2018

Impact of Oyster Mushroom Mycelium on the Growth of Kale and Forage Radish

Levi Lilly

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A Thesis Presented By

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Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

May 2018

Plant Biology

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ACKNOWLEDGEMENTS

I would like to thank members of the Jung lab between 2015 and 2018; Dr. Geunhwa Jung, Jay Popko, Dr. Hyunkyu Sang, Dr. Elisha Allen-Perkins, Robert Green, Jaemin Lee, Solomon Maerowitz-McMahan and Georgios Hatzipetrou. The UMass Crop and Animal Research and Education Farm; Neal Woodard, Keith Lilly, Zachary Zenk, and Jason Silverman & Amanda Brown from the UMass Student Farm Enterprise. The UMass CNS Research and Education Greenhouse; Chris Joyner and David O'Neil. The UMass Vegetable Team; Katie Campbell-Nelson and Susan Schuefele. Committee members Dr. Marco Keiluweit and Dr. Sergey Savinov. And, my dear friends who have helped me complete these projects; Danny MacDonald, Michael Montuori, Nick Steiman, Sean Sullivan, Jefferson Lu, Rachel Burns, Rachelle LaCroix, Jing Li, Dr. Hesham Abdullah, Dr. Lindsey Hoffman and last but not least Mr. Willie Crosby.

ABSTRACT

IMPACT OF OYSTER MUSHROOM MYCELIUM ON THE GROWTH OF KALE AND FORAGE RADISH

MAY 2018

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Saprophytic fungi can be paired with companion crops in interplant systems to increase production efficiency. However, fungal species/strain, substrate, and inoculation rate can affect the growth of companion crops. This project investigated the viability of open-field mushroom production by interplanting three strains of *Pleurotus ostreatus* (Elm A, Elm B, and 8801) with kale (*B. oleracea* var. *acephala*) and forage radish (*Raphanus raphanistrub* sub. *sativus*), and measured the effect of interplanting on plant yield over two field seasons. In the field, Elm A showed an increase in plant yield at a low inoculation rate and decrease in plant yield at a high inoculation rate, compared to the untreated. Conversely, 8801 showed a reduction in plant yield at high and low inoculation rates in the field. Elm B at a high rate showed a reduction in plant yield both in the field and greenhouse. Kale was grown in hydroponics with fungal secretions added at a range of concentrations (10, 100, 1,000 and 10,000 ppm). Elm A showed an overall increase in plant yield in hydroponics, and Elm B showed an overall decrease in plant yield, compared to the untreated. Mushroom production was low in field plots and was not a commercially viable option with the strains and methods used.

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CHAPTER 1

OBSERVING THE INTERACTION BETWEEN *PLEUROTUS OSTREATUS* AND FALL VEGETABLE CROPS

Introduction

In 2017, the United States produced over 11 million pounds of fresh oyster mushrooms (*Pleurotus* spp.) at a value of $34 million (USDA). Oyster mushrooms are a commodity that can be utilized by local farmers, but most mushroom production occurs in controlled environment rooms or outside on hardwood logs. Open-field mushroom production is an option for farmers to utilize the production environment already in place. Open-field environments create challenges for mushroom production since most filamentous fungi prefer humid environments with no direct sunlight. Lack of humidity and heat allow the fungi and their associated substrate to dry out. A solution to this problem is to interplant mushrooms with companion field crops that create a favorable environment for mushroom production. Interplanting oyster mushrooms in the field with commodity crops would allow farmers to increase production efficiency without increasing land use. Limited research exists to determine if co-production is a viable option. Research is needed to determine the efficiency of oyster mushroom growth in the field along with the impact oyster mushrooms may have on plant health and yield.

A limited number of studies have been conducted examining the impact of oyster mushroom substrate on plant health. For example, Abdullah et al. (2000) showed a 21.8% increase in cabbage yield (*Brassica oleracea* var. *capitata* L.) when crop rows were interplanted with oyster mushroom spawn from *P. columbinus*, using a straw

substrate. Similarly, a 27% increase in dry seed yield of faba bean (*Vicia faba* L.) was detected in plants grown in the field with either *P. columbinus* and *P. ostreatus* (Mohamed et al., 2014). However, Stamets (2005) showed a decrease in Brussels sprout (*Brassica oleracea* var. *gemmifera*) yield when interplanted with *P. ostreatus*, grown in a mixture of straw and sawdust, but also observed a significant increase in Brussels sprout yield when interplanted with *H. ulmariu*s (elm oyster mushroom).

Oyster mushrooms are the fruiting body of *Pleurotus* spp., a saprophytic wood decomposer. Saprophytic fungi release enzymes and acids into the environment to break down large complex organic molecules into smaller more available ones, in turn reabsorbing the nutrients, minerals, salts, and other compounds necessary for their survival (Stamets, 2005). Wood decaying saprophytic fungi are classified into two main groups, white rot and brown rot, characterized by their ability to decompose. White rot fungi secrete cellulolytic and lignolytic enzymes and metabolites, giving the fungus the ability to break down lignin, cellulose, and hemicellulose. After the fungus decomposes lignin and cellulose, wood is left bleached white (Goodell et al. 2008). According to Stamets (2005), *Pleurotus* oyster mushrooms are white rot fungi, which secrete very complex compounds consisting of laccases, manganese peroxidases, veratryl oxidases, organic acids, mediators and accessory enzymes (Bezalel et al.1996; Dashtban et al. 2010). These secretions could influence plant growth by carrying nutrients directly to the plant roots, recruiting microbes to the rhizosphere that in turn affect plant roots, interacting with soil particles and mineral nutrients, or indirectly affecting plant roots.

