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Effect of benomyl on soil fungi associated with rye. 1. Effect on the incidence of sharp eyespot caused by Rhizoctonia cerealis

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Abstract

Effects of benomyl on incidence of pathogens affecting the culm base of rye were studied in field trials and growth chamber experiments. Spraying of the crop with the fungicide at a high dosage (2.4 kg.ha^{-1}) resulted in a tenfold increase of sharp eyespot caused by *Rhizoctonia cerealis* and reduced foot rot symptoms caused by fusaria by 50%. In a field trial at a low dosage (0.24 kg.ha^{-1}) a slight increase of sharp eyespot was observed. In one year, probably because of wet conditions during the infection period, sharp eyespot did not occur in either benomyl-treated or untreated plots, but eyespot caused by *Pseudocercosporella herpotrichoides* was abundant. Its occurrence was reduced from 74% affected culm bases in untreated plots to 8% and 1% in plots that received 0.24 and 2.4 kg.ha^{-1} of the fungicide, respectively.

In growth chambers seedlings were grown in two sandy soils inoculated with *R. cerealis*. The soil was kept dry at about 35% of the moisture holding capacity. In plots with benomyl (1 mg.kg^{-1}; moisture content 11% of fresh weight), fewer seedlings emerged than in plots without the fungicide. This result was highly significant ($P < 0.01$) for one soil but not for the other. The number of seedlings that remained free of disease symptoms was higher ($P < 0.01$) in untreated than in fungicide-treated plots of both soils.

Isolates of pathogens obtained from diseased culms were tested for their sensitivity to benomyl. Growth of all of them including *R. cerealis* was inhibited, although not always completely suppressed, at 10 μg.ml^{-1} on potato-dextrose agar. ED_{50} values of most isolates of *R. cerealis* were between 2.2 and 3.1 μg.ml^{-1}. The fungus was slightly but consistently less sensitive than *F. culmorum*. Mycelial growth of *F. nivale* was appreciably more sensitive than that of the other *Fusarium* spp. from cereals. *P. herpotrichoides* and *F. nivale* were the most sensitive pathogens tested with ED_{50} values of < 1 μg.ml^{-1}. Accordingly, *F. nivale* was absent on culms from treated plots. In a growth chamber experiment, seedlings were protected from infection by supplying the fungicide (1 mg.kg^{-1}) to previously inoculated soil.

In a laboratory assay the effect of benomyl on microbial antagonism to *R. cerealis* was estimated for rhizosphere soil. Enhanced incidence of sharp eyespot in treated crops was associated with adverse effects of the fungicide on microbial antagonism. There is presumptive evidence that *R. cerealis* is suppressed by bacteria after wet periods during the vegetation period of the crop and by fungi after dry periods. Only fungal antagonism, which may be less effective, is affected by benomyl. The response to benomyl of the microflora in different soils varied. Reasons for this inconsistency are suggested.

Additional keywords: Fusarium culmorum, F. nivale, Pseudocercosporella herpotrichoides, microbial antagonism, soil moisture.

Introduction

During the last few years the use of MBC fungicides in the control of cereal diseases has become common practice in West European countries (Tempel, 1977). The main objectives are the control of eyespot caused by *Pseudocercosporella herpotrichoides* and of diseases of the ears caused by *Septoria* spp. and *Fusarium* spp. In Germany, Fehrmann and Schrödter (1972) developed a procedure for the control of eyespot on wheat in which the fungicide application was timed according to epidemiological data. In the control of diseases of the ears a combination of MBC fungicides and maneb is used in the Netherlands. This combination is especially effective against those *Septoria* and *Fusarium* species causing symptoms that show up predominantly during ripening (Defloor and Scholtens, 1973; Rapilly et al., 1973).

Sprays with MBC fungicides at dosages even up to 5.6 $kg.ha^{-1}$ of the active ingredient (a.i.) did not appreciably control root and foot rot of cereals caused by *Fusarium culmorum* (Bruehl and Cunfer, 1972; Duben, 1978) and *Gaeumannomyces graminis* (Huber and Mulanax, 1972; Prew and McIntosh, 1975).

Our first study on the effect of benomyl on diseases of the culm base of rye dates back to 1970. The plant material came from experimental fields of the Department of Field Crops at Wageningen. The experiments formed part of a study on the cause of yield depressions in continuous rye cultivation. The results of this study were recently published by Scholte and Kupers (1977, 1978). In the experiments of 1970 high dosages of systemic fungicides were included to find out whether pathogenic soil-borne fungi might play a role in yield depression. In a preliminary account on the effect of the fungicide on the mycoflora of the culm base, we reported that fusarium foot rot was suppressed, but that in benomyl-treated plots there was ten times as much sharp eyespot caused by *Rhizoctonia* (Fig. 2) than in untreated plots (Van der Hoeven and Bollen, 1972). A detailed account will be given in this article. Increase of sharp eyespot as a result of treatment with benzimidazole fungicides was also observed in wheat (Jenkyn and Prew, 1973; Prew and McIntosh, 1975; Obst et al., 1977; Reinecke, 1977). Because of morphological and cytological differences between isolates of *Rhizoctonia* causing sharp eyespot on cereals and those of *R. solani* Kühn, the pathogen was described as *R. cerealis* Van der Hoeven (in Boerema and Verhoeven, 1977; cf. Fig. 3).

Observations on rye crops during 1971 to 1975 showed that application of benomyl was not always followed by a high incidence of sharp eyespot. Hence, the study was continued by experiments under standard conditions in a climate room. From extensive studies on the environmental conditions favouring the disease, Pitt (1964) concluded that it only occurred in crops that were sown and grew under dry conditions during autumn and spring. Therefore, soil moisture was maintained at a low level. The results of these experiments are also presented here.

Material and methods

Field experiments. Trials 1 (1970) and 2 (1973) were carried out at Wageningen-Hoog and Trial 3 (1974) at Droevendaal, an experimental farm near Wageningen. Soil characteristics are given in Table 1.

Winter rye (cv. Dominant) was grown after rye or potatoes in Trial 1 or after potatoes only in Trials 2 and 3. Crops were treated with benomyl at stages 4, 7 and 10.5

Table 1. Characteristics of the sandy soils used in the experiments.

