Integrated approaches for the management of *Phytophthora* spp. of strawberry

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Summary

Phytophthora crown rot (PhCR), caused by *Phytophthora cactorum*, is currently managed mainly by applications of RidomilGold® (mefenoxam). However, the emergence of mefenoxam resistance has made this disease even more challenging for Florida strawberry. In the 2018-19 season, resistance was found in 16% of the isolates tested. Whole genome sequencing of *P. cactorum* isolates indicates that resistance mechanisms might be associated with three point mutations in the same gene. Therefore, alternative management strategies need to be investigated. Heat treatment of strawberry transplants inoculated with *P. cactorum* isolates reduced mortality under field conditions from 30% (non-treated control) to 2.5% (heat-treated). Phosphite products were able to control *P. cactorum* mefenoxam-resistant isolates *in vitro* and control the disease in fruit assays. However, foliar sprays in the field did not reduce disease incidence. On the other hand, drip applications of Orondis® Gold (oxathiapiprolin), a fungicide from Syngenta not registered yet for strawberry, reduced mortality in field experiments from 43.8% (non-treated control) to 12.7%.

Methods

Monitoring for resistance to mefenoxam (RidomilGold®)

Mycelial discs (4-mm diameter) of each isolate were grown on Potato dextrose agar for 4-7 days and transferred to plates containing V8 medium amended with 0, 0.5, 5 and 100 µg/ml of mefenoxam. Plates were incubated at room temperature and mycelial growth was assessed after 4 days by measuring the colony diameter. Based on the discriminatory doses tested, isolates were separated into two groups: sensitive and resistant.

Resistance mechanism to mefenoxam (RidomilGold®).

Unlike resistance to the strobilurin fungicides where the mechanism of resistance is well studied, the mechanism of resistance to mefenoxam (RidomilGold®) in *P. cactorum* isolates is unknown. Mefenoxam targets the RNA polymerase gene and primers were developed to sequence the gene and identify target mutations in resistant isolates. In addition, the whole genome of sensitive and resistant isolates was sequenced to identify possible variations and alteration of gene expressions.

Heat treatment trials.

In field trials, 'FL 127' (Sensation) plants were inoculated with zoospore and oospore (a more resistant structure) suspensions of *P. cactorum* isolates (a mixture of two sensitive and two resistant isolates to RidomilGold®). Transplants were subjected to heat treatment using an aerated steam chamber, a.k.a. *plant sauna*, at 37°C [98.6°F] for 1 hour, followed by 44°C [111.2°F] for 4 hours. Treated and non-treated plants were planted in the field at GCREC and plant mortality was assessed weekly throughout the strawberry season.

Efficacy of phosphite materials in controlling mefenoxam resistant isolates.

To determine the sensitivity of Phytophthora isolates to phosphites, isolates recovered from symptomatic plants in commercial strawberry fields during the 2018-2019 and past seasons were tested in media

amended with phosphite. Methods used were the same as described for mefenoxam; however, phosphite rates were 0, 10, 50, 100, 150 and 300 µg/ml. Isolates with reduced sensitivity were used for in vivo tests conducted in plants and fruit by applying the label rate of the product to confirm field resistance.

Fungicide and biological products to control Phytophthora crown rot.

'FL 127' (Sensation) plants were inoculated with a mixture of isolates resistant and sensitive to mefenoxam (RidomilGold®) and planted in the field at GCREC. Plants were treated with different products, such as RidomilGold®(mefenoxam), Helena Prophyt (phosphite), Actinovate (biological), and Orondis® Gold (oxathiapiprolin) by dipping, drip application or foliar spray. Plant mortality was evaluated biweekly for 15 weeks.

Results

Monitoring for resistance to mefenoxam (RidomilGold®)

During this past season (2018-19), among 43 *Phytophthora* samples received by the Plant Diagnostic Clinic at GCREC, seven isolates were resistant to RidomilGold® (Figure 1). It is important to note that resistance to mefenoxam has been linked to specific nursery sources and continued monitoring will be needed to determine the effectiveness of Ridomil for controlling Phytophthora crown rot.

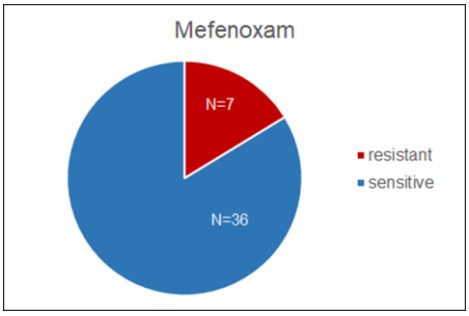

Figure 1. Sensitivity profile of *Phytophthora* spp. isolates from 2018-2019 strawberry season to mefenoxam (RidomilGold®).

Resistance mechanism to mefenoxam (RidomilGold®).

Point mutations in the RNA polymerase gene, commonly associated with the mefenoxam target site, were not found in isolates resistant to mefenoxam (RidomilGold®). Whole genome sequencing showed that there is one probable candidate gene associated with mefenoxam resistance. Three point mutations were identified in resistant isolates. Currently, our group has been working on identifying this gene and designing molecular markers to develop methods for quick detection of mefenoxam resistance.

