Efficacy and Baseline Sensitivity of Succinate-Dehydrogenase-Inhibitor Fungicides for Management of *Colletotrichum* Crown Rot of Strawberry

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Abstract

The current management of *Colletotrichum* crown rot (CCR) of strawberry, caused by *Colletotrichum gloeosporioides* sensu lato, relies on the use of a few fungicide classes, particularly QoI fungicides. Since resistance to QoI fungicides has recently been detected, alternative fungicide groups are needed to control this disease. Our objective was to evaluate the efficacy of succinate-dehydrogenase-inhibitor (SDHI) fungicides in managing CCR. Five SDHI fungicides, fluopyram, isofetamid, penthiopyrad, fluxapyroxad, and benzovindiflupyr, were applied 2 days before or 1 day after inoculation of cultivar Strawberry Festival. SDHI treatments were compared with the most common fungicides used for

CCR management, i.e., thiophanate-methyl, pyraclostrobin, and captan. Benzovindiflupyr applied 1 day after inoculation was effective in reducing plant mortality and disease development. The baseline sensitivity of *C. gloeosporioides* isolates was determined in vitro using a spiral gradient dilution assay. The EC₅₀ for benzovindiflupyr and penthiopyrad varied from 0.08 to 1.11 and 0.45 to 3.17 µg/ml, respectively, whereas the other SDHI fungicides did not inhibit fungal growth. If registered, benzovindiflupyr could serve as an alternative to manage CCR in Florida.

Keywords: fungi, small fruits, disease management, chemical

Florida is the second largest strawberry producer in the United States with a harvested area of approximately 4,330 ha annually (USDA 2018). Every season, Florida's strawberry production is affected by major diseases, including fruit and crown rots. *Colletotrichum* crown rot (CCR) of strawberry, caused by *Colletotrichum gloeosporioides*, is an important disease for warm strawberry-growing regions (MacKenzie et al. 2009; Peres et al. 2017). *C. gloeosporioides* was recently studied and reclassified as a species complex that includes three species related to strawberry: *C. siamense*, *C. fructicola*, and *C. theobromicola* (syn. *C. fragariae*) (Weir et al. 2012). Since the species causing CCR on strawberry in Florida is not known, we used *C. gloeosporioides* sensu lato in our study. In Florida, the primary sources of inoculum of *C. gloeosporioides* are non-cultivated hosts around strawberry fields, i.e., oak trees, wild grape, and common weeds (MacKenzie et al. 2008; Xiao et al. 2004). Pathogen dispersal is favored by water, especially windy rains and overhead irrigation, which is commonly used for about 10 days after planting to aid plant establishment in Florida.

The symptoms of the disease start with plant wilting, similar to water stress, and develop until plant collapse. A vascular discoloration is observed when the crown is cut in longitudinal section (Peres et al. 2017). Such symptoms might be mistaken for those caused by *Phytophthora* spp. (Smith 1998) and *Macrophomina phaseolina* (Peres et al. 2018), and confirmation of the diagnosis should be done by pathogen identification in a laboratory.

Management of CCR in Florida relies on the use of resistant cultivars. Although there are no completely resistant cultivars to CCR, different levels of susceptibility exist among them. For instance, the two most planted cultivars in the past years ('Florida Radiance'

and Sweet Sensation 'Florida 127') are moderately resistant to the disease, whereas 'Strawberry Festival' and 'Florida Beauty' are susceptible (Chandler et al. 2009; Mangandi et al. 2015; Whitaker et al. 2015, 2017). Cultural practices that involve limited use of water for overhead irrigation or selection of nitrate sources of nitrogen rather than ammonium for fertilization might help suppress pathogen dispersal and disease development (Smith 2009). However, such practices might pose practical constraints on the strawberry production system. Therefore, commercial management of CCR relies on the use of fungicides. The most recent list of fungicides recommended for CCR includes captan, thiophanate-methyl, pyraclostrobin, and azoxystrobin (Whitaker et al. 2018). With the extensive use of azoxystrobin in strawberry production to manage other diseases such as anthracnose fruit rot and root necrosis (Forcelini et al. 2016), resistance of *C. gloeosporioides* to this active ingredient was reported in 2015 (Oliveira et al. 2016). Hence, it is crucial to conduct research to screen for resistance and identify alternative fungicides for CCR management.

