Inoculum sources and alternative strategies for management of charcoal rot caused by *Macrophomina phaseolina*

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Summary

Management of charcoal rot, caused by *Macrophomina phaseolina*, relies on reduction of pathogen inoculum in the field. Besides the soil, we have determined that *M. phaseolina* can survive on old strawberry crowns and serve as source of inoculum to new transplants, especially when old crowns are disposed in between beds in fields where the plastic is reused the following season. Pre-plant fumigation with KPam, Pic80, and Telone C35 to a lesser extent, have shown to reduce *M. phaseolina* in the soil, however these fumigants are not as effective in reducing the pathogen population in the old-infected strawberry crowns and do not distribute uniformely in the soil. Removal of crop debris from fields with low to moderate incidence of charcoal rot reduced *M. phaseolina* populations in the soil, but had no significant effect on plant mortality. However, the use of white-stripped plastic mulch reduced plant mortality and might be an effective tool to manage charcoal rot.

Methods

Potential sources of inoculum of *M. phaseolina*. A) Nursery transplants: Strawberry transplant from the main nurseries used by Florida strawberry growers were sampled. Attempts were made to detect the presence of *M. phaselina* on symptomless plants, using a molecular technique called RPA. Sub-samples were planted in the field at GCREC (Balm, FL) and plant mortality was evaluated throughout the strawberry season. Isolations were made to identify the causal agent. B) Crop residue (strawberry crowns): Strawberry crowns were collected from fields where charcoal rot was reported at four different times: after the end of the 2016-17 season, during the summer, and before and after pre-plan soil fumigation at the beginning of the 2017-18 season. Crowns were processed in the laboratory and the survival of *M. phaseolina* inoculum was evaluated.

Infection/Dissemination mechanisms of *M. phaseolina*. Experiments were conducted in greenhouses and experimental fields at GCREC (Balm, FL). Strawberry cultivars with different levels of susceptibility to charcoal rot were used ('Strawberry Festival', 'Florida Beauty' and Winterstar™) and plants were monitored weekly for development of typical symptoms A) Greenhouse trials: Strawberries were planted in pots and inoculated with two different *M. phaseolina* isolates from our culture collection, or ground infected crowns from two commercial farms that had issues with charcoal rot in the 2016-2017 season. Three inoculation methods were used: (i) spraying *M. phaseolina* sclerotia or crown suspensions on leaves, (ii) adding 50 ml of suspension to the soil, and (iii) dipping trimmed roots in a suspension for 2 minutes. B) Field trials: To simulate commercial strawberry production on second year plastic-mulches, where strawberry plants from the previous season are maintained on the beds or disposed in between beds, strawberries were planted and old crowns infected with *M. phaseolina* were buried next to the transplants or placed on the ground in between beds. In the positive control treatments, plant roots were trimmed and dipped in

a suspension with a mixture of three *M. phaseolina* isolates commonly used in GCREC trials.
Plants were overhead irrigated for 10 days.

Heat treatment and white-striped plastic mulch. The effect of different combinations of temperature and time periods on the survival of four different isolates of *M. phaseolina* was evaluated in the laboratory. These same isolates were used to inoculate 'Strawberry Festival' plants prior heat-treatment using an aerated steam chamber, a.k.a. plant sauna, at GCREC (37 °C [98.6 °F] for 1 hour, followed by 44 °C [111.2 °F] for 4 hours). Along with the heat-treatment trial in the field, the effect of white-striped plastic-mulch covered beds (black with a central 20-in white stripe) was also evaluated.

Crop debris removal in Floral City, FL. The impact of crop residue removal (mainly old strawberry crowns) on the survival of *M. phaseolina* populations in the soil and on control of the disease were assessed on a commercial farm in Floral City, FL. Soil samples were collected during the fallow period (between April and August 2017 when *Crotalaria juncea* was used as cover crop), before and after pre-planting soil fumigation with Telone C35, and at the end of the 2017-218 strawberry season. Strawberry crowns were also collected from the areas where crop residue was not removed during the fallow period, and at pre-fumigation. Each treatment consisted of four plots with 50 plants of 'Radiance' and four plots with 50 plants of 'Florida Beauty'. Plant mortality was evaluated monthly from November 2017 to March 2018.

