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Sensitivity of populations of *Botrytis cinerea* to triazoles, benomyl and vinclozolin

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Abstract

Sensitivity of field isolates (121) of *Botrytis cinerea* from France (1992), Germany (1979–1992), Israel (1990) and the Netherlands (1970–1989) to the triazoles tebuconazole and triadimenol, the benzimidazole benomyl and the dicarboximide vinclozolin were tested in radial growth experiments. Resistance to benomyl (in 21 to 100% of isolates tested) and vinclozolin (in 25 to 71% of isolates tested) was common in most countries. EC_{50}s (concentrations of fungicides inhibiting radial mycelial growth of *B. cinerea* on B5-agar by 50%) for tebuconazole and triadimenol ranged between 0.01–1.64 and 0.4–32.6 $\mu g\ ml^{-1}$, respectively, and were log-normally distributed. The variation factor (ratio between EC_{50}s of the least and most sensitive isolate tested) amounts 164 and 82 for tebuconazole and triadimenol, respectively. These values are comparable to those for azole fungicides applied in control of other pathogens. Hence, variation in sensitivity to triazoles can probably not explain limited field performance of triazoles towards *B. cinerea*. Isolates from south west Germany (1992) were significantly less sensitive to tebuconazole than isolates collected earlier in Germany, Israel and the Netherlands. Such less sensitive populations may contribute to the limited field performance of DMI fungicides towards *B. cinerea*. The sensitivity of isolates from south west Germany to tebuconazole was similar to that of DMI-resistant mutants generated in the laboratory. These mutants displayed stable resistance with Q-values (ratio between EC_{50} of resistant mutant and wild type isolate) between 5 and 20. Sensitivity of field isolates and laboratory mutants to tebuconazole and triadimenol was correlated.

Introduction

Botrytis cinerea Pers.; Fr., the causal agent of grey mould, is a pathogen with an extensive geographic distribution and host range [Jarvis, 1977]. Chemical control of the fungus is achieved by treatments with protectant, benzimidazole and dicarboximide fungicides. Protectants are not affected by development of resistance because of their multi-site effects, whereas benzimidazoles selected rapidly for highly resistant pathogen populations [Bollen and Scholten, 1971; Schuepp and Lauber, 1977]. Dicarboximides, which generally replaced the benzimidazoles during the late 1970s and early 1980s, also severely suffered from resistance development [Holz, 1979; Lorenz and Eichhorn, 1980; Leroux *et al.*, 1982].

Triazoles, which have been introduced from 1973 onwards, are widely used for disease control in a variety of crops but rarely for control of grey mould [Gullino, 1992; Kataoka, 1992]. These fungicides inhibit sterol 14α-demethylation during synthesis of ergosterol, the main sterol of fungal membranes [Buchenauer, 1987; Vanden Bossche, 1988; Köller, 1992]. This specific mode of action implies a risk of resistance development. Indeed, strains resistant to sterol demethylation inhibitors (DMIs) can be readily isolated under laboratory conditions. However, the risk of resistance development towards DMIs in the field

is considered to be lower than that of benzimidazoles and dicarboximides [Köller and Scheinpflug, 1987; De Waard, 1993], although eroding performance due to resistance development has been reported for a number of pathogens [De Waard, 1994].

Previous studies demonstrated that triazole fungicides inhibit effectively sterol 14α-demethylase activity and *in vitro* mycelial growth of *B. cinerea*, and development of grey mould on tomato leaves and grape berries under growth chamber conditions [Pontzen and Scheinpflug, 1989; Elad, 1992; Stehmann *et al.*, 1994; Stehmann and De Waard, 1995; Stehmann and De Waard, accepted]. However, no definite data were presented to explain their relatively limited field performance towards the pathogen. The aim of the present study is to identify factors involved in the limited field performance of triazole fungicides towards *B. cinerea*. Triazole sensitivity of field isolates of *B. cinerea* from different sites in Europe and Israel were studied in order to determine whether the limited field performance can be attributed to a relatively large variation in sensitivity of the pathogen populations to these fungicides. The experiments were carried out with tebuconazole, a DMI fungicide with high *in vitro* and *vivo* activity towards *B. cinerea*. Sensitivity towards triadimenol was also tested, since *B. cinerea* is a common non-target pathogen in crops treated with this fungicide. For comparison, a limited number of DMI-resistant laboratory mutants were included in radial growth experiments. In addition, isolates were tested for resistance to the benzimidazole benomyl and the dicarboximide vinclozolin.

