Monitoring fungicide resistance in *Botrytis cinerea*, *Phytophthora* spp., *Colletotrichum acutatum* and *C. gloeosporioides*

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Summary

In recent years, our program has been continually monitoring the population of important pathogens affecting Florida strawberry fields for their resistance to fungicides. Unfortunately, resistant populations to at least one fungicide group have been found for *Botrytis cinerea, Phytophthora* spp., *Colletotrichum acutatum*, and *C. gloeosporioides*. During the 2019-20 season, an increase in resistance to Luna® Tranquility (fluopyram) and Switch® (fludioxonil) with frequencies of 62.1 and 34.5%, respectively, were observed in Botrytis isolates collected from commercial fields. However, resistance frequencies to Kenja® (isofetamid) and Miravis® Prime (pydiflumetofen) were below 3%. For Phytophthora, resistance to mefenoxam was observed in 9% of the isolates causing crown rot, but no resistance was found for isolates causing leather rot disease on the fruit. Furthermore, *C. gloeosporioides* isolates collected from symptomatic strawberry crowns had a low resistance frequency to Abound® (azoxystrobin) and Topsin® (thiophanate-methyl). However, isolates resistant to Abound® were also resistant to Topsin®.

Methods

Objective 1. Monitoring resistance of *Botrytis cinerea* isolates to fungicides used for BFR control.
Isolates were collected from nine different strawberry fields in Central Florida during the 2019-20 strawberry season. Fungicide resistance evaluation was conducted using a conidial germination essay. After isolation, isolates were incubated on HA culture medium for 7 days at ~23˚C to attain profuse sporulation. Conidia were collected in water and diluted to 10^6 conidia/ml. A 7-microliter drop of the spore suspension of each isolate was placed on 40 to 50 ml of Yeast Bacto Agar (YBA) growth medium for SDHIs (Kenja®, Luna®, and Miravis®) and Malt Extract Agar (MEA) for the Phenyl Pyrrole (Switch®) fungicides. YBA was amended with 2 or 5 µg/ml of fluopyram (Luna® Tranquility), 1 or 5 µg/ml of isofetamid (Kenja®), and 1, 3 or 5 µg/ml of pydiflumetofen (Miravis® Prime) and MEA with 0.1 or 10 µg/ml of fludioxonil (Switch®) to monitor their resistance levels. Fungicide resistance was determined based on the combination of the number of conidia germinated and germ tube elongation. The assay was conducted for all fungicides to determine the resistance profile for each isolate and obtain the frequency of fungicide resistance.

Objective 2. Monitoring resistance of *Phytophthora* species for resistance to mefenoxam (RidomilGold®).
Mycelial discs (4-mm diameter) of isolates from different fields were grown on P_5ARP medium for 4-7 days and transferred to plates containing V8 medium amended with 0, 5 and 100 µg/ml of mefenoxam. Three plates were used as replications for each isolate-fungicide combination tested and the experiment was repeated once. Plates were incubated at room temperature and mycelial growth assessed after 4 days by measuring the colony diameter (two perpendicular measurements). Based on their growth on the discriminatory doses tested,

isolates were separated in two groups, sensitive or resistant.

Objective 3. Monitoring resistance of *Colletotrichum acutatum* isolates to azoxystrobin.

C. acutatum isolates were recovered on general isolation medium from symptomatic samples. Pure cultures were obtained, and isolates were challenged with azoxystrobin. Mycelial plug discs (4-mm diameter) of each isolate were grown on potato dextrose agar (PDA) for five days and transferred to azoxystrobin-amended (3 and 100 µg/ml) and non-amended medium (control). Two plates (replications) per each combination of isolate-fungicide concentration were tested and the experiment was repeated once. Plates were incubated at room temperature and mycelial growth assessed after 72 hours by measuring colony diameter.

Objective 4. Monitoring *C. gloeosporioides* isolates for sensitivity to the most commonly used fungicides for CCR management.

The crowns of wilting strawberry plants received by the Plant Diagnostic Clinic at the GCREC were cut in half to make isolations onto general isolation medium. The isolates that were identified as *C. gloeosporioides* were transferred to Potato Dextrose Agar (PDA). In total, 52 isolates were collected and tested in vitro for their sensitivity to Abound® (azoxystrobin) and Topsin® (thiophanate-methyl) at 3 and 100 µg/ml.

