Fungicide resistance monitoring and management alternatives for Botrytis fruit rot of strawberries

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Summary

During the past season, an increase in resistance to fluopyram (Luna Tranquility), fludioxonil (Switch), and emergence of resistance to isofetamid (Kenja) was observed in Botrytis isolates collected from six commercial fields. It is important for growers to know the resistance profile of *B. cinerea* population in Florida strawberry fields so that alternative disease management options can be recommended. Besides fungicide choice, proper fungicide spray coverage and penetration is also important to avoid further selection of resistance. However, none of the different spray patterns evaluated in our trial reduced Botrytis fruit rot incidence. A non-fungicide control strategy with applications of ultraviolet C (UVC) was also evaluated to manage Botrytis post-harvest and prolong the fruit shelf-life. Disease incidence was reduced with the higher UVC doses, but further studies are needed to determine the effect on naturally infected fruit.

Methods

Monitoring of *B. cinerea* isolates for fungicide resistance. During the 2018-2019 strawberry season, 194 *B. cinerea* isolates were collected from six commercial farms in Florida, and from two strawberry nurseries in North Carolina and one nursery in California. Resistance evaluation was conducted using a conidial germination assay. After isolation, colonies were incubated on HA medium for 7 days at ~23˚C until sporulation was profuse. 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 (FRAC 7) and Malt Extract Agar (MEA) for the PhenylPyrroles (FRAC 12) fungicides. YBA was amended with 2 or 5 µg/ml of Luna Tranquility (fluopyram) and 1 or 5 µg/ml of Kenja (isofetamid), and MEA with 0.1 or 10 µg/ml of Switch (fludioxonil) to evaluate the sensitivity of each isolate. 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 and each isolate, and the frequency of fungicide resistance in the populations was determined.

Evaluation of spray patterns and reduced flow rates. A field experiment was conducted on a commercial farm in Plant City to determine the effectiveness of different spray patterns and reduced flow rates on the management of BFR. Transplants of 'Florida Radiance' from a California nursery were used. Four types of spray patterns (hollow-cone, flat-fan, air-induction flat-fan, and twin flat-fan) applied at two flow rates (50 and 100 GPA) were tested. Treatments-specific descriptions are presented in Table 1. Treatments were applied using a 4-stroke gas powered back-pack Solo sprayer to reach spray pressures achieved by a tractor-towed sprayer. An experimental standard treatment was sprayed using a CO2 back-pack sprayer. In total, four applications were made base on the Strawberry Advisory System (http://agroclimate.org/tools/sas/fl/) and the same fungicides were applied for all treatments. The fungicides Captan Gold 4L (28 Nov 2018 and 3 Dec 2018), Switch 62.5WG (17 Dec 2018 and 29 Jan 2019), and Kenja 400SC (2 Jan 2019) were used. Fruit were harvested twice weekly from 4 Dec 2018 to 12 Feb 2019 (19 harvests) to determine yield and BFR incidence. Yield was expressed as pounds of marketable fruit per acre, and BFR incidence was expressed as the percentage of diseased fruit relative to the total number of fruit.

Efficacy of ultraviolet-C light (UV-C) on BFR postharvest.

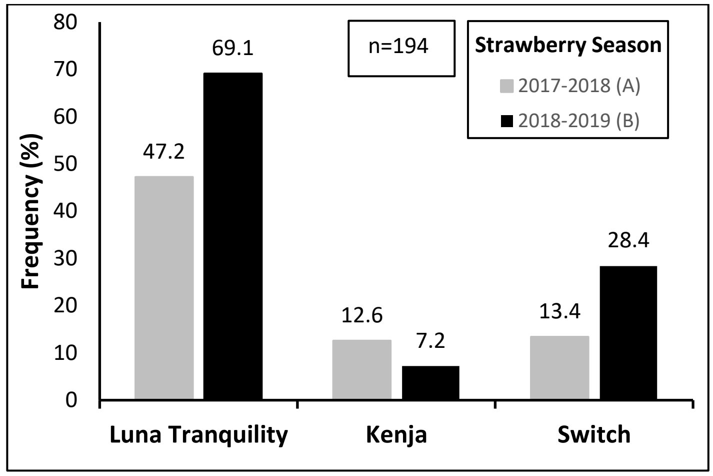
A post-harvest experiment was conducted to evaluate the efficacy of UV-C for post-harvest management of BFR. Healthy strawberry fruit were harvested before they reached standard commercial maturity, and disinfested using a 0.02% solution of sodium hypochlorite for 2 min and rinsed twice with sterile water. Fruit were wounded and inoculated with 7 µl of a conidial suspension of 10^6 spores/ml. Inoculum was prepared using a mixture of four isolates with different fungicide resistance profiles. One and twelve hours after inoculation, fruit were exposed to 0.25, 1.0 and 2.5 $kJ.m^{-2}$ of UV-C irradiation using a designed table top hemicylindrical array with ten fluorescent lamps with peak emission at 254 nm. Fruit were stored in the dark at 10˚C and BFR incidence (%) was evaluated after 7 days.