Materials and methods

Chemicals

Benomyl (Du Pont de Nemours & Co., Wilmington, Del., USA), tebuconazole and triadimenol (Bayer AG, Leverkusen, Germany) and vinclozolin (BASF AG, Ludwigshafen, Germany) were kindly supplied by their respective manufacturers. They were used as pure active ingredients. Gamborg's B5-medium was purchased from DUCHEFA (Haarlem, the Netherlands).

Fungal isolates

Isolate SAS56, obtained from the ascospore progeny of a cross from WS55 (a field isolate from roses) and another undefined parent [Faretra *et al.*, 1988; Faretra *et al.*, 1991], was used as a reference in all experiments. It was kindly supplied by Dr. F. Faretra (University of Bari, Italy). Isolates NF1 to NF10 and SF1 to SF20 were isolated in 1992 from untreated grapes (control plots) in experimental vineyards in northern and southern France, respectively. They were generously provided by Dr. R. Pontzen (Bayer AG, Leverkusen, Germany). Isolates SD1 to SD38 were collected from control plots in experimental vineyards in south west Germany in 1992. Isolates D1 to 15 were isolated from vine (grapes, leaves and stems), lettuce and strawberry in Germany during 1979–1992. Isolates SD1–38 and D3–6, 8, 9 and 11–13 were kindly provided by Dr. R. Pontzen (Bayer AG, Leverkusen, Germany) and isolates D1, 2, 7, 10, 14 and 15 by Dr. G. Lorenz (BASF AG, Ludwigshafen, Germany). Isolates I1 to I30 were isolated during January–March 1990 from tomato and cucumber in commercial plastic greenhouses in Israel [Elad, 1992]. They were generously provided by Dr. Y. Elad (Agricultural Research Organization, Bet Dagan, Israel). Isolates N1 to N7 were isolated during 1970–1989 from gerbera, tomato and roses in commercial greenhouses in the Netherlands [Salinas and Schots, 1994]. They were kindly supplied by Dr. J. Salinas (LMA-IPO, Wageningen, the Netherlands). All isolates are bulk-isolates derived from crops not treated with tebuconazole. However, other DMIs (e.g. triadimenol) were used frequently for control of other diseases in the same or other crops in the same region.

Isolate B3 is a DMI-sensitive strain isolated from tomato in Greece. The monospore isolates G25, G39, G66 and G68 are laboratory-generated mutants with reduced sensitivity to DMIs, obtained by selection with triadimefon (100 μg ml^{-1}) after a 4-h MNNG (10 μg ml^{-1}) treatment (B. N. Ziogas, pers. comm.). They were kindly provided by Dr. B. N. Ziogas (University of Athens, Greece).

Preservation and culture conditions

Isolates were cultured on tomato agar prepared as described by Salinas (1992) supplied with 1.2% technical agar (Oxford, Basingstoke, UK) and grown under near UV-light at 20 °C. Conidia were harvested from 2–3-week-old cultures with 0.1% Tween 20 solution in sterile distilled water. Conidia were separated from mycelium by filtration through sterile glass wool. Conidial concentrations were determined with a haemacytometer.

Mycelium and conidia were harvested from tomato agar cultures with 10% glycerol in sterile distilled water and preserved at −80 °C in Eppendorf vials.

Sensitivity tests

Dose response relationships for inhibition of germination of conidia and mycelial growth by the triazoles tebuconazole and triadimenol were assessed in radial growth experiments on fungicide-amended B5-agar (Gamborg's B5-medium supplied with 1.5% technical agar) using drops (5 μl) of conidial suspensions in sterile water (10^6 ml^{-1}) as inoculum. After incubation in the dark at 20 °C for three days colony diameters were measured. Average diameters of colonies on fungicide-amended agar were calculated as percentages of colony diameters in control treatments. Percentages were plotted against fungicide concentrations on a logarithmic scale and EC_{50}s (concentrations of fungicides inhibiting radial mycelial growth by 50%) were determined by regression analysis of inhibitor response data. Experiments were performed in duplicate. Average EC_{50}s of isolates from different regions were tested for significant differences after log transformation according to the ANOVA method.