To investigate the interaction between oyster mushroom mycelium and agricultural crops, kale (*B. oleracea* var. *acephala*) and forage radish (*Raphanus raphanistrub* sub. *sativus*) were chosen. These crops are commonly grown in the northeast where favor the same environmental conditions as *Pleurotus* spp. Both crops create a humid microclimate and shaded canopy that is low to the ground to support fungal growth. In the field and greenhouse, *P. ostreatus* was interplanted with kale and forage radish and differences in plant yields and soil nutrition were observed. A range of *P. ostreatus* secretion exudate concentrations were added to a hydroponic system to further examine *P. ostreatus'* effect on kale growth. The experimental objectives were to determine (i) the effect of fungal strain on crop yield, (ii) the effect of inoculation rate on crop yield, and (iii) the effect of fungal secretions on kale growth hydroponically.

Materials and Methods

Field Trials

Plant materials

Forage radish (Johnny's' Selected Seeds, Winslow, ME) was direct seeded via grain drill with 7.5-inch row spacing at 10 kg ha^{-1} the third week of August in 2016 and 2017. Every second row was hand-pulled upon germination to create 15-inch row spacing. The kale cultivar Winterbor (Johnny's Selected Seeds, Winslow, ME) was established from seed in 128-cell trays containing Pro-Mix BX potting soil (Premier Horticulture, Quakertown, PA) 5 weeks prior to transplanting to the field. During the germination and establishment period, kale was maintained under optimal conditions in

a greenhouse and fertilized with Peters Professional 20-10-20 fertilizer (JR Peters, Allentown, PA) at 1:100 injector ratio bi-weekly. Following the 5-week period, kale was transplanted into the field by hand with 12-inch plant-spacing and 15-inch row-spacing during the first week of September. The field site was at the University of Massachusetts Crop and Animal Research and Education Farm in South Deerfield, MA and the soil was a Winooski silt-loam soil (Coarse-silty, mixed, superactive, mesic Fluvaquentic Dystrudepts) based on the USDA web soil survey. Field plots were broadcast fertilized with 112 kg ha^{-1} nitrogen, phosphorus and potassium (13-13-13) and incorporated by hand-tillage the second week of September.

Treatments

Fungal strains were originally supplied by local mushroom producer, and then supplied by the producer's supplier. Treatments consisted of three strains of *P. ostreatus* tested at 2 concentrations. Specifically, Elm A, Elm B, and 8801 fungal strains were obtained from Aloha Medicinals Culture Bank (Carson City, NV) and sub-cultured onto Potato Dextrose Agar (PDA) in 100 x 15 mm petri plates. Spawn was made in 2.26 kg blocks using 73.0% hardwood sawdust, 24.6% wheat bran, and 2.4% $CaSO_4$, hydrated to 65% moisture, and sterilized at 121*C for 2 hrs in polypropylene life science bags (Tufpak, Ossipee, NH). Bags were inoculated from 100 x 15 mm colonized PDA plates and were allowed to colonize for 3-4 weeks until the whole substrate was filled with white mycelium. Preliminary tests were conducted to determine the optimal substrate for field mushroom production. Chopped straw soaked in hydrated lime and supplemented with hardwood sawdust was optimal for inoculating fungal isolates in the

field. Straw substrate was prepared by soaking pre-chopped straw (Semican, Plessisville, Qb) in hydrated lime ($Ca(OH)_2$) at 0.01 g ml^{-1} H_2O for 16 hrs and drained immediately prior to inoculation. Plots with fungal treatments were covered with soaked-straw at 1.5 kg $m^{2\,-1}$, fungal treatment from spawn bags was added on top, then both were covered with straw again at 1.5 kg $m^{2\,-1}$ for a total of 3.0 kg $m^{2\,-1}$ straw plus fungal treatment. Plots were irrigated immediately and received a daily light irrigation for the duration of the experiment. Treatments consisted of three main effects: two inoculation rates (244 g $m^{2\,-1}$ and 488 g $m^{2\,-1}$); three fungal strains (Elm A, Elm B and 8801); and two crop species (kale and forage radish). The 244 g $m^{2\,-1}$ inoculation rate and *P. ostreatus* 8801 isolate were both used in the 2016 field trial only. Treatments in the 2017 field trial consisted only of 488 g $m^{2\,-1}$ inoculation rate and the isolates used were *P. ostreatus* Elm A and Elm B.

Soil analysis

Soil samples were taken every other week starting at the time of kale transplanting in 2017 only. For each sampling date, three soil plugs were taken per treatment-plot and homogenized. Biomass measurements were taken after 8 weeks. Measurements were made fresh in whole-plant (above soil surface) and harvest-yield (marketable portions) distinctions.

