Fungicide Sensitivity Assessed in Monilinia vaccinii-corymbosi Isolates from Lowbush Blueberry Fields in Maine

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ABSTRACT


The fungicide sensitivity in populations of Monilinia vaccinii-corymbosi (Reade) Honey, the fungus that causes mummy berry, may be affected by the use of fungicides for control of this fungus. The sensitivity of M. vaccinii-corymbosi isolates from conventionally and organically managed fields and an unmanaged, wild area was determined for the fungicides propiconazole and fenbuconazole. Propiconazole has been used for more than 14 years to control mummy berry, and fenbuconazole has become more widely used in the last 4 years than previously. The baseline EC50 for isolates from the unmanaged area was significantly higher for propiconazole (0.016 µg/ml) than fenbuconazole (0.006 µg/ml). The propiconazole EC50 for isolates from conventionally managed fields (0.020 µg/ml) was significantly higher than for isolates from the unmanaged area (0.016 µg/ml), but not from the organically managed fields (0.018 µg/ml). No significant differences in the fenbuconazole EC50 were found among management types. The biennial long-term use of the fungicide propiconazole on populations of M. vaccinii-corymbosi may contribute to the development of fungicide insensitivity over time.

INTRODUCTION

Natural variation in lowbush blueberry plants (Vaccinium angustifolium Ait.) (2) and conventional disease management practices may affect populations of Monilinia vaccinii-corymbosi (Reade) Honey, the fungal cause of mummy berry of blueberry. Mummy berry is an economically important disease of highbush, lowbush and rabbiteye blueberries (4). Mummy berry is characterized by “mummified” fruit, pseudosclerotia, that overwinter and germinate in the early spring to produce apothecia whose ascospores cause the primary infection of the young leaves and flowers (1). The infected leaves and flowers of lowbush blueberry die within eight to ten days and produce conidia which are dispersed by wind or insects to infect open flowers (1,9). The fungus colonizes the developing fruit, resulting in pseudosclerotia (1,7,9). Crop losses due to mummy berry vary in severity and among fields each year, depending on inoculum level, environmental conditions, and host susceptibility (4). In lowbush blueberries, an entire year’s crop of berries can be lost due to high levels of primary infection of leaves and flowers early in the season.

Lowbush blueberries are typically grown on a two-year crop cycle with a vegetative year followed by a crop the next year. Since 1998, most conventional growers of lowbush blueberries in Maine control mummy berry disease by two to three applications of a propiconazole-based fungicide (demethylation inhibitor fungicide, DMI) each biennial crop year. No in situ fungicide resistance has been reported for M. vaccinii-corymbosi in blueberry fields as yet (8). Long-term use of fungicides with a risk for development of resistance could increase the possibility of more fungicide insensitive genotypes in conventionally managed lowbush blueberry fields than in fields where these fungicides are not used. Monilinia vaccinii-corymbosi may have less risk for developing fungicide insensitivity because of the single sporulation events in its lifecycle and the biennial nature of applications of fungicides for its control. However, crop fields are usually adjacent to vegetative year fields, apothecia can be produced 1 or 2 years after pseudosclerotia fall to the ground (S. L. Annis, unpublished data), and ascospores can be carried by wind currents for at least 30 m (3); therefore, it is conceivable that the fungus may be exposed to propiconazole multiple times each year, not just biennially. Fungicides are not applied after the risk of ascospore infections has passed in lowbush blueberries, and therefore conidia produced on infected leaf and flower tissues may not be directly exposed to fungicides but may be affected by fungicides still present systemically in plant tissues. Conidia are spread by insects, and possibly wind, to healthy flowers. Conidia may be another way in which fungal genotypes could be spread to new areas. The propiconazole sensitivity status of M. vaccinii-corymbosi populations has not been reported previously. Understanding the current status of fungicide sensitivity in M. vaccinii-corymbosi is important for maintaining mummy berry control over time in lowbush blueberries. This study aims to answer two important questions: Does the continual use of a single fungicide, propiconazole, for several years affect the sensitivity of M. vaccinii-corymbosi to this fungicide? Does M. vaccinii-corymbosi exhibit less sensitivity to the widely applied fungicide propiconazole than to fenbuconazole, which has been used for fewer years?

