MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON NOVEMBER 19, 1970

D. H. WIERINGA-BRANTS (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Infection of pythium sp. with tobacco mosaic virus

Cultures of *Pythium sp. in vitro* were inoculated with a sterile concentrated suspension of Tobacco Mosaic Virus (TMV). The cultures were shaken continuously. Virus could be demonstrated in the mycelia beginning with the seventh day after inoculation. Virus-containing mycelia were subcultured in virus-free media for a period up to 2 months and tested regularly for virus content. Virus was still present in the mycelium after 2 months of subculture, but the concentration was low.

After growth on solid medium, infected mycelium still contained virus, an indication that the virus is able to persist in the mycelium. When hyphal tips were used for subculturing the cultures eventually became virus-free. It is likely that virus particles lag behind the growing hyphal tips because of the rapid mycelial growth.

TMV could be demonstrated also in cultures of *Pythium sylvaticum* (Campbell & Hendrix) *in vitro* 11 days after inoculation.

Results show that it is possible to infect a *Pythium* culture *in vitro* with TMV. There is no clear evidence up till now that the virus is able to multiply within these fungus cells but further studies are in progress.

L. C. VAN LOON (*Afd. Plantenfysiologie van de Landbouwhogeschool, Wageningen*)

The relation of peroxidase to the hypersensitive reaction in tobacco var. "Samsun NN" after infection with tobacco mosaic virus.

Peroxidase activity in Samsun NN tobacco plants inoculated with a U 1 strain of Tobacco mosaic virus (TMV W U1) at first decreased, but sharply increased at the formation of visible local lesions. A new peroxidase isoenzyme with an optimum at low pH appeared by day 5. It reached maximal activity at day 6, and disappeared gradually thereafter. This isoenzyme was not present in the young leaves developing after infection, but total peroxidase activity was comparatively high in these tissues and correlated with systemic acquired resistance. In Samsun NN plants inoculated with the Holmes' ribgrass strain of TMV, no symptoms developed under the conditions used. In this combination peroxidase activity increased up to day 3 and levelled off afterwards. A new isoenzyme was not detected. Systemic acquired resistance was not achieved.

Injection with actinomycin D one day before inoculation with TMV W U1 resulted in both an increase in peroxidase activity and a decrease in lesion size. These results indicate that the hypersensitive reaction is not directly dependent on DNA-directed RNA synthesis and that peroxidase plays a role in limiting lesion enlargement.

G. A. KAMERBEEK and A. J. B. DURIEUX (*Laboratorium voor Bloembollenonderzoek, Lisse*)

Abscission and blasting of flower-buds of Lilium "Enchantment", a physiological aberration, possibly correlated with endogenous production of ethylene by the buds.

SUMMARY NOT RECEIVED

C. KLIFFEN (*Instituut voor Plantenziektenkundig Onderzoek, Wageningen*).

Investigation on biochemical aspects of potato leaves to *Phytophthora infestans*.

Three potato varieties were used to study some biochemical aspects of resistance. The first was the very susceptible variety "Bintje", the second was "Libertas" with a high degree of field resistance and the third one was "Maritta" with a high degree of field resistance but a hypersensitivity to fysio 4.

The lower sides of the detached leaves were sprayed with a concentrated spore suspension. The leaves were incubated in a dark and humid room at 15° during 24 hours. Subsequently, the leaves were placed with the petioles in water and kept under a plastic cover for another 24 hours under light conditions. Leaves sprayed with water instead of a spore suspension were used as a control.

The leaves were homogenized in a solution containing: 10 % w/v polyethylene glycol – 6000 (PEG–6000), 0.004 M magnesium acetate, 0.4 M sucrose and 0.005 M tris (hydroxymethyl) aminoethane-acetic acid buffer (pH 7). The homogenate was mixed with white sand and cellulose powder. The slurry was brought on top of a column consisting of a mixture of sand and cellulose, and then the following solutions were passed through the column:

1. 10% PEG–6000, 0.004 M Mg-acetate, sucrose-buffer
2. 0.004 M Mg-acetate, sucrose-buffer.
3. sucrose-buffer.

