Title
Occurrence and distribution of resistance to Elevate and Switch in populations of *Botrytis cinerea* from strawberries in the Carolinas

Final Report SRSFC- Research Proposal

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Objective
Determine resistance to fungicides fenhexamid (Elevate) and cyprodinil+fludioxonil (Switch) in the Botrytis gray mold fungus from different locations in North and South Carolina.

Justification
Control of gray mold disease caused by *B. cinerea* is largely dependent on the use of fungicides (Leroux et al., 2004). However, in view of fungicide resistance development, *B. cinerea* represents a classical high-risk pathogen due to its high genetic variability, its abundant sporulation and polycyclic nature, its wide host range and the high number of sprays required for its successful control (Leroux et al., 2002). Over the last 20 years, resistance of *B. cinerea* to anilinopyrimidine, benzimidazole, dicarboximide, hydroxianilide and phenylpyrrole has been reported in different countries soon after their introduction for the control of gray mold disease (Baroffio et al., 2003; Gullino et al., 1987; Ziogas and Klamarakis, 2001). Resistance of *B. cinerea* to fungicides can often result in the failure of disease control. For this reason, it is generally recommended that fungicides with different modes of action be applied alternately, as co-formulations, or as tank mixes to prolong their usefulness.

The usefulness and efficacy of these fungicides is dependent on many things, including timing, the applications system, but also the existing genotypes of the pathogen in the field. Resistance to fungicides may develop, which can eliminate much of the usefulness of products of an entire chemical class. Very little is known about the resistance situation in strawberry fields from NC and SC despite the fact that all above listed fungicides have been used extensively over the last years. But there were reports of control failure in experimental research fields from NC and FL. A field survey was performed in different commercial strawberry growing areas of North and South Carolina during the spring-summer 2011. In vitro fungicide sensitive assays revealed alarming fungicide resistance problems for the active ingredients of Pristine, boscalid and pyraclostrobin. Among the 216 isolates from 11 locations tested, 121 (56%) were resistant; 73 (33.8%) sensitive and 22 (10.2%) highly sensitive. With regard to pyraclostrobin, 136 isolates (63%) were identified as resistant and 80 (37%) sensitive to this fungicide. A total of 119 isolates were resistant to both fungicides; 72 were sensitive to both and 25 were still sensitive to boscalid but resistant to pyraclostrobin.

This development is reflective of the widespread and frequent use of Pristine by our growers. Our latest research shows that point mutations in well characterized fungal target proteins are responsible for the resistance described above. These point mutations basically provide qualitative resistance, which means that even an increase in dosage will not provide much control. It is now critical to explore the resistance situation for the remaining Botryticides in order to fully understand the resistance problem and eventually come up with better disease management recommendations.

Methodologies

Two hundred and fourteen *B.cinerea* field strains from NC and SC were analyzed using a fungicide sensitivity test assay described previously by Weber and Hahn (2011). Technical-grade cyprodinil, fenhexamid and fludioxonil were used for *in vitro* assays. Discriminatory concentrations were utilized for all fungicides based on conidial germination method (Weber and Hahn, 2011). For each isolate and each fungicide concentration, a 40µL drop of spore suspension were streaked out across the length of the corresponding medium to ensure a good spread of conidia. For fenhexamid and fludioxonil, autoclaved 1% (wt/vol) malt extract agar (MEA) medium were amended with 1 and 50 µg mL^{-1} fenhexamid or 0.1 and 10 µg mL^{-1} fludioxonil. However for cyprodinil, the growth media 0.5% (wt/vol) sucrose agar (SA) was augmented with 1 and 25 µg mL^{-1} of this fungicide. Tests for each isolate were replicated three times per concentration of each fungicide and results were analyzed under microscope.

Results

Of the 214 isolates collected, 16.8% were resistant to fenhexamid. Resistance was found in three of four locations from North Carolina and in four of seven locations from South Carolina indicating that resistance was widespread. Mutations in Erg27 (T63I, F412S, F412C, and F412I) were associated with resistance with F412S the predominant and most widespread mutation. Mutations T63I and F412C in field isolates of *B. cinerea* are described for the first time (Fig. 1).

