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Authors: Martini, Xavier, Pelz-Stelinski, Kirsten S., and Stelinski, Lukasz L.

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Factors affecting the overwintering abundance of the Asian citrus psyllid (Hemiptera: Liviidae) in Florida citrus (Sapindales: Rutaceae) orchards

Xavier Martini, Kirsten S. Pelz-Stelinski, and Lukasz L. Stelinski*

Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of the pathogen presumably responsible for causing huanglongbing, a devastating disease affecting citrus (Sapindales: Rutaceae). It is well established that *D. citri* populations decrease significantly during winter because of the diminution of young emerging leaves, which are the only site of egg laying and nymphal development. Therefore, this period could be a weak link in the phenology of *D. citri*, and populations appear to be more sensitive to responsive actions such as insecticide sprays. However, little is known about the distribution of *D. citri* adults within citrus orchards during winter. We surveyed populations of *D. citri* in commercial citrus groves in central Florida over the course of 2 yr. In 4 groves, we sampled the citrus canopy at 3 heights and at the 4 cardinal directions. We also investigated the potential for alternative habitats for *D. citri* and sampled non-crop vegetation and potential alternative hosts over 2 seasons. We did not find a consistent pattern in the distribution of *D. citri* adults during winter in citrus, with one exception; canopies facing south harbored more psyllids than those facing north. We found that the presence of young emerging leaves was the major factor driving *D. citri* population increases during winter, but the abundance of *D. citri* adults was also positively correlated with relative humidity. We also found that the proportion of psyllids with the green-blue morphotype (the morph with greatest dispersal capability and likely the major driver of spring grove infestations) increased after insecticide application and after cold weather. Finally, we investigated the potential existence of alternative hosts on which *D. citri* could feed and/or reproduce in the absence of young emerging citrus leaves during winter. We did not find evidence of an alternative host during winter for *D. citri* in central Florida.

Key Words: suction sampler; alternative host; weed; abiotic factor; *Diaphorina citri*; color morph

Resumen

El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es el vector del patógeno responsable de causar Huanglongbing, que es una enfermedad devastadora que afecta a los cítricos (Sapindales: Rutaceae). Está bien establecido que las poblaciones de *D. citri* disminuyen significativamente durante el invierno debido a la disminución de las hojas jóvenes emergentes, que son el único sitio de la puesta de huevos y el desarrollo de las ninfas. Por lo tanto, este período podría ser un eslabón débil en la fenología de *D. citri,* y las poblaciones parecen ser más sensibles a las acciones de respuesta tales como aplicación de insecticida. Sin embargo, poco se sabe acerca de la distribución de *D. citri* en huertos de cítricos durante el invierno. Se hizó un sondeo de las poblaciones de *D. citri* en plantaciones comerciales de cítricos en el centro de Florida durante un periodo de 2 años. En 4 huertos, se muestreó el dosel de los cítricos en 3 alturas y en los 4 puntos cardinales. También se investigó la posibilidad de hábitats alternativos de *D. citri* y la vegetación no cultivada muestreado y los posibles hospederos alternativos durante 2 temporadas. No se encontró un patrón consistente en la distribución de los adultos de *D. citri* durante el invierno en los cítricos, con una excepción; los doseles orientados al sur tenian más psílidos que los orientados hacia el norte. Hemos encontrado que la presencia de hojas jóvenes emergentes fue el principal factor que impulsa el aumento de la población de *D. citri* durante el invierno, pero la abundancia de adultos de *D. citri* también se correlacionó positivamente con la humedad relativa. También, se encontró que la proporción de psílidos con el morfotipo verde-azul (la metamorfosis con mayor capacidad de dispersión y probablemente el impulso principal de las infestaciones en los huertos en la primavera) aumentó después de la aplicación de insecticidas y después de clima frío. Por último, se investigó la posible existencia de otros hospederos que *D. citri* pudiera alimentarse y/o reproducirse en ausencia de hojas de cítricos jóvenes emergentes durante el invierno. No se encontró evidencia de un hospedero alternativo de *D. citri* durante el invierno en el centro de la Florida.

Palabras Clave: muestreador de succión; hospedero alternativo; hierba; factor abiótico; *Diaphorina citri*; morf de colores

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of the bacterial pathogen, *Candidatus* Liberibacter asiaticus Jagoueix et al. (Rhizobiales: Rhizobiaceae). This bacterium is the potential causal agent of the citrus disease huanglongbing (HLB) also named citrus greening (Grafton-Cardwell et al. 2013). HLB is considered the most destructive disease of citrus (Sapindales: Rutaceae) crops worldwide (Bové 2006). Trees infected with HLB may die 3 to 5 yr after infection, produce small, bitter-tasting fruit, and suffer significant fruit drop (Wang & Trivedi 2013). *Diaphorina citri* was first detected in Florida in 1998 (Halbert 1998) and quickly became established throughout the state in all citrus production areas (Wang & Trivedi 2013).

Control of *D. citri* is based on intensive insecticide treatments (Qureshi et al. 2014; Boina & Bloomquist 2015). Application of insecti-

University of Florida, Entomology and Nematology Department, Citrus Research and Education Center, 700 Experiment Station Rd, Lake Alfred, Florida 33850, USA
*Corresponding author; E-mail: xmartini@ufl.edu

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cides during the dormant winter season most effectively reduces outbreaks of *D. citri* during spring (Qureshi & Stansly 2010). Given that *D. citri* populations are lowest during winter (Tsai et al. 2002; Hall et al. 2008; Hall & Hentz 2011), this period is likely the weakest link in the phenology of *D. citri* for commercial management of citrus. *Diaphorina citri* has been observed overwintering as adults within citrus groves (Hodkinson & Bird 2000; Sétamou et al. 2012); however, investigations of its distribution during this period have been sparse. A better understanding of the distribution of *D. citri* adults during winter could improve optimization of dormant-season insecticide sprays and monitoring of *D. citri* populations.