Isolates of M. vaccinii-corymbosi from three types of sites, conventionally and organically managed fields, and an unmanaged, wild area of lowbush blueberry in Maine were tested for their fungicide sensitivity. Isolates from unmanaged, wild areas can provide a baseline of fungicide sensitivity because they have not been exposed to fungicides in the past, and therefore are unlikely to have developed fungicide resistance (6,10). According
to the field managers, the organically managed areas had not been treated with any fungicides for at least 10 years, while the conventionally managed fields had been treated with propiconazole approximately twice every crop year since 1998 (Table 1). Fungicide sensitivity levels seen in conventionally managed and organically managed fields were compared to the baseline level established from the unmanaged area to determine if fungicide insensitivity was occurring in managed fields.

### TABLE 1

<table>
<thead>
<tr>
<th>Field</th>
<th>Fungicide</th>
<th>Year of First Application</th>
<th>Applications per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB1</td>
<td>propiconazole</td>
<td>1999</td>
<td>2</td>
</tr>
<tr>
<td>DB2</td>
<td>propiconazole</td>
<td>1999</td>
<td>2</td>
</tr>
<tr>
<td>COL</td>
<td>propiconazole</td>
<td>1998</td>
<td>2</td>
</tr>
<tr>
<td>CR</td>
<td>propiconazole</td>
<td>1998</td>
<td>1.7</td>
</tr>
</tbody>
</table>

**ISOLATION OF MONILINIA VACCINII-CORYMBOSI**

*Monilinia vaccinii-corymbosi* isolates were grown from pseudosclerotia collected from three types of sites in Washington, Waldo, and Hancock counties in Maine: conventionally managed fields; organically managed fields; and an unmanaged, wild area. Conventionally managed field sites were COL and CR, located near Cherryfield and Wesley, Washington County, Maine, respectively; and DB1 and DB2, both located near Deblois, Washington County, Maine. Organic fields are certified organic by the Maine Organic Farmer and Gardeners Association (MOFGA), meaning that they were not treated with conventional fungicides for 3 years prior to certification. The three organically managed field sites were: HK (certified in 2004) in Jonesboro, Washington Country, Maine; HB (certified in 1999) in Stockton Springs, Waldo County, Maine; and PM (certified in 2001) in Dedham, Hancock County, Maine. Pseudosclerotia from these fields were collected during the spring and late summer of 2010. In each field, 5 to 7 pseudosclerotia were collected every 15 m along each of two 100-m transects that intersected in approximately the middle of the field. Pseudosclerotia from an unmanaged, wild area were collected near Sebec Lake, Piscataquis County, Maine in a previous year. Pseudosclerotia are difficult to find in unmanaged, wild areas. We were unable to obtain pseudosclerotia from other areas since the number of berries and therefore pseudosclerotia were low in unmanaged areas in 2010. Pseudosclerotia were stored in paper envelopes at 11°C in the dark before use.

Pseudosclerotia of *M. vaccinii-corymbosi* were surface sterilized in batches of three pseudosclerotia from each collection location by submerging them in 70% ethanol for 5 min, 0.6% sodium hypochlorite solution for 5 min, and three rinses of sterile water for 1 min each. Pseudosclerotia were periodically agitated every few minutes during sterilization. Each sterilized pseudosclerotium was cut into quarters and placed in a Petri dish containing malt yeast extract agar (MYA) (1% w/v malt extract, 0.3% w/v yeast extract, and 1.5% w/v agar) and grown in the dark at 20°C. Hyphae of *M. vaccinii-corymbosi* were transferred to new MYA dishes after 10 to 14 days. One isolate per pseudosclerotium was used in subsequent experiments. A total of 81 pseudosclerotia produced isolates of *M. vaccinii-corymbosi*: 39 from the 4 conventional fields; 26 from the 3 organic fields; and 16 isolates from the one wild area.

