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Resistance to carbendazim in Pseudocercosporella herpotrichoides from Dutch wheat fields

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Abstract

Culm base samples were collected in August 1984, from winter wheat fields in Groningen, Flevoland and near Wageningen in Gelderland. In contrast to fields in the latter two areas, fields in Groningen were characterized by intensive wheat cultivation and yearly applications of benzimidazole fungicides for eyespot control. Seventy-seven isolates of *Pseudocercosporella herpotrichoides* were recovered. Forty-three percent of recovered isolates were carbendazim-resistant, all of which originated from fields in Groningen. Resistant isolates were detected among both 'rye-' and 'wheat-types' of the pathogen. 'Rye-type' isolates were identified as *P. herpotrichoides* var. *acuformis* and those of the 'wheat-type' as *P. herpotrichoides* var. *herpotrichoides*.

Introduction

Eyespot occurs on the basal culm or foot of cereals grown in cool, moist climates. Wheat is most susceptible and winter cereals are more frequently damaged than spring cereals. The disease may kill plants outright, but more frequently it reduces yield by decreasing kernel size and number and by causing lodging of infected culms (Booth and Waller, 1973). Lesions occur at or near the soil, and appear as light-coloured elliptical areas with dark margins, that are oriented longitudinally on the stem. These areas become necrotic and darken with age. Sometimes a weft of gray mycelium appears in the lumen of the culm (Wiese, 1977).

Eyespot is caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton. Cultural characteristics and fungal morphometrics are variable among isolates. 'Wheat-' and 'rye-types' have been described, which are distinguished chiefly by colour, morphology and growth rate in culture (Scott et al., 1975). Nirenberg (1981), however, has associated two varieties *(P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis)* and two additional species *(P. anguioides* and *P. aestiva)* with eyespot of cereals. Taxonomic criteria were those mentioned above, plus conidial morphometry.

Control of eyespot is possible with a spring application of benzimidazole fungicides. In 1981 two control failures were reported in the U.K. (Griffin and Yarman, 1983). High proportions of benzimidazole-resistant *P. herpotrichoides* were found at the control failure sites. Surveys conducted in 1982, 1983 and 1984 in the U.K. showed fields with benzimidazole-resistant isolates increasing from 40 to 75 to 90% over the

period, and resistant isolates recovered increasing from 20 to 80% (Griffin and King, 1985). Benzimidazole resistance in *P. herpotrichoides* has also been reported in France (Cavelier and Leroux, 1983), Denmark and Germany (Fehrmann, 1984; Griffiths, 1985; Rashid and Schlösser, 1977).

The purpose of the present study was to determine the level of carbendazim sensitivity in isolates of *P. herpotrichoides* from selected fields in the Netherlands. Knowledge of extant sensitivity of these pathogen populations to carbendazim-generating fungicides is necessary to make sound recommendations for eyespot management.

Materials and methods

Isolation and identification. Culm base samples were obtained from winter wheat fields with various cropping histories and varying durations of benzimidazole use (Table 1). Fields sampled in Groningen where characterized by continuous wheat cultivation, yearly application of benomyl for eyespot control, and twice-yearly application of EBI fungicides for control of other wheat diseases. The incidence of eyespot in these fields was reported to have been relatively high in recent years. Fields in Flevoland and in Wageningen were included as a reference since chemical disease control has never been employed in these areas. Samples (c. 20 straws per sample; one sample per field) were taken non-random in August 1984 and stored in plastic bags at −22 °C for five months until processing began in January 1985. Eight culm pieces were selected from each sample for isolation. Straw sections, 1-2 cm in length, were cut, each containing typical eyespot lesions. In the absence of typical lesions, discoloured internodal pieces were used. Pieces were split lengthwise, and half of each piece was processed. The opposite stem halves were placed in separate numbered test tubes, sealed with Parafilm and returned to low-temperature storage.

Isolations were made using a method described by Bateman et al. (1983). Culm halves were surface sterilized for 45 seconds in sodium hypochloride solution (1% available chlorine), rinsed in sterile water and placed on moist filter paper in separate plastic Petri dishes. Dishes were sealed with Parafilm and placed under near-UV light at 10 °C to induce conidial production. After two weeks, culm pieces were removed from

Table 1. Location, cropping history and benomyl use in sampled sites.

