

## Effect of benomyl on soil fungi associated with rye. 2. Effect on fungi of culm bases and roots

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### Abstract

The mycoflora of culm bases and roots of rye was assessed in field trials, where benomyl was applied at dose rates ranging from 0.24 to 4.80 kg ha<sup>-1</sup>. Samples of culm bases were taken three times during the growing season, those of roots only at the harvest date. On culms with various symptoms from untreated plots, *Pseudocercospora herpotrichoides*, *Alternaria* spp. and *Gerlachia nivalis* were prevalent and on those from benomyl-treated plots *Alternaria* spp. and *Fusarium culmorum*. In later stages of growth, *G. nivalis* sharply declined and *Alternaria* spp. and *F. culmorum* increased. At the end of the season, *Periconia macrospinoso* and *Typhula incarnata* appeared in treated plots.

In samples of roots taken more than two months after the last spray, porosporous dematiaceous species (*Alternaria*, *Ulocladium* and *Dendryphion*), *Mortierella* spp. and other resistant fungi were prevalent in plots treated with 1.20 kg ha<sup>-1</sup> or more, but not in those that received 0.24 kg ha<sup>-1</sup>, which is recommended for disease control. Lower counts were recorded only for species that are highly sensitive in vitro, e.g. *Microdochium bolleyi* and *Trichoderma* spp. Some fusaria were either not affected or tended to be slightly stimulated by the treatment. An attempt was made to attribute the incidence of these moderately sensitive fungi to the effect of the fungicide on non-pathogens.

*Additional keywords:* microbial balance, antagonist, dynamics of mycoflora, *Alternaria*, *Fusarium culmorum*, *Gerlachia nivalis*, *Microdochium bolleyi*, *Mortierella*, *Periconia macrospinoso*, *Pseudocercospora herpotrichoides*, *Trichoderma*, *Typhula*.

### Introduction

Information on effects of benomyl on the composition of the fungal flora deals mainly with its parasitic component. Data on parasites of the culm base in treated and untreated crops are available for various cereals, especially wheat. Examples of comprehensive studies on these fungi are those of Reinecke (1977) and Duben and Fehrmann (1979). However, the saprophytes among fungi on culms and roots have received little attention. The effects of benomyl on rhizosphere- and root-colonizing fungi under wheat were studied by Weber-Czerwińska (1977, 1979). She reported that treatments at dose rates up to 0.6 kg ha<sup>-1</sup> only slightly affect the composition of the population. An analysis of the root-colonizing mycoflora of rye, grown in a greenhouse, was made by Platenkamp and Bollen (1973). The results are in line with

those obtained for wheat by Weber-Czerwińska in that few fungi remained inhibited or stimulated for a long period after the treatment. Three weeks after application of the fungicide, the population was markedly affected, but six weeks later this effect had almost disappeared. At that time, a significant inhibition was only recorded for *Microdochium bolleyi* and *Papulaspora* sp.

In a study on the influence of benomyl on fungi affecting the culm bases of rye, both parasites and saprophytes were isolated from culms and roots. The effect on the incidence of *Rhizoctonia cerealis* and a few other parasites was reported in a previous paper (Van der Hoeven and Bollen, 1980). The fungicide caused a shift in the population of parasites. Evidence was obtained that a decrease in microbial antagonism was one of the reasons. In first instance, this may be due to a change in inhibition of fungal antagonists, since benomyl is a specific fungicide with little or no effect on bacteria. The association of a shift in population of parasites with a decrease in microbial antagonism prompted us to further study of the mycoflora. The results presented in this article were obtained by running through lists of isolates made from culms and roots of rye in various field trials in different years.

## Materials and methods

*Experiments and sampling.* The culm bases and roots used for the analysis of the mycoflora came from field plots with winter rye cv. Dominant at Wageningen-Hoog and Droevendaal, an experimental farm near Wageningen. For properties of soil the reader is referred to Van der Hoeven and Bollen (1980).

The crops were treated with benomyl suspended in 600 l water ha<sup>-1</sup> at stages 4 (in the various trials ranging from 14 to 22 April), 7 (from 1 to 11 May) and 10.5 (from 20 to 25 May). Numbers of replicates and total amounts of active ingredient applied in the various experiments are given in the headings of the tables. The replicate plots varied from three to nine and were randomized over the blocks. Each plot was 4 × 4 m including a border of 1 m wide from which no culms or roots were sampled.

