Acta Bot. Neerl. 22(2), April 1973, p. 166–172.

MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PHYTOPATHOLOGY ON 16 NOVEMBER, 1972

B. SCHIPPERS and A. BOUMAN (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn*)

Inhibition of germination and mycelial growth of Fusarium solani f. cucurbitae and Aspergillus flavus by volatiles from soil

Conidia of *Aspergillus flavus* and macroconidia of *Fusarium solani* f. *cucurbitae* were incubated on water agar-disks above a sandy loam soil (pH 7.4) in closed petri dishes at 15 °C.

Their germination and subsequent growth was retarded over a two day period when compared with those incubated above distilled water (D.W.).

Retardation of germination and growth was increased when chitin (1 g/100 g soil) had been added to the soil one week in advance. Germination after 18 hrs above chitin-amended soil amounted to 25% and 58% for *Aspergillus* and *Fusarium*, respectively. Spores of both fungi germinated for 95–100% above D.W. Inhibition of spore germination presented a more permanent character above chitin-amended soil than above non-amended soil or D.W. Inhibition of germination of the *Fusarium* correlated with an increase in number of germ tubes per germinating macroconidum.

The mean number of germ tubes per germinating macroconidum amounted to 2–5, 1–3, and 1, when incubated above chitin-amended soil, non-amended soil, and D.W., respectively.

It is concluded that volatile substances are responsible for the inhibition of germination and mycelial growth. They may play a prominent role in the fungistatic phenomena that have been reported by SCHIPPERS (1972) for macroconidia of *Fusarium solani* f. *cucurbitae* in non-amended and chitin-amended soil.

SCHIPPERS, B. (1972): Reduced chlamydospore formation and lysis of macroconidia of Fusarium solani f. cucurbitae in nitrogen-amended soil. *Neth. J. Pl. Path.* **78**: 189–197.

D. MULDER (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Spikeness disease of Guatamala grass (Tripsacum laxum Nash)

Guatamala Grass is grown as a cover crop after tea has been roded in Ceylon. Individual plants grown from cuttings show a disease syndrome described as spikiness (MULDER, 1962). The symptoms are: leaf deformations, upright growth habit (spikiness), and local waxy cover of leaves. Healthy plants could be obtained by treating cuttings for 10 minutes at 50° C. A virus was supposed to be the cause of the disease. However, neither virus nor mycoplasma particles could be detected.

Further research about the origin of the waxy deposit on the leaves showed this to be the dried-up and deformed remnants of a mycelium loosely attached to the leaf surface. This same deposit can be clearly identified as a mycelium on the youngest unfolded leaf.

Among still folded leaves an extensive network of hyphae can be found running over the surface of the leaves apparently without penetrating into the leaf. Masses of one-celled hyaline spores measuring 1.5 micron by 25 micron are connected with this mycelium.

The isolation of this fungus proved to be difficult but was at last successful. The growth of the fungus is extremely slow on the usual culture media under laboratory conditions. According to provisional identification the fungus appears to be a *Microdochium* (Dr. J. A. VON ARX, personal communication) and has been named *Microdochium tripsaci* nom. ined.

The relation between the presence of this fungus and the disease symptoms has not yet been elucidated by results of inoculation experiments. It seems likely, however, that *M. tripsaci* is the cause of the spikiness disease of Guatamala Grass in Ceylon.

As no penetration of the fungus into the leaf has been observed it is supposed that a metabolic product of the fungus influences the growth of the plant.

MULDER, D. (1962): Diseases, pests and disorders of Guatemala Grass in Ceylon. *FAO Plant Protection Bulletin* **10**: 57–60.

D. M. ELGERSMA, W. E. MACHARDY and C. H. BECKMAN (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn and Univ. of R.I., Kingston, R.I., U.S.A.*)

Growth and distribution of Fusarium oxysporum f. lycopersici in resistant and susceptible tomato plants

See *Phytopathology* **62**: 1232–1237 (1972).

