CITRUS GREENING IN GEORGIA: DETECTION AND GENETIC CHARACTERIZATION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' AND MONITORING FOR ASIAN CITRUS PSYLLIDS WITHIN COMMERCIAL GROVES

by

CARLTON FITZ-PATRICK COLLINS

(Under the Direction of Alejandra Jimenez Madrid)

ABSTRACT

Huanglongbing (HLB), is caused by '*Candidatus* Liberibacter asiaticus' (CLas), and vectored by the Asian Citrus Psyllid (ACP). HLB represents a major concern for the continued growth of Georgia's citrus industry. To further assess this potential threat, six commercial groves from Georgia were selected based on previous CLas detections in nearby areas for surveys during 2023 and 2024 to determine the in-grove distribution of CLas. Six groves were also selected for monitoring the prevalence of ACP. Among the 804 citrus trees tested, 35 (4.35%) located in clusters of four groves in Wayne, Ware and Pierce counties were determined to be CLas positive. ACP were only recovered from Pierce County, and 14 out of 127 (11%) tested positive for CLas via qPCR. Analysis of the CLas 16S rDNA region revealed no major variations; however, nine strains were associated with prophage type 1, while five had type 1-2.

INDEX WORDS: Citrus greening, Huanglongbing, *Candidatus* Liberibacter asiaticus, Citrus, Asian citrus psyllid, *Diaphorina* citri

CITRUS GREENING IN GEORGIA: DETECTION AND GENETIC CHARACTERIZATION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' AND MONITORING FOR ASIAN CITRUS PSYLLIDS WITHIN COMMERCIAL GROVES

by

CARLTON FITZ-PATRICK COLLINS

B.S., Brigham Young University Idaho, Rexburg, ID, 2023

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2025

CITRUS GREENING IN GEORGIA: DETECTION AND GENETIC CHARACTERIZATION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' AND MONITORING FOR ASIAN CITRUS PSYLLIDS WITHIN COMMERCIAL GROVES

by

CARLTON FITZ-PATRICK COLLINS

Major Professor:	Alejandra M. Jimenez Madrid
Committee:	Jonathan E. Oliver
	Apurba K. Barman

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2025

ACKNOWLEDGEMENTS

All glory be to Christ.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Economic importance of citrus production. Citrus is one of the oldest crops grown worldwide with records of its introduction into Europe dating back to 310 BC (Ramon-Laca 2003). Yet, citrus is known to have originated in China and has been cultivated there for thousands of years (Ramon-Laca 2003). Today, global citrus production tops 100 million tons and is grown in Brazil, China, Mexico, the European Union, Egypt and the United States of America (Foreign Agricultural Service 2025). In the United States, citrus is mainly produced in California, Florida, and Texas. The overall production for 2023 was estimated to be 5.24 million tons, however, in 2000, almost 17.40 million tons were produced in the United States (Cooper 2024; USDA NASS 2025; 2000). This reduction in citrus production in the United States in mainly attributed to losses caused by citrus greening or Huanglongbing (HLB) disease (Singerman and Rogers 2020).

Georgia has not traditionally been a large producer of citrus especially when compared to other major citrus producing US states such as Florida and California. As of 2023, there were about 4,000 acres planted commercially, which is around 600,000 trees (Price 2023). Today, citrus ranks 5th in Georgia among fruits and nuts for farmgate value (above peaches) at an estimated $40 million (University of Georgia, College of Agriculture and Environmental Science 2025).

Citrus production in the United States. The current citrus production in the United States is divided among four major states with California accounting for 79%, Florida for 17% and the remaining 4% produced by Texas, Arizona and other states, including Georgia (Cooper 2024). In terms of acreage in 2024, California had 267,000 acres of bearing citrus trees, Florida had 298,000,

Texas had 16,400 and Arizona had 6,400 (Cooper 2024). Although citrus remains a significant commodity in the country, its production has been on a downward trend. In Florida, citrus production has reduced from a forecasted 170 million boxes produced in 2005 to 14 million boxes in 2025, representing over a 90% reduction (USDA NASS 2005). This trend is attributed to several challenges to citrus production in the United States, including harsh climatic conditions and major citrus pests and diseases, such as citrus canker and HLB (Aregbe 2024; Singerman and Rogers 2020).

Challenges to citrus production in the United States. Environmental conditions play a critical role in citrus production. Temperatures between -6.7 and -1.1 °C will likely damage citrus trees regardless of the variety, although some varieties experience more severe damage than others, dependent on the maturity of the trees (Price and Westerfield 2009). Satsumas are some of the most cold-hardy varieties of citrus and are commonly grown in Georgia (Price and Westerfield 2009). Trees that get damaged in the winter do not manifest the extent of the damage until spring when pruning can be carried out to remove severely damaged branches. In summer months, constant exposure to sunlight can cause scalding of fruits (Price 2024). This can also make fruit unmarketable.

Citrus pests, such as leafminers (*Phyllocnistis citrella*), are a major challenge for citrus producers. Adult leafminer females lay eggs on the underside of leaves and hatched larvae burrow through the leaves causing leaves to become misshapen and in severe cases result in reduced citrus production (Vanaclocha et al. 2016). In Georgia, their life cycle is usually disrupted by the winter (Price 2019). Leafminers are arguably the most pervasive challenge in citrus production; however, the main concern is the creation of wounds for *axonopodis pv. citri* to infect plants (Gottwald et al. 2002). Among the main citrus diseases, citrus canker, caused by *Xanthomonas axonopodis* pv.

citri, citrus tristeza, caused by the citrus tristeza virus (CTV) and HLB are the most concerning. In Florida, citrus canker resulted in an eradication mandate that resulted in the removal of trees from residential and commercial locations (Chamberlain et al. 2004). In Georgia, citrus canker was reported for the first time in commercial groves in 2022 in Decatur County (Oliver 2022). However, satsumas (*Citrus reticulata* 'Owari'), which are the main type of citrus grown in Georgia (Price 2023) are known to be tolerant of citrus canker.

Citrus tristeza virus was also recently reported in Georgia in 2021 (Ali et al. 2021). There are several strains of CTV designated as T36, T3, T30, T68, VT, and RB, which are linked to disease severity (Dawson et al. 2015). The strain T30 was reported in Georgia, and this strain is considered a mild strain (Ali et al. 2021). Neither CTV nor citrus canker are known to be widespread throughout Georgia (Oliver, *personal communication*).

HLB disease is the most important disease affecting citrus and is a global threat to citrus production (da Graça et al. 2016). Symptoms of HLB include uneven chlorosis referred to as blotchy mottling, uneven ripening of fruit, stunting and dieback. HLB originated in Southeast Asia where the disease was first described as yellow shoot disease and yellow dragon disease or huanglongbing (HLB) (Gottwald et al. 2007). To date, HLB has been reported in over 60 countries (Gottwald et al. 2007). In the United States, HLB was first detected in Florida in 2005 (Bové 2006). Since then, HLB infected trees have been reported in Alabama, Arizona, Florida, Georgia, Louisiana, Puerto Rico, South Carolina, Texas and the U.S Virgin islands(Citrus Greening | Animal and Plant Health Inspection Service 2025). In most of the citrus producing states, HLB has been observed in restricted areas. However, in Florida, HLB is considered endemic (Wang 2019).

***Candidatus* Liberibacter asiaticus**. HLB is caused by '*Candidatus* Liberibacter asiaticus' (CLas) and transmitted by the Asian Citrus Psyllid (ACP). CLas is a gram-negative bacterium belonging to the alpha-proteobacteria family. There are two other *Candidatus* species that cause HLB found in this genus, *Candidatus* Liberibacter africanus (CLaf), vectored by the African citrus psyllid (*Trioza erytreae*) and primarily found in Africa and *Candidatus* Liberibacter americanus (CLam), vectored by the Asian citrus psyllid (*Diaphorina citri*), primarily found in Brazil (Teixeira et al. 2005). The genus *Candidatus* Liberibacter was first described by Jagoueix et al., 1994 using the 16S ribosomal DNA sequence. Prior to this, the causal agent of HLB was considered a BLO (bacteria-like organism) (Jagoueix et al., 1994).

CLas has been described as a "plant immune disease" as it does not encode its own pathogenicity factors. Rather, it triggers a plant immune response, which causes a buildup of callose and hydrogen peroxide within sieve elements inside phloem vessels, which is toxic to the plants resulting in programmed cell death (Ma et al. 2022). CLas is primarily transmitted by ACP that feed on sap produced within infected plants and subsequently feed on healthy plants.

The Asian citrus psyllid (*Diaphorina citri* Kuwayama) is a hemipteran insect belonging to the family Psyllidae. ACP is primarily found in Asia, South America, the Caribbean and some areas of Central America and the Middle East (Mead and Fasulo 2017). ACP was first found in Florida in 1998 on Orange Jasmine (*Murraya paniculata*) plants and has since been reported in all citrus-producing regions throughout North America (Citrus Greening | Animal and Plant Health Inspection Service 2025). In many of these areas, the pest is regulated by quarantine enforced by the USDA-APHIS (Mead and Fasulo 2017).

Adult ACP usually have variegated wings with three segmented mouthparts and ten segmented antennae. There are five nymphal stages, and nymphs are typically ovoid, yellow orange with wing pads that become prominent in later stages (Frank W. Mead and T.R. Fasulo 2017). CLas circulates through infected ACP who transmit CLas in a persistent propagative manner, meaning that CLas can multiply within ACP, and ACP are able to transmit CLas until they die. Psyllids that acquire CLas as nymphs are better at transmitting it once they become adults than those who acquire CLas as adults (Mead and Fasulo 2017).

HLB Regulations and Management. CLas is classified as a select agent by the United States government because of its far-reaching impact. The Animal Plant Health Inspection Service (APHIS) is an entity of the United States Department of Agriculture (USDA) responsible for the regulation of these select agents to limit their entry and spread. They have developed and/or adapted diagnostic protocols to ensure that testing for the pathogen of intertest is standardized and accurate. These protocols must be followed, especially in areas where the pathogen has not previously been reported. Therefore, diagnostic laboratories that test for the pathogen, and personnel conducting diagnosis must be certified by USDA-APHIS. In addition, quarantine boundaries have been established as an exclusionary strategy to limit the spread of HLB (USDA APHIS 2025). Growers are encouraged to plant USDA certified disease-free trees, remove infected trees, and spray insecticides to control populations of ACP within commercial groves. Removing infected trees presents a financial burden to growers who invest capital to plant these trees and to maintain them. This is arguably one of the greatest burdens of citrus production for growers who may be hesitant to test trees for fear of having to remove them. Insecticide applications are recommended during winter months when ACP populations are lower (Diepenbrock 2024). Both

foliar and soil applications are used for suppression of ACP populations. Foliar products such as Exirel (cunantraniliprole), Apta 15 SC (Tolfenpyrad), VoliamFlexi (chloraniliprole+thiamethoxam) and Sivanto 200 SL (flupyradifurone) have been reported to be most effective against adult ACP, while Baythroid XL (beta-cyfluthrin), Midan 70 W (phosmet), Lorsban 4 E (chlorpyrifos), and Exirel (cyantraniliprole) were most effective against nymphs (Qureshi et al. 2014). Similarly, soil applications of NUQ 05054b (imidacloprid), Verimarkc (cyantraniliprole), Belay 2.13 SC (clothianidin) and Temik 15 Gb (aldicarb) were ranked highest for efficacy against nymphs and adults (Qureshi et al. 2014).

Antibiotics such as oxytetracycline and streptomycin have also been labelled for use on CLas infected trees and have been reported to reduce CLas acquisition by ACP when applied as foliar sprays, however, there are mixed results about the ability of these antibiotics to reduce CLas infection in trees (Roldán et al. 2023). Trunk injections of antibiotics have shown more promising results for reducing CLas titer within infected trees (Killiny et al. 2020). Biological control agents such as the parasitoid wasp (*Tamarixia radiata*) have also been used to reduce ACP populations, though their presence alone has not led to complete suppression of ACP within fields (Qureshi et al. 2009).

Detection of CLas. Accurate and rapid detection of CLas and diagnosis of HLB are essential to controlling the spread of the disease. This is especially true in areas where HLB is not endemic. Detecting CLas presents several challenges, which necessitate the use of molecular diagnostic assays. Originally, CLas was detected via the use of electron microscopy and dot-blot hybridization assay before the first Polymerase Chain Reaction (PCR) assay was developed in 1996 (Jagoueix et al. 1996). Subsequently, the first quantitative PCR (qPCR) assay for detection

of CLas was developed in 2006 (Li et al. 2006). Today, detection of CLas primarily relies on qPCR, although there are other assays, such as LAMP (Loop-mediated isothermal amplification), digital PCR and the use of monoclonal antibodies, which have shown some promise (Pagliaccia et al. 2017; Stolowicz et al. 2022). The USDA-APHIS has developed a standard protocol based on findings of Zheng et al. (2016), which uses qPCR targeting the 5 copy RNR gene for the detection of CLas. This protocol is required to be used by National Diagnostic Laboratories for detection of CLas.

Genetic characterization of CLas. Genetic characterization provides information on how a pathogen infects, multiplies, and spreads within its host and in a geographical region and in some cases, may connect genotypic observations to phenotypic traits. One essential component of genetic characterization is determining the genetic diversity among bacterial strains. Several studies have been conducted to determine the genetic diversity of CLas in India (Adkar-Purushothama et al. 2009), Brazil (De Paula et al. 2019), and China (Gao et al. 2022). These studies have included sequences of the 16S rDNA, the deoxy-ribonucleotide reductase gene (*nrdB*), outer membrane protein (*omp*) gene, tandem repeats and hypervariable prophage regions (Adkar-Purushothama et al. 2009; Bastianel et al. 2005; Gao et al. 2022).

Hypervariable prophage regions have been used to classify strains of CLas in what has been termed prophage typing. Prophages are regions of DNA left in the genome of CLas from its association with certain bacteriophages. Three major prophage types have been identified with CLas, designated prophage type 1, type 2 and type 3. So far, prophage types have been used to infer the origin and movement of CLas strains, such as in the case of California where the presence of prophage type 3 has been used to infer that strains found in California originated from Asia, while in Florida, strains have not been found to contain prophage type 3 (Dai et al. 2019).

Additionally, prophages have the potential to lead to future management strategies as they are linked to pathogenicity and the ability of CLas to adapt to new environments and hosts (Gao et al. 2022). The characterization of CLas in Georgia will provide information on the migration patterns of the different strains as well as provide insights into differences between strains that occur in America. This may help researchers investigating the development of resistance to HLB and provide insights into management of the disease (De Paula et al. 2019).

CLas in Georgia. HLB was first reported in Chatham County, Georgia in 2009. As a result, the entire state was placed under federal quarantine (Animal Plant Health Inspection Service (APHIS) 2009). At that point, commercial citrus in Georgia was essentially non-existent. Subsequently, surveys conducted between 2019 and 2022 confirmed the presence of CLas in residential trees in Bryan, Camden, Lowndes and Pierce Counties (Oliver et al. 2020). A regional survey has also reported the presence of ACP in residential areas in Georgia in coastal and southern counties including Charlton and Lowndes counties (Martini et al. 2020; Oliver et al. 2020). CLas was detected in commercial citrus groves in Wayne and Pierce counties in 2022 (Oliver, *Personal Communication*). Additionally, an ACP found in Chatham County was confirmed positive with CLas and ACP was reported for the time in a commercial grove in Georgia in Pierce County in 2023 (Collins et al. . These findings confirm that HLB is a significant risk factor for the continued growth of Georgia's citrus industry and raise questions about the incidence of CLas and ACP within commercial groves.

Rationale and Objectives. Citrus is a small but rapidly growing industry in Georgia, and it is imperative that threats to the industry are monitored closely to allow for continued growth. HLB has proven to be the greatest threat to the survival of the industry in Florida where HLB is considered endemic and is likely to pose a significant hurdle to growth if it is left unchecked in

Geogia. The Asian citrus psyllid is the foremost vector of CLas in North America and control of ACP is one of the few ways that HLB can be managed. Prior to this study, there were no reports of ACP in commercial groves, though recent surveys conducted in commercial groves confirmed the presence of CLas infected trees in 2022. Consequently, it is not clear whether ACP are currently existing within commercial groves and transmitting CLas to these trees and the incidence of CLas within these groves remains unknown. Additionally, there has been no work done to identify genetic variations between strains of CLas in Georgia. This project was designed to address these gaps in knowledge that will elucidate the actual threat of HLB in Georgia and provide a baseline for future studies into this disease. **The objectives of this study are to**:

1. Confirm '*Candidatus* Liberibacter asiaticus' in Asian Citrus Psyllids in Georgia and detect Asian Citrus Psyllids in commercial citrus in Georgia (Chapter 2; Collins et al. 2025)
2. Determine the incidence of CLas in symptomatic and asymptomatic leaf tissues from commercial citrus (Chapter 3)
3. Characterize the genetic diversity of CLas strains found in Georgia to better understand adaptation and evolutionary traits within Georgia populations (Chapter 3)
4. Monitor the prevalence of ACP in commercial plantings for molecular detection of CLas (Chapter 4).

LITERATURE CITED

Adkar-Purushothama, C. R., Quaglino, F., Casati, P., Gottravalli Ramanayaka, J., and Bianco, P. A. 2009. Genetic 'Candidatus Liberibacter asiaticus' in 16S rRNA and Ann. Microbiol. 59:681–688. https://doi.org/10.1007/BF03179208.

Ali, E., Bennett, A., Stackhouse, T., Waliullah, S., and Oliver, J. E. 2021. First Report of Citrus tristeza virus Infecting Citrus Trees in Georgia, USA. Plant Dis. https://doi.org/10.1094/PDIS-02-21-0365-PDN.

Animal Plant Health Inspection Service (APHIS). 2009. Confirmation of Citrus Greening in Chatham County, Georgia. .

Aregbe, I. 2024. Citrus Greening, Hurricanes, and the Decline of the Florida Citrus Industry. Southern Ag Today. https://southernagtoday.org/2024/01/05/citrus-greening-hurricanes-and-the-decline-of-the-florida-citrus-industry/ (accessed 16 Jun 2025).

Bastianel, C., Garnier-Semancik, M., Renaudin, J., Bové, J. M., and Eveillard, S. 2005. Diversity of " *Candidatus* Liberibacter asiaticus," Based on the *omp* Gene Sequence. Appl. Environ. Microbiol. 71:6473–6478. https://doi.org/10.1128/AEM.71.11.6473-6478.2005.

Bové, J. M. 2006. Huanglongbing: A Destructive, Newly-Emerging, Century-Old Disease of Citrus. J. Plant Pathol.

Chamberlain, H. L., Roberts, P. D., Timmer, L. W., and Zekri, M. 2004. Status of the Citrus Canker Eradication Program in Florida and University of Florida Citrus Canker Extension Program. .

Citrus Greening | Animal and Plant Health Inspection Service. https://www.aphis.usda.gov/plant-pests-diseases/citrus-diseases/citrus-greening (accessed 17 Jun 2024).

Collins, C. F., Oliver, J. E., Barman, A. K., Munoz, G., & Madrid, A. J. 2025. Confirmation of 'Candidatus Liberibacter asiaticus' in Asian Citrus Psyllids and Detection of Asian Citrus Psyllids in Commercial Citrus in Georgia (U.S.A.). Plant disease, 109(4), 800–803. https://doi.org/10.1094/PDIS-07-24-1424-SC

Cooper, D. 2024. U.S. Citrus Production and Value by State - Production Citrus Industry Magazine. Citrus Ind. Mag. https://citrusindustry.net/2024/11/20/u-s-citrus-production-value-state/ (accessed 13 Jun 2025).

Dai, Z., Wu, F., Zheng, Z., Yokomi, R., Kumagai, L., Cai, W., Rascoe, J., Polek, M., Chen, J., and Deng, X. 2019. Prophage Diversity of ‘Candidatus Liberibacter asiaticus’ Strains in California. Phytopathology® 109:551–559. https://doi.org/10.1094/PHYTO-06-18-0185-R.

Dawson, W. O., Bar-Joseph, M., Garnsey, S. M., and Moreno, P. 2015. Citrus Tristeza Virus : Making an Ally from an Enemy. Annu. Rev. Phytopathol. 53:137–155. https://doi.org/10.1146/annurev-phyto-080614-120012.

De Paula, L. B., Lin, H., Stuchi, E. S., Francisco, C. S., Safady, N. G., and Coletta-Filho, H. D. 2019. Genetic of ‘Candidatus Liberibacter asiaticus’ in Brazil Analyzed Different Geographic Regions Citrus Varieties. Eur. J. Plant Pathol. 154:863–872. https://doi.org/10.1007/s10658-019-01695-1.

Diepenbrock, L. M. 2024. 2024–2025 Florida Citrus Production Guide: Asian Citrus Psyllid. Ask IFAS - Powered EDIS. https://edis.ifas.ufl.edu/publication/CG097 (accessed 22 Apr 2025).

Frank W. Mead and T.R. Fasulo. 2017. Asian citrus psyllid - Diaphorina citri Kuwayama. . https://entnemdept.ufl.edu/creatures/citrus/acpsyllid.htm (accessed 24 Feb 2024).

Gao, F., Wu, B., Zou, C., Bao, Y., Li, D., Yao, W., Powell, C. A., and Zhang, M. 2022. Genetic Diversity of " *Candidatus* Liberibacter asiaticus" Based on Four Hypervariable Genomic Regions in China ed. Lindsey Price Burbank. Microbiol. Spectr. 10:e02622-22. https://doi.org/10.1128/spectrum.02622-22.

Gottwald, T. R., Graça, J. V. D., and Bassanezi, R. B. 2007. Citrus Huanglongbing: The Pathogen and Its Impact. Plant Health Prog. 8:16, 18. https://doi.org/10.1094/PHP-2007-0906-01-RV.

Gottwald, T. R., Sun, X., Riley, T., Graham, J. H., Ferrandino, F., and Taylor, E. L. 2002. Geo-Referenced Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida. Phytopathology® 92:361–377. https://doi.org/10.1094/PHYTO.2002.92.4.361.

da Graça, J. V., Douhan, G. W., Halbert, S. E., Keremane, M. L., Lee, R. F., Vidalakis, G., and Zhao, H. 2016. Huanglongbing: An overview of a complex pathosystem ravaging the world's citrus. J. Integr. Plant Biol. 58:373–387. https://doi.org/10.1111/jipb.12437.

Jagoueix, S., Bové, J. M., and Garnier, M. 1996. PCR detection of the species associated with greening disease of citrus. Mol. Cell. Probes 10:43–50. https://doi.org/10.1006/mcpr.1996.0006.

Killiny, N., Hijaz, F., Gonzalez-Blanco, P., Jones, S. E., Pierre, M. O., and Vincent, C. I. 2020. Effect of Adjuvants on Oxytetracycline Uptake upon Foliar Application in Citrus. Antibiotics 9:677. https://doi.org/10.3390/antibiotics9100677.

Li, W., Hartung, J. S., and Levy, L. 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. J. Microbiol. Methods 66:104–115. https://doi.org/10.1016/j.mimet.2005.10.018.

Ma, W., Pang, Z., Huang, X., Xu, J., Pandey, S. S., Li, J., Achor, D. S., Vasconcelos, F. N. C., Hendrich, C., Huang, Y., Wang, W., Lee, D., Stanton, D., and Wang, N. 2022. Citrus Huanglongbing is a pathogen-triggered immune disease that can be mitigated with antioxidants and gibberellin. Nat. Commun. 13:529. https://doi.org/10.1038/s41467-022-28189-9.

Martini, X., Malfa, K., Stelinski, L. L., Iriarte, F. B., and Paret, M. L. 2020. Distribution, Phenology, and Overwintering Survival of Asian Citrus Psyllid (Hemiptera: Liviidae), in Urban and Grove Habitats in North Florida. J. Econ. Entomol. 113:1080–1087. https://doi.org/10.1093/jee/toaa011.

Mead, F. W., and Fasulo, T. R. 2017. Asian citrus psyllid - Diaphorina citri Kuwayama. FDACS/DPI Entomology Circular No. 180. Pub. Num. EENY-33 https://entnemdept.ufl.edu/creatures/citrus/acpsyllid.htm

Oliver, J. 2022. ALERT: Citrus Canker Confirmed in Georgia | UGA Citrus Blog. . https://site.caes.uga.edu/citrus/2022/06/alert-citrus-canker-confirmed-in-georgia/ (accessed 13 Jun 2025).

Oliver, J. E., Ali, M. E., Waliullah, S., Price, J., Warren, J., Jacobs, J., Hoppers, A., Evans, R., Dowdy, M., and Curry, S. 2020a. Huanglongbing, Caused by ‘ *Candidatus* Liberibacter asiaticus,’ Detected in New Locations Across Southern and Coastal Georgia. Plant Health Prog. 21:31–35. https://doi.org/10.1094/PHP-09-19-0064-S.

Oliver, J. E., Ali, M. E., Waliullah, S., Price, J., Warren, J., Jacobs, J., Hoppers, A., Evans, R., Dowdy, M., and Curry, S. 2020b. Huanglongbing, Caused by 'Candidatus Liberibacter asiaticus,' Detected in New Locations Across Southern and Coastal Georgia. Plant Health Prog. 21:31–35. https://doi.org/10.1094/PHP-09-19-0064-S.

Pagliaccia, D., Shi, J., Pang, Z., Hawara, E., Clark, K., Thapa, S. P., De Francesco, A. D., Liu, J., Tran, T.-T., Bodaghi, S., Folimonova, S. Y., Ancona, V., Mulchandani, A., Coaker, G., Wang, N., Vidalakis, G., and Ma, W. 2017. A Pathogen Secreted Protein as a Detection Marker for Citrus Huanglongbing. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02041.

Price, J., and Westerfield, B. 2009. Citrus Fruit for Southern and Coastal Georgia | UGA Cooperative Extension. . https://extension.uga.edu/publications/detail.html?number=B804&title=citrus-fruit-for-southern-and-coastal-georgia (accessed 18 Jun 2025).

Price, J. 2024. Maintaining Commercial Citrus in Georgia. https://extension.uga.edu/publications/detail.html?number=B1520&title=maintaining-commercial-citrus-in-georgia (accessed 18 Jun 2025).

Price, J. 2023. Citrus Plantings in Georgia Continue to Increase. UGA Citrus Blog. https://site.caes.uga.edu/citrus/2023/09/citrus-plantings-in-georgia-continue-to-increase/ (accessed 29 Jan 2025).

