Baseline Sensitivity of *Botrytis cinerea* to Pyraclostrobin and Boscalid and Control of Anilinopyrimidine- and Benzimidazole-Resistant Strains by These Fungicides

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ABSTRACT

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Fifty-five isolates of *Botrytis cinerea* collected from vegetable crops were used to determine the pathogen's baseline sensitivity to two new fungicides: boscalid, which inhibits the enzyme succinate dehydrogenase in the electron transport chain, and pyraclostrobin, which blocks electron transport between cytochrome *b* and cytochrome *c*₁. Measurement of sensitivity to boscalid was based on both inhibition of mycelial growth and spore germination, while measurement of sensitivity to pyraclostrobin was based only on inhibition of spore germination. For both fungicides, the sensitivity distribution was a unimodal curve, with a mean EC₅₀ value (effective concentration that reduces mycelial growth or spore germination by 50%) of 0.033 µg ml⁻¹ for pyraclostrobin and 2.09 and 2.14 µg ml⁻¹ for boscalid based on the inhibition of mycelial growth and spore germination, respectively. No cross-sensitivity relationship was observed between the two fungicides (*r* = 0.09). In addition, no cross-resistance relationship was observed between these two fungicides with other botryticides: cyprodinil, pyrimethanil, fenhexamid, fludioxonil, and iprodione. Moreover, the control efficacy of the two fungicides was tested against two anilinopyrimidine-resistant and two benzimidazole-resistant isolates, and two of wild-type sensitivity. Both pyraclostrobin and boscalid provided satisfactory control of all six isolates that was independent of the isolate sensitivity to benzimidazoles and anilinopyrimidines. In contrast, carbendazim failed to control sufficiently the benzimidazole-resistant isolates, while cyprodinil failed to provide satisfactory control of the anilinopyrimidine-resistant isolates.

Additional keywords: fungicide resistance, gray mold

Gray mold, caused by *Botrytis cinerea* Pers:Fr., is a severe disease causing significant yield losses in vegetable crops such as tomato, eggplant, pepper, and cucumber in greenhouses throughout the Mediterranean basin. Since resistant varieties do not exist for these crops, control of the disease relies mostly on fungicide sprays. Growers around the Mediterranean basin apply fungicides on a calendar schedule, starting the applications in late October and ending them in late February to early March at 7- to 10-day intervals.

The fungicides used against gray mold belong to several classes. Until the mid-1990s, control of the disease was mainly achieved by applications of site-specific fungicides belonging to the benzimidazole, dicarboximide, and *N*-phenylcarbamate groups, while multisite inhibitors such as chlorothalonil, dichlofluanid, iminactodine, and captan were used in tank mixtures or in rotation with site-specific in-

hibitors (7). In the recent past, new botryticides possessing new mechanisms of action were developed and registered for use against gray mold worldwide. These new botryticides were the anilinopyrimidines, pyrimethanil and cyprodinil, the phenylpyrrole derivative, fludioxonil, and the hydroxylanilide derivative, fenhexamid (26). However, intensive use of these site-specific inhibitors has led to a rapid selection of pathogen strains resistant to benzimidazoles, dicarboximides, and anilinopyrimidines in many countries worldwide (4,8,9,15,19-21,23). In these cases, the introduction into spray programs of new fungicides belonging to different chemical groups with no cross-resistance with botryticides already in use could aid in overcoming limitations in disease control caused by fungicide resistance. Two new active ingredients, pyraclostrobin and boscalid, could play such a role.

Pyraclostrobin is among the newer members of the group of Quinone outside inhibitors (QoIs), a fungicide class that was developed from natural fungicidal derivatives such as strobilurin A and oudemansin A (1,5). The mechanism of action of this fungicide class is the inhibition of mitochondrial respiration by binding at the Qo site of the cytochrome *b*, causing the blocking of electron transport

between cytochrome *b* and cytochrome *c*₁. Inhibition of mitochondrial respiration leads to a disruption of the energy cycle (5). Pyraclostrobin possesses an extremely broad spectrum of activity, including fungal species such as *B. cinerea* and oomycetes (1,13,17).

Boscalid is a new broad-spectrum fungicide belonging to the carboxamide (anilide) class of fungicides. These fungicides possess a different mode of action, the inhibition of the enzyme succinate ubiquinone reductase, also known as succinate dehydrogenase (SDH) (6). The enzyme plays a crucial role in the tricarboxylic cycle and the mitochondrial transport chain, and its inhibition leads to a block of the cell energy cycle. Boscalid is active against several ascomycetous fungal species attacking fruit, vegetables, and vines, including *B. cinerea* (18,29).