Field number	Location	Years of continuous wheat	Years of benomyl use
1	Zuurdijk (Groningen)	9	9
2	Nieuwolda (Groningen)	7	7
3	Nieuwolda (Groningen)	5	5
4	Nieuw-Scheemda (Groningen)	5	5
5	Nieuw-Beerta (Groningen)	12	5
6	Stedum (Groningen)	6	7
7	Finsterwolde (Groningen)	7	5
8	Field NZ 28 (Flevoland)	1	0
9	Field NZ 24 (Flevoland)	1	0
10	Field NZ 17 (Flevoland)	1	0
11	IPO field (Wageningen)	1	0

the Petri dishes, placed in test tubes containing 10 ml of 0.01% Tween 80 in sterile water and mixed for 10-15 seconds on a Vortex mixer. Serial dilutions of 1 : 10, 1 : 100 and 1 : 1000 were made in sterile water and 1 ml of each suspension was uniformly pipetted onto two PDA dishes (diameter 9 cm): one fungicide-free and one containing carbendazim (1 μg ml^{-1}). The PDA was prepared at $^1/_5$ strength (4.8 g dehydrated Difco potato dextrose broth and 12 g technical grade agar per liter distilled water) and contained streptomycin sulfate (0.3 g l^{-1} added after autoclaving and cooling to 60 °C). Carbendazim-containing PDA was made by adding 5 ml of a 0.02% solution in chloroform/methanol (5 : 95) to each liter of autoclaved, cooled PDA.

Petri dishes containing conidial suspensions were allowed to remain in a horizontal position for 2-3 h to allow conidia to settle to the agar surface. Plates were then gently slanted to an angle of approximately 20 °, and, after remaining in this position for several hours, accumulated liquid was poured from each plate. This procedure was found to be effective in drying the agar surface and minimizing bacterial contamination. Plates were incubated at room temperature for 1 to 5 days. At the end of this period, germinated conidia or colonies typical of *P. herpotrichoides* were counted and representative single spore isolates were transferred to PDA plates for further testing. Isolates that grew on the medium containing 1 μg carbendazim ml^{-1} were considered to be resistant. This was verified in minimum inhibitory concentration (MIC) tests. Identification of recovered isolates was done according to the taxonomic criteria of Nirenberg (1981).

Minimum inhibitory concentration of carbendazim. All recovered isolates were tested on a range of carbendazim concentrations (0, 0.1, 1, 10 and 50 μg ml^{-1}) in PDA, prepared as previously described. Inoculum for MIC tests was prepared using a modification of the method of Chang and Tyler (1964). Individual isolates were grown on water agar in 6 cm plastic Petri dishes at 10 °C under near-UV light for at least two weeks. Dishes were flooded with 6 ml of sterile distilled water and hand-agitated to dislodge conidia. One ml of spore suspension from individual isolates was dispersed over the agar surface of each of the 5 Petri dishes in the carbendazim test series (0, 0.1, 1, 10 and 50 μg ml^{-1}). 'Mother' dishes were returned to the 10 °C, near-UV environment for re-sporulation. Excess liquid was decanted from the dishes after spores had been allowed to settle to the agar surface, as previously described. Following four days' incubation at room temperature, growth of the test isolates in the presence of the various carbendazim concentrations was assessed.

Pathogenicity to wheat seedlings. Pathogenicity of recovered isolates was tested on 'Okapi' winter wheat. Twenty seeds were planted per 7 × 7 cm plastic pots with pasteurized greenhouse soil. At seven days post-seeding, seedlings were inoculated by spraying stem bases with conidial suspensions in sterile water. One pot with twenty seedlings was inoculated per isolate. Ten pots with seedlings were sprayed with sterile water. Conidial suspensions were prepared, as previously described, form 'mother' plates which had been 're-sporulated' for two weeks at 10 °C under near-UV light. After inoculation, seedlings were kept at 18-22 °C and maintained at a height of approximately 10 cm above the soil surface by trimming twice a week with scissors. At eight weeks after inoculation, ten seedlings from each pot were rated for disease development. A 0-7 rating scale was employed, which corresponded to the number of leaf sheaths (including

the cotyledon) showing visible necrosis. Mean disease rating were calculated for each isolate.

Results and discussion

Table 2 presents a summary of the carbendazim reaction and identification of *P. herpotrichoides* isolates recovered from the sampled sites. Using the taxonomic criteria of Nirenberg (1981), isolates of the 'rye-type' were identified as *P. herpotrichoides* var. *acuformis* and those of the 'wheat-type' as *P. herpotrichoides* var. *herpotrichoides*. Incidence of eyespot in field 9 was extremely low, and no isolates were obtained from this field. All isolates from the remaining fields in Flevoland and Wageningen (fields 8, 10 and 11) were sensitive. Carbendazim MIC's for these isolates were less than 0.1 $\mu g\ ml^{-1}$. Isolates resistant to carbendazim, however, were obtained from five of the seven fields in Groningen. The carbendazim MIC's for all resistant isolates were greater than 10 $\mu g\ ml^{-1}$ and for 82% were in excess of 50 $\mu g\ ml^{-1}$. From three of the Groningen sites (fields 2, 4 and 5), carbendazim-resistant isolates exclusively were recovered.

No relationship was found in the Groningen samples (fields 1-7) between the percentage of resistant isolates recovered and history of benomyl use. For example in fields 3, 4, 5 and 7 where benomyl had been used in five years, 33, 100, 100 and 0%, respectively, of recovered isolates were carbendazim-resistant. This finding is more probably related to the random occurrence of infection foci in the fields, than to the actual nature of the *P. herpotrichoides* population in the sampled sites.

