The role of the soil microbiome in plant growth and defense against Colorado Potato Beetles

Ziyan Jin

Department of Entomology - Purdue University - West Lafayette, IN 47907

Abstract

Microbes have built connections with all kinds of creatures since they exist. Microbes related to insects can facilitate digestion as well as spread diseases to the host plants. Plants respond to biotic challenges either by internal ways such as to synthesize chemical compounds as signals to alert, or external ways like seeking more nutrients or help from other organisms. Microbes living in the soil can strengthen plants' root system and increase growth or can cause disease. The goal of this research was to analyze the growth response of tomato plants in different soil types under the attack of Colorado Potato Beetles and to test how a beneficial microbial product affects plant growth and defense against CPB in these soil types. Results show the commercial microbial product improved plant growth in potting mix, but not field soil. Soil type had no effect on CPB growth, but there was an interaction of soil type and microbial treatment on plant growth.

Introduction

The interactions between herbivores and plants have been occurring for million years. Plants recognize and defend against individual attackers through defense hormone signaling (Casteel, 2014). The synthesis and distribution of defensive signaling depends on the health of the plant. But it is not only necessary to have suitable abiotic factors (e.g. climate, temperature, soil conditions), additional biotic factors can also influence plant-insect interactions. Microbes are invisible but they play a significant role in the relationships between herbivores and plants in many ways. The involvement of microbial communities (i.e. the microbiome) can alter the expression of plant defenses, plant toxicity and plant tissue digestion during plant-insect interaction (Mason, 2018). Therefore, the soil microbiome is a key component of plant immunity (Raaijmakers, 2016).

There are many microbiome products used in integrated pest management to control insects and plant pathogens. However, compared to the fast and immediate chemical control, environmental friendly biological control options are not as widely applied. Not a lot of cases have shown the effective use of microbiome based products as a treatment against damage from insect herbivores. Also, it is unknown whether different soil compositions may have impacts on the treatment result. Arbuscular mycorrhizal fungi (AMF) is a phylum of fungi that have been studied for a long time. AMF can be found in the rhizosphere and be applied as biological fertilizer to increase the root system development and improve nutrient uptake (Carine, 2017). However, it is unkown whether AMF can then improve plant defense against insect pests.

Colorado potato beetle (*Leptinotarsa decemlineata)* is a common pest to many *Solanaceae* crops including tomatoes. The larvae and adults will feed on the leaves and cause complete defoliation. The goals of this study were to test the effect of microbiome treatment, in this case apply AMF, on plant growth in different soil types and secondly, to see whether different soils will affect the ability of AMF to help tomato plants defend against Colorado potato beetles. Different soils contain different microbiomes, which can influence the performance of plants growing in them (Pineda, 2017). However, in both home gardens and in commercial nurseries, tomatoes are grown in potting mix that contains only the basic nutrients for plant growth. Loose potting mix is helpful for roots to breathe and develop, and for water to run through, but potting mix does not have many microbes that affect plant growth. My hypothesis is that after adding the AMF treatment, the growth of CPB on the tomatoes will be reduced in all of three soil types, and the tomatoes growing in the treated soil will be stronger and more tolerant to herbivore damage.

Method and Materials

Soil Type, Plants and Microbial Treatments

The soil types used in this experiment were a standard potting mix, agricultural soil collected from Meigs Purdue Agricultural Center, and wild soil collected from the Purdue Wildlife Area. To prevent the soil from compressing in the pots, the wild soil and Meigs soil were mixed with the autoclaved potting mix in a 1:1 ratio. In total, 20 pots of wild soil, 20 pots of Meigs soil and 20 pots of non-autoclaved potting mix were prepared.

Solanum lycopersicum cv. M82 was used for the experiment because it is a widely used, commercial variety of tomato and it has a similar germination time to all other types of tomatoes. Before putting seeds in the pots, they were first immersed in 50% bleach for 30 min and then washed with distilled water for 3 to 4 times in the centrifuge tubes. Four seeds were planted in each pot. After germination, each pot was thinned to one seedling. During the experiment, in the total of 60 pots, 16 out of 20 wild soil pots, 16 out of 20 Meigs pots and 13 out of potting mix pots were germinated.

