Seasonal Movement Patterns and Long-Range Dispersal of Asian Citrus Psyllid in Florida Citrus

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ABSTRACT The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the vector of the bacterial pathogen, *Candidatus* Liberibacter asiaticus, which is the causal agent of huanglongbing (HLB) in the United States. Both short-range and long-range dispersal of *D. citri* adults affect the spread of HLB; however, little is known about the long-range dispersal capabilities of *D. citri* in the field or the seasonality of flight behavior. In the present study, an in situ protein marking technique was used to determine the dispersal of *D. citri* by trapping marked adults under natural field conditions. *D. citri* movement from abandoned citrus groves to adjacent managed citrus groves was greatest during the spring and summer months and decreased significantly during the colder months (September–March). *D. citri* were able to traverse potential geographic barriers such as roads and fallow fields. In an experiment conducted to determine long-range dispersal capacity in the absence of severe weather events, *D. citri* were able to disperse at least 2 km within 12 d. Wind direction was not correlated with the number of marked psyllids captured, indicating substantial flight capability by *D. citri*. Finally, the number of marked psyllids captured increased with the density of emerging young leaves on surrounding trees. The results confirm that abandoned citrus groves in Florida serve as reservoirs for *D. citri*, which can disperse across long distances despite geographical barriers.

KEY WORDS citrus greening, mark–recapture, huanglongbing, immunomarking, ELISA

Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is a major citrus pest in the United States given that it transmits *Candidatus* Liberibacter asiaticus (Las), which is the likely bacterial pathogen responsible for causing huanglongbing (HLB). HLB is a worldwide devastating disease of citrus groves (Bové 2006). *D. citri* was first detected in Florida in 1998 (Halbert 1998) and quickly became established throughout the state and continues to spread to new citrus production areas (Wang and Trivedi 2013). HLB-infected trees show symptoms of fruit drop, off-season bloom, and twig dieback. Fruit from infected trees is often small, misshapen, and bitter tasting (Halbert and Manjunath 2004). Tree death may eventually occur as a result of HLB infection because of altered gene expression in the plants resulting in blockage of the phloem (Gottwald et al. 2007, Kim et al. 2009, Folimonova et al. 2009). Importantly, *D. citri* development is linked to the presence of newly emerging leaves termed "flush," which is the only site of nymphal development (Hall and Albrigo 2007).

An important factor in the spread of HLB is the role of abandoned citrus as a reservoir for *D. citri* and HLB. The number of abandoned groves in Florida has increased in the past decade because of socioeconomic pressures, freezing events, and the loss of production because of canker and HLB diseases (Cumming and George 2009). Abandoned groves are not treated with insecticides or managed in other ways to limit the populations of *D. citri*. Previous work has suggested that abandoned groves may provide a continuous source of *D. citri* and Las (Boina et al. 2009, Tiwari et al. 2010). Consequently, there are recommendations to remove and destroy abandoned citrus (FDACS 2013). As a result, the acreage of abandoned groves in Florida has only recently decreased from 56,055 to 51,386 ha over the past 3 yr (U.S. Department of Agriculture [USDA] 2012, 2013).

Spread of the disease is associated with the movement of *D. citri* between Las-infected and uninfected trees (Gottwald et al. 1991a,b). Therefore, the dispersal range of *D. citri* directly affects the rate and range of HLB dissemination both within a grove and between groves (Halbert and Manjunath 2004). Under laboratory conditions, it has been quantified that *D. citri* are capable of flying continuously up to 2.4 km without wind assistance (Martini et al. 2014). In addition, long-range dispersal of psyllids might be facilitated by wind. Gottwald et al. (2007) investigated HLB infection data and hypothesized that wind-assisted *D. citri* dispersal

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in Florida ranges from 90–145 km. In addition, Sakamaki (2005) suggested that *D. citri* could have dispersed up to 470 km, throughout the Okinawan islands, mediated by lower jet airstreams. Using a mark–release–recapture method with fluorescent powders, Kabori et al. (2011) recorded 5–12 -m dispersal distances for laboratory reared psyllids and suggested that *D. citri* move infrequently for the initial few days following colonization of a host plant. However, in Florida, *D. citri* are known to move at least 100 m from one citrus grove to an adjacent citrus grove over a 3-d period (Boina et al. 2009). In addition, some adults moved at least once from their grove of origin to an adjacent grove and then back to the original grove (Boina et al. 2009). Hall and Hentz (2011) found that adult psyllids dispersed from citrus up to 150 m throughout the year in east central Florida with the greatest dispersal in the spring. Geographical barriers may have little impact on dispersal of *D. citri* given that this psyllid has been captured in a dense forest 2.3 km away from the nearest citrus grove, which was abandoned (Martini et al. 2013). *D. citri* has also been captured in an urban environment (Chong et al. 2010, Godfrey et al. 2013).

To further quantify the movement of adult *D. citri* in the field, a protein marking technique (Jones et al. 2006) was used to mark wild populations of *D. citri* in situ. The specific objectives of this study were to experimentally quantify the dispersal capabilities of *D. citri* under normal field conditions in central Florida and to identify factors influencing dispersal such as wind direction, flush availability, geographical constraints, and grove type (abandoned vs. managed). In addition, experiments were conducted throughout the year to determine the seasonal variation in the dispersal of *D. citri*.

