Optimizing guided selection for microbiome-mediated protection from aphid herbivory

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Introduction and Research Questions

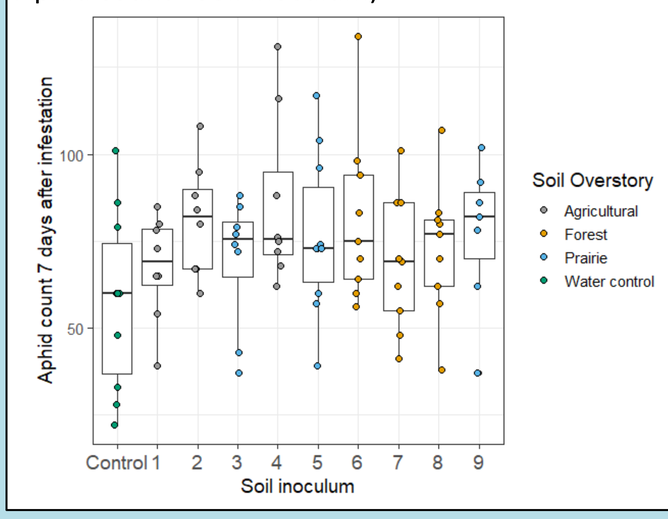
- Guided microbiome selection is an emerging method of modifying plant traits through iterative selection of whole soil microbial communities that could lead to the development of microbial consortia that improve or protect plant health[1-3].
- However, choice of initial soil microbial community, levels of exposure to stress and appropriate methods of microbial transfer needed for successful selection of beneficial microbiomes have yet to be determined (Figure 1 questions).
- The **goal** of this study was to optimize guided selection for microbiome-mediated defense against an insect pest.
- We chose to optimize guided selection using *Macrosiphum euphorbiae* (Potato aphid) on *Solanum pimpinellifolium*, the closest wild relative of tomato, as a model system to capture pre-domestication microbiome interactions with a specialist insect.

Figure 1 Guided microbiome selection conceptual methodology and questions for optimization

Question One: Starter Microbiomes

- We measured variation in aphid performance in wild tomato plants grown in sterile soil mix inoculated with soil slurries collected from multiple locations in central Indiana with different overstories. 2.5 week old plants were infested with 10 adult aphids. Aphid performance was assessed by counting the number of aphids present after seven days.
- **Because we saw no variation in aphid performance across soil types, we decided to mix multiple soils to maximize variation from which to select in the initial round of selection experiment.**

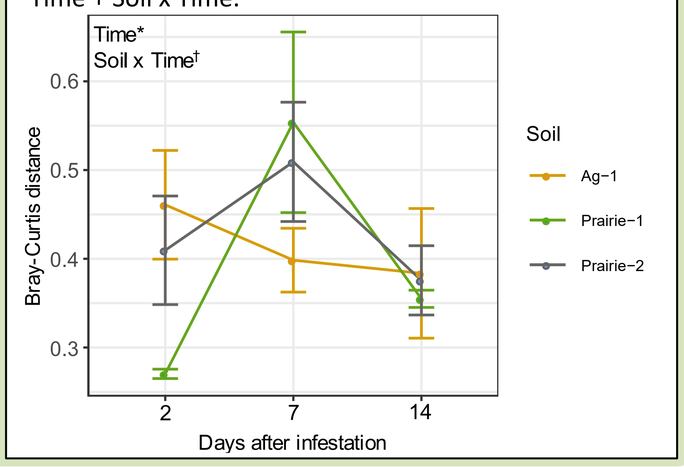
Figure 2 Aphid performance did not vary among diverse live soil slurry inoculums. (ANOVA Model: Aphid count ~Soil inoculum)



Question Two: Insect Pressure

- Using 16S rRNA sequencing, we identified differences in rhizosphere bacterial communities of control and Potato aphid-infested wild tomato plants across three soil sources (2 prairie, 1 agricultural), three time points (2, 7, 14 days after infestation), and two levels of pest exposure (2 or 10 initial adult aphids).
- **High initial aphid infestation resulted in significant changes to bacterial community composition in two soil types, peaking at seven days after infestation. We saw minimal impacts of low initial aphid infestation on the microbiome (not shown).**

Figure 3 Differences in rhizosphere community composition between aphid-infested and un-infested controls, measured by Bray-Curtis distance, peaked at seven days after infestation. Significant factors (*p <0.05, †p <0.1) for ANOVA model: Distance ~ Soil + Time + Soil x Time.



