Fungicide Resistance Among *Botrytis cinerea* Isolates from California Strawberry Fields

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**Abstract**

The resistance to four fungicides used for the management of Botrytis rot (gray mold) in strawberry was evaluated among 65 isolates of *Botrytis cinerea* from coastal California. Fungicide resistance was tested by agar diffusion assay on Czapek-Dox agar. Isolates not showing inhibition zones around wells at a discriminatory concentration were considered resistant. Concentrations of active ingredients for detecting resistance were 50 mg a.i./liter for thiophanate-methyl, 10 mg a.i./liter for fenhexamid, 50 mg a.i./liter for cyprodinil and fludioxonil (Switch pre-mix), and 100 mg a.i./liter for bosalid and pyraclostrobin (Pristine pre-mix). Most isolates (92%) in all fields surveyed were resistant to thiophanate-methyl. Resistance to the more recent products was also widespread and occurred in most fields. Overall, incidence of resistance among isolates was 25% for fenhexamid, 28% for cyprodinil/fludioxonil, and 66% for bosalid/pyraclostrobin. Resistant isolates remained uninhibited by higher concentrations, suggesting that they had become insensitive to these products when re-tested at twice and four times the discriminatory concentration. Among isolates of *B. cinerea*, 85% were resistant to at least two products. This widespread occurrence of resistance to single site mode of action fungicides suggests that their effectiveness to control Botrytis rot might become impaired.

**Introduction**

California is the most important producer of strawberries in the USA, with 14,780 ha (36,520 acres) producing 809 million kg in 2008 (6). Production is almost continuous throughout the year with summer fruit harvested from April to October in the northern central coast and winter fruit produced in Southern California from November to April. A major cause of fruit decay in the field and postharvest is Botrytis rot caused by *Botrytis cinerea*. Infection usually occurs in flowers and then appears later as fruit develop and mature (4,5).

Botrytis rot of strawberry, also known as gray mold, is normally managed by field applications of fungicides. During the 1980s and 1990s, the control of Botrytis rot in the United States relied on fungicides such as iprodione, thiram, captan, and benomyl (1,12). The use of some of these chemistries has become more restricted: benomyl is no longer available, iprodione can no longer be used during fruit production, and thiram can only be used with a 3-day pre-harvest interval in the United States and Canada. In addition to captan and thiophanate-methyl, which are still available for Botrytis rot management, several newer active ingredients are now being used extensively because of their efficacy in protecting flowers and fruit (13,16). These include fenhexamid, bosalid/pyraclostrobin pre-mix, cyprodinil/fludioxonil pre-mix, and pyrimethanil.

The development of fungicide resistance in pathogen populations from repeated exposure is always a concern as it can negatively affect fungicide efficacy (3). Products targeting single or limited metabolic pathways as most recently introduced fungicides do are especially at risk. At this time, we know little about the occurrence of resistance to the more recently registered products.
used for controlling Botrytis rot. The objective of this research was to determine the prevalence of fungicide resistance in populations of *B. cinerea* from strawberry fields in California. We collected *B. cinerea* isolates from production fields and tested their sensitivity to four fungicides used for managing Botrytis rot. Three of these fungicides are relatively recent: fenhexamid (Elevate), boscalid/pyraclostrobin pre-mix (Pristine), and cyprodinil/fludioxonil pre-mix (Switch). The Fungicide Resistance Action Committee (FRAC) rates these actives as medium to high resistance risk and places them in resistance group 17 for fenhexamid, 7 for boscalid, 11 for pyraclostrobin, 9 for cyprodinil, and group 12 for fludioxonil (8). In addition, we included an older product, thiophanate-methyl (Topsin M), considered as a high resistance risk and placed in the FRAC group 1, with a known history of resistance in various populations of *B. cinerea* (11,15,19).

**Evaluation of Fungicide Sensitivity**

*B. cinerea* isolations were made from infected strawberries collected in four fields in southern California’s Oxnard district (Ventura Co.) during the winter in early 2008 (total of 38 isolates) and two fields in the California central coast’s Watsonville district (Monterey and Santa Cruz counties) during the summer of 2008 (total of 27 isolates). All fields were managed with conventional chemical fertilizers and pesticides. Isolates were transferred to half-strength potato dextrose agar and allowed to sporulate at room temperature under fluorescent light (12-h photoperiod). Conidial suspensions were made in 15% glycerol and stored at -80°C until use.