Resistance of isolates to benomyl and vinclozolin was determined in radial growth experiments on B5-agar amended with discriminating concentrations (1 to 5 μg ml^{-1}) allowing growth of resistant but fully inhibiting growth of sensitive isolates. Petri dishes were inoculated with drops (5 μl) of conidial suspension in sterile water (10^6 ml^{-1}). After incubation in the dark at 20 °C for three days, colony diameters were assessed. Isolates barely affected in growth by 5 μg ml^{-1} were defined as highly resistant. Isolates affected in growth by 5 μg ml^{-1} but not by 1 μg ml^{-1} were defined as moderately resistant. Isolates slightly affected in growth by 1 μg ml^{-1} were defined as low-resistant.

Results

Sensitivity of field isolates to tebuconazole

The EC_{50} of the reference isolate SAS56 for tebuconazole was 0.16 ± 0.02 μg ml^{-1} (n = 18). Sensitivity distribution of all isolates tested (n = 121) was log-normal in character with an average EC_{50} of 0.38 ± 0.3 μg ml^{-1}. As an example dose-response curves of the reference isolate SAS56 and isolates collected in Germany during 1978–1992 are presented in Fig. 1A. Isolates were arbitrarily classified in categories with EC_{50}s < 0.1 (I), 0.1–0.25 (II), 0.25–0.5 (III), 0.5–1.0 (IV) and > 1.0 μg tebuconazole ml^{-1} (V). The distribution of isolates from different countries over the categories is given in Table 1. Highest sensitivity levels of isolates range from 0.01 (Israel, class I) to 0.16 μg ml^{-1} (south west Germany, class II). Similarly, lowest sensitivity levels of isolates range from 0.3 (the Netherlands, class III) to 1.64 μg ml^{-1} (Germany, class V). Hence, the variation factor (ratio between the highest and lowest EC_{50} found) of the populations tested varies between 2 (the Netherlands) and 62 (Israel) and amounts 164 for all isolates tested. The average EC_{50} determined for the 39 isolates collected in south west Germany during 1992 differed significantly from the average EC_{50}s of the isolates collected in Germany (1979–1981), Israel and the Netherlands. Isolates NF7 from northern France (EC_{50} 1.3 μg ml^{-1}), D12 from Germany (EC_{50} 1.6 μg ml^{-1}) and SD29 from south west Germany (EC_{50} 1.5 μg ml^{-1}) showed the lowest sensitivity to tebuconazole.

Sensitivity of field isolates to triadimenol

The EC_{50} of the reference isolate SAS56 for triadimenol was 2.2 ± 0.4 μg ml^{-1} (n = 18). The sensitivity distribution of all isolates tested (n = 121) was log-normal in character with an average EC_{50} of 4.1 ± 3.7 μg ml^{-1}. As an example dose-response curves of the reference isolate SAS56 and isolates collected in Germany during 1978–1992 are presented in Fig. 1B. Isolates were arbitrarily classified in categories with EC_{50}s < 1.0 (I), 1.0–2.5 (II), 2.5–5.0 (III), 5.0–10.0 (IV) and > 10.0 μg triadimenol ml^{-1} (V). The distribution of isolates from different countries over the categories is given in Table 1. Highest sensitivity levels of isolates range from 0.4 (Israel, class I) to 1.8 μg ml^{-1} (south west Germany, class II). Similarly, lowest sensitivity levels of isolates range from 3.3 (the Netherlands, class III) to 32.6 μg ml^{-1} (Germany, class V). Hence, the variation factor of populations tested varies between 2 (the Netherlands) and 31 (Germany) and amounts 82 for all isolates tested. Average EC_{50}s of the isolates collected in south west Germany and northern France in 1992 differed significantly from the average EC_{50} of isolates collected in the Netherlands during 1970–1989. Isolates NF7 (EC_{50} 18.7 μg ml^{-1}) and D12 (EC_{50} 32.6 μg ml^{-1}) showed the lowest sensitivity to triadimenol.

