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Patterns of habitat use by the Asian citrus psyllid, *Diaphorina citri*, as influenced by abiotic and biotic growing conditions

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Abstract

1 The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is an economically important pest of citrus throughout Asia and the Americas because it transmits *Candidatus* Liberibacter asiaticus (*C*Las), which is the presumed causal agent of citrus greening disease.

2 We investigated whether biotic and abiotic characteristics can be used to predict *Diaphorina citri* population abundance and assessed whether agricultural intensity explained the distribution of *D. citri* populations during winter dormant periods (December to March).

3 Over two consecutive winters, we examined the abundance of *D. citri* in groves throughout Florida in response to four different management regimes, defined as: conventional, intermittent, unmanaged and organic.

4 During both years, the winter abundance of *D. citri* in groves with intermittent management was greater than in groves with other management regimes. Latitude and row orientation both had a significant effect on psyllid density during winter. *Diaphorina citri* abundance was higher when more than 20% of the surrounding landscape was urbanized.

5 These findings suggest that only conventional management of groves reduced *D. citri* populations during winter periods. By contrast, intermittent management was associated with higher *D. citri* populations. These results might be of some concern in light of the economic and environmental costs of repeated insecticide applications and the dramatic decline of citrus production in the U.S.A.

Keywords Citrus greening disease, geographic information system, huanglongbing, psylloidea.

Introduction

Understanding the ecological components of an agricultural ecosystem is critical for developing sustainable pest management strategies (Lewis *et al.*, 1997). One approach for improving pest management is the identification of abiotic and biotic factors that influence the distribution of a given pest throughout the year, rather than during the growing season. During winter dormant periods, pests employ various strategies to optimize survival during deleterious environmental conditions, including movement to alternative hosts (Norris & Kogan, 2005; Kristoffersen & Anderbrant, 2007), burrowing into leaf litter or sediments (Rozsypal *et al.*, 2013), or entering diapause (Bale & Hayward, 2010). Overwintering can occur in insects living in subtropical climates, despite warmer winter temperatures (Yasuda, 1990; Gangwar *et al.*, 2008). In subtropical climates, insects may experience short diurnal freezing periods (Turnock & Fields, 2005). In Florida, regular short periods of below freezing temperatures between December and March may have some impact on the behaviour and mortality of citrus pests, such as the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). For example, dispersal of *D. citri* is significantly reduced during autumn and winter compared with spring and summer (Hall & Hentz, 2011; Lewis-Rosenblum *et al.*, 2015). Oviposition by *D. citri* is also reduced as temperatures decrease (Hall *et al.*, 2011).

Diaphorina citri transmits a phloem-limited bacterium *Candidatus* Liberibacter asiaticus (*C*Las), which is associated with citrus greening disease, also called huanglongbing (HLB) (Grafton-Cardwell *et al.*, 2013), and is considered to be the most destructive disease of citrus crops worldwide (Wang & Trivedi, 2013). *Diaphorina citri* populations decline in Florida during the

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winter period (from December to March) in correlation with low minimum temperatures and reduced precipitation (Tsai *et al.*, 2002). This is despite tolerance to periodical cold temperatures experienced by *D. citri* adults. For example, 50% of *D. citri* adults survive up to 48 h of exposure to 0 °C (El-Shesheny *et al.*, 2016). *Diaphorina citri* overwinters on citrus hosts in all life stages (Hodkinson, 2009); however, reproduction is limited in winter months as a result of the low abundance of young emerging leaves (called ‘flush’ in citrus horticulture) needed for oviposition and nymph development (Hall & Albrigo, 2007). Consequently, populations decrease significantly during the winter season, suggesting that this period is the most biologically challenging to *D. citri* survival (Tsai *et al.*, 2002). Winter temperatures appear to provide the ‘weakest link’ in an annual cycle of *D. citri* populations that may be exploited in an integrated programme for management of the pathosystem. Florida has a subtropical climate dominated by hot and wet summers with warm and dry winters. There is also a risk of sustained temperatures below 0 °C (Waylen, 1988) that may lead to citrus tree loss and *D. citri* population reduction.

Management of HLB relies primarily on insecticide applications, removal of infected trees to reduce *C*Las inoculum and replanting of trees with certified pathogen-free nursery stocks (Grafton-Cardwell *et al.*, 2013). Understanding seasonal population fluctuations of *D. citri* has allowed the development of pest management programmes that minimize spring population peaks of the vector (Qureshi & Stansly, 2010). Management programmes typically consist of winter insecticide applications (dormant spraying generally from December to March, depending on the weather) of pyrethroid and organophosphate insecticides (Qureshi & Stansly, 2010). One constraint of such programmes is that these chemistries cannot be used during citrus bloom, resulting in delayed insecticide application to protect pollinators found in citrus groves during ‘early bloom’ events (as frequently seen during mild winters in Florida). Thus, insecticide applications during dormant periods must occur prior to bloom (Qureshi & Stansly, 2010).

Insect management regimes vary among citrus groves, largely as a result of economic constraints among growers, resulting in variation in the degree of *D. citri* management within the Florida citrus crop landscape (Cumming & George, 2009). Citrus production costs have considerably increased during the past decade because of citrus canker and HLB, increasing from 1906 to 3950 US$ per ha between 2005 and 2009 (Muraro, 2012). Unmanaged or abandoned citrus groves accounted for 53 010 ha during 2012 (USDA, 2012) and are likely important reservoirs for *C*Las and *D. citri* (Boina *et al.*, 2009; Tiwari *et al.*, 2010; Lewis-Rosenblum *et al.*, 2015). *Diaphorina citri* actively move between unmanaged and productive groves; however, the majority of migration is directed primarily from the unmanaged into productive groves (Boina *et al.*, 2009). Almost 20% of groves receive ‘intermittent management’, consisting of five or fewer insecticide treatments per year, compared with twelve annual applications in ‘conventionally’ managed groves (Grafton-Cardwell *et al.*, 2013; Futch & Singerman, 2015).