Materials and Methods

Protein Markers. A protein marking technique developed by Jones et al. (2006) and modified for optimal use under Florida weather conditions by Boina et al. (2009) was used to mark experimental plots. Two different food proteins were used to mark *D. citri* adults. Bovine casein (20% solution in water) in the form of whole milk (All Natural Whole Milk, Publix Super Markets, Lakeland, FL) was used as one of the markers. The second marker used was chicken egg albumin protein from egg whites (All Whites, Papetti Foods, Elizabeth, NJ) prepared as a 10% solution in water. A surfactant was added to both protein solutions to lower the surface tension of spray solutions (either Silwett L-77 [2,000 ppm] [Helena Chemicals, Collierville, TN] or Sylgard 309 [Wilber-Ellis Co., Fresno, CA]). The solutions were applied in the field using an all-terrain vehicle mounted handgun sprayer (model 5275016; Fimco Industries, North Sioux City, SD) calibrated at ~250 psi until visible leaf runoff (~7.5 liter per tree). *D. citri* acquired a protein mark either from direct contact during spraying or through contact with dried residue on leaves (Boina et al. 2009). It has been shown that over 90% of psyllids marked with egg and milk proteins remained marked for at least 10 d under field conditions (Boina et al. 2009).

Indirect ELISA. The methods used for the indirect enzyme-linked immunosorbent assay (ELISA) were modified from Jones et al. (2006) and Boina et al. (2009). Adult *D. citri* were individually removed from traps using disposable wooden toothpicks to minimize the risk of cross-contamination and placed into 1 ml of protein extraction buffer (Tris-buffered saline [TBS], pH 8.0 + 0.3 g/liter sodium ethylenediamine tetraacetic acid [EDTA]) (Sigma-Aldrich, St. Louis, MO) for 5 min. Eighty microliter of extraction buffer was removed and placed into a single well of a 96-well microplate (Nunc Polysorp; Fisher Scientific, Pittsburgh, PA). For each microplate, one adult *D. citri* from a greenhouse colony was soaked for 5 min in 1 ml of extraction buffer, and from this, eight wells were each filled with 80 µl to serve as negative controls. On each plate, eight wells of 80 µl extraction buffer alone served as blanks. Eighty microliter of a 0.001% dilution of milk or egg protein in TBS-EDTA was added to three wells per plate as a positive control. The microplates were covered with aluminum foil and incubated for 2 h at 37°C. After this incubation, each plate was washed five times with 300 µl of phosphate-buffered saline (PBS) pH 7.4 + 0.09% Triton X-100 (Sigma-Aldrich) (PBST) per well. Washing was accomplished with a microplate washer (Wellwash 4 Mk 2, Thermo Electron Corporation, Vantaa, Finland). After this wash, 300 µl of the blocking solution (StartingBlock, Fisher Scientific) was added to each well, followed by 1-h incubation at 37° C. Plates were then washed once with 300 µl of PBST. The primary antibodies were then discarded and each plate was washed five times with PBST. The secondary antibody used to detect egg protein was donkey anti-rabbit IgG (H + L) with a peroxidase conjugate (Pierce Biotechnology, Rockford, IL), and donkey anti-sheep IgG (Sigma Aldrich) was used for milk. Eighty microliter of the secondary antibodies, diluted in the blocking solution, were added to each well. Following 2-h incubation at 37°C, plates were washed three times with 300 µl of PBS + 2.3 g/liter sodium dodecyl sulfate (Sigma-Aldrich) and twice with 300 µl of PBS. Thereafter, 80 µl of 1-Step Ultra TMB-ELISA (Pierce Biotechnology, Rockford, IL) was added to each well, and the plate was covered with foil and placed on a shaker (IKA* MTS 2/4 Digital Microtiter Plate Shaker, [Fisher Scientific]) at room temperature for 10 min. Eighty microliter of 2N H_2SO_4 was added to each well to stop the reaction. The optical density (OD) from each well was read twice at 450 nm with 490 nm as a reference standard on an Emax microplate reader (Molecular Devices, LLC, Sunnyvale, CA), and the two values for each well were averaged. Error correction was accomplished by subtracting the mean OD value from the blank wells from each OD value of extracts from the trapped and laboratory psyllids. Any OD values higher than four standard deviations above the mean of the control were considered positive for having a protein mark (Jones et al. 2006).

