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Effects of Lobster Shell Meal as a Soil Amendment on Verticillium Wilt and Potato Growth

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EFFECTS OF LOBSTER SHELL MEAL AS A SOIL AMENDMENT ON VERTICILLIUM WILT AND POTATO GROWTH

by

Ross Sousa

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Botany)

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ABSTRACT

Potatoes (*Solanum tuberosum* L.) are the most valuable crop in the state of Maine. Despite the crop's success in the state, potato growers still face the challenges of various abiotic and biotic stresses, including diseases such as potato early dying, caused by the soilborne fungal pathogen, *Verticillium dahliae*. The disease has been controlled by soil fumigation and fungicides. As an alternative method, organic byproducts, such as lobster shell meal (LSM) or compost, can be used. The benefit of using LSM is thought to occur through the promotion of beneficial chitinolytic soil microbes which can degrade LSM. The derivatives from the LSM degradation can be a food source for some beneficial microbes that consequently suppress *V. dahliae*. To evaluate the efficacy of compost and LSM as a disease-suppressive tool, a greenhouse study was performed. Potato 'Shepody' seed pieces were planted in soil mixed with compost (10% pot volume) and/or LSM (1 lb/cu yd), with some being infested with *V. dahliae*. Plants were evaluated for emergence, disease symptoms, height over time, and biomass during the growth stage and after harvest. The soil was sampled from the pots during the growing period, and the survival of *V. dahliae* was examined using soil dilution plating and quantitative polymerase chain reaction (qPCR). Results showed there was little difference in plant emergence and plant height between treatments. However, compost with or without LSM was found to increase root biomass. LSM alone showed a decrease in the number of tubers, yet an increase in total tuber mass. Stem lesions caused by *V. dahliae* were larger on the compost treatments, while all treatments had similar disease ratings. Both soil plating and qPCR were inconclusive due to challenges in their protocols. Further investigation is needed to determine if LSM is a useful tool in *V. dahliae* management.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Potatoes in Maine

The potato (*Solanum tuberosum* L.) has been a staple crop since emerging from the New World. As a member of the Solanaceae family, its underground tubers are an important food source. The importance of the potato began 7,000 years ago as indigenous South Americans discovered the plant's unique traits and potential as an agricultural crop. Eventually, the tubers would make their first appearance in Europe and North America in the 16th and 17th centuries, due to Western colonialism (Navarre and Pavek 2014). In the state of Maine, the potato has become an essential part of the state's agricultural economy. In 2020 alone, it was reported that cash receipts for potato farmers in Maine amounted to over $160 million, more than any other agricultural commodity in the state (NASS USDA). In 2021, Maine farmers harvested over 18.7 million pounds of potatoes, ranking eighth in the United States (NASS USDA). Although these are impressive figures, growers in the potato industry continue to battle a variety of stressors that could reduce potato yield and quality.

Verticillium Wilt in Potatoes

Although these are impressive figures, growers in the potato industry continue to battle a variety of stressors that could reduce potato yield and quality. One of these stressors is *Verticillium dahliae*, a fungal pathogen that causes the disease known as potato early dying or Verticillium wilt (Fig. 1). *Verticillium dahliae* is known to infect a substantial range of hosts, including potatoes (Berlanger and Powelson 2005). Although symptoms vary, making the disease difficult to diagnose, there are common symptoms in potatoes such as unilateral leaf chlorosis and necrosis, stem lesions, browning of vascular

tissue, and browning of the vascular ring in the tubers (Berlanger and Powelson 2005). A monocyclic disease, the plant is infected when the fungus' hyphae invade the roots, eventually allowing for the hyphae to grow through the root cortex to access the vascular system. Vessel elements in the xylem will then be infected, which allows the fungus to systematically spread throughout the xylem by hyphae and conidia (Subbarao 2020). Wilting and chlorotic leaves appear due to this infection, as water flow is inhibited by fungal growth (Berlanger and Powelson 2005). Eventually, after vascular tissue senescence, *V. dahliae* will produce microsclerotia that will be dispersed into the soil, living in dormancy for as long as 14 years (Subbarao 2020). Microsclerotia are a mass of cells containing melanin and protective cell layers on the outer layers that allow for this dormancy period and they germinate only in ideal conditions (Fradin and Thomma 2006). The key for controlling *V. dahliae* is to reduce or eliminate the microsclerotia in soil. Despite the economic loss and other detrimental effects *V. dahliae* and other pathogens may impose on a potato field, research is ongoing for both conventional and sustainable management strategies to mitigate these negative effects.

Figure 1. Unilateral leaf chlorosis and necrosis in potatoes caused by *Verticillium dahliae*.