Fungicide resistance was evaluated on Czapek-Dox agar (19) supplemented with 100 ppm chloramphenicol using an agar diffusion assay (Fig. 1). After applying and spreading 150 μl of conidial suspension to standard petri plates, 3 wells (8 mm diameter) were cut in the agar of the edge of each plate and 100 μl of fresh fungicide test solution was applied to each well. Sterile water was used as control. All tests were done in duplicate plates. Fungicides tested were commercial formulations of thiophanate-methyl (Topsin M 70 WP, Nisso, New York, NY), fenhexamid (Elevate 50WDG, Arysta LifeScience, Cary, NC), boscalid/pyraclostrobin pre-mix (Pristine, BASF, Research Triangle Park, NC), and cyprodinil/fludioxonil pre-mix (Switch 62.5WDG, Syngenta, Greensboro, NC). A discriminatory concentration was used for each fungicide, based on a concentration sufficient to produce consistently a clear inhibition zone without hyphal growth in the agar diffusion assay in a subset of sensitive isolates exposed to a series of two-fold concentrations. Discriminatory concentrations, based on active ingredients, were 50 mg a.i./liter for thiophanate-methyl, 10 mg a.i./liter for fenhexamid, 50 mg a.i./liter for Switch (30 mg a.i. cyprodinil + 20 mg a.i. fludioxonil per liter), and 100 mg a.i./liter for Pristine (66 mg a.i. boscalid + 34 mg a.i. pyraclostrobin per liter). Test fungicide solutions were made with sterile water. After 4 to 6 days, the plates were scored for the presence of inhibition zones: isolates having an inhibition zone with no hyphal growth or with some restricted hyphal growth around wells were considered inhibited by the test solution while wells with normal fungal growth (not different from the water control) were considered resistant (Fig. 1). Up to twenty resistant isolates were subsequently retested at the same discriminatory concentration, as well as two and four times higher, to determine the extent of their insensitivity to the fungicides.
Fig. 1. Example of agar diffusion assay. Left plate shows clear inhibition zone by fenhexamid (Elevate) at 10 ppm (mg/liter) but not at 2.5 and 5 ppm. Right plate shows isolate insensitive to thiophanate-methyl (Topsin) at both 10 and 50 ppm, comparable to the water control.

Table 1. Percentage of resistant isolates showing resistance when retested at the 1x, 2x, and 4x discriminatory fungicide concentration. Up to 20 isolates were retested for each fungicide.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Discriminatory (1x) a.i. concentration (mg/liter)</th>
<th>% isolates showing resistance at each concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1x</td>
<td>2x</td>
</tr>
<tr>
<td>boscalid/pyraclostrobin</td>
<td>100*</td>
<td>100</td>
</tr>
<tr>
<td>cyprodinil/fludioxonil</td>
<td>50*</td>
<td>94</td>
</tr>
<tr>
<td>fenhexamid</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>thiophanate-methyl</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

* Concentration of combined a.i.
**Incidence of Fungicide Resistance**

**Thiophanate-methyl.** Most isolates (92%) in all fields surveyed were resistant to thiophanate-methyl at 50 mg a.i./liter (Fig. 2). Furthermore, most resistant isolates were not inhibited when tested at 100 or 200 mg a.i./liter (Table 1), suggesting that they were completely immune to this fungicide. Because of such prevalent resistance, this fungicide is unlikely to be effective for the management of Botrytis rot. This is not surprising as resistance to thiophanate-methyl in *B. cinerea* is widespread and has been found for some time to be prevalent in populations from greenhouses (11,19) and strawberry fields (15). It is also found in populations of other fungal plant pathogens (7,10,17,18).

![Bar chart](https://example.com/bar_chart.png)

**Fig. 2.** Effect of thiophanate-methyl (Topsin) at 50 mg a.i./liter on the growth of *B. cinerea* isolates from six strawberry field.

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**Fenhexamid.** Resistance to fenhexamid was found in all fields surveyed, with about 25% of isolates showing resistance (Fig. 3). All resistant isolates remained insensitive when tested at higher concentrations (Table 1). Because of this resistance to fenhexamid in the California population, care should be taken to prevent selection pressure so resistant isolates do not become more prevalent.

