Long-Term Monitoring for Resistance of *Botryotinia fuckeliana* to Anilinopyrimidine, Phenylpyrrole, and Hydroxyanilide Fungicides in Switzerland

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ABSTRACT

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In Switzerland, the use of phenylpyrrole, anilinopyrimidine, and hydroxyanilide fungicides for control of *Botryotinia fuckeliana,* causal agent of gray mold in grapes, has been restricted to one treatment per fungicide class per year as part of an anti-resistance strategy. Resistance development in *B. fuckeliana* was monitored from 1995 to 2001 for the anilinopyrimidine cyprodinil and the phenylpyrrole fludioxonil and from 1997 to 2001 for the hydroxyanilide fenhexamid in experimental vineyards in Richterswil and Stäfa, Switzerland. In total, over 2,400 field isolates were tested. In 1996, the first case of field resistance to anilinopyrimidines was encountered in Richterswil. Efficacy of the anilinopyrimidine cyprodinil decreased significantly, and 54% of the isolates were resistant to anilinopyrimidines. During 7 years of monitoring, one field isolate was found that showed a slightly decreased sensitivity to the phenylpyrrole fludioxonil. Resistance to the hydroxyanilide fenhexamid was not found in 1997 and 1998. From 1999 to 2001, the level of fenhexamid-resistant isolates increased to 100% in Stäfa. The analysis of monitoring and efficacy data showed that subpopulations of *B. fuckeliana* resistant to cyprodinil and fenhexamid have increased significantly; however, the efficacy of a mixture of fludioxonil and cyprodinil and of fenhexamid alone was still effective. The objective of this study was to initiate long-term monitoring in order to establish an early resistance-detection system as a tool to assess the effectiveness of the anti-resistance strategy used in Switzerland.

Botryotinia fuckeliana (de Bary) Whetzel, anamorph *Botrytis cinerea* Pers., causes severe damage in vineyards to yield as well as to quality of the grapes at harvest (26). Three groups of fungicides with different modes of action have been used recently to control gray mold, the disease caused by this pathogen (18).

The anilinopyrimidines were registered in Switzerland in 1995 to control gray mold in grapes. A dual mode of action has been described involving the inhibition of methionine biosynthesis (25) and the inhibition of hydrolytic enzyme secretion (23). The anilinopyrimidines inhibit germ tube elongation and mycelial growth. Hilber and Schüepp (12), Hilber and Hilber-Bodmer (10), and Forster and Staub (5) reported that intensive use of anilinopyrimidines may result in reduced sensitivity and loss of efficacy. Recently, a reduction in sensitivity to anilinopyrimidines in *B. fuckeliana* strains was also reported from France (21).

Fludioxonil's structure is related to pyrrolnitrin, an antibiotic produced by several species of bacteria (24). The mode of action of this phenylpyrrole is not fully understood. Jespers and de Waard (14) hypothesized that the phenylpyrrole fenpiclonil inhibits the transport-associated phosphorylation of glucose. Fludioxonil inhibits germination and induces an alteration of germ tubes (8,22). A mixture of the active ingredients fludioxonil, a phenylpyrrole, and cyprodinil, an anilinopyrimidine (Switch), was registered in 1995 in Switzerland. Pathogen resistance to phenylpyrroles has not been demonstrated in the field to date. However, in the laboratory, isolates with cross resistance between phenylpyrroles and dicarboximide fungicides could be produced (8,10,30). Leroux et al. (22) reported that some field isolates showed a slightly decreased sensitivity to anilinopyrimidines and phenylpyrroles in in vitro conidial germination tests.

In 1999, fenhexamid (Teldor), a hydroxyanilide fungicide (16), was registered in Switzerland. It inhibits germ tube formation and mycelial growth of *B. fuckeliana*. Although its mode of action is not understood, several modes of action that were excluded were the inhibition of pectinases, fungal respiration, and mitochondrial transport of electrons (28). The baseline population of *B. fuckeliana* contains a small proportion of isolates with reduced sensitivity to fenhexamid in vitro (29). In France, few isolates with reduced sensitivity in vitro have been reported (20). Cross resistance to other fungicides has not been described.