Compost in Agriculture

Compost has been extensively studied for disease control, but the efficacy varies depending on many conditions (Larkin and Tavantzis 2013; Koivunen et al. 2018; Entry et al. 2005). Using composts with bark, peat, conifer, or hardwood blends has mixed results when managing potato diseases, specifically that they increased yield and reduced stem canker caused by *Rhizoctonia solani* but failed to reduce black scurf on the tubers caused by the same pathogen (Larkin and Tavantzis 2013). Even a common opportunistic pathogen such as *Botrytis cinerea* may become prolific when introduced to leaf compost (Koivunen et al. 2018). Specifically for *V. dahliae*, dairy compost has been found to not be beneficial for reducing symptoms, which contrasts with vegetable compost that showed reduced symptoms for potato plants when applied (Entry et al. 2005). Despite this variability, a combination of a crustacean shell and compost application may be well suited for managing fungal diseases such as *V. dahliae*. In the case of *B. cinerea*, a mixture of crab shell and leaf compost not only provided nutrients in the soil, but also may encourage the degradation of sclerotia and growth of microbial communities associated with this degradation that inhibits sclerotia survival (Koivunen et al. 2018).

Shellfish By-products Used in Agriculture

One sustainable management strategy garnering more attention is the use of lobster shell meal (Fig. 2) to manage plant diseases. *Homarus americanus*, the American lobster, has been the center of this conversation. In general, there has been evidence supporting lobster shell use as an agricultural fertilizer for thousands of years, ranging from indigenous people in North America to Europeans in the Enlightenment during the 17th and 18th centuries (Spanier et al. 2015). However, in recent years, research has

shifted towards the soil health benefits that lobster shell and other shellfish exoskeletons provide when managing plant pathogens. Like fungal cell walls, the lobster exoskeleton is partially composed of chitin, with the exoskeleton's organic matrix being composed of up to 95% chitin (Mergelsberg et al. 2019). Crab shell, which has similar agricultural uses and composition to lobster shell, has also been used in a similar management strategy. Crab shell meal has been found to not only decrease symptoms caused by *V. dahliae*, but also can decrease the soil's microsclerotia density as well as increase the metabolic activity of microorganisms involved in producing chitinase, which is the enzyme that breaks down chitin (Inderbitzin et al. 2018). Lobster shell has been shown to have a similar microbial effect, with soil bacteria such as *Streptomyces* displaying high rates of chitinolysis that could pose a threat to plant pathogenic fungi (Ilangumaran et al. 2017).

Figure 2. Lobster shell meal.

Objectives

The successful management of V. dahliae using crab shell and vegetable compost in separate studies poses the question of whether lobster shell, food waste-based compost, or a combination of these two soil amendments may be an effective management strategy. This project aims to address this question by conducting a greenhouse study with potatoes infested with *V. dahliae* with the soil amendments mentioned above. The objectives are to a) determine if *V. dahliae* survival and infection is affected by soil amendments such as lobster shell meal or compost and b) discuss what this may mean for the potato industry and management of early dying caused by *V. dahliae*, especially if similar results are seen in future field studies. Researching new plant disease management strategies is key to determine the best practices for managing diseases on an industrial scale. Lobsters are a massive commodity in Maine, which may aid in introducing lobster-based soil amendments on an industrial scale if found to be beneficial. The project also addresses disease management practices that are alternatives to chemical management, which may allow growers to diversify their management practices and use less fungicides in their integrated pest management program.

METHODS AND MATERIALS

Greenhouse Trial

The greenhouse trial was conducted in the Roger Clapp Greenhouse on the University of Maine Orono campus. Treatments with five replications included: 1) no soil amendment and not infested 2) no soil amendment, 3) LSM, 4) compost, and 5) a combination of LSM and compost. In treatments 2 to 5, *V. dahliae* was mixed into the potting soil at a rate of 10 microsclerotia per gram of soil mix (ms/g). Microsclerotia were produced from two- to three-week-old *V. dahliae* cultures from the strain PV4 from continuously grown colonies in the Hao laboratory. The fungal cultures were grown on potato dextrose agar (PDA) at 23°C. Microsclerotia were harvested from the plate by adding distilled water, disturbing the microsclerotia with a spreader bar, and pouring the now water-suspended microsclerotia off the plate. This suspension was mixed in vermiculite and 20 grams was mixed into the soil in each one gallon pot. The same amount of uninoculated vermiculite was added to the control treatment. The compost, primarily made of fruits, vegetables, and processed grains (bread, pasta, etc.) from University of Maine Dining Services was applied at 10% in volume/volume in a mix of potting and garden soils (ProMix and MiracleGro, respectively). LSM was applied at 1 lb/cu yd of soil mix. The LSM was prepared by obtaining shells from Ready Seafood (Saco, Maine, USA), drying them in the greenhouse for one week, and grinding them using a blender and grain mill machine from Wondermill (South Korea). LSM was applied to compost at a rate of 10 lb/cu yd of compost for the lobster compost treatment, equivalent to the rate of the LSM only treatment as the lobster compost was only 10% of the pot's composition (Fig. 3).