Culms were sampled at three growth stages: 10.5.4 (just after flowering, 14 June), 11.1 (milky ripe, 6 July) and 11.4 (harvest, 30 July). At the first and second sampling date, 0.75 m<sup>2</sup> of each of five replicate plots was harvested for estimation of yields. The remaining 2.5 m<sup>2</sup> was used for the final estimation of yields on 30 July. On the same date samples of about 100 culms of each plot were taken for assessing disease symptoms and estimation of the fungus flora. Stubbles of which the fungus flora of the roots was investigated were collected immediately after harvest of the crop by sampling at a distance of 1 m in the rows, irrespective of whether they showed symptoms or not.

*Isolation procedure.* For the isolation of fungi from culm bases c. 5-mm pieces of culms showing symptoms were washed in running water for 30 min, surface-sterilized in 1% sodium hypochlorite for 2 min, rinsed in three changes of sterile water and dried on sterile filter paper. Segments of about 1 by 2 mm were plated out onto potato dextrose agar (pH 5.6) containing oxytetracycline (50 µg ml<sup>-1</sup>) for suppressing bacterial growth.

In the isolation of fungi from roots no hypochlorite or other sterilizing agent was used. Adhering soil was removed by washing the root segments in water that was stir-

red by means of a vertical agitator (Vibromischer) for two hours. The water was changed three times. Then the segments were washed in sterile water, dried on filter paper and cut into smaller segments (2 mm), which were plated out onto soil extract agar (pH 6.6).

The plates with segments of culms and roots were incubated at 15 °C. Sub-cultures were made from the fifth day on.

Sensitivity of mycelial growth to benomyl was measured on potato dextrose agar (PDA) according to Bollen and Fuchs (1970).

## Results

*Fungi of the culm base.* The effect of benomyl on these fungi was studied in only one field experiment at Wageningen-Hoog. The symptoms appearing on the culm

Table 1. Percentages of culms with symptoms in benomyl-treated and untreated plots at three sampling times<sup>1</sup>.

Symptoms	Benomyl (kg ha <sup>-1</sup> )											
	0			0.24			1.20			2.40		
	1	2	3	1	2	3	1	2	3	1	2	3
dark discolorations	23	4	10	10	13	21	4	24	30	2	25	21
eyespot symptoms	68	80	74	17	19	8	7	5	3	3	3	1
elongate lesions	4	4	5	11	12	17	13	19	16	11	13	13
light discolorations	0	13	11	10	37	51	9	29	50	18	36	62
sound	5	1	0	52	19	2	67	22	2	66	25	2

<sup>1</sup> Sampling was done on (1) 14 June, (2) 6 July and (3) 30 July (harvest).

The samples comprised 478 to 612 culms, about 100 from each of five replicate plots.

*Tabel 1. Percentage halmen met symptomen in met benomyl behandelde en onbehandelde proefveldjes.*

Table 2. Number of culms and yield of rye from benomyl-treated and untreated plots.

Yield <sup>1</sup>	Benomyl (kg ha <sup>-1</sup> )				
	0	0.24	1.20	2.40	L.S.D. ( $\alpha = 0.05$ ) <sup>2</sup>
number of culms (m <sup>-2</sup> )	335	346	347	339	51
dry matter (ton ha <sup>-1</sup> )	7.28	7.83	7.68	8.18	0.90
dry weight grains (ton ha <sup>-1</sup> )	3.45	3.66	3.74	3.88	0.43

<sup>1</sup> Estimated in each of five replicate plots.

<sup>2</sup> According to the Studentized range test of Tukey.

*Tabel 2. Aantal halmen en de opbrengst van rogge van met benomyl behandelde en onbehandelde proefveldjes.*

bases are mentioned in Table 1. Data on yield are given in Table 2. Sharp eyespot caused by *R. cerealis* was completely absent probably due to wet conditions during the infection period. On the other hand, the wet spring was conducive to infection by *Pseudocercospora herpotrichoides* resulting in a high incidence of eyespot symptoms in untreated plots. The categories of symptoms seemed to overlap. This is especially apparent for dark discolorations and eyespot symptoms in the untreated plots as the same pathogens were prevalent among the fungi isolated from culms of both categories.

