Neth. J. Pl. Path. 92 (1986) 21-32

Variation in sensitivity to fungicides which inhibit ergosterol biosynthesis in wheat powdery mildew

M.A. DE WAARD, E.M.C. KIPP, N.M. HORN and J.G.M. VAN NISTELROOY

Laboratory of Phytopathology, Agricultural University, Binnenhaven 9, 6709 PD Wageningen, the Netherlands

Accepted 24 October 1985

Abstract

Fungicides which inhibit ergosterol biosynthesis have been in use for control of wheat powdery mildew *(Erysiphe graminis* f. sp. *tritici)* in the Netherlands since 1978. Mildew populations were tested for their variation in sensitivity to triadimefon from 1982 to 1984.

In 1982 isolates from the province Limburg, with a triazole spray-regime history, were less sensitive to triadimefon than isolates from the provinces Gelderland and Noord-Brabant, where triazoles had not been used. In the following years isolates with reduced sensitivity were also detected in the latter provinces and other parts of the country. This spread correlates with the increased use of triazoles, both in frequency and space, from 1983 onwards. The reduced sensitivity can as a whole or in part be responsible for the decline in field performance of triazoles, observed during these years.

Cross-sensitivity to the triazoles triadimefon and propiconazole was established, but not to triazoles and the morpholine fungicide fenpropimorph. Effectiveness of the latter compound was similar to all isolates from Limburg tested in 1984. Field performance of fenpropimorph, introduced in 1983, appeared to be normal. It is recommended to counteract further development of resistance by sequential use of fenpropimorph early in the season (May) and triazoles at the end (June-July).

Additional keywords: fungicide resistance, fenpropimorph, propiconazole, triadimefon, *Erysiphe graminis* f.sp. *tritici.*

Introduction

Agricultural practice for powdery mildew control in wheat in the Netherlands usually includes one or two foliar spray applications with fungicides which inhibit ergosterol biosynthesis (EBIs). The triazole triadimefon has been used since 1978; from 1982 a second triazole for this purpose became available: propiconazole. Both chemicals inhibit fungal ergosterol biosynthesis at the same site of action. Up to 1982 use of triadimefon and propiconazole remained mainly restricted to areas with a high mildew incidence, such as the province Limburg (Fig. 1). In 1983 a severe wheat powdery mildew epidemic occurred all over the country. This has contributed to the sharply increased use of triazole-fungicides from that year onwards both in space and frequency. In many fields mildew was even exclusively controlled by two triazole sprays during the growing season. A restricted number of fields were sprayed with the newly approved fungicide fenpropimorph. Use of the latter chemical significantly increased in 1984. This

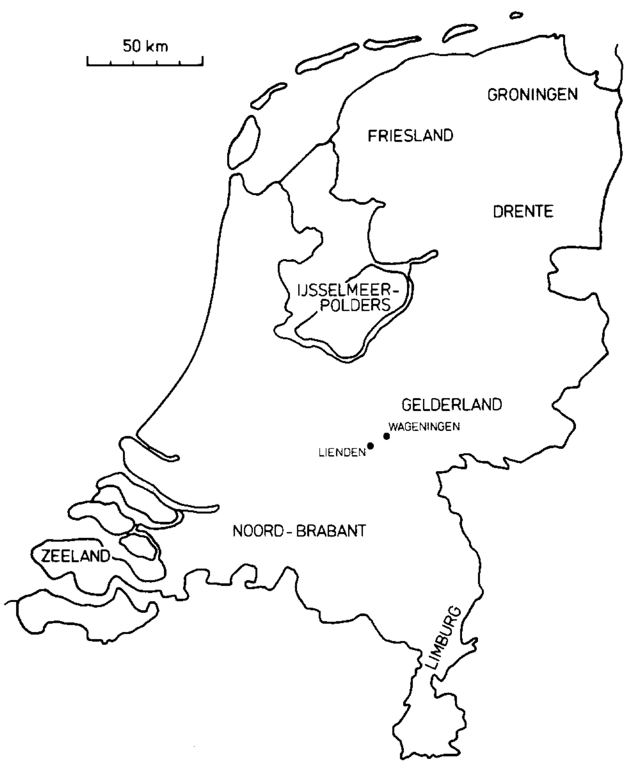

Fig. 1. Areas in the Netherlands surveyed for variation in sensitivity of wheat powdery mildew to EBIs.

chemical also inhibits fungal ergosterol biosynthesis but at a site different from that of triazoles.