Price, J. 2019. How to Control Citrus Leafminers. https://extension.uga.edu/publications/detail.html?number=C1145&title=how-to-control-citrus-leafminers (accessed 13 Jun 2025).

Qureshi, J. A., Kostyk, B. C., and Stansly, P. A. 2014. Insecticidal Suppression of Asian Citrus Psyllid Diaphorina citri (Hemiptera: Liviidae) Vector of Huanglongbing Pathogens. PLoS ONE 9:e112331. https://doi.org/10.1371/journal.pone.0112331.

Qureshi, J. A., Rogers, M. E., Hall, D. G., and Stansly, P. A. 2009. Incidence of Invasive Diaphorina citri (Hemiptera: Psyllidae) and Its Introduced Parasitoid Tamarixia radiata (Hymenoptera: Eulophidae) in Florida Citrus. J. Econ. Entomol. 102:247–256. https://doi.org/10.1603/029.102.0134.

Ramon-Laca, L. 2003. The Introduction of Cultivated Citrus to Europe via Northern Africa and the Iberian Peninsula. Economic Botany 57:502–514.

Roldán, E. L., Stelinski, L. L., and Pelz-Stelinski, K. S. 2023. Foliar Antibiotic Treatment Reduces Candidatus Liberibacter asiaticus Acquisition by the Asian Citrus Psyllid, Diaphorina citri (Hemiptera: Liviidae), but Does not Reduce Tree Infection Rate. J. Econ. Entomol. 116:78–89. https://doi.org/10.1093/jee/toac200.

Singerman, A., and Rogers, M. E. 2020. The Economic Challenges of Dealing with Citrus Greening: The Case of Florida. Journal of Integrated Pest Management 11:3. https://doi.org/10.1093/jipm/pmz037.

Stolowicz, F., Larocca, L., Werbajh, S., Parma, Y., Carrillo, C., Ogas, L., Agostini, J. P., Redes, J., Welin, B., Castagnaro, A., and Vojnov, A. 2022. A colorimetric, sensitive, rapid, and simple diagnostic kit for the HLB putative causal agent detection. Front. Agron. 4. https://doi.org/10.3389/fagro.2022.984360.

United States Department of Agriculture Foreign Agricultural Service. 2025. *Citrus: World Markets and Trade Global Market Analysis*.

University of Georgia, College of Agriculture and Environmental Science. 2025. 2025 Georgia Ag Impact Report. . https://discover.caes.uga.edu/georgiaagimpact/ (accessed 30 Apr 2025).

Vanaclocha, P., Jones, M. M., Monzó, C., and Stansly, P. A. 2016. Placement Density and Longevity of Pheromone Traps for Monitoring of the Citrus Leafminer (Lepidoptera: Gracillariidae). Fla. Entomol. 99:196–202. https://doi.org/10.1653/024.099.0207.

Wang, N. 2019. The Citrus Huanglongbing Crisis and Potential Solutions. Mol. Plant 12:607–609. https://doi.org/10.1016/j.molp.2019.03.008.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., and Deng, X. 2016. Unusual Five Copies and Dual Forms of nrdB in "Candidatus Liberibacter asiaticus": Biological Implications and PCR Detection Application. Sci. Rep. 6:39020. https://doi.org/10.1038/srep39020.

CHAPTER 2

CONFIRMATION OF CANDIDATUS LIBERIBACTER ASIATICUS IN ASIAN CITRUS PSYLLIDS AND DETECTION OF ASIAN CITRUS PSYLLIDS IN COMMERCIAL CITRUS IN GEORGIA (U.S.A.)[1]

---

[1]Collins, C. F., Oliver, J. E., Barman, A. K., Munoz, G., and Jimenez Madrid, A.M. 2025. Confirmation of *'Candidatus* Liberibacter asiaticus' in Asian Citrus Psyllids and Detection of Asian Citrus Psyllids in Commercial Citrus in Georgia (U.S.A.). *Plant disease*, 109: 800–803. Reprinted here with permission of the publisher

ABSTRACT

The Asian citrus psyllid (ACP) is the vector of *Candidatus* Liberibacter asiaticus (CLas), the causal agent of citrus greening or Huanglongbing (HLB), one of the most devastating citrus diseases worldwide. The citrus industry in Georgia (U.S.A.) is in the process of a rapid expansion, and based on experiences with HLB in Florida, there is great concern about the potential impacts of HLB on this emerging industry. Prior to 2023, ACP had been identified in residential citrus trees in isolated Georgia counties but little to no testing of psyllids for CLas had occurred. However, in 2023, one individual psyllid collected from Chatham County was confirmed positive for CLas by PCR and sequencing. Furthermore, during 2023, ACP adults and nymphs were identified for the first time in a Georgia commercial citrus grove. The finding of ACP in a commercial planting represents a significant risk for CLas dissemination, and thereby has the potential to stall the rapid expansion of Georgia's citrus industry. In the coming years, surveillance and testing of ACP from commercial groves will be essential for the early detection and management of HLB and its vector to reduce HLB spread within Georgia's commercial groves.

SHORT COMMUNICATION

The Asian citrus psyllid (ACP), *Diaphorina citri (*Kuwayama*)*, is the foremost vector of the bacterium *Candidatus* Liberibacter Asiaticus (CLas), which causes citrus greening or Huanglongbing (HLB) disease. ACP was first detected in the United States (U.S.A.) in Florida on orange jasmine (*Murraya* paniculata) in 1998, while CLas was first detected in 2005 (Halbert 2005). Since then, Florida's citrus production has been reduced by 85% according to recent

estimates (USDA NASS 2023). HLB is the most devastating disease affecting citrus in the U.S.A., with estimated annual losses of over $1 billion per year (Li et al. 2020). Citrus trees infected with CLas typically become unproductive in 2-5 years, and many eventually die in the absence of treatment (Li et al. 2020). Management of the disease is based on planting CLas-free citrus germplasm, monitoring and eradicating infected citrus trees, and control of the vector with systemic insecticides. In other parts of the world, HLB is also caused by *Candidatus* Liberibacter americanus (CLam) and *Candidatus* Liberibacter Africanus (CLaf), vectored by *Trioza erytreae*, the African citrus psyllid. However, CLas is the only species that has been found in the U.S.A.

The ACP originated in India (Capoor et al. 1974), but it is currently found in several countries and territories, including Afghanistan, Saudi Arabia, France, Mauritius, Central and South America, Asia, Mexico, the Caribbean, and the U.S.A. In the United States, ACP has been observed in either commercial or residential trees in Florida, Arizona, Texas, Alabama, California, Louisiana, Mississippi, South Carolina, Hawaii, and Georgia (Mead et al. 2017). The ACP was intercepted 170 times at U.S.A ports on plant material between 1985 and 2003 (Grafton-Cardwell et al. 2006). Due to the risk of the presence and spread of this vector in citrus-producing regions, very strict protocols have been implemented to prevent movement of this insect. The USDA Animal Plant Health Inspection Service (APHIS) has implemented quarantine regulations in the U.S.A since June 2010 for ACP, restricting movement of any material including host plants and plant parts that may aid in the dissemination of ACP. Following the identification of ACP and CLas-infected residential trees near the city of Savannah, the entire state of Georgia has been quarantined for both HLB and ACP since 2009. However, Georgia's commercial citrus industry at that time was practically nonexistent, and commercial citrus acreage in Georgia did not begin to rapidly grow until the mid-to-late 2010s. Subsequent findings of CLas and ACP have been

minimal in Georgia, except within 10 counties, primarily in coastal areas (Oliver 2020). However, the Asian citrus psyllid has not been previously reported in commercial groves in Georgia (USDA APHIS 2023).

In California, ACP monitoring efforts include the establishment of quarantine zones that are delineated based on the risk that exists in specific geographical regions. Detection surveys are routinely carried out via visual inspection and the use of yellow panel traps. If psyllids are found, trapping is focused within the 4 square miles surrounding that area at a rate of approximately 50 traps per square mile. Chemical and biological control treatments are also implemented in the area surrounding the confirmed ACP detection. These efforts may continue for years after the initial detection (California Department of Food and Agriculture. 2024).

ACP usually enters new commercial plantings from abandoned fields or residential trees, including orange jasmine that may serve as reservoirs for the insect (Boina et al. 2009). Under laboratory conditions, Martini et al. (2014) found that ACP is able to travel up to 2.4 km at a time without the aid of wind, and it may travel even further when aided by wind (Gottwald et al. 2007). In Florida, psyllids move at distances greater than 2000 m from one grove to another over a 12-day period (Lewis-Rosenblum et al. 2015). They are also moved by humans and with infested plants. Although testing of psyllids for CLas is not commonly performed, a survey conducted in 2017-2018 in North Florida counties, which included Lowndes and Decatur counties in Georgia, revealed that the infection rate of psyllids ranged from 0 to 28% (Martini et al. 2020). In addition, an ongoing study in California indicates that 3.5% of all psyllids collected are infected with CLas (Callies 2022).

ACP feed on the phloem sap of plants and are thus able to acquire CLas. The insect can acquire the pathogen after just 15-30 minutes of feeding on previously infected plants, however,

the transmission rate may vary according to ACP stage (nymphs vs adults) (Capoor et al. 1974; Pelz-Stelinski et al. 2010). Following this, there is a 16-18-day latent period, after which the insect can transmit the bacterium to other plants for the rest of its life (Canale et al. 2017). One characteristic symptom that comes with the presence of psyllids is a whitish waxy secretion from nymphs (**Figure 2.1a**).

Between 2018 and 2023, individual ACP detections were tested on the UGA-Tifton Campus for the presence of CLas. In September 2018, ACP adults (*n*=10) were identified for the first time in Lowndes County, Georgia on satsuma (*Citrus unshiu*) and 'Glenn Navel' (*Citrus* × *sinensis*) citrus trees located in the courtyard of a public school in a residential neighborhood, and in June 2023, adult ACP (*n*=3) were observed from a residential satsuma tree symptomatic for HLB in Chatham County. Insect morphology matched the description of ACP as described by Mead et al. (2017) (**Figure 2.1b**). DNA was extracted from individual psyllids following the protocol of Pelz-Stelinski et al. (2010), with a few modifications. Briefly, the incubation period was adjusted to 4.5 hours and the insect tissue disruption was conducted with a disposable pellet pestle. The DNA concentration and quality was confirmed using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE). A conventional PCR assay was performed using the OI1/OI2c primer set (Jagoueix et al. 1996) to amplify the 16S rDNA region from the CLas bacterium. While the CLas testing results from the Lowndes County psyllids collected in 2018 were negative, an amplicon (~ 1,200 bp) was observed from one of the psyllids collected from Chatham County in 2023. This amplicon (**Figure 2.2**) was purified and sequenced. Sequence quality and pairwise alignment was conducted using the Geneious Prime Program (version 2024.0.7; GraphPad software, LLC., Boston, MA). Sequence comparison revealed that the ACP from Chatham County was carrying the CLas bacterium. The obtained consensus

sequence was >99% identical (100% query coverage) to multiple publicly available CLas sequences based upon comparisons using the BLASTn function (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in the NCBI database. The derived sequence from this ACP has been deposited in GenBank as Accession No. PP829198.

In September 2023, during scouting operations, ACP were observed in an approximately 10-year-old commercial planting of satsumas located in Pierce County, GA. Five (*n*=5) psyllid adults and twenty (*n*=20) nymphs were found on a single asymptomatic tree (**Figures 2.1a**). ACP morphology was confirmed according to the previous description by Mead et al. (2017). DNA extraction was conducted on each individual psyllid and nymph and a PCR assay was conducted as described above. DNA concentrations ranged from 10 ng/µL to 56.2 ng/µL. No amplification was observed from any of the psyllids and nymphs tested (data not shown), indicating that either they were not carrying CLas or that the CLas titer was below the detection limit of the test. Between 2018 and 2023, ACP were found only on residential citrus trees in Georgia. Therefore, to our knowledge, this finding represents the first identification of ACP in a commercial citrus planting in Georgia (**Figure 2.3**). The citrus tree on which the ACP were found was tested for CLas via qPCR in 2023 and 2024. A composite sample of leaves (*n*=12) were randomly collected from the tree. Midribs and petioles were removed and chopped before DNA extraction using a commercial plant extraction kit. A multiplex real-time-PCR was conducted on a Bio-Rad CFX Opus 96 thermal cycler (Bio-Rad Lab Inc., Hercules, CA) with primers and probes targeting the RNR gene from CLas (Zheng et al. 2016) following USDA-APHIS standard protocols. No amplification was observed indicating that the tree was either not infected with CLas or that the bacterial titer was below the detection threshold. This test was repeated twice.

The detection of CLas in ACP in Georgia and the first confirmation of ACP within a commercial planting in Georgia is alarming. This confirmation of ACP within a commercial citrus grove poses a serious and significant risk to the citrus industry in Georgia due to the role of this insect in the spread of the devastating HLB disease. While the commercial citrus industry in Georgia is young, with most plantings being under 5 years old across the state, some established commercial plantings are now greater than 10 years old. Young trees that become infected with HLB will not be productive for growers or provide them with the ability to get a return on their investment. This has the potential to discourage existing and future growers from expanding citrus plantings within the state. To ensure that HLB does not spread in Georgia, it will be vital to monitor and manage ACP to prevent vector spread (Diepenbrock et al. 2023). Growers need to remain alert for the risk of ACP and must implement proactive measures, such as adopting a monitoring protocol, regular insecticide spray programs against ACP, and removal of CLas-infected citrus trees (Bassanezi et al. 2013). Area wide management programs (Grafton-Cardwell et al. 2018.) may also be beneficial for implementation in areas where ACP has been found within or close to commercial groves, although these programs have not been efficient in Florida. Monitoring efforts, including ACP scouting, trapping, and testing need to be employed in both commercial and nearby residential citrus to ensure that ACP, and consequently HLB, does not continue to spread unchecked throughout the state.

Funding: This research was partially supported by funding from the USDA/AMS Specialty Crop Block Grant Program (SCBGP) administered by Georgia Department of Agriculture.

LITERATURE CITED

Aubert, B. 1987. Trioza erytreae del Guercio and Diaphorina citri Kuwayama (Homoptera: Psylloidea), the two vectors of Citrus Greening Disease: Biological aspects and possible control strategies. Fruits. 42:149-162.

Bassanezi, R. B., Belasque, J., and Montesino, L. H. 2013. Frequency of symptomatic trees removal in small citrus blocks on citrus huanglongbing epidemics. Crop Protection. 52: 72-77. https://doi.org/10.1016/j.cropro.2013.05.012

Boina, D. R., Meyer, W. L., Onagbola, E. O., and Stelinski, L. L. 2009. Quantifying Dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by Immunomarking and Potential Impact of Unmanaged Groves on Commercial Citrus Management. Environ. Entomol. 38:1250-1258.

California Department of Food and Agriculture. 2024. California Statewide Action Plan for Asian Citrus Psyllid and Huanglongbing. https://www.cdfa.ca.gov/citrus/docs/committee/ActionPlan.pdf

Callies, T. 2022. California Psyllids Carrying HLB Bacterium in Groves. Citrus Industry AgNet. https://citrusindustry.net/2022/11/22/california-psyllids-carrying-hlb-bacterium-in-groves/.

Canale, M. C., Tomaseto, A. F., Haddad, M. D. L., Della Coletta-Filho, H., and Lopes, J. R. S. 2017. Latency and Persistence of '*Candidatus* Liberibacter asiaticus' in Its Psyllid Vector, *Diaphorina citri* (Hemiptera: Liviidae). Phytopathology 107:264-272.

Capoor, S. P., Rao, D. G., and Viswanath, S. M. 1974. Greening Disease of Citrus in the Deccan Trap Country and its Relationship with the Vector, Diaphorina citri Kuwayama. Int. Organ. Citrus Virol. Conf. Proc. 1957-2010:6. https://escholarship.org/uc/item/6rm6x1tw

Diepenbrock, L. M., Qureshi, J., and Stelinski, L. 2023. 2023-2024 Florida Citrus Production Guide: Asian Citrus Psyllid: CG097, rev. 5/2023. EDIS. https://doi.org/10.32473/edis-cg097-2023.

Gottwald, T. R., Graça, J. V. D., and Bassanezi, R. B. 2007. Citrus Huanglongbing: The Pathogen and Its Impact. Plant Health Prog. 8:31.

Grafton-Cardwell, E. E., Godfrey, K. E., Rogers, M. E., Childers, C. C., and Stansly, P. A. 2006. Asian Citrus Psyllid. Publication 8205. University of California, Agriculture and Natural Resources. https://www.cdfa.ca.gov/plant/acp/docs/anr/8205.pdf

Grafton-Cardwell, E., and Garcia-Figuera, S. 2018. Area-wide management of ACP to limit the spread of HLB in California. University of California, Davis Agriculture and Natural Resources. https://ucanr.edu/sites/scienceforcitrushealth/Research_Snapshots/Psyllid_Management/Area-Wide-Management/

Halbert, S. 2005. The Discovery of Huanglongbing in Florida. Second International Citrus Canker and Huanglongbing Research Workshop H3 p. 50 https://swfrec.ifas.ufl.edu/hlb/database/pdf/22_CankerHuang_05.pdf

Halbert, S. E., and Manjunath, K. L. 2004. Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: A Literature Review and Assessment of Risk in Florida. Florida Entomologist. 87:330–353.

Jagoueix, S., Bové, J. M., and Garnier, M. 1996. PCR detection of the two “Candidatus” Liberobacter species associated with greening disease of citrus. Mol. Cell. Probes. 10:43-50.

Lewis-Rosenblum, H., Martini, X., and Tiwari, S. 2015. Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. Journal of Economic Entomology, 208, 3–10. https://doi.org/10.1093/jee/tou008

Li, S., Wu, F., Duan, Y., Singerman, A., and Guan, Z. 2020. Citrus Greening: Management Strategies and Their Economic Impact. HortScience. 55:604-612.

Martini, X., Hoyte, A., and Stelinski, L. L. 2014. Abdominal Color of the Asian Citrus Psyllid (Hemiptera: Liviidae) is Associated with Flight Capabilities. Annals of the Entomological Society of America. 107:842–847.

Martini, X., Malfa, K., Stelinski, L. L., Iriarte, F. B., and Paret, M. L. 2020. Distribution, Phenology, and Overwintering Survival of Asian Citrus Psyllid (Hemiptera: Liviidae), in Urban and Grove Habitats in North Florida. J Econ Entomol. 113:1080–1087.

Mead, F. W., and Fasulo, T. R. 2017. Asian citrus psyllid - Diaphorina citri Kuwayama. FDACS/DPI Entomology Circular No. 180. Pub. Num. EENY-33 https://entnemdept.ufl.edu/creatures/citrus/acpsyllid.htm

Oliver, J. 2020. UGA Citrus Greening Survey Results (Spring 2019). UGA Citrus Blog. UGA Citrus Greening Survey Results (Spring 2019) | UGA Citrus Blog

Pelz-Stelinski, K., Brlansky, R., and Rogers, M. 2010. Transmission parameters for Candidatus Liberibacter asiaticus by Asian citrus psyllid (Hemiptera: Psyllidae). Journal of Economic Entomology, 103, 1531–1541.

Qureshi, J. A., Kostyk, B. C., and Stansly, P. A. 2014. Insecticidal Suppression of Asian Citrus Psyllid Diaphorina citri (Hemiptera: Liviidae) Vector of Huanglongbing Pathogens ed. Kun Yan Zhu. PLoS ONE. 9:e112331.

Tsai, J. H., and Liu, Y. H. 2000. Biology of Diaphorina citri (Homoptera: Psyllidae) on Four Host Plants. ec. 93:1721–1725.

Wulff, N. A., Daniel, B., Sassi, R. S., Moreira, A. S., Bassanezi, R. B., Sala, I., Coletti, D. A., Rodrigues, J. C. 2020. Incidence of Diaphorina citri Carrying Candidatus Liberibacter asiaticus in Brazil's Citrus Belt. Insects. 11:672.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., and Deng, X. 2016. Unusual Five Copies and Dual Forms of nrdB in "Candidatus Liberibacter asiaticus": Biological Implications and PCR Detection Application. Sci Rep. 6:39020.

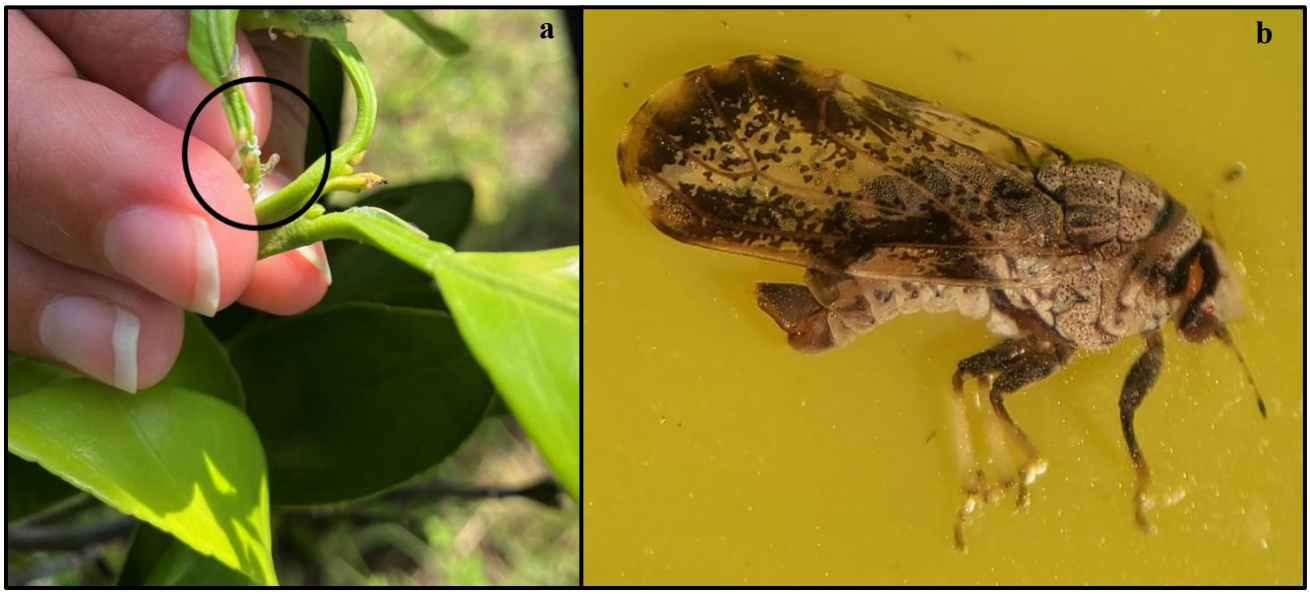

Figure 2.1. Morphological characteristics of nymphs (black circle; Fig. 2.1a) and adult (Fig. 2.1b) Asian citrus psyllid, *Diaphorina citri* Kuwayama found in a commercial citrus grove in Georgia in 2023.

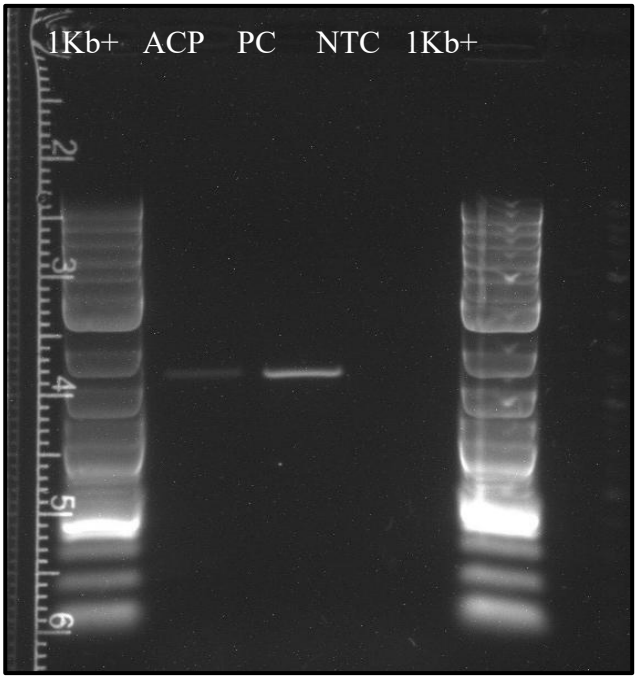

Figure 2.2. Gel electrophoresis (0.7%) visualization of the amplified 16S rDNA gene from CLas obtained from the Asian citrus psyllid (ACP) found in Georgia. PC: Positive control. NTC: non-template control (water).

