Screening for anthracnose and Botrytis latent infections and fungicide resistance for improved disease control

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Summary

The intensive use of single-site fungicides in strawberry nurseries and the consequent selection for resistant pathogens has been a problem for FL strawberry growers. Widespread distribution of *Botrytis cinerea* and *Colletotrichum acutatum* populations resistant to fungicides in Florida can reduce the effectiveness of fungicide applications. For *C. acutatum*, early visual diagnosis is difficult, and rapid detection could lead to better control. The objectives of this project aimed (i) to determine the baseline sensitivity of Botrytis isolates in FL strawberry fields to the new fungicides Kenja and Luna; (ii) to determine the level of cross resistance between Fontelis and Merivon with Kenja and Luna; (iii) to develop a rapid detection assay for *C. acutatum* that combines sensitivity, low cost and ease of use; and (iv) to develop a rapid detection assay to determine whether C. acutatum isolates are resistant to the strobilurin fungicides Abound, Cabrio, Flint, and Evito.

Methods

Botrytis Fruit Rot. One hundred and forty one isolates were collected from five different commercial farms in Florida during the 2015 - 2016 strawberry season (first season of Kenja use and before use of Luna). In addition, 188 isolates collected from nine different nurseries during the 2014-2015 season were also tested. Isolates were incubated at 23˚C for 5 to 7 days and conidia were collected to prepare a suspension at 2.5 x 10^6 conidia/ml. A 7µl drop of spore suspension was placed on 40 to 50 ml of growth medium (Yeast Bacto Agar-YBA) amended with fungicides. YBA was amended with 5 µg/ml of boscalid (one of the ingredients of Pristine), penthiopyrad (Fontelis), fluopyram (Luna), benzovindiflupir (Syngenta product not registered for strawberries), and isofetamid (Kenja). The percentage of germinated spores was evaluated twice for all fungicides to determine frequency of resistant isolates to the fungicides tested.

Anthracnose Fruit Rot. During the 2016- 2017 strawberry season, petioles from 215 transplants from multiple cultivar/nursery combinations were sampled and used for detection of *C. acutatum* by molecular and biological assays. DNA was extracted from petiole tissue and *C. acutatum* amplicons were detected with a High-Resolution Melting (HRM) assay. Petioles were incubated for a week and signs of *C. acutatum* spores confirmed the presence of the pathogen. Transplants from which petioles were sampled were transplanted into the field and plant mortality was evaluated after plant establishment.

Results

Botrytis Fruit Rot. Isolates recovered from the commercial farms in Florida (Fig. 1) showed 95.7, 13.5, 11.3, 5.7, and 0% frequency of resistance to boscalid (Pristine), penthiopyrad (Fontelis), fluopyram (Luna), benzovindiflupir (Syngenta product not registered for strawberries), and isofetamid (Kenja), respectively. Similar results were observed for isolates recovered from nursery samples (Fig. 2). Thus, resistance to fluopyram (Luna) and penthiopyrad (Fontelis) is still at low levels and no

isolate resistant to isofetamid (Kenja) was detected. Resistance to boscalid (Pristine), however, is still at a high frequency even though this fungicide doesn't seem to have been much used in Florida strawberry fields during the past couple seasons.

The frequency of cross-resistance among isolates collected from different farms in Florida is shown in Fig.3-A where 116, 9, 14, and 2 isolates were resistant to 1, 2, 3, and 4 fungicides, respectively. Cross-resistance for the five fungicides tested was not observed since all isolates were sensitive to isofetamid (Kenja). Isolates from nursery samples had similar results except that cross-resistance to 4 fungicides was not observed (Fig. 3-B).

Anthracnose Fruit Rot. Out of 215 samples, 115 tested positive for *C. acutatum* with the HRM assay. Signs of *C. acutatum* on petioles were observed on 15 out of the 115 samples that had tested positive (HRM). Plant mortality did not correlate well with laboratory trials, and rate of plant mortality due to *C. acutatum* infections was relatively low in the field (Table 1).