The fungi obtained from untreated plots were in order of frequency for culms with dark discolorations *P. herpotrichoides*, *Gerlachia nivalis* (syn. *Fusarium nivale*), *Fusarium culmorum*, with eyespot symptoms *P. herpotrichoides*, *G. nivalis*, *Microdochium bolleyi*, with elongate lesions *G. nivalis*, *P. herpotrichoides* and with

Table 3. Fungi isolated from culm bases with symptoms in benomyl-treated and untreated plots.

	Benomyl (kg ha <sup>-1</sup> )					
	0			2.40		
	14 June	6 July	30 July	14 June	6 July	30 July
Number of culms	108	61	81	75	82	95
Number of isolates	154	101	128	74	124	133
Taxa	Relative frequency (%)					
<i>Alternaria</i> spp., including						
<i>Pleospora infectoria</i> (r) <sup>1</sup>	9	37	35	18	46	45
<i>Cladorrhinum</i> sp. (ss)	6	0	0	8	0	1
<i>Drechslera</i> spp., including						
<i>Cochliobolus sativus</i> (r)	0	0	1	1	5	8
<i>Fusarium culmorum</i> (s)	3	8	9	0	11	19
Other <i>Fusarium</i> spp. (s)	0	1	3	0	5	8
<i>Gerlachia nivalis</i> (ss)	34	21	0	0	0	0
<i>Microdochium bolleyi</i> (ss and s)	4	3	14	1	0	8
<i>Mucor</i> spp. (r)	9	9	3	2	1	0
<i>Periconia macrospinoso</i> (s)	0	0	2	0	0	11
<i>Pseudocercospora</i>						
<i>herpotrichoides</i> (ss)	81	65	74	1	0	1
<i>Typhula incarnata</i> (r)	0	0	0	0	0	8
Sterile basidiomycetes (r)	0	2	2	0	2	5
Sterile isolates of other fungi	1	1	5	0	6	15
Other species	0	32	17	1	11	9

<sup>1</sup> r, s and ss – resistant, sensitive and highly sensitive to benomyl corresponding to ED<sub>50</sub> for mycelial growth on PDA of > 5, 1-5, and < 1 µg ml<sup>-1</sup>, respectively.

Tabel 3. Schimmels geïsoleerd uit halmvoeten met symptomen in met benomyl behandelde en onbehandelde proefvelden.

light discolorations *Alternaria* spp., *M. bolleyi*. For fungicide-treated plots this order was for culms with dark discolorations *Alternaria* spp., *F. culmorum*, *Typhula* sp., with eyespot symptoms *P. herpotrichoides*, *F. culmorum*, *Alternaria* spp., with elongate lesions *Alternaria* spp., *Cladorrhinum* sp., *F. culmorum* and with light discolorations *Alternaria* sp., *F. culmorum*, *Cochliobolus sativus*.

An attempt to estimate the quantitative effect of the fungicide on fungi in the tissue of the culm bases was made in the following way. For each of the four categories of culms with symptoms mentioned in Table 1 the relative frequency (F) of a species was estimated from the isolates obtained from segments of diseased tissue:

$$F = \frac{\text{number of isolates of the species}}{\text{total number of fungal isolates}} \times \text{percentage of affected culms.}$$

The frequency of a species given in Table 3 was assessed by adding the F values estimated for the four categories. The total of percentages in one treatment on each sampling date may exceed 100% since from many lesions more than one species had been isolated.

Table 4. Fungal colonization of roots from benomyl-treated and untreated plots sampled on the harvest date (Wageningen-Hoog).

	Benomyl (kg ha <sup>-1</sup> )	
	0	4.8
Number of segments	250	250
Number of isolates	248	255
Colonization ratio (%)	99	102
Taxa	Percentage of root segments colonized	
<i>Coniothyrium cerealis</i> (s) <sup>1</sup>	2.8	0.0
<i>Fusarium avenaceum</i> (s)	18.6	17.6
<i>F. culmorum</i> (s)	19.6	20.4
Other <i>Fusarium</i> species (ss and s)	3.6	0.0
<i>Gaeumannomyces graminis</i> (ss)	5.2	1.2
<i>Microdochium bolleyi</i> (ss)	12.0	0.4
<i>Mortierella</i> spp. (r)	4.8	19.2
<i>Mucor</i> spp. (r)	12.0	26.0
<i>Phoma leveillei</i> (s)	2.0	2.4
<i>Trichoderma</i> spp. (ss)	1.2	3.2
Sterile basidiomycetes (r)	0.4	3.6
Sterile isolates of other fungi	10.0	5.0
Species with colonization < 2.0% in each treatment	6.0	0.4

<sup>1</sup> r, s and ss, see Table 3.