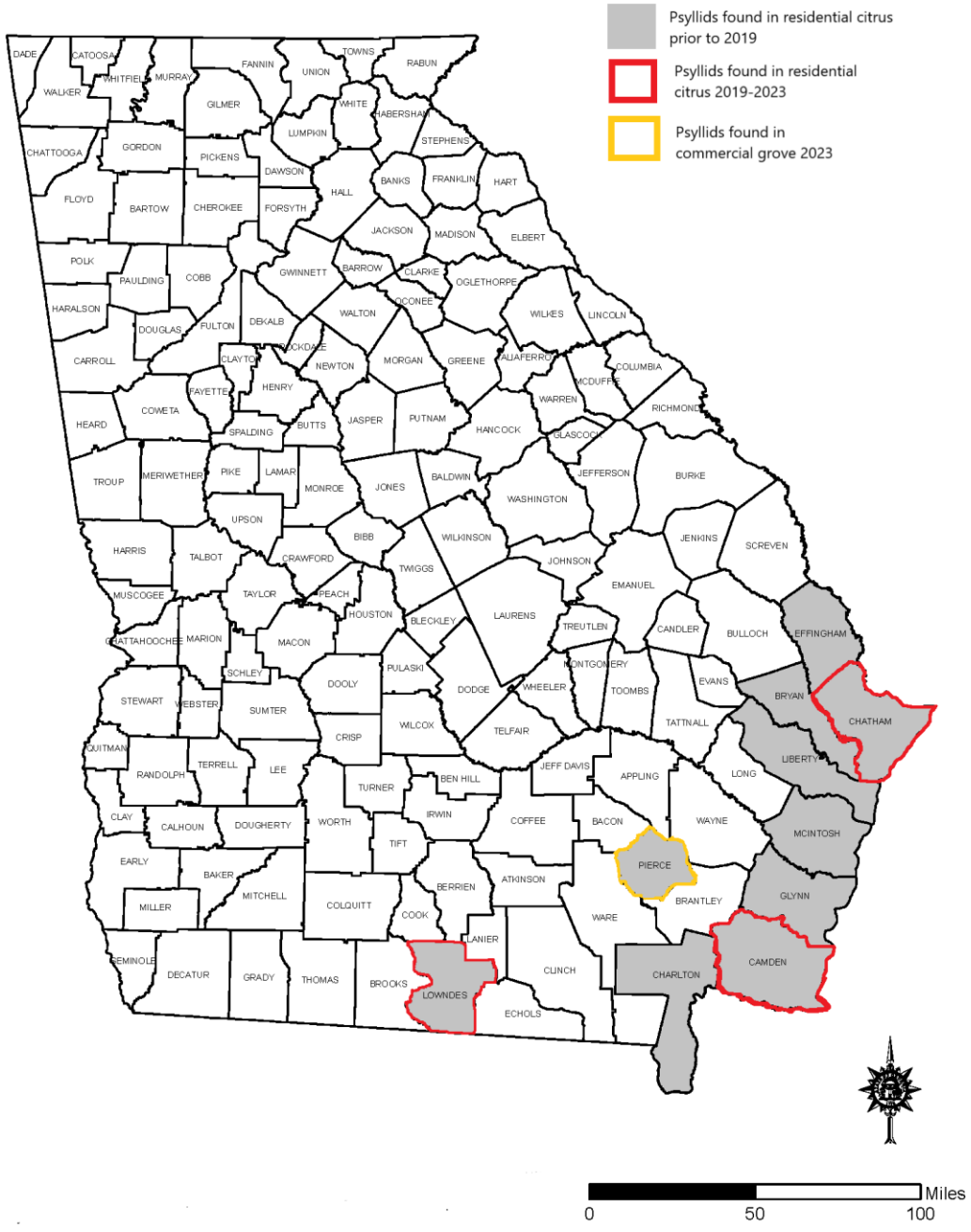

Figure 2.3. Map of Georgia showing the counties where the Asian citrus psyllid had been observed in residential trees prior to 2019 (gray color), residential trees during 2019-2023 (red outline), and in commercial citrus during 2023 (yellow outline).

CHAPTER 3

GROVE-LEVEL DETECTION AND GENETIC DIVERSITY OF *CANDIDATUS* LIBERIBACTER ASIATICUS IN GEORGIA'S COMMERCIAL CITRUS[1]

[1]Collins, C., Oliver, E. J., Barman A., Jimenez Madrid A.M. 2025. To be submitted to *Plant Disease.*

ABSTRACT

'Candidatus Liberibacter asiaticus' (CLas), the causal agent of Huanglongbing (HLB) or citrus greening disease, is a phloem limited bacteria transmitted by the Asian Citrus Psyllid (ACP). In North America, ACP is the only known vector of HLB, which is considered the most destructive disease affecting citrus. There are about 4,000 acres of commercial citrus planted in Georgia (GA), but the industry is growing rapidly. Regional sampling and testing efforts conducted between 2019 and 2023 have revealed that both CLas positive trees and ACP are present within commercial groves in GA. The goal of this study was to determine the distribution of CLas within selected commercial groves and to genetically characterize strains of CLas found in GA. Six commercial groves were selected for testing of CLas from plant tissue. Grove selection was based on previous detections of CLas and proximity to CLas-infected groves. From Fall 2023 to Fall 2024, 804 trees were tested via qPCR. Thirty-five of the 804 trees (4.35%) have been determined positive for CLas. Based on analysis of the CLas 16S rDNA region, no major genetic variations were observed; however, prophage typing revealed that five CLas strains had prophage type 1 and 2, while nine strains had type 1 only.

INTRODUCTION

'Candidatus Liberibacter asiaticus' (CLas) is a phloem limited, gram-negative alpha-proteo bacterium, and the main causal agent of HLB disease, also known as huanglongbing (HLB), in North America (Folimonova and Achor 2010; Tatineni et al. 2008). CLas is transmitted by the Asian citrus psyllid (ACP; *Diaphorina citri*) and has been described as the most destructive pathogen affecting citrus in the United States (Ghosh et al. 2018; Halbert and Manjunath 2004). HLB has led to losses of over 4.5 billion dollars in Florida, which was the world's largest citrus-producing region just two decades ago (Hodges and Spreen 2012).

Georgia has not been a large producer of citrus, however the production throughout the state has been steadily rising since 2018 when the number of new trees planted in the state increased from 29,000 to 71,000 (Oliver et al. 2020; Price 2023). Different factors have influenced the increasing interest in citrus production in Georgia including the development of cold hardy varieties and the perceived gap in the market due to the over 90% reduction in Florida's citrus production (USDA NASS 2005, 2025; Jake Price 2019). With this rapid increase, managing important diseases, including HLB, is essential. CLas was first detected in Georgia in 2009 and the state has since been under quarantine by the federal regulations (Animal Plant Health Inspection Service; APHIS 2009). A regional survey conducted from May to August 2019 reported CLas positive trees from residential areas in several counties throughout the state (Oliver et al., 2020). CLas has not been reported in commercial groves in Georgia and growers are required to plant trees that are free from CLas, however most of the commercial citrus groves are established in the southern region, often in proximity to Florida's northern border. The potential for infection to occur and even go unnoticed is high; therefore, detection of CLas will be essential to the survival and continued growth of the industry in Georgia. Additionally, the genetic diversity of CLas in Georgia is unknown and therefore characterizing the strains found in the state will provide insights into their potential geographic origin and will enable the monitoring of any genetic changes over time.

Characteristic HLB symptoms include asymmetrical chlorosis of leaves, known as blotchy mottling, fruit that is misshapen, lopsided, and unevenly ripened, aborted seeds, and stunted trees. These symptoms may appear months to years after the tree is initially infected with CLas (Coletta-Filho et al. 2014; Lee et al. 2015). Since symptoms such as chlorosis and stunting can often be caused by other diseases or abiotic factors such as micronutrient deficiency, visual identification

of symptoms is not a 100% effective method for diagnosis of the disease (Futch et al. 2009; Ghosh et al. 2018). Consequently, detection of CLas and diagnosis of HLB relies on molecular assays (Ghosh et al. 2018).

Detection assays for CLas, such as PCR and real time PCR, have generally utilized primers specific to target the 16S ribosomal regions (Chaves-Sierra et al. 2024; Jagoueix et al. 1996b; Li et al. 2006). In 1994, Jagoueix et al. developed the OI1/OI2c primer set to be used in PCR assays for CLas detection. A quantitative PCR (qPCR) assay was later developed by Li et al. (2006), which also utilized primers and probes specific to regions of the CLas 16S rDNA region. The 16S rDNA is a highly conserved region within bacteria species and sequencing of this region is frequently utilized to identify bacteria to the genus level (Janda and Abbott 2007). Additional genomic regions, such as the ribosomal protein genes, prophage genes, or intergenic regions have been explored for molecular detection of CLas (Morgan et al. 2012; Wang et al. 2006; Zheng et al. 2016). In recent years, Zheng et al. (2016) observed the presence of 5 copies of the RNR gene that offered greater sensitivity for CLas detection versus the 3 copies of the 16S rRNA. This highly sensitive protocol using the RNR gene has been adopted by USDA-APHIS for use in molecular detection of CLas via quantitative PCR.

Other diagnostic methods that have been developed for CLas include serological assays targeting secreted proteins, colorimetric LAMP (Loop-mediated isothermal amplification) kits and detection assays utilizing CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas12 systems (Pagliaccia et al. 2017; Stolowicz et al. 2022; Wheatley et al. 2021). However, these assays are not widely adopted, and qPCR remains the standard method for CLas detection. Because of its devastating nature, CLas is regulated by the USDA Animal Plant Health Inspection

Service (APHIS) and plant diagnostic laboratories in the US are required to be certified for routine testing of plant material.

Management options are very limited, and there is no cure once the trees are infected with CLas (Folimonova et al. 2009; Grafton-Cardwell et al. 2013). Prevention is the best strategy, and growers are encouraged to plant disease free trees from USDA certified nurseries (USDA APHIS 2025). Removal of infected trees is highly recommended, as is the routine spraying of insecticides for psyllid control (Ghosh et al. 2022; Grafton-Cardwell et al. 2013). In an effort to combat the disease, alternative management options have been explored including the use of thermotherapy, antibiotics, and foliar nutritional programs (Li et al. 2020). Unfortunately, none of the strategies evaluated by Li et al (2020) were determined to be cost-effective, while the only management option that remains effective in that study was the use of insecticides. Antibiotics are rarely used and/or labeled for crop protection due to concerns of resistance development and high costs (Roldán et al. 2023; Sundin and Wang 2018). However, antibiotics such as oxytetracycline and streptomycin sulfate were approved for use on citrus in Florida in 2016 and have been adopted as an “expensive” therapeutic measure against CLas (Ghosh et al. 2022, 2018).

HLB was first confirmed in Florida in 2005, followed by Louisiana in 2008, Georgia in 2009, Texas and California in 2012, Alabama in 2017 and most recently in Arizona in 2024 (Halbert 2005; Kumagai et al. 2013; Kunta et al. 2012; Mayo 2017; Singh 2014; Wang and Trivedi 2013). Since its initial confirmation, the HLB incidence has risen significantly in Florida with estimates of up to 100% of trees infected (Singerman and Useche 2019; Wang 2019). In California, the disease remains restricted to the Los Angeles basin in Los Angeles, Orange, and Riverside counties, and has mostly been reported in residential areas (Graham et al. 2020). In Texas, HLB was first confirmed in two commercial groves in 2012 and in seven additional groves in 2013

(Sétamou et al. 2020). However, a survey conducted in 2017 reported that 38.7% of trees tested were infected with CLas (Graham et al. 2020; Sétamou et al. 2020). HLB was also confirmed for the first time in Arizona in 2025 after citrus leaves and ACPs from a residential tree in Nogales were tested for CLas (Cooper 2025).

CLas was first classified as a bacterial like organism (BLO) due to the inability of researchers to complete Koch's postulates with this organism (Garnier 1983; Jagoueix et al. 1994). Since then, it has been grouped as a *Candidatus* Liberibacter species, since it is unable to be cultured in artificial media and thus difficult to characterize (Jagoueix et al. 1994). CLas has been reported in more than 40 countries from multiple hosts, mostly belonging to the Rutaceae family (Ghosh et al. 2022). The complete genomes of 183 strains of this species have been published and are available in the National Center for Biotechnology Information (NCBI) database. Since most CLas genomes sequenced have had very low variation within conserved regions, which are generally used to identify diversity within bacterial species, many studies have relied on analysis of sequences from a combination of polymorphic genetic markers to differentiate strains. These include short tandem repeats, single nucleotide polymorphisms (SNPs), microsatellite markers, hypervariable genomic regions (HGR) within chromosomal and prophage regions and the 16S/23S ribosomal spacer regions (Adkar-Purushothama et al. 2009; Dai et al. 2019; De Leon et al. 2024; Gao et al. 2022; Islam et al. 2012; Katoh et al. 2011).

In the United States, prophage typing has been conducted for strains found in Texas, California, and Florida (Dai et al. 2019; De Leon et al. 2024). The prophage system has been used to infer potential origin and determine any relationships between strains based on location and host (Dai et al. 2019; Gao et al. 2022). Prophages have also been associated with aspects of ecological adaptation, host selectivity, and pathogenicity (Gao et al. 2022). The proposed prophage type (type

1, type 2, type 1-2 and type 1-3) is based on the presence of one prophage or a combination of prophages based on identification by sequencing. The phage structural gene is used to identify type 1, while the endolysin gene is used to identify phage type 2 and the *hsdR* gene to identify phage type 3 (Dai et al. 2019; Gao et al. 2022). Dai et al. 2019 analyzed the complete genome sequences of 10 isolates from California and found that 60% had type 1, 20% had type 2, 10% had type 1-2, and 10% had type 1-3. A similar study, which used phage-specific primers to identify phage types in Florida and Texas found that 98.1% and 95.7% of isolates from Florida and Texas, respectively, were type 1-2 (De Leon et al. 2024). These findings suggest that CLas strains in Texas may have originated from Florida (De Leon et al. 2024). Genetic characterization has not been previously performed for CLas strains found in Georgia. Therefore, conducting this characterization will provide valuable information to aid in understanding both the potential origin of CLas in Georgia and the distribution and movement of CLas throughout the state.

MATERIALS AND METHODS

Collection of Leaf Tissue from Citrus Groves

To determine the incidence of HLB within commercial groves in Georgia, a total of six groves were selected for sample collection in the Fall of 2023 and Spring and Fall of 2024. Leaf samples were collected in September 2023 from groves located in Pierce (P1), Wayne (W1) and Ware (WR1) counties (**Table 3.1**). Leaf samples were collected again on April 23, 2024, from the same groves, Pierce (P1), Wayne (W1), and Ware (WR1), with the addition of another Pierce County grove (P2), adjacent to P1, and a grove in Bacon County (B1) (**Figure 3.5**). Finally, on October 11, 2024, samples from P2, W1, and a second grove in Wayne (W2) were collected. These locations were chosen on the basis of either having a CLas positive sample in testing during

previous years or due to their proximity to previously detected CLas-positive trees (Oliver, *personal communication*). A small number of additional samples received by the Molecular Diagnostics Lab (UGA-Tifton) during 2024 from residential citrus were also included in this study.

Within each commercial grove, all trees were visually assessed for HLB-related symptoms such as yellow veins, corky veins or leaf mottling prior to determining the sampling approach. For example, in Fall 2023, all trees from P1 were sampled due to the presence of multiple trees with potential HLB symptoms at this site, while in W1 and WR1 leaf samples were arbitrarily targeted and collected (**Table 3.1**). Two surveyors, one on each side of the tree canopy, collected at least 12 leaves from each tree by carefully cutting the leaves using classic steel bypass hand pruners (Fiskars, Finland) and pooling the sample in plastic zipper bags. Clippers were sanitized (sprayed) with 70% ethanol between cuttings to prevent cross-contamination. Pooled samples were placed in plastic Ziploc® (26.8cm x 27.3 cm) labeled with a unique sample identifier (row and tree number), collection date, and grove location. Samples were stored in coolers for transportation to the UGA Plant Molecular Diagnostic Laboratory in Tifton, Georgia. Samples were kept in a refrigerator until processed.

DNA Extraction from Leaf Tissue

DNA was extracted following the approved USDA-APHIS protocol for CLas testing. Briefly, midribs and petioles were removed from leaves (n=12) and chopped into pieces measuring 1-2 mm with a sterile razor blade. For each sample, 200 mg were weighed and transferred into Lysing matrix A tubes (Qbiogene, San Carlos, CA) and an additional ceramic bead was added. DNA was then extracted using a Qiagen Plant Mini Kit (Qiagen, Germantown, MD) following the manufacturer recommendations, except that twice the volume of RNase A and Buffer AP1 were utilized, as instructed in the USDA-APHIS protocol. Each tube containing DNA was labelled with

the sample ID for identification throughout processing and subsequent testing. To assess DNA quality, DNA concentration and nucleic acid A_{260}/A_{280} ratio were measured using the Nanodrop Lite (Thermo Fisher Scientific Inc, Wilmington, DE) and recorded before storage at -20 °C.

CLas Detection via quantitative PCR

Extracted DNA was tested for CLas via (qPCR) using a Bio-Rad CFX9 (Bio-Rad, Hercules, CA). The ribonuclease reductase (RNR) gene was used to detect CLas (Zheng et al., 2016) and the mitochondrial cytochrome oxidase gene (COX) gene was used as an internal control for citrus. Primers and probes (**Table 3.2**) specific to the RNR gene (RNR-1F, RNR-1R, RNR-P) and the COX gene (COXf, COXr, COXp) were used to create a primer-probe mix by combining 20 µL of 100 µM stock of each primer, 10 µL of each probe, in 900 µL of total volume with molecular grade (MG) water.

For each reaction, 12.5 µL of 2X pre-made master mix (PerfeCTa MultiPlex qPCR SuperMix Low ROX Quantabio), 7.5 µL of MG water, 3 µL of the primer probe mix and 2 µL of DNA were combined for a total reaction volume of 25 µL. For each reaction, a non-template (MG water) and a positive control (provided by USDA-APHIS) were included.

The cycling parameters of the qPCR reaction included an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 3 s and annealing/extension at 60°C for 40 s. The threshold cycles (Cq) in the HEX (Hexachloro-fluorescein) channel, used for COX, was established at 30 relative fluorescence units (RFU), while FAM (6-Carboxyfluorescein), used for RNR, was set up at 94 RFU. After the internal control (COX amplification), positive and non-template controls were validated based on their expected amplification or lack thereof, and the Cq values for samples were evaluated to determine if they were positive or negative. Samples with Cq values ≤ 38 in the FAM channel were considered positive for CLas. Samples with a Cq value >38

were considered questionable, and the assay was repeated once to obtain a second Cq value. If the Cq value was consistently <40 in the repeated run, the sample was considered positive (**Figure 3.4**). A Cq value >40 for FAM was considered negative for CLas. Samples with a positive outcome (Cq value >38) were confirmed positive for CLas in two individual (repeat) runs. DNA aliquots (20 ul) from the positive samples were also sent to the Plant Pathogen Confirmatory Diagnostics Laboratory- USDA (Laurel, MD) for final confirmation, as indicated in the HLB testing certification agreement. For Fall 2024 sample testing (*n*=474), samples with Cq values < 35 were provisionally considered positive until further confirmatory testing can be performed on the remainder of samples with Cq values >35≤38 (*n*=168).

Growers and county extension agents were notified of positive trees for management recommendations (tree removal). If groves were visited before trees were removed, a second sampling was done targeting CLas positive trees. These samples were collected and tested using the same methods as described above to determine if sampling times would provide consistent diagnoses (Table 2.5).

Genetic characterization of CLas via 16S Sequencing

To identify the genetic characteristics of CLas strains from Georgia, a conventional PCR was performed to amplify the 16S rDNA of positive CLas samples using the primer pair OI1/OI2c, which amplifies a 1,160 bp region (Jagoueix et al. 1996). The PCR mixture contained 12.5 µL of Go-Taq green master mix 2X (Promega, Madison WI), 9.5 µL of MG water, 1 µL of each forward and reverse primers (10 µM) and 1µL of DNA for a total of 25 µL. PCR was completed with 35 cycles of 92°C for 30 s, followed by 54°C for 30 s, and 72°C for 90 s performed on a T100 Thermocycler Machine (Bio-Rad Laboratories, Singapore) .

To confirm successful amplification, the PCR product was visualized on a 0.7% agarose gel using Gel-Red nucleic acid stain (10,000x stock reagent, Biotium, Fremont, CA) (Adkar-Purushothama et al. 2009; Jagoeix et al. 1996a). Gel electrophoresis was carried out in 1X Tris Borate EDTA (TBE) buffer for 90 min at 90 V and visualized under UV light with the Benchtop UVP Transilluminator (Analytik Jena US, Upland CA). The image was captured using Visionworks software version 4.16. Once amplification was confirmed, PCR products were cleaned up using ExoSAP-IT (ThermoFisher Scientific, Wilmington, DE) according to the manufacturer's instructions. Purified products were Sanger sequenced by Eurofins Genomics (Eurofins genomics, Louiseville, KY). Sequence reads were analyzed and trimmed, and consensus sequences were assembled using Geneious Prime Program (version 2024.0.7; GraphPad software, LLC., Boston, MA). Multiple alignment was conducted using the MUSCLE alignment function. Sequence data were compared to sequences published in GenBank (ncbi.nlm.nih.gov/genbank/) using the Basic Local Alignment Search Tool (BLAST) function. Reference sequences used in the analyses were selected from previously published strains deposited in GenBank database. Sequences included strains from Florida (DQ471900; EU265646), California (JX455745), Louisiana (FJ750458), and Georgia (ACP; PP829198) as well as strains from Mexico (MK031940), Cuba (OQ892130), Colombia (MG976240), India (OK562742), Iran (KY990821) and China: Hainan (ON080846).

Genetic characterization of CLas via Phage Identification and Typing

All CLas isolates were also genetically characterized via prophage sequence analysis. Primer pairs T1-2F/R, T2-2F/R, and 891-2F/R (**Table 3.3**) were used to amplify prophage regions to identify phage type 1, type 2 and type 3, respectively (De Leon et al. 2024; Zheng et al. 2016, 2018). Three individual PCR assays were conducted, one for each primer pair, as follow: mixtures

were created by adding 0.5 µL of each primer (10 µM), 12.5 µL of Go-Taq green master mix 2X (Promega, Madison WI), 9.5 µL of MG water and 2 µL of DNA for a total reaction mixture of 25 µL. PCR was completed in a Bio-Rad T100 Thermocycler (Bio-Rad, Hercules, CA) using the following program: 3 minutes at 94°C; 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 minute; and then a final extension at 72°C for 10 minutes. The PCR products from these reactions were separated and stained via gel electrophoresis as described above on a 1% agarose gel. DNA amplification was visualized under UV light with the Benchtop UVP Transilluminator (Analytik Jena US, Upland CA) and the image was captured with a Visionworks software version 4.16 (**Figure 3.1**).

An amplification observed with any of the primer sets was considered to contain that specific phage type (**Figure 3.1**). PCR products were cleaned and sequenced as described above. Sequence reads were analyzed and trimmed, and consensus sequences were assembled with the De Novo Assemble function using Geneious Prime Program (version 2024.0.7; GraphPad software, LLC., Boston, MA). Sequence data were compared to the reference sequences deposited in GenBank database. Sequences used as reference included SC1 (Type 1) and SC2 (Type 2) (GenBank accession numbers NC_019549 and NC_019550, respectively) of strain UF506 (Zhang et al. 2011). In addition, sequences from Brazil (PQ160487), India (MN650714; MN650715) and China (PP116527) were included. A phylogenetic tree was generated in Geneious Prime using the Tamura-Nei genetic distance model with a bootstrap method with 1000 replicates. The support threshold was 90%.

RESULTS

Incidence of CLas in commercially-grown citrus trees

Leaf samples from six commercial groves from Pierce, Wayne, Ware and Bacon counties and one diagnostic sample (residential tree from Lowndes County) were included in this study. A total of 140 leaf samples were first collected and tested in Fall of 2023 from WN (n=23), P1 (n=111) and WR1 (n=6) commercial groves. From those, two samples tested positive for CLas, one from P1 and another from WR1. Therefore, the incidence of CLas from Fall 2023 sampling was 1.4% (2 of 140). Of the 239 leaf samples collected in the Spring of 2024, three of 72 tested positive from WR1 and three of 139 from P2. None of the samples from P1 (n=14), BA (n=10) or WN (n=4) tested positive for CLas. Accordingly, the incidence from Spring 2024 sampling was 2.5% (6 of 239). Finally, in the Fall of 2024, of the 474 trees that were sampled from WN (n=98), P2 (n=365) and WN2 (n=11), 27 (5.69%) tested positive for CLas. Of those positive samples, seven were collected from P2 and 20 from WN. While 853 samples were collected, with some trees being tested twice, 804 trees were tested from all six commercial groves, and 35 (4.35%) tested positive for CLas (**Table 3.4**). No positive trees were observed among those tested from Bacon County.

Throughout this study, CLas positive trees were found within four groves from Pierce, Wayne and Ware counties (**Table 3.4**). From P1, one of 111 trees tested positive (0.9%). For P2, 10 of 501 (2 %) tested positive. For WR1, four of 72 (5.6%) tested positive. Finally, for W1, 20 of 99 (20.2%) tested positive. No infected trees were found among those sampled from W2 and B1 in the Spring and Fall of 2024 (**Table 3.4**).

Location of CLas positive trees within commercial groves

Between Fall 2023, Spring 2024 and Fall 2024, all trees within P1, P2, WN and WR1 were tested at least once. The only positive tree in P1 occurred 4 rows into the grove (**Figure 3.2C**), however in P2 the 10 positive trees occurred in three clusters, two of which were on the edge of the grove (**Figure 3.2D**). This trend was also true in the Ware County grove where all four positive trees were adjacent to each other on one edge of the grove (**Figure 3.2A**) and in Wayne County where the two positive trees with Cq values <30 were seperated by just one row, also along the edge of the grove (**Figure 3.2B**).

Seven of the eight samples that tested positive for CLas by qPCR between Fall 2023 and Spring 2024 were successfully confirmed via conventional PCR targeting a portion of the 16S rDNA region and visualized via gel electrophoresis. Sample WA41, which had an average Cq value of 36.23 (**Table 3.7**), did not amplify when the PCR product was visualized. Nevertheless, USDA-APHIS Plant Pathogenic Confirmatory Diagnostic Laboratory (PPCDL) confirmed that all 8 samples were positive for CLas via positive amplification of qPCR assays targeting the 16S rRNA gene, Ribonuclease reductase (RNR) and Heat Shock Protein (HSP) genes (**Table 3.8**). In Fall 2024, there were 27 positive samples, however only seven had average Cq values <30 (**Table 3.5**). Six of those seven were successfully confirmed via conventional PCR targeting 16S rDNA and visualized via gel electrophoresis (**Figure 3.7**). Overall, 13 of the 35 positive trees from commercial groves were able to be confirmed via conventional PCR and sequencing using the primer pairs OI1/I2C targeting the 16S region. In addition, the only sample from a residential tree was also confirmed positive via conventional PCR and sequencing using the primer pairs OI1/I2C targeting the 16S region (**Table 3.5).**

Genetic diversity and CLas Characterization based on 16S sequence analysis

To genetically characterize CLas, the 16S region of the DNA from 13 positive trees from commercial groves and 1 positive tree from a residential tree was sequenced. The obtained consensus sequence from all positive samples were >99.7% identical (100% query coverage) to multiple publicly available CLas sequences based upon comparisons using the BLASTn function in the NCBI database. Consensus sequences were compared with strains of CLas from California, Florida, China, Mexico, Cuba, Colombia, Iran, and India. The sequence of the ACP recovered from the residential tree that was confirmed positive for CLas (Collins et al. 2025) was also included in the analysis. Analysis of the sequencing data revealed there were no differences in the 16S region (~1,200 bp) of the strains compared, except for the sequence of the CLas infected ACP, which had 2 single nucleotide polymorphisms (SNPs) occurring at 168 bp and at 208 bp (**Figure 3.6**).