The succinate-dehydrogenase-inhibitors (SDHIs) is a group of fungicides already used in strawberry production to manage major fruit rot diseases such as Botrytis and Anthracnose fruit rots (Amiri et al. 2019; Whitaker et al. 2018). Among the SDHIs registered for strawberry use are boscalid, penthiopyrad, fluopyram, fluxapyroxad, and isofetamid. Boscalid, for example, was registered for use in strawberry production in 2003. Soon after its registration, resistance of *Botrytis cinerea* and *Colletotrichum* spp. to boscalid and other SDHI fungicides was reported in the U.S. (Fernández-Ortuño et al. 2012; Hu et al. 2016; Ishii et al. 2016). SDHI fungicides act by inhibiting cellular respiration and their target is the mitochondrial complex II (FRAC 2019). One of the steps prior to registration of a new SDHI fungicide against a certain pathogen involves investigating the baseline sensitivity of fungal species to the new products. To determine the baseline sensitivity, one needs to calculate the effective dose of the fungicide that inhibits 50% of the fungal growth (EC₅₀), and this calculation may be facilitated by the use of a spiral gradient dilution assay, previously described by Förster et al. (2004).

Previous work by Ishii et al. (2016) demonstrated in vitro activity of the SDHIs against *Colletotrichum* species, but this work only included a single isolate from a strawberry field in Japan. Thus, there was a need to determine the efficacy of SDHI fungicides for control of CCR and the baseline sensitivity of *C. gloeosporioides* isolates from strawberry. To test our hypothesis that some of the SDHI fungicides could be effective for management of CCR in Florida, our

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objectives were to: 1) determine the sensitivity of *C. gloeosporioides* from Florida fields to six SDHIs in vitro, 2) determine the baseline sensitivity profile of *C. gloeosporioides* isolates to the SDHI fungicides found effective in the prior screening, i.e., benzovindiflupyr and penthiopyrad, and 3) evaluate the efficacy of five different SDHI fungicides applied pre- or postinoculation of *C. gloeosporioides* on CCR management in a field trial.

Materials and Methods

In vitro screening of SDHIs. Eighty-two isolates of *C. gloeosporioides* from strawberry were selected from the Gulf Coast Research and Education Center fungal culture collection. Isolates were recovered from symptomatic strawberry crowns from 1988 to 2018 and stored both at -80°C on 30% glycerol and at -20°C on sterile filter paper. To test for sensitivity to six SDHI fungicides, isolates were grown on 50% potato dextrose agar (PDA) (Oxoid, ThermoFisher Scientific, Wilmington, DE) for 5 days at 23°C under fluorescent light. A 4-mm diameter mycelial plug from each isolate was transferred to a Petri dish each containing PDA (Difco, Becton Dickinson & Company, Sparks, MD) amended with a different SDHI fungicide. PDA was amended with 100 $\mu\text{g}/\text{ml}$ of benzovindiflupyr (Aprovia, Syngenta Crop Protection, Greensboro, NC), fluopyram (Luna Privilege, Bayer Crop Science, St. Louis, MO), fluxapyroxad (Xemium, BASF, Research Triangle Park, NC), isofetamid (Kenja, Summit Agro USA, Durham, NC), penthiopyrad (Fontelis, Corteva Agriscience, Wilmington, DE), or pydiflumetofen (Miravis, Syngenta Crop Protection, Greensboro, NC). The concentration of SDHI fungicide amended to the medium and methodology for evaluation of *C. gloeosporioides* sensitivity was previously determined by Ishii et al. (2016). Mycelial growth of the isolates was evaluated after 4 days and compared with a nonamended control plate. An isolate was considered resistant if its mycelia grew at 100 $\mu\text{g}/\text{ml}$. One plate per isolate/fungicide was used and the assay was repeated once.

Baseline and fungicide sensitivity assay. The initial screening of SDHI fungicides showed that only benzovindiflupyr and penthiopyrad were effective for in vitro control of *C. gloeosporioides* at 100 $\mu\text{g}/\text{ml}$. Therefore, we determined the baseline and fungicide sensitivity of strawberry isolates that were collected before and after the introduction of these SDHI fungicides. EC_{50}s were determined by mycelial growth assays using the spiral gradient dilution (SGD) method as described by Förster et al. (2004).