We hypothesized that *D. citri* populations shelter in unmanaged and intermittently managed groves during winter as a result of the absence or low levels of pest control that present fewer challenges to *D. citri* survival during winter conditions. Additionally, we investigated the effects of several biotic and abiotic challenges, pest management regimes, winter temperatures, landscape and host characteristics on *D. citri* population density, survival and host selection during winter dormant periods.

Materials and methods

Grove classification

Fifty-two citrus groves (38 sites in 2013 and 28 sites in 2014) were sampled during January and February across nine Florida counties: Polk (27.861722, 81.691167) (27°51′42.2″N, 81°41′28.2″W); Lake (28.702833, 81.778694) (28°42′10.2″N, 81°46′43.3″W); Orange (28.4845, 81.251883) (28°29′4.20″N, 81°15′ 6.78″W); Osceola (28.101983, 81.075467) (28°6′ 7.14″N, 81°4′31.68″W); Highlands (27.340028, 81.340028) 27°20′24.10″N, 81°20′24.10″W); Indian River (27.694772, 80.543844) (27°41′41.18″N, 80°32′37.84″W); St Lucie (27.493611, 80.342222) (27°29′37″N, 80°20′ 32.00″W); Volusia (29.027983, 81.075467) (29°1′40.74″N, 81°4′31.68″W); and Miami Dade (25.551603, 80.625556) (25°33′5.77″N, 80°37′57.69″W) counties. Groves were classified according to management regime: (i) unmanaged (receiving no insecticide or fertilizer treatments for at least 2 years); (ii) organic management (organic pest management and fertilization); (iii) intermittent management (a maximum of five insecticides per year, as well as fertilization treatments); and (iv) conventional management (at least nine neurotoxic insecticide treatments per year, as well as standard fertilization). Groves were classified based on data stored on the University of Florida Citrus Health Management Area (CHMA) program (http://www.crec.ifas.ufl.edu/extension/chmas/index.shtml) database and, in some cases, through grower surveys. The groves under the conventional management regime usually followed the recommendations of the University of Florida Citrus Pest Management Guide and therefore included two applications of neurotoxic insecticide during winter (dormant spray). Although CHMA guidelines suggest at least nine annual insecticide applications, some growers applied up to 13 sprays per year, according to CHMA surveys. Organic groves were United States Department of Agriculture (USDA) certified. Abandoned and intermittently treated groves were determined by direct field observation and grower surveys. Grove characteristics, including area, latitude, row orientation, citrus variety, shape and number of gaps as a result of missing trees, were determined from onsite observations and from aerial photography images taken during the sampling periods. Grove shape was scored according to the number of straight edges in each grove. Soil type, grove area and edge lengths were calculated using ARCGIS, version 10.2 (ESRI, Redlands, California). In addition, 480 groves included in the statewide surveillance for the CHMA program (http://www.crec.ifas.ufl.edu/extension/chmas/index.shtml) were analyzed separately.

Abundance of D. citri

For the 52 groves sampled during the present study in 2013 and 2014, the abundance of *D. citri* was assessed in 10 randomly selected trees within an eight row transect of each grove, using

Table 1 Meteorological data collected from weather stations nearest to focus groves

		Day of sampling				Over previous 7 days				Over previous 14 days			
Location	Month	Temp	Wind speed	Wind direction	Rainfall	Temp	Wind speed	Wind direction	Rainfall	Temp	Wind speed	Wind direction	Rainfall
Lakeland	January	21.1	2.8	S	0.0	23.0	6.4	SE	0.0	19.9	6.0	NE	0.0
Orlando	January	22.8	4.7	S	0.0	22.4	5.6	E–SE	0.0	19.2	5.9	N–NE	0.0
Leesburg	January	22.2	3.0	SE	0.0	22.4	4.0	E–SE	0.0	18.7	6.2	N–NE	0.0
Orlando	January	22.2	4.2	S–SE	0.0	22.3	5.3	E–SE	0.0	19.5	6.1	E–NE	0.1
Miami	January	20.6	5.2	E	0.0	20.9	5.0	N–NW	0.0	23.9	9.0	E–SE	0.0
Punta Gorda	February	20.0	5.4	NW	0.0	22.8	7.6	W–SW	0.0	15.9	6.6	N–NW	0.3
Lakeland	February	15.6	7.6	W–NW	0.0	21.6	7.5	SW	0.0	16.3	6.3	W–SW	0.0
Fort Pierce	February	20.6	5.5	S–SW	0.0	23.3	7.5	S–SW	0.0	15.7	6.3	E	0.0
Lakeland	February	15.0	5.5	W	0.0	13.7	6.4	W	0.0	23.3	6.7	S–SW	0.0
Lakeland	February	11.1	8.7	W–NW	0.0	19.6	7.5	W	0.0	17.8	6.1	SW	0.0
Vero Beach	February	19.4	5.6	S–SE	0.0	23.2	8.3	S–SE	0.0	15.2	7.1	W–NW	0.0

Meteorological data were collected from National Climate Data Center (NDCC) and National Oceanic and Atmospheric Administration (NOAA) weather stations closest to 38 individual groves around Florida for winter months of December, January and February 2012–2013. Data shown are mean values for each time period. These data were compiled to assess the impact of temperature (°C), rainfall (mm), wind speed (m/s) and wind direction on abundance of *Diaphorina citri* within 38 groves. Within the analyses, we determined that temperature, wind direction and wind speed had no significant impact on *D. citri* abundance.

