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Authors: Martini, Xavier, Addison, Thomas, Fleming, Barry, Jackson, Ian, Pelz-Stelinski, Kirsten, et al.

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OCCURRENCE OF *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE) IN AN UNEXPECTED ECOSYSTEM: THE LAKE KISSIMMEE STATE PARK FOREST, FLORIDA

XAVIER MARTINI*, THOMAS ADDISON, BARRY FLEMING, IAN JACKSON, KIRSTEN PELZ-STELINSKI AND LUKASZ L. STELINSKI
University of Florida, Entomology and Nematology Department, Citrus Research and Education Center,
700 Experiment Station Rd., Lake Alfred, FL 33850, USA

*Corresponding author; E-mail: xavier.martini@voila.fr

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of the bacterial pathogen, '*Candidatus* Liberibacter asiaticus' (Las), which is the putative causal agent of the citrus disease, huanglongbing (HLB) in the U.S.A. (Grafton-Cardwell et al. 2013). Although ACP typically reproduce on plants in the family Rutaceae, reproduction on *Ficus carica* L. (Rosales: Moraceae) (Thomas & De Leon 2011), and feeding on hackberry (*Celtis* spp.; Rosales: Ulmaceae) and potato (*Solanum tuberosum* L.; Solanales: Solanaceae) (Thomas 2011) has been reported. Therefore, ACP may feed on a wider array of alternative plants beyond the Rutaceae. Although some of these alternative plants may not be suitable for reproduction, they may serve as temporary or partial hosts for adult feeding, prolonging survival. This strategy would enhance survival of ACP in the absence of reproductive hosts, and thus may explain long distance movement impacting colonization of citrus groves (Tiwari et al. 2010).

On 12 July 2012, we captured 13 psyllids on 8 white sticky traps (Elm bark beetle traps, Great Lakes IPM, Vestaburg, Michigan) deployed 1 wk prior to collect redbay ambrosia beetles (*Xyleborus glabratus* Eichhoff; Coleoptera: Curculioidae), in Lake Kissimmee State Park forest (Polk county, Florida). These traps were located in a wet flatwood ecosystem (27°55'11.64"N; 81°22'25.38"W). The specimens were sent to the Florida Department of Agriculture and Consumer Services (FDACS) and were all identified as *D. citri*. They have been conserved as voucher specimens (sample number E2012-5211-1).

Through 8 Oct 2012, we monitored the ACP population at this location and 2 additional locations 60 m and 220 m east of the initial site where ACP were collected. Two yellow sticky traps (Trece AM traps, Trece Inc. Adair Oklahoma) were deployed randomly within a 500 m^2 area at both of these locations and monitored weekly until 17 Sep 2012. Thereafter, trap density was increased to 6 traps per location. A peak capture of 1.3 ACP per trap and per wk was recorded on 19 Jul 2012; average weekly capture of ACP decreased thereafter (Fig. 1A). Except for those specimens that were sent to FDACS for species confirmation, all ACP captured were individually isolated in 70% ethanol, and DNA was extracted. Quantitative polymerase chain reaction (qPCR) was conducted to determine if any of the psyllids carried Las bacteria [protocol in Pelz-Stelinski et al. (2010)]. Of the 12 ACP analyzed, 3 (25%) were positive for Las.