In the last 25 years, *B. fuckeliana* developed resistance to virtually all the specific fungicides used to control gray mold. Field resistance to benzimidazoles, phenylcarbamates, and dicarboximides was detected shortly after their introduction (17). Therefore, an anti-resistance strategy was recently introduced in Switzerland for the current fungicides (2,26). This study was initiated as a long-term monitoring program for resistance of *B. fuckeliana* to the three classes of modern fungicides, the phenylpyrrole fludioxonil, the anilinopyrimidine cyprodinil, and the hydroxyanilide fenhexamid. This information will be useful for establishing an early resistance detection system and to assess the effectiveness of the anti-resistance strategy imposed in Switzerland.

MATERIALS AND METHODS

Fungicide active ingredients and formulated products. Technical grade cyprodinil (anilinopyrimidine class) and fludioxonil (phenylpyrrole class) (Syngenta, Basel, Switzerland) and fenhexamid (hydroxyanilide class) (Bayer, Monheim, Germany) were used in all laboratory tests.

Switch (Syngenta) is composed of 37.5% cyprodinil a.i. and 25% fludioxonil a.i. Teldor (Bayer) is composed of fenhexamid 50% a.i. In the spraying program, Switch was used at a rate of 1.2 kg ha^{-1} (0.1%), while Teldor was applied at a dose of 1.5 kg ha^{-1} (0.125%). All field experiments were conducted using formulated products.

Media. The following media were used: water agar supplemented with antibiotics (agar 15 g $liter^{-1}$ distilled water, streptomycin sulfate and tetracycline each 100 μg ml^{-1}); malt agar (malt extract 15 g $liter^{-1}$, agar 15 g $liter^{-1}$ distilled water); synthetic medium (7), modified according to Hilber and Schüepp (12); pea agar (160 g homogenized frozen green peas $liter^{-1}$, 5 g saccharose $liter^{-1}$, 15 g agar $liter^{-1}$ distilled water, pH was adjusted to 6.0 using HCl). Fludioxonil and fenhexamid were used at the discriminatory dose of 0.1 μg ml^{-1}, and cyprodinil at 0.03 μg ml^{-1}. Fungicide sensitivity categories (sensitive and resistant) were defined according to the discriminatory doses that differentiate resistant from sensitive isolates as follows: cyprodinil resistant ($ED_{50} \geq 0.03$ μg ml^{-1}); fludioxonil resistant ($ED_{50} \geq 0.1$ μg ml^{-1}) and fen-

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hexamid resistant ($ED_{50} \geq 0.1$ µg ml^{-1}). Fludioxonil and cyprodinil were dissolved in 10 ml of ethanol or acetone, respectively, and mixed with 1 liter of malt agar or synthetic medium, respectively. Fenhexamid was dissolved in 1 ml of acetone and mixed with 1 liter of malt agar medium. In the control plates, the final ethanol and acetone concentration did not exceed 1% (vol/vol).

Field plots and spray programs. Monitoring was performed in Richterswil plot I (experimental vineyard ca. 30 km east of Zürich, Switzerland, on the south shore of Lake Zürich) and in Stäfa plots II and III (experimental vineyard ca. 30 km east of Zürich, Switzerland, on the north shore of Lake Zürich). A spraying program with seven to eight treatments per year against powdery and downy mildew was applied uniformly to all plots with an axial fan sprayer following the official Swiss recommendations for integrated production in viticulture (27). Fungicides for control of *B. fuckeliana* were sprayed with a knapsack atomizer (300 liter ha^{-1}) at bunch closure *(77)* and at veraison *(81)*. Fungicides were applied in a randomized plot design with four replications. Each replication consisted of at least 25 vine plants.