S, south; E, east; N, north; W, west; SE, southeast; NE, northeast; NW, northwest; SW, southwest.

tap sampling methods (Qureshi & Stansly, 2007; Hall & Hentz, 2011). Ten branches per tree were tapped five times at the same time as a grid board was held below to collect and enumerate dislodged psyllids. *Diaphorina citri* abundance data were also collected from 480 groves during statewide surveillance for the CHMA program (http://www.crec.ifas.ufl.edu/extension/chmas/index.shtml). Surveillance was conducted by the USDA Animal and Plant Health Inspection Service (USDA-APHIS) and the Florida Department of Agriculture and Consumer Services for the Citrus Health Response Program in Florida (www.flchma.org). *Diaphorina citri* were quantified from ten trees at each corner and 10 trees in the centre of each grove, using the tap sampling method described above. The data from 480 groves that we selected were added as a data layer in ArcView to assess *D. citri* abundance within a 1000-m buffer of each focus grove during sampling. In addition, we used these data to examine the seasonal abundance of *D. citri* over 3 years between summer 2011 and spring 2014.

Plant nutrient analysis

To assess the nutrients available to *D. citri* within leaves, five fully expanded young leaves were randomly collected from each tree sampled in the 52 groves. Leaves were thoroughly washed in 3% H_2SO_4, rinsed in deionized water, air dried and placed in a drying oven at 65 °C for 48 h. Leaves were combined into two pooled samples of equivalent mass per site and subjected to elemental level analysis (Waters Agriculture Laboratories Inc., Camilla, Georgia). Dry weight percentages or parts per million of certain minerals were assessed per site each year: nitrogen (N), potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sulphur (S), boron (B), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu).

Meteorological data analysis

Weather conditions in groves at the time of sampling were collected from the nearest National Climate Data Center (NDCC) and National Oceanic and Atmospheric Administration (NOAA) weather stations (http://www.ncdc.noaa.gov/). Rainfall, temperature, wind speed and wind direction were determined for each site on the day of sampling. Mean conditions during the 1- and 2-week periods prior to sampling were also included in the analysis (Table 1). Using these data, we compiled climate data from each day of sampling, and the mean conditions over the week and 2 weeks prior to the sampling date for each sampled grove. Meteorological data were analyzed to identify correlations between climate conditions within groves and *D. citri* abundance.

Landscape characteristics

To examine the effect of local grove characteristics on *D. citri* abundance, grove area and length were calculated using ARCVIEW, version 10.1 (ESRI) with aerial photography (Google, 2013). Digitized polygons were applied manually to the 52 sampled groves to calculate grove area and edge length. The proportion of landscape encompassed by each descriptor was calculated within 1000-m wide concentric buffers around each focus grove using ARCVIEW. Digitized polygons were manually assigned within these buffers to landscape descriptors such as: water bodies (lakes, waterways and seasonal marshes), open land (agricultural land, plains and woods), citrus groves and urban area (roads, dwellings, gardens and industrial landscape). To examine the impact of landscape characteristics on grove selection by *D. citri*, we analyzed surrounding landscape characteristics of the 52 focus groves. Using ARCGIS, we assigned digitized polygons to water bodies (lakes, waterways and seasonal marshes), open land (agricultural land, plains and woods), citrus groves and