Development of field resistance to EBIs was expected to be unlikely because laboratory mutants of several fungi with EBI resistance were characterized by reduced fitness and a relatively low degree of resistance (Fuchs and De Waard, 1982). However, reduced sensitivity to EBIs of cereal and cucumber powdery mildews in practice became gradually apparent (e.g. Fletcher and Wolfe, 1981; Schepers, 1983). Unconfirmed reports on reduced wheat powdery mildew control by triazole-EBIs in the Netherlands started to circulate during the 1981 growing season and gave rise to the present study. It gives an account of the variation in sensitivity of wheat powdery mildew populations in the Netherlands, mainly in the province Limburg. The study covers the period from 1982 up to 1984 and includes data on field performance of EBIs in 1984. In addition, cross-sensitivity to various EBIs was tested.

Materials and methods

Plants. Wheat seedlings cv. Okapi used for maintenance of mildew isolates and foliar spray tests were grown in pots (180 ml; 20 seedlings per pot) with Trio-potting mixture under glasshouse conditions at 16-22 °C for 7 days. Seedlings used for production of inoculum were grown in 9-cm diam. pots (c. 60 seedlings per pot) under otherwise similar conditions.

Fungicides. Bayleton (a.i. triadimefon, 25% WP) was kindly provided by Bayer Nederland B.V. (Arnhem, the Netherlands); Corbel (a.i. fenpropimorph, 75% EC) by BASF Nederland B.V. (Arnhem, the Netherlands) and Tilt (a.i. propiconazole, 25% WP) by Ligtermoet Chemie B.V. (Roosendaal, the Netherlands). Chemically pure triadimefon was generously supplied by Bayer A.G. (Leverkusen, Fed. Rep. Germany), propiconazole by Ciba Geigy A.G. (Basle, Switzerland) and fenpropimorph by Dr. R. Maag A.G. (Dielsdorf, Switzerland).

Sampling and maintenance of isolates. Wheat leaves with powdery mildew symptoms were usually collected from EPIPRE fields in Gelderland, Limburg and Noord-Brabant (Fig. 1) EPIPRE is a computer based pest and disease advisory system for winter wheat (Rabbinge and Rijsdijk, 1983). In this way relevant data on mildew occurrence and fungicide spray regimes were easily available. The number of samples collected per field in 1982, 1983 and 1984, usually amounted to 1, 3 and 6, respectively. Samples from Gelderland predominantly originated from experimental fields at Lienden (Schuilenburg) or Wageningen. Samples from other provinces of the country were collected by extension officers from fields (three samples per field) suspected from insufficient mildew control by triazole fungicides. Samples of 1984 were exclusively obtained from fields with cv. Okapi; in the previous years some isolates were also collected from other wheat cultivars, mainly cv. Arminda.

The samples were collected in plastic bags, brought to the laboratory on the same day and kept at 4 °C. The next day mildew conidia were transferred to leaves of wheat seedlings. Cross-contamination between isolates was prevented by sealing each pot of seedlings with a cellophane bag (18-26 cm; Agentur Heimann, Wahlenstrasse 44, 5 Köln 30, Fed. Rep. Germany). The seedlings were incubated in climate rooms at 18 °C under fluorescent light (7000 lux) during 16 h a day at 80% RH. After 10 days the seedlings were stored at 4 °C under otherwise identical conditions. Subculturing of isolates was carried out every 6 weeks.

Mass-production of conidia was carried out by dry inoculation of seedlings in 9-cm-diam. pots with fresh conidia from diseased seedlings. Cross-contamination was prevented by placing a cellophane bag attached onto a PVC cylinder (height 10 cm; diam. 9 cm) on the soil surface over the seedlings. Seedlings outside the cylinder were removed. Conidia produced after c. 12 days of incubation were used as inoculum in foliar spray tests.

Foliar spray and leaf segment tests. Foliar spray tests were carried out with the formulated products Bayleton (a.i. triadimefon) and Corbel (a.i. fenpropimorph). Bayleton suspensions (250 μg a.i. ml^{-1}) were freshly prepared in water and diluted with a suspension of the blind formulation of Bayleton (750 μg ml^{-1}). The concentration range finally adopted for dosis-response tests was 0, 0.1, 0.3, 1.0, 3.0, 10, 30 and 100 μg triadimefon ml^{-1}. Corbel suspensions were always freshly made in water. The concentration range used in foliar spray tests was 0, 0.125, 0.25, 1.0, 2.5, 5 and 10 μg fenpropimorph ml^{-1}.

