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

**C. K. Myresiotis, G. A. Bardas,** and **G. S. Karaoglanidis,** Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, POB 269, 54124, Thessaloniki, Greece

**ABSTRACT**

Myresiotis, C. K., Bardas, G. A., and Karaoglanidis, G. S. 2008. Baseline sensitivity of *Botrytis cinerea* to pyraclostrobin and boscalid and control of anilinopyrimidine- and benzimidazole-resistant strains by these fungicides. Plant Dis. 92:1427-1431.

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 $EC_{50}$ 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 inhibitors (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 hydroxyanilide 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.

Corresponding author: G. S. Karaoglanidis
E-mail: gkarao@agro.auth.gr

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