Orthophosphate, ammonium, and nitrate were analyzed using 96-well microplate colorimetric assays based on the methods of Ringuet *et al.* (2010), Rhine *et al.* (1998), and Miranda *et al.* (2001), respectively and with modifications. Briefly, reagents were used to facilitate chemical reactions with extracted soil-nutrients, and

nutrient standards were used to create a calibration curve. K_2SO_4 was used to extract mineral nutrients from 0.5 grams of dry soil. Conical tubes with soil and K_2SO_4 solution were shaken for 1 hour at 300 rpm, followed by centrifugation at 4000 G for 1 hour. For orthophosphate, the standard was potassium phosphate monobasic (KH_2PO_4) and there were 4 reagents. Reagent 1 was 51 mM ammonium molybdate, reagent 2 was 5 N sulfuric acid, reagent 3 was 0.1 M ascorbic acid, and reagent 4 was 1.2 mM antimony potassium tartrate. 1.5 mL reagent 1, 5.0 mL reagent 2, 3.0 mL reagent 3, and 0.5 mL reagent 4 were mixed thoroughly to mage a working reagent immediately prior to analysis. 200 µL of sample or standard was added to microplates. 50 µL of working reagent was added. Microplate was vortexed then shaken for the 30-minute reaction time. The absorbance was read at 880 nm by Epoch microplate spectrophotometer (BioTek, Winooski, VT). For ammonium, reagents were PPS-nitroprusside, 0.17 M citrate, and 0.03 M buffered hypochlorite solution. The standard was ammonium sulfate ($(NH_4)_2SO_4$). Reagents were added in this order: 50 µL sample or standard, 50 µL citrate reagent was added and allowed to react for 1 minute, 50 µL PPS- nitroprusside, 25 µL buffered hypochlorite reagent, and 100 µL DIH_2O. Microplate was shaken on vortex for 30s. Microplate was then sealed and stored in dark for 45-minute reaction time, then absorbance was read at 660nm. The reagents used for nitrate were Griess 1 (N-napthyl ethylenediamine dihydrochloride “NED”), Griess 2 (sulfanilamide), and Vanadium Chloride. 25 mL Griess 1 and 25 mL Griess 2 were mixed before starting. 100 µL of sample or standard was added to microplate, followed by 100 µL VCL_3, then 100 µL of combined Griess reagents (quickly). Microplate was incubated at 37C for 60-minute

reaction time. The standard used was potassium nitrate (KNO_3) and absorbance was read at 540nm.

Experimental design and statistics

The experiment had a randomized complete block design with 4 replications. All data was subject to analysis of variance (ANOVA) by JMP Pro (v 13.0; SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected Least Significant Difference test (LSD, $P \leq 0.05$).

Greenhouse Trials

Plant materials

Kale cultivar seeds were seeded into germination trays in Fafard premium topsoil (Sun Gro Horticulture, Agawam, MA). Seedlings were transplanted into 1020 trays filled 80% with topsoil with even plant spacing, 10 plants per tray. *P. ostreatus* Elm A, Elm B and 8801 fungal isolates and chopped straw were used and prepared the same as the field trials. Kale was inoculated with fungal treatments when plants had 2-3 sets of true leaves. Biomass measurements were taken 6-weeks after seeding.

Experimental design and statistics

This experiment had a completely randomized design with 3 replications. All data was subject to analysis of variance (ANOVA) by JMP Pro (v 13.0; SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected least significant difference test ($P \leq 0.05$).

Hydroponic Trials

Plant materials

Kale (*B. oleracea* var. *acephala*) cultivar Winterbor (Johnny's Selected Seeds, Winslow, ME). Seeds were germinated on germination paper for 5 days, and young seedlings were transplanted to 50% Hoagland modified basal salt solution (PhytoTechnology Laboratories, Shawnee Mission, KS) for 3 days, before moving into treatment media and kept there for 10 days before harvest. Plants were grown in small reservoirs that held 250 mL media. Reservoirs were not aerated, but media were changed every third day to replenish dissolved oxygen saturation.

Treatments

Fungal strains *P. ostreatus* Elm A and Elm B (supplied and cultured per experiment 1) were grown on Potato Dextrose Agar in 100 x 15 mm Petri dishes. When the plates were fully colonized (10-14 days), mycelium and agar were transferred into 1 quart wide-mouth mason jars. Jars contained 200 g of dried rye berries in 200 ml H_2O (1:1 rye, water). Jars with contents were autoclaved at 121*C for 1 hour, shaken and left to cool. Mycelium completely colonizes jars within 2 weeks and secretions start to collect around the base 4 weeks after inoculation. Secretions were collected 6 weeks after inoculation and vacuum filtered through 9 cm P8 filter paper. Filtered secretions were collected into 50ml conical tubes and cold sterilization was performed using 2x centrifugation at 4000 G for 1 hour. The secretions were then decanted into clean conical tubes and stored at 4C. Treatments were made by adding mycelial secretions to 100% Hoagland's solution at levels 10 ppm, 100 ppm, 1000 ppm, and 10,000 ppm. Final

solutions were diluted 50% with water prior to planting to make a modified 50% Hoagland's solution.

Experimental design and statistics

P. ostreatus strain (Elm A and B) and concentration were considered effects. The experiment had a completely randomized design with 5 technical replicates and 3 biological replications. All data were subject to analysis of variance using the MIXED procedure in the Statistical Analysis System (SAS v. 9.2; SAS Institute Inc., Cary, NC). The strain*concentration interaction was assessed using the SLICE option in the LSMEANS statement and means were separated using Fishers protected least significant difference test ($P \leq 0.05$).

