Integrated approaches for the management of *Phytophthora spp.* in strawberries

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Summary

***Phytophthora cactorum* and *P. nicotianae*, causal agents of Phytophthora crown rot and leather rot, are mainly introduced into Florida production fields by infected nursery transplants. Although both *P. cactorum* and *P. nicotianae* were recovered from soil, frequency was very low. *Phytophthora* spp. are usually controlled by fungicide applications. However, in the 2017-18 season, resistance to RidomilGold® (mefenoxam) was found in 25% of crown rot isolates tested, whereas none of the leather rot isolates were resistant to Abound® (azoxystrobin). Heat treatment of strawberry transplants artificially inoculated with *P. cactorum* reduced mortality in field condition from 80% (non-tretated control) to 2.5% (heat-treated), whereas the use of white-striped plastic much showed no effect.**

Methods

Potential sources of inoculum of *Phytophthora* spp.

a) Nursery transplants: At the beginning of the 2017-2018 strawberry season, transplant samples were collected from the main strawberry nurseries from which *Phytophthora* species were isolated from plants in previous seasons. The trial was conducted at GCREC using plants collected from different boxes. When symptoms were observed, isolations were performed to identify the causal agent.

b) Soil: Using a calibrated drill, soil was sampled (20-cm depth) from commercial and research strawberry fields where the pathogen had been reported. Apple baits were used to detect the presence of *Phytophthora* species in the soil. Some of the isolates recovered were tested for pathogenicity by inoculation of a susceptible strawberry cultivar in the greenhouse. Roots of strawberry transplants cv. Winterstar™ were inoculated with a suspension adjusted to 10^4 zoospores/ml, while control plants were dipped in water. Five plants per each isolate were used and plant mortality was evaluated weekly. If symptoms were observed, re-isolations were made in order to confirm the causal agent. Isolates from plants and soil were morphologically and molecularly characterized as *Phytophthora cactorum* or *P. nicotianae*.

Heat treatment trials

In the laboratory, the effect of different combinations of temperature (40 [104°F], 44 [111.2°F], and 48°C [118°F]) and exposure periods (1, 5, 10, 30, 60, 120, and 240 min) on the survival of reproductive structures (zoospores) of *P. cactorum* was evaluated. Four isolates, two sensitive and two resistant to RidomilGold®, were used. In field trials, 'FL 127' (Sensation®) plants were inoculated with the same isolates, and submitted 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.

White-striped plastic-mulch trial

The effect of white-striped plastic mulch to cover the beds was assessed as a cultural alternative for management of Phytophthora crown rot. Black plastic and white-striped (black with a central 20-in white stripe) plastic mulch (previously tested by the

Dr. Shinsuke Agehara group) was used. 'FL 127' (Sensation®) plants were inoculated with a mixture of the same isolates used in the heat treatment trials. Control treatments were not inoculated. Symptom development and plant mortality were evaluated weekly throughout the strawberry season.

Monitoring for resistance to the fungicides mefenoxam (Ridomil Gold®) and azoxystrobin (Abound®)

Isolates recovered from symptomatic plants and fruits in commercial strawberry fields in 2017-2018 were tested for sensitivity to mefenoxam (RidomilGold®) and azoxystrobin (Abound®). EC_{50} (effective concentration that inhibits the pathogen growth in 50%) was determined to separate the isolates in two groups, sensitive or resistant.

Results

Potential sources of inoculum of *Phytophthora* spp.

a) Nursery transplants: Plant mortality varied from less than 1% to almost 15% depending on the nursery (Table 1). *P. cactorum* was isolated from all *symptomatic* nursery transplants. Mortality also varied within the same nursery depending on the strawberry cultivar (i.e., Nursery E – Sensation: 0% and Radiance: 12%). Moreover, variation was also observed even within the same nursery and cultivar. For example, Box 1 from nursery K had 0% mortality whereas Box 4 from the same nursery had 50%. These differences might be related to different planting areas within a nursery, for instance, an area with poor drainage.

b) Soil: From approximately 130 soil samples collected at commercial and research strawberry fields, *P. cactorum* and *P. nicotianae*, respectively, were recovered from 4 and 2 samples, during the summer, and from 2 and 0 samples after soil fumigation (Table 2).

Our results support the evidence that *Phytophthora* species barely survive in the soil during Florida summer, and that infected nursery transplants are likely the main source for yearly introduction of *Phytophthora* spp. into production fields.

Heat treatment trials. In the laboratory, results showed that germination of *P. cactorum* zoospores were totally inhibited at 40°C (104°F) after 60 min, and at 44 (111.2°F) and 48°C (118°F) after 5 min of exposure (Figure 1).

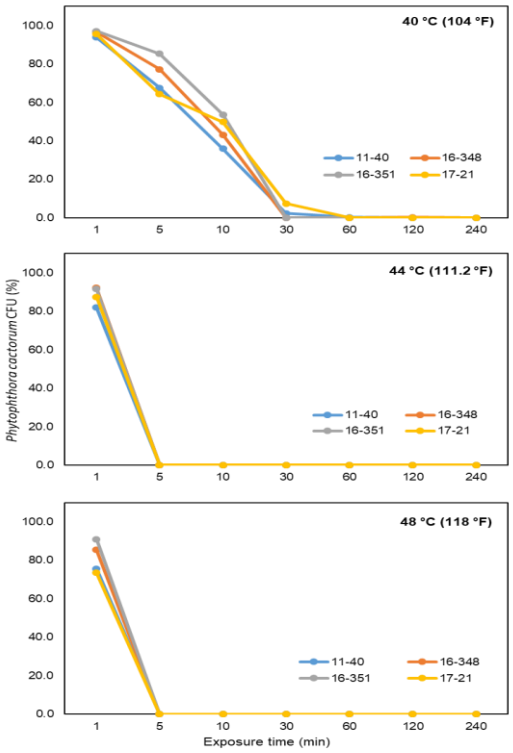

Figure 1. Percentage of *P. cactorum* colony-forming units (CFU) in relation to the non-treated control of four isolates (11-40, 16-348, 16-351 and 17-21) at 40, 44 and 48 °C during 7 exposure times (min).