Soil fumigant trials at the FSGA research field in Dover, FL. The efficacy of soil fumigants was determined by evaluating the survival of *M. phaseolina* inoculum on infested corn-cob litter buried in the centers and sides of plastic-covered beds as well as strawberry crowns infected with *M. phaseolina* buried in the sides of the beds. Samples were disinfested, plated on semi-selective medium and incubated at 30 °C in the dark. *M. phaseolina* inoculum was recovered and expressed as colony forming units per bag or gram of crown – CFU/bag or CFU/g of crown.

Results

Potential sources of inoculum. A) Nursery transplants: *M. phaseolina* detection using molecular techniques (RPA) was only possible when typical vascular ring discoloration symptoms were present. B) Crop residue (strawberry crowns): *M. phaseolina* was recovered from all the crown samples colleted at a range of 150 to 3000 colony-forming units per gram of crown (CFU/g), demonstrating that the fungus can survive on old-strawberry crowns during the Florida summer.

Infection/Dissemination mechanisms of *M. phaseolina*. A) Greenhouse trials: Results indicated that new strawberry transplants could be infected and develop charcoal rot symptoms from infecions starting from the soil or from the top (Figure 1). Furthermore, these trials confirmed different levels of susceptibility to charcoal rot among the cultivars used, with 'Strawberry Festival' being very susceptible and Winterstar™ being moderately resistant.

Figure 1. Internal symptoms of charcoal rot, including reddish-brown necrotic areas on the top (white arrow) of strawberry crowns.

B) Field trials: As observed in greenhouse trials, infected strawberry crowns could act as source of inoculum and infect new strawberry transplants, especially when disposed in between beds, regardless of the cultivar used (Table 1).

Heat treatment and white-stripped plastic mulch. Laboratory results showed that temperatures as high as 52 °C (126 °F) and 56 °C (133 °F) were able to completely inhibit the development of *M. phaseolina* colonies especially after 30 and 10 minutes of exposure, respectively (Figure 2).

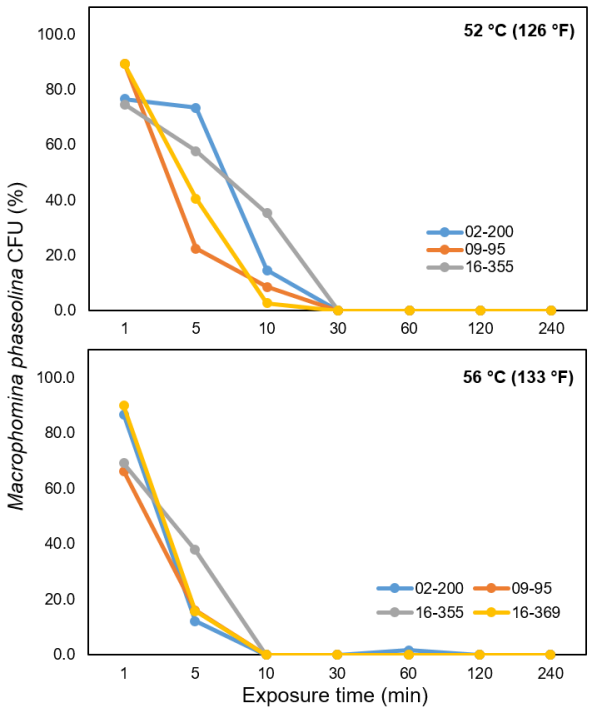

Figure 2. Percentage of *M. phaseolina* colony-forming units (CFU) in relation to the non-treated control of four isolates (02-200, 09-95, 16-355 and 16-369) at 52 and 56 °C during 7 exposure times (min).