When samples tested positive for *C. acutatum*, extracted DNA was used for detection of mutations that confer resistance to the strobilurin fungicides (Abound, Cabrio, etc.), with the use of molecular markers. Detection of resistant isolates was observed when DNA was extracted from fungal colonies using a DNA extraction kit. However, since DNA from samples were extracted using a fast extraction method, contaminants present on petiole surface may have interfered with detection, which was not consistent among samples. Thus, methodology improvements are required to optimize the detection of *C. acutatum* on transplants and whether the isolates are resistant to the strobilurin fungicides.

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Figure 1. Frequency of resistant isolates collected in Florida during 2015-2016 strawberry season. Boscalid = Pristine, penthiopyrad = Fontelis, fluopyram = Luna, benzovindiflupir = Syngenta product not registered for strawberries, and isofetamid = Kenja.

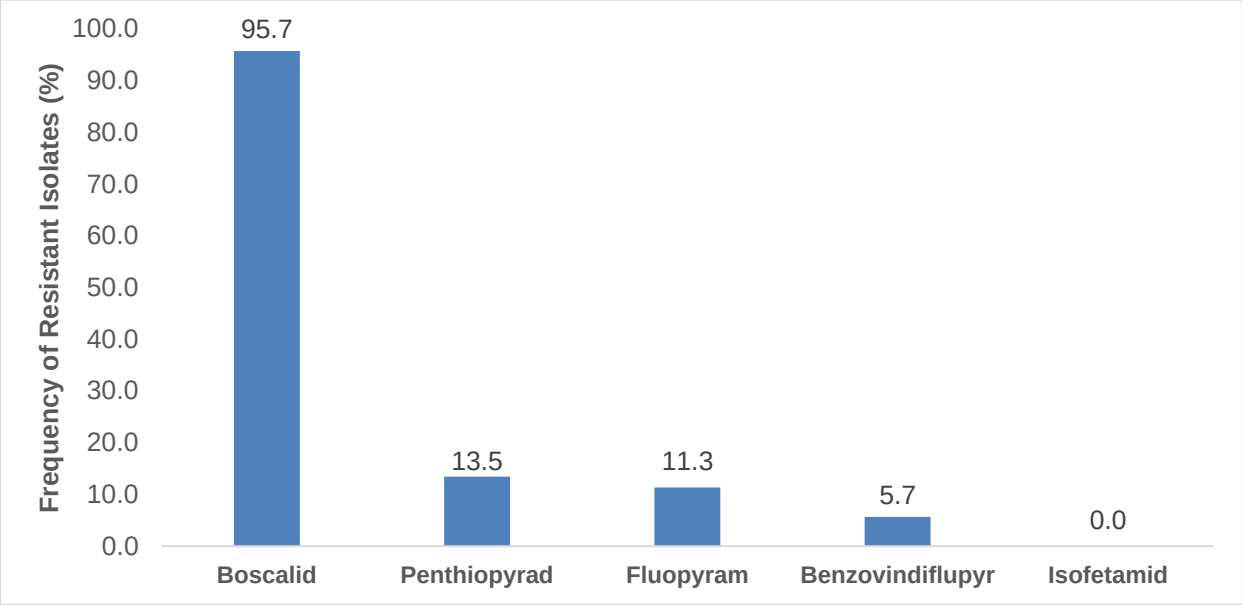


Figure 2. Frequency of resistant isolates collected from nursery samples during 2014-2015 strawberry season. Boscalid = Pristine, penthiopyrad = Fontelis, fluopyram = Luna, benzovindiflupir = Syngenta product not registered for strawberries, and isofetamid = Kenja.