For the microbial treatment, a commercial product called Platform Pure® (Nutri-Tech Solutions) was used. From the description, the company claims the product can help root system growth and improve nutrient uptake for plants. The composition includes three species of Arbuscular Mycorrhizal Fungi (AMF) in a concentration of 0.0196 g/L. In total 50 ml of the microbial solution was applied to the 10 pots of each soil for a total 30 pots (about 1 gram of AMF in each pot). To avoid contamination between treated pots and untreated pots, pots with the microbial treatment were kept separate from untreated pots. (Fig.1)

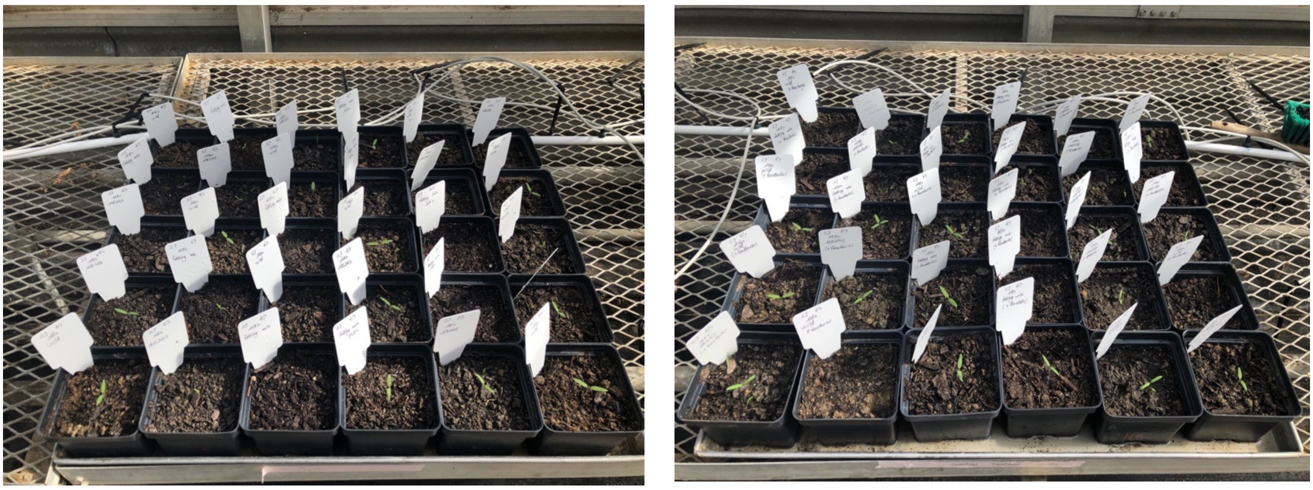


Figure 1: Experimental set up showing pots without AMF treatment (left) and pots with AMF treatment (right). Pictures were taken after seeds germination, but the AMF solutions were treated before seeds germinated. All the pots were randomly arranged.

Insect Treatments

Colorado potato beetles (CPB) were reared in lab from eggs to larvae by feeding with tomato leaves. After about 20 days, the average height of all M82 plants were 16.4cm and they were ready for the next step. Two CPB larvae (2^{nd} or 3^{rd} instar) were weighed and placed on each plant. All pots were covered with plastic bags (with holes for air circulation), including the pots with no larvae, to isolate each plant for 48 hours in a controlled growth chamber with a temperature of 23°C and 60% humidity (Fig.2).