	Wageningen-Hoog	Droevendaal
organic matter (%)	2.4	2.9
fraction < 16μ (%)	9	6
pH – 1N KCl	4.4	4.9
moisture-holding capacity (%)	26.0	25.2

Tabel 1. Eigenschappen van de zandgronden die in de proeven zijn gebruikt.

of the Feekes scale. Total rates were 2.4 and 0.24 kg.ha^{-1} of the active ingredient in Trials 1 and 3, respectively. In Trial 2 three dosages were applied, viz. 0.24, 1.2 and 2.4 kg.ha^{-1}. Differently treated plots were randomized over the blocks in Trials 1 and 2 or arranged in a Latin square in Trial 3.

Isolations. Segments of culm bases with foot rot, eyespot or sharp eyespot were washed in running water for 30 min, surface-sterilized for 2 min in 1% sodium hypochlorite and rinsed in three changes of sterile distilled water. After drying on sterile paper, 2 mm segments of diseased tissue were plated out on potato-dextrose agar (pH 5.6) containing Vendarcin (50 μg.ml^{-1}), an oxytetracyclin antibiotic that prevents bacterial growth. The plates were incubated at 22°C.

Seedling tests. Samples from the above-mentioned soils were taken a few days before use. The soil was passed through a 10 mm sieve and dried to 40% moisture-holding capacity (MHC). The inoculum was prepared by growing *R. cerealis* for 8 weeks at 22°C in a soil-oatmeal mixture prepared from Droevendaal or Wageningen-Hoog soil (50% MHC) with oatmeal at 2% (w/w). The mixture was autoclaved twice and subsequently inoculated with a recent isolate obtained from rye at Wageningen-Hoog. The two experimental soils were incorporated with inoculum at rates of 1 and 5% (w/w). The inoculum was prepared from the same soil that was inoculated. Control pots were supplemented with inoculum killed by autoclaving. An aqueous suspension of benomyl (100 μg.ml^{-1}) was thoroughly mixed with the soil to a final rate of 1 mg.kg^{-1} (based on the active ingredient, water content of the soil was 11% of fresh weight). For each treatment four multipot plates of 50 pots (60 ml) each were used. In each pot five surface sterilized rye seeds (cv. Dominant) were sown. By weighing and watering three times a week the soil water content was adjusted to 40% MHC. Because of evaporation, the soil moisture content was about 35% MHC on average. The plants were kept in a growth chamber at 10°C, 80% relative air humidity (vapour pressure deficit 1.84 mm Hg) and a 12 h day.

The effect of inoculation was estimated after 36 days by determining emergence of seedlings and the numbers of seedlings with symptoms. The data were processed by analysis of variance.

Estimation of benomyl sensitivity. The in vitro sensitivity to benomyl of the fungi isolated from diseased culm bases was tested at 22°C on PDA as described earlier (Bollen and Fuchs, 1970).

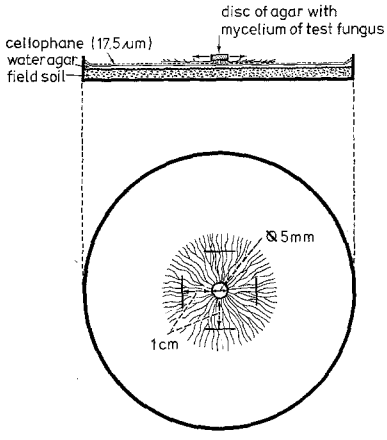

Fig. 1, Assay of antagonistic activity of soil microflora.

Fig. 1. Bepaling van de antagonistische activiteit van de bodemmicroflora.

Assay of antagonistic activity of the soil microflora. About 25 ml of the soil to be tested was saturated with handwarm water agar in a Petri dish. Subsequently, the surface was smoothened by a thin layer of water agar and covered with a cellophane membrane (17.5 µm thick) on which an inverted disc of agar (5 mm diam.) with mycelium of *R. cerealis* was placed. Growth was estimated by measuring the area of the colony. Density of mycelium was estimated by counting the number of hyphae crossing a one centimeter line at a distance of 1 cm from the agar disc (Fig. 1).

The soil was used immediately after sampling from the field or after having been stored at 3°C. Details on experimental conditions are given in the headings of Tables 5,6 and 7.

Results

Effect of the fungicide on incidence of sharp eyespot in the experimental fields. In the crop of 1970 the incidence of disease symptoms on culm bases was assessed at harvest time. The results are presented in Table 2. The benomyl treatment resulted in a reduction of

Table 2. Effect of benomyl sprays on incidence of disease symptoms on the culm base of rye in Trial 1. Each treatment included 9 plots from each of which 100 culms were examined.

Symptoms	Mean numbers of culms affected and standard deviation	
	without benomyl	with benomyl
sharp eyespot	2.9 ± 2.3	35.0 ± 15.4***
discoloration	65.7 ± 6.7	30.9 ± 10.0***
– light	49.1 ± 4.8	22.7 ± 7.4***
– dark	16.6 ± 7.5	8.2 ± 4.9*
none	31.4 ± 6.0	34.1 ± 9.7$^{n.s.}$

*, ***, and n.s., significantly different at 5 and 0.1% level and not significantly different from numbers from untreated plots (Student's t-test).

Tabel 2. Invloed van bespuiting met benomyl op het voorkomen van ziektesymptomen op de halmvoet van rogge. Per behandeling werden van 9 veldjes elk 100 halmen onderzocht.

Fig. 2. Sharp eyespot on the culm base of rye.

Fig. 3. Hyphae of *R. cerealis* and *R. solani* stained in 1% aniline blue in 50% glycerin. A – binucleate cells of *R. cerealis*; B – multinucleate cells of *R. solani*.

