BIOAg Final Project Report

Title: Can lignin degrading organisms reduce Verticillium wilt in pumpkin cropping systems?

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ABSTRACT

Raised beds in pumpkin cropping systems are frequently mulched with polyethylene (PE), providing numerous benefits. However, PE mulch has negative environmental consequences since it can remain in the soil for years. Biodegradable plastic mulch films (BDM) that are soil incorporated and biodegraded by microorganisms may present a sustainable alternative. *Verticillium dahliae* causes Verticillium wilt on cucurbits and is difficult to manage because the pathogen produces highly durable survival structures in the form of melanized microsclerotia. Recently, grafting of a susceptible scion onto a resistant rootstock has been shown to be an effective practice for cucurbits. Crop rotation is another effective management strategy to reduce Verticillium wilt especially when rotations include high lignolytic crops. Organisms that degrade lignin produce laccases and peroxidases that may degrade BDMs and fungal melanin. This study will determine if: (1) microsclerotia survival decreases when soils are amended with BDM in the presence of *Streptomyces viridisporus*, a lignin degrading organism, and (2) if Verticillium wilt severity and microsclerotia survival are reduced when a high-lignin, non-host crop (sweet corn) is followed by a susceptible host (either grafted or non-grafted), and coupled with soil incorporation of certain BDM and *S. viridisporus*.

Two trials have been completed to evaluate the effect of BDM on microsclerotia survival. Sachets containing microsclerotia were buried within Deepot conetainers amended with one of four mulch treatments (polyethylene, Organix, experimental PLA/PHA, or WeedGuard Plus) or non-amended, and amended with *S. viridisporus* or non-amended. Microsclerotia viability was evaluated on NP-10 medium for germination and percent non-germinated microsclerotia (%NGM) was calculated. No significant differences were observed for %NGM harvested from the conetainers regardless of the amended BDM (P =0.675) and whether *S. viridisporus* was amended into the field soil or non-amended (P = 0.885).

Two trials, each with two crop rotations, have been completed to evaluate the effect of crop rotation on *V. dahliae* microsclerotia. Sachets containing microsclerotia were buried within nursery pots filled with field soil and amended with *S. viridisporus* and *V. dahliae* or non-amended, and planted with 'Kickstarter' corn, 'Secretariat' watermelon, 'Secretariat' watermelon grafted onto 'Tetsukabuto' squash, or fallowed. Dried plant debris was incorporated into the soil, and the experiment was repeated for the second crop rotation. Only fallow-fallow rotations resulted in significantly reduced %NGM. All other treatments resulted in high %NGM. The observed decrease in microsclerotia viability (or high %NGM) in all treatments except those that were fallow was most likely due to a direct interaction with the crop, such as root exudates.

The results of this study provide foundational data for future studies where lignin degrading organisms in the soil are identified and their breakdown of BDM is quantified. Results will also expand information on the role of lignin-degrading organisms on BDM degradation and effects of crop rotation.

Project Description:

Raised beds in pumpkin cropping systems are frequently mulched with polyethylene (PE), which provides benefits such as reducing weeds, conserving water, and raising soil temperatures (Miles et al., 2012). However, PE use results in large amounts of waste and high costs for disposal. Biodegradable

plastic mulch films (BDM) that are soil incorporated and biodegraded by microorganisms present a desirable alternative to PE.

Verticillium dahliae causes Verticillium wilt on cucurbits and is a significant and difficult to manage pathogen. *V. dahliae* forms resilient survival structures (microsclerotia) that can survive for long periods of time in the absence of a host. These microsclerotia are melanized, which protects them from oxidative stress, desiccation, and UV radiation; moreover, they serve as the primary inoculum for the following growing season. Management practices, such as soil fumigation and crop rotation, are largely focused on reducing primary inoculum. However, grafting susceptible scions onto tolerant rootstocks is also an effective practice for cucurbits, although not suitable for other *V. dahliae* hosts. Recent studies on crop rotations report that Verticillium wilt incidence can be reduced when rotations include crops that have high lignin content. One hypothesis for this result is that organisms that degrade lignin produce laccases and peroxidases that not only degrade lignin but also BDM and fungal melanin.

