vein virus |
| | clover (alsike) mosaic virus |
| | clover (white) mosaic virus* |
| | clover (red) mottle virus |
| | clover (red) necrotic mosaic virus |
| | clover (alsike) vein mosaic virus |

Table 1 (continued 5)

| *Phaseolus* species | Viruses |
|---|---|
| | clover (red) vein mosaic virus |
| | clover yellow mosaic virus |
| | clover yellow vein virus* |
| | cowpea aphid-borne mosaic virus |
| | cowpea mild mottle virus |
| | cowpea mosaic virus |
| | cowpea mottle virus |
| | cowpea severe mosaic virus* |
| | cucumber mosaic virus* |
| | *Cymbidium* ringspot virus |
| | *Desmodium* yellow mottle virus |
| | elm mottle virus |
| | *Euonymus* mosaic virus |
| | *Galinsoga* mosaic virus |
| | garden bean crinkle mosaic virus* |
| | grapevine fanleaf virus |
| | grapevine Hungarian chrome mosaic virus |
| | groundnut (peanut) stunt diesease virus* |
| | *Hippeastrum* tumor virus |
| | hop latent virus |
| | horsegram yellow mosaic virus* |
| | *Iris* ringspot virus |
| | *Kennedya* yellow mosaic virus |
| | lilac chlorotic leafspot virus |
| | lettuce mosaic virus |
| | lucerne mottle dwarf virus |
| | *Melandrium* yellow fleck virus |
| | milk vetch dwarf virus* |
| | mung bean mosaic mottle virus |
| | myrobalan latent ringspot virus |
| | narcissus latent virus |
| | narcissus mosaic virus |
| | okra mosaic virus |
| | onion yellow dwarf virus |
| | passionfruit woodiness virus* |
| | pea common mosaic virus |
| | pea early-browning virus* |
| | pea enation mosaic virus |
| | pea leaf roll virus* |
| | pea necrosis virus |
| | pea streak virus |
| | peach rosette mosaic virus |
| | peanut clump virus |
| | peanut green mosaic virus |
| | peanut mottle virus* |
| | *Pelargonium* zonate spot virus |
| | plum line pattern virus |
| | potato virus M |
| | potato virus T |

Table 1 (continued 6)

| *Phaseolus* species | Viruses |
|---|---|
| | *Prunus* necrotic ringspot virus |
| | quail pea mosaic virus |
| | raspberry bushy dwarf virus |
| | raspberry ringspot virus |
| | *Robinia* mosaic virus |
| | rose mosaic virus |
| | satellite virus* |
| | Southern bean mosaic virus* |
| | soybean dwarf virus* |
| | soybean mosaic virus |
| | spinach latent virus |
| | strawberry latent ringspot virus |
| | strawberry vein clearing virus |
| | subterranean clover stunt virus* |
| | sunn-hemp mosaic virus |
| | *Tephrosina* symptomless virus |
| | tobacco mosaic virus |
| | tobacco necrosis virus* |
| | tobacco rattle virus* |
| | tobacco ringspot virus* |
| | tobacco streak virus |
| | tobacco yellow dwarf virus |
| | tomato aspermy virus |
| | tomato black ring virus* |
| | tomato bushy stunt virus |
| | tomato mosaic virus |
| | tomato ringspot virus* |
| | tomato spotted wilt virus* |
| | tulare apple mosaic virus |
| | tulip halo necrosis virus |
| | tulip white streak virus |
| | turnip crinkle virus |
| | urd bean mosaic mottle virus |
| | watermelon mosaic virus |
| | *Wisteria* vein mosaic virus |
| *P. vulgaris* var. *aborigineus* | cucumber mosaic virus |
| *P. vulgaris* var. *humilis* | bean common mosaic virus |
| *P. vulgaris*× *P. coccineus* | bean yellow mosaic virus |

far spontaneous disease in bean plants – as far as we know – however, since there is a 12–40 per cent possibility of transmitting the virus with the seeds of bean (Lister and Murant, 1967), an occasional appearance of the virus in bean plants may represent a great danger. Almeida et al. (1984) found that the average yield of dry beans was reduced by 73 per cent in early and by 43 per cent in late bean golden mosaic virus infections under field conditions in Brazil. The spontaneous occurrence of viruses (Table 1) as well as the complex virus infections cause great

symptomatological difficulties in virus identification. The etiology of a relatively large number of diseases of virus character has not been sufficiently cleared up, and many economically important relations between viruses and beans are supposed to be unknown as yet. A large proportion of the relations between bean plants and viruses are, however, sufficiently known, and this fact makes it possible to identify the different viruses (cf. Schmelzer and Pop, 1957; Horváth, 1972*a*, *b*, Horváth and Beczner, 1972; Saettler and Trujillo, 1972; Beczner, 1973, 1974; Horváth 1976; Drijfhout, 1978). As it is known, the *Phaseolus vulgaris* cv. *Monroe* as effective local lesion host is excellently suitable for the identification of bean common mosaic virus (Saettler and Trujillo, 1972; Castano et al., 1982) or clover yellow vein virus (Dwadash-Shreni and Stavely, 1984), and the variety *Red Kidney* can be used to point out potato virus M (Hiruki, 1970). According to the data of Lima Neto (1984) the *Calima* bean variety is useful as a local lesion test plant for soybean mosaic virus. In the course of studying the responses of some 50 *Phaseolus vulgaris* varieties we found them suitable not only for the identification of many viruses but also for their differentiation (Horváth, 1972*a*, *b*, 1973, 1983*a*, 1985). It is necessary to clear up the insufficiently known host-virus relations and detect the unknown ones, because when knowing the conditions of reaction we can use the *Phaseolus* plants in plant virology diagnostics on the one hand, and as possible resistance gene carriers in breeding for virus resistance, on the other.

*Virus Resistance of Phaseolus Species*

To our best knowledge the resistante bean-virus relations cover 20 different *Phaseolus* plants and some 93 viruses (Table 2).

The breeding of bean for virus resistance began relatively early, in the 1910s in the United States of America. By the twenties two varieties (*Robust* and *Great Northern*) resistant to bean common mosaic virus were already subjected to selection. The first hybridization was carried out in the thirties, when by crossing the varieties *Wisconsin Refugee* and *Idaho Refugee* varieties resistant to bean common mosaic virus were produced (Pierce and Walker, 1933; Pierce, 1934). As a result of a thirty-year work of breeding since 1940 most of the bean variaties grown in the United States have been resistant to bean common mosaic virus (cf. Silbernagel and Zaumeyer, 1975 Zaumeyer and Meiners, 1975). Since 1958 no important data on the inheritance of resistance to bean common mosaic virus have been reported, except the result of the excelletn work of Drijfhout (1978). He gave new ideas of and contributed new results to the genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for virus strain identification and breeding for resistance. This work was of special importance because none of the earlier investigations had studied the inheritance of resistance to different bean common mosaic virus strains. According to the data of Schmidt et al. (1980) 308 bean common mosaic virus resistant *Phaseolus vulgaris* var. *nanus*, 65 bean common mosaic virus resistant *P. vulgaris* var. *vulgaris*, 16 bean yellow mosaic virus resistant *P. vulgaris* var. *nanus*, and 28 bean common mosaic virus and bean yellow

Table 2

Insusceptible *Phaseolus* species to viruses

| *Phaseolus* species | Viruses |
|---|---|
| *Phaseolus aconitifolius* | bean common mosaic virus |
| | bean mild mosaic virus |
| | bean yellow stipple virus |
| | cowpea mosaic virus |
| | pea mosaic virus |
| *P. acutifolius* var. *acutifolius* | cucumer mosaic virus |
| *P. acutifolius* var. *latifolius* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean yellow mosaic virus |
| | clover (red) mosaic virus |
| | cucumber mosaic virus |
| | pea streak virus |
| *P. angularis* | bean common mosaic virus |
| | bean mild mosaic virus |
| | bean pod mottle virus |
| | bean yellow mosaic virus |
| | bean yellow stipple virus |
| | clover (red) mosaic virus |
| | pea streak virus |
| | Southern bean mosaic virus |
| | tobacco ringspot virus |
| | tobacco streak virus |
| *P. anisotrichus* | bean mild mosaic virus |
| | cucumber mosaic virus |
| *P. aureus* (syn.: *P. radiatus)* | bean common mosaic virus |
| | bean pod mottle virus |
| | bean yellow mosaic virus |
| | bean yellow stipple virus |
| | broad bean mottle virus |
| | clover (alsike) mosaic virus |
| | clover (red) vein mosaic virus |
| | cowpea mosaic virus |
| | pea enation mosaic virus |
| | pea mosaic virus |
| | pea streak virus |
| | potato aucuba mosaic virus |
| | potato virus Y |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco etch virus |
| | tobacco mosaic virus |
| *P. calcaratus* | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean yellow mosaic virus |
| | clover (red) mosaic virus |
| | cucumber mosaic virus |
| | mung bean leaf crinkle virus |

Table 2 (continued 1)

| *Phaseolus* species | Viruses |
|---|---|
| | pea streak virus |
| | tobacco streak virus |
| | urd bean leaf crinkle virus |
| *P. coccineus* (syn.: *P. multiflorus*) | *Anemone* brown ring virus |
| | bean pod mottle virus |
| | bean yellow mosaic virus |
| | bean yellow stipple virus |
| | beet mosaic virus |
| | cauliflower mosaic virus |
| | cucumber mosaic virus |
| | pea mosaic virus |
| | pea virus 2 |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco etch virus |
| | tobacco mosaic virus |
| | tobacco rattle virus |
| | turnip mosaic virus |
| | urd bean leaf crinkle virus |
| *P. coccineus* var. *albiflorus* | bean yellow mosaic virus |
| *P. coccineus* var. *coccineus* | Southern bean mosaic virus |
| *P. coccineus* var. *polyanthus* | Southern bean mosaic virus |
| *P. dumosus* (syn.: *P. lunatus*, *P. limensis*) | cucumber mosaic virus |
| *P. filiformis* | bean mild mosaic virus |
| *P. lathyroides* | bean yellow stipple virus |
| | cucumber mosaic virus |
| | tobacco streak virus |
| | urd bean leaf crinkle virus |
| *P. limensis* (syn.: *P. dumosus*, *P. lunatus*) | bean yellow mosaic virus |
| | *Canna* mosaic virus |
| | cucumber mosaic virus |
| | *Datura* distortiion mosaic virus |
| | pea mosaic virus |
| | tobacco etch virus |
| | tobacco mosaic virus |
| | tomato ringspot virus |
| *P. limensis* var. *limenanus* | squash mosaic virus |
| *P. lunatus* (syn.: *P. dumosus*, *P. limensis*) | alfalfa mosaic virus |
| | bean common mosaic virus |
| | bean mild mosaic virus |
| | bean yellow mosaic virus |
| | bean yellow stipple virus |
| | beet yellows virus |
| | belladonna mottle virus |
| | cacao swollen shoot virus |
| | clover (alsike) mosaic virus |
| | clover (red) mosaic virus |
| | cowpea mosaic virus |
| | cucumber mosaic virus |

Table 2 (continued 2)

| *Phaseolus* species | Viruses |
|---|---|
| | mung bean yellow mosaic virus |
| | muskmelon mosaic virus |
| | papaw mosaic virus |
| | pea streak virus |
| | potato virus Y |
| | potato yellow dwarf virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | urd bean leaf crinkle virus |
| *P. lunatus* var. *macrocarpus* | alfalfa mosaic virus |
| | bean pod mottle virus |
| | bean yellow stipple virus |
| | clover (red) mosaic virus |
| | *Datura* distortion mosaic virus |
| | pea streak virus |
| | Southern bean mosaic virus |
| *P. mungo* (syn.: *P. radiatus)* | bean common mosaic virus |
| | bean pod mottle virus |
| | bean yellow mosaic virus |
| | clover (red) mosaic virus |
| | cowpea mosaic virus |
| | pea streak virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | tobacco streak virus |
| *P. polyanthus* | cucumber mosaic virus |
| *P. trilobus* | cowpea mosaic virus |
| | mung bean yellow mosaic virus |
| *P. vulgaris* | *Abutilon* infectious variegation virus |
| | alfalfa mosaic virus |
| | *Anemone* brown ring virus |
| | *Anemone* mosaic virus |
| | barley stripe mosaic virus |
| | bayberry yellow virus |
| | bean yellow mosaic virus |
| | beet curly top virus |
| | beet mosaic virus |
| | beet yellows virus |
| | beet yellow wilt virus |
| | belladonna mottle virus |
| | *Bidens* mottle virus |
| | broad bean mottle virus |
| | cacao swollen shoot virus |
| | carnation mosaic virus |
| | carnation mottle virus |
| | carnation vein mottle virus |
| | cauliflower mosaic virus |
| | chilli (pepper) mosaic virus |
| | *Chrysanthemum* B (mild mosaic) virus |

Table 2 (continued 3)

| *Phaseolus* species | Viruses |
|---|---|
| | *Chrysanthemum* latent virus |
| | *Chrysanthemum* ringspot virus |
| | *Chrysanthemum* stunt virus |
| | *Citrus* tristeza virus |
| | clover (red) mosaic virus |
| | clover (red) vein mosaic virus |
| | clover yellow vein virus |
| | cowpea mosaic virus |
| | cucumber mosaic virus |
| | *Datura* distortion mosaic virus |
| | *Desmodium* mosaic virus |
| | dodder latent virus |
| | eggplant mosaic virus |
| | filaree red leaf virus |
| | *Hibiscus* yellow vein virus |
| | *Hydrangea* ringspot virus |
| | lettuce mosaic virus |
| | mung bean yellow mosaic virus |
| | muskmelon mosaic virus |
| | *Nicotiana glutinosa* root necrosis virus |
| | *Nothoscordum* mosaic virus |
| | onion yellow dwarf virus |
| | orchid *(Cymbidium)* mosaic virus |
| | orchid *(Vanda)* mosaic virus |
| | papaw mosaic virus |
| | pea die back virus |
| | pea enation mosaic virus |
| | pea mosaic virus |
| | pea stem streak virus |
| | pea streak virus |
| | pea wilt virus |
| | pea yellow mosaic virus |
| | *Pelargonium* ringspot virus |
| | pigeon pea sterility mosaic virus |
| | potato virus S |
| | potato virus X |
| | potato virus Y |
| | radish mosaic virus |
| | red currant ringspot virus |
| | ribgrass mosaic virus |
| | Southern bean mosaic virus |
| | soybean mosaic virus |
| | squash mosaic virus |
| | stone fruit virus |
| | sweet potato mosaic virus |
| | tobacco coarse etch virus |
| | tobacco etch virus |
| | tobacco green mosaic virus |
| | tobacco mosaic virus |

Table 2 (continued 4)

| *Phaseolus* species | Viruses |
|---|---|
| | tobacco ringspot virus |
| | tomato aspermy virus |
| | tomato mosaic virus |
| | tomato ringspot virus |
| | tomato spotted wilt virus |
| | turnip crinkle virus |
| | turnip rosette virus |
| | turnip yellow mosaic virus |
| | urd bean leaf crinkle virus |
| | *Wisconsin* pea streak virus |
| *P. vulgaris* var. *aborigineus* | bean mild mosaic virus |

mosaic virus resistant *P. vulgaris* var. *nanus* are known in the world as yet. However, the resistance of these plants to viruses is, in fact, a transition between tolerance and resistance. Further, we have to point out that the resistant varieties are resistant only to a certain strain of a virus given.