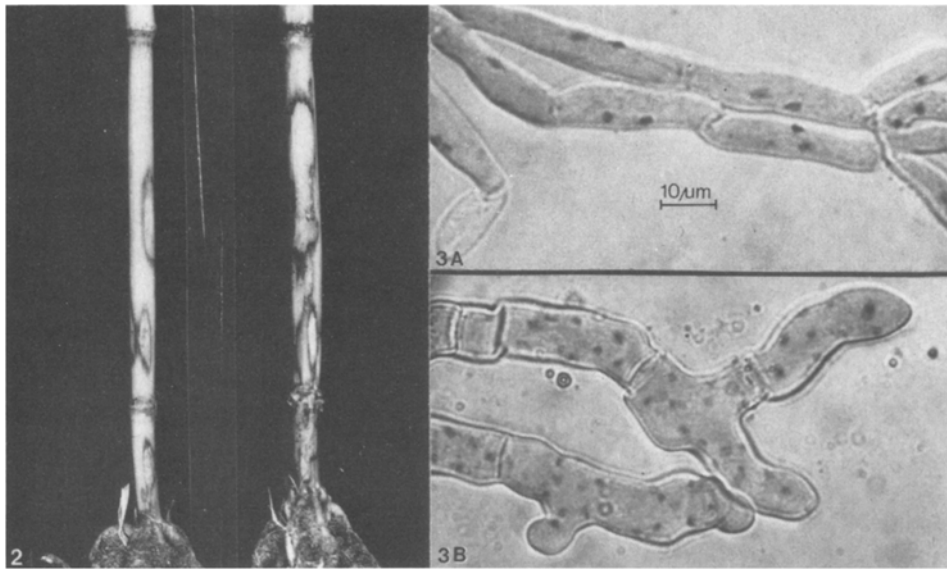

Fig. 2. Scherpe oogvlekken op de halmvoet van rogge.

Fig. 3. Hyfen van R. cerealis en R. solani die gekleurd zijn met 1% anilineblauw in 50% glycerine. A – twee kernige cellen van R. cerealis; B – meerkernige cellen van R. solani.

the number of discoloured culm bases by about 50%. On the other hand, incidence of sharp eyespot had increased tenfold. Among the fungi isolated from sharp eyespots, *R. cerealis* was by far the most common one. The growing season of the crop was characterized by dry periods at sowing time and in spring. rainfall in October 1969 was 17.2 mm (mean of last 30 years was 65.7 mm). In 1970 a wet period during March and April was followed by dry months May and June with 21.6 and 22.4 mm rain (mean over last 30 years 52.5 and 56.4 mm).

The isolates of *R. cerealis* were easily distinguished from those of *R. solani* by their slower growth rate, narrower hyphae and binucleate cells (Fig. 3). The discoloured culm bases yielded mainly *Fusarium* species. The most common species was *F. culmorum*, followed by *F. avenaceum* and *F. graminearum*. *F. nivale* was not isolated probably because sampling was done at the end of the vegetation period. In 1973, when the mycoflora of discoloured culm bases was assessed at various growth stages of the crop, *F. nivale* was frequently isolated earlier in the season, but then mainly from the untreated crop. At harvest time, the fungus was not obtained from culm bases taken from the same plots as from those from which it had been frequently isolated three weeks earlier.

In 1973, sharp eyespot did not occur on rye grown at the same location in both benomyl-treated and untreated plots probably because of wet wheather during the infection period in spring with 76.7 mm rainfall in April (mean rainfall in April of last

30 years was 51.3 mm). On the other hand, eyespot caused by *P. herpotrichoides* was abundantly present. In plots treated with benomyl at 0.24, 1.2 and 2.4 kg.ha^{-1}, eyespots were observed on 8, 3 and 1 % of the culm bases, respectively; in the untreated ones 74 % of the culm bases showed eyespot symptoms. Pieces of infected tissue were plated on PDA. *P. herpotrichoides* was obtained from 112 (89 %) out of 126 lesions on culms from untreated plots and from 6 (15 %) out of 40 lesions on culms from treated plots. *R. cerealis* was isolated neither from these lesions nor from those of 250 culms showing other symptoms.

In 1974, rainfall during the growing season was normal, except during April, when rainfall was only 9.5 mm. In this year, on rye grown at Droevendaal, a benomyl treatment (0.24 kg.ha^{-1}) resulted in a slight increase in sharp eyespot. Symptoms were observed on 5.3 % of the culms from untreated plots and on 12.3 % of those from treated plots. This increase, however, was not significant at $P < 0.05$. *P. herpotrichoides* did not occur among the 200 isolates made from diseased culms.

Effect of benomyl on incidence of symptoms on seedlings grown under controlled conditions in inoculated soil. In a preliminary experiment, rye was sown in Wageningen-Hoog soil in which 5 % (w/w) soil-oatmeal inoculum of *R. cerealis* was incorporated. Emergence was about 30 % and the seedlings showed severe damping-off. Therefore, in the final experiment the amount of inoculum was lowered to 1 % (w/w). The symptoms that appeared on the seedlings were similar to those described in detail by Pitt (1964).

The results are shown in Table 3 and Fig. 4. The main conclusions are:

1. In the Droevendaal soil more seedlings emerged than in the Wageningen-Hoog soil ($P < 0.01$).
2. Benomyl treatment resulted in lower numbers of emerging seedlings with high significance for seedlings grown in Droevendaal soil ($P < 0.01$), but not for those grown in Wageningen-Hoog soil.
3. Inoculation of the soil with *R. cerealis* resulted in a high incidence of symptoms ($p < 0.01$). Some seedlings grown in uninoculated soil also showed disease symptoms. Apparently, pathogens were already present in both field soils. From the seedlings grown in soils with and without benomyl *F. culmorum* and *R. cerealis* were isolated, but *F. nivale* was only isolated from seedlings grown in soil without the fungicide.
4. Addition of benomyl to the soil favoured the incidence of disease symptoms. Heavily affected seedlings occurred more frequently in benomyl-treated than in untreated plots.

Experiments similar to those with *R. cerealis* were carried out with *F. culmorum* and *F. nivale*. The soil-oatmeal inoculum was added to the soil at a high rate (5%, w/w). *F. nivale* proved to be suppressed by benomyl: percentages of healthy seedlings increased from 25.5 % in untreated soil to 84.9 % and 93.5 % in soil to which the fungicide was added at 1 mg.kg^{-1} and at 2.5 mg.kg^{-1}, respectively. Two isolates of *F. culmorum*, indicated as A17 and B14, were used. After inoculation with isolate A 17, more healthy plants were counted in soil with benomyl at a rate of 2.5 mg.kg^{-1} than in untreated soil, percentages being 78.4 and 52.7, respectively. No significant difference was recorded when the fungicide was added at a rate of 1.0 mg.kg^{-1}. After inoculation with isolate B14, numbers of healthy seedlings were not affected by the fungicide at both of the dosages used.