Figure 3. Combined treatment of lobster shell meal and compost.

Potato ‘Shepody,’ which is highly susceptible to potato early dying, was planted as seed pieces two inches below the soil surface. The plants were observed weekly for watering and measuring plant height and stem lesions caused by early dying for three months. During harvest, aboveground growth, roots, and tubers were all collected for measuring biomass. Each tuber was cut open to observe its vascular ring. Early dying disease was determined by the percent coverage of the vascular ring in the tuber. Any other disease observations were also noted (common scab, Rhizoctonia, abnormal morphology, etc.).

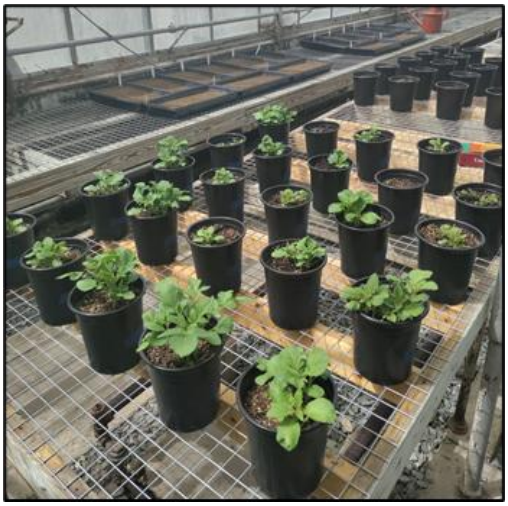

Figure 4. Planting of potatoes in Roger Clapp Greenhouse.

Soil Plating for Quantifying *Verticillium dahliae*

Soil samples were collected monthly during the greenhouse trial. Only the last month of sampling was used for soil plating. The soil was placed in paper bags and allowed to dry at room temperature (22 °C). After drying, 1 gram of the soil sample was added to 9 mL of 1X phosphate buffered saline (PBS) solution in a flask containing a magnetic bar and mixed for a minimum of 5 minutes on a spinning base. Each sample was plated onto ten sodium polypectate agar (NP-10; Xiao and Subbarao 1998) plates by pipetting 100 µL and spreading using a spreader bar (Fig. 5). To allow for the soil particles to be pipetted onto the plates, pipette tips were cut at the 200 µL graduation mark and autoclaved before use. All the plates were incubated at 23°C for 2 to 3 weeks. Soil was removed from the agar plates by gently rubbing the surface under tap water. Under a compound microscope at 40x magnification, the number of germinated microsclerotia per plate were counted.

Figure 5. Soil dispersed on an NP-10 agar plate using a spreader bar.

DNA Extraction and qPCR for Quantifying *Verticillium dahliae*

Soil samples were frozen at -20°C until the DNA extraction. DNA was extracted from 0.25 g of soil using the DNeasy® PowerSoil® Pro Kit (Qiagen, Hilden, Germany, Borza et al. 2018). The soil was put in a PowerBead Pro Tube (which is pulse centrifuged to settle beads towards the bottom of the tube) along with 800 µl of the CD1 solution (sodium thiocyanate). The samples were briefly vortexed and then placed in a Vortex-Genie 2 vortex adapter (Scientific Industries, New York, USA, Fig. 6) to be mixed for 10 minutes at maximum speed. Samples were then centrifuged for 1 min at 15,000 × g. The manufacturer's instructions were followed for the rest of the DNA extraction protocol and DNA was eluted into 100 µl elution buffer

A NanoDrop (Thermo Fisher Scientific, Delaware, USA) device was used to quantify DNA, with ultrapure water being used as a blank between samples. DNA samples were stored at -20°C until qPCR was performed. A colony of *V. dahliae* was used as a positive control for qPCR, and sterile Milli-Q® (Millipore Sigma, Darmstadt, Germany) water was used as the negative control. The *V. dahliae* DNA sample was

extracted using a FastDNA® Spin Kit (MP Biomedicals, California, USA). Microsclerotia were harvested from the culture by adding distilled water, disturbing the microsclerotia with a spreader bar, and pouring off the now water-suspended microsclerotia off the plate into a test tube. From this test tube, 200 µl of the suspended microsclerotia were added to a Lysing Matrix A Tube. An aliquot of 1,000 µl of cell lysis solution (CLS) was added to the Lysing Matrix A Tube, specifically with CLS-Y for fungal cell lysis. The sample was then loaded onto a FastPrep® (MP Biomedicals, California, USA) where it was mixed at the 6.0 setting for 40 seconds. The rest of the manufacturer's instructions were followed, with the exception that DNA was eluted into 100 µl of sterile distilled water. The DNA sample was stored at -20°C until qPCR was performed.

