Biological control of Huanglongbing by the bacterium *Paraburkholderia phytofirmans* PsJN

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Year 2 of 3 (33% Complete)

Objectives

1) Optimize the delivery method for PsJN into citrus trees
2) Demonstrate the efficacy of PsJN to reduce CLas titers and slow down symptom formation on CLas-grafted citrus trees
3) Test the timing of PsJN delivery in relation to a CLas infection event and assess the impact of PsJN on Asian Citrus Psyllid (ACP)-mediated CLas infection
4) Quantify the effect of tree inoculation with PsJN on acquisition and transmission of CLas by ACPs

Problem and Significance

Novel solutions are needed to protect California citrus growers from the threat imposed by the ongoing huanglongbing (HLB) epidemic. In this project, which addresses Citrus Research Board (CRB's) high-priority area 3 (develop tools to prevent '*Candidatus* Liberibacter asiaticus' (CLas) transmission or suppress HLB disease), we probed the potential of the bacterium *Paraburkholderia phytofirmans* PsJN (PsJN) as a biological control agent of CLas. This research was motivated by reports that PsJN is able to suppress Pierce's disease of grapevines, caused by the bacterium *Xylella fastidiosa.*

Benefit to Industry

A successful demonstration of PsJN-based tolerance to CLas infection would offer a path towards a temporary and practical solution for the current lack of resistance to HLB in citrus trees. Furthermore, a demonstration that inoculation of trees with PsJN might curtail the ACP-dependent spread of CLas between trees has potential to benefit the California citrus industry by slowing down or preventing the introduction of CLas from residential into commercial areas.

Progress Summary

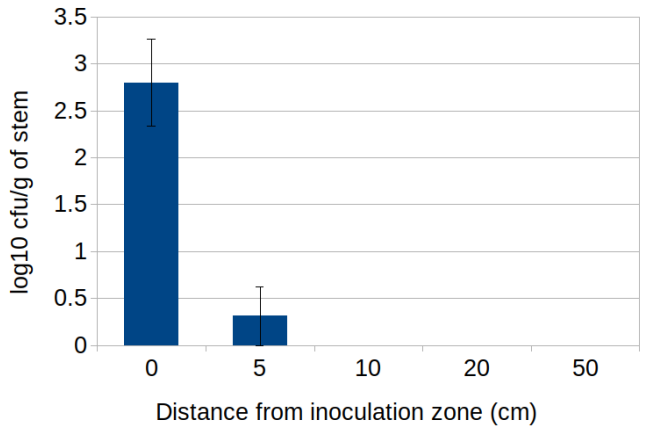
Work in the first year of the project (2019-2020) was delayed due to the Covid-19-related shutdown of the Leveau lab and UC Davis campus. For the second year of the project (2020-2021), CRB approved a budget to finalize our work on Objective 1. We report here on the completion of that work, which assessed the petiole puncture and derma-roller inoculation methods for delivering PsJN into citrus treelings in order to see if and how PsJN would survive and/or move in citrus treelings.

The petiole puncture method involved placing a 5-µL suspension of PsJN (10^8 cells per mL) on a single petiole and subsequently puncturing the petiole with a needle to allow infiltration of the PsJN suspension. At 20 days post inoculation, we sampled stem sections at 0, 5, 10, 20, and 50 cm from the point of inoculation. We performed three replicated trials for this inoculation method; each trial involved 8 Meyer Lemon treelings with 1 petiole on each tree. In the first trial, we quantified population sizes of PsJN by plating stem tissue (**Figure 1A**). For the second and third trials, we determined the presence/absence of PsJN in stem sections in selective enrichment cultures (**Figure 1B**). We found that PsJN persisted around the point of inoculation for at least 20 days after inoculation but did not disperse far (5 cm at most) from the initial inoculation point.

We also assessed the derma-roller method of inoculation. In this approach we used a derma-roller device to puncture small holes in the trunk of trees, after which a paper tissue soaked in a bacterial suspension of PsJN (10^8 cells per mL) was placed over these holes and sealed on the trunk with Parafilm. PsJN was able to survive at least 20 days around the inoculation zone but did not disperse far (5 cm at most) beyond this zone (**Figure 2A and**

2B). The derma-roller method seemed to do a slightly better job than the petiole puncture in terms of achieving 5-cm movement in a larger fraction of inoculated stems/petioles. In order to test whether PsJN needs more time to move systemically, we left two inoculated trees for 10 weeks and were able to recover PsJN from the zone of inoculation, but not 5, 10, 20, or 50 cm away from the point of inoculation (results not shown).

A.



B.