Diaphorina citri may also benefit from the presence of alternative hosts for survival during the dormant winter season. Indeed, *D. citri* has been described reproducing on Old World fig (Rosales: Moraceae), a non-Rutaceae plant (Thomas & De Leon 2011), and can survive on a wide range of plants (Thomas 2011; Martini et al. 2013). Other psyllid species are known to have alternative hosts during winter, especially in areas where the major host is unsuitable or absent during winter (Mead 1966; Kristoffersen & Anderbrant 2007; Čermák & Lauterer 2008). In the case of *D. citri*, development is linked to the presence of newly emerging leaves termed "flush," which is the only site of nymphal development (Hall & Albrigo 2007). During winter, citrus trees in Florida produce few or even no flush (Hall & Albrigo 2007). Consequently, the density of potential oviposition sites for *D. citri* during winter is dramatically reduced. Therefore, we hypothesized that *D. citri* could benefit from alternative hosts located near or within commercial citrus groves when oviposition sites are less abundant among citrus trees. The possibility of alternative hosts required investigation because of the possible implementation of cultural control to remove alternative hosts or habitats of *D. citri*, if they exist.

Here, we present a 2 yr investigation of the distribution of *D. citri* adults within citrus groves and the effects of biotic and abiotic factors on the abundance of *D. citri* adults during winter. We also examined the fluctuation of *D. citri* populations characterized by a green-blue abdomen morphotype within populations of *D. citri* during winter. This morphotype is known to have the highest fitness among existing color morphs (Wenninger & Hall 2008; Wenninger et al. 2009; Tiwari et al. 2013) and is the only color morph capable of long-duration flights (Martini et al. 2014). Therefore, psyllids with green-blue abdomens likely have the most potential for infesting new groves during the spring flushing season. Finally, we investigated the possibility that *D. citri* could reproduce and/or overwinter on alternative hosts near citrus groves during winter in Florida. Our results provide new insight into the distribution and abundance of *D. citri* adults in citrus groves during winter in Florida that might be useful for HLB management.

Materials and Methods

INSECT VACUUM SAMPLING DEVICE

Based on previous studies (Tsai et al. 2002; Hall et al. 2008; Hall & Hentz 2011), we anticipated a small number of *D. citri* adults within groves during winter. Consequently, we employed a high-power vacuum insect sampler (D-Vac Vacuum Insect Net – Model 24, Rincon-Vitova Insectaries, Ventura, California) to evaluate densities of *D. citri* populations. Thomas (2012) and Monzo et al. (2015) proved that this method is particularly effective for *D. citri*. Our sampling device had an air flow capacity of 21.23 m^3/min at the collection head with a 3.75 H.P engine. The original collection cone was replaced by a 0.23 m^2 custom-made steel collection cone. For each sample, the collection cone was applied for 10 s onto approximately 2 m^2 of citrus canopy. The insects were collected into mesh bags that were replaced for each sample. Mesh bags were brought back to the laboratory and placed in a freezer (−4 °C) for 24 h. The number of psyllids collected within each bag was determined under a stereomicroscope.

TWO-DIMENSIONAL DISTRIBUTION OF *D. CITRI* WITHIN CITRUS GROVES

The objective was to investigate the distribution of *D. citri* adults in groves depending on canopy height and canopy exposure to sunlight. We also tested the hypothesis that the abundance of *D. citri* adults could be correlated with various biotic and abiotic conditions, such as ambient temperature, leaf nutrient content, or presence of flush. We conducted this study during 2 consecutive winters (2013 and 2014) in central Florida. The groves selected were in Lake Alfred, Florida (28.0885833°N, 81.7554806°W). Two consisted of *Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae) 'Hamlin' sweet orange trees, and the 2 others consisted of 'Valencia' sweet orange trees. Two groves had rows oriented north to south (citrus trees facing east and west), and the 2 others had rows oriented east to west (citrus trees facing north and south) (Fig. 1). Within each of these groves, 4 blocks were delineated that consisted of 6 rows among which 6 trees were selected for sampling (1 tree per row). To avoid an edge effect (Gottwald 2010), all trees were at least 3 rows within the interior of the citrus grove. A minimum buffer of 5 rows was left between replicate blocks.

Each tree was assigned to 1 of the 6 treatments consisting of the combination of 2 factors: 1) three canopy heights (high, middle, and low) and 2) orientation with respect to exposure to sunlight (east and west or north and south, depending on the orientation of the rows). The low (up to 1.6 m), middle (>1.6 m to 3.2 m), and high (>3.2 to 5 m) positions of the canopy were determined by dividing the average size of the trees present within a grove by 3. The middle and high positions were accessed using a truck-mounted platform. The groves investigated were all managed by the same grower with identical agrochemical treatment regimes. Standard cultural practices employed by citrus growers in central Florida were used for fertilization, irrigation, and pest management. All of the groves received the same treatments at approximately the same time intervals. In 2013, citrus groves were not sprayed during the sampling period, whereas in 2014, fenpropathrin (Danitol®, Valent, Walnut Creek, California) was applied on 28 Jan at 1.17 L/ha and foliar fenpyroximate (Portal®, Nichino America, Wilmington, Delaware) was applied on 3 Mar at 4.64 L/ha.

