Colletotrichum crown rot: screening for alternative fungicides and monitoring for fungicide resistance in Florida strawberry fields

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Summary

In the 2018-2019 Florida strawberry season, *Colletotrichum gloeosporioides* was one of the most prevalent pathogens diagnosed from crown rot samples received at our diagnostic clinic. In a field trial, the not yet labeled succinate-dehydrogenase inhibitor (SDHI) fungicide Approvia (benzovindiflupyr), successfully reduced Colletotrichum crown rot (CCR). Preliminary results from our pathogenicity tests demonstrated that three species within the *C. gloeosporioides* species complex cause CCR in Florida, *C. siamense* *C. fructicola*, and *C. theobromicola*. However, fungicide resistance was only found within isolates of *C. siamense*.

Methods

Screening of SDHI fungicides for CCR management.
In a field trial at the Gulf Coast Research and Education Center, 'Strawberry Festival' plants were inoculated with *Colletotrichum gloeosporioides* 4 weeks after planting. Sixteen fungicide treatments were tested (Table 1) and were either applied 2 days before or one day after inoculation. CCR incidence was evaluated weekly for 20 weeks by counting the number of wilted and dead plants. Fungicide treatments were compared with an inoculated and a non-inoculated control.

Baseline sensitivity for fungicides effective for *in vitro* control of *C. gloeosporioides*.
During the 2017-2018 strawberry season, we screened *C. gloeosporioides* isolates for their *in vitro* sensitivity to SDHI fungicides (FRAC 7) at 100 µg/mL. The fungicides containing penthiopyrad (Fontelis) and benzovyndiflupyr (Approvia) were the most effective against *C. gloeosporioides* in vitro. However, the initial fungicide concentration used was high (100 ppm). In 2018-2019, we selected 100 *C. gloeosporioides* isolates and tested lower concentrations of the two fungicides. Using the spiral gradient dilution assay, we developed a baseline sensitivity profile for *C. gloeosporioides* isolates from Florida.

Optimizing a resazurin-based assay to evaluate the sensitivity of *C. gloeosporioides* to the respiration-inhibitor fungicides.
Previous results with *C. acutatum* in 2017-2018 demonstrated that the resazurin-reduction assay can be used to evaluate sensitivity to QoI fungicides pyraclostrobin and azoxystrobin (Cabrio and Abound). To optimize the same assay for *C. gloeosporioides*, we used 11 isolates with variable sensitivity profiles. The parameters tested were conidial concentrations, culture medium, and time of evaluation of the microplates.

Characterizing the newly described *C. gloeosporioides* species according to their aggressiveness to strawberry transplants and their sensitivity to fungicides.
Strawberry plants of 'Strawberry Festival' (susceptible to CCR) were inoculated with four different species within the *C. gloeosporioides* species complex in a greenhouse. Plant wilt and mortality were evaluated weekly for four weeks. The inoculated isolates were grown in vitro under fluorescent light for 7-10 days and their morphology,

production of conidia and other characteristic fungal structures were observed. Isolates representing the different species were tested in vitro for their sensitivity to thiophanate methyl and azoxystrobin.

Results

Screening of SDHI fungicides for CCR management.

Results from the field trial indicated that Approvia (benzovyndiflupyr) applied either 2 days before inoculation or 1 day after inoculation was highly effective in reducing plant wilt and mortality. Omega (fluazinam) was also highly effective. These results reinforce the potential post-infection activity of Approvia and that the product could serve as an alternative to manage CCR in Florida if registered in the near future. However, it is uncertain at this point if Syngenta will register Approvia for strawberry. A special request for registration of Omega for nursery usage has been submitted since this fungicide is unlikely to be registered for use on strawberry production fields. Miravis, another SDHI from Syngenta that was included in the 2018-2019 trial, was not effective against *C. gloeosporioides*.

Table 1. Colletotrichum crown rot incidence in different fungicide treatments applied 2 days before inoculation (2 dbi) or 1 day after inoculation (1 dai).

Treatment	Time of application	11 wks after inoculation Mortality (%)
Control, non-inoculated	na	1.0 e
Omega[x]	2 dbi	6.0 de
Omega[x]	1 dai	6.0 de
Approvia[x]	1 dai	6.0 de
Cabrio	1 dai	7.0 de
Captan	2 dbi	8.0 de
Approvia[x]	2 dbi	9.0 cde
Fontelis	1 dai	11.0 bcde
Topsin	2 dbi	23.0 abcd
Luna T	1 dai	24.0 abcd
Miravis[x]	2 dbi	24.0 abcd
Fontelis	2 dbi	27.0 abc
Kenja	1 dai	28.0 ab
Control, inoculated	na	29.0 ab
Kenja	2 dbi	30.0 ab
Topsin	1 dai	36.0 a
Luna T	2 dbi	37.0 a
Miravis[x]	1 dai	39.0 a
	P-value	0.0003

x Not registered for strawberry

y Columns with the same letter are not significantly different based on least significant difference (LSD) test (α=0.05).

