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MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 21 JANUARY 1988

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MOVEMENT OF APOPLASTIC FLUORESCENT DYES THROUGH TOMATO ROOTS INFECTED BY *MELOIDOGYNE INCOGNITA*

In many sedentary nematode/plant combinations the nematode, once in the root, pierces the endodermis and induces feeding cells (giant cells) within the central cylinder. These cells develop extensive wall ingrowths adjacent to the vascular elements, suggesting that the bulk of solutes enters the giant cells from the apoplast. Physiological changes of the root system following infection, such as enhanced mineral/metabolite leakage and an increased uptake rate of ions assumed to be transported mainly via the apoplast, have been demonstrated. It has been suggested that the nematode maintains an apoplastic pathway for solute movement into, as well as out of the central cylinder. To trace whether there is such an apoplastic pathway between the cortex and the central cylinder of tomato roots (*Lycopersicon esculentum* Mill cv. Moneymaker) infected by *Meloidogyne incognita* (Kofoid & White), the movement of two fluorescent dyes into the central cylinder and their possible translocation to the giant cells was followed.

Disodium, 4,4′-bis (sulfostyryl) biphenyl (Tinopal CBS), a blue fluorescent dye which binds strongly to cellulose, and the more mobile dye trisodium, 3-hydroxy-5,8,10-pyrene trisulphonate (PTS), a green fluorescent dye which does not bind to the cell walls, were applied to uninfected and infected root systems. The uptake of both dyes is restricted to the apoplast. The Tinopal CBS- and PTS-treated roots were rinsed in tap water for 1 h and for a few seconds, respectively. Handcut sections were viewed with a fluorescence microscope with suitable filters.

PTS was less accurate as a marker than Tinopal CBS because of its enhanced mobility and its fluorescence, which was similar to the yellow autofluorescence of the plant tissues (epidermis, cortex and xylem). In uninfected, as well as in infected, roots the endodermis prevented the entry of both dyes into the central cylinder. The dyes entered the main roots only via the cortex and the central cylinder of branch roots. During transport in the xylem vessels, the dyes left the xylem at the sites of giant cells but remained inside the central cylinder. In some sections there was a sharp contrast between the fluorescent and the non-fluorescent tissues indicating that there is little lateral diffusion of Tinopal CBS during the translocation from the xylem compartment to the giant cells. The water suction of the giant cells, together with their associated nematodes, must be considerable, since in experiments in which the root system was partly submerged in the dye solution, the water movement in some xylem vessels in infected branch roots above the solution was reversed.

We conclude that there is no mass flow of water and solutes into or out of the central cylinder along the body of the nematode. At the time of feeding there is water flow within the central cylinder from the xylem compartment to the giant cells. The resistance to water movement out of the xylem vessels to the giant cells is probably lower than for water movement out of neighbouring cells.

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ENZYMES AND OTHER PROTEINS OF APOPLASTIC FLUIDS FROM THE INTERACTION *CLADOSPORIUM FULVUM*–TOMATO

The interaction between the intercellularly growing fungus *Cladosporium fulvum* and tomato is used as model system to study the accumulation of host-, pathogen- and interaction-specific proteins in leaf apoplastic fluids of various compatible and incompatible combinations.

Fungus-mediated accumulation of pathogenesis-related (PR) proteins of plants has been reported to occur in the apoplastic fluid of *C. fulvum*-infected tomato. Also race-specific elicitors of necrosis have been isolated from apoplastic fluids obtained from compatible interactions between *C. fulvum* and tomato.

Analysis of apoplastic fluids by polyacrylamide gel electrophoresis (PAGE) under low pH, non-denaturing conditions revealed a soluble protein, present in all compatible interactions but not detectable in incompatible interactions, nor in uninoculated controls. The protein was purified by ion-exchange chromatography, followed by chromatofocusing, and was used to raise antibodies. The protein (M_r 14 000) could not be induced by several types of plant stress or fungal infections other than *C. fulvum* and therefore is unrelated to the class of PR proteins. Although it is not yet known whether the protein is of host or fungal origin, it might be an important factor of basic compatibility.