One hundred *C. gloeosporioides* isolates from strawberry were selected from the fungal culture collection at the Gulf Coast Research and Education Center and separated into two groups: nonexposed to SDHI fungicides (collected before 2003, $n = 42$) and exposed to SDHI fungicides (collected after 2003, $n = 58$). The isolates were grown on 50% PDA for 7 days at 23°C until full sporulation. For each isolate, 1 ml of spore suspension of 10^5 conidia/ml was prepared and evenly spread onto a 15-cm diameter Petri dish containing 45 ml of PDAA⁺ medium (39 g PDA + 7 g agar per liter). The plates were incubated for 2 days at 23°C under constant fluorescent light. Using an agar slicer designed by Amiri et al. (2013), mycelial strips were cut and transferred to a new plate containing PDA amended with fungicide, placing the strip across the fungicide gradient. Two strips for each isolate were used on three different plates. The stock suspension or solution concentration for each fungicide was calculated using the spiral gradient endpoint (SGE) software (Spiral System, Autoplate 4000; Spiral Biotech) and was 185.32 $\mu\text{g}/\text{ml}$ for benzovindiflupyr and 1,789.96 $\mu\text{g}/\text{ml}$ for penthiopyrad. PDA was amended with fungicides using the Spiral System Autoplate 4000 (Spiral Biotech) and the final range of concentrations were 0.01 to 1 $\mu\text{g}/\text{ml}$ for benzovindiflupyr and 0.1 to 10 $\mu\text{g}/\text{ml}$ for penthiopyrad.

After 3 days, the mycelial growth of the control plate containing nonamended PDA was measured and divided by two. The value corresponding to 50% growth of the isolate was marked on the fungicide plate. The distance between the 50% growth and the center of the plate was measured and that number was used in the SGE software to calculate the EC_{50} . Data were tabulated and histograms were created for each fungicide using Excel spreadsheets and general formulas to determine data range, number of class interval, and interval class width. The experiment was conducted twice. The least square

means EC_{50} values of each isolate were determined by analyzing the data in a mixed model using the statistical software SAS (Procedure PROC MIXED) (SAS Institute, Cary, NC). The model was constructed using EC_{50} values as response variable, 'isolates' as fixed, and 'experiment' and 'repetition' as random variables, respectively. The least square means EC_{50} values are presented in the supplementary table of this manuscript.

Field assay. Two experiments were conducted in the 2017–18 and 2018–19 strawberry seasons to evaluate the efficacy of SDHI fungicides for CCR management. Strawberry transplants of 'Strawberry Festival' were obtained from a California nursery. Field experiments were planted in mid-October in both years, in raised beds covered with black plastic mulch. Strawberry transplants were planted in two staggered rows in a 20-plant plot and spaced 38 cm within and between rows. Gaps of 76 cm without plants were left between plots. Overhead irrigation was provided for 10 days after planting to facilitate plant establishment and further irrigation and fertilization were delivered via a single drip tape located in the center of the bed.

On 15 November 2017 and 2018, the plants were inoculated with a mixture of four *C. gloeosporioides* isolates pathogenic to strawberry, 97-15A, 95-63A, 98-285 (MacKenzie et al. 2006), and 16-128. The isolates were grown for 7 days as described above, and a spore suspension at 2×10^5 conidia/ml was prepared by scraping the conidia from the colony and suspending them in deionized water. Final volume was adjusted by calculating about 5 ml of spore suspension per inoculated plant, which was foliar applied to each plant using a CO_2 pressurized backpack sprayer mounted with a single nozzle and operating at 6.9 kpa of pressure. Strawberry plants were treated with fungicides either 2 days before inoculation (DBI) or 1 day after inoculation (DAI). Treatments are listed in Table 1 and consisted of 12 fungicide treatments in 2017–18, 16 fungicide treatments in 2018–19, one noninoculated control (CNI), and one inoculated control (CI). SDHI treatments were compared with the most common fungicides used for CCR management, i.e., thiophanate-methyl, pyraclostrobin, and captan. Omega (a.i. fluazinam), a fungicide of the FRAC group 29 not yet registered for strawberry production, was also included in our field trial for comparison.