Tabel 4. Schimmels op wortels afkomstig uit met benomyl-behandelde en onbehandelde proefveldjes (Wageningen-Hoog). De monsters werden op de oogstdatum genomen.

Fungal analysis of culms from treatments not included in Table 3 showed that even at the lowest dose rate ( $0.24 \text{ kg ha}^{-1}$ ) the fungicide suppressed the development of *P. herpotrichoides* and *G. nivalis*. In untreated plots, the latter species disappeared completely from the culms towards the end of the vegetation periode.

Only few species were favoured by the treatments. *Alternaria* species, mainly *A. alternata*, were slightly stimulated. Incidence of *A. alternata* increased during the vegetation period. *Typhula* was only found in treated plots and then at harvest time, albeit at a low level. Moderately sensitive species, like *F. culmorum* and *Periconia macrospinoso*, also tended to be more represented in treated plots.

The category of 'other species' included various species of nine genera with low frequency, e.g. *Epicoccum purpurascens*, *Monilia pruinosa*, and *Dendryphion nanum*.

*Fungi of the roots.* In contrast to culms, roots were only analyzed at the end of the crop, immediately after harvest date. The results for a crop treated with a high dosage

Table 5. Fungal colonization of roots from benomyl-treated and untreated plots sampled on the harvest date (Droevendaal). A and B are samples from different plots.

	Benomyl ( $\text{kg ha}^{-1}$ )					
	0		0.24		1.20	
	A	B	A	B	A	B
Number of segments	275	275	150	150	275	275
Number of isolates	280	297	154	153	299	293
Colonization ratio (%)	102	108	103	102	109	107
Taxa	Percentage of root segments colonized					
<i>Alternaria</i> , <i>Dendryphion</i> and <i>Ulocladium</i> spp. (r) <sup>1</sup>	1.8	1.1	6.0	2.0	13.1	5.8
<i>Fusarium</i> spp. (s)	13.5	10.2	21.3	10.7	22.9	15.6
<i>Microdochium bolleyi</i> (ss)	50.5	36.7	30.0	26.0	14.9	24.4
<i>Mortierella</i> spp. (r)	4.0	4.7	1.3	4.0	4.0	12.0
<i>Mucor</i> and <i>Zygorhynchus</i> spp. (r)	4.7	1.4	8.7	8.7	5.8	8.7
Pycnidial fungi (s) <sup>2</sup>	2.9	2.6	4.0	2.7	1.8	3.3
Pycnidial fungi (r)	0.0	1.8	6.0	1.3	5.5	4.0
<i>Pythium</i> spp. (r)	0.0	0.0	0.0	0.0	0.4	4.0
<i>Trichoderma</i> spp. (ss)	10.9	31.6	12.7	29.3	8.4	5.5
Sterile grey isolates	3.3	6.6	5.3	6.0	10.6	10.6
Species with colonization < 4% in each treatment	9.9	10.5	7.4	9.7	21.1	13.1

<sup>1</sup> r, s and ss, see Table 3.

<sup>2</sup> The pycnidial fungi included *Phoma* and *Coniothyrium* spp.

Tabel 5. Schimmels op wortels afkomstig uit met benomyl-behandelde en onbehandelde veldjes (Droevendaal). De monsters werden op de oogstdatum genomen. A en B zijn monsters uit verschillende proefveldjes.

of the fungicide are given in Table 4. The last one of the three sprayings ( $1.6 \text{ kg ha}^{-1}$ ) was given 65 days before sampling date. The colonization ratio of roots was unaffected, but the species composition was still markedly influenced by the treatment. Fungi that are highly sensitive to benomyl in vitro, e.g. *Microdochium bolleyi* with an  $\text{ED}_{50}$  value for mycelial growth of  $0.04 \mu\text{g ml}^{-1}$  (Van Tuyl, 1977), were suppressed. Resistant fungi, e.g. *Mucor* spp. and *Mortierella* spp. with  $\text{ED}_{50} > 10 \mu\text{g ml}^{-1}$ , were stimulated. Among these fungi *Mortierella elongata* was the most prevalent species. No differences in incidence were found for fungi with an  $\text{ED}_{50}$  value between 1 and  $2 \mu\text{g ml}^{-1}$ , e.g. *F. avenaceum* and *F. culmorum*.

