

# Rapid Diagnosis and Monitoring Fungicide Resistance of Major and Emerging Strawberry Diseases in Florida and Nursery Production Fields

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## Summary

The strawberry pathology lab at the UF-GCREC has successfully developed a high-throughput high-resolution melting (HRM) assay, which allows preliminary diagnostic results to be sent 24 to 48 h after sample arrival, followed by final reports that are validated by standard culture procedures in the clinic. In total, 379 strawberry samples were processed during the 2020-21 season, of which 154 exhibited crown and root symptoms and 167 leaf spot symptoms. In recent years, our program has been continually monitoring populations 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., and *Colletotrichum acutatum*. During the 2020-21 strawberry season, an increase in resistance to Luna® Tranquility (fluopyram) and Switch® (fludioxonil) with frequencies of 99.0 and 39.0%, respectively, were observed in *Botrytis* isolates collected from commercial fields. Resistance frequency to Kenja® (isofetamid) was 19 %, followed by Miravis® Prime (pydiflumetofen) with a frequency of less than 9 %. For *C. acutatum*, only 6 samples were received, but they were all resistant to azoxystrobin. For *Phytophthora*, resistance to mefenoxam occurred in 16% of the isolates received. This information will help guide disease management programs in the next season.

## Methods

**Objective 1. To provide rapid and accurate diagnosis of strawberry diseases and monitoring emerging pathogen populations in strawberry fruit production.**

Our HRM assay developed for the detection of common crown rot pathogens of strawberry and *Neopestalotiopsis* spp. is advantageous to growers because of its simplicity, accuracy, reproducibility, and low cost. With the kind donation of a Light Cycler 480 system by the FSREF, this assay is implemented in the plant diagnostic clinic and becomes part of standard diagnostic procedures for strawberry samples, particularly with crown rot and leaf spot symptoms. We plan to continue performing rapid diagnosis for strawberry samples and providing growers with preliminary results within 24 to 48 h. Strawberry samples provided by growers or consultants or delivered by mail were processed for the HRM assay and validated by standard culture procedures.

**Objective 2. Monitoring resistance of *Botrytis cinerea* isolates to fungicides used for BFR control.**

Isolates were collected from four different strawberry commercial fields in Central Florida during the 2020-21 season. Fungicide resistance evaluation was conducted using a conidial germination assay. After isolation, isolates were incubated on HA culture medium for 7 days at ~23°C to obtain profuse sporulation. Conidia were collected to prepare suspensions of 10<sup>6</sup> conidia/ml. A 7-microliter drop of

the conidial suspension of each isolate was placed on 40 to 50 ml of Yeast Bacto Agar (YBA) growth medium used to test SDHIs (Kenja<sup>®</sup>, Luna<sup>®</sup>, and Miravis<sup>®</sup>) and on Malt Extract Agar (MEA) for the Phenyl Pyrrole (Switch<sup>®</sup>) fungicides. YBA was amended with 2 or 5 µg/ml of fluopyram (Luna<sup>®</sup> Tranquility), 1 or 5 µg/ml of isofetamid (Kenja<sup>®</sup>), and 1 or 3 µg/ml of pydiflumetofen (Miravis<sup>®</sup> Prime) and MEA with 0.1 or 10 µg/ml of fludioxonil (Switch<sup>®</sup>) 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 twice for all fungicides to determine the resistance profile for each isolate and obtain the frequency of fungicide resistance.

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

*C. acutatum* isolates were recovered on general isolation (GI) 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 was assessed after 72 h by measuring colony diameter.

### **Objective 4. Monitoring resistance of *Phytophthora* species for resistance to mefenoxam (RidomilGold<sup>®</sup>).**

Mycelial discs (4-mm diameter) of isolates from different fields were grown on P<sub>5</sub>ARP 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 was assessed after 4 days by measuring the colony diameter (two perpendicular measurements). Based on their growth on the discriminatory doses tested,

isolates were separated into two groups, sensitive or resistant.

