Expanding suppressive microbial communities to manage bacterial spot of tomato

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Project Summary:
This project was initiated in the summer of 2021 with two objectives: 1) Acquire 6-12 microbial communities from Pennsylvania and 2) repeatedly transfer communities from objective 1 to select for those that suppress bacterial spot in a greenhouse setting.

We were successful in recovering 6 microbial communities each from both high tunnel and field grown tomatoes. Additionally, using a passaging approach, which we've previously employed for bacterial speck, we saw similar dynamics in that over successive passages, disease severity decreased. Additionally, we were able to show that disrupting the microbial community resulted in increased disease.

The results of this project show that the passaging approach are generally effective against different foliar bacterial diseases of tomato. This approach may also be effective against other foliar diseases, as well.

Results:
Objective 1 | *Acquire 6 – 12 distinct microbial communities from different tomato sources in Pennsylvania and New York*

We initially wanted to acquire microbial communities from both Pennsylvania and New York, however it was only feasible to acquire communities from Pennsylvania. We were able to acquire 6 communities from field grown tomatoes and 6 communities from high tunnel grown tomatoes.

Objective 2 | *Repeatedly transfer communities from objective 1 to select for those that suppress bacterial spot in a greenhouse setting*

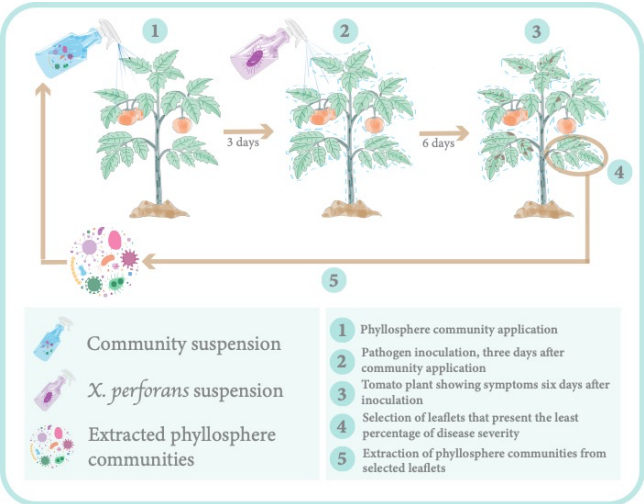

Figure 1. Passaging approach to select for disease suppressive communities.

Our approach of community passaging (Figure 1) showed similar results to our previous work with bacterial speck of tomato, though not exactly the same. In the case of bacterial speck, disease increased considerably over the first ~4 cycles of passaging, before declining and remaining low over the remaining passages. For bacterial spot, this was different in that the disease levels never increased significantly

over the early passages. Beginning with passage 6 , however, the disease for the passaged treatments did decline compared to the pathogen only treatment (Figure 2). This was in addition to and overall decline in disease for the pathogen only treatment as well. Although we were

