CALIFORNIA LEAFY GREENS RESEARCH PROGRAM ANNUAL REPORT

Project title: Application of soil amendments for improved soil health and management of fusarium wilt of lettuce

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Abstract:
Soilborne diseases are a significant constraint on lettuce production in California. Among these diseases, fusarium wilt of lettuce has become more common in the Central Coast production region since its initial detection in CA in the 1990s. Fusarium wilt of lettuce is caused by a soilborne fungus called *Fusarium oxysporum* f. sp. *lactucae*. The disease can co-occur in fields where other common lettuce diseases like verticillium are established. To reduce the negative impacts of fusarium wilt and other diseases, growers need management strategies that can be integrated into conventional and organic production systems. Application of soil amendments to alter microbial activity in soil or introduce beneficial microorganisms has potential to reduce pressure from these soilborne diseases and improve soil health. The primary goal of this project is to test if soil amendments can be used to reduce fusarium wilt and other co-occurring diseases of lettuce, while improving microbial characteristics of soil health. A secondary goal is to test efficacy of commercial microbe-based soil amendments for reducing fusarium wilt disease. Compost will be applied as a pre-plant treatment in a field infested with the fusarium wilt pathogen and the causal agent of verticillium wilt. The incidence and severity of fusarium and verticillium wilt diseases will be measured at harvest. Soil from the same field will be used to measure microbial metrics of soil health and abundance of the two wilt pathogens using molecular methods. The efficacy of commercial microbe-based amendments will be evaluated under controlled conditions on highly susceptible and moderately susceptible lettuce cultivars. The outcomes of this project will provide data on the potential use of soil amendments to manage soilborne diseases in CA lettuce production.

Objectives:

1. Test the effect of compost on fusarium wilt and co-occurring soilborne diseases of lettuce. Deliverable: Measurement of the effects of compost on fusarium wilt disease pressure in soil with co-occurring verticillium wilt disease under field conditions.
2. Evaluate effect of compost on microbial characteristics of soil health and abundance of soilborne lettuce pathogens. Deliverable: Measurement of the effects of compost on microbial metrics of soil health and abundance of co-occurring fusarium and verticillium wilt pathogens under field conditions.

3. Measure efficacy of microbe-based soil amendments for control of fusarium wilt of lettuce. Deliverable: Measurement of the effects of commercial microbe-based soil amendments on fusarium wilt disease pressure under controlled conditions conducive to disease.

Procedures:

Objective 1. Test effect of compost on fusarium wilt and co-occurring diseases of lettuce

Soil will be amended with compost at 28 Mg/ha prior to planting lettuce in a field with known presence of fusarium and verticillium wilt diseases. Lettuce plants will be maintained using standard commercial lettuce production practices. A goal of at least two trials will be pursued during the growing season to represent different environments (contingent on pressure from INSV disease). Treatments will be established within the field in a completely randomized design. At harvest, plants will be uprooted and internal tissue of tap-roots will be rated for symptoms of fusarium and verticillium on a standard 0-5 scale used by the USDA lettuce breeding program. Effect of compost on fusarium and verticillium disease incidence and severity will be tested using analysis of variance (ANOVA). Correlations between fusarium and verticillium disease measurements will be tested using linear regression.

Objective 2. Evaluate effect of compost on soil health and soilborne pathogens

Compost will be applied in field trails as described in Objective 1. Soil samples will be collected mid-season and at harvest for each treatment. Microbial metrics of soil health (e.g., microbial diversity and composition) will be characterized using metagenome sequencing of extracted soil DNA. The fusarium and verticillium wilt pathogens will be measured in the same soil samples using established qPCR molecular diagnostic assays. The effects of compost application on microbial diversity and soilborne pathogen abundance will be tested using ANOVA. Standard non-parametric methods will be used to test for an effect of compost on the composition of microorganisms in soil. In addition, correlations between microbial diversity, microbial taxa, and abundance of fusarium and verticillium wilt pathogens will be evaluated using linear regression.