Fig. 3. Effect of fenhexamid (Elevate) at 10 mg a.i./liter on the growth of *B. cinerea* isolates from six strawberry fields.
Cyprodinil/fludioxonil (Switch) pre-mix. Resistance to the Switch pre-mix product was found in five out of six fields surveyed, with 29% of isolates showing resistance to 50 mg/liter combined a.i. (30 mg a.i. cyprodinil + 20 mg a.i. fludioxonil per liter) (Fig. 4). However, resistant isolates tended to be sensitive to higher fungicide concentrations, indicating that they had retained sensitivity to at least one component of the pre-mix and were probably not immune to the pre-mix combination (Table 1). It is noteworthy that resistance to one of the pre-mix components, cyprodinil, has been found for some time in B. cinerea isolates from Swiss vineyards (9).

Fig. 4. Effect of cyprodinil/fludioxonil pre-mix (Switch) at 50 mg/liter combined a.i. (30 mg a.i. cyprodinil + 20 mg a.i. fludioxonil per liter) on the growth of B. cinerea isolates from six strawberry fields.
**Boscalid/pyraclostrobin (Pristine) pre-mix.** Resistance to the Pristine pre-mix was found in all fields surveyed, with 66% of isolates showing no inhibition at 100 mg/liter combined a.i. (66 mg a.i. boscalid + 34 mg a.i. pyraclostrobin per liter) (Fig. 5). Furthermore, all resistant isolates were not inhibited when exposed to a dose twice or four times higher than the discriminatory concentration (Table 1). It is likely that the reason for this is that only one active ingredient in the Pristine pre-mix, boscalid, has inhibitory activity against *B. cinerea* while pyraclostrobin, a strobolin, has only suppressive activity on this pathogen. For this reason, this pre-mix fungicide can be considered as having a single active ingredient against *B. cinerea*, which can easily develop resistance to boscalid. The very high prevalence of this resistance in the population raises the question of possible failure of this product to control Botrytis rot in the field.

*Fig. 5. Effect of boscalid/pyraclostrobin pre-mix (Pristine) at 100 mg/liter combined a.i. (66 mg a.i. boscalid + 34 mg a.i. pyraclostrobin per liter) on the growth of *B. cinerea* isolates from six strawberry fields.*

**Resistance to multiple fungicides.** A large proportion of isolates (85%) had resistance to at least two fungicides (Fig. 6). This is to be expected given the prevalence of the resistance to thiophanate-methyl and the boscalid/pyraclostrobin pre-mix. Several isolates had resistance to three and even four products even though all products tested belong to different FRAC groups and there is no chance of cross resistance among them (8). It might become more difficult to manage resistant populations of *B. cinerea* with the limited options available to control Botrytis rot. In such situation, low resistance risk fungicides such as captan (FRAC number M4), thiram (FRAC number M3), and Serenade (FRAC number F6) might help relieve selection pressure.
Considerations for Botrytis Rot Control

This widespread occurrence of resistance to single-site active fungicides suggests that there is a possibility that their efficacy against Botrytis rot is becoming impaired and that more aggressive resistance management will be required. Several factors compound the problem. In California, the very long fruiting season and continuous cultivation of strawberries throughout the year allow resistant isolates to persist in strawberry plantings and maintain selection pressure for resistant isolates as the same few products are often used repeatedly. In addition, caneberrries and vegetables that are grown in strawberry production areas could harbor resistant B. cinerea populations, especially if they are exposed to some of the same fungicides. For example, fenhexamid, as well as the boscalid/pyraclostrobin and cyprodinil/fludioxonil pre-mixes are also used in raspberry production. Senescent foliage and other plant parts are a source of inoculum in fruit production fields (2,14) and could help shelter resistant isolates until the next growing season. Since some of these fungicides are also used in strawberry transplant production, even though they are usually targeting other pathogens such as Colletotrichum spp., resistant B. cinerea isolates could originate from the nursery and be carried to the production field in transplants. In our study, we found no relation between fungicide use history and the occurrence of resistance. For example, the Watsonville #2 field had received no thiophanate-methyl, fenhexamid or Switch pre-mix the year before and during this survey, while it had isolates resistant to all products tested. It is possible that these resistant populations occur regionally and do not necessarily reflect the fungicide usage history of a given field or a nursery source. Periodic monitoring could help us understand whether the prevalence of resistant isolates changes over the years. Knowledge of such a pattern could help us predict risks of product failures. Meanwhile, it would be prudent to be proactive and promote prevention of fungicide resistance with growers.

Acknowledgments

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Literature Cited


