Resistance to Cyprodinil and Lack of Fludioxonil Resistance in *Botrytis cinerea* Isolates from Strawberry in North and South Carolina

Dolores Fernández-Ortuño, Fengping Chen, and **Guido Schnabel,** School of Agricultural, Forestry & Life Sciences, Clemson University, Clemson, SC 29634

Abstract

Fernández-Ortuño, D., Chen, F., and Schnabel, G. 2013. Resistance to cyprodinil and lack of fludioxonil resistance in *Botrytis cinerea* isolates from strawberry in North and South Carolina. Plant Dis. 97:81-85.

Chemical control of gray mold of strawberry caused by *Botrytis cinerea* is essential to prevent pre- and postharvest fruit decay. For more than 10 years, the anilinopyrimidine (AP) cyprodinil and the phenylpyrrole fludioxonil (Switch 62.5WG) have been available to commercial strawberry producers in the United States for gray mold control. Both active ingredients are site-specific inhibitors and, thus, prone to resistance development. In this study, 217 single-spore isolates of *B. cinerea* from 11 commercial strawberry fields in North and South Carolina were examined for sensitivity to both fungicides. Isolates that were sensitive (53%), moderately resistant (30%), or resistant (17%) to cyprodinil were identified based on germ tube inhibition at discriminatory doses of cyprodinil at 1 and 25 mg/liter at 10 of the 11 locations. None of the isolates was fludioxonil resistant. Phenotypes that were moderately resistant or resistant to cyprodinil were not associated with fitness penalties for mycelial growth rate, spore production, or osmotic sensitivity. Detached fruit assays demonstrated cross resistance between the two AP fungicides cyprodinil and pyrimethanil, and that isolates that were characterized in vitro as moderately resistant or resistant were equivalent in pathogenicity on fruit sprayed with pyrimethanil (currently the only AP registered in strawberry as a solo formulation). This suggests that the in vitro distinction of moderately resistant and resistant isolates is of little if any field relevance. The absence of cross-resistance with fludioxonil, iprodione, cycloheximide, and tolnaftate indicated that multidrug resistance in the form of multidrug resistance phenotypes was unlikely to be involved in conferring resistance to APs in our isolates. Implications for resistance management and disease control are discussed.

Botrytis cinerea Pers. is the causal agent of gray mold of strawberry and causes pre- and postharvest fruit decay worldwide (35). Infections of flowers and fruit are caused primarily by airborne conidia, which are produced on dead tissue of overwintering plants (5). The fungus can also form quiescent infections on young strawberry leaves only to subsequently colonize senescent tissue and sporulate as the leaves die (6). Fruit infections begin as small, firm, light-brown lesions that enlarge quickly and become covered with a gray fuzzy mass of spores followed by a soft rot. The disease, favored by prolonged periods of high humidity and temperatures around 15 to 22°C, can also spread by contact from infected to healthy fruit (34,35).

Fungicide sprays during and after bloom remain the main measure to reduce the incidence of gray mold (25); however, *B. cinerea* has the ability to develop resistance to commonly used site-specific fungicides. That ability is, in part, a result of its relatively large genetic diversity and enormous capacity for asexual reproduction by means of conidia (25). Repeated applications of fungicides with single-site modes of action may select for mutations in the gene targeted by the fungicide. Some of these mutations result in reduced fungicide affinity and, thus, efficacy, which may ultimately result in control failure. Examples include resistance to benzimidazole (10), demethylation inhibitor (11), dicarboxamide (8), hydroxyanilide (4), quinone outside inhibitor (QoI) (20), and succinate dehydrogenase inhibitor (SDHI) fungicides (26). Multidrug resistance (MDR) development based on a single mechanism of resistance such as overexpression of genes encoding drug efflux transporters (ATP-binding cassette [ABC]) and major facilitators (MFS) has also been observed in isolates of *B. cinerea* resistant to different classes of fungicides (30,36).

