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

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Extension Proposal
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Objectives

Determine location-specific resistance profiles and provide realtime fungicide resistance management recommendations

Justification and Description:

Gray mold is one of the most economically important diseases of commercially produced strawberry fruit. In the southeastern United States, the disease is caused by *Botrytis cinerea* Pers. The pathogen may cause blight on leaf or petal tissues, crown rot, stem cankers, cutting rot, and damping-off. The fungus produces germ tubes from conidia that can infect through natural openings or wounds. It is a cool season disease and infection is favored under wet conditions with temperatures below 22^{o}C. Besides actively causing disease during the growing season, the fungus is also able to cause latent infections leading to disease after harvest, either during storage, transit, in the store, or after purchase by the consumer.

Seven classes of site-specific fungicides are currently available for the control of gray mold disease in the United States. They include anilinopyrimidines (APs), dicarboximides (DCs), hydroxyanilides (HAs), methyl benzimidazole carbamates (MBCs), phenylpyrroles (PPs), quinone outside inhibitors (QoIs; disease suppression only) and succinate dehydrogenase inhibitors (SDHIs). The first QoI, SDHI, AP and HA fungicides were registered within 1 to 4 years from each other in the US; in 2001, 2003, 2001, and 1999 for disease control of strawberries. While most active ingredients are sold as solo products, some are sold as mixtures, such as the QoI pyraclostrobin and the SDHI boscalid in form of Pristine 38 WG and Merivon (BASF Corporation, Research Triangle Park, NC) or the AP cyprodinil and the PP fludioxonil in form of Switch 62.5 WG (Syngenta Crop Protection, Inc. Greensboro, NC). Most growers use several different chemical classes of fungicides during the season in form of tank mixtures or alternations, which over time may give rise to populations with multifungicide resistance. Some isolates recovered from strawberries in Germany and France exhibited multidrug resistance in form of ATP-binding cassette (ABC) transporter and major facilitator superfamily (MFS) transporter activity. But in the southeastern United States, resistance to many fungicide classes, including the MBCs, QoIs, SDHIs and HAs is based on target gene alterations.

In previous studies we determined that populations with strains resistant to multiple fungicides are readily selected in fields with frequent (weekly) spray exposure. In addition, each farm has not only its own fungicide resistance profile, but also a single, most dominant genotype (Table 1).

As a result of this work and with the help of previous year's SRSFC funds, the sensitivity to seven chemical classes of fungicides was investigated in more than 800 *B. cinerea* isolates collected from primarily strawberry blossoms of 80 strawberry fields from at least 7 southern states in the United States during 2014. Ten isolates were examined from each field. Fungicide sensitivity assays were carried out based on visual assessment of diametrical mycelial growth after 4 days of incubation on media amended with discriminatory doses of fungicides in microtiter plates. Results of visual assessments were verified with selected isolates using a previously published germination assay and by inoculating representative isolates with resistant phenotypes on fungicide-sprayed fruit. Results were shared within 5 days of receiving the samples with the person that submitted the sample and the grower. Attached to this grant proposal is an example of a report. It contains:

1. Information about the sample origin

2. Information about the resistance risk to each of the 7 chemical classes based on lab results
3. A recommendation for managing resistance and optimize gray mold control

TABLE 1. Phenotypic variation in *Botrytis cinerea* isolates from blackberry and strawberry fields in North and South Carolina

			Number of isolates															
Phenotype		Single resistances (SR)	Blackberry field[b]					Strawberry field[b]										
No.	Resistance to fungicide[a]		CB^{0}	WM$^{<5}$	KC$^{>12}$	MC$^{>12}$	CO$^{>12}$	WIC^{0}	MV$^{<5}$	HP$^{<5}$	FLOR$^{<5}$	NC$^{N/A}$	GIK$^{>12}$	MOD$^{>12}$	SBY$^{>12}$	JEY$^{>12}$	KUD$^{>12}$	Total
1	None	0SR	51	4	1	0	0	8	8	29	10	5	3	2	0	0	0	**121**
2	Py	1SR	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
3	Tm	1SR	0	24	0	0	0	0	0	3	0	0	1	0	0	0	0	**28**
4	Tm-bo	2SR	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
5	Tm-cy	2SR	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	**3**
6	Tm-py	2SR	0	2	1	0	0	3	0	0	0	0	2	0	1	0	0	**9**
7	Tm-ip-fl	3SR	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
8	Tm-py-bo	3SR	0	7	33	3	19	2	1	0	1	0	6	3	3	2	8	**88**
9	Tm-py-cy	3SR	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	**3**
10	Tm-py-fe	3SR	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
11	Tm-py-bo-cy	4SR	0	0	0	4	8	6	2	0	2	3	5	8	11	18	12	**79**
12	Tm-py-bo-fe	4SR	0	1	14	0	4	0	0	0	0	1	0	0	8	1	1	**30**
13	Tm-py-bo-ip	4SR	0	1	0	7	3	0	0	0	0	0	0	0	1	0	0	**12**
14	Tm-py-bo-cy-ip	5SR	0	1	0	1	0	0	0	0	0	0	0	0	1	2	0	**5**
15	Tm-py-bo-ip-fe	5SR	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	**1**
16	Tm-py-bo-cy-fe	5SR	0	0	2	0	0	0	0	1	0	3	3	5	11	2	0	**27**
17	Tm-py-cy-ip-fl	5SR	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	**2**