Prophage diversity based on prophage type associated with each strain

Gel electrophoresis to determine prophage types revealed that in Fall 2023, the strain from WR1 had type 1-2, while the strain from P1 had type 1 only. In Spring 2024, all three strains from P1 had phage type 1, while the two strains from WR1 were type 1-2, and type 1, respectively. The additional strain from WR1 was not able to be amplified with any prophage primer set and therefore no phage type was assigned. For Fall 2024, P2 had four strains with type 1, one with type 1-2, and two which didn't have any type assigned due to lack of amplification. One strain from W1 had phage type 1-2, but the remaining nineteen strains did not have any phage type amplification (**Table 3.5**). The strain from the residential tree had phage type 1-2. In total, there were nine isolates with type 1 (P1=1, P2=7, WR1=1) and five with type 1-2 (WR1=2, W1=1,

P2=1, R=1). Phage type 3 was not detected in any of the strains from Georgia. Percentage of each phage type detected was calculated and compared to phage type detected in Florida, Texas and California (**Table 3.6**). Phylogenetic analysis confirmed the prophage typing results obtained with the conventional PCR using the prophage specific primers. However, strains 3P2018, 3P1922 and WA61 had similar sequence reads to the reference strain from Brazil and are clustered in the same group (**Figure 3.3**). Those three strains have a few SNPs when compared to the Florida strain UF506 used as a reference.

DISCUSSION

Our results indicate that even though CLas has been found in commercial groves since 2022 (Oliver, *personal communication)*, the disease is not yet widespread within those commercial groves. Only 4.35% (35/804) of trees tested were determined to be positive for CLas with the majority of these being detected in Pierce and Wayne counties. This contrasts with Florida where the disease is considered endemic with the most recent estimates nearing 100% incidence (Futch et al. 2009; Wang 2019). This was, however, not always the case in Florida, and in the years following the first reported positive HLB trees in Florida in 2005, HLB incidence remained low. In 2008 the incidence was reported between 1.6% and 2.3% based on surveys sent to growers (Irey et al. 2011; Graham et al. 2020), while 6.4% was reported later in 2009 (Irey et a. 2011). Disease incidence increased dramatically to 43.3% by 2011, although it is unclear whether these trees were tested via qPCR and the threshold utilized to determine positive detection of CLas (Graham et al. 2020; Irey et a. 2011). Unfortunately, this rapid rise is a clear example of how HLB can spread if not detected and managed properly.

By contrast, in California, CLas infection has been contained in several parts of the state and has had very low incidence within commercial groves since the first HLB observation in 2012 (Futch et al. 2009). Testing efforts in California on those initial surveys also utilized qPCR assays with Cq value <32 being considered CLas positive (Graham et al. 2020). This threshold was increased to 36.99 by the California Department of Food and Agriculture (CDFA) and USDA-APHIS in 2018 (Graham et al. 2020). Current USDA-APHIS PPQ protocols established for CLas testing have a Cq threshold of 38, which is more sensitive than earlier protocols. The more stringent threshold used in early testing may have led to under-reporting of positive trees during early testing effort. The differences observed in the incidence rate between Georgia and other states may be due to environmental conditions, citrus varieties planted in Georgia, and the distribution of the Asian citrus psyllid throughout Georgia. The first ACP was reported in a commercial grove in Pierce County in 2023, the same grove referred to as P1 in this study (Collins et al. 2025). Our study found that the majority of CLas positive trees with Cq <30 (n=9 of 15) were from groves in Pierce County (P1 and P2). It is plausible that ACPs within these groves are actively transmitting CLas leading to greater incidence of HLB. Another consideration is that these groves have likely been infected with CLas for longer leading to higher titer of CLas and therefore, lower Cq values.

Sampling methods including tissue used and timing of sampling are decisive factors that influence detection of CLas (Braswell et al. 2020; Hajeri and Yokomi 2020). CLas moves through the phloem of plants to sink tissues including roots, shoots and fruits resulting in varying bacterial titers in each tissue (Hajeri and Yokomi 2020). The current standard is to use leaf midribs and petioles, however recent studies have shown that root sampling may provide greater sensitivity, consistency, and accuracy for CLas detection than other parts of the plant (Braswell et al. 2020; Johnson et al. 2014). Additionally, CLas titer has been shown to vary depending on the season or

timing of sampling (Hajeri and Yokomi 2020). This results in inconsistent detection, particularly in trees where CLas titer is low, as we observed for sample WA41, which had an average Cq of 36.23 after sampling in the Spring of 2024 and no Cq value (indicating no amplification) when sampled again just a few weeks later. It may be possible that trees with a low bacterial titer, depending on when they were sampled, may test negative but still may serve as an inoculum source for ACP and allow transmission of CLas. Hence, sampling time and tissue selection may need to be evaluated for future survey efforts in Georgia.

The location of the positive trees within groves provides valuable insights into the transmission of CLas, especially at this early stage of transmission in the state. We found that most of the CLas-infected trees were located along the edges of groves in Ware, Pierce and Wayne counties. These trees were often in clusters or "hotspots" and may be influenced by the "edge effect" observed by Gottwald et al. (2008); and Sétamou and Bartels (2015) where ACP populations were consistently higher on border trees within groves. The edge effect has been partly attributed to the movement of ACP into groves, which starts on the borders and may indicate the point of introduction of external inoculum (Sétamou and Bartels 2015). In P2 where there were 3 separate "hotspots", two occurred along a pathway, which is consistent with observations that CLas infected trees occur adjacent to any "internal voids" and suggests that limiting such voids may reduce CLas incidence within a grove (Gottwald et al. 2008). Targeted management along the edges of fields has also been suggested as a potential strategy (Sétamou and Bartels 2015). These considerations may be especially valuable in Georgia where new plantings are currently expanding.

Since Georgia is a new commercial market for citrus, it is essential to understand the genetic diversity of CLas to identify any differences that may necessitate a tailored approach to

HLB management in Georgia. Prophage regions within CLas have been used to identify and delineate strain origins to track transmission of CLas. Regarding prophage grouping types, four group types have been identified in the United States, including Type 1, Type 2, Type 1-2 and Type 1-3. In this study, we observed that prophage types varied, particularly in the case of P1 and P2. In P2, seven out of eight strains found in that grove contained prophage typing group (PTG) 1 only and the remaining strain contained PTG 1-2. In P1, the only strain recovered contained PTG1. Overall, 89% of CLas strains recovered for Pierce County were PTG1 only.

Four CLas strains, from Ware and Wayne counties, belong to PTG1-2. Similarly, the only CLas strain from a residential tree included in this study, from Lowndes County, was PTG1-2 (**Table 2.5**). There seems to be some uniformity among prophage types geographically as the only strain outside of Pierce County that was PTG-1 only was from the Ware County grove, which was less than 3 miles away from the Pierce County groves. These observations suggest that transmission of CLas between groves in Georgia is low, if it is happening at all, though further research would have to be conducted to confirm this hypothesis.

Strains of CLas from Georgia only contained prophage type 1 and type 2, with most of the strains having type 1 only. This is consistent with findings in Florida and Texas where only these two prophage types have been found, which alludes to the possibility of relatedness of these strains (De Leon et al. 2024). The ratios of prophage types in Georgia were compared to ratios from Florida, Texas and California (De Leon et al. 2024; Dai et al. 2019). However, it is important to emphasize that these studies were focused on small sample sizes and therefore, may not be representative of entire states. In addition, CLas strains in our study were found from citrus trees, while in those studies they used CLas strains from ACP and plant tissue, including roots (De Leon et al. 2024; Dai et al. 2019). Nevertheless, comparison of these ratios reveals that Georgia has a

higher percentage of isolates that are type 1 only (64.3%), compared to Florida (1.1%) and Texas (3.5%), while no strains in Georgia were detected with type 1-3 prophage and that prophage has only been reported in California.

CONCLUSIONS

CLas was first reported in Georgia in 2009, and the entire state has since been under USDA quarantine for HLB. Commercial citrus production has grown rapidly over the last 8 years, increasing from less than 100,000 trees planted in 2018 to an estimated 600,000 trees planted in 2023 (Price 2023). In 2022, two commercial groves were confirmed to have CLas infected trees prompting this work (Oliver, *Personal Communication*). Between Fall 2023, spring 2024 and Fall 2025 a total of 804 trees were tested via qPCR from 6 groves in Ware, Wayne, Pierce and Bacon Counties. Thirty-five trees (4.35%) from two groves in Pierce, 1 in Ware and 1 Wayne Counties Georgia were CLas positive based on being below the Cq threshold. CLas positive trees occurred in clusters along the edges of groves. These findings suggest that CLas, while present within commercial groves, occurs at a relatively low incidence and provides valuable clues regarding how introduction and transmission takes place. No major genetic differences were noted among isolates from Georgia based on 16S sequencing, except for two SNPs that occurred in a CLas strain from an infected ACP. Additionally, prophage typing revealed that 9 of 14 typed strains had prophage type 1 only, most of which were from Pierce County. The remaining 5 of 14 strains had both prophage types 1 and 2, including the only strain included from a residential tree in Lowndes County. Prophage types associated with Georgia CLas strains are also found in Florida and Texas, strengthening the assumption the CLas in Georgia was introduced from Florida. To safeguard citrus in Georgia, testing needs to continue and expand throughout Georgia's commercial groves

and further research needs to be conducted using novel approaches to elucidate the genetic diversity of CLas strains.

ACKNOWLEDGMENTS

We would like to thank the members of the UGA-MDL laboratory, and Dr. Oliver's and Dr. Barman's laboratory personnel for assisting with sample collection, as well as the County extension coordinator, Mr. Jake Price, and UGA Citrus Extension Specialist Dr. Mary Sutton. We also express our gratitude to the citrus growers for allowing us to sample their fields and to the Georgia extension agents for coordinating sampling. Funding for project was made possible by a grant/cooperative agreement from the U.S. Department of Agriculture (USDA) Agricultural Marketing Service. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

LITERATURE CITED

Adkar-Purushothama, C. R., Quaglino, F., Casati, P., Gottravalli Ramanayaka, J., and Bianco, P. A. 2009. Genetic diversity among ‘Candidatus Liberibacter asiaticus’ isolates based on single nucleotide polymorphisms in 16S rRNA and ribosomal protein genes. Ann. Microbiol. 59:681–688. https://doi.org/10.1007/BF03179208.

Animal Plant Health Inspection Service (APHIS). 2009. Confirmation of Citrus Greening in Chatham County, Georgia. https://www.aphis.usda.gov/sites/default/files/da-2009-26.pdf.

Braswell, W. E., Park, J.-W., Stansly, P. A., Kostyk, B. C., Louzada, E. S., Da Graça, J. V., and Kunta, M. 2020. Root samples provide early and improved detection of Candidatus Liberibacter asiaticus in Citrus. Sci Rep 10:16982. https://doi.org/10.1038/s41598-020-74093-x.

Chaves-Sierra, C., Rodriguez-Cruz, M. C., Mejia-Alvarado, F. S., Ramírez-Higuera, C., Mejía-Eslava, A., and Romero, H. M. 2024. Identification of ‘Candidatus Liberibacter asiaticus’, the Huanglongbing Bacterium, in Citrus from Colombia. Plant Disease 108:1169–1173. https://doi.org/10.1094/PDIS-10-23-2003-SC.

Coletta-Filho, H. D., Daugherty, M. P., Ferreira, C., and Lopes, J. R. S. 2014. Temporal Progression of ‘ Candidatus Liberibacter asiaticus’ Infection in Citrus and Acquisition Efficiency by Diaphorina citri. Phytopathology® 104:416–421. https://doi.org/10.1094/PHYTO-06-13-0157-R.

Cooper, D. 2025. HLB Confirmed in Arizona for First Time - Diseases Citrus Industry Magazine. Citrus Industry Magazine. https://citrusindustry.net/2025/03/07/hlb-confirmed-arizona-first-time/ (accessed 19 May 2025).

Dai, Z., Wu, F., Zheng, Z., Yokomi, R., Kumagai, L., Cai, W., Rascoe, J., Polek, M., Chen, J., and Deng, X. 2019. Prophage Diversity of 'Candidatus Liberibacter asiaticus' Strains in California. Phytopathology® 109:551–559. https://doi.org/10.1094/PHYTO-06-18-0185-R.

De Leon, V. S., Chen, J., McCollum, G., Park, J.-W., Louzada, E. S., Setamou, M., and Kunta, M. 2024. Diversity of 'Candidatus Liberibacter asiaticus' Strains in Texas Revealed by Prophage Sequence Analyses. Plant Disease 108:1455–1460. https://doi.org/10.1094/PDIS-09-23-1994-SR.

Folimonova, S. Y., and Achor, D. S. 2010. Early Events of Citrus Greening (Huanglongbing) Disease Development at the Ultrastructural Level. Phytopathology® 100:949–958. https://doi.org/10.1094/PHYTO-100-9-0949.

Folimonova, S. Y., Robertson, C. J., Garnsey, S. M., Gowda, S., and Dawson, W. O. 2009. Examination of the Responses of Different Genotypes of Citrus to Huanglongbing (Citrus Greening) Under Different Conditions. Phytopathology® 99:1346–1354. https://doi.org/10.1094/PHYTO-99-12-1346.

Futch, S., Weingarten, S., and Irey, M. 2009. Determining HLB Infection Levels using Multiple Survey Methods in Florida Citrus. .

Gao, F., Wu, B., Zou, C., Bao, Y., Li, D., Yao, W., Powell, C. A., and Zhang, M. 2022. Genetic Diversity of " Candidatus Liberibacter asiaticus" Based on Four Hypervariable Genomic Regions in China ed. Lindsey Price Burbank. Microbiol Spectr 10:e02622-22. https://doi.org/10.1128/spectrum.02622-22.

Garnier, M. 1983. Transmission of the Organism Associated with Citrus Greening Disease from Sweet Orange to Periwinkle by Dodder. Phytopathology 73:1358. https://doi.org/10.1094/Phyto-73-1358.

Ghosh, D. K., Motghare, M., and Gowda, S. 2018. Citrus Greening : Overview of the Most Severe Disease of Citrus. .

Ghosh, D., Kokane, S., Savita, B. K., Kumar, P., Sharma, A. K., Ozcan, A., Kokane, A., and Santra, S. 2022. Huanglongbing Pandemic: Current Challenges and Emerging Management Strategies. Plants (Basel) 12:160. https://doi.org/10.3390/plants12010160.

Gottwald, T., Irey, M., and Gast, T. 2008. The plantation edge effect of HLB: A geostatistical analysis. Proc. Int. Res. Conf. Huanglongbing 305–308.

Grafton-Cardwell, E. E., Stelinski, L. L., and Stansly, P. A. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annu Rev Entomol 58:413–432. https://doi.org/10.1146/annurev-ento-120811-153542.

Graham, J., Gottwald, T., and Setamou, M. 2020. Status of Huanglongbing (HLB) outbreaks in Florida, California and Texas. Trop. plant pathol. 45:265–278. https://doi.org/10.1007/s40858-020-00335-y.

Hajeri, S., and Yokomi, R. K. 2020. Reliable Sampling Tissue and Seasonality for Consistent Detection of ‘Candidatus Liberibacter asiaticus’ by qPCR. Curr Agri Res Jour 8:01–03. https://doi.org/10.12944/CARJ.8.1.01.

Halbert, S. 2005. The Discovery of Huanglongbing in Florida. .

Halbert, S. E., and Manjunath, K. L. 2004. Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) And Greening Disease Of Citrus: A Literature Review And Assessment of Risk in Florida.

Florida Entomologist 87:330–353. https://doi.org/10.1653/0015-4040(2004)087[0330:ACPSPA]2.0.CO;2.

Hodges, A. W., and Spreen, T. H. 2012. Economic Impacts of Citrus Greening (HLB) in Florida, 2006/07–2010/11: FE903/FE903, 1/2012. EDIS 2012. https://doi.org/10.32473/edis-fe903-2012.

Irey, M. S., Morris, R. A., and Estes, M. 2011. Survey to Estimate the Rate of HLB Infection in Florida Citrus Groves. In 2nd International Research Conference on Huanglongbing, , p. 73.

Islam, M.-S., Glynn, J. M., Bai, Y., Duan, Y.-P., Coletta-Filho, H. D., Kuruba, G., Civerolo, E. L., and Lin, H. 2012. Multilocus microsatellite analysis of "Candidatus Liberibacter asiaticus" associated with citrus Huanglongbing worldwide. BMC Microbiol 12:39. https://doi.org/10.1186/1471-2180-12-39.

Jagoueix, S., Bové, J. M., and Garnier, M. 1996a. PCR detection of the two "Candidatus" Liberobacter species associated with greening disease of citrus. Mol Cell Probes 10:43–50. https://doi.org/10.1006/mcpr.1996.0006.

Jagoueix, S., Bové, J. M., and Garnier, M. 1996b. PCR detection of the two«Candidatus»liberobacter species associated with greening disease of citrus. Molecular and Cellular Probes 10:43–50. https://doi.org/10.1006/mcpr.1996.0006.

Jagoueix, S., Bove, J.-M., and Garnier, M. 1994. The Phloem-Limited Bacterium of Greening Disease of Citrus Is a Member of the α Subdivision of the Proteobacteria. International Journal of Systematic and Evolutionary Microbiology 44:379–386. https://doi.org/10.1099/00207713-44-3-379.

Jake Price. 2019. Maintaining Commercial Citrus in Georgia. .

Janda, J. M., and Abbott, S. L. 2007. 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. J Clin Microbiol 45:2761–2764. https://doi.org/10.1128/JCM.01228-07.

Johnson, E. G., Wu, J., Bright, D. B., and Graham, J. H. 2014. Association of 'Candidatus Liberibacter asiaticus' root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. Plant Pathology 63:290–298. https://doi.org/10.1111/ppa.12109.

Katoh, H., Subandiyah, S., Tomimura, K., Okuda, M., Su, H.-J., and Iwanami, T. 2011. Differentiation of "Candidatus Liberibacter asiaticus" Isolates by Variable-Number Tandem-Repeat Analysis. Appl Environ Microbiol 77:1910–1917. https://doi.org/10.1128/AEM.01571-10.

Kumagai, L. B., LeVesque, C. S., Blomquist, C. L., Madishetty, K., Guo, Y., Woods, P. W., Rooney-Latham, S., Rascoe, J., Gallindo, T., Schnabel, D., and Polek, M. 2013. First Report of Candidatus Liberibacter asiaticus Associated with Citrus Huanglongbing in California. Plant Dis 97:283. https://doi.org/10.1094/PDIS-09-12-0845-PDN.

Kunta, M., Setamou, M., Skaria, M., Rascoe, J. E., Li, W., Nakhla, M. K., and Da Graça, J. V. 2012. First report of citrus huanglongbing in Texas. . https://www.apsnet.org/meetings/Documents/2012_Meeting_Abstracts/aps12abP393.htm (accessed 19 May 2025).

Lee, J. A., Halbert, S. E., Dawson, W. O., Robertson, C. J., Keesling, J. E., and Singer, B. H. 2015. Asymptomatic spread of huanglongbing and implications for disease control. Proc Natl Acad Sci U S A 112:7605–7610. https://doi.org/10.1073/pnas.1508253112.

Li, S., Wu, F., Duan, Y., Singerman, A., and Guan, Z. 2020. Citrus Greening: Management Strategies and Their Economic Impact. horts 55:604–612. https://doi.org/10.21273/HORTSCI14696-19.

Li, W., Hartung, J. S., and Levy, L. 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. Journal of Microbiological Methods 66:104–115. https://doi.org/10.1016/j.mimet.2005.10.018.

Mayo, D. 2017. Citrus Greening Confirmed in Alabama Panhandle Agriculture. https://nwdistrict.ifas.ufl.edu/phag/2017/06/30/citrus-greening-confirmed-in-alabama/ (accessed 14 Jul 2025).

Morgan, J. K., Zhou, L., Li, W., Shatters, R. G., Keremane, M., and Duan, Y.-P. 2012. Improved real-time PCR detection of 'Candidatus Liberibacter asiaticus' from citrus and psyllid hosts by targeting the intragenic tandem-repeats of its prophage genes. Molecular and Cellular Probes 26:90–98. https://doi.org/10.1016/j.mcp.2011.12.001.

Oliver, J. E., Ali, M. E., Waliullah, S., Price, J., Warren, J., Jacobs, J., Hoppers, A., Evans, R., Dowdy, M., and Curry, S. 2020. Huanglongbing, Caused by ' Candidatus Liberibacter asiaticus,' Detected in New Locations Across Southern and Coastal Georgia. Plant Health Progress 21:31–35. https://doi.org/10.1094/PHP-09-19-0064-S.

Pagliaccia, D., Shi, J., Pang, Z., Hawara, E., Clark, K., Thapa, S. P., De Francesco, A. D., Liu, J., Tran, T.-T., Bodaghi, S., Folimonova, S. Y., Ancona, V., Mulchandani, A., Coaker, G., Wang, N., Vidalakis, G., and Ma, W. 2017. A Pathogen Secreted Protein as a Detection Marker for Citrus Huanglongbing. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02041.

Price, J. 2023. Citrus Plantings in Georgia Continue to Increase. UGA Citrus Blog. https://site.caes.uga.edu/citrus/2023/09/citrus-plantings-in-georgia-continue-to-increase/ (accessed 29 Jan 2025).

Roldán, E. L., Stelinski, L. L., and Pelz-Stelinski, K. S. 2023. Foliar Antibiotic Treatment Reduces Candidatus Liberibacter asiaticus Acquisition by the Asian Citrus Psyllid, Diaphorina citri (Hemiptera: Liviidae), but Does not Reduce Tree Infection Rate. Journal of Economic Entomology 116:78–89. https://doi.org/10.1093/jee/toac200.

Sétamou, M., Alabi, O. J., Kunta, M., Dale, J., and da Graça, J. V. 2020. Distribution of Candidatus Liberibacter asiaticus in Citrus and the Asian Citrus Psyllid in Texas Over a Decade. Plant Disease 104:1118–1126. https://doi.org/10.1094/PDIS-08-19-1779-RE.

Sétamou, M., and Bartels, D. W. 2015. Living on the Edges: Spatial Niche Occupation of Asian Citrus Psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae), in Citrus Groves. PLoS One 10:e0131917. https://doi.org/10.1371/journal.pone.0131917.

Singh, R. 2014. Citrus Greening (Huanglongbing). Louisiana Plant Pathology Disease Identification Series. https://www.lsuagcenter.com/~/media/system/4/d/f/e/4dfec7eb84c03892627d36f35403bf98/pub3359citrusgreeningfinal.pdf (accessed 14 Jul 2025).

Singerman, A., and Useche, P. 2019. Impact of Citrus Greening on Citrus Operations in Florida. Ask IFAS - Powered by EDIS. https://edis.ifas.ufl.edu/publication/FE983 (accessed 9 Jun 2025).

Stolowicz, F., Larocca, L., Werbajh, S., Parma, Y., Carrillo, C., Ogas, L., Agostini, J. P., Redes, J., Welin, B., Castagnaro, A., and Vojnov, A. 2022. A colorimetric, sensitive, rapid, and

simple diagnostic kit for the HLB putative causal agent detection. Front. Agron. 4. https://doi.org/10.3389/fagro.2022.984360.

Sundin, G. W., and Wang, N. 2018. Antibiotic Resistance in Plant-Pathogenic Bacteria. Annual Review of Phytopathology 56:161–180. https://doi.org/10.1146/annurev-phyto-080417-045946.

Tatineni, S., Sagaram, U. S., Gowda, S., Robertson, C. J., Dawson, W. O., Iwanami, T., and Wang, N. 2008. In Planta Distribution of ‘ Candidatus Liberibacter asiaticus’ as Revealed by Polymerase Chain Reaction (PCR) and Real-Time PCR. Phytopathology® 98:592–599. https://doi.org/10.1094/PHYTO-98-5-0592.

USDA APHIS. 2025. Citrus Greening and Asian Citrus Psyllid | Animal and Plant Health Inspection Service. . https://www.aphis.usda.gov/plant-pests-diseases/citrus-diseases/citrus-greening-and-asian-citrus-psyllid (accessed 31 May 2025).

Wang, N. 2019. The Citrus Huanglongbing Crisis and Potential Solutions. Molecular Plant 12:607–609. https://doi.org/10.1016/j.molp.2019.03.008.

Wang, Z., Yin, Y., Hu, H., Yuan, Q., Peng, G., and Xia, Y. 2006. Development and application of molecular-based diagnosis for ‘Candidatus Liberibacter asiaticus’, the causal pathogen of citrus huanglongbing. Plant Pathology 55:630–638. https://doi.org/10.1111/j.1365-3059.2006.01438.x.

Wang, N., and Trivedi, P. 2013. Citrus Huanglongbing: A Newly Relevant Disease Presents Unprecedented Challenges. Phytopathology 103:652–665. https://doi.org/10.1094/phyto-12-12-0331-rvw.

Wheatley, M. S., Duan, Y.-P., and Yang, Y. 2021. Highly Sensitive and Rapid Detection of Citrus Huanglongbing Pathogen (‘Candidatus Liberibacter asiaticus’) Using Cas12a-Based

Methods. Phytopathology® 111:2375–2382. https://doi.org/10.1094/PHYTO-09-20-0443-R.

Zheng, Z., Bao, M., Wu, F., Van Horn, C., Chen, J., and Deng, X. 2018. A Type 3 Prophage of 'Candidatus Liberibacter asiaticus' Carrying a Restriction-Modification System. Phytopathology® 108:454–461. https://doi.org/10.1094/PHYTO-08-17-0282-R.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., and Deng, X. 2016. Unusual Five Copies and Dual Forms of nrdB in "Candidatus Liberibacter asiaticus": Biological Implications and PCR Detection Application. Sci Rep 6:39020. https://doi.org/10.1038/srep39020.

Table 3.1. Grove identity, location, size, and number of trees sampled in Fall 2023, Spring 2024 and Fall 2024.

County	Grove ID	Size of grove (ha)	# Trees within grove	Samples collected Fall 2023	Samples collected Spring 2024	Samples collected Fall 2024
Pierce	P1	0.42	111	111	14	*
Pierce	P2	0.85	501	*	139	363
Wayne	W1	0.39	99	23	4	99
Wayne	W2	0.39	88	*	*	11
Ware	WR1	0.26	72	6	72	*
Bacon	B1	1.13	--	*	10	*
Total				140	239	473

*Indicates that no leaf samples were collected from the grove in that sampling period.
--unknown number of trees.