The primers, VertBt-F (AAC AAC AGT CCG ATG GAT AAT TC) and VertBt-R (GTA CCG GGC TCG AGA TCG), were used for the detection of *V. dahliae*, targeting the β-tubulin gene (Atallah et al. 2007). The master mix for qPCR was made with 10 µl iQ Supermix SYBR-Green, 1 µl of 200nM VertBt-F, 1 µl of 200nM VertBt-R, 7 µl of water, and 1 µl of DNA (total volume of reaction was20 µl). The conditions of the qPCR room were dark while handling the samples on ice. The thermal cycler (Bio-Rad, Hercules, California) was set up with an initial denaturation at 95°C for 3 minutes, then 40 cycles of the following: 95°C for 10 seconds and 63°C for 35 seconds. To differentiate between primer dimers and nonspecific products, a melt curve analysis was used.

Statistical Analysis

Statistical analysis was done using the coding program R. Data was analyzed with an analysis of variance (ANOVA) test to determine any significant difference. Least

significant difference (LSD) tests were performed on any data that was deemed to be significantly different ($\alpha = 0.05$) to examine differences among means.

RESULTS

Plant Growth and Disease Evaluation

Plant emergence was not significantly affected by any treatments ($P > 0.05$; Fig. 6). Under both compost treatments (compost or compost mixed with LSM), plants emerged at an average of 8.6 days, while it took 9.4 to 10 days under the other treatments.

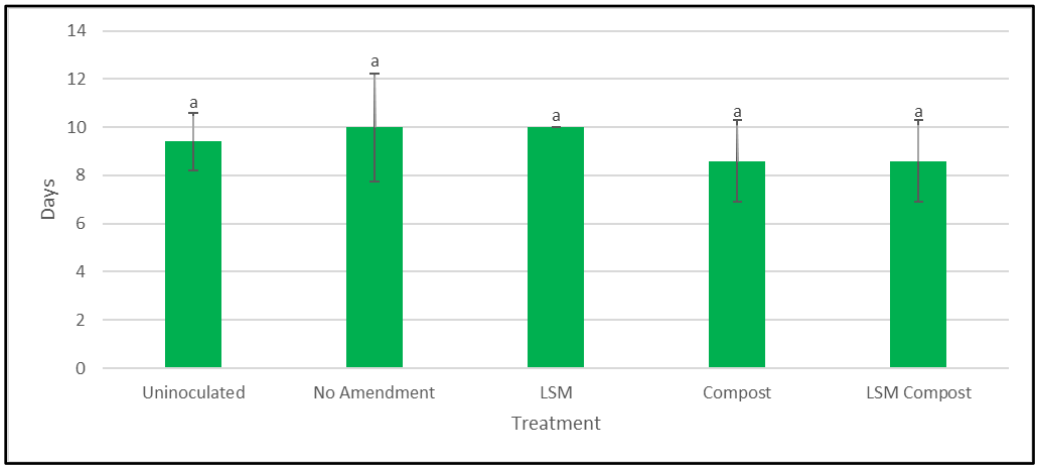

Figure 6. Potato emergence under different soil treatments in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost. Columns with different letters were significantly different at $p<0.05$).

For plant height, there was no significant difference among treatments in the final plant height at the end of the study where they ranged between an average of 33.5 to 39.5 cm ($P > 0.05$) (Fig. 7).

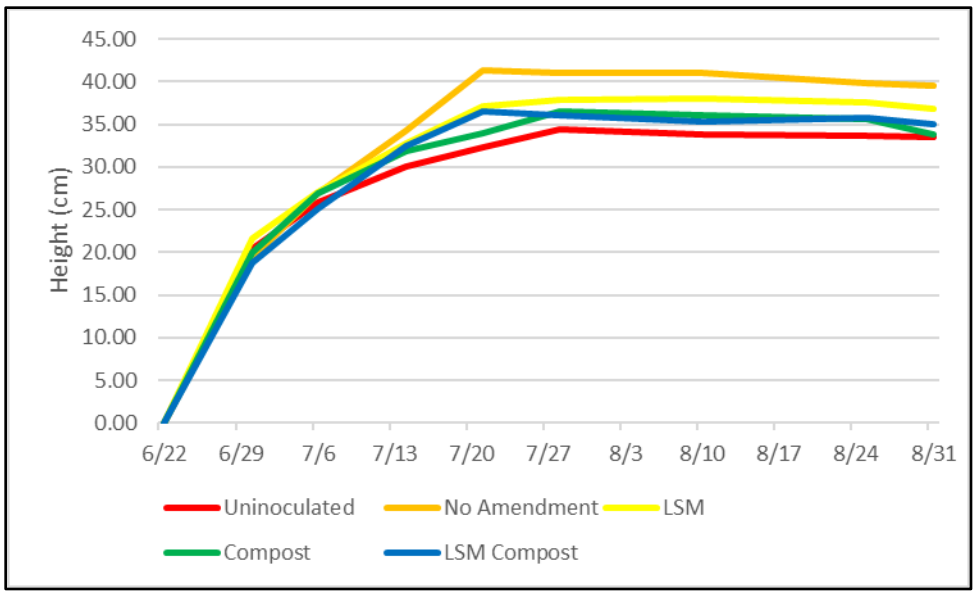

Figure 7. Plant height measured at various stages in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost.