Table 3. Effect of benomyl on incidence of symptoms caused by *R. cerealis* on rye seedlings grown in previously inoculated soil. Disease assessment 36 days after sowing date. Incubation at 10 °C and under 80 % relative air humidity. Soil moisture was 35 % MHC on average.

Soil	Inoculation with *R. cerealis*	Benomyl (1 $\mu g.g^{-1}$)	Mean numbers of seedlings and standard error				
			emergence from 250 seeds	completely sound	brown discoloration at the base of the shoot		dead
					light	dark	
Droevendaal	–	–	208.5 ± 4.0	195.0 ± 3.5	8.8 ± 2.9	2.0 ± 0.8	2.3 ± 1.3
	–	+	197.5 ± 5.6	179.5 ± 16.2	14.0 ± 9.6	2.0 ± 1.0	2.0 ± 2.6
	+	–	187.5 ± 7.7	35.5 ± 9.8	80.3 ± 4.4	35.5 ± 2.4	36.5 ± 10.3
	+	+	177.5 ± 7.6	6.3 ± 1.5	42.5 ± 6.5	44.8 ± 6.3	84.0 ± 12.8
L.S.D. $\alpha = 0.05$			4.7	12.6	–	–	15.0
$\alpha = 0.01$			6.8	18.0	–	–	21.5
$\alpha = 0.001$			10.0	26.5	–	–	31.7
Wageningen-Hoog	–	–	161.0 ± 11.6	150.3 ± 8.1	7.0 ± 4.1	1.8 ± 1.0	2.0 ± 1.4
	–	+	163.3 ± 15.9	148.5 ± 14.2	9.5 ± 3.7	3.0 ± 2.2	2.3 ± 1.9
	+	–	160.0 ± 12.9	46.0 ± 6.8	68.3 ± 7.0	26.0 ± 2.8	19.8 ± 5.4
	+	+	144.0 ± 9.1	23.0 ± 1.4	56.8 ± 7.7	33.8 ± 3.6	30.5 ± 7.6
L.S.D. $\alpha = 0.05$			21.6	15.1	–	–	14.0
$\alpha = 0.01$			31.1	21.7	–	–	20.1
$\alpha = 0.001$			45.7	31.9	–	–	29.5

Tabel 3. Invloed van benomyl op het voorkomen van symptomen op kiemplanten van rogge die zijn opgegroeid in met R. cerealis geënte grond. De beoordeling gebeurde 36 dagen na de zaaidatum. Incubatie bij 10 °C en onder 80 % relatieve luchtvochtigheid. Het vochtgehalte van de grond was 35 % van het waterhoudend vermogen.

Fig. 4. Effect of benomyl on emergence of rye seedlings and disease symptoms caused by *R. cerealis*. Living (R) or dead (S) inoculum was added to soil as soil-oatmeal culture at 1% (w/w). B and O – soil with and without benomyl (1 $\mu g.g^{-1}$).

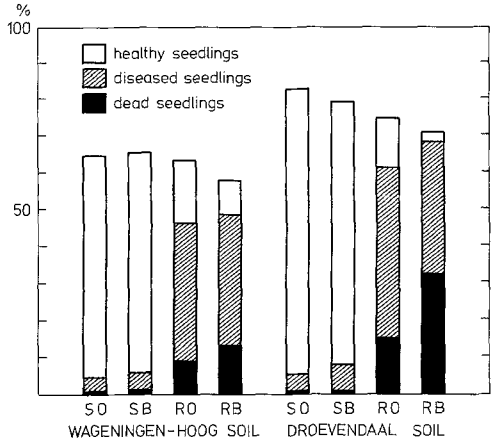

Fig. 4. Invloed van benomyl op de opkomst van kiemplanten van rogge en op symptomen, die door R. cerealis worden veroorzaakt. Levend (R) of dood (S) inoculum werd als aardemeelcultuur in een hoeveelheid van 1% op basis van versgewicht aan de grond toegediend. B en O-grond met en zonder benomyl (1 $\mu g.g^{-1}$).

Differential sensitivity to benomyl of pathogens isolated from diseased culm bases. The predominance of fusaria among fungi obtained from affected culm bases in the untreated crop and that of *R. cerealis* in the benomyl-treated crop prompted us to compare the sensitivity to benomyl of these fungi. All isolates tested were sensitive to

Table 4. Differential sensitivity to benomyl of fungi isolated from diseased culm bases of rye. Mycelial growth at 20°C was estimated in three replicates. I = number of isolates tested. (L) = number of locations from which the isolates came.

	I	(L)	Number of isolates with an ED_{50} of mycelial growth on PDA ($\mu g.ml^{-1}$) within the range given				
			< 1.0	1.0–2.5	2.5–5.0	5.0–10.0	> 10
F. avenaceum	2	(2)	0	2	0	0	0
F. culmorum	15	(4)	0	12	3	0	0
F. graminearum	6	(3)	0	6	0	0	0
F. nivale	5	(3)	5	0	0	0	0
P. herpotrichoides	3	(3)	3	0	0	0	0
R. cerealis	53	(16)	0	25	27	1	0

Tabel 4. De gevoeligheid voor benomyl van schimmels die uit aangetaste halmvoeten van rogge werden geïsoleerd. De myceliumgroei bij 20°C werd in drie herhalingen gemeten. I – aantal getoetste isolaten, L – aantal plaatsen van herkomst.

Fig. 5. Sensitivity to benomyl of mycelial growth on PDA. R – *R. cerealis* F1 – *F. culmorum* and F2 – *F. avenaceum. Figures indicate concentrations of fungicide in* $\mu g.ml^{-1}$.

