Dispersal patterns of *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae) as influenced by citrus grove management and abiotic factors

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Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), remains the most economically important and difficult to manage pest in citrus throughout Florida. To improve existing control methods, the potential for Asian citrus psyllid to evade insecticide sprays and engage in short-term dispersal was investigated. Dispersal was evaluated to better understand responses of Asian citrus psyllid to citrus grove management, and how these factors affect population dynamics when citrus flush is scarce. To determine the impact of insecticide applications and other abiotic factors on Asian citrus psyllid movement, psyllid captures were measured on yellow sticky-card traps placed along the citrus grove borders. Additional suction trap and citrus grove stem-tap samples were collected and compared with sticky card catches, then correlated with data taken from the Florida Automated Weather Network. More Asian citrus psyllids were caught after than directly before insecticide sprays (t = 3.096; df = 27; P = 0.005), with the highest catches occurring from Mar to May. Both solar radiation and wind direction were positively correlated with Asian citrus psyllid dispersal, whereas humidity was negatively correlated across all sampling methods. Significant psyllid catches in suction traps located 500 m from a citrus grove provide additional evidence that Asian citrus psyllids tend to migrate when host conditions are unfavorable, returning to previous or neighboring groves after dispersal.

Key Words: insecticides; sticky card; suction trap; correlation; tap sampling

Resumen

El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama, sigue siendo la plaga más importante y difícil de manejar en los cítricos de la Florida. Para mejorar los métodos de control existentes, se investigó el potencial del psílido asiático de los cítricos para evadir los aerosoles de insecticida y habilidad de dispersarse a corto plazo. Se evaluó la dispersión para comprender mejor las respuestas del psílido asiático al manejo de los cítricos, y cómo estos factores afectan la dinámica de la población cuando los brote de nuevas hojas son escasos. Para determinar el impacto de las aplicaciones de insecticidas y otros factores abióticos en el movimiento de los psílidos asiáticos de los cítricos, se midieron las capturas de psílidos en trampas amarillas de tarjetas adhesivas colocadas a lo largo de los bordes del huerto de cítricos. Se recolectaron muestras adicionales de los arboles de cítricos con trampas de succión y de pega en las ramas y se compararon con las capturas de tarjetas adhesivas, estos resultados posteriormente se correlacionaron con los datos tomados de la Red Meteorológica Automatizada de Florida. Se capturaron más psílidos asiáticos de cítricos después de la aplicación de los insecticidas que antes (t = 3.096; df = 27; P = 0.005), con las capturas más altas ocurriendo desde marzo hasta mayo. Tanto la radiación solar como la dirección del viento se correlacionaron positivamente con la dispersión del psílido cítrico asiático, mientras que la humedad se correlacionó negativamente en todos los métodos de muestreo. Numeros significativos de psílidos capturados en las trampas de succión ubicadas a 500 m de los huertos de cítricos provee evidencia adicional de que el psílido asiático de los cítricos tiende a migrar cuando las condiciones del hospedero son desfavorables, y regresan a los huertos donde estaban anteriormente o un huerto cercano después de la dispersión.

Palabras Clave: insecticidas; tarjeta adhesiva; trampa de succión; correlación; toma de muestras

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the primary vector of the pathogens causing huanglongbing, also known as citrus greening disease (Bové 2006; Manjunath et al. 2008). Originally described from Taiwan, Asian citrus psyllid was first detected in Florida in 1998, and huanglongbing in 2005 (Halbert & Manjunath 2004; Hall 2008). The causal agent of huanglongbing, *Candidatus* Liberibacter asiaticus (*C*Las), is usually acquired by Asian citrus psyllid nymphs during phloem feeding on infected trees. These then remain infective during the adult stage, capable of transmitting *Candidatus* Liberibacter asiaticus to citrus after dispersal to uninfected trees occurs (Bové 2006). Adult psyllids also may acquire *Candidatus* Liberibacter asiaticus from feeding on infected citrus, albeit at much lower rates than nymphs, and these adults are not likely to transmit the bacteria. However, adult psyllids must acquire *Candidatus* Liberibacter asiaticus during the nymphal stage to become a competent vector (Ammar et al. 2011). Once adults become infected with *Candidatus* Liberibacter asiaticus, Asian citrus psyllid can spread huanglongbing throughout a citrus grove via salivary gland excretions as they feed on the flush of uninfected trees (Lee et al. 2015). Because controlling Asian citrus psyllid is critical in mitigating the spread of huanglongbing, understanding the underlying factors that influence psyllid movement plays a key role in disease management.