Baseline sensitivity for fungicides effective for in vitro control of *C. gloeosporioides*. The EC_{50} for benzovindiflypyr and penthiopyrad varied from 0.08 to 1.11 and 0.45 to 3.17 µg/mL, respectively (Fig 1 and 2), whereas the other SDHI fungicides did not inhibit fungal growth. The EC_{50} baseline is useful information for these SDHIs so that pathogen shifts in sensitivity can be monitored in the future.

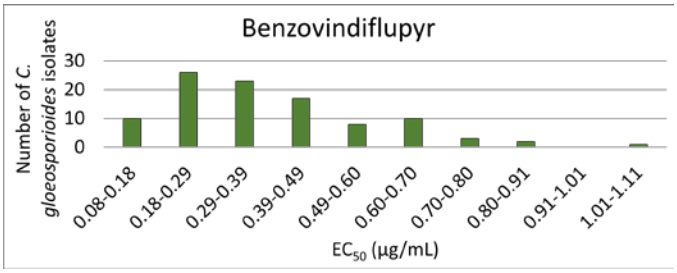

Fig 1. Distribution of the effective concentration of benzovindiflupyr (Approvia) needed to reduce the mycelial growth of *C. gloeosporioides* isolates (n=100) by 50% (EC_{50}).

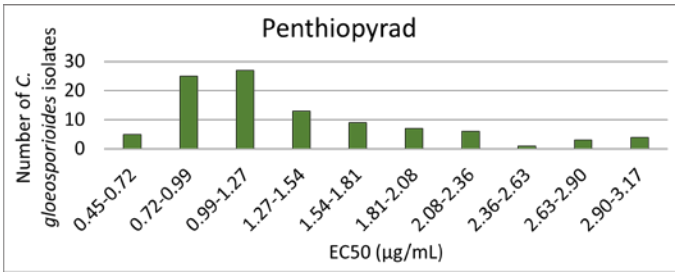

Fig 2. Distribution of the effective concentration of penthiopyrad (Fontelis) needed to reduce the mycelial growth of *C. gloeosporioides* isolates (n=100) by 50% (EC_{50}).

Optimizing a resazurin-based assay to evaluate the sensitivity of *C. gloeosporioides* to the respiration-inhibitor fungicides. The optimal conditions for evaluation of resazurin reduction by *C. gloeosporioides* were 10^5 conidia/ml, complete medium, and evaluation at 24h after preparation of the microplates. We are currently working on the optimization of the medium for the assay since the preparation of the complete medium is expensive and time-consuming.

Characterizing the newly described *C. gloeosporioides* species according to their aggressiveness to strawberry transplants and their sensitivity to fungicides.

This aggressiveness trial is still in progress of evaluation in the greenhouse. Preliminary results show that *C. siamense* and *C. theobromicola* isolates are pathogenic to strawberry. The in vitro

assays demonstrate clear morphological differences between the species (Fig 3) with a growth rate of about 5 mm per day for all species. *C. siamense* isolates were the first to produce spores at 3 days under fluorescent light. Thus far, resistance to thiophanate-methyl and azoxystrobin was found only in isolates of *C. siamense* (Table 2).

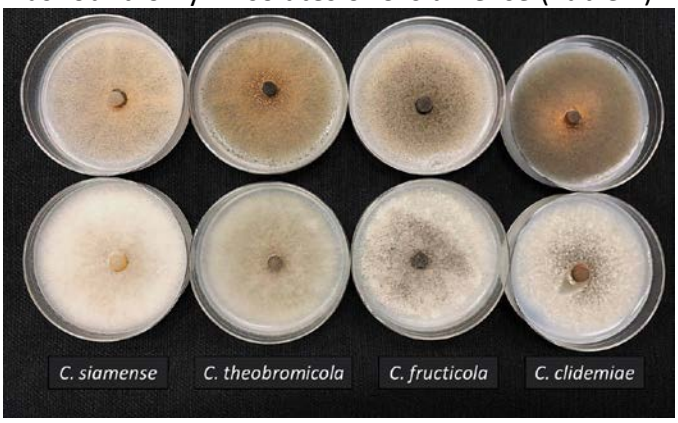

Fig 3. Differences in colony morphology of four species within *Colletotrichum gloeosporioides* species complex found on strawberry. The same isolate was grown under constant fluorescent light (top) and in complete darkness (bottom).

Table 2. Phenotype of resistance of strawberry isolates from three species within *Colletotrichum gloeosporioides* species complex.

	Phenotype (thiophanate-methyl/azoxystrobin) [x]			
Species	R/R	R/S	S/R	S/S
C. siamense	11	1	6	53
C. fructicola	-	-	-	1
C. theobromicola	-	-	-	12

[x] Resistance to thiophanate-methyl (Topsin) and azoxystrobin (Abound) was tested at 3 and 100 µg/mL. R=resistant and S=sensitive.

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