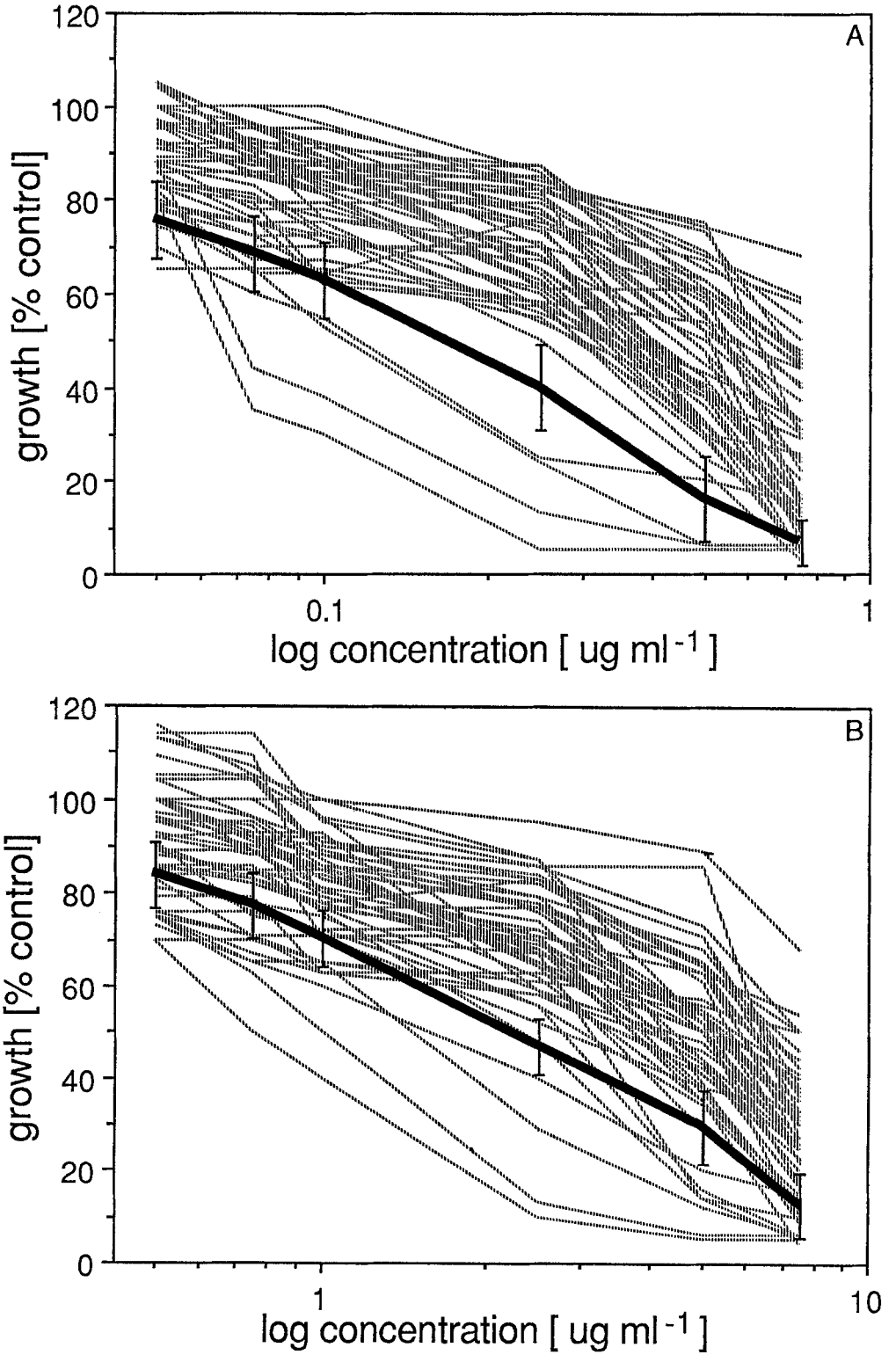

Fig. 1. Effect of tebuconazole (**A**) and triadimenol (**B**) on radial growth of 53 field isolates of *Botrytis cinerea* collected in Germany during 1979–1992 (dotted lines) and reference isolate SAS56 (bold line).

Table 1. Inhibition of radial mycelial growth of *Botrytis cinerea* isolates on B5-agar by tebuconazole and triadimenol

Country	Year of isolation	Number of isolates	Fungicide Teb/Tri	EC_{50} range I[1]	II	III	IV	V	Average EC_{50} ±SD ($\mu g\ ml^{-1}$)	Lowest and highest EC_{50}	VF[3]
France (north)	1992	10	Teb	0[2]	5	3	1	1	0.4 ± 0.3 ab[3]	0.13–1.28	10
			Tri	0	3	4	2	1	5.1 ± 5 B	1.7–18.7	11
France (south)	1992	20	Teb	0	7	10	3	0	0.3 ± 0.2 ab	0.12–0.65	5
			Tri	0	7	11	1	1	3.6 ± 3 AB	1.6–13.7	9
Germany (south west)	1992	39	Teb	0	7	18	9	6	0.6 ± 0.4 b	0.16–1.51	9
			Tri	0	5	21	12	1	4.6 ± 3 B	1.8–13.7	8
Germany	1979–1991	14	Teb	1	6	6	0	1	0.3 ± 0.4 a	0.07–1.64	23
			Tri	0	7	6	0	1	5.0 ± 8 AB	1.1–32.6	30
Israel	1990	31	Teb	2	13	15	1	0	0.3 ± 0.1 a	0.01–0.62	62
			Tri	1	10	13	6	1	3.7 ± 2 AB	0.4–12.4	31
The Netherlands	1970–1989	7	Teb	0	5	2	0	0	0.2 ± 0.07 a	0.13–0.3	2
			Tri	0	5	2	0	0	2.2 ± 0.7 A	1.5–3.3	2
Total	1970–1992	121	Teb	3	42	54	14	8	0.4 ± 0.3	0.01–1.64	164
			Tri	1	37	57	21	5	4.1 ± 3.7	0.4–32.6	82

