Webinar Q&A

Biotic and abiotic stress distinctly drive the phyllosphere microbial community structure

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The webinar recording is available on the Phytobiomes Alliance YouTube channel at https://youtu.be/8uChM8j7HHs?si=4_KyToo_ED3_99OG

Q: Do Capsaicin levels in cultivars have an impact on the microbial community structure and interactions? What's the current understanding there, if any?

We did not look at levels of capsaicin on microbial communities in our work.

Answered live. Timestamp: 1:00:09

Q: How were the leaves inoculated? How did you decide on that inoculation method? Is there any evidence for the mode of action for pathogen invasion based on your inoculation method?

The leaves were inoculated by using dip inoculation method as shown in slide 10. As our model pathogen Xanthomonas is a foliar epiphyte, it first establishes itself in the phyllosphere followed by apoplastic colonization for pathogenesis.

Q: Based on your research, how do we prepare for climate change? What the focus of our research should be?

Answered live. Timestamp: 52:15

Q: Davis and Aususbel showed in the 1990s that Ozone induces salicylic acid biosynthesis and thus increased defense against biotrophic pathogens. How did you consider this data in your system?

Answered live. Timestamp: 58:06

Q: What experiment can one perform to test the requirement for your hub species in driving any of the measured phenotypes?

This is a great question. Network analysis is used to statistically identify the keystone or hub taxa in the network based on compositional data, it is important to link such taxa to ecosystem processes. These taxa can be isolated from the environment using culture-based methods followed by co-culturing multiple taxa to mimic the structure and function of a microbiome under particular stress. Moreover, we can also use the genomic information and gene expression profiles from these selected microbes with beneficial functions of design the syntheric communities.

Q: Are there examples of either public or private sector groups that have successfully developed and deployed any biocontrol products that contain more than one strain?

We did not remove anything from the soil, just brought it to the lab and used it as is.

Q: You noted that under ozone, the % of diseased plants (or leaves?) goes up but the bacterial titer remains the same. How did you differentiate from disease and ozone damage?

As the disease severity was not associated with Xanthomonas population, we hypothesize it might be due to various factors such as altered plant immune response, altered in pathogen response with secretion of different effectors, and loss in microbiota mediated protection etc. As the ozone damage symptoms are different compared to bacterial leaf spot and we also had the plants under elevated ozone but not inoculated with pathogen, it helped us to differentiate the symptoms between the ozone damage and pathogen.

Q: For your microbial communities, roughly how much was bacterial vs. fungal vs. nematode, etc.? Did any of the individual taxa respond differently to treatments?

Answered live. Timestamp: 55:19

Q: For shotgun metagenomics, how did you mitigate host plant contamination?

Answered live. Timestamp: 45:36

Q: Was there any difference in the yield in response to single and combined stress at the end of season?

We did not look at the yield on this particular experiment but that will be something interesting to look at in the future.

Q: Setting aside all of the data from ozone treatments and the end of season data, did you look at the mid-season data under ambient conditions to see if any specific taxa were enriched, or present only, in the resistant cultivar compared to the sensitive cultivar?

Answered live. Timestamp: 48:11

Q: Slide 26 and 27, was this also analysed for resistant and susceptible cultivars separately?

The network analysis was not performed separately for the cultivars because of the limited sample size. They were performed by combining both the cultivars under similar stress.

Q: How can we analyse MENA for 3 samples per treatment?

As one of the issues in microbial studies is small sample size, molecular network ecological analysis is also greatly affected. Small sample size can result in lower power for network analysis and sometimes it will be hard to find the association between the replicate sample resulting in no network. The bigger the sample size, the more robust the analysis will be.

Q: Did you use MAGS or contigs for taxonomic classification? If you used MAGS, what was the level of contamination and MAGS quality was used?

We used metagenomic reads for our classification. While using Kraken2 and bracken for taxonomic classification, we can directly perform the taxonomic placement in the quality trimmed reads. MAGs can be a good way of classification too but with MAG, we will miss the low abundance microbial communities whose MAG cannot be assembled.