The presence of UV-absorbing substances was demonstrated in the three effluents. The tailing effect of the first eluate was the reason to subject the second fraction to a passage through a second column according to the same procedure as described above.

The results were expressed in the extinction of 100 ml of the fraction 2 and 3 per 10 g fresh plant material.

The results showed that the extinctions of the fractions 2 and 3 from "Bintje" plants treated with the spore suspension were lower than the extinctions of the corresponding fractions from plants of the same variety treated with water. In case of field resistance the extinctions of the fractions 2 and 3 were similar or slightly higher as compared to the extinctions of the fractions obtained from corresponding control leaves. The extinctions of the effluents 2 and 3 from "Maritta" plants treated with a spore suspension of fysio 4 were much higher than those from plants of this variety treated with water.

It is tentatively concluded that the degree of field resistance can be determined by the use of this method. More important is the possibility to distinguish a high degree of field resistance from resistance caused by hypersensitivity.

The latter is very useful for breeding programmes.

A. VAN DIJKMAN (*Organisch Chemisch Instituut TNO, Utrecht*)

A biochemical explanation for the gene-for-gene resistance of tomatoes to *Cladosporium fulvum* Cooke.

In model experiments with the tomato varieties Moneymaker (no resistance genes), Leaf Mould Resister No. 1 (resistance gene Cf 1), Vetomold (resistance gene Cf 2) and V 473 (resistance genes Cf 1 and Cf 2) leaking of 32P from labelled leaf discs was obtained on infiltration with high-molecular-weight excretion products from incompatible races of *C. fulvum* but not with those from compatible races. These products were obtained by Sephadex G-25 gel filtration of culture filtrates. The observations are in line with our hypothesis that the gene-for-gene relation existing between tomato and *C. fulvum* is based on interaction of specific fungal excretion products with specific receptor sites in the host which may be located in the cell membrane. We expect that the hypersensitivity reaction in vivo is effected by membrane damage.

B. G. VAN 'T LAND (*Afd. Fytopathologie van de Landbouwhogeschool, Wageningen*)

Can cucumber powdery mildew (*Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll) be grown in vitro?

Growth of *Sphaerotheca fuliginea* on cucumber callus tissue and on nutrient agar media was studied.

On white callus tissue development of hyphae was very much restricted, but up to 20% of the green and yellow callus tissue pieces not only supported fungal growth, but this also gave rise to abundant sporulation. The mildew infected callus pieces, moreover, proved to be able to infect cucumber cotyledons.

On Czapek-Dox medium enriched with 0.4% yeast extract and solidified with 0.8% agar the spore germination percentage was about 20–30%. The maximum growth rate of the fungus was estimated to be 2.0–2.5 hyphal cells per spore per day, one hyphal cell being about 50–60 μm long. The hyphae ceased to grow after 3–4 weeks of incubation; consequently the maximum number of hyphal cells arisen from one spore proved to be 30–40. After two weeks of incubation on this nutrient agar medium the mycelium proved still to be infectious, about 80% of cucumber cotyledons becoming infected. Because haustoria and viable spores were completely absent the infection must have been accomplished by mycelial hyphae alone. Addition of IAA or kinetin to the nutrient agar resulted in a decreased growth rate at concentrations of more than 5×10^{-7}M and 5×10^{-8}M, respectively. Complete inhibition of fungal growth was observed at concentrations of 5×10^{-5}M and 5×10^{-6}M, respectively. The effect of IAA-kinetin mixtures resembled that of the most effective component alone.

These observations seem to justify the conclusion that powdery mildew can be maintained on agar media for at least some weeks; it not only preserves its viability under these circumstances, but it even grows to a certain extent.

AFKE C. VAN DER BURG and CARLA DE GOEDE-DIEPEVEEN (*Botanisch Laboratorium, Vrije Universiteit, Amsterdam*)

Yeasts and wax on reed leaves.