There are no previous studies that have looked at how rotational cropping combined with BDM affect soilborne pathogen inoculum density through the promotion of lignin degrading organisms. This project examined the effects of *S. viridisporus*, a lignin degrading bacterium, on BDM, and crop rotation on *V. dahliae* microsclerotia viability in two greenhouse experiments. The first experiment investigated the interaction of *S. viridisporus* and BDM, on microsclerotia viability whereas the second included the effects of crop rotation and resistant rootstocks on microsclerotia viability and plant colonization.

Outputs

Objective 1: Impact of Biodegradable plastic mulches on Microsclerotia survivorship (Conetainer Study)

Work completed. Soils were collected from a field not previously cropped with corn or other high lignolytic crops. Due to the high moisture content of the soil, soil was divided and placed into 8-inch deep stainless-steel pans and placed on a greenhouse bench to dry. Soils were then screened and heat pasteurized to prepare to evaluate microsclerotia survivorship in a Deepot experiment.

A *V. dahliae* sand inoculum was initiated on December 18, 2018 and September 18, 2019 as described in Wheeler and Johnson 2016, where *V. dahliae* isolate JAW13-114 was cultured in Czapek Dox Broth and incubated on a rotary shaker at 150 rpm for 7 days. Autoclaved play sand in 1 L conical flasks was then infested with the liquid culture in a laminar flow hood. Sand was left to dry in the hood for 7 days, after which it was transferred to a chemical fume hood for 1 month to allow for microsclerotia formation. Inoculum was quantified in triplicate by shaking 1 g of sand inoculum across 10 NP-10 agar plates, incubating for 3 weeks, washing the plates, and counting the number of microsclerotia.

V. dahliae isolate JAW 132-114 (Wimer et al. 2015) was placed onto a Verticillium inoculum medium (VIM) on December 12, 2018 and incubated at room temperature for 3 weeks to allow for microsclerotia formation. Microsclerotia were extracted from the medium by gently blending five agar plates in 500 mL of lukewarm, sterile deionized water. Microsclerotia were collected by filtration and airdried. Two-cm^2 nylon mesh sachets with 41 µm openings (Elko Filtering Co., Miami, FL) were filled with microsclerotia aggregates and heat sealed. Sachets were then placed into Deepots (D60L, 984 cm3) filled with heat-pasteurized field soil amended with one of four lab-weathered BDMs (PE, experimental PLA/PHA, WeedGuard Plus, a cellulose based product, and Organix, a PLA+PBAT blend) and either vermiculite inoculated with *S. viridisporus* or non-inoculated.

Deepots were filled with a ratio of 40% v/v *S. viridisporus* infested vermiculite to pasteurized field soil on June 5, 2019 and August 8, 2019, and amended with *V. dahliae* sand inoculum to a concentration of 40 colony forming units (CFU) per g soil: vermiculite. Deepots were arranged in a RCBD with five replications for a total of 40 experimental units, (4 mulch treatments x 2 *S. viridisporus* treatments, infested or non-infested x 5 replications). One microsclerotia sachet was buried in each

Deepot. Soil was kept adequately moist and Deepots were maintained under controlled conditions in a growth chamber with 16 hours light, 8 hours dark at 27 °C for 8 weeks to allow for colonization by *S. viridisporus*. Microsclerotia sachets were collected from the Deepot, emptied into 10 mL of 1% water agar and briefly vortexed. One hundred µL were spread-plated onto an NP-10 medium and incubated at room temperature, in the dark for 3 weeks. CFU and non-germinated microsclerotia were counted using a stereo microscope.

Data analyses: Since data were non-normal, Kruskal-Wallis tests were completed in RStudio v.1.2.1335 (Allaire 2012) using the R package agricolae (De Mendiburu 2019).

In Progress. Due to significant differences in %NGM between trials 1 and 2, microsclerotia from both trials were replated onto NP-10 medium on December 12, 2019. Plates will be evaluated on January 12, 2019.