Seasonality of *D. citri* Dispersal. To quantify the movement of *D. citri* from abandoned groves into managed groves at various points throughout the year, four replicate plots were established in adjacent abandoned and conventionally managed commercial citrus groves [sweet orange, *Citrus sinensis* (L.) Osbeck] located in Polk County, FL. The USDA (2012) considers a grove abandoned when there has been "no production care during the past two years, no weed control or grass mowing, livestock present, weather damage, neglected trees that are not economically feasible to maintain, or no commercial harvest during the last two seasons." In our case, the abandoned groves followed these conditions for at least three years. The experiment was initiated in June 2009 and concluded in March 2010. Each plot consisted of an abandoned subplot and an adjacent regularly managed commercial subplot (Fig. 1A). The abandoned and the regularly managed commercial subplots were separated by 50 to 100 m. A subplot consisted of a replicated area of 1 ha (10 rows by 30 trees) delineated within the abandoned and the regularly managed groves. Replicate blocks were separated by a minimum of 40 m. Once a month throughout the experiment, the 15th tree of each row in the abandoned subplots was sprayed with egg protein solution (Fig 1A). The 1st tree of each row (closest to the managed subplots) in the abandoned subplots was sprayed with milk protein (Fig 1A). Eight unbaited Pherocon AM yellow sticky traps (Trécé Inc., Adair, OK) were deployed in each managed and abandoned subplot after spraying with the protein marker. Four traps were placed on the 1st trees and four more on the 15th trees in rows 2, 4, 6, and 8. In addition, four traps were placed between the two subplots (Fig 1A). Traps were removed 4 d after treatment and the psyllids captured on each trap were collected. Each psyllid was placed into a 1.5-ml microcentrifuge tube containing 1 ml of the protein extraction buffer in preparation for the ELISA described previously. This experiment was repeated monthly for 10 months. The *D. citri* population within the study area during winter was estimated by stem tap counts 4 d after the application of protein markers beginning in September 2009, when the number of marked *D. citri* collected began to decline. Tap counts were taken on 10 trees on the border of each grove and 10 trees immediately adjacent to the border (Hall et al. 2007). In addition, another 10 trees from the 14th and 15th tree in the interior of the plot (~50 m from the border) were sampled. The number of adult psyllids dislodged onto 21 by 28 cm white

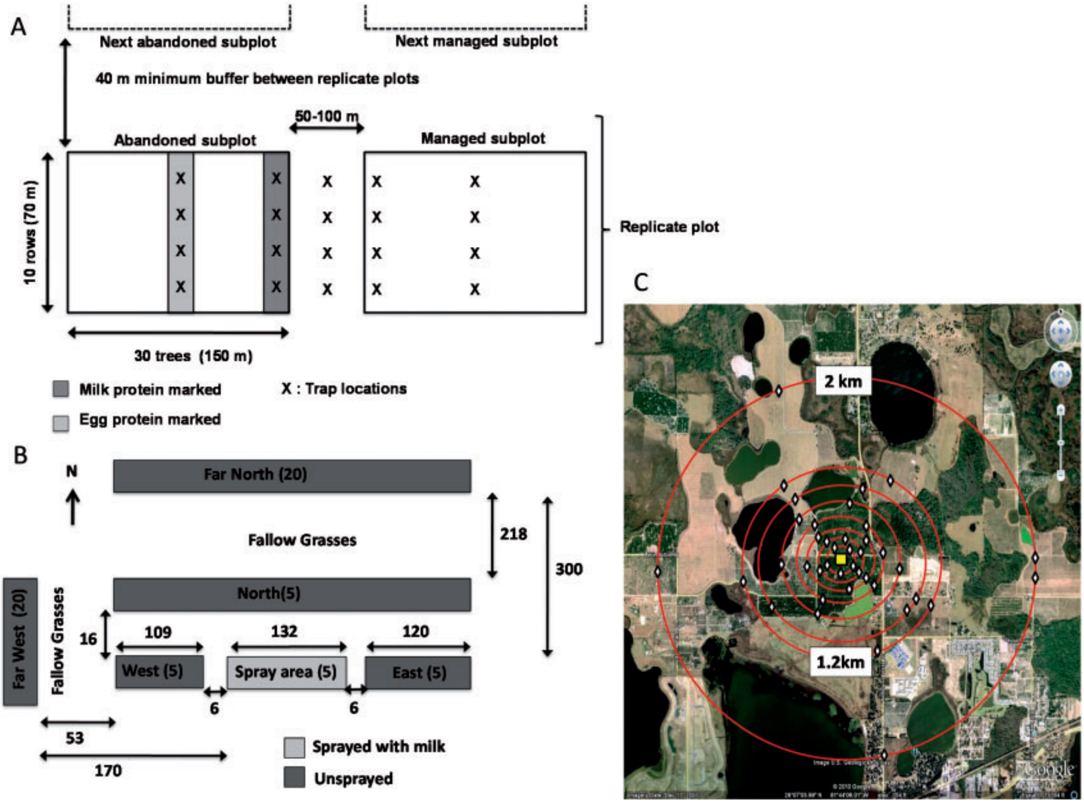

Fig. 1. Schematic maps of field plots for the three field experiments. (A) Field experiment setup to investigate seasonality of *D. citri* dispersal; (B) Design of the "fallow field experiment." The central block was sprayed with milk protein solution and the dispersal of psyllids was observed after 7 d. The numbers in brackets indicate the number of traps per block. Unit: m. (C) Field setup for the long-range dispersal experiment (Google Earth, imagery date: 17 December 2007). The central block (yellow square) was sprayed with egg protein solution and the dispersal of psyllids was observed after 11 d. The positioning of traps is indicated by white diamonds (traps positioned in the sprayed zone are not indicated).

cards for every two taps with a 0.45 m piece of polyvinyl chloride (PVC) tubing (2.5 cm in diameter) was recorded.

