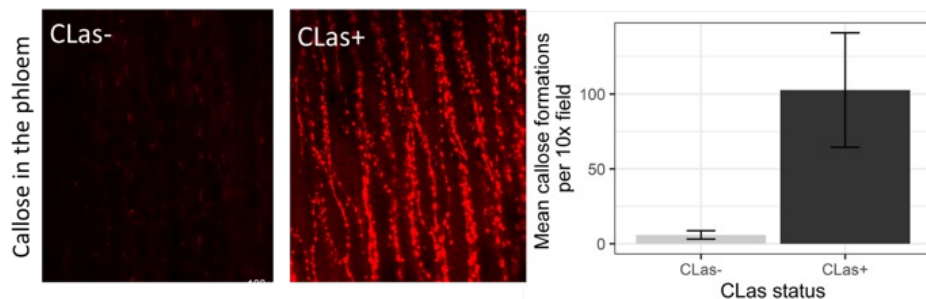


Unraveling *Candidatus Liberibacter Asiaticus* and Citrus Tristeza Virus-Phloem Interactions

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Accumulation of callose (red) in the phloem of HLB infected citrus infected with CLAs.

Our goal is to identify targets for gene editing to generate citrus tolerance to huanglongbing (HLB). HLB progression is tightly associated with phloem plugging in the stem, leading to inhibition of sugar and nutrient transport into sink tissues, including the fruit. Eliminating these plugs will presumably result in renewed sugar transport and increased yields. A second phloem-limited pathogen is citrus tristeza virus, the causal agent of the decline and stem-pitting diseases. Citrus tristeza virus (CTV), the causal agent of the tristeza disease, is the major current threat. Both HLB and tristeza caused the death

of millions of trees. However, there is a significant gap in our understanding of *Candidatus Liberibacter asiaticus* (CLAs) and CTV-phloem interactions in citrus, which has been a major limiting factor for controlling the diseases. This gap results from the phloem being a narrow tissue buried inside the stem, and the low and inconsistent titers of CLAs in tissues studied so far. To address these challenges, we established a novel seed coat-based system that supports in-depth analyses of phloem dynamics and CLAs and CTV-phloem interactions in HLB-affected citrus. This system is based on extracting vasculature

from HLB trees seed coat with 100% of the tissues containing extraordinarily high titers of CLAs. We are employing this system to identify key players required for phloem plugging, host immune response and CLAs and CTV colonization inside the phloem. These key players will become novel targets for manipulation with gene editing techniques that can be translated into usable products, such as transgene-free CRISPR/Cas9 edited plants to block CLAs propagation and movement or increase sugar and nutrient translocation into fruit thus increasing tolerance or resistance to HLB.

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