J. VAN DEN HEUVEL and H. D. VAN ETTEN (*Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn and Department of Plant Pathology, Cornell University, Ithaca, N.Y., U.S.A.*)

Studies on the alteration of phaseollin by Fusarium solani f. sp. phaseoli

In a previous report (VAN DEN HEUVEL & VAN ETTEN 1972) it has been demonstrated that the phytoalexin phaseollin is readily metabolized in shake cultures of actively growing mycelium of the bean pathogen *Fusarium solani* f. sp. *phaseoli*. Disappearance of ^{14}C-phaseollin from these cultures is accompanied by the appearance of at least three labeled metabolic products.

The most prominent of these, compound B ($C_{20}H_{18}O_5$), differs from phaseollin in that its mass is 16 units higher and it has a carbonyl group, which suggests that it is an oxidation product of phaseollin. Compound B is less fungitoxic than phaseollin. Germinating spores also can metabolize phaseollin to the same products. It is suggested that both spores and mycelium of *F. solani* f. sp. *phaseoli* are able to detoxify phaseollin by an inducible oxidizing system.

VAN DEN HEUVEL, J. & H. D. VAN ETTEN (1972): *Phytopathology* **62**: 794 (Abstr.).

W. J. DE MUNK (*Laboratorium voor Bloembollenonderzoek, Lisse*)

Flower-bud blasting of tulips caused by ethylene

Exogenous ethylene caused blasting of the flower buds during dry storage of the bulbs and forcing in the glasshouse.

Damage was greater as exposure occurred later in the storage period, the storage temperature and the ethylene concentration became higher and the periods of exposure were made longer. The amount of injury differed much between cultivars.

Exposure of the bulbs immediately after lifting, followed by treatment for early flowering (i.e. planting in boxes, cooling at 9 °C and 5 °C, and forcing at 18 °C in a glasshouse), often resulted in blasting during the phase of rapid growth before flowering. The same symptoms

were observed when tulips were exposed to ethylene in the glasshouse or when *Fusarium*-infected bulbs had been planted between healthy specimens. The susceptibility of tulips during this final growth phase of the flower was very high: 64 and 100% blasting occurred after exposure to 0.5 ppm for three and a half, and seven days, respectively.

Blasting symptoms evoked by exogenous ethylene closely resembled those caused by improper temperature treatments ("heating in transit"). This raises the question of whether "heating in transit" is stimulated by endogenous ethylene. Although the ethylene content of air extracted under vacuum from tulip bulbs stored at 30 °C (after which "heating" occurred) was higher than for bulbs stored at 20 °C (buds unaffected), the results did not point to a significant relationship.

C. J. A. BAREL (*Instituut voor Plantenziektenkundig Onderzoek en Laboratorium voor Entomologie, Landbouwhogeschool, Wageningen*).

Studies on dispersal of Adoxophyes orana (Lepidoptera: Tortricidae) in relation to the sterile male technique

In Dutch pome fruit growing, *Adoxophyes orana* and *Panonychus ulmi* (red spider mite) are the most important pests. Recent investigations have given good indications that *P. ulmi* can be controlled by the predacious fauna of an orchard, provided this fauna is not destroyed by insecticide spraying. Against *A. orana* on the average three sprayings are necessary. This makes biological control of *P. ulmi* impossible. Hence other modes of control of *A. orana* have been looked for.

The sterile male technique is one of the possibilities. Mass rearing and sterilization are possible. A problem is its polyphagy and its high population density. To which extent this is compensated by poor dispersal capacities is now being studied. In a simulation model the impact of reinfestation of the treated area on the feasibility of a sterilization program is demonstrated.

For dispersal there are three possibilities: flight, transport by man, and transport of caterpillars by wind. It is shown that flight is probably not important. It is demonstrated that without transport by man, *A. orana* colonizes new areas very quickly. The possibility of aerial transport of larvae is left open. Qualitatively this mode of transport is demonstrated. Quantitatively nothing can be said about it.

L. H. KAASTRA-HÖWELER and W. GAMS (*Centraalbureau voor Schimmelcultures, Baarn*)

The effect of benomyl on the fungal flora in a fallow greenhouse soil

Soil samples from a greenhouse in Naaldwijk were treated with 20 ppm Benlate 6 months, 3 months, 6 and 3 months, and 1 week, respectively before fungal analysis (soil washing technique). Contrary to the expectations no alteration in the fungal spectrum was found. This may be explained by the low metabolic activity of the soil fungus flora.

