Seasonal variability of Ct Values in *C*Las-positive samples collected in Southern California

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Introduction

The removal of infected citrus trees is considered an integral element of HLB management, particularly during the early phases of a disease epidemic. In Southern California, the quantitative polymerase chain reaction (qPCR) test is used to detect infected trees. This test is reliant on cycle threshold (Ct) values, which refers to the number of PCR amplification cycles that are completed before *C*Las is detected. If more than 37 cycles are completed without detection of *C*Las occurring, the sample is considered negative for *C*Las. Generally, if fewer cycles are needed before *C*Las can be detected, the concentration of bacteria in the sample is higher than if more cycles are needed.

Various research has shown seasonal changes in the concentration of bacteria (titer) in *C*Las-positive plant samples, as well as the proportion of samples that test positive. In Mexico and Brazil, for example, research has observed higher titers in samples that were collected during periods of milder weather, relative to samples collected under higher environmental temperatures. Climate-controlled experiments in Brazil observed similar results (Lopes, 2013; Lopes et al., 2017; Sauer et al., 2015). In Florida, the greatest percentage of samples submitted for analysis tested positive from midsummer – midwinter (Irey et al., 2011); bacterial titer was observed to be lowest during hotter periods of the year (Gottwald et al., 2012). In Pakistan, where annual weather patterns are more similar to citrus-growing areas of California, researchers reported more *C*Las-positive plant samples were collected in summer than in spring (Razi et al., 2013). This was in contrast to psyllid populations, which were observed to peak twice, in spring and in fall, although *C*Las-positive psyllids were mostly detected in spring (Razi et al., 2013).

Such seasonal differences may impact when, or how often, sampling for *C*Las should occur. Currently, the California Department of Food and Agriculture "Action Plan for Asian Citrus Psyllid and Huanglongbing (Citrus Greening) in California" instructs that the perimeters of a commercial grove should be sampled by qPCR biennially after the detection of *C*Las within 400 m of that grove, under the assumption that *C*Las titers would vary seasonally, as has been observed in other growing regions. However, little research has been done on the bacterial titers of *C*Las-positive plant samples in California. Differences between the growing environments of California citrus, compared to the commercial growing environments of other regions, may affect the seasonality of Ct value variability. The purpose of this analysis was to determine if similar seasonal patterns of Ct values were present in *C*Las-positive plant samples collected in California, and if biennial sampling was therefore justified.

Plant samples and analysis

From March 2012 through December 2018, 1,047 plant samples tested positive for *C*Las out of over 275,000 samples, based on PCR testing. Statistical analysis of Ct values was conducted in R (R Studio Team, 2018). An ANOVA of the Ct values of all positive samples and the season the positive sample was collected (spring, summer, winter, or autumn) showed no significant seasonal differences in the Ct values for plant samples collected from the residential areas of California ($p = 0.27$, $F = 1.3$) (Figure 1). Ct Values were slightly higher in the spring then in the rest of the year, indicating a lower

bacterial titer, but the difference was not significant (Figure 2). Overall, the greatest proportion of HLB+ plant samples have been collected in the summer (Table 1).
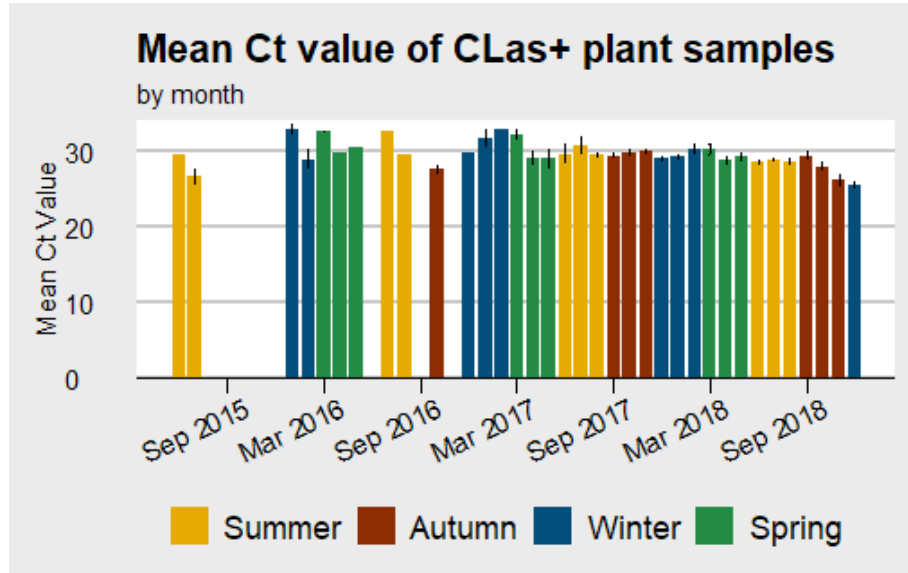

Figure 1. Mean Ct Value each month for CLas-positive plant samples collected in California residential areas, colored by the season the samples were collected. Data before the summer of 2015 is excluded for ease of visualization. Bars indicate SE.
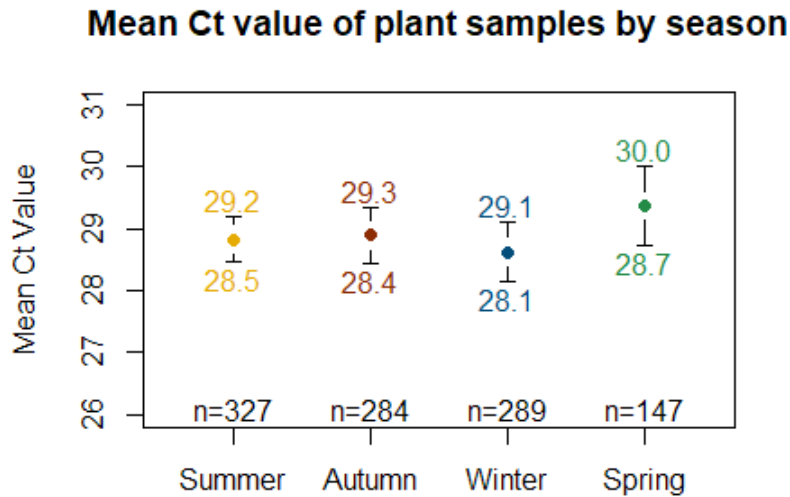

Figure 2. Mean Ct Value of CLas-positive plant samples collected by season. Bars indicate the 95% confidence interval (CI). A lower Ct Value indicates a higher bacterial titer.

Table 1. Plant samples collected in California residential areas and tested for HLB.

Season	No. of *C*Las+ Plant Samples	Total Plant Samples Collected	Proportion of all samples that were *C*Las+
Summer	327	74,306	.0044
Autumn	284	72,047	.0039
Winter	289	68,909	.0042
Spring	147	59,836	.0025
Total	1,047	275,098	.0038

Conclusions

In summary, *C*Las-positive plant samples collected from residential areas in southern California show no significant seasonal differences in Ct values. Resampling commercial groves for disease detection biennially is therefore unnecessary on the basis of seasonal variability; disease detection is equally as likely during any season.

This conclusion should be revisited in the future if significant numbers of *C*Las-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.

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