According to our knowledge extensive research work concerning the virus resistance of bean is being carried on in the United States, South America, the Netherlands and the German Democratic Republic (cf. Zaumeyer and Meiners, 1975; Drijfhout, 1978; Schmidt et al., 1980; Anonymus, 1983). An immediate task is to bring about resistance to bean common mosaic virus, bean yellow mosaic virus, beet curly top virus, bean pod mottle virus, tobacco ringspot virus, bean chlorotic mottle virus and bean golden mosaic virus. The main objective is, however, to develop resistance to bean common mosaic virus and bean yellow mosaic virus. A very important source of resistance to bean yellow mosaic virus was detected by Provvidenti and Schroeder (1973) *Phaseolus vulgaris* cv. *Great Northern 1140*. For the resistance a single recessive gene (by-3) is responsible. Tu (1981, 1983) pointed out resistance to the necrotic strain of bean yellow mosaic virus and severe bean strain of tobacco ringspot virus in *Phaseolus vulgaris* cv. *Kentwood*. Breeding for resistance to bean golden mosaic virus began in 1975 with the evaluation of germplasm accessions in Central America and Brazil (Anonymous, 1983). Most bean golden mosaic virus resistant varieties developed by CIAT (Centro International de Agricultura Tropical, Cali, Colombia) have proved to be also resistant to bean chlorotic mottle virus, which, like the bean golden mosaic virus, is transmitted by the whitefly.

The current methods of breeding for virus resistance fundamentally differ from those used in earlier years. The changed view and techniques of virus strain determination render it possible to study the resistance on the host plant and differentiate the virus strains simultaneously. The genetic study of host plants is only possible when sufficient information is available for the differentiation of the virus

strains. The scientific work that demands an international cooperation consists of three phases: (*1*) establishing the interactions of resistance genes in bean plants, (*2*) determining the pathogenicity of genes occurring in the different virus strains, (*3*) pointing out the inheritance of the resistance genes. In this extensive scientific work important role is played by the Centro Internacional de Agricultura Tropical (Cali, Colombia) which possesses some 32,532 *Phaseolus* accessions, and the US Deartment of Agriculture, Plant Introduction Station (Pullman, Washington) where about eight thousand lots of bean (varieties and wild species) can be found, on the one hand, and by the International Working Group for Legume Viruses which makes it possible to standardize the viruses and determine the differences in their strains, on the other hand. The international gene bank of plants (*Phaseolus* spp.) and virus strains enables a successful virological and genetic work to which Hungary most also contribute. In the identification and analysis of viruses and virus strains pathogenic for beans great progress has recently been made (Hubbeling, 1963, 1972; Silbernagel, 1966, 1969; Moreno et al., 1968; Beczner, 1968; Alconero et al., 1972; Drijfhout and Bos, 1977; Drijfhout, 1978; Schmidt, 1982; Hampton et al., 1983; Schmidt and Weissenstein, 1983; Schmidt and Zobywalski, 1984).

In the course of breeding beans for virus resistance a basic task is to seek out the resistant initial forms, determine the types of resistance and find the way of transferring the resistance genes into cultivated bean plants. Breeding for resistance requires internationally determined viruses (virus strains). There are different types of virus resistance (tolerance, infection resistance, hypersensitivity, immunity, field- or epidemiological resistance; cf. Horváth, 1968, 1984; Schmidt et al., 1980). Resistance can be transmitted by one or more dominant major genes and by polygenes (cf. Ali, 1950; Hubbeling, 1972; Walkey and Innes, 1979; Innes and Walkey, 1979, 1980).

In his paper Drijfhout (1978) points out that the resistance to bean common mosaic virus is controlled by at least two genes indicated as *a*/A and *s*/S, and I/1 for systemic necrosis. Whether a plant will show systemic mosaic or systemic necrosis depends on the combinations of recessive and dominant alleles of these three genes, on the virus strain used, and perhaps to some extent on the climatic conditions during the tests.

Investigations into the *Phaseolus*-virus relations have mostly consisted of the examination of varieties (e.g. Beczner, 1968; Hubbeling, 1972; Horváth and Beczner, 1972, 1983; Horváth, 1972*a*, *b*; Nene, 1972; Provvidenti and Schroeder, 1973; Bos and Maat, 1974; Zaumeyer and Meiners, 1975; Drijfhout, 1978; Walkey and Innes, 1978, 1979; Innes and Walkey, 1979; Tu, 1980; Schmidt et al., 1981; Cupertino et al., 1982; Jayasinghe, 1982; Horváth, 1983*a*, *b*; Horváth et al., 1983; Dwadash–Shreni and Stavely, 1984; Horváth, 1985). Recently increasing attention has been paid to the wild *Phaseolus* species (Schmelzer and Pop, 1957; Dickson and Natti, 1968; Provvidenti, 1976; Meiners et al., 1977; Walkey and Taylor, 1979; Horváth, 1982*a*, *b*, 1983*a*, *b*; Jayasinghe, 1982; Schmidt and Weissenstein, 1983; Horváth, 1985). Experiments carried out so far concerning the resistance of wild

species suggest that American species are more resistant to New World diseases, and Asiatic species to Old World diseases (cf. Leppik, 1970).

We think that by describing new relations between *Phaseolus* species and viruses we supply useful information for the diagnostics of plant virology and for the work of breeding for virus resistance. In our experiments we set the aim of examining *Phaseolus* species known in plant virology for responses to viruses not studied so far on the one hand, and testing new *Phaseolus* species for responses to viruses of which there are no data in world literature, on the other.

In the second part of this study series we are going to deal with the new compatible host-virus relations, and in the third part with the incompatible relations.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 319—327 (1986)*

# Compatible and Incompatible Relations between *Phaseolus* Species and Viruses

## II. New Compatible Host-Virus Relations (Susceptible Plants)

J. HORVÁTH

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

In the present second part of the publication series responses of 11 *Phaseolus* species and 5 *Phaseolus* varieties to 8 viruses are discussed. Between the *Phaseolus* plants and viruses examined 49 local and systemic relations were established. Special attention is worth being paid to the virus susceptibility of those *Phaseolus* plants (*Phaseolus caffer*, *P. delphus*, *P. gonospermus*, *P. hysterinus*, *P. riccardianus*, *P. semierectus*, *P. tuberosus*, *P. vulgaris* var. *nanus-nonscandens*) of which — to our best knowledge — no relevant data are found in the literature.

Among the new host-virus relations remarkable is the susceptibility of the *Phaseolus* species to cucumber mosaic virus. No less importance is attached to the detection of a new host-virus relation between *Phaseolus* species (e. g. *Phaseolus coccineus*, *P. gonospermus*, *P. hysterinus*, *P. lunatus*, *P. mungo*, *P. semierectus*, *P. tuberosus*) and peanut stunt virus.

In the first part of the publication series we gave a survery of the virus susceptibility and virus resistance of *Phaseolum* species. We pointed out that some 40 different *Phaseolus* plants had so far been known to be susceptible to 137 viruses; 35 of the 137 viruses infected the *Phaseolus* plants under natural conditions. Incompatible host-virus relations were established in the case of 20 different *Phaseolus* plants and 93 viruses (Horváth, 1986*b* and references therein).

Considering that the *Phaseolus* species are highly important from the point of view of virus diagnosis (cf. Hiruki, 1970; Horváth, 1972*a*, *b*, 1973; Saettler and Trujillo, 1972; Castaño et al., 1982; Horváth, 1983*a*, *b*; Horváth and Beczner, 1983; Lima Neto, 1984; Dwadash—Shreni and Stavely, 1984; Horváth, 1985) and virus resistance (e.g. Provvidenti and Schroeder, 1973; Zaumeyer and Meiners, 1975; Provvidenti, 1976; Drijfhout, 1978; Schmidt et al., 1980; Horváth and Beczner, 1982; Schmidt and Weissenstein, 1983), for some years we have studied the responses of virologically little or not at all known *Phaseolus* species and accessions to various viruses. Our experiments were aimed at supplying new information on the virus susceptibility of some *Phaseolus* species on the one hand, and pointing out new resistant host-virus relations, on the other. Present paper deals with the new susceptible host-virus relations. A well-known characteristic of the compatible (susceptible) host-virus relations is that the plants inoculated with the virus become infected and the virus multiplies and spreads in them (cf. Provvidenti, 1976; Provvidenti et al., 1978; Cooper and Jones, 1983; Gáborjányi and Tóbiás, 1984; Horváth, 1986*a*).

## Materials and Methods

In the course of our experiments we studied 11 *Phaseolus* species and 5 *Phaseolus* varieties for response to 8 viruses (Tables 1 and 2). Primary leaves of bean plants were inoculated with the carborundum spatula technique. Evaluation was carried out 3–10, then 20–30 days after inoculation. The virus was then reisolated from both the inoculated and the subsequently developed leaves to virus specific indicator plants (cf. Horváth, 1976, 1977).

Table 1

*Phaseolus* species and varieties investigated in the experiments

| *Phaseolus* species and varieties[1] | Source[2] |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* spp. *aborigineus)* | Hau |
| *P. caffer* (syn.: *Vigna capensis)** | K |
| *P. coccineus* (syn.: *P. multiflorus)* | Hal, K, Le, Lo, N, V |
| *P. coccineus* var. *albiflorus* | G |
| *P. coccineus* var. *bicolor* | G |
| *P. coccineus* var. *coccineus* | G |
| *P. coccineus* var. *rubronanus* | G |
| *P. delphus** | K |
| *P. gonospermus** | Hau |
| *P. hysterinus** | Hau, N, V |
| *P. lunatus* (syn.: *P. dumosus, P. limensis)* | C, G, Hau, K, Le, O |
| *P. mungo* (syn.: *P. radiatus)* | C, Hau, K, Lo, V |
| *P. riccardianus** | K, Le, V |
| *P. semierectus** | G, Hau |
| *P. tuberosus** | K, V |
| *P. vulgaris* var. *nanus-nonscandens** | N |

[1] Plants marked with asterisks are species new to experimental virology.

[2] Abbreviations used for sources of seed: *C* = Jardin Botanique de Caen (*C*aen, France), *G* = Zentralinstitut für Genetik und Kulturpflanzenforschung (*G*atersleben, German Democratic Republic), *Hal* = Botanischer Garten, Sektion Biowissenschaften (*Hal*le, German Democratic Republic), *Hau* = Universitetets Botaniske Have (Cobenhavn K, Danmark) *K* = Lietuvos TSR Mokslu Akademijos Botanikos, Instituto Botanikos Sodo (*K*aunas, Soviet Union), *Le* = Botanischer Garten, Sektion Biowissenschaften, Karl-Marx-Universität (*Le*ipzig, German Democratic Republic), *Lo* = University of London, Botanical Supply Unit (*Lo*ndon, England), *N* = Botanische Tuin van de Universiteit te Nijmegen (*N*ijmegen, The Netherlands), *O* = Botanical Gardens, University of Oulu (*O*ulu, Finland), *V* = MTA, Botanikai Kutató Intézet (*V*ácrátót, Hungary).

Table 2

Viruses used in the experiments

| Viruses | Strains or isolates | Literature |
|---|---|---|
| *Arabis* mosaic virus | Phil. 2 | Schmelzer (1968, 1974) |
| Bean common mosaic virus | F23 | Horváth (1976, 1977) |
| Bean yellow mosaic virus | S | Horváth (1976) |
| Carnation ringspot virus | GDR | Fritzsche and Schmelzer (1967) |
| Cucumber mosaic virus | U/246 | Schmidt and Horváth (1982) |
| Peanut stunt virus | Tp | Beczner and Devergne (1979) |
| Tobacco rattle virus | H | Horváth (1976, 1977) |
| Tomato ringspot virus | Bonn | Nienhaus (Virus gene bank, Bonn, GFR) |

## Results and Discussion

Between the 11 *Phaseolus* species and 5 *Phaseolus* varieties on the one hand, and the 8 viruses on the other, discussed in this paper we detected 49 local and systemic compatible host-virus relations characterized by chlorotic and/or necrotic lesions (in some cases rings or veinal necrosis) appearing on the inoculated leaves, and by chlorotic and/or nectrotic lesions, mosaic, epinasty, malformation, rings, tumours, veinal necrosis and top necrosis on the subsequently developed leaves. In some host-virus relations total (local and systemic) latency could be observed: *Phaseolus caffer*, *P. coccineus* var. *bicolor* – cucumber mosaic virus, (see Table 3).

The virus susceptibility of *Phaseolus aborigineus* is relatively little known by the literature. Besides its susceptibility to bean common mosaic virus, bean yellow mosaic virus and cucumber mosaic virus (cf. Provvidenti, 1976; Horváth, 1986*b*) we have lately found the above plant to be susceptible to carnation ringspot virus (see Table 3). Considering that Provvidenti (1976) pointed out differences in the susceptibility of various *Phaseolus* species to two cucumber mosaic virus strains, in some cases we studied the responses of *Phaseolus* species to the Hungarian bean strain of cucumber mosaic virus too. In our experiments *Phaseolus aborigineus* (see Table 3), *P. aconitifolius*, *P. angularis*, *P. coccineus* and *P. lunatus* (Horváth, unpublished results) equally responded with local lesions and systemic mosaic to infection by the bean strain of cucumber mosaic virus. In the course of experiments with the above *Phaseolus* species Provvident (1976) found that while the isolate of cucumber mosaic virus obtained from *Cucurbita pepo* var. *Zucchini* (CMV – B) caused systemic mosaic to the plants (to the P. I. 201389 accession of *Phaseolus coccineus* necrotic local lesions too), the cucumber mosaic virus isolated

Table 3

Reactions of *Phaseolus* species and varieties to various viruses

| *Phaseolus* species and varieties[1] | Viruses[2] and symptoms[3] |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* spp. *aborigineus)* | CaRSV: NLL(ri), SN(ri, vn) |
| *P. caffer* (syn.: *Vigna capensis)** | BCMV: LL, SM<br>BYMV: NLL(vn), SM<br>CMV: LL, SM |
| *P. coccineus* (syn.: *P. multiflorus)* | PSV: CLL, SM(mal) |
| *P. coccineus* var. *albiflorus* | BCMV: LL, SM |
| *P. coccineus* var. *bicolor* | BCMV: NLL, SM<br>BYMV: NLL, SM<br>CMV: LL, LS<br>CaRSV: NLL, SN(ri, vn) |
| *P. coccineus* var. *coccineus* | BCMV: NLL, SM<br>BYMV: NLL, SM |
| *P. coccineus* var. *rubronanus* | BCMV: NLL(vn), SM<br>BYMV: NLL, SM |
| *P. delphus** | CaRSV: C-NLL(ri), C-NSL(ri, vn) |
| *P. gonospermus** | BCMV: NLL, SM(mal)<br>BYMV: LL, SM<br>CMV: NLL, SM<br>CaRSV: C-NLL, SN(ri, vn)<br>PSV: C-NLL(ri), SM(mal)<br>TomRSV: NLL, SN |
| *P. hysterinus** | AraMV: LL, SM(mal)<br>BCMV: C-NLL, SM<br>BYMV: C-NLL, SM<br>CMV: NLL(vn), SM<br>CaRSV: LL, SN(ri, vn)<br>PSV: C-NLL, SM(ep) |
| *P. lunatus* (syn.: *P. dumosus*, *P. limensis)* | CaRSV: C-NLL(ri), SN(ri, vn)<br>PSV: CLL, SM(ml) |
| *P. mungo* (syn.: *P. radiatus)* | CMV: C-NLL, SN<br>CaRSV: C-NLL, SN<br>PSV: C-NLL, SN(tn, tu)<br>TRV: C-NLL, SN<br>TomRSV: NLL, SM |
| *P. riccardianus** | BCMV: NLL, SM<br>BYMV: NLL, SM<br>CMV: LL, SM<br>CaRSV: C-NLL, SN(ri) |
| *P. semierectus** | BCMV: C-NLL, SM(mal)<br>BYMV: C-NLL, SM<br>PSV: CLL, SM(mal)<br>TRV: NLL, SN |

Table 3 continued

| *Pheseolus* species and varieties[1] | Viruses[2] and symptoms[3] |
|---|---|
| *P. tuberosus** | BCMV: NLL, SM(mal)<br>BYMV: NLL, SM<br>CMV: NLL(vn), SM<br>CaRSV: C-NLL, SN(ri)<br>PSV: C-NLL, SM |
| *P. vulgaris* var. *nanus-nonscandens** | BCMV: LL, SM<br>BYMV: NLL(vn), SM |

[1] Plants marked with asterisks are species new to experimental virology.