[a]py = pyraclostrobin (QoI), tm = thiophanate methyl (MBC), bo = boscalid (SDHI), cy = cyprodinil (AP), ip = iprodione (DC), fl = fludioxonil (PP), fe =

[b]the numbers in superscript following field names indicate the estimated number of sprays with site-specific fungicide per season.

In 2014 multifungicide resistant isolates of *B. cinerea* were again widespread in southern states. Resistance to thiophanate-methyl and pyraclostrobin was found in 70 to 80% of the samples collected. Resistance to fenhexamid, cyprodinil, and iprodione is on the rise compared to 2013 (data not shown), but fludioxonil and boscalid resistance was rarely found. Maybe resistance to dicarboxamide fungicide Rovral increased because of the favorable resistance profile in regard to this fungicide over the last few years encouraged many growers to start using it prior to bloom. Surprisingly, resistance to some major players has not increased. For example, resistance to SDHI fungicides is still very low and additional monitoring suggests that the new generation SDHIs expected to be registered in 2015 (fluxapyroxad from Bayer) are not necessarily cross resistant to other SDHIs (data not shown). The data also show that fungicide resistance in *B. cinerea* was already present in blossoms, indicating that resistance management needs to be implemented early in the season.

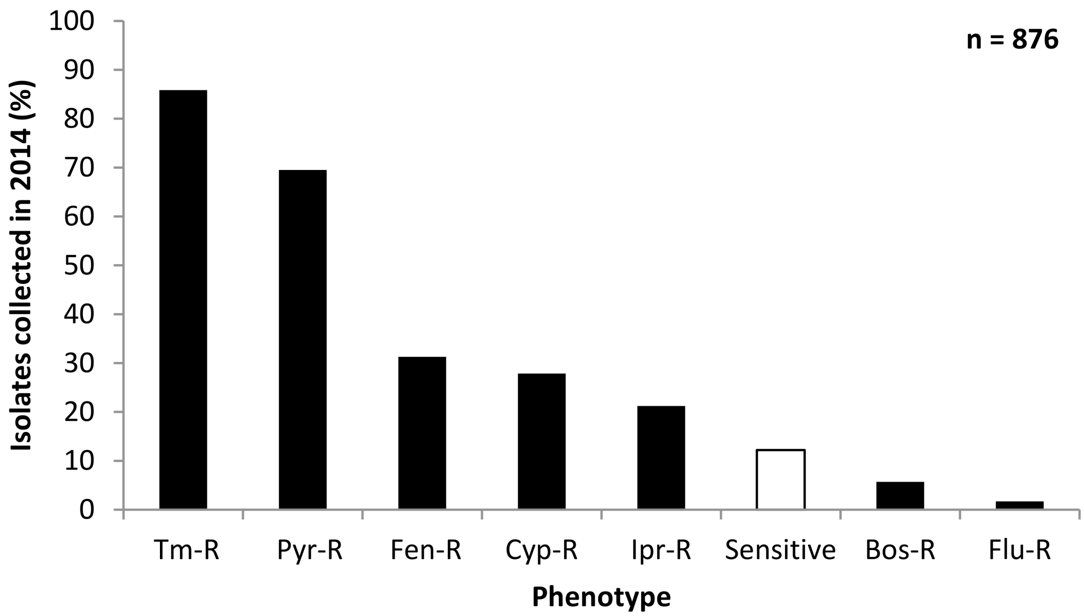


Fig. 1. Summary of fungicide resistance in *Botrytis cinerea* from commercial strawberry fields to 7 chemical classes. Tm = thiophanate methyl; pyr = pyraclostrobin; fen = fenhexamid; cyp = cyprodinil; ipr = iprodione; bos = boscalid; and flu = fludioxonil.