SDS–PAGE of apoplastic fluids revealed that several PR proteins accumulated 2 or 4 days earlier in incompatible interactions than in compatible ones. Two of the accumulating PR proteins could be purified by ion-exchange chromatography, followed by chromatofocusing, and were used to raise polyclonal antibodies. One of the proteins (M_r 25 000) showed chitinase activity, while the other (M_r 35 000) was able to degrade laminarin (1,3-β-glucanase activity).

The early accumulation of both proteins in incompatible interactions, visualized by SDS–PAGE (and/or western blotting) of apoplastic fluids obtained at different times after inoculation, could also be shown by measuring enzyme activities in total apoplastic fluids. The rapid accumulation of these enzymes, potentially capable of degrading the hyphal walls of *C. fulvum*, at the site of penetration, might play an important role as an active plant defence mechanism.

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STRUCTURE AND FUNCTION OF VIRUS-INDUCIBLE PLANT GENES

Plants which react hypersensitively to infection by pathogens are known to accumulate 'pathogenesis-related' (PR) proteins in the intercellular space of the leaf. The ten acidic PRs induced by tobacco mosaic virus (TMV) infection of Samsun NN tobacco have been studied in great detail, i.e. the proteins 1a, 1b, 1c, 2, N, O, P, Q, R and S. Available data on the function of PRs are consistent with the assumption that these proteins play a role in various defence mechanisms of the plant. At least three groups of closely related proteins have been recognized: the PR-1 proteins of unknown function, the β-1,3-glucanases 2, N and O, and the chitinases P and Q. Basic counterparts of all three groups of acidic PRs have been identified (for a review, see: Bol & Van Kan 1988).

To gain further insight into the function and mode of induction of PR proteins, we have cloned cDNA to the mRNAs of the tobacco acidic PRs (with the exception of PR-R) and their basic homologues. Probing genomic blots of tobacco with these cDNAs revealed that the respective

groups of PR proteins are each encoded by families of four to eight genes. Moreover, the cDNAs were used as probes to isolate the corresponding genomic clones. Of the four to eight genes for acidic PR-1 proteins, one expressed gene and two putatively silent genes were sequenced (Cornelissen *et al.* 1987). In addition, four tobacco genes were cloned encoding a TMV-inducible 13·5 kD protein that does not correspond to any of the known PRs and two of these genes were sequenced. This protein appears to contain a high proportion of glycine residues (25%) suggesting that it may be a cell-wall component.

Bol, J.F. & Van Kan, J.A.L. (1988): *Microbiological Sciences* **5:** 47–54.
Cornelissen, D.B.J.C., Horowitz, J., Van Kan J.A.L., Goldberg, R.B. & Bol, J.F. (1987): *Nucleic Acids Res.* **15:** 6799–6811.

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ISOLATION AND CHARACTERIZATION OF VIRUS–RIBOSOME COMPLEXES FROM CELL-FREE TRANSLATION SYSTEMS SUPPLEMENTED WITH COWPEA CHLOROTIC MOTTLE VIRUS PARTICLES

Initial interactions between viruses and plant cells are topics in plant virus research to elucidate resistance mechanisms. These interactions may be crucial in determining whether plants are susceptible or resistant to a specific virus. Possible candidates for interactions between host components and virus particles are ribosomes. Therefore, virus–ribosome interaction studies were initiated using cowpea chlorotic mottle virus (CCMV), a bromovirus, in combination with cell-free translation systems derived from wheat germ or rabbit reticulocytes.

Both extracted (unencapsidated) RNA and RNA supplied as intact virus particles appeared to be translated into viral specific proteins with molecular masses of 105, 100, 35 and 23 kDa. The rate of protein synthesis on addition of encapsidated RNA was much slower than that of extracted RNA. The quantity of oligopeptide formed, however, was also only 10% of that of extracted RNA.

