

Research Article

Within-orchard edge effects of the azimuth of the sun on *Diaphorina citri* adults in mature orchards.

DJ Anco^{1,2*} and TR Gottwald²

¹ North Carolina State University, National Science Foundation Center for Integrated Pest Management, 1730 Varsity Drive, Suite 110, Raleigh, NC 27606, USA; ² Agricultural Research Service, US Department of Agriculture, US Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA.

*Correspondence to: dan.anco@ars.usda.gov

Citation: Anco DJ, Gottwald TR. 2015. Within-orchard edge effects of the azimuth of the sun on *Diaphorina citri* adults in mature orchards. J Cit Pathol. iocv_journalcitruspathology_28535.

Abstract

Huanglongbing (HLB) is considered the most devastating disease of citrus. The bacterium and vector associated with HLB in Florida are *Candidatus Liberibacter asiaticus* and *Diaphorina citri* (Asian citrus psyllid), respectively. *D. citri* is positively phototropic, and higher populations have been found along edges of orchards exposed to the sun. A survey was designed to determine if *D. citri* adult populations along edges of orchards varied according to time-of-day and time-of-year in relation to the azimuth of the sun. The survey was conducted twice. Citrus orchards, each divided into 9 sampling areas, were surveyed for *D. citri* via stem-tap sampling. Orchards were sampled 3 times per day (near sunrise, solar noon, and sunset) and 4 times per year (near the summer solstice, autumnal equinox, winter solstice, and vernal equinox). Time-of-year and sampling area significantly affected psyllid counts ($P = 0.0518$ and 0.0630 , respectively). *D. citri* adults were most prevalent during the summer solstice sampling period. No overall significant time-of-day effect was observed ($P > 0.6$). Localization of adult *D. citri* in sampled citrus orchards did not significantly change in relation to time-of-year ($P = 0.0907$). Linear mixed regression was used to fit a quadratic equation to log *D. citri* abundance data in relation to elevation-corrected azimuth at the time of sampling; the fitted model was significant and predicted log *D. citri* abundance to exhibit a concave-up pattern with increasing elevation-corrected azimuth. This relationship represented in a new form how population counts of *D. citri* adults in Florida were greatest during the summer.

Keywords: Asian citrus psyllid, huanglongbing

Introduction

Huanglongbing (HLB) is considered the most devastating disease of citrus (Bové 2006; Gottwald 2010; McClean and Schwarz 1970). While promising progress is being made, there is currently no cure for this disease. The bacterium and vector associated with HLB in Florida are *Candidatus Liberibacter asiaticus* and *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Asian citrus psyllid), respectively. *D. citri* is positively phototropic, and the presence of light has been shown to strongly affect its preference for host plants under laboratory conditions (Sétamou et al. 2012). Within orchards in Texas, Sétamou et al. (2008) reported the greatest populations of *D. citri* nymphs were located in southeastern quadrants on an individual tree scale but no significant canopy quadrant preference was detected for *D. citri* adults. In Bangalore, India, Dharajothi et al. (1989) observed the most nymphs and adults in eastern canopy quadrants but these populations were not

significantly different from those in other quadrants. In China, *D. citri* adults were reported to preferentially localize in warmer south-facing sections of the canopy (Xu et al. 1994; Yang et al. 2006). Changes in canopy location in relation to time-of-day (Prokopy et al. 1972; Yee 2002) or time-of-year (Stork et al. 2001) have been reported for various insects (e.g., *Rhagoletis indifferens* Curran, *R. pomonella* (Walsh), and *Adalia decempunctata* (L.)). Higher psyllid populations were detected along orchard edges more exposed to the sun (Sétamou et al. 2008; Xu et al. 1994). While these reports provide evidence that the localization of insects is influenced by the direction of incident light and time, there have been no reports of temporal changes in localization of *D. citri* on an orchard scale. Thus, the current study was conducted in order to increase understanding of *D. citri*'s phototropism within Florida orchards so that efficiency of scouting and site-specific management might be enhanced.