The fungicide Switch 62.5WG (Syngenta Crop Protection), registered in 2001 in the United States to control gray mold of strawberry contains two active ingredients, the anilinopyrimidine (AP) cyprodinil and the phenylpyrrole (PP) fludioxonil. Both active ingredients are site-specific inhibitors and, thus, prone to resistance development. Although numerous studies have been carried out, the primary mode of action of either chemical class has not yet been clarified. It has been hypothesized that AP fungicides inhibit the biosynthesis of methionine and other amino acids and the secretion of hydrolytic enzymes involved in the infection process of *B. cinerea* (9,18,28), and that PP fungicides interfere with the osmotic signal transduction pathway, resulting in an abnormal accumulation of glycerol (33).

B. cinerea isolates resistant to AP fungicides have been described in many studies (7,14,19,22,24,31), indicating a high risk for the build-up of resistance. Although resistance to APs in *B. cinerea* from strawberry has been reported in Florida and California (1,29), detailed peer-reviewed research studies are nonexistent. Field isolates with resistance to PPs have not been reported in the United States, although reduced sensitivity to fludioxonil has been mentioned (4,38). The objectives of this study were to (i) determine the sensitivity of *B. cinerea* isolates from several commercial strawberry fields in North and South Carolina to cyprodinil and fludioxonil, (ii) examine potential mechanisms of resistance, and (iii) investigate fitness and field relevance of isolates that are moderately resistant and resistant to cyprodinil.

Materials and Methods

Fungal isolates and culture conditions. Between April and June 2011, 217 *B. cinerea* isolates were obtained from strawberry fruit with gray mold symptoms from commercial fields encoded HP, MV, NC, and SBY in North Carolina and FLOR, GIK, JEY,

Corresponding author: G. Schnabel, E-mail: schnabe@clemson.edu

Technical contribution number 6042 of the Clemson University Experiment Station. This material is based upon work supported by the CSREES/USDA, under project number SC-1000642 and the USDA SCRI Grants Program no. 2010-51181-21113.

Accepted for publication 24 July 2012.

http://dx.doi.org/10.1094/PDIS-06-12-0539-RE

KUD, MER, MOD, and WIC in South Carolina. Most of these isolates were used in a previous study to investigate the sensitivity and molecular basis of resistance to QoI and SDHI fungicides (12). Single-spore isolations were performed as previously described (12,13). To prepare spore suspensions, mycelium was incubated for 10 days at 22°C with a 14-h photoperiod on potato dextrose agar (PDA) medium (Difco Laboratories) in 90-mm petri dishes. Spores were harvested by flooding the dish surface with 2 ml of sterile distilled water and pipetting the suspension gently off the dish surface. Spore suspensions were filtered through a 34-µm nylon mesh to remove fungal mycelium, and adjusted to the desirable concentration for the in vitro (2 to 4 × 10^5 spores/ml) or in vivo (10^6 spores/ml) assay.

In vitro fungicide sensitivity tests. The sensitivity of *B. cinerea* isolates was investigated as described previously (37) using cyprodinil formulated as Vangard WG fungicide (Syngenta Crop Protection, Inc.) and fludioxonil formulated as Scholar SC fungicide (Syngenta Crop Protection, Inc.). The conidia germination assay distinguishes sensitive, moderately resistant, and resistant isolates to cyprodinil at discriminatory doses of 0, 1, and 25 mg/liter. Germ tubes of conidia of sensitive isolates did not grow more than 50% of the rate of the control on 0.5% sucrose agar at 1 mg/liter and were completely inhibited at 25 mg/liter; germination tubes of moderately resistant isolates grew more than 50% at 1 mg/liter and up to 10 to 25% at 25 mg/liter; and germ tubes of resistant isolates grew uninhibited at 1 mg/liter and more than 25% of the control at 25 mg/liter. Discriminatory concentrations of 0, 0.1, and 10 mg/liter were used for fludioxonil. Experiments were conducted in triplicate. Germination was assessed visually under a light microscope at ×40 magnification.

