Resistance of *Botrytis cinerea* to Multiple Fungicides in Northern German Small-Fruit Production

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Abstract

Weber, R. W. S. 2011. Resistance of *Botrytis cinerea* to multiple fungicides in Northern German small-fruit production. Plant Dis. 95:1263-1269.

During the vegetation period 2010, 353 isolates of *Botrytis cinerea* from 23 Northern German strawberry, raspberry, highbush blueberry, and redcurrant fields were examined for sensitivity to the benzimidazole derivative thiophanate-methyl and the dicarboximide iprodione, as well as five fungicides currently used against gray mold in Germany. Of all isolates, 40.5% were highly resistant to thiophanate-methyl, 64.0% to iprodione, 45.0% to fenhexamid, 76.8% to trifloxystrobin, 21.5% to boscalid, and 14.7% to cyprodinil. No high resistance to fludioxonil was observed but medium resistance was recorded to fludioxonil as well as cyprodinil (41.1 and 27.2% of all isolates, respec-

tively). In all, 63 isolates were sensitive to all five of the currently registered botryticides whereas 43, 81, 94, 49, and 23 isolates were medium or highly resistant to one, two, three, four, and five fungicides, respectively. Isolates resistant to five fungicides in vitro were capable of causing fruit rot on wounded apple pretreated with any one of the three commercially available products containing fenhexamid, pyraclostrobin plus boscalid, or cyprodinil plus fludioxonil. These results question the sustainability of the current gray mold control strategy relying exclusively on fungicides with specific, single-site modes of action.

Small-fruit production is an important component of Northern German agriculture, covering some 4,000 ha of strawberry (*Fragaria* L.), 100 ha of raspberry (*Rubus idaeus* L.), and 1,500 ha of highbush blueberry (*Vaccinium corymbosum* L.) as well as smaller acreages of redcurrant (*Ribes rubrum* L.), gooseberry (*Ribes uva-crispa* L.), and blackberry (*Rubus* L.). Under the cool and humid regional climatic conditions, several fungicide treatments at bloom are essential in order to control phytopathogenic fungi, notably the gray mold pathogen *Botrytis cinerea* Pers. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) which is now regarded as an aggregate of several morphologically similar but reproductively isolated species (12,13). In February 2007, tolylfluanid, the only available broad-spectrum fungicide, was withdrawn from use by emergency legislation due to concerns about its environmental toxicity. Since then, control of *Botrytis cinerea* in German small-fruit production has been reliant exclusively on compounds with specific, single-site modes of action. Representatives of five different fungicide classes are currently registered for use against *B. cinerea* in Germany. These include the hydroxylanilide compound fenhexamid; anilinopyrimidines (cyprodinil, pyrimethanil, and mepanipyrim); the quinone outside inhibitors (QoIs) pyraclostrobin, trifloxystrobin, and azoxystrobin; the carboxamide compound boscalid, which is a succinate dehydrogenase inhibitor (SDHI); and the phenylpyrrole compound fludioxonil. Three products in widespread current use as botryticides are Teldor (active ingredient [a.i.] fenhexamid; Bayer CropScience, Langenfeld, Germany), Signum (a.i. pyraclostrobin and boscalid; BASF, Ludwigshafen, Germany), and Switch (a.i. cyprodinil and fludioxonil; Syngenta, Maintal, Germany). Furthermore, single applications of anilinopyrimidines as well as QoIs are frequently made in order to control a range of other fungal pathogens during the vegetation period. In view of the high risk of resistance development in *B. cinerea* to these and other single-site fungicides (5,22), the sustainability of the current gray mold control strategy is uncertain.

There are different perceptions of the term “resistance” in the scientific literature. In the current study, an isolate was considered to display high resistance (HR) if the effective fungicide concentration necessary for a 50% inhibition of germ-tube growth (EC_{50}) was higher by a resistance factor (RF) of at least 50 compared with the average EC_{50} value of sensitive isolates (33). However, the precise RF cut-off point may depend on the distribution of EC_{50} values within a population and has to be identified for each fungicide (33). The genetic basis of HR phenotypes is usually a mutation in the gene encoding the putative target protein (8,15,22,23,35). Reduced in planta efficacies against HR strains of *B. cinerea* have been demonstrated for QoI fungicides (15), fenhexamid (37), anilinopyrimidines (27), benzimidazoles (36), and dicarboximides (36).

