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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT PATHOLOGY ON NOVEMBER 18, 1975.

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Systemic symptoms in Nicotiana species reacting hypersensitively to infection with tobacco mosaic virus

Since July 1974 tobacco plants, which react hypersensitively to infection with tobacco mosaic virus (TMV), have occasionally been found to develop necrotic symptoms on non-inoculated upper leaves. A similar phenomenon was reported recently by Shimomura (Japan) who was unable to detect virus in the systemically reacting plant parts.

In our laboratory systemic symptoms occurred on all hypersensitively reacting *Nicotiana* species tested by TMV inoculation: *N. tabacum* "Samsun NN", *N. tabacum* "Xanthi nc" and *N. glutinosa*, although much less frequently in the last case. Varying percentages of "Samsun NN" plants developed systemic symptoms. A minimal dose of 0.2 µg TMV/ml was required for expression of the symptoms which increased in severity with higher virus doses. When either "Samsun NN" or *N. glutinosa* were grafted on "Samsun NN", and the rootstocks were subsequently inoculated with TMV, systemic necrosis developed in the scions of both species. After re-inoculation with TMV of systemically infected leaves of "Samsun NN", the usual systemic acquired resistance was expressed.

Contrary to Shimomura's findings, however, substantial amounts of virus could be isolated from the systemically affected plant parts. No differences could be established between this virus and virus isolated from the inoculated leaves. Mutants could not be demonstrated when single lesion transfers were made. As the systemic symptoms very much resembled those caused by tobacco rattle virus, a possible contamination with this virus was investigated. Host plant studies and electron microscopic investigations did not reveal the presence of the tobacco rattle virus.

The occurrence of systemic symptoms did not appear to be influenced by the origin of tobacco seeds, soil, TMV isolates, carborundum or the use of pesticides.

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Some properties of lilac ring mottle virus

Lilac ring mottle virus (LRMV) causes small faint rings on leaves of artificially infected lilac seedlings. Symptoms are very weak and in summer they are completely masked. On several lilac cultivars LRMV does not cause symptoms. The virus is readily transmitted to herbaceous hosts and it was purified from one of these, *Chenopodium quinoa*, by a procedure which included precipitation with polyethylene glycol 6,000 and differential centrifuging. An antiserum with a titre of 1024 was prepared. Purified preparations of LRMV which gave clear reactions with its homologous antiserum did not react with any of the antisera of the 32 isometric plant viruses tested.

LRMV is very sensitive to buffers of high ionic strength and is visible in the electron microscope only after fixation with glutaraldehyde. The virus then appears as irregularly shaped isometric particles with an average diameter of about 27 nm. In rate-zonal centrifuging the virus precipitates in two zones (top and bottom component). In polyacrylamide

gel electrophoresis a separation into two bands also occurs. The fast moving band corresponds to the top component, the slow moving one to the bottom component. The protein coats of both centrifugal components are serologically identical. The buoyant density of the particles and the molecular mass of the coat protein subunit are the same for the top and the bottom component.

After separation of top and bottom components by density-gradient centrifuging, only the bottom component is infectious. The top component is neither infectious nor does it enhance the infectivity of the bottom component. The virus contains 3 major and 1 minor RNA components, but it is not yet completely clear how these components are distributed over the top and bottom components.

AMELITA W. DOORNIK (*Laboratorium voor Bloembollenonderzoek, Lisse*)

Relations between temperature and virulence of Rhizoctonia solani in irises

Bulbous irises may be infected by various strains of *R. solani* originating from a diversity of host plants, such as irises, tulips, hyacinths, gladioli, lettuce, potatoes and carnations. Development of the disease is mainly influenced by the temperature requirements of the strain used for inoculation; the influence of the temperature on the rapidity of development of the host does not affect the results of the parasitic activity of the fungus.

The relation between temperature and parasitic activity of various strains is distinctly different. Some strains infect irises at soil temperature between 2 and 13 °C, whereas others usually cause symptoms at temperatures of 13 °C and higher. However, symptoms caused by all of those strains are identical.

The temperature- virulence relation of the various strains for irises is also valid for anemones and partly for tulips, lettuce and young radish plants, especially with the strains pathogenic at high temperatures. Both the parasitic and the saprophytic activity of the fungus is considerably reduced at temperature ranges, other than the soil temperature which is favourable for parasitic activity.

W. KAMERMAN (*Laboratorium voor Bloembollenonderzoek, Lisse*)

Some results of investigations on a bacterial disease ("hell fire") in tulip leaves

Hell fire is a conspicuous disease found occasionally in tulip leaves. Locally the colour of the leaf becomes silvery-greyish, the epidermis cracks and curls slightly. These symptoms may be found in the field in the springtime, when the young sprouts have been exposed to temperatures below zero.