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Asian citrus psyllids are diurnal insects, hence photoperiod and visual stimuli strongly affect movement and dispersal patterns (Paris et al. 2015). Sétamou et al. (2012) and Paris et al. (2015) reported that nearly all Asian citrus psyllid activity occurs during the day with peak movement occurring in midafternoon, between 12:00 Noon and 3:00 PM. Along with sunlight, Asian citrus psyllids also are sensitive to certain wavelengths in the ultraviolet-spectrum that can be exploited for management using ultraviolet-reflective mulch to effectively disorient psyllids during flight (Croxton 2015; Paris et al. 2015). In addition to light, color may influence Asian citrus psyllid dispersal and settlement on a host. Studies have shown that Asian citrus psyllids are more attracted to yellow and ultraviolet wavelengths that have a similar reflectance pattern to citrus flush on the visible spectrum, and may stimulate flying behavior (Sétamou et al. 2014; Paris et al. 2015). These observations are further confirmed by the greater numbers of psyllids captured in yellow sticky-card traps than other colors (Aubert & Hua 1990; Hall & Albrigo 2007).

Several other seasonal and abiotic factors influence Asian citrus psyllid dispersal, such as temperature, humidity, and wind (Lewis-Rosenblum et al. 2015) that may further complicate evaluation of effectively timed insecticide applications. Like all poikilotherms, temperature can have a direct effect on Asian citrus psyllid activity and movement (Tsai & Liu 2000). Consequently, Asian citrus psyllid activity appears to be greatest during the spring and summer mo coinciding with warmer temperatures and greatest citrus flushing (Martini et al. 2016). During the colder winter mo, Asian citrus psyllid activity is considerably reduced and movement tends to be limited, thus presenting an excellent opportunity to target populations with dormant winter sprays (Qureshi & Stansly 2010). In addition to temperature, relative humidity and precipitation can influence Asian citrus psyllid dispersal; however, the exact reasons for the correlations between some abiotic factors remains unknown (Hall & Hentz 2011).

Once Asian citrus psyllid populations have become established in a citrus grove, dispersal tends to be limited; however, there are still factors that may induce searching for a new host (Kobori 2011). Most notably, the lack of young flush, which is often scarce when growing conditions are unfavorable, such as during the dormant winter season, may cause Asian citrus psyllid to disperse to search for new citrus that contains the proper foliage on which to lay their eggs (Lewis-Rosenblum et al. 2015). During periods of high-density infestation, intraspecific competition also may pressure a percentage of the psyllid population to search for a new host (Croxton 2015). Finally, physical disturbances in the grove, such as spray operations, have the potential to induce Asian citrus psyllid dispersal, because treated citrus may create a temporarily unsuitable habitat. Although insecticides primarily have been considered a control measure that kills a targeted pest on contact, considerably less attention has been given to the repellency that these chemical residues leave behind. Insecticidal residues, whether repellent by olfactory or tactile means, can become important, especially when a significant proportion of the pest population has developed resistance and can survive initial effects of the spray. Whiteflies, which are closely related to psyllids, have exhibited repellency to pyrethroids and other chemicals, affecting oviposition on tomato plants (Liu & Stansly 1995). Similarly, a host of chemicals, including volatiles of essentials oils, have been shown to repel psyllids when co-presented with citrus leaves (Mann et al. 2012). Even imidacloprid, a commonly used insecticide, has been shown to exhibit antifeedant effects on psyllids feeding on plants sprayed with sublethal concentrations (Boina et al. 2009). Understanding the effects that insecticides have on dispersal will lead to optimal timing of intervals between applications, and better control practices overall.