Isolates of *B. cinerea* showing an RF of 10 to 50 were classified as possessing medium or moderate resistance (MR) for the purposes of the current monitoring (33). Such MR phenotypes may be caused in this pathogen by a genetic mutation (8,35) or by the activity of effective multidrug resistance (MDR) mechanisms, the latter giving rise to resistance factors up to 25 (19). In some instances, reduced field efficacies of fungicides due to MR strains have been suggested (30), although few detailed tests have been performed as yet. Minor reductions of sensitivity ($RF < 10$) have been observed in *B. cinerea* against several fungicides (19,33) but such cases of low resistance were not further considered in the present study.

In viticulture and vegetable production, strains of *B. cinerea* resistant to benzimidazoles (21,29,36) and dicarboximides (16,20,25,26) have been frequently described. Less common are reports on resistance to QoI fungicides (3,15,23), anilinopyrimidines (6,14,28), boscalid (21,23), and fenhexamid (21,31). In the case of fludioxonil, no HR isolates have been detected thus far in the field, although MR strains possessing an effective MDR mechanism may occur (19,21).

Since 2008, Northern German farmers have been voicing concerns about increasing problems with gray mold control, especially in strawberry and raspberry. As a first step toward a large-scale resistance-monitoring program, a simple method based on conidial germination assays to identify MR and HR phenotypes was developed for all five active compounds currently in use against *B. cinerea* (32,33). The dicarboximide iprodione and the benzimidazole derivative thiophanate-methyl were also included because fungicides belonging to these two classes have been heavily used against

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Accepted for publication 29 May 2011.

B. cinerea in Northern German small-fruit production during the periods 1979 to 2009 and 1971 to 1976, respectively. The aims of the current study were to (i) perform resistance monitoring for seven fungicides in *B. cinerea* populations from small-fruit fields of Northern Germany, (ii) characterize individual isolates for resistance to multiple fungicides, and (iii) assess the potential impact of MR and HR phenotypes on gray mold management.

Materials and Methods

Resistance monitoring. Samples were collected from 23 Northern German strawberry, raspberry, blueberry, and redcurrant fields, most of them belonging to two major production areas south of

Fig. 1. A, Map of Northern German sampling sites and frequency of *Botrytis cinerea* isolates sensitive (white areas), medium resistant (hatched areas), and highly resistant (black areas) to B, thiophanate-methyl; C, iprodione; D, fenhexamid; E, trifloxystrobin; F, boscalid; G, cyprodinil; and H, fludioxonil. Strawberry fields are indicated by circles and raspberry, highbush blueberry, and redcurrant by squares.

Oldenburg and southwest of Hamburg (Fig. 1A). Data on the number of fungicide sprays during the growing season preceding sampling were obtained from the fruit farmers (Table 1). A previously described conidial germination method (33) was used as the fungicide resistance assay. In brief, aqueous conidial suspensions were obtained directly from infected sporulating plant material or from pure cultures growing on potato dextrose agar, and 20- μ l aliquots were incubated at 20°C on agar media amended with discriminatory concentrations of fungicides. These were obtained as commercial formulations of thiophanate-methyl (Cercobin FL; BASF), iprodione (Rovral WG; BASF), fenhexamid (Teldor; Bayer CropScience), trifloxystrobin (Flint; Bayer CropScience), boscalid (Cantus; BASF), cyprodinil (Chorus; Syngenta), and fludioxonil (Saphire; Syngenta). The incubation time was 12 to 14 h unless otherwise indicated.