OBJECTIVE 2: EFFECTS OF BDM, HIGH LIGNOLYTIC ROTATION CROP, AND *S. VIRIDISPORUS* ON *V. DAHLIAE* MICROSCLEROTIA VIABILITY (CROP ROTATION STUDY)

Work completed. Two greenhouse trials, with two crop rotation cycles, have been completed. Vermiculite (Therm-o-rock, West Inc., Chandler AZ) was sieved through a number 6 sieve (3.35 mm openings) to retain the larger pieces. One thousand cm^3 of vermiculite was placed into a mushroom spawn bag (Unicorn bag, 20 in. x9 in., Plano Tx) with 50 mL sterile, deionized water. Bags were sealed and autoclaved for 30 minutes three times, with 48 hours in between each autoclave period. Spores were gently scraped from actively growing *S. viridisporus* (ATCC 39115) cultures and suspended in 50 mL 2X SAY (sucrose L-asparagine yeast extract) solution. Inoculum was adjusted to 10^7 spores. Autoclaved vermiculite was then infested with the *S. viridisporus* inoculum and incubated for 14 days. Bags were gently shaken and agitated approximately every 48 hours. A *V. dahliae* sand inoculum was prepared and quantified as described above.

Seeds of Verticillium wilt-susceptible watermelon 'Secretariat' were sown in soilless medium (Sunshine Redi-Earth Plug and Seedling mix, SunGro Horticulture, Agawam, MA) on May 16, 2018. To increase germination rates, seeded flats were placed on heat mats, domed, and covered with black landscape fabric for 5 days. Domes were removed from the flats and flats were removed from heat. Seed of Verticillium wilt-resistant squash rootstock, 'Tetsukabuto' were sown on May 30, 2018. When stems of both cucurbit varieties were similar in diameter, 100 'Secretariat' scions were grafted onto 'Tetsukabuto' rootstocks using the one-cotyledon grafting method (Miles et al. 2017)

Field soil not previously cropped with corn or other high lignolytic crops was collected and air-dried. Baseline *V. dahliae* CFU/g soil was quantified by shaking 1 g of soil across 10 plates of NP-10 medium. Plates were incubated at room temperature, in the dark, for 4 weeks. Plates were gently washed in running tap water to remove soil, and baseline *V. dahliae* CFU counts were 14.5 CFU/g field soil.

One hundred, 2.8 L black nursery containers were each filled with field soil and amended with one of four mulch treatments (polyethylene, Organix, experimental PLA/PHA, or WeedGuard Plus) or received no mulch. The amount of plastic film added per experimental unit was based on the calculated area of field recovered mulch/cubic meter of soil following soil incorporation after one growing season (Table 1). Soils were also either amended with approximately 40 % v/v *S. viridisporus* vermiculite: field soil mix, or non-infested vermiculite: field soil mix (0.6 L vermiculite: 1.5 L field soil), and *V. dahliae* sand inoculum to achieve approximately 40 CFU/g of *V. dahliae*, or non-amended. Two-cm^2 nylon mesh sachets with 41 µm openings (Elko Filtering Co., Miami, FL) were filled with microsclerotia aggregates and heat sealed. Two sachets were buried in each nursery container. Subsequently, pots were either fallowed, or planted with either 'Kickstarter' corn, 'Secretariat' watermelon, or 'Secretariat' watermelon grafted onto 'Tetsukabuto' squash rootstock. Nursery containers were maintained in the greenhouse, and fertilized and watered as needed for 8 weeks. Containers were completely randomized but treatments were not replicated in this experiment due to the large number of experimental units.

The first rotation of trial one was taken down on August 16, 2018. For each nursery pot, soil was emptied into a large, gusseted plastic bag (25.4 x 10.6 X 50.8 cm) and soil was separated from roots. Plant biomass was stored in large #16 paper sacks, weighed, and then placed into a dryer set at 22 °C for 48 hours, and dry biomass was recorded. One microsclerotia sachet was removed from soil and stored in wax paper bags. Two small handfuls of soil were placed into small paper bags for later soil plating onto NP-10 medium. Microsclerotia sachets were emptied into 10 mL of 1% water agar and briefly vortexed. One hundred microliters were then spread plate onto an NP-10 medium and incubated at room temperature, in the dark for 3 weeks. CFU and non-germinated microsclerotia were counted using a stereo microscope.

