Impact of *Leptoglossus phyllopus* Population Density on Free-Living Symbiont-*Caballeronia*

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Introduction

- There is increased interest in agricultural pests and controlling them to protect crop yield.
- Several important pest species are true bugs, such as *L. phyllopus*, and engage in promiscuous symbiotic association with the free-living soil bacteria- *Caballeronia* [1].
- *L. phyllopus* acquires the bacteria from its environment every generation in its early developmental stage, called the 2nd instar; this acquisition is essential for the insects' survival [1].
- *Caballeronia* colonizes the gut and grows to high abundance, then the insect digests excess cells, obtaining B vitamins and amino acids [2].
- Previous research has shown that **live *Caballeronia*** cells are transmitted to the environment through the feces of *Anasa tristis*, another pest in the same family [3].
- Our research sought to further describe the Bug-*Caballeronia* symbiotic association and the agricultural impacts thereof.

Figure 1: *Leptoglossus Zonatus*, a close relative of *L. phyllopus*, developmental stages (instars), from 1st (left) to adult (right)

Results

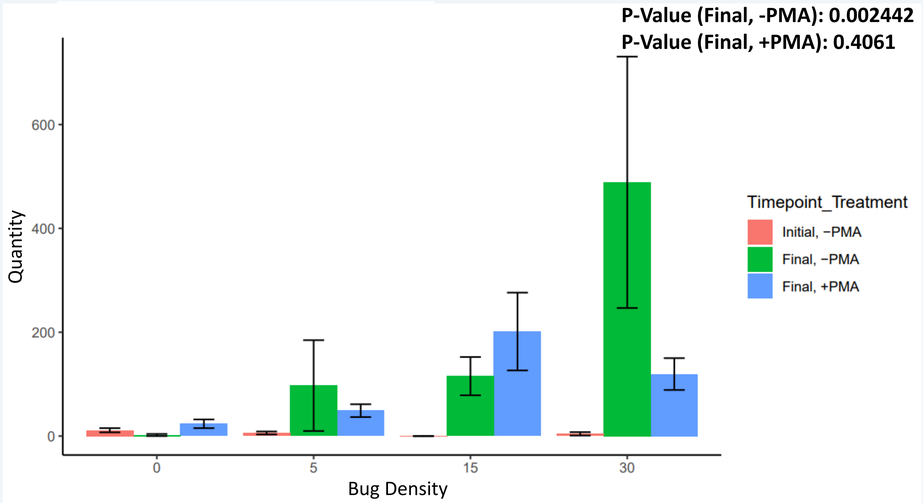

Figure 3: **Effect of Bug density on *Caballeronia***. Mean abundance of *Caballeronia* in soil samples. Comparison between initial soil samples, final samples treated with PMA, and final samples not treated with PMA.

Result Summary

- Our results suggest that the final abundance of *Caballeronia* **increases** with bug density.
- When the soil samples were treated with PMAxx to remove dead cells; our results suggest that final abundance of *Caballeronia* was not related to bug density.

Research Question: How does bug density affect symbiont transmission to soil?
Hypothesis: The soil microcosms exposed to higher densities of bugs would be colonized with more *Caballeronia*, the free-living symbiont.

Methods

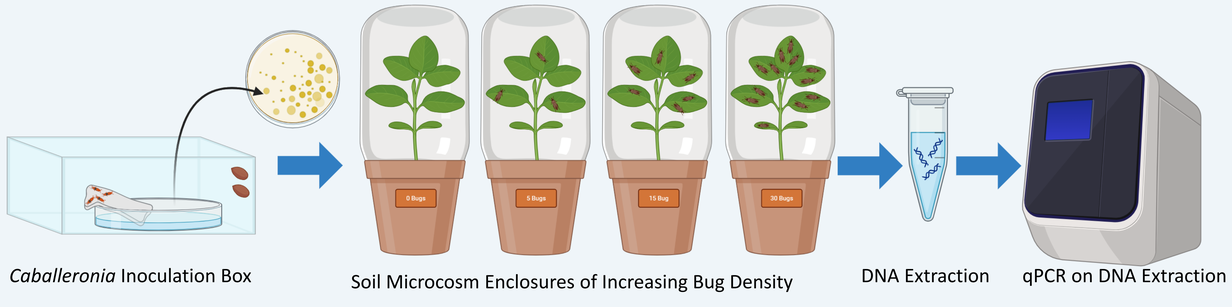

Figure 2**: Overview of Experimental Design**. 2nd Instar nymphs were inoculated with *Caballeronia* for 72 hours and transferred to sterile soil microcosm enclosures. Each enclosure had a cowpea plant and peanuts. We ran 5 replicates of 4 treatments: 0 bugs, 5 bugs, 15 bugs, and 30 bugs, for a total of 250 *L. phyllopus* reared. Bugs were reared to adulthood in enclosures and soil samples were collected from each enclosure. One set of soil sample was treated with PMAxx to remove dead cells. And another set of soil was not treated with PMAxx. Soil/ Fecal DNA extraction was performed on samples to extract *Caballeronia* DNA. The DNA was quantified with qPCR using specific primers that target the Lep1A1 strain of *Caballeronia.*

Discussion

- We cannot conclusively determine that the higher abundance of *Caballeronia* at higher density bug exposure was due to live cells being excreted.
- This may be because PMA was not made specifically for use in soil samples and may not have been as accurate.
- If *L. phyllopus* migrates to a new area and transmits the symbiont to the soil, this could cause a pest boom because the next generation of insects could more easily acquire the symbiont.
- Understanding the bug-*Caballeronia* symbiosis can inform the development of more effective pest control strategies that account for *Caballeronia's* role in the proliferation of *L. phyllopus*.

Future Work

- Future projects could include doing DNA extractions on leaf tissues of plants in the microcosm to evaluate how the symbiont is transmitted to other portions of the bug's environment.

References

1. Hunter MS, Umanzor EF, Kelly SE, Whitaker SM, Ravenscraft A. 2022. Development of Common Leaf-Footed Bug Pests Depends on the Presence and Identity of Their Environmentally Acquired Symbionts. Appl Environ Microbiol 88:e01778 21.https://doi.org/10.1128/aem.01778-21
2. Tsubasa Ohbayashi, Ryo Futahashi, Mia Terashima, Quentin Barrière, Florian Lamouche, Kazutaka Takeshita, Xian-Ying Meng, Yasuo Mitani, Teruo Sone, Shuji Shigenobu, Takema Fukatsu, Peter Mergaert, Yoshitomo Kikuchi, Comparative cytology, physiology and transcriptomics of Burkholderia insecticola in symbiosis with the bean bug Riptortus pedestris and in culture, The ISME Journal, Volume 13, Issue 6, June 2019, Pages 1469–1483, https://doi.org/10.1038/s41396-019-0361-8
3. Scott M. Villa, Jason Z. Chen, Zeeyong Kwong, Alice Acosta, Nicole M. Vega, Nicole M. Gerardo, Specialized acquisition behaviors maintain reliable environmental transmission in an insect-microbial mutualism, Current Biology, Volume 33, Issue 13, 2023, Pages 2830-2838.e4, ISSN 0960-9822,https://doi.org/10.1016/j.cub.2023.05.062.
Figure 2 Created in BioRender.

Acknowledgments