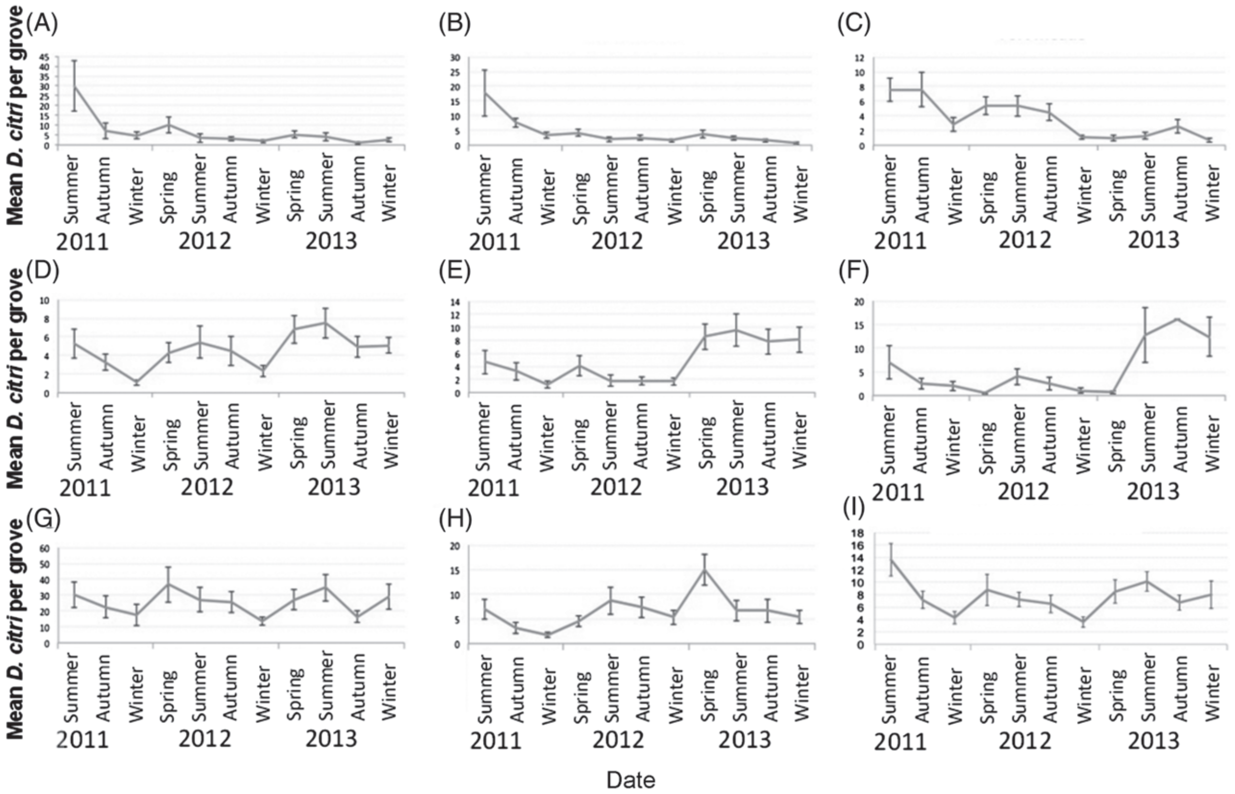

Figure 1 Populations of *Diaphorina citri* declined significantly during winter in Florida. Psyllid numbers were obtained for 480 citrus groves within eight Citrus Health Monitoring Areas (CHMA) of Florida. Groves were monitored for *D. citri* every 3 weeks and mean counts of psyllids found in all groves on seven CHMAs are highlighted: (A) Auburndale, (B) Babson Park, (C) Fort Meade, (D) Indian River county, St Lucie, (F) Volusia, (G) South Lake & West Orange, (H) Bears Den and (I) average of the eight CHMAs.

urban area (roads, dwellings, gardens and industrial landscape) within 1000 m radius of the centre each of our 52 groves.

Statistical analysis

Because the present study included a large number of groves, meteorological and landscape variables that may influence *D. citri* abundance within groves, it was not viable to include all explanatory variables into one model. Therefore, we grouped explanatory variables in models to test specific hypotheses. The analyses of many experiments include counts of *D. citri* as the response variable; therefore, analyses of deviance were performed using Poisson distributions in GENSTAT, version 16 (VSN, U.K.). Wald tests were performed to identify explanatory variables in models with no significant effect for exclusion from models.

We first analyzed the effect of abiotic and biotic grove characteristics for the 39 groves sampled in 2013. This included area, latitude, row orientation, citrus variety, grove shape and number of gaps per ha as a result of missing trees. Citrus varieties were pooled into three categories: sweet orange (Hamlin, Valencia, Navel, Ambersweet), Grapefruit and small citrus fruit (Tangerine, Mandarin and Lime). The grove shape was transformed into a score that was based on the number of edges within a particular citrus grove. Second, we investigated the effect of grove management, flush abundance and weather conditions on the abundance of *D. citri* during the winters of 2013 and 2014. Third, the effects of surrounding landscape within a 1000-m radius of commercial groves on *D. citri* abundance were determined.

Finally, leaf nutrient data were analyzed by principal component analysis using the package FACTOMINER (Lê *et al.*, 2008) in R (R Foundation for Statistical Computing, Austria). Because of the multicollinearity of these variables, predicted values from the first and second principal components (PC1 and PC2, respectively) were used as explanatory variables in a generalized linear model (GLM) of *D. citri* abundance with a log link function for Poisson distribution. In this case, data were transformed using a quasi-GLM model, where the variance is given by $\varphi \times \mu$, where φ is the dispersion parameter and μ the mean, and SEs multiplied by the square root of φ (Zuur *et al.*, 2009). Scores from PC1 and PC2 were subjected to analysis of variance for comparisons of grove management regimes.

Results

Diaphorina citri *populations exhibit seasonal fluctuations and differed between regions*

Count data of *D. citri* from 480 groves, selected from a data set representing all CHMAs, indicated a significant reduction in psyllid abundance during winter periods between late December and the end of February ($F_{16,90} = 12.303$, $P \leq 0.001$)

Table 2 Effects of abiotic and biotic factors on *Diaphorina citri* abundance during winter in Florida

Explanatory variable	d.f.	Mean deviance	*P* value
Management regime	3	98.271	< 0.001
Row orientation	1	5.148	0.023
Latitude	1	10.966	< 0.001
Area	1	3.431	0.064
Elevation	1	1.175	0.027
Citrus culture	2	1.789	0.409
Grove shape	1	0.775	0.384
Gaps per ha	1	0.328	0.567
Elevation × Area	1	4.174	0.041
Residual	25	369.198	1.083

Nonsignificant interactions ($\alpha < 0.10$) were removed from the model.

(Fig. 1), which is similar to the findings of Hall *et al.* (2008); however, *D. citri* abundance differed significantly among locations ($F_{6,90} = 309.83$, $P \leq 0.001$). *Diaphorina citri* populations declined in three locations: Auburndale/Lake Alfred, Babson Park and Fort Meade, during late summer of 2011, and remained low for the remainder of the year (Fig. 1A–C). In St Lucie, Indian River and Volusia CHMAs, *D. citri* exhibited a similar significant increase in numbers in spring and low numbers during the rest of the year (Fig. 1D–F). In addition, in these locations, numbers of psyllids were significantly higher in 2013 year round (including winter) than in 2011 and 2012. Two CHMAs, Bears Den and South Lake/West Orange, had significantly higher *D. citri* populations during the spring and summer compared with other locations sampled during this sample period (Fig. 1G,H). In addition, South Lake/West Orange had significantly more *D. citri* compared with other locations for the entire duration of the present study.

Influence of grove characteristics on D. citri *distribution*

Analysis of the psyllid distribution within the 39 groves sampled in 2013 indicated that *D. citri* abundance was significantly affected by row orientation. More *D. citri* were found on tree rows oriented East–West (exposed North South) and at southern latitudes (Table 2) than at other sampled locations. By contrast, *D. citri* abundance was not significantly affected by the overall shape of a grove, citrus cultivar or the number of gaps between rows per ha (Table 2). We found a significant interaction between grove area and elevation, with fewer *D. citri* found in large groves at higher elevations. However, there was no difference between large and small groves at lower elevations (Table 2). The parameter that had the most important effect on abundance of *D. citri* was the management regime within a grove (Table 2).