Usually eight pots with seedlings were sprayed simultaneously on a turn-table with

40 ml of a fungicide suspension. Corbel was sprayed outdoors because of its high vapour phase activity. The seedlings were allowed to dry for c. 2-4 h. Then, seedlings sprayed with a series of fungicide concentrations were placed together and simultaneously inoculated by tapping c. 20 freshly sporulating leaves above the pots and by passing the leaves gently through the seedlings. This procedure was repeated five times, thereby using, in total, inoculum produced in two 9-cm-diam. pots. Inoculated seedlings were covered with cellophane bags and incubated in climate rooms under conditions as described above. In order to avoid interaction between different treatments, seedlings in climate rooms were grouped per fungicide concentration on different trays. The incubation period was 14 days.

Leaf segment tests were carried out by floating five leaf segments (3 cm) of second leaves of seedlings on Knop's nutrient solution in open petri dishes under climate room conditions as described above (in duplicate). The nutrient solution contained EBIs at various concentrations. Leaf segments were inoculated as in foliar spray tests before transfer to the nutrient solution.

Assessment and calculations. Assessment of mildew severity in foliar spray tests was carried out for 10 leaves per treatment taken at random from each pot with seedlings. 'Standard area' diagrams according to Grainger (1947) were used to estimate the percentage of the inoculated leaf area with powdery mildew symptoms. Values for control treatments ranged between 50-80%. Values estimated for treatments were expressed as percentages of the control treatment (= 100%) and transformed to probits. EC_{50} and EC_{95} values of fungicides were calculated by linear regression. Mildew assessment in leaf segment tests and calculations of EC_{50} and EC_{95} values were carried out in a similar way. In wheat fields mildew severity was determined by assessing the leaf surface area covered with mildew of the three top leaves of 40 plants, selected at random.

Results

Survey of sensitivity to triadimefon in 1982. Control of wheat powdery mildew by triazole-EBIs in the Netherlands from 1978 onwards was rather intensive in the province Limburg. Therefore, this province was chosen as the target area for the survey studies. Mildew isolates were collected from 28 different fields. Reference mildew isolates from this province with a definite wild-type sensitivity to triadimefon were not available. Therefore, mildew isolates from fields in the provinces Gelderland and Noord-Brabant in which EBIs were hardly ever applied, were taken as substitute references (n = 25). Most of these isolates were obtained from samples of experimental fields around Lienden (Schuilenburg) and Wageningen in which EBIs were never used.

Results, summarized in Tables 1 and 2, indicate that isolates from Limburg had a significantly lower sensitivity than isolates from Gelderland and Noord-Brabant. Average EC_{50} and EC_{95} values (n = 10) of triadimefon for isolates from an experimental station in Gelderland (Schuilenburg, Lienden) were 1.1 ± 0.9 and 6.3 ± 6.2 μg triadimefon ml^{-1}, respectively. One representative isolate of this field was maintained for further studies and was indicated as 'reference isolate LH'.

Survey of sensitivity to triadimefon in 1983. The 1983 season was characterized by an early and severe mildew epidemic over the whole country. EBIs were intensively used

Table 1. Sensitivity to triadimefon of isolates of *Erysiphe graminis* f. sp. *tritici* collected in 1982, 1983 and 1984 in the Netherlands in foliar spray tests.

Year	Province	Sampling period	Fungicide regime	Number of fields sampled	Number of isolates tested	Triadimefon (μg ml^{-1})	
						EC_{50}	EC_{95}
1982	Gelderland[5]	April-July	None	25	25	1.7 ± 1.7[2]	9 ± 8[2]
1982	Limburg	April-July	Triazoles	28	28	4.2 ± 2.0[3]	21 ± 11[3]
1983	Reference isolate LH	—	—	—	15[1]	0.7 ± 0.7	8 ± 6
1983	Limburg	April-May	None	21	63	1.4 ± 1.6[4]	25 ± 22[4]
1983	Limburg	June-July	Triazoles	34	102	1.8 ± 1.5[4]	70 ± 95[4]
1983	Gelderland	April	None	9	27	1.0 ± 4.1	18 ± 11[4]
1984	Reference isolate LH	—	—	—	19[1]	0.7 ± 0.4	7 ± 4
1984	Reference isolate 67	—	—	—	11[1]	4.2 ± 1.6[4]	320 ± 240[4]
1984	Limburg	May	None	8	46	2.8 ± 3.9[4]	41 ± 55[4]
1984	Limburg	July	Corbel and triazoles	6	36	2.9 ± 2.3[4]	39 ± 27[4]
1984	Limburg	July	Triazoles	2	12	3.0 ± 2.7[4]	26 ± 9[4]
1984	Gelderland	May-June	None	3	17	2.8 ± 2.6[4]	40 ± 48[4]
1984	Gelderland	July	None	3	14	2.9 ± 3.0[4]	27 ± 15[4]
1984	Gelderland	July	Triazoles	2	9	1.2 ± 0.8[4]	34 ± 22[4]
1984	Drente-Friesland	July	Triazoles	3	9	2.4 ± 0.9[4]	30 ± 19[4]
1984	IJsselmeer-polders	July	Triazoles	5	15	2.0 ± 1.0[4]	38 ± 22[4]
1984	Zeeland	July	Triazoles	2	6	1.6 ± 1.1[4]	41 ± 29[4]