Resistance responses of *B. cinerea* to all seven fungicides were described in detail by Weber and Hahn (33), who produced highly resolved fungicide inhibition curves for isolates with widely differing sensitivities. From these, critical concentrations were identified which enabled the discrimination between sensitivity (S), MR, and HR to each fungicide. In the case of fenhexamid, HR isolates with EC_{50} values > 100 μ g/ml and RFs > 1,000 displayed uninhibited germ-tube growth on 1% malt extract agar (MEA) augmented with fenhexamid at 1 and 50 μ g/ml. In all cases, the Hyd R3 phenotype, known to be caused by a mutation of the target gene (10,22), was confirmed by uninhibited germ-tube growth after 24 h of incubation in tap water agar containing fenhexamid at 10 μ g/ml. Isolates with MR to fludioxonil (EC_{50} = 0.30 to 0.65 μ g/ml, RF = 12 to 25) showed 0 and 50% inhibition of germ-tube growth on MEA with fludioxonil at 0.1 and 10 μ g/ml, respectively. Isolates with MR (EC_{50} = 4.7 to 16, RF = 50 to 178) and HR (EC_{50} = 28 to 436 μ g/ml, RF > 300) phenotypes to thiophanate-methyl were not inhibited at 1 μ g/ml and slightly inhibited (MR) or uninhibited (HR) at 100 μ g/ml. Germ-tube growth of isolates with MR (EC_{50} = 9.2 to 24 μ g/ml, RF = 16 to 42) and HR (EC_{50} = 44 to 96 μ g/ml, RF = 76 to 170) phenotypes to iprodione was <50% (MR) or $\geq$ 50% (HR) at 50 μ g/ml on MEA. Both resistance phenotypes grew uninhibited at 5 μ g/ml. Trifloxystrobin represented QoI fungicides, and HR isolates (EC_{50} > 100 μ g/ml, RF > 1,000) showed uninhibited germ-tube growth at 0.1 and 10 μ g/ml on MEA amended with salicyl hydroxamic acid, an alternative oxidase inhibitor, at 100 μ g/ml (24,34). Sensitivity of *B. cinerea* to the anilinopyrimidine fungicide cyprodinil was assayed on 0.5% sucrose agar. Both MR (EC_{50} = 0.75 to 1.4 μ g/ml, RF = 14 to 27) and HR (EC_{50} = 1.9 to 23 μ g/ml, RF = 36 to 427) isolates showed strong growth with cyprodinil at 1 μ g/ml but were distinguished by the absence (MR) or presence (HR) of growing germ tubes at 25 μ g/ml after 16 h. On 0.5% yeast extract agar augmented with boscalid, HR isolates (EC_{50} = 3.4 to 55 μ g/ml, RF = 50 to 790) displayed uninhibited germ-tube growth at 1 μ g/ml and >25% growth at 50 μ g/ml after 16 h.

In planta tests of fungicide resistance. For each fungicide, two representative isolates with MR or HR phenotypes as well as two S isolates were chosen for inoculation tests on apple ('Jonagold'). Fruit were surface sterilized by immersing them for 10 s in 70% (vol/vol) ethanol, followed by drying in a laminar flow cabinet. Four wounds per apple (2 mm in diameter, 1 mm deep) were created by turning the sterilized tip of a pocket knife under gentle pressure. Fungicides were applied with a hand-held pump sprayer until a continuous surface film was obtained (approximately 0.6 ml/fruit). Concentrations of active ingredients were the same as those applied by fruit farmers for thiophanate-methyl (437 μ g/ml), iprodione (500 μ g/ml), fenhexamid (500 μ g/ml), trifloxystrobin (150 μ g/ml), boscalid (240 μ g/ml), cyprodinil (375 μ g/ml), and fludioxonil (250 μ g/ml). A spray with water was used as a control. After the spray cover had been dried in a laminar flow cabinet, apple fruit were inoculated by placing a 20- μ l drop of conidial suspension (10^6 spores/ml) at the surface of each wound. The diameter of fruit rot was recorded after 7 days of incubation at 20°C. This experiment was performed in triplicate, each repeat comprising five inoculated wounds for each isolate–fungicide combination.

In a similar experiment, the efficacy of the two combination products Signum and Switch was tested using two isolates with multiple resistance (HR against trifloxystrobin, boscalid, and cyprodinil; MR against fludioxonil) and two fully sensitive isolates for comparison. The concentrations were those commonly sprayed by fruit farmers; that is, 0.09% (wt/vol) Signum (containing 67 g of pyraclostrobin and 267 g of boscalid per kilogram) and 0.1% (wt/vol) Switch (375 g of cyprodinil and 250 g of fludioxonil per kilogram).

Data analysis. For each field sampled, the percentage of isolates belonging to different fungicide resistance categories was determined. The computing environment R-project (4) was used for all statistical analyses. An analysis of variance test of arcsin-transformed percentages of lesion diameter on fungicide-treated apple fruit relative to the zero-fungicide control was performed in order to evaluate whether there were significant differences in the effects of fungicides on sensitive and resistant isolates. Averages were separated using the Tukey test (7).