In 2013, only the 2 Hamlin groves were sampled 4 times from 9 to 29 Jan and twice thereafter in Mar. In addition to sampling *D. citri* adults, 10 leaves from each tree were collected on 18 Feb to evaluate their nutritional status. Leaves were washed initially in water, then an aqueous solution of 5% H_2SO_4, and finally washed with water, before being dried for 48 h at 60 °C. The nutritional status of citrus leaves was analyzed by a commercial laboratory (Water Agricultural Laboratories,

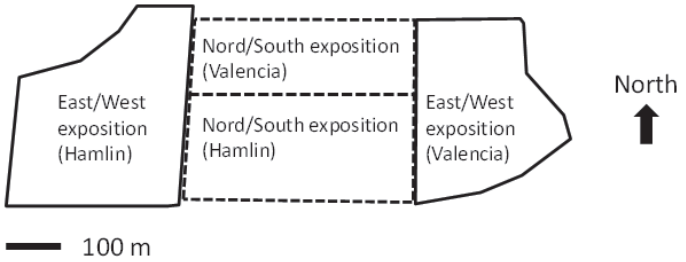


Fig. 1. Schematic diagram of the citrus groves used to investigate the distribution of *Diaphorina citri* adults in Florida during winter.

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Camilla, Georgia). Leaf phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), boron (B), and nitrogen (N) concentrations were determined by inductively coupled plasma atomic emission spectroscopy.

In order to measure temperature and humidity at the sampling locations, Hobo® data loggers (Onset, Cape Cod, Massachusetts) were attached within the canopy of trees at the 12 sampling location treatments and within randomly chosen trees at the 2 conventional groves. Degree days were calculated based on the development threshold of Liu & Tsai (2000) following a sine-wave curve model, and they were calculated with the software HOBOware Pro v3 (Onset, Cape Cod, Massachusetts).

In 2014, the experiment was conducted in both Hamlin and Valencia sweet orange groves. The groves were sampled 7 times from 17 Jan to 17 Mar. In 2014, we also collected temperature and relative humidity data with Hobo® data loggers as described above but did not measure leaf nutrients. Instead, we recorded flush abundance within each sampled area to examine a possible correlation with population densities of *D. citri*. Flush abundance was recorded within each sample area based on the following ranking system: 0: no flush; 1: flush on less than 10% of branches; 2: flush on 10 to 50% of branches; 3: flush on more than 50% of the branches. In 2014, we also recorded the color of the abdomen of the collected *D. citri* adults to determine seasonal variability of morphotypes in our investigation.

INVESTIGATION OF POSSIBLE DORMANT-SEASON ALTERNATIVE HOSTS

The objective of this part of the study was to investigate the possibility that *D. citri* might be present and possibly reproducing on alternative hosts in Florida during the dormant winter season. In 2013, we investigated 5 citrus groves. Two groves were conventionally managed and were located in Auburndale (variety: Hamlin) and Winter Park (variety: Navel). Two other groves were organic groves and were both located in Clermont (varieties: Navel and Grapefruit). The last one was an abandoned grove located in Lake Alfred (variety: Valencia). Six blocks of 3 mature citrus trees were designated at least 3 rows within the interior of each grove. All trees within these blocks were randomly assigned to 4 sampling treatments: A) sampling on citrus canopy (control); B) sampling in the grass within alleys; C) sampling in the vegetation found under the citrus trees; and D) sampling of vegetation on the closest edge surrounding the grove. There was a wide variety of plant species among the 5 groves and the 4 sampling treatments. However, our objective was to sample as extensively as possible on a diversity of plants and habitats to determine if an alternative overwintering habitat for *D. citri* may exist. Each sampling area was flagged so that we could go back and identify plant species in possible cases where we would find *D. citri*. In 2014, we modified our sampling plan given that we did not find *D. citri* on non-citrus hosts (see results). Within 3 conventional citrus groves in Lake Alfred (variety: Navel), Auburndale (variety: Hamlin), and Winter Park (variety: Navel), we sampled 11 plants found on the edge of citrus groves (adjacent to windbreaks or fallow land) resulting in 33 plants sampled covering 17 plant species.

STATISTICAL ANALYSES

All statistical analyses were conducted with the statistical software R v3.02 (The R Foundation for Statistical Computing, Vienna, Austria). Initially, we evaluated the effect of canopy height and cardinal direction on *D. citri* population density. Because of non-normal distribution of count data and temporal replication, counts of *D. citri* adults were analyzed using a general linear mixed model (GLMM) with Laplace approximation (lmer function in R, lme4 package). Model simplification