Grove management has a significant impact on D. citri *abundance*

During 2013, *D. citri* abundance was significantly greater in intermittently managed groves during winter compared with organic or conventionally managed groves (GLM with Poisson distribution: $\chi = 68.519$, d.f. = 3, $P \leq 0.001$) (Fig. 2A). Furthermore, intermittently managed groves exhibited very little variability in psyllid abundance. Similarly, *D. citri* abundance was significantly greater during the winter of 2014 in organic and intermittently managed groves compared with conventionally managed groves (GLM with Poisson distribution: $\chi = 68.79$, d.f. = 3, $P \leq 0.001$) (Fig. 2B). A CHMA database of *D. citri* populations during the period from the summer of 2011 to the winter of 2014 in 480 groves with known management regimes indicated a greater abundance of *D. citri* during winter in intermittently managed groves and significantly higher populations occurring in organically managed groves during the spring compared with populations in conventionally managed groves ($F_{3,49} = 13.543$ $P \leq 0.001$) (Fig. 3).

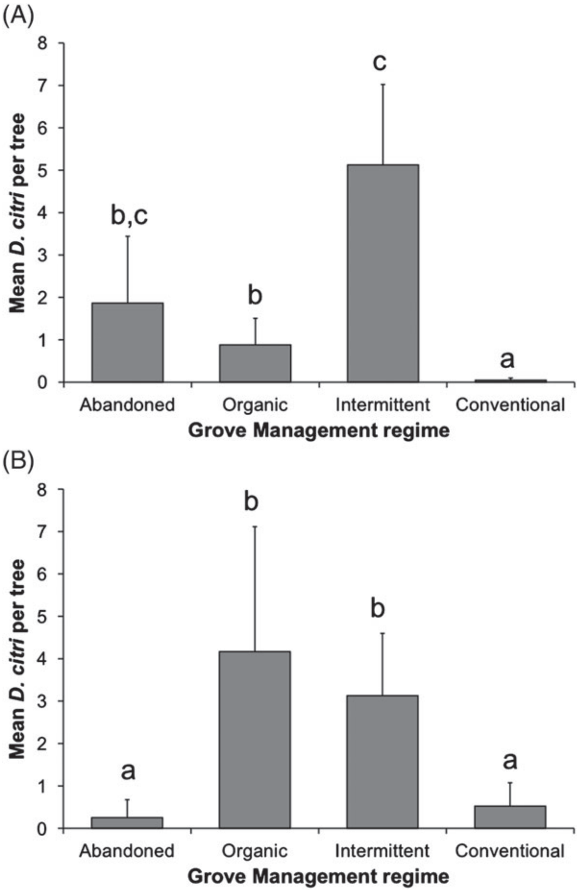

Figure 2 Distribution of *Diaphorina citri* during winter months (January to February) in groves sampled under different management regimes in (A) 2013 and (B) 2014.

Finally, neither new plant growth, nor flush abundance affected the winter abundance of *D. citri* in 2013 or 2014 ($F_{2,33} = 7.5893$, $P = 0.201$) in the present study. Furthermore, there was no correlation between management practice and flush abundance ($F_{3,27} = 12.421$, $P = 0.08$). Temperature, wind direction and wind speed also had no significant effect on *D. citri* abundance in the 52 groves sampled during the winter ($F_{2,20} = 0.883$, $P = 0.502$; $F_{2,20} = 2{,}555$, $P = 0.322$; $F_{1,20} = 8.918$ $P = 0.08$, respectively).

Landscape characteristics

Citrus groves with intermittent or no psyllid management (26.2% and 32.7%, respectively) were surrounded by more

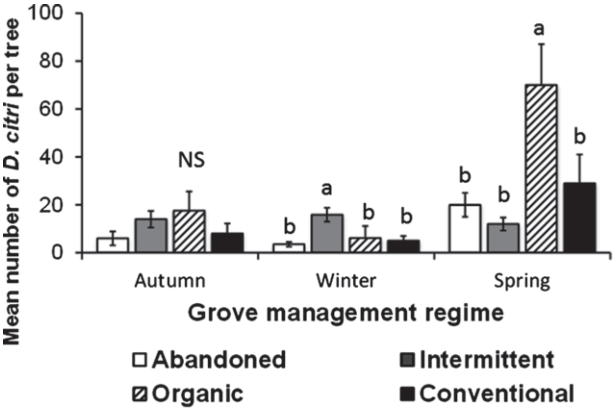

Figure 3 Seasonal abundance of *Diaphorina citri* among groves with different management regimes in a subset of 26 groves from the Citrus Health Monitoring Areas (CHMA) data set. Different letters indicate significant differences between grove management within each season.

Table 3 Percentage of landscape area associated with *Diaphorina citri* infestation and grove management type

Number of *Diaphorina citri* per tree	Mean ± SEM landscape area (%)			
	Open	Groves	Water	Urban
< 1	29.2 ± 4.4^{a}	53.1 ± 5.7^{a}	6.6 ± 2.0^{a}	11.12 ± 3.2^{b}
2.5	38.9 ± 13.7^{a}	33.7 ± 17.3^{a}	5.5 ± 5.4^{a}	22.1 ± 4.7^{a}
> 6	24.6 ± 7.9^{a}	49.4 ± 12.5^{a}	3.3 ± 2.6^{a}	22.9 ± 6.4^{a}
Grove management				
Abandoned	$40.8 \pm 27.6^{a,b}$	23.6 ± 22.0^{b}	2.8 ± 1.4^{a}	$32.7 \pm 2\ 4.2^{b}$
Organic	41.6 ± 7.0^{a}	35.7 ± 8.5^{b}	7.7 ± 4.0^{a}	$15.1 \pm 3.2^{a,b}$
Intermittent	$27.6 \pm 11.0^{a,b}$	$42.1 \pm 16.7^{a,b}$	4.3 ± 3.7^{a}	26.2 ± 8.6^{b}
Conventional	21.1 ± 3.1^{b}	65.2 ± 4.9^{a}	5.9 ± 2.2^{a}	7.8 ± 2.2^{a}

