**Title**

Helping southern strawberry growers control gray mold in light of widespread fungicide resistance

**Progress Report SRSFC- Research Proposal**

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**Objective**

Determine resistance to 7 important classes of fungicides in *Botrytis cinerea* from commercial strawberry fields in South Carolina, North Carolina, Florida, Georgia, and other southern states, analyze the data, and provide farmers with timely, location-specific resistance management recommendations

**Justification**

Chemical control of gray mold of strawberry caused by *Botrytis cinerea* Pers. is essential to prevent pre- and postharvest fruit decay; however, resistance to multiple chemical classes of fungicides including anilinopyrimidines (APs; cyprodinil and pyrimethanil), dicarboxamides (DCs; iprodione), hydroxyanilides (HAs; fenhexamid), methyl benzimidazole carbamates (MBCs; thiophanate-methyl), quinone outside inhibitors (QoIs; pyraclostrobin), or succinate dehydrogenase inhibitors (SDHIs; boscalid) was found recently in *B. cinerea* from strawberry fields in North Carolina and South Carolina. A significant proportion of isolates were resistant to thiophanate-methyl, pyraclostrobin, boscalid, and cyprodinil.

Multifungicide resistance in *B. cinerea* isolates is also well known in Florida, the largest strawberry production region on the USA east coast. In a study carried out between 2010 and 2012, 392 *B. cinerea* isolates were collected from Florida strawberry fields and evaluated for sensitivity to registered site-specific fungicides. The study documented widespread resistance to boscalid, pyraclostrobin, fenhexamid, cyprodinil, and pyrimethanil. Isolates resistant to two, three and four fungicides from different chemical groups were also observed. Reports from other states are rare or focus on just one chemical class. For example, resistance to DCs in *B. cinerea* was described in the west coast and North Carolina in the 1990´s, and Louisiana in 2000, 2002, and 2010. Resistance to HAs, QoIs and SDHIs has been documented only in California and resistance to MBCs in *B. cinerea* from strawberry was reported from the west coast, North Carolina, and Louisiana.

Field resistance to phenylpyrroles fungicides (PPs, fludioxonil) in fungal plant pathogens is uncommon and has been reported only in a few studies. Resistance to fludioxonil has been described only in isolates from grapevines in Germany, from apple groves in Washington State, from strawberry a field in Virginia and from blackberry fields in South Carolina. Some isolates with resistance to fludioxonil possessed fitness penalties, which may at least partly explain the low frequency of fludioxonil resistant isolates in *B. cinerea* populations in the field. In southeastern USA, strawberry fields are sprayed weekly during the flowering period, which typically continues well into the production season, resulting in multiple fungicide treatments during the season. The appearance of gray mold despite applications of fungicides has been reported by strawberry growers in several states especially during highly favorable conditions, enhancing concerns about the presence of fungicide resistant isolates of *B. cinerea* in the region.

**Methodologies**

In 2012 and 2013, a total of 1,810 bulk conidial isolates of *B. cinerea* were collected from 181 strawberry fields (10 isolates per field, designated a 'sample' in this study) in 7 states, including Arkansas (110 isolates), Florida (250 isolates), Georgia (90 isolates), Maryland (310 isolates), North Carolina (190 isolates), South Carolina (660 isolates), and Virginia (200 isolates; Table 1). Isolates were collected from sporulating blossoms (660 isolates) and fruit (1,150 isolates). Most (77%) of the flower and fruit samples did not originate from the same farm. Blossoms and fruit samples from the same farms (23%) were not necessarily from the same field, which is why these datasets were considered as not dependent. To obtain conidia from blossom samples, a total of 20 to 40 strawberry blossoms with a degenerated, black torus (likely caused from freeze damage) were obtained from each strawberry field tested. After petals were removed, the blossoms were surfaced sterilized with 10% bleach for 1 min, rinsed with sterile water for 1 min, and allowed to air dry for 5 min. Then the blossoms were placed into 15 cm diameter Petri dishes containing sterile filter paper imbibed with 2 ml of sterile water. The blossoms were kept at 22°C for 2 to 4 days after which many became symptomatic for gray mold. During the first 24 hrs, the dishes were sealed with plastic bags to keep the relative humidity at 98 to 100%. For fruit samples, 10 to 12 individual strawberries with small (young) gray mold lesions were obtained from commercial fields; each fruit from a different plant with at least 5 buffer plants between sampled plants. Conidia were collected using individually wrapped sterile cotton swabs (Fisher Scientific, Pittsburgh, PA). The cotton tip was rubbed gently against the youngest area of sporulation (periphery of the lesion) of a fruit to capture conidia. The white cotton tip turned from pure white to lightly gray, indicating that sufficient conidia were collected; then, the swab was returned to its wrapper. Fungicide sensitivity of the bulk conidial isolates was defined using a novel mycelial growth assay.