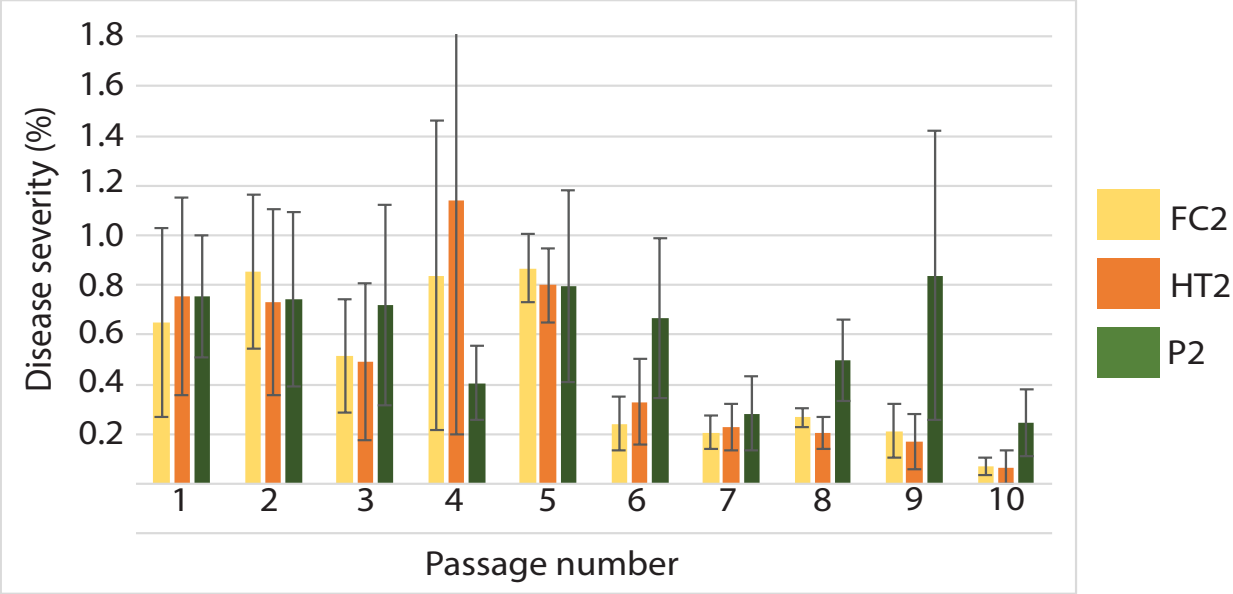

Figure 2. Average disease severity of bacterial spot on tomato leaves at each passage. From passage 6 onward, the average disease for the pathogen only (P2, green bars) control is higher than either the field tomato community (FC2, yellow bars) or high tunnel tomato community (HT2, orange bars).

happy to see these trends, because they indicate that the passaging does result in reduced disease, the overall level of disease was very low. We think this is because we did not have the right conditions that were conducive for bacterial spot (maybe not warm enough in the greenhouse) and our passaging cycle, which was developed for bacterial speck, was maybe too short for bacterial spot, which takes a little longer to develop.

In addition to having a lower disease severity on leaves that showed some disease symptoms, we also observed that at later passages, the number of leaflets that showed any symptoms decreased for the passaged treatments compared to the pathogen only control, as well (Figure 3). In total, there was an average reduction in disease severity by ~60% from passage 6 on for the community-treated compared to the pathogen only control. Additionally, there was an average reduction of ~20% for the total number of leaves affected for passage 6 on for the community-treated compared to the pathogen only control.

To confirm that the disease suppression was the result of the microbial community, we heat killed the community and tested whether the disease suppression was reduced. Figure 4 shows that heat killing the communities results in disease severity that is greater than the untreated communities.

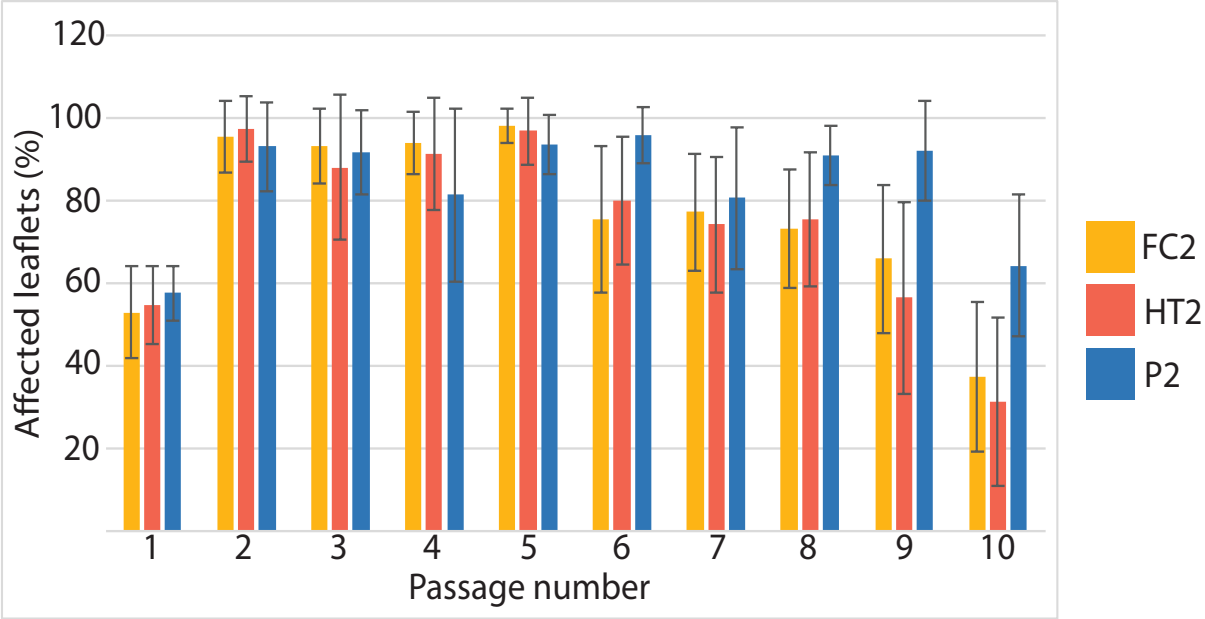

Figure 3. Average number of tomato leaflets showing disease symptoms. From passage 6 onward, the average number of affected leaflets for the pathogen only (P2, blue bars) control is higher than either the field tomato community (FC2, yellow bars) or high tunnel tomato community (HT2, orange bars).