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 80% in the non-treated inoculated control (Table 3 and Figure 2A). We envision heat treatment can be use as alternative for nurseries to reduce disease inoculum load from mother plant stock.

Figure 2. 'FL 127' (Sensation) plants inoculated with a mixture of sensitive and resistant isolates of *P. cactorum* and planted over black plastic 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 white-striped plastic (B).

White-striped plastic mulch trial. The use of white-striped (black with a central 20-in white stripe) plastic mulch did not reduce plant mortality caused by *P. cactorum* (75%) compared to the inoculated control planted on black plastic (80%) (Table 3 and Figure 2B).

Monitoring for resistance to the fungicides mefenoxam (RidomilGold®) and azoxystrobin (Abound®).
In the 2016-17 season, resistance to RidomilGold® (mefenoxam) was found in 14% of crown rot isolates, whereas in the 2017-18 season, 25% of the isolates tested were resistant to this fungicide (Figure 3). None of the leather rot isolates tested were resistant to Abound®(azoxystrobin). 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.

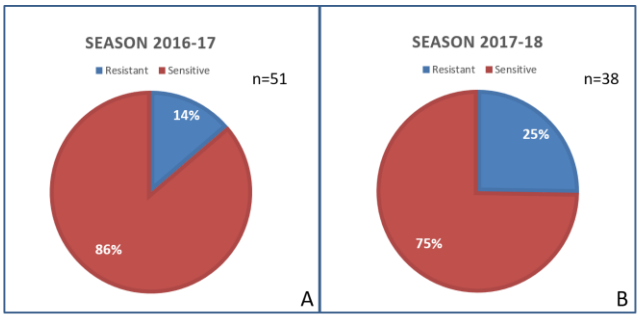

Figure 3. Percentage of RidomilGold®-sensitive and -resistant isolates of *P. cactorum* evaluated during the 2016-17 and 2017-18 strawberry seasons.

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Table 1. Percentage of mortality (%) of transplant samples collected from the main strawberry nurseries serving Florida during the 2017-18 season due to Phytophthora crown rot.

Nursery	Cultivar	Mortality (%)[a*]	
A	Yasmin	0	d
B	Yasmin	0	d
B	Radiance	0	d
C	Radiance	0	d
D	Radiance	0	d
E	Sensation	0	d
D	Florida Beauty	0.6	cd
F	Sensation	0.6	cd
G	Winterstar	0.6	cd
D	Sensation	0.6	cd
H	Florida Beauty	1.3	bcd
A	Radiance	1.3	bcd
I	Radiance	2.6	abcd
J	Radiance	2.6	abcd
K	Radiance	2.6	abcd
G	Sensation	5.3	abcd
G	Radiance	7.5	ac
H	Radiance	7.5	abc
K	Sensation	10.0	abc
E	Radiance	12.2	ab
L	Sensation	14.6	a

[a] Average mortality of seven 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).

Table 2. Detection of Phytophthora sp. from soil samples from different farms.

Farm	City	Pre-fumigation				Post-fumigation			
		Number		Positive results[a]		Number		Positive results[a]	
		Samples[b]	Sub-samples[c]	PC	PN	Samples[b]	Sub-samples[c]	PC[d]	PN[e]
A	Floral City	20	60	0	0	20	60	0	0
B	Dover	15	45	1	0	20	60	0	0
C	Parish	20	60	1	0	18	54	1	0
D	Dover	20	60	0	0	15	45	0	0
E	Lakeland	18	54	0	0	17	51	0	0
F	Lithia	20	60	1	1	20	60	1	0
G	Balm	20	60	1	0	20	60	0	0

* Samples were collected between August and September, 2017.

[a] per sample number; [b] composed by three samples in a same bag; [c] sub-samples for bait technique; [d] PC: Phytophthora cactorum; [e] PN: *Phytophthora nicotianae*.

Table 3. Plant diameter and plant mortality of plants inoculated with *Phytophthora cactorum* and heat treated or planted on white-striped plastic-covered beds.

Treatments	**Plant diameter (cm)** [a]	**Plant mortality (%)**[a]
White-striped plastic + inoculated	4.7 c	75.0 a
Black plastic + inoculated	3.2 c	80.0 a
Black plastic + heat treated + inoculated	26.1 b	2.5 b
Controls		
White-striped plastic + non-inoculated	30.0 a	0.0 b
Black plastic + non-inoculated	29.0 a	0.0 b
Black plastic + heat treated + non-inoculated	28.3 ab	0.0 b

'Florida 127' (Sensation®) plants were inoculated on 10/10/17, heat-treated at 44 °C for 4 hours (pre-heat at 37 °C for 1 hour) on 10/11/17, and planted on 10/12/17. Plant diameter was evaluated on 11/30/17 (49 days after planting) and percentage plant mortality was assessed on 12/04/17 (53 days after planting). Average of 10 plants per repetition and 4 repetitions 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).