A χ^2 test was performed in order to examine whether resistance to either iprodione or thiophanate-methyl was distributed randomly among isolates classed according to their number of resistances against zero, one, two, three, four, or all five of the currently used fungicides (4).

Results

Resistance monitoring. In 2010, 353 isolates of *B. cinerea* were collected from 23 Northern German strawberry, raspberry, redcurrant, and highbush blueberry fields (Table 1; Fig. 1A) and examined for resistance to seven fungicides. Isolates with MR or HR to thiophanate-methyl were present in all sampling sites but one, representing >25% of the population in most fields (Fig. 1B) and 40.5% of all isolates (Table 2). Similarly, isolates with MR or HR to iprodione were widespread and very common across Northern Germany (Fig. 1C), representing 64.0% of all isolates examined.

Isolates of *B. cinerea* with HR or at least MR were also observed for the other five compounds. In the case of fenhexamid, HR isolates showed an uneven distribution, being abundant in many fields but absent in some others (Fig. 1D). The frequency of HR isolates was higher where fenhexamid had been used during the preceding vegetation period, with 59.3% after two previous applications, 46.9% after one previous application, and 27.5% on fields where

this compound had not been used during the preceding vegetation period. Apparent exceptions to this trend were fields 7 to 9, showing a high proportion of HR isolates without fenhexamid treatments preceding sampling, but all of these older plantations (>7 years) had experienced up to three annual fenhexamid sprays until and including the 2008 season. QoI resistance was even more widespread and abundant (Fig. 1E), being present in 22 of 23 fields and averaging 76.1% of all isolates examined (Table 2). Although boscalid HR isolates were also found across the Northern German plain, their occurrence in individual fields was more variable, ranging from 0 to 80% (Fig. 1F). Isolates with MR to the anilino-pyrimidine compound cyprodinil were found in all fields, with the additional presence of HR isolates in many fields at variable frequencies (Fig. 1G). No isolates with HR to fludioxonil were obtained in the current study, although MR isolates were common in most fields of both major Northern German small-fruit production areas (Fig. 1H).

Multiple fungicide resistance in individual isolates. Of 48 possible combinations of sensitivities to fenhexamid, trifloxystrobin, boscalid (all S or HR), fludioxonil (S or MR), and cyprodinil (S, MR, or HR), 31 were actually observed, albeit at variable frequencies (Table 3). Twelve of the missing combinations concerned the coupling of boscalid resistance with QoI sensitivity, which was never detected. Of 353 isolates, 17.8% were sensitive to all five of the abovementioned compounds; 12.2, 23.0, 26.6, 13.9, and 6.5% were MR or HR to one, two, three, four, and five fungi-

Table 2. Frequency of fungicide-resistant isolates of *Botrytis cinerea* in Northern German small-fruit production areas

Fungicide	Frequency of resistant isolates (%) ^z	
	MR	HR
Thiophanate-methyl	18.7	21.8
Iprodione	34.8	29.2
Fenhexamid	–	45.0
Trifloxystrobin	–	76.8
Boscalid	–	21.5
Cyprodinil	27.2	14.7
Fludioxonil	41.1	–

^z MR = medium resistant, HR = highly resistant (total *n* = 353 isolates).

Table 1. Origin of *Botrytis cinerea* isolates and number of seasonal fungicide applications during the vegetation period preceding sampling