Results

Objective 1

In the 2019-20 season, 261 isolates were collected from nine different commercial fields. The resistance frequencies were 62.1, 34.5, 2.7, and 0.4% for fluopyram, fludioxonil, isofetamid, and pydiflumetofen, respectively (Fig. 1). The high resistance frequency observed for fluopyram (Luna®), in particular of highly resistant isolates, indicating that the efficacy of this fungicide in commercial strawberry fields has been compromised. The frequency of moderately resistant isolates to fludioxonil (Switch®) increased 6.1% compared to the 2018-19 season. Fortunately, highly resistant isolates have not been found, and the fungicide is still performing well in our field efficacy trials. Resistance frequencies to isofetamid (Kenja®) and pydiflumetofen (Miravis®) remain low. Our results suggest that the use of fluopyram should be limited to preserve the efficacy of other fungicides within the same group, such as isofetamid (Kenja®) and pydiflumetofen (Miravis®). Fludioxonil (Switch®) should be used cautiously to prevent further selection of moderately resistant isolates.

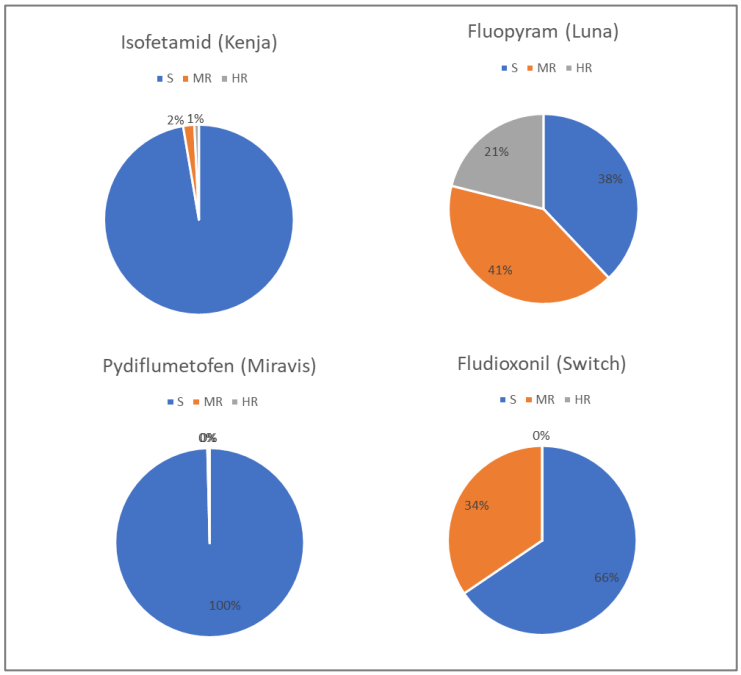

Figure 1. Sensitivity profile of *Botrytis cinerea* isolates to Luna Tranquility, Switch, Kenja and Miravis® Prime during 2019-2020 strawberry season in Florida. S=Sensitive; MR=Moderately Resistant; HR=Highly Resistant

Objective 2

In the 2019-20 season, 25% of the strawberry samples with crown rot symptoms were infected by *Phytophthora* spp. Among 46 samples received in the Plant Diagnostic Clinic at GCREC, four (from different farms) were resistant to RidomilGold® (Fig. 2). In addition, 10 isolates (five from two different farms) caused leather rot disease, but they were all sensitive to mefenoxam. Since resistance to mefenoxam was detected, the continuous monitoring of resistance to this active ingredient, should be considered for disease management recommendations.

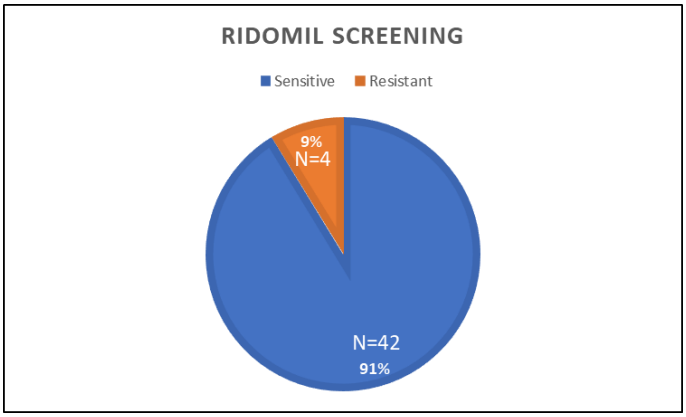

Figure 2. Sensitivity profile of *Phytophthora* spp. isolates to mefenoxam (RidomilGold®), causing strawberry crown rot during 2019-2020 season.