In experimental plot I in Richterswil (1,600 m^2), *Vitis vinifera* var. Räuschling and var. Pinot noir, grafted on rootstock 3309 and 5 C, respectively, were planted in 1983 with a planting density 2 × 0.9 m. From 1995 to 2001, cyprodinil was applied in a mixture with the phenylpyrrole fludioxonil once or twice per year (Tables 1 and 2). Controls were treated with a combination of Folpet (Phthalimidderivate class) and copper following the official Swiss recommendations for integrated production in viticulture (27).

In experimental plots II and III in Stäfa (5,000 m^2), *V. vinifera* var. Pinot noir (clone 10/5) (plot II) and var. Müller-Thurgau (plot III), grafted on rootstock 5 C, were planted in 1990 and 1994, respectively, with a planting density 2 × 0.9 m. Since 1995, one or two treatments, respectively, with Switch were applied every year in plot II (Tables 1 and 2). Controls (included in the random block design) were treated with Folpet–Cu. Since 1997, plot III was sprayed with Teldor (fenhexamid) once or twice, respectively, per year.

Infection of gray mold on bunches was assessed at harvest. Disease severity (=measure of damage done by B. fuckeliana in percent infected berries per bunch) and incidence (=percent infected bunches) were both assessed from 50 to 100 bunches per replicate. Control plots and fungicide treated plots were assessed. The efficacy of the fungicide was calculated according to Abbott (1) with respect to disease severity: Efficacy of treatment = 100 – (100 * mean severity$_{\text{treated grapes}}$/mean severity$_{\text{untreated control grapes}}$).

Monitoring procedure. The monitoring was performed following the Fungicide Resistance Action Committee (FRAC) methods for resistance monitoring (3,11) with the following modifications: Diseased berries were collected randomly per treatment, in autumn shortly before harvest (sample sizes are shown in Tables 1 to 3). Grape berries with sporulating *B. fuckeliana* were picked individually and put into petri dishes (one berry per dish). In the laboratory, conidia were isolated in order to obtain one isolate per lesion from each berry. Conidia were transferred to water agar plates amended with antibiotics, followed by a transfer onto malt agar plates and then to pea agar slants. Pea agar slants were incubated for 10 days at 18°C with a cycle of 12 h black light/12 h darkness. To assess fungicide sensitivity, inhibition of mycelial growth was tested as follows: Agar disks (8 mm diameter) were cut from malt agar or synthetic medium, respectively. The agar disks were incubated with 20 µl of conidia suspension (10^5 conidia ml^{-1}; prepared from the pea agar slants) and incubated for 17 h at 20°C in the dark. These disks were then placed upside-down onto plates containing either malt agar or synthetic medium without fungicide or with fludioxonil, fenhexamid, or cyprodinil. For the monitoring tests, one discriminatory dose was chosen for each fungicide as described above. Isolates were

Table 1. Monitoring for resistance of *Botryotinia fuckeliana* to cyprodinil ($ED_{50} \geq 0.03$ µg ml^{-1}) and to fludioxonil ($ED_{50} \geq 0.1$ µg ml^{-1}) in a vineyard treated with the mixture of fludioxonil and cyprodinil (Switch) at bunch closure and veraison in Richterswil and in Stäfa, Switzerland

			Cyprodinil			Fludioxonil				
Year	AP[a]	n[b]	$ED_{50} \geq 0.03$ µg ml^{-1}	FR[c]	*P*[d]	$ED_{50} \geq 0.1$ µg ml^{-1}	FR[e]	*P*[d]	Eff[f]	Sev[g]
Baseline[h]	0	140	0			0				
Richterswil plot I										
1995	8	20	1	5	0.04	0	0	1	nd[i]	nd
1996	10	39	21	54	0	0	0	1	nd	nd
1997	12	30	12	40	0	0	0	1	65	9
1998	14	50	19	38	0	0	0	1	85	13
1999	16	50	9	18	0	0	0	1	90	5
2000	18	18	2	11	0	0	0	1	91	14
2001	20	50	11	22	0	0	0	1	91	46
Stäfa plot II										
1996	4	20	0	0	1	0	0	1	98	32
1997	6	11	2	18	0	0	0	1	100	9
1998	8	20	6	30	0	1	5	0.04	97	31
1999	10	1	1	100[j]	nd	0	0	1	99	11
2000	12	30	5	17	0	0	0	1	80	39
2001	14	50	42	84	0	0	0	1	73	76
Richterswil winter monitoring[k]										
1997	8	90	2	2	0.05	0	0	1		
1998	10	97	1	1	0.18	0	0	1		
1999	12	175	4	2	0.02	0	0	1		