Results

2016 Field Experiment

The treatments measured in the 2016 field experiment were fungal strain (Elm A and 8801), inoculation rate (244 g $m^{2\,-1}$ and 488 g $m^{2\,-1}$) and crop (kale and forage radish) (Table 1). Significant harvest yield differences were observed among treatments for forage radish ($P = 0.0166$). The Elm A strain (244 g $m^{2\,-1}$) recorded the highest yield, and the 488 g $m^{2\,-1}$ rate recorded the lowest yield (Fig. 1b). The 8801 strain (244 g $m^{2\,-1}$ and 488 g $m^{2\,-1}$) recorded yields lower than the untreated. Strain 8801 produced higher mushroom yields than Elm A for both crops (Table 1). However, there were no significant differences in plant yield for kale (p=0.9428), however for kale, the Elm A strain (244 g $m^{2\,-1}$) recorded the highest yield, and the Elm A 488 g $m^{2\,-1}$ rate recorded

the lowest yield. The 8801 strain (244 g $m^{2\ -1}$ and 488 g $m^{2\ -1}$) recorded yields lower than the untreated (Table 1).

2017 Field Experiment

Strains Elm A and Elm B were interplanted with kale in the field at the 488 g $m^{2\ -1}$ rate. Significant harvest yield differences were observed between fungal strains (P = 0.0117). Elm A showed the lowest harvest yield and was significantly lower than the untreated. Also significant differences in nutrient level for orthophosphate (P < 0.0001) and nitrate (P < 0.0001) were observed in the soil, but not soil ammonium (P = 0.3683) (Table 1). No significant differences were observed in soil pH (P = 0.3480). Mushroom count showed Elm B to produce significantly higher mushroom yields than Elm A (Table 1).

Greenhouse Experiment

Strains Elm A and 8801 at the 488 g $m^{2\ -1}$ rate were interplanted with kale in the greenhouse. Significant harvest yield differences were observed among fungal strains (P = 0.037). Lower harvest yields were recorded for both strains (Elm A and 8801) compared to the untreated, but only Elm A was significantly lower than the untreated (Figure 1).

Hydroponics Experiment

Significant harvest yield differences were observed among the main effects strain (P < 0.0001), fungal exudate concentration (P = 0.0282), and the strain*concentration interaction (P = 0.032). Strain Elm A was significantly higher than the untreated, and Elm B was significantly lower than the untreated (Figure 4a). When the interaction was partitioned, significant harvest yield differences among concentrations within both Elm

A (P = 0.0543) and Elm B (P = 0.0091) were observed. Significant harvest yield differences were observed among Elm A within fungal exudate concentrations 100 ppm (P < 0.0001), 1000 ppm (P = 0.0225) and 10,000 ppm (P = 0.0002).

Discussion

The results from all trials (field, greenhouse and hydroponic) showed promotion and inhibition of plant growth depending on the strain and rate of *P. ostreatus.* These inconsistencies were reported in the literature and are likely due to limited knowledge on the strains being used and other unknown interactions. Abdullah et al. (2000) and Mohamed et al. (2014) observed an increase in crop yield using *P. ostreatus* in field production of cabbage and faba bean, respectively, yet Stamets (2005) showed a decrease in brussel sprout yield. Mohamed and Abdullah both used straw as a substrate for fungal growth, while Stamets used predominantly sawdust with straw used to retain moisture. This can be a source of variation among the results of these studies. Salama (2016) showed increased nutrient levels in sawdust substrate after inoculation and fruiting of *P. ostreatus* compared to straw types. Fungal substrates' effect on plant yield was not shown in this study and is still unknown.

Another source of variation among the data in this study and the previous research of Mohamed, Abdullah, and Stamets is a fungal strain. Although each study contained *P. ostreatus*, strains were only identified to the species level and their sequence data was not provided. Strain differences at the subspecies level can lead to differences in plant yield, as indicated by results in this study. The amount of different *P. ostreatus* strains on the market has not been identified, however keeping the same

strain across research is important. At the onset of this research project, the objective was to compare the *Pleurotus* spp. to *Hypsizygus* spp. oyster mushrooms' effect on plant growth. From the culture supplier, the Elm A strain was originally identified as *Hypsizugus ulmarius*, which Stamets (2005) showed to have an improvement of crop yield, and the 8801 strain was identified as *P. ostreatus*, which Stamets (2005) showed to reduce crop yield. The 2016 field experiment used Elm A and 8801 strains to compare *H. ulmarius* to *P. ostreatus*. However, after the 2016 field experiment the culture supplier informed that Elm A was actually *P. ostreatus*, and provided a new strain Elm B, as *H. ulmarius*. The strains used in 2017 were Elm A and Elm B, again to test *P. ostreatus* vs. *H. ulmarius*. After the 2017 field experiment when both strains showed a reduction in plant yield, isolates were sequenced (no information on how in materials and methods) to confirm species. Sequence results indicated that both Elm A and Elm B were both *P. ostreatus* (data not shown) and that different strains within *P. ostreatus* have different effects on harvest yields.