Materials and Methods

Sampling

Experimental design was a split-split plot, with time-of-year assigned to whole-plots, time-of-day assigned to sub-plots, and sampling area assigned to sub-sub-plots. Orchard site was considered a blocking factor. Two experimental replications were conducted from 2012 to 2014 (e.g., 2 years of seasonal data: summer 2012 through spring 2013 and summer 2013 through spring 2014). Time-of-year sampling periods (summer solstice, autumnal equinox, winter solstice, and vernal equinox) were centered to maximize differences in the azimuth (360° range of compass direction perceived from a point of observation [e.g., the center of an orchard]) of the sun (during solstice sampling periods) as well as to sample during times of inflection as azimuth values change from one extreme to the other (during equinox sampling periods). Time-of-year sampling periods surrounding each solstice or equinox maintained an azimuth error range of 5° . This error range yielded an interval within which to conduct sampling, since resources typically restricted sampling to one orchard per day, while allowing an extent of accommodation for sampling-restrictive events (e.g., inclement weather and pesticide applications). During each time-of-year sampling, orchards were sampled during 3 times of the day (the 3 hours after sunrise, surrounding solar noon, and before sunset). Orchards were divided into nine sampling areas (Fig. 1).

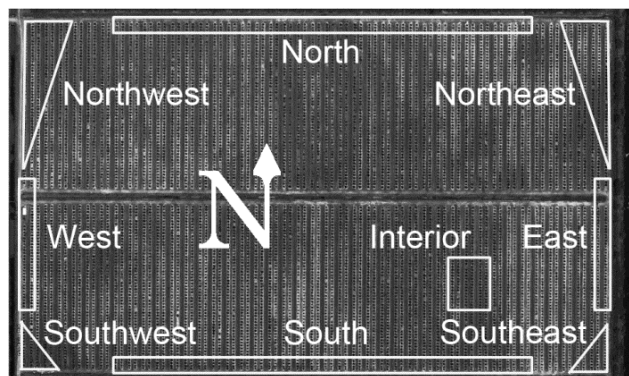


Fig 1. Example orchard with delineated and labeled sampling areas.

Sampling areas per orchard represented the 4 cardinal directions (N, E, S, and W), 4 intercardinal directions (NE, SE, SW, and NW), and an interior sampling area (I) at least 10 rows (>34 m) away from the orchard's perimeter. The interior sampling area was included as a control area whose only unobstructed and direct light came from overhead. Trees were selected to optimize the orientation of intercardinal sampling areas with respect to incident sunlight, thus maximizing the chance of observing temporal changes in psyllid localization within orchards. Selection of trees in NE, NW, SE, and SW sampling areas was based on right triangles with hypotenuses perpendicular to the average azimuth of the

sun during specific time-of-day and time-of-year intervals overlaid on satellite imagery (Google Earth 6.0.3.2197, Mountain View, CA) of each orchard. With each intercardinal sampling area's respective corner as an anchor, triangles were sized to encompass 30 trees along the orchard perimeter, with trees distributed according to the leg-length ratio of each triangle. The NE and NW sampling areas were based on average azimuths during the summer solstice. Sunrise and sunset time-of-day azimuths then were 72° and 288° , respectively, with true north = 0° . Triangles for NE and NW areas possessed a leg width-to-height ratio of 1:3.11 along their respective orchard corners. The SE and SW sampling areas were based on azimuths during the winter solstice. Sunrise and sunset time-of-day azimuths then were 128° and 232° , respectively. The sampling area of delimiting triangles had a leg width-to-height ratio of 1:1.28 along the respective orchard corners. Trees within cardinal sampling areas were selected from those spanning their respective orchard perimeters.