Corynebacterium oortii Saaltink and M. Geesteranus can be readily isolated from diseased leaves. Dipping of the bulbs in a suspension of this bacterium before planting may result in a severe incidence of the disease in the following spring.

It has been shown that the bacteria do not induce the development of symptoms when plants are exposed separately to either frost or humid conditions. However, a combination of both factors were found to cause symptoms in a naturally infected stock under experimental conditions Plants of cv. Paul Richter were grown in boxes which were transferred daily, over a period of several weeks, from a storage room at −2 °C to a glasshouse at 18 °C, and vice versa. When plants were sprayed with water immediately before transfer to the cold room, "hell fire" symptoms developed in 10–15% of the plants irrespective of daytime or nighttime exposure.

These results indicate that these external factors either influenced the activity of the bacteria, or put a strain on the leaves which became more sensitive to any toxic substance produced by the bacteria which are nearly always present.

In these experiments many flower stalks showed a longitudinal fissure, a phenomenon which is rare under field conditions.

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Does ethylene function in soil fungistasis?

Ethylene, up to several ppm, is a common constituent of the soil atmosphere of many soils. SMITH (1973) considered it to play an important role in soil fungistasis. His hypothesis is based on the inhibitory effect of 1 ppm C_2H_4 on germination of sclerotia of *Sclerotium rolffsii*.

To check this hypothesis, spores of *Botrytis cinerea*, *Helminthosporium sativum*, *Trichoderma viride* and *Zygorrinchus vuilleminii* were incubated on moist membrane filters suspended in closed vials (37 ml). C_2H_4 was injected and adjusted to 1 and 10 ppm. Percentages of germinating spores were determined at intervals up to 24 hr and compared with controls. No significant differences were observed.

Nine soils (15 g) of different origin, organic content and pH were incubated in the closed vials. Their production of C_2H_4 measured after 24 hr by gas chromatography was far below 1 ppm.

Spores of *B. cinerea* and *H. sativum* on moist membrane filters were suspended above the soil samples or water in the closed vials to check whether volatile fungistatic factors were produced. Significant inhibition of spore germination of both fungi occurred above seven soils and was enhanced by chitin and lime amendments. Because of low C_2H_4 levels above these soils and the insensitivity of spores to 1 and 10 ppm C_2H_4, the fungistatic effects could not be attributed to ethylene; they must be caused by other volatile substances.

SMITH, A. M. (1973): Ethylene as a cause of soil fungistasis. *Nature, Lond.* **246**: 311–313.

A. FUCHS, M. PLATERO SANZ and F. W. DE VRIES (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

In vitro conversion of pisatin by Fusarium oxysporum f. pisi

Pathogenicity of pea pathogens has been ascribed to their ability to convert the pea phytoalexin pisatin to less fungitoxic compounds (DE WIT-ELSHOVE, 1968, 1969; DE WIT-ELSHOVE & FUCHS, 1971). One of these metabolites produced by *Ascochyta pisi* (VAN 'T LAND et al., 1975) and *Fusarium solani* f. *pisi* (VAN ETTEN et al., 1975) has recently been identified as 6a-hydroxyinermin (6a-HI; 3,6a-dihydroxy-8,9-methylenedioxypterocarpan). Preliminary in vitro experiments with *Aphanomyces euteiches*, *A. laevis*, *Fusarium oxysporum* f. *lycopersici* and *F. oxysporum* f. *pisi* have shown that these fungi are also able to degrade pisatin, within 24 hours, to 6a-HI or even further (PLATERO SANZ & FUCHS, 1975). In vivo experiments, however, produced evidence for pisatin breakdown only in the case of *A. euteiches* and *F. oxysporum* f.*pisi*; neither of the two non-pea pathogens proved to degrade pisatin to 6a-HI under such conditions, although both induced the synthesis of large amounts of pisatin.

Time-course studies were made with both *Fusarium* species to obtain more conclusive information about the degradative pathway of pisatin in these two taxonomically related fungi. Both fungi were first grown in shake cultures in Erlenmeyer flasks, which contained 200 ml LILLY and BARNETT'S medium (1951) with 1% glucose, for one week. The contents of each flask together with 10 ml fresh medium (containing 0.1% glucose) were then transferred to Erlenmeyer flasks, in which either ^{14}C-pisatin (calc. amount 19.24 mg; 226900 dpm) or ^{14}C-6a-HI (calc. amount 3.18 mg; 33900 dpm) was dried down. Samples were taken after 40.5, 66 and 98 hours and separated into supernatant and mycelial fractions. After extraction with petroleum ether and ethyl acetate, the extracts were analysed by spectrophotometry, TLC-radioscanning and LSC for pisatin, 6a-HI and other degradation products. In this experiment, *F. oxysporum* f. *lycopersici* did convert neither pisatin nor 6a-HI to any other product. However, whereas pisatin became increasingly incorporated into the mycelial fraction, 6a-HI was found only in the supernatant. On the other hand, *F. oxysporum* f. *pisi* metabolized both pisatin and 6a-HI. With ^{14}C-pisatin, after 40.5 hours, only pisatin and 6a-HI