In addition to *P. ostreatus* strain being a major influence on crop growth, the inoculation rate of the strain also shows importance in multiple studies. The Elm A 488 g/m^2 treatment was associated with a significant decrease in crop yield in both field studies and the greenhouse study (Table 1; Figure 1, 2). In addition, the Elm B strain significantly reduced yields in the 2017 field experiment (Figure 2) and in the hydroponic trials (Figure 3). In contrast, the Elm A strain (244 g/m^2) increased forage radish yield in the 2016 field trial compared to the other oyster strains tested, but not the untreated. Furthermore, the Elm A strain in hydroponics showed a significant increase in kale yield

compared to the Elm B and the untreated (Figure 3). Differences in plant yield response of the Elm A strain in the field and in hydroponics were influenced by the inoculation rate or treatment concentration, respectively. However, an expanded range of concentrations has not been tested in hydroponics, and concentrations above 10,000 ppm should be evaluated to determine full dose-response range. The treatment concentration of Elm A (10 - 10,000 ppm) in hydroponics could be representative of a low application rate in the field, but further experiments are needed to confirm how field inoculation and crude exudate rates would relate. Overall, these results suggest the fungal strain and rate of application in intercropping systems can both effect plant growth.

Previous research did not provide supporting data nor discuss mechanisms on how saprophytic fungi affect crop yield; these mechanisms are still widely unknown. Results from 2017 soil nutrient analysis show a significant reduction in nitrate and orthophosphate from Elm A and Elm B treatments (Table 2). Although the reduction in orthophosphate is significant in plots treated with Elm A and Elm B, the change in concentration is not substantial (-8%). The recommended rate of phosphorus for brassica crop production in New England is 112 – 168 kg ha^{-1} (NEVMG). An 8% loss keeps phosphorus within the optimum range. However, the reduction in nitrate among the Elm A plots in 2017 is 25%, bringing the nitrogen levels below the recommended rate. A reduction in the soluble nitrate at the time of measurement could be a main factor affecting crop yield, but closer examination is needed to further understand the main

effect causing changes in plant growth. Give some explanations/theory on lower nitrate with growth reduction.

Finally, we observed that mushroom production in field and greenhouse experimental plots was less than optimal (Table 1). We also observed that plots with increased mushroom production seemed to have lower crop yields. Fungal substrate and inoculation rate need to be optimized before *Pleurotus* spp. interplanting with vegetable crops is a viable option in commercial production.

Conclusion

The effects of *Pleurotus* on crop production in an interplant system are dependent on fungal strain, inoculation rate, and likely substrates used. Oyster mushrooms can be used in agricultural systems when correctly prescribed, however, the methods used in our experiments did not produce enough marketable mushrooms nor increase crop production enough to justify this as a viable practice for production farms. This research lays the groundwork needed for further improvement of the methods described and may one day be a suitable method of double cropping and increasing grower profitability. Although it has been shown that an increase in crop yields can be made, methods should be refined before this practice should be utilized by farmers in a production setting. In the field, further experiments testing a range of fungal species/strains, substrates, inoculation rates, and companion crops are needed. In the lab, further experiments are needed to identify interacting factors and understand effects at the cellular level. Co-culture techniques described by Goers et al. (2014)

should be used to remove all possible variables and to examine a complex interaction between plant and fungal cells. Fungi and plants are highly complex organisms whose phenotypes depend on genetics, environment, their interactions, and how they are managed. Fine tuning independent variables to unravel a symbiotic relation between the two systems is no trivial task, but can have valuable upside in a future with uncertain food security and increased scrutiny on synthetic inputs.

Table 1. 2016 field trial plant harvest yield and mushroom count.

	Kale		Forage radish	
Treatment	Yield (g)	Mushroom no.	Yield (g)	Mushroom no.
Elm A 244 g	531	0	317 a	0 b
Elm A 488 g	422	0	104 c	3 ab
8801 244 g	456	7	195 bc	8 a
8801 488 g	450	16	163 c	9 a
Untreated	499	0	286 ab	0 b
P - value	0.9428	0.1124	0.0166	0.0549

Kale and forage radish interplanted with two strains of *Pleurotus ostreatus* (Elm A and 8801) at two rates (244 g $m^{2\,-1}$ and 488 g $m^{2\,-1}$).

Table 2. 2017 field trial soil nutrients (mg/g)

Treatment	Nitrate	Ammonium	Orthophosphate
Elm A	50.392 c	0.0086	0.0236 b
Elm B	59.406 b	0.0087	0.0242 b
Untreated	63.178 a	0.0088	0.0259 a
P value	<0.0001	0.3683	<0.0001

Soil nutrients presented in mg/g soil extracted by K_2SO_4 from 2017 field trial experimental plots.