Stem-tap sampling, i.e., tapping a branch 3 times while holding a $44 \times 29 \times 8$ cm tray underneath to catch and count all adult psyllids (Hall and Hentz 2010; Qureshi et al. 2009), was used. One branch with a foliar flush (feather-flush when available) was sampled per tree. Thirty trees were sampled per orchard survey area (adapted from Sétamou et al. 2008). No attempt was made to sample the same shoots per tree per orchard sampling area during different sampling times of the day or year. The tree canopy quadrant sampled was selected arbitrarily as previous research (Dharajothi et al. 1989; Sétamou et al. 2008) and observations within the current study did not provide evidence for such a preference by *D. citri* adults. With the exception of the interior sampling area, sampling was conducted on trees from the 2 outer-most rows of perimeter sampling areas per orchard. Zigzag pattern sampling was employed, i.e., 1 tree was sampled from the outer-most row, the next in the adjacent, second out-most row within that area, and then alternating back to the outer-most row as sampling proceeded within an area. This reduced the likelihood of counting individual psyllids twice due to psyllids disturbed from a previous sampled tree landing on a shoot on the nearest adjacent tree. The order of surveying sampling areas was arbitrary, and individual surveys were conducted by an average of 3 trained samplers each dispatched to different areas of the orchard concurrently.

Survey data was collected from 4 orchards (A, B, C, and D) in St Lucie County, Florida, over the course of the survey (Table 1). In the first year of the survey, 3 orchards (A, B, and C) were sampled. At the conclusion of the first year of the survey, orchards B and C were abandoned by their managers and converted into cow pasture. Two orchards (A and D) were sampled in the second year of the survey. Orchards were sampled a minimum of 3 weeks post insecticide application to minimize adverse effects on psyllid counts while still allowing time for sampling when not restricted by inclement weather or impending insecticide applications.

Table 1
Sampled orchards.

Orchard	Planting date	Cultivar	Tree spaces	No. of blocks	Hectares
A	2006	Mixture of 'Valencia' orange scions on 'Carrizo' and 'Swingle' rootstocks	9408	2	34.84
B	1998	'Marsh' white grapefruit	5694	2	21.09
C	1960 to 1966	Mixture of 'Flame', 'Roble', and 'Marsh' grapefruit and 'Pineapple' orange	8844	3	49.87
D	2002	'Valencia' orange scions on 'Volk' rootstocks	10505	2	31.46

Size of voids (e.g., canals, roads, and vacant fields) adjacent to sampling areas in each orchard were classified according to the following criteria using Google Earth satellite imagery: none (0 m, interior sampling areas only), small (≤ 100 m), medium (100 to ≤ 300 m), and large (> 300 m). Void size intervals were empirically chosen to classify voids. Sampling areas adjacent to both small and large voids (e.g., intercardinal corners) were assigned a medium void size. Alternative classifications of sampling areas adjacent to multiple void classes did not affect fit of the model.

Weather data

To evaluate variation in *D. citri* counts explained by weather variables (incorporated as covariates or fixed effects), weather data was obtained from the Florida Automated Weather Network (FAWN, <http://fawn.ifas.ufl.edu/>) and Weather Underground (<http://www.wunderground.com/>). Examined variables included temperature, relative humidity, precipitation, wind speed (in addition to wind being expressed as area under the wind speed curve per direction), solar radiation, and pressure. Hourly weather data for each orchard sampling area was spatially interpolated via universal kriging according to an isotropic exponential variogram in PROC MIXED (SAS 9.3, Cary, NC) (Littell et al. 2007), factoring into account correlations with elevation above sea level, distance to the Atlantic Ocean, distance to Lake Okechobee, and distance to the nearest water body. Anisotropic relationships were examined but did not produce superior fits in the current study. Distances were calculated in ArcMap 10 (ESRI, Redlands, CA) using the World Water Bodies layer package (DeLorme Publishing Company Inc, Yarmouth, ME).

Data analysis

The GLIMMIX procedure of SAS was used to examine fits of generalized linear mixed models to the survey data. The best fitting model was a linear mixed model fit to (natural) $\log(\text{total } D. citri \text{ adults/sampling area} + 1)$, with fixed-effect factors time-of-year, sampling area, and the interaction thereof. Neither its simple effect nor interactions involving time-of-day were significant: $P > 0.6$; thus, time-of-day was excluded from the final model. Random effects in the model included a sub-plot (replication \times orchard \times time-of-year), block (replication \times orchard) (Littell et al. 2007), void size (void size \times