The analyses were performed with the statistical software R (v. 3.0.2; The R Foundation for Statistical Computing, Vienna, Austria). The number of marked psyllids captured consisted of repeated measure; consequently, these data were first log transformed to account for non-normal distribution, and then compared with a linear mixed model where grove type (managed or abandoned) and location of the trap (interior vs. edge) were used as fixed variables and the trap number as a random variable. For each sampling period, the proportion of psyllids marked in the interior of the abandoned grove (marked with egg protein) versus the proportion of psyllids marked on the edge of the abandoned grove (marked with milk protein) were compared with a chi-square test.

Fallow Field Experiment. The objective of this experiment was to investigate dispersal of *D. citri* from a given plot toward nearby plots in relation to wind direction. This experiment was conducted in a managed sweet orange grove of mixed varieties in Winter Park, FL. The grove consisted of six citrus blocks (*C. sinensis*) of various sizes. A single 1-ha block of "Ambersweet" trees was sprayed once on 3 September 2009 with 20% whole milk solution (Fig. 1B). After the spray dried, five un-baited Pherocon AM traps were placed evenly along the border of the sprayed area. Five traps were deployed in the three blocks immediately adjacent to the sprayed area: the east block ("Valencia" trees), the north block ("Ambersweet" trees), and the west block ("Valencia" trees). These blocks were separated from the sprayed area by 6 m (east and west) or 16 m (north) (Fig. 1B). In addition, 20 traps were placed in planted blocks 170 m west (the "Far west block, "Valencia" and "Hamlin" trees) and 300 m north (the "Far north" block, "Hamlin" trees) of the sprayed area (Fig. 1B). Traps were removed from the field on 9 September 2009, or 6 d after protein had been sprayed. The *D. citri* were removed, as described above, and analyzed by ELISA for the presence of the protein mark. Weather data were obtained from the Florida Automated Weather Network station located approximately 1 km from the experimental site.

Because the number of marked psyllids found in the six locations was not normally distributed, we used a generalized linear model (GLM) with a Poisson distribution for analysis. The dependent variable was the number of marked psyllid captured and the fixed variable was block location. We calculated *P*-values of the selected model using the likelihood ratio test that approximately follows a chi-square distribution. We subsequently performed post hoc tests to determine if the captures of marked psyllids in the six locations differed.

Long-range Dispersal Experiment. An area (~1,250 ha) was chosen to investigate the long-range movement capabilities of *D. citri* (Fig. 1C). The study area was made up of managed and abandoned citrus groves, fallow land, and residential properties. At the beginning of each experiment trial, a central area of 200 mature sweet orange trees (*C. sinensis* "Hamlin") (~0.45 ha) in a well-managed, 40-ha grove in Lake Alfred, FL, was sprayed with 10% egg protein with 2,000 ppm Sylgard 309. After spraying with the protein marker, eight un-baited Pherocon AM traps were placed within the protein-sprayed area: two each on the northern, eastern, southern, and western borders. At each of the eight concentric circles from the focal sprayed area (100, 300, 400, 500, 650, 1,000, 1,200, and 2,000 m), one pair of traps was deployed in five separate locations (Fig. 1C; Table 1). Traps placed at distances of ≥500 m extended beyond the border of the sprayed grove. Therefore, positions of traps >500 m were dictated by the availability of orange trees at each distance. Some traps were consequently placed into nearby managed and abandoned groves. There were 22 traps in abandoned groves and 58 traps in managed groves (Table 1). In addition, the relative abundance of citrus leaf flush was evaluated on the final day of the experiment in July 2010. A PVC pipe frame (16 by 16 by 16 cm) was randomly placed into the tree canopy and the number of flush shoots within the cube frame was counted (Hall and Albrigo 2007). At each trap site, two samples were taken from each of five adjacent trees and the 10 values were averaged to estimate relative flush abundance. Traps were removed 11 d after application of the marker protein. *D. citri* were removed from traps with a toothpick, separated by sex, and placed in a 1.5-ml microcentrifuge tube and analyzed for the presence of protein with ELISA.

The average daily wind direction data during the experiment were obtained from a Florida Automated Weather Network weather monitoring station located 4 km from the experimental area. The compass degree coordinates from the database were categorized into eight categories: N (338–22°), NE (23–67°), E (68–112°), SE (113–157°), S (158–202°), SW (203–247°), W (248–292°), and NW (293–337°). A compass graph was created and superimposed over a satellite map of the study area and used to determine how many days a trap was downwind from the protein-sprayed area. This experiment was conducted twice, once in June 2010, and again in July 2010.

