

Great variability in the infection rate of ‘*Candidatus Liberibacter asiaticus*’ in field populations of *Diaphorina citri* (Hemiptera: Liviidae) in Florida

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Huanglongbing (HLB) is a devastating disease of citrus, causing rapid decline and ultimate death of infected trees (Bové 2006). HLB has caused significant economic loss to the Florida citrus industry (Hodges & Spreen 2013) and now threatens those in Texas and California. At this time, there is no cure for this disease, and the only option to maintain productivity of infected trees is through treatments to assuage disease symptoms and intensive vector management. The presumptive causal pathogen of HLB infection is a gram negative α -proteobacterium, ‘*Candidatus Liberibacter asiaticus*’ (CLas). This pathogen is transmitted through feeding activity by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), on citrus phloem sap. Because HLB is widespread throughout Florida citrus, it is presumed that CLas infection may approach 100% in insect vector populations (e.g., Neff 2014). Citrus growers, researchers, and policy makers are interested in knowing the levels of CLas infection of both plant and insect samples in the field. To investigate the levels of CLas infection in the insect vector, we collected psyllids from six sites across central and southern Florida in the summer months of 2014 (Table 1 and Fig. 1). At each location, psyllids were collected from 200 - 250 mature Valencia citrus trees by D-Vac (Rincon-Vitova Insectaries; Ventura, CA) and brought back to the laboratory for testing. Insects were maintained under laboratory conditions for 24 hours on 20-30 cm high Kuharski Carrizo rootstock in Plexiglas cages under controlled conditions (T:27 °C; RH:55%; 10:14 h L:D) prior to assays. DNA was isolated from each of 48 independent adult psyllids of mixed gender for each sampled site using established protocols described in Coy et al. (2014). To determine the percentage of CLas-positive psyllids, a highly sensitive, nested-qPCR method for CLas detection was used, which can detect as few as three molecules of template per sample (Coy et al. 2014). A positive control with a known C_q value was used as a standard in all assays to evaluate the robustness of the assay and all negative controls were devoid of amplification signal. The infection rates for the six surveyed sites are shown in Fig. 1.

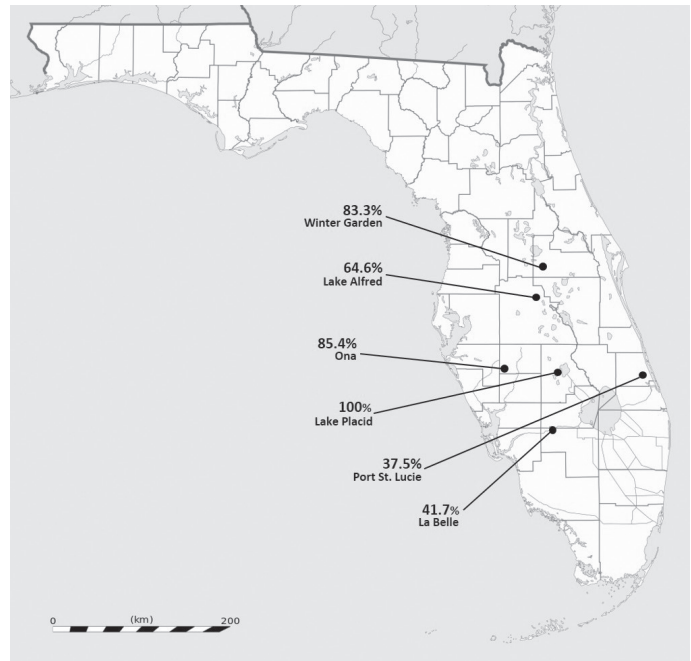


Fig. 1. Percent infection rates of *Diaphorina citri* with ‘*Candidatus Liberibacter asiaticus*’ in groves across central and southern Florida as determined from 48 independent adult psyllids per site.

Surprisingly, there were 2 relatively low rates of infection; both found in the southern part of the Florida citrus growing region, with 37.5 and 41.7% for Port St. Lucie and LaBelle, respectively. The range of infection was broad, ranging from 37.5–100%, with an average of 68.8% (SD 25.3%). There was no significant correlation between latitude and rate of infection based on this sample size. However, the lowest rates of infection were found at the two most southerly locations sampled (Fig. 1). If a correlation exists between infection rate and latitude, this would be congruent with the reported temperature sensitivity of CLas; i.e. greater mortality at higher temperatures (Jagoueix 1994; Hoffman 2013). However, the influence of factors such as geographical features and management practices cannot be discounted and requires further investigation. The insects were collected over the course of several months, and therefore time cannot be ruled out as a factor that might influence infection rates. It is possible that as the season progresses, infection rates change. However, we do not believe that this was the

Table 1. Collection sites and dates of *Diaphorina citri*.