Yeasts are mostly considered as non pathogenic for plants. In our study of the phyllosphere of *Phragmites communis*, *Sporobolomyces* was isolated in the same period in which the substrate supplies many nutrients by means of leaching out of the leaves and by excretion of honeydew by the mealy plum aphid, *Hyalopterus pruni*.

Although the occurrence of *Sporobolomyces roseus* is well correlated with the nutritional state of the substrate we wondered if this organism really behaves like a saprophyte. Therefore the ultrastructure of the leaves and the influence of yeasts on it was studied. Leaves grown at the end of April and in May were freeze-dried and the upper-side covered with a yeast suspension or with sterile water as a control.

The leaves were incubated in moist petri-dishes at a temperature of 25 °C and afterwards rinsed with sterile deionized water. Subsequently carbon replicas were prepared and examined with a Zeiss E.M. 9A electron microscope. The yeasts, viz. *Cryptococcus laurentii, Rhodotorula glutinis, Sporobolomyces roseus, Bullera alba* and *Tilletiopsis minor*, were previously isolated from reed leaves.

The examination showed a wax layer which, in the case of yeast-treated leaves, was corroded to a large extent, while the leaves covered with sterile water displayed the unaffected wax structures.

The corroding capacity of *Cryptococcus* and *Tilletiopsis* seemed to be greater than that of *Rhodotorula* and *Sporobolomyces* while the smallest effect was found with *Bullera*. The significance of the results is discussed in relation with the plant's defence mechanism against pathogens.

N. J. FOKKEMA (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Influence of pollen on pathogenic fungi on rye leaves.

When rye leaves were inoculated with a spore suspension of *Helminthosporium sativum* to which pollen was added, the percentage of necrotic leaf surface was about five times as high as that of leaves inoculated without pollen. Stimulation of germination by pollen was only slight because the spores germinated well on leaves without pollen. Three days after inoculation, however, the superficial mycelium development was increased from $\pm$ 0.5 mm/mm^2 to $\pm$ 3.5 mm/mm^{2}on leaves with pollen. Also more young lesions were found on leaves with pollen.

Pollen influenced germination of, superficial mycelial growth of, and infection by *Septoria nodorum* in a similar way. Infection by the obligate parasite, *Puccinia recondita*, was not increased by pollen. Pollen did not enhance the germination and superficial growth of *P. recondita*.

Stimulation of superficial mycelial growth by pollen may be an important causal factor in the enhancement of infection. However, some other influence of pollen on the enlargement of the lesions is not excluded. Pectolytic and cellulolytic enzyme activity of culture filtrates of *H. sativum* was much higher if pollen diffusate was added to the cultures.

Inoculations with *H. sativum* of rye leaves in the field, just before and just after flowering, revealed that the necrotic area of leaves with a natural pollen deposit was about five times as high as that of the leaves without pollen. This effect of pollen on necrosis diminished 3 weeks after flowering. Artificial addition of pollen did not have any stimulatory effect on the infection. The results suggest that the development of *H. sativum* is antagonized by the increased microflora in the phyllosphere.

G. A. VAN DEN BERG and G. J. BOLLEN (*Afd. Fytopathologie van de Landbouwhogeschool, Wageningen*)

Effect of benomyl on incidence of wilting of *Callistephus chinensis*, caused by *Phytophthora cryptogea*.

In order to determine whether application of benomyl to soil might have consequences for the growth of pathogenic fungi belonging to resistant groups[1], the incidence of wilting of chinese aster, *Callistephus chinensis*, caused by *Phytophthora cryptogea* in benomyl-treated soil (B) was compared with that in untreated soil (N).

To that end benomyl was applied to a Trio-17 potting-mixture by spraying a suspension of Benlate 50% W.P. After mixing thoroughly, a final concentration of 10 ppm active ingredient of the fungicide (based on dry weight of the soil) was attained. The soil was inoculated with a pure culture of *Phytophthora cryptogea* in autoclaved soil (2% of inoculum). At 9 days, 1, 2 and 3 months after inoculation part of the soil (further to be called BP) was diluted with Trio-17 mixture to 50, 10, 1 and 0.2%. Of untreated inoculated soil (NP) the same series were made. Hundred pots of each dilution were used; in each pot one aster seedling was planted.