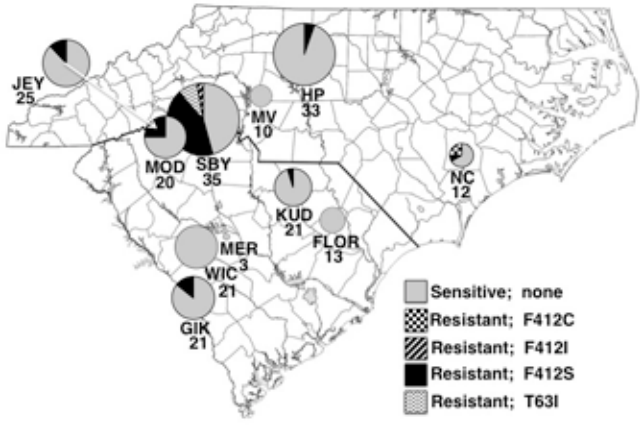

Fig. 1. Occurrence and frequency of Erg27 amino acid genotypes associated with resistance to fenhexamid in *Botrytis cinerea* isolates from the Carolinas. Sensitive isolates are represented in gray and resistant isolates with mutations F412C, F412I, F412S, and T63I are represented with patterns checker board, black and white stripes, solid black, and waived lines, respectively. The circle diameter correspond to the number of isolates collected in each location, which is indicated below the locations' name. The white arrow indicates the center position of the JEY circle.

Among all 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 3 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. 2). 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.

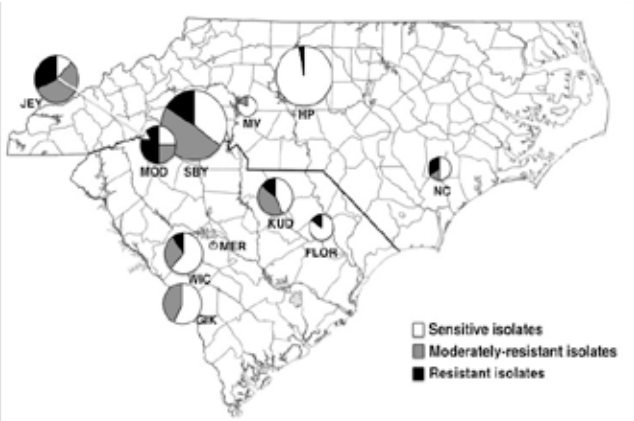

Fig. 2. Frequency of *Botrytis cinerea* isolates sensitive, moderately resistant, or resistant to cyprodinil. The circle diameter corresponds to the number of isolates tested in each location. The white arrow indicates the center of the JEY circle.

Conclusions

Fenhexamid and cyprodinil resistance are widespread in the Carolinas but most often accounted for only a small percentage of the local population. Interestingly, fenhexamid resistance was based on one of four different target site mutations. Our study strongly suggests that fenhexamid and cyprodinil resistance in *B. cinerea* needs to be monitored in commercial strawberry fields of the Carolinas. Growers need to implement resistance management strategies such as rotating chemical classes or tank mixing fenhexamid and cyprodinil with other products from different chemical classes for gray mold disease management.

Impact Statement

Fungicide resistance makes fungicide applications ineffective and exposes the crop to crop diseases. With this study we established emerging and existing resistance to fenhexamid and cyprodinil, two commonly used products for gray mold control. This and other information has initiated a regionwide resistance monitoring service for commercial growers, which will be critical to avoid pre-and postharvest disease control failure.

Citation(s) for any publications arising from the project

- Grabke, A. Fernández-Ortuño, D. and G. Schnabel 2012. Fenhexamid resistance in *Botrytis cinerea* from strawberry fields in the Carolinas is associated with four target gene mutations. Plant Dis. In press.
- Fernández-Ortuño, D., F. Chen, and G. Schnabel 2012. Distribution and characterization of resistance to cyprodinil and lack of fludioxonil resistance in *Botrytis cinerea* isolates from strawberry in North and South Carolina. Plant Dis. In press.