These two new fungicides, with modes of action distinct from those of botryticides already in use, could play a significant role in the control of the disease and in the management of resistance developed to other fungicide classes. However, it is well known that *B. cinerea* represents a classical high-risk pathogen for fungicide resistance development due to its high genetic variability, the abundance of sporulation, the short generation time, and the wide host range. Moreover, recent studies indicated a high risk for resistance development in this pathogen to pyraclostrobin (17) and a moderate risk for resistance development to boscalid (32). Consequently, the current study was conducted: (i) to determine the baseline sensitivity of *B. cinerea* isolates obtained from vegetable crops in Greece to these two fungicides, (ii) to determine the cross-sensitivity/resistance patterns between boscalid and pyraclostrobin and with active ingredients from several other chemical classes, and (iii) to test the efficacy of pyraclostrobin and boscalid in controlling benzimidazole- and anilinopyrimidine-resistant strains of *B. cinerea*.

MATERIALS AND METHODS

Pathogen isolates. The *B. cinerea* isolates used in the current study were isolated from diseased vegetable crops grown in greenhouses on the Crete island, Greece, during the winter period of 2005. The isolates were collected during a monitoring program to determine the sensitivity of pathogen isolates to several fungicides.

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Details on the isolation procedure and the determination of sensitivity to these fungicides have previously been described (21). The isolates were stored on potato dextrose agar (PDA) slants at 4°C until use. In total, 55 pathogen isolates were used.

Baseline sensitivity to pyraclostrobin and boscalid. Pure technical grades of the fungicides, provided by the manufacturer BASF Hellas (Athens, Greece), were used. The pure technical grades of these two fungicides were dissolved in pure acetone and ethanol, respectively, to prepare stock solutions.

For the measurement of sensitivity to pyraclostrobin, based on the inhibition of spore germination, autoclaved 2% water agar medium (Oxoid, Unipath Ltd., Basingstoke, England) was amended with 0.001, 0.005, 0.01, 0.05, and 0.1 µg ml⁻¹ pyraclostrobin, as well as salihydroxamic acid (SHAM) in methanol at 100 µg ml⁻¹. Control plates were amended only with acetone and methanol. The concentration of the solvents did not exceed 0.1% by volume in fungicide-amended and non-amended media.

For the measurement of sensitivity to boscalid that was carried out based both on inhibition of spore germination and mycelial growth, the growth media were amended with 0.05, 0.1, 0.5, 1, and 5 µg ml⁻¹ boscalid. Control plates were amended with ethanol at a concentration of 0.1% by volume. For the measurement of sensitivity based on inhibition of spore germination, the growth medium used was 2% water agar, while for the measurement based on inhibition of mycelial growth, a minimal medium was prepared containing 10 g glucose, 1.5 g K₂HPO₄, 2 g KH₂PO₄, 1 g (NH₄)₂SO₄, 0.5 g MgSO₄·7H₂O, 2 g yeast extract, and 12.5 g agar per liter. Tests for each isolate were replicated three times per concentration of each fungicide.

For the measurement of sensitivity based on inhibition of spore germination, conidia of each isolate from 10- to 12-day cultures grown on PDA were used to prepare suspensions containing 1 × 10³ conidia ml⁻¹. Aliquots of the conidial suspension were spread on 6-cm petri dishes containing the fungicides. After 12 h of incubation at 20°C, the number of germinated conidia was measured. A conidium was considered germinated if the germ

tube was at least half the length of the conidium. In total, 100 conidia were scored per petri dish, and there were three petri dishes per isolate and fungicide concentration.

For the measurement of sensitivity based on inhibition of mycelial growth, mycelial plugs were removed with the aid of a 5-mm cork borer from the margins of actively growing, 72-h-old colonies and placed upside down on the centers of 9-cm plastic petri dishes containing the fungicide-amended or unamended media. Cultures were incubated at 20°C in the dark for 3 days, and then mean colony diameters were measured. Colony diameters on a fungicide-amended medium were expressed as percentage of the mean diameter on the unamended medium.