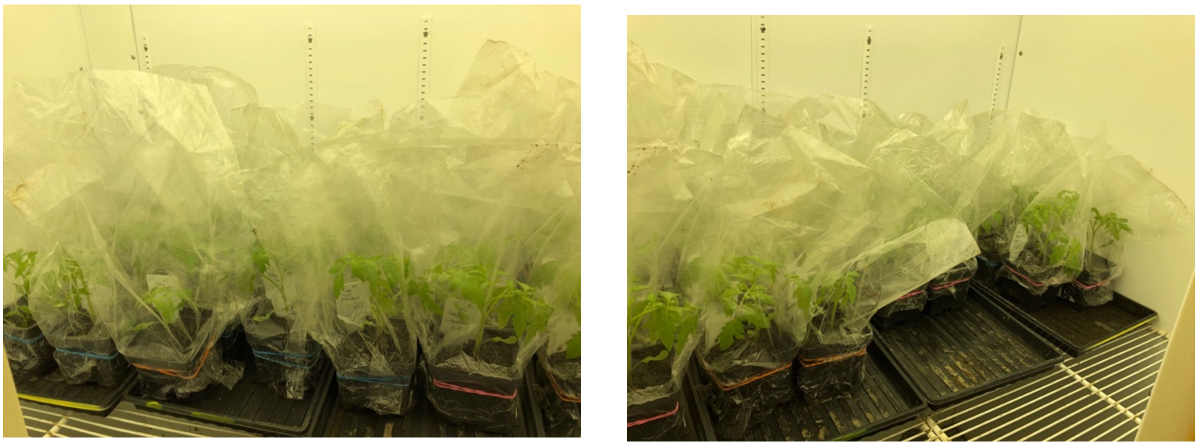


Figure 2: 20-day old M82 plants with CPB treatments. All plants were isolated with plastic bags and arranged randomly in the growth chamber.

Data Collection and Analysis

Beetle larvae were weighed before and after feeding on the tomato plants for 48hrs. After weighing the beetles twice, plant shoots were weighed by cutting off the plant stems above soil. The "one cut, one weight" rule was followed to avoid inaccuracy caused by water loss. The average weight of the control plants without CPB was calculated, assuming the growth of plants would not have be too different since they are all under the same environment. The percentage difference from controlled plant weight and the percentage difference of beetle weight were also calculated. To estimate beetle growth across the experiment the following formula was used:

$$\% \textit{ difference in beetle weight} = \frac{beetle\ weight\ after\ feeding(g) - beetle\ weight\ before\ feeding(g)}{beetle\ weight\ before\ feeding(g)}$$

All data was entered to Excel and the percentage difference of plant weights was calculated. Data was visualized into graphs by using JMP and Microsoft Excel. The percentage difference from controlled plant weight and plant weight after beetle feeding can be used to estimate how much of a difference there is before and after putting the beetle larvae on plants. And it is the same for the percentage difference of beetle weight, to find out how much measurements differ before feeding and after feeding on the plants. We can measure the effects of microbial treatment by comparing the weight changes of the plants with the microbial treatment and without the treatment either before putting the larvae or after putting the larvae. The effects of CPB can be measured by weight loss of the plants comparing before putting the larvae and after putting the larvae.

I used JMP was used as the statistic software to test fixed effects: CPB, AMF treatment, and soil types through three-way ANOVA statistical test and tukey test. Significant differences were determined using a p value threshold of greater than 0.05, and accepting the significance when p value is smaller than 0.05.

Results

Beetle Growth

The beetles varied in weight but statistically, there is no difference for the growth of CPB fed on plants grown in different soil types (p value >0.05). The comparison of CPB weight under MEIGS, Wild and Potting mix soil types did not show a lot of difference and there did not appear to be a major effect of AMF treatment (Fig.3, p values >0.05).

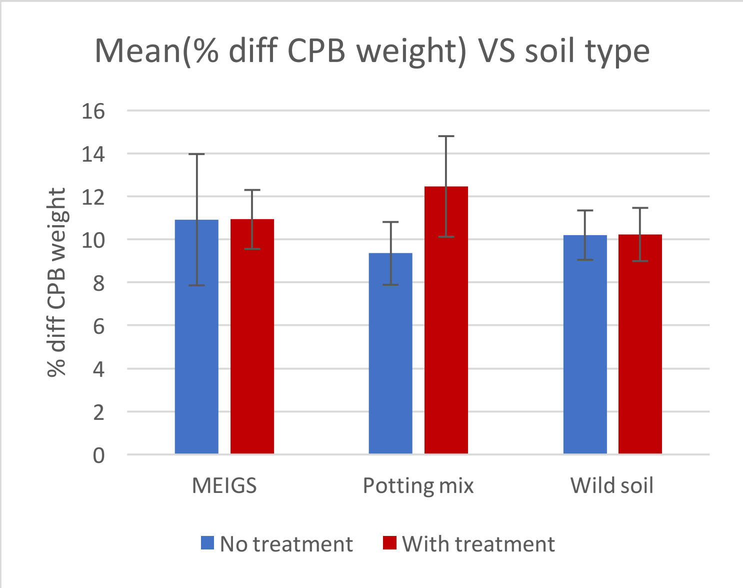


Figure 3: The change in CPB weight (before and after feeding for 48hrs) on plants in different soil types with or with out microbial (AMF) treatment.