Data corresponding to the number of marked psyllids captured were not normally distributed. Consequently, we performed a GLM with a log link function for Poisson distribution. The data also showed overdispersion and we consequently corrected the SEs

Table 1. Number of traps placed in abandoned and managed groves during the "long range dispersal" experiment

Trap distance (m)	No. of traps in abandoned groves	No. of traps in managed groves
Protein marked area	0	8
100	0	10
300	0	10
400	0	10
500	2	8
650	4	6
1,000	4	6
1,200	4	6
2,000	8	2

using a quasi-GLM model where the variance is given by $\varphi \times \mu$, where φ is the dispersion parameter and μ the mean (Zuur et al. 2009). To analyze the number of marked psyllid captured, we developed two different models for sampling in June and July to avoid pseudoreplication. Models included distance, number of days a trap was downwind of the marked area, trap placement in abandoned or managed areas of the study site, and flush abundance (July only) on the neighboring citrus trees: *Number of psyllids captured* ~ *distance* + *wind direction* + *management* + *flush*.

Results

Seasonality of *D. citri* Dispersal. The greatest number of both trapped and protein-marked *D. citri* occurred in June, July, and August of 2009 (Fig. 2). After August 2009, the total number of trapped and protein-marked *D. citri* decreased dramatically. In July, *D. citri* that moved from the abandoned to the managed grove came principally from the interior of the abandoned grove ($\chi^2 = 35.33$; df = 1; $P < 0.001$; Fig. 2). In June ($\chi^2 = 0.35$; df = 1; $P = 0.55$) and September ($\chi^2 = 0.44$; df = 1; $P = 0.50$), the difference in *D. citri* that moved from the interior or from the edge of the abandoned grove was not significant. From June to August, we found significantly more marked psyllids in the managed grove than in the abandoned grove ($F = 35.93$; df = 1,12; $P < 0.001$). We found no marked psyllids on the traps placed between the two groves. The numbers of marked psyllids collected on the edge or in the interior of the groves were not significantly different ($F = 0.76$; df = 1,12; $P = 0.40$). There were no significant interactions between the management of the grove and the numbers of marked psyllids collected on the edge or in the interior of the groves ($F = 0.32$; df = 1,12; $P = 0.58$). Between December 2009 and March 2010, no marked *D. citri* were trapped in either abandoned or managed groves (data not shown), although tap counts indicated that adult *D. citri* were present in the groves; 51.6 ± 23.1 *D. citri* were found on average per sampling day from September to March 2009.

Fallow Field Experiment. The predominant wind direction was to the north throughout the experiment (mean wind direction ranged between 351° and 21°, with 0° representing north), except for 5 September 2009, when the wind direction averaged 280° or west. The daily average wind speed for the period of this experiment was 7.32 km/h. The number of protein-marked psyllids recovered differed significantly between the various locations sampled ($\chi^2 = 70.93$; $P < 0.001$; df = 5; Fig. 4). We found significantly more marked psyllids in the far north, west, and east blocks than in the far west block (Fig. 3).

Long-Range Dispersal Experiment. For the experiment conducted in June 2010, 179 adult *D. citri* were trapped, and 19% were marked with protein. Protein-marked adult *D. citri* were trapped within the marked area, and at all distances except at 1,000 m. In total, 10 marked *D. citri* were trapped at a distance of 2,000 m. The number of days that a trap was in the downwind direction from the marked area was not found to be significant in determining the number of

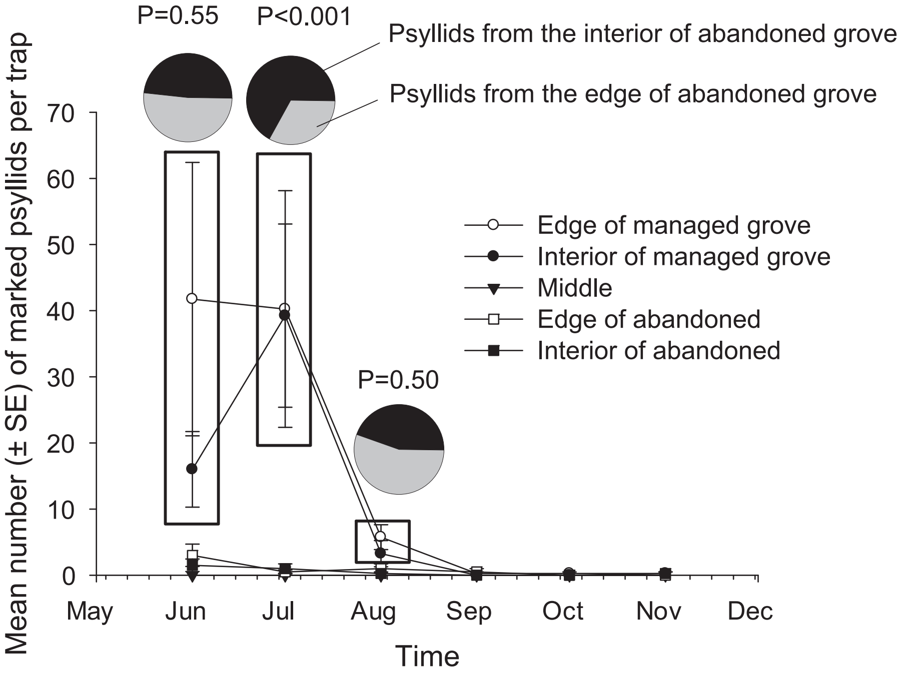

Fig. 2. Mean number of marked *D. citri* captured on yellow sticky traps from June to November 2009. Pie charts indicate the origin of psyllids captured in the managed groves (data points inside the boxes).

