Title: Effect of soil amendments and solarization on plasticulture strawberry growth and yield and soil borne pathogen communities in Arkansas.

Progress Report (SRSFC Project #2010-05)

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Research Proposal

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Objectives:
The objectives of this project are: 1) to determine the effect of incorporation of fresh brassica species or mustard seed meal for biofumigation and solarization on the severity of soil borne diseases and nematodes of strawberry 2) to characterize changes in soil health and microbial composition that result from these practices in an annual strawberry production system, 3) to determine the impact of these practices on insects, foliar diseases, and weeds in the production system, and 4) to estimate the economic feasibility of these practices in a plasticulture system.

Justification:
This request is for a second year of support for the project that was funded in 2009 (currently underway). We would like to expand our focus to include a considerably more in-depth characterization of the soil microbial community and quantification of the pathogens that are associated with the various treatments using both selective media and a molecular approach using DGGE analysis.

There are approximately 300 acres of plasticulture strawberries in Arkansas, and acreage is increasing. A standard practice is to produce the crop in raised beds that have been methyl bromide-fumigated and covered with black plastic mulch. Improved crop growth and yield are expected to result from the use of methyl bromide as it controls all major soil borne plant pathogens of strawberry as well as certain insects, nematodes and weeds (1). However, the use of methyl bromide in strawberry production systems as well as for all other crops is being phased out because it is an ozone depleting substance (2). Alternatives to fumigation with methyl bromide are needed, but these alternatives must be efficacious in controlling soil borne pests, compatible with current production practices, and economically feasible.

Biofumigation is a term that describes the use of certain *Brassica* species in rotation or as green manure crops to suppress soil-borne pests (3). *Brassica* spp. produce glucosinolates which decompose into chemicals including isothiocyanates, thiocyanates, nitriles, or other compounds by enzymatic hydrolysis as they decompose in the soil (4). These compounds are volatile and can be toxic to many organisms including bacteria, fungi, nematodes, insects, and germinating seeds (5, 6). A supposed mechanism of disease suppression has been based on the release of these chemicals as volatiles. However, certain products of glucosinolate hydrolysis are detected in soil for longer periods of time, with reports ranging from 24 hours (7) to 12 days(8). In studies where brassica seed meal was incorporated into soil four weeks prior to inoculation with pathogenic *Rhizoctonia*, there was still significant reduction in apple seedling infection compared to non-amended soil, suggesting other factors besides glucosinolate hydrolysis products also play a role in suppressing disease (7). Furthermore, Brassica seed meal amendments suppressed *Pratylenchus pentrans* and increased fluorescent Pseudomonas spp., actinomycetes, and total bacterial populations. Biofumigation has been studied for suppression of soil borne pathogens on soybean (9), pea (10), tomato (11) and cotton (12). Many factors influence the biofumigation potential of the brassicas and include environment and ontogeny (13).

Solarizarion is another technique that is used for suppression of soilborne plant pathogens and other pests including, insects and weeds and weed seeds. The effectiveness of solarization when compared to chemical controls methods in strawberries varies according to location, time of

year, and type of plastic being used. Some studies indicate positive results (14) while others indicating a lack of effectiveness (15).

Common soilborne diseases of strawberries are black root rot (*Pythium ultimum, Rhizoctonia fragariae*, and *Cylindrocarpon* spp.), crown and leather rot (*Pythopthora cactorum*), red stele (*Phytophthora fragariae*) and Verticillium wilt *(Verticillium dahliae*). Rotation of strawberry with broccoli led to a reduction in Verticillium wilt and decreased the number of microsclerotia in the soil compared to non-brassica lettuce (16). Additionally, *in vitro* studies have shown that macerated roots of brassica suppressed six different soil borne pathogens of strawberry including *Pythium ultimum, Rhizoctainia fragariae, Phytophthora cactorum, Fusarium oxysporum, Alternaria alternate, Colletotrichum dematium* and *Cylindrocarpon destructans* (17). Also, a Brassica-strawberry cropping sequence resulted in increased fruit yield compared to a non-treated control with yields that were numerically lower but not significantly different from methyl bromide treated plots (18).

Materials and Methods:

This research will be conducted at two sites in Arkansas. Site 1: UA Vegetable Research Station- Kibler, located in Arkansas River Valley; Site 2: Southwest Research and Extension Center- Hope in the southwestern part of the state. At each site, the experiment will be set up as a randomized complete block design with four replications of each treatment. Individual plots will be a single bed 20 feet long. Six treatments will be included at each site. Plastic mulch will be standard plastic (4 mil) at all locations and in all treatments except for the Midas treatment where VIF film will be used. Soil moisture will be monitored in all plots and temperature will be measured continuously at 2 and 10 cm depth in all treatments in one replication.