Field	Host	Tissue	Isolates ^z	Sampling date	Number of fungicide applications ^y					
					Year	FH	QoI	Bos	AP	Flu
1	Raspberry	Cane	8	19 April 2010	2009	–	3	1	2	2
2	Blueberry	Fruit	11	5 September 2010	2010	–	1	1	2	2
3	Strawberry, greenhouse	Fruit	20	3 June 2010	2010	–	6	1	4	3
4	Strawberry	Fruit	15	16 June 2010	2010	2	3	1	3	3
5	Blueberry, greenhouse	Fruit	10	18 May 2010	2010	–	2	2	2	2
6	Raspberry	Cane	21	3 May 2010	2009	–	2	2	2	2
7	Raspberry	Fruit	22	13 August 2010	2010	–	2	2	3	3
8	Raspberry	Fruit	29	13 August 2010	2010	–	5	3	3	3
9	Redcurrant	Fruit	13	1 August 2010	2010	–	3	3	3	3
10	Strawberry	Fruit	12	10 June 2010	2010	1	2	1	4	3
11	Strawberry	Fruit	19	9 July 2010	2010	1	2	1	2	2
12	Strawberry	Fruit	13	16 July 2010	2010	1	2	–	3	3
13	Strawberry	Fruit	14	16 July 2010	2010	1	2	–	3	3
14	Strawberry	Fruit	18	16 July 2010	2010	1	2	–	3	3
15	Strawberry	Fruit	12	15 July 2010	2010	1	2	–	3	3
16	Strawberry	Fruit	20	25 June 2010	2010	1	3	1	4	3
17	Strawberry, autumn	Fruit	14	6 September 2010	2010	2	6	3	2	2
18	Strawberry	Fruit	15	7 July 2010	2010	1	2	1	3	2
19	Raspberry	Cane	8	8 December 2010	2010	–	1	1	2	2
20	Blueberry	Fruit	18	5 September 2010	2010	–	2	2	2	2
21	Strawberry, autumn	Fruit	20	18 November 2010	2010	2	4	2	3	3
22	Raspberry	Cane	10	15 November 2010	2010	–	2	1	1	1
23	Redcurrant	Mummy	11	15 November 2010	2010	–	5	1	2	2

^y FH = fenhexamid, QoI = quinone outside inhibitor, Bos = boscalid, AP = anilino-pyrimidines, Flu = fludioxonil.

^z Number of isolates.

cides, respectively. The occurrence of iprodione resistance (MR/HR) was not random within these six categories ($P < 0.001$), rising steeply from 3.2 to 80% in populations with dual resistance and climbing to 100% in populations with quintuple resistance (Fig. 2). Similarly, the distribution of thiophanate-methyl resistance was not random ($P < 0.001$) among isolates exhibiting resistance to multiple fungicides. The frequency of thiophanate-methyl-resistant isolates stayed fairly constant in populations with triple, quadruple, and quintuple resistance (Fig. 2).

Apple inoculation tests. All compounds applied individually provided effective control when fruit was inoculated with sensitive isolates. In contrast, isolates with MR or HR caused disease on fruit pretreated with the respective fungicides (Table 4). Therefore, the resistance phenotypes identified in the conidial germination test were substantiated using inoculation assays on apple. Furthermore, the ability of the combination products Signum and Switch to control *B. cinerea* infections was compromised by isolates with resistance to the constituent active ingredients (Table 5).

Discussion

In the present study, isolates characterized by MR or HR to all seven fungicides were found to be abundant in Northern German small-fruit production areas (Fig. 1). Because both MR and HR isolates displayed a significantly enhanced ability to infect wounded apple pretreated with the respective fungicide (Table 4), and because of unclear or missing distinctions between these two groups in the literature, MR and HR may be considered together for practical purposes. The high frequencies of isolates with MR/HR phenotypes to thiophanate-methyl or iprodione were in line with previous observations for benzimidazoles (up to 90%; 9,20,21,26,28,36) and dicarboximides (up to 77%; 21,26,28,36) in *B. cinerea* populations of horticultural crops and vineyards. Resis-

tance to both fungicide classes may be combined (26) and is known to be stable for several years after termination of use (16,25). In the current work, the frequency of benzimidazole-resistant *B. cinerea* isolates was 40.5% even though this fungicide class had not been used during the vegetation period in regional small-fruit production for the past 34 years.

Fig. 2. Occurrence of medium and high resistance to iprodione (Ipr, hatched bars) and thiophanate-methyl (TM, black bars) in *Botrytis cinerea* isolates with multiple (0 to 5) resistance phenotypes to fenhexamid, trifloxystrobin, boscalid, cyprodinil, and fludioxonil.