[1] Number of repetitions with the same reference isolate.
[2] Average EC_{50} and EC_{95} values of isolates from Lienden (Schuilenburg) (n = 10) 1.1 ± 1.0 and 6.3 ± 6.2 μg triadimefon ml^{-1}, respectively.
[3] Significantly different from isolates from Gelderland and Noord-Brabant (P = 0.05).
[4] Significantly different from reference isolate LH (P = 0.05) tested in the same year.
[5] Including Noord-Brabant

in all provinces and as a result untreated reference fields in any province of the country were hard to find. In addition, the dynamics of mildew populations with decreased EBI sensitivity would make it uncertain whether wild-type isolates could really be collected from untreated fields. It was, therefore, decided to use isolate LH of the 1982 survey as a reference wild-type. Fields monitored were again located in Limburg. Samples were collected in April-May before application of any EBI and in June-July, when most of the fields had been sprayed once or twice with triazoles. Results, presented in Table 1, show that average EC_{50} and EC_{95} values of triadimefon for all Limburg isolates significantly differed from those for the reference isolate LH. Differences be-

Table 2. Distribution of the sensitivity to triadimefon of isolates of *Erysiphe graminis* f. sp. *tritici* collected in 1982, 1983 and 1984 from various parts of the Netherlands in foliar spray tests.

Year	Province	Fungicide regime	Number of isolates tested	EC_{50} or EC_{95}	Frequency (%) of EC_{50} or EC_{95} values in sensitivity categories 1-11[1]										
					1	2	3	4	5	6	7	8	9	10	11
1982	Gelderland[2]	None	25	EC_{50}	44	24	16	0	12	0	4	0	0	0	0
				EC_{95}	60	32	4	4	0	0	0	0	0	0	0
1982	Limburg	Triazoles	28	EC_{50}	4	11	11	18	25	18	4	8	0	4	0
				EC_{95}	11	39	36	11	0	4	0	0	0	0	0
1983	Reference isolate LH	—	15	EC_{50}	73	13	13	0	0	0	0	0	0	0	0
				EC_{95}	73	20	7	0	0	0	0	0	0	0	0
1983	Limburg	None	63	EC_{50}	62	19	0	5	10	5	0	0	0	0	0
				EC_{95}	14	52	5	10	5	10	0	0	0	5	0
1983	Limburg	Triazoles	102	EC_{50}	32	35	24	6	0	0	0	0	0	0	0
				EC_{95}	0	6	18	24	3	12	9	12	3	3	12
1984	Reference isolate LH	—	19	EC_{50}	84	11	5	0	0	0	0	0	0	0	0
				EC_{95}	84	16	0	0	0	0	0	0	0	0	0
1984	Reference isolate 67	—	11	EC_{50}	0	9	9	27	9	36	9	0	0	0	0
				EC_{95}	0	0	0	0	0	0	0	0	0	9	91
1984	Limburg	None	46	EC_{50}	41	28	11	0	2	0	2	4	2	2	6
				EC_{95}	17	24	20	9	7	9	2	0	0	0	11
1984	Limburg	Corbel and triazoles	36	EC_{50}	3	47	17	8	6	11	3	3	0	0	3
				EC_{95}	0	14	28	28	8	11	6	0	0	3	3

[1] Sensitivity categories 1-11 represent EC_{50} values of 0-1, 1-2 --- 9-10 and higher than 10 μg triadimefon ml^{-1} and EC_{95} values of 0-10, 10-20 --- 90-100 and higher than 100 μg triadimefon ml^{-1}, respectively.