[1] EC_{50} of tebuconazole (Teb): I < 0.1, II = 0.1–0.25, III = 0.25–0.5, IV = 0.5–1.0 and V > 1.0 $\mu g\ ml^{-1}$, EC_{50} of triadimenol (Tri): I < 1.0, II = 1.0–2.5, III = 2.5–5.0, IV = 5.0–10.0 and V > 10.0 $\mu g\ ml^{-1}$.
[2] Number of isolates.
[3] Variation factor: ratio between highest and lowest EC_{50} determined.
[4] Average EC_{50}s for the same fungicide followed by the same letter(s) do not differ significantly (P = 0.05).

Cross-sensitivity to tebuconazole and triadimenol

Regression analysis of EC_{50}s of tebuconazole and triadimenol for all field isolates tested (y = 0.91304 + 0.741244 x) indicates that sensitivity to tebuconazole and triadimenol are correlated (R^2 = 0.86; Fig. 2). Cross-sensitivity is not absolute, since resistance factors of particular isolates for tebuconazole and triadimenol vary considerably (data not shown).

Resistance of field isolates to benomyl and vinclozolin

In all experiments, mycelial growth of the sensitive reference isolate SAS56 was completely inhibited on agar amended with benomyl (1 $\mu g\ ml^{-1}$) or vinclozolin (1 $\mu g\ ml^{-1}$). Of all 121 isolates tested, 17 were benomyl resistant and vinclozolin sensitive, and 30 isolates were benomyl sensitive and vinclozolin resistant. The highest frequency of benzimidazole (HR) or dicarboximide (LR, MR) resistant isolates was detected in northern France and Germany, respectively (Table 2). Of all isolates tested, 38 were resistant to both benomyl and vinclozolin. Highest frequencies of isolates with double resistance was detected in northern France (*c.* 70%), Germany (*c.* 60%) and the Netherlands (*c.* 60%). In the other regions, between *c.* 10 (southern France, south west Germany) and *c.* 40% (Israel) of the isolates tested were resistant to both fungicides. Isolates D12 and NF7 were resistant to benomyl and vinclozolin and had a relatively low sensitivity to triazoles.

Characterization of laboratory mutants

Isolate B3 with wild-type sensitivity to triazoles was also sensitive to benomyl but had a low degree of resistance towards vinclozolin. EC_{50}s of tebuconazole and triadimenol for inhibition of radial mycelial growth were 0.07 and 1.1 $\mu g\ ml^{-1}$, respectively (Table 3). Of the laboratory mutants, G25 had the lowest degree of sensitivity to tebuconazole with a Q-value (ratio between EC_{50} of resistant mutant and sensitive wild-type) of 13. Isolate G66 had the lowest sensitivity