Table 3. Resistance profiles to five botryticides (left), and distribution of thiophanate-methyl (TM) and iprodione (Ipr) resistance (right) within these groups

Resistance profile ^y						Number of isolates ^x			
						TM ^S Ipr ^S	TM ^{MR/TM^{HR}} Ipr ^S	TM ^S Ipr ^{MR/Ipr^{HR}}	TM ^{MR/TM^{HR}} Ipr ^{MR/Ipr^{HR}}
FH	TFS	Bos	Cyp	Flu	Total ^z				
S	S	S	S	S	63	57	4	0	2
HR	S	S	S	S	2	1	0	0	1
S	HR	S	S	S	26	18	4	3	1
S	S	S	S	MR	2	1	0	0	1
S	S	S	MR	S	10	6	1	0	3
S	S	S	HR	S	3	2	0	1	0
HR	HR	S	S	S	22	4	1	1	16
HR	S	S	S	MR	5	0	0	0	5
S	HR	HR	S	S	22	2	4	8	8
S	HR	S	S	MR	22	2	0	7	13
S	HR	S	MR	S	7	3	1	2	1
S	HR	S	HR	S	1	1	0	0	0
S	S	S	MR	MR	2	0	0	0	2
HR	HR	HR	S	S	1	1	0	0	0
HR	HR	S	S	MR	33	1	0	16	16
HR	HR	S	MR	S	19	0	0	9	10
HR	HR	S	HR	S	6	0	0	6	0
HR	S	S	MR	MR	2	0	0	0	2
HR	S	S	HR	MR	1	0	0	0	1
S	HR	HR	S	MR	3	0	0	1	2
S	HR	HR	MR	S	12	2	0	3	7
S	HR	HR	HR	S	6	1	0	5	0
S	HR	S	MR	MR	4	1	0	0	3
S	HR	S	HR	MR	7	2	0	1	4
S	HR	HR	HR	MR	2	0	0	2	0
HR	HR	S	MR	MR	27	0	0	9	18
HR	HR	S	HR	MR	13	1	0	11	1
HR	HR	HR	MR	S	2	0	0	0	2
HR	HR	HR	S	MR	5	0	0	1	4
HR	HR	HR	MR	MR	11	0	0	3	8
HR	HR	HR	HR	MR	12	0	0	11	1

^x S = sensitive, MR = medium resistant, HR = highly resistant.

^y FH = fenhexamid, TFS = trifloxystrobin, Bos = boscalid, Cyp = cyprodinil, Flu = fludioxonil.

^z Total number of isolates.

Resistance to the five currently registered botryticides was also found at unexpectedly high frequencies. Matching literature data have been published only for anilinopyrimidines such as cyprodinil (up to 57%; 18,21,26,28), and for QoI fungicides (up to 57%; 1,3,15). In contrast, high levels of resistance to boscalid corresponding to the HR phenotype (RF > 50) have not been reported for *B. cinerea* until 2010 (3,17,21,23). In this context, the current finding of 21.5% boscalid-resistant isolates across a large sampling area such as Northern Germany is notable because sampling was performed during and after the 2010 vegetation period, which was only the second season of use of this fungicide class in German small-fruit production.

In most *B. cinerea* populations from a range of horticultural crops, highly fenhexamid-resistant strains belonging to the Hyd R3 phenotype were either absent altogether (3) or occurred at frequencies below 10% (18,21,28,30), rendering the frequency of 45.1% recorded in the current study exceptionally high. In a previous survey confined to fenhexamid resistance during the 2009 season, 18.5% of all Northern German *B. cinerea* isolates belonged to the Hyd R3 category (31), indicating a growing importance of these HR strains in regional small-fruit production.

No highly fludioxonil-resistant field isolates of *B. cinerea* have been found as yet (3,21,33). However, resistance factors up to 25 may be conferred by the activity of effective MDR mechanisms (19,33). Such MDR strains have been isolated from French and German vineyards (19,21) and are likely to be responsible for MR to cyprodinil and fludioxonil in Northern German small-fruit production areas (33). The abundance (27 to 41%) of MDR isolates as

observed in the present study is cause for concern and is significantly higher than that reported from other German surveys (18,19,21). Further, isolates with MR to fludioxonil and HR to cyprodinil showed a partial resistance to the cyprodinil-fludioxonil combination product Switch in the apple infection test, indicating that the dual resistance in those isolates is of field relevance.