Heat treatment trials.

In field trials, the treatment commonly used in our '*plant sauna*', which has been shown to reduce *Colletotrichum acutatum* and *Botrytis cinerea*, reduced plant mortality caused by *P. cactorum* to 2.5% compared to 30% in the non-treated inoculated with oospores and 17.5% in the non-treated inoculated with zoospores (Figure 2). We envision that heat treatment can be used as an alternative for nurseries to reduce disease inoculum load from planting stock.

Figure 2. 'FL 127' (Sensation) plants inoculated with a mixture of sensitive and resistant isolates of *P. cactorum* and planted after being heat-treated at 37°C [98.6°F] for 1 hour, followed by 44°C [111.2°F] for 4 hours (A) or not-treated (B).

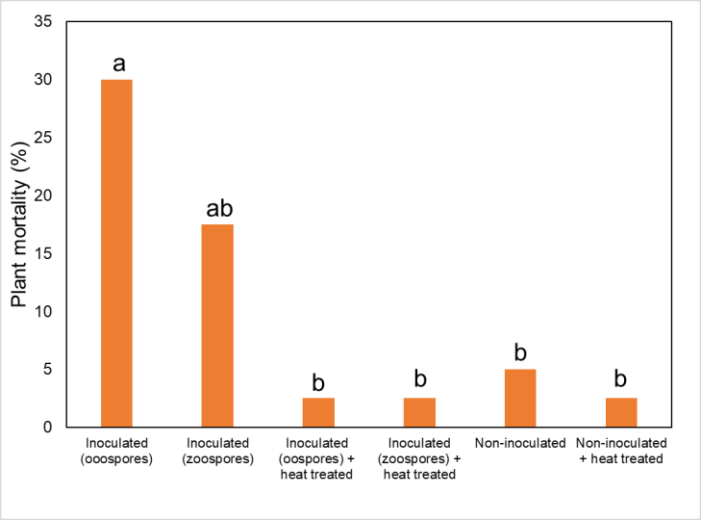

Figure 2. Plant mortality (%) of 'FL 127' (Sensation) plants inoculated or not with oospore and zoopore suspensions of *Phytophthora cactorum* and heat treated in the '*plant sauna*'.

*Florida 127 (Sensation) plants were inoculated on 10/08/18, heat-treated at 44 °C for 4 hours (pre-heat at 37 °C for 1 hour) on 10/09/18, and planted on 10/10/18. Plant diameter was evaluated on 12/20/17 (70 days after planting) and percentage plant mortality was assessed on 01/03/17 (84 days after planting). Average of 10 plants per replication and 4 replications per treatment.
[a] Means in a column followed by the same letter are not significantly different according to Fisher's Protected LSD test (a = 0.05).

Efficacy of phosphite materials in controlling mefenoxam-resistant isolates.

Most of *Phytophthora* spp. isolates were sensitive to phosphite materials and were inhibited by 100 µg/ml. However, some isolates required more than 300 µg/ml to reduce their growth in vitro (Table 2). Fruit assays showed that phosphite controlled disease caused by *P. cactorum* isolates, including the ones with low in vitro sensitivity. However, phospite materials had no effect on *P. nicotianae*. Thus, poor activity of phosphite materials in field conditions might be related to the species of Phytophthora present in the field.

Table 2. Sensitivity of *Phytophthora* spp. isolates from commercial strawberry fields from 1997 to 2018 against different concentrations of phosphite.

Concentration range (µg ml^{-1})	# isolates		Total
	P. nicotianae	*P. cactorum*	
0-100	21	120	141
>100	3	8	11
Total	24	128	152

Fungicide and biological product alternatives to control Phytophthora Crown Rot.

Orondis® Gold (oxathiapiprolin) applied through drip 2, 6, and 10 weeks after planting (WAP) reduced plant mortality caused by *P. cactorum* to 12.7% compared to 43.8% in the inoculated control (Table 2). However, Helena Prophyt, RidomilGold®, and Actinovate did not reduce disease symptoms. Due to the presence of isolates resistant to mefenoxam, applications of RidomilGold® did not provide disease control as observed in some commercial fields.

Table 2. Plant mortality (%) of strawberry transplants inoculated with a mixture of isolates resistant and sensitive to mefenoxam (RidomilGold®) and treated with different products.

Treatment (rate/A)	Application type	Application timing (WAP)*	Plant mortality (%)[a]
Non-inoculated control	na	na	7.5 b
Inoculated control	na	na	43.8 a
Actinovate AG (0.12 and 6 oz/gal)	plant dip and drip	2,4,6,8,10	30.0 a
Helena Prophyt (2 pt)	spray	2,4,6,8,10	30.3 a
Ridomil Gold (16 fl oz)	drip	2,6,10	26.4 a
Orondis Gold (20 fl oz)	drip	2,6,10	12.7 b

a Average mortality of 15 weeks after transplanting. Means in a column followed by the same letter are not significantly different according to Fisher's Protected LSD test (a = 0.05).
* WAP = weeks after planting

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