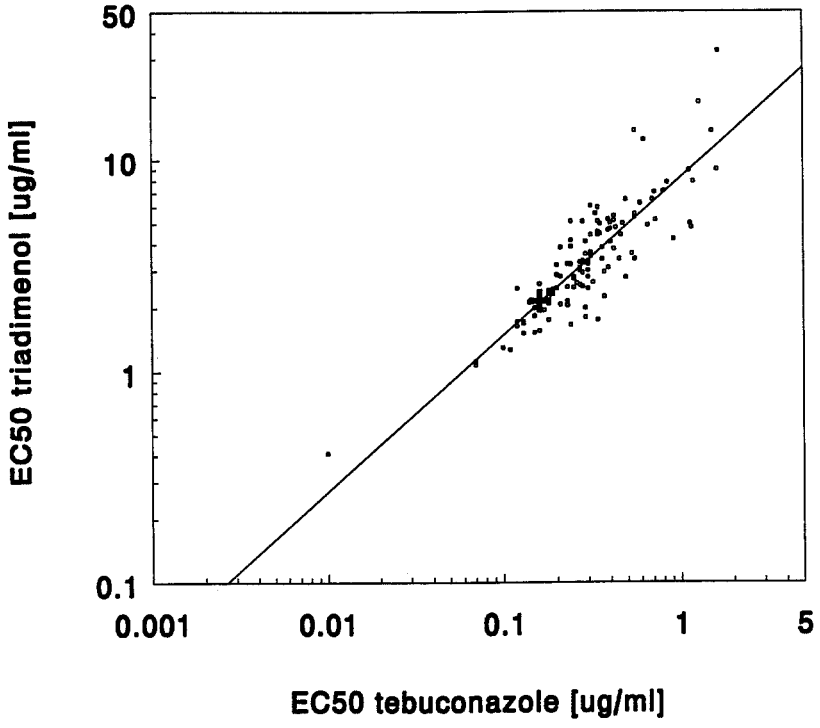

Fig. 2. Correlation between sensitivity of the reference isolate SAS56 (+) and 121 field isolates (■) of *Botrytis cinerea* towards tebuconazole and triadimenol.

towards triadimenol (Q value 19). Sensitivity of B3 and DMI-resistant mutants to benomyl and vinclozolin did not differ significantly.

Discussion

The sensitivity distribution of 121 field isolates of *B. cinerea* from different regions in Europe and Israel was based on EC_{50}s for inhibition of radial mycelial growth by the DMI fungicide tebuconazole. EC_{50}s were log-normally distributed and ranged from 0.01 to 1.64 $\mu g\ ml^{-1}$. The variation factor for all isolates tested was 164, indicating a considerable variation in sensitivity of *B. cinerea* populations towards tebuconazole. Similar variations in sensitivity to the same and other classes of fungicides were demonstrated earlier [Grindle, 1981; Malathrakis, 1989; Elad, 1992]. Experiments performed at BASF AG indicated that sensitivity to DMIs in populations of *B. cinerea* varied regardless whether populations were treated with DMI fungicides or not [G. Lorenz, pers. comm.]. A similar phenomenon was described for *Erysiphe graminis* f. sp. *tritici*, by Schulz *et al.* [1986] who discussed the influence of environmental factors on sensitivity of the pathogen to triazoles. Heterokaryosis is widely accepted as an important source of genetic variation in *B. cinerea* [Lorbeer, 1980; Summers *et al.*, 1984; Grindle, 1987], although its role in natural variability is not well understood and evidence for vegetative incompatibility, which would limit formation of heterokaryons has been published for *B. cinerea* [Beever and Parks, 1993] and other members of the *Sclerotiniaceae* [Kohn *et al.*, 1990].

In order to judge whether the variation in sensitivity to triazoles may account for the limited field performance towards *B. cinerea*, literature data on variations in sensitivity of other pathogens to DMI fungicides were collected (Table 4). These data indicate, that the variation in sensitivity of *B. cinerea* populations to tebuconazole and triadimenol is in the same order of magnitude as that of other pathogens for the same or related DMI fungicides (Table 4). For most pathogens listed, disease control by DMIs was achieved in the periods investigated. Hence, it is concluded that variation in sensitivity of *B. cinerea* populations to triazoles can probably not explain the limited field performance of triazoles in grey-mould control.

Table 2. Inhibition of radial mycelial growth of *Botrytis cinerea* isolates on B5-agar by benomyl and vinclozolin

Country	Year of isolates	Number of isolates	Fungicide Ben/Vin	Sensitivity[1,2] S	LR	MR	HR
France (north)	1992	10	Ben	0	0	0	10
			Vin	3	6	1	0
France (south)	1992	20	Ben	14	0	0	6
			Vin	12	6	2	0
Germany (south west)	1992	39	Ben	30	1	0	8
			Vin	19	5	15	0
Germany	1979–1991	14	Ben	5	1	0	8
			Vin	3	4	6	1
Israel	1990	31	Ben	15	2	0	14
			Vin	14	8	9	0
The Netherlands	1970–1989	7	Ben	2	0	0	5
			Vin	2	4	1	0
Total	1970–1992	121	Ben	66	4	0	51
			Vin	53	33	34	1

[1] Sensitive (S): radial growth fully inhibited at 1 μg ml^{-1} B5-agar; low resistance (LR): radial growth slightly inhibited by 1 μg ml^{-1} B5-agar; Moderate resistance (MR): radial growth inhibited by 5 μg ml^{-1} B5-agar; high resistance (HR): radial growth not inhibited by 5 μg ml^{-1} B5-agar.
[2] Number of isolates.