Groves with more *D. citri* present had significantly more urban area within the surrounding 1000-m radius. Of the 52 groves investigated, those under intermittent or no management (abandoned) have significantly more urban area within a 1000-m radius. Using ARCGIS mapping software, we assigned polygons to water bodies, open land, citrus groves and urban area within a 1000-m radius of the centre of each grove. Urban area was defined as roads, dwellings including gardens and industrial landscape.

urban habitats than conventional groves (8%) (Kruskal–Wallis: $H = 6.548$, $P = 0.038$, with abandoned and intermittent groves pooled) (Table 3). Conventional groves were surrounded on average 65.2% of the time by citrus groves, whereas abandoned groves were surrounded by citrus 23.6% of the time within a 1000-m radius (Kruskal–Wallis: $H = 8.135$, $P = 0.043$) (Table 3). Abandoned groves were typically isolated more from other groves than conventional groves, which tended to be included within larger contiguous citrus growing areas. In addition, significantly more *D. citri* were present in groves when the surrounding landscape consisted of at least 20% urban habitat (Table 3).

Using ARCGIS, *D. citri* abundance data were sampled from 480 surrounding groves comprising the CHMA database and layered on the map of the groves sampled by us. Mean *D. citri* numbers were collated from groves that fell within 1000 m of each sampled grove. There was no significant relationship between *D. citri* abundance in our 52 sampled groves and groves within a 1000-m radius ($F_{3,33} = 53.642$, $P = 0.09$), suggesting the movement of *D. citri* is greatly reduced between groves during winter.

Nutritional analysis

Leaf nutrient concentrations from sampled groves were analyzed using principal component analysis to identify associations between leaf nutrition and density of *D. citri*. Scores from PC1 and PC2 were used as explanatory variables in a GLM model. We found that the density of *D. citri* collected was negatively associated with PC1 ($F_{1,37} = 4.21$, $P = 0.047$) and not impacted by PC2 ($F_{1,37} = 1.32$, $P = 0.259$). We found a significant difference in the predicted values of PC1 depending on the various management regimes explored ($F_{3,35} = 7.33$, $P = 0.001$). Managed groves exhibited a higher PC1 than groves under organic ($P = 0.012$) or intermittent management ($P = 0.001$) (Fig. 4B). In 2013, the two first principal components of the PC analysis accounted for 54.47% of the variation (40.39% and 15.45% for PC1 and PC2, respectively) (Fig. 4A). PC1 was positively correlated with Cu ($r = 0.79$, $P \leq 0.001$), S ($r = 0.87$, $P \leq 0.001$), Zn ($r = 0.86$, $P \leq 0.001$), Mn ($r = 0.92$, $P \leq 0.001$) and Ca ($r = 0.88$, $P \leq 0.001$), as well as negatively correlated with P ($r = -0.49$, $P \leq 0.001$) and K ($r = -0.65$, $P \leq 0.001$). PC2 was positively correlated with B ($r = 0.56$, $P = 0.002$), Mg ($r = 0.82$, $P \leq 0.001$) and N ($r = 0.40$, $P = 0.011$), as well as negatively with K ($r = -0.37$, $P \leq 0.021$) and P ($r = -0.50$, $P \leq 0.001$) (Fig. 4A).

Similarly, in 2014, the two first principal components (PC) of the principal component analysis accounted for 57.90% of the variation (42.66% and 15.24% for PC1 and PC2, respectively) (Fig. 4A). PC1 was positively correlated with Cu ($r = 0.89$, $P \leq 0.001$), S ($r = 0.66$, $P \leq 0.001$), Zn ($r = 0.81$, $P \leq 0.001$), Mn ($r = 0.73$, $P \leq 0.001$) Ca ($r = 0.84$, $P \leq 0.001$) and B ($r = 0.30$, $P = 0.027$), as well as negatively correlated with P ($r = -0.74$, $P \leq 0.001$), K ($r = -0.51$, $P \leq 0.001$) and N ($r = -0.51$, $P \leq 0.001$). PC2 was positively correlated with Mg ($r = 0.81$, $P \leq 0.001$) and Fe ($r = 0.78$, $P = 0.001$), as well as negatively correlated with P ($r = -0.40$, $P = 0.003$) (Fig. 4C). We used the scores of PC1 and PC2 as an explanatory variable in a GLM model and observed that the densities of *D. citri* collected were negatively correlated with PC1 ($F_{1,49} = 17.56$, $P \leq 0.001$) and not impacted by PC2 ($F_{1,49} = 0.50$, $P = 0.48$). We found a significant difference in the predicted values of PC1 depending on the different management regimes investigated ($F_{3,48} = 5.61$, $P = 0.002$). Managed groves displayed higher PC1 scores than groves with organic ($P = 0.004$) or intermittent management ($P = 0.019$) (Fig. 4B,D).