[2] AraMV = *Arabis* mosaic virus, BCMV = bean common mosaic virus, BYMV = bean yellow mosaic virus, CaRSV = carnation ringspot virus, CMV = cucumber mosaic virus, PSV = peanut stunt virus, TRV = tobacco rattle virus, TomRSV = tomato ringspot virus.

[3] CLL = chlorotic local lesions, C-NLL = chlorotic-necrotic local lesions, C-NSL = chlorotic-necrotic systemic lesions, LL = latent local (symptomless), LS = latent systemic (symptomless), NLL = necrotic local lesions, SM = systemic mottle or mosaic, SN = systemic necrosis, (ep) = epinasty; (mal) = malformation, (ri) = rings, (tn) = top necrosis, (tu) = tumours, (vn) = veinal necrosis.

from *Lake Shasta* (CMV–C) induced necrotic local lesions in *Phaseolus aborigineus*, and systemic mosaic in *Phaseolus aconitifolius* and *P. angularis* plants. On *Phaseolus coccineus* (P. I. 176675, P. I. 201389) and *P. lunatus (Henderson Bush, Thaxter)* plants necrotic local lesions, systemic mosaic and necrosis developed. On the other hand, *Phaseolus lunatus (Fordhook U. S. 242)* was resistant to the isolate C of cucumber mosaic virus. Meiners et al. (1977) described vein banding symptoms for *Phaseolus coccineus* when inoculating it with the Puerto Rico strain of cucumber mosaic virus. On the basis of Provvidenti's (1976) and our own results the relation between *Phaseolus* species and cucumber mosaic virus strains is considered to be highly varying.

*Phaseolus caffer* – a new experimental plant in virology according to our knowledge – responded equally with local lesions and systemic mosaic to infecton by the most important viruses (bean common mosaic, bean yellow mosaic and cucumber mosaic viruses (Table 3). In the case of bean yellow mosaic virus veinal necrosis appeared on the inoculated leaves.

*Phaseolus coccineus* is susceptible to many viruses (see Horváth, 1986*b*). To our best knowledge it is a natural host for *Arabis* mosaic virus and bean common mosaic virus. In the experiments it also proved susceptible to peanut stunt virus. As it is known the preanut stunt virus susceptibility of *Phaseolus vulgaris* was reported by Silbernagel et al. (1966), Mink et al. (1967) and Echandi and Hebert (1970, 1971). On the peanut stunt virus susceptibility of various *Phaseolus* species Echandi and Hebert (1971), Horváth and Beczner (1982) published data. The data by Schmelzer et al. (1977) on the susceptibility of *Phaseolus coccineus* to *Arabis*

mosaic virus were confirmed in our experiments. Six days after inoculation the plant in question showed chlorotic-necrotic lesions followed by systemic mosaic (Horváth, unpublished results).

*Phaseolus coccineus* var. *bicolor*, *P. coccineus* var. *coccineus* and *P. coccineus* var. *rubronanus* equally responded with necrotic local lesions and systemic mosaic to infection by bean common mosaic virus and bean yellow mosaic virus (see Table 3). The leaves of *Phaseolus coccineus* var. *albiflorus* inoculated with bean common mosaic virus showed latent infection. From the inoculated leaves the virus could be reisolated. Bean common mosaic virus even caused systemic infection to the plant in question; the disease manifested itself in mosaic symptoms. As known from the experiments of Schmidt and Weissenstein (1983) the *Phaseolus coccineus* var. *albiforus* while resistant to many strains of bean yellow mosaic virus is susceptible to the *Fresia* (Fr Dr 2/78) and *Lupinus* (Tr 52/80) strains of the virus. In our own experiments (Horváth, unpublished results) *Phaseolus coccineus* var. *albiflorus* responded to bean yellow mosaic virus with necrotic local lesions and systemic mosaic. *Phaseolus coccineus* var. *bicolor* proved locally and systemically susceptible to cucumber mosaic and carnation ringspot viruses (Table 3).

Three *Phaseolus species (Phaseolus delphus*, *P. gonospermus* and *P. hysterinus)* unknown to plant virology as far as we know proved equally susceptible to carnation ringspot virus. *Phaseolus gonospermus* and *P. hysterinus* responded with characteristic symptoms to bean common mosaic, bean yellow mosaic, cucumber mosaic and peanut stunt viruses. Besides the host-virus relations mentioned above we found the *Phaseolus gonospermus* to be susceptible to tomato ringspot virus and the *P. hysterinus* to peanut stunt virus too (Table 3).

*Phaseolus lunatus*, one of the *Phaseolus* species best known in virology is susceptible to some 26 viruses (see Horváth, 1986*b*). In recent experiments we found the plant in question to be susceptible to infection by carnation ringspot virus and peanut stunt virus as well. Its susceptibility to cucumber mosaic virus first pointed out by Provvidenti (1976) was verified in our experiments.

In the case of *Phaseolus mungo* we detected 5 new host-virus relations (see Table 3). On leaves inoculated with four of them (cucumber mosaic virus, carnation ringspot virus, peanut stunt virus, tobacco rattle virus) chlorotic-necrotic lesions appeared, while the subsequently developed leaves showed systemic necrosis. It is remarkable that in the plants inoculated with peanut stunt virus tumours and top necrosis developed.

Further three *Phaseolus* species *(Phaseolus riccardianus*, *P. semierectus*, *P. tuberosus)* unknown in virology responded with necrotic local lesions and systemic mosaic to infection by bean yellow mosaic virus and cucumber mosaic virus. The only exception was *Phaseolus riccardianus* whose inoculated leaves did not exhibit symptoms following inoculation with cucumber mosaic virus, though the virus could be reisolated from them (see Table 3). Susceptibility was shown by *Phaseolus riccardianus* and *P. tuberosus* to carnation ringspot virus and by *P. semierectus* and *P. tuberosus* to peanut stunt virus as well. These new experiment data prove the importance of peanut stunt virus in bean cultivation on the one hand,

and call attention to the necessity of breeding for resistance to viruses, on the other. We also founda new compatible relation, and that between *Phaseolus semierectus* and tobacco rattle virus.

In experiments with *Phaseolus vulgaris* var. *nanus-nonscandens*, another plant unknown so far in virology, we found it to be locally and systemically susceptible to infection by bean common mosaic virus and bean yellow mosaic virus. It should be noted that bean common mosaic virus did not induce symptoms in the inoculated leaves of the plant, though the virus could be reisolated from them.

## Acknowledgements

I am indebted to the following botanical gardens and institutes: Jardin Botanique de Caen (Caen, France), Zentralinstitut für Genetik und Kulturpflanzenforschung (Gatersleben, German Democratic Republic), Botanischer Garten, Sektion Biowissenschaften (Halle, German Democratic Republic), Universitetets Botaniske Have (Cobenhavn K, Dankmark) Lietuvos TSR Mokslu Akademijos Botanikos, Instituto Botanikos Sodo (Kaunas, Soviet Union), Botanischer Garten, Sektion Biowissenschaften, Karl-Marx-Universität (Leipzig, German Democratic Republic), University of London, Botanical Supply Unit (London, England), Botanische Tuin van de Universiteit te Nijmegen (Nijmegen, The Netherlands), Botanical Gardens, University of Oulu (Oulu, Finland), MTA, Botanikai Kutató Intézet Vácrátót, Hungary) for putting the seeds of the experimental *Phaseolus* species and varieties at our disposal.

Thanks are due to Miss K. Molnár and Mrs É. Bősze for their technical assistance in the experiments.

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*Acta Phy pathologica et Entomologica Hungarica, 21 (3—4), pp. 329—336 (1986)*

# Compatible and Incompatible Relations between *Phaseolus* Species and Viruses

## III. New Incompatible Host-Virus Relations (Resistant and Immune Plants)

J. HORVÁTH

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

In the last part of the publication series 48 resistant host-virus relations between 17 *Phaseolus* species and varieties on the one hand and 10 viruses on the other are described. Of the resistant plants *Phaseolus hysterinus* and *P. mungo* proved suitable for the quantitative examination of tobacco necrosis virus, *Melandrium* yellow fleck virus and tobacco rattle virus, too.

Immunity was pointed out between 10 *Phaseolus* species and 10 viruses in 27 cases. The bean yellow mosaic virus immunity of *Phaseolus occoineus* may be very important for breeding. *Phaseolus* species immune of various viruses are also important as separator plants in virus differentiation.

In the first part of the publication series the incompatible *Phaseolus*-virus relations were also surveyed besides the compatible ones. According to our present knowledge incompatible relations have so far been demonstrated between some 20 fifferent *Phaseolus* plants and 93 viruses (see Horváth, 1986*a*, and literature therein). Earlier research results greatly helped in producing virus resistant bean varieties. The role and damage of viruses in bean cultivation have recently increased to a considerable extent. This fact makes it reasonable to continue studying the host-virus relations and seek out further resistant and immune *Phaseolus* species. Our experiments were aimed at detecting new incompatible host-virus relations between various *Phaseolus* species and viruses. Present paper discusses the new resistant and immune host-virus relations.

## Materials and Methods

In the course of studying the *Phaseolus*-virus relations we pointed out resistance to or immunity of virus(es) in 18 *Phaseolus* species and varieties, respectively (Table 1). The viruses included in the experiments were obtained from the gene bank of our laboratory (Table 2). We inoculated the primary leaves of the *Phaseolus* plants with various viruses using the carborundum-spatula technique, then sprayed the leaves with tap water. The inoculated plants were grown in a vector-free greenhouse. From the 3rd to the 30th day following inoculation the plants were repeatedly checked on symptomatologically and back-tested with viruses. In order to determine the virus infection or virus immunity the viruses were reisolated from the

inoculated plants (both the inoculated leaves and the subsequently developed leaves). Before reisolation the leaves of the plants were surface disinfected with 2 per cent NaOH then washed with a strong jet of water. After that the water diluted (1 : 1, v/v) tissue sap of inoculated and non-inoculated leaves virus reisolation was carried out to specifically responsive indicator plants (see Horváth, 1976, 1977). Plants reacting only with local chlorotic-necrotic lesions (with or without leaf drop), or showing only local latent infection were considered resistant, while those which showed no symptoms and the virus could not be re-isolated from them were regarded as immune (cf. Zaumeyer and Meiners, 1975; Provvidenti, 1976; Walkey and Innes, 1978; Cooper and Jones, 1983; Horváth 1986*b*).

Table 1

*Phaseolus* species and varieties investigated in the experiments

| *Phaseolus* species and varieties[1] | Source[2] |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* ssp. *aborigineus)* | Hau |
| *P. aconitifolius* | Hau |
| *P. acutifolius* | G, K |
| *P. angularis* | G, V |
| *P. atropurpureus* | G |
| *P. calcaratus* | G |
| *P. coccineus* (syn.: *P. multiflorus)* | Hal, K, Le, Lo |
| *P. coccineus* var. *albiflorus* | G |
| *P. delphus** | K |
| *P. gonospermus** | Hau |
| *P. hysterinus** | Hau, N, V |
| *P. lunatus* (syn.: *P. dumosus, P. limensis)* | C, G, Hau, K, Le, O |
| *P. mungo* (syn.: *P. radiatus)* | C, Hau, K, Lo, V |
| *P. occoineus** | N |
| *P. riccardianus** | K, Le, V |
| *P. semierectus** | G, Hau |
| *P. tuberosus** | K, V |
| *P. vulgaris* var. *nanus-nonscandens** | N |

[1] Plants marked with asterisks are species new to experimental virology.

[2] Abbreviation used for sources of seed: *C* = Jardin Botanique de Caen (*C*aen, France), *G* = Zentralinstitut für Genetik und Kulturpflanzenforschung (*G*atersleben, German Democratic Republic), *Hal* = Botanicher Garten, Sektion Biowissenschaften (*Hal*le, German Democratic Republic), *Hau* = Universitetets Botaniske Have (Cobenhavn K, Danmark), *K* = Lietuvos TSR Mokslu Akademijos Botanikos, Instituto Botanikos Sodo (*K*aunas, Soviet Union), *Le* = Botanischer Garten, Sektion Biowissenschaften, Karl-Marx-Universität (*Le*ipzig, German Democratic Republic), *Lo* = University of London, Botanical Supply Unit (*Lo*ndon, England), *N* = Botanische Tuin van de Universiteit te Nijmegen (*N*ijmegen, The Netherlands), V = MTA, Botanikai Kutató Intézet (*V*ácrátót, Hungary).

Table 2

Viruses used in the experiments

| Viruses | Strains or isolates | Literature |
|---|---|---|
| Alfalfa mosaic virus | $K_2$ | Beczner (1972) |
| *Arabis* mosaic virus | Phil. 2 | Schmelzer (1968, 1974) |
| Belladonna mottle virus | H | Horváth et al. (1976) |
| Bean yellow mosaic virus | S | Horváth (1976) |
| *Melandrium* yellow fleck virus | LB | Hollings et al. (1978), Hollings and Horváth (1981) |
| Potato virus M | Bie | Horváth (1971), Horváth and De Bokx (1972) |
| Potato virus S | Fort | Horváth (1972) |
| Tobacco mosaic virus | $U_1$ | Siegel and Wildman (1954) |
| Tobacco necrosis virus | f | Szirmai (1964) |
| Tobacco rattle virus | H | Horváth (1976, 1977) |
| Tobacco ringspot virus | D | Horváth (1976, 1977) |
| Tomato mosaic virus | H | Horváth and Beczner (1973), Mamula et al. (1974), Juretić et al. (1977) |
| Tomato ringspot virus | Bonn | Nienhaus (Virus gene bank, Bonn, GFR) |
| Turnip mosaic virus | All | Horváth et al. (1975*a*) |
| Watermelon mosaic virus | PW | Horváth et al. (1975*b*) |

## Results and Discussion

*Resistant plants*

In the course of our experiments we found 48 resistant host-virus relations of 17 *Phaseolus* species and varieties to 10 viruses (Table 3). On the plants tested *Phaseolus delphus*, *P. gonospermus*, *P. hysterinus*, *P. riccardianus*, *P. semierectus*, *P. tuberosus*, *P. vulgaris* var. *nanus-nonscandens* can be regarded as experimental plants new to virology. Although the resistant plants in the given host-virus relation could be infected with the virus, but from the inoculated leaves the virus could not spread in the plant. Several days following the infection the inoculated leaves of the plants exhibited chlorotic lesions, veinal necrosis, and in some cases dropped (e.g. *Phaseolus aborigineus* – tobacco necrosis virus; *P. acutifolius*, *P. coccineus*, *P. coccineus* var. *albiflorus* – tomato ringspot virus; *P. gonospermus*, *P. hysterinus*, *P. riccardianus*, *P. tuberosus* – tobacco necrosis virus, and others). It is remarkable

Table 3

Resistant *Phaseolus* species and varieties to different viruses
(Virus infection and/or replication, and/or invasion is restricted to the inoculated leaves)

| *Phaseolus* species and varieties[1] | Viruses[2,3] |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* spp. *aborigineus)* | MYFV: NLL<br>TNV: NLL(ld) |
| *P. aconitifolius* | MYFV: NLL<br>TMV: NLL<br>TNV: NLL<br>ToMV: NLL |
| *P. acutifolius* | MYFV: NLL<br>TNV: NLL<br>TomRSV: C-NLL(ld) |
| *P. angularis* | TMV: NLL<br>TNV: NLL |
| *P. atropurpureus* | TNV: NLL |
| *P. calcaratus* | TMV: LL |
| *P. coccineus* | MYFV: NLL<br>TomRSV: C-NLL(ld) |
| *P. coccineus* var. *albiflorus* | MYFV: NLL<br>TomRSV: C-NLL(ld) |
| *P. delphus** | TNV: NLL/vn, ld) |
| *P. gonospermus** | AMV: NLL<br>MYFV: NLL<br>TMV: NLL<br>TNV: NLL(vn, ld)<br>ToMV: NLL |
| *P. hysterinus** | AMV: NLL(vn)<br>MYFV: NLL<br>TMV: LL<br>TNV: NLL(ld)<br>TRV: NLL<br>TRSV: NLL(vn)<br>ToMV: LL |
| *P. lunatus* (syn.: *P. dumosus, P. limensis)* | MYFV: NLL<br>TRV: NLL<br>TomRSV: C-NLL(vn) |
| *P. mungo* (syn.: *P. radiatus)* | AraMV: NLL<br>MYFV: NLL(vn)<br>PVM: NLL<br>TMV: LL<br>TNV: NLL<br>ToMV: LL |

Table 3 continued

| *Phaseolus* species and varieties[1] | Viruses[2,3] |
|---|---|
| *P. riccardianus** | PVM: NLL<br>TNV: NLL(vn, ld) |
| *P. semierectus** | TMV: NLL<br>TNV: NLL |
| *P. tuberosus** | MYFV: NLL<br>TNV: NLL(vn, ld)<br>ToMV: LL |
| *P. vulgaris* var. *nanus-nonscandens** | TMV: LL<br>TNV: NLL(vn) |

[1] Plants marked with asterisks are species new to experimental virology.