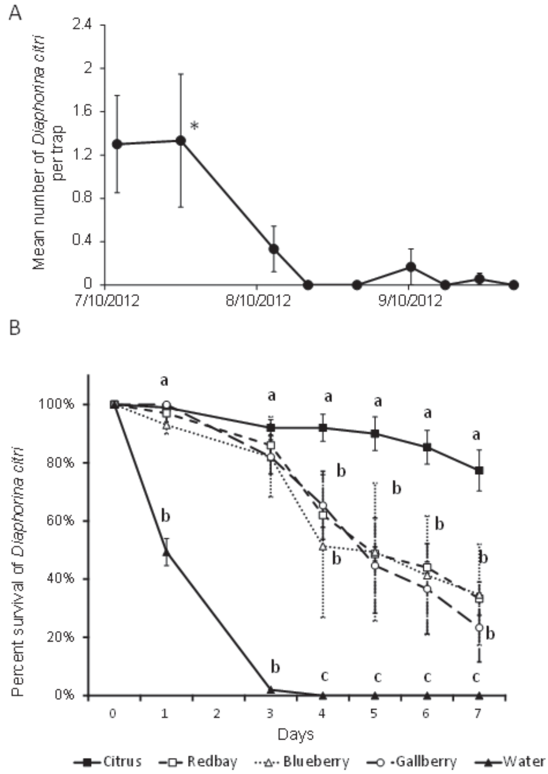

Fig. 1. (A) Mean number (± standard error) of *Diaphorina citri* captured per sticky trap in Lake Kissimmee State Park forest (LKSP). The asterisk indicates the date when the three Las-infected psyllids were captured. (B) Percent survival (± standard error) of adult *D. citri* on three native plant species found in LKSP. Days indicated by different letters indicate significant differences between *D. citri* survival at the $\alpha < 0.01$ level.

The closest citrus grove (13 ha) from the location in the forest was 2.3 km southwest, but this grove was abandoned during the trapping period

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and in significant decline (Fig. 2). After thorough exploration of the surrounding area, we found 4 non-cultivated tangerine plants (*Citrus reticulata* Blanco) within a 25 m transect along Rosalie Creek in a floodplain forest ecosystem approximately 1 km west of where the ACP were collected during this investigation (Fig. 2). DNA was extracted from 6 leaves of these trees and subjected to qPCR analysis [protocol in Pelz-Stelinski et al. (2010)] to determine Las infection. All of the leaves tested were negative for the pathogen. On 20 Aug 2012, 4 yellow sticky traps were deployed on these citrus trees and evaluated for ACP capture at weekly intervals until 8 Oct 2012. ACP were not captured at this location throughout the monitoring interval.

To determine whether alternative plants may harbor ACP in this forest, we sampled 2 locations where ACP had been captured. On 30 Jul and 6 Aug 2012, 3 persons conducted sweep net sampling of 4 potential habitats in the surrounding area: 1) grass, 2) small bushes (up to 1.5 m), 3) small trees (up to 2 m), 4) large trees (over 2 m). Each sample consisted of 10 min of continuous sampling. Following the 48 samples (3 samplers × 2 locations × 4 habitats × 2 days), no ACP were found. Species of plants for sampling were chosen at random; however, the most abundant ones found were later evaluated in the laboratory as described below.

In addition to the field surveys, no-choice feeding assays were conducted in the laboratory to determine if ACP could survive on the three most abundant non-host plants found in the area where ACP were captured and where sweep netting was conducted. The plants tested were gallberry *Ilex glabra* (L.) Gray (Aquifoliales: Aquifoliaceae), Darrow's blueberry *Vaccinium darrowii* Camp (Ericales: Ericaceae), and redbay, *Persea borbonia* (L.) Spreng. (Laurales: Lauraceae). We used 2-year old *Citrus sinesis* (L.) Osbeck 'Valencia' citrus plants as positive controls. We also included a negative control where plants were replaced with cups (266 mL) filled with cotton and water, and covered with a fine mesh, as a water source for ACP without other sources of food. Plants were bought at local nurseries and were not treated with pesticides. For each species, 2 plants were placed into fine mesh cages (90 × 60 × 30 cm), at 25 ± 1 °C, 55-65% RH, and 14:10 h L:D. Fifty field-collected ACP of unknown age were introduced into each cage and the numbers of dead ACP were counted over the course of 1 wk. There were 3 replications (150 ACP per treatment). ACP were observed to settle and apparently feed on all of the plant species tested. ACP that settled on the negative control treatment were found on the top of the humidified cup, but all were dead three days after the assay was initiated. Survival of ACP on each non-host plant was similar to that