The second rotation of trial one was set up on August 22, 2018. Watermelon scions and rootstocks were grafted as described above. Dried corn plant material was cut into fragments, approximately one to two inches in length, and pulverized in a coffee grinder for approximately 15 seconds. Dried watermelon and grafted watermelon tissue were broken up manually. Soils from crop rotation 1 were amended with corresponding plant debris, or non-amended. Nursery containers were re-filled with amended field soil and planted with either 'Kickstarter' corn, 'Secretariat' watermelon, or 'Secretariat' watermelon grafted onto 'Tetsukabuto' or remained fallow. Plants were maintained as described above.

Plants were grown for 9 weeks and the experimental take down was similar to crop rotation 1. Soil was stored for plating onto NP-10 medium to quantify soil inoculum, the remaining microsclerotia sachet was recovered, and plant fresh weights were recorded. Plants were dried for 48 hours and plant dry weights were recorded. Microsclerotia sachets were emptied into 10 mL of 1% water agar, spread plated onto NP-10 medium, and incubated for 3 weeks. CFUs and non-germinated microsclerotia counts were recorded. Percent non-germinated microsclerotia were calculated by dividing non-germinated microsclerotia counts by total microsclerotia plated. Since no replications could be included in this trial because due to the numerous factors and limitations on greenhouse space, only exploratory data analyses were completed.

To minimize the number of experimental units in trial 2, BDM treatments were eliminated from the study. 'Secretariat' and 'Tetsukabuto' seeds were sown as described above on March 27, 2019 and April 8, 2019, respectively, with grafting occurring on April 19, 2019. Healing of the graft union was unsuccessful and there was an insufficient number of surviving seedlings remaining to repeat the grafting. Therefore, only non-grafted 'Secretariat' plants were used for the crop rotations in this trial. 'Kickstarter' corn was sown on May 6, 2019. Trial 2, rotation 1 was planted on May 15, 2019 as described above and maintained for 8 weeks in the greenhouse. Rotation 2 was set up on July 11, 2019 with the experimental takedown occurring on November 4, 2019.

Data analyses: Only exploratory data analyses for experimental units that were non-amended with BDM were completed for trial 1 since no replications were included and so that comparisons could be made with trial 2. For trial 2, Kruskal-Wallis tests were completed in RStudio v.1.2.1335 (Allaire 2012) using the R package agricolae (De Mendiburu 2019).

In Progress. Since grafting could not be completed for trial 2, a third trial is currently underway with non-grafted 'Secretariat' plants.

Results

Conetainer study. Mulch treatments did not affect microsclerotia survival. No significant differences were observed for %NGM harvested from the conetainers regardless of the amended BDM (P =0.675) or whether the field soil was amended with *S. viridisporus* or non-amended (P = 0.885). However, %NGM was significantly greater for trial 1 than for trial 2 (Figure 1).

Crop rotation study. In trial 1, %NGM was lower for a fallow-fallow rotation compared to other rotations regardless of whether soils were also amended with *S. viridisporus* or non-amended (Table 1). No other trends in %NGM were associated with rotation or amendment with *S. viridisporus*. In trial 2, a fallow-fallow rotation resulted in a significantly lower %NGM ($P < 0.0001$) regardless of whether experimental units were amended with *S. viridisporus*, *V. dahliae*, or non-amended (Table 1). No significant differences in %NGM were observed between microsclerotia collected from field soil amended with *S. viridisporus* than the non-amended soil.

Discussion and Impacts:

Lignin peroxidases were previously reported to degrade melanin (Woo et al. 2004), which is a component of *V. dahliae* microsclerotia. Previous studies examining green manure effects on Verticillium wilt of potato showed that *V. dahliae* inoculum densities were significantly less after a 3-year consecutive planting of Sudan grass followed by 2 years of potato than in fallow fields (Davis et al. 1996). In a more recent study, a 2-year Sudan grass rotation with potato resulted in reduced Verticillium wilt incidence by 20-31% (Larkin et al. 2011). One hypothesis for this response is that Sudan grass is high in lignin content. In another study (Debode et al. 2005), lignin content was inversely associated with the viability of *V. longisporum* (a species related to *V. dahliae*) due to supporting an environment favorable to lignin degrading organisms. These organisms, which are primarily fungi that are Basidiomycetes and Streptomyces species such as *S. viridisporus*, produce laccases and peroxidases that not only degrade lignin, but also have the capacity to biodegrade plastics as well as fungal melanin (Bailes et al. 2013, Butler and Day 1998). However, *S. viridisporus* had no effect on microsclerotia viability in either the conetainer or crop rotation study. In the conetainer study, amending field soils with BDM also had no effect. Due to significant differences in %NGM between trials 1 and 2 in this conetainer study, results will be validated. The microsclerotia from both trials have been re-plated and are currently incubating.