Control of anilinoypyrimidine- and benzimidazole-resistant strains. Six *B. cinerea* isolates—two benzimidazole-resistant, two anilinoypyrimidine-resistant, and two of wild-type sensitivity—were selected for the experiment carried out to determine the efficacy of pyraclostrobin and boscalid in controlling benzimidazole- and anilinoypyrimidine-resistant strains of the pathogen. The isolates had been collected for the requirements of a monitoring program to determine the sensitivity of pathogen isolates to several fungicides (21). Details on the sensitivity of each of the six isolates to carbendazim, cyprodinil, pyraclostrobin, and boscalid are given in Table 1.

Cucumber seedlings (cv. Cetriolo Telegrafo) were grown in plastic pots (18 cm diameter) containing a 2:1 mixture of peat and perlite. After sowing, pots were kept in the growth chamber at 23°C. After appearance of the cotyledons, plants were treated with the fungicides, and 24 h later, they were inoculated with the six selected isolates.

Formulations of boscalid (F510 50WG) and pyraclostrobin (F500, 25EC) were provided by the manufacturer (BASF Hellas, Athens, Greece). In addition, applications of commercial formulations of cyprodinil (Chorus 50 WG, Syngenta Hellas, Athens, Greece) and carbendazim (carbendazim 50WP, Papoikonomou Agrochemicals SA, Thessaloniki, Greece) were included in the study as reference treatments. Carbendazim was applied at a

concentration of 100 µg ml⁻¹, cyprodinil at 50 and 100 µg ml⁻¹, and boscalid and pyraclostrobin at 25, 50, and 100 µg ml⁻¹. The fungicides were applied to runoff with a hand-sprayer 24 h before inoculation of the cucumber seedlings. Control plants were sprayed with sterile tap water.

The center of each cotyledon was punctured by a needle, and a 3-mm mycelial plug from the edge of an actively growing 3-day-old culture on PDA was placed on the wound. Six plants (12 cotyledons) were inoculated per isolate and fungicide treatment. The inoculated plants were transferred to a moisture chamber at 23°C for 4 days. After this period of incubation, diseased plants were removed from the moisture chamber and the infection was recorded by evaluating the lesion on each cotyledon. The evaluation was carried out by using a disease-index scale suggested by Ziogas et al. (33) as follows: 0, no infection; 0.5, rot only under the inoculum; 1, less than 20% rot; 2, 21 to 50% rot; and 4, rot on more than 50% of the cotyledon surface. The experiment was replicated twice.

Data analysis. Values of disease severity on inoculated cucumber cotyledons were subjected to analysis of variance, and means were compared by Duncan's multiple range test. Because data from the two replications did not differ significantly ($P > 0.05$), the mean disease severity values from the two experiments for each isolate and treatment were used in the data analysis. The EC₅₀ values (effective concentration that reduces mycelial growth or spore germination by 50%) of each isolate to boscalid and pyraclostrobin were calculated by regressing the relative inhibition of growth or spore germination against the Log₁₀ fungicide concentration using JMP (JMP, SAS Institute, Cary, NC). The mean value of the three replicate tests per isolate and concentration were used to calculate the EC₅₀ values. To measure cross-resistance between pairs of fungicides, the EC₅₀ values were correlated and the correlation coefficients (r) were calculated. The EC₅₀ values were transformed to Log₁₀ values before analysis.

RESULTS

Baseline sensitivity to boscalid and pyraclostrobin. A total of 55 single-spore

Table 1. Level of sensitivity to several fungicides (in terms of EC₅₀ values) of *Botrytis cinerea* isolates used in the study

Isolates	Host of origin	Characterization ^z	EC ₅₀ (µg ml ⁻¹) ^y			
			Carbendazim	Cyprodinil	Pyraclostrobin	Boscalid
13g	Tomato	BENR	>100	0.004	0.042	3.55
15d	Tomato	BENR	>100	0.003	0.072	1.17
1d	Eggplant	ANIR	0.08	5.0	0.022	1.65
7e	Eggplant	ANIR	0.05	10.0	0.020	2.40
16e	Tomato	WTBC	0.10	0.004	0.025	4.35
6	Tomato	WTBC	0.07	0.004	0.025	3.30

^y Measurement of effective concentration values that reduce mycelial growth or spore germination by 50% (EC₅₀) was based on mycelial growth test for carbendazim, cyprodinil, and boscalid and on spore germination test for pyraclostrobin.