Three different classes of resistance to multiple drugs (MDR) based on a single mode of action in *B. cinerea* (MDR1, MDR2, and MDR3) have been described previously (21). Isolates from each class display increased tolerance to cyprodinil and the squalene epoxydase inhibitor tolnaftate (used only in medicine) but have varying levels of sensitivity to fludioxonil, iprodione, and cycloheximide depending on the MDR class (7,21). To assess North and South Carolina *B. cinerea* isolates for MDR, the fungicide concentration (effective dose) that reduced mycelial growth by 50% (EC_{50}) was determined for cycloheximide, cyprodinil, fludioxonil, iprodione, and tolnaftate in three cyprodinil-sensitive (GIK1, HP17, and JEY18), three moderately resistant (GIK13, MOD4, and SBY21), and three resistant (MOD11, SBY36, and WIC18) isolates. The EC_{50} values for cycloheximide, cyprodinil, fludioxonil, iprodione, and tolnaftate were determined as described previously (21), with minor modifications. Briefly, 1,000 spores were transferred to 0.1 ml of 96-microplate cultures using threefold drug dilutions. Final concentrations for all compounds were 10, 3, 1, 0.3, 0.1, 0.03, and 0.01 µg/ml. Tests were performed in malt extract broth, except for cyprodinil (Gamborg B5 minimal medium supplemented with 10 mM KH_2PO_4 and 50 mM glucose, pH 5.5). After 96 h of incubation at 22°C, the optical density at wavelength with absorbance at 600 nm was determined.

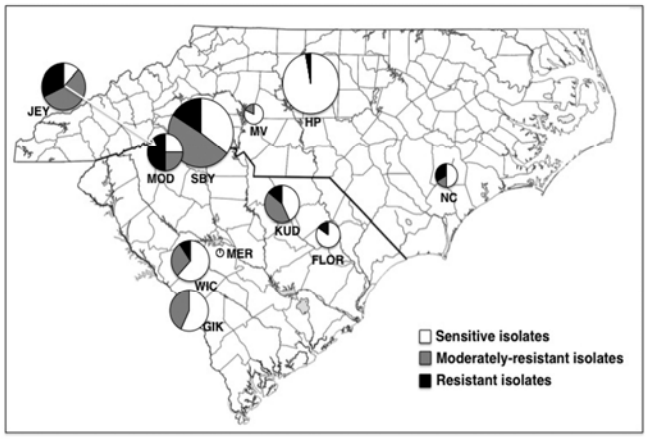

Fig. 1. Frequency of *Botrytis cinerea* isolates sensitive, moderately resistant, or resistant to cyprodinil. The circle diameter corresponds to the number of isolates tested in each location. The white arrow indicates the center of the commercial field JEY circle.

Fitness evaluation based on mycelial growth rate on PDA. Mycelial plugs (5 mm in diameter) from 1-week-old cultures grown on water agar (WA; EMD Chemicals Inc.) were transferred to the center of PDA dishes. The dishes were incubated at 22°C in the dark. The colony diameter was measured after 3 days of incubation, with three replicates per isolate. Mean colony diameter was calculated for growth rate determination. The experiment was conducted twice.

Osmotic sensitivity. Osmotic sensitivity was assessed by determining radial growth of isolates on PDA amended with 2, 4, 6, or 8% sodium chloride (NaCl). Colony diameter was measured after 2 days of incubation at 22°C in the dark. The percentage of mycelial growth inhibition was calculated using the formula RGI% = (C – N)/(C – 5) × 100, where C represents the colony diameter of the control (not amended with NaCl) and N represents the treatment with NaCl (27). Three replicate dishes per isolate were used and the experiment was performed twice.

Spore production in vitro. To assess the ability to produce spores, PDA dishes were inoculated with a 5-mm-diameter mycelial plug taken from a 5- to 7-day-old culture grown on WA and incubated for 10 days at 22°C with a 14-h photoperiod. Sporulating colonies were rinsed with 20 ml of distilled sterile water and the conidia suspension was filtered through 34-µm nylon mesh to remove fungal mycelium. The spore concentration in the suspension was estimated using a hemacytometer. Four assessments were made and averaged for each plate to calculate the number of spores produced in each plate. Three replicate dishes were used per isolate. The experiment was conducted twice.