[a] Cumulative number of anilinopyrimidine treatments.
[b] Number of isolates tested.
[c] Frequency of anilinopyrimidine-resistant isolates in percentage.
[d] Comparison of baseline counts of isolates classified as sensitive or resistant with respective counts obtained for the populations specified (log-linear models according to Köller et al. [15]).
[e] Frequency of fludioxonil (phenylpyrrole class) resistant isolates in percentage.
[f] Efficacy data calculated according to Abbott (1) with respect to disease severity.
[g] Disease severity in percentage.
[h] Baseline from Hilber and Hilber-Bodmer (9).
[i] Not determined.
[j] Only one diseased berry found in the vineyard.
[k] Winter monitoring was done on the whole vineyard in Richterswil, which included the experimental plot and all surrounding vines.

considered resistant when, compared with the control growth on agar not supplemented with fungicides, their relative growth on fungicide amended agar was ≥50%. (The relative growth of sensitive isolates was <50%.) All cultures were incubated for 3 days at 20°C. The mean colony diameter minus the diameter of the inoculation disk was measured and expressed as the percentage of the mean colony diameter of the untreated control. Each test was performed three times.

To assess the fitness of resistant subpopulations, the ability to overwinter on grape wood canes was studied in Richterswil from February 1997 to 1999. Pieces, 5 cm long, were cut randomly from canes of the previous year and incubated for 2 to 5 days at 20°C in a sealed petri dish containing a wet cotton plug to induce sporulation of *B. fuckeliana*. Conidia from sporulating *B. fuckeliana* isolates were collected from each cane and processed as described above for isolates from grape berries. In total, fungicide sensitivity of 362 randomly collected isolates from the whole vineyard, which included the experimental plots and surrounding plants, was tested. Isolates were grouped into the categories sensitive and resistant according to their growth rate at the discriminatory dose of the respective fungicide.

Data analysis. Categorical fungicide sensitivity data obtained for different populations were compared by fitting log linear models supported by JMP (SAS Institute, Cary, NC) with numbers of isolates grouped into the categories fungicide sensitive and fungicide resistant. The model assumes that sensitivity categories are multinomially distributed within vineyards, and it evaluates the homogeneity of the distributions, with nonhomogeneity indicating differences in distribution among populations (15). Cyprodinil and fludioxonil baseline data published by Hilber and Schüepp (12) and Hilber and Hilber-Bodmer (10) were used. Fenhexamid baseline data were obtained in Stäfa in 1997.

RESULTS

From Richterswil plot I treated with the combination of cyprodinil and fludioxonil (Switch) twice a year at bunch closure *(77)* and at veraison *(81)*, a total of 257 isolates of *B. fuckeliana* were tested for fludioxonil and cyprodinil sensitivity between 1995 and 2001 (Table 1). In 1995, the level of resistance to cyprodinil was 5%. In 1996, it increased to 54%. From 1997 on, it decreased every year, reaching 11% in 2000. In 2001, however, the level increased again to 22%. The frequencies of these anilinopyrimidine-resistant isolates were significantly different from the baseline in all of the years tested ($P < 0.05$). No fludioxonil-resistant isolates were found.

From 1998 to 1999 and in 2001, a subplot within Richterswil plot I was treated with Switch once per year and compared with the plot treated twice per year. In total, 105 isolates of *B. fuckeliana* were tested from the population treated once per year and 150 isolates were tested from the population treated twice per year (Table 2). The population treated once per year showed a lower level of cyprodinil resistance than the population treated twice per year (between 0 and 8% compared with 18 to 38%). The difference was significant in the years 1998 and 1999 ($P < 0.05$). In 2001, the same experiment was repeated in Stäfa plot II (Table 2). Fifty isolates each were tested from the populations treated once or twice with Switch. The population treated with Switch once per year showed a significantly lower level of cyprodinil resistance (26%) compared with the population treated twice per year with Switch (84%, $P < 0.05$) (Table 2). This confirmed the results obtained in the Richterswil plot.