A second analysis of fungi on roots was done for a crop grown at Droevendaal (Table 5). Like in the previous experiment, the colonization ratio was unaffected. For surveyability, related species with equal sensitivity to the fungicide are classified under one category, e.g. the porosporous dematiaceous species including *Alternaria*, *Dendryphion* and *Ulocladium*, that are highly resistant to the fungicide. Among the sensitive pycnidial fungi *Phoma eupyrena* and *P. leveillei* predominated and among the resistant ones an unidentified *Phoma* species. The category of sterile grey isolates probably harboured *Phoma* species too. *F. culmorum* and *F. sambucinum* were the most common fusaria in control and treated plots as well. *G. nivalis* was not isolated from roots.

Table 6. Fungi sporulating on segments of roots from benomyl-treated and untreated plots (Droevendaal).

	Benomyl ( $\text{kg ha}^{-1}$ )	
	0	1.20
Number of segments examined	550	550
Total number of sporulating colonies	355	421
Taxa	Percentage of segments with sporulations	
<i>Alternaria</i> spp. (r)	9.6	26.6
<i>Dendryphion nanum</i> (r)	2.4	2.2
<i>Drechslera</i> sp. (r)	0.2	1.3
<i>Epicoccum purpurascens</i> (s)	2.6	1.8
<i>Fusarium</i> spp. (s)	4.7	12.2
<i>Mortierella</i> spp. (r)	7.8	17.5
<i>Mucor</i> spp. (r)	0.6	1.7
<i>Phoma</i> spp. (s)	8.6	4.6
<i>Trichoderma</i> spp. (ss)	26.0	2.4
<i>Ulocladium</i> sp. (r)	0.2	5.3
Other fungi	2.0	0.9
Total of porosporous fungi	12.4	35.4

<sup>1</sup> r, s and ss, see Table 3.

Tabel 6. Schimmels welke sporuleerden op worteldeeltjes afkomstig uit met benomyl-behandelde en onbehandelde proefveldjes (Droevendaal).

The frequent occurrence of *M. bolleyi* in plots treated with 1.20 kg ha<sup>-1</sup> at Droevendaal is noteworthy because of its high sensitivity to the fungicide. In previous analyses of material from Wageningen-Hoog, this species was suppressed in treated plots. One reason may have been the use of lower dose rates of the fungicide in the plots at Droevendaal. A second reason probably was a change in sensitivity. Eight fresh isolates from treated plot B (1.20 kg ha<sup>-1</sup>) were tested. Three of them proved to be less sensitive (ED<sub>50</sub> > 1 µg ml<sup>-1</sup>) than those that had been tested before (ED<sub>50</sub> < 0.5 µg ml<sup>-1</sup>).

Although the number of *Trichoderma* isolates differed highly for replicate plots, the population was only inhibited in plots treated at the highest dose rate. This was, however, not statistically significant because of the large variation in numbers obtained from the untreated plots (30 and 87 isolates).

*Pythium* was only rarely isolated. This was probably due to the use of an oxytetracycline in the isolation medium. Antibiotics of this group do not only suppress bacterial growth, but also inhibit many pythiaceae fungi.

After isolations had been made, the plates with the segments were placed in daylight. Three weeks later the segments were examined for sporulating fungi with a dissecting microscope. In such examinations only species with conspicuous fructifications are recognized and consequently many species remain unnoticed (e.g. *Microdochium*) or are underestimated (e.g. *Fusarium*). The results (Table 6) show the same tendency as those presented in Table 5.