Since the initial Florida outbreak in 2005, and as pest management practices to combat huanglongbing have continued to evolve, a rotation of foliar-applied insecticides remains an essential component of most control programs (Stansly et al. 2014). Foliar insecticides reduce Asian citrus psyllid populations by 5- to 13-fold, whereas the combined effect of nymphal and adult suppression can provide > 90% population reduction for up to 68 d (Qureshi et al. 2014; Stansly et al. 2014). Despite the relative success of insecticides, no control method alone has proven capable of eliminating Asian citrus psyllid at a regional or even statewide level. Concerns have arisen over psyllid populations now exhibiting insecticide resistance, especially to neonicotinoids used for their control (Tiwari et al. 2011; Vázquez-García et al. 2013). As resistance in populations increases, the likelihood of Asian citrus psyllids surviving contact with insecticides and evading applications also increases, particularly if those resistant psyllids disperse to new citrus groves. The objective of this study was to monitor Asian citrus psyllid dispersal before and after application of insecticides along the border of a citrus grove. Seasonality, temperature, and abundance of Asian citrus psyllid populations in surrounding groves also were examined as factors that may influence dispersal. These findings should help improve current control methods by increasing our understanding of Asian citrus psyllid behavioral ecology and dispersal in light of existing pest management programs.

Materials and Methods

FIELD PLOT

A block of 'Hamlin' orange bud grafted on 'US802' rootstock, a hybrid of pomelo, *Citrus grandis* (L.) Osbeck (Rutaceae), with trifoliate orange, *Poncirus trifoliata* (L.) Raf. (Rutaceae), and planted in May 2010 at the Southwest Florida Research and Education Center in Immokalee, Florida, was selected to evaluate psyllid movement in response to insecticide sprays. Trees were planted in 8 rows at 5.5 m between rows and 2.5 m within the row for a total of 123 trees per ha, each approximately 2.3 m (90 in) tall and 1.5 m (60 in) in diameter.

DISPERSAL IN RELATION TO MANAGEMENT

For each sampling period, a total of 7 Yellow Corn Rootworm sticky cards, IPM-CRW-100 (Great Lakes IPM, Vestaburg, Michigan, USA), stapled to wooden tomato stakes 1.38 m (4.5 ft) in height were placed at mid-canopy height (approximately 1.0 m) around the border of the grove. All traps were placed at a minimum distance of 1 m away and facing the nearest citrus tree; 1 trap was positioned on the northern border, 1 trap on the eastern border, 2 traps on the southern border, and 3 traps on the western border (Fig. 1). Placement of sticky cards was arranged in a manner to maximize trap catch across an open field border; however, the sticky card trap on the eastern border also was adjacent to a neighboring citrus field and was primarily used for comparison to the other 6 traps. Once placed, sticky card traps remained in the field for 1 wk prior to insecticide applications. After 1 wk, sticky cards were collected and replaced with new sticky cards in preparation for spraying the following day. All sticky cards were visually inspected, and Asian citrus psyllids counted and confirmed underneath a high intensity illuminator (41723-series, Cole Parmer, Vernon Hills, Illinois, USA). Visual inspections were repeated 1 wk later for the sticky cards that collected Asian citrus psyllid after insecticide applications. These trials were repeated at regular monthly intervals starting on 13 Oct 2016 and ending 27 Oct 2017.

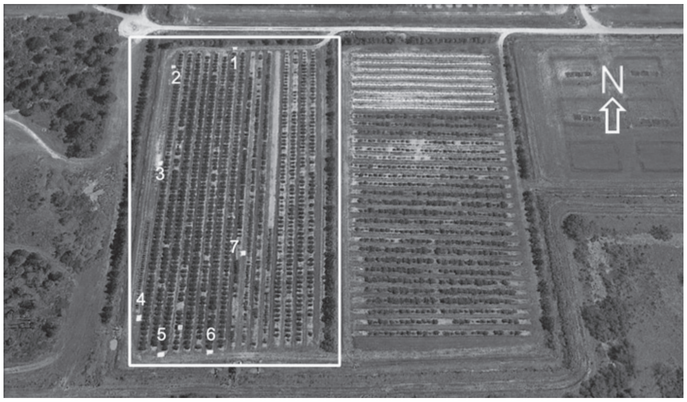

Fig. 1. Yellow sticky card trap locations at a Southwest Florida Research and Education Center citrus grove numbered and outlined in white. Yellow markers indicate trap locations.