Table 3. Inhibition of radial mycelial growth of the wild-type isolate B4 and DMI-resistant laboratory mutants of *Botrytis cinerea* by tebuconazole and triadimenol

Isolate	EC_{50} (μg ml^{-1})[1,2] Tebuconazole	Triadimenol
B3	0.07	1.1
G25	0.09 (13)	10.0 (9)
G39	0.4 (6)	5.1 (5)
G66	0.4 (6)	20.5 (19)
G68	0.5 (7)	5.5 (5)

[1] Concentration of fungicide inhibiting radial mycelial growth on B5-agar by 50%.
[2] Between brackets: Q-value (ratio between EC_{50} of resistant mutant and wild-type isolate B3).

The average EC_{50} of tebuconazole for isolates collected in south west Germany during 1992 was significantly higher than average EC_{50}s of populations from Germany (1979–1991), Israel (1990) and the Netherlands (1970–1989). This may be due to selection for resistance to DMI fungicides by treatments with tebuconazole or other DMIs. In France, Germany and Israel tebuconazole (10%) is registered in combination with dichlofluanid (40%) for control of *B. cinerea*, *Uncinula necator* and *Plasmopara viticola* in grape vine. Tebuconazole and other DMIs (e.g. triadimenol) have also been used for control of other pathogens (e.g. powdery mildews) in other crops, which can also be infected by *B. cinerea*. These treatments could have exerted a selection pressure on *B. cinerea*, which may have resulted in reduced sensitivity of the pathogen population to DMI fungicides. Similar conditions led Elad [1992] assume that resistance to DMI fungicides developed in Israel. Such a resistance development may be caused by quantitative shifts in sensitivity of *B. cinerea* populations as described for other pathogens [Schulz *et al.*, 1986; Skylakakis and Hollomon, 1987]. The rapid selection of DMI-resistant isolates of *B. cinerea* under laboratory conditions may corroborate this hypothesis. However,

Table 4. Literature data on the sensitivity distribution to DMIs in populations of plant pathogens

Pathogen	Fungicide	Sensitivity distribution	VF[1]	Reference
Populations not treated with DMIs				
Venturia inaequalis	Flusilazole	0.0006–0.17[2]	283	Smith *et al.*, 1991
Puccinia recondita f. sp. *tritici*	Cyproconazole	0.07–2.0[3]	29	Ohl and Gisi, 1994
Populations treated with DMIs				
Botrytis cinerea	Tebuconazole	0.1–4.0[2]	40	Elad, 1992
Botrytis cinerea	Tebuconazole	0.01–1.6[2]	164	Stehmann and De Waard, this paper
Botrytis cinerea	Triadimenol	0.4–33.0[2]	82	Stehmann and De Waard, this paper
Erysiphe graminis f. sp. *hordei*	Triadimenol	< 0.025–> 0.625[4]	> 25	Fletcher and Wolfe, 1981
Puccinia recondita f. sp. *tritici*	Cyproconazole	0.05–9.0[3]	180	Ohl and Gisi, 1994
Rhizoctonia spp.	Cyproconazole	0.2–14.0[5]	70	Kataria *et al.*, 1991
Rhizoctonia spp.	Triadimenol	1.0–23.0[5]	23	Kataria *et al.*, 1991
Rhynchosporium secalis	Triadimenol	1.0–> 30.0[6]	> 30	Hunter *et al.*, 1986
Pseudocercosporella herpotrichoides	Prochloraz	0.03–0.9[3]	30	Leroux and Marchegay, 1991
	Prochloraz	0.002–0.08[7]	40	Leroux and Marchegay, 1991
Septoria tritici	Flutriafol	0.04–40.0[2]	1000	Hollomon, pers. comm.
Septoria tritici	Cyproconazole	0.01–0.9[2]	90	Gisi and Hermann, 1994
Septoria tritici	Flutriafol	0.04–6.3[2]	158	Gisi and Hermann, 1994
Sphaerotheca fuliginea	Fenarimol	< 0.007–> 0.2[8]	> 32	Schepers, 1985
Uncinula necator	Triadimenol	0.1–10.0[9]	100	Steva *et al.*, 1990
Venturia inaequalis	Flusilazole	0.0007–0.14[2]	200	Smith *et al.*, 1991
Venturia inaequalis, V. piri	Bitertanol	< 0.1–> 7.5[2]	> 75	Creemers *et al.*, 1988
Venturia inaequalis, V. piri	Fenarimol	< 0.25–> 2.5[2]	> 10	Creemers *et al.*, 1988
Venturia nashicola	Triflumizole	< 0.2–> 1.0[2]	> 5	Ishii *et al.*, 1990
Venturia nashicola	Triflumizole	1.56–25.0[6]	16	Ishii *et al.*, 1990
Venturia nashicola	Bitertanol	0.78–25.0[6]	32	Ishii *et al.*, 1990