[2] Including Noord-Brabant.

came more prominent upon comparison of frequency distribution figures of EC_{50} and EC_{95} values (Table 2). The most evident shift was observed for the EC_{95} values for isolates collected after the triazole sprays. For four isolates tested EC_{95} values above 100 $\mu g\ ml^{-1}$ were found. One of these isolates, number 67, was maintained after the survey study on seedlings sprayed with 25μg triadimefon ml^{-1}. Transfer of the isolates on such triadimefon-sprayed seedlings took place every four weeks.

Survey of sensitivity to triadimefon in 1984. The occurrence of mildew isolates with decreased sensitivity to triazole-EBIs prompted the majority of growers in Limburg to use fenpropimorph for the first mildew spray. In five out of six fields tested the fenpropimorph spray was followed by a second treatment with triazoles. These fields were sampled before and after both fungicide applications; isolates LH and 67 were used as references. Again, sensitivity of all Limburg isolates to triadimefon was lower than that of the wild-type; isolate 67 displayed the lowest sensitivity (Table 1). Figures on the frequency distribution of EC_{50} and EC_{95} values of triadimefon lead to the same conclusion (Table 2).

In addition to the fields mentioned above, a limited number of fields with other fungicide spray regimes, often located in other provinces of the country, were studied. Most of these fields were exclusively treated with triazole-EBIs. Samples from Drente, Friesland, IJsselmeerpolders and Zeeland came from fields in which extension officers had observed poor mildew control by triazole-EBIs. Isolates from Gelderland originated from experimental fields around Wageningen. All these isolates showed on average a decreased sensitivity to triadimefon as compared with the reference isolate LH (Table 1). Decreased sensitivity of isolates from triazole-treated fields in Gelderland appeared relatively low. This may be an experimental artefact due to the low number of fields and isolates tested.

Survey of sensitivity to fenpropimorph in 1984. Because of the increased use of fenpropimorph in 1984 the variation in sensitivity to this particular fungicide was also studied. Isolates were collected from the same fields in Gelderland and Limburg sampled for the survey of the sensitivity to triadimefon. References tested were isolates LH and 67. Results presented in Table 3 indicate that EC_{50} and EC_{95} values of fenpropimorph for all isolates tested did not differ significantly.

Field performance. The efficacy of EBI fungicides in practice was studied by assessing mildew development in the surveyed fields in time. Data presented in Table 4 indicate that the efficacy of Corbel was superior to that of Bayleton or Tilt. The leaf area with powdery mildew symptoms was on average 0.3% on June 12-14 in Corbel-sprayed fields and 5.2% in Bayleton or Tilt-sprayed fields.

Mildew development in triazole-treated fields was assessed by extension officers by estimating the percentage of leaves with mildew on the day of fungicide treatment and about four weeks later at the day the isolates were sampled. In all fields tested mildew development was not arrested but increased approximately from 60 to 86%.

Table 3. Sensitivity to fenpropimorph of isolates of *Erysiphe graminis* f. sp. *tritici* collected from fields with various fungicide spray regimes in 1984.

Province	Sampling period	Fungicide regime	Number of fields sampled	Number of isolates tested	Fenpropimorph (μg ml^{-1})	
					EC_{50}	EC_{95}
Reference isolate LH	—	—	—	4	0.9 ± 0.5	3.1 ± 1.1
Reference isolate 67	—	—	—	4	0.8 ± 0.2[1]	3.2 ± 0.6[1]
Gelderland	May-July	None	3	15	1.0 ± 0.3[1]	3.4 ± 0.9[1]
Limburg	May-July	Triazoles	4	20	1.0 ± 0.3[1]	3.6 ± 1.3[1]
Limburg	May-June	Corbel	3	18	0.8 ± 0.3[1]	3.0 ± 0.8[1]

[1] Not significantly different from reference isolate LH (P = 0.05).

Table 4. Development of powdery mildew in wheat fields in 1984 with various fungicide regimes for mildew control.

Province	Fungicide regime	Leaf area with mildew (%)[1]			
		May 20-24	June 12-15	July 9-11	July 26-30
Gelderland	None	0	3	14	14
Gelderland	None	0	3	13	14
Gelderland	Bayleton June 6 Bayleton June 21	0	7	8	6
Gelderland	Bayleton May 14 Bayleton June 21	0	4	12	9
Limburg	Tilt May 25 Bayleton June 26	0	4	11	8
Limburg	Bayleton June 11 Corbel July 19	0	6	13	18
Limburg	Corbel June 6 Bayleton June 29	0	1	1	1
Limburg	Corbel May 9 Tilt June 12	0	0	3	4
Limburg	Corbel May 10 Bayleton June 15	0	0	1	1
Limburg	Corbel May 16 Bayleton June 22	0	0	4	4
Limburg	Corbel May 9	1	1	2	8
Limburg	Corbel June 15 Bayleton July 5	0	0	1	10
Limburg	Corbel May 5 Tilt June 18	0	0	1	9

[1] Average percentages on the three upper leaves.