were present. The latter, in contrast to pisatin, was found for the greater part in the supernatant. After 66 hours, pisatin and 6a-HI had been completely replaced by another compound (X), which had an R_f-value in chloroform/methanol 97:3 similar to that of 6a-HI (0.23 versus 0.25), but a different UV-spectrum (λ_{max} at 291 nm, shoulder at 300 nm versus λ_{max} at 282, 287 and 309 nm). Clearly, compound X must be quite different from both pisatin and 6a-HI. After 98 hours, no more radioactivity was found in the extracts investigated. With ^{14}C-6a-HI, only the sample taken at 40.5 hours still contained an appreciable amount of radioactivity, which was virtually confined to the ethyl acetate extract of the supernatant fraction. The only compound (Y), found to be present in this case, behaved in many respects like 6a-HI, with only distinct differences in the relative absorptions at the λ_{max}s, which in Y were at 288 and 306 nm, respectively.

Preliminary NMR and mass spectral analytical data suggest that substance X is an isoflavan (or a mixture of two closely related isoflavans), whereas Y seems to be an anhydro-derivative of 6a-HI.

ETTEN, H. D. VAN, S. G. PUEPPKE & T. C. KELSEY (1975): 3,6a-Dihydroxy-8,9-methylenedioxypterocarpan as a metabolite of pisatin produced by Fusarium solani f. sp. pisi. *Phytochemistry* **14:** 1103–1105.

LAND, B. G. VAN 'T, E. D. WIERSMA-VAN DUIN & A. FUCHS (1975): In vitro and in vivo conversion of pisatin by Ascochyta pisi. *Acta Bot. Neerl.* **24:** 251.

LILLY, V. G. & H. L. BARNETT (1951). In: *Physiology of the Fungi*, p. 427. McGraw-Hill Book Co., Inc., New York.

PLATERO SANZ, M. & A. FUCHS (1975): Degradación de pisatina, un compuesto antimicrobiano producido por Pisum sativum L. *Proc. V Congreso Nacional de Microbiologia*, Salamanca (Spain).

WIT-ELSHOVE, A. DE (1968): Breakdown of pisatin by some fungi pathogenic to Pisum sativum. *Neth. J. Pl. Path.* **74**: 44–47.

WIT-ELSHOVE, A. DE (1969): The role of pisatin in the resistance of pea plants – some further experiments on the breakdown of pisatin. *Neth. J. Pl. Path.* **75:** 164–168.

WIT-ELSHOVE, A. DE & A. FUCHS (1971): The influence of the carbohydrate source on pisatin breakdown by fungi pathogenic to pea (Pisum sativum). *Physiol. Pl. Path.* **1**: 17–24.

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Symptom expression of Verticillium wilt of Chrysanthemums was found to be influenced by both the temperature and the flowering moment

Chrysanthemums, cv. Yellow Bonni Jean, naturally infected with *Verticillium* were used as mother plants and stored at 2–6 °C during the winter months. Only 5% of the cuttings, taken from plants under these conditions, showed *Verticillium*. However, 85% of the cuttings, taken from the same mother plants when kept at 18–23 °C, were found to be infected at 5 cm from the growing point. Visual differences between infected and healthy cuttings could not be seen at the cutting stage. Wilting symptoms mostly became visible at the flowering moment and, even then, the temperature appeared to be a very important factor.

When naturally infected chrysanthemums were grown under different temperature conditions, i.e. 10–13 °C, 18–23 °C and 22–27 °C, the most severe wilt symptoms were observed at the highest temperatures, even though it was possible to isolate *Verticillium* from plants grown at 10–13 °C.

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

Some environmental factors limiting the growth of Sporobolomyces roseus on wheat leaves

Under controlled environmental conditions *S. roseus* developed on green wheat leaves equally well in the presence of simulated dew as under conditions of high ambient relative humidity. Relative humidities varying from 95–65% had a differential effect on the *Sporobolomyces* population. At 65% r.h. the population decreased, but started growing again as soon as the plants were exposed to 95% r.h.

Under optimal humidity conditions, the population reached a steady state at densities between 5.10^3 and 10^4 cells/cm^2. Spraying the leaves with nutrients (Czapek Dox and yeast extract) resulted in additional growth and a steady state was reached again within 10 days at a population density of c. 5.10^5 cells/cm^2. The nutrients were equally available under dew as under high r.h. conditions. *Sporobolomyces* could only profit from the nutrients if exposed to relative humidities of at least 90% during a part of the day.

