



### Seasonal variability of Ct Values in CLAs-positive samples collected in Southern California: Update

Holly Deniston-Sheets<sup>1</sup>  
Lukasz Stelinski<sup>2</sup>  
Greg McCollum<sup>3</sup>  
Neil McRoberts<sup>4</sup>

<sup>1</sup> Citrus Research Board

<sup>2</sup> Department of Entomology and Nematology, University of Florida

<sup>3</sup> US Horticultural Research Laboratory, Agricultural Research Service, USDA

<sup>4</sup> Quantitative Biology & Epidemiology Lab, Plant Pathology Department, UC Davis

#### SUMMARY

In the summer of 2019, DATOC published a report highlighting an absence of significant seasonal differences in the Ct values of HLB+ plant samples in California. In the most current dataset available at that time, 1,047 HLB+ trees had been detected. Now, a year later, an additional 843 diseased trees have been added to the dataset and we have completed a similar analysis to update the results. As in 2019, there remains no significant effect of season on bacterial titers, although they are otherwise increasing over time.

#### BACKGROUND

In other areas of the world, researchers have suggested optimal times to sample for *Candidatus Liberibacter asiaticus* (CLAs) detection based on observed seasonal variations of CLAs titers in citrus trees. We investigated whether this was the case in California, to help guide the regulatory sampling plan.

#### EVIDENCE

We examined the effects of year, season, and city on titers using ANOVA. Variety was not included, as differences were not clearly defined in the dataset. We found that year and city were significant, but season was not ( $p > 0.4$ ). We also examined the effects of sample size to determine if fewer samples earlier in the epidemic could be confounding results, as the number of samples tested yearly has tripled since earlier years. However, we found no significant effect of sample size using stepwise model selection.

**Bacterial load of HLB+ samples over time**  
from 2016

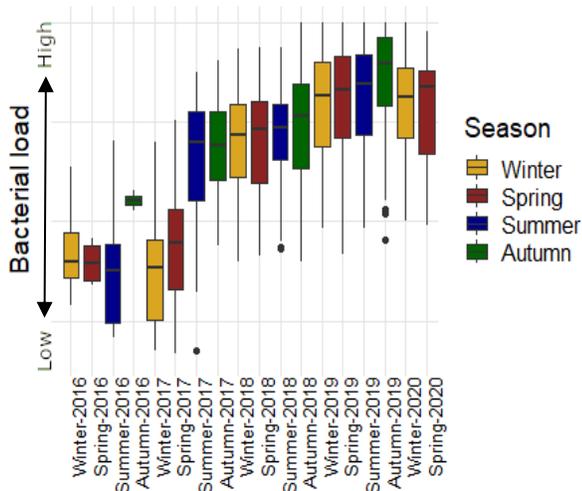


Figure 1. Log copy number distribution of CLAs+ plant samples indicating the median (horizontal line), first and third quartiles (lower and upper hinges) and largest and smallest value less than 1.5 \* the interquartile range. Points represent outliers. For samples tested with 16S primers, calculated as  $11.5 - (0.33 * Ct)$ . For samples tested with RNR primers,  $11.5 - (0.27 * Ct)$ .

**Bacterial load of HLB+ samples**  
by season

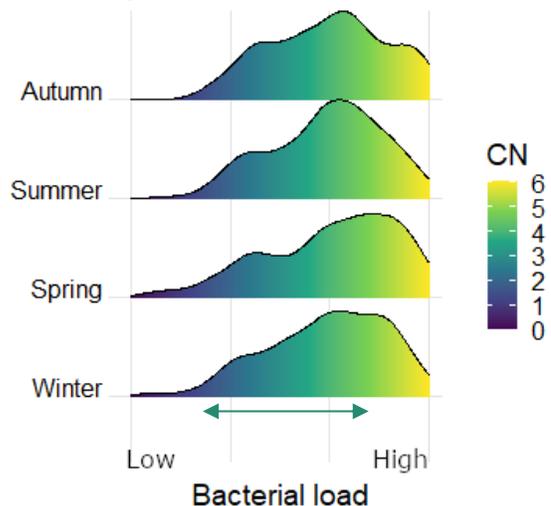


Figure 2. Log copy number density by the season in which the sample was collected.

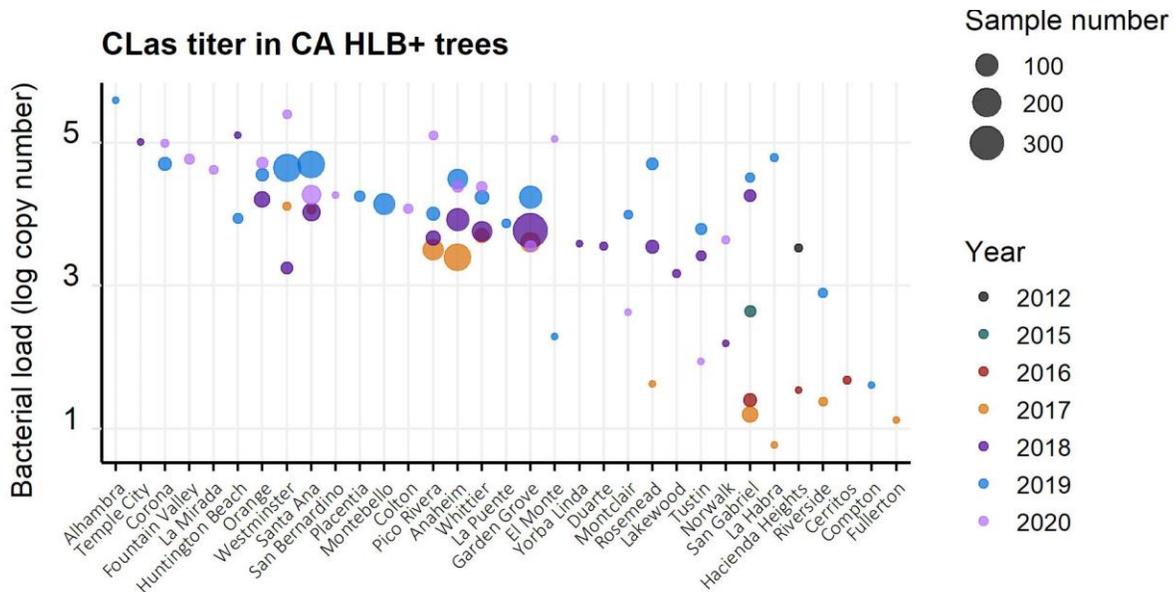


Figure 3. Mean log copy number of HLB+ plant samples in CA cities each year. The size of the point indicates the number of samples taken per year in that city. Cities are ordered by mean log copy number (high to low).

## CONCLUSIONS & CONSIDERATIONS

In summary, CLas-positive plant samples collected from residential areas in southern California show no significant seasonal differences in bacterial titers. This diversion from patterns observed elsewhere could be due to differential growth patterns under California climatic conditions and/or variable residential watering regimes, which could in turn affect bacteria movement within the phloem.

This conclusion should be revisited in the future if significant numbers of CLas-positive plant samples are collected from commercial groves in California, as the differences in the growing environment between residential areas and commercial groves could affect the results of the analysis.