Table 5. Cross-sensitivity of isolates of *Erysiphe graminis* f. sp. *tritici* to triadimefon, propiconazole and fenpropimorph in leaf segment tests.

Isolate	Triadimefon ($\mu g\ ml^{-1}$)		Propiconazole ($\mu g\ ml^{-1}$)		Fenpropimorph ($\mu g\ ml^{-1}$)	
	EC_{50}	EC_{95}	EC_{50}	EC_{95}	EC_{50}	EC_{95}
LH	0.3 ± 0.1	3.9 ± 0	0.7 ± 0.2	14.6 ± 4.0	4.7 ± 2.5	18.2 ± 1.1
60	2.2 ± 1.4	15.2 ± 9.1	2.0 ± 0.1	17.0 ± 0.1	3.9 ± 1.0	32.5 ± 5.7
56e	7.4 ± 4.5	24.3 ± 3.7	12.4 ± 8.0	83.9 ± 0.5	3.3 ± 0.9	17.3 ± 3.3
H7	8.9 ± 0.4	26.8 ± 3.8	2.1 ± 0.6	52.2 ± 15.0	3.7 ± 2.3	17.8 ± 2.8
64	10.3 ± 5.5	50.1 ± 14.1	3.0 ± 0.4	36.8 ± 5.7	5.1 ± 4.5	18.3 ± 6.3
67	29.2 ± 6.4	116.8 ± 3.0	11.5 ± 1.8	64.9 ± 4.2	5.6 ± 2.1	22.8 ± 3.5

Cross-sensitivity. Cross-sensitivity to EBIs was tested in leaf segment tests. Results show that field isolates with reduced sensitivity to triadimefon also displayed to a varying degree reduced sensitivity to propiconazole (Table 5). It indicates cross-sensitivity to different triazole-EBIs. Sensitivity to fenpropimorph of all isolates tested did not differ significantly indicating absence of cross-sensitivity to triazoles and this particular fungicide. Absence of cross-sensitivity to triadimefon and fenpropimorph in isolate 67 became also apparent in foliar spray tests (compare Tables 1 and 3).

Discussion

The present study indicates a significant variation in sensitivity to triazole-EBIs of powdery mildew populations over the years 1982-1984. In 1982, isolates with an abnormally low sensitivity to triadimefon were found in the province Limburg, but not in Gelderland and Noord-Brabant. In 1983 and 1984 less sensitive isolates were also demonstrated in the latter provinces and in others parts of the country. The presence of such isolates correlated with the increased use of triazoles from 1983 onwards. The results are in agreementt with similar observations in wheat or barley powdery mildew in England and the Fed. Rep. of Germany (Buchenauer et al., 1984; Butters et al., 1984; Gilmour, 1984; Heany et al., 1984; Hunter et al., 1984; Wolfe et al., 1984).

The degree of reduced sensitivity of mildew populations, expressed as the ratio between EC_{50} (or EC_{95}) values for field and reference isolates was rather low and varied from 2-6 (Table 1). Average EC_{50} and EC_{95} values of triadimefon for the 1984 mildew population in Limburg did not differ from those for the 1982 population. In 1983 slightly lower values were observed (Table 1). These results indicate that a continued selection pressure of triazoles in space and time did not readily select for significantly higher degrees of resistance. A reason for this stable level of reduced sensitivity may be the presence of long periods without selection pressure, since as a rule treatment of sowing-seed with triazoles is not common in the Netherlands and eyespot control with the EBI prochloraz, which may also select for resistance to triazole-EBIs in mildew, is still very limited. As a consequence selection pressure is only present from about May to July. Other reasons may be the increasing use of fungicides which lack cross-sensitivity (fenpropimorph) and the influx of conidia from refugee areas where sensitive isolates can survive. This situation differs significantly from cucumber powdery mildew control in Dutch glasshouses where an almost continuous selection pressure by EBIs in time led to selection of isolates with gradually higher degrees of resistance in the course of three years (Schepers, 1985). The discontinuous selection pressure in wheat powdery mildew may also explain why the frequency of isolates from Limburg in different sensitivity categories varied over years and during the course of one growing season (Table 2). Apparently such changes in the frequency distribution of the sensitivity of isolates did not necessarily affect the average EC_{50} or EC_{95} values of triadimefon (Table 1). Therefore, a comparison of the sensitivity of mildew populations should preferentially be based on the frequency distribution of the sensitivity of isolates in different populations.