For stem disease evaluation, the compost treatments had the largest lesions in the second month of the study, measuring up to 4.7 cm. However, there was no significant difference between treatments for lesion size at the end of the study where they ranged between an average of 1.26 to 3.00 cm ($P > 0.05$; Fig. 8).

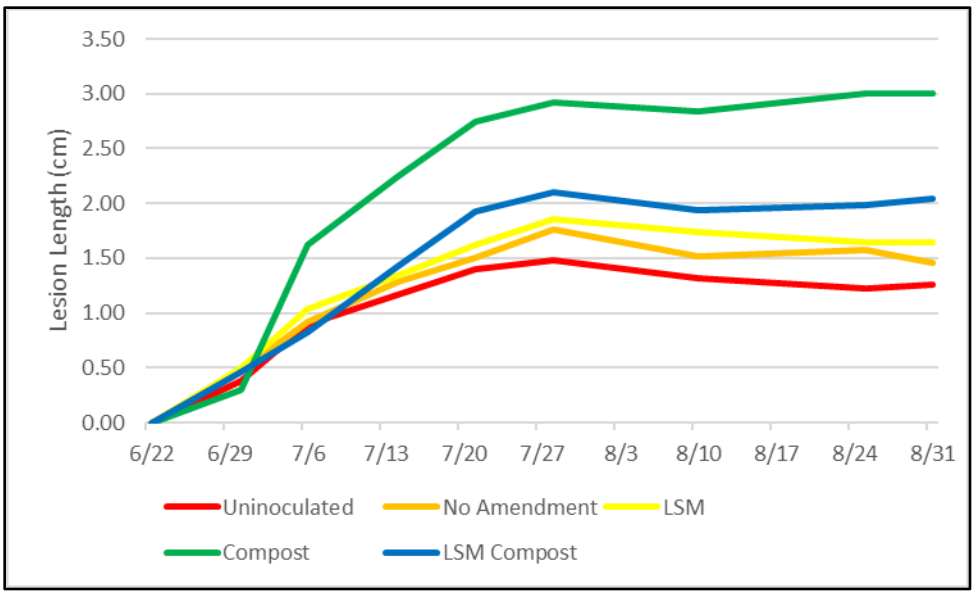

Figure 8. Disease lesion size on potato plants caused by *Verticillium dahliae* in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost.

Plant Biomass

For shoot biomass, there was no significant difference ($P > 0.05$) among treatments. The compost-only treatment had the highest average mass of 7.99 g. Root biomass was not significant ($P > 0.05$), but it was observed that both compost treatments, LSM compost and compost alone, had greater average biomasses (12.43 and 14.81 g, respectively) than the other treatments (ranging from 7.29 to 11.62 g; Fig. 9). The LSM-only treatment had the least number of tubers with an average of 3.4 tubers per replication, while the uninoculated treatment had the greatest number of tubers with an average of 6 tubers per replication (Fig. 10). However, no significant difference in tuber number was found between treatments ($P > 0.05$). Average tuber biomass was found to be significantly different among the treatments ($P < 0.05$; Fig. 9), with the average tuber biomass of the LSM treatment (20.28 g) being significantly greater than the uninoculated

and compost-only treatments (11.98 and 10.24 g, respectively) and the average tuber biomass of the inoculated-only treatment (16.42 g) being significantly greater than the compost-only treatment. However, no significant difference was found between the total tuber mass yielded by each treatment on average ($P > 0.05$, Fig. 11). The compost-only treatment yielded the lowest total tuber mass at 59.38 g, while all other treatments ranged between 68.96 g and 71.87 g on average.