Location	County	GIS Coordinates (DD)	Collection Date
Lake Placid	De Soto	27.256303, -81.462028	June 16 th , 2014
Pt. St. Lucie	St Lucie	27.36868, -80.550495	June 9 th , 2014
LaBelle	Hendry	26.693667, -81.439167	July 14 th , 2014
Lake Alfred	Polk	28.088565, -81.750880	August 11 th , 2014
Ona	Hardee	27.477922, -81.984619	September 3 rd , 2014
Winter Garden	Orange	28.465871, -81.659135	May 5 th , 2014

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major factor in our observations because the insects with the lowest infection rate, 37.5%, and those with the highest infection rate, 100%, were collected one week apart. In addition, there is no correlation between percent infection and order in which the insects were collected.

The results presented here show that the infection rate of *D. citri* with CLas is not 100% throughout Florida, and that in some groves it is significantly lower, despite the fact that HLB is widespread across the state. The basis of this variability should be investigated because it may provide clues for reducing potential of CLas infection rates in field psyllids, thereby reducing the transmission of the pathogen to trees. The variability may be due to a factor that can be controlled, such as management practices. However, if due to a factor that cannot be controlled, such as latitude or geographic location, this information would be useful in furthering understanding of HLB disease dynamics. This could thus lead to the development of an approach to exploit this factor in order to reduce CLas levels in field populations of psyllids. We are initiating future investigations to address how the interactions between current management practices, geographical location, and sampling protocols may affect the assessment of *D. citri* infection rate under field conditions.

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Summary

Citrus growers, researchers, and policy makers are commonly interested in knowing the rate of CLas pathogen infection in populations of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in Florida and elsewhere. Given that HLB is widespread across Florida, a common presumption is that nearly 100% of *D. citri* are currently carrying the pathogen. We investigated this presumption and found that instead, in 2014 – nearly a decade after HLB was officially detected in Florida – the rate of CLas infection in populations of *D. citri* varies widely between commercial citrus growing locations. Across the state, infection rates ranged from 37.5 to 100%, with an average of 68.8% (SD 25.3%). We suggest that there may be yet unknown factors responsible for the lower rates of infection found at certain sites, particularly in South Florida, that may be useful for management of this disease when determined. Our future goal is to identify the potential factors that affect CLas infection rates within field populations of *D. citri* across Florida under varying management practices and geographical locations.

Key words: citrus greening, detection methods, geographic variability, insect vector

Resumen

Los productores de cítricos, los investigadores y los elaboradores de política están comúnmente interesados en conocer la tasa de infección por patógenos CLas en las poblaciones de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) en Florida y en otros lugares. Puesto que la enfermedad Huanglongbing (HLB o enverdecimiento de los cítricos) es generalizada en Florida, una presunción común es que casi el 100% de *D. citri* lleva actualmente el patógeno. Estamos investigando esta presunción y encontramos que al contrario, en 2014 - casi una década después de HLB se detectó oficialmente en la Florida - la tasa de infección CLas en las poblaciones de *D. citri* varía ampliamente entre los lugares de cultivo de cítricos comerciales. En todo el estado, la tasa de infección varía desde 37.5 hasta 100%, con un promedio de 68.8% (DS 25.3%). Sugerimos que puede haber factores aún desconocidos responsables para las tasas más bajas de infección que se encuentran en ciertos sitios, sobre todo en el sur de la Florida, que pueden ser útiles para el manejo de esta enfermedad, cuando sean determinados. Nuestra meta futura es identificar los posibles factores que afectan las tasas de infección CLas dentro de las poblaciones de campo de *D. citri* en toda la Florida bajo diferentes prácticas de manejo y localidades geográficas.

Palabras Clave: enverdecimiento de los cítricos, métodos de detección, variabilidad geográfica, insecto vector

References Cited

- Bové JM. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* 88: 7-37.
- Coy MR, Hoffmann M, Kingdom Gibbard HN, Kuhns EH, Pelz-Stelinski KS, Stelinski LL. 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 Microbiological Methods* 102: 15-22.
- Hodges AW, Spreen TH. 2012. Economic impacts of citrus greening (HLB) in Florida, 2006/07 – 2010/2011. Gainesville, FL: University of Florida Department of Food and Resource Economics, University of Florida; [Electronic Data Information Source (EDIS) Update FE903 2012].
- Hoffman MT, Doud MS, Williams L, Zhang M, Ding F, Stover E, Hall D, Shang S, Jones L, Gooch M, Fleites L, Dixon W, Gabriel D, Duan Y. 2013. Heat treatment eliminates '*Candidatus* *Liberibacter asiaticus*' from infected citrus trees under controlled conditions. *Bacteriology* 103(1): 15-22.
- Jagoueix S, Bové JM, Garnier M. 1994. The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology* 44: 379-386.
- Neff E. 2014. Protecting young trees. *Citrus Industry Magazine* 95(8): 50.