Approximately half of the isolates obtained were identified as *P. herpotrichoides*

Table 2. Carbendazim reaction and identification of *Pseudocercosporella herpotrichoides* isolates recovered from sampled sites.

Field number	Number of *P. herpotrichoides* isolates recovered				Total
	var. *acuformis*		var. *herpotrichoides*		
	sensitive	resistant	sensitive	resistant	
1	4	12	0	0	16
2	0	2	0	0	2
3	2	2	2	0	6
4	0	7	0	0	7
5	0	6	0	4	10
6	2	0	0	0	2
7	3	0	1	0	4
8	0	0	2	0	2
9	0	0	0	0	0
10	0	0	4	0	4
11	0	0	24	0	24
Total	11	29	33	4	77
% of total	14	38	43	5	

var. *acuformis* or *P. herpotrichoides* var. *herpotrichoides*. Carbendazim-resistant isolates were detected among both varieties, but only in the Groningen samples. Occurrence of resistant isolates appeared to be most prominent in the *acuformis* isolates. The recovery of *acuformis* also appeared to be associated with sites where fungicides have been intensively used. These findings support the association between benomyl resistance and pathogen type reported in England, where there is a clear tendency for 'rye-type' isolates to be resistant and for resistant isolates to be 'rye-type' (King and Griffin, 1985; Hollins et al., 1985).

Laboratory studies by Griffin and King (1985) with benzimidazole-sensitive and --resistant 'types' of *P. herpotrichoides* showed consistent differences in sensitivity to various EBI fungicides. Radial growth of benzimidazole-resistant 'rye-type' isolates was less inhibited by EBI fungicides than was growth of benzimidazole-sensitive 'rye-types' or either 'wheat-type'. These findings would appear to indicate that use of EBI fungicides alone would exert a selection pressure that would favour 'rye-types' generally, and benzimidazole-resistant 'rye-types' in particular. In fact, field surveys in the U.K. have shown that the percentage of 'rye-type' isolates of *P. herpotrichoides* increased with increased use of EBI fungicides (Griffin and King, 1985). Our recovery of *acuformis* isolates from only fields 1-7 agrees with the foregoing reports. Therefore, in addition to benzimidazoles, the EBI fungicide treatments in the Groningen fields might have been of selective advantage for such isolates.

All isolates produced visible leaf sheath necrosis on winter wheat seedlings. Under the conditions of this test, the *P. herpotrichoides* var. *herpotrichoides* isolates were more virulent as a group than the *P. herpotrichoides* var. *acuformis* isolates. Although the range of mean disease ratings was similar for *herpotrichoides* (6.2-3.0) and *acuformis* (6.2-2.7), the group mean ratings were 5.2 and 4.0, respectively. For *herpotrichoides* isolates, 70% of the mean disease ratings were greater than 5.0, 25% were between 5.0 and 4.0 and 5% were less than 4.0. For *acuformis* isolates, these values were 18, 29 and 53%, respectively. Mean disease rating for 100 uninoculated seedlings was 1.0.

Scott and Hollins (1985) have reported that 'rye-type' isolates are more virulent on winter barley than on winter wheat. Therefore, increased areas of winter barley grown in Europe may also have selected for 'rye-type' isolates in *P. herpotrichoides* populations (Hollins et al., 1985). Findings of the present work, where 'rye-type' isolates predominate in Groningen fields of continuous wheat cultivation, suggest that fungicide applications, including both carbendazim-generating and EBI fungicides may be of major importance in this respect.

Although failure to control eyespot with benomyl has not been reported in the Netherlands, the presence in Groningen of highly resistant *P. herpotrichoides* in fields where benomyl has been used in five or more years emphasizes the necessity for more diversity in prophylactic chemicals. In light of these findings, a more complete characterization of *P. herpotrichoides* populations in Dutch wheat fields may be warranted.

Samenvatting

Resistentie tegen carbendazim bij Pseucocercosporella herpotrichoides van Nederlandse tarwepercelen

Monsters van de halmbasis van tarwestro werden in augustus 1984 verzameld van per-

celen met wintertarwe in Groningen, Flevoland en bij Wageningen in Gelderland. In tegenstelling tot de percelen in de twee laatstgenoemde gebieden, waren de percelen in Groningen gekenmerkt door intensieve tarweteelt en door jaarlijkse bespuitingen met benzimidazool fungiciden tegen de oogvlekkenziekte. In totaal werden 77 isolaten van *Pseudocercosporella herpotrichoides* verkregen. Drieënveertig procent van de isolaten was resistant tegen carbendazim en alle resistente isolaten waren afkomstig van percelen in Groningen. Resistente isolaten werden zowel bij het 'rogge-' als bij het 'tarwetype' van het pathogeen aangetroffen. Isolaten van het 'roggetype' werden geidentificeerd als *P. herpotrichoides* var. *acuformis* en die van het 'tarwetype' als *P. herpotrichoides* var. *herpotrichoides.*

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