## **Results**

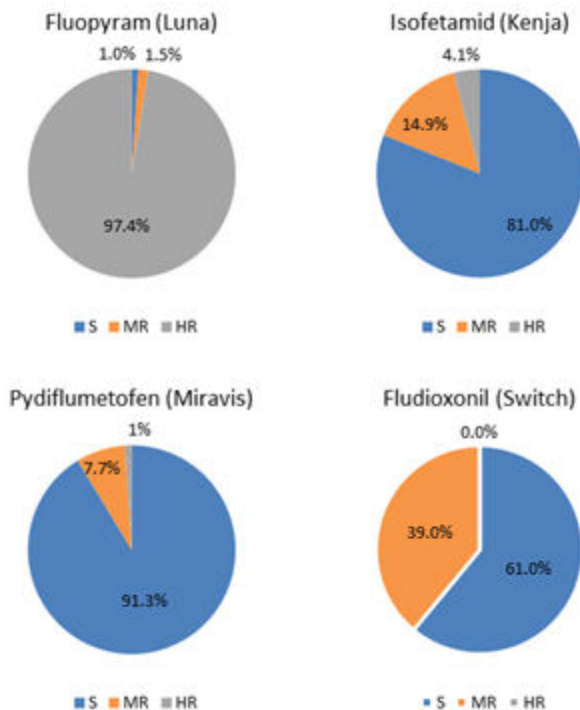
### **Objective 1**

During the past season (2020-2021), we processed 379 strawberry samples, of which 154 exhibited crown and root symptoms and 167 leaf spot symptoms (Appendix, Table 1). The number of samples processed during the 2020-21 season was 50% higher than we usually received in previous seasons (n = 250). Likely, this increase was due to the concern about *Neopestalotiopsis* sp. and is consistent with the increased number of leaf spot samples. With the aid of our HRM assay, preliminary diagnostic results were provided to growers within 24 to 48 h of sample arrival, followed by final reports that were validated by standard culture procedures in the clinic. Consequently, this technique largely improves the decision-making for the timely deployment of effective disease management practices.

### **Objective 2**

During the 2020-21 season, 195 Botrytis isolates were collected from commercial strawberry fields. Overall, the combined frequencies of moderately and highly resistant isolates were 99, 19, 8.7, and 39% for fluopyram (Luna), isofetamid (Kenja), pydiflumetofen (Miravis), and fludioxonil (Switch), respectively (Fig. 1). An increase of 34.8 percentage points was observed for fluopyram (Luna<sup>®</sup>) compared to the 2020-21 resistance frequency. Furthermore, a high frequency of highly resistant isolates was observed, indicating an increase in the resistant *B. cinerea* population in commercial fields. Resistance frequencies to isofetamid (Kenja<sup>®</sup>) and pydiflumetofen (Miravis<sup>®</sup>) remained low. However, the corresponding increases of 17.9 and 8.3 percentage points were observed when compared to the 2019-20 season. Moderately resistant isolates to fludioxonil (Switch<sup>®</sup>) increased by 3.2 percentage points compared to the previous season, but highly resistant isolates were not found during the 2020-21 season. Interestingly, even with the increase in moderate resistance, the fungicide Switch<sup>®</sup> was the best performing in our fungicide efficacy trial conducted at Fancy Farms during 2020-21 (Appendix

2), reducing BFR incidence by more than 50% compared to the non-treated control. Our results indicate that the use of fluopyram (Luna®) 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 future selection of resistant isolates. We recommend fungicides Kenja®, Miravis®, Switch® to be applied only during weeks with high risk for infection, as determined by the Strawberry Advisory System (StAS, <http://sas.agroclimate.org>). We also suggest further investigation to better understand the increase of moderately resistant isolates to Switch® *in vitro*, yet with high efficacy in the field.



**Figure 1.** Sensitivity profile of *Botrytis cinerea* isolates to Luna Tranquility, Kenja, Miravis® Prime, and Switch during the 2020-2021 strawberry season in Florida. S=Sensitive; MR=Moderately Resistant; HR=Highly Resistant.

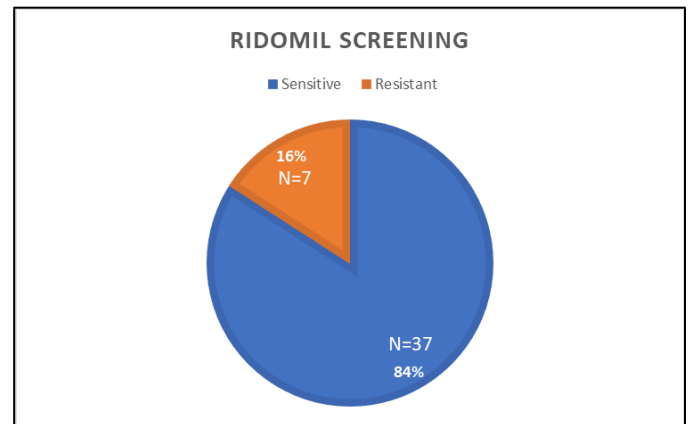
### Objective 3

In the 2020-21 season, 2% (6 samples) of the strawberry samples received by the diagnostic clinic at the GCREC were infected with *C. acutatum*, of which only one *C. acutatum* was causing crown rot, and the other 5 were anthracnose fruit rot. All 6

samples received were resistant to azoxystrobin. Resistance to azoxystrobin was first detected in 2014, and the continuous monitoring of resistance has shown that resistance seems to be widespread now.

### Objective 4

In the 2020-21 season, 45% of the strawberry samples with crown rot symptoms were infected by *Phytophthora* spp. Of 44 samples received in the Plant Diagnostic Clinic at the GCREC, seven (from four different farms) were resistant to mefenoxam (Ridomil Gold®) (Fig. 2). 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 (Ridomil Gold®), causing strawberry crown rot during the 2020-21 season.

## Summary and recommendations

Based on our results, management recommendations for Botrytis fruit rot 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. In addition, 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. 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 a control failure is observed.