^z BENR: benzimidazole-resistant isolates; ANIR: anilinoypyrimidine-resistant isolates; WTBC: wild-type sensitivity *B. cinerea* isolate.

isolates of *B. cinerea* were tested for sensitivity to boscalid on the basis of mycelial growth and spore germination and to pyraclostrobin on the basis of spore germination. For both pyraclostrobin and boscalid, the frequency distribution of the EC_{50} values was a unimodal curve (Figs. 1 and 2). The EC_{50} values for each fungicide are shown in Table 2. The individual EC_{50} values for pyraclostrobin ranged from 0.005 to 0.077 $\mu\text{g ml}^{-1}$ and thus were separated by a factor of 15, while the mean EC_{50} had a value of 0.033 $\mu\text{g ml}^{-1}$. The intrinsic activity of boscalid was lower compared with that of pyraclostrobin. The individual EC_{50} values for boscalid, when sensitivity was measured based on inhibition of spore germination, ranged from 0.09 to 5.60 $\mu\text{g ml}^{-1}$ with a mean value of 2.14 $\mu\text{g ml}^{-1}$, while the sensitivity measured on the basis of mycelial growth ranged from 0.075 to 5.05 $\mu\text{g ml}^{-1}$ with a mean value of 2.09 $\mu\text{g ml}^{-1}$. In the first case, the individual EC_{50} values were separated by a factor of 62, while in the second they were separated by a factor of 67 (Table 2).

Cross-resistance relationships. The sensitivity to boscalid was plotted versus the sensitivity to pyraclostrobin, and the log-transformed EC_{50} values were analyzed by establishing correlation and linear regression. In addition, the sensitivity to these two fungicides was plotted versus the sensitivity to cyprodinil, pyrimethanil, fludioxonil, fenhexamid, and iprodione, which had been determined for the same isolates in a previous study (21). Correlation coefficients between all the fungicide pairs are presented in Table 3. For all combinations, the correlation coefficients were very low, ranging from 0.03 to 0.19, and none were significant ($P > 0.05$).

Control of anilinopyrimidine- and benzimidazole-resistant strains. Data on disease severity and control efficacy obtained by the several treatments are summarized in Table 4. Specifically, applications of both pyraclostrobin and boscalid at the higher concentrations of 50 and 100 $\mu\text{g ml}^{-1}$ significantly suppressed the disease caused by all isolates compared with the control treatments ($P < 0.05$), and the control efficacy had values higher than 90%. In contrast, cyprodinil failed to control satisfactorily the anilinopyrimidine-resistant strains. Disease severity on the plants treated with cyprodinil, either at 50 or 100 $\mu\text{g ml}^{-1}$, and inoculated with anilinopyrimidine-resistant isolates was significantly higher ($P < 0.05$) compared with the disease severity on plants treated with either boscalid or pyraclostrobin. Similarly, applications of carbendazim at 100 $\mu\text{g ml}^{-1}$ failed to suppress satisfactorily the disease caused by the benzimidazole-resistant strains, while they efficiently controlled the anilinopyrimidine-resistant isolates and the isolates of wild-type sensitivity. Disease severity on cucumber seed-

lings treated with carbendazim and inoculated with benzimidazole-resistant strains was significantly higher ($P < 0.05$) compared with that observed on pyraclostrobin- or boscalid-treated plants, but lower ($P < 0.05$) than that observed on the untreated control plants.

DISCUSSION

Emergence of resistance to botryticides such as the benzimidazole, dicarboximide,

and anilinopyrimidine fungicides has become a limitation to the sustained control of gray mold caused by *B. cinerea* on vegetable crops and grapevines worldwide. The development of new fungicide compounds with good efficacy against the disease and novel biochemical mechanisms of action, and their introduction into the spray programs applied against gray mold, is a prerequisite for successful disease management, particularly in crops

Fig. 1. Baseline sensitivity of *Botrytis cinerea* isolates to pyraclostrobin based on inhibition of spore germination. EC_{50} = effective concentration that reduces mycelial growth or spore germination by 50%.

Fig. 2. Baseline sensitivity of *Botrytis cinerea* isolates to boscalid based on the inhibition of A, spore germination, and B, mycelial growth. EC_{50} = effective concentration that reduces mycelial growth or spore germination by 50%.

with high disease pressure and where problems of resistance to site-specific fungicides have emerged.

