Colletotrichum crown rot: fungicide sensitivity monitoring and genetic characterization of recent populations affecting Florida strawberry fields

Michelle S. Oliveira and Natalia A. Peres

Summary

In the 2017-2018 Florida strawberry season, *Colletotrichum gloeosporioides* was the most prevalent pathogen diagnosed from crown rot samples received at our diagnostic clinic. Isolates collected from these samples presented a low resistance frequency to Abound (azoxystrobin) and Topsin (thiophanate-methyl). In a field trial, Captan applied preventively was effective in reducing plant mortality. Two other fungicides not yet labelled for strawberry, Approvia (benzovindiflupyr) and Omega (fluazinam), were also effective in managing Colletotrichum crown rot (CCR). Preliminary results from our phylogenetic analysis demonstrated that four different species within the *C. gloeosporioides* complex cause CCR in Florida.

Methods

Monitoring of *C. gloeosporioides* to the most common fungicides used for CCR management. Wilting strawberry plants were received by the Plant Diagnostic Clinic at GCREC and the pathogen causing the disease was isolated from the symptomatic infected tissue. Forty-three *C. gloeosporioides* isolates were recovered and tested in vitro for their sensitivity to Abound (azoxystrobin) and Topsin (thiophanate-methyl) at 3 and 100 µg/ml.

Screening of SDHI fungicides for CCR management. Isolates collected were also tested for their sensitivity to Luna (fluopyram), Kenja (isofetamid), Fontelis (penthiopyrad), Merivon (fluxapyroxad), and Approvia (benzovindiflupyr) at 100 µg/ml.

In a field trial, 'Strawberry Festival' plants were inoculated with a mixture of 4 *Colletotrichum gloeosporioides* isolates 4 weeks after planting. Of the 4 isolates, 1 was resistant to Topsin and one was resistant to Cabrio. Twelve fungicide treatments were tested (Table 1) and were either applied 2 days before or one day after inoculation. CCR incidence was evaluated once a week for 24 weeks by counting the number of wilted and dead plants. Fungicide treatments were compared with an inoculated and a non-inoculated control.

Genetic characterization of *C. gloeosporioides* isolates causing CCR. The objective of our study was to phylogenetically characterize *C. gloeosporioides* isolates from strawberry and other non-cultivated plants around Florida strawberry fields. Five genomic regions were sequenced from 53 strawberry isolates and seven isolates from non-cultivated hosts (balsom apple, Brazilian pusley, smilax, magnolia, and wax myrtle). Sequences were analysed and aligned with type species sequences obtained from the online library (GenBank). Phylogenetic trees were created and the species of *C. gloeosporioides* causing CCR in Florida were determined.

Results

Monitoring of *C. gloeosporioides* to the most common fungicides used for CCR management. In the 2017-2018 strawberry season, 35% of crown rot samples received by the Plant Diagnostic Clinic (Balm-FL) were infected with *C. gloeosporioides.*

Among the 43 isolates tested, 6 isolates (14%) were resistant to Abound, and 2 (5%) isolates were resistant to Topsin (Figure 1).
It is important to note that isolates resistant to Abound are also resistant all other fungicides in the same chemical group, i.e. Cabrio, Flint, and Evito.

Screening of SDHI fungicides for CCR management. In vitro sensitivity assays showed that Fontelis (penthiopyrad) and Approvia (benzovindiflupyr) were the most effective among the SDHI fungicides tested in reducing growth of *C. gloeosporioides*.
Results from the field trial indicate that Approvia applied 1 day after inoculation was highly effective in reducing plant mortality as well as disease development (AUDPC) (Table 1). Fontelis, however, was not as effective in the field as it was in vitro. Captan and Omega were the most effective when applied preventively, i.e. before inoculation. Despite only one of the isolates being resistant to Topsin or Cabrio, treatments where these fungicides were applied did not perform well.
These results indicate potential post-infection activity of Approvia but it is uncertain at this point if Syngenta will register this fungicide for strawberries. Omega is unlikely to be registered for use on strawberry production fields, but we have submitted a special request for its registration for nursery usage. We have determined in other trials that Omega is also effective for controlling anthracnose caused by *C. acutatum*. Thus, the registration for nursery would provide them with a fungicide from a different chemical group than the ones currently labeled for fruit production.
We plan to repeat this field experiment in the 2018-2019 strawberry season to confirm efficacy results. We also plan to also include Miravis, another SDHI from Syngenta that is currently being considered for registration on strawberry.