**FUNGICIDE SENSITIVITY EXPERIMENTS**

Propiconazole and fenbuconazole stock solutions of 1 µg/ml were made by diluting with water the fungicide products Bumper 41.8EC, containing 41.8% propiconazole (Makhteshim Agan of North America, Raleigh, NC), and Indar 2F, containing 23.5% fenbuconazole (Dow AgroScience, Canada). These stock solutions were used to amend MYA agar plates with 5 concentrations, 0.01, 0.03, 0.07, 0.1, and 0.3 µg/ml, of propiconazole or fenbuconazole, and unamended control plates containing 0 µg/ml fungicide. The concentrations used were similar to the range used in previous fungicide resistance studies of two related species, *M. fructicola* and *M. oxyccoci* (5,6,10). Three replicates of each treatment were performed during each trial.

All fungal cultures were grown in the dark at 20°C. Fungicide plates were inoculated with *M. vaccinii-corymbosi* mycelial plugs (0.8 cm diameter) grown on MYA for 14 days. Fungal colony diameters were measured after 7 days’ growth. Two diameters were measured through the center of the plug horizontally and vertically and these were averaged to accommodate the irregular growth of the fungus. The plug diameter was included in measurements; however, when no hyphae were visible, 0 cm of growth was recorded. All measurements were made using a Darkfield Quebec colony counter model #3330 (American Optical Co., Southbridge, MA).

The average growth of each isolate in each repetition was transformed using the natural log after 0.045 cm (10 times smaller than the lowest growth measured) was added to each average growth measurement to allow for transformation of data points where no growth was measured. The fungicide sensitivity levels were expressed in terms of the concentration of fungicide necessary to reduce mycelial growth by 50% (EC50) (10). The EC50 was determined for each replicate of each isolate using line equations obtained by nonlinear regression analysis using General Linear Models (procGLM, SAS 9.2, SAS Institute Inc., Cary, NC, USA). The three replicates for each isolate were averaged to determine the average EC50 for that isolate. The baseline EC50 was the average EC50 for all isolates from the unmanaged area, Sebec Lake. ANOVA analysis with Tukey’s HSD test for mean comparisons (procGLM, SAS 9.2) was performed to compare EC50 among fields, management types for each fungicide, and between fungicides in each field.

**EC50 RESULTS FOR M. VACCINII-CORYMBOSI FROM DIFFERENT MANAGEMENT REGIMES**

Fenbuconazole reduced mycelial growth more than propiconazole at each of the concentrations tested (data not shown). The EC50 ranges for both fenbuconazole and for propiconazole were similar for isolates from all fields treated with the same fungicide (Table 2 and 3), though the percentage of isolates over the EC50 differed among the management types. Overall, the EC50 for propiconazole was significantly higher (P < 0.0001) than the EC50 for fenbuconazole in all fields and management types (Tables 2, 3, and 4). The baseline EC50 was significantly higher (P < 0.0001) for propiconazole than for fenbuconazole for the isolates from the unmanaged area at Sebec Lake, which had never been treated with fungicides (Table 4).

The propiconazole EC50 for conventionally managed fields was significantly higher (P < 0.05) than for the unmanaged area (Table 4). However, the propiconazole EC50 for organically managed fields was intermediate between the other management types and was not significantly different from them. The percentage of isolates with an EC50 above the baseline EC50 for propiconazole varied among management type with more isolates...
fenbuconazole EC$_{50}$ was found among the three management fungicide sensitivity data (Table 2). A range and average EC$_{50}$ (μg/ml) for fenbuconazole found for isolates of Monilinia vaccinii-corymbosi from each field (Table 2).

