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

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Summary

Integrated approaches to manage charcoal rot, caused by *Macrophomina phaseolina* were investigated, such as alternative soil fumigants, use of cover crops, and determing if the fungus can be disseminated with nursery transplants. Alternative fumigants such as Kpam® and Pic-Clor 80 were effective in reducing the pathogen population in the soil. Cover crop trials were inconclusive, since low natural inoculum was recovered from the soil. *M. phaseolina* was mostly isolated from transplants coming from six main nurseries.

Methods

Alternative Soil Fumigants Trials. Two trials were conducted, one on a commercial farm with high infestation of *M. phaseolina*, and one at the FSGA research field in Dover.

A) Commercial farm: Natural inoculum of *M. phaseolina* was determined by collecting soil samples prior to and after fumigation with Vapam® (75 gpta) or Telone EC (18 gpta) at crop termination (Feb/Mar, 2016) and prior to and after pre-plant fumigation (Aug, 2016) with Kpam® (63 gpta) or Inline® (35 gpta). Soil samples were disinfested, plated on semi-selective medium and incubated at 30 °C in the dark. Plant mortality of cultivars 'Albion' and 'Radiance' was determined at three different times. Strawberry crowns from the 2015-16 season that were disposed on the ground between the reused plastic-covered beds for the season 2016-17 (Fig. 1a) were also collected and evaluated for fungus survival.

B) FSGA research field: the efficacy of alternative soil fumigant treatments 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.

Cover Crop Trials. The effect of plastic reuse, secondary and cover crops on the survival of *M. phaseolina* was evaluated in trials at Balm and Dover. Soil samples were collected at different periods from plot treatments described in Table 1. Soil samples were analyzed as previously described.

Detection of Inoculum from Nursery Plants. At the beginning of the 2016-17 season, crown rot samples from different strawberry farms were processed and isolations were made to identify the causal agent.

Results

Alternative Soil Fumigants Trials. A) Commercial farm: The amount of inoculum in the Vapam-Kpam area was lower than the Telone-Inline area in all soil-sampled periods. Reduction of soil inoculum was observed after the crop termination treatment with Vapam®, but not with Telone EC. For the Telone-Inline area, a decrease of population was observed over the summer, which could be related to the residual effect of the fumigant or the soil temperature during the summer (Table 2). Pre-plant fumigations with Kpam®and Inline® did not further affect the survival of *M. phaseolina* inoculum since the inoculum levels were already low. Although inoculum was reduced, plant mortality from 13 to 35% was observed with no differences according to the different fumigant treatments (Table 3). We later found that the pathogen survived over the summer in strawberry crowns from the previous season (Fig. 1a). Thus, further investigation is needed to determine if these strawberry crown residues from the previous season serve as the major source of inoculum for areas where plastic is re-used.

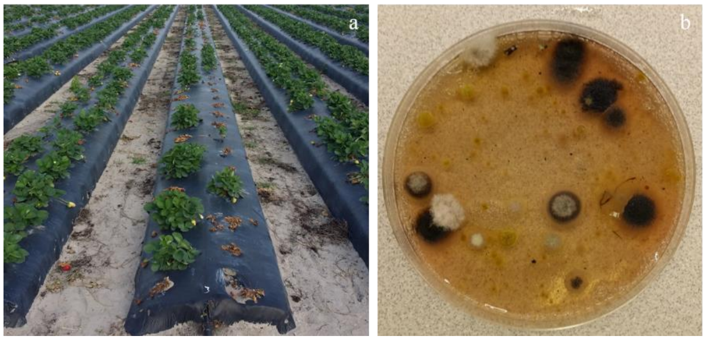

Figure 1. (a) Crop residue, i.e. strawberry crowns, from the 2015-16 season in between the reused plastic-covered beds of season 2016-17. (b) *M. phaseolina* (black colonies) growing on semi-selective medium after processing of infected strawberry crowns.

B) <u>FSGA research field</u>: Shank applications of Telone C35 and Pic-Clor80 under VIF and drip applications of Kpam® and Dominus were as effective as MeBr50. However, Dominus was not effective in controlling *M. phaseolina* on the sides of the beds. DMDS + Pic applied via shank was less effective than applied through the drip (Table 4).

Cover Crop Trials. Our results show that few pathogen colonies were recovered in both locations and sampling periods. The low inoculum levels do not allow for definite conclusions, and further experiments are being planned with inoculated plants under controlled conditions in the greenhouse.