In the crop rotation study, which evaluated the effect of crop rotation and *S. viridisporus* on *V. dahliae* microsclerotia germination, only fallow-fallow rotations resulted in significantly reduced %NGM. All other treatments resulted in very low CFU counts, regardless of rotation or amendment with *S. viridisporus*. In field soils that have been fallowed for at least 2 years, lower soil microbial biomass have been observed (Nielsen and Calderón 2011). Microbial biomass may decline as organic matter declines, due to reduced sustenance for the microbial population, resulting in fewer microbial antagonists to *V. dahliae.* However, it is not clear whether this decrease in biomass had occurred within the short time period that these rotations were maintained. More likely, the observed decrease in microsclerotia viability, or high %NGM, was due to a direct interaction with the crop, such as root exudates. The field soils used in this study had not been previously cropped with a lignolytic crop, so the time for which the rotations were maintained may have been too short to allow for sufficient establishment of *S. viridisporus* and for the soil to become suppressive to *V. dahliae*. For instance, soils that were suppressive to *Rhizoctonia solani* on radish required at least four consecutive plantings so that the Trichoderma sp. population could increase (Henis et al. 1978). Longer crop rotations and allowing crop residues to degrade rather than manual incorporation may be needed to further elucidate the interaction of *S. viridisporus* with *V. dahliae*.

Short term: This project will expand the limited information currently available on how BDM affect *V. dahliae* inoculum density in soil as well as how lignin degrading organisms impact *V. dahliae* microsclerotia integrity using *S. viridisporus* as the model melanin-degrading organism. The results of this study will provide foundational data for future studies where lignin degrading organisms in the soil are identified and their breakdown of BDM is quantified. Results will also expand information on the role of lignin-degrading organisms on BDM degradation.

Intermediate term: The results of this project may alter rotational crop selection in the Skagit Valley, and elsewhere where BDM are commonly used. Additionally, this project could be expanded in the future to investigate longer rotations, and other rotational crops such as broccoli, which is documented to reduce Verticillium wilt due to the presence of glucosinolates. Lastly, the project could be coupled with rotations using cucurbits grafted onto Verticillium wilt resistant rootstocks in order to significantly reduce Verticillium wilt on susceptible cucurbit varieties.

Long term: Improving the efficacy of crop rotation as a means to managing *V. dahliae* reduces the grower need for soil fumigation, thereby minimizing the economic and environmental impacts associated with fumigation. These as well as the above stated positive outcomes could lead to future collaborations and leveraged funded projects with other researchers who are interested in the role of lignolytic enzymes and survival of soilborne plant pathogens.

ADDITIONAL FUNDING APPLIED FOR / SECURED: Additional funding was applied for through the NIFA methyl bromide transition program, but funding was denied.

GRADUATE STUDENTS FUNDED: None

RECOMMENDATIONS FOR FUTURE RESEARCH

Longer crop rotation studies are needed to investigate the direct interaction between *S. viridisporus* and *V. dahliae*. Future studies should also investigate the interactions of V. dahliae with other lignin-degrading organisms such as *Phanerochaete chrysosporium* since the peroxidases and laccases produced are different. A survey of endemic lignin-degrading organisms should be completed to identify other potential antagonists.