In vivo pathogenicity test and sensitivity to pyrimethanil. Because cyprodinil was not available as a formulated, single-active-ingredient product in strawberry, we used formulated pyrimethanil, which is also an AP fungicide, in the form of Scala SC (Bayer CropScience) for our detached-fruit assays. Commercially grown ripe strawberry fruit were rinsed with sterile water three times for 30 s each and allowed to air dry. Then, they were placed into plastic boxes (eight berries per box for each of the three replicates of each treatment). Fruit were sprayed to runoff 4 h prior to inoculation with Scala SC at 2.8 ml/liter using a hand mister. Untreated fruit were sprayed with sterile distilled water. When the fruit surface had dried off, each fruit was stabbed at three equidistant points, each about 1 cm apart and all facing up to a depth of 9.5 mm using a 26G3/8 9.5-mm beveled syringe tip (Becton Dickson & Co.). Immediately thereafter, the wounds were injected with a 30-µl droplet of conidia suspension prepared in distilled sterile water (10^6 spores/ml) using the same type of syringe. Most of the conidia suspension formed a droplet on top of the wounded area. Untreated strawberry fruit inoculated with sterile water were used as negative controls. After inoculation, the boxes were kept at 22°C for 4 days. During the first 24 h, the boxes were sealed with plastic bags to keep the relative humidity at 98 to 100%. Lesion diameters were measured after 4 days. The experiment was performed twice.

In vivo spore production. Strawberry fruit were prepared, sprayed, and inoculated as described above. After 2, 3, and 4 days, the presence or absence of conidiophores and conidia on each lesion was visually assessed. Conidia and conidiophores form a characteristic gray color that can easily be distinguished from white aerial mycelium not containing conidia. The percentage of fruit with sporulating lesions was calculated for each isolate. Two replicates of this experiment were conducted.

Data analysis. A statistical model was developed that related the in vitro and in vivo responses (radial growth, sporulation on PDA, lesion size, and sporulating lesions after 2, 3, and 4 days) to the experimental replication, isolate, fungicide treatment, and their combination. The method of least squares was used to estimate the

model terms associated with the factors, and analysis of variance was used to test for a significant effect of the factors on the response means. If a factor was found to be significant, mean separation (using Student's *t* test) was used to further determine the nature of the effect of the factor on the responses. All calculations were performed using the statistical package JMP (version 9.0.0; SAS Institute Inc.) and all tests were performed with $\alpha = 0.05$.

Results

Sensitivity of *B. cinerea* isolates to cyprodinil and fludioxonil. Among the 217 field isolates, 36 (17%) were resistant, 65 (30%) were moderately resistant, and 116 (53%) were sensitive to cyprodinil. Resistant phenotypes were found in every location with the exception of MER, which included just three isolates and, therefore, may not have represented this location accurately. Resistant or moderately resistant isolates were always found on the same farms as sensitive isolates (Fig. 1). All 217 isolates were sensitive to fludioxonil. The highest frequency (75% and higher) of isolates that were moderately resistant or resistant to cyprodinil was observed in a location cluster in the western part of the Carolinas.

MDR phenotypes. As expected, the isolates classified as cyprodinil-sensitive had a lower average EC_{50} value ($P < 0.001$; 0.6 mg/liter) for cyprodinil than the moderately resistant isolates (EC_{50} = 12.9 mg/liter) which, in turn, were more sensitive to cyprodinil than the resistant isolates ($P < 0.001$; EC_{50} = 23.0 mg/liter). The groups of isolates moderately resistant and resistant to cyprodinil were not more resistant to cycloheximide, fludioxonil, iprodione, and tolnaftate than the group of sensitive isolates (Table 1), indicating that MDR was not the cause of cyprodinil resistance.

Fitness components. The data of the two experiments were combined due to the absence of experiment–isolate, experiment–treatment, and experiment–isolate–treatment effects ($P \geq 0.97$ for all three combinations). The isolates moderately resistant and resistant to cyprodinil grew as fast on PDA and produced as many spores in vitro as the sensitive group (Table 2). Although there were significant differences in the number of sporulating lesions between groups of resistance phenotypes 2 days after inoculation ($P < 0.001$), these differences on untreated fruit disappeared 3 and 4 days after inoculation. The three groups produced the same mean lesion size on untreated fruit 4 days after inoculation. In the Scala SC treatment, there were significant differences between isolates moderately resistant to cyprodinil as a group compared with the resistant isolates 2 days after inoculation, which was probably due to the smaller lesion development during the first 48 h. However,

Table 1. Sensitivity of *Botrytis cinerea* isolates sensitive (S), moderately resistant (MR), and resistant (R) to cyprodinil to different inhibitors that are routinely used to identify multidrug resistance MDR1, MDR2, and MDR3 phenotypes