In 1999, the sensitivity of 40 isolates from Richterswil was tested against cyprodinil and fenhexamid (data not shown). Two isolates were resistant to both fungicides. In 2001, 4 out of 50 isolates from Stäfa were double resistant to cyprodinil and fenhexamid (data not shown).

Table 3. Monitoring for resistance of *Botryotinia fuckeliana* to fenhexamid (Teldor) ($ED_{50} \geq 0.1$ µg ml^{-1}) in an experimental vineyard in Stäfa, Switzerland, treated with Teldor twice per year at bunch closure and veraison

Year	Treat[a]	n[b]	Fenhexamid $ED_{50} \geq 0.1$ µg ml^{-1}	FR[c]	*P* [d]	Eff [e]	Sev[f]
Baseline	0	20	0				
Stäfa plot III							
1997	2	20	0	0	1	100	4
1998	4	20	0	0	1	91	26
1999	6	23	4	17	0.02	92	10
2000	8	25	3	12	0.06	98	26
2001	10	50	50	100	0	72	48

[a] Cumulative number of fenhexamid (hydroxyanilide class) treatments.
[b] Number of isolates tested.
[c] Frequency of fenhexamid-resistant isolates in percentage.
[d] Comparison of baseline counts of isolates classified as sensitive or resistant with respective counts obtained for the populations specified (log-linear models according to Köller et al. [15]).
[e] Efficacy data collected in the field before harvest calculated according to Abbott (1) with respect to disease severity.
[f] Disease severity in percentage.

Table 2. Monitoring for resistance of *Botryotinia fuckeliana* to cyprodinil ($ED_{50} \geq 0.03$ µg ml^{-1}) in two experimental vineyards in Richterswil and in Stäfa, Switzerland, treated with Switch (mixture of fludioxonil and cyprodinil) twice per year at bunch closure and veraison versus once per year at bunch closure or veraison

	Two sprays per year[a]					One spray per year[b]					
Year	n[c]	Cyprodinil $ED_{50} \geq 0.03$ µg ml^{-1}	FR[d]	Eff [e]	Sev[f]	n[c]	Cyprodinil $ED_{50} \geq 0.03$ µg ml^{-1}	FR[d]	Eff [e]	Sev[f]	*P* [g]
Richterswil plot I											
1998	50	19	38	85	13	40	0	0	80	13	0.00
1999	50	9	18	90	5	40	0	0	90	5	0.00
2001	50	11	22	91	46	25	2	8	90	46	0.11
Stäfa plot II											
2001	50	42	84	73	76	50	13	26	78	76	0.00

[a] Mixture of cyprodinil (anilinopyrimidine class) and fludioxonil (phenylpyrrole class) (Switch) sprayed twice per year at bunch closure and veraison (*77* and *81*).
[b] Mixture of cyprodinil and fludioxonil (Switch) sprayed once per year at bunch closure or at veraison (*77* or *81*).
[c] Number of isolates tested.
[d] Frequency cyprodinil-resistant isolates in percentage.
[e] Efficacy data collected in the field before harvest calculated according to Abbott (1) with respect to disease severity.
[f] Disease severity in percentage.
[g] Comparison of counts of isolates classified as sensitive or resistant from population sprayed twice with respective counts obtained for the populations sprayed once (log-linear models according to Köller et al. [15]).