Plant Growth

There was no effect of soil types for plant weight (p value =0.70579). The comparison of plant weight between all the soil types with AMF treatment and all the soil types without AMF treatment showed that AMF did help plants to gain more weight, as it claims to help root system to grow better and uptake more nutrients. When comparing the percentage of plant weight change with AMF and without AMF, Figure 4 shows positive growth under different growing soils when tomato plants were under attack of CPB. Although the average weight of tomatoes in MEIGS soil was still declining, the number is much smaller with the AMF treatment than without the AMF treatment (Fig 4). And statistically, there was a significant effect of AMF on the plant growth (p=0.01020), and marginal significant effects of the interaction between AMF and CPB (p=0.08088) (Fig.5).

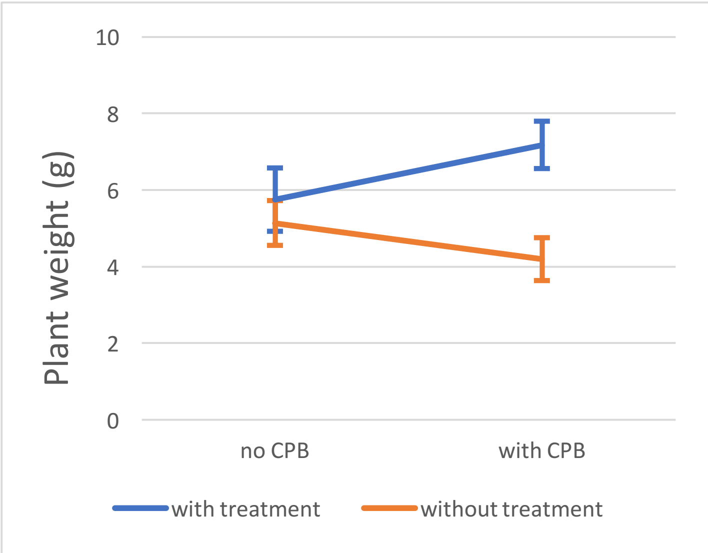
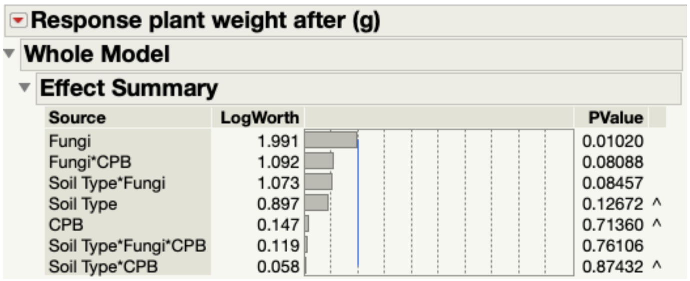


Figure 4: Weight of tomato plants grown in soils treated with AMF or not (controls) and with or without CPB feeding on them. Plants are grouped together across all soil types.



Response plant weight after (g)

Whole Model

Effect Summary

Source	LogWorth	PValue
Fungi	1.991	0.01020
Fungi*CPB	1.092	0.08088
Soil Type*Fungi	1.073	0.08457
Soil Type	0.897	0.12672 ^
CPB	0.147	0.71360 ^
Soil Type*Fungi*CPB	0.119	0.76106
Soil Type*CPB	0.058	0.87432 ^

Figure 5: Results of statistical tests comparing plant weight across different soils, AMF treatment and CPB treatment.

However, when breaking down the result into different soil types, rather than showing great improvements after the AMF treatment in all 3 soil types only the tomato plants in the potting mix changed their average weight from 3.5g to 7.5g. The average weight of tomato in MEIGS showed little difference, and the average weight of tomato in wild soil only changed from 6g to 7g (Fig.6).