In a restricted number of fields isolates with relative high levels of reduced sensitivity were detected, e.g. the 1983 isolate 67. Average EC_{50} and EC_{95} values of triadimefon for this isolate were 4.2 ± 1.6 and 320 ± 240 μg a.i. ml^{-1}, respectively. One may wonder whether this reduced sensitivity level represents the maximum which can be obtained by the pathogen. The EC_{95}/EC_{50} ratio for this isolate was significantly higher than for the wild-type isolate LH and for field isolates with a low degree of reduced sensitivity. Development of resistance apparently does not only affect EC_{50} values but also the slope of the dosis-response curve. The high EC_{95} value of triadimefon for this isolate equals the practical field rate of the fungicide (250 μg a.i. ml^{-1}). The occurrence of isolates like 67 in practice may, therefore, well reduce the field performance of triazole-EBIs. A sound fungicide management should, therefore, prevent the selection of mildew populations which exclusively consist of these type of isolates. The judgement whether isolates with a lower degree of reduced sensitivity are involved in decreased disease control is difficult. On the one hand the EC_{95} values of triadimefon for these isolates are far below the field rate but on the other hand the efficacy of triadimefon in laboratory spray tests is higher than in practice. This difference may amount to a factor 5-10. In that case occurrence of such isolates may also contribute to the relatively weak field performance of triazoles (e.g. Table 4) and explain why such isolates appeared one year after the use of triazoles in Gelderland (Table 1). The occurrence of similar isolates in other provinces may have the same reason.

Sensitivity to fenpropimorph of all isolates tested did not differ significantly from each other (Table 3). In addition, in all fields sampled satisfactory mildew control by fenpropimorph was observed (Table 4). Therefore, the EC_{50} and EC_{95} values obtained most probably represent the baseline sensitivity of wheat powdery mildew to fenpropimorph under the test conditions used (Table 3). The same sensitivity of the reference isolates LH and 67 also suggests absence of cross-sensitivity to triazoles and fenpropimorph. This was confirmed in leaf segment tests for a larger number of isolates (Table 5). Lack of cross-sensitivity to triazole EBIs and fenpropimorph in cereal powdery mildews has also been demonstrated by others (Buchenauer et al., 1984; Butters et al., 1984; Lorenz and Pommer, 1984).

A decrease in field performance of EBIs against wheat powdery mildew cannot only be caused by reduced sensitivity to these fungicides but also by unfavourable conditions like low temperatures, use of mildew-sensitive wheat varieties, curative control strategies and intensive fertilization. Such unfavourable conditions may well determine to what extent decreased sensitivity to triazoles reduces disease control. A strategy recommended to counteract further development of resistance by reduction of the selection pressure is the alternating use of fenpropimorph and triazoles. Fenpropimorph is advised as the first mildew spray since it has the best curative action of all EBIs available. Strong curative action is a prerequisite for a fungicide used in disease management systems (EPIPRE). Triazoles are recommended as the second spray, since they extend the green leaf period of the flag leaf, thereby enhancing grain yield (Bayer, 1985). Late application of triazoles is especially relevant for triadimefon since its mildew action seems to be dependent on relatively high temperatures. In order to avoid further increase of selection pressure on mildew by EBIs their application during other phases of growth should be discouraged. Integrated control measures like the use of mildew-resistant varieties (Wolfe, 1984) and moderate use of fertilizers should make allowance for the other conditions which may hamper mildew control.

Acknowledgements

The authors thank Dr L.C. Davidse, Dr J. Dekker, Dr A. Fuchs, Dr H. Scheinpflug, Dr Th. Staub and Dr P. Zobrist for valuable discussions and critically reading the manuscript. Financial support from BASF A.G., Bayer A.G., Ciba Geigy A.G. and Dr. R. Maag A.G. is gratefully acknowledged.

Samenvatting

Variatie in gevoeligheid voor fungiciden die de ergosterolbiosynthese remmen bij tarwemeeldauw

Fungiciden die de ergosterolbiosynthese remmen worden in Nederland sinds 1978 gebruikt bij de bestrijding van tarwemeeldauw *(Erysiphe graminis* f. sp. *tritici)* Meeldauwpopulaties werden getoetst op hun variatie in gevoeligheid voor triadimefon van 1982 tot 1984.