Using sucrose and caesium-chloride gradient analysis, virus–ribosome complexes, with up to four ribosomes per virus particle, were isolated from translation mixtures supplemented with CCMV particles. These complexes, with densities intermediate to those of the virus (1·36 g cm^{-3}) and ribosomes (1·58 g cm^{-3}), were visualized in the electron microscope. Less than 5% of the particles was found in association with ribosomes.

From these results it can be concluded that a small fraction of CCMV particles interacts with ribosomes, which results in translation of the viral genome into viral-specific proteins in a process of co-translational disassembly. The implications of this process for the structure of the particle, as well as the role of this process *in vivo*, will be discussed.

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INVOLVEMENT OF HOST PLANT IN PRODUCTION OF VOLATILE SPIDER-MITE KAIROMONE THAT ATTRACTS PREDATORY MITES?

Plants may defend themselves against pathogens or herbivores through constitutive or induced defensive features (Rhoades 1985). Well-known examples of induced defence are, for example,

production of phytoalexins or proteinase inhibitors after attack by pathogens or phytophagous insects, respectively. Apart from direct induced defence against pathogens or herbivores, plants may also use indirect ways, through mobilizing natural enemies of the herbivores after they attack the plant.

When a plant is damaged by the spider mite *Tetranychus urticae* Koch, a volatile infochemical (Dicke & Sabelis 1988) is emitted that attracts predatory mites (Sabelis & Van de Baan 1983; Sabelis *et al.* 1984). This infochemical is not emitted by spider mites isolated from the plant but after removal of the spider mites the plant remains attractive for several hours (Sabelis & Van de Baan 1983). The infochemical involved, which is called a kairomone in the interaction between spider-mite and predatory mite, is phytophage-species specific (Sabelis & Van de Baan 1983; Dicke & Groeneveld 1986), and is emitted from the infested plant. In contrast, clean host plants do not attract predatory mites. Would the plant be involved in production of the kairomone after attack by the phytophagous mites? Chemical analysis of the compounds emitted from clean and infested host plants show that all chemicals are well-known in the plant kingdom. Three of the chemicals that are emitted from infested plants (linalool, E-β-ocimene and methyl salicylate) attract the predatory mite *Phytoseiulus persimilis*, which indicates that these chemicals are components of the kairomone. To gain more insight into the involvement of the plant in production of the volatile kairomone, an analysis of the biosynthetic pathway of the compounds is needed.

To understand the evolution of plant-produced kairomones after herbivore attack, it is crucial to assess how they are produced and to quantify the costs in terms of energy and the benefits in terms of a lowered probability of damage by herbivores.

Dicke, M. & Groeneveld, A. (1986): *Ecol. Entomol.* **11:** 131–138.
Dicke, M. & Sabelis, M.W. (1988): *Func. Ecol.* **2:** (in press).
Rhoades, D.F. (1985): *Am. Nat.* **125:** 205–238.
Sabelis, M.W. & van de Baan, H.E. (1983): *Entomol. Exp. Appl.* **33:** 303–314
Sabelis, M.W., Afman, B.P. & Slim, P.J. (1984): *Acarology*, **VI, I:** 431–440.

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THE BIOCHEMICAL RESPONSE OF DIFFERENT CULTIVARS OF *DIANTHUS CARYOPHYLLUS* L. TO INFECTION BY *FUSARIUM OXYSPORUM* F. SP. *DIANTHI*

Microscopical investigations have shown that the physiological response of the host in vascular wilt in carnations varies with the degree of susceptibility (Baayen & Elgersma, 1985). Resistance in carnations to *Fusarium oxysporum* f. sp. *dianthi* is governed by several gene pairs and possibly includes those that govern gel formation and production of phytoalexins.