REIJNA G. H. PLATENKAMP and G. J. BOLLEN (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

The effect of benomyl sprays on the root mycoflora of rye

Rye grown under greenhouse conditions was sprayed with Benlate 50% w.p. (active ingredient benomyl: 160 mg/m^2). Analyses of the root mycoflora were performed 3, 6, and 9 weeks after the treatment with the fungicide. To this end the roots were washed thoroughly and 500 2 mm-

pieces from each sample were plated out onto soil extract agar, to which an antibioticum was added to prevent bacterial growth.

Three weeks after treatment some of the fungi, which *in vitro* proved to be very sensitive to benomyl, were significantly less frequently isolated from roots of sprayed plants than from those of untreated ones viz. *Chaetomium spp.* ($\alpha < 0.001$), *Cylindrocarpon sp.* ($\alpha < 0.02$), and *Fusarium spp.* ($\alpha < 0.05$). This effect had almost disappeared nine weeks after treatment. At that time for frequently isolated fungi significant differences were only recorded for *Aureobasidium bolleyi* ($\alpha < 0.001$) and *Papulaspora sp.* ($\alpha < 0.05$). Both species were less common on roots of benomyl-treated plants than on those of control plants.

In greenhouse and field experiments it appeared that spraying of the above-ground plant parts did not result in an appreciable downward transport of fungitoxicant to the roots. Most probably the low concentration in the roots of the rye plants in field experiments (on a sandy soil) was caused by leakage of the fungicide along the haulms. As an additional result of this study the complete absence of fungicide in the kernels of plants, in which relatively high concentrations of fungitoxicant were found in other parts, deserves special mention.

H. G. VAN FAASSEN (*Instituut voor Bodemvruchtbaarheid, Haren (Gr.)*)

Effect of the fungicide benomyl on some metabolic processes and on numbers of bacteria and actinomycetes in the soil

Treatment of a sandy glass-house soil with benomyl resulted in increased numbers of bacteria plus actinomycetes; an accompanying shift in the bacterial flora cannot be excluded. However, addition of benomyl to agar media reduced the number of bacteria plus actinomycetes that could be isolated on these media, starting from soil-suspension dilutions of untreated soil. When starting from benomyl treated soil this reduction was smaller or non-existent.

The conversion of soil organic matter and of added cellulose and chitin was not seriously affected by benomyl concentrations of 50 and 200 ppm in the soil, judged by the CO_2-evolution from the soil.

Nitrogen mineralization and nitrification were hardly influenced by benomyl-concentrations in the soil of 10, 25 and 100 ppm. However, addition of benomyl at a level of 200 mg/l to liquid media with a mixed culture of *Nitrosomonas* and *Nitrobacter* caused a delayed oxidation of ammonium to nitrite and prevented the oxidation of nitrite to nitrate. With 20 mg/l benomyl in the medium only the oxidation of nitrite was delayed.

A more detailed paper will be published elsewhere.

LEONOOR VAN DOMMELEN and G. J. BOLLEN (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Antagonism between benomyl-resistant fungi on cyclamen sprayed with benomyl

In a lot of benomyl-treated cyclamen plants seriously diseased by a resistant strain of *Botrytis cinerea* some plants were relatively slightly affected. From these plants strains of *Penicillium brevicompactum* and *P. stoloniferum* were isolated which were even more resistant to the fungicide than the strain of *Botrytis*. In order to learn whether these *Penicillia* might have contributed in a "biological" control of *Botrytis* on the plants sprayed with the fungicide, the antagonistic activity of these strains towards the pathogen was studied.

In vitro antibiosis towards *Botrytis* could clearly be demonstrated on malt agar. The strain of *Penicillium* causing the largest inhibition of mycelial growth of *Botrytis* was chosen for an experiment "*in vivo*". Three lots of 30 densely foliated plants each were treated with 0.2% Benlate. They were sprayed with water (C), a spore suspension of *Botrytis* (B), and a spore

suspension of *Penicillium* plus one of *Botrytis* (PB), respectively. All plants were kept under humid conditions.

Using the Fischer test no significant difference in soft rot caused by *Botrytis* was found for the treatments C and PB during the first month after inoculation. However, plants of both treatments were significantly less diseased than those of the B-treatment. For instance, 32 days after inoculation percentages of affected leaves for the C, the B, and the PB-treatments were 9.6, 20.2 and 11.3%, respectively. Forty days after inoculation the difference between the B- and PB-treatment was less pronounced.