Plant wilt and mortality was evaluated once a week for 18 weeks after inoculation by counting the number or symptomatic plants. Wilting plants were sampled four times during the evaluation period to confirm infection by *C. gloeosporioides*. Data of plant mortality was analyzed at 11 weeks after inoculation, when the noninoculated control started showing CCR symptoms. Plant mortality data were used to calculate area under disease progress curve (AUDPC), treatments were compared and means separated by Fisher's Protected LSD test ($\alpha = 0.05$) using the statistical software SAS.

Results

In vitro screening of SDHIs. All *C. gloeosporioides* isolates from strawberry were insensitive (naturally resistant) to fluopyram, fluxapyroxad, isofetamid, and pydiflumetofen at 100 $\mu\text{g}/\text{ml}$ (Table 2). The SDHIs benzovindiflupyr and penthiopyrad, at 100 $\mu\text{g}/\text{ml}$, completely inhibited the mycelial growth of *C. gloeosporioides* isolates. Inhibition of mycelial growth on PDA amended with fluopyram varied from 18.2 to 30.3%, with fluxapyroxad varied from 48.6 to 67.5%, with isofetamid from 29.5 to 41.8%, and with pydiflumetofen from 31 to 49.1%. The inhibition was similar for all the isolates independent of fungal species or year collected.

Baseline and fungicide sensitivity assay. The EC_{50} of *C. gloeosporioides* isolates nonexposed to SDHI fungicides ($n = 42$) varied from 0.08 to 1.11 $\mu\text{g}/\text{ml}$ on benzovindiflupyr (Fig. 1A) and from 0.45 to 3.17 $\mu\text{g}/\text{ml}$ on penthiopyrad amended media (Fig. 1C). *C. gloeosporioides* isolates exposed to SDHI fungicides ($n = 58$) had EC_{50}s varying from 0.08 to 0.70 $\mu\text{g}/\text{ml}$ when treated with benzovindiflupyr (Fig. 1B), and from 0.70 to 3.06 $\mu\text{g}/\text{ml}$ when treated with penthiopyrad (Fig. 1D). For both fungicides, the histogram of *C. gloeosporioides* EC_{50}s was skewed left, meaning that most of the isolates were grouped at the lower ranges of EC_{50} (Fig. 1). For benzovindiflupyr, most of the isolates independent of exposure to SDHIs had EC_{50}s between 0.15 and 0.40 $\mu\text{g}/\text{ml}$, whereas only one isolate had an EC_{50} higher than 0.8 $\mu\text{g}/\text{ml}$ (Fig. 1). In contrast,

for penthiopyrad, more than 90% of the exposed isolates had an EC₅₀ higher than 1.02 µg/ml ($n = 50$, Fig. 1D), whereas most of the non-exposed isolates had EC₅₀s between 0.45 and 1.29 µg/ml ($n = 31$, Fig. 1C). Fourteen *C. gloeosporioides* isolates nonexposed to penthiopyrad had an EC₅₀ to penthiopyrad between 0.45 and 0.87 µg/ml and 20 exposed isolates had EC₅₀s between 1.02 and 1.33 µg/ml. The highest EC₅₀ to penthiopyrad was 3.17 µg/ml from a *C. gloeosporioides* isolate collected in 1998 whereas the highest EC₅₀ to benzovindiflupyr was 1.11 µg/ml from an isolate collected in 2001 (Supplementary Table).

Field assay. In both seasons, we observed the greatest differences between treatments at 11 weeks after inoculation (Table 1). In the 2018–19 season, after 11 weeks of evaluation, plants from the CNI started to show disease symptoms (Fig. 2). The first symptoms of plant wilt and mortality were observed within 2 weeks after inoculation and increased until the last evaluation at 18 weeks after inoculation.