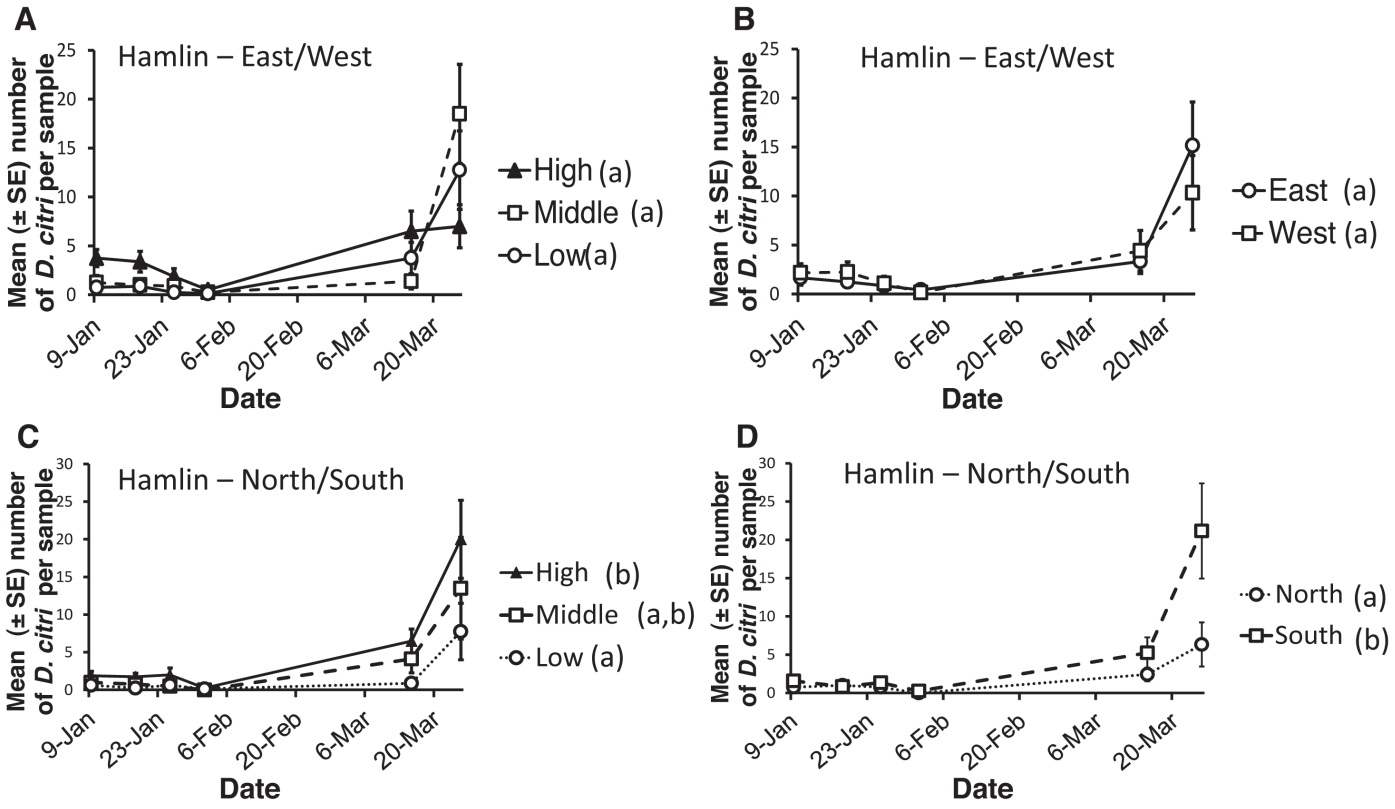


Fig. 2. Mean number of *Diaphorina citri* adults collected per vacuum sample as a function of citrus height and cardinal direction of exposure during winter in 2013. Different letters in brackets indicate significant differences ($P < 0.05$) between treatments.

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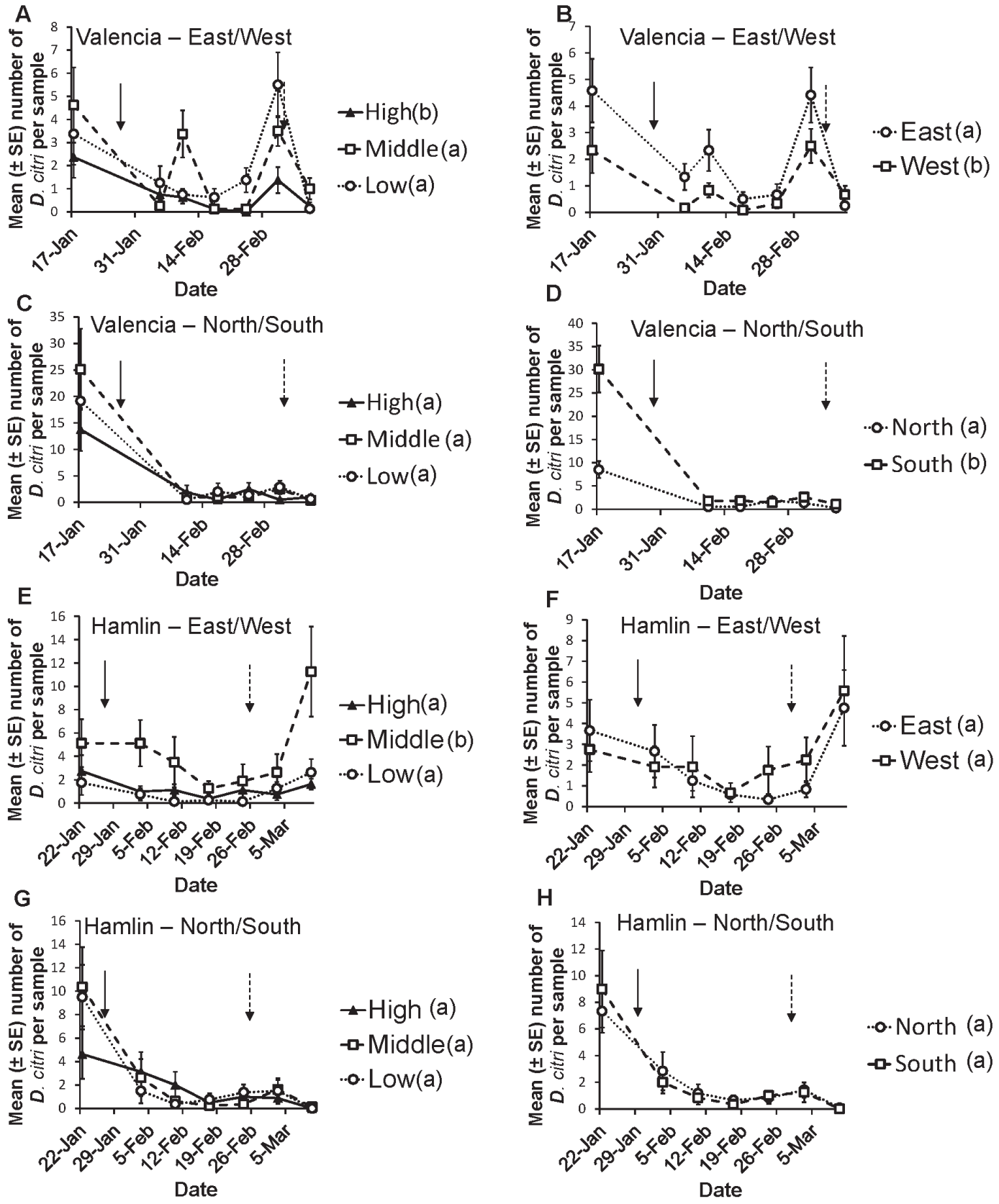


Fig. 3. Mean number of *Diaphorina citri* adults collected per vacuum sample as a function of citrus height and cardinal direction of exposure during winter in 2014. Solid arrows indicate application of Danatol®, dashed arrows indicate application of Portal®. Different letters in brackets indicate significant differences ($P < 0.05$) between treatments.