Objective 3

In the 2019-20 season, 1% of the strawberry samples received by the diagnostic clinic at GCREC were infected with *C. acutatum*. Among those 3 samples, two (from different farms) were resistant to azoxystrobin, whereas the other was sensitive. Resistance to azoxystrobin was first detected in 2014, and the continuous monitoring of resistance should be considered for disease management recommendations.

Objective 4

During the 2019-20 season, fifty-two *C. gloeosporioides* isolates were recovered from strawberry crowns. Isolates were evaluated for sensitivity to azoxystrobin and thiophanate-methyl. Results indicate that 9.6% (n=5) of the isolates were resistant to azoxystrobin (Abound®) (Fig. 3A), and 7.7% (n=4) were resistant to thiophanate-methyl (Topsin®) (Fig. 3B). Similar to the previous season (2018-19), all isolates showing resistance to thiophanate-methyl were also resistant to azoxystrobin. Thus, it is important to introduce new formulations from different chemical groups or test alternative fungicides to control CCR in Florida strawberry fields.

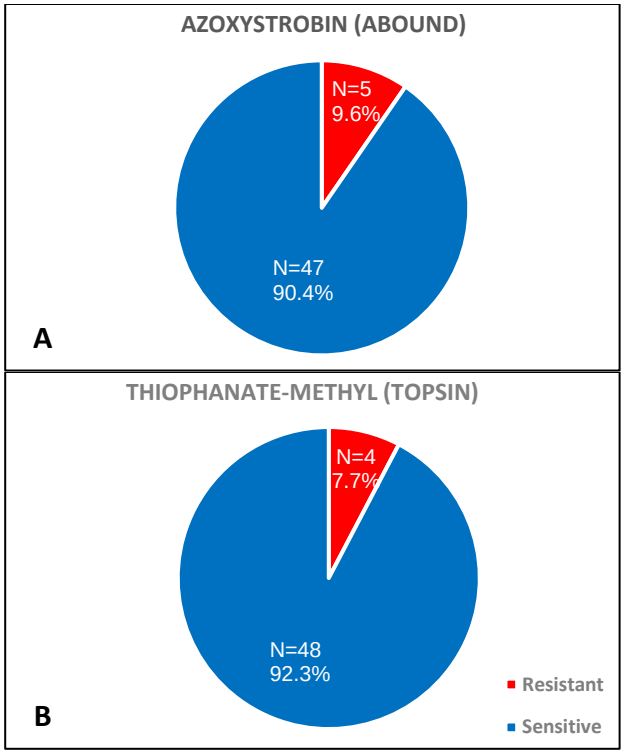

Figure 3. Sensitivity profile of *Colletotrichum gloeosporioides* isolates from strawberry to **A.** azoxystrobin (Abound®) and **B.** thiophanate-methyl (Topsin®) at 3 or 100 µg/ml.

Summary and recommendations

Based on our results, management recommendations for Botrytis are that Switch®, Miravis®, and Kenja® are saved for periods when weather conditions are highly favorable in particular during peak bloom. It is important to note that both Switch® and Miravis® contain fludioxonil, and no more than 4 applications of fludioxonil should be made in a season. Moreover, the Miravis® label is limited to two applications per season. Pydiflumetofen, the other ingredient of Miravis® is in the Group 7 fungicide class, the same as Kenja®, Luna®, Fontelis®, and Merivon® and no more than 4 applications of Group 7 fungicides altogether should be used in a season. To minimize resistance selection, multi-site fungicides such as thiram and captan should be used during periods of moderate or low disease pressure. Thiram has been shown highly effective for Botrytis management in our field efficacy trials. For the diseases caused by Colletotrichum spp., anthracnose fruit rot and crown rot, preventive captan applications are recommended since resistance to Abound® and other QoI fungicides limit their efficacy. Finally, Ridomil® applications are still recommended for control of

Phytophthora, but samples should be submitted for resistance testing if control failure is observed.

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