In 2017–18, benzovindiflupyr applied 1 DAI and captan and fluazinam applied 2 DBI were the best treatments for reducing plant mortality compared with the controls ($P \leq 0.0002$; Table 1). In 2018–19, fluazinam and benzovindiflupyr applied either 1 DAI or 2 DBI, pyraclostrobin applied 1 DAI, and captan applied 2 DBI were the best treatments for reducing plant mortality ($P \leq 0.0003$). Captan was able to reduce plant mortality by 21 to 22% in both seasons, demonstrating it to be a good protectant fungicide. The SDHI fungicides

fluopyram, isofetamid, and pydiflumetofen were not effective for CCR management. Strawberry plants treated with these three SDHIs had over 46% mortality in the first season and over 23% mortality in 2018–19. Mortality in the treatment with thiophanate-methyl, a fungicide currently used for CCR management, was not significantly different from the inoculated control. Penthiopyrad was not effective for CCR management in the 2017–18 season but had some effectiveness in 2018–19 when applied 1 DAI.

AUDPC in the 2017–18 season was higher than in 2018–19 (Table 1). For the inoculated control, the AUDPC was 9.86 in 2017–18 and 7.32 in 2018–19, after 11 weeks of evaluation. Mean separation for AUDPC showed that in 2017–18, fluazinam applied 2 DBI and benzovindiflupyr applied 1 DAI were the most effective fungicides for reducing the progress of the disease ($P \leq 0.0001$). In 2018–19, the most effective fungicides for reducing CCR progress were the same as those that reduced plant mortality, plus penthiopyrad ($P \leq 0.0014$).

Discussion

This study demonstrates the efficacy of the SDHI fungicide benzovindiflupyr for inhibiting *C. gloeosporioides* and managing Colletotrichum crown rot of strawberry in the field. To our knowledge, this is the first study of fungicide sensitivity of *C. gloeosporioides* from strawberry before and after exposure to SDHI fungicides in the U.S.

Table 1. Plant mortality and area under the disease progress curve (AUDPC) of fungicide treatments applied for the management of Colletotrichum crown rot (CCR) of strawberry in Florida during the 2017–18 and 2018–19 seasons

Time of application ^w	Treatment (a.i.) ^x	2017–18		2018–19	
		% Plant mortality ^y	AUDPC	% Plant mortality	AUDPC
2 days before inoculation	Aprovia (benzovindiflupyr)	37.5 bcde	6.82 defg	9.0 cde	1.35 cd
	Captan (captan)	20.0 def	3.50 efg	7.8 de	1.97 bcd
	Fontelis (penthiopyrad)	40.0 abcd	11.37 abcd	27.0 abc	6.52 ab
	Kenja (isofetamid)	52.5 ab	13.82 abc	30.0 ab	5.60 abc
	Luna Tranquility (fluopyram+pyrimethanil)	48.8 abc	13.56 abc	36.8 a	9.37 a
	Miravis (pydiflumetofen)	–	–	24.0 abcd	4.87 abcd
	Omega (fluazinam)	21.3 def	3.06 fg	6.3 de	2.23 bcd
	Topsin (thiophanate-methyl)	–	–	23.0 abcd	5.53 abc
	Aprovia (benzovindiflupyr)	16.3 ef	2.19 g	6.5 de	2.15 bcd
	Cabrio (pyraclostrobin)	27.5 cde	5.77 defg	7.5 de	1.40 cd
1 day after inoculation	Fontelis (penthiopyrad)	33.8 bcde	8.84 cdef	11.5 bcde	2.26 bcd
	Kenja (isofetamid)	60.0 a	17.67 a	28.3 ab	5.41 abc
	Luna Tranquility (fluopyram+pyrimethanil)	46.3 abc	11.64 abcd	23.8 abcd	6.60 ab
	Miravis (pydiflumetofen)	–	–	38.8 a	9.36 a
	Omega (fluazinam)	–	–	6.3 de	0.96 cd
	Topsin (thiophanate-methyl)	55.0 ab	16.36 ab	36.0 a	8.87 a
	Control noninoculated	5.0 f	1.21 g	1.3 e	0.26 d
	Control inoculated	42.0 abcd	9.86 bcde	28.8 ab	7.32 a
	Probability of a greater F value ^z	0.0002	<0.0001	0.0003	0.0014
N.A.					
N.A.					

^w Time of application refers to the timing during which plants were sprayed with the respective fungicide treatments relative to pathogen inoculation.

^x Treatment refers to the brand name and active ingredient of the fungicides tested for CCR management.

^y Percent plant mortality evaluated 11 weeks after inoculation was calculated using the proportion of dead plants relative to the total number of plants for each treatment.