Discussion

Although the dormant winter season has been demonstrated as a 'weak link' for psyllid population growth (Hall *et al.*, 2008), the variability in abundance between Florida citrus growing regions has not been demonstrated fully. We analyzed *D. citri* abundance data from 480 groves within eight CHMAs, spanning eight counties, and found significant variation among groves. This sample is highly representative of citrus production for the the entire state. We found that abundance of *D. citri* was affected by

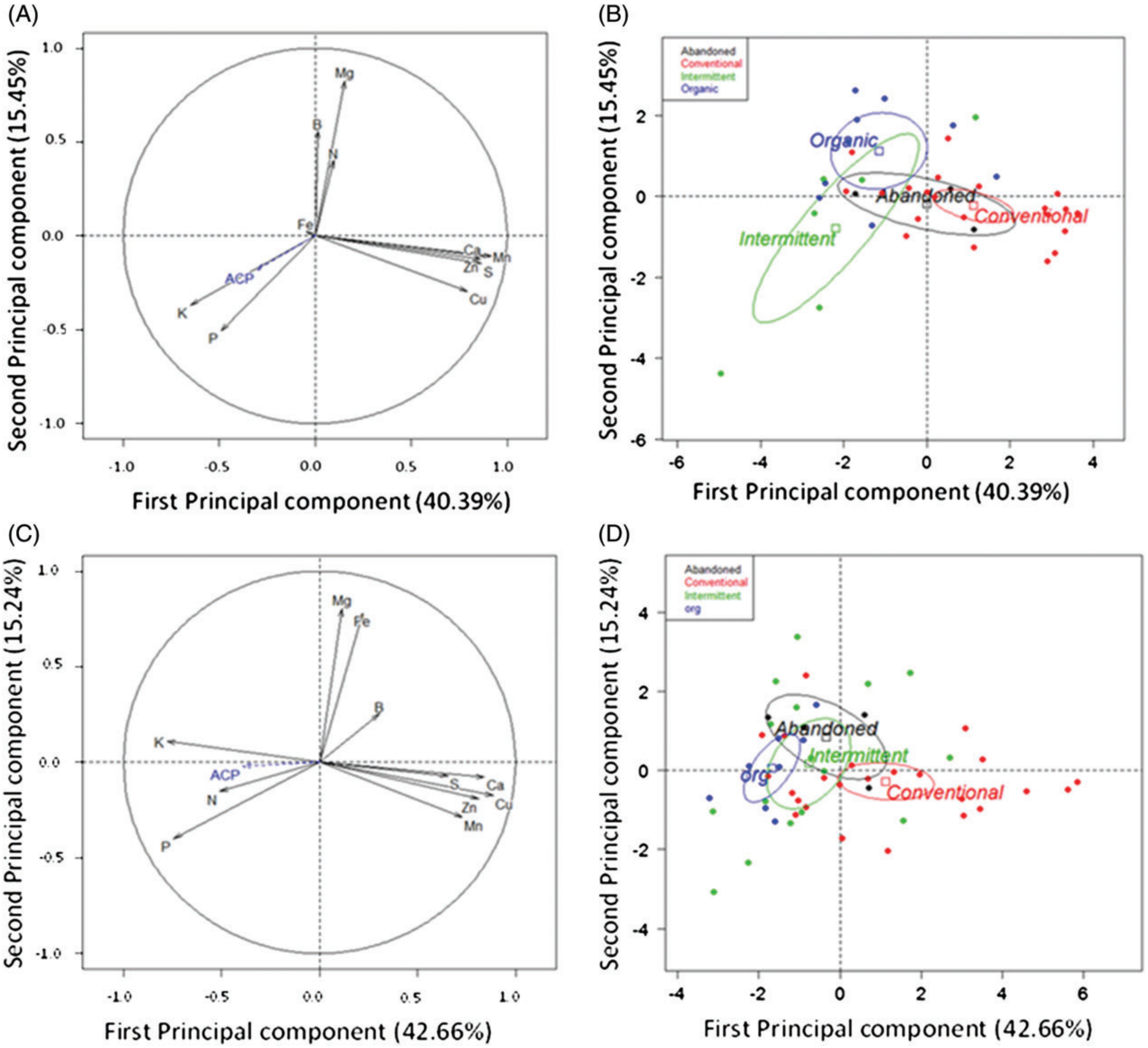

Figure 4 Abundance of *Diaphorina citri* was correlated with leaf macro elements. Principal component analysis of macro minerals in citrus leaf samples from different groves in (A) 2013 and (C) 2014 revealed correlation between *D. citri* abundance and available leaf nutrients. The contributions of different nutrients are highlighted in the first and the second principal component: 40.39% and 15.45% in winter of 2013 (A) and 42.66% and 15.24% in winter of 2014 (C). Blue arrows denote how *D. citri* densities are correlated with each principal component. Density of *D. citri* was used as a supplementary variable and had no influence on the principal components of the analysis (Lê *et al.*, 2008). Individual groves are highlighted in red, blue, green and black (conventional, organic, intermittent and abandoned, respectively) depending on the management regime and their score within each principal component: (B) 2013 and (D) 2014. Confidence ellipses in (B) and (D) highlight management correlations with each principle component, indicating variances in nutrient availability between groves under different management regimes.

abiotic and biotic attributes of individual groves or surrounding areas.

We hypothesized that an insecticide regime for managing groves would be a significant factor influencing *D. citri* abundance during winter. We found that major variation in *D. citri* abundance was a result of grower management practices. CHMA guidelines suggest at least nine annual insecticide applications, including four area-wide applications, for which growers coordinate applications of the same insecticide mode of action. Two coordinated applications are recommended during dormant winter periods to target overwintering *D. citri*. Grower surveys revealed significant variation in psyllid management regimes, ranging from no treatment (unmanaged groves) to 13 treatments per year (data not shown). Insecticide treatments targeting *D. citri* have increased the average annual cost of citrus production by $240–400 per ha (Spreen *et al.*, 2014). The type of insecticide regime highlights the patchwork of grove environments within which *D. citri* survival and colonization could be correlated.