Table 3.2 Primers and probes used for detection and characterization of CLas using qPCR and PCR.

qPCR Primers and Probes				
Primer/Probe	**Sequence 5'-3'**	**Target gene/phage**	**Size (bp)**	**Reference**
RNR-1F	CAT GCT CCA TGA AGC TAC CC	Ribonucleotide reductase	390	Zheng et al. 2016
RNR-1R	GGA GCA TTT AAC CCC ACG AA			
RNRP	5' 6FAM/CCT CGA AAT CGC CTA TGC AC/3' BHQ-1			
COXf	GTA TGC CAC GTC GCA TTC CAG A	Mitochondrial cytochrome oxidase	68	Li et al. 2006
COXr	GCC AAA ACT GCT AAG GGC ATT C			
COXp	5' HEX/ATC CAG ATG CTT ACG CTG G/3' BHQ-1			
Conventional PCR primers				
OI1	GCGCGTATGCAATACGAGCGGCA	16S rRNA gene	1160	Jagoueix et al. 1996
OI2c	GCCTCGCGACTTCGCAACCCAT	16S rRNA gene	1160	

Table 3.3. Phage-specific primers used for prophage typing of CLas

Phage Type	Primer name	Sequence 5'-3'	Target	Size (bp)	Reference
1	T1-2F	TGGCTCGGGTTCAGGTAAAT	Phage structural protein gene	975	Zheng et al. 2016
	T1-2R	AAGGGCGACGCATGTATTTC			
2	T2-2F	ACCCTCGCACCATCATGTTA	Endolysin	813	Zheng et al. 2016
	T2-2R	TCGTCTTGATTGGGCAGAGT			
3	891-2F	ACCGCGATCTACCCGTAATT	hsdR	884	Zheng et al. 2018
	891-2R	TGTGTTTTGCGAGTGAAGGG			

Table 3.4. Groves, counties, number of trees and incidence of *Candidatus* Liberibacter asiaticus in Georgia commercial citrus

County	Grove ID	# Trees within grove	CLas +ve Trees (%)[a]	CLas -ve Trees (%)[b]	Total Trees tested
Pierce	P1	111	1 (0.9%)	110 (99.1%)	111
Pierce	P2	501	10 (2.0%)	491 (98%)	501
Wayne	W1	99	20 (20.2%)	79 (79.8%)	99
Wayne	W2	88	0	11 (100%)	11
Ware	WR1	72	4 (5.56%)	68 (94.4%)	72
Bacon	B1	--	0	10 (100%)	10
Totals			35 (4.35%)	769 (95.65%)	804

[a]+ve= Positive. Percent incidence shown in parenthesis.
[b]-ve= Negative. Percent incidence shown in parenthesis.
-- unknown.

Table 3.5. DNA concentration, quality, Cq values and phage type of CLas positives samples from Georgia commercial citrus groves

Sample ID	Season	Grove ID	DNA (ng/µL)	A_{260}/A_{280}	Average Cq OG[b]	Average Cq RP[cd]	Phage Type[e]
P64	Fall 2023	P1	48.2	1.8	22.76	23.22	1
3P2018	Spring 2024	P2	113.3	1.74	36.15	23.13	1
P3R15T4	Fall 2024	P2	70.3	1.75	24.51	-	1-2
2P92	Spring 2024	P2	53.1	1.73	24.74	20.06	1
3P1922	Spring 2024	P2	89.9	1.8	27.12	22.27	1
P2R10T3	Fall 2024	P2	123.4	1.8	21.01	-	1
P3R21T20	Fall 2024	P2	147.8	1.73	21.12	-	1
P3R22T15	Fall 2024	P2	110.4	1.78	21.82	-	1
P3R21T21	Fall 2024	P2	215.5	1.78	22.04	-	1
WA42	Fall 2023	WR1	68.6	1.78	18.75	22.05	1-2
WA52	Spring 2024	WR1	75.4	1.77	22.38	21.57	1-2
WA61	Spring 2024	WR1	48.9	1.76	25.32	19.63	1
WN81	Fall 2024	W1	146.3	1.67	20.26	-	1-2
JP084	Spring 2024	R[a]	110.5	1.69	21.44		1-2
WN62	Fall 2024	W1	117.7	1.74	28.99	-	N/A
WA41	Spring 2024	WR1	62.1	1.67	36.23	N/A	N/A
P3R16T6	Fall 2024	P2	106.7	1.58	33.63	-	N/A
P3R20T14	Fall 2024	P2	163.4	1.52	35.16	-	N/A
WN42	Fall 2024	W1	80.4	1.72	32.09	-	N/A
WN44	Fall 2024	W1	70.4	1.75	32.97	-	N/A
WN51	Fall 2024	W1	91.6	1.82	33.07	-	N/A
WN45	Fall 2024	W1	39.4	1.74	33.36	-	N/A
WN211	Fall 2024	W1	58.3	1.77	33.52	-	N/A
WN39	Fall 2024	W1	48.2	1.76	33.77	-	N/A
WN36	Fall 2024	W1	55.8	1.78	33.89	-	N/A
WN31	Fall 2024	W1	100.4	1.7	34.15	-	N/A
WN85	Fall 2024	W1	91.3	1.81	34.16	-	N/A
WN34	Fall 2024	W1	131.9	1.73	34.2	-	N/A
WN33	Fall 2024	W1	59	1.77	34.24	-	N/A
WN38	Fall 2024	W1	75.3	1.76	34.49	-	N/A
WN92	Fall 2024	W1	78.8	1.65	34.71	-	N/A
WN35	Fall 2024	W1	93.4	1.79	34.88	-	N/A
WN47	Fall 2024	W1	52.7	1.68	34.9	-	N/A
WN43	Fall 2024	W1	117.3	1.77	34.95	-	N/A
WN310	Fall 2024	W1	52	1.78	34.99	-	N/A
WN510	Fall 2024	W1	103.1	1.8	35.23	-	N/A

[a]R sample was from a residential tree

[b]Average Cq value from original (first) samples collected.

[c]Average Cq value from repeated (additional) samples collected

[d] – indicates that trees were not sampled twice

[e] N/A no phage type amplification

Table 3.6. Comparison of prophage types of CLas strains collected from Florida, Texas, California and Georgia

State[a]	Type 1-2	Type 1	Type 2	Type 1-3	Total	Reference
Georgia	5 (35.7%)	9 (64.3%)	0 (0%)	0	14	This Study
Florida	623 (98.1%)	7 (1.1%)	5 (0.8%)	0	635	De Leon et al. 2024)
Texas	488 (95.7%)	18 (3.5%)	4 (0.8%)	0	510	De Leon et al. 2024
California	1 (10%)	6 (60%)	2 (20%)	1 (10%)	10	Dai et al. 2019

[a]GA: CLas isolated from leaves only. CA: CLas from leaves and ACP. FL and TX: CLas from leaves, roots and ACP.

CHAPTER 2: SUPPLEMENTAL FIGURES AND TABLES

Table 3.7. Cq values of original plant tissue collected in Fall 2023 from CLas positive trees and testing targeting the RNR gene.

Sample ID	Cq 1	Cq 2	Cq3	Cq 4	Cq 5	Average Cq
3P1922	27.36	28.01	26			27.12
P64	25.42	21.96	22.21	22.1	22.09	22.75
3P2018	36.73	37.11	34.61			36.15
2P92	24.86	25.55	23.82			24.74
WA61	25.68	26.4	23.88			25.32
WA52	22.66	23.34	21.14			22.38
WA41	37.95	35.47	35.28			36.23
WA42	18.3	18.99	18.96			18.75

Table 3.8. Cq for samples sent to USDA-APHIS PPCDL for confirmatory tests.

Target-channel:	**COX-VIC**	**16SG-JUN**	**RNR-FAM**	**HSP-ABY**	
Sample ID		Average Cq value			*n*=
3P1922	16.31	28.08	27.59	29.66	2
P64	16.22	22.94	22.20	24.62	2
3P2018	15.31	24.30	23.50	25.87	2
2P92	16.35	25.29	24.66	26.94	2
WA61	17.14	26.24	25.65	27.72	2
WA52	15.90	23.26	22.58	24.73	2
WA41	16.22	38.02	35.51	37.04	4

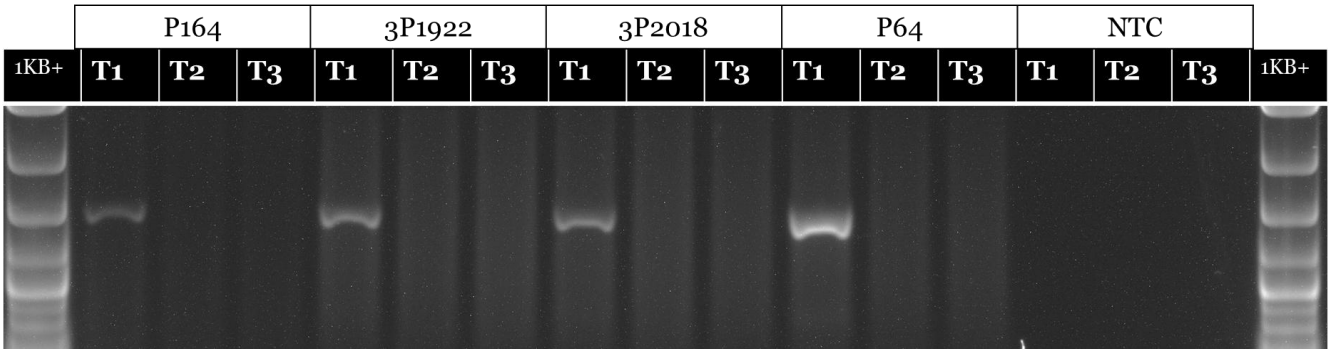

Figure 3.1. DNA amplification visualized on agarose gel (1%) for identification of prophage types (T1: type 1; T2: type 2; T3: type 3). Example of prophage type 1 amplifying (975 bp) in 4 CLas strains (P146, 3P1922, 3P2018 and P64) from Georgia. Non-template control (NTC; water)

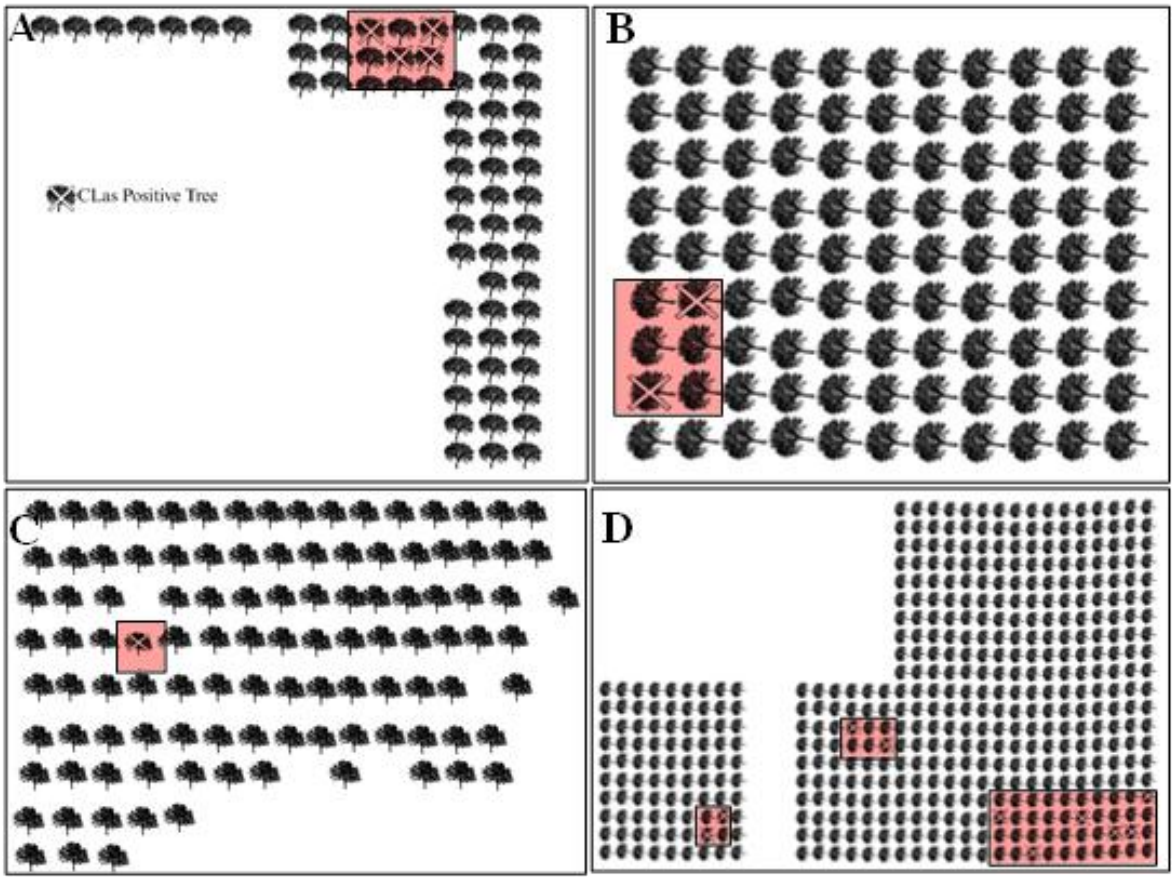

Figure 3.2. Sections of maps showing relative location of CLas positive trees (marked with white X) in commercial groves in WR1 (A), W1 (B), P1 (C) and P2 (D) in GA. Proposed "hotspots" of CLas positive trees highlighted in red.

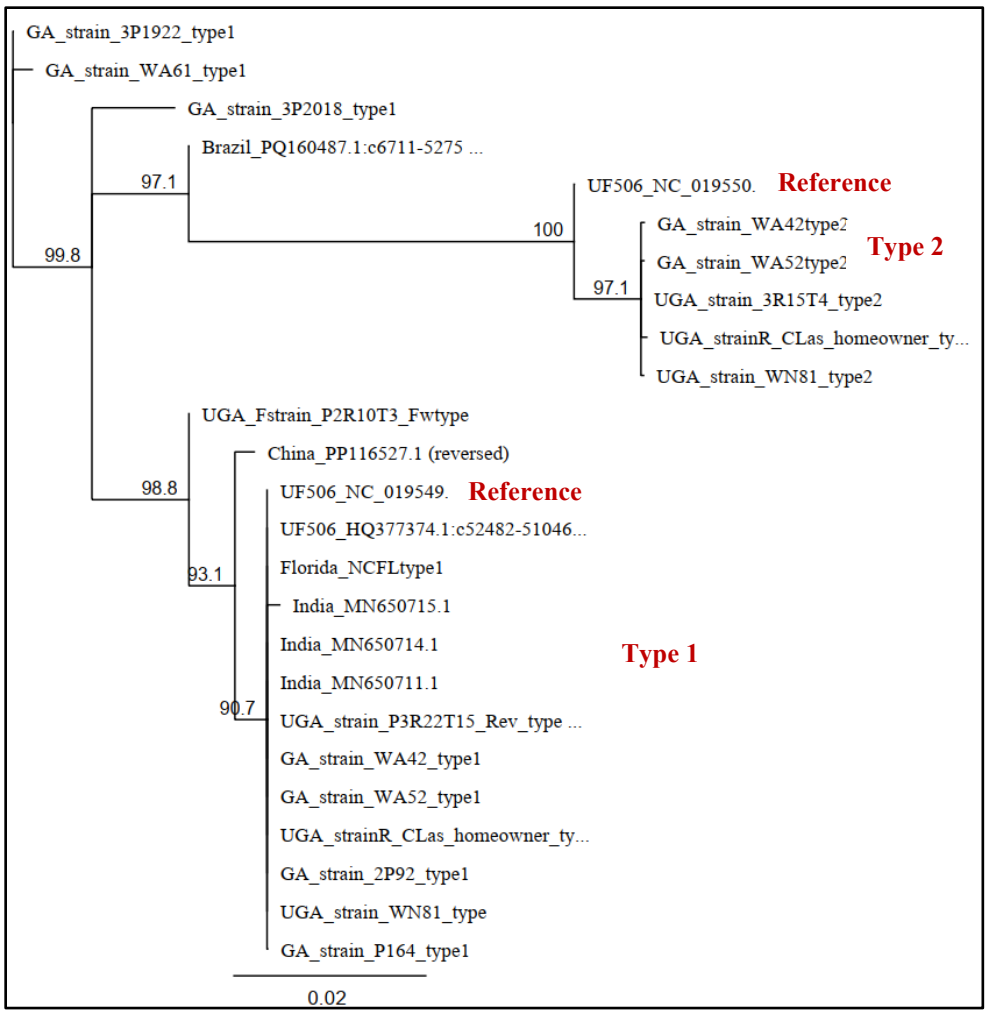

Figure 3.3. Phylogenetic tree showing relationships between GA CLas strains and reference strains based on prophage sequences. Numbers at nodes are bootstrap values.

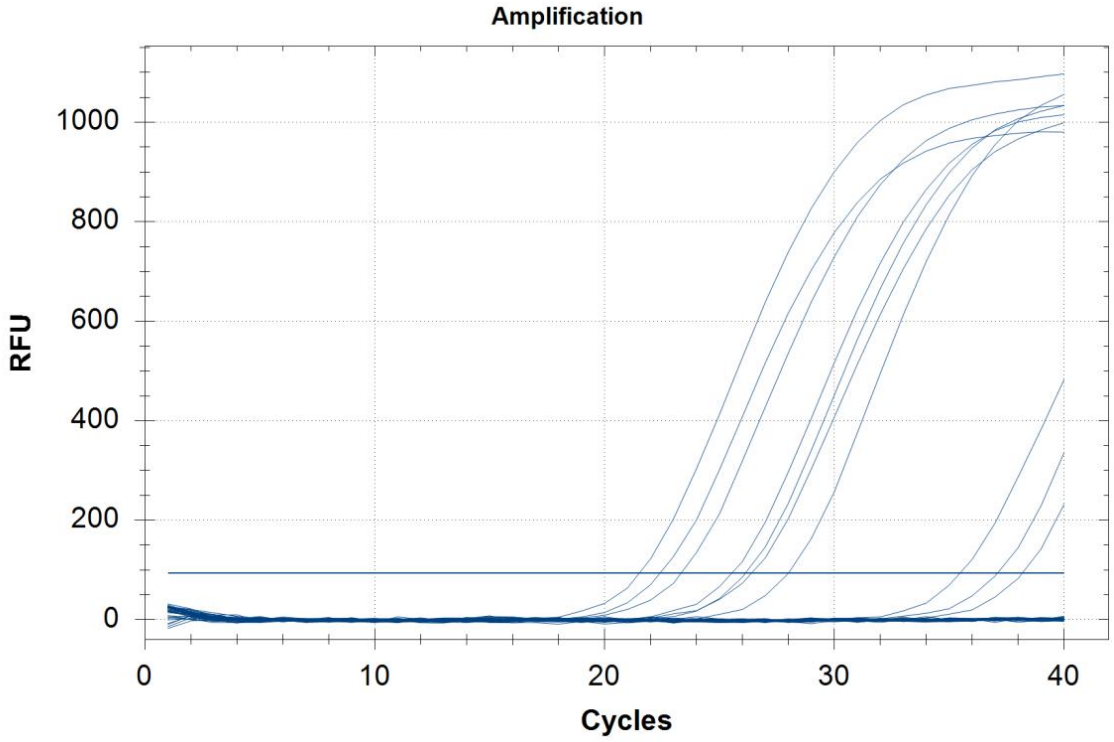

Figure 3.4. Example of amplification plot generated by Bio-Rad CFX Opus showing amplification of the RNR gene for CLas positive samples.

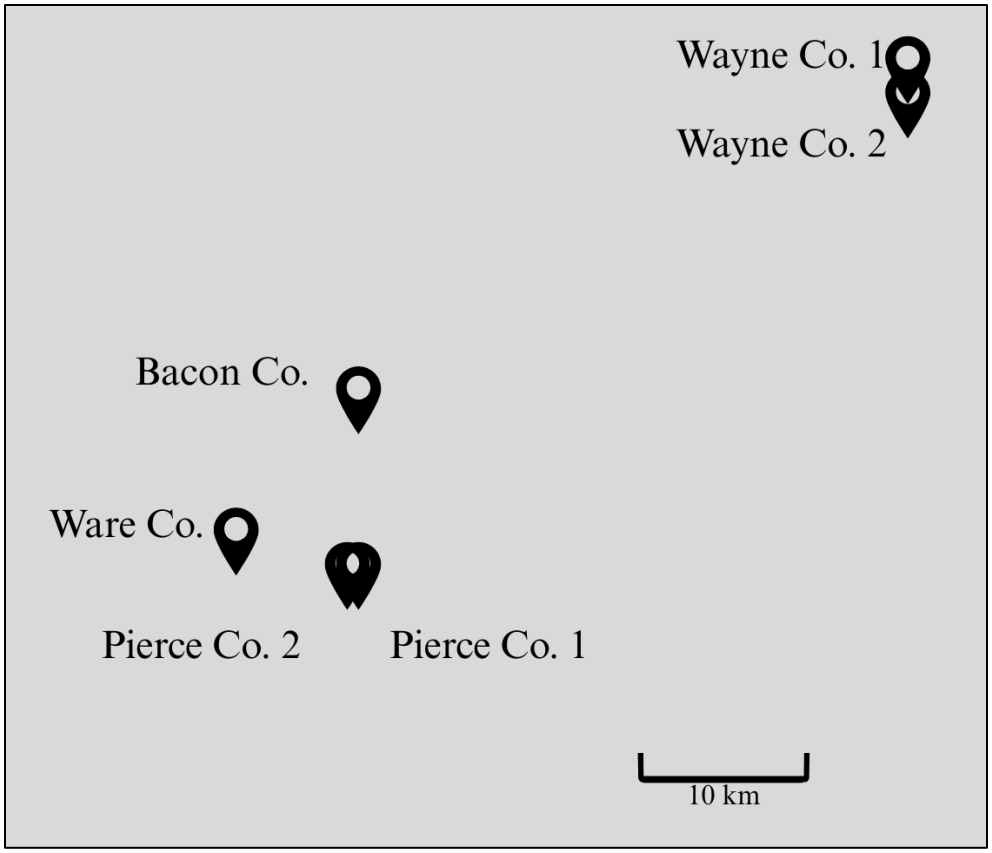

Figure 3.5. Map showing relative location of citrus groves sampled in this study.

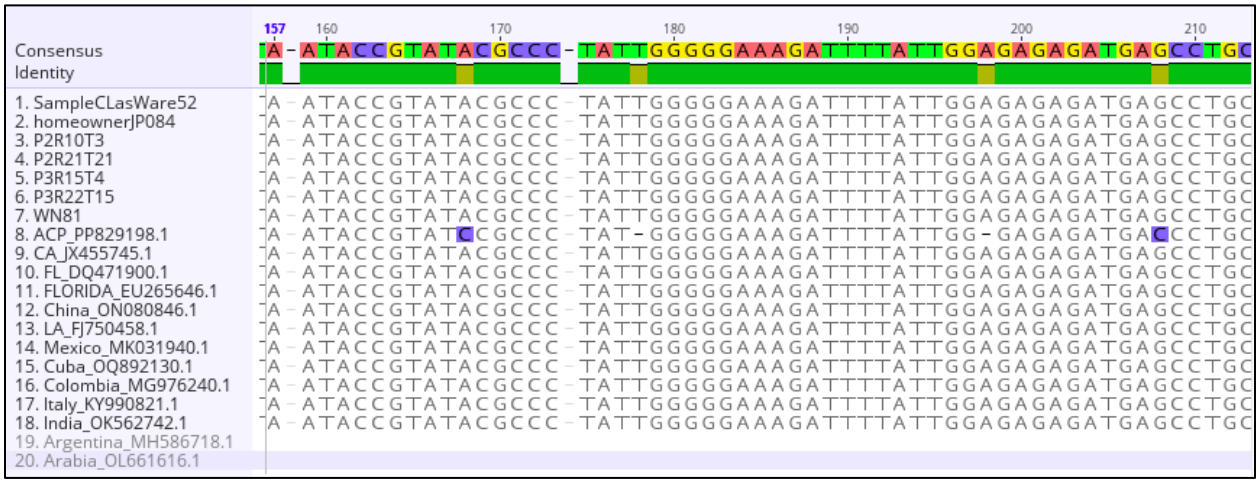

Figure 3.6. Partial 16S sequences showing no genetic variation among CLas strains from different locations, except for SNPs found in CLas isolated from ACP in Georgia.

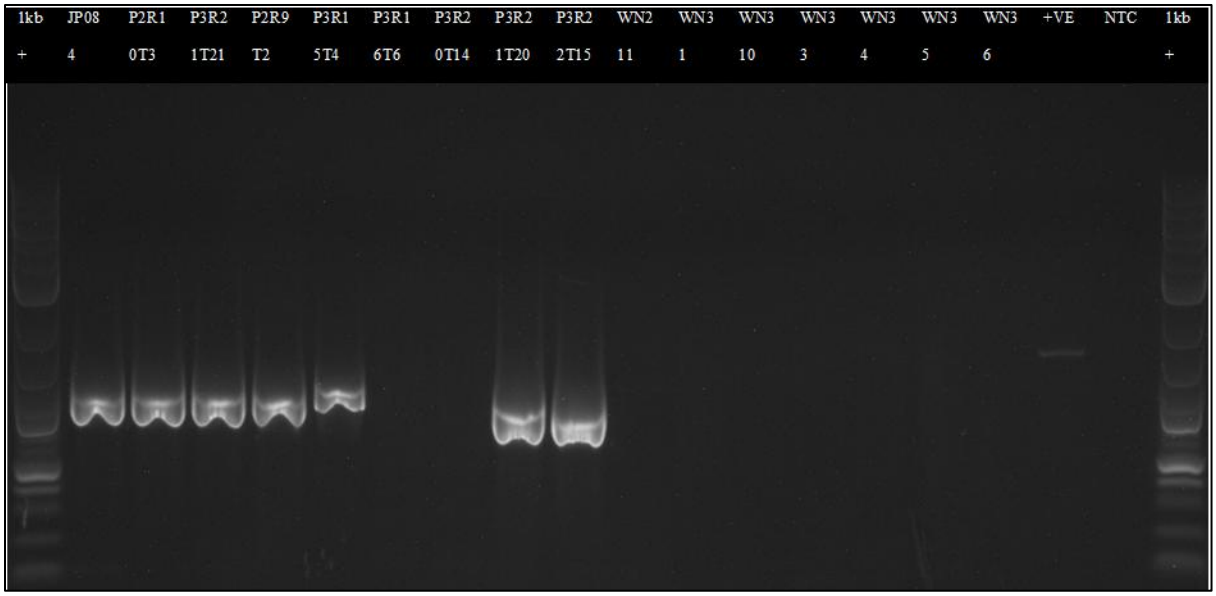

Figure 3.7. Agarose gel (0.7%) confirms amplification of 16S rDNA (~1,200 bp amplicon) from CLas positive trees from Fall 2024.