Question Three: Microbiome Transfer

- We tested two methods of microbiome transfer comparing transferring soil from lowest-aphid performing pots to randomly selected pots. Mixed soil inoculum was used for both methods.
- **Only Method Two resulted in successful selection for low aphid performance.**

Figure 4 Selecting for low aphid performance with method one resulted in no change to aphid performance phenotype after two generations of selection. (ANOVA model: Aphid count ~ Selection Line)

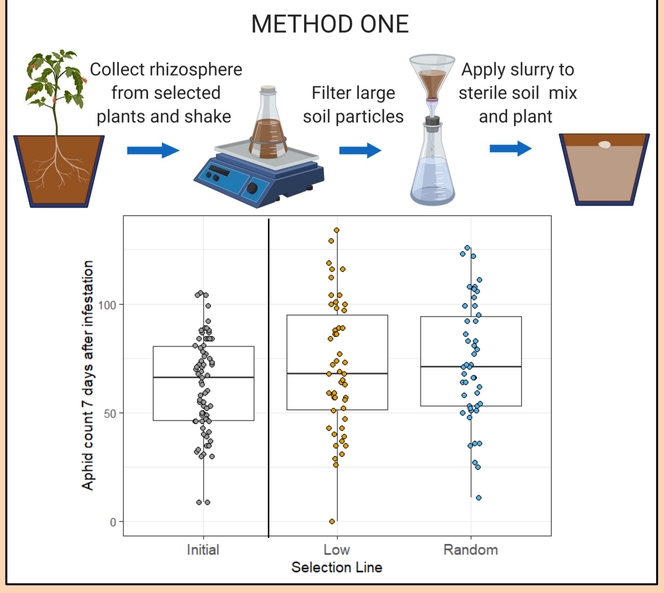
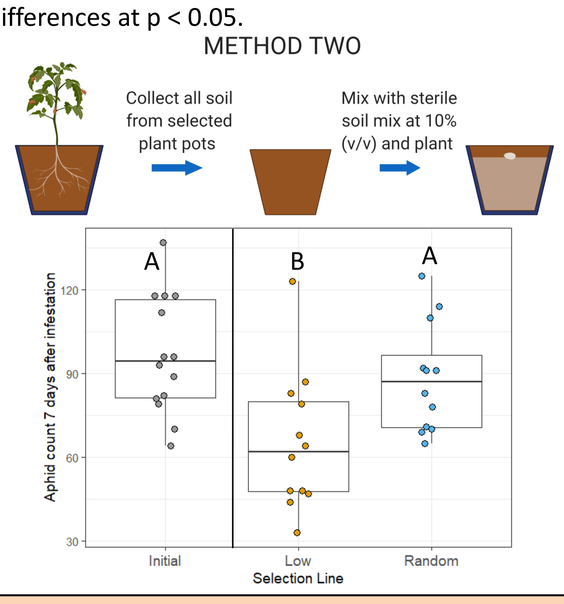


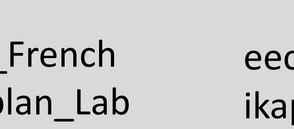
Figure 5 Selecting for low aphid performance with method two resulted in a significantly decreased aphid performance phenotype after one generation. (ANOVA model: Aphid count ~ Selection Line). Differing letters indicate significant differences at $p < 0.05$.



Conclusions and Future Directions

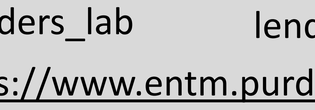
We successfully optimized guided microbiome selection in wild tomato for microbiome-mediated protection against Potato aphids.

Question 1 Soil microbiome variation for selection.



→ **Grow plants in randomly varying mixed soil microbiomes.**

Question 2 Insect pressure for maximum microbiome variation



→ **Exposure to ten initial adult aphids for seven days maximizes the impact of herbivory on the rhizosphere bacterial community.**

Question 3 Soil transfer to conserve selection phenotype

→ **Transfer whole soil from selected pots at a 10% rate (v/v) into sterile soil mix.**

Currently, we are running the optimized experiment with multiple independent lines and will use metagenomic sequencing and follow-up assays to identify selected microbial communities and the mechanism of plant protection.

References

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2. Mueller UG, Sachs JL (2015) *Trends Microbiol* 23:606–617. doi: 10.1016/j.tim.2015.07.009
3. Jochum MD, McWilliams KL, Pierson EA, Jo YK (2019) *PLoS One* 14:1–16. doi: 10.1371/journal.pone.0225933
4. Methods and conceptual figures made in BioRender.
5. Aphid picture taken by Joseph Berger, bugwood.org

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