The high frequency of MR and HR isolates as well as frequent applications of the corresponding single-site fungicides by fruit

Table 5. Development of fruit rot by sensitive and resistant *Botrytis cinerea* isolates on wounded apple fruit treated with fungicides Signum and Switch

Isolate ^y	Resistance category ^z	Lesion diameter (%) ^x	
		Signum	Switch
RW.B51-4	TFS ^S Bos ^S Cyp ^S Flu ^S	0	0
RW.10-03a	TFS ^S Bos ^S Cyp ^S Flu ^S	0	0
RW.B53-21	TFS ^{HR} Bos ^{HR} Cyp ^{HR} Flu ^{MR}	91.5 (3.5)	45.0 (20.8)
RW.B54-18	TFS ^{HR} Bos ^{HR} Cyp ^{HR} Flu ^{MR}	85.1 (6.1)	29.7 (11.4)

^x Mean values and standard deviation in parentheses of the lesion diameter on fungicide-treated apple fruit 7 days after inoculation. Signum (a.i. pyraclostrobin, 67 g/kg; boscalid, 267 g/kg) was sprayed at 0.09% (wt/vol) and Switch (a.i. cyprodinil, 375 g/kg; fludioxonil, 250 g/kg) at 0.1% (wt/vol) according to manufacturers' recommendations and regional practice.

^y For details of isolates, see Weber and Hahn (33).

^z Resistance categories: S (sensitive), MR (medium resistant), and HR (highly resistant) to trifloxystrobin (TFS), boscalid (Bos), cyprodinil (Cyp), and fludioxonil (Flu).

Table 4. Development of fruit rot by sensitive and resistant *Botrytis cinerea* isolates on wounded, fungicide-treated apple fruit

Fungicide ^v	Isolate ^w	Resistance ^x	EC ₅₀ (µg/ml) ^y	Lesion diameter (%) ^z
Thiophanate-methyl	RW.08-24	TM ^S	0.0532	15.2 a
	RW.10-03e	TM ^S	0.126	0 a
	RW.B50-8	TM ^{MR}	16.1	75.2 b
	RW.B52-8	TM ^{MR}	6.1	74.0 b
	RW.08-27	TM ^{HR}	>100	95.4 b
	RW.08-18	TM ^{HR}	>100	97.8 b
Iprodione	RW.08-18	Ipr ^S	0.614	0 a
	RW.08-24	Ipr ^S	0.399	0 a
	RW.B42-8	Ipr ^{MR}	23.7	43.4 b
	RW.B43-7	Ipr ^{MR}	16.1	52.5 bc
	RW.B50-21	Ipr ^{HR}	68.7	89.2 cd
	RW.B50-20	Ipr ^{HR}	96.1	75.0 d
Fenhexamid	RW.B62-11	FH ^S	0.153	0 a
	RW.B63-22	FH ^S	0.232	0.1 a
	RW.B53-21	FH ^{HR}	>100	102.0 b
	RW.B54-18	FH ^{HR}	>100	95.7 b
Trifloxystrobin	RW.B50-3	TFS ^S	0.0133	2.8 a
	RW.B46-15	TFS ^S	0.0080	1.5 a
	RW.B62-9	TFS ^{HR}	>100	124.6 b
	RW.10-04a	TFS ^{HR}	>100	105.6 b
Boscalid	RW.B50-3	Bos ^S	0.0787	0 a
	RW.B41.19	Bos ^S	0.0753	0 a
	RW.10-04a	Bos ^{HR}	12.5	67.9 b
	RW.B54-2	Bos ^{HR}	13.6	81.1 b
Cyprodinil	RW.10-03e	Cyp ^S	0.0499	0 a
	RW.B50-22	Cyp ^S	0.0344	0 a
	RW.B43-7	Cyp ^{MR}	1.11	65.7 b
	RW.B50-20	Cyp ^{MR}	1.05	84.6 c
	RW.B54-18	Cyp ^{HR}	3.95	74.3 bc
	RW.B53-21	Cyp ^{HR}	22.7	79.2 bc
Fludioxonil	RW.08-18	Flu ^S	0.0140	0 a
	RW.08-24	Flu ^S	0.0133	0 a
	RW.B53-21	Flu ^{MR}	0.465	29.4 b
	RW.B53-8	Flu ^{MR}	0.647	25.0 b

^v Concentrations of active ingredients were those sprayed by regional farmers as single or combination products: thiophanate-methyl (437 µg/ml), iprodione (500 µg/ml), fenhexamid (500 µg/ml), trifloxystrobin (150 µg/ml), boscalid (240 µg/ml), cyprodinil (375 µg/ml), and fludioxonil (250 µg/ml).