References:

1. Allaire, J. 2012. RStudio: integrated development environment for R. Boston, MA 770.
2. Bailes, G., Lind, M., Ely, A., Powell, M., Moore-Kucera, J., Miles, C., Inglis, D., and Brodhagen, M. 2013. Isolation of native soil microorganisms with potential for breaking down biodegradable plastic mulch films used in agriculture. JoVE.
3. Butler, M. J., and Day, A. W. 1998. Destruction of fungal melanins by ligninases of *Phanerochaete chrysosporium* and other white rot fungi. Int. J. Plant Sci. 159:989-995.
4. Davis, J., Huisman, O., Westermann, D., Hafez, S., Everson, D., Sorensen, L., and Schneider, A. 1996. Effects of green manures on Verticillium wilt of potato. Phytopathology 86:444-453.
5. De Mendiburu, F. 2019. Agricolae: statistical procedures for agricultural research. R package version 1.3-1.
6. Debode, J., Clewes, E., De Backer, G., and Höfte, M. 2005. Lignin is involved in the reduction of *Verticillium dahliae* var. *longisporum* inoculum in soil by crop residue incorporation. Soil Biol. Biochem. 37:301-309.
7. Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of Rhizoctonia solani damping-off of radish: effect of successive plantings, PCNB, and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68:900-907.
8. Larkin, R. P., Honeycutt, C. W., and Olanya, O. M. 2011. Management of Verticillium wilt of potato with disease-suppressive green manures and as affected by previous cropping history. Plant Dis. 95:568-576.

9. Miles, C., Devi, P., Zhao, X., and Guan, W. 2017. Grafting manual: How to produce grafted vegetable plants.
10. Nielsen, D. C., and Calderón, F. J. 2011. Fallow Effects on Soil. US Department of Agriculture/ University of Nebraska-Lincoln, Lincoln, Nebraska. 10.2136/2011.soilmanagement.c19.
11. Woo, S. H., Cho, J. S., Lee, B. S., and Kim, E. K. 2004. Decolorization of melanin by lignin peroxidase from *Phanerochaete chrysosporium*. Biotechnology and bioprocess engineering 9:256.

Table 1. Percent non-germinated microsclerotia (2018) and mean % non-germinated microsclerotia recovered from crop rotation study sachets. Sachets were buried in field soil that was either amended with *S. viridisporus* and *V. dahliae*, or non-amended. Soils were planted with one rotation combination with 'Kickstarter' corn, grafted 'Secretariat' watermelon on 'Tetsukabuto' rootstocks, 'Secretariat' watermelon only, or fallow.

Rotation[a]	*S. viridosporus*[b]	*V. dahliae*[c]	% Non-germinated microsclerotia
Corn-Corn	N	N	0.857
Corn-Corn	N	Y	0.800
Corn-Corn	Y	N	1.0
Corn-Corn	Y	Y	1.0
Corn-Grafted Watermelon	N	N	1.0
Corn-Grafted Watermelon	N	Y	0.750
Corn-Grafted Watermelon	Y	N	0.853
Corn-Grafted Watermelon	Y	Y	1.0
Fallow-Fallow	N	N	0.449
Fallow-Fallow	N	Y	0.552
Fallow-Fallow	Y	N	0.846
Fallow-Fallow	Y	Y	1.0
Grafted Watermelon-Grafted Watermelon	N	N	1.0
Grafted Watermelon-Grafted Watermelon	N	Y	1.0
Grafted Watermelon-Grafted Watermelon	Y	N	0.667
Grafted Watermelon-Grafted Watermelon	Y	Y	1.0
Watermelon-Watermelon	N	N	0.857
Watermelon-Watermelon	N	Y	0.875
Watermelon-Watermelon	Y	N	0.935
Watermelon-Watermelon	Y	Y	1.0

Rotation[d]	*S. viridosporus*[b]	*V. dahliae*[c]	Mean % Non-germinated microsclerotia
Corn-Corn	N	N	0.916 a
Corn-Corn	N	Y	0.983 a
Corn-Corn	Y	N	0.992 a
Corn-Corn	Y	Y	0.979 a
Corn-Watermelon	N	N	0.904 a
Corn-Watermelon	N	Y	0.966 a
Corn-Watermelon	Y	N	1.0 a
Corn-Watermelon	Y	Y	0.807 a
Fallow-Fallow	N	N	0.536 b
Fallow-Fallow	N	Y	0.640 b
Fallow-Fallow	Y	N	0.589 b
Fallow-Fallow	Y	Y	0.444 b
Watermelon-Watermelon	N	N	0.910 a
Watermelon-Watermelon	N	Y	0.938 a
Watermelon-Watermelon	Y	N	0.956 a
Watermelon-Watermelon	Y	Y	0.993 a

[a] Trial 1 (2018) data shown only for experimental units non-amended with biodegradable mulch. % non-germinated microsclerotia values are for single experimental units.