In the first series (9 days after inoculation) benomyl gave some protection, but in following ones (1 and 2 months after inoculation) wilting of asters was much higher in the BP-soil, especially in the 1% and 0.2% dilutions. However, the difference in incidence of wilting in BP- and NP-soil decreased with time. In the last series (3 months after inoculation) no significant difference was found. Hence, the conclusion could be drawn that benomyl had no effect on the final level of infestation of the soil by *Phytophthora*; however, this level was attained much earlier in the presence of benomyl.

The mycoflora of BP-soil was compared with that of NP-soil at 6 weeks after application of benomyl using the soil dilution plate technique and the washing technique. Some groups had sharply increased, e.g. yeasts, *Doratomyces spp.*, *Mucor spp.*, *Zygorhynchus spp.* and also *Fusarium spp.* The increase of the last species was rather unexpected, because of their susceptibility to the fungicide[1]. A marked decrease of the following fungi was found: *Aureobasidium sp.*, *Acremonium spp.*, *Aspergillus spp.*, *Chaetomium spp.*, *Cylindrocarpon spp.*, *Penicillium spp.*, *Phialophora spp.*, *Sesquicillium candelabrum* and *Trichocladium asperum.*

[1] Bollen, G. J. and A. Fuchs (1970). Neth. J. Pl. Path. 76: 299–312.

J. W. L. VAN VUURDE and B. SCHIPPERS (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Chlamydospore formation and lysis of macroconidia of *Fusarium solani* f. *cucurbitae* in chitin-amended and non-amended soil.

One week after the addition of chitin to soil (1 g/100 g soil), macroconidia were introduced into chitin-amended and non-amended soil (3×10^6 macroconidia/g soil). The fate of the macroconidia in both soils was studied during a two-month period by use of the soil-dilution-plate method with a *Fusarium*-selective agar medium. Acid-fuchsin-stained soil smears were examined qualitatively and quantitatively with a light microscope.

In the non-amended soil the number of (colony-producing) propagules of the pathogen dropped during the first five days to 65%, due to lysis of macroconidia. The number of remaining propagules, mainly chlamydospores, thereafter slowly decreased to about 55%.

In the chitin-amended soil, however, during the first 40 days the number of propagules slowly decreased to about 90%. These were mainly macroconidial cells. During the next 20 days there was a rapid decrease in the number of these macroconidial structures to about 25%. There followed a period of slow decrease when the remaining propagules were mainly chlamydospores.

The breakdown of the chitin seems to inhibit formation of chlamydospores and lysis of macroconidial cells in the initial phase. The rapid decline of viable macroconidial structures coincided with the end of detectable production of ammonia in chitin-amended soil.

G. J. BOLLEN (*Afd. Fytopathologie van de Landbouwhogeschool, Wageningen*)

Antagonism after pasteurization of greenhouse soil.

Colonization rates of pathogens after inoculation in pasteurized soils proved to be very dependent on the temperature of pasteurization e.g. colonization rate of *Phytophthora cryptogea* in a potting mixture was very high in soil treated at 60°C (30 min.) as compared to untreated soil or to soil heated at 50 or 80°C.

In order to determine whether these phenomena are due to antagonistic effects an attempt has been made to study the development of antagonism to three soilborne plant pathogens, expressed by the microflora, which survived pasteurization of the soil. For this purpose a modification of the method, designed by WASTIE (1961)[1] had been used. On large petri dishes (ø = 15 cm) growth rates of the pathogens, inoculated in the centre, were measured on cellophane film overlying media, which were previously inoculated with pasteurized soil on seven loci at equal distance from the centre of the plate. The test fungi were *Phytophthora cryptogea*, *Rhizoctonia solani* and *Fusarium solani f. cucurbitae*. On selective media the antagonism expressed by bacteria, fungi and cellulolytic fungi was measured from soil directly after pasteurization and from soil that had been kept under sterile conditions for two weeks after pasteurization.