	Phenotype[y]		EC_{50} (mg/liter)[z]				
Isolates	Cyprodinil	Fludioxonil	Cyprodinil	Cycloheximide	Fludioxonil	Iprodione	Tolnaftate
GIK1	S	S	0.9	14.5	0.2	5.1	21.5
HP17	S	S	0.1	13.0	0.1	2.7	10.7
JEY18	S	S	0.9	7.5	0.1	4.4	25.6
GIK13	MR	S	15.2	6.5	0.1	1.7	18.3
MOD4	MR	S	14.1	3.7	0.1	4.0	7.4
SBY21	MR	S	9.4	6.0	0.1	1.9	4.3
MOD11	R	S	20.2	8.9	0.1	6.0	10.2
SBY36	R	S	31.7	6.9	0.1	6.4	13.1
WIC18	R	S	17.0	3.9	0.1	2.4	11.6
Mean EC_{50}	S	S	0.6 c	11.7 a	0.1 a	4.1 a	19.3 a
Mean EC_{50}	MR	S	12.9 b	5.4 b	0.1 a	2.5 a	10.0 a
Mean EC_{50}	R	S	23.0 a	6.6 ab	0.1 a	4.9 a	11.6 a

[y] Differentiation between isolates that are S, MR, and R to cyprodinil was based on a germination assay using cyprodinil at 1 and 25 mg/liter; differentiation between fludioxonil S and R isolates was based on the same assay with fludioxonil at 0.1 and 10 mg/liter.

[z] EC_{50} = the fungicide concentration that reduced mycelial growth by 50%. Numbers in each column separately followed by the same letter are not significantly different at $\alpha = 0.05$ as determined by analysis of variance. Mean separation was conducted using student's *t* test.

Table 2. Fitness components for *Botrytis cinerea* isolates sensitive (S), moderately resistant (MR), and resistant (R) to cyprodinil

		Fitness components[w]									
		In vitro experiments		In vivo experiments							
				Sporulating lesions (%)						Lesion size (cm)	
		3 dai	10 dai	2 dai		3 dai		4 dai		4 dai	
Isolates	Type[x]	Growth (cm)[y]	*N*[z]	Control	Scala	Control	Scala	Control	Scala	Control	Scala
GIK1	S	6.4 abc	376.7 bc	30.0 c	0.0 e	75.0 f	0.0 i	100.0 a	0.0 e	3.0 a	0.2 e
HP17	S	4.7 g	24.0 d	45.0 b	0.0 e	90.0 c	0.0 i	100.0 a	0.0 e	2.8 abc	0.3 e
JEY18	S	5.4 ef	610.7 a	40.0 b	0.0 e	75.0 f	0.0 i	98.0 ab	0.0 e	2.9 ab	0.0 e
GIK13	MR	6.9 a	510.0 abc	15.0 d	30.0 c	70.0 g	100.0 a	95.0 bc	100.0 a	2.8 abc	2.5 cd
MOD4	MR	5.8 de	333.3 c	45.0 b	6.0 e	85.0 d	53.0 h	100.0 a	87.0 d	3.0 a	2.5 cd
SBY21	MR	6.3 bc	493.3 abc	30.0 c	3.0 e	90.0 c	53.0 h	100.0 a	100.0 a	2.9 ab	2.7 abc
MOD11	R	6.8 ab	430.0 abc	45.0 b	13.0 d	73.0 fg	53.0 h	95.0 bc	93.0 c	2.6 cd	2.3 d
SBY36	R	6.2 cd	533.3 ab	54.0 a	30.0 c	95.0 b	94.0 b	100.0 a	100.0 a	3.0 a	2.7 abc
WIC18	R	5.3 f	336.7 c	43.0 b	33.0 c	76.0 f	80.0 e	100.0 a	100.0 a	3.0 a	2.6 bcd
Mean	S	5.5 a	337.1 a	38.4 b	0.0 e	80.0 a	0.0 c	99.3 a	0.0 c	2.9 a	0.2 c
Mean	MR	6.3 a	445.6 a	30.0 c	13.0 d	81.7 a	68.7 b	98.4 ab	95.7 b	2.9 a	2.6 b
Mean	R	6.1 a	433.3 a	47.3 a	25.3 c	81.3 a	75.7 ab	98.3 ab	97.7 ab	2.9 a	2.5 b

[w] Numbers in each column of the in vitro experiments and in control and treatment columns for each dai section separately followed by the same letter are not significantly different at $\alpha = 0.05$ as determined by analysis of variance. Mean separation was conducted using Student's *t* test; dai = days after inoculation.