Between 2 May 2017 and 2 Sep 2017, 3 stem tap samples were taken with each sticky card for a total of 21 taps per trial. Stem tap samples were obtained from the citrus grove border closest to each suction trap using the protocol of Arevalo et al. (2011) in order to compare Asian citrus psyllid abundance from stem taps with those of suction traps.

SEASONAL MOVEMENT

In conjunction with Asian citrus psyllid movement across the citrus grove border, 3 suction traps also were set to evaluate the seasonal effects on dispersal. Suction traps were placed in different locations throughout the Southwest Florida Research and Education Center: the first in the experimental block (26.4620°N, 81.4460°W), the second in a nearby open field (26.4620°N, 81.4370°W) with no citrus within 500 m, and the third in a neighboring citrus grove (26.4650°N, 81.4440°W). Suction traps were built on a design from Allison and Pike (1988), and constructed from a PVC pipe 1.8 m long and 30.5 cm diam on three 1.5 m lengths of 10.2 × 10.2 cm lumber set 0.9 m into the ground for a total height of 2.4 m. The third suction trap labeled the "tall" trap had a similar design but was 8.5 m tall. All traps contained an AC power driven fan inside the pipe to draw air from the top to a funnel below where a jar filled with ethylene glycol would capture and preserve insects. Jars were changed each wk from 2 Aug 2016 to 27 Nov 2017, and the contents strained, rinsed, and identified by Susan Halbert, Florida State Collection of Arthropods, Gainesville, Florida.

STATISTICAL ANALYSIS

Mean Asian citrus psyllid abundance on traps before and after insecticide applications were compared for the entire study period and 4 separate trial periods (Oct/Nov 2016, Mar 2017, May/Jun 2017, and Sep/Oct 2017) using paired *t* tests ($\alpha = 0.05$) (R 3.3.1, RStudio, Boston, Massachusetts, USA). Pearson's correlation test was conducted to test for possible relationships between trap counts (sticky card and suction trap), and geospatial weather data collected by the Florida Automated Weather Network (RStudio, Boston, Massachusetts). A 1-way analysis of variance (ANOVA) was conducted to detect significant differences between mean Asian citrus psyllid abundance in stem tap samples and sticky card traps before and after insecticide applications (R 3.3.1, RStudio, Boston, Massachusetts). In addition, a line chart was generated in Microsoft Excel to compare combined sticky card trap and stem tap sample results by mo. Correlations also were conducted to compare sticky cards and suction trap data collected 1 wk prior to the date sticky card traps were collected.

Results

DISPERSAL IN RELATION TO MANAGEMENT

Captures of Asian citrus psyllids on traps were greater after than before insecticide applications ($t = 3.096$; df = 27; $P = 0.005$) for combined sticky trap data across all seasons. However, this was not consistent across seasons (Table 1). Mean captures on sticky cards were greater after applications for trials conducted in Oct/Nov and Mar, but not for those conducted in May/Jun and Sep/Oct. We found a difference in average Asian citrus psyllid count between sampling methods for samples taken before insecticide application ($F = 4.861$; df = 1; $P = 0.048$), whereby stem tap samples yielded an average of 3.71 psyllids per tap, and sticky cards collected 1.43 psyllids per trap. Despite higher Asian citrus psyllid counts, no difference was detected between sampling methods after insecticide applications ($F = 1.707$; df = 1; $P = 0.216$) where stem tap samples yielded an average of 4.86 psyllids per tap, and sticky cards collected 2.57 psyllids per trap.

SEASONAL MOVEMENT

Seasonal abundance and dispersal patterns of Asian citrus psyllid were reflected in trap samples where both sticky cards and suction traps caught high numbers of psyllids in Mar; however, unlike suction traps, sticky cards exhibited an additional peak catch in late Apr (Figs. 2, 3). Data collected from the tall suction trap is not reported because almost no Asian citrus psyllids were collected by this trap. In addition to suction and sticky card traps, stem tap samples directly reflected a similar pattern of Asian citrus psyllid abundance in the experimental block during the summer and fall (Fig. 4).