[2] Abbreviation used for viruses: AMV = alfalfa mosaic virus, AraMV = *Arabis* mosaic virus, MYFV = *Melandrium* yellow fleck virus, PVM = potato virus M, TMV = tobacco mosaic virus, TNV = tobacco necrosis virus, TRV = tobacco rattle virus, TRSV = tobacco ringspot virus, ToMV = tomato mosaic virus, TomRSV = tomato ringspot virus.

[3] Abbreviation used for local symptoms: C-NLL = chlorotic-necrotic lesions, LL = latent local, NLL = necrotic local lesions, (ld) = leaf drop, (vn) = veinal necrosis.

that in certain host-virus relations the inoculated leaves of the *Phaseolus* plants did not show symptoms, still the virus could be reisolated from them. That was the case when inoculating *Phaseolus calcaratus*, *P. hysterinus*, *P. mungo* and *P. vulgaris* var. *nanus-nonscandens* with tobacco mosaic virus and *P. hysterinus*, *P. mungo* and *P. tuberosus* with tomato mosaic virus (see Table 3). Latent local infection was pointed out by Bos (1970) for *Phaseolus vulgaris* cv. *Bataf* inoculated with pea leafroll mosaic virus (E 210) and lettuce mosaic virus; by Drijfhout and Bos (1977) for *P. vulgaris* cv. *Michelite* and *Monroe* inoculated with bean common mosaic virus, and by Jayasinghe (1982) for *P. lunatus* inoculated with bean mild mosaic virus. Schmidt and Weissenstein (1983) found that in one of the accessions of *Phaseolus vulgaris* bean yellow mosaic virus could induce only local and latent infection. Among the resistant host-virus relations those with tobacco necrosis virus are remarkable. As it is known tobacco necrosis virus occurs in *Phaseolus* plants under natural conditions (cf. Szirmai, 1962; Schmelzer et al., 1977), therefore the tobacco necrosis virus resistance pointed out for 13 *Phaseolus* species and varieties, respectively, in our experiments may be highly important. The necrotic local lesions brought about by tobacco necrosis virus in the inoculated leaves of *Phaseolus hysterinus* and *P. mungo* made the quantitative examination of the virus also possible. For quantitative examinations of *Melandrium* yellow fleck virus *Phaseolus hysterinus* and *P. mungo*, and of tobacco rattle virus *P. mungo* proved to be similarly good test plants. The tobacco ringspot virus resistance of *Phaseolus hysterinus* – unknown to plant virology according to our knowledge – is also

remarkable, since tobacco ringspot virus has recently spread to a considerable extent in bean cultures which makes breeding for virus has cultures which makes breeding for virus resistance increasingly important (cf. Tu, 1980, 1981, 1983).

*Immune plants*

We pointed out immune host-virus relations between 10 *Phaseolus* species (four of which can be regarded as experimental plants new to virology) and 10 viruses (Table 4). Particularly important is the bean yellow mosaic virus immunity of *Phaseolus coccineus*, and *P. occoineus*. These plants were found to be immune of potato virus M, potato virus S and tobacco necrosis virus as well. The new potato virus M and potato virus S immune *Phaseolus* plants (see Table 4) represent valuable contribution to the differentiation of plant viruses (cf. Hiruki, 1970; Horváth, 1972, 1973, 1983, 1986*b*).

Table 4

Immune *Phaseolus* species to different viruses
(Plants cannot be infected)

| *Phaseolus* species[1] | Viruses[2] |
|---|---|
| *Phaseolus aborigineus* (syn.: *P. vulgaris* spp. *aborigineus)* | PVM, PVS |
| *P. acutifolius* | TMV |
| *P. calcaratus* | TNV |
| *P. coccineus* | BYMV, PVM, PVS, TMV, TNV, TRV, ToMV, TuMV, WMV |
| *P. delphus** | PVM, PVS |
| *P. gonospermus** | PVM, PVS |
| *P. hysterinus** | BMV, PVS |
| *P. lutanus* (syn.: *P. dumosus*, *P. limensis)* | PVM, PVS |
| *P. mungo* (syn.: *P. radiatus)* | PVS, WMV |
| *P. occoineus** | BYMV, PVM, PVS, TNV |

[1] Plants marked asterisks are species new to experimental virology.

[2] Abbreviation used for viruses: BMV = belladonna mottle virus, BYMV = bean yellow mosaic virus, PVM = potato virus M, PVS = potato virus S, TMV = tobacco mosaic virus, TNV = tobacco necrosis virus, TRV = tobacco rattle virus, ToMV = tomato mosaic virus, TuMV = turnip mosaic virus, WMV = watermelon mosaic virus.

## Acknowledgements

I am indebted to the following botanical gardens and institutes: Jardin Botanique de Caen (Caen, France), Zentralinstitut für Genetik und Kulturpflanzenforschung (Gatersleben, German Democratic Republic), Botanischer Garten, Sektion Biowissenschaften (Halle, German Democratic Republik), Universitetets Botaniske Have (Cobenhavn K, Danmark), Lietuvos TSR Mokslu Akademijos Botanikos, Instituto Botanikos Sodo (Kaunas, Soviet Union), Botanischer Garten, Sektion Biowissenschaften, Karl-Marx-Universität (Leipzig, German Democratic Republic), University of London, Botanical Supply Unit (London, England), Botanische Tuin van de Universiteit te Nijmegen (Nijmegen, The Netherlands), MTA, Botanikai Kutató Intézet (Vácrátót, Hungary) for putting the seeds of the experimental *Phaseolus* species and varieties at our disposal.

Thanks are due to Miss K. Molnár and Mrs É. Bősze for their technical assistance in the experiments.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 337–340 (1986)*

# Tobacco Mosaic Virus in Danube Water in Hungary

J. HORVÁTH,[1] N. JURETIĆ,[2] M. KRAJAČIĆ[3] and D. MAMULA[4]

University of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, P. O. Box 71, Hungary,[1] University of Zagreb, Biology Department, YU-41000 Zagreb, Marulićev trg 20/II, Yugoslavia[2,3,4]

Tobacco mosaic virus (TMV) was isolated from water samples of river Danube in Hungary. The identification was based on infectivity tests, electron microscope analysis, type of cell inclusions and serological assay.

So far from rivers and lakes over the world more various plant viruses have been isolated (reviewed by Koenig, 1986). Tobacco necrosis virus (TNV) has been found in England (Tomlinson et al., 1983*a*). Often in rivers and lakes occurs tomato bushy stunt virus (TomBSV), Tomlinson et al., 1982, 1983*b*). Koenig and Lesemann (1985) have isolated from German rivers and lakes some tumbusviruses, a potexvirus and carnation mottle virus (CarMV).

Tobamoviruses were also found in rivers, lakes and draining water. From water in the Netherlands Van Dorst (1969) has isolated cucumber green mottle mosaic virus (CGMMV). Ribgrass mosaic virus (RMV) was isolated from Zala river in Hungary (Juretić et al., 1986). Koenig and Lesemann (1985) have found in 6 various water samples in Germany tobamoviruses other than tobacco mosaic virus (TMV) which were not identified. Regarding TMV, it was found in river water in Yugoslavia, Italy and USA (Tošić and Tošić, 1984).

In this report the identification of a tobamovirus originating from Danube river in Hungary will be presented.

## Materials and Methods

Danube water was taken in Budapest at Margit (Margaret) bridge. Water samples of 250 ml were ultracentrifuged for 90 min at 90 000 g. The sediments were resuspended in a few drops of water and centrifuged 15 min at 3.000 g. The supernatant was inoculated on *Chenopodium quinoa* Willd. and *Nicotiana megalosiphon* Heurck. et Muell. These plants were the source of inoculum in further inoculations. The virus isolate was marked *D*. The light microscopical analysis of the virus cell inclusions was performed on living tobacco hair cells. The virus was partially purified after the method of Gooding and Hebert (1967). The serological experiments were carried out by means of the agar gel double-diffusion tests. An antiserum to common TMV (titre 1 : 2048) was used.

## Results and Discussion

The virus isolate *D* was mechanically transmitted to some test plants. Symptoms caused on these plants are presented in Table 1. They were similar to those caused by TMV.

Table 1

Symptoms in test plants inoculated by D isolate

| Test plants | Symptoms |
|---|---|
| *Chenopodium amaranticolor* Coste et Reyn. | necrotic local lesions |
| *C. quinoa* Willd. | necrotic local lesions |
| *Datura stramonium* L. | necrotic local lesions |
| *Nicotiana glutinosa* L. | necrotic local lesions |
| *N. megalosiphon* Heurck et Muell. | necrotic local lesions |
| *N. tabacum* cv. *Samsun* | systemic mosaic |
| *Phaseolus vulgaris* L. | chlorotic local lesions |

The analysed of infected sap and purified virus suspension in the electron microscope showed that the *D* isolate has rod-shaped virus particles about 300 nm long (Fig. 1).

Light microscope investigations of infected tobacco plants showed that *D* isolate produced, similarly to TMV, crystalline inclusions in form of hexagonal prisms (Fig. 2A). Sometimes paracrystalline needles were also visible.

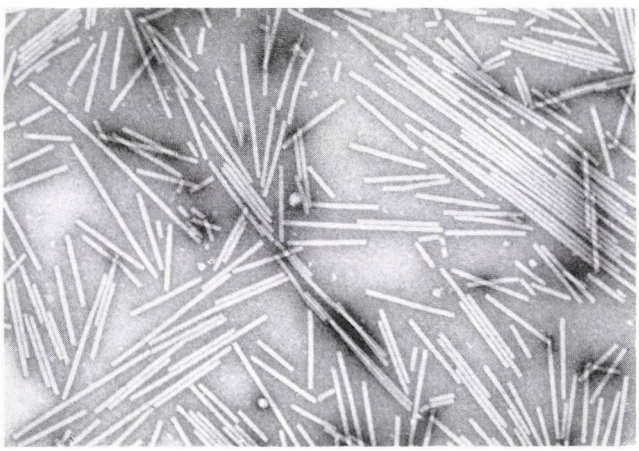


Fig. 1. Particles of TMV-D about 300 nm long in a purified preparation treated with phosphotungstic acid. Courtesy of Dr. N. Ljubešić, Rudjer Bošković Institute, Zagreb, Yugoslavia

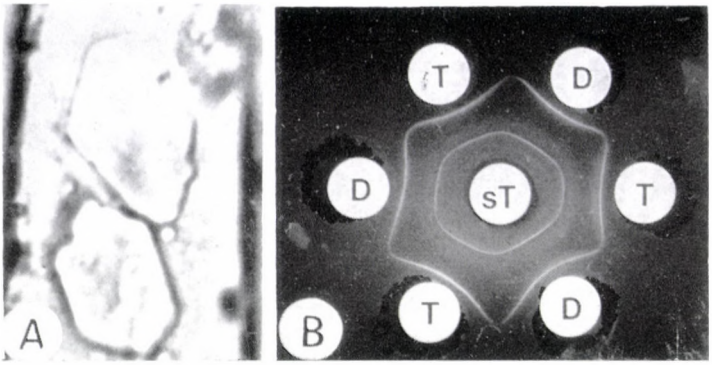



Fig. 2. Hexagonal crystalls in tobacco hair cell caused by TMV-D (A). Serological comparison of TMV-D (*D*) and TMV (*T*) in agar gel diffusion test (B). Abbreviations used: sT, antiserum to TMV (T), inner precipitin ring formed by A-protein

When *D* isolate was compared serologically in agar gel diffusion tests with typical TMV using TMV antiserum, the precipitin bands coalesced without any spur (Fig. 2B).

On the basis of these data it has been established that *D* isolate belongs to TMV (TMV–D).

Occurring of TMV in water of the river Danube was firstly stated by Tošić and Tošić (1984) in Yugoslavia. It would be interesting to compare TMV isolated from Danube by Tošić and Tošić and our TMV–D found in the same river. Water from Danube is much used for irrigation of fields in Danubian countries. Therefore it can be the significant source of TMV infections of some crops in the mentioned countries.

## Acknowledgements

We are indebted to K. Molnár and É. Bősze, Keszthely and A. Škof, Zagreb, for technical assistance.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 341–346 (1986)*

# Die Wirkung der Mineraldüngung auf die Abundanz von Gramineenthripsen

K. Czencz und K. Gerber

Agrarwissenschaftliche Universität Keszthely, Institut für Pflanzenschutz, H-8361 Keszthely, P.O.B. 71, Ungarn

In this study, the authors are going to give an account of their observations on the connection between plant feeding and density of grass thrips. Based on the experiments it has been verified that number of thrips individuals significantly increased with higher N-doses.

When only K (potassium) levels were raised, it did not show any explicit effects. Dosing N- and K-stuffs together had, however, a greater influence on increasing individual of thrips than separately. The interaction was significant on the level $P = 0.1\%$

Es ist bekannt, daß sich die Zusammensetzung der Inhaltsstoffe von angebauten Pflanzen durch die Anwendung von Mineraldünger ändern kann. Bei Grünland haben stickstoffhaltige Mineraldünger die größte Wirkung. Verschiedene Autoren (Bánszky 1973, Barcsák et al., 1978) sind eindeutig der Meinung, daß diese Mineraldünger vor allem auf den Proteingehalt der Gräser Einfluß haben. Debreczeniné und Hajas (1980) stellten in Düngungsversuchen an Knäuelgras fest, daß der N-Gehalt der Pflanze durch steigende N-Gaben (100–500 mg/kg Boden) allmählich auf das Doppelte angestiegen war.