In the present investigation the following 13 cultivars with different degrees of resistance to *F. oxysporum* were compared for vacuolar and cell-wall-bound compounds of phenolic nature found at different times before and after inoculation: 'Novada', '78618-12', 'Revada', 'Niky', 'Carrier 929', 'Elsy', 62093-G', 'Pallas', 'Silvery Pink', 'Scania', 'Lena', 'Sam's Pride' and 'Early Sam'. In all the cultivars infection with *F. oxysporum* led to the production of at least six new compounds; four were mildly toxic to *F. oxysporum*. By co-chromatography, two of the compounds were found to be identical to the phytoalexins dianthelexin and dianthramide A. All compounds were present in the cell sap (with the exception of dianthalexin) but, in addition, also appeared to occur bound to the cell wall residue from which they were released by mild degradation with sodium hydroxide.

With three cultivars time-course experiments showed, for most phytoalexins, a fast increase in concentration in the period from 24 h to 5–7 days after inoculation. The compounds were still present in considerable amounts after 1·5 months. In these experiments the more resistant cultivars accumulated phytoalexins to a higher degree than more susceptible ones; for dianthramide A this also occurred at a higher rate.

In some susceptible cultivars the comparative concentrations of the phytoalexins were different from those of resistant cultivars. A comparatively low concentration of dianthramide A, the 4-methoxyanthranilic acid amide of salicylic acid, together with a higher incidence of a compound with the retention time of salicylic acid, might indicate a blockade of the conversion of salicylic acid into its amide in these susceptible cultivars.

Baayen, R.P. & Elgersma, D.M. (1985): *Neth. J. Pl. Pathol.* **91**: 119–135.

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MORPHOLOGY OF PRIMARY INFECTION STRUCTURES AS A CRITERIUM FOR THE TAXONOMIC CLASSIFICATION OF RUST FUNGI OF CEREALS AND GRASSES

The rust fungi are an important and large group of pathogens in agricultural and horticultural crops. The species or intraspecific taxa typically have very narrow host ranges. The taxonomy of rust fungi is complex. The criteria used to distinguish species or intraspecific taxa in the dikaryotic phase include host range (main and alternate hosts), sorus morphology, urediospore and teliospore morphology (including spore colour) and dimensions, and dormancy of the teliospores. These criteria are either impractical to determine or are subjective or are not sufficiently distinctive because of overlap between taxa. The lack of distinctive morphological traits not only hampers a meaningful taxonomic classification but also the identification of rust samples (quarantine).

Research on rust samples collected from cereals and grasses indicated that the infection structures (substomatal vesicles, primary infection hyphae and haustorial mother cells) are considerably less conservative in their morphology than the spores and sores. Interspecific differences between the ten rust species studied were very large. Formae speciales or varieties of some species did not differ in morphology of the structures (e.g. isolates of *P. striiformis* from barley, wheat, wheat grass and *Bromus*). Isolates of *P. striiformis* collected from *Poa* species differed markedly from those collected from the other plant species. The form from *Poa* may deserve a distinct taxonomic status. In *P. recondita* and *P. brachypodii* samples differed according to the host from which they were collected.

Within the lowest taxonomic units there may or may not be variation for the morphology of infection structures. In *P. hordei* such variation in Moroccan isolates was associated with a spectacular difference in virulence spectrum on barley.

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ANALYSIS OF EPIDEMICS OF *PHYTOPHTHORA INFESTANS* IN POTATO VARIETIES DIFFERING IN LEVEL OF RESISTANCE

Field plots of three potato varieties were inoculated by a spray with a complex fysio of *P. infestans*. One week after inoculation the genotypes differed widely in the percentage of leaf area covered by

lesions. The lesion coverage showed a similar logistical increase in rate in the following weeks, thus differences between genotypes were maintained. The magnitude of the differences and the earliness of their appearance (during the first disease cycle) did not allow an explanation based on different latent periods or sporulation intensities.

In greenhouse experiments the same ranking of genotypes was found, with regard to the area covered by individual lesions. Again genotypic differences were established within the first week of inoculation. Later the lesions showed similar relative growth rates until they reached the edge of the leaflets.