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Table 1. Effect of canopy height and canopy cardinal direction of exposure on the density of *Diaphorina citri* populations within citrus groves during winter in Florida.

Field	Exposition	Year	Exposure (df = 1)	Height (df = 2)	Exposure × Height (df = 2)
Hamlin	North–south	2013	$\chi^2 = 3.80^a$	$\chi^2 = 14.979^{***}$	$\chi^2 = 1.90$
Hamlin	East–west	2013	$\chi^2 = 0.12$	$\chi^2 = 2.66$	$\chi^2 = 4.64$
Valencia	North–south	2014	$\chi^2 = 6.77^{**}$	$\chi^2 = 0.31$	$\chi^2 = 3.24$
Valencia	East–west	2014	$\chi^2 = 8.60^{**}$	$\chi^2 = 22.86^{***}$	$\chi^2 = 4.36$
Hamlin	North–south	2014	$\chi^2 = 0.05$	$\chi^2 = 0.30$	$\chi^2 = 7.21^{*}$
Hamlin	East–west	2014	$\chi^2 = 0.05$	$\chi^2 = 7.77^{*}$	$\chi^2 = 1.94$

[a]$P = 0.052$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

was undertaken using stepwise deletion (Crawley 2009). Initial fixed terms of the model consisted of canopy height, cardinal direction, and the interaction between these variables. The random terms were time and plot identification number. Post hoc contrasts were used to determine significant differences between canopy heights.

Subsequently, we tested the effect of abiotic and biotic factors on the abundance of *D. citri* adults. To do so, we built a dataset that included the 4 groves sampled in 2014. Those data were analyzed with a GLMM with Poisson distribution with the Laplace approximation. This model included the effects of grove, average temperature, average humidity, flush abundance, degree day accumulated during the 10 d preceding sampling, and days elapsed since the most recent application of insecticide spray. The random terms were time and plot identification number. We used the same type of model but with a binomial distribution to investigate the effect of those factors on the proportion of green-blue morphotypes found in the sample that we collected. Finally, the nutrient data obtained from leaves coming from the various heights and canopy cardinal directions in 2013 were analyzed using 2-way ANOVA.

Results

WEATHER PATTERNS

During 2013, the minimum and maximum temperatures averaged 12.22 and 23.89 °C, respectively, and the minimum temperature recorded was 2.22 °C. Mean monthly rainfall was 0.10 cm (total: 5.60 cm). During 2014, the minimum and maximum temperatures averaged 11.67 and 22.7 °C, respectively, and the minimum temperature recorded was 2.78 °C. Mean monthly rainfall was 0.20 cm (total: 12.8 cm).

TWO-DIMENSIONAL DISTRIBUTION OF *D. CITRI* WITHIN CITRUS GROVES

Overall, we did not find a consistent pattern in the distribution of *D. citri* adults among the 3 canopy heights (Figs. 2 and 3; Table 1). There was also no difference in *D. citri* population densities found between canopy edges exposed to west versus east directions in the groves examined (Figs. 2 and 3; Table 1). More consistently, *D. citri* was found with greater frequency on canopy edges exposed to south versus north directions (Figs. 2D and 3D; Table 1). Flush density did not differ significantly between canopy heights or as a function of canopy orientation. The temperature on the canopy edges exposed to the south direction was on average 1.41 ± 0.14 °C warmer than on canopy edges exposed to the north direction during daytime. Relative humidity was similar between canopies exposed to the north and south. In contrast, there was no difference in temperature and humidity between canopy edges exposed to east and west directions.

EFFECTS OF BIOTIC AND ABIOTIC FACTORS ON *D. CITRI* ABUNDANCE

The percentage of branches containing flush on the citrus trees sampled varied between 0 and 10% (corresponding to a score between