From Stäfa plot II treated with Switch twice a year at bunch closure *(77)* and at veraison *(81)*, 132 isolates were tested for fludioxonil and cyprodinil sensitivity between 1996 and 2001 (Table 1). In 1996, no cyprodinil-resistant isolates were found. The level of resistance increased to 18% in 1997 and 30% in 1998. In 1999, the disease incidence was low, with only one diseased berry found in the entire plot. This isolate was resistant to cyprodinil. In 2000, the level of cyprodinil resistance decreased to 17%. However, in 2001, 84% of the isolates tested were resistant (Table 1). In the years 1997 to 2001, the frequencies of anilinopyrimidine-resistant isolates were significantly different from the baseline ($P < 0.05$). In plot II treated with Switch twice a year, one isolate with low resistance to fludioxonil was found in 1998.

From the Stäfa plot III (Table 3) treated with fenhexamid (Teldor) at bunch closure *(77)* and at veraison *(81)*, 138 isolates were tested between 1997 and 2001 for sensitivity to fenhexamid. No resistant isolates were found from 1997 to 1998. In 1999, 17% of the isolates were resistant to fenhexamid. In 2000, the resistance level was 12%, but in 2001 100% of the isolates were resistant to fenhexamid. In the years 1999 and 2001, frequencies of fenhexamid-resistant isolates were significantly different from the baseline ($P < 0.05$).

Winter monitoring. The overwintering ability of resistant isolates was monitored in the entire 1.5-ha Richterswil vineyard. Between 1997 and 1999, 362 isolates were tested for sensitivity to fludioxonil and cyprodinil (Table 1). A low and constant level of resistance to cyprodinil in isolates survived the winter period on canes. In February 1997, 2% of the isolates sampled from wood were resistant to cyprodinil. Compared to the 54% resistance on grape samples 4 months earlier, a significant reduction of resistance was found. Only one resistant isolate was also found in a surrounding plot. In 1998, one cyprodinil-resistant isolate was found in our experimental plot. In 1999, no resistant isolates were found in the experimental plot, whereas four resistant isolates were detected in the surrounding plots. All resistant isolates detected outside the experimental plot were found in the same area, which corresponds to the main orientation of the wind. The frequency of anilinopyrimidine-resistant isolates collected during the winter monitoring was significantly different from the baseline in 1997 and 1999 ($P \leq 0.05$). All isolates tested were sensitive to fludioxonil.

Efficacy data. Efficacy data are summarized in the tables. Both strategies, application of Switch at bunch closure *(77)* and/or at veraison *(81)*, were compared. The efficacy of Switch in the field in 1997 was 65% (Table 1). In the following years (1998 to 2001), efficacy ranged between 85 and 91%. In Stäfa, the efficacy of Switch in plot II was greater than 97% in the years 1996 to 1999. It decreased, however, in the following 2 years, reaching 73% in 2001. In 2001, disease severity was high (76%) (Table 1). The efficacy of Teldor in plot III was greater than 90% in the years 1997 to 2000 but decreased to 72% (Table 3) in 2001.

DISCUSSION

In Switzerland, the use of modern fungicides with high activity against *B. fuckeliana* has been restricted due to the implementation of a stringent anti-resistance strategy (2,3,9,26,27). In grapes, the strategy has consisted of the integration of three classes of fungicides with different modes of action and the limitation to one application per class per year. In two locations in Switzerland, Richterswil and Stäfa, a long-term monitoring program for resistance of *B. fuckeliana* to anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides has been initiated. Over 7 years, more than 2,400 isolates of *B. fuckeliana* were examined. The monitoring approach described in this study differs from the monitoring programs run by the chemical industry. While their standard monitoring programs focus on monitoring for resistant isolates of *B. fuckeliana* in a large area and therefore include only a limited number of samples per location, our program focused on two locations where high numbers of samples were tested. The comparatively large sample size allowed us to analyze changes in the resistant subpopulations of *B. fuckeliana*. The results of the selection process in the populations surveyed differed for all three classes of fungicides.

Field resistance to cyprodinil was encountered in 1996 in the experimental vineyard in Richterswil, Switzerland. Forster and Staub (5) and Hilber et al. (9) demonstrated that decreased efficacy in the field coincided with a high level of cyprodinil-resistant isolates in the laboratory tests. Although the occurrence of anilinopyrimidine-resistant isolates has been described by various authors (6,10,19,20), to our knowledge field resistance to anilinopyrimidines is not widespread.