These results warrant further study of the processes of infection and early lesion growth.

The three genotypes showed similar spatial distributions of lesions in the field experiment. Leaf lesions were most prominent at low levels within the canopy while the opposite was found for stem lesions. Stem lesions, therefore, were probably not important as initiators of leaf infection.

Apart from lesion coverage, leaf yellowing led to loss of green leaf area. Comparison with control plots showed that leaf yellowing was stimulated by infection, especially in the lower leaves of the most heavily infected variety.

Tuber yield was determined in both inoculated and control plots. The results were examined by means of a simulation model of potato crop growth and yield reduction due to infection by *P. infestans* could be explained in all three genotypes by the loss of green leaf area alone. Thus the production capacity of green leaves did not seem to be influenced by infection. The same model showed a minor effect of the observed leaf yellowing stimulation compared to the effect of lesion coverage.

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REDUCED EFFECT OF FUNGICIDES ON PERTHOTROPHIC LEAF PATHOGENS ON WHEAT IN THE PRESENCE OF NUTRIENTS

The influence of nutrients on the effect of fungicides on perthotrophic leaf pathogens of wheat was investigated on flag leaves of spring wheat, cv. Minaret, in a controlled environment. Different fungicides were tested in combination with *Cochliobolus sativus* and *Septoria nodorum.*

The pathogens were sprayed on the leaves in combination with different concentrations of fungicides, with or without nutrient addition (2% sucrose + 0·5% yeast extract).

In all the experiments the average germ tube length per spore on Day 2 was significantly larger when nutrients were added compared to the treatments without nutrients. This stimulation occurred both in the absence and in the presence of the fungicides tested. In treatments with both nutrients and fungicides the germ tube growth was stimulated compared to the treatment without nutrients and without fungicide. Thus the effect of the fungicides was neutralized by the nutrients.

In the absence of added nutrients, infection (percentage necrotic leaf area) by *C. sativus* was reduced by captafol (400 mg a.i. l^{-1}), by triadimefon (125 mg a.i. l^{-1}) and by benomyl (500 mg l^{-1}) to about 20, 15 and 30% of the infection of control leaves (pathogen only), respectively. Added nutrients neutralized the effect of the fungicides completely. Generally more infection occurred in the presence of fungicides plus nutrients than in the absence of both additions.

In the absence of added nutrients, infection of wheat leaves by *Septoria nodorum* by captafol (160 mg a.i. l^{-1}), by benomyl (500 mg a.i. l^{-1}) and by prochloraz (45 mg a.i. l^{-1}) was reduced to 4, 7 and 7% of the infection of the control leaves, respectively, whereas in the presence of added nutrients the reduction of infection was limited to 14, 48 and 60%, respectively. Control

experiments demonstrated that the nutrients did not alter the susceptibility of the leaves. There is thus a direct effect of nutrients on the pathogens.

Under field conditions the concentration of fungicides will decrease after application. Our results indicate that the concentration which is no longer effective might be considerably higher in the presence of exogenous nutrients such as aphid honeydew and pollen grains.

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INTERACTION OF SOIL PATHOGENIC FACTORS, VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHATE AVAILABILITY IN THE RHIZOSPHERE OF *PLANTAGO MAJOR* SSP. PLEIOSPERMA