In the current study, the baseline sensitivity of *B. cinerea* to two novel compounds was established using isolates collected from vegetable crops grown in greenhouses on Crete island, Greece. The isolates were collected during the winter period of 2005 before the introduction of boscalid and pyraclostrobin into the spray programs applied against the disease. The number of isolates used in this study fell within the limits provided by Russel (27), who suggests that 50 isolates will likely give a reasonable picture of the baseline sensitivity of a fungal population. Establishment of baseline sensitivity data is of great importance in fungicide resistance management, since they can be used to illustrate shifts in pathogen sensitivity and provide evidence that control failures occurring in the future can be correlated with

the appearance of resistance in the population (12,27). Determination of the isolates' sensitivity to pyraclostrobin was based on the inhibition of spore germination, since strobilurin fungicides show a dominant effect on this developmental stage (5,25,31). Sensitivity distribution to pyraclostrobin was unimodal with a relatively narrow sensitivity range. A similar narrow baseline sensitivity range to strobilurin fungicides has been observed in several other pathogens (10,22,30). To the best of our knowledge, this is the first report of the baseline sensitivity of *B. cinerea* isolates to pyraclostrobin.

Sensitivity to boscalid was determined based both on inhibition of spore germination and mycelial growth since it is known that in addition to being a strong inhibitor of spore germination, this fungicide is also very effective against all other major stages of fungal growth (28). In both cases, the sensitivity distribution to boscalid was

unimodal and similar to that observed for pyraclostrobin. The baseline sensitivity distribution that we obtained, based on the inhibition of mycelial growth, appears to be consistent with a previously reported range of EC₅₀ values of 0.09 to 3.69 µg ml⁻¹ (32). These values are higher than those provided by Stammeler and Speakman (29), but this is probably due to differences in the experimental approach for the EC₅₀ measurement. In their study, Stammeler and Speakman (29) determined EC₅₀ values based on a microtiter method combining the inhibition of both spore germination and mycelial growth; the growth substrate was also different. The level of sensitivity to boscalid, in terms of EC₅₀ values, based on the inhibition of spore germination for most of the isolates tested, was similar to that based on the inhibition of mycelial growth. However, it should be mentioned that in the current study, mycelial growth for most isolates was not completely inhibited even at the higher concentration of 5 µg ml⁻¹. This is in accordance with the findings presented by Zhang et al. (32), who reported higher levels of sensitivity of *B. cinerea* to boscalid based on the inhibition of spore germination compared with that based on the inhibition of mycelial growth. However, in a recent report regarding the sensitivity of *Alternaria alternata* isolates from pistachio to boscalid, it was found that mycelial growth in that fungal species was more sensitive than spore germination for evaluating the inhibitory effects of boscalid against the pathogen (3).

Regarding the cross-sensitivity relationship between pyraclostrobin and boscalid, the results of the current study showed that there was no correlation between the sensitivity to these two fungicides. A similar conclusion was obtained in a recent study of cross-resistance relationships with pyraclostrobin-resistant isolates of *A. alternata* from pistachio (3). In contrast, two previous reports suggested that strobilurin-resistant strains of *Alternaria solani* and *B. cinerea* were more sensitive to boscalid

Table 2. Baseline sensitivity of *Botrytis cinerea* isolates, obtained from vegetable crops, to boscalid and pyraclostrobin

Fungicide	Stage of inhibition	EC ₅₀ (µg ml ⁻¹)		VF ^z
		Range	Mean	
Pyraclostrobin	Spore germination	0.005 – 0.077	0.033	15
Boscalid	Mycelial growth	0.075 – 5.050	2.09	67
Boscalid	Spore germination	0.090 – 5.600	2.14	62

^z Variation factor = highest EC₅₀ value (effective concentration that reduces mycelial growth or spore germination by 50%) divided by the lowest EC₅₀ value.

Table 3. Cross-resistance patterns between pyraclostrobin, boscalid, cyprodinil, pyrimethanil, fludioxonil, fenhexamid, and iprodione in *Botrytis cinerea* isolates obtained from vegetable crops

Fungicide	Pyraclostrobin		Boscalid	
	r ^y	P ^z	r	P
Boscalid	0.09	0.47	–	–
Cyprodinil	0.001	0.98	0.12	0.35
Pyrimethanil	0.03	0.81	0.11	0.41
Fludioxonil	0.05	0.68	0.19	0.16
Fenhexamid	0.13	0.33	0.08	0.54
Iprodione	0.04	0.73	0.08	0.55

^y r = correlation coefficient value.

^z Correlation coefficients are significant at P < 0.05.