Fludioxonil-resistant isolates were rarely found in the field, although the selection of phenylpyrrole-resistant isolates, cross resistant to dicarboximides, can be demonstrated in the laboratory (8,13,30). Vignutelli et al. (30) demonstrated that fludioxonil resistance is influenced by at least two different genes, responsible for field and laboratory resistance, respectively. Field resistance at the population level as defined by Delp and Dekker (4) has not been reported for fludioxonil to date.

The level of isolates resistant to fenhexamid, the most recently introduced fungicide with high activity against *B. fuckeliana*, in baseline populations has been reported to be elevated (29). In our monitoring, we found a steady increase of the resistant subpopulation from 1999 to 2001. In 2001, only resistant isolates were found in the monitoring program. However, the efficacy of Teldor (fenhexamid) in the field was still acceptable taking into account the high levels of disease.

To our knowledge, in Swiss vineyards the use of all three classes of novel fungicides and the restriction of the number of applications to one spray per class per year has resulted in acceptable control efficacy of gray mold. Nevertheless, our monitoring data revealed a significant increase in resistance to anilinopyrimidines and fenhexamid but not to fludioxonil in the population. In years where the weather conditions were favorable for *B. fuckeliana* (e.g., 1996 and 2001), the control of gray mold was inferior to the years where weather conditions were less favorable for the pathogen. This suggests that disease control as described in Table 1, which was observed despite the resistance development against cyprodinil, was due to the activity of fludioxonil in the mixture. However, this activity may not be sufficient in the case of critical weather conditions. It is unlikely that cyprodinil-resistant isolates were controlled by field rates of cyprodinil. When the first case of field resistance to cyprodinil was reported in 1996, it was shown that this coincided with loss of control.

In the population at Richterswil, a significant decrease in the frequency of cyprodinil-resistant isolates occurred in the late 1990s (Table 1). Monitoring *B. fuckeliana* after the winter period demonstrated a reduction of the cyprodinil-resistant subpopulation. We hypothesize that the buildup of the anilinopyrimidine-resistant subpopulation might be slowed down or prevented due to the reduced potential of the resistant isolates to overwinter. The acquisition of resistance may have caused a fitness penalty. Besides the selection pressure due to the treatments, the resistance buildup after the winter period is influenced by external factors such as the climate. Studies on resistant isolates produced in the laboratory have demonstrated the fitness deficiencies of fludioxonil-resistant isolates (8,13,31). The acquisition of fludioxonil resistance under field conditions might also have detrimental effects on the survival of *B. fuckeliana.* This may be why a fludioxonil-resistant subpopulation has not yet been selected under field conditions. In 2001, the total population of *B. fuckeliana* showed a reduced sensitivity to fenhexamid in the laboratory test. This result did correspond with reduced efficacy (72%) obtained for Teldor in the field; however, it did not lead to a total control failure. In order for laboratory data demonstrating a decreased sensitivity of the population to be relevant, it must coincide with a total or partial failure of disease control

in the field (4). With respect to the development of fenhexamid-resistant isolates in 2001, this is the case. Our long-term monitoring has provided an early detection of fenhexamid resistance. Further studies on the development of fenhexamid resistance in the populations under investigation are warranted.

Our monitoring and efficacy data have demonstrated that the Swiss anti-resistance strategy has prevented the selection of fludioxonil-resistant isolates but did not prevent the selection of anilinopyrimidine- or hydroxyanilide-resistant subpopulations. The limitation of the number of treatments slowed the selection process for anilinopyrimidine-resistant isolates. Our data show that frequent treatments resulted in an increase of the resistant subpopulation, which may eventually lead to failure of disease control as encountered in 1996 for the anilinopyrimidines. Alternating these three classes of fungicides (anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides), which have different modes of action and high activity against *B. fuckeliana,* together with limiting the number of fungicide treatments, is suggested as an effective way to control *B. fuckeliana*.

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