The appearance of the *Sporobolomyces* colonies under the various conditions was studied with the Scanning Electron Microscope. Large flat colonies (c. 2 mm in diameter) had developed in the presence of exogenous nutrients. Mostly numerous balistospores were formed on the colony surface and individual cells appeared to be embedded in mucilage. Under dew conditions more small colonies could be observed than under 95% r.h., indicating a better distribution of *Sporobolomyces* over the leaf surface.

H. J. MILLER and D. M. ELGERSMA (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

A scanning electron microscopical examination of the development of aggressive and non-aggressive strains of Ophiostoma ulmi in elms

The search for elms resistant to the Dutch elm disease, caused by *Ophiostoma ulmi* (Buisman) Nannf., has so far cost five decades. The isolation of an aggressive strain of *O. ulmi* in 1972, which can cause the death of a number of these so-called resistant elms, prompted this study with the scanning electron microscope (SEM).

Two year old rooted callus cuttings of the susceptible elm, *Ulmus hollandica* cl. 'Belgica' and *U. hollandica* cl. 390, a resistant clone, were inoculated with either a non-aggressive strain or an aggressive strain of *O. ulmi* and left in the greenhouse. Wood samples were collected after 2, 3, 5, 7 and 10 days between 10–14 cm above the site of inoculation. The small wood blocks were fixed in osmium tetroxide and glutaraldehyde directly after sampling. They were then dehydrated in an acetone series and liquid CO_2 for critical point drying.

The non-aggressive strain of *O. ulmi* was observed within two days of inoculation in cl. 'Belgica' whereas it was not detected until the 5th day in cl. 390. Conidia and numerous hyphae of the aggressive strain, however, were found in cl. 390 within three days of inoculation.

In the early infection stage, therefore, it would appear that the aggressive strain of *O. ulmi* has a more rapid and active development than the non-aggressive strain in cl. 390. This parallels the more rapid growth of the aggressive strain on culture media. The retarded development of the non-aggressive strain in cl. 390 probably is related to the functioning of the resistance mechanism in that clone.

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Light and scanning electron microscopy of the host pathogen relation Cladosporium fulvum and tomato

Infection of tomato by *Cladosporium fulvum* was studied with the light and scanning electron microscope.

Races 1.2.3. and 4. (HUBBELING 1971) of *Cladosporium fulvum* were used, whereas the tomato varieties Vetomold (resistance gene Cf_2; resistant to race 4. and susceptible to race 1. 2. 3.) and Purdue 135 (resistance gene Cf_4; resistant to race 1, 2, 3, and susceptible to race 4.) served as differentials. After inoculation no differences in growth were observed between compatible and incompatible combinations during germination and subsequent formation of runner hyphae. Penetration only occurred through stomata. No directed growth (chemotropism) of runner hyphae to stomata was observed. Penetration usually occurred on the fourth or fifth day after inoculation, possibly earlier.

In the compatible combination the fungus grew intercellularly in the spongy mesophyll in intimate contact with the cells without visible damage to either the plant cells or the fungus during the early stages of infection. Ten to twelve days after inoculation conidiophores emerged through stomata and sporulation followed.

In the incompatible combinations fungal growth was confined to stomata and surrounding cells. Often the penetrating hyphae did not pass the stomata, but became swollen at the tips, causing damage to the yellow coloured guard cells. The guard cells did not stain with an iodine solution (light microscope) and sometimes appeared to be collapsed (scanning electron microscope).

HUBBELING, N. (1971): Determination trouble with new races of Cladosporium fulvum Cooke. *Mededelingen Fakulteit Landbouwwetenschappen Gent* **36** (1): 300–305.

MARIAN W. VAN MAARSCHALKERWEERD and K. VERHOEFF (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Lignification as a possible defense mechanism in tomato fruits after infection by Botrytis cinerea

Infection of young tomato fruits by germ tubes of conidia of *B. cinerea* leads to the development of 'ghost spots": small necrotic lesions usually surrounded by a white halo. Cross sections through penetration sites showed infection hyphae in epidermal cells of the host. The infected cells were found to have dark coloured, thickened cell walls. A few days after inoculation, infected cells showed an increase in total phenolic compounds, compared with non infected cells. An increase in simple phenols such as coumaric acids, caffeic acid, ferulic acid, chlorogenic acid (possibly) caempherol and polymerisation products of some of these phenols were also found, as well as lignin and "lignin-like" substances.

Lignification of host cell walls could form a barrier to infection by *B. cinerea* in tomato fruits as has already been demonstrated for other host-pathogen combinations.