The results suggest that the natural control of *Botrytis* by its antagonists, which are normally present on cyclamen, is destroyed by the benomyl treatment. The fungicide favours the development of the resistant strain of *Botrytis*. However, the antagonism can be restored by the appearance of resistant strains of antagonistic saprophytes, i.e. *Penicillia*.

A. FUCHS, J. HAANSTRA-VERBEEK and F. W. DE VRIES (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

Formation of benzimidazole carbamic acid, methyl ester (BCM) from 2-aminobenzimidazole in plants

In several literature reports (e.g. SEWELL et al. 1972; SIEGEL 1972) 2-aminobenzimidazole (2-AB) has been considered as a possible metabolic breakdown product of benzimidazole carbamic acid, methyl ester (BCM), the hydrolysis product of benomyl. According to the latter author, 2-AB was unequivocally shown to be a metabolite in strawberry plants root-treated with BCM. These observations and reports on chemical breakdown of BCM to 2-AB led us to investigate the reverse reaction, viz. synthesis of BCM from 2-AB. To this end barley, beet, broad bean, cucumber, pea, Petunia, and tomato plants were grown in Hoagland solution and root-treated during 9–18 days with a 50 ppm solution of 2-AB (in Hoagland). Control plants were left untreated. After the treatment period the plants were homogenized and extracted with methanol. Aliquots were taken to dryness, and then either taken up in chloroform or, after first being dissolved in distilled water pH 9.0, extracted with chloroform. Aliquots were bioassayed for the presence of BCM on silicagel plates, using *Penicillium brevicompactum* as test organism (HOMANS & FUCHS 1970). Extracts of all 2-AB treated plants produced inhibition zones co-chromatographing with that of BCM. Concentrations, however, were quite low, amounts per g fresh weight ranging from 2–50 ng, depending on species and duration of administration.

To account for this synthesis of BCM a reaction analogous to carbamyl phosphate synthesis could be surmised, leading to a benzimidazole carbamyl phosphate, which in its turn would be transformed into BCM. Whether this transformation is performed biochemically by the 2-AB treated plants as such, or is taking place chemically upon methanol extraction is, as yet unknown.

Contrary to 2-AB neither imidazole nor o-phenylene diamine and benzimidazole, when administered to barley plants in 50 ppm concentrations, gave rise to detectable amounts of BCM in plant samples of up to 13 g fresh weight.

HOMANS, A. L. & A. FUCHS (1970): *J. Chromatog.* **51**: 327–329.

SEWELL, G. W. F. *and others* (1972) in: *East Malling Research Station Report for 1971*: 122–123.

SIEGEL, M. R. (1972): *Phytopathology* **62**: 789 (Abstr.)

B. G. VAN 'T LAND and A. FUCHS (*Laboratorium voor Fytopathologie, Landbouwhogeschool, Wageningen*)

A hypothetical gene-for-gene relationship between Ascochyta pisi and pea

According to the concept of PERSON (1959) it must be possible to describe any host-parasite relation in terms of the gene-for-gene hypothesis of FLOR (1955). The relationship between *Ascochyta pisi* and pea has been studied by HUBBELING & HUIJBERTS (1969). They could distinguish five groups of physiological races of *A. pisi* and five groups of pea differentials. It proved to be possible to integrate their data into a hypothetical genetical scheme based on four gene-for-gene pairs. In most cases the empirical groups of both hosts and parasites allow for more than one interpretation. The most simple representation is given in *table 1*.

The biochemical implications of this genetical model are currently under study in order to investigate whether there is an analogy between the *Ascochyta pisi* – pea relation and the combination *Cladosporium fulvum*-tomato as studied by VAN DIJKMAN (1972).

Table 1. Relation between the pea varieties used as selective hosts and the physiological races of *Ascochyta pisi*. R: resistant; S: susceptible.