Results

Fungicide resistance monitoring

Resistance frequencies during the 2018-2019 strawberry season of *B. cinerea* isolates to Luna Tranquility, Kenja, and Switch were 69.1, 7.2, and 28.4%, respectively (Fig. 1). Fungicide resistance for Luna Tranquility increased by 22% compared to the previous season (2017-2018). An increase of resistance was also observed for Switch, but results from other field experiments indicated that it is still effective for controlling BFR. Resistance frequency to Kenja remains low (7.2%), but its use should be limited to reduce the further selection of *B. cinerea*-resistant populations. Based on these results, our recommendation is that Kenja and Switch should be used only during highly favorable conditions for disease (red alerts), whereas Luna Tranquility might be applied when environmental conditions are moderately conducive (orange alert) according to the Strawberry Advisory System.

Fig. 1. Resistance frequencies during 2018-2019 (A) and 2017-2018 (B) strawberry seasons in Florida.



Evaluation of spray patterns and reduced flow rates.

BFR incidence was analyzed for the entire season with an average of 39.4%. Yield ranged from 3,650 to 7,170 pounds per acre. The weather conditions were very suitable for BFR development during the 2018-2019 season. However, none of the treatments significantly reduced BFR incidence nor increased yield relative to the non-treated control (NTC) (Table 1). The lack of effectiveness of treatments in this trial is likely due to a spray that was missed during a high alert that occurred on 14 Feb 2019.

Table 1. Evaluation of spray patterns and reduced flow rates to manage Botrytis fruit rot of strawberry for three periods of evaluation during the 2018-2019 season.

Nozzles	Pressure (psi)	GPA	Pattern[w]	Yield (lb/A)[x]	BFR (%)[y]
-	-	-	NTC	3650	39.4
TXVK-18	260	100	Hollow-cone	6078	30.4
XR8003VK	260	100	Flat-Fan	7170	28.1
AIC11005	90	100	Air-induction flat	5317	23.9
TTJ60-11006	70	100	Twin flat spray	5381	29.0
TXVK - 10	260	50	Hollow-cone	5623	29.9
TP80015VK	260	50	Flat-Fan	5072	25.2
AIC110025	90	50	Air-induction flat	5366	32.3
TTJ60-11003	70	50	Twin flat spray	5283	30.1
T-Jet 8002 (standard)	60	100	Hollow-cone	5501	34.1
Probability of a greater F value				0.356	0.206

[w] Pattern corresponds to the spray pattern resulting from each nozzle. GPA= flow rate in gallons per acre. Pressure corresponds to the application pressure at the nozzle. Nozzles correspond to the code of each nozzle found in the Teejet catalog.

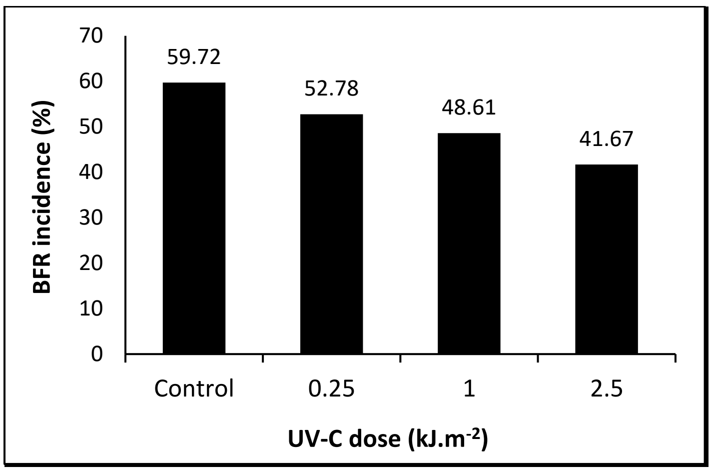
[x] Yield from 22 harvests made from 4 Dec 2018 to 12 Feb 2019 (whole season).

[y] Average Botrytis fruit rot (BFR) incidence during the whole season (all harvests).

Efficacy of ultraviolet-C light (UV-C) on BFR postharvest.

The highest BFR incidence was observed in the NTC with 59.7% of symptomatic fruit followed by the UV-C treatment at the lowest dose of 0.25 kJ.m^{-2} with 52.8% (Fig. 2). The UV-C doses of 1 and 2.5 kJ.m^{-2} significantly reduced BFR incidence compared to the NTC with 48.6 and 41.7%, respectively. Further investigation is needed to determine whether UV-C applications at these rates on the plant can delay Botrytis postharvest symptoms. Studies from other authors have shown that UV-C applications can induce resistance by increasing the level of phenylalanine ammonia-lyase (PAL) in the fruit 12h after UV-C application. PAL is a key enzyme in the biosynthesis of phenolic compounds, and is usually considered to have antifungal activity.

Fig. 2. Postharvest Botrytis fruit rot incidence after application of ultraviolet-C light (UV-C).



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