Objective 3. Measure efficacy of microbe-based soil amendments for fusarium wilt control

Effect of microbe-based soil amendments on fusarium wilt disease will be evaluated under controlled conditions. Amendments will include commercial products containing single beneficial microorganisms and multiple beneficial microorganisms. Amendments will be applied at recommended application rates, prior to inoculation of lettuce plants with the fusarium wilt pathogen. Treatments will be tested on a highly susceptible lettuce cultivar (cv. Patriot or equivalent) and moderately susceptible cultivar (cv. Salinas or equivalent) maintained in controlled conditions conducive to fusarium wilt disease. Negative control treatments will include water and autoclaved (i.e., sterilized) microbe-based amendments to account for potential effects of non-biological materials in amendments. Experiments will be maintained in a randomized complete block design. Effect of amendments on disease will be tested using ANOVA, followed by Dunnett's test to identify significant differences between individual treatments and negative controls.

Results:

Objective 1: The compost field trial was conducted at a location with known presence of verticillium and fusarium wilt diseases. As shown in Figure 1, harvested lettuce plants showed

characteristic symptoms of verticillium and fusarium wilt at the end of the trial. At harvest, verticillium wilt disease severity nor lettuce yield was significantly different between control and compost treated experimental plots (Table 1). Fusarium wilt disease symptoms were too infrequent to conduct statistical analyses.

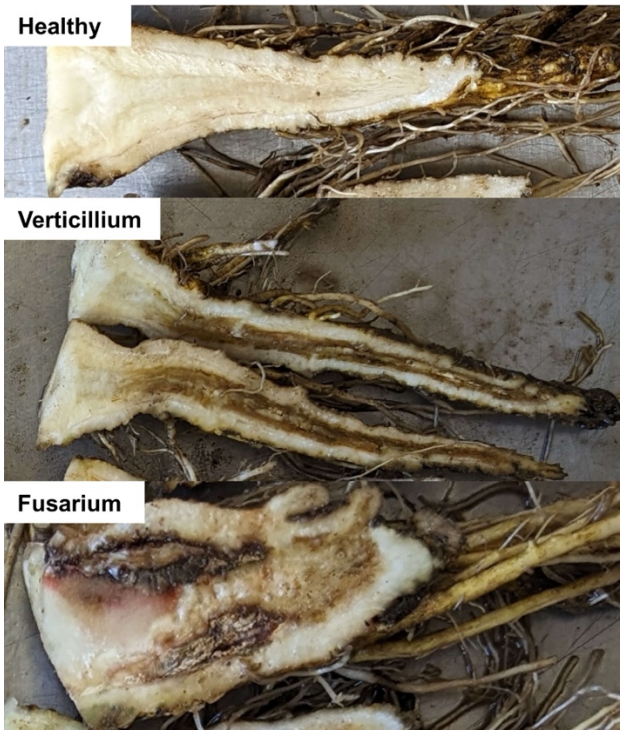

Figure 1. Symptoms of soilborne diseases in lettuce tap-roots in harvested lettuce from compost field trial.

Measurement	Control	Compost	p-value
Yield	462.22±143.29	490.56±164.97	0.7025
Disease severity	1.96±1.31	1.93±1.26	0.8641

Table 1. Estimates of lettuce yield (head fresh weight) and verticillium wilt disease severity in compost field trial. Values represent average ± standard deviation in non-amended ("Control") and compost-amended ("Compost") treatments. P-values are from a t-test comparing control and compost treatments.

Objective 2: Soil samples from the same compost field trial were used to analyze soil bacterial communities as a microbial metric of soil health and the amount of soilborne pathogens in control and compost treated plots. As shown in Figure 2, compost significantly increased bacterial diversity in soil at mid-season and harvest. Compost also had a significant effect on the

types and amounts of bacteria within soil (Figure 3). There was also a significantly higher amount of *V. dahliae* in soil treated with compost, though this was only observed at harvest (Figure 4). There was no significant correlation between individual bacteria in soil and the abundance of *V. dahliae. Fusarium oxysporum* f. sp. *lactucae* was too infrequent to plot data or conduct statistical analyses (data not shown).