We studied the effect of micro-organisms in the rhizosphere of a population of *Plantago major* ssp. pleiosperma, which was situated on a location with different phosphate availabilities. In a pot experiment with sterilized soil from this location, the effect of the addition of 2·5% rhizosphere soil on the growth of *P. major* ssp. pleiosperma was highly dependent on the phosphate availability: a growth stimulating effect at low phosphate availability and a growth reducing effect at high phosphate availability. In all the treatments with rhizosphere soil, the roots were infected with vesicular-arbuscular mycorrhizal (VAM) fungi. Therefore, a second experiment was set up in order to separate the effect of the indigenous VAM fungi from the total rhizosphere population. Independent variables in this full-factorial experiment were phosphate availability, the addition of rhizosphere soil, and the addition of VAM inoculum. At low-phosphate availability, both the VAM fungi and the rhizosphere soil increased the shoot growth of *P. major* ssp. pleiosperma, whether applied separately or in combination. At a higher (but still growth limiting) phosphate availability, however, there was no significant effect of the addition of VAM inoculum, whereas the addition of rhizosphere soil again showed a reduction in growth. It was concluded that this reduction in growth could not only be due to mycorrhizal infection. Moreover, the combined addition of VAM inoculum and rhizosphere soil decreased the reduction in growth. The total acquisition of phosphate (and nitrogen) was well correlated with dry-matter production, and, therefore, was lower in the treatments which showed a reduced growth. The addition of VAM inoculum increased the phosphate uptake and growth. The results suggest that unknown pathogens in the rhizosphere of *P. major* ssp. pleiosperma affect the uptake of phosphate, which leads to an induction of phosphate stress, and a subsequent growth reduction. Furthermore, it is suggested that VAM-infection decreases this phosphate stress by creating a higher phosphate uptake capacity.

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THE RELATIONSHIP BETWEEN SOIL MICRO-ORGANISMS AND THE OCCURRENCE OF SELF-INTOLERANCE IN *AMMOPHILA ARENARIA* (MARRAM GRASS)

Degeneration of *Ammophila arenaria* on coastal fore-dune ridges is caused by harmful biotic soil factors. To determine which groups of organisms induce this self-intolerance, soil samples from the root zone of a vigorous (V) as well as from a degenerating (D) *Ammophila* vegetation were treated with biocides. In a greenhouse experiment the growth of *Ammophila* seedlings on these treated sand types was compared with the growth on both untreated and γ-irradiated (2·5 Mrad) sand.

A mixture of bactericides (streptomycin, 50 mg kg^{-1} dry sand + penicillin, 25 mg kg^{-1}) did not affect the growth of the seedlings significantly. A mixture of Benlate® (benomyl, 20 mg kg^{-1}) and Previcur N® (propamocarb, 20 mg kg^{-1}), two fungicides, enhanced the growth of seedlings on V sand only.

In a second experiment, Benlate® and Previcur N® were applied separately to both V and D sand. Only seedlings on V sand treated with Benlate® showed a significantly higher biomass production than untreated sand. In the same experiment, application of Vydate® (oxamyl, 100 mg kg^{-1}), a nematocide, enhanced the biomass production significantly on both V and D sand. Nematod countings showed that the roots of the plants in untreated sand and in sand with Previcur N contained low numbers of *Heterodera* sp. (*Avenae* group) and of *Meloidogyne graminis*, whereas roots in D sand also contained *Pratylenchus* sp. In sand treated with Vydate® the roots were free of plant parasitic nematods and with Benlate® the roots contained neither *Heterodera* nor *Meloidogyne*.

It is obvious that plant parasitic nematods are involved in the degeneration of *A. arenaria*. Because of the nematocidal activity of Benlate®, no conclusions could be drawn with respect to the role of soil fungi.

In a third experiment, three fungus species (*Microdochium bolleyi, Fusarium culmorum*, and *F. oxysporum*) were isolated from the roots of a natural *Ammophila* vegetation. Nematods were also collected from this location. Fungi, nematods, and a mixture of both were inoculated in pots with sterile sand in which *Ammophila* seedlings were grown in the greenhouse. *M. bolleyi* caused severe growth reduction. The mixture of the three fungus species caused significantly reduced growth only in combination with the nematods. Nematods alone did not cause any reduction in growth.

These results explain the strongly enhanced growth of *Ammophila* on V and D sand treated with Vydate®. Even low numbers of nematods may intensify the pathogenicity of the soil fungi and, consequently, seedling growth is reduced severely. It seems likely, therefore, that a combination of soil fungi and plant parasitic nematods is involved in the degeneration of *Ammophila*.