However, temperatures higher than 48 °C (118 °F) can cause damage to strawberry plants, especially after long periods of exposure. Therefore, the treatment used in the *plant sauna* (37 °C [98.6 °F] for 1 hour, followed by 44 °C [111.2 °F] for 4 hours) did not reduce plant mortality caused by *M. phaseolina* compared to the non-treated inoculated control. However, the use of white-striped plastic mulch reduced the incidence of charcoal rot (Figure 3 and Table 2).

Figure 3. 'Strawberry Festival' plants inoculated with *M. phaseolina* and planted over white-striped plastic (A) or 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 (B).

Crop debris removal in Floral City, FL. Although plant mortality was higher in the area from where crop residue (old strawberry crowns) was removed for both 'Radiance' and 'FL Beauty', the amount of *M. phaseolina* colonies recovered from the soil was lower. Fumigation with Telone C35 reduced the amount of inoculum at all sampled areas (post fumigation). The high temperatures during the summer had no impact on the survival of *M. phaseolina* on strawberry crowns, as the colony-forming units were practically the same for both periods of sampling (Table 3). However, at the end of the season, the Macrophomina population in the soil was 10 fold higher where old crowns were not removed.

Soil fumigant trials at FSGA research field in Dover, FL. All treatments were effective in reducing *M. phaseolina* on artificial inoculum (infested corn-cob litter) placed in the centers and sides of the beds, whereas the Kpam and DMDS + Pic treatments did not reduce inoculum on infected strawberry crowns placed at the side of the beds compared to the non-treated control. All treatments also reduced plant mortality, and no difference was observed among them (Table 4). Nonetheless, the cause of plant mortality was attributed to a combination of nematodes and different pathogenic fungi, as *Colletotrichum* sp., *Phytophthora* sp., and *M. phaseolina* were isolated from crowns of wilting plants.

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Table 1. Charcoal rot incidence (%) of strawberry cultivars 'Strawberry Festival' 'Florida Beauty' and Winterstar™ after different inoculation methods in the field, 10 weeks after planting.

Inoculation method	Charcoal rot Incidence (%)					
	Cultivar					
	Strawberry Festival		FL Beauty		Winterstar	
Inoculated control	100.0	a	100.0	a	93.0	a[y]
Old crowns thrown in the aisles	57.0	b	43.0	b	90.0	ab
Old crowns buried next to new plants	43.0	bc	27.0	b	73.0	bc
Non-inoculated control	27.0	c	13.0	b	60.0	c

[y] Treatments followed by the same letter within a column are not significantly different according to Fisher's Protected LSD test (a = 0.05).

Table 2. Plant mortality (%) of 'Strawberry Festival' plants inoculated or not (control) with *M. phaseolina* and heat treated or planted on white-striped plastic mulch covered beds

Treatments	Plant mortality (%)[x]	
	Experiment 1[y]	Experiment 2[z]
Multi-colored plastic + inoculated	**25.0 b**	**10.0 de**
Black plastic + inoculated	**72.5 a**	**57.5 ab**
Black plastic + heat-treatment + inoculated	**90.0 a**	**77.5 a**
Controls		
Multi-colored + non-inoculated	2.5 c	2.5 e
Black plastic + non-inoculated	0.0 c	22.5 cd
Black plastic + heat-treatment + non-inoculated	15.0 bc	35.0 bc

[x] Final percentage of plant mortality was taken on 60 days after planting. Average of 10 plants per repetition and 4 repetitions per treatment.

[y] Bare-root plants of 'Strawberry Festival' 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.

[z] Cut-top plants of 'Strawberry Festival' were inoculated on 03/05/18, heat-treated at 44 °C for 4 hours (pre-heat at 37 °C for 1 hour) on 03/06/18, and planted on 03/07/18.