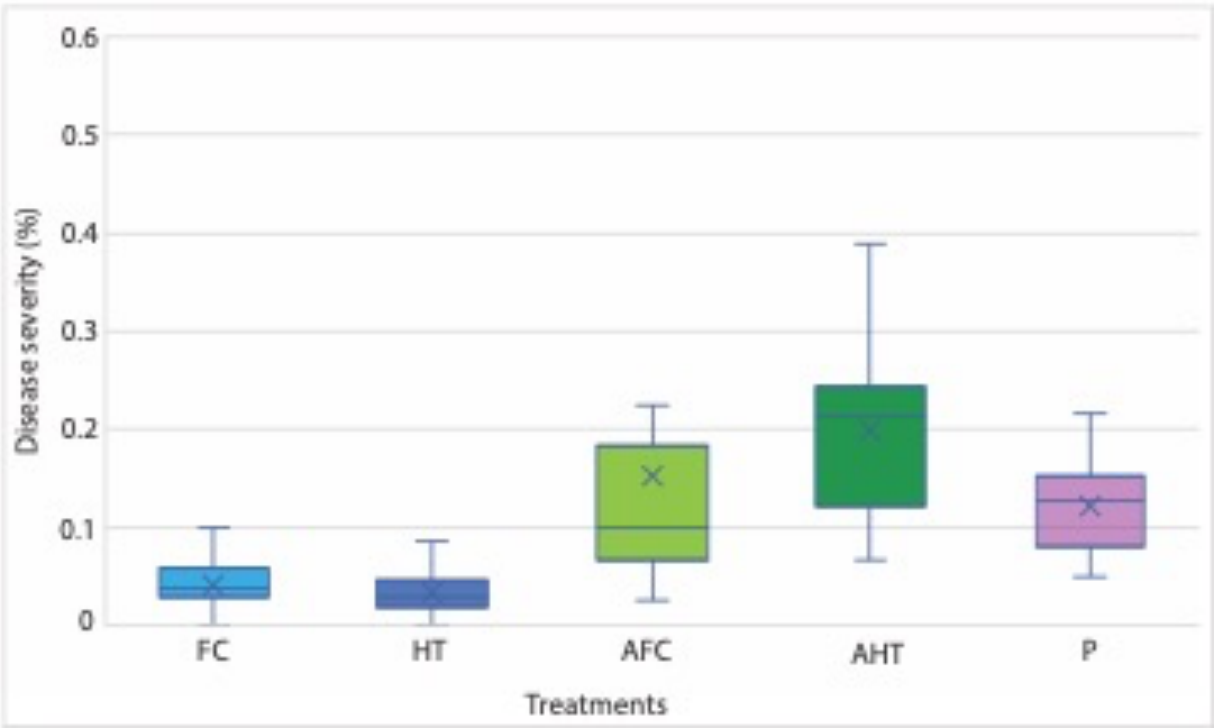

Figure 4. Average disease severity for tomato leaves treated with either a passaged field tomato community (FC), a passaged high tunnel community (HT), an autoclaved (heat killed) field community (AFC), an autoclaved high tunnel community (AHT), or the pathogen only. Both the field community and high tunnel community treatments resulted in lower disease, than the other three treatments.

Conclusions

Our results show that our passaging approach results in microbial communities that are able to suppress both bacterial speck (previous research) and bacterial spot (this research) of tomato.

A limitation of our findings is the low overall level of disease that we achieved throughout the project. As noted above there are likely several reasons for this (greenhouse conditions weren't conducive to disease and not giving the plants long enough to develop disease), which will be addressed in future research.

Now that we've shown as proof-of-concept that the passaging approach can successfully result in disease suppressive communities, we plan to assess whether they may be effective in a tomato transplant setting, where the plants are more dense and the plants are top watered. We believe both conditions will help promote disease development and will make it easier to assess the potential value of our approach to stakeholders.

We submitted a proposal to follow up on this research to the USDA NIFA in 2021. Although the proposal was rated as *high priority*, it was ultimately unfunded. These results will be included a revised USDA NIFA proposal that will be submitted in October 2022.