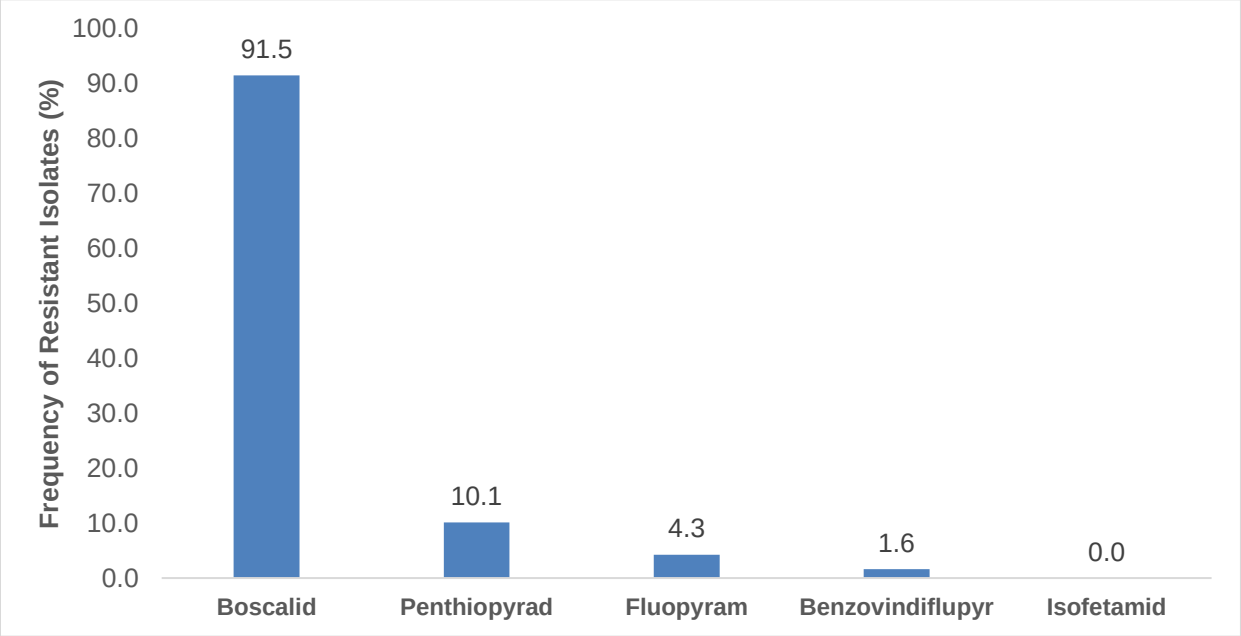
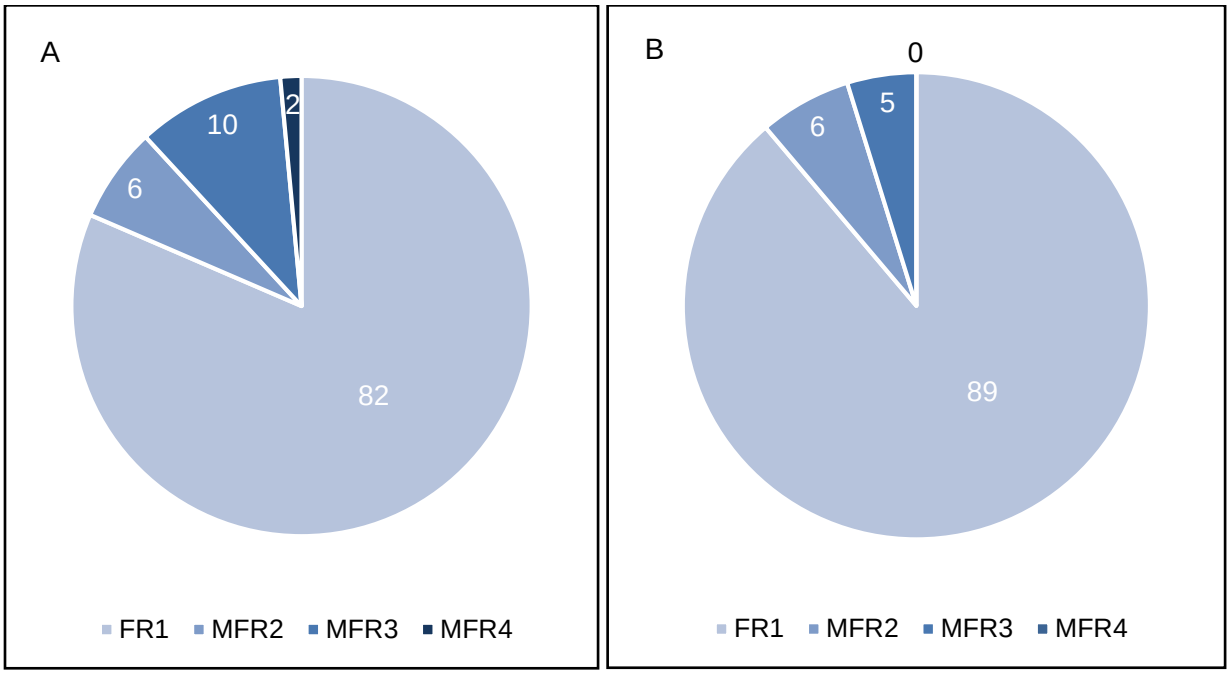