Counts from sticky cards and suction traps showed a significant positive correlation ($R = 0.531$; df = 17; $P = 0.019$) with each other across all seasons. Sticky cards and suction traps indicated that the greatest Asian citrus psyllid population surge and dispersal activity occurred from mid-Mar to early May, then progressively tapered off throughout the summer (Fig. 5). These results were further supported by data from stem tap samples.

The number of psyllids captured was negatively correlated with humidity for sticky cards (Table 2) and suction traps ($R = -0.3149$; $P = 0.0101$). Solar radiation and wind direction were positively correlated with Asian citrus psyllid capture on sticky cards that were oriented in a manner facing the nearest citrus tree (Table 2). Regarding wind direction, Asian citrus psyllids tended to move against the wind where sticky cards collected greater numbers in southern traps when the wind was coming from a higher angle and more southerly direction. This trend was observed in other sticky card samples as well, depending on the average wind direction during a given month. Further analysis revealed that wind direction averaged between 82 to 157°, with 0° or 360° indicating a

Table 2. Correlation of abiotic factors over 2-wk periods with Asian citrus psyllid, *Diaphorina citri*, captures on yellow sticky card traps.

	Sticky Card Correlation		
	R	P	N
Temperature	−0.0957	0.2733	96
Humidity	−0.3093	0.0003	96
Rainfall	−0.0427	0.6257	96
Solar Radiation	0.2924	0.0006	96
Wind Speed	0.0804	0.3575	96
Wind Direction	0.3021	0.0004	96
Barometric Pressure	−0.1082	0.2151	96

Table 1. Paired *t*-test analysis on Asian citrus psyllid, *Diaphorina citri*, captures on sticky card traps before (μ_1) and after (μ_2) insecticide applications for four 2-wk periods.

	Paired Differences							
				95% Confidence Interval				
Pair	μ_1	μ_2	Diff. in μ	Lower	Upper	*t*	df	*P*
Before - After (Oct/Nov)	0.143	0.571	0.428	0.219	1.495	3.286	6	0.017
Before - After (Mar)	0.286	1.143	0.857	−1.021	3.307	3.159	6	0.020
Before - After (May/Jun)	2.286	8.857	6.571	1.482	11.661	1.292	6	0.244
Before - After (Sep/Oct)	1.429	2.571	1.142	−0.474	1.331	1.162	6	0.289
Before - After (all)	1.036	3.286	2.25	0.759	3.741	3.096	27	0.005

predominant wind directed due north. Other abiotic factors such as temperature, rainfall, wind speed, and barometric pressure did not significantly correlate with Asian citrus psyllid catches on those traps (Table 2).

Discussion

More Asian citrus psyllids were captured on sticky cards after insecticide application, which supports grower speculation that spraying induces Asian citrus psyllid dispersal, at least temporarily. Because complete knockdown of psyllid populations is almost never achieved with a single insecticide application, the remaining population surviving control efforts represents the portion of Asian citrus psyllids that have the potential to disperse to nearby groves with available flush. Surviving Asian citrus psyllids represent an even greater problem for management, because the dispersing insects have a greater likelihood of resistance to commonly used insecticides such as imidacloprid (Vázquez-García et al. 2013). Though an overall relationship with insecticide application could be observed, sticky card catches also were influenced by seasonal factors. During the May/Jun trial, Asian citrus psyllid populations were at their peak already, and intraspecific competition may have induced dispersal independent of management actions. Sticky card collections for the Sep/Oct trial occurred directly after Hurricane Irma, and mass movement in relation to rapidly emerging flush partly could explain the higher numbers of Asian citrus psyllids obtained during this period. The phenomenon of psyllid dispersal due to hurricane-induced flushing also is illustrated by the high Asian citrus psyllid counts in suction traps from Oct/Nov 2017 compared to the previous year (Fig. 3).

In past studies, yellow sticky cards have been used to gauge the abundance of Asian citrus psyllid populations over time (Hall & Hentz 2011; Monzo et al. 2015). Our study used suction traps in conjunction with sticky cards to determine seasonal abundance that yielded similar results, because the 2 methods are well correlated. Suction and sticky traps caught high Asian citrus psyllid numbers beginning in Mar at the end of spring flush, sustaining high catches until the end of May (Qureshi et al. 2018). Additionally, the suction trap positioned in the citrus field block captured greater numbers of Asian citrus psyllids than the open field trap located about 500 m away. Low trap catches in the third suction (tall) trap confirmed reports that Asian citrus psyllid does not fly at heights much greater than 7 m during dispersal (Aubert & Hua 1990). Psyllid populations also showed a brief resurgence in Oct and Nov 2017, likely a response to flushing induced by defoliation from Hurricane Irma on 10 Sep 2017 (Qureshi et al. 2018).