Table 3. Survival of *Macrophomina phaseolina* in the soil and strawberry crowns collected at three different areas of a commercial farm with charcoal rot in Floral City, FL during the fallow period, before and after pre-planting soil fumigation, and at the end of the 2017-18 strawberry season. Plant mortality (%) of strawberry cultivars 'Florida Radiance' and 'Florida Beauty'

Area[z]	*M. phaseolina* (CFU g^{-1})[x]							Plant Mortality (%)[y]	
	Soil					Crowns			
	Fallow period	Pre-fumigation	Post fumigation	End of season		Fallow period	Pre-fumigation	End of season	
				Radiance	FL Beauty			Radiance	FL Beauty
Plants removed	6.3	3.1	2.4	3.2	1.6	-	-	11.5	18.5
Grower standard	3.0	9.2	4.9	4.4	16.1	2914.3	2130.0	3.5	15.0

Fallow period corresponds to the period between April and August 2017 when *Crotalaria juncea* was used as cover crop. Soil was fumigated with Telone C35 shank applied at 35 gpta on August 30 and 31, 2017. 'Rad' = 'Radiance'. 'FL' = 'FL Beauty'.

[x] *M. phaseolina* colony-forming units per gram of soil and strawberry crown (CFU g^{-1}). Each value is the average of three samples (4 subsamples) collected during the fallow period (07/17/17) with three replicates of two dilutions per sample, and four samples (with 4 subsamples) at pre-fumigation (08/22/17), post fumigation (09/25/17) and end of the season (03/06/18) at plots having either 'Radiance' or 'FL Beauty' cultivars, with two replicates of two dilutions per sample.

[y] Incidence of plant mortality (%). Each value is the average of four plots for each one of the cultivars. Each plot contained 50 plants. 'Radiance' plants come from a nursery in Canada and 'FL Beauty', from a nursery in California.

[z] "Plants removed" = area where strawberry plants were removed out of the field at the end of the 2016-2017 season before tillage; "Grower standard" = area where plants were incorporated to the soil through tillage after the end of the season.

Table 4. Effect of products applied to the soil on populations of *Macrophomina phaseolina* and on plant mortality (%) in a strawberry field in Dover, FL (FSGA) in the 2017-18 season

Treatments		*M. phaseolina* (CFU bag^{-1})[a]			Pr>F	*M. phaseolina* (CFU g^{-1} crown)[a]	Plant Mortality (%)[b]
		3" center	8" center	3" side		3" side	
Telone C35 (30gpta)	Shank	0 c[c]	0 c	0 d	NS	0 b	8.97 b
PicClor60 (300lb/ta)	Shank	0 c	0.4 c	0.1 cd	NS	0 b	8.72 b
PicClor80 (320 lb/ta)	Shank	0 c	0.3 c	0 d	NS	0 b	9.41 b
Pic100 (320 lb/ta)	Shank	0.1 c	3.3 c	24.8 bc	NS	0.6 b	10.84 b
Kpam (60 gpta)	Drip	0.3 c	0.1 c	1.3 cd	NS	1.3 ab	9.41 b
DMDS + Pic (40 gpta)	Shank	35.8 b	85.4 b	57.5 b	NS	32.5 ab	10.44 b
Untreated control	n.a.	197.4 a	192.4 a	519.5 a	NS	189.2 a	35.25 a

"Telone C35" = 1,3-Dichloropropene:chloropicrin (63/35), "Pic-Clor 60" = 1,3-Dichloropropene:chloropicrin (39/60), "Pic-Clor 80" = 1,3-Dichloropropene:chloropicrin (20/80), "Pic-Clor 100" = chloropicrin, "Kpam" = potassium N-methyldithiocarbamate, "DMDS + Pic" = dimethyl disulfide:chloropicrin (79/21), "n.a" = not applicable.

[a] *M. phaseolina* colony-forming units per bag (CFU bag^{-1}) or per gram of crown (CFU g^{-1} crown). Each value is the average of two beds, four plots and three replicates of three dilutions per bag or crown.

[b] Percentage of plant mortality (%). Each value is the average of the count of wilted and dead plants at two beds and four plots per treatment.

[c] Treatments followed by the same letter within a column are not significant different according to the LSD test ($p \leq 0.05$). NS: Not significant