Detection of Inoculum from Nursery Plants. From all the samples with symptomatic crown rots, 12% were confirmed as *M. phaseolina*. In the 2015-16 season, most of the strawberry samples in which *M. phaseolina* was isolated from came from nurseries “C” (21%), “A” (14%), “L” (14%) and “D” (14%) (Fig. 2a). In 2016-17, samples diagnosed with charcoal rot came from “C” (14%), “D” (14%), “E” (13%) and “F” (13%) (Fig. 2b).

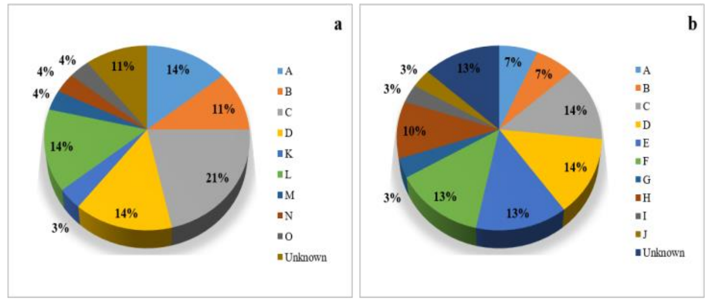

Figure 2. Strawberry nurseries from where strawberry plant samples with crown rot symptoms were diagnosed with charcoal rot and *M. phaseolina* was recovered during the 2015-16 (a) and 2016-17 (b) seasons.

Comments on Fusarium wilt: Fusarium wilt, caused by *F. oxysporum* f. sp. *fragariae*, has been a problem in strawberry fields in California during the last decade, but has never been reported in Florida. However, *Fusarium* spp. are commonly isolated within *M. phaseolina* from plants with crown rot symptoms. A molecular diagnostic tool was used to identify *Fusarium* spp. isolates collected in 2013, 2014, 2016 and 2017. Thus far, none of the samples has been confirmed as *F. oxysporum* f. sp. *fragariae*.

Disclaimer

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Table 1. Treatments evaluated by Dr. Nathan Boyd for control of weeds for which soil samples were collected to evaluate the effect of fumigation, cover/secondary crop or fallow period on *Macrophomina phaseolina* inoculum levels.

Trt (main plot/subplot)	1st strawberry crop	2nd crop	fallow period	2nd strawberry crop
11	fumigated with C35 and transplanted with Radiance in October - no herbicide	none	Terminate strawberries with gramoxone. Leave plastic in place. Apply glyphosate /gramoxone on same schedule as used in treatment 21	Inject fumigant TBD. No herbciide
12	fumigated with C35 and transplanted with Radiance in October - no herbicide	A cucurbit (cantaloupe) will be planted in February. Strawberries will be removed by hand - hand weeding	Terminate crop with gramoxone. Leave plastic in place. Apply glyphosate /gramoxone on same schedule as used in treatment 21	Inject fumigant TBD. No herbciide
21	fumigated with C35 and transplanted with Radiance in October - no herbicide	none	Terminate strawberries with gramoxone. Remove plastic and disk. Alternate gly. /gram. and cultivation. Apply herbicides as needed to control weeds	Fumigated with C35 or Pic-Clor 60. No herbicide
22	fumigated with C35 and transplanted with Radiance in October - no herbicide	A cucurbit (cantaloupe) will be planted in February. Strawberries will be removed by hand - hand weeding	Terminate crop with gramoxone. Remove plastic and disk. Alternate gly. /gram. and cultivation. Apply herbicides as needed to control weeds	Fumigated with C35 or Pic-Clor 60. No herbicide
31	fumigated with C35 and transplanted with Radiance in October - no herbicide	none	Terminate strawberries with gramoxone. Remove plastic, plant Sunn Hemp. Mow once to encourage branching.	Fumigated with C35 or Pic-Clor 60. No herbicide
32	fumigated with C35 and transplanted with Radiance in October - no herbicide	A cucurbit (cantaloupe) will be planted in February. Strawberries will be removed by hand - hand weeding	Terminate strawberries with gramoxone. Remove plastic, plant Sunn Hemp. Mow once to encourage branching.	Fumigated with C35 or Pic-Clor 60. No herbicide

Table 2. Effect of products applied to soil as crop termination and pre-planting treatments in a commercial strawberry field in Floral City, FL on natural populations of *M. phaseolina* in the soil.