Table 3. Hydroponics experiment ANOVA

Source	DF	SS	MS	F	P
Conc.	4	3.6879	0.8422	2.8277	0.0282
Strain	1	5.7113	5.7113	17.5166	<0.0001
Conc.*Strain	4	3.5801	0.8950	2.7451	0.0320

Concentrations include 10, 100, 1000, and 10,000 ppm, of Elm A and Elm B

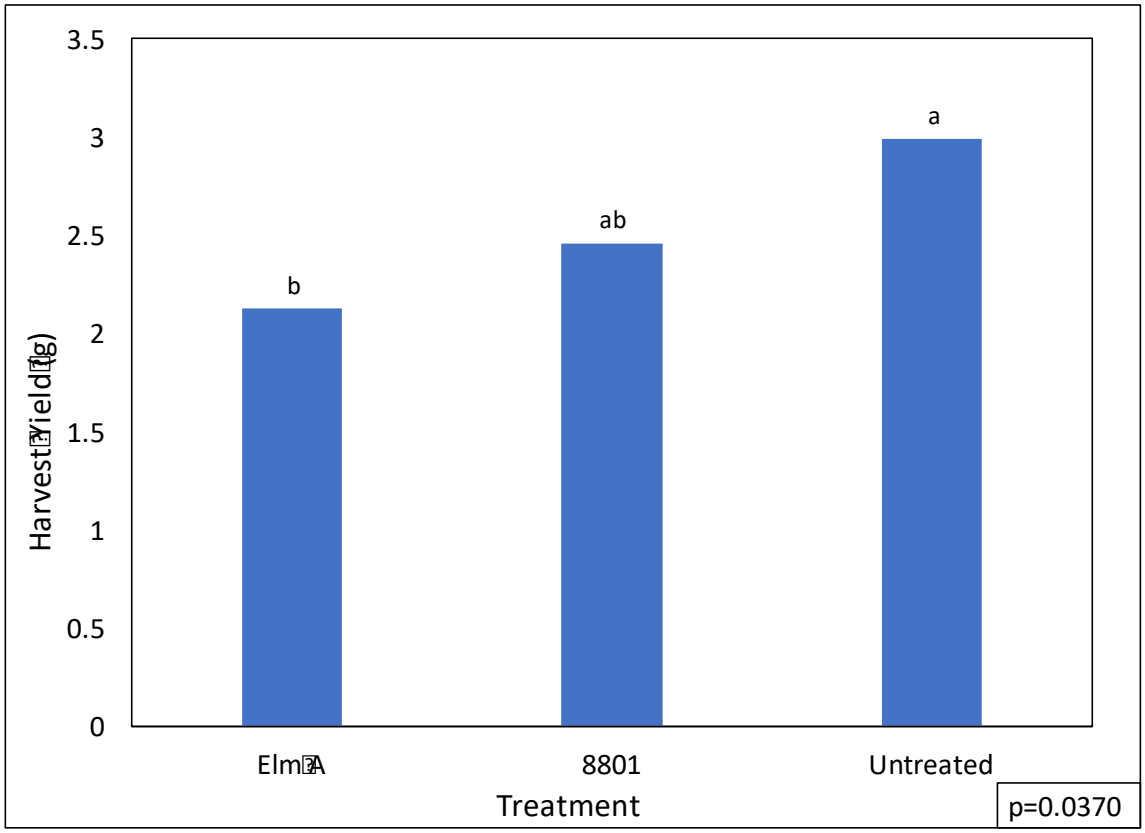

Figure 1. Kale interplanted with two strains of *Pleurotus ostreatus* (Elm A and 8801) at 488 g $m^{2\ -1}$ on Fafard premuim topsoil at the University of Massachusetts College of Natural Science Research and Education Greenhouse in Amherst, MA in 2016.