TRT 1- Control: No pre-plant fumigation, solarization, or biofumigation. Beds will be formed and tarped about August 1.

TRT 2- Standard fumigation with methyl iodide (Midas®) fumigation (75 lb/a) in early August following standard commercial application procedures.

TRT 3- Biofumigation: Turnip (Seven Top) will be sown broadcast (6 kg/ha) over the rows in early June and allowed to grow until approximately August 1. Biomasss cover will be estimated by harvesting plant material from a 1 ft^2 quadrat in each plot. The plants will then be shredded with a flail-type mower and incorporated by disking immediately before beds are formed and covered with plastic.

TRT 4- Solarization: Beds will be formed about August 1 and covered with clear plastic. Soil moisture will be maintained near field capacity until planting time. Immediately prior to transplanting, the plastic will be spray painted black to moderate soil temperatures after planting.

TRT 5. Combination of biofumigation and solarization: The *Brassica* will be sown, shredded, and incorporated as in TRT 3. Immediately after incorporation (August 1), beds will be formed and covered with clear plastic mulch. At the time of transplanting (ca. October 1 in Kibler and October 10 at Hope), plastic will be spray painted black as in TRT 4.

TRT 6. Mustard seed meal (Green Envy®) at 1,000 lb/a. will be incorporated into beds about August 1, immediately before bed formation and tarping.

Pre-plant land preparation and other cultural practices will follow standard commercial recommendations (19). 'Chandler' or 'Camarosa' strawberries (according to availability) will be used. Irrigation will be via drip, and nitrogen applications will be according to leaf and petiole

assay. Fruit yield and size, number of crowns, disease incidence and severity, arthropod damage, and weed presence and density will be monitored and recorded. Insects and foliar diseases will be managed within season as needed according to standard control practices. Input costs associated with each treatment will be tabulated for evaluation of profitability.

Nematode population densities will be recorded in all plots at transplanting, and when the crop is terminated the following year. Soil samples will be collected at turnip planting, immediately after incorporation, and at strawberry planting. Pathogens involved in black root rot (*Pythium* and *Rhizoctonia*) will be assayed from soil samples. *Phytophthora* populations will not be monitored from these samples due to lack of a suitable soil isolation method. The soil borne pathogens, *Pythium* and *Rhizoctonia*, will be cultured on P_5ARP medium using dilution plating and Ko and Hora medium by the multiple-pellet technique, respectively. Roots of strawberries will be assayed at the time of strawberry planting, the beginning of flowering, and harvest. Roots will be visually inspected and plated for isolation of pathogens involved in black root rot (*Pythium*, *Rhizoctonia* and *Cylindrocarpon)*, red stele (*Phythophthora fragariae*) and crown and leather rot (*P. cactorum*) to determine treatment effects. Roots will be plated on the following media: water agar for isolation of *Pythium*, *Rhizoctonia* and *Cylindrocarpon*, P_5ARP with benomyl and hymexazol for isolation of *P. cactorum* and P_5ARP with hymexazol for isolation of *P. fragariae*.

Bacteria, fungi and actinomycete populations will be determined by plating soil dilutions on the appropriate media. Bacteria will be plated on 10% TSA and grown for three days, fungi will be plated on Martin's Rose Bengal + streptomycin sulfate and grown for four days and actinomycetes will be plated on chitin agar and grown for 14 days. As culturable methods may not show population shifts, a molecular approach using DGGE will also be applied to soil samples. Additional sampling times, 4 and 15 days after each Brassica termination, will also be taken and subjected to DGGE analysis. DGGE amplifies a common template among a group of organisms, and then the amplification products are separated on a polyacrylimide gel based on sequence variation. DGGE will be used to amplify the 16S rRNA gene of bacteria and the 18S rRNA gene of fungi. Differences in banding patterns will reveal sequence variations within a group of organisms and give an indication of species differentiation. Therefore, this method will show if soil microbial populations differ among treatments and within treatments. Isolated DNA bands of interest can be sequenced and specific organisms which are enriched or suppressed can be identified using sequence databases. Additionally, if certain treatments show promising results, DGGE may also be applied to organisms that have been associated with soil disease suppression such as *Streptomyces*, *Pseudomonas*, *Bacillus*, or *Trichoderma* populations.

2009-2010 Results.