[1] VF: variation factor (ratio between lowest and highest sensitivity detected).
[2] EC_{50} mycelial growth on agar ($\mu g\ ml^{-1}$).
[3] EC_{50} leaf piece test ($\mu g\ ml^{-1}$).
[4] EC_{50} seed treatment ($g\ kg^{-1}$ seed).
[5] EC_{50} mycelial growth on agar ($\mu g\ ml^{-1}$).
[6] MIC mycelial growth on agar ($\mu g\ ml^{-1}$).
[7] EC_{50} germtube elongation on agar ($\mu g\ ml^{-1}$).
[8] EC_{50} leaf disc test ($\mu g\ ml^{-1}$).
[9] MIC (*in vivo*) in $\mu g\ ml^{-1}$.

it remains unclear why selection for reduced sensitivity would have occurred exclusively in south west Germany. Data on the selection pressure exerted by tebuconazole and other DMIs in the sampling regions are not available, but it seems unlikely that it has been highest in south west Germany. Another explanation for the relatively low sensitivity of the *B. cinerea* population in south west Germany to tebuconazole may be a high local variation in natural sensitivity. Conclusive evidence for both hypotheses is lacking since data on the baseline sensitivity of *B. cinerea* populations in south west Germany and other sampling regions are not available. The facts underline the necessity to establish baseline sensitivities of pathogen populations.

It is suggested, that for isolates with a relatively low *in-vitro* sensitivity to DMIs *in-vivo* sensitivity may be considerably lower than described for the reference isolate SAS56 (Stehmann and De Waard, accepted). This is especially relevant, since field rates of DMIs recommended were assessed to be relatively low for control of grey mould [Stehmann and De Waard, accepted]. Hence, the existence of populations with relatively low sensitive isolates may contribute to the limited field performance of DMI fungicides against

B. cinerea. Results emphasize the need to use reference isolates in screening programmes for candidate fungicides which represent the mean or even the lowest sensitivity of isolates in a pathogen population.

Distribution of isolates tested over the EC_{50} categories designed for tebuconazole and triadimenol was similar, suggesting a positive correlation between sensitivity to tebuconazole and triadimenol. Regression analysis confirms that isolates with reduced sensitivity to tebuconazole are cross-resistant to triadimenol (Fig. 2). However, cross-resistance is not absolute, since resistance factors for tebuconazole and triadimenol may vary considerably. Similar results were published for other pathogens such as *Septoria tritici* and *Erysiphe graminis* f. sp. *tritici* treated with DMIs [De Waard, 1992; Gisi and Hermann, 1994]. Until the mechanism of field resistance to DMIs is not understood, the basis of cross-resistance and its variation will remain unclear. Results indicate that studies on cross-resistance between DMI fungicides should be performed at a population level and not with single isolates.

Since resistance to benzimidazoles and dicarboximides has a qualitative character [Georgopoulos and Skylakakis, 1986], resistance in *B. cinerea* populations to benomyl and vinclozolin was studied with discriminating concentrations (1 or 5 μg ml^{-1}). Frequency of benzimidazole-resistant isolates varied from 21 to 100% in the different sampling regions. The degree of resistance was generally high, although a few low resistant phenotypes were identified. This is ascribed to the presence of distinct alleles (*Mbc1HR* and *Mbc1LR*) at the *Mbc1* locus [Beever and O'Flaherty, 1985; Faretra and Pollastro, 1991]. Between 25 and 60% of the isolates collected at different sites were moderately resistant to vinclozolin. Part of the isolates (32%) was multiple resistant to benomyl and vinclozolin. Resistance to benomyl, triazoles and vinclozolin was not correlated (data not shown). The data confirm that a major part of the *B. cinerea* population in Europe and Israel has developed resistance to both benzimidazoles and dicarboximides, which will seriously hamper grey mould control. Results also emphasize the need for registration of new fungicides for grey mould control.

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