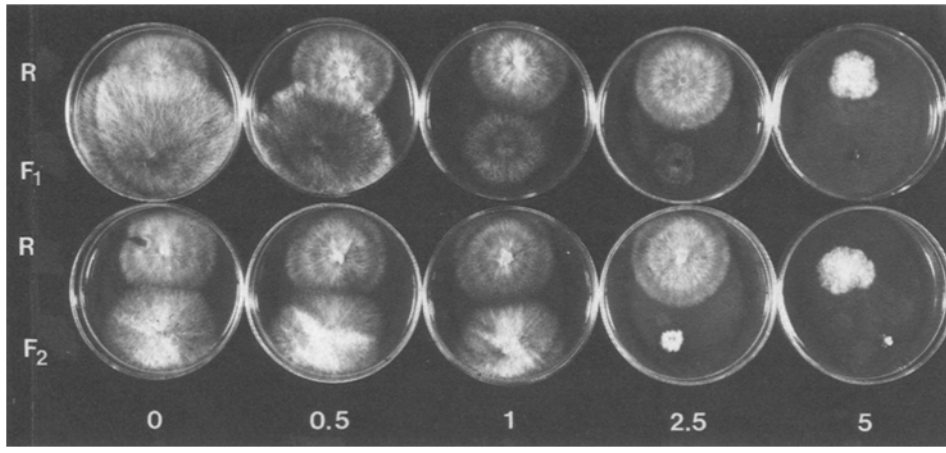

Fig. 5. Gevoeligheid voor benomyl van myceliumgroei op aardappel-glucose-agar. R – R. cerealis, F1 – F. culmorum en F2 – F. avenaceum.

the fungicide including those of *R. cerealis* obtained from crops in which sharp eyespot was favoured by the application of the fungicide. There was, however, a slight but consistent difference, mycelial growth of *R. cerealis* being less sensitive than that of fusaria (Table 4, Fig. 5). On PDA with 10 $\mu g.ml^{-1}$ of benomyl, growth of all isolates of *F. culmorum* was completely inhibited, while few isolates of *R. cerealis* still showed some growth.

Sensitivity of the isolates used for inoculation of soil in the seedling tests was estimated in detail. On PDA, ED_{50} values of *R. cerealis*, *F. culmorum* A17, *F. culmorum* B14 and *F. nivale* were 3.2, 1.3, 1.7 and < 0.5 $\mu g.ml^{-1}$, respectively.

Treatment of the crop with the fungicide does not seem to enhance resistance of *R. cerealis*. In 10 isolates from crops on experimental fields, where an extremely high dose rate (3.9 kg benomyl.ha^{-1}) was applied, the ED_{50} values ranged from 2.2 to 3.1, with a mean of 2.8 $\mu g.ml^{-1}$. Those of 7 isolates obtained from untreated plots were between 2.1 and 3.3, with a mean of 2.7 $\mu g.ml^{-1}$. That the sensitivity of *R. cerealis* was retained in presence of the fungicide, was also demonstrated when seedlings were grown in inoculated soil in a growth chamber. After termination of the experiment, isolates were made from diseased seedlings grown in fungicide-treated plots. Their sensitivity was equal to that of the original isolate.

Effect of benomyl on the antagonistic activity of the soil microflora to R. cerealis. The predominance of *R. cerealis* in crops sprayed with the fungicide may not only be due to differences in benomyl sensitivity between the fungus and other pathogens but also to a decrease in microbial antagonism.

A preliminary estimation of the effect on the antagonistic activity of the rhizosphere flora was made for soil from plots of Trial 1. Mycelial growth over soil from benomyl-treated plots was significantly stronger than that over soil from untreated plots

(Table 5). Treatment with the fungicide had obviously decreased the antagonistic activity of the microflora.

Absence of sharp eyespot in the 1973 crop prompted us to study whether in the soil under this crop, microbial antagonism to the pathogen was affected also by the fungicide. In this experiment, the same isolate was used as in the previous one. However, experimental conditions differed in two respects from those in 1970: the plates were incubated at 15 °C instead of 20 °C and the samples of the rhizosphere soil were taken three weeks before harvest time instead of immediately after harvest. The results shown in Table 6 contradict those obtained in 1970. Mycelial growth over soil from benomyl-treated plots was less than that over soil from untreated plots. This result is not likely to be due to inhibition caused by fungicide residues in the soil. Firstly, because the rate of inhibition is not proportional to the dosage of the fungicide applied to the crop. Secondly, because mycelial growth over autoclaved soil from treated and untreated plots did not differ. It is known that MBC, the fungistatic hydrolysis product of benomyl, is not broken down by autoclaving in aqueous solution (Fuchs et al., 1972) and its concentration was in fact highest in the autoclaved soil (Table 6).

Growth of an isolate of *F. culmorum* over natural soil was very restricted, especially when the soil was taken from benomyl-treated plots. Obviously, the rhizosphere of rye harbours organisms that are strongly antagonistic to the fungus.

In 1974, sandy soils of five rye fields situated in different regions in the Netherlands were used twice to test the effect of benomyl on microbial antagonism to *R. cerealis*. Before the samples were used the moisture content was adjusted to 50% of field capacity. The plates were incubated at 15 °C. Addition of benomyl to soil (1 $\mu g.g^{-1}$ on the base of dry weight) significantly decreased antagonistic activity for two of the five soils ($P < 0.05$, t-test). Mycelial growth over the other three soils was not appreciably affected by the fungicide.

In order to know whether the enhanced incidence of symptoms on seedlings in benomyl-treated soils as presented in Table 3 coincided with a decrease in antagonism to the pathogen, a sample from the Wageningen-Hoog soil was included. A portion of each lot of soil used for the seedling test was stored in the refrigerator at 3 °C for one

Table 5. Effect of benomyl on mycelial growth of *R. cerealis* on rhizosphere soil of rye in Trial 1. Last spray with benomyl May 25. Samples were taken at July 31. The soil plates were incubated for 7 days at 20 °C.

	Benomyl	Colony size mean ± s.d. (cm^2)	Density of mycelium (number of hyphae.cm^{-1})
natural soil	–	20.9 ± 11.1	19.8 ± 4.1
	+	58.7 ± 9.2*	52.7 ± 1.8***
autoclaved soil	–	> 64	> 100

* and ***, significantly different at 5 and 0.1% level from growth on rhizosphere soil from untreated plots (Student's t-test).