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GROWTH AND SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN THE RHIZOSPHERE OF HOST AND NON-HOST PLANTS

The growth of *Xanthomonas campestris* pv. *campestris* (Xcc) strain 107 was studied in the rhizosphere of axenically grown plants. The plants, *Brassica oleracea* var. *gemnifera* cv. *Adeline, Medicago lupulina* and *Solanum nigrum*, were grown on vermiculite in wide tubes. The *Brassica* sp. is known as a host for Xcc; *M. lupulina* and *S. nigrum* are described as hosts for other *Xanthomonas campestris* pathovars (resp. pv. *alfalfae* and pv. *vesicatoria*). Xcc was inoculated onto the roots by adding 0·5 ml suspensions containing 5×10^4 and 5×10^7 cfu ml^{-1}. The growth of the bacterial population was studied by assessing the number of microscope fields which showed colonies on the roots by direct observation 0, 7 and 14 days after inoculation. A staining procedure was used which was based on the method described by Van Vuurde & Elenbaas (1978). The results indicate that Xcc establishes itself on the roots of the plants used in this study, as shown by the formation of colonies. In the case of *Brassica* and *Medicago*, the number of microscope fields showing colonies increased to 50·5 and 22·6%, respectively, during the observation period. While on *Solanum* the number of microscope fields with colonies stabilized on 8%.

The impact of growth of Xcc on host plants, without causing symptoms, as well as on non-host plants for the survival of this pathogen is discussed.

Van Vuurde, J.W.L. & Elenbaas, P.F.M. (1978): *Can. J. Microbiol.* **24:** 1272–1275.

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BACTERIA IN HYDROPONICS: EFFECTS ON PLANT GROWTH

Artificial substrates are widely used in greenhouse cropping. Development and the role of the microflora in relationship to plant development has received scant attention. As only low numbers of bacteria ($2{\cdot}0 \times 10^2$ cfu ml) were present at the beginning of our experiments, compared to $2{\cdot}0 \times 10^8$ cfu g^{-1} soil, it was hypothesized that the rhizosphere microflora in soilless cultures is less balanced than the more complex soil. Therefore, an excessive development of certain deleterious micro-organisms has to be anticipated.

Bacterial infection of lettuce, tomato, cucumber and potato stem cuttings with selected *Pseudomonas* spp. strains resulted in an increase in growth. This growth promotion was thought to be due to the suppression of growth or activity of a deleterious microflora. Simulation of microbial development was acquired by inoculation of tomato plants with rhizosphere suspensions of plants that had previously been grown for different lengths of time (14 and 30 days) in cultures without soil. A reduction of 17% in plant fresh-weight was found in the treatment where a rhizosphere suspension of plants (grown for 30 days in hydroponic culture) was added to the tomato plants.

Bacterial root colonization demonstrated no relationship between densities in the ecto-rhizosphere and plant growth. The fluorescent pseudomonas population in the endo-rhizosphere, however, showed a positive correlation with growth reduction. Growth reduction could be counteracted by bacterization with the plant growth-promoting *Pseudomonas* sp. strain WCS417.

The role of bacteria that particularly inhabit the endo-rhizosphere of plants grown hydroponically, and their interactions with plant-growth promoting bacteria, is subject to further study.

MEETING OF THE SECTION FOR PLANT SYSTEMATICS AND GEOGRAPHY ON 13 NOVEMBER 1987

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DELIMITATION OF GENERA IN MICROSOROID FERNS (POLYPODIACEAE)

A major taxonomic problem in the microsoroids is the delimitation of supraspecific taxa. Since its revival in 1929, *Microsorum* has been associated in various ways with a considerable number of other genera. Several authors disagree on the (genealogical) relationships between these genera or their species and the way these should be classified. This disagreement is rooted in differences in the used selection of characters and species (e.g. in regional revisions), the interpretation of character distributions (e.g. recognition of parallel developments) and, above all, the criteria used for generic delimitation.