Although the method described rendered information only about the antagonism expressed by a part of the microflora, the following conclusions could be drawn:

1. After elimination of most organisms by pasteurization of soil at 60°C (30 min.) and at higher temperatures, the antagonistic activity had been taken over partly by survivors and in some cases this even reached a higher level than the original antagonism.
2. For the greater part the antagonism had been restored within two weeks, especially the antagonism expressed by spore-forming bacteria. The fungal antagonism to the test pathogens had not been restored in the soil within two weeks incubation at 20°C.
3. The antagonism proved to be very specific, e.g. *Phytophthora cryptogea* was very susceptible to fungal antagonism, but *Fusarium solani* and *Rhizoctonia solani* to bacterial antagonism. Moreover *Fusarium* proved to be more inhibited than *Rhizoctonia* by cellulolytic fungi.

[1] Wastie, R. L. (1961). Trans. Brit. Mycol. Soc. 44: 145–159.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON OCTOBER 3rd, 1970

H. DOING (*Laboratorium voor Plantensystematiek en -geografie der Landbouwhogeschool, Wageningen*)

Vegetation formations in Australia

The usual classification of Australian vegetation upon a physiognomical basis (BEADLE and COSTIN 1952; WEBB 1959) is rightly based on Australian conditions only. On the other hand, its interpretation in textbooks (*e.g.* POLUNIN 1960; STRASBURGER 1967; SCHMITHÜSEN 1968) is often unsatisfactory. Some Australian formations, e.g. tropical rain forest or savanna woodlands, can easily be fitted into the usual world system, but others should be indicated as separate, exclusively Australian formations even on extremely small-scale world vegetation maps. The problem discussed in this paper is the way in which Australian vegetation can be fitted into a simple world system of formations. Suggestions for an improvement in interpretation are listed below.

The rainforests can roughly be divided into tropical, subtropical and temperate rainforests.

A more detailed description and classification can be found in WEBB (1959). On world maps the Australian areas of rainforest are frequently drawn much too extensively.

The savanna woodlands (tropical deciduous and tropical, subtropical and temperate evergreen) and evergreen tree savannas under natural conditions have a dense, tall grass cover and an open canopy or a layer of more or less widely scattered trees, mostly of Eucalypts. A special case is the evergreen savanna woodland (e.g. of *Eucalyptus niphophila*) with a dwarf shrub layer near timber line altitudes. None of these formations is unique to Australia. However, it may be noted that on some world maps parts of the savanna areas have erroneously been indicated as natural grasslands (*e.g.* "steppe"), based on general climatic comparisons instead of botanical observations (cf. BEADLE 1951).

The Eucalyptus forests are the most obvious example of an Australian formation that cannot be fitted into a system developed in other parts of the world. They are physiognomically homogeneous throughout the humid and sub-humid parts of the warm and cool temperate, mediterranean and subtropical areas (cf. DOING 1970). Therefore, their division into "Durisilvae", "Laurisilvae" etc. (RÜBEL 1930) is misleading. The "mallee" in the drier mediterranean areas is more or less closed, tall, evergreen Eucalypt shrub and may also be regarded as a separate formation.

"Saltbush" and "bluebush" are types of open dwarf shrub vegetation mainly consisting of Chenopodiaceae. As a formation they are not unique to Australia, nor is this true for the "mulga", a tree desert dominated by thornless phyllodineous Acacia species. The indication of large areas of "thorn shrub" in Australia is another error occurring in world formation maps.

The final major Australian formation is the "spinifex" or porcupine grass desert. This is an open, sclerophyllous, perennial grass vegetation dominated by species of *Triodia*. It has no counterparts in other continents, but climatologically it is comparable to the succulent deserts in America and Africa. It should be indicated as the third exclusively Australian formation.

Finally there are a number of minor formations, mainly of extreme environments, e.g. water and bog vegetation, coastal vegetation, inland salt vegetation, heaths and high mountain vegetation. Physiognomically, and sometimes even floristically, they are similar to comparable formations in other parts of the world.

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