## Disclaimer

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## APPENDIX 1

### REPORT ON STRAWBERRY SAMPLES PROCESSED IN THE DIAGNOSTIC LABORATORY (GCREC)

**Table 1.** An overview of the strawberry samples processed during the 2020-21 season by disease and pathogen diagnosed.

Disease / Pathogen	Number of samples
<b>Leaf spot</b>	
<i>Botrytis cinerea</i>	4
<i>Cercospora</i> spp.	2
<i>Diplocarpon earliana</i>	4
<i>Gnomonia</i> sp.	38
<i>Mycosphaerella fragariae</i>	1
<i>Phomopsis</i> sp.	7
<i>Podosphaera aphanis</i>	8
<i>Xanthomonas fragariae</i>	6
<i>Neopestalotiopsis</i> spp.	97
<b>Crown rot</b>	
<i>Colletotrichum gloeosporioides</i>	56
<i>Macrophomina phaseolina</i>	20
<i>Phytopythium helicoides</i>	2
<i>Phytophthora</i> sp.	69
<i>Neopestalotiopsis</i> spp.	6
<i>Collectotrichum acutatum</i>	1
<b>Fruit rot</b>	
<i>Collectotrichum acutatum</i>	5
<i>Collectotrichum gloeosporioides</i>	1
<i>Neopestalotiopsis</i> spp.	7
<b>Nematodes</b>	
<i>Belonolaimus longicaudatus</i>	2
<i>Pratylenchus</i> sp.	1
<b>Mites</b>	
<i>Tetranychus urticae</i>	1
<b>Phytoplasma</b>	
<i>Phytoplasma</i>	2
<b>No pathogen found</b>	
NPF	39
<b>Total number of samples</b>	<b>379</b>

## APPENDIX 2

### FUNGICIDE EFFICACY TO CONTROL BOTRYTIS FRUIT ROT OF STRAWBERRY DURING THE 2020-2021 SEASON

An experiment was conducted at a strawberry commercial field in Plant City to evaluate the efficacy of products to control Botrytis fruit rot (BFR). Treatment application was performed weekly, but the single-site fungicides were applied based on conducive environmental conditions (17 to 25°C and ≥ 12 h leaf wetness) for Botrytis infection following the Strawberry Advisory System (StAS, <http://sas.agroclimate.org>). Fruit were harvested twice a week to determine yield and BFR incidence. After each harvest, marketable fruit were weighed to determine yield in pounds per acre, and BFR incidence was quantified as the percentage of infected fruit of the total number of fruit harvested. Yield and disease incidence were analyzed by fitting a generalized linear mixed model using the statistical software SAS and means were separated according to Fisher's Protected LSD test ( $\alpha = 0.05$ ).

In table 1, the average disease incidence for the whole season is presented. BFR incidence in the non-treated control (NTC) averaged 14.8%. All treatments reduced BFR incidence, but the most effective included Switch rotated with Captan Gold 80WDG or Sil-Matrix, Kenja, Thiram SC at 2.5 qt and Thiram 1.5 qt + Captan Gold 4L. Furthermore, Switch rotated with Captan Gold 80WDG was associated with the highest yield during the experiment. The treatments of Switch rotated with Sil-Matrix, and Thiram SC at 2.5 qt and Thiram 1.5 qt + Captan Gold 4L also increased yield compared to the NTC.

Treatment (products and rates/A)	Application timing <sup>z</sup>	Yield (lb/A) <sup>y</sup>	BFR incidence (%) <sup>x</sup>
Switch 62.5WG 14 oz	4, 7, 10, 12, 13	22979 a <sup>w</sup>	4.4 c
Captan Gold 80WDG 1.9 lb	1, 2, 3, 5, 6, 8, 9, 11, 14		
Thiram SC 2.5 qt	weekly	18266 b	4.8 c
Kenja 400SC 15 fl oz	4, 7, 10, 12, 13	16360 bc	5.2 bc
Captan Gold 80WDG 1.9 lb	1, 2, 3, 5, 6, 8, 9, 11, 14		
Switch 62.5WG 14 oz	4, 7, 10, 12, 13	18416 b	5.4 bc
Sil-Matrix 1%	1, 2, 3, 5, 6, 8, 9, 11, 14		
Thiram SC 1.5 qt + Captan Gold 4L 1.5 qt	weekly	18068 b	5.9 bc
Luna Tranquility 20 oz	4, 7, 10, 12, 13	16255 bc	8.0 b
Captan Gold 80WDG 1.9 lb	1, 2, 3, 5, 6, 8, 9, 11, 14		
Non-treated control	-	13201 c	14.8 a
Probability of a greater F value		0.0016	0.0001

<sup>z</sup> Week of product application over the 14 weeks from 24 Nov 2020 to 23 Feb 2021.

<sup>y</sup> Yield from 21 harvests made from 1 Dec 2020 to 26 Feb 2021.

<sup>x</sup> Average of Botrytis fruit rot (BFR) incidence for the whole season (1 Dec 2020 to 26 Feb 2021).

<sup>w</sup> Values in a column with the same letter are not significantly different based on least significant difference (LSD) test ( $\alpha = 0.05$ ).