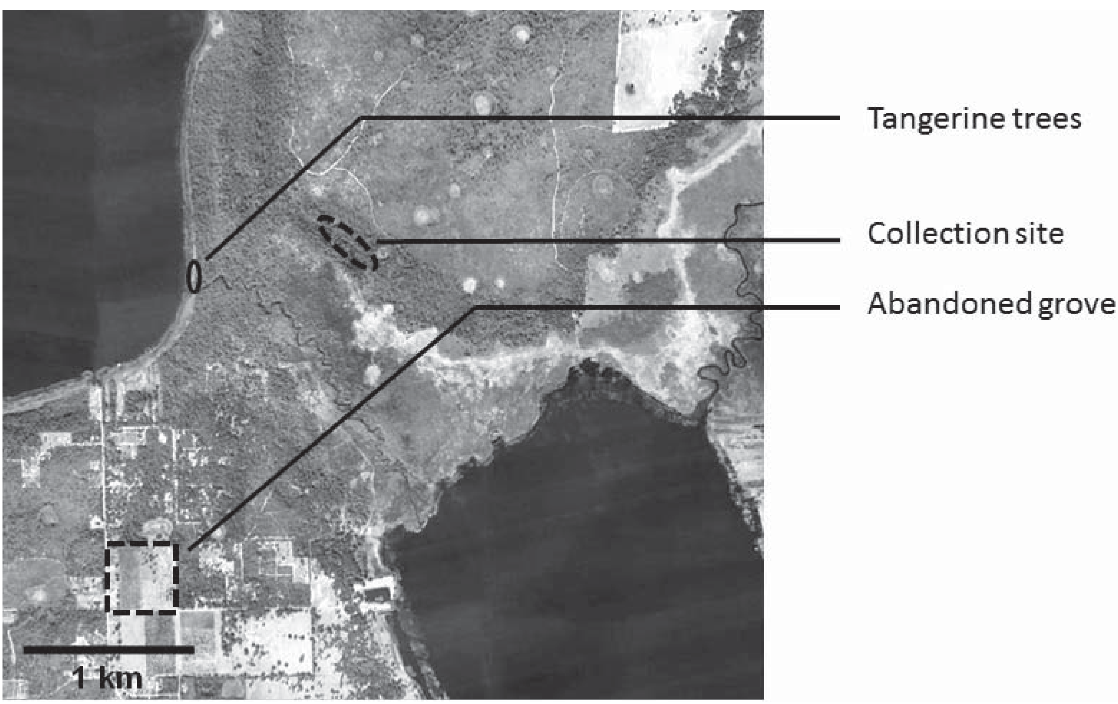

Fig. 2. Satellite view (Google Earth©, imagery date: 11 Jan 2011) of the collection area, indicating the abandoned grove (2.3 km from collection site) and the tangerine trees in border of Lake Rosalie (1 km from collection site).

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observed on citrus for the initial 3 days of the experiment and began to decrease significantly by the fourth day ($\chi^2 = 63.88$, df = 3, $P < 0.001$) (Fig. 1B). However, there was some ACP survival on all non-host plants evaluated for up to 7 days after the experiment was initiated (Fig. 1B). It is possible that the transfer of ACP from the field to the laboratory may have impacted survival in our assays. Consequently, we cannot rule out that ACP may, under field conditions, survive for longer intervals on the plants tested. Moreover, we only tested the three most abundant plant species within the collection area. Further investigation of alternative plants that occur in lower densities and that may serve as feeding or shelter sites for ACP is warranted.

Summary

The current investigation describes the capture of Asian citrus psyllid (ACP), *Diaphorina citri*, in an unexpected ecosystem, the Lake Kissimmee State Park forest. In this forest, occurrence of citrus is particularly rare, and no Rutaceae were found within the trapping locations or in nearby (≈500 m) areas. In addition to trap capture data, complementary laboratory no-choice feeding bioassays suggest that ACP may have a broader alternative plant feeding range than previously thought, which may allow for significant dispersal even through dense forests in Florida up to 2.3 km from large-area plantings of citrus.

Key Words: alternate host, '*Candidatus* Liberibacter asiaticus' *Ilex glabra, Vaccinium darrowii, Persea borbonia,* sticky traps

Resumen

La presente investigación describe la captura del psílido asiático de los cítricos (PAC), *Diaphorina citri*, en un ecosistema inesperado, el bosque del Parque Estatal del Lago Kissimmee. En este bosque, la aparición de los cítricos es particularmente raro, y no se encontraron plantas de Rutaceae en los lugares de captura o en las áreas cercanas (≈ 500 m). Además de los datos de captura, los bioensayos complementarios de alimentación sin opción versus con opciones hechos en laboratorio indican que el PAC puede tener una gama de plantas alternativas más amplia sobre las que se alimentan de lo que se pensaba anteriormente, lo que puede permitir una dispersión significativa, incluso a través de los densos bosques de la Florida hasta 2.3 km de las áreas grandes donde se siembran los cítricos.

Palabras Clave: hospederos alternativos, '*Candidatus* Liberibacter asiaticus', *Ilex glabra*, *Vaccinium darrowii*, *Persea borbonia*, trampas pegajosas

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