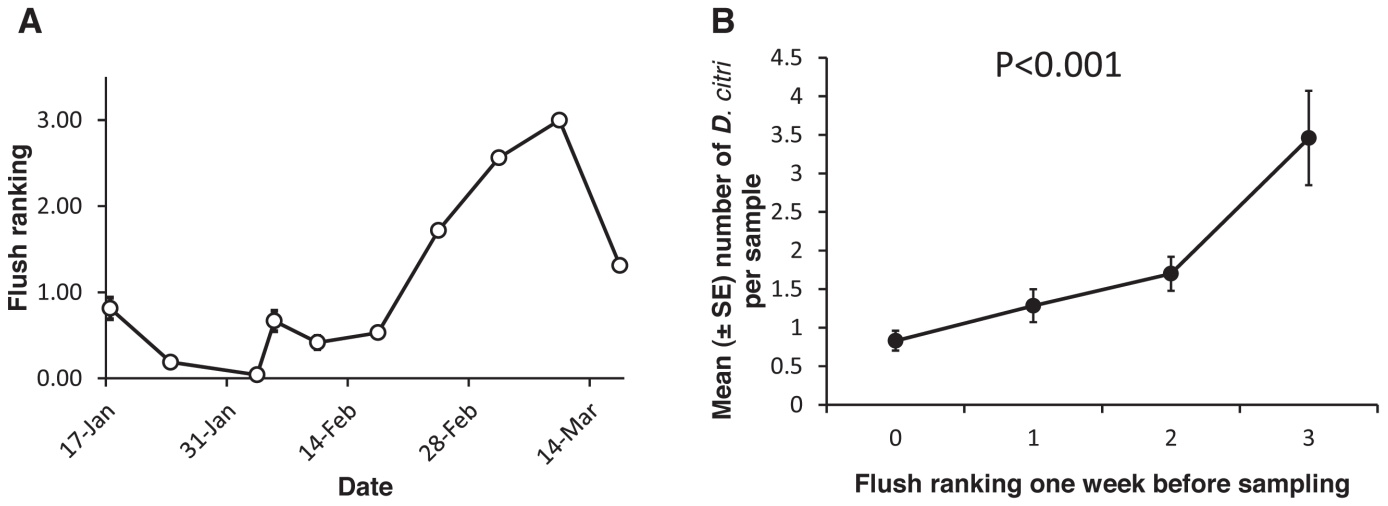


Fig. 4. (A) Mean flush abundance (measured with a visual ranking: 0: no flush; 1: isolated flush on less than 10% of branches; 2: flush on 10 to 50% of branches; 3: flush on more than 50% of branches) in 4 citrus groves sampled in 2014. (B) Average number of *Diaphorina citri* adults per sample as a function of flush ranking of the sample area 1 wk before actual sampling. *P*-value refers to the output from the generalized linear mixed model with Poisson distribution.

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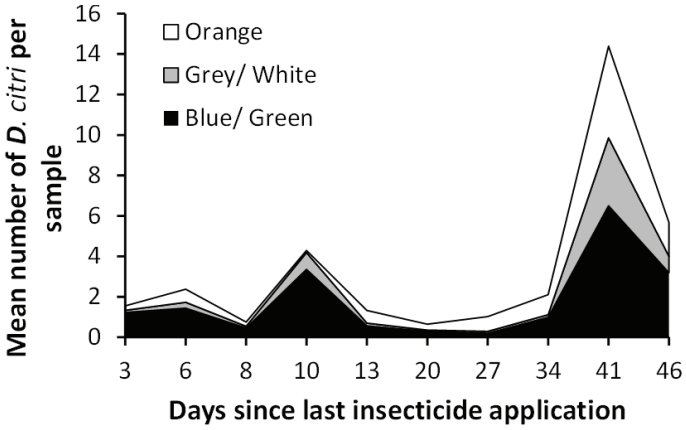


Fig. 5. Mean number of *Diaphorina citri* adults as a function of their color morphotype during the interval following the most recent insecticide spray application in 2014. The legend refers to the color morphotypes of the *D. citri* adults sampled.

0 and 1 with our visual ranking method) during winter, and started to increase in Mar (Fig. 4A). Not surprisingly, we found that the density of flush on trees was positively correlated with the density of psyllids (Table 2; Fig. 4B). There was a 1 wk delay between the appearance of new flush and the effective increase of psyllid populations (Fig. 4B). There was also a significant positive effect of relative humidity on the number of psyllids collected (Table 2). As expected, we found that *D. citri* populations were significantly reduced on days following insecticide application (Fig. 5; Table 2). Temperature on the day of sampling and degree days accumulated during the previous 10 d did not impact psyllid abundance (Table 2).

COLOR MORPH VARIATION

The green-blue morphotype is the only color morph capable of long-duration flight (Martini et al. 2014). It is also the color morph with the greatest reproductive potential (Wenninger at al. 2009). Therefore, we postulated that the green-blue morphotype has the greatest potential for infesting new groves as new flush appears in the spring. The proportion of the green-blue morphotype among *D. citri* populations was approximately at 80% up to 11 d following an insecticide application. Afterward, the proportion of the green-blue morphotype dropped to 40% (Fig. 5; Table 2). In contrast, the proportion of the orange-yellow morphotype was significantly reduced for 10 d following insecticide application as compared with the proportion of this morphotype 11 to 47 d following insecticide application (coefficient estimate: 0.033, SE = 0.01, $P < 0.001$). The proportion of the green-blue morphotype was positively correlated with the abundance of flush 1 wk before the sampling event (Table 2). Humidity did not affect the proportion of the green-blue morphotype within psyllid populations (Table 2). The degree days accumulated during the previous 10 d significantly decreased the proportion of the green-blue morphotype within populations (Table 2), suggesting that the proportion of the green-blue color morph was particularly large after a period of cold weather.

NUTRIENT ANALYSIS

Nutrients did not vary significantly between canopy heights (Table 3). However, N and P elements were higher in north–south exposed blocks than in east–west exposed blocks (Table 3). Conversely, Mg, B, and Cu were lower in north–south exposed blocks than in east–west exposed blocks (Table 3). We did not find any significant correlation ($P > 0.05$) between psyllid density and the leaf concentration of any element.

INVESTIGATING THE ALTERNATIVE HOST HYPOTHESIS

In 2013, we collected *D. citri* adults in conventional and organic groves. However, no significant numbers of *D. citri* adults were found in any alternative habitat sampled among all of the fields sampled (Table 4). In 2014, we found no *D. citri* adults in any of the potential alternative hosts sampled (Table 5).