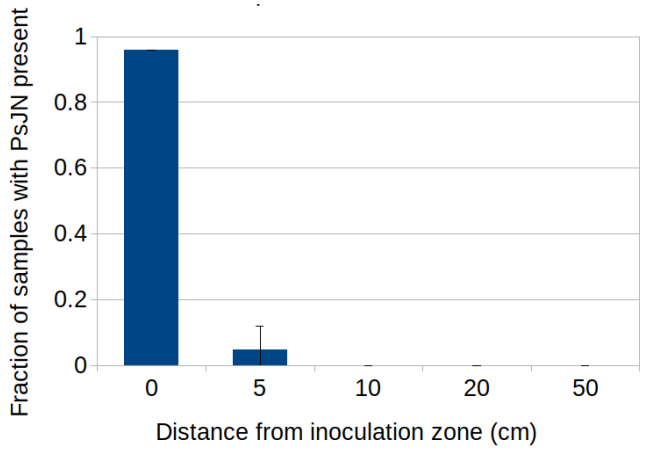
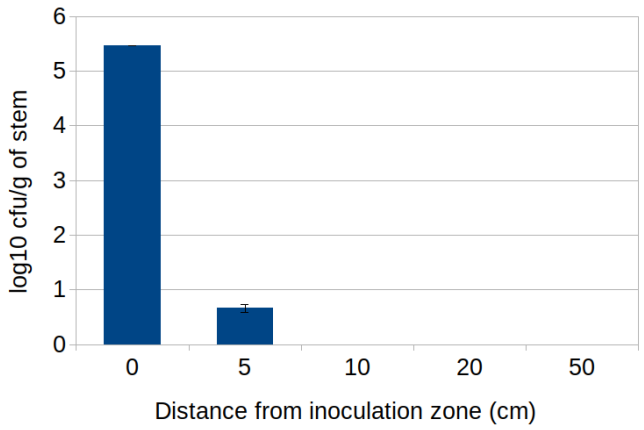


Figure 1. *Petiole puncture inoculation trial results* ***A.*** *Trial 1 - endophytic population sizes of Paraburkholderia phytofirmans PsJN. Values of 0 were adjusted to 1 prior to log transformation. The error bars represent the standard error.* ***B.*** *Presence/absence of PsJN at various distances from the inoculation zone in trials 1, 2 and 3 of the petiole puncture method. Error bar represents the standard error. In each trial, data were obtained from 8 PsJN inoculated plants sampled 20 days post inoculation at increasing distances (0, 5, 10, 20, or 50 cm) from the zone of inoculation. Shown is the fraction of stem samples in which PsJN could be detected.*

A.



B.

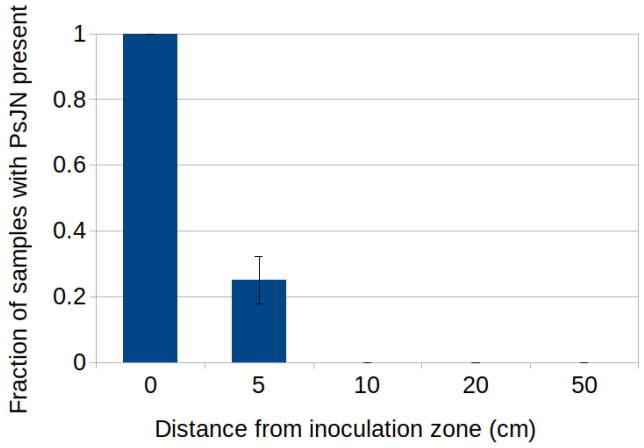


Figure 2*. Derma-roller inoculation trial results* ***A.*** *Trial 1 - endophytic population sizes of Paraburkholderia phytofirmans PsJN. Values of 0 were adjusted to 1 prior to log transformation. The error bars represent the standard error.* ***B.*** *Presence/absence of PsJN at various distances from the inoculation zone in trials 1, 2, and 3 of the derma-roller method. Error bar represents the standard error. In each trial, data were obtained from 8 PsJN inoculated plants sampled 20 days post inoculation at increasing distances (0, 5, 10, 20, or 50 cm) from the zone of inoculation. Shown is the fraction of stem samples in which PsJN could be detected.*

In our first year of work, using the leaf infiltration method, we observed that PsJN-inoculated leaves, but not buffer-inoculated leaves,

turned yellow at the point of infiltration about 20 days post inoculation. Yellowing coincided with the onset of PsJN population size decline. Our observation suggests that the plant recognizes PsJN and responds to its presence. Therefore we were interested to find out what this response is, whether it moves, unlike the bacterium itself, to other parts of the plant, and whether it offers protection from CLas or ACP.

To start addressing these questions, we infiltrated 4 leaves each on 6 Meyer Lemon trees with a PsJN cell suspension and another 4 leaves each on 6 Meyer Lemon trees with phosphate buffer only. After 20 days, we sampled these 48 leaves along with a nearby, uninfiltrated leaf, either directly above or below each one of the infiltrated leaves on the same stem. All 96 leaves were stored at -80°C and are awaiting analysis by comparative metabolomics in collaboration with the lab of Cristina Davis at UC Davis. We expect to see differences in the metabolite profile of leaves that were or were not inoculated with PsJN. Also, any differences in metabolite profile between distal leaves from inoculated or uninoculated treelings would suggest that the PsJN-induced response moves into other parts of the tree canopy. We will share the results of the metabolomics analysis when they become available.

Conclusions

We have demonstrated that petiole puncture and derma-roller inoculation are successful in delivering the potential biocontrol bacterium *Paraburkholderia phytofirmans* PsJN into citrus trees. With all three methods, we observed that PsJN persists for at least 20 days in the tree tissue, and in some cases was detected even 10 weeks after inoculation. However, we did not see movement of PsJN to other parts of the tree. This result is consistent with the findings of a collaborator of ours, Dr. Ozgur Batuman at the Southwest Florida Research & Education Center, who did similar inoculation PsJN experiments (with a derma-roller type instrument) and also concluded that PsJN does not disperse far from the point of inoculation in citrus trees.

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