Diese Veränderungen in den Inhaltssotffen der Pflanzen werden auch von den Schädlingen, die sich von den Pflanzen ernähren, wahrgenommen. Laut Schwenke (1962) wird die Vermehrung der über stechend-saugende Mundwerkzeuge verfügenden Insekten (also auch der von uns beobachteten Thripsarten) von gewissen Faktoren, die den Turgor der Pflanze erhöhen, begünstigt. Ein solcher Faktor ist z. B. die durch Stickstoffdüngung hervorgerufene Zunahme des Zuckergehalts.

Laut Stroms (1969) nimmt die Eierproduktion von Webermilben bei einer Erhöhung des N-Gehalts von Apfelblättern zu. Ähnliche wurde auch von Tulisalo (1971) für die Erhöhung der freien Aminosäuren festgestellt. Fritzsche (1958) bemerkte eine stärkere Vermehrung von Milben an kaliumarmen Pflanzen. Für Blattläuse hatte Baran (1971, 1972) bei steigenden P- und K-Dosen dagegen eine Abnahme an Fertilität und Individuenzahl wahrgenommen.

Im Laufe unserer Beobachtungen wurden die Abundanzverhältnisse von Thripsen auf Gramineen, die bei verschiedenen N- und K-Niveaus gezogen wurden, untersucht.

## Material und Methoden

Die Untersuchungen wurden 1984 und 1985 in West-Transdanubien (Ungarn) an Knäuelgras (Dactylis glomerata L.) und Straußgras (Agrostis alba L.) durchgeführt. Die Pflanzen wurden auf Ramman-Braunerde gezogen und dienten der Staatguterzeugung; somit setzte die Ernte Anfang Juli den Untersuchungen ein Ende.

Die Beobachtungen wurden auf 2 N-Niveau ($N_1$ = 200 kg/ha; $N_2$ = 300 kg/ha N) und 2 K-Niveaus ($K_1$ = 200 kg/ha; $K_2$ = 300 kg/ha $K_2O$) in 9 Kombinationen und 4 Wiederholungen auf je 100 $m^2$ großen Parzellen in split-plot Anordnung durchgeführt. Die Kombinationen Waren folgende:

$N_0N_0$, $N_0K_1$, $N_1K_1$, $N_2K_1$, $N_1K_0$, $N_0K_2$, $N_1K_2$, $N_2K_2$, $N_2K_0$.

Die Abundanz der Thripse wurde in jeder Wiederholung anhand der Zahl der Individuen, die sich nach je 10 Schlägen im Netz vefanden, festgestellt. Die Proben wurden, vom Wetter abhängig, an jedem zehnten Tag genommen.

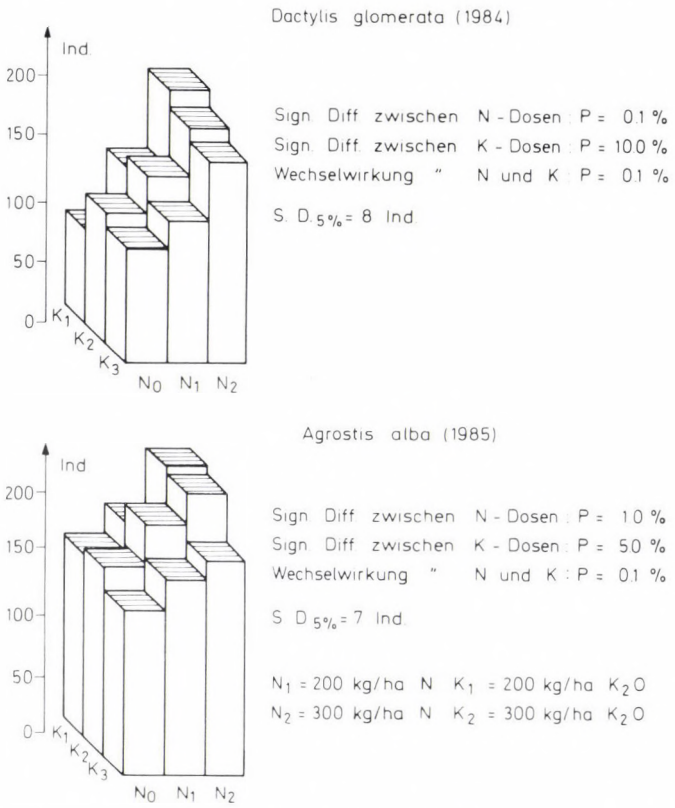



Abb. 1. Individuelzahlen der Thripse bei verschiedenen N- und K-Niveaus

## Ergebnisse und Diskussion

Die Wirkung der Mineraldüngung auf die Abundanz der Thripse wurde nach mehreren Gesichtspunkten ausgewertet.

Die Gesamtzahl der Thripse, die von auf untarschiedlichen N- und K-Niveaus gezogenen Gramineen eingesammelt werden (Abb. 1) zeigt, daß die Erhöhung der N-Dosen zu einer Zunahme der Individuenzahl führte. Die abundanzverändernde Wirkung einer höheren K-Dosis war nicht so eindeutig nachzuweisen. Bei K-Dosen von 300 kg/ha zeigten beide Gramineenarten eine Abundanzverminderung der Thripse.

Die Abbildung zeigt, daß die kombinierte Anwendung von N- und K-Dünger eine höhere Abundanz der Thripse zur Folge hatte. Diese Tatsache ist wohl auf

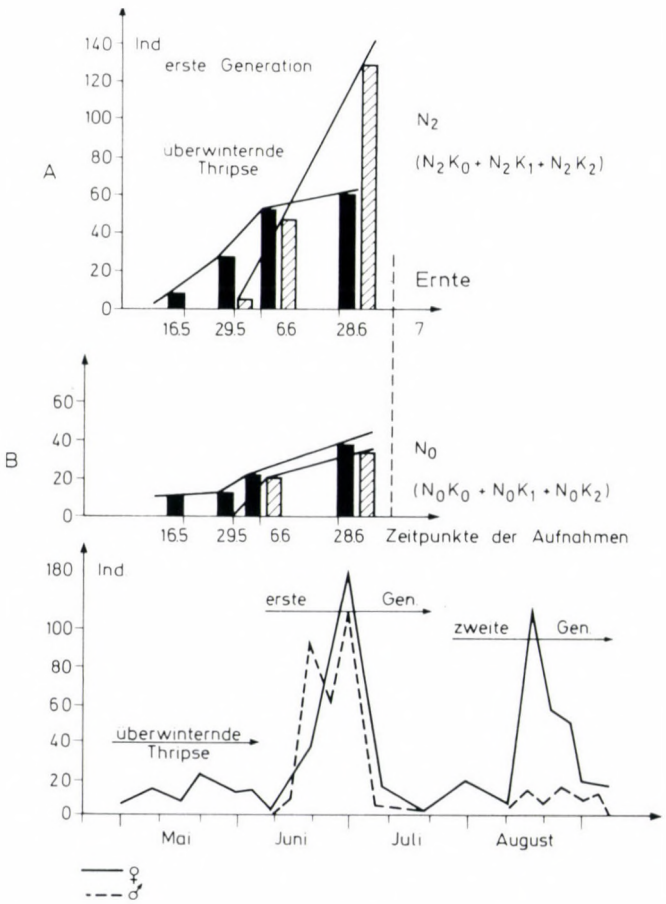



Abb. 2. Abundanzveränderungen N-Niveaus (A, B) Keszthely, 1985

den günstigeren Gehalt der Pflanzen an Inhaltsstoffen zurückzuführen. Die Wechselwirkung erwies sich auf einem Niveau von p = 0.1 % als signifikant.

Eine eingehendere Auswertung der Wirkung von Mineraldünger ist bei Insekten nur möglich, wenn die Anzahl der Larven- und Imagines der neuen Population auf der betreffenden Pflanze analysiert wird.

Aus Tabelle 1 und 2 geht hervor, daß nach der Überwinterung schlüpfende Populationen in jeder Parzelle, unabhängig von den Varianten, nur eine geringe Anzahl von Individuen aufwiesen. Die zu späteren Zeitpunkten gefundenen Abundanzen von Larven und Imagines wiesen schon auf die unterschiedlichen Ernährungszustande der Pflanzen hin. Ein wesentlicher Unterschied zwischen den Zahlen der Larven der neuen Population ist vor allem bei den Varianten $N_0$ und $N_2$ von Dactylis glomerate auffallend.

Tabelle 1

Anzahl der Imagines (I) und Larven (L)
(Dactylis glomerata L., 1984)

| Variante | Zeitpunkte des Sammelns | | | | | | Ind. insgesamt |
|---|---|---|---|---|---|---|---|
| | 7.5 | 14.5 | 28.5 | 9.6 | 18.6 | 5.7 | |
| $N_0K_0$ | | | | | | | |
| I | 2 | 3 | 12 | 13 | 20 | 20 | 70 |
| L | 0 | 0 | 3 | 2 | 5 | 7 | 17 |
| $N_1K_0$ | | | | | | | |
| I | 1 | 7 | 22 | 6 | 26 | 25 | 87 |
| L | 0 | 0 | 7 | 4 | 9 | 8 | 28 |
| $N_2K_0$ | | | | | | | |
| I | 3 | 3 | 8 | 10 | 28 | 16 | 68 |
| L | 0 | 0 | 9 | 9 | 10 | 64 | 92 |
| $N_0K_1$ | | | | | | | |
| I | 6 | 1 | 6 | 8 | 13 | 44 | 78 |
| L | 0 | 0 | 5 | 3 | 7 | 10 | 25 |
| $N_0K_2$ | | | | | | | |
| I | 2 | 2 | 10 | 7 | 13 | 23 | 57 |
| L | 0 | 0 | 7 | 2 | 3 | 7 | 19 |
| $N_1K_1$ | | | | | | | |
| I | 1 | 1 | 16 | 8 | 29 | 22 | 77 |
| L | 0 | 0 | 8 | 9 | 15 | 23 | 55 |
| $N_1K_2$ | | | | | | | |
| I | 2 | 2 | 8 | 5 | 29 | 19 | 65 |
| L | 0 | 0 | 9 | 7 | 10 | 30 | 56 |
| $N_2K_1$ | | | | | | | |
| I | 2 | 2 | 10 | 5 | 47 | 21 | 87 |
| L | 0 | 0 | 10 | 10 | 12 | 52 | 84 |
| $N_2K_2$ | | | | | | | |
| I | 1 | 2 | 8 | 8 | 45 | 27 | 91 |
| L | 0 | 0 | 8 | 10 | 13 | 62 | 93 |

Tabelle 2

Anzahl der Imagines (I) und Larven (L)
(Agrostis alba L., 1985)

| Variante | Zeitpunkte des Sammelns | | | | Ind. insgesamt |
|---|---|---|---|---|---|
| | 16.5 | 29.5 | 6.6 | 28.6 | |
| $N_0K_0$ | | | | | |
| I | 6 | 23 | 40 | 11 | 80 |
| L | 0 | 5 | 4 | 8 | 17 |
| $N_1K_0$ | | | | | |
| I | 9 | 30 | 54 | 31 | 124 |
| L | 0 | 8 | 10 | 16 | 34 |
| $N_2K_0$ | | | | | |
| I | 11 | 24 | 42 | 51 | 128 |
| L | 2 | 10 | 10 | 20 | 42 |
| $N_0K_1$ | | | | | |
| I | 19 | 43 | 29 | 41 | 132 |
| L | 6 | 3 | 4 | 5 | 18 |
| $N_0K_2$ | | | | | |
| I | 16 | 53 | 48 | 20 | 137 |
| L | 2 | 1 | 3 | 5 | 11 |
| $N_1K_1$ | | | | | |
| I | 12 | 28 | 78 | 40 | 158 |
| L | 2 | 4 | 10 | 15 | 31 |
| $N_1K_2$ | | | | | |
| I | 10 | 30 | 67 | 35 | 142 |
| L | 0 | 4 | 8 | 18 | 30 |
| $N_2K_1$ | | | | | |
| I | 9 | 56 | 53 | 58 | 176 |
| L | 1 | 4 | 12 | 16 | 33 |
| $N_2K_2$ | | | | | |
| I | 10 | 30 | 60 | 75 | 175 |
| L | 4 | 5 | 10 | 20 | 39 |

Die Wirkung, die unterschiedlich hohe N- und K-Dosen auf eine bestimmte Thripsart ausüben, wird an Beispiel der Art Chirothrips manicatus Hal., die nahezu die Hälfte der gesammelten Individuen ausmacht, dargestellt (Abb. 2). Diese Art hat zwei Generationen (s. Diagramm der interzyklischen Abundanzveränderung am unteren Drittel der Abbildung). Da die Gramineen Anfang Juli geerntet wurden, beschränkten sich unsere Beobachtungen nur auf die Wirkung des Mineraldüngers auf die erste Generation. Da bei der Art Chirothrips manicatus Hal. nur die Weibdchen überwintern, können die Wirkungen des Düngers auf die Vermehrung (durch Vergleich der Varianten $N_0$ und $N_2$) as Erscheinen der Männchen eindeutig abgemessen werden.

## Zusammenfassung

Die Lebensfunktionen von Organismen, die sich von Pflanzen ernähren, so auch die von tierischen Schädlungen, werden durch die Veränderung in den Inhaltsstoffen der Pflanzen durch Mineraldünger beträchtlicht beeinflußt.

Im Laufe unserer Untersuchungen wurde an Dactylis glomerata und Agrostis alba die Wirkung von hohen N- und K-Dosen (200—300 kg/ha) auf die Abundanz von Thripsen analysiert. Es wurde festgestellt, daß durch die Erhöhung der N-Gabe die Anzahl der Thripse zugenommen hatte. Die Abundanzwerte von Larven und Imagines der neuen Insektenpopulation, die sich auf den gedüngten Pflanzen (Parzellen) ausbildete, wiesen eindeutig darauf hin.

Zusammenfassend ist festzustellen, daß hohe N-Gaben zu Futtergäsern zwar einen höheren Ertrag bringen können, doch führt dies zu einer Abnahme des Anteils an essentiellen Aminosäuren und damit zur Vermehrung von Thripsschädlingen, die die Pflanzen durch Saugen schädigen.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 347—354 (1986)*

# Study of Migratory *Lepidoptera* on the Balcan Peninsula

Z. MÉSZÁROS

Plant Protection Institute of the Hungarian Academy of Sciences,
1525 Budapest, P.O. Box 102, Hungary

The author studied in Bulgaria the migratory routes and the conditions of migration of *Lepidoptera* that are unable to overwinter in Middle Europe, although occasionally cause grave damage there. Based on his observations the author draws conclusions regarding the factors that bring about and modify the process of migration.

In the lepidopteran fauna of the Carpathian Basin many species are known that do not overwinter in Middle Europe. These are migratory species that arrive mostly from South Europe to the central and northern territories mostly in the first half of the season. In course of the vegetation period they produce one or two generations and the portion of the population that developed to adult leaves again towards the Mediterranean area. These species are able to overwinter under Hungarian conditions only in special cases, like in glasshouses. The regular annual changes in ecological factors (e.g. photoperiod) do not trigger off a diapause in these species that do not react to the length and intensity of the daily illumination with a quiescent period. This is corroborated also by the fact that the development of the progeny of specimens that had found their way into glasshouses, does not stop during the winter. Other *Lepidoptera* displace their populations within their area of distribution and the "unexpected" damage of these species is also explained by the migratory habits of local populations.

## Literature Review

The classification of migratory butterflies that became more or less a classic system is linked with the name of Warnecke (1950). By studying the migratory Lepidoptera from a Middle European standpoint, Warnecke erected the following categories:

1. More or less occasional immigrants ("guests"),
2. More or less regular immigrants, that are unable to survive the Middle European winter under normal conditions,
3. Indigenous species, that may receive "reinforcements" from smaller or larger immigrant populations,
4. Indigenous populations, whose populations become displaced within their Middle European area.