Tabel 5. Invloed van benomyl op de myceliumgroei van R. cerealis *over rhizosfeergrond van rogge in proef 1. Laatste bespuiting met benomyl 25 mei. De monsters werden op 31 juli genomen. De grondplaten werden gedurende 7 dagen bij 20 °C geïncubeerd.*

Table 6. Effect of benomyl on mycelial growth of *R. cerealis* over rhizosphere soil of rye in Trial 2. Last spray with benomyl May 25. The samples of the rhizosphere soil that were autoclaved were taken on June 16, the others on July 16. The soil plates were incubated for 8 days at 15°C.

Rhizosphere soil	MBC-residue in soil ($\mu g.g^{-1}$ dry weight)	*R. cerealis*		*F. culmorum*	
		colony size (cm^2)	density of mycelium (number of hyphae.cm^{-1})	colony size (cm^2)	density of mycelium (number of hyphae.cm^{-1})
B_0	< 0.1	47.6	33.6	3.4	74.9
$B_{0.24}$	< 0.1	34.4*	24.9*	2.7*	–
$B_{1.20}$	0.1	43.3*	37.8$^{n.s.}$	2.7*	–
$B_{2.40}$	0.1	39.2*	34.7$^{n.s.}$	2.7*	–
B_0 autoclaved	< 0.1	45.4	> 100	27.2	> 100
$B_{2.40}$ autoclaved	0.3	46.3	> 100	26.3	> 100

* and n.s., significantly different and not significantly different at 5% level from values obtained for untreated soil (Student's t-test).

Tabel 6. Invloed van benomyl op de myceliumgroei van R. cerealis *over rhizosfeergrond van rogge in proef 2. Laatste bespuiting met benomyl 25 mei. De monsters van de geautoclaveerde rhizosfeergrond werden op 16 juni genomen, de andere monsters op 16 juli. De grondplaten werden gedurende 8 dagen bij 15°C geïncubeerd.*

Table 7. Mycelial growth of *R. cerealis* over benomyl-treated soil. For data on soil moisture and treatment with the fungicide see heading of Table 3.

Treatment	Incubated at 10°C for 9 days		Incubated at 15°C for 8 days	
	colony size (cm^2)	density of mycelium (number of hyphae.cm^{-1})[1]	colony size[2] (cm^2)	density of mycelium (number of hyphae.cm^{-1})
B_0	22.2 ± 3.6	23.5 ± 7.2	27.4 ± 5.5	32.2 ± 5.6
$B_{1.0}$	26.7 ± 2.2**	32.8 ± 6.0**	34.7 ± 4.7**	40.3 ± 4.6**

[1] Number of hyphae crossing a one centimeter line at a distance of 2 cm from the inoculum disk.
[2] In some plates the colony had covered the medium entirely during the incubation period. Therefore, the colony size was not measured to the outermost margin. Only the area of dense mycelium was measured, thus excluding the marginal zone of the colony.
** Significantly different at 1% level from growth over untreated control (Student's t-test).

Tabel 7. Myceliumgroei van R. cerealis *over met benomyl behandelde grond. Gegevens over de vochtigheid van de grond en de behandeling met het fungicide zijn bij Tabel 3 gegeven.*

year. Even after this long period of storage, the benomyl-treated soil was significantly less suppressive to *R. cerealis* than the untreated soil (Table 7).

Discussion

Changes of the dominant pathogen following treatment of a crop with a pesticide, like the exchange of *F. culmorum* for *R. cerealis* in benomyl-treated rye, have been reported mainly for cases associated with soil treatments (cf. Kreutzer, 1960). The phenomenon was termed 'disease trading' and defined by Kreutzer (1965) as 'a situation in which a dominant pathogen is controlled by soil treatment, and a minor pathogen is elevated to major importance, thus becoming the new dominant pathogen'.

The use of selective fungicides may result in replacement of sensitive pathogens by tolerant ones and thus lead to a change in dominance. The exchange of *Rhizoctonia* for *Fusarium* and *Pythium* in soil treated with quintozene is a well-known example (Gibson et al., 1961).

Most recent reports on the appearance of non-target pathogens in pesticide-treated crops concern the incidence of pythiaceous and other benomyl-resistant fungi after treatment with benzimidazole fungicides (Bollen, 1979; Papavizas and Lewis, 1979). Tolerant pathogens appearing in cereals treated with fungicides of this type were *Typhyla incarnata* (Hossfeld, 1974) and *Cochliobolus sativus* (Saur and Schönbeck, 1975). The latter observation was made in a crop grown under greenhouse conditions. In a field study, Rashid et al. (1978) found that after treatment of wheat and barley at recommended dosages the incidence of *C. sativus* was not increased. However, in fields where barley was attacked by the related pathogen, *Pyrenophora graminea*, treatment with benzimidazole fungicides had increased the disease.

The increase of sharp eyespot in benomyl-treated cereals is a special case, as the pathogen is not resistant to the fungicide in vitro. Whereas mycelial growth of Pythiaceae and other resistant fungi is not or only slightly inhibited on media containing 100 $\mu g.ml^{-1}$ of the fungicide (Bollen and Fuchs, 1970; Edgington et al., 1971), yet growth of *R. cerealis* was retarded at concentrations below 5 $\mu g.ml^{-1}$. The difference in sensitivity between *R. cerealis* and the dominant pathogen in the untreated crop, *F. culmorum*, was relatively slight (Table 4).

A higher disease incidence in a fungicide-treated crop than in an untreated crop can be caused in different ways. The mechanisms involved have been discussed elsewhere (Bollen, 1979) and are only briefly mentioned here:

1. Appearance of resistant strains in the population of the pathogen, that are more virulent than the wild type fungus. This may incidentally occur and was reported for *Verticillium fungicola*, a parasite of commercial mushrooms (Bollen and Van Zaayen, 1975). The reasons why there is little chance that these strains will maintain themselves on the long term in untreated crops are considered in detail by Dekker (1979).
2. An inhibition of host resistance mechanisms by the fungicide (Swinburne, 1975).
3. A decrease in microbial antagonism to which the pathogen is exposed in the soil or on the surface of roots and other plant organs.

The first mechanism is irrelevant in the case of *R. cerealis* since no marked differences were found in sensitivity of strains from treated and untreated crops.