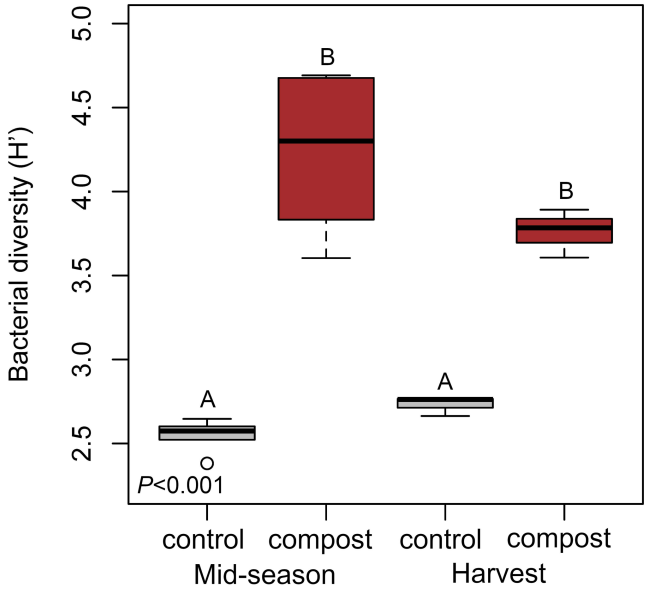

Figure 2. Boxplots showing variation of bacterial diversity in soil of compost field trial. The y-axis shows bacterial diversity. The x-axis shows control and compost treated plots at mid-season and harvest. P-value represents results from analysis of variance testing for significant differences among treatments. Plots with different letters are significantly different from each other based on post-hoc comparisons (Tukey's HSD).

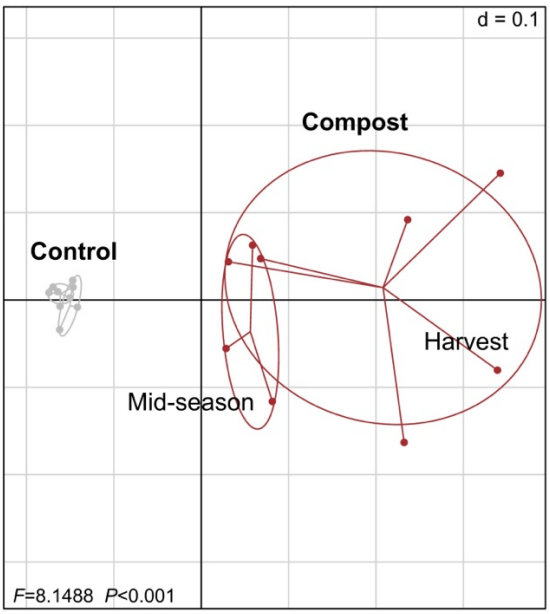

Figure 3. Ordination plot showing variation of bacterial communities in soil of compost field trial. Each point represents a soil sample and the composition of bacteria within. Points closer together have more similar types and amounts of bacteria in them. Lines and ellipses represent variation within a given treatment (control versus compost) and timepoint (mid-season versus harvest). Control plot samples are in grey and compost treated samples are in brown. P-value represents results from a non-parametric analysis testing for significant differences among treatments.