A single dose for each fungicide was applied in mycelial growth assays to distinguish sensitive and resistant strains. The active ingredients, media and doses are listed in **table 1**. Inoculated plates were incubated at 22 ºC for 4 days and diametric colony growth was visually assessed in each well: (sensitive, S) for absence of growth, (low resistant, LR) for less than 20% diametrical growth, (medium resistant, MR) for less than 50% but more than 20% diametrical growth, and (resistant, R) for more than 50% diametrical growth compared to the control wells.

**Table 1.** Discriminatory concentrations and media used in spore germination and mycelial growth assays to monitor resistance in *Botrytis cinerea*

| | **Spore germination assay**[y] | | **Mycelial growth assay** | |
|---|---|---|---|---|
| **Fungicides** | **Concentration (µg/ml)** | **Medium**[z] | **Concentration (µg/ml)** | **Medium**[z] |
| Boscalid | 1, 50 | 0.5% YEA | 75 | 1% YBA |
| Cyprodinil | 1, 25 | 0.5% SA | 4 | CzA |
| Fenhexamid | 1, 50 | 1% MEA | 50 | 1% MEA |
| Fludioxonil | 0.1, 10 | 1% MEA | 0.5 | 1% MEA |
| Iprodione | 5, 50 | 1% MEA | 10 | 1% MEA |
| Pyraclostrobin | n/d | n/d | 10 | 1% MEA+100 µg/ml SHAM |
| Trifloxystrobin | 0.1, 10 | 1% MEA+100 µg/ml SHAM | n/d | n/d |
| Thiophanate-methyl | 1, 100 | 1% MEA | 100 | 1% MEA |

[y] Fungicide concentrations and media were previously described by Weber and Hahn (2011).

[z]CzA= CzapeK-Dox agar medium; MEA= Malt extract agar ; n/d= not determined; SA= Sucrose agar; SHAM= the alternative oxidase inhibitor salicyl hydroxamic acid; YEA= Yeast extract agar; and YBA= Yeast bacto acetate agar.

Resistance monitoring results were shared with growers and specialists in form of an official report detailing the resistance profile based on 10 strains examined to each chemical class together with resistance management recommendations.

**Results**

The overall resistance frequencies of 750 isolates collected in 2012 for thiophanate-methyl, pyraclostrobin, boscalid, cyprodinil, fenhexamid, iprodione, and fludioxonil were 76, 42, 29, 27, 25, 3, and 1%, respectively. Frequencies of 1060 isolates collected in 2013 were 85, 59, 5, 17, 26, 2, and 1%, respectively. Resistance to thiophanate-methyl and pyraclostrobin was found in virtually every location in both years, whereas resistance to iprodione and fludioxonil was rarely found. Resistant isolates were resistant to either one (23%), two (18%), three (19%), four (14%), five (3%), or six (0.1%) chemical classes of fungicides in 2012. In 2013 this distribution was 24, 29, 26, 8, 2, and 0.3%, respectively (**Fig. 1**). Multifungicide resistant isolates of *B. cinerea* were widespread in southern states and evidence suggests that the frequency of isolates with multifungicide resistance increased from 2012 to 2013.

Resistance monitoring results were shared with growers and specialists. A survey handed out to participating growers in SC, MD, PA, VA, and NC revealed that all 16 growers who returned the survey implemented the location-specific advice and that control success was very high:

1. All growers followed our recommendations and experienced either good or very good gray mold control
2. All growers want the service to continue
3. All established growers (defined as not being in their first year of strawberry production) thought they did a better job controlling the disease
4. All growers were more aware of the need for fungicide resistance management
5. All growers learned more about chemical control options
6. All established growers improved resistance management tactics