An effect on host resistance mechanisms cannot be excluded. At first sight, it seems

unlikely that host resistance was essentially affected by the fungicide, since treatment of the crop resulted in a decrease of infection by other pathogens like *F. culmorum* and *P. herpotrichoides*. However, direct inhibition of the development of these pathogens might have masked an effect on host resistance.

Substantial evidence was obtained for the third mechanism, a decrease in microbial antagonism. This antagonism includes suppression of *R. cerealis* by the soil microflora and, according to Reinecke (1977), also the competition for host tissue with other pathogens on the culm base. The assay used in our study provides data on only a part of the microbial antagonism to which *R. cerealis* is exposed in the field. Firstly, the assay was confined to the antagonism exerted by the microflora of the soil, in particular that of the rhizosphere. In the field, infection of the crop will be influenced mainly by antagonism of micro-organisms in soil adjacent to the culm base. The microflora of this habitat may differ from that of the rhizosphere. A second limitation arises from the technique used for the assay. Since the test fungus is separated from soil by a cellophane membrane, the test supplies information only on those interactions with the soil microflora that are brought about by diffusible substances passing through the membrane. Consequently, the test provides data on competition for soluble nutrients and amensalism by diffusible antibiotics and other inhibitors. Direct parasitism and predation of *R. cerealis* by soil-inhabiting organisms are not estimated. Even though only a part of the antagonism to which the fungus is subjected under field conditions is examined by the assay, a marked effect on it was noticed.

Pitt (1964) observed that sharp eyespot is highly favoured by dry conditions during early stages of crop growth. The incidence of the disease in 1970 and 1974 can be related to periods of dryness during the growing season. A higher incidence of root and foot diseases in dry than in wet soils can be caused by (1) an adverse effect on host resistance by water stress and (2) a diminished microbial antagonism to the pathogen (cf. Cook and Papendick, 1972). Our study provides only information on the latter aspect. Under conditions favouring the disease, the increase of its incidence by benomyl treatment was associated with a decrease of antagonism (Tables 5 and 7). In the crop of 1973, where sharp eyespot did not occur – propably due to wet conditions during the infection period – the effect of benomyl on microbial antagonism was contrary to that recorded for 'disease-conducive' soils (Table 6). These results together with the observations (unpublished) that hyphae of *R. cerealis* on wet soil are surrounded by bacterial masses and subsequently lysed, suggest that in wet soil *R. cerealis* is suppressed by bacteria, whereas in dry soil fungal antagonism predominates. The contrast in response to benomyl of antagonism in field soils after dry and wet periods was used to support this hypothesis, because of the difference in response between the bacterial and the fungal component of the microflora. Numbers of heterotrophic bacteria can be enhanced by treatment of soil with benomyl (Hofer, 1971; Van Faassen, 1974) and consequently bacterial antagonism may be favoured by the fungicide. When fungal antagonism is operating, a specific fungicide like benomyl may reduce its level. In this case, the fungal antagonists as a group seem to be more sensitive to the fungicide than the pathogen.

Like antagonism by saprophytic fungi, interactions between *R. cerealis* and other pathogens on the culm base may be affected by the fungicide. Reinecke (1977) attributed the incidence of sharp eyespot in benomyl-treated cereals to a reduced competition for host tissue at the culm base. This conclusion was based on his extensive

study on the causal organisms of the foot-rot disease complex. In field trials, the numbers of culms with sharp eyespot lesions were inversely correlated with those of culms with eyespot lesions caused by *P. herpotrichoides*. A main reason for this phenomenon should be sought in a difference in ecological requirements of the pathogens, *R. cerealis* being favoured by dry conditions and *P. herpotrichoides* by wet conditions during the infection period. Moreover, competition between the pathogens is involved as has been shown in experiments where one of both pathogens was suppressed by a chemical or – the reverse – was promoted by inoculation (Reinecke, 1977). Inoculation of wheat with *R. cerealis* resulted in a decrease of symptoms caused by *P. herpotrichoides* and, as has been reported by Prillwitz and Bauerman (1977), also of those caused by *F. culmorum*. Conversely, inoculation of wheat with *P. herpotrichoides* suppressed incidence of sharp eyespot (Mielke, 1978). In field trials, where treatment of rye with benodanil resulted in a decrease of sharp eyespot, the number of culms with eyespot caused by *P. herpotrichoides* had increased (Reinecke, 1977). In conclusion, incidence of *R. cerealis* seems to be highly hampered by antagonistic activity of the microflora present on and around the culm base, where saprophytes as well as pathogens can operate as antagonists. Suppression of either component of the microflora by environmental conditions or chemicals, enhances the chance of infection by the pathogen.

Records on the effect of MBC fungicides on incidence of sharp eyespot are rather inconsistent. Whereas most authors (Van der Hoeven and Bollen, 1972; Prew and McIntosh, 1975; Hanuss and Oesau, 1977; Hoffmann, 1977; Obst et al., 1977; Reinecke, 1977) have reported an increase of incidence, Baker (1975) and Reschke (1975) did not find an effect on disease incidence in wheat treated with thiophanate-methyl. A first cause of a difference in responses may be a difference in moisture conditions. As has been discussed above, soil moisture constitutes a main factor for incidence of the disease and for the effect of the fungicide on microbial antagonism to the pathogen. Another source of a difference in responses arises from the specificity of microbial antagonism. Out of five soils sampled at the same time and adjusted to the same moisture content, two were affected in their antagonistic activity, the other three were not. The response to the fungicide depends on the sensitivity of those species which predominate in the population of antagonists. Because the microflora differs from soil to soil, it is easily understood that the effect of selective inhibitors (like the MBC fungicides) on microbial antagonism is specific for the soil. A highly specific response to these fungicides was also reported for non-target pathogens of turfgrasses (Joyner and Couch, 1976).