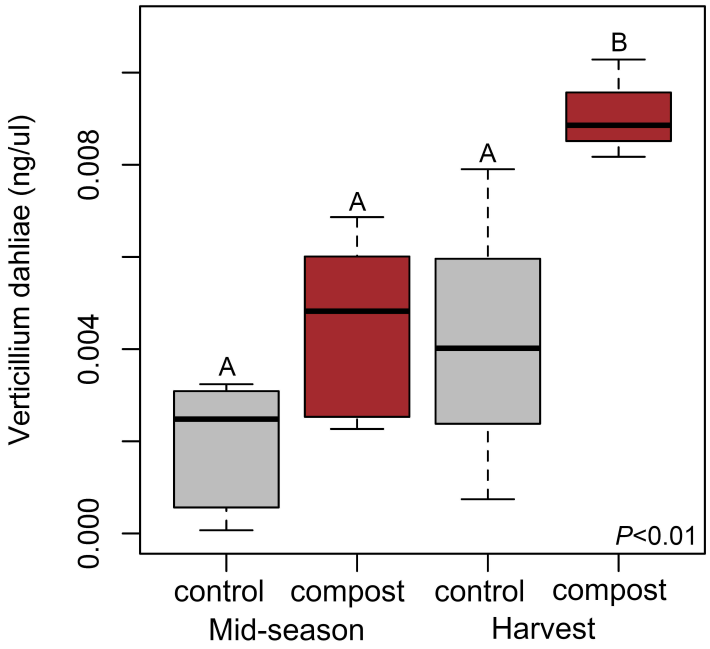

Figure 4. Boxplots showing variation of *Verticillium dahliae* in soil of compost field trial. The y-axis shows *V. dahliae* abundance (measured using qPCR). The x-axis shows control and compost treated plots at mid-season and harvest. P-value represents results from analysis of variance testing for significant differences among treatments. Plots with different letters are significantly different from each other based on post-hoc comparisons (Tukey's HSD).

Objective 3: Commercially available biofungicides (i.e., beneficial microorganisms) were tested for their effects on lettuce yield and the disease fusarium wilt of lettuce. The biofungicides included Actinovate SP (active ingredient *Streptomyces lydicus* WYEC108), Cease (active ingredient *Bacillus subtilis* QST713), RootShield Plus WP (active ingredients *Trichoderma harzianum* T22 and *T. virens* G41), and Trianum-P (active ingredient *T. harzianum* T22). Biofungicides were applied as root dips to lettuce seedlings at the high-end of labelled application rates in all experiments.

An initial greenhouse experiment was conducted to test effects of biofungicides on growth of lettuce cv. Salinas and Patriot in the absence of the pathogen *F. oxysporum* f. sp. *lactucae*. In this experiment, there was no significant effect of biofungicide application on fresh weight of lettuce cv. Patriot (P=0.938) or Salinas (P= 0.301). A subsequent experiment tested the effects of the same biofungicides on fusarium wilt disease severity under conditions conducive to disease and inoculating the pathogen at the same time as the biofungicides. As shown Figure 5, all treatments showed symptoms of disease, except the water (negative control) treatment.

Statistical analyses showed there was an overall significant variation among at least two treatments (P<0.001). Post-hoc comparisons showed these differences were only between treatments where the pathogen was inoculated and the water negative control. There was no significant (P>0.8) difference in disease severity among the treatments treated with the pathogen only and the pathogen plus the biofungicides. Two additional experiments were conducted using both lettuce cultivars and inoculating the pathogen one week after biofungicide application. However, due to an absence of disease pressure in any treatment, data were not collected or reported.