A brassica summer cover crop (turnip) , mustard seed meal, solarization or a combination of the cover crop and solarization were compared to no soil treatment prior to establishing the strawberry crop. General microbial and suspect pathogen populations from soils were quantified by plate count methods. Additional soil samples were taken after cover crop incorporation to generate denatured gradient gel electrophoresis (DGGE) profiles for bacterial and fungal populations. Roots of strawberry plants, including the initial transplants, were also analyzed for the isolation frequency of plant pathogens.

Excessive rainfall combined with an unusually cold winter resulted in virtually no marketable yield at either location in 2010 – a situation also encountered by most commercial growers in the state. Plants did, however, survive and grew vegetatively, so soil microbial populations were monitored as planned.

Pythium populations were numerically higher in soils following the brassica cover crop compared to the control, but results were only significant (P=0.05) at Kibler (Table 1). *Pythium* was also increased when brassica cover crop was followed by solarization and in mustard seed meal amended soils compared to the control. *Rhizoctonia* was only isolated from soil at the Hope location, where binucleate and multinucleate *Rhizoctonia* populations were higher in soils that received a brassica cover crop compared to no soil treatment. However, soil treatments usually did not significantly affect the frequency of *Pythium*, *Rhizoctonia* or *Colletotrichum* isolated from the roots of strawberry (Table 2). The only exception was in plots that had been planted with a brassica cover crop and then solarized prior to strawberry planting. In these plots, 15.3% percent of strawberry plants were found to be colonized by *Rhizoctonia solani* compared to only 5.0% in control plots. Approximately 80% of strawberry transplants were colonized by *Pythium*, *Rhizoctonia* or *Colletotrichum* before they were planted into the test plots. Root ratings, which were used to monitor disease severity based on the percent discoloration of roots, did not differ among the treatments (Table 3). Along with contamination of transplants, unusually high rainfall amounts prior to and shortly after strawberry transplanting likely contributed to a compromised root system for all plants.

Soil treatments did affect the level of bacterial, fungal and actinomycete populations in the soil at the time of brassica cover crop termination and at strawberry transplant (Table 4). In all cases, there was a trend for higher bacterial, fungal and actinomycete populations in brassica, brassica plus solarization and mustard seed meal amended soils compared to solarized only and control soils, yet results were not always significant at both test locations. Total culturable, bacterial populations were significantly higher in soils that had been planted with a brassica cover crop followed by solarization and soils receiving mustard seed meal amendments at both locations at the time of strawberry transplanting.

Conclusions:

This project has successfully shown how including soil treatments such as a brassica cover crop, solarization or mustard seed meal application as a practice in annual strawberry production can enhance the soil microflora, especially the bacterial community. Since changes could be observed in both the bacterial and fungal communities throughout the sampling times, this system has the potential to produce a soil that is more diverse and possibly reduce populations or colonization of roots by soilborne pathogens. The study is being repeated a second year at both locations to see how the crop responds under more favorable growing conditions as well as to monitor microbial population dynamics after a second year.

Impact: This work may lead to a more biorational approach for managing soilborne pathogens in plasticulture systems, particularly for small and mid-scale growers who do not have sufficient land to rotate crops appropriately.

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Table 1. Soil populations of select pathogenic genera for different preplant strawberry treatments

		Brassica termination			Strawberry transplant
Location	Treatment	Pythium[1]	binucleate Rhizoctonia[2]	Rhizoctonia solani[2]	Pythium[1]
Kibler					
	None	148.8 a[3]	ND[4]	ND	242.1 b
	Brassica	293.5 a	ND	ND	389.7 a
	Brassica +solarization	NT[5]	NT	NT	368.0 a
	Solarization	NT	NT	NT	227.1 b
	Mustard seed meal	NT	NT	NT	568.8 a
Hope					
	None	177.7 a	1.2 b	0.0 b	154.2 ab
	Brassica	344.7 a	15.3 a	17.7 a	232.6 a
	Brassica +solarization	NT	NT	NT	86.1 b
	Solarization	NT	NT	NT	74.5 b
	Mustard seed meal	NT	NT	NT	143.0 ab

Soil samples were taken at the time the brassica cover crop was terminated and when the strawberry plants were transplanted.

[1] Colony forming units per g soil, oven-dry weight equivalent

[2] Colony forming units per 100g soil, oven-dry weight equivalent

[3] Means in the same column for a location followed by the same letter are not significantly different, LSD ($P < 0.05$)

[4] ND= None detected

[5] NT= Not tested

Table 2. Isolation frequency of select pathogenic genera from strawberry roots at flowering and at the end of strawberry harvest.