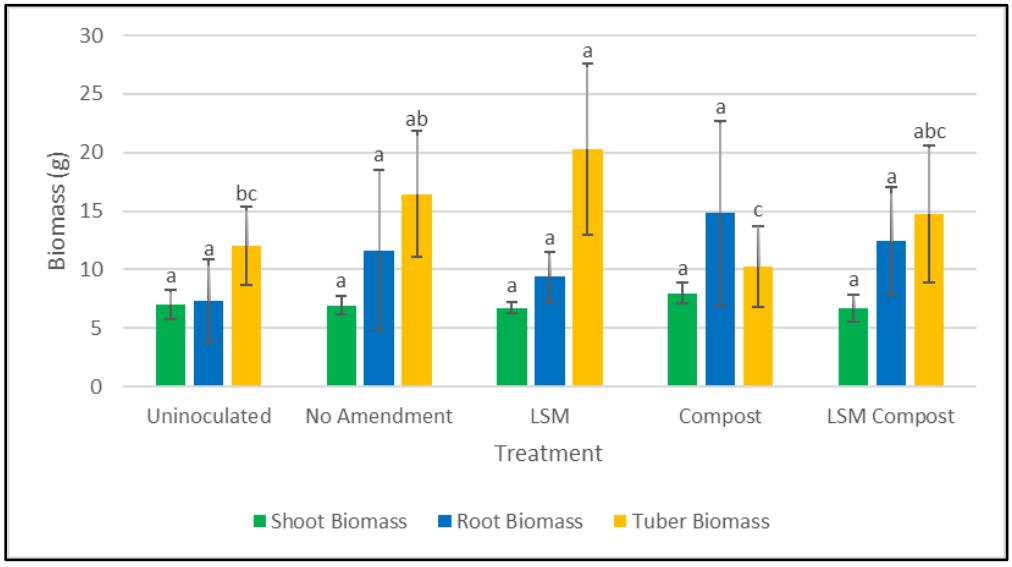

Figure 9. Potato biomass of shoots, roots, and tubers under different soil treatments in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost. Columns with different letters were significantly different at $p<0.05$).

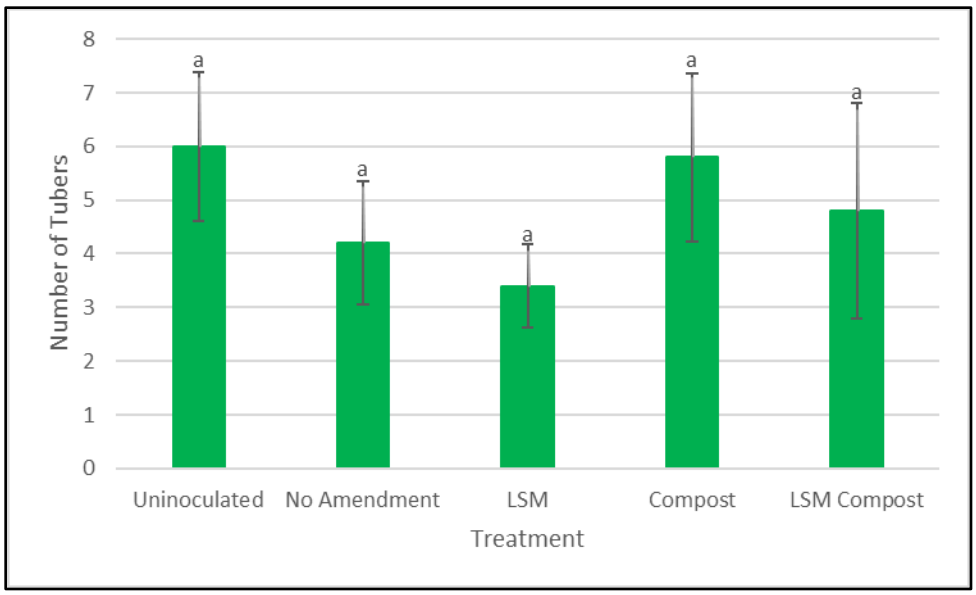

Figure 10. Number of potato tubers yielded under different soil treatments in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost. Columns with different letters were significantly different at $p<0.05$).