Genetic characterization of *C. gloeosporioides* isolates causing CCR. Phylogenetic analysis revealed that most of the isolates from Florida strawberry (77%) were closely related to *C. siamense*, whereas two of the strawberry isolates were closely related to *C. theobromicola (syn. C. fragariae)*. The biological importance of these different *Colletotrichum* species within the *C. gloeosporioides* species complex as well as fungicide resistance profiles needs to be investigated to determine whether different management strategies are needed in strawberry production fields in Florida.

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Contact

Dr. Natalia A. Peres
UF/IFAS Gulf Coast Research and Education Center
P: 813. 419-6602
E: nperes@ufl.edu

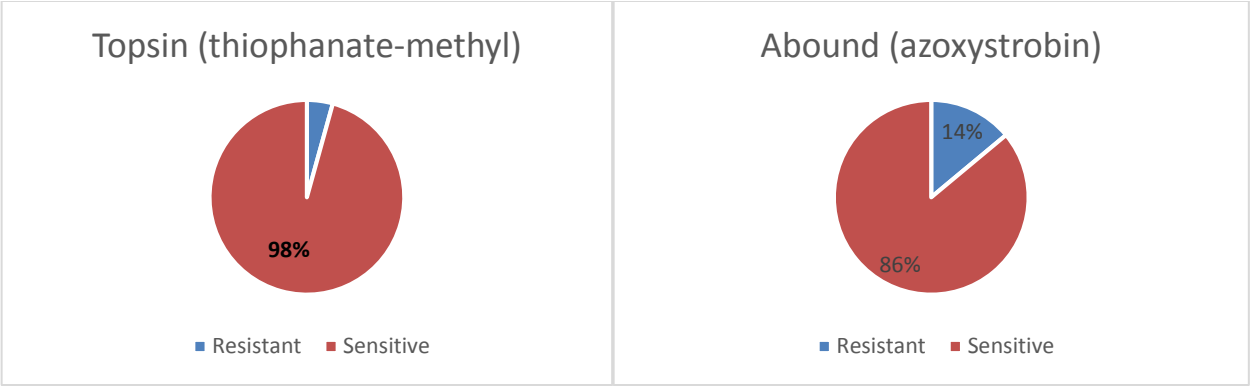

Figure 1. Percentage of Topsin and Abound -sensitive and -resistant isolates of *C. gloeosporioides* evaluated during the 2017-18 strawberry season.

Table 1. Colletotrichum crown rot incidence of different fungicide treatments applied 2 days before inoculation (2dbi) or 1 day after inoculation (1dai) and evaluated 13 weeks after planting.

Treatment	Time of application	Disease incidence (%)		
		Wilt	Mortality	AUDPC[w]
Captan 80WDG	2dbi	12.5	13.8 efg[y]	22.3 cde
Omega [x]	2dbi	18.8	12.5 fg	23.6 cde
Fontelis	2dbi	5.0	33.8 bcde	33.0 bcd
Kenja	2dbi	15.0	40.0 abcd	40.8 ab
Approvia[x]	2dbi	22.5	23.8 cdefg	34.82 abc
Luna Tranquility	2dbi	11.3	43.8 abc	42.7 ab
Topsin	1dai	10.0	51.3 ab	48.6 a
Cabrio 20EG	1dai	7.5	21.3 defg	24.0 cd
Fontelis	1dai	11.3	31.3 bcdef	33.4 bcd
Kenja	1dai	11.3	55.0 a	48.7 a
Approvia[x]	1dai	15.0	6.3 g	20.0 de
Luna Tranquility	1dai	16.3	36.3 abcd	39.4 ab
Control, inoculated	na	14.8	34.6 abcde	36.1 abc
Control, non-inoculated	na	2.5	7.4 g	8.3 e
Probability of a greater F value		0.2516	0.0001	

[w] Area under disease progress curve represents an average of disease progress from weeks 7 to 19 after planting.

[x] Not registered for strawberry; na: not applicable

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