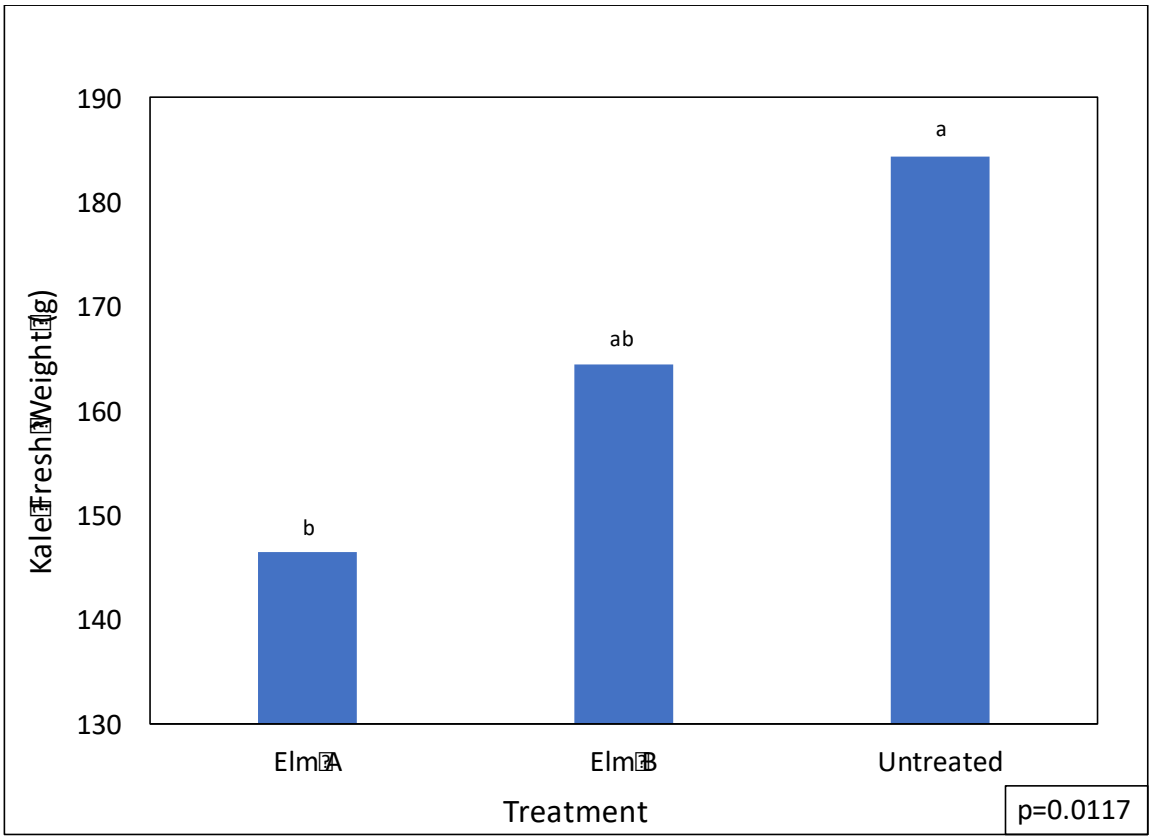

Figure 2. Measurement of kale yield when interplanted with two strains of *Pleurotus ostreatus* (Elm A and Elm B) on Winooski silt loam at the UMass Crop and Animal Research and Education Farm in South Deerfield, MA in 2017

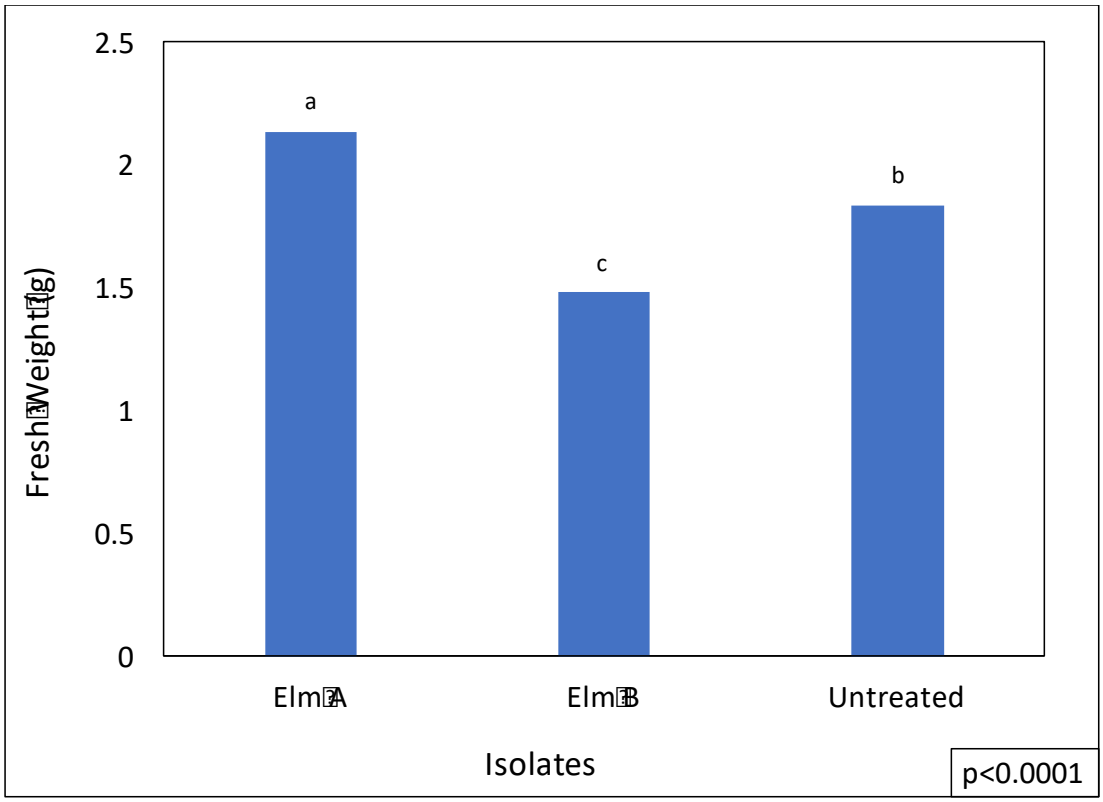

Figure 3. Hydroponic kale in 50% Hoagland's solution with fungal secretions added at a range of concentrations. Data shown is separated by isolate and pooled across treatment concentrations.