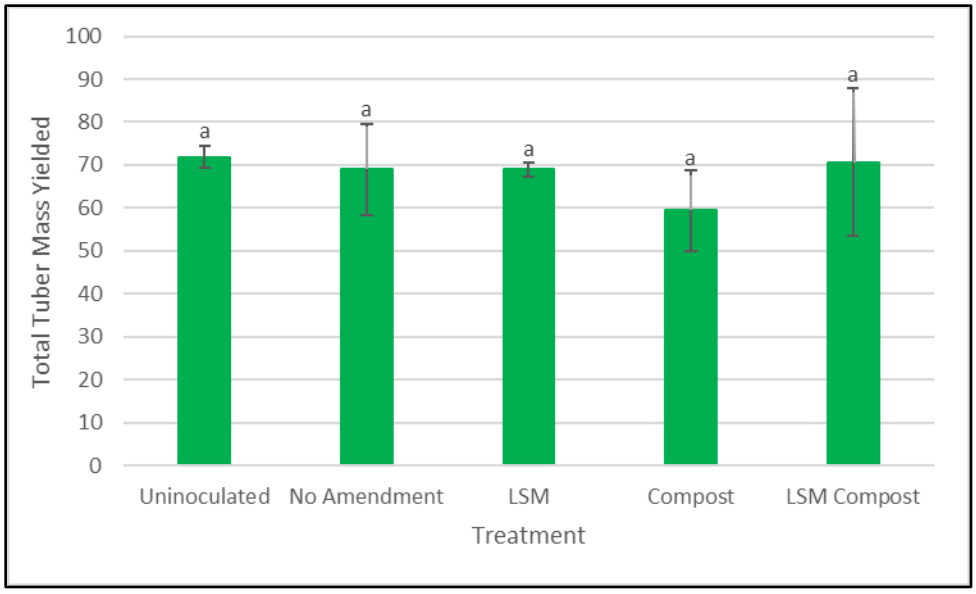

Figure 11. Total tuber mass yielded under different soil treatments in 2022. Treatments included uninoculated and no amendment control, V. dahliae-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost. Columns with different letters were significantly different at $p<0.05$).

Tuber Disease Evaluation

When observing the vascular ring of the tubers, the highest severity, 49.17% of the vascular ring, was observed in the compost treatment with LSM (Fig. 12). This was followed by the *Verticillium*-treatment without amendments (40.33%), *Verticillium*-treatment with the compost (36.93%), *Verticillium*-treatment with LSM (35.29%), and the uninoculated without amendments (27.2%). However, there was no significant difference found in disease severity between any of the treatments ($P > 0.05$).

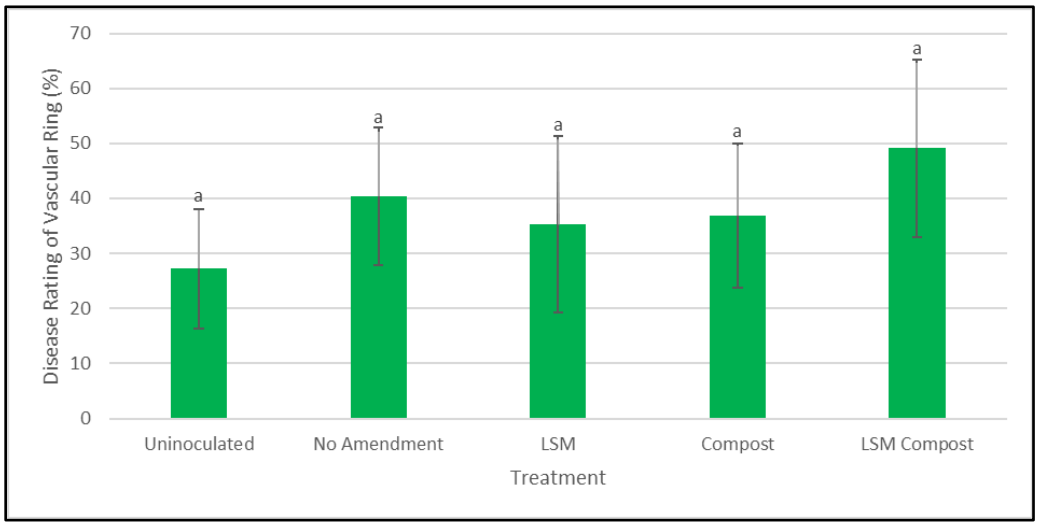

Figure 12. Disease severity of Verticillium wilt in vascular tissues of potato tubers under different soil treatments in 2022. Treatments included uninoculated and no amendment control, *V. dahliae*-inoculated soil amended with either no amendment, lobster shell meal (LSM), compost, or LSM mixed with compost. Columns with different letters were significantly different at $p<0.05$).

Verticillium dahliae Quantification

No *V. dahliae* growth was found in any of the plated soil samples. Various fungal morphologies were observed on the plates, but none resemble the morphology of *V. dahliae*. When testing qPCR, the positive samples of *V. dahliae* were not easily detected by the primer, with Ct values ranging from 37.24 to 38.07 (Table 1). When checking the DNA quality of the samples, it was found that there was a substantial concentration of

nucleic acids in the positive samples, yet the 260/280 ratio of samples were lower than the ideal value ≈1.80, ranging from 1.44 to 1.56 (Table 2).

Table 1. Cycle of the threshold for SYBR-Green-based qPCR to detect *Verticillium dahliae* targeting the β-tubulin gene.

Sample	Replicate	Ct
Verticillium	1	37.32
Verticillium	2	38.07
Verticillium	3	37.24
Water	1	None
Water	2	None
Water	3	None

Table 2. NanoDrop measurements of nucleic acid concentration and absorbance at 260 nm and 280 nm of Verticllium dahliae DNA samples.

