3.7 Optimizing qPCR for Detection of Candidatus Liberibacter Species in Plant and Psyllid Samples

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Although there are approved PCR-based methods for Candidatus Liberibacter (CL) detection, we have shown that they grossly under-estimate the presence of this pathogen due to detection sensitivity limitations associated with the assay procedure. The lack of sensitivity means that current survey methods are not accurately predicting the total number of infected trees and that research on acquisition and transmission of CL cannot be accurately conducted. We have used a set of multivariate statistical strategies called minimum run and response surface methodology to identify the components of the PCR-based detection methods that influence sensitivity. Significant influences of the buffer/salt composition and temperature profiles, have been identified and interactions among these were apparent. The information is being used to develop the most cost-effective assay that does not compromise assay sensitivity. Optimized methods for CL detection in both citrus and psyllids, including DNA preparation methods for each organism, were developed that take into account minimal sample handling as a means of reducing the risk of sample cross-contamination.