## Discussion

The samples for analysis of the root-colonizing mycoflora were taken at harvest; that was more than two months after the last spraying of the crop had been given. At that time, a tendency to a higher frequency of resistant fungi, e.g. *Alternaria* spp. and *Mortierella* spp., was observed for plots treated with 1.20 kg ha<sup>-1</sup> or more, but not for those that received 0.24 kg ha<sup>-1</sup>, being the recommended dose for control of eyespot. Lower numbers were only recorded for very sensitive fungi like *Trichoderma* spp. None of the treatments resulted in a decreased fungal recolonization of roots as reported for benomyl-treated onions by De Bertoldi et al. (1978). This probably occurs only when extremely high dose rates are used. In our trials, the colonization ratio of roots in treated plots equalled that of those in untreated plots, but the species composition was different.

Data on the response of the root mycoflora to treatment of the crop with benomyl are inconsistent. Malone et al. (1978) reported that the mycoflora of grass roots was unaffected even after application of 25 kg ha<sup>-1</sup>, which is more than fivefold the highest dose rate used in our trials. The increased yield of grass swards obtained in their trials was therefore attributed to other effects than control of root-colonizing fungi. Weber-Czerwińska (1977, 1979) applied similar amounts to winter wheat as used in our trials with rye. She found that amounts of 0.15 to 0.60 kg ha<sup>-1</sup> left most rhizosphere and root-colonizing fungi unaffected, but that the *Penicillium* population was markedly increased in treated plots. The increase in these sensitive fungi might have been due to development of resistance, as has been reported for some species of this genus (Bollen, 1971; Kuramoto, 1976). In contrast to their frequent occurrence on wheat roots in Weber-Czerwińska's plots, penicillia were rare on roots of rye in



our plots. On the other hand, *Mortierella* species were not recorded in her study, but were consistently observed in our fields, especially in benomyl-treated plots. The prevalent species, *M. elongata*, was reported to be one of the most common rhizosphere fungi of wheat or potatoes grown after wheat in soils studied by Van Emden (1972).

Fusaria were unaffected (Table 4) or showed a tendency to increase in treated plots (Tables 5 and 6). In previous trials, *F. culmorum* was only reduced at a high dose rate of the fungicide (Van der Hoeven and Bollen, 1980). Hanuss and Oesau (1977) observed that application of a low dose rate of carbendazim to wheat ( $0.18 \text{ kg ha}^{-1}$ ) resulted in an increased incidence of fusarium foot rot. This is in line with the results of Weber-Czerwińska (1979), who found that, on roots of wheat, fusaria were stimulated by a treatment with benomyl at  $0.15 \text{ kg ha}^{-1}$  but were inhibited at  $0.60 \text{ kg ha}^{-1}$ . This can be explained by the moderate sensitivity of predominant *Fusarium* species, like *F. avenaceum* and *F. culmorum* ( $\text{ED}_{50}$  of mycelial growth  $1\text{--}2 \mu\text{g ml}^{-1}$ ); when low dose rates are applied, they may benefit from inhibition of more sensitive competitors. One of them is *M. bolleyi*, a very sensitive species common on roots in both of our experimental fields (Table 4 and 5). In earlier observations, it was the most inhibited species in benomyl-treated rye plots over a period of two months (Platenkamp and Bollen, 1973). Reinecke (1978) demonstrated that this fungus is an effective antagonist of *Fusarium* species affecting culm bases of wheat and rye. The observation that some isolates from treated plots at Droevendaal, where the fungus was less suppressed than in previous trials, were only moderately sensitive in vitro show that not only pathogens but also their antagonists may become resistant to fungicides (Bollen, 1982).

Other potential antagonists of fusaria are *Trichoderma* species. Uoti (1979) obtained encouraging results in controlling *F. culmorum* by applying a suspension of *Trichoderma* spores either as a seed treatment or as a soil drench. The results in Table 5 show that *Trichoderma* spp. were only suppressed on roots when a dose rate higher than the recommended  $0.24 \text{ kg ha}^{-1}$  had been used. Because of run-off along the culm after spraying, more effect is to be expected on fungi at the culm base than on those of the roots. *Trichoderma* was, however, only rarely obtained from culms, probably because of the isolation procedure, by which fungi colonizing the surface were eliminated by the sterilization agent.