^z Probability of a greater F value refers to the probability value for the effect of treatment on plant mortality and AUDPC.

Table 2. Average mycelial growth inhibition by succinate dehydrogenase inhibitor (SDHI) fungicides of *Colletotrichum gloeosporioides* isolates from strawberry

Year collected ^y	Number of isolates	Mycelial growth inhibition (%) ^z					
		Fluopyram	Isofetamid	Penthiopyrad	Fluxapyroxad	Pydiflumetofen	Benzovindiflupyr
1988	1	19.9	29.5	100.0	67.5	38.8	100.0
1995	5	18.2	32.4	100.0	48.6	36.4	100.0
1996	11	26.3	38.9	100.0	49.5	42.3	100.0
1997	8	24.5	35.6	100.0	50.7	41.2	100.0
1998	2	24.9	40.4	100.0	54.7	43.7	100.0
1999	3	29.1	35.5	100.0	50.0	49.1	100.0
2000	4	30.3	41.8	100.0	54.0	31.0	100.0
2001	2	18.7	30.7	100.0	50.8	31.0	100.0
2017	46	23.2	34.3	100.0	49.5	38.8	100.0

^y Year in which isolates of *Colletotrichum* were collected from strawberry fields.

^z Mycelial growth was evaluated in potato dextrose agar amended with fungicides at 100 µg/ml and compared with nonamended control.

Currently, benzovindiflupyr is not labeled for strawberry. None of the registered SDHI fungicides were effective in controlling the disease.

We tested the *in vitro* efficacy of fluopyram, isofetamid, penthiopyrad, fluxapyroxad, pydiflumetofen, and benzovindiflupyr at 100 µg/ml for inhibiting mycelial growth of *C. gloeosporioides*. Benzovindiflupyr and penthiopyrad completely inhibited mycelial growth, whereas the other SDHIs showed partial inhibition. Fluxapyroxad, one of the most recent SDHIs released for strawberry use, inhibited mycelial growth by about 50%. The use of products containing fluxapyroxad as an active ingredient, e.g., Merivon, a premixture of fluxapyroxad and the QoI fungicide pyraclostrobin, might further reduce pathogen growth and disease development due to the additive effect of both compounds, but this fungicide combination was not included in our trials. Previous work with *Botrytis cinerea* from strawberry (Amiri et al. 2014) reported a high correlation between resistance to fluxapyroxad and penthiopyrad; in our work, we did not observe such a correlation between these two SDHIs. Penthiopyrad completely inhibited *C. gloeosporioides* mycelial growth at 100 µg/ml, whereas fluxapyroxad did not.

Pydiflumetofen, the most recent SDHI registered for strawberry production, was not effective against *C. gloeosporioides* *in vitro* or under field conditions. In previous work with *C. acutatum* (Haack et al. 2018), pydiflumetofen did not inhibit mycelial growth at concentrations of up to 80 µg/ml, similar to our results with *C. gloeosporioides*. Even though pydiflumetofen had little to no efficacy against CCR, it is registered for strawberry production in a mixture

with fludioxonil (Miravis Prime) due to its efficacy against *Botrytis* fruit rot (Cordova et al. 2019). In screening of 30 of our *C. gloeosporioides* isolates to fludioxonil at 100 µg/ml, mycelial growth was inhibited by 62.9 to 82.3%. However, none of the isolates was completely inhibited by fludioxonil (data not shown).

Before registration of a new fungicide against a certain pathogen, the baseline sensitivity of a diverse collection of isolates must be determined by calculating their EC₅₀ values. Most of the *C. gloeosporioides* isolates had EC₅₀s to benzovindiflupyr between 0.16 and 0.44 µg/ml, and only one isolate collected in 2001 had an EC₅₀ higher than 1.0 µg/ml. This variation seems to occur naturally in *Colletotrichum* spp. isolates. The fact that the EC₅₀ of *C. gloeosporioides* isolates is higher for penthiopyrad than for benzovindiflupyr indicated that the latter might work better for CCR management. This was confirmed in our 2017–18 season field trial, but no significant differences were observed between the two fungicides in the 2018–19 season.