Figure 3. Frequency of resistance to multiple SDHI fungicides (MFR). A. Isolates collected in Florida during the 2015-2016 strawberry season. B. Isolates collected from nursery samples during the 2014-2015 strawberry season.



FR1: resistant to one SDHI fungicide; MFR2: resistance to two SDHI fungicides; MFR3: resistance to three SDHI fungicides; MFR4: resistance to four SDHI fungicides (boscalid, penthiopyrad, fluopyram, and benzovindiflupir).

Table 1. Summary of results of high-resolution melting (HRM) analyses, petiole tests, and plant mortality in the field for samples collected from various nurseries in the U.S. and Canada.

Cultivar	Country	Location	Nursery	% HRM (Y/N)	% Petiole test (Y/N)	% Mortality (Y/N)[a]
Radiance	USA	North Carolina	MT	50.0% (8/16)	12.5% (2/16)	2.5% (1/40)
Radiance	USA	North Carolina	MS	12.5% (1/8)	12.5% (1/8)	12.5% (5/40)
Radiance	USA	California	CR	25.0% (1/4)	ND (0/4)	N/A
Radiance	USA	California	CP	50.0% (6/12)	ND (0/12)	ND (0/40)
Radiance	Canada	Ontario	EG	14.3% (1/7)	ND (0/7)	17.6% (6/34)
Radiance	Canada	Nova Scotia	KD	75.0% (12/16)	6.3% (1/16)	87.5% (35/40)
Radiance	Canada	Québec	LT	25.0% (4/16)	ND (0/16)	2.5% (1/40)
Radiance	Canada	Nova Scotia	GA	18.8% (3/16)	12.5% (2/16)	7.8% (3/39)
Radiance	USA	North Carolina	TL	75.0% (12/16)	31.3% (5/16)	ND (0/39)
Radiance	Canada	Ontario	ST	62.5% (10/16)	ND (0/16)	ND (0/40)
Radiance	Canada	Ontario	KC	100.0% (4/4)	25.0% (1/4)	N/A
Sensation®	USA	California	CP	25.0% (3/12)	8.3% (1/12)	2.5% (1/40)
Sensation®	Canada	Nova Scotia	BM	50.0% (2/4)	ND (0/4)	N/A
Sensation®	Canada	Nova Scotia	GA	68.8% (11/16)	ND (0/16)	10.0% (4/40)
Sensation®	USA	North Carolina	TL	31.3% (5/16)	6.3% (1/16)	ND (0/40)
WinterStar™	Canada	Nova Scotia	GA	50.0% (8/16)	ND (0/16)	ND (0/40)
Portola	USA	California	NC	100.0% (16/16)	ND (0/16)	2.5% (1/40)
Camarosa	Canada	Nova Scotia	GA	100.0% (4/4)	100.0% (4/4)	N/A
				51.6% (111/215)	8.4% (18/215)	9.6% (53/552)

[a], N/A, not planted in the field; ND, not detected.