In 1982 bleken isolaten afkomstig uit de provincie Limburg, waar voordien triazolen werden toegepast, minder gevoelig te zijn voor triadimefon dan isolaten uit de provincies Gelderland en Noord-Brabant, waar nog geen triazolen werden gebruikt. In de daaropvolgende jaren werden isolaten met een verminderde gevoeligheid ook in laatstgenoemde provincies en in andere delen van het land gevonden. Deze uitbreiding is gecorreleerd met een toename in het gebruik van triazolen vanaf 1983 in areaal en frequentie. De afname in gevoeligheid kan geheel of gedeeltelijk verantwoordelijk zijn voor de verminderde meeldauwwerking van triazolen die gedurende deze jaren werd waargenomen.

Kruisgevoeligheid werd vastgesteld voor de triazolen triadimefon en propiconazool, maar niet voor triazolen en het morfoline-derivaat fenpropimorf. De werking van dit middel was tegen alle isolaten uit Limburg die in 1984 werden getoetst, gelijk. De meeldauwwerking van fenpropimorf, dat in 1983 werd geïntroduceerd, was normaal. Aanbevolen wordt om verdere resistentie-ontwikkeling tegen te gaan door afwisselend gebruik van fenpropimorf vroeg in het groeiseizoen (mei) en een triazool aan het eind (juni-juli).

References

Bayer, 1985. Bayfidan, een nieuw fungicide in de topklasse. Agro Chemie Koerier, p. 1.

Buchenauer, H., Budde, K., Hellwald, K.-H., Taube, E. & Kirchner, R., 1984. Decreased sensitivity of barley powdery mildew isolates to triazole and related fungicides. Proc. Br. Crop Prot. Conf. 2: 483-488.

Butters, J., Clark, J. & Hollomon, D.W., 1984. Resistance to inhibitors of sterol biosynthesis in barley powdery mildew. Meded. Fac. Land.wet. Rijksuniv. Gent 49: 143-151.

Fletcher, J.T. & Wolfe, M.S., 1981. Insensitivity of *Erysiphe graminis* f. sp. *hordei* to triadimefon, triadimenol and other fungicides. Proc. Br. Crop Prot. Conf. 2: 633-640.

Fuchs, A. & Waard, M.A. de, 1982. Resistance to ergosterol biosynthesis inhibitors. I. Chemistry and phenomenological aspects. In: Dekker, J. & Georgopoulos, S.G. (Eds), Fungicide resistance in crop protection. Pudoc, Wageningen, p. 71-86.

Gilmour, J., 1984. Comparison of some aspects of mildew fungicide use on spring barley in South-East Scotland in 1982 and 1983. Proc. Br. Crop Prot. Conf. 1: 109-114.

Grainger, J., 1947. The ecology of *Erysiphe graminis* D.C. Trans. Br. mycol. Soc. 31: 54-65.

Heaney, S.P., Humphreys, G.J., Hutt, R., Montiel, P. & Jegerings, P.M.F.E., 1984. Sensitivity of barley powdery mildew to systemic fungicides in the U.K. Proc. Br. Crop Prot. Conf. 2: 459-464.

Hunter, T., Brent, K.J. & Carter, G.A., 1984. Effects of fungicide regimes on sensitivity and control of barley mildew. Proc. Br. Crop Prot. Conf. 2: 471-476.
Lorentz, G. & Pommer, E.-H., 1984. Investigations into the sensitivity of wheat powdery mildew populations towards fenpropimorph. Proc. Br. Crop Prot. Conf. 2: 489-493.
Rabbinge, R. & Rijsdijk, F.H., 1983. EPIPRE, a disease and pest management system for winter wheat, taking account of micro-meteorological factors. EPPO Bull. 13: 297-309.
Schepers, H.T.A.M., 1983. Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. Neth. J. Pl. Path. 89: 185-187.
Schepers, H.T.A.M., 1985. Changes over a three-year period in the sensitivity to ergosterol biosynthesis inhibitors of *Sphaerotheca fuliginea* in the Netherlands. Neth. J. Pl. Path. 91: 105-118.
Wolfe, M.S., 1984. Trying to understand and control powdery mildew. Pl. Path. 33: 451-466.
Wolfe, M.S., Minchin, P.N. & Slater, S.E., 1984. Dynamics of triazole sensitivity in barley mildew, nationally and locally. Proc. Br. Crop Prot. Conf. 2: 465-470.