CHAPTER 4

MONITORING OF ASIAN CITRUS PSYLLID IN SIX COMMERCIAL CITRUS GROVES IN GEORGIA AND DETECTION OF '*CANDIDATUS* LIBERIBACTER ASIATICUS' IN INDIVIDUAL PSYLLIDS[1]

[1]Collins, C., Oliver, J. E., Barman A., Jimenez Madrid A. M. 2025. To be submitted to *Plant Disease*

ABSTRACT

The citrus industry in Georgia is rapidly growing and one of the major concerns for its survival is HLB or citrus greening disease. HLB is caused by '*Candidatus* Liberibacter asiaticus' (CLas), which is transmitted by the Asian citrus psyllid (ACP; *Diaphorina citri*). Management strategies for this disease are often aimed at reducing the population of ACP. ACP were first detected in one commercial grove in Georgia in September 2023, and the distribution and prevalence of ACP in commercial groves was not known. To understand the prevalence, infection status, and survival ability of ACP in Georgia, monitoring and scouting were performed in six groves from five counties. From September 2023 to October 2024, yellow sticky traps were set along borders of the groves, replaced biweekly, and individual psyllids removed and tested for the presence of CLas. Green-blue and gray-brown morphotypes were observed within groves, although the entire morphotype population was not assessed. ACP were consistently detected within groves despite temperatures dipping below -5°C in the winter as well as several applications of pesticides throughout the year. A total of 127 ACP were recovered from two commercial groves in Pierce County, (including 98 adults and 29 nymphs) and DNA was tested for the presence of CLas using conventional PCR and qPCR. None of the ACP tested positive via PCR, however testing via qPCR revealed that 14 ACP were infected with CLas. These findings indicate the need for coordinated efforts to monitor and control the spread of ACP to ensure that the Georgia citrus industry survives.

INTRODUCTION

The citrus industry in the state of Georgia is estimated to have a farmgate value of $39 million with more than 50% of the farmgate value (21.2 million) occurring in Echols, Thomas, Mitchell, Decatur and Coffee counties (Price 2023; UGA-CAES 2025). The Georgia citrus industry is

considered small compared to other commodities, but it is rapidly growing, and its survival will rely on management of significant pests and pathogens. *'Candidatus* Liberibacter asiaticus' (CLas), the causal agent of Huanglongbing or citrus greening disease, is considered the most important citrus pathogen because of its devastating impacts to citrus production. The Asian citrus psyllid (ACP; *Diaphorina citri* Kuwayama) is the only reported vector of CLas in North America and is the primary means of bacterial transmission (Bové 2006; Halbert and Manjunath 2004). Because of this, the ACP is considered a major citrus pest wherever it is found.

The Asian citrus psyllid is a hemipteran insect with adults measuring up to 4 mm (Halbert and Manjunath 2004). They are divided into morphotypes based on colors on the abdomen: gray-brown, green-blue, and orange-yellow, which have been linked to fitness and fecundity (Skelley and Hoy 2004; Wenninger and Hall 2008). Nymphs are ovoid and light orange with prominent wing pads in later stages. ACPs complete their life cycle in as much as 47 days and as little as 15 days and females lay ~800 eggs on average and up to 1,400 eggs in a lifetime (Aubert 1987; Halbert and Manjunath 2004; Tsai and Liu 2000).

Adult ACPs survive and reproduce best at around 28°C, while nymphs develop best in temperatures ranging from 25°C to 28°C (Tsai and Liu 2000). However, ACP can survive at lower temperatures. Martini et al. (2020) found that ACP were able to survive temperatures below -5.5°C in field conditions, consistent with observations of ACP surviving -5°C in Gainesville, Florida (Halbert and Manjunath 2004). Although ACP are able to survive freezing temperatures, a high mortality rate has been reported if exposed to those temperatures for a long time. A study conducted by Hall et al. (2011) found that under laboratory conditions, adults ACP collected from temperature-controlled greenhouses had 95% mortality at -4.5°C after 7 hours of exposure. They also found a 95% mortality at -9.2°C after 2 hours exposure (Hall et al. 2011).

ACP acquire CLas as they feed on infected trees and transmit in a persistently propagative manner (Hung et al. 2004; Canale et al. 2017), as supported by findings of CLas in multiple tissues and its multiplication within organs (Ammar et al. 2016, 2019). CLas propagates primarily within nymph ACP and must reach sufficient titers for transmission as adults (Ammar et al. 2019). Consequently, ACP that acquire CLas as nymphs are better able to transmit CLas as adults (Mead and Fasulo 2017; Ammar et al. 2020). Additionally, CLas must overcome barriers within ACP to efficiently propagate throughout the psyllid and be transmitted (Ammar et al. 2016; Inoue et al. 2009). Infected insects can also transmit CLas to offspring via transovarial transmission; however, this occurs at a low rate (Hung et al. 2004; Pelz-stelinski et al. 2010). Studies have reported variability in infection rates of ACP from areas with infected trees. One greenhouse study reported infection rates above 90% after being reared on CLas infected tissue (Ammar et al. 2020), while field studies conducted in North Florida report infection rate between 0% and 28% (Martini et al. 2020).

There are no varieties of citrus with resistance to CLas, and antibiotics or bactericides have been used cautiously due to limited availability and efficacy as well as a high risk of antibiotic resistance development (Roldán et al. 2023; Sundin and Wang 2018). Management of HLB relies on exclusion of the pathogen through quarantine regulations, removing infected trees, and applying insecticides for control of ACP. Insecticides labelled for use against ACP include soil drenches and foliar chemical sprays belonging to the organophosphate (group 1B), and pyrethroid (group 3A) groups, which are recommended to be applied as dormant sprays to reduce impacts to bees (Diepenbrock et al. 2023). The frequency and interval of insecticide applications are based on the specific product used and their period of efficacy, with many being used between 2 to 4 applications within a season with a recommended spray interval of 10-14 days (Diepenbrock

2024). Qureshi et al (2014) found that products representing 12 modes of action (9 known and 3 unknown) from the Insecticide Resistance Action Committee (IRAC) reduced adult psyllid populations for varying durations after application. For example, they found that 38 of 42 chemicals tested as foliar sprays provided average population reductions of adult psyllids ranging from 90% to 100% over 24-57 days (Qureshi et al. 2014).

ACP was first identified in Florida in 1998 on orange jasmine (*Murraya paniculata*) plants and has since been reported in all citrus-producing regions throughout North America including Texas and California (French et al. 2001; Grafton-Cardwell et al. 2013; Halbert and Manjunath 2004). In many of these areas, this insect vector is regulated by quarantine enforced by USDA-APHIS. In Georgia, most of the commercial citrus groves are established in the southern region, often in close proximity to the northern border of Florida. Commercial growers are advised to plant disease free trees obtained from reputable sources. Nonetheless, the proximity to Florida and the frequent passage of hurricanes provide ACP with many opportunities to move and establish in Georgia's commercial groves.

CLas was first detected in Georgia in 2009 in residential trees (Animal Plant Health Inspection Service; APHIS 2009). At that time, the commercial industry in Georgia was non-existent. However, today citrus is commercially produced in 23 counties on over 1,618.7 Ha of land (Price 2023; UGA-CAES 2025). Scouting for ACP prior to 2022 revealed that ACP is present in all coastal counties in the state, including residential areas (Oliver et al. 2020). However, ACP was also detected for the first time in commercial groves in Pierce County, Georgia in 2023 (Collins et al. 2025). In addition, CLas-positive trees have been detected in Pierce and Wayne counties in a previous survey conducted from 2019-2022 (Oliver, *personal communication*). The presence of ACP and the detection of CLas-positive trees within commercial groves demand

research to determine the prevalence of ACP within commercial groves throughout the year and to understand the overwinter survival ability of ACP in Georgia. Previous published studies conducted in Georgia have only included ACP monitoring on residential trees with none conducted on commercial citrus (Martini et al. 2020). The objectives of this study were to assess the prevalence of ACPs on selected commercial citrus groves and detect CLas on each individual ACP. In addition, this study aimed to examine some environmental factors with the potential to influencing the survival of the vector in Georgia conditions. This will aid to further our understanding of the spread and impact of ACP in Georgia and provide a baseline for further research into this pest in the state.

MATERIALS AND METHODS

ACP Monitoring and Identification

To determine the prevalence of adult and nymph ACPs in commercial groves, six commercial citrus groves from five Georgia counties were selected. These groves were identified as follows: Pierce County groves (P1 and P2), Ware County (WA), Wayne County (WN), Bacon County (BA) and Coffee County (CO) (**Table 4.1**). These commercial groves were selected due to their relative proximity to the previous identification of CLas-positive trees within two commercial groves in 2023 (P1 and WN). The distance between the commercial groves monitored for ACP in this study is represented in **Figure 4.1**.

Yellow sticky cards (7.62×11.43 cm; BASF, Ludwigshafen, Germany) were placed strategically on the edges of each grove to maximize the likelihood of attracting/intercepting ACP (Sétamou and Bartels 2015). When applicable, sticky cards were also placed near trees that previously tested positive for CLas. Eight to twelve sticky cards were placed within P1 (**Figure**

3.2), eleven to sixteen (varied by placement date) within P2, six within WA, five within WN, six within CO and three within BA.

Traps were attached to citrus trees using twist ties and labelled based on the row and tree number (tree ID). The first traps were placed on September 20th, 2023 within P1 and WN and in CO on April 16th, 2024. On April 23rd, 2024, traps were placed within WA, P2, and BA. After placement, between September 2023 and December 2024, traps were collected every 2-3 weeks with some disruptions in this schedule due to weather events (Table 3.5). Traps were carefully removed from the tree and placed in 17.8*17.8 cm transparent polybags (Uline, Pleasant Prairie, WI) and sealed before being placed into plastic zipper bags (26.8 cm x 27.3 cm). Traps were then transferred at room temperature to the UGA Plant Molecular Diagnostics Laboratory in Tifton, Georgia for detailed inspection under the dissecting microscope. At the time of trap collection, each trap was replaced with a new trap in the same tree ID as described above. In addition to the bi-weekly replacement of traps, a quick scouting was conducted in each grove by visually examining new flush on trees.

The collected sticky cards were stored at -20°C before examination using a dissecting microscope to determine if there were any ACP present. ACP were identified via morphological characteristics including variegation on wings and general color (**Figure 4.4**). Pictures were taken for ACP collected on September 29, 2023, April 11, 2024, May 2, 2024, May 17, 2024, June 14, 2024, August 28, 2024, and Sept 18, 2024. Morphotypes observed from these pictures were recorded based on abdominal color. Once identified, individual ACPs were removed from sticky traps by adding 10 µL of histo-clear solution and removing with toothpick (Butterwort et al. 2022; Miller et al. 1993). ACP were then assigned a unique identifier and placed in 2 mL tubes filled

with 50 µL of 70% ethanol for storage prior to DNA extraction or were immediately processed for DNA extraction.

ACP DNA Extraction

DNA from individual ACPs was extracted using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) according to the USDA-APHIS approved protocol. Briefly, each ACP was removed from 70% ethanol and dried on absorbent paper towels and was processed by first placing it in a Lysing Matrix A tube (Qbiogene, San Carlos, CA) with 360 µL of 1X PBS (phosphate buffer saline) 7.4 pH. Each tube was labeled with the unique identifier and placed in a FastPrep-24 5G Bead beating device (M.P. Biomedicals, LLC Irvine CA) at a speed of 6 m/s for 40 seconds. The DNA was eluted in 100 µL of AE buffer. The DNA concentration and quality was evaluated using a Nanodrop Lite (Thermo Fisher Scientific Inc., Wilmington, DE) and stored at -20°C prior to polymerase chain reaction (PCR).

The DNA concentration and quality of the first ACPs processed (n=88 of 123) were often low, ranging from 3.8 to 78.9 ng/µL and an A_{260}/A_{280} average of 1.55. Accordingly, the DNA extraction process was slightly modified for the remaining ACP samples (n=35) by following the protocol as described by Pelz-Stelinski et al. 2010. Briefly, the ACP was first placed in a 1.5 ml conical tube in 360 µL of 1X PBS 7.4 pH and macerated using a plastic pellet pestle before incubation at 56°C for 4.5 hours. The extraction was resumed following the QIAGEN Blood and Tissue Kit instructions. The DNA was eluted in 100 uL of AE buffer and concentrations were checked as described above.

Conventional PCR Using 16S rDNA for Detection of CLas within ACP

The primer pair OI1/OI2c (Adkar-Purushothama et al. 2009) was used to perform conventional PCR to amplify a 1,160 bp region of the CLas 16S rDNA. The PCR mixture included

12.5µL of GoTaq green master mix 2X (Promega, Madison WI, USA), 9.5 µL of molecular grade (MG) water, 1 µL of forward and reverse primers (10 µM) and 1 µL of ACP DNA for a total reaction volume of 25 µL. DNA from a previously confirmed CLas-positive ACP from Georgia was used as positive control (Collins et al. 2025) and sterilized MG water was used as the non-template control. PCR was completed using the program: 35 cycles of 92℃ for 30 seconds, 54° for 30 seconds and 72℃ for 90 seconds in a BioRad T100 Thermocycler machine (Bio-Rad Laboratories, Singapore). The PCR product (5 µl) was examined on a 0.7% agarose gel with GelRed nucleic acid stain (10,000x stock reagent; Biotium, Fremont, CA) (Adkar-Purushothama et al. 2009). Electrophoresis was carried out in Tris-Borate-EDTA (TBE) buffer (pH 8.3) for 90 minutes at 90V. DNA was visualized with the Benchtop UVP Transilluminator (Analytik Jena US, Upland CA), and images were captured using Visionworks software version 4.16. If no amplicon was observed, it was concluded that either the ACP did not contain CLas, or the titer was too low for detection with this assay.

Detection of CLas in ACP via Quantitative qPCR targeting the RNR gene

For greater sensitivity, all ACP were also tested using qPCR. CLas was confirmed within ACP by using the RNR-1 gene while the WG gene was used as an internal control for ACP (**Table 3.1)**. Primer Probe mix (PPmix) was first created by mixing 20 µL of 100 µM stock of each primer and 10 µL of 100 µM stock of each probe in 900 µL of MG water. For each qPCR reaction, 3 µL of PPmix, 12.5 µL of 2X premade master mix (PerfeCTa MultiPlex qPCR SuperMix Low ROX Quantabio) and 7.5 µL of MG water were combined with 2 µL of ACP DNA for total reaction volume of 25 µL. The final concentration was 240 nM for each primer and 120nM for each probe. Cycling parameters were 95°C for 3 minutes followed by 40 cycles of 95°C for 3 seconds and 60°C for 40 seconds. Baseline thresholds for RNR-1 based on the HEX (Hexachloro-fluorescein)

channel and WG based on the FAM (6-Carboxyfluorescein) channel were 106 and 30 relative Fluorescence Units (RFU), respectively. Samples were determined to be positive if Cq values were less than 38 in the HEX channel with amplification observed in the FAM channel after the test was repeated twice.

Pesticide Application Schedule from Pierce County Groves

Insecticides applications records were obtained from growers to determine products applied, rate and date of applications (**Table 4.2**). Only applications from groves where ACPs were recovered were obtained to correlate with number of ACP recovered at various timepoints throughout the monitoring period.

Collection of Weather Data

Weather data was collected from the UGA Weather Network (accessed April 21, 2025) to identify climate parameters that may have influenced insect vector populations. Information collected include daily minimum and maximum temperatures between September 15, 2023 and January 15, 2025. Data was retrieved from four weather stations corresponding with the six groves where ACP monitoring was conducted. The weather station in Waycross, Georgia was used for P1, P2, and WA, the weather station in Alma, Georgia was used for BA, and the Odum weather station was used for WN. The Douglas weather station was used for CO **(Table 4.3)**.

RESULTS

ACP Prevalence in Two Commercial Groves in Pierce County Georgia

Between September 2023 and December 2024, a total of 681 sticky traps were placed in the six commercial groves and evaluated for the presence of ACP (Table 3.4). ACP (n=5) were first observed in traps collected in P1 on September 29, 2023, and subsequently observed in traps collected on March 19, 2024 (n=1), April 11, 2024 (n=3), May 17, 2024 (n=1) and October 4, 2024 (n=1) (**Figure 4.3**). In addition to the ACP recovered from sticky traps, ACP were found during scouting operations on citrus trees. On September 20, 2023, 20 nymphs and 5 adults were observed on P1 and collected in vials (Collins et al, 2025). Similarly, in P2, 4 adults and 9 nymphs were observed and collected during scouting on October 11, 2024. Scouting activities in Spring 2024 did not result in the observation of any ACP.

On May 2, 2024, ACP were observed in P2 (n=7) and subsequently on May 17, 2024 (n=11), May 31, 2024 (n=4), June 14, 2024 (n=2), June 28, 2024 (n=1), July 12, 2024 (n=3), August 12, 2024 (n=2), August 21, 2024 (n=9), August 28, 2024 (n=14) September 18, 2024 (n=2), October 4, 2024 (n=13), October 11, 2024 (n=8) and October 18, 2024 (n=3) (**Figure 4.3**).

From October 6th, 2023, to December 2023 no ACP were observed in P1 despite six trap replacements during that period. Therefore, trapping was paused for the months of January and February of 2024 before resuming in March (**Figure 4.3**). In total, by the end of monitoring, 127 ACP had been recovered from P1 (n=36) and P2 (n=91) **(Table 4.4)**. No ACP were observed in any of the other four monitored groves.

While the complete population of ACP collected in this study were not evaluated for morphotype, some individuals were assessed. Gray-brown morphotypes were observed throughout

the study in both P1 and P2. Blue- green morphotypes were also observed in P2 on May 2, 2024 (n=1), May 17, 2024 (n=2), June 14, 2024 (n=1) and August 28th, 2024 (n=3) (**Figure 4.4**).

We also noted number of ACP recovered in relation to the four flush cycles (February-March, May-June, July-August and September-October) as described by Setamou and Bartels (2015). Scouting efforts were only successful at recovering ACP during September of 2023 and October of 2024 which correspond with the September-October flush cycle. In regard to ACP recovered from traps, we observed some spikes in the number recovered during May and August of 2024 which also correspond with known flush cycles (**Figure 4.4**).

DNA Concentration of Individual ACP

DNA concentrations from ACP extracted with the standard USDA protocol ranged from 0.1 ng/µL to 73.1 ng/µL and A_{260}/A_{280} ratios ranged from 0.83 to 6.39 with an average of 1.55 and standard deviation of 0.58. When extracted via the method including incubation (Pelz-Stelinski et al. 2010), DNA concentrations ranged from 3.8 ng/µL to 78.9 ng/µL and the A_{260}/A_{280} ratio ranged from 1.52 to 3.56 with an average of 2.01 and standard deviation of 0.47. An ideal nucleic acid ratio is 1.8, while values below 1.6 can indicate that DNA is contaminated by proteins and values above 1.9 that DNA is contaminated by RNA. Approximately 17% of the DNA extraction products from ACP using the protocol established by USDA (n=88) had A_{260}/A_{280} ratios ranging from 1.6 to 2.0. In contrast, 71% of samples extracted with the modified incubation procedure (n=35) were between 1.6 and 2.0.

Detection of CLas in individual ACPs using Conventional PCR and qPCR

The 127 ACP recovered from commercial groves, were tested via PCR and qPCR. Conventional PCR did not result in amplification of the expected 1160 bp amplicon from any of the ACP other than the positive control **(Figure 4.7**). From qPCR, amplification was observed

from 14 ACP with Cq values ranging from 34.2 to 37.6 (**Figure 4.5**). Of the 127 ACPs tested, 4 ACP had an average Cq value ≤ 35 and 10 ACP had a Cq value between 36 and 38. None of the remaining 113 ACP had detectable CLas (**Table 4.5**). Thirteen of the ACP that tested positive for CLas were from grove P2 representing 14.3% of ACP from that grove, while 1 was from P1 representing 2.8% (**Table 4.5**). The only CLas positive psyllid in P1 was found during initial scouting on September 20, 2023. In P2, 2 infected psyllids were found in August 12, 2024, 10 on August 21, 2024 and 1 on October 18, 2024.

Minimum and Maximum Temperatures Observed Close to Pierce County Groves

For late 2023 and early 2024, weather station data indicated that the first frost occurred on November 29, 2023, with a temperature of -1.7°C. There were 17 additional days during this period when the temperature dipped below freezing with the lowest temperature of -5.1°C occurring on Jan 21, 2024 (**Figure 4.6**). For late 2024 through early 2025, the first freeze occurred on Nov 30th, 2024, at -2°C with 21 additional days below freezing before Jan 15, 2025. The lowest temperature during this period was -5.2°C which occurred on both Dec 4, 2024, and Jan 12, 2025. ACPs were not observed from October to December 2023. The scouting operations were paused due to the winter conditions and were resumed on March 19, 2024, when a single ACP was found. The mean temperature prior to this ACP observation (from January to March 2024) was 18.56°C with the lowest being -5.1°C on January 21, 2024 (**Figure 4.6**).

Pesticide Applications on the Pierce County Grove

During the 2024 growing season, the grower made six foliar applications of insecticides to P1 and P2. The products applied inlude Sivanto Prime (Flupyradifurone; Bayer Crop Science), Mustang Maxx (Zeta-cypermethrin; FMC corporation) and ABBA Ultra (Abamectin; AMVAC corporation) (**Table 4.2**). The insecticides Mustang Maxx and AbbA ultra were applied at a rate

of 4 ounces per acre while Sivanto Prime was applied at a rate of 14 ounces per acre (**Table 4.2**). All applications were made to both P1 and P2, on the same dates. After applications of Flupyradifurone (Sivanto prime) were made on April 17, 2024, no ACPs were found on traps or via scouting 6 days later April 23rd, however at 15 days post application, 7 ACPs were recovered from P2 (**Figure 4.3**). A follow-up application on May 6th saw similar results at 11 days post application when 11 ACP were recovered from traps in P2 and 1 from traps in P1. Following the application of Zeta-cypermethrin (Mustang Maxx) in Pierce groves very few ACP were recovered from traps and scouting at 8 days (n=2) and 22 days (n=1) after application. After the application of Abamectin (AbbA Ultra) only 3 ACPs were recovered 6 days after the July 6 application but 13 were recovered from P2 12 days after the September 22 application.

DISCUSSION

Surveillance of ACP and determining the CLas infection status of ACP provide essential information for HLB management to prevent losses due to HLB. In this study, 127 ACPs were observed in two commercial groves in Pierce County, Georgia collected between September of 2023 and October of 2024. ACP were first detected in Georgia in one of these commercial groves in 2023 (Collins et al. 2025) and this observation highlighted the need to keep monitoring ACP in this region. The numbers of ACP recovered from traps and scouting may not be directly correlated with the population of ACP within groves but provides a means to identify how populations fluctuate. The fluctuation in the number of ACP recovered from each grove at each time point during this study may be a function of several factors including the placement of traps, pesticides applied, environmental conditions and fitness of ACP (Hall et al. 2011; Lewis-Rosenblum et al. 2015; Martini et al. 2014, 2020; Sétamou and Bartels 2015).

Identifying the best placement of yellow sticky traps in a citrus grove can maximize the likelihood of capturing ACP. Sétamou and Bartels (2015) found that ACP populations, determined from lime-green sticky traps and scouting in the Rio Grande Valley of Texas were consistently higher on perimeter trees versus interior trees. Consequently, in our study we placed most of our traps om perimeter trees (**Figure 4.2**).

ACP populations will vary according to seasonal temperatures. Martini et al. (2020) found that nymph population peaks in north Florida occurred in July-August followed by peaks in the adult ACP population. This is consistent with our recovery of ACP during the fall scouting and peaks in number of ACP including nymphs and adults recovered in September and October of 2023 and 2024. Observations of higher number of ACP from trapping and successful observations from scouting in September 2023 and October 2024 is also consistent with findings by Sétamou and Bartels (2015) that among four major flush cycles observed within groves ACP were observed in the highest densities in the September-October flush cycle (Sétamou and Bartels 2015). Monitoring and scouting were not conducted during the putative Feb-March flush cycles, however in May and August we also observed more recoveries of adult ACP from traps, consistent with the proposed May-June and July-August flush cycles.

Although a morphotype description was not recorded for each ACP recovered in this study, we clearly observed two distinct morphotypes- grey-brown and blue-green- within P1 and P2 commercial groves. However, blue-green morphotypes were found exclusively in P2 during 2024. The presence of the blue-green morphotype in P2 may explain why the number of ACP recovered from that grove was consistently higher than P1 despite both groves being sprayed with the same chemicals on the same dates. The ACP with blue-green morphotype have been reported to have greater fitness, mass, ability to reproduce and fly longer distances than gray-brown morphotypes

(Martini et al. 2014; Wenninger et al. 2009; Wenninger and Hall 2008). In addition, abdominal color has also been associated with susceptibility to insecticides with grey-brown and orange yellow being more susceptible than blue-green (Tiwari et al. 2013), which may also be contributing to these findings. These observations are relevant because they highlight diversity among ACP within groves in Georgia, which may indicate their ability to adapt, maintain and increase their population, thus increasing the risk of HLB spread into and within commercial groves.

All 127 Psyllids in this study were first tested for CLas via conventional PCR, and no amplification was observed with this assay. The lack of amplification observed when testing via conventional PCR is not surprising due to the lower sensitivity of this assay compared to qPCR (Li et al. 2005). Other important considerations such as the potential low titer of CLas within adult ACP, or poor DNA quality, may also influence CLas detection via conventional PCR.