Area/Fumigants	*M. phaseolina* (CFU g^{-1}) [a]			
	Crop termination		Pre-planting	
	Pre fumigation	Post fumigation	Pre fumigation	Post fumigation
Vapam-Kpam	1.96 A[b]	0.22 B	0.01 B	0.01 B
Telone-Inline	5.8 AB	7.17 A	1.88 BC	1.67 C
Pr>F[c]	0.027	0.002	0.013	0.032

"Vapam-Kpam" = area where strawberries were terminated with Vapam® (75 gpta) and pre-planting fumigation was made with Kpam® (63 gpta), "Telone-Inline" = area where strawberry plants were killed with Telone EC (18 gpta) and Inline® (35 gpta) was applied as pre-planting fumigant.

[a] *M.phaseolina* colony-forming units per gram (CFU g^{-1}). Each value is the average of five beds and four plots for the Vapam-Kpam area and six beds and one plot for the Telone-Inline area, and three replicates of two dilutions per soil sample of 10 g.

[b] Means in the row followed by the same letter are not significantly different by LSD test ($p \leq 0.05$).

[c] Probability of a greater F value, p values < 0.05 were considered significant.

Table 3. Effect of products applied to soil as crop termination and pre-planting treatments in a commercial strawberry field in Floral City, FL on plant mortality caused by *M. phaseolina* in strawberry cultivars 'Radiance' and 'Albion'.

Area/Fumigants	Plant mortality (%) [a]			
	Radiance			Pr>F[c]
	6-Dec	25-Jan	3-Mar	
Vapam-Kpam	13.6 a[b]	15 a	15.7 a	0.722
Telone-Inline	13.6 a	17.3 ab	18.6 b	0.057
Pr>F[c]	0.932	0.578	0.325	
	Albion			Pr>F[c]
	6-Dec	25-Jan	3-Mar	
Vapam-Kpam	27.4 a	29.4 a	30.3 a	0.728
Telone-Inline	27.1 a	31.6 a	35.1 a	0.179
Pr>F[c]	0.848	0.445	0.359	

"Vapam-Kpam" = area where strawberries were terminated with Vapam® (75 gpta) and pre-planting fumigation was made with Kpam® (63 gpta), "Telone-Inline" = area where strawberry plants were killed with Telone EC (18 gpta) and Inline® (35 gpta) was applied as pre-planting fumigant.

[a] Incidence of plant mortality (%). Each value is the average of five beds and four plots for the Vapam-Kpam area and six beds and one plot for the Telone-Inline area. Each plot contained 77 plants.

[b] Means in the row followed by the same letter are not significantly different by LSD test ($p \leq 0.05$).

[c] Probability of a greater F value, p values < 0.05 were considered significant.

Table 4. Effect of products applied to soil on artificial inoculum of *M. phaseolina* at different depths in a strawberry field in Dover, FL during the 2016-17 season.

Treatments	Application methods	Rate		*M. phaseolina* (CFU bag^{-1}) [a]			Pr>F[c]
				7.6 cm center	20.3 cm center	7.6 cm side	
1. MeBr50	Shank + Vapor safe	320	lb/ta	0.0 d	0.0 d	0.0 d[b]	1.000
2. Telone C35	Shank + VIF	30	gpta	0.1 d	0.1 d	0.0 d	0.983
3. Pic-Clor 60	Shank + VIF	300	lb/ta	122.1 b	559.3 bc	76.5 bcd	0.057
4. Pic-Clor 80	Shank + VIF	23	gpta	0 d	0.0 d	0 d	1.000
5. Pic-Clor 100	Shank + VIF	21.6	gpta	10.9 c	48.3 c	23.6 bcd	0.568
6. Kpam	Drip + VIF	62	gpta	0.3 d	0.5 d	0.6 cd	0.963
7. DMDS + Pic	Shank + TIF	30	gpta	247.9 a	518.1 ab	374.1 a	0.757
8. DMDS EC + Pic EC	Drip + TIF	30	gpta	0.1 d	0.4 d	76.0 bc	0.012
9. Dominus	Drip + VIF	30	gpta	0.4 d	0.0 d	137.5 b	0.004
10. Untreated control	n.a.	n.a.		529.4 a	577.0 a	483.8 a	0.976
Pr>F[c]				<0.0001	<0.0001	<0.0001	

"MeBr50" = Methyl bromide + Chloropicrin (50/50), "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), "DMDS EC + Pic EC" = Dimethyl disulfide:chloropicrin (79/21), "Dominus" = Allyl isothiocyanate, "n.a" = not applicable.

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

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

[c] Probability of a greater F value, p values < 0.05 were considered significant.