Pea variety	Presumed genes for resistance (single set)	Usual nomen clature	Physiological races of *A. pisi* as described by HUBBELING & HUIJBERTS (1969)				
			A	B	C	D	E
			presumed genes for avirulence				
			A_1A_2 A_3A_4	a_1A_2 A_3A_4	a_1A_2 a_3a_4	a_1A_2 a_3A_4	a_1a_2 A_3A_4
			possible indices				
			0	1	1.3.4	1.3	1.2
Dark skin Perfection	ap 1 ap2 Ap3 ap 4	Ap 3	R	R	S	S	R
Kelvedon wonder	ap 1 ap 2 Ap 3 Ap 4	Ap 3 Ap 4	R	R	S	R	R
Dik Trom	ap 1 Ap 2 ap 3 ap 4	Ap 2	R	R	R	R	R
Ceres	Ap 1 ap 2 ap 3 ap 4	Ap 1	R	S	S	S	S
Stirling	ap 1 Ap 2 Ap 3 ap 4	Ap 2 Ap 3	R	R	R	R	R

DIJKMAN, A. VAN (1972): Natural resistance of tomato plants to Cladosporium fulvum, a biochemical study. Thesis, Utrecht.
FLOR, H. H. (1955): *Phytopathology* **45**: 680–685.
HUBBELING, N. & N. HUIJBERTS (1969): *Jubileumuitgave 30 jaren P.S.C.*: 42–47.
PERSON, C. (1959): *Can. J. Botany* **37**: 1101–1130.

MEETING OF THE SECTION FOR PLANT PHYSIOLOGY ON 1 OCTOBER, 1971

J. C. M. BEIJERSBERGEN and B. H. H. BERGMAN (*Laboratorium voor Bloembollenonderzoek, Lisse*)

The influence of ethylene on the possible resistance mechanism of the tulip (Tulipa spp.) against Fusarium oxysporum

Extracts of tulip bulb tissues (*Tulipa* spp.) contain the fungitoxic substance tulipalin A (α-methylenebutyrolactone) (BERGMAN et al. 1967). This substance may play a role in the resistance mechanism of the white bulb skin of tulips against infection by *Fusarium oxysporum* f. *tulipae* (BERGMAN 1966; BERGMAN & BEIJERSBERGEN 1968).

In the outermost layer of the outer tulip bulb scale, the concentration of the hypothetical *in vivo* precursor of tulipalin A found after lifting rises in about four days from a very low level to a level comparable with that present in the white skin (BERGMAN & BEIJERSBERGEN 1971). This rise in the concentration of the precursor under discussion is inhibited completely when 2 or more ppm ethylene are present in the air surrounding the bulbs. If the concentration of the precursor in the tissue is already high, as is the case for the white skin, ethylene does not influence that concentration. Because *Fusarium oxysporum* growing on bulb tissue (DE MUNK & DE ROOY 1971) or artificial growth media produces ethylene, it is supposed that the supposed barrier, in which tulipalin A plays a crucial role, cannot be built up in the bulb scale near the site of the infection.

Although other factors are involved, the phenomenon described here could be the main reason why in general the fungus is incapable of invading white skin tissue. Details will be published elsewhere.

BERGMAN, B. H. H. (1966): Presence of a substance in the white skin of young tulip bulbs which inhibits growth of Fusarium oxysporum. *Neth. J. Pl. Path.* **72**: 222–230.

BERGMAN, B. H. H. & J. C. M. BEIJERSBERGEN (1968): A fungitoxic substance extracted from tulips and its possible role as a protectant against disease. *Neth. J. Pl. Path.* **74** (suppl. 1): 175–162.

BERGMAN, B. H. H. & J. C. M. BEIJERSBERGEN (1971): A possible explanation of variations in susceptibility of tulip bulbs to infection by Fusarium oxysporum. *Proc. Symp. on Flower Bulbs*, Noordwijk/Lisse 30 March–4 April 1970. vol **2**: 225–229.

BERGMAN, B. H. H., J. C. M. BEIJERSBERGEN, J. C. OVEREEN & A. KAARS SIJPESTEIJN (1967): Isolation and indentification of – methylenebutyrolactone, a fungitoxic substance from tulips. *Recl Trav chim Pays-Bas Belg.* **86**: 709–714.

MUNK, W. J. DE & M. DE ROOIJ (1971): The influence of ethylene on the development of 5 °C-precooled 'Apeldoorn' tulips during forcing. *Hort Science* **6**: 40–41.