[x] Phenotype.

[y] Mycelial growth.

[z] Number (N) $\times 10^4$ spores/ml.

much like in the untreated control, those differences disappeared after 3 days of incubation and even lesion size after 4 days of inoculation was indistinguishable from the two resistance phenotypes. The isolates sensitive to cyprodinil as a group did not produce sporulating lesions 2, 3, or 4 days after inoculation (Table 2). There was no significant difference in osmotic sensitivity among the groups at any of the NaCl concentrations (*data not shown*).

Discussion

In this study, *B. cinerea* isolates with increased tolerance to the AP fungicide cyprodinil were found in almost every field we sampled, indicating that resistance is widespread in the Carolinas. The history of AP use in these strawberry fields was determined by survey. Producers indicated they had not used Scala SC, the only product on the market prior to 2012 with an AP fungicide as sole active ingredient. The majority of strawberry producers reported having used Switch 62.5WG, which was the only premixture of an AP fungicide (cyprodinil) and PP fungicide (fludioxonil) registered prior to 2012, at least once per season between 2008 and 2011. Although it is possible that resistant isolates were native in baseline populations, it is more likely that selection for cyprodinil resistance has occurred in the Carolinas despite the presence of fludioxonil in the spray tank. Selection for AP resistance was also observed in *B. cinerea* populations from Chilean vineyards (22), where 38.5% of all isolates were resistant to cyprodinil following four applications in a 2-year period. Low proportions of resistant strains (0.1 to 0.4%) were found in nontreated populations in Switzerland but, after AP applications, the frequency of resistant isolates built up to frequency values of 53 to 93% (14). Similar observations following multiple applications of AP fungicides per year were found in *B. cinerea* populations from Swiss vineyards (19) and vegetable crops from 18 greenhouses on the island of Crete in Greece (32). In contrast, some studies indicate that resistance levels can stay comparatively low despite up to nine accumulative applications (19) and that a single application of an AP fungicide per season may not select for resistance. For example, sensitivity monitoring of *B. cinerea* strains collected annually in France and Italy, where pyrimethanil was used only once per year, showed no shift in sensitivity after 10 years of use (16).

The in vitro distinction between isolates that are moderately resistant and resistant to cyprodinil made by Weber and Hahn (37) and that was used in this study may be of only academic value because our data indicate that both types of resistance may possess equal field relevance. Moderately resistant and resistant isolates were equally competitive in vitro and in vivo and were equally pathogenic and virulent on Scala SC-treated strawberry. Previous studies also documented that resistance in *B. cinerea* to AP fungicides is stable and without fitness cost (2,39). The use of cyprodinil in vitro and pyrimethanil in vivo in this study also underscores existing cross-resistance of the two resistance types (moderately resistant and resistant) to AP fungicides. Cross-resistance among APs has been well documented (14,22,32).

In this study, no resistance to the PP fungicide fludioxonil was found, indicating continued low resistance risk to this fungicide. Low risk for resistance development to fludioxonil was documented in a study from Switzerland showing that, during 7 years of monitoring, only one *B. cinerea* isolate with reduced sensitivity to fludioxonil was isolated from AP-treated grapevines (4). The lack of documented field resistance to PP fungicides may be a result of an associated fitness penalty that such mutants may suffer. For example, one *B. cinerea* isolate from apple with reduced sensitivity to fludioxonil also suffered a fitness penalty in the form of sensitivity to osmotic stress in vitro and less pathogenicity and virulence (38). Likewise, laboratory mutants resistant to fludioxonil were compromised in fitness (40). Although PP resistance was not observed in the this study, we reported the presence of QoI and SDHI resistance in the same isolates from this collection (12). In total, from the two studies, 116 isolates (53.4%) were sensitive to APs, QoIs, and SDHIs; 3 isolates (1.4%) were resistant to APs but sensitive to QoIs and SDHIs; 3 isolates (1.4%) were resistant to APs and QoIs but not to SDHIs; and 95 isolates (43.8%) were resistant to all three chemical classes (*data not shown*). The presence of resistance to multiple chemical classes is of great concern given the limited number of chemical classes available to control gray mold of strawberry.