Discussion

We examined the distribution of *D. citri* adults in commercial citrus groves in Florida during 2 consecutive winter seasons. In each year, we observed an increase of *D. citri* populations in Mar. This was 1 mo earlier than what Tsai et al. (2002) and Hall & Hentz (2011) observed in experimental groves with no insecticide application 223 km (Fort Lauderdale, Florida) and 73 km (Fort Pierce, Florida), respectively, south of our sampling area. Hall et al. (2008) found an increase in numbers of *D. citri* adults in groves in Fort Pierce in Mar 2005 but not in March 2006. Our field sampling differed from previous investigations, and the use of a vacuum sampler may have increased the number of psyllids captured, allowing detection of *D. citri* at lower densities than in previous studies. Recently, Monzo et al. (2015) determined that vacuum sampling was the most effective sampling method for *D. citri* compared with tap sampling or visual examination, but it was also the most time consuming. The use of vacuum sampling in Mar to detect early increases of *D. citri* populations may be useful for predicting the need for post-dormant season sprays in Florida before and after the bloom when insecticide use is limited. However, the cost of specialized equipment and labor must be considered and may hinder practical application of this sampling technique.

Table 2. Factors influencing the abundance of *Diaphorina citri* adults during winter, and proportion of green-blue morphotype during winter in central Florida. If the coefficient estimate is positive, the factor has a positive effect on the abundance of *D. citri* adults at $P < 0.05$. If the coefficient estimate is negative, the factor has a negative effect on the abundance of *D. citri* adults at $P < 0.05$.

	Abundance of *D. citri* adults			Proportion of green-blue morphotypes		
Factor	Coefficient	SE	*P*	Coefficient	SE	*P*
Temperature on sampling day	−0.014	0.04	0.731	0.163	0.09	0.071
Degree days accumulated during 10 previous days	0.005	0.01	0.472	−0.038	0.02	0.017
Relative humidity on day of sampling	0.013	<0.01	<0.001	0.010	0.01	0.262
Flush abundance on day of sampling	−0.045	0.07	0.471	−0.180	0.14	0.216
Flush abundance the week before sampling	0.531	0.06	<0.001	0.440	0.12	<0.001
Days since last insecticide application	0.02	<0.01	<0.001	−0.042	0.01	<0.001

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Table 3. Differing levels of various nutrients in citrus trees from the Hamlin plot in 2013 depending on canopy height and cardinal direction of exposure. *F*-values from 2-way ANOVA conducted for each element are presented in the bottom part of the table.

Canopy exposure	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	S (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
East	2.61 ± 0.02a	1.06 ± 0.03a	0.10 ± 0.00	0.44 ± 0.01a	3.48 ± 0.07	0.32 ± 0.01	113.50 ± 4.24a	27.33 ± 1.31	27.58 ± 1.31	80.08 ± 4.16	13.83 ± 1.24a
West	2.60 ± 0.03a	1.06 ± 0.05a	0.09 ± 0.00	0.41 ± 0.01a	3.41 ± 0.12	0.31 ± 0.01	113.00 ± 3.99a	27.42 ± 1.59	28.33 ± 1.50	83.92 ± 6.11	13.33 ± 0.82a
North	2.83 ± 0.05b	1.09 ± 0.06ab	0.10 ± 0.00	0.36 ± 0.01b	3.69 ± 0.12	0.31 ± 0.01	95.58 ± 2.51b	30.42 ± 2.22	28.33 ± 1.31	82.17 ± 2.19	8.17 ± 0.67b
South	2.93 ± 0.06b	1.15 ± 0.07b	0.11 ± 0.00	0.34 ± 0.01b	3.77 ± 0.15	0.31 ± 0.01	94.25 ± 2.55b	29.00 ± 2.64	30.42 ± 2.66	85.67 ± 5.92	8.50 ± 0.53b
ANOVA *F*-values											
Height x Exposure	0.665	1.820	0.999	0.493	0.437	0.614	0.764	1.511	1.223	0.845	0.230
Height	0.073	0.727	0.421	1.257	0.015	0.136	0.655	0.107	0.571	0.631	0.320
Exposure	13.103***	5.584**	0.573	13.449***	1.871	0.329	9.147***	0.547	0.455	0.233	10.856***

Nutrient values in a column followed by a different letter are significantly different at a < 0.01 when *F*-values are labeled with ** and at a < 0.001 when *F*-values are labeled with *** (2-way ANOVA).

We found no significant pattern in the distribution of *D. citri* adults with respect to canopy height. In contrast, we found significantly more psyllids located on canopy with southern exposure than northern exposure during 2 consecutive years in the majority of plots sampled. These results are congruent with those of Sétamou et al. (2008), who found more *D. citri* nymphs during spring and summer in southeastern quadrants of trees than on other parts of the canopy. Nutrient analyses and flush rating did not reveal significant differences between north- and south-oriented canopies; therefore, the slight increase in psyllid density on the south-oriented canopies compared with the north-oriented canopies might be explained by the slight increase in temperature observed on the southern compared with the northern location. Also, greater sun exposure on the south side compared with the north side during the day may explain this difference in psyllid abundance (Sétamou et al. 2012).

Regarding other biotic and abiotic factors, presence of flush was the main factor that drove density of *D. citri* populations during winter. Mature trees in Florida typically produce major flush during early spring and summer and sometimes minor flush during late summer and fall. During winter, the production of new leaves is reduced (Hall & Albrigo 2007), but sporadic flushing does occur (Hall et al. 2008). Without surprise, our study revealed that a slight presence of flush (from no flush to 10% of the branches) during winter was sufficient to significantly increase the density of *D. citri* populations within citrus trees. Flush has been found to be a strong attractant for *D. citri* in the field (Lewis-Rosenblum et al. 2015). Humidity was also an important factor that increased capture of *D. citri* adults during winter. However, changes in humidity may drive flush production and thus indirectly affect densities of *D. citri* populations.

