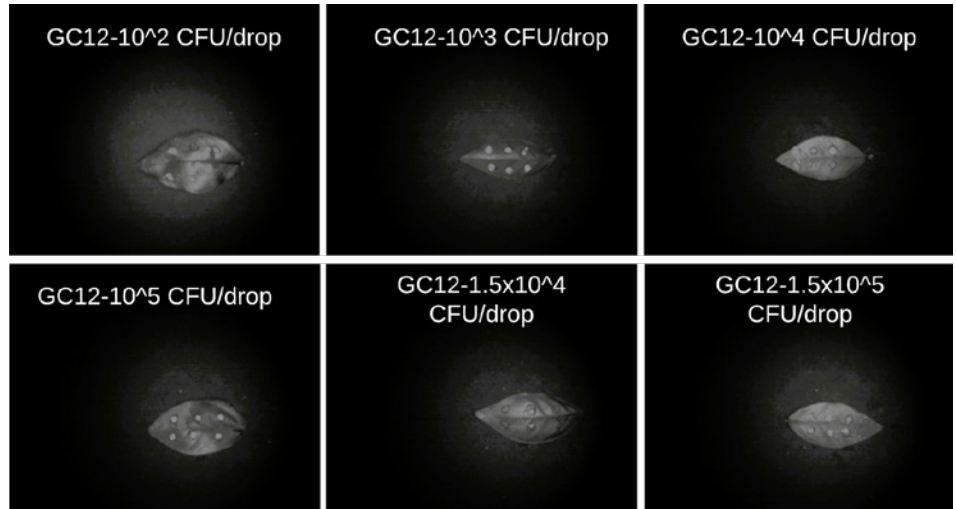


Finding *Phyllosticta citricarpa* when Citrus Black Spot Cannot be Seen

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Effort Statement: More confirmatory experiments to ensure the data gathered was correct. More data analysis.

Summary: Citrus black spot (CBS) is a quarantinable fungal disease caused by *Phyllosticta citricarpa*. It was first discovered in Florida in 2010 in a grove near Immokalee. The disease has spread from the initial location through the southwestern citrus production region and Polk County. New locations were identified in Polk County in 2023 and Manatee County in 2024. One of the challenges with CBS is detecting low levels of infection or early infection. The disease is mostly asymptomatic in leaves and fruit mostly become symptomatic once ripening, long after the infection

event. Since CBS is a regulated disease, the inadvertent movement of the fungus on plant material is concerning. We are investigating hyperspectral imaging tools to see if the fungus can be detected on and within immature fully expanded leaves. There are two challenges, first, a very common second *Phyllosticta spp.* fungus that harmlessly infects leaves naturally in Florida may confuse the results of any detection not based on PCR and secondly, once the *P. citricarpa* infects the leaf and becomes embedded as a tiny fungal ball, it is not known when the fungus grows within the leaf extensively. We conducted two experiments where multiple concentrations of spores of both the pathogen and the harmless colonizer were visualized on the leaf

surfaces. We have also collected leaves from a grove with a known CBS problem and conducted qPCR to identify which leaves are infected. Imaging was done with the leaves to match the PCR data. We were able to detect the fungi when deposited as spores on the leaf surfaces but natural infections were much more challenging. Further analysis is underway to confirm these results.

Take Home Message:

- *Phyllosticta* species can be detected with remote imaging but so far only on leaf surfaces.
- *Phyllosticta* remains difficult to detect when in the asymptomatic phase.
- Citrus black spot continues to move within the state of Florida.

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