These criteria are rarely made explicit or used consistently. If genera and other supraspecific taxa should reflect historical events in evolution, they have to be monophyletic. Interpretation of hypotheses of genealogical relationships obtained phylogenetic analysis results in recognition of monophyletic groups. The decision to formalize these groups as taxa of a certain rank is not made arbitrarily but should be based on a choice of pragmatic or practical criteria, such

as tradition, stability, surveyability and suitability for keys. The criterium of monophyly may not, of course, be overruled.

In applying these principles to the microsoroids, it is concluded that only after phylogenetic analysis of the involved species can a supraspecific classification be set up. *Microsorum* is suspected of being a polyphyletic genus, involving at least three groups of species that are more related to species from *Colysis, Diblemma* and *Neocheiropteris*, respectively, than to the rest of *Microsorum*. This, first, has to be confirmed by phylogenetic analysis, then the choice between one large genus or two smaller genera will remain to be solved on pragmatical and practical grounds.

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CONFLICTING EVIDENCE CONCERNING THE TAXONOMIC STATUS OF *RACOPILUM INTERMEDIUM* HAMPE (RACOPILACEAE, BRYOPSIDA)

The pantropical genus *Racopilum* has two representatives in tropical America, namely *R. tomentosum* (Hedw.) Brid. and *R. intermedium* Hampe. Gametophytically *R. intermedium* can only be distinguished by its longer, more regularly pinnate stems. Sporophytically, however, there are very distinct differences. *R. intermedium* has a smooth capsule (versus longitudinally furrowed in *R. tomentosum*), a much longer rostrate lid, and much longer peristome teeth. The basal membrane of the endostome of *R. intermedium* is very low, compared with half the length of the peristome in *R. tomentosum* and other species of the same genus. Similar distinctions in peristome structure have been the basis for the description of separate genera in several families (e.g. the Bryaceae). Moreover, the two species concerned are spatially separated: *R. intermedium* is a montane, rain forest species (not below *c.* 2000 m), and *R. tomentosum* a lowland, submontane species (not above *c.* 2000 m) which prefers more open situations.

In the past few years the first author has managed to collect living material of 11 different *Racopilum* species (including *R. intermedium* and *R. tomentosum*). This material was used for electrophoretic studies (De Vries *et al.* 1983). Based on the analysis of seven presumptive enzyme loci (Bramer 1986), Nei's index of genetic identity between the *Racopilum* species was calculated to range from 0.91, between closely related species, to 0.14 between distantly related pairs of species. The genetic identity between populations within a species ranged from 0.88 to 0.98. The genetic identity between *R. intermedium* (only one population) and three different populations of *R. tomentosum* varied between 0.91 and 0.95. These data suggest differentiation at the population level only and question the species status of *R. intermedium*, let alone its classification into two different genera.

Morphological characters are thought to be determined by many loci. Therefore, large changes in sporophyte morphology, as mentioned above, would generally occur only after mutations at many of these loci. In that case, however, one would also expect a considerable differentiation at the enzyme level. This contradiction between morphological and electrophoretic data can possibly be explained by assuming that, in the ancestral population of *R. intermedium*, a mutation occurred in a regulatory gene which caused this large change in peristome morphology. This mutation could have been spread throughout the population because either the population went through a bottleneck, or the mutation had some selective advantage (adaptation to spore dispersal in a rain forest environment ?), or was closely linked to another (mutated) gene with selective advantage.

In conclusion, based on morphological criteria, *R. intermedium* could be separated as a new genus but, by stressing the electrophoretic data, *R. intermedium* and *R. tomentosum* could even be united into one species. At the moment we do not have a practical solution for this apparent conflict in evidence.

Vries, A. de, B.O. van Zanten & H. van Dijk (1983): *Lindbergia* **9**: 73–80.