Sample	Nucleic Acid Concentration (ng/µl)	A260	A280	A260/A280
Positive Verticillium Sample #1	69.4	1.389	0.967	1.44
Positive Verticillium Sample #2	56.3	1.127	0.779	1.45
Positive Verticillium Sample #3	51.7	1.034	0.664	1.56

DISCUSSION

Compost and LSM had a trend in enhancing plant growth, but the effects were not statistically significant. Compost treatments slightly promoted plants to emerge faster than other treatments, but not significantly. As the plants in this study were not fertilized, the additional fertility provided to the plant by compost may be a consideration for this occurrence. Compost may provide a small increase in nutrient availability such as nitrogen or potassium as it breaks down in the soil (Wilson et al. 2019). Plant height was not affected by either a compost or lobster shell meal amendment, so plant height may have not been a factor in nutritional benefits from these amendments.

Treatments containing LSM did display lower shoot biomass compared to other treatments. However, this was not significant and did not exhibit any negative consequences for aboveground plant growth. Although it was not significant, there was a noticeable increase in root biomass in treatments containing compost. In potato production, it has been noted that over time, compost can significantly increase root biomass (Larkin et al. 2021). These systems were usually implemented with the goal of improving soil health (which can include additional practices such as crop rotations and cover cropping), matching the purpose of this study when incorporating a compost amendment. Neither LSM nor compost increased the number of tubers yielded per pot. The published studies have very controversial results and conclusions on whether compost can increase the number of marketable tubers, as separate studies have found it to increase tuber yield (Salem et al. 2010) or have no impact on yield (Wilson et al 2019). This variability may be attributed to the vast variety of composts, which often have different physical and chemical compositions. The compost-only treatment had a

significantly lower tuber biomass of 10.24 g, which leads to the question of whether it is a good idea to rely on this specific compost amendment for marketable tubers. The compost treatment differs from the treatment containing only LSM, which averaged at a biomass of 20.28 g, which may have been a marketable size if replicates were planted in larger pots or in the field. Interestingly, the combination of these amendments resulted in an average biomass of tubers in between these values, measuring 14.73 g. This could be the balance between the compost decreasing tuber mass and the LSM increasing the tuber mass. Despite these potential effects, each treatment yielded similar total tuber masses on average.

Lesion size on the stems were slightly larger under compost treatments compared to other treatments, but not significantly different. Some compost applications can result in favorable conditions for *V. dahliae* infection such as low C/N ratios (higher availability of nitrogen may lead to greater chance of infection) and large concentrations of unsaturated fatty acids (Cocozza et al. 2021). Compost analysis at a soil testing service could indicate whether nitrogen or unsaturated fatty acids were high in this compost application, therefore encouraging *V. dahliae* inoculum in the soil to cause infection.

The control treatment had a questionable tuber disease rating as well as lesions on the stem. This may indicate two different issues in the study. One issue may have been that *V. dahliae* from the other treatments may have spread to the uninoculated control replicates, leading to infection. The other issue that could have occurred is that seedborne *V. dahliae* may have already infected the seed pieces planted in the study, leading to infection occurring in all individuals, including the uninoculated. If the study is repeated,

larger pots, clean seed, and wider spacing between pots may be required to ensure the uninoculated control is not contaminated by *V. dahliae*.

When considering the results of the soil plating, there was evidently an error as no *V. dahliae* grew on the plate. If this part of the methodology were to be repeated, there are a couple of changes that could be made to optimize the protocol. One solution could be changing the media. *Verticillium dahliae* was able to grow on NP-10 from pure culture, however, it was a challenge to plate it using soil, which may introduce contamination from a variety of other microorganisms. Another consideration would be to increase the volume of the soil solution that was plated onto a replicate. It is possible that 100 µl was too low of an amount to successfully plate a microsclerotium and observe a *V. dahliae* colony. Otherwise, other methods of quantifying *V. dahliae* should be further investigated to find a reliable protocol.

Regarding the qPCR analysis to quantify *V. dahliae* from the soil, the protocol needs to be modified in the future. Many factors should be considered for establishing a robust and reliable qPCR protocol. One factor would be ensuring the primer is effective in the given conditions in the thermal cycler. Another factor may be the age of the primer or its efficiency. The DNA extraction protocol should also be reconsidered on whether the DNA samples of *V. dahliae* were sufficient. The 260/280 values of these samples were lower than optimum, with all the samples under the generally accepted minimum of 1.60 (Lucena-Aguilar et al. 2016). This may indicate there are contaminants that have a high absorbance at 280 nm, such as proteins or phenols.

In conclusion, more experimentation is needed to determine whether LSM or compost can be used as a soil amendment to manage Verticillium wilt in potatoes. LSM

should continue to be studied in the lab, greenhouse, and field to determine its potential benefits in disease management. Research should determine its effect on chitinolytic microbial populations and if this effect results in the suppression of fungal pathogens such as *V. dahliae* in the field. Different types of compost would need further experimentation to determine if there are better types of compost for managing *V. dahliae.* However, LSM and compost show promise to improve plant growth by possibly providing soil nutrients such as nitrogen to the crop. Soil plating may be an unreliable method to quantify *V. dahliae* and methods such as qPCR need further testing to establish a reliable method.

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