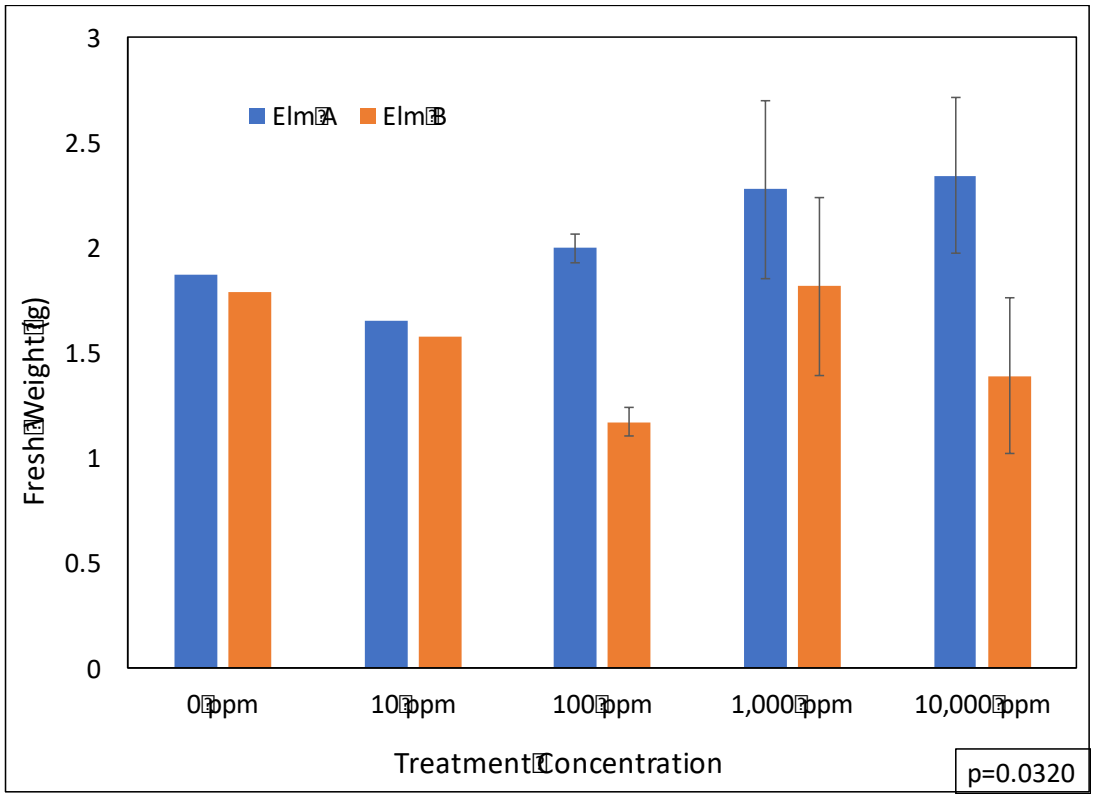

Figure 4. Hydroponic kale in 50% Hoagland's solution with fungal secretions added at a range of concentrations. Strain is shown within concentration for the strain*concentration interaction.

BIBLIOGRAPHY

Abdallah, M. M. F., Emara, M.F.Z., and T.F. Mohammady. (2000). Open field interplanting of oyster mushroom with cabbage and its effect on the subsequent eggplant crop. Annals of Agricultural Science Cairo 45(1): 281-293.

Bezalel L, Hadar Y., and Cerniglia CE. (1996). Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol., 62(1): 292-295.

Gianotti, B. Cleaver, M. Cleaver, P. C. Bailey, and J. C. Holliday. (2009). Diversified agriculture part 1: Simplified and lower cost methods for mushroom cultivation in Africa.

Chaparro J.M., Badri D.V., Bakker M.G., Sugiyama A., Manter D.K., and Vivanco J.M. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS ONE, 8:e55731.

Dashtban, G., Schraft, H., Syed, T., and Qin W. (2010). Fungal biodegradation and enzymatic modification of lignin. International Journal of Biochemistry and Molecular Biology, 1(1): 36-50.

F. Mohamed, M., Nassef, D. M. T., A. Waly, E., and M. Kotb, A. (2014). Production of oyster mushroom (*Pleurotus* spp.) intercropped with field grown faba bean (*Vicia faba* L.). Asian Journal of Crop Science, 6(1): 27-37.

Goers, L., Freemont, P., and K.M. Polizzi. (2014). Co-culture systems and technologies: taking synthetic biology to the next level. J. R. Soc. Interface, 11: 20140065.

Goodell, B., Qian, Y., and J. Jellison. (2008). Fungal Decay of Wood: Soft Rot-Brown Rot-White Rot. Development of Commercial Wood Preservatives, 2: 9-31.

Jonathan, S.G., Lawal, M.M., and O.J. Oyetunji. (2011a). Effect of spent mushroom compost of *Pleurotus pulmonarius* on growth performance of four Nigerian vegetables. Mycobiology, 39(3): 164-169.

Jordan, S.N., Mullen, G.J., and M.C. Murphy. (2008) Composition variability of spent mushroom compost in Ireland. Bioresour Technol., 99: 411–418.

Miranda, K., Espey, G.M., and D. Wink. (2001). A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. Nitric Oxide, 5(1): 62-71.

New England Vegetable Management Guide. (2014). University of Massachusetts Extension Vegetable Program. www.nevegetable.org

Ringuet, S., Sassano, L., and Z. Johnson. (2011). A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate and silicate concentrations for aquatic nutrient monitoring. J. Environ. Monit., 13: 370-376.

Rhine, E.D., G.K. Sims, R.L. Mulvaney, and E.J. Pratt. (1998). Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal, 62(2): 473-480.

Salama A.N.A., Abdou A.A.K., Helaly A.A., and E.A. Salem. (2016). Effect of Residues Agricultural Wastes on the Productivity and Quality of *Pleurotus colombinus* L. by Using Polyethylene Bags Wall Technique. Adv. Plants Agric. Res., 5(3): 00181.

Stamets, P. (2005). Mycelium running: How mushrooms can help save the world. Berkeley, Calif: Ten Speed Press.