^w For details of isolates, see Weber and Hahn (33).

^x Resistance categories = sensitive (S), moderately resistant (MR), or highly resistant (HR) to thiophanate-methyl (TM), iprodione (Ipr), fenhexamid (FH), trifloxystrobin (TFS), boscalid (Bos), cyprodinil (Cyp), or fludioxonil (Flu).

^y Data from Weber and Hahn (33). EC₅₀ values are the effective fungicide concentrations at which germ-tube growth was reduced by 50%.

^z Mean values of the lesion diameter on fungicide-treated apple fruit 7 days after inoculation. Values followed by the same letter within the same fungicide treatment were not significantly different according to the Tukey test.

farmers strongly suggest that a selection for resistance has taken place in Northern German small-fruit production areas since the introduction of fenhexamid (1999), QoI fungicides (2001), boscalid (2009), anilinopyrimidines (1999), and fludioxonil (1999) for the control of *B. cinerea*. Resistant populations have probably built up over several years but, unfortunately, spray schedules were available only for the vegetation period preceding sampling (Table 1); therefore, more precise correlations between number of treatments and frequency of resistant isolates could not be drawn. However, it is noteworthy that isolates with MR or HR to all five current botryticides were collected from only a few fields; this is, sites 3 (2 times), 7 (2 times), 9 (1 times), 17 (3 times), and 21 (15 times). All of these fields were among those receiving the most fungicide treatments, and this was particularly the case with the autumn strawberry fields 17 and 21 (Table 1). Specific surveys have been initiated in order to correlate the cumulative number of fungicide applications over several years with the occurrence of resistant strains.

In contrast to strawberry fields, which are turned over after two to three harvests in Northern Germany, raspberry fields are commonly managed for more than 10 years and, therefore, are suitable for long-term studies of the dynamics of fungicide resistance development. Forced selection experiments (5) are under way to explore this issue in *B. cinerea* under regional conditions. Conversely, long-term surveys are being conducted to monitor the fate of resistant isolates in fields where the use of a fungicide has been discontinued. In the case of fenhexamid, no decline in Hyd R3 frequency has been observed as yet in geographically isolated raspberry fields where this fungicide was last used in spring 2008 (Table 1, fields 7 and 8; R. W. S. Weber, unpublished data). This persistence of fenhexamid-resistant strains may be a result of competitive fitness, as has been shown to be the case for resistance to benzimidazoles, iprodione, and anilinopyrimidines (2,16,20,25; this article).

A 25% share of HR or even MR isolates in a field population may be sufficient to compromise the efficacy of a fungicide (11,30). In the current study, this share was reached or exceeded by *B. cinerea* populations in 22 of 23 fields for at least one fungicide, indicating that all of the products examined here are potentially under threat from loss of efficacy. Regional small-fruit farmers have already reported increasing problems in regard to gray mold control before harvest and during storage. More studies are needed to quantify the effect of HR and especially MR in *B. cinerea* populations on the efficacy of fungicides under controlled conditions.

To this author's best knowledge, no previous report has described the occurrence of resistances against so many different fungicide classes at such high frequencies in individual isolates or populations of *B. cinerea*. Future prospects for successful chemical control of *B. cinerea* in Northern Germany are uncertain in light of the documented resistance of a relatively large percentage of isolates to multiple chemical classes of fungicides. Resistance management is further hampered by the lack of broad-spectrum protective fungicides such as tolylfluanid, and by additional pressure imposed by food retailers' demands for nearly residue-free produce. Therefore, gray mold control will have to be achieved with reduced fungicide spray programs accompanied by site-specific monitoring of the resistance prior to bloom, when botryticides are routinely applied. There is room for improvement in aspects of cultivation and phytosanitation (e.g., by removing infected raspberry canes in winter or fruit with gray mold during harvest, by avoiding long periods of leaf wetness during irrigation, or by exercising moderation in nitrogen fertilization).

Acknowledgments

I thank regional small-fruit consultants A.-P. Entrop, K. Loock, A. Nordmann, and R. Faby for help with sampling; J. Wiechers for technical assistance; D. Zabel for useful advice concerning data analysis; and two anonymous reviewers and the assigned senior editor for constructive suggestions for improvement.

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