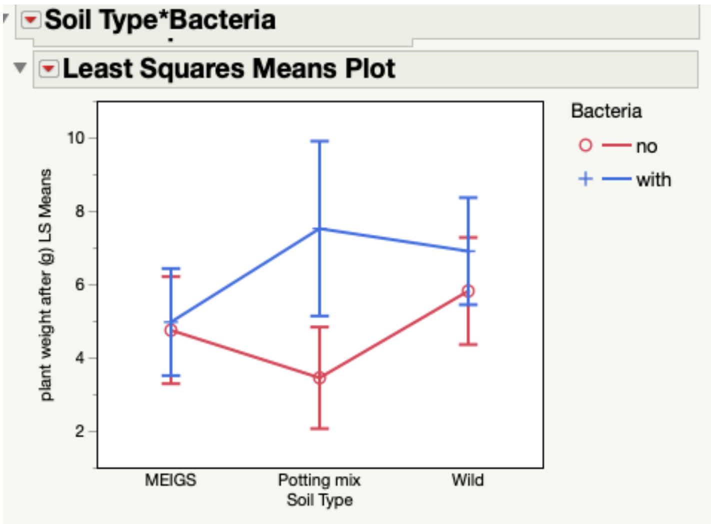


Figure 6: Least Square Mean is estimated from a linear model. It has been adjusted for unequal observations, in this case is different numbers of pots for each soil type.

Discussion

This experiment aimed to test how a beneficial soil microbial treatment affects the growth of tomato plants especially under the attack of CPB larvae. The results show that AMF fungi can help plants gain more weight, and especially in the potting mix soil type, the performance of AMF product is greatly different from the other soil type, MEIGS and wild soil. The orginal hypothesis was not supported, instead the weight of CPB did not show a lot of differences in all of three soil types or with the microbial treatment. One possible explanation for only seeing the significant effect of AMF treatment in the potting mix on plant size could be the result of the existing and stabilized microbiome community in the other two soil types preventing the AMF from being functional. There may need to be a larger concentration of the fungi solution to make it work in different soil conditions. But more experiments will need to be conducted to support my assumption.

Soil is the key for plant growth. Not only the nutrients it can provide to the plants, but also the possible attack of soil microbiomes can “teach” plants how to protect themselves by producing chemicals, or to make them become their little helper to defend other organisms. Studies have shown that all kinds of soil organisms include bacteria, fungi, root herbivores can affect plant performance to handle above ground herbivores by changing the concentration of primary and secondary metabolites (Kos, et al., 2015). Microbiomes are invisible, but they play a significant role in many ways. Soil microbiomes can either accelerate decomposition and provide more nutrients to the plants, or attach to the plants and help transform and fix more vital elements for plants (example like soybean and Rhizobium). Soil microbiomes are essential to plants.

More research is needed testing whether the damage caused by CPB can be controlled after applying beneficial microbial solutions. More variables other than plant weight should be collected over multiple time points. Since I cannot weigh a plant before putting on CPB, the before-and-after weight comparison can be less accurate due to different growth condition for plants. More soil types should be collected and tested as well. Colonies of microbiomes in the soil should also be collected to determine their function in plant health and defense against insect pests like CPB.

Acknowledgments

Many thanks to Dr. Laramy Enders for acting as my research mentor and providing me laboratory equipment. I appreciate the help and constructive advice from Laramy Enders, Elizabeth French, Thor, and Marian.

References

1. Temegne, Nono Carine, et al. "Effect of Phosphorus Fertilization on Arbuscular Mycorrhizal Fungi in the Bambara Groundnut Rhizosphere." *African Journal of Microbiology Research*, vol. 11, no. 37, July 2017, pp. 1399–1410., doi:10.5897/ajmr2017.8680.

2. Casteel, Clare L., and Allison K. Hansen. "Evaluating Insect-Microbiomes at the Plant-Insect Interface." *Journal of Chemical Ecology*, vol. 40, no. 7, 2014, pp. 836–847., doi:10.1007/s10886-014-0475-4.

3. Kos, Martine, et al. "Species-Specific Plant–Soil Feedback Effects on above-Ground Plant–Insect Interactions." *Besjournals*, John Wiley & Sons, Ltd, 9 Apr. 2015, besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.12402.

4. Mason, Charles J., et al. "Co-Option of Microbial Associates by Insects and Their Impact on Plant-Folivore Interactions." *Plant, Cell & Environment*, vol. 42, no. 3, 2018, pp. 1078–1086., doi:10.1111/pce.13430.

5. Pineda, Ana, et al. "Steering Soil Microbiomes to Suppress Aboveground Insect Pests." *Trends in Plant Science*, vol. 22, no. 9, 2017, pp. 770–778., doi:10.1016/j.tplants.2017.07.002.

6. Raaijmakers, J. M., and M. Mazzola. "Soil Immune Responses." *Science*, vol. 352, no. 6292, 2016, pp. 1392–1393., doi:10.1126/science.aaf3252.