Field populations of *B. cinerea* from French vineyards were previously categorized into three AP-resistant phenotypes, Ani^{R1}, Ani^{R2}, and Ani^{R3} (7) and both of our resistance phenotypes appear to be consistent with Ani^{R1}, which discards the presence of MDR as mechanism of resistance. Phenotype Ani^{R1} is characterized as moderately to highly resistant to AP fungicides with resistance factors (RFs) of 14 to 222 and retaining a similar level of sensitivity to PPs and other classes of fungicides compared with sensitive strains. Ani^{R2} phenotypes possess low to moderate resistance to APs (RF 6 to 13) and exhibit low levels of resistance to the PP fludioxonil. Phenotype Ani^{R3} shows low levels of AP resistance (RF 2) and reduced sensitivity to the dicarboxamide iprodione. Ani^{R2} and Ani^{R3} are MDR phenotypes (24) and were reclassified by Kretschmer et al. (21) as MDR1 and MDR2 phenotypes, respectively. In our study, the RF values for cyprodinil-resistant and moderately resistant isolates ranged between 20 and 36, which matched best with the RF range of 14 to 222 described for Ani^{R1} strains. Also, our isolates had a similar level of sensitivity to fludioxonil and iprodione compared with the sensitive isolates. The lack of cross resistance between AP and PP fungicides observed in this study is consistent with observations in other studies (3,14,32). Some studies, however, document cross resistance of low levels of resistance to AP fungicides with PP fungicides (7,21,23,24). Ani^{R1} strains are not associated with MDR (7) and, therefore, resistance may be based on either target gene overexpression or target gene mutations.

Ani^{R1} phenotypes pose a high risk to strawberry crops due to the suspected qualitative nature of resistance. As opposed to quantitative resistance, qualitative resistance is generally related to the reduced affinity of the target site to the fungicide (17) and may cause complete control failure in a short period of time. The qualitative nature of Ani^{R1} resistance was demonstrated in an analysis of sexual progenies of Ani^{R1} strains. Results showed that resistance to APs segregated in a 1:1 ratio, indicating a monogenic basis of resistance (7). This result, as well as the phenotypic characteristics of Ani^{R1} strains, suggests that AP resistance is qualitative. The primary target site of APs is not yet known but its discovery would greatly assist in the development of early detection methods of resistant strains and aid in our understanding of the Ani^{R1} resistance phenotype.

This study provides a strong argument against marketing premixtures rather than products containing only one active ingredient. Most premixtures contain two compounds that typically belong to different chemical classes such as an SDHI and a QoI. Companies are registering such premixtures at an accelerated pace with the argument that the two products will provide increased disease control, provide protection if resistance has developed but has not been detected, and aid in resistance management (15). Such products are often sold at a premium price compared with solo products. If strawberry producers in the Carolinas wanted to continue using fludioxonil for gray mold control, they have no choice but to apply the combination product (Switch 65WG), thereby applying another product (cyprodinil) that now is virtually ineffective in controlling gray mold. This does not make economic sense, nor does the unnecessary, continued selection for AP resistance make scientific sense. In our opinion, fludioxonil should be made available as a solo active ingredient to producers so that it can be tank mixed at a reasonable price with chemical classes other than APs for resistance management. Also, if growers continued to use Switch 65WG in fields harboring strains with AP resistance, the selection pressure for fludioxonil resistance would likely rise. That is because of the ineffectiveness of its mixing partner, cyprodinil, and because the relatively high price of Switch would prevent many growers from adding another active ingredient to the spray tank for resistance management purposes.

Acknowledgments

We thank X. P. Li., P. K. Bryson (Clemson University, Clemson, SC), and F. Wang (Huazhong Agricultural University, Wuhan, China) for technical support; and E. Schnabel for providing valuable comments on the manuscript.

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