### TABLE 2

<table>
<thead>
<tr>
<th>Field</th>
<th>Management type</th>
<th>Propiconazole EC$_{50}$ Range (μg/ml)</th>
<th>Average Propiconazole EC$_{50}$ (μg/ml) (± SEM)$^x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB1</td>
<td>Conventional</td>
<td>0.015 - 0.028</td>
<td>0.021 (0.00047)$^a$</td>
</tr>
<tr>
<td>DB2</td>
<td>Conventional</td>
<td>0.014 - 0.032</td>
<td>0.021 (0.00014)$^a$</td>
</tr>
<tr>
<td>COL</td>
<td>Conventional</td>
<td>0.012 - 0.037</td>
<td>0.022 (0.0003)$^a$</td>
</tr>
<tr>
<td>CR</td>
<td>Conventional</td>
<td>0.013 - 0.037</td>
<td>0.019 (0.00015)$^a$</td>
</tr>
<tr>
<td>PM</td>
<td>Organic</td>
<td>0.014 - 0.029</td>
<td>0.019 (0.0003)$^a$</td>
</tr>
<tr>
<td>HK</td>
<td>Organic</td>
<td>0.009 - 0.029</td>
<td>0.018 (0.0002)$^a$</td>
</tr>
<tr>
<td>HB</td>
<td>Organic</td>
<td>0.011 - 0.033</td>
<td>0.019 (0.0003)$^a$</td>
</tr>
<tr>
<td>Sebec Lake</td>
<td>Unmanaged</td>
<td>0.011 - 0.031</td>
<td>0.016 (0.00012)$^a$</td>
</tr>
</tbody>
</table>

$^x$ SEM is the standard error of the mean.

$^y$ Different letters in a column indicate significant differences at $\alpha = 0.05$.

The significant differences in propiconazole sensitivity seen between conventionally managed fields and the unmanaged area in this study suggests that a decrease in sensitivity to propiconazole may be occurring in conventionally managed fields, although no fungicide resistance to propiconazole has been reported in M. vaccinii-corymbosi in lowbush blueberry fields (8). Continual use of propiconazole every crop year with multiple applications per year may be driving insensitivity in conventionally managed fields. The propiconazole EC$_{50}$ for organically managed fields was not significantly different from that of the conventionally or unmanaged field types. This may be because some of the fields were treated with fungicides, probably propiconazole, prior to transitioning to organic. Some isolates of M. vaccinii-corymbosi from organic populations were less sensitive to propiconazole but these less sensitive isolates were not predominant within these fields.

Similar EC$_{50}$ results for both propiconazole and fenbuconazole have been found in studies of Monilinia oxycocci (6). Much like the EC$_{50}$ results for M. vaccinii-corymbosi, M. oxycocci was less sensitive to propiconazole than fenbuconazole (6). The propiconazole median baseline EC$_{50}$ results for M. oxycocci are similar to the mean baseline EC$_{50}$ exhibited by M. vaccinii-corymbosi, and the fenbuconazole EC$_{50}$ for M. vaccinii-corymbosi was approximately 1.5 times higher than that of M. oxycocci (6). Neither M. vaccinii-corymbosi nor M. oxycocci has exhibited insensitivity to propiconazole or fenbuconazole (6). The similar fungicide sensitivity to propiconazole exhibited by M. vaccinii-corymbosi and M. oxycocci is probably due to similar life cycles with a single generation of ascospores and conidial spores produced each year.

In conclusion, isolates of M. vaccinii-corymbosi from conventionally managed lowbush blueberry fields in Maine are exhibiting early signs of reduced sensitivity to propiconazole, although no resistance has been seen in the field. There are other fungicides available to protect against mummy berry disease, but most are DMI fungicides in the same class as propiconazole. These other azoles are poor candidates for alternation with propiconazole, or for use in place of propiconazole, due to the possibility of cross-resistance occurring. Including a non-azole fungicide in rotation with propiconazole or fenbuconazole could also slow the development of decreased sensitivity to these fungicides. Fungicide resistance in the field should continue to be monitored in the future.
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LITERATURE CITED