Due to low numbers of Asian citrus psyllids in preliminary stem tap samples throughout the winter, stem tap sampling paired with sticky card catch was not conducted until after the first seasonal flush in 2017. Psyllid counts were expected to be comparatively higher on tapped citrus trees before insecticide applications than after. These differences also were expected to be inversely correlated with sticky card catch since existing grove populations represented by stem tap samples are expected to be greater than dispersing populations represented by sticky cards. However, this relationship between stem tap sampling and sticky cards was observed only in "before" groups, with higher average counts for stem tap samples. A combination of seasonal timing coincident with low Asian citrus psyllid field populations in Jun 2017 (Fig. 2) may partially explain why no differences attributable to spray effect were found with either stem tap samples or sticky card traps after insecticide applications. Mortality associated with various insecticides also should be considered. Depending on insecticide evasion and resistance, low Asian citrus psyllid populations along the border of the citrus grove due to drift would likewise yield low sticky card and stem tap sample counts despite elevated dispersal activity.

In addition to physical disturbances such as insecticide or herbicide applications, several abiotic factors also may influence dispersal.

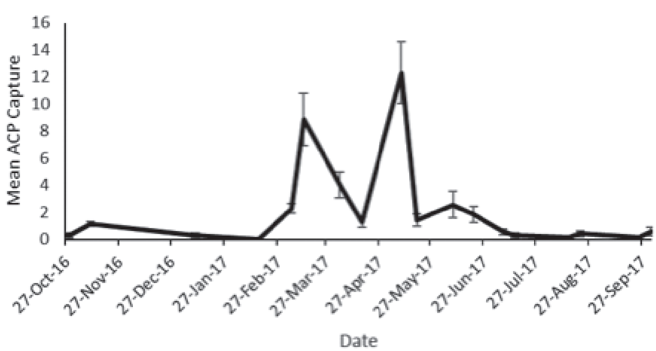

Fig. 2. Mean capture (± SEM) of Asian citrus psyllid, *Diaphorina citri*, on yellow sticky card traps over a 1-yr period.

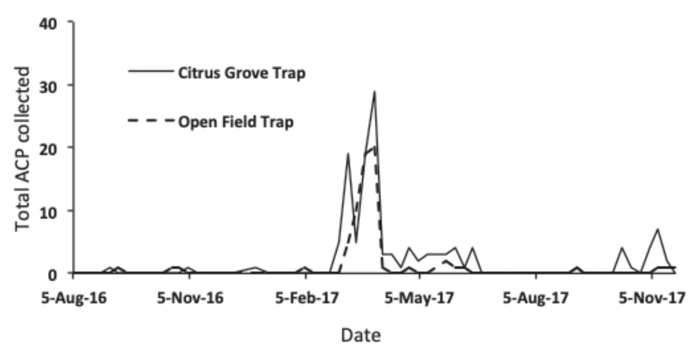

Fig. 3. Asian citrus psyllid, *Diaphorina citri*, counts for citrus grove and open field suction traps. Asian citrus psyllid counts for the tall trap were excluded due to the low number of collected samples.

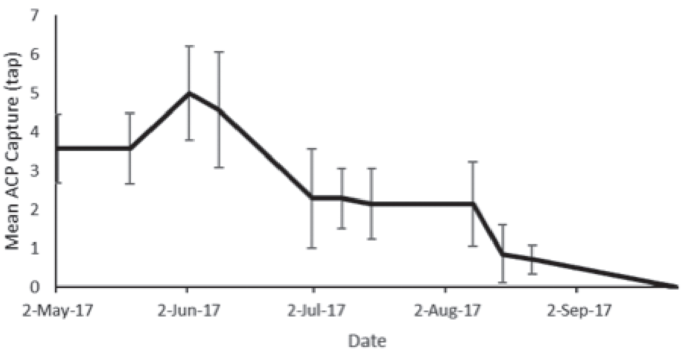

Fig. 4. Mean capture (± SEM) of Asian citrus psyllid, *Diaphorina citri*, in stem tap samples paired with corresponding yellow sticky card traps. All samples were collected in a citrus grove at Southwest Florida Research and Education Center, Immokalee, Florida.