The mycoflora of the culm base seems to be more prone to effects of the fungicide than that of the roots. This can be inferred from the frequencies of culms with symptoms from plots treated at low dose rates (Table 1) and the enumeration of fungi isolated from these culms. A more detailed analysis was only made for untreated plots and those that received a high dose rate. Conspicuous differences were recorded only for very sensitive species (Table 3). The suppression of *P. herpotrichoides* by benzimidazoles is well documented, e.g. in the comprehensive study on eyespot control by Fehrman and Schrödter (1972). In comparison with the shift in the fungal populations of the culm bases of rye and wheat observed by Reinecke (1977), the response was weak in our trials, the more since we applied a larger amount of the fungicide. In the fields investigated by Reinecke, *Alternaria* spp., pycnidial fungi and *R. cerealis* were significantly stimulated. Reasons for different responses under different situations, especially in relation to *R. cerealis*, have been discussed elsewhere (Van der Hoeven and Bollen, 1980).

The appearance of *Typhula incarnata* and *Drechslera* spp. in treated plots at the end of the season is in line with observations of Hossfeld (1974) for *Typhula* in wheat and Fokkema et al. (1975) for *D. sorokiniana* in rye that these pathogens benefit in the field from their resistance.

The dynamics of the mycoflora of the culms show an increase in *Alternaria* spp., and *Periconia macrospinoso* towards the end of the season (Table 3). A similar pattern was found for winter wheat by Hoes (1962). The occurrence of *P. macrospinoso* is noteworthy as this fungus was recently considered to cause 'crater disease' on wheat in South Africa (Scott et al., 1979). Table 3 shows that *G. nivalis* was only prevalent on culms during the earlier stages of the crop. This does not seem to be a general rule. In their extensive survey on fusaria in wheat, Duben and Fehrmann (1979) observed that *F. culmorum* and *F. avenaceum* became predominant after tillering and *G. nivalis* remained present at a low level but did not decline as in our trials.

Our study contributes only fragmentary knowledge to the effect of benomyl on fungi associated with rye. The analysis was confined to the mycoflora of roots and culm bases. Occasionally an effect on non-pathogens was probably related to the incidence of pathogens. We realize that an important niche has not been included in our study: the caulosphere at the stem base and the subcoronal internodes of healthy and diseased plants. Some pathogens operate in this zone. A study of the effect of fungicides on the microflora at these sites may add further information on the kind of antagonists that are effective against pathogens in the field.

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## Samenvatting

*De invloed van benomyl op bodemschimmels in rogge. 2. De invloed op schimmels van de halmvoet en de wortels*

De schimmelflora van de halmvoet en de wortels werd bepaald in veldproeven, waar benomyl was toegepast in doseringen variërend van 0,24 tot 4,80 kg ha<sup>-1</sup>. De monsters van de halmvoeten werden drie maal gedurende het groeiseizoen genomen, die van de wortels alleen op het tijdstip van de oogst. Op halmen met diverse symptomen uit onbehandelde proefveldjes kwamen *Pseudocercospora herpotrichoides*, *Gerlachia nivalis* en *Alternaria*-soorten het meest voor, op die uit behandelde domineerden *Alternaria*-soorten en *Fusarium culmorum*. Tijdens het afrijpen liep *G. nivalis* sterk terug en namen *Alternaria* en *F. culmorum* toe. Aan het eind van het groeiseizoen verschenen *Periconia macrospinoso* en *Typhula incarnata* in behandelde veldjes.

De wortelmonsters werden ruim twee maanden na de laatste bespuiting genomen. Resistente schimmels, zoals de porospore Dematiaceae (*Alternari*, *Ulocladium* en *Dendryphion*) en *Mortierella*-soorten waren toegenomen in proefveldjes die met 1,20

kg ha<sup>-1</sup> of meer waren behandeld. Dat was niet het geval in de veldjes behandeld met de geadviseerde praktijkdosering van 0,24 kg ha<sup>-1</sup>. Een vermindering van de populatie werd alleen voor zeer gevoelige soorten als *Microdochium bolleyi* en *Trichoderma*-soorten waargenomen. De *Fusarium*-soorten, hoofdzakelijk *F. culmorum* en *F. avenaceum*, werden niet beïnvloed of bleken te tenderen naar een lichte toename tengevolge van de behandeling. In de discussie is getracht om het zich handhaven van deze betrekkelijk gevoelige schimmels in verband te brengen met een effect van het fungicide op de niet-pathogene schimmelflora.

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