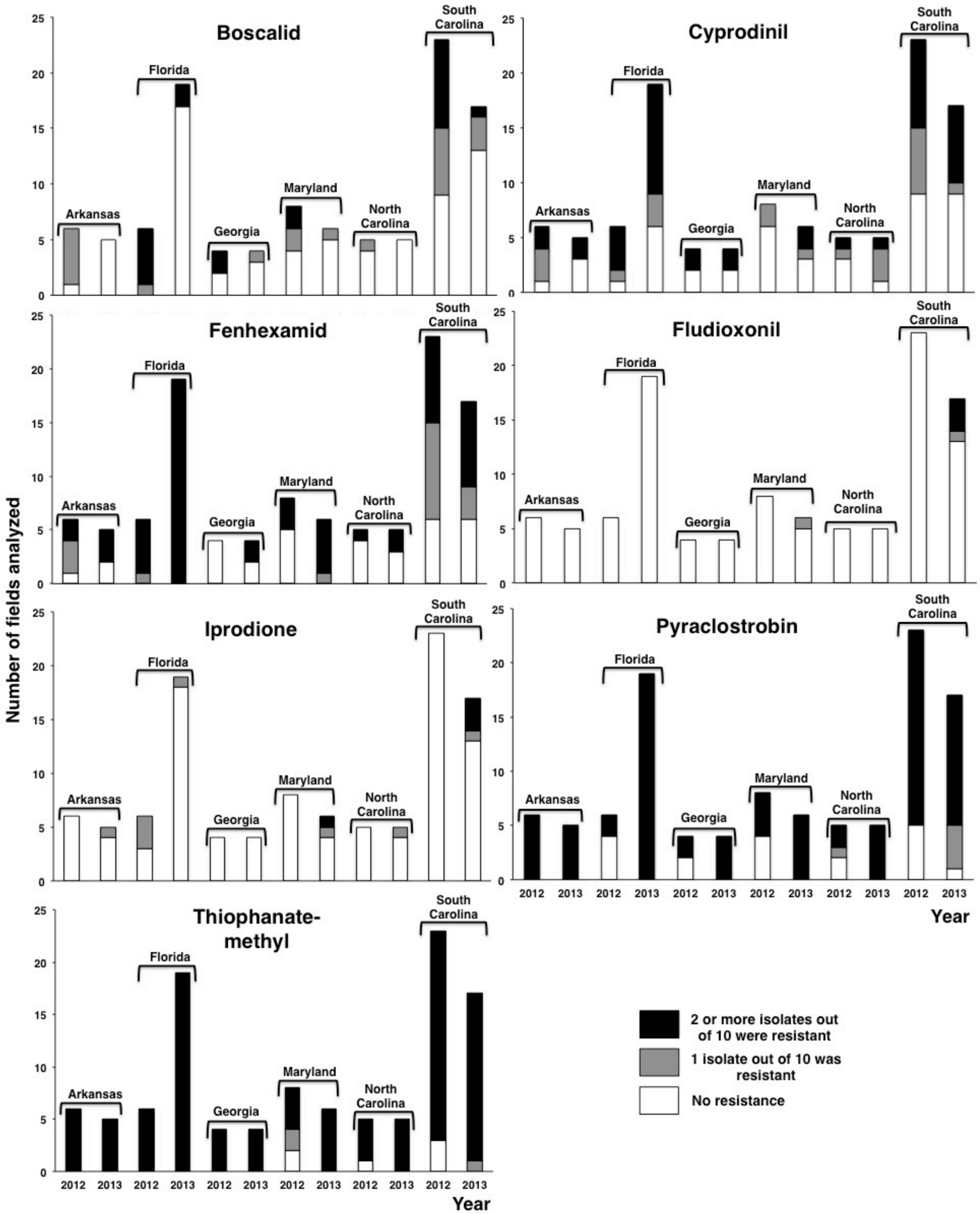


**Fig.1.** Origin and frequency of strawberry fields with fruit-derived *Botrytis cinerea* isolates sensitive or resistant to seven classes of fungicides collected in 2012 and 2013. Results of blossom-derived samples are not included in this report.

## Conclusions

Our data show that fungicide resistance in *B. cinerea* was already present in blossoms, indicating that resistance management needs to be implemented early in the season. The information provided through this monitoring program benefited all growers in that resistance was managed, unnecessary sprays were avoided, and gray mold was effectively controlled. The research provides further insights into the evolution and spread of fungicide resistance and will improve current anti-resistance management strategies.

## Impact Statement

This resistance monitoring program serves all southeastern strawberry growers and provides critical, location-specific disease management recommendations. This service extends the life span of reduced risk fungicides, reduces pesticide input (through avoidance of ineffective sprays), catches resistance built up before an epidemic can cause preharvest and postharvest yield loss, makes producers aware about the need for resistance management, and teaches producers about novel resistance management options.

## Citation(s) for any publications arising from the project

Fernández-Ortuño, D., A. Grabke, A. Amiri, X. Li, N. Peres, P. Smith and G. Schnabel 2013. Fungicide resistance profiles in *Botrytis cinerea* from Southern State Strawberry Fields. **Plant Dis.** Accepted pending revisions.

Grabke, A., D. Fernández-Ortuño, A. Amiri, X. Li, N. Peres, P. Smith and G. Schnabel 2013. Characterization of iprodione resistance in *Botrytis cinerea* from strawberry and blackberry. **Phytopathology**. In press.