Table 4. Disease severity and control efficacy (%) of gray mold caused by *Botrytis cinerea* isolates with different levels of sensitivity to fungicides on cucumber seedlings treated with cyprodinil, carbendazim, boscalid, and pyraclostrobin

Treatment	Dose ^w	Benzimidazole-resistant isolates				Anilino-pyrimidine-resistant isolates				Wild-type sensitivity isolates			
		15D		13G		1D		7E		16E		6	
		DS ^x	CE ^y	DS	CE	DS	CE	DS	CE	DS	CE	DS	CE
Cyprodinil	50	0.78 c ^z	81	0.87 c	78	2.37 e	41	3.00 d	25	0.12 a	97	0.43 d	89
Cyprodinil	100	0.40 b	90	0.43 b	89	1.59 d	60	1.75 c	56	0.15 a	96	0.12 a	97
Carbendazim	100	3.00 d	25	2.93 d	27	0.59 bc	85	0.21 a	95	0.37 b	91	0.34 b-d	92
Pyraclostrobin	25	0.50 b	88	0.37 ab	91	0.84 c	79	0.40 ab	90	0.25 ab	94	0.25 a-d	94
Pyraclostrobin	50	0.25 ab	94	0.31 ab	92	0.59 bc	85	0.25 ab	94	0.12 a	97	0.21 a-c	95
Pyraclostrobin	100	0.12 a	97	0.21 ab	95	0.40 ab	90	0.18 a	96	0.12 a	97	0.15 ab	96
Boscalid	25	0.50 b	88	0.87 c	78	0.90 c	69	0.53 b	87	0.37 b	91	0.37 cd	91
Boscalid	50	0.21 ab	95	0.25 ab	94	0.37 ab	91	0.34 ab	92	0.25 ab	94	0.09 a	98
Boscalid	100	0.06 a	99	0.15 a	96	0.12 a	97	0.28 ab	93	0.15 a	96	0.15 ab	96
Control		4.00 e	–	4.00 e	–	4.00 f	–	4.00 e	–	4.00 c	–	4.00 e	–

^w µg a.i. ml⁻¹.

^x DS: disease severity. Measurements of disease severity were based on a disease scale from 1 to 5 as suggested by Ziogas et al. (33).

^y CE: control efficacy. Percent control efficacy values were calculated as 100 – disease severity (treated) / disease severity (untreated) × 100.

^z Means followed by different letters in a column are significantly different according to Duncan's multiple range test at P = 0.05.

than the strobilurin-sensitive strains of these pathogens (17,24). However, the data presented in our study were derived using baseline isolates that had never been exposed to the fungicides tested, and there was no resistance to either of the two fungicides. In addition, we found no correlation of sensitivities between sensitivity to boscalid or pyraclostrobin and sensitivity to cyprodinil, pyrimethanil, fenhexamid, fludioxonil, and iprodione. In a recent study carried out using isolates of *B. cinerea* with laboratory-induced resistance to pyraclostrobin, a negative cross-resistance relationship between pyraclostrobin and cyprodinil was observed, while there was no correlation between the level of sensitivity to pyraclostrobin and the level of sensitivity to fenhexamid or to fludioxonil (17).

In this study, the control efficacy provided by boscalid and pyraclostrobin also was investigated. The results showed that boscalid and pyraclostrobin can successfully control anilinopyrimidine- and benzimidazole-resistant strains of *B. cinerea*. Under controlled environmental conditions, applications of both boscalid and pyraclostrobin, even at the lowest concentration tested of 25 µg ml⁻¹, provided satisfactory control of the disease on plants inoculated with either the benzimidazole-resistant (BENR) or the anilinopyrimidine-resistant (ANIR) isolates of the pathogen. At the same time, cyprodinil and carben-dazim failed to protect satisfactorily plants inoculated with the ANIR and the BENR isolates, respectively.

Such results suggest that both boscalid and pyraclostrobin could play a key role in gray mold management in the near future and encourage their introduction into the spray programs. However, the highly specific mode of action indicates a high risk for rapid selection of resistant genotypes among pathogen populations. Field resistance to strobilurin fungicides has been developed in several pathogens more rapidly than expected (11,14,16), while resistance to boscalid has been observed in field isolates of *A. alternata* from pistachio (2,3). This potentially high risk for resistance development suggests the need for strategies to reduce the risk. Consequently, further research is required to design the most effective fungicide spray schedules and to select the most effective partners for use in mixtures.

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