In our field trial, we evaluated the efficacy of SDHIs as protectant or curative fungicides for CCR management. Benzovindiflupyr applied 1 day after inoculation of *C. gloeosporioides* was the best among the SDHI treatments. Data for the field experiments were analyzed at 11 weeks after inoculation, when the noninoculated control started showing CCR symptoms indicating that the pathogen was likely spreading to the neighboring plots.

CCR development was faster during 2017–18 than in the 2018–19 season. That might be because the temperature from inoculation time until the next morning ranged from 15 to 25°C in 2017, whereas in

Fig. 1. Distribution of the concentration of benzovindiflupyr (A and B) and penthiopyrad (C and D) needed to inhibit 50% (EC₅₀) of mycelial growth of *Colletotrichum gloeosporioides* isolates from strawberry collected before (A and C) and after (B and D) exposure to SDHI fungicides.

2018 the temperature ranged from 8 to 20°C (FAWN 2019). The colder temperatures might have slowed down *C. gloeosporioides* infection and colonization, and consequent CCR symptom development. The slower disease development might also explain the fact that more fungicides were found to be effective in the 2018–19 season, including no difference between applying benzovindiflupyr applied before or after inoculation.

Another product added to our field trial, Omega (a.i. fluazinam), was effective against CCR in both seasons. A special request has been

submitted for its registration for strawberry nursery use. With the alarming situation of fungicide-resistant pathogens coming with nursery transplants (Forcelini and Peres 2018; Oliveira et al. 2017), it is important to search for different fungicides to be used in nursery and fruit production systems to avoid overuse of the same active ingredients.

SDHI fungicides, such as boscalid, have been available for the management of Botrytis fruit rot, caused by *Botrytis cinerea*, on Florida strawberry production since 2003, but resistance has become widespread (Amiri et al. 2014; Zuniga et al. 2020). Other SDHIs also

Fig. 2. *Colletotrichum* crown rot incidence of strawberry treated with succinate-dehydrogenase-inhibitor (SDHI) fungicides 2 days before inoculation (DBI) and 1 day after inoculation (DAI) with *Colletotrichum gloeosporioides* during the 2017–18 and 2018–19 seasons.

registered for strawberry use are penthiopyrad, fluopyram, fluxapyroxad, and isfetamid (Whitaker et al. 2018). However, none of them was ever recommended for CCR in Florida. We screened *C. gloeosporioides* isolates from strawberry to SDHI fungicides and found natural resistance to fluopyram, fluxapyroxad, isfetamid, and pydiflumetofen. A previous study by Ishii et al. (2016) also found natural resistance of *C. gloeosporioides* from apple, grapevine, strawberry, and peach to boscalid, fluxapyroxad, and fluopyram. We did not test the sensitivity of our isolates to boscalid but would expect that our isolates would have had the same natural resistance as reported by Ishii et al. (2016).

SDHI fungicides are categorized as fungicides with medium to high risk of resistance development (FRAC 2019). Different levels of resistance to the SDHI fungicides might be conferred by at least eight known mutations in the *sdhB* mitochondrial gene. Previous studies found the mutations H272R, H272Y, H272L, P225F, and N230I in *B. cinerea* from strawberry (Amiri et al. 2014; Fernández-Ortuño et al. 2017; Hu et al. 2016; Zuniga et al. 2020). However, Ishii et al. (2016) sequenced the *sdhB*, *sdhC*, and part of *sdhD* genes of *Colletotrichum* spp. and did not find mutations causing resistance to SDHI fungicides, describing it as natural resistance or insensitivity. In our study, we observed the same natural resistance reported by Ishii et al. (2016), but the *sdhB* gene was not sequenced.

In summary, *C. gloeosporioides* from strawberry appear to have natural resistance (insensitivity) to some SDHI fungicides such as fluopyram, fluxapyroxad, isfetamid, and pydiflumetofen. Penthiopyrad inhibited mycelial growth of *C. gloeosporioides* in vitro but was not as effective in reducing CCR development on strawberry plants. The most effective SDHI fungicide for CCR management was benzovindiflupyr and strawberry growers would benefit from its registration. Fluazinam was also highly effective against CCR and could serve as a good disease management alternative for nursery growers. With the low number of fungicides available for CCR management on strawberry and the risk of fungicide resistance selection, further studies with other alternative fungicides are still necessary.

Acknowledgments

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