Koch (1964, 1965) modified this classification by separating the group of "seasonal migrants" from the "internal migrants".

Vojnits (1966) composed the following groups that are generally accepted in the Hungarian literature, by combining with minor modifications the two beforementioned systems:

1. True migratory *Lepidoptera*, that are regularly present during the vegetation period, but which are not, or only occasionally able to overwinter, especially in the northern areas of Middle Europe,

2. Guests. These are occasional immigrants that, owing to favourable weather conditions spread from time to time beyond the limits of their regular area, appearing thus also in Middle Europe,

3. Internal migrants, that displace themselves within the limits of their area. Their populations may receive occasionally additional numbers from groups that immigrated from the south, southeast or east.

More recently, Gatter (1981) brought into correlation the different types of migration with the diapause conditions of the species involved. It was a merit of his work migrating groups as well. In the grouping of Gatter, however, the relationships to a given geographical area are mostly disregarded.

The migrational groups of Gatter are the following:

1. Anemomigration – drifting. The insects are swept away by the wind, without conscious orientation,

2. Dismigration – spreading. It is not an active migration, but a passive dispersion. Two types are distinguished: expensive and seasonal dismigration.

3. Migration – wandering into a definite direction. This migration is endogenously programmed with a definite orientation and combined with a navigating capacity of the animals. Two types are distinguished: expansive and seasonal migration.

## Aims of our present studies

Among the migrating Lepidoptera some are significant pests of agriculture, whose "unexpected" damage, spreading over many countries, is connectied to their migrational habit.

By begin of the sixties a light trap network has been established in Hungary. This network became divided in the seventies into two parts, one of them operated by the Plant Protection Service, the other by forestry experimentalstations. The informations on migrating Lepidoptera originate mostly from these two networks or from the centres where their data are analized, respectively. The population dynamics of the most important lepidopterous pests have been continuously observed since 1960 and the forecastings on their damage are being transmitted to the producers via different broadcast media (Benedek et al., 1974).

It is hardly disputable that it is impossible to prepare a reliable damage forecasting of the migrating species appearing in the Carpathian Basin if one observes the changes in the Hungarian populations alone. The "unexpected" damages

extending over many countries are brought about by the progenies of immigrated adults. This happened in 1962 with *Autographa gamma* L., in 1965 and 1983 with *Agrotis ipsilon* Hufn. and in 1975 with *Loxostege sticticalis* L. (Mészáros and Nagy, 1968; Herczig and Schütz, 1985).

In course of our own studies we aimed to find answers to the following questions:

– is it possible to recognize the routes of populations migrating from the Mediterranean area into the Carpathian Basin?

– how and under what conditions do the populations overcome the obstacles of mountain ranges laying across their migration directed from south to north or conversely?

– how do they continue their way after passing the mountains?

– what are the connections between the migration and the meteorological situations?

– is it possible to designate localities where forecasingt stations carried out observations with light traps on the migrating lepidopterous pests that would damage in the Carpathian Basin?

## Areas and Methods of Observation

The migrating Lepidoptera that damage in the Carpathian Basin arrive from the Mediterranean and Pontomediterranean territories. Populations originating from the Mediterranean arrive from two main directions: the Aegean Sea and the Adriatic Sea. For the observations therefore two countries: Bulgaria and Yugoslavia were considered. Regrettably, surveys in Yugoslavia had to be left out from two reasons: on one hand, Yugoslavia is not readily accessible to us due to currency difficulties, on the other hand the collection of insects is made there unlawful and the procurement of necessary permissions seemed to be hopeless. Thus the Balkan Mountains (Stara Planina) and the valley of the Struma river were chosen for observation areas.

In course of our first journey (22–31st October 1980) the passes of Stara Planina were visited to observe the autumn migration through the high mountain. By utilizing the experiences of the first surveys our subsequent expeditions were directed into the Struma Valley southward from the pass that lies farthest to the west (Petrohanski Pass). We journeyed each time along this valley until the Greek border.

The observations and collections were made both during the daytime and the night. In the nocturnal collections Honda generators connected to Tungsram mercury-vapour bulbs (125 and 250 W HGL) or combined light bulbs (160 W HMLI) were used. From among the climatic factors the air temperature and wind velocity were measured, other factors were estimated and also the frontal meteorological situation was considered.

## Results

At our first journey the passes of the main ridge of Stara Planina were visited that lays in the east-west direction. The experiences were the following:

1. Petrohanski Pass, 24th October 1980

In the pass at noontime on a sunny day (temperature 10–12 °C) individuals of *Colias croceus* Fourcr. were flying southward against a light southern wind. The number of passing animals could be well estimated: 50–100 per hour. Some hundred meters from the highest point the migrating butterflies scattered again and continued singly their journey. Simultaneously also a sporadic migration of *Vanessa atalanta* L. was observed.

2. Zlatica Pass, 24th October 1980

The collection was made in the evening hours on the southern slope in the beech forest zone. In a rather cool weather (8 °C at 6 p. m., 5 °C at 9 p. m.), after the onset of darkness the mountain breeze blowed downhill, southward. After dark a sporadic flight of *Autographa gamma* L. adults was observed, the moths flew towards the south, before the light wind. On the light souce the activity was low, some individuals of *Agrotis ipsilon* Hufn., *A. segetum* Den. et Schiff., *Phlogophora meticulosa* L. and *Autographa gamma* L. were attracted by light.

3. Sipka Pass, 26th October 1980

– Up in the pass itself the temperature was 10–12 °C in the noon hours, with a strong southern wind (8, 10 and 12 m/sec). Sporadic, south-bound flight of *Vanessa atalanta* L. and *Autographa gamma* L. adults was oberved against the wind. Because of the strong wind the animals avoided the open terrain and flew at low altitudes (1 m high) in the protection of trees and bushes.

– In a side valley on the southern slope the migration towards south was well visible in the early afternoon. Within 30 minutes the appearance of 14 *Colias croceus* Fourcr., 1 *Vanessa atalanta* L., 2 *Vanessa cardui* L. and 1 *Autographa gamma* L. was noted. They were observed to stop for feeding on the flowers.

– Collection in the evening in a lateral valley on the southern slope, in a height of about 800 m. After nightfall sporadic southward flight of *Autographa gamma* L. was observed. Later a strong northern wind got up that spoiled the flight. In the dark some *A. gamma* L. were attracted by the light, alighted on the white collection sheet but soon after left again, contrary to other, non-migrating Noctuids that stayed on the sheet.

4. North Bulgaria:

Between Belogradčik and Dolni Lom, 24th October 1980

In a karst region in the afternoon hours a south-bound sporadic flight of *Colias croceus* Fourcr. and *Autographa gamma* L. was observed.

– 28th October 1980

In the noon hours a sporadic southward flight of *Colias croceus* Fourcr., *Vanessa cardui* L. and *Autographa gamma* L. was observed.

Based on these preliminary observations we started the regular surveys on the north-south migraton route, north and south from the Petrohanski Pass

(Belogradčik) and the Struma river valley, respectively. The collecting and observation sites were:

1. North Bulgaria: the area of Belogradčik,
2. the area of Sofia-Kostinbrod,
3. Struma river valley: Kresna and Melnik

In the localities mentioned at two occasions autumnal (end of September and end of October 1981) and at two occasions spring-early summer observations and collections were carried out (the latter by begin of June 1982 and mid-May 1983).

The results of the *spring-early summer* observations were the following:

*a)* From among the species migrating during the daytime the migration or the begin of wandering of *Pieris brassicae* L. and of *Colias croceus* Fourcr. could be observed. In the Struma valley an anticyclonic weather situation prevalied between 3 and 5 June 1982 with southern wind or calm. The two species mentioned were present in the area, the adults were feeding, but their movement did not indicate a migration. On the 6th June the same two species flew northward in a cyclonic weather situation against a northern wind. The same was observed also in the following days, in a light northern breeze.

*b)* Sporadic occurrence of migarting day-flyers was observed between 9th and 17th May 1983: *Pieris brassicae* L., *Colias croceus* Fourcr., *Lampides pirithous* L., *Vanessa cardui* L., *Vanessa atalanta* L., *Macroglossa stellatarum* L.

*c)* In eventing- and night collections individuals of the following species were trapped: *Celerio livornica* Hbn., *Agrotis segetum* Den. et Schiff., *Agrotis ipsilon* Hufn., *Xesthia c-nigrum* L., *Phlogophora meticulosa* L., *Peridroma saucia* Hbn., *Mythimna l-album* L., *Mythimna vitellina* Hbn., *Helicoverpa armigera* Hbn., *Melicleptria scutosa* Den. et Schiff., *Autographa gamma* L., *Autographa circumflexa* L., *Grammodes stolida* F., *Grammodes bifasciata* Pet., *Dysgonia algira* L., *Cyclophora pupillaria* Hbn.

The results of the *autumnal observations* can be summarized as follows:

*a)* The migration of the day-flyer species was hardly visible at the end of September 1981. By 27 September 1981 a sporadic, south-bound flight of only one species, *Colias croceus* Fourcr. could be observed in the Petrohanski Pass.

*b)* At the end of October of the same year the diurnal migration was already well noticeable. On suitable days (sunny weather, southern wind) the south-bound migration was well expressed. On the 21st October 1981 in the Petrohanski Pass the migration of the following species was observed in the noon hours, at 14–15 °C and against a strong wind (velocity 6–8 m/sec. with gusts of 10 m/sec.): *Vanessa atalanta* L., *Vanessa cardui* L., *Autographa gamma* L. In the Struma valley in many cases the sporadic migration of *Colias croceus* Fourcr. and *Autographa gamma* L. was noted in the period 21st–24th October 1981.

*c)* In trapping in the evening and night hours the abults of the following migratory species were collected: *Agrotis segetum* Den. et Schiff., *Agrotis ipsilon* Hufn., *Xestia c-nigrum* L., *Mythimna vitellina* Hbn., *Mythimna l-album* L., *Phlogophora meticulosa* L., *Heliothis peltigera* Den. et Schiff., *Autographa gamma* L., *Chrysodeixis chalcites* Esp.

## Discussion

From the results of our journeys the following conclusions may be drawn on the migration of *Lepidoptera* (the results were earlier briefly reported at the 10th SIEEC Conference held in Budapest Mészáros, 1983):

The two comprehensive tables (Table 1 and 2) contain not only the data of our journeys described above but also those of the Hungarian light-trap network, the latter collected in a period of 20 years (Mészáros et al., 1979).

1. In day-flyer migrating species the process of migration is well observable. Populations with displacement and feeding within a given habitat can positively be distinguished from migrating ones (and feeding in course of their migration).

2. Species with nocturnal migration can readily be collected by artificial light sources, however, their local populations feeding within a given area cannot be distinguished unequivocally from the ones passing through in course of their migration.

3. In contrast to the last conclusion, individuals of *Autographa gamma* L. attracted by the light on cool autumn nights behaved differently from other, but non-migratory noctuid moths. The individuals of the former were sitting on the collecting sheet with vibrating wings and some moments later flew away from the

Table 1

Grouping of migratory Lepidoptera, according to Vojnits, 1966 (slightly modified)

| Groups | Relationship to area | Diapause | Species |
|---|---|---|---|
| 1. True migrants | Periodical change of area, the re-migration is marked, regular | There is no diapause, independently of photoperiod during the development | Agrotis ipsilon Hufn.<br>Autographa gamma L.<br>Colias croceus Fourcr.<br>Vanessa atalanta L.<br>Vanessa cardui L.<br>(Pieris brassicae L.?) |
| 2. Guests | The widening of area occurs both regularly or occasionally, the evidences of re-migration are not inconclusive | Facultative (?) diapause occurring within the regular area | Heliothis peltigera Den. & Schiff.<br>Helicoverpa armigera Hbn.<br>Spodoptera exigua Hbn. (?)<br>Celerio livornica F.<br>Daphnis nerii L.<br>Nomophila noctuella Den. & Schiff. |
| 3. Internal migrants | Migration or displacement occurs within the area, no evidence of re-migration | Facultative diapause (brought about by short day conditions) | Agrotis segetum Den. & Schiff.<br>Xestia c-nigrum L.<br>Mythimna albipuncta Den. & Schiff.<br>Mythimna l-album L.<br>(Pieris brassicae L.?) |

illuminated area whereas the body temperature of the other noctuids decreased soon in the cool night and they became unable to fly.

4. The flight of the migrating populations is well visible in passes of mountain ranges that lay across their direction. In passes the migrating populations crowd together, their densities become estimable, their individual numbers can even be counted. After passing the mountain ranges the populations scatter again.

Table 2

Connections between the environmental factors and the migration

| Phases of migration | Triggering or modifying factors |
|---|---|
| 1. Determination of direction (northward or southward) | Changes in daylength or the tendency of change, respectively (lenghtening illumination: northward, shortening illumination: southward) |
| 2. Begin of migration ("impulse" for flight begin) | Changes in the weather situation, anticyclonic calm (or light breeze) followed by a cyclonic wind (headwind) |
| 3. Orientation (navigation) | Eart magnetism? It is independent of the position of the Sun, firmament or local winds, but may be influenced by main wind |
| 4. Termination of migration | The population arrived among suitable conditions (i.e. the place is optimal for feeding, reproduction or rest) |

5. The autumnal, south-bound migration takes place in a definite, sometimes strong, headwind. In some especially windy places the *Lepidoptera* are repeatedly swept back in trying to pass the area. Downwind migration is far less common and was mostly the case of a local, downhill mountain wind.

6. The northbound migration in the spring or early summer also takes place in a headwind. The begin of migration is very likely brought about by a northerly atmospheric motion starting following an anticyclonic weather situation.

7. Properly placed light traps are by all means suitable to detect the movement of migratory agricultural pests. The forecating of these species can be achieved only by international co-operation that would be especially important to the agriculture of Middle- and North Europe.

In Table 1 the grouping of migratory Lepidoptera is presented together with some characteristics of the species. Table 2 points to connections between some environmental factors and the migration.

## Acknowledgements

Our studies were carried out in all cases as a team-work. Special acknowledgements are due to all colleagues who participated in the journeys, in determinations and in putting their data to the disposal of the author: L. Ronkay (Museum of Natural History), B. Nagy, Éva Visnyovszky (Plant Protection Institute of Hungarian Academy of Sciences), Cs. Szabóky (Central Institute of Food Industry), B. Herczig (Station of Plant Protection and Agrochemistry, Tata, K. Szeőke (Station of Plant Protection and Agrochemistry, Velence), P. Gyulai (Station of Plant Protection and Agrochemistry, Miskolc), Cs. Thuróczy (Jurisich Museum, Kőszeg) and L. Balásházy.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 355–361 (1986)*

# Composition of Heteropteran Populations in Hungary in Apple Orchards Belonging to Different Management Types and the Influence of Insecticide Treatments on the Population Densities*

VERA RÁCZ

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

In Hungarian apple orchards receiving different levels of chemical treatment 184 heteropteran species were detected. Actually 90 species were collected on the apple trees themselves, most of these were present in low individual numbers. From among the species established in the orchards 15 regularly occurred or were found in high population densities, while 27 species were living both on apple trees and on the weed undergrowth. The heteropteran populations of apple orchards are considerably influenced by the insecticidal treatments and by the vegetation neighbouring with the experimental plots. The former affect also the heteropteran populations living on the undergrowth of apple trees.

The ecological investigations in different agricultural crops and planned for a 10-year period were commenced in Hungary on the suggestion and under the guidance of Jermy (1977, 1979). The research has been carried out since 1976 in the Department of Zoology of the Plant Protection Institute of the Hungarian Academy of Sciences.