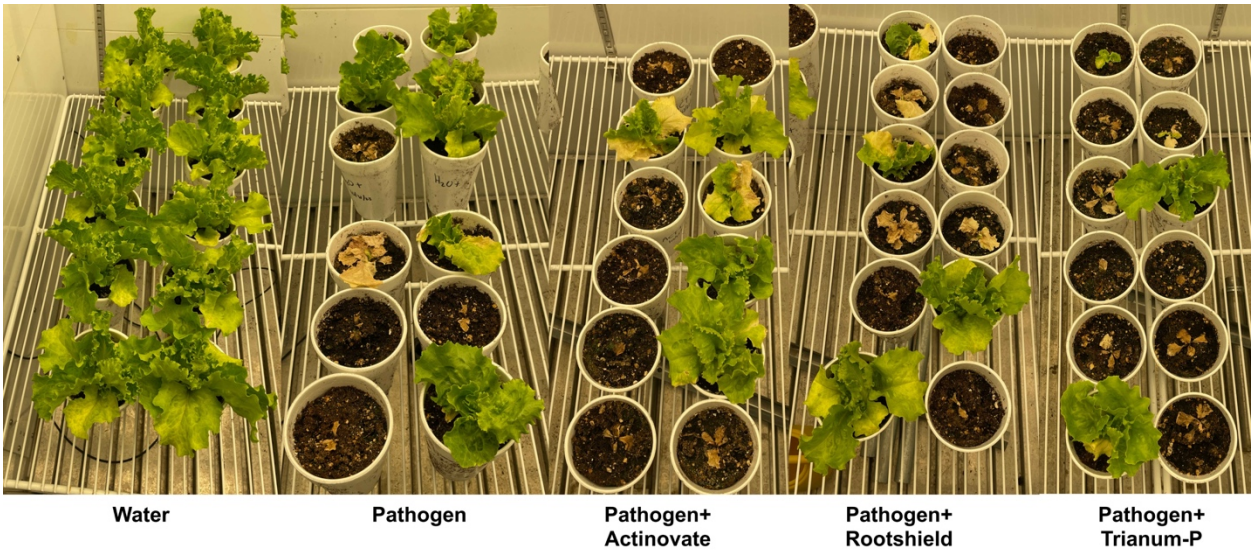

Figure 5. Images from growth chamber experiment testing effects of biofungicides on fusarium wilt of lettuce disease severity. Treatments are labelled under each subsection and "Water" indicates plants that were not inoculated with the pathogen *F. oxysporum* f. sp. *lactucae*. Based on analysis of variance (ANOVA) and post-hoc comparisons, there was no significant differences among treatments that were inoculated with the pathogen.

Discussion:

Long-term management of soilborne diseases impacting California lettuce production requires a combination of multiple strategies in an Integrated Pest Management (IPM) approach. Using compost to alter the soil environment or introducing beneficial microorganisms into soil as biofungicides are two potential approaches for reducing pressure from soilborne diseases of lettuce. This research tested pre-plant application of plant-based compost in a pathogen infested field and generated preliminary data on potential use of biofungicides for managing fusarium wilt of lettuce.

Compost increased bacterial diversity and altered the composition of bacterial communities (i.e., type and amounts of different bacteria) in soil. Increased microbial diversity is often cited as a positive characteristic of soil health. Increased microbial diversity has also been proposed as an indicator of soilborne pathogen and disease suppression (Van Bruggen and Semenov 2000). However, there was no clear relationship between bacterial diversity and the abundance of the pathogen *V. dahliae*. There was also no correlation between individual types of bacteria and the abundance of *V. dahliae* in soil. Based on these results, blanket generalizations

should be avoided and used to inform production practices focused on linking microbial characteristics of soil health and soilborne disease management.

Compost had no significant effect on verticillium wilt disease severity, but increased the abundance of the soilborne pathogen *V. dahliae*. Increased *V. dahliae* was especially evident later at the end of field trial close to harvest. This delayed increase in *V. dahliae* could explain why there was no corresponding increase in verticillium wilt disease severity in compost treated plots. However, it seems likely that increased abundance of *V. dahliae* could lead to greater disease pressure in a subsequent cycle of lettuce planting in the same field. Since different types of compost – including the tested plant-based compost – have been shown to reduce other soilborne diseases and pathogens, plant pathogens present in a field should be determined prior to integrating compost into production practices.

A secondary focus of this year's research was on testing commercially available biofungicides for managing fusarium wilt of lettuce. The experiments conducted were under conditions that increased the ability of the pathogen to cause disease. *Fusarium oxysporum* f. sp. *lactucae* was applied at high concentrations and biofungicides were only applied once. Plants were also grown under elevated temperatures that promote fusarium wilt disease. The outcomes of these experiments indicate that under these worst-case scenarios of high pathogen pressure and elevated temperature, the tested biofungicides will not reduce fusarium wilt of lettuce.

Overall, this research shows that compost can have positive effects on microbial metrics of soil health, like microbial diversity, while also increasing soilborne pathogen abundance. The ability to link microbial soil characteristics and disease is still a poorly understood phenomenon and requires more research (Fierer et al. 2021). This research also indicates biofungicides are not effective for managing fusarium wilt of lettuce when the pathogen is abundant and the environment favors disease. Pending research will evaluate if biofungicides are effective for managing fusarium wilt and other soilborne diseases of lettuce under environmental conditions that are less conducive to disease.

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References:

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