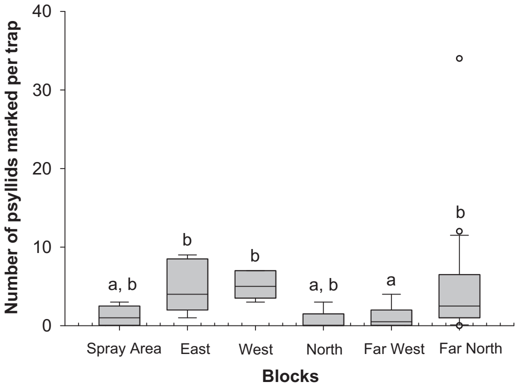

Fig. 3. Number of marked psyllids captured in each block during the "fallow field experiment." Different letters indicate significant differences ($\alpha < 0.05$).

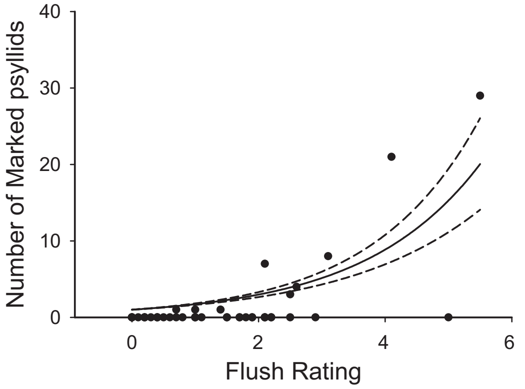

Fig. 4. Number of marked psyllids captured during the "long-range dispersal" experiment depending on the flush rating of citrus trees surrounding the traps. For each site, the flush rating consisted of the number of flush within 4.1 dm^3 averaged for 10 samples. We included the curve from a nonlinear regression $y = e^{0.55 \times X}$, $R^2 = 0.5$).

marked psyllids found on that trap (Estimate = −0.10; SE: 0.55; $\chi^2 = 0.03$; df = 1; $P = 0.854$), nor was the distance of a trap from the marked area (Estimate: <0.001; SE: <0.001; $\chi^2 = 0.34$; df = 1; $P = 0.567$). Grove type was the only variable found to be significant (abandoned = 2.00 ± 0.91 marked psyllids per trap; managed = 0.33 ± 0.17 marked psyllids per trap; Estimate = 1.70; SE = 0.64; $\chi^2 = 7.50$; df = 1; $P = 0.006$).

In July 2010, 541 *D. citri* were sampled and 18% were protein-marked. Protein-marked *D. citri* were trapped at all distances except 1,000 m. Two traps located 2,000 m from the marked area in abandoned fields contained over 1,000 psyllids. A subsampling showed an abnormal number of marked psyllids (29 and 21 marked psyllids out of two samples of 50 psyllids). It is possible that the high number of marked psyllids was because of cross contamination by contact between psyllids that occurred at such a high density of capture. These two data points consisted of two outliers compared to the rest of the data set where the number of marked psyllids averaged 0.69 ± 0.17 per trap, with a maximum of 10. Consequently, even if these two traps demonstrated that psyllids can travel up to 2,000 m, these two data points were removed from the model. After removing the outliers from the model, distance was no longer a significant factor (Estimate: <0.001; SE: <0.001; $\chi^2 = 0.12$; df = 1; $P = 0.724$); significance of other factors did not change. Flush abundance was positively correlated with the number of marked psyllids captured (Estimate = 0.66; SE = 0.23; $\chi^2 = 6.73$; df = 1, $P = 0.009$; Fig. 4). Wind direction was not found to be a significant factor in *D. citri* dispersal at these distances of recapture (Estimate: −0.098; SE = 0.32; $\chi^2 = 0.10$; df = 1; $P = 0.756$). In contrast to the June trial, we did not find a significant difference between the number of marked psyllids captured in abandoned and managed groves in July (abandoned = 0.11 ± 0.11 marked psyllids per trap; managed = 0.74 ± 0.34 marked psyllids per trap; Estimate −1.098; SE = 1.82; $\chi^2 = 0.49$; df = 1; $P = 0.482$).

Discussion

Local dispersal of *D. citri* was found to be greatest during the spring and summer months and decreased greatly during the colder months (September–March). During the warmer months, *D. citri* were consistently capable of dispersing at least 300 m within 4 d from inner rows of abandoned groves to inner rows of managed groves. These results are consistent with those found in a study by Hall and Hentz (2011) where movement of resident psyllids in small blocks of citrus was monitored by trapping in areas up to 150 m away from the block. Use of protein marking in the present investigation allowed us to pinpoint the origin of marked psyllids, thereby providing a direct measure of dispersal over time. Interestingly, we captured fewer marked psyllids on traps in abandoned groves than in adjacent managed groves during the seasonal survey. This suggests that psyllids left protein-marked trees in the abandoned grove plots and tended to move over longer distances to find better hosts rather than finding a new host within the same abandoned grove. Another interesting result was that psyllids that moved from the abandoned to managed grove plots did so from both the edge and the interior of the abandoned plots. This is despite the previously observed edge effect in the distribution of *D. citri* within groves (Boina et al. 2009).