## Materials and Methods

*Localities and dates of surveys*

The investigations and surveys were carried out in the following apple orchards, belonging to different types and levels of treatment:

1. Farming-scale experimental orchard of the Plant Protection Institute in Nagykovácsi-Julianna major, near Budapest. The orchard is bordering to a cropland and to a natural oak forest. Half of the orchard was left untreated (received only soil cultivation and fungicidal treatments) while the other half was occassionally treated (2–3 times per vegetation period) with insecticides.

2. Farming-scale (100 hectares) apple orchard Nyíregyháza-Ilona tanya (County Szabolcs-Szatmár), treated intensively with phosphorester insecticides, by using the most up-to-date production methods.

3. Smaller, traditional-type apple orchard of 5 hectares, receiving less intensive plant protection (8 insecticides) treatments than in No. 2. The orchard lies in Újfehértó (County Szabolcs-Szatmár).

* Apple Ecosystem Research, No. 56.

4. Small-scale apple orchard (half-hectare), receiving intensive plant protection treatment. The orchard lies in Nyíregyháza-Füzesbokor (County Szabolcs-Szatmár).

5. Scattered, abandoned orchard of 0.2 hectare, without any treatment. The orchard lies in Nyíregyháza-Sóskút (County Szabolcs-Szatmár).

*Survey Methods*

1. Jermy-type light-trap (100 Watt normal light bulb). With the exception of No. 5 (scattered orchard) the light-traps operated in all experimental orchards.

2. Beating of apple tree branches. The surveys were carried out weekly in all experimental orchards. The insect material was caught in a circular funnel of 0.5 m diameter.

3. Investigation of individual apple trees. Study of a determined number of shoots collected at random in the field.

4. Application of corrugated paper belts on the tree trunks.

5. Weekly use of a sweeping net (opening 0.1 $m^2$) on the weed undergrowth. With the exception of weed-free orchards and periods.

6. Soil-trapping of faunal elements active on the soil surface (5–20 plastic cups per orchard, with their openings kept at soil level). Weekly change of cups.

Table 1

Number of heteropteran species grouped according to families, collected with different methods in Hungarian apple orchards (1976—1979)

| Family | Number of species | | | | Total |
|---|---|---|---|---|---|
| | from apple | light-trap | sweeping | soil-trap | |
| Miridae | 37 | 44 | 10 | 0 | 91 |
| Lygaeidae | 20 | 2 | 3 | 2 | 27 |
| Pentatomidae | 9 | 0 | 5 | 0 | 14 |
| Corizidae | 3 | 1 | 6 | 0 | 10 |
| Corixidae | 1 | 7 | 0 | 0 | 8 |
| Anthocoridae | 5 | 0 | 1 | 0 | 6 |
| Nabidae | 6 | 0 | 0 | 0 | 6 |
| Cydnidae | 2 | 0 | 1 | 1 | 4 |
| Coreidae | 2 | 0 | 1 | 0 | 3 |
| Tingidae | 2 | 0 | 1 | 0 | 3 |
| Piesmidae | 0 | 0 | 2 | 0 | 2 |
| Reduviidae | 0 | 1 | 1 | 0 | 2 |
| Scutelleridae | 1 | 0 | 1 | 0 | 2 |
| Alydidae | 0 | 0 | 1 | 0 | 1 |
| Plataspidae | 0 | 0 | 1 | 0 | 1 |
| Saldidae | 0 | 1 | 0 | 0 | 1 |
| Pyrrhocoridae | 0 | 0 | 0 | 1 | 1 |
| Berytidae | 2 | 0 | 0 | 0 | 2 |
| Total | 90 | 56 | 34 | 4 | 184 |

## Results and Discussion

With the survey methods briefly presented above, 184 heteropteran species were collected in total (Mészáros et al., 1984) that were grouped in Table 1 according to their families. The species belonged to 18 families, of which the family Miridae was most abundantly represented with 91 species. As a matter of fact, this bug family contains the highest number of species in Hungary.

From all orchards studied 90 species were collected from the apple trees themselves. A considerable number of species (94) was established with other methods (e.g. light-trapping, sweeping, soil-trapping) as shown in Table 1.

The species number showed variations according to the intensity of insecticide treatments and to plant stands neighbouring to the individual apple orchards (Table 2).

Table 2

Number of heteropteran species collected in apple orchards of different types (several collecting methods, 1976–1979)

| Locality | Species number |
|---|---|
| Farming-scale orchard — Nagykovácsi, half of it untreated, bordering to an oak forest and cropland | 128 |
| Scattered, abandoned orchard, Nyíregyháza—Sóskút, untreated, collection without light-trap | 67 |
| Small-scale orchard, Nyíregyháza—Füzesbokor | 51 |
| Traditional, farming-scale orchard, Újfehértó | 47 |
| Intensively treated farming-scale orchard, Nyíregyháza-Ilona tanya | 32 |

In No. 1 farming-scale, partly treated orchard (bordering to an oak forest and a cropland) 128 species were found, in the No. 5 scattered orchard (without light-trap!) 67 species, in the No. 4 small-scale orchard 51, in the No. 3 traditional apple orchard 47 and in the intensively treated No. 2 farming-scale orchard only 32 species.

These data indicate that by using the method of light-trapping the range of species established could considerably be increased, at the same time, however, the neighbouring vegetation and the intensity of pesticide treatments may cause extreme variations in the species number of *Heteroptera*. This is shown also in Table 3 that contains the numbers of species collected from the apple trees by beating or individual collections and of those occurring regularly or in high densities, respectively (Table 4).

In accordance with the opinion of Solomon (1983) these data demonstrated that the surrounding vegetation influences at least as deeply the heteropteran popu-

Table 3

Number of heteropteran species collected by beating and individual collections on apple trees (1976–1979)

| Locality | Species number |
|---|---|
| Farming-scale orchard — Nagykovácsi, half of it untreated, bordering to an oak forest and cropland | 49 |
| Scattered, abandoned orchard, Nyíregyháza—Sóskút, untreated | 13 |
| Small-scale orchard, Nyíregyháza—Füzesbokor | 4 |
| Traditional, farming-scale orchard, Újfehértó | 2 |
| Intensively treated farming-scale orchard, Nyíregyháza-Ilona tanya | 1 |

Table 4

Heteroptera collected regularly or in high numbers on apple trees either by beating or by individual collections (1976–1979)

| Species | Locality, No. of orchard | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Deraeocoris ruber L. | + | — | — | — | — |
| Deraeocoris putoni Mont. | + | — | — | — | — |
| Phytocoris reuteri Saund. | + | — | — | — | — |
| Exolygus rugulipennis Popp. | + | — | — | — | — |
| Pilophorus perplexus Doug. et Scott | + | — | — | — | — |
| Atractotomus mali Meyer-Dürr | + | — | — | — | — |
| Anthocoris spp. | + | — | — | — | — |
| Orius minutus L. | + | — | — | — | + |
| Himacerus apterus F. | + | — | — | — | — |
| Nabis spp. | + | — | — | — | — |
| Stephanitis pyri F. | — | — | — | — | + |
| Nysius senecionis Schill. | + | — | — | — | + |
| Rhyparochromus vulgaris Schill. | + | — | — | — | — |
| Dolycoris baccarum L. | + | — | — | — | — |
| Palomena prasina L. | + | — | — | — | — |
| Total | 14 | — | — | — | 3 |

lations as the insecticides themselves. In the partly untreated No. 1. orchard the number of species occurring regularly or in high densities was 14 (as found by beatings and individual collections). This number sunk to 3 in the No. 5 scattered, untreated orchard and to zero in the other orchards receiving intensive pesticide treatments.

Both on the apple trees and the undergrowth 20 phytophagous and 7 predatory bug species occurred (Table 5).

Table 5

Heteropteran species occurring both on apple trees and on the weed undergrowth (total in all ocrhards) (1976–1979)

| Phytophags: 20 species | Predators: 7 species |
|---|---|
| More important ones of these: | |
| Dolycoris baccarum L. | Nabis spp. |
| Stephanitis pyri F. | Deraeocoris ruber L. |
| Palomena prasina L. | Atractotomus mali Meyer-Dürr |
| Exolygus rugulipennis Popp. | Phytocoris ulmi L. |
| | Orius minutus L. |
| | Picromerus bidens L. |

The results of insecticide applications are shown in Tables 6 and 7 in the untreated vs. treated halves of No. 1 orchard.

The data of Table 6 refer to the apple trees, of Table 7 to the undergrowth. The comparatively high species numbers and low individual densities showed that there were many species only represented by a few specimens but that may be present on the trees even in the intervals of infrequent insecticide treatments. The same applies to the undergrowth (weed cover) with the difference that here the influence of pesticides seemed to be more pronounced than on the trees themselves.

The effect of insecticides on the more common species that occur on the apple trees for more prolonged periods and that display more important ecological potentials seem to be more convincing if the quantitative data of species found on 100 or 200 apple shoots were compared (Table 8).

Table 6

Influence of insecticides on the heteropterans living on apple trees (beating and individual collections) (1976–1979)

| Family | Untreated orchard | | Treated orchard | |
|---|---|---|---|---|
| | species number | individual number | species number | individual number |
| Miridae | 18 | 81 | 13 | 36 |
| Anthocoridae | 4 | 31 | 2 | 30 |
| Nabidae | 4 | 14 | 4 | 15 |
| Lygaeidae | 6 | 15 | 4 | 8 |
| Coreidae | 1 | 1 | 0 | 0 |
| Corizidae | 1 | 1 | 2 | 3 |
| Pentatomidae | 5 | 16 | 6 | 14 |
| Total | 39 | 159 | 31 | 106 |

Table 7

Influence of insecticides on the Heteroptera living on the weed undergrowth of apple orchards (sweeping, 1976–1979)

| Family | Untreated orchard | | Treated orchard | |
|---|---|---|---|---|
| | species number | individual number | species number | individual number |
| Pentatomidae | 6 | 24 | 2 | 5 |
| Scutelleridae | 0 | 0 | 1 | 1 |
| Coreidae | 2 | 3 | 0 | 0 |
| Nabidae | 4 | 14 | 4 | 13 |
| Corizidae | 4 | 24 | 2 | 3 |
| Miridae | 18 | 230 | 16 | 80 |
| Anthocoridae | 2 | 5 | 2 | 4 |
| Piesmidae | 1 | 1 | 0 | 0 |
| Lygaeidae | 6 | 99 | 4 | 19 |
| Total | 43 | 400 | 31 | 125 |

Table 8

Influence of insecticides on the Heteroptera living on apple trees, based on regular surveys of shoots (Farming-scale orchard, Nagykovácsi) (1980–1981)

| Species | Individual number in | |
|---|---|---|
| | untreated part | treated part |
| Atractotomus mali Meyer-Dürr | 113 | 1 |
| Himacerus apterus F. | 24 | 4 |
| Anthocoridae fam. | 49 | 2 |
| Miridae fam. | 54 | 9 |
| Deraeocoris ruber L. | 22 | 4 |
| Pilophorus spp. | 2 | 0 |
| Phytocoris spp. | 2 | 0 |
| Pentatomidae fam. | 2 | 0 |
| Total | 268 | 20 |

The reduction in the individual numbers was significant in the species *Atractotomus mali*, in the species belonging to the families *Anthocoridae* and *Miridae* (in the latter the genus Deraeocoris) and in Himacerus apterus. These being predators, besides the insecticidal effect also the possibility of relative starving can not be excluded. This needs to be further studied but may apply foremost to species overwintering in the egg stage *(Atractotomus mali, Deraeocoris* spp., *Himacerus apterus)* and less to members of the *Anthocoridae.*

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3—4), pp. 363—366 (1986)*

# Plant Growth Retarding Properties of Some Heterocyclic Quaternary Ammonium Salts Under Various Field Conditions

Y. DARWISH,[1] GY. KEREKES,[2] G. MATOLCSY[2] and S. AFIFY[3]

[1]Mansoura University, Faculty of Science, Chemistry Department, Mansoura, Egypt; [2] Plant Protection Institute, Hungarian Academy of Sciences, 1526 Budapest, P.O. Box 102, Hungary; [3]Mansoura University, Faculty of Agriculture, Mansoura, Egypt

Some quaternary ammonium salts revealed growth retardant activity of bean plants in the greenhouse. The effect of the most active compounds was tested under two different climatic conditions. The results were compared and calculated by mathematical statistical methods. Among the compounds tested, compound N-benzyl-N-methyl-3,3,5-trimethyl azepinium chloride showed the best activity it was also superior to the CCC used as a standard in both experiments.

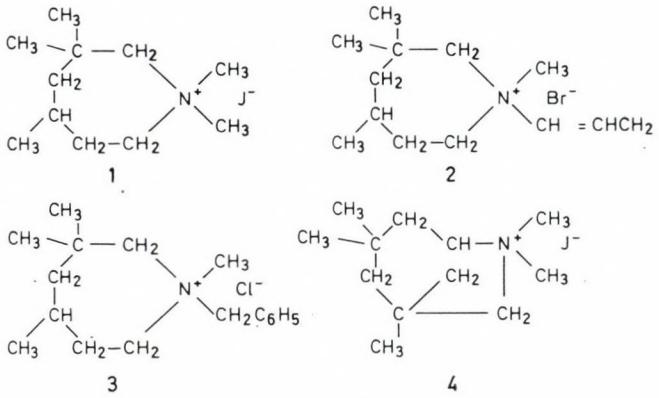
The biological activity of quaternary ammonium salts as fungicides, insecicides, and bactericides has been reported extensively in the literature (De Benneville, 1956). Certain quaternary ammonium compounds showed the ability to reduce stem elongation of bean plants (Mitchell et al., 1949). Many works have been reported on the synthesis and testing of quaternary ammonium derivatives as plant growth retardants (Krewson et al., 1959; Tolbert, 1959; Newhall and Piernger, 1966; Knight et al., 1969; Haruta et al., 1974; Kwang et al., 1978). In our previous work (Darwish et al., 1981), some aliphatic heterocyclic tertiary amines and their quaternary ammonium salts containing seven-membered and bicyclic ring structures with trimethyl groups were prepared and have been tested in the greenhouse as plant growth retardants on bean plants (*Phaseolus vulgaris* cv. Pinto), most of the quaternary ammonium salts revealed to be active.

The purpose of this study was to test the most active compounds under two different climatic conditions in field experiment i.e. in Hungary and in Egypt and to compare the results obtained.

## Materials and Methods

The synthesis of quaternary ammonium salts used in this investigation was described previously (Darwish et al., 1981). The chemical structure of the compounds examined is shown in Fig. 1.

The field experiment was conducted during April, 1983 and April, 1984 in Hungary and Egypt, respectively. The data presented are representative of at least two similar experiments. Each experiment was a randomized complete block design with four replicate. The tested bean plants (Phaseolus vulgaris cv) were arranged as micro parcels ($2 \times 2$ m $= 4$ m$^2$) in four repetitions. The distances be-



tween the rows and plants were 40 cm alike, and in one hole there were 5–6 seeds. The number of the bean stocks were the same in each parcel. When the bean plants reached the two true leaf stage, they were treated with 10 ml solutions (per plant) containing 0.1 % of the effective substances and 0.01 % cytowett as a wetting agent. Three weeks later when the plants were of three-four leaf stage, two plants from each parcel and eight pieces (4 × 2) from each treatment were harvested.

Rating for plant growth retarding activity based on measuring the following parameters: length of internodes, length of pods, and weight of pods (crop yield). Phytotoxicity evaluations of the compounds tested was based on the total surface of the leaves and the surface of the damaged leaves during the experiments. In each case chlorocholine chloride (CCC) was applied for comparison in the same concentration.