[b, c] Experimental units were either amended (Y) or non-amended (N) with *S. viridisporus* and *V. dahliae*.

[d] Trial 2 (2019) included five replications for each treatment. % non-germinated microsclerotia values are means for each treatment.

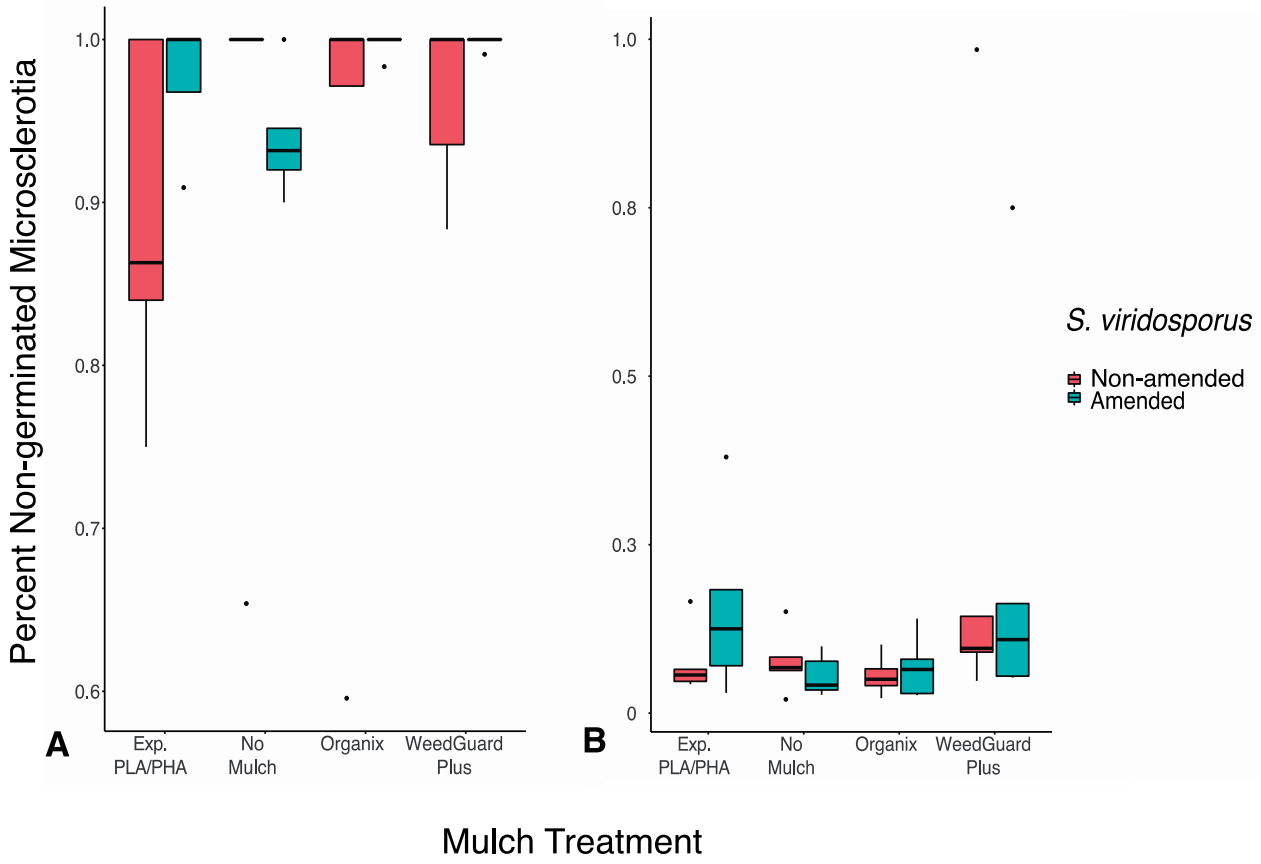

Figure 1. (A) Trial 1, (B) Trial 2. Percent non-germinated microsclerotia recovered from the conetainer study sachets. Sachets were buried in field soil that was either amended with *S. viridisporus* and either experimental PLA/PHA, Organix, or WeedGuard Plus biodegradable mulches, or non-amended.