The results indicate that *D. citri* can disperse from abandoned groves into nearby managed groves within only a few days, corroborating previous findings (Boina et al. 2009). In addition, our data suggest that *D. citri* may exhibit more limited dispersal during the winter months. *D. citri* adults were present in the groves, as indicated by tap counts, but marked psyllid captures on traps declined to zero from October 2009–March 2010. This is in accordance with Hall and Hentz (2011), who found evidence of only short distance movement (2 m from citrus trees) of *D. citri* during winter. In the fallow field experiment, adult *D. citri* were found to disperse into adjacent blocks and at least 300 m over fallow ground. This is twice the distance recorded by Hall and Hentz (2011) and much more than the average 5–12 m reported by Kobori et al. (2011). The average wind direction over the course of this experiment was northerly and a significant number of protein-marked *D. citri* were found in the block 300 m to the north of the protein sprayed area. These data suggest that psyllids can disperse long distances over fallow ground and might be assisted by wind. Further studies with a shorter time scale under more controlled conditions are needed to unravel the effect of wind assistance on dispersal capability of *D. citri*.

The results of the long-range dispersal experiment showed that adult *D. citri* are capable of traveling at least 2,000 m within 12 d. Surprisingly, distance of trap placement from the sprayed area did not influence the number of marked psyllids captured per trap. It suggests that psyllids can travel long distances to find necessary resources such as young emerging leaves ("flush"). The primary factors affecting *D. citri* dispersal were availability of new leaf flush and the presence or absence of insecticide treatment (in June only). Flush is a necessary resource for *D. citri* reproduction, and thus it is not surprising that this would affect *D. citri* dispersal. Hall and Hentz (2011) also noted that flush abundance might have been a factor in dispersal, with fewer psyllids trapped at distant locations when flush was abundant in the trees of possible dispersal origin.

The present study expands on the work initiated by Boina et al. (2009) where an in situ protein marking technique was shown to be an effective method for tracking the relatively short distance dispersal (~300 m) of *D. citri* from abandoned groves into proximal managed groves over the course of 3 d. In east central Florida, Hall and Hentz (2011) found that *D. citri* disperse from citrus at any time of the year with consistent dispersal activity during the spring. They found no correlations with wind speed or wind direction, but suggested that relative humidity and crowding may be factors influencing the propensity for flight. It has been shown that a different psyllid vector of the HLB-causing pathogens, African citrus psyllid [*Trioza erytreae* (Del Guercio)], can disperse up to 1.52 km within 7 d in the absence of host plants (van den Berg and Deacon 1988). Thus, it was determined that in geographical locations where *T. erytreae* is the primary vector of HLB, groves could only be considered isolated if they were at least 1.5 km away from any other grove. In the present study, in only one of the two experiments was there a correlation between prevailing wind direction and capture of marked *D. citri*. This suggests that wind may assist *D. citri* migration, but is not a key factor for dispersal distances of up to 2 km. This is similar to the results of van den Berg and Deacon (1988), who postulated that active psyllid movement occurred during periods when the wind was calm. Moreover, *D. citri* are capable of flying up to 2.4 km without wind assistance as measured by laboratory flight mills (Martini et al. 2014).

It is clear that abandoned groves can act as reservoirs of *D. citri* (Tiwari et al. 2010). The extent of the impact of these abandoned groves on the commercial citrus industry in Florida is difficult to quantify. Knowing how devastating HLB can be to citrus production, the presence of these areas of refuge should be of immediate concern, especially in locations such as Texas and California, where quarantine and eradication methods are still being pursued. This issue should also be considered with respect to citrus grown in residential areas by homeowners. While intensity and coordination of control measures for *D. citri* are an important step in mitigating the damage caused by HLB, the presence of large numbers of abandoned groves in Florida may undermine these efforts (USDA 2013).

Area-wide management practices to control HLB and its psyllid vector in Florida may help to mitigate the impact of immigration of infected *D. citri* into commercial citrus. Citrus groves that are within 2 km of any other citrus plantings are at risk for *D. citri* infestation and HLB disease introduction from those areas. Further studies should seek to identify if *D. citri* are capable of non-wind or human-assisted dispersal greater than the 2 km recorded here (but see Martini et al. 2013). Results from this study should also serve as further evidence of the threat of *D. citri* infestation and HLB infection posed by abandoned groves in proximity to managed citrus.

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References Cited

Boina, D. R., W. L. Meyer, E. O. Onagbola, and L. L. Stelinski. 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of abandoned groves on commercial citrus management. Environ. Entomol. 38: 1250–1258.

Bové, J. M. 2006. Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

Chong, J.-H., A. L. Roda, and C. M. Mannion. 2010. Density and Natural Enemies of the Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the Residential Landscape of Southern Florida. J. Agric. Urban Entomol. 27: 33–49.

Cumming, G. S., and A. George. 2009. Historical influences dominate the composition of regenerating plant communities in abandoned citrus groves in north-central Florida. Landsc. Ecol. 24: 957–970.