Table 1

Effect of quaternary ammonium salts on the growth of bean plants in Hungary

| Compound No. | Internodes length, cm | % effect of control | Pods length, cm | % effect of control | Pod weight, kg | % effect of control |
|---|---|---|---|---|---|---|
| 1 | 5.2 | −22.4 | 9.1 | −3.2 | 1.8 | +12.5 |
| 2 | 5.8 | −13.5 | 9.3 | −1.1 | 1.7 | +6.2 |
| 3 | 3.8 | −43.3 | 8.6 | −8.6 | 2.0 | +25.0 |
| 4 | 6.7 | ±00.0 | 8.9 | −5.4 | 1.3 | −18.8 |
| CCC | 4.3 | −35.9 | 8.7 | −7.5 | 1.9 | +18.7 |
| Control | 6.7 | — | 9.4 | — | 1.6 | — |
| SD 5 % | 2.0 | | 1.1 | | 0.6 | |

## Results and Discussion

No phytotoxicity of the tested compounds on bean plants was observed at any time during the experiments. In addition to the growth retarding activity observed, the plants showed a dark green color, a thickening of leaves.

The rate of growth retarding activity of the compounds in Hungary is shown in Table 1. Compounds 1, 2, and 3 revealed appreciable activity on the been plants at the applied concentration (0.1 %). Unexpectedly, compound 4 which showed a good activity on bean plants in the greenhouse was found to be completely inactive under the field conditions and its behaviour was similar to that of the control.

The data in Table 2 indicate the rate of growth retardation of the above mentioned compounds in Egypt. Again compounds 1, 2, and 3 showed relatively high growth retardant activity on bean plants, while compound 4 revealed to be inactive in the field.

Table 2

Effect of quaternary ammonium salts on the growth of bean plants in Egypt

| Compound No. | Internodes length, cm | % effect of control | Pods length, cm | % effect of control | Pods weight, kg | % effect of control |
|---|---|---|---|---|---|---|
| 1 | 9.7 | −9.3 | 11.7 | −17.9 | 2.2 | +18.8 |
| 2 | 7.1 | −34.0 | 13.4 | −6.4 | 1.7 | −7.1 |
| 3 | 8.3 | −22.3 | 11.8 | −17.0 | 2.4 | +28.6 |
| 4 | 10.0 | −7.0 | 12.8 | −10.4 | 1.2 | −35.4 |
| CCC | 9.8 | −8.1 | 13.5 | −5.5 | 2.2 | +20.5 |
| Control | 10.7 | — | 14.3 | — | 1.8 | — |
| SD 5 % | 2.6 | | 1.0 | | 0.6 | |

In general, it was found that the activity of a compound is not of the same under the two different climatic conditions. No linear correlation was found between the inhibitory effects of compounds on internode and pot lengths in Egypt and in Hungary ($r = 0.3352$ and 0.5186, respectively). The pod weights (crop yield) showed highly significant linear correlation:

$$\text{Pod weight}_{(\text{Egypt})} = -1.01 + (1.74 \pm 0.23) \cdot \text{Pod weight}_{(\text{Hungary})} \quad r = 0.9666$$

According to the results obtained, it can be confirmed that among this type of the quaternary ammonium salts, compounds containing one heterocyclic ring structure are more active as plant growth retardants than those of the bicyclic ones as mentioned in our previous work, 1981. Among the compounds tested, com-

pound N-benzyl-N-methyl-3,3,5-trimethyl azepinium chloride (3) showed the best activity as plant growth retardant and its activity was also superior to that of CCC used as a standard in both experiments in the same concentration. To increase the group yield, the effect of compound 3 on plant growth when treated twice (at the two-leaf state as usual and then at the full flowering state) is being studied further.

## Acknowledgment

We thank Dr. T. Cserháti for mathematical evaluation of this study.

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*Acta Phytopathologica et Entomologica Hungarica, 21 (3–4), pp. 367–371 (1986)*

# Book Review

R. N. Goodman, Z. Király and K. R. Wood: *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, Columbia, Mo. 65211, USA. p. 433. 1986.

This well illustrated book written by three international experts from USA, Hungary and England is the only textbook of its kind to present the latest advances on biochemical and physiological aspects of diseases caused by viruses, bacteria and fungi. The volume provide both students and research workers of plant pathology and plant physiology with basic concepts and current research of host parasite interaction.

As an introduction to almost each chapters, the reader finds a summary of the physiology of the healthy plant. This is extremely useful for students in plant pathology. The ten chapters comprise: infection process, photosynthesis, respiration, cell wall composition and metabolism, nucleic acid and protein metabolism, secondary metabolites, growth regulators, transcellular and vascular transport, toxins and resistance to infection.

The text is written in an eminently readable style, thus it is highly recommended to students and young scientists as an introductory text as well as an up-to-date source book.

R. G.

J. V. Groth and W. R. Bushnell (editors): *Genetic Basis of Biochemical Mechanisms of Plant Disease*. Amer. Phytopathol. Society Press, St. Paul, Minnesota, USA. p. VII + 57. 1985.

This book is based on presentations from a symposium held in Guelph, Ontario in 1984. It has been reproduced directly from the typewritten manuscripts of the authors. It contains the lectures of five authors on the recognition phenomenon, general resistance, race-specific resistance, non-host resistance and on using recombinant DNA technology to study the host-parasite interaction.

The paper of W. R. Bushnell on The Role of Nonself Recognition in Plant Disease Resistance (pp. 1–24) is a speculative summarizing article. The main conclusion is that plants do not possess a general ability to recognize either nonself individuals or nonself species. Recognition is not a component of plant disease resistance. Bushnell believes that plants develop defences only in response to threats encountered in the course of their evolution. In other words, defence responses are more likely triggered by certain substances and activities of attacking microorganisms.

As is known, in plants neither the histocompatibility complex nor the humoral immunological system exist as in higher animals. The occurrence of common antigens (shared proteins) in the pathogen and host seems to help the pathogen species to cause disease in a host species. However these cross-reactions do not relate to specificity at the race or forma specialis levels. We can not explain why antigenic similarity to a plant host confers an advantage on a parasite.

The author then treats evidences against nonself recognition in plants, such as protoplast fusion, tissue co-culture with paired species and grafts. After discussing somatic incompatibility in fungi and pistil-pollen compatibility and incompatibility in higher plants he deals with the defence responses triggered by the pathogen. It is known that reactions lead to disease resistance of plants are controlled by unknown interactions of specific molecules of host and parasite. Knowing too few about those specific molecules, one cannot say too much at the moment on the mechanism of induced defence.

The second article by Michele C. Heath treats the Implications of Nonhost Resistance for Understanding Host-Parasite Interaction (pp. 25–42). It would seem that understanding nonhost resistance will help in understanding specific host-parasite interactions. She concludes that plant resistance should be durable if it is multi-component and based on several defence mechanisms, furthermore, if it is negated by a product of the pathogen with a high degree of specificity. This article too, seems to be rather speculative.

The next chapter is on The Implications of General Resistance for Physiological Investigations by B. C. Clifford, T. L. W. Carver and H. W. Roderick. This is the most original paper in this book. Dealing with rust and powdery mildew diseases they identified different barriers against fungal development: reduced penetration, prevention of haustorium formation, restriction of haustorium size and efficiency resulting in lower infection frequency, slower colony development and reduced or delayed sporulation. This type of resistance seems race non-specific and governed by complex genetic systems.

The chapter on Progress in Understanding the Biochemistry of Race-specific Interactions by N. T. Keen deals with recognitions in gene-for-gene systems, the elicitor-suppressor hypothesis, the elicitor-receptor model of resistance connected with phytoalexin production and with the cloning of pathogen avirulence genes. He speculates on the usefulness of recombinant DNA technology which will permit in the future to understand of the relationship between phytoalexin elicitors and pathogen avirulence genes, whether or not they are primary (or secondary) avirulence gene products, how do suppressors relate to the function of virulence/avirulence alleles, are plant resistance gene products the protein receptors for pathogen-produced specific elicitors, as the elicitor-receptor model predicts? According to Keen it is unlikely that single genes conferring race-specific disease resistance will be useful because they are too specific. It would seem more reasonable to isolate a plant gene which confers general, race non-specific, resistance to an entire pathogen species and introduce it into a plant that is susceptible to the pathogen.

The last chapter was written by A. H. Ellingboe on Prospects for Using Recombinant DNA Technology to Study Race-specific Interactions between Host and Parasite. He believes that recombinant DNA technology already influences plant pathology in the form of diagnostic kits for viruses, by engineered biocontrol agents, by cloned avirulence genes of pathogens and by routine genetic transformation in host plants.

Ellingboe points out that plant breeders and pathologists have manipulated genes for disease resistance for practical gain for more than 75 years. But the products of these genes are still unknown. Genes that control host-parasite specificity will be cloned in both pathogens and host plants probably soon. However, the products of these genes should be known in the very near future. Obviously, this information will have great effects on both plant pathology and plant breeding.

The book contains 239 citations and a subject index. This volume certainly will be widely used by students as well as researchers. It contains a great number of new data, new ideas and stimulating speculations.

*Z. Király*

D. G. A. Walkey: Applied Plant Virology. Heinemann Ltd., (London), 1985. p. 329.

In his book of 329 pages the author gives highly important and quite new pieces of information in 12 chapters. At the end of each chapter references and further selected reading are found.

In Chapter 1 (Plant virology: An introduction) the author deals with the definition, economic importance and structure of viruses. In Chapter 2 (Plant virus classification) describes the most important characteristics of some 27 groups of viruses, and discusses the virus strains, the virus-like diseases, the viroids, mycoplasmas and rickettsias. Chapter 3 (Virus symptoms) deals with general problems of virus symptomatology. The chapter is enriched by excellent figures. Descriptions are given of the cytological and histological changes; further on the effect of environment on the symptoms and the genetic factors are discussed. Chapter 4 (Mechanical transmission and virus isolation) describes the processes of virus transmission, discusses problems related with the transmission and mentions the storage of viruses. In Chapter 5 (Virus purification) the propagation of viruses and the methods of purification are dealt with. Chapter 6 (Virus identification) makes the readers acquainted with the concept of host-range, with in vitro properties of viruses, cross protection, electron microscopy, serology, cytopathology, sedimentation properties, electrophoretic mobility, chemical composition and molecular hybridization. The subject of Chapter 7 (virus transmission by biological means) is the spontaneous transmission of viruses, but transmission by grafting and vectors are also mentioned. Special sections deal with virus transmission by seed and pollen as well as by dodder (Cuscuta spp.), and with transmission in a vegetative way. Chapter 8 (Ecology and epidemiology of plant viruses) discusses the role of weeds and other alternative hosts in virus transmission, the virus infection of new areas, the appearance of new virus strains, and viruses infecting new cultivars. Chapter 9 (Basic control measures) is concerned with such problems of international importance as the production of virus-free plants, the chemical and non-chemical control of vectors, and the role of cross protection in disease control. Chapter 10 (Control through resistant cultivars) deals with various methods of achieving virus resistance, with the sources of resistance, the screening- and breeding methods, the production of resistant plants by cell manipulation, further with somatic hybridization and DNA transformation. Chapter 11 (The production of virus-free plants) gives information on thermotherapy, tissue culture, chemotherapy. Chapter 12 (Practical information and introductory exercises), the last chapter of the book acquiants the readers with laboratory techniques and methodological questions. This kind of information is highly useful for all those enganged in experimental virology.

The book is completed with a glossary and an index. Dr. D. G. A. Walkey's book "Applied Plant Virology" will be used by undergraduates and postgraduates in plant virology, plant pathology, microbiology and general biology.

*J. Horváth*

K. Maramorosch and H. Koprowski (editors): *Methods in Virology*. Academic Press (New York and London), Vol. VI., 1977. p. 542., Vol. VII., 1984. p. 332., Vol. VIII., 1984. p. 396.

The five volumes of the fundamental work "Methods in Virology" published between 1967 and 1971 which discussed the most important methodological questions of general virology in 81 chapters on some 3293 pages was followed by further three volumes (VI., VII. and VIII.) between 1977 and 1984. In the 31 chapters of the last 3 volumes of 1190 pages in total information is given of the latest methods and technical procedures the knowledge of which is indispensable in research and education. The orientation is made easy by a subject index of about 58 pages. At the end of each chapter a selected list of the most important literary works is found. The latest three volumes of the "Methods in Virology" conatin some 3529 literary references and 183 figures. The three volumes were written by 67 internationally

acknowledged prominent virologists. It is the editors' merit to have chosen the right authors, whereby they could compile a work that no virological workshop can dispense with.

The volumes edited by K. Maramorosch and H. Koprowski belong to the most outstanding literary works on plant virology. The latest volumes of "Methods in Virology" contain the following chapters:

Vol. VI: *1.* Application of immunofiuorescence to diagnosis of viral infections (R. W. Emmons and J. L. Riggs), *2.* Experimantal analysis of effector thymus-derived lymphocyte function in viral infections (P. C. Doherty, J. C. Palmer and R. F. Zinkernagel), *3.* Methods for assays of RNA tumor viruses (V. Klement and M. O. Nicolson), *4.* Assay methods for viral pseutotypes (J. Závada), *5.* Methods for assaying reverse transcriptase (D. L. Kacian), *6.* Methods for studying viroids (T. O. Diener, A. Hadidi and R. A. Owens), *7.* Electron microscopy of viral nucleic acids (D. P. Evenson), *8.* Rapid immune electron microscopy of virus preparations (R. G. Milne and E. Luisoni), *9.* Transfection methods (V. M. Zhdanov), *10.* Invertebrate cell culture methods for the study of invertebrate-associated animal viruses (D. L. Kundson and S. M. Buckley), *11.* Techniques of invertebrate tissue culture for the study of plant viruses (D. V. R. Reddy), *12.* Use of protoplasts for plant virus studies (S. Sarkar), *13.* Nucleic acid hybridization technology and detection of proviral genomes (E.-S. Huang and J. S. Pagano).

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The volumes of "Methods in Virology" have been received in the international scientific world with an enthusiasm which they merited: this worthy appreciation may be attributed with good reasons not only to their pioneering character but also to the high standard of their content.

*J. Horváth*

P. Singh and R. F. Moore (editors): *Handbook of Insect Rearing* Elsevier Science Publishers, Amsterdam—Oxford—New York—Tokyo, 1985. pp. VIII+488, VIII+514.

For up-to-date entomological research and for integrated pest management projects the successful rearing of insects is indispensable. Especially the sterile insect technique and other biological control methods need huge amount of laboratory reared insects, therefore, efforts continued to develop mass-rearing systems. In the last 15 years increasing progress has been made in relation to the artificial diets and rearing procedures. Literature on rearing techniques has grown up and a quick orientation in this field has became difficult.

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The larger part of the work are concerned with practical guide for rearing of a series of insect species inside the order *Coleoptera*, *Collembola*, *Dictyoptera*, *Hemiptera (Heteroptera) Hymenoptera*, *Neuroptera*, *Orthoptera* (in the 1st volume), *Diptera* and *Lepidoptera* (in the 2nd volume). These papers offer detailed information on the insect rearing techniques in a "cookbook-style", wich make it usable also for non-professional entomologists. Unfortunately, references attached to each chapter of the species are unequal, sometimes indeed poor (e. g. *Aphidiae*, *Ostrinia nubilalis)*. Rearing of some omitted, but important species (e. g. *Locusta migratoria*, *Bacillus rossius*, *Dermaptera*, *Grapholitha funebrana*, *Sitotroga cerealella)* surely will be discussed in the promised third volume.

This standard work offers very useful and up-to-date informations in insect-rearing and will be a vaulable help for biologists, geneticists, toxicologists, but, first of all for entomologists.

The two volumes are well presented and illustrated and completed by different indexes.

*B. Nagy*

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**HU ISSN 0001—6780** **Index: 26.032**