Testing individual ACP for CLas is not a common practice for diagnostics purposes, however, a study from Florida found that when tested via qPCR 79.5% of nymphs raised on infected tissue in greenhouse conditions were infected with CLas while 91.4% of adults infected (Ammar et al. 2020). A recent field survey in China also found that 19.8% of individual ACPs tested via reverse transcriptase (RT) PCR were infected with CLas (Liu et al. 2024). Similarly, in our study, we found 11% of ACP were infected with CLas when tested via qPCR. Our observations are similar to prior field observations in Florida where CLas infection ranged from 0% to 28%, when tested via qPCR (Martini et al. 2020).

All CLas infected ACP in this study were found in groves P1 and P2 where CLas infected trees have also been detected (Chapter 2). In P1 where one infected ACP was found, there was one positive tree found in 2023 (Chapter 2) and two from previous testing in 2022 (Oliver, personal communication). In P2, where 13 infected ACP were found, 10 CLas positive trees were found in

2023. The presence of infected trees in these fields suggests that transmission has happened in the past and is likely actively taking place within these groves. It also suggests that infected trees may be providing sources of inoculum for non-infected ACP to acquire CLas (Chapter 2).

The high number of psyllids found in grove P2 correlates with higher incidence of CLas infected trees when compared to the neighboring grove P1. It is notable that the 14.3% infection rate of the ACP collected from P2 is fairly high considering the lower incidence of CLas infected trees found in this same grove (2%) (Chapter 2). Interestingly, only a 2.7% (n=1) of ACP infection rate was found and <1% (n=1) infected trees in P1 (chapter 2).

Additionally, psyllid populations were active in P2 for at least eight months of the year, meaning there are many opportunities for infected ACP to feed on non-infected trees and transmit CLas throughout the groves. It also means that ACP will undergo many life cycles which allows for better acquisition and transmission of ACP across generations. These findings are important and highlight the imminent risk that HLB poses to Georgia's citrus industry.

Regarding DNA quality, the DNA extraction methods we employed showed variability in effectiveness when judged by the absorbance of nucleic acids (A_{260}/A_{260}) and DNA concentration. Oure results suggest that, in general, DNA quality was improved using the modified incubation procedure. Since most samples examined in this study were extracted with the USDA protocol, the relatively lower quality of DNA that resulted from utilizing this method for so many samples, may have affected the ability to detect CLas within psyllids when tested via PCR and qPCR.

Martini et al. (2020) found that ACP on residential trees in northern Florida were able to survive overwintering temperatures as low as -5.5 °C. The observation of ACP on March 19th even after extended period of colder weather and temperatures dipping to below -5°C indicate that ACP were likely able to survive these temperatures within the grove, consistent with the findings in

northern Florida. It is also possible that ACP survived in nearby locations such as on residential trees during the winter and subsequently migrated into the groves where ACP were recovered.

Throughout the study ACP were only recovered from two adjacent groves despite the fact that other monitored groves less than ~3 to 4 km away did not have detectable ACP. ACP are reported to fly up to 2.4 km in one continuous flight (Martini et al 2014) and possibly further when aided by wind. Hurricane Helene occurred on September 27, 2024, with wind gusts up to 134 km/h. Subsequent visits to groves as close as 3 km from P2 did not indicate that the ACP had moved into those groves. In Florida, even without the aid of high winds, ACP were reported to travel between groves but showed lower dispersal rates during the winter (Lewis-Rosenblum et al. 2015). Further studies will be needed to determine the exact impact of temperature and wind on ACP survival and dispersal in Georgia over time.

The Florida Citrus Production Guide recommends targeting overwintering ACPs with broad spectrum insecticides and warns that applications used during ACP reproduction on new flush may not be effective (Diepenbrock 2024). The grower spraying within this period may have contributed to the inefficiency of pesticide applications, especially as applications were not made during winter months for which we did not receive records. Another consideration is the proximity of residential trees commercial groves, which may have served as reservoirs for ACP survival. In this study, residential trees were not monitored or scouted for ACP, however ACP have been regularly found in residential areas in Georgia and confirmed to be infected with CLas (Collins et al. 2025).

A study conducted by Qureshi et al. (2014b) at the southwest Florida Research and Education Center found that Sivanto 200 EC ranked 4th for suppression of adult ACP and achieved 90% reduction over 52 days when applied a 14 oz/acre, which is the same rate applied to groves

in this study. It was also able to suppress nymphs (rank 14 of 42) with a 99% reduction for up to 33 days. Our observations suggest that suppression of adult ACP with Sivanto Prime may not be lasting as long as was observed in that study in at least one of the Georgia groves. This could be due to multiple variables not recorded in this study such as poor spray coverage, insecticide resistance and presence of external reservoirs. In the study by Qureshi et al., tap sampling was used to record populations of ACP which may also contribute to differences observed.

The same study reported that Mustang Maxx 1.5 EC (Zeta-cypermethrin) at a rate of 4 oz/acre, which corresponds with the rate applied to the groves in this study, was able to reduce 44% of adult ACPs over 18 days. Increasing the rate to just 4.3 oz/ac resulted in 97% reduction in population over 44 days (Qureshi et al. 2014b). We observed that following the application of Mustang Maxx in Pierce groves very few ACP were recovered from traps and scouting at 8 days (n=2) and 22 days (n=1) after application suggesting that the lower rate of 4 oz/ac may have been able suppress populations more effectively than reported in the previous study. Abamectin (AgriMek 0.15 EC) ranked 15th of 42 insecticides tested by Qureshi et al. (2014b) and reduced 87% of the population of adults for 42 days when applied at 20 oz/Acre, however at 4.3 oz/ac, which is closer to the 4 oz/ac rate used in this study only 32% of adult ACP population was suppressed for 24 days. For nymphs, it ranked 29th of 42 and reduced nymph populations at 84% for 20 days at 20 oz/ac and had no suppression at 4.3 oz/ac. Our observations indicate that the application of abamectin (AbbA Ultra) at 4 oz/ac may have varying effectiveness as only 3 ACPs were recovered 6 days after the July 6 application but 13 were recovered from P2 12 days after the September 22 application.

Though the number of ACP recovered following applications of pesticides were generally lower, this trend was not consistent. Specifically for the applications of Sivanto Prime made on

May 6 and AbbA Ultra on Sept 22, 2024, the number of ACP recovered increased. This finding may be due to the ACP being in traps after they were placed but before pesticides were applied. This highlights a limitation to the interpretation of these results as new traps were not placed immediately after each application. Additionally, the methods used in this study vary considerably from ours and so these comparisons may serve only as further points due for additional investigation.

CONCLUSION

Management of HLB disease as well as the insect vector remains a top priority to the citrus industry worldwide. Proactive surveillance programs are highly effective in reducing disease outbreaks and may contribute to protecting the growing citrus industry in Georgia. In this study, ACP were found only in two adjacent groves in Pierce County, and populations were able to "rebound" after temperatures dipping below -5°C and the application of groups 3A, 4D insecticides for up to six applications within the study period. These findings suggest that ACP are likely reproducing within commercial groves and can survive harsh environmental conditions or that ACP are actively migrating into these groves from external sources. Although the method used in this study to monitor the insect is very common, the number of ACP collected from these groves are only a representation of their presence and cannot be used to estimate ACP populations in Georgia as a whole. Nevertheless, our observations provide valuable insights into the prevalence of ACP within Georgia commercial groves across seasons. ACPs are not known to be widely distributed in citrus groves throughout the state as they have only been reported and observed in one county. However, the findings of infected ACP within these groves, previously reported to have CLas infected trees, should raise the concern to the heightened risk of HLB spreading to more

commercial groves. There are currently over 4,000 acres of commercial citrus in Georgia, and it is expected to increase. Therefore, further research needs to be conducted to understand the population of ACP in Georgia including frequency of morphotypes, presence of symbionts, sensitivity to insecticides and the effect of weather conditions on ACP dispersal in Georgia. Growers also need to be informed of these findings and be encouraged to implement more robust insecticidal controls and increased monitoring and scouting to prevent spread of ACP into their groves. They should also consider becoming familiar with any commercial and residential neighbors who may have trees infested with ACP to coordinate area wide management strategies. Overall, these findings represent a baseline for continued monitoring of ACP populations in Georgia and the development of surveillance programs for safeguarding the survival of the commercial citrus industry in Georgia.

LITERATURE CITED

Ammar, E.-D., Achor, D., and Levy, A. 2019. Immuno-Ultrastructural Localization and Putative Multiplication Sites of Huanglongbing Bacterium in Asian Citrus Psyllid Diaphorina citri. Insects 10: 422.

Ammar, E.-D., George, J., Sturgeon, K., Stelinski, L. L., and Shatters, R. G. 2020. Asian citrus psyllid adults inoculate huanglongbing bacterium more efficiently than nymphs when this bacterium is acquired by early instar nymphs. Sci Rep 10.18244.

Ammar, E.-D., Ramos, J. E., Hall, D. G., Dawson, W. O., and Jr, R. G. S. 2016. Acquisition, Replication and Inoculation of Candidatus Liberibacter asiaticus following Various Acquisition Periods on Huanglongbing-Infected Citrus by Nymphs and Adults of the Asian Citrus Psyllid. PLOS ONE 11:e0159594.

Aubert, B. 1987. Trioza erytreae Del Guercio and Diaphorina citri Kuwayama (Homoptera : Psylloidea), the two vectors of Citrus Greening Disease : Biological aspects and possible control strategies. Fruits. 42:149-162.

Bové, J. M. 2006. Huanglongbing: A Destructive, Newly-Emerging, Century-Old Disease of Citrus. Journal of Plant Pathology. 88: 7-37.

Callies, T. 2022. California Psyllids Carrying HLB Bacterium in Groves. Citrus Industry Magazine. https://citrusindustry.net/2022/11/22/california-psyllids-carrying-hlb-bacterium-in-groves/ (accessed 12 Jun 2024).

Collins, C., Oliver, J. E., Barman, A., Munoz, G., and Jimenez Madrid, A. M. 2025. Confirmation of Candidatus Liberibacter asiaticus in Asian Citrus Psyllids and Detection of Asian Citrus Psyllids in Commercial Citrus in Georgia (U.S.A.). Plant Disease. 109:800-803.

Coy, M. R., Hoffmann, M., Kingdom Gibbard, H. N., Kuhns, E. H., Pelz-Stelinski, K. S., and Stelinski, L. L. 2014. Nested-quantitative PCR approach with improved sensitivity for the detection of low titer levels of Candidatus Liberibacter asiaticus in the Asian citrus psyllid, Diaphorina citri Kuwayama. Journal of Microbiological Methods 102:15–22.

Diepenbrock, L. M. 2024. 2024–2025 Florida Citrus Production Guide: Asian Citrus Psyllid. Ask IFAS - Powered by EDIS. https://edis.ifas.ufl.edu/publication/CG097 (accessed 22 Apr 2025).

French, J. V., Kahlke, C. J., and de Graca, J. V. 2001. First Record of the Asian Citrus Psylla, Diaphorina citri Kuwayama (Homoptera:Psyllidae), in Texas. Subtropical Plant Science 53: 14-15.

Grafton-Cardwell, E. E., Stelinski, L. L., and Stansly, P. A. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annu Rev Entomol 58:413–432.

Halbert, S. E., and Manjunath, K. L. 2004. Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) And Greening Disease Of Citrus: A Literature Review And Assessment of Risk in Florida. Florida Entomologist 87:330–353.

Hall, D. G., Wenninger, E. J., and Hentz, M. G. 2011. Temperature Studies with the Asian Citrus Psyllid, *Diaphorina citri* : Cold Hardiness and Temperature Thresholds for Oviposition. Journal of Insect Science 11:1–15.

Inoue, H., Ohnishi, J., Ito, T., Tomimura, K., Miyata, S., Iwanami, T., and Ashihara, W. 2009. Enhanced proliferation and efficient transmission of Candidatus Liberibacter asiaticus by adult Diaphorina citri after acquisition feeding in the nymphal stage. Annals of Applied Biology 155:29–36.

Jagoueix, S., Bové, J. M., and Garnier, M. 1996. PCR detection of the two "Candidatus" Liberobacter species associated with greening disease of citrus. Mol Cell Probes 10:43–50.

Li, W., Hartung, J. S., and Levy, L. 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. Journal of Microbiological Methods 66:104–115. https://doi.org/10.1016/j.mimet.2005.10.018.

Lewis-Rosenblum, H., Martini, X., Tiwari, S., and Stelinski, L. L. 2015. Seasonal Movement Patterns and Long-Range Dispersal of Asian Citrus Psyllid in Florida Citrus. Journal of Economic Entomology 108:3–10.

Liu, L., Chen, J., Jiang, J., Liang, J., Song, Y., Chen, Q., Yan, F., Bai, Z., Song, Z., and Liu, J. 2024. Detection of Candidatus Liberibacter asiaticus and five viruses in individual Asian citrus psyllid in China. Front. Plant Sci. 15:1357163.

Martini, X., Hoyte, A., and Stelinski, L. L. 2014. Abdominal Color of the Asian Citrus Psyllid (Hemiptera: Liviidae) is Associated with Flight Capabilities. Annals of the Entomological Society of America 107:842–847.

Martini, X., Malfa, K., Stelinski, L. L., Iriarte, F. B., and Paret, M. L. 2020. Distribution, Phenology, and Overwintering Survival of Asian Citrus Psyllid (Hemiptera: Liviidae), in Urban and Grove Habitats in North Florida. J Econ Entomol 113:1080–1087.

Oliver, J. E., Ali, M. E., Waliullah, S., Price, J., Warren, J., Jacobs, J., Hoppers, A., Evans, R., Dowdy, M., and Curry, S. 2020. Huanglongbing, Caused by ' *Candidatus* Liberibacter asiaticus,' Detected in New Locations Across Southern and Coastal Georgia. Plant Health Progress 21:31–35.

Price, J. 2023. Citrus Plantings in Georgia Continue to Increase. UGA Citrus Blog. https://site.caes.uga.edu/citrus/2023/09/citrus-plantings-in-georgia-continue-to-increase/ (accessed 29 Jan 2025).

Animal Plant Health Inspection Service (APHIS). 2009. Confirmation of Citrus Greening in Chatham County, Georgia. .

Butterwort, V., Dansby, H., Zink, F. A., Tembrock, L. R., Gilligan, T. M., Godoy, A., Braswell, W. E., and Kawahara, A. Y. 2022. A DNA Extraction Method for Insects From Sticky Traps: Targeting a Low Abundance Pest, Phthorimaea absoluta (Lepidoptera: Gelechiidae), in Mixed Species Communities. Journal of Economic Entomology 115:844–851.

Diepenbrock, L. M., Qureshi, J., and Stelinski, L. 2023. 2023–2024 Florida Citrus Production Guide: Asian Citrus Psyllid: CPG ch. 22, CG097, rev. 5/2023. EDIS.

Miller, R. S., Miller, R. S., Passoa, S., Waltz, R. D., and Mastro, V. 1993. Insect Removal From Sticky Traps Using A Citrus Oil Solvent. Entomological news 104:209--213.

Pelz-Stelinski, K. S., Brlansky, R. H., Ebert, T. A., and Rogers, M. E. 2010. Transmission parameters for Candidatus liberibacter asiaticus by Asian citrus psyllid (Hemiptera: Psyllidae). J Econ Entomol 103:1531–1541.

Roldán, E. L., Stelinski, L. L., and Pelz-Stelinski, K. S. 2023. Foliar Antibiotic Treatment Reduces Candidatus Liberibacter asiaticus Acquisition by the Asian Citrus Psyllid, Diaphorina citri (Hemiptera: Liviidae), but Does not Reduce Tree Infection Rate. Journal of Economic Entomology 116:78–89.

Sundin, G. W., and Wang, N. 2018. Antibiotic Resistance in Plant-Pathogenic Bacteria. Annual Review of Phytopathology 56:161–180.

Qureshi, J. A., Kostyk, B. C., and Stansly, P. A. 2014a. Insecticidal Suppression of Asian Citrus Psyllid Diaphorina citri (Hemiptera: Liviidae) Vector of Huanglongbing Pathogens ed. Kun Yan Zhu. PLoS ONE 9:e112331.

Roldán, E. L., Stelinski, L. L., and Pelz-Stelinski, K. S. 2024. Reduction of *Wolbachia* in *Diaphorina citri (Hemiptera: Liviidae)* increases phytopathogen acquisition and decreases fitness ed. Arash Rashed. Journal of Economic Entomology 117:733–749.

Sétamou, M., and Bartels, D. W. 2015. Living on the Edges: Spatial Niche Occupation of Asian Citrus Psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae), in Citrus Groves. PLoS One 10:e0131917.

Tiwari, S., Killiny, N., Mann, R. S., Wenninger, E. J., and Stelinski, L. L. 2013. Abdominal color of the Asian citrus psyllid, Diaphorina citri, is associated with susceptibility to various insecticides. Pest Management Science 69:535–541.

Tsai, J. H., and Liu, Y. H. 2000. Biology of Diaphorina citri (Homoptera: Psyllidae) on Four Host Plants. ec 93:1721–1725.

University of Georgia, College of Agriculture and Environmental Science. 2025. 2025 Georgia Ag Impact Report. https://discover.caes.uga.edu/georgiaagimpact/ (accessed 30 Apr 2025).

Wenninger, E. J., and Hall, D. G. 2008. Daily and Seasonal Patterns in Abdominal Color in Diaphorina citri (Hemiptera: Psyllidae). Annals of the Entomological Society of America 101:585–592.

Wenninger, E. J., Stelinski, L. L., and Hall, D. G. 2009. Relationships Between Adult Abdominal Color and Reproductive Potential in *Diaphorina citri* (Hemiptera: Psyllidae). Annals of the Entomological Society of America 102:476–483.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., and Deng, X. 2016. Unusual Five Copies and Dual Forms of nrdB in “Candidatus Liberibacter asiaticus”: Biological Implications and PCR Detection Application. Sci Rep 6:39020.

Table 4.1. Primers and probes used for detection of CLas within ACP collected from Georgia commercial groves via qPCR.

Target Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'	Probe	Reference
ACP: WG	GCT CTC AAA GAT CGG TTT GAC GG	GCT GCC ACG AAC GTT ACC TTC	TTACTGACCATCAC TCTGGACGC	Coy et al. 2014
CLas: RNR-1	CAT GCT CCA TGA AGC TAC CC	GGA GCA TTT AAC CCC ACG AA	CCT CGA AAT CGC CTA TGC AC	Zheng et al. 2016

Table 4.2. Dates of pesticide application provided by the grower, rate, active ingredient and IRAC group for each insecticide applied to commercial groves (P1 and P2) from April to October 2024

Date of application	Product applied	Active Ingredient	IRAC Group[a]	Rate (oz/Acre) applied
April 17, 2024	Sivanto Prime	Flupyradifurone	4D	14
May 6, 2024	Sivanto Prime	Flupyradifurone	4D	14
June 5, 2024	Mustang Maxx	Zeta-cypermethrin	3A	4
July 6, 2024	AbbA Ultra	Abamectin	6	4
September 22, 2024	AbbA Ultra	Abamectin	6	4
October 29, 2024	Mustang Maxx	Zeta-cypermethrin	3A	4

[a]IRAC: Insecticide Resistance Action Committee

Table 4.3. UGA weather stations closest to each of the groves monitored and their distance from commercial grove

Grove ID	Location, county	Weather station and location	~ Distance between citrus grove and weather station (km)
P1	Pierce	Waycross, Ware Co. GA	17.07
P2	Pierce	Waycross, Ware Co. GA	17.4
WA	Ware	Waycross, Ware Co. GA	16.82
BA	Bacon	Alma, Bacon Co. GA	20.47
WN	Wayne	Odom, Wayne Co. GA	6.35
CO	Coffee	Douglas, Coffee Co. GA	13.9

Table 4.4. Grove ID, size, date of initial monitoring and number of nymph and adult ACP recovered from traps and scouting in each commercial grove in this study.

Georgia County	Grove ID	Size of grove (Ha)	Date of initial monitoring[a]	Number of Traps[b]	Total number of traps evaluated[c]	Adult ACP recovered[d]	Nymphs recovered[e]
Pierce	P1	0.42	Sept 25, 2023	9-12	207	16	20
	P2	0.85	April 23, 2024	11-16	143	82	9
Ware	WA	0.26	April 23, 2024	6	84	0	0
Wayne	WN	0.39	Sept 25, 2023	5	115	0	0
Bacon	BA	1.13	April 23, 2024	3	42	0	0
Coffee	CO	5.46	April 15, 2024	6	90	0	0
Total				**40**	**681**	**98**	**29**

[a]Refers to the first day that scouting was performed, or traps were set within each grove
[b]Indicates number of traps set in each grove at each time point
[c]Combined total number of traps recovered from each grove (number of traps x number of times collected or exchanged), including traps that were not placed for entire span of study
[d]Includes adult ACP found in scouting
[e]Nymphs only recovered during scouting. No nymphs were recovered from traps

Table 4.5. Extraction method, DNA concentration and A_{260}/A_{280} ratio for each psyllid collected from commercial groves in Georgia

Date Set	Date Collected	Grove	Trap Location (Tree ID)	Psyllid ID	DNA ng/µL	A260/ A280	Extraction Protocol	Average Cq Value
NA	20-Sep-2023	P1	Adult	CPsyllid1	21.4	2.01	USDA	36.74
NA	20-Sep-2023	P1	Adult	CPsyllid2	17.4	2.05	USDA	
NA	20-Sep-2023	P1	Adult	CPsyllid3	35.8	2.01	USDA	
NA	20-Sep-2023	P1	Adult	CPsyllid4	56.2	1.94	USDA	
NA	20-Sep-2023	P1	Adult	CPsyllid5	36.1	1.99	USDA	
NA	20-Sep-2023	P1	Nymph	N1	42	1.48	USDA	
NA	20-Sep-2023	P1	Nymph	N2	16.7	1.85	USDA	
NA	20-Sep-2023	P1	Nymph	N3	15.3	1.75	USDA	
NA	20-Sep-2023	P1	Nymph	N4	23.3	1.66	USDA	
NA	20-Sep-2023	P1	Nymph	N5	45.1	1.44	USDA	
NA	20-Sep-2023	P1	Nymph	N6	8	1.57	USDA	
NA	20-Sep-2023	P1	Nymph	N7	6.3	1.45	USDA	
NA	20-Sep-2023	P1	Nymph	N8	7.3	1.62	USDA	
NA	20-Sep-2023	P1	Nymph	N9	9.4	1.79	USDA	
NA	20-Sep-2023	P1	Nymph	N10	20.5	1.78	USDA	
NA	20-Sep-2023	P1	Nymph	N11	5.4	1.66	USDA	
NA	20-Sep-2023	P1	Nymph	N12	18.6	1.73	USDA	
NA	20-Sep-2023	P1	Nymph	N13	14.7	1.61	USDA	
NA	20-Sep-2023	P1	Nymph	N14	22.1	1.68	USDA	
NA	20-Sep-2023	P1	Nymph	N15	19	1.75	USDA	
NA	20-Sep-2023	P1	Nymph	CPsyllid6	18.9	1.97	USDA	
NA	20-Sep-2023	P1	Nymph	CPsyllid7	28.5	2.1	USDA	
NA	20-Sep-2023	P1	Nymph	CPsyllid8	9.7	1.83	USDA	
NA	20-Sep-2023	P1	Nymph	CPsyllid9	11.8	1.9	USDA	
NA	20-Sep-2023	P1	Nymph	CPsyllid10	39.5	2.12	USDA	
20-Sep-2023	29-Sep-2023	P1	R5T15	ACP2	7.8	1.34	USDA	

20-Sep-2023	29-Sep-2023	P1	R5T15?	ACP3	3.7	1.57	USDA
20-Sep-2023	29-Sep-2023	P1	R8T16	ACP1	5.6	1.53	USDA
20-Sep-2023	29-Sep-2023	P1	R8T16	ACP4	3.2	1.37	USDA
20-Sep-2023	29-Sep-2023	P1	R8T16	ACP5	7.7	1.32	USDA
15-Dec-2023	19-Mar-2024	P1		A31924	1.6	1.32	USDA
19-Mar-2024	11-Apr-2024	P1	R3T12	R3T12-4/4/2024	6.7	1.68	USDA
19-Mar-2024	11-Apr-2024	P1	R7T17	R7T17-4/4/2024	3.8	1.39	USDA
19-Mar-2024	11-Apr-2024	P1	R9T7	R9T7-4/4/2024	4.6	1.52	USDA
18-Apr-2024	2-May-2024	P3	R1T1	11A1	55.7	1.36	USDA
18-Apr-2024	2-May-2024	P3	R1T15	15A1	48.1	1.39	USDA
18-Apr-2024	2-May-2024	P3	R1T15	15A2	0.4	6.39	USDA
18-Apr-2024	2-May-2024	P3	R1T15	15A3	0.1	1.39	USDA
18-Apr-2024	2-May-2024	P3	R1T15	15A4	10.7	1.49	USDA
18-Apr-2024	2-May-2024	P2	R4T8	48A1	6.4	2.28	USDA
18-Apr-2024	2-May-2024	P2	R5T1	51A1	26.3	1.43	USDA
2-May-2024	17-May-2024	P3	R11T1	P3111B1	1.9	1.33	USDA
2-May-2024	17-May-2024	P2	R1T1	P211B1	1.6	1.35	USDA
2-May-2024	17-May-2024	P3	R1T15	P3115B1	1.7	0.83	USDA
2-May-2024	17-May-2024	P3	R1T15	P3115B2	3.1	1.41	USDA
2-May-2024	17-May-2024	P3	R1T15	P3115B3	2.2	1.35	USDA
2-May-2024	17-May-2024	P3	R1T15	P3115B4	27.7	1.36	USDA
2-May-2024	17-May-2024	P3	R1T15	P3115B5	48.2	1.33	USDA