		Isolation frequency (%)[1]							
		Flowering				Harvest			
Location	Treatment	Pythium	binucleate Rhizoctonia	Rhizoctonia solani	Colletotrichum	Pythium	binucleate Rhizoctonia	Rhizoctonia solani	Colletotrichum
Kibler									
	None	57.5 a[2]	47.5 a	5.0 a	2.5 a	20.7 a	55.6 a	0.0 a	58.9 a
	Brassica	62.5 a	45.0 a	0.0 a	0.0 a	25.2 a	48.6 a	0.0 a	37.4 a
	Brassica +Solarization	64.2 a	41.1 a	15.3 a	5.3 a	10.0 a	45.0 a	0.0 a	15.0 a
	Solarization	57.5 a	35.0 a	2.5 a	5.0 a	2.5 a	20.0 a	0.0 a	6.7 a
	Mustard seed meal	60.0 a	55.0 a	2.5 a	0.0 a	32.5 a	50.0 a	0.0 a	27.5 a
Hope									
	None	90.0 a	60.0 a	0.0 b	5.0 a	NT[3]	NT	NT	NT
	Brassica	65.0 a	75.0 a	15.0 a	20.0 a	NT	NT	NT	NT
	Brassica +Solarization	50.0 a	85.0 a	10.0 b	15.0 a	NT	NT	NT	NT
	Solarization	60.0 a	70.0 a	10.0 b	35.0 a	NT	NT	NT	NT
	Mustard seed meal	60.0 a	65.0 a	0.0 b	20.0 a	NT	NT	NT	NT

[1]Isolation frequency is based on the mean percent isolation from 4 root segments sampled from each of 10 strawberry plants per plot at Kibler and 5 strawberry plants per plot at Hope.

[2] Means in the same column for a location followed by the same letter are not significantly different, LSD ($P < 0.05$)

[3] NT= Samples not taken

Table 3. Strawberry root discoloration ratings of plants for different preplant strawberry treatments.

	Root rating[1]		
	Flowering		Harvest
Treatment	Kibler	Hope	Kibler
None	3.6 ab[2]	3.6 a	2.9 a
Brassica	3.4 b	3.6 a	3.7 a
Brassica +solarization	3.8 a	3.7 a	3.4 a
Solarization	3.9 a	3.2 a	2.9 a
Mustard seed meal	3.5 b	3.4 a	3.4 a

Plant samples were collected at flowering and at the end of strawberry harvest.

[1]Ratings were based on the amount of discoloration of the roots, with a scale of 0-5, where 0 = 0-10%, 1 = 10-30%, 2 = 30-50%, 3 = 50-70%, 4 = 70-90% and 5 = 90-100% discoloration.

[2]Values are the mean rating of roots from 10 plants per plot at Kibler and 5 plants per plot at Hope. Means within the same column and followed by the same letter are not significantly different, LSD ($P < 0.05$)

Table 4. Soil populations of select microbial groups for different preplant strawberry treatments[1]

		Brassica termination			Strawberry transplant		
Location	Treatment	Bacteria (x 10^7)	Fungi (x 10^4)	Actinomycetes (x 10^6)	Bacteria (x 10^7)	Fungi (x 10^4)	Actinomycetes (x 10^6)
Kibler							
	Control	1.5 a[2]	4.8 b	3.3 b	2.4 b	17.1 a	4.8 a
	Brassica	2.5 a	13.4 a	4.0 a	3.9 a	30.8 a	9.7 a
	Brassica +Solarization	NT[3]	NT	NT	3.6 a	22.2 a	8.9 a
	Solarization	NT	NT	NT	2.7 b	10.8 a	5.8 a
	Mustard seed meal	NT	NT	NT	3.2 a	15.9 a	8.1 a
Hope							
	Control	2.0 b	12.3 a	4.6 a	1.9 b	6.5 a	10.0 b
	Brassica	12.7 a	45.1 a	5.7 a	2.9 b	18.5 a	17.0 a
	Brassica +Solarization	NT	NT	NT	9.8 a	19.4 a	19.5 a
	Solarization	NT	NT	NT	2.9 b	12.3 a	10.4 b
	Mustard seed meal	NT	NT	NT	8.0 a	31.0 a	14.4 ab

Soil samples were taken at the time of termination of the brassica cover crop and at the time of strawberry transplant.

[1]All microbial populations are reported as colony forming units per g soil, oven-dry weight equivalent.
[2]Values in the same column of a location and followed the same letter are not signfiicantly different, LSD ($P < 0.05$)

[3] NT= Samples not taken