We found an interesting pattern regarding the proportion of green-blue color morphs within populations of *D. citri*. Green-blue color morphs are known to have greater fitness, with greater fecundity, greater flight capability, greater mass, and greater resistance to insecticides compared with other color morphs (Wenninger & Hall 2008; Wenninger et al. 2009; Tiwari et al. 2013; Martini et al. 2014). It is therefore possible that the green-blue morphotype of *D. citri* has the greatest potential for infesting new flush in the spring. We indeed found an increase in the proportion of green-blue *D. citri* morphs following insecticide applications, which confirms laboratory toxicology assays indicating that these are less susceptible to insecticides than orange-yellow morphs (Tiwari et al. 2013). It appears that the repetitive use of insecticide favors the selection of the green-blue morphotype, which is incidentally the morphotype with the greatest reproductive and migration potential. Additionally, we found a larger proportion of the green-blue morph after extended periods of cold weather than after periods of warm temperatures, suggesting a high level of cold tolerance in this color morph. Collectively, these data suggest that the green-blue morphotype may be an indicator of *D. citri* infestation of groves during the cool spring temperatures as citrus flushing begins. However, difference in cold tolerance between the various morphotypes requires further investigation.

Although densities of *D. citri* populations were lower in Jan and Feb as compared with Mar, psyllids were continuously found in citrus groves. These results are congruent with those previously reported by Hall et al. (2008). Although *D. citri* was present throughout the winter within citrus groves, we did not find that surrounding non-citrus habitats served as refuges during winter. These results demonstrate that citrus is the primary and probably only host of *D. citri* in commercial groves during winter in Florida and therefore an ideal weak link to target with insecticide sprays as demonstrated by Qureshi & Stansly (2010). Also, these results are congruent with previous reviews of *D. citri* biology (Hodkinson & Bird 2000) and indicate that

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Table 4. Mean (± SE) number of *Diaphorina citri* adults per sampling found in 5 groves with differing management practices in 2013 in central Florida. We did not find any significant alternative hosts in proximity to groves that may harbor *D. citri* during this investigation.

Grove location	Management	Sampling date	Citrus	Grass	Litter	Hedges
Auburndale	Conventional	20 Feb 2013	0.50 ± 0.34	0	0	0
Winter Park	Conventional	20 Feb 2013	0.67 ± 0.33	0	0	0
Clermont	Organic	22 Feb 2013	1.33 ± 0.80	0	0	0
Lake Alfred	Abandoned	22 Feb 2013	19.00 ± 3.96	0	0	0
Clermont	Organic	4 Mar 2013	3.00 ± 1.13	0	0.33 ± 0.21	0

Table 5. List of the potential alternative host plants with location sampled from 3 Feb to 5 Mar 2014. All plants were neighboring or within citrus groves in central Florida. We did not find *Diaphorina citri* adults in any of these samples.

Common name	Family	Species	Number of samples
Brazilian pepper	Anacardiaceae	*Schinus terebinthifolius*	3
Grape	Vitaceae	*Vitis* sp.	3
Oak	Fagaceae	*Quercus* sp.	3
Sand pine	Pinaceae	*Pinus clausa*	3
Grass (between citrus rows)	Poaceae	mix of different species	2
Maple	Aceraceae	*Acer rubrum*	2
Mulberry	Moraceae	*Morus* sp.	2
Salt myrtle	Myricaceae	*Baccharis halmifolia*	2
Wax myrtle	Myricaceae	*Myrica cerifera*	2
Willow	Salicaceae	*Salix caroliana*	2
Blackberry	Rosaceae	*Rubus* sp.	2
Carolina laurelcherry	Rosaceae	*Prunus caroliniana*	2
Elderberry	Adoxaceae	*Sambucus* sp.	1
Eucalyptus	Myrtaceae	*Eucalyptus* sp.	1
Holly	Aquifoliaceae	*Ilex* sp.	1
Primrose	Primulaceae	*Primula* sp.	1
Wild cherry	Rosaceae	*Prunus* sp.	1

non-Rutaceae alternative hosts upon which *D. citri* is able to feed (Martini et al. 2013) or even reproduce (Thomas & De Leon 2011) have likely little or no impact on the abundance of *D. citri* adults during winter, at least in central Florida. Combined with the apparent absence of significant dispersal by *D. citri* during winter (Hall & Hentz 2011; Lewis-Rosenblum et al. 2015), the current results indicate that *D. citri* likely settles in a sessile manner during winter within groves that were infested during fall. In this regard, groves with little or no control measures implemented, such as abandoned groves, may be significant overwintering sites for *D. citri* during winter in Florida, despite their minimal flush production. Given that the winter dormant season is the ideal weak link for timing insecticide application (Qureshi & Stansly 2010), influx of *D. citri* from over 50,000 ha of abandoned groves in the spring remains a likely problem (Tiwari et al. 2010; Lewis-Rosenblum et al. 2015)

Our results may have several implications for management of *D. citri* in Florida. Sampling of *D. citri* will likely be more efficient if efforts are simply focused on the southern edges of groves during the winter. Also, height of sampling does not appear to affect catch of *D. citri* adults; therefore, any logistically manageable location for sampling on the southern borders of groves should effectively predict infestation. Not surprisingly, the presence of flush was correlated to the presence of psyllids; therefore, sampling should also be concentrated in areas where flush appears during winter. For instance, young trees produce flush continuously (Hall & Albrigo 2007); therefore, it is possible that young plantings, and particularly solid set new plantings (blocks of young citrus trees planted within the past 3 yr), would be susceptible to infestation during winter, in addition to spring and summer (Martini et al. 2015). Our data indicate that *D. citri* is present on only citrus during winter and does not benefit from alternative hosts or habitats in proximity to citrus in Florida during the dormant period. Therefore, there is no need to spray areas beyond citrus, and non-citrus plants may be employed to favor natural enemy biodiversity in citrus groves (Silva et al. 2010). For instance, cover crops could provide refuge and alternative food for the *D. citri* parasitoid, *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae). Also, our results suggest that targeted supplemental border sprays for *D. citri* during the dormant winter period should likely focus on southern borders of groves; however, this suggestion will require further investigation.

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