Groves with fewer than five insecticide applications per year (intermittent management) had significantly more *D. citri* during winter than groves under any other management regime.

We would expect that, if insecticide applications alone were affecting *D. citri* abundance, unmanaged groves would harbor higher *D. citri* numbers, followed by intermittent and organic groves, whereas conventionally managed groves would have the lowest population abundance. A higher abundance in intermittently managed groves suggests that *D. citri* can overcome challenges of intermediate insecticide use within these groves. This is supported by the ability of *D. citri* to quickly develop resistance to various insecticides (Tiwari *et al.*, 2011). Additionally, intermittent pesticide application likely reduced the density of natural enemies, which may explain why intermittently managed groves had greater psyllid densities than organic and abandoned groves. The resurgence of pests after pesticide application is a well-known, unwanted side effect of pesticide application and has been demonstrated in multiple systems (Dutcher, 2007). It also suggests that organic insecticides such as neem oils (Weathersbee & McKenzie, 2005) and biological control agents could be as effective for managing *D. citri* as the intermittent use of synthetic insecticides.

To determine how biotic and abiotic factors may influence *D. citri* abundance of particular groves during winter, we examined a number of landscape characteristics potentially associated with *D. citri* abundance. Winter in Florida is a variable season with periods at around 23 °C to brief overnight frosts as low as −10 °C, which are damaging to citrus trees (Waylen, 1988). During cold nights, the coolest layer of air is between the ground and 1.5 m above, which can be 2–6 °C cooler than ambient temperature (Bill *et al.*, 1977). Hall *et al.* (2011) showed that *D. citri* can become acclimated to brief periods of cold temperatures to survive and overwinter under these conditions and Martini *et al.* (2016) showed that temperature was not a major driver of psyllid abundance during winter in central Florida. Similarly, we found that variation in temperature during 11 January 2013 to 28 February 2013 had no effect on *D. citri* abundance between groves. In addition, mean temperatures at the time of sampling were between 15 and 23 °C, which represent conditions potentially conducive to *D. citri* survival and oviposition if flush were available (Hall *et al.*, 2011; Kobori *et al.*, 2011).

Interestingly, the abundance of *D. citri* within groves was positively correlated with potassium and phosphorous within leaves. Insecticide management alone had no significant effect on phosphorous or potassium; however, groves under conventional management receive more fertilizer than intermittent groves and more efficient fertilizer than organic groves. Therefore, the nutritional quality of citrus differed significantly between the different management programs. N-K and N-K-P blended fertilizers are predominantly used by Florida citrus growers on a calendar basis; however, P is often omitted if soil levels already contain high levels of P (Obreza & Schumann, 2010). Sandy entisols accounted for 79% of soil type of the 52 groves sampled, which have low nutrient retention and are prone to nutrient leaching (Mattos *et al.*, 2003; Morgan & Hanlon, 2011). Large-scale producers increasingly utilize enhanced efficiency fertilizers that contain N, K, P and other micronutrients; such products minimize nutrient losses to leaching (Obreza & Schumann, 2010). Growers with varying insecticide regimes also operate varying fertilizer regimes, which have an effect on leaf nutrient availability to *D. citri*, thus affecting settling, feeding and colonization of groves. Our data suggest that the application of fertilizers with high concentrations of K and P should be associated with greater scouting for *D. citri* to identify greater potential risk of grove infestation.

Factors that influence *D. citri* presence appear to vary throughout the year. For example, in spring and summer months, *D. citri* abundance was correlated with flush abundance, whereas there was no significant correlation between *D. citri* and flush during winter. This suggests that survival rather than oviposition is a priority for *D. citri* during winter (Hall *et al.*, 2008). However, these results are in contrast to the results obtained by Martini *et al.* (2016) who reported greater densities of *D. citri* during winter correlated with the presence of leaf flush. Further investigations are needed to determine the frequency and influence of sporadic flush emergence during winter on *D. citri* ecology.

We also found that abiotic factors such as latitude or row orientation affected *D. citri* density in winter. It is not surprising to find more *D. citri* on southern edges of groves; however, it was interesting to note that groves oriented East–West (exposed North–South) had higher densities than groves oriented North–South (exposed East–West). This confirms the results of other studies showing higher densities of psyllids on south-exposed citrus canopies (Sétamou *et al.*, 2008; Martini *et al.*, 2016). The size of the grove also had an impact on psyllid density, although this was correlated with the management strategy of the grove. Conventionally managed groves were significantly larger than abandoned or intermitted managed groves (12.95 ha versus 7.27 ha and 6.33 ha, respectively). This difference in size reflects the economic difficulties smaller growers face with HLB.

Also, the proximity of groves to urban areas appeared to increase *D. citri* populations during winter. This may be because most of the groves with a high proportion of surrounding urban area sampled were abandoned or under intermittent management. We hypothesize this difference may be a result of the smaller size of groves near urban areas and lesser opportunity to spray insecticides near urban areas. Urban areas might also have more unmanaged hosts of *D. citri* such as *Murraya*, *Bergera* and *Severinia*, which are common ornamental plants. Additionally, urban environments have fewer living windbreaks, a landscape element that is known to reduce population densities of *D. citri* (Martini *et al.*, 2015). Understanding how *D. citri* overwinters in Florida has important implications for management of HLB. The present study has revealed gaps in control within some groves allowing populations of *D. citri* to survive during the winter dormant season. It also highlights landscape features that could increase psyllid densities during winter. Our results indicate the need for intensive management of the *D. citri* vector on a contiguous and large scale. The results also strongly suggest that abandoned citrus should be eradicated and that half-measures of inputs for managing this pathosystem will likely be wasted investment. Urban growing landscapes that prevent or interfere with contiguous and intensive management of the vector may undermine effective citrus production in growing areas where HLB is endemic.

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