The *Fusarium* species of the foot-rot disease complex seem to be hardly affected by MBC fungicides at recommended dosages (Bruehl and Cunfer, 1972; Baker, 1975; Duben, 1978). A slight control of fusarium foot rot in rye by treatment with thiophanate-methyl was reported by Reschke and Rieth (1978). Application of carbendazim at a low dosage (180 $g.ha^{-1}$) resulted in an increase of fusarium foot rot along with sharp eyespot and a decline of eyespot caused by *P. herpotrichoides* (Hanuss and Oesau, 1977). On the other hand, in our first field experiment treatment with benomyl at an extremely high dose rate reduced the number of diseased culms by c. 50%. This observation illustrates that the response of moderately sensitive fungi like *F. culmorum* and *F. avenaceum* (ED_{50} of mycelial growth on agar about 1–2 $\mu g.ml^{-1}$) is mainly determined by the dose rate present in their natural microhabitat. At low concen-

trations they may increase because of reduced antagonism by more sensitive fungi (*Penicillium*, *Aspergillus*, *Trichoderma*, etc.). When the fungicide reaches a level at which the fusaria themselve are inhibited, they are superseded by more resistant competitors. Carter and Price (1974) made use of the moderate sensitivity of *F. lateritium* in their experiments on integrated control of *Eutypa armeniacae*, a vascular pathogen of apricot. When invading wounded surfaces after pruning, the pathogen is effectively antagonized by *F. lateritium*. The antagonist is less sensitive to benomyl than the pathogen. Conidia of *F. lateritium* were added to a suspension of benomyl used to cover wounded surfaces. The conidia germinated and the fungus colonized the wounded tissue, thus resulting in a control supplementary to that by the fungicide.

Among the fusaria obtained from diseased culm bases in rye, *F. culmorum* ranked first. The fusaria isolated by Duben (1978) from wheat with foot rot symptoms were mainly *F. avenaceum*, *F. culmorum* and *F. nivale*. The ambiguous response of fusaria to benomyl as set forth above does not apply for *F. nivale*. This fungus was only rarely isolated from culm bases of benomyl-treated rye, but frequently from those of untreated crops. As has been shown in the seedling test, the fungus was already suppressed at low dose rates of the fungicide applied to soil. From its high sensitivity to the fungicide in vitro (Table 4), it can be inferred that this will be due to direct inhibition of the pathogen in soil. The high sensitivity of *F. nivale* was confirmed by Van Tuyl (1977), who recorded an ED_{50} value of 0.1 $\mu g.ml^{-1}$ on malt agar for the isolate that had been used in the seedling test.

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Samenvatting

De invloed van benomyl op bodemschimmels in rogge. 1. De invloed op het optreden van scherpe oogvlekkenziekte veroorzaakt door Rhizoctonia cerealis

In veldproeven en in een klimaatkamer werd de invloed van benomyl op het optreden van voetziekten in rogge onderzocht. In veldjes die bespoten waren met een hoge dosis van het fungicide (in totaal 2.4 $kg.ha^{-1}$) bleken tienmaal zoveel halmen met scherpe oogvlekken, veroorzaakt door *Rhizoctonia cerealis*, voor te komen dan in onbespoten veldjes. Daarentegen was voetrot veroorzaakt door *Fusarium*-soorten met 50% verminderd. In een volgende veldproef, waarbij een voor de praktijk geadviseerde dosis (0.24 $kg.ha^{-1}$) was toegepast, werd een lichte toename van scherpe oogvlekken waargenomen.

In een ander jaar trad scherpe oogvlekkenziekte in het geheel niet op, ook niet in met benomyl behandelde veldjes. De vochtige omstandigheden tijdens de infectieperiode

zijn daarvan waarschijnlijk de oorzaak. Daarentegen werd de oogvlekkenziekte, welke door *Pseudocercosporella herpotrichoides* werd veroorzaakt, veel aangetroffen. In de onbehandelde veldjes waren 74% van de halmen aangetast tegen 8 en 1% in de veldjes die met het fungicide waren behandeld in doseringen van 0.24 en 2.4 kg.ha^{-1}.

De invloed van het fungicide op de aantasting van kiemplanten werd in klimaatkamerproeven onderzocht. Daartoe werden twee zandgronden met *R. cerealis* geënt. De grond werd droog gehouden (op 35% van het waterhoudend vermogen). In grond met fungicide (1 mg.kg^{-1}) was de opkomst minder dan in grond zonder fungicide. Dit was zeer significant ($P < 0.01$) voor één van de beide zandgronden, maar niet voor de andere. Het aantal gezonde kiemplanten was in beide gevallen duidelijk hoger ($P < 0.01$) voor de onbehandelde grond.

De isolaten van ziekteverwekkers uit aangetaste halmen werden op hun gevoeligheid voor het fungicide getoetst. Op aardappel-glucoseagar werden alle isolaten in hun groei geremd bij een benomyl-concentratie van 10 µg.ml^{-1}. *R. cerealis* was iets minder gevoelig dan *F. culmorum*. Voor het overgrote deel van de isolaten van *R. cerealis* lag de ED_{50} waarde tussen 2,2 en 3,1 µg.ml^{-1}. De myceliumgroei van *F. nivale* werd meer geremd dan die van de andere *Fusarium*-soorten. *P. herpotrichoides* en *F. nivale* waren met een ED_{50} waarde van < 1 µg.m.$^{-1}$ de gevoeligste pathogenen die uit de halmvoeten werden geïsoleerd. Dat de populatie van *F. nivale* in benomylhoudende grond wordt onderdrukt, blijkt uit (1) het feit dat de schimmel niet voorkwam op halmen uit behandelde veldjes en (2) de bescherming tegen infectie van kiemplanten als aan de besmette grond fungicide (1 mg.kg^{-1}) was toegevoegd.

In laboratoriumproeven werd de invloed van benomyl op het microbiële antagonisme in rhizosfeergrond tegen *R. cerealis* bepaald. Een toename in het optreden van scherpe oogvlekkenziekte in behandelde gewassen bleek gepaard te gaan met een remming van het antagonisme tegen de ziekteverwekker. Er zijn sterke aanwijzingen dat *R. cerealis* na vochtige perioden tijdens de vegetatieperiode door bacteriën wordt onderdrukt en na droge perioden door schimmels. Het antagonisme van de laatste groep lijkt minder effectief te zijn en alleen dit antagonisme wordt door benomyl verlaagd. Tenslotte wordt een mogelijke oorzaak aangegeven voor de ongelijke respons op het fungicide van het microbieel antagonisme in verschillende gronden.

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