Although relative humidity was negatively correlated with sticky card and suction trap samples (Tables 2, 3), the impact of relative humidity on psyllid dispersal may be more complex, because this abiotic factor also is dependent on additional factors such as temperature and solar radiation. Past studies also have found negative correlations between Asian citrus psyllid dispersal and relative humidity, although the exact causes for this relationship remain uncertain (Hall & Hentz 2011; Croxton 2015). The positive correlation between solar radiation and Asian citrus psyllid movement further confirms that dispersal occurs during peak daylight h, and that visual cues strongly influence these insects (Paris et al. 2015). Another abiotic factor to consider is wind direction and its positive correlation to dispersal. Further analysis of trap catches showed that prevailing wind direction was primarily from a southeasterly direction when Asian citrus psyllid movement was at its peak seasonally. During the colder mo when Asian citrus psyllid is less active, the prevailing wind direction was more northeasterly in direction, thus a lower wind angle was associated with a lower trap catch, and subsequently, a positive correlation in wind direction (see Fig. 1 for reference). However, this finding indicates that psyllids orient themselves in the direction facing the wind and engage in short-range dispersal against the wind gradient. This flight behavior might be adaptive allowing Asian citrus psyllids to follow odor plumes in search of host and mate resources and avoiding wind resistance during high wind speeds. As demonstrated in wind tunnel experiments, Asian citrus psyllids will orient their streamlined bodies in a direction facing the wind to prevent air from circulating under their wings (Croxton 2015).

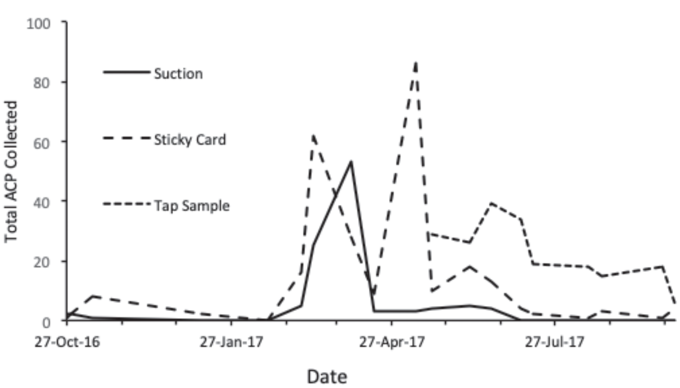

Fig. 5. Comparison of 3 sampling methods for collecting Asian citrus psyllid, *Diaphorina citri*. Total numbers are reported over a yr-long period where stem tap sampling was conducted beginning in May 2017.

During intervals of wind cessation, adults will engage in short bursts of flight in the direction they were oriented.

Overall, many different factors were found to influence Asian citrus psyllid dispersal, including within-grove disturbances related to insecticide applications to citrus groves. As resistance to insecticides will increase over time, short-range Asian citrus psyllid dispersal may lead to re-introduction of psyllids into previous or neighboring citrus groves once new "unsprayed" flush emerges. As Asian citrus psyllid movement in this study was shown to be correlated with season, solar radiation, humidity, and wind direction, existing control tactics may require further adjustment during these periods, especially during mo of peak citrus flushing. Adhering to a strict mode of action rotation for insecticides during peak seasonal activity and natural events that lead to flushing (such as hurricanes) will help narrow the window of opportunity for re-infestation of commercially managed groves. Though the full extent of long-range dispersal is unknown, relatively high counts in the open field suction trap comparable with the suction trap placed within our citrus field plot, indicate that Asian citrus psyllids can travel great distances in search of a suitable citrus host. These findings also indicate that communication regarding the timing of insecticide applications between neighboring groves also may be crucial in implementing effective management programs. Future studies conducted in a wider variety of locations will be essential to fully evaluate the effects of short-range and long-range dispersal of Asian citrus psyllids in response to foliar-applied insecticides.

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