FDACS. 2013. Citrus health response program update abandoned grove initiative. DACS-P-01613. (http://freshfromflorida.s3.amazonaws.com/CHRP_abandoned_grove_update.pdf) (accessed 3 December 2014).

Folimonova, S. Y., C. J. Robertson, S. M. Garnsey, S. Gowda, and W. O. Dawson, 2009. Examination of the responses of different genotypes of citrus to Huanglongbing

(Citrus Greening) under different conditions. Phytopathology 99: 1346–1354.

Godfrey, K. E., C. Galindo, J. M. Patt, and M. Luque-Williams. 2013. Evaluation of color and scent attractants used to trap and detect Asian citrus psyllid (Hemiptera: Liviidae) in urban environments. Fla. Entomol. 96: 1406–1416.

Gottwald, T. R., B. Aubert, and H. K. Long, 1991a. Spatial pattern analysis of citrus greening in Shan-tou, China, pp. 421–427). *In* R. H. Brlansky, R. F. Lee, and L. W. Timmer (eds.), Proceedings of 11th Conference of the International Organization of Citrus Virologists. University of California, Riverside, CA.

Gottwald, T. R., C. I. Gonzales, and B. G. Mercado. 1991b. Analysis of the distribution of citrus greening in groves in the Philippines, pp. 414–420. *In* R. H. Brlansky, R. F. Lee, and L. W. Timmer (eds.), Proceedings of 11th Conference of the International Organization of Citrus Virologists. University of California, Riverside, CA.

Gottwald, T. R., J. V. Da Graça, and R. B. Bassanezi. 2007. Citrus Huanglongbing: The pathogen and its impact. Plant Management Network. Plant Health Progress. (http://www.plantmanagementnetwork.org/pub/php/review/2007/huanglongbing (accessed 3 December 2014)).

Halbert, S. E. 1998. Entomology Section. Tri-ology 37: 6–7.

Halbert, S. E., and K. L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Fla. Entomol. 87: 330–353.

Hall, D. G., and L. G. Albrigo, 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. Hort. Sci. 42: 364–368.

Hall, D. G. and M. G. Hentz. 2011. Seasonal flight activity by the Asian citrus psyllid in east central Florida. Entomol. Exp. Appl. 139: 75–85.

Hall, D. G., M. G. Hentz, and M. A. Ciomperlik. 2007. A comparison of traps and stem tap sampling for monitoring adult Asian citrus psyllid (Hemiptera: Psyllidae) in citrus. Fla. Entomol. 90:327–334.

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ. Entomol. 35: 827–836.

Kim, J.-S., U. S. Sagaram, J. K. Burns, J.-L. Li, and N. Wang. 2009. Response of sweet orange (*Citrus sinensis*) to *Candidatus* Liberibacter asiaticus infection: microsopy and microarray analyses. Phytopathology 99: 50–57.

Kobori, Y., T. Nakata, Y. Ohto, and F. Takasu. 2011. Dispersal of adult Asian citrus psyllid, *Diaphorina citri* Kuwayama (homoptera: Psyllidae), the vector of citrus greening disease, in artificial release experiments. Appl. Entomol. Zool. 46: 27–30.

Martini, X., T. Addison, B. Fleming, I. Jackson, K. Pelz-Stelinski, and L. L. Stelinski. 2013. Occurrence of *Diaphorina citri* (Hemiptera: Liviidae) in an unexpected ecosystem: the Lake Kissimmee State Park Forest, Florida. Fla. Entomol. 96: 658–660.

Martini, X., A. Hoyte, and L. L. Stelinski. 2014. Abdominal color of the Asian citrus psyllid (Hemiptera: Liviidae) is associated with flight capabilities. Ann. Entomol. Soc. Am. 107: 842–847.

Sakamaki, Y. 2005. Possible migration of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) between and within islands. Occas. Pap. Kagoshima Univ. Res. Cent. 42: 121–125.

Tiwari, S., H. Lewis-Rosenblum, K. Pelz-Stelinski, and L. L. Stelinski. 2010. Incidence of *Candidatus* Liberibacter asiaticus infection in abandoned citrus occurring in proximity to commercially managed groves. J. Econ. Entomol. 103:1972–1978.

(USDA) U.S. Department of Agriculture. 2012. Abandoned Acres (ed. by N.A.S. Service). (http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/aban/CitAA12.pdf) (accessed 3 December 2014).

(USDA) U.S. Department of Agriculture. 2013. Abandoned Acres (ed. by N.A.S. Service). (http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/aban/CitAA13.pdf) (accessed 3 December 2014).

Van Den Berg, M. A., and V. E. Deacon. 1988. Dispersal of the citrus psylla, *Trioza erytreae* (Hemiptera:Triozidae), in the absence of its host plants. Phytophylactica 20: 361–368.

Wang, N., and P. Trivedi. 2013. Citrus Huanglongbing: A Newly Relevant Disease Presents Unprecedented Challenges. Phytopathology 103: 652–665.

Zuur, A. F., E. N. Ieano, N. J. Walker, A. A. Saveliev and G. M. Smith. 2009. GLM and GAM for count data. In: Mixed effects models and extension in Ecology with R. Springer Science, New York, NY.

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