2-May-2024	17-May-2024	P3	R1T15	P3115B6	17.7	1.35	USDA
2-May-2024	17-May-2024	P3	R22T20	P32120B1	36.7	1.21	USDA
2-May-2024	17-May-2024	P2	R5T1	P251B1	27.1	1.34	USDA
2-May-2024	17-May-2024	P2	R5T1	P251B2	49.4	1.35	USDA
2-May-2024	17-May-2024	P1	R7T17	P1717B1	15.5	1.4	USDA
17-May-2024	31-May-2024	P3	R11T1	R11T1C	1.7	1.17	USDA
17-May-2024	31-May-2024	P3	R1T15	R0T20C	46	1.37	USDA
17-May-2024	31-May-2024	P3	R22T9	R21T6C	3.4	1.41	USDA
17-May-2024	31-May-2024	P2	R5T1	R5T1C	26.8	1.27	USDA
31-May-2024	14-Jun-2024	P3	R1T15	R0T20D	33.2	1.56	USDA
31-May-2024	14-Jun-2024	P3	R22T9	R21T6D	55	1.36	USDA
14-Jun-2024	28-Jun-2024	P2	R4T8	P248	23.5	1.26	USDA
28-Jun-2024	12-Jul-2024	P3	R22T20	P32120-1	6.6	1.29	USDA
28-Jun-2024	12-Jul-2024	P3	R22T20	P32120-2	54.2	1.32	USDA
28-Jun-2024	12-Jul-2024	P3	R22T20	P32120-3	8.9	1.3	USDA
12-Jul-2024	12-Aug-2024	P3	R22T20	P32120-1	7.4	1.45	USDA
12-Jul-2024	12-Aug-2024	P3	R22T20	P32120-2	5.6	1.33	USDA
12-Aug-2024	21-Aug-2024	P2	R11T4	S16	11.1	1.33	USDA
12-Aug-2024	21-Aug-2024	P2	R11T4	S17	6.8	1.29	USDA
12-Aug-2024	21-Aug-2024	P2	R11T4	S18	7.4	1.33	USDA

12-Aug-2024	21-Aug-2024	P2	R11T4	S19	11.2	1.36	USDA	
12-Aug-2024	21-Aug-2024	P3	R15T1	S12	7.4	1.32	USDA	34.43
12-Aug-2024	21-Aug-2024	P3	R22T20	S13	3.6	1.69	USDA	34.00
12-Aug-2024	21-Aug-2024	P3	R22T20	S14	4.2	1.34	USDA	
12-Aug-2024	21-Aug-2024	P3	R22T20	S15	5.8	1.32	USDA	
12-Aug-2024	21-Aug-2024	P3	R22T9	S20	4.1	1.33	USDA	
21-Aug-2024	28-Aug-2024	P2	R11T4	S3	13.1	1.36	USDA	36.01
21-Aug-2024	28-Aug-2024	P2	R11T4	S4	12.6	1.36	USDA	36.65
21-Aug-2024	28-Aug-2024	P2	R11T4	S5	31.7	1.35	USDA	35.90
21-Aug-2024	28-Aug-2024	P2	R11T4	S6	8.3	1.35	USDA	34.28
21-Aug-2024	28-Aug-2024	P2	R11T4	S7	6.5	1.47	USDA	36.29
21-Aug-2024	28-Aug-2024	P3	R13T3	S11	17.3	1.32	USDA	37.71
21-Aug-2024	28-Aug-2024	P2	R1T1	S1	18.7	1.35	USDA	37.34
21-Aug-2024	28-Aug-2024	P2	R1T1	S2	13.1	1.35	USDA	
21-Aug-2024	28-Aug-2024	P3	R1T10	S9	44.9	1.27	USDA	35.70
21-Aug-2024	28-Aug-2024	P3	R1T15	S21	44.4	1.33	USDA	
21-Aug-2024	28-Aug-2024	P3	R22T20	S10	20.5	1.37	USDA	37.04
21-Aug-2024	28-Aug-2024	P3	R22T9	S8	73.1	1.3	USDA	36.19
21-Aug-2024	28-Aug-2024	P2	R4T8	S23	1.2	1.22	USDA	
28-Aug-2024	18-Sep-2024	P2	R11T4	S59	4.0	2.01	Incubation	
28-Aug-2024	18-Sep-2024	P2	R11T9	S22	9.6	1.34	USDA	
18-Sep-2024	4-Oct-2024	P2	R11T4	S33	32.8	1.52	Incubation	
18-Sep-2024	4-Oct-2024	P2	R11T4	S34	18.4	1.62	Incubation	
18-Sep-2024	4-Oct-2024	P2	R11T4	S35	21.1	1.56	Incubation	
18-Sep-2024	4-Oct-2024	P2	R11T4	S36	78.9	1.59	Incubation	
18-Sep-2024	4-Oct-2024	P2	R11T4	S37	30	1.52	Incubation	
18-Sep-2024	4-Oct-2024	P3	R16T22	S24	22.6	2.04	Incubation	
18-Sep-2024	4-Oct-2024	P3	R16T22	S25	11.7	1.75	Incubation	
18-Sep-2024	4-Oct-2024	P3	R1T1	S28	19.2	1.64	Incubation	

18-Sep-2024	4-Oct-2024	P3	R1T1	S29	24.6	1.8	Incubation	
18-Sep-2024	4-Oct-2024	P3	R1T10	S27	3.8	3.56	Incubation	
18-Sep-2024	4-Oct-2024	P3	R1T15	S30	15.3	1.97	Incubation	
18-Sep-2024	4-Oct-2024	P3	R1T15	S31	6.6	1.84	Incubation	
18-Sep-2024	4-Oct-2024	P3	R22T9	S26	33.7	1.69	Incubation	
18-Sep-2024	4-Oct-2024	P1	R5T15	S32	31.6	1.81	Incubation	
NA	11-Oct-2024	P3	Adult	S46	36.2	1.85	Incubation	36.73
NA	11-Oct-2024	P3	Adult	S47	34.4	1.8	Incubation	
NA	11-Oct-2024	P3	Adult	S48	22	1.76	Incubation	
NA	11-Oct-2024	P3	Adult	S49	22.5	1.93	Incubation	
NA	11-Oct-2024	P3	Nymph	S50	4.6	2.7	Incubation	
NA	11-Oct-2024	P3	Nymph	S51	6.6	2.82	Incubation	
NA	11-Oct-2024	P3	Nymph	S52	11.1	2.49	Incubation	
NA	11-Oct-2024	P3	Nymph	S53	7.1	2.19	Incubation	
NA	11-Oct-2024	P3	Nymph	S54	7.3	1.81	Incubation	
NA	11-Oct-2024	P3	Nymph	S55	4.9	3.41	Incubation	
NA	11-Oct-2024	P3	Nymph	S56	21.8	2.2	Incubation	
NA	11-Oct-2024	P3	Nymph	S57	28.7	2.22	Incubation	
NA	11-Oct-2024	P3	Nymph	S58	40.5	1.96	Incubation	
4-Oct-2024	11-Oct-2024	P2	R11T4	S41	51	1.9	Incubation	
4-Oct-2024	11-Oct-2024	P2	R11T4	S42	32.5	2.01	Incubation	
4-Oct-2024	11-Oct-2024	P2	R11T4	S43	23.9	1.97	Incubation	
4-Oct-2024	11-Oct-2024	P2	R11T4	S44	17.9	1.96	Incubation	
4-Oct-2024	11-Oct-2024	P2	R11T9	S45	25.7	2.1	Incubation	
4-Oct-2024	11-Oct-2024	P3	R1T1	S39	24.3	1.7	Incubation	
4-Oct-2024	11-Oct-2024	P3	R1T10	S40	56.1	2	Incubation	
4-Oct-2024	11-Oct-2024	P3	R22T9	S38	35.4	1.84	Incubation	
				+ Control				**31.95**
11-Oct-2024		P2	R11T4	S60	4.7	1.98		

11-Oct-2024	P3	R16T22	S61	9.0	1.93
11-Oct-2024	P3	R16T22	S62	7.0	1.96

+Control: Residential ACP reported in Collins et al. 2025

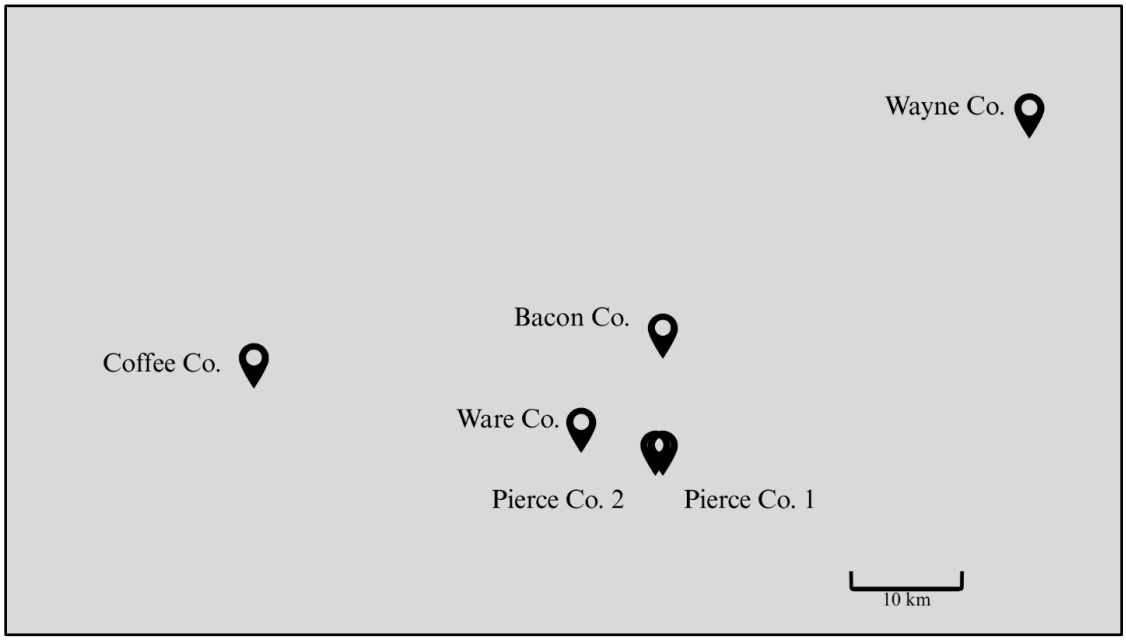

Figure 4.1. Map showing distance between citrus groves where ACP monitoring and scouting were conducted in this study

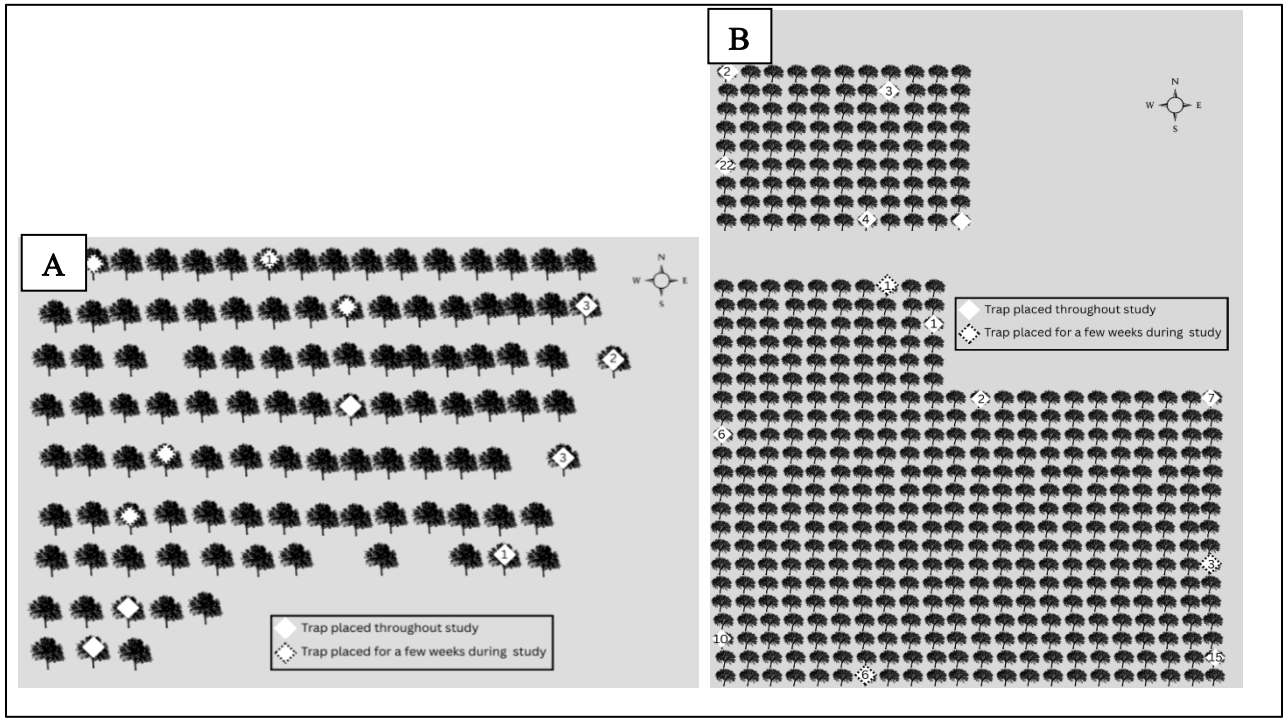

Figure 4.2. Map showing placement of traps within P1(A) and P2 (B) and number of ACP (within the symbol) recovered from each trap between Sept. 2023 and Oct. 2024

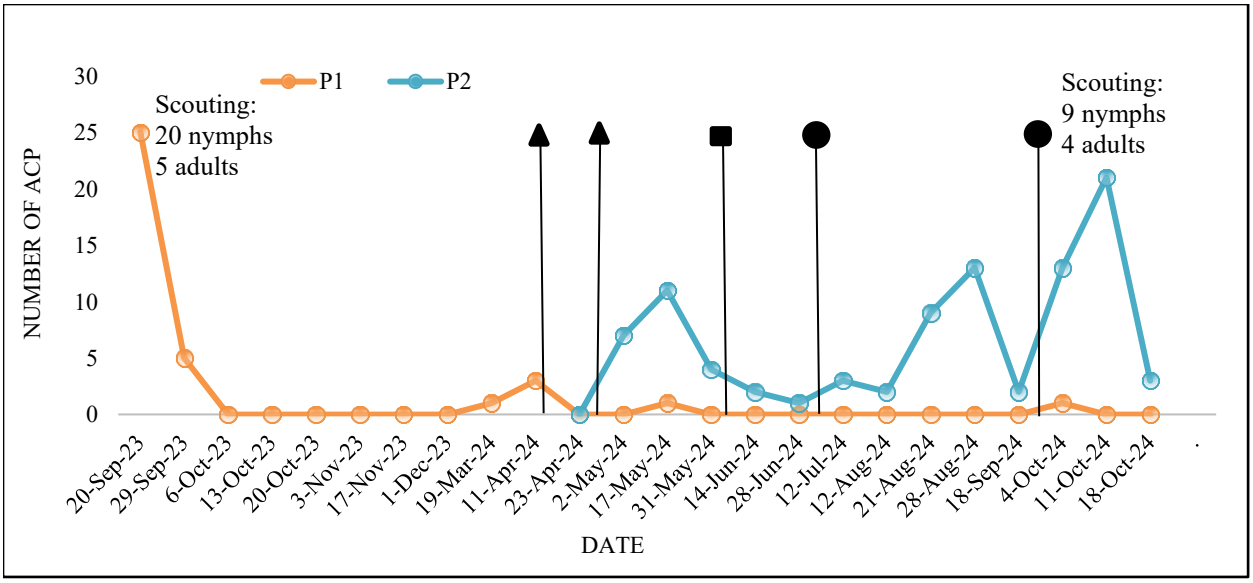

Figure. 4.3 Total number of ACP (nymphs and adults) collected from Pierce County, Georgia. P1= Pierce 1 (green line) September 2023 to October 2024. P2= Pierce 2 (blue line) April 2024 to October 2024 from scouting and trapping. Each date represents a timepoint when traps were recovered and scouting conducted. Insecticide application dates are represented by black line with triangles (Sivanto Prime), square (Mustang Maxx) and circles (AbbA Ultra).

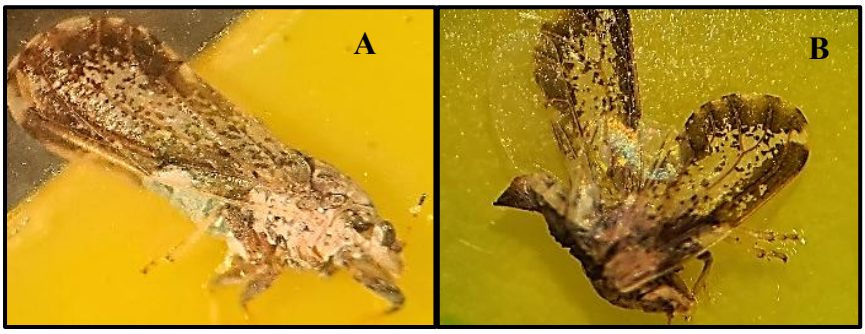

Figure. 4.4 Morphotypes of ACP observed in commercial groves. Blue, green (A). Gray, brown (B)

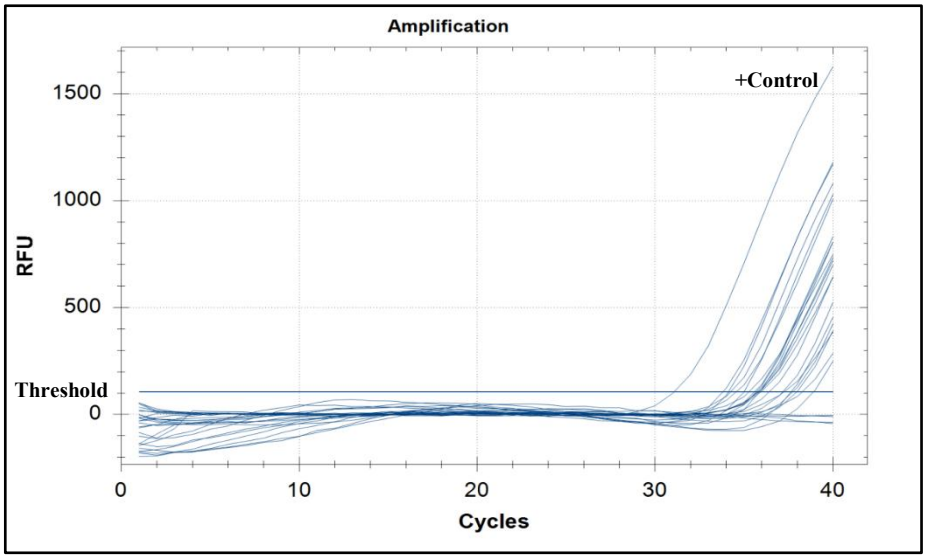

Figure 4.5. Cq values following a qPCR assay for CLas detection in Asian Citrus Psyllids recovered from Georgia.

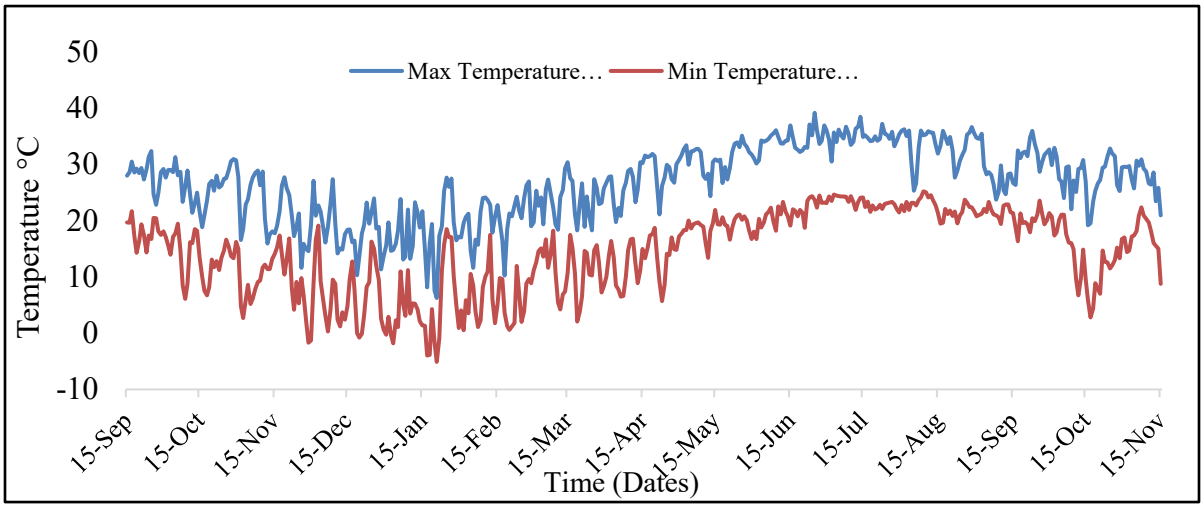

Figure 4.6. Maximum and minimum daily temperatures observed from September 15, 2023, to November 15, 2025, at the Waycross weather station.

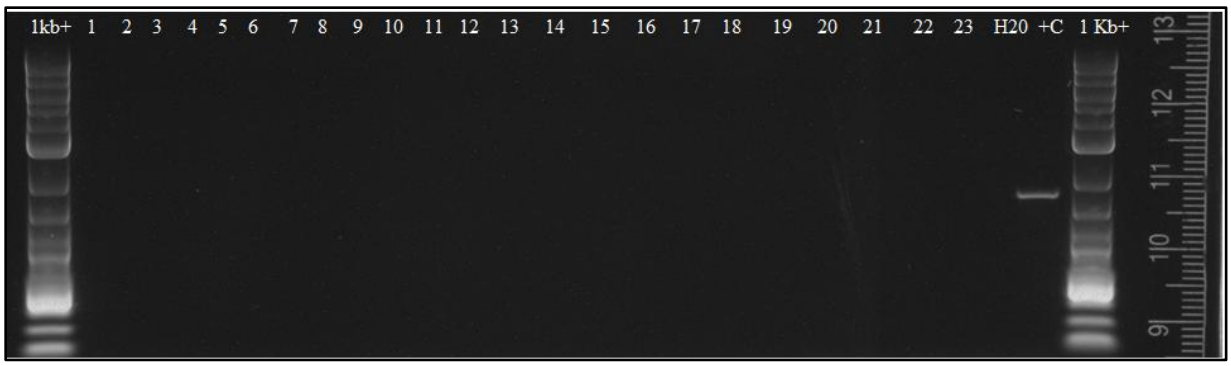

Figure 4.7. Example of 0.7% agarose gel with no 1,160 bp amplicon when testing ACP via conventional PCR for detection of CLas. +C: Positive control.

CHAPTER 5

CONCLUSIONS

Georgia holds enormous potential for citrus production, especially with the growing popularity of non-traditional types of citrus including Satsumas (*Citrus reticulata* 'Owari'). The recent rapid growth of Georgia's citrus production, which has surpassed the peach production for which the state is well known, alludes to growing excitement and confidence among growers. Safeguarding the future of the citrus industry needs to be a priority for all involved. Citrus greening, caused by '*Candidatus* Liberibacter asiaticus' has resulted in a 90% reduction in Florida's citrus production and is arguably the biggest threat to the future of the commercial citrus industry in Georgia.

In this study, commercial trees were tested for CLas using quantitative PCR based on protocols developed by USDA-APHIS. Of the 804 trees surveyed, 4.35% tested positive for CLas. Those positive trees were in Pierce, Ware and Wayne Counties. Infected trees were located mostly along the edges of groves and occurred in clusters. DNA sequencing revealed no genetic variations withing the 16S rRNA region among CLas strains collected from commercial groves. However, prophage type 1 was associated with all strains and prophage type 2 was associated with five CLas strains. Understanding the incidence and distribution of CLas from these selected commercial groves will allow the implementation of grove specific or statewide management strategies. The genetic diversity among CLas strains in Georgia was unknown, therefore this study is fundamental in initiating an understanding on the potential origin and movement of CLas between groves.

Some of the major challenges that we encountered when carrying out this research include the time-consuming nature of the extraction protocols for plant tissue when processing hundreds of samples. This protocol, authorized by the USDA, reduces the risk of contamination, however testing large batches of samples can pose a problem. This will be particularly true when these efforts expand to include larger groves that have thousands of trees. The solution to this issue is unclear, however there are automatic extraction machines that may help speed up the process. Another challenge was the sensitivity of the qPCR assay for testing plant tissue. Even the slightest contamination may register a Cq value below the threshold and create false positives. Additionally, the adaptation of the USDA protocol for testing using the thermal cycler Bio-Rad CFX opus 96 was prompted based on our request to utilize this machine and consequently baseline thresholds were suggested. These thresholds should be revisited to ensure maximum testing efficiency. Other things to consider include timing of sampling and sampling tissue selection to maximize the likelihood of detecting CLas especially in trees that may have low titer. More frequent sampling in smaller batches to reduce time that leaf tissue is stored before extraction of DNA and using hydrogen peroxide instead of acetyl alcohol to spray pruners to reduce cross contamination at time of sampling should also be considered. One potentially interesting area of research will be to do a more detailed study of the diversity of CLas in Georgia using next generation sequencing technologies and including strains from residential samples and ACP.

Asian citrus psyllids are the major vectors of CLas in North America. Scouting and monitoring were done between Fall 2023 and Fall 2024 in commercial groves in Pierce, Wayne, Ware, Bacon and Coffee Counties to understand the prevalence, distribution and infection rate of ACP. Among the 127 ACP recovered from two commercial groves in Pierce County 11% were determined to be CLas positive based on qPCR, and blue-green and gray-brown morphotypes were

observed. The number of ACP observed on yellow sticky traps and via scouting fluctuated throughout the year, with the observed fluctuation likely influenced by temperature, leaf flush periods, and the application of insecticides. Our findings confirm that CLas-infected ACP are present within commercial groves and suggest that ACP may be able to overwinter and survive despite low temperatures.

In this study, the major way that ACPs were recovered from groves was on yellow sticky traps. This method of trapping was very useful, however the frequency of replacing these traps presented an issue as ACP may have been trapped the day of setting but were not retrieved until weeks later. More frequent retrieval of ACP and/or the use of other trapping mechanisms may be necessary to improve freshness and quality of DNA. It is also well documented that ACP is associated with a number of symbionts that may disrupt the detection of CLas. Protocols have been developed to improve the quality of the DNA extracted from individual ACP and their use should be explored. Identification of these symbionts, some of which have been postulated to affect the acquisition and transmission of CLas by ACP, may also provide valuable information for CLas characterization in Georgia. Future research to better understand the ACP population in Georgia in regard to survivability, fecundity, morphotype diversity and sensitivity to insecticides will provide significant information to help manage this pest.

Together, the findings of this research provide valuable information regarding the current prevalence of CLas and ACP withing Georgia commercial groves and suggest that further monitoring and testing is needed to prevent a widespread or establishment of this disease in the State. Relevant stakeholders also have valuable information that should be considered for the development of policies that can ultimately save Georgia's citrus industry.