A questionnaire filled out by 16 strawberry producers in 2013 from SC, MD, PA, VA, and NC revealed that the service not only helps with preharvest and postharvest control but also adds an educational component. Here is a summary of the survey results:

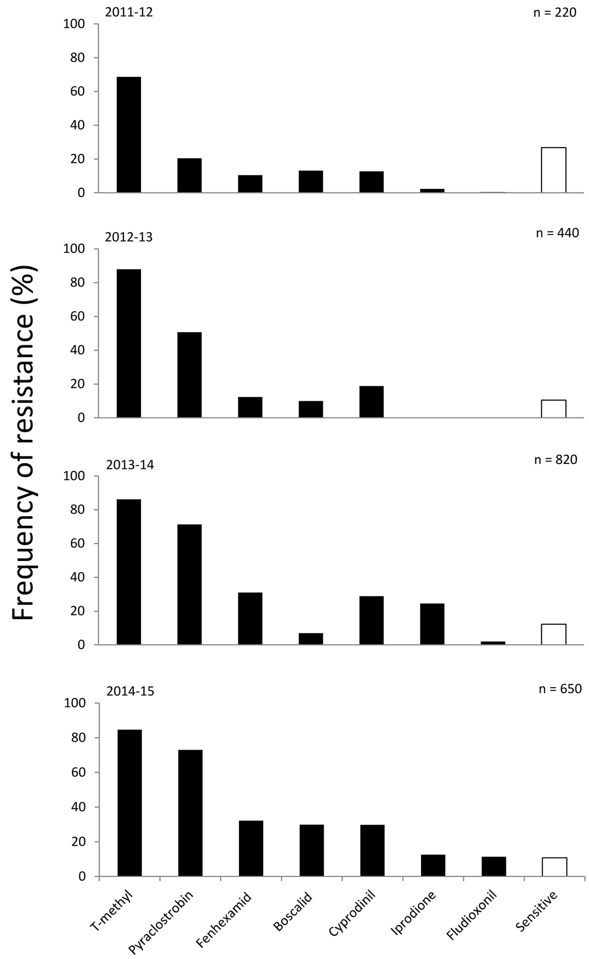
1. All growers followed our recommendations and experienced either good or very good gray mold control
2. All of the growers want the service to continue
3. All established growers though they did a better job controlling the disease
4. All are more aware of the need for fungicide resistance management
5. All learned more about chemical control options
6. All established growers improved resistance management tactics

We propose to continue the locations-specific fungicide resistance profiling service for growers in the southern states. Due to its popularity, the number of early season flower samples continued to rise over the last few years. With the help of the SRSFC and matching funds from strawberry associations and other small grants, we can provide this service for free for our growers.

Results and Conclusions

The frequency of 650 *Botrytis cinerea* isolates resistant to individual fungicide classes in strawberry fields from South Carolina and other states was investigated. In South Carolina, the frequencies of isolates with resistance to thiophanate-methyl, pyraclostrobin, fenhexamid, boscalid, cyprodinil, or iprodione were 96.7, 18.9, 5.6, 7.8, 21.1, and 1.1% in the 2011-12 season, 98.8, 51.8, 14.1, 5.3, 11.8, and 0.6%, in the 2012-13 season, 92.0, 69.7, 25.7, 4.7, 27.0, 24.0, and 3.0%, in the 2013-14 season, and 92.1, 81.8, 31.5, 28.2, 25.5, 8.2, and 6.4% in the 2014-15 season, respectively (Fig. 1). The frequencies of isolates sensitive to all seven fungicides tested were 3.3, 1.2, 6.7, and 3.3% in 2011-12, 2012-13, 2013-14, and 2014-15, respectively (Fig. 1). Resistance to thiophanate-methyl was consistently found at high frequencies, whereas resistance to fludioxonil was found only in the isolates collected in the 2013-14 (3.0%) and 2014-15 (6.4%) seasons. Frequencies of isolates with resistance to pyraclostrobin, fenhexamid, or fludioxonil consistently increased from the 2011-12 to the 2014-15 seasons. Resistance to boscalid increased dramatically in season 2014-15 (28.2%) compared to seasons 2011-12 (7.8%), 2012-13 (5.3%) and 2013-14 (4.7%). Isolates collected in 2014-15 season had the highest resistance frequencies for pyraclostrobin, fenhexamid, boscalid, and fludioxonil (Fig. 1).

Similar to the results obtained for South Carolina, resistance profiles of isolates collected from 10 eastern states revealed an increase of resistance to pyraclostrobin, fenhexamid, and cyprodinil from 2012 to 2015 (Fig. 1). Resistance to thiophanate-methyl was found in nearly every location in all four seasons and resistance to fludioxonil was rarely found with the exception of isolates collected in season 2014-15 (11.3%). Isolates collected in the 2014-15 season showed the highest resistance frequencies for pyraclostrobin, fenhexamid, boscalid, cyprodinil, and fludioxonil (Fig. 1).



Impact Statement

Fungicide resistance makes fungicide applications ineffective and exposes the crop to diseases that normally would be controlled with fungicides. With our service we established emerging and existing resistance to fenhexamid, fludioxonil, pyraclostrobin, boscalid, iprodione, thiophanate methyl, and cyprodinil, all commonly used products for gray mold control. This regionwide resistance monitoring service and associated communication of recommendations to growers provides benefit for commercial growers because it allows them to identify ineffective products, design more effective spray programs, and by applying resistance management practices extends the life of reduced risk fungicides.

Peer reviewed papers supported by this research and extension effort:

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