orchard \times sampling area), and a residual error term. The whole-plot error term (replication \times orchard \times time-of-year \times time-of-day) and the random effect of replication did not improve fit of the model to the data and were thus excluded. The Kenward-Roger method of determining denominator degrees-of-freedom (Kenward and Roger 1997; SAS Institute Inc 2011) was utilized due to the presence of missing data. During 2012 autumnal equinox sampling, biweekly insecticide application prevented sampling of orchard A. In 2012 and 2013, inclement weather interfered with the autumnal equinox sunset sampling of orchard B and the sunrise and sunset samplings of orchard D, respectively. Biweekly insecticide application during this time prevented sampling on subsequent days. Because of some missing data, significance of results was evaluated at a slightly less stringent cutoff ($\alpha = 0.075$). Among the interpolated weather variables, incorporation of wind speed and precipitation as covariates most improved the fit of the model to the data; however, this resulted in nonsensical predictions of negative amounts of *D. citri*; thus, an analysis excluding weather variables was used to examine survey data.

Linear mixed regression was used to examine the relationship between $\log(D. citri \text{ adults/sampling area} + 1)$ with (solar) elevation-corrected azimuths ($[90 - \text{elevation}]/90 \times \text{azimuth}$) (determined theoretically), which give less weight to azimuths during solar noon samplings (when sunlight comes primarily from above), during the time of sampling. The quadratic equation $y_{deijw} = b_1 z_{de} + b_2 z_{de}^2 + b_3 A_w + \beta + G(T)_{ij} + \varepsilon_{deijw}$ fit the data best, where y_{deijw} is $\log(D. citri \text{ adults/sampling area}_w + 1)$ at time-of-day d , time-of-year e , orchard i , and year j , b_1 , b_2 , and b_3 are parameters, z_{de} is elevation-corrected azimuth at time-of-day d and time-of-year e , A is sampling area w (numerically ordered as per Table 2 estimates), β is an intercept, $G(T)_{ij}$ is the random effect of orchard i within year j (with mean 0 and variance $s_{G(T)}^2$), and ε_{deijw} is the error associated with time-of-day d , time-of-year e , orchard i , year j , and sampling area w (with mean 0 and variance s_e^2). Model appropriateness and goodness of fit to the data were assessed using residual plots, fit diagnostics, robust pseudo- R^2 (via the ROBUSTREG procedure of SAS with the MM estimation method [Yohai 1987]), and concordance correlation coefficient (Lin 1989; Madden et al. 2007).

Table 2

Estimated abundance of $\log(\text{Diaphorina citri}$ adults/sampling area + 1) in orchard sampling areas.

Sampling area	Estimate (estimated standard error) ^a
N	2.1163 (0.4291) a
NE	1.9158 (0.4291) ab
E	1.6698 (0.4291) abc
NW	1.6000 (0.4291) abc
S	1.4632 (0.4291) bc
W	1.3427 (0.4291) c
SW	1.3317 (0.4291) c
SE	1.2762 (0.4291) c
I	1.2031 (0.4291) c

^a Estimates followed by different letters are significantly different ($\alpha = 0.075$) according to t tests of least squares means (BLUPs) performed utilizing the LINES options of PROC GLIMMIX in SAS, with denominator degrees of freedom determined according to the Kenward-Roger method (Kenward and Roger 1997; SAS Institute Inc, 2011).

Results

Time-of-year significantly affected *D. citri* counts ($P = 0.0518$) (Table 3), with *D. citri* adults most abundant during summer solstice (Fig. 2A–E). Estimates of *D. citri* counts were lowest during vernal equinox, with the exception of the S sampling area being slightly less during autumnal equinox (approximately 0.69 compared to approximately 0.78). Autumnal equinox samplings generally had more psyllid adults per sampling area than vernal equinox but generally less than winter solstice (Table 4). *D. citri* counts in N and I sampling areas during autumnal equinox only decreased slightly from levels during summer solstice samplings. Estimates of N and I counts (log-scale) decreased 0.77 and 0.51 ($P > 0.11$), respectively, whereas the average decrease for all other sampling areas during this time was approximately 1.41 ($P = 0.014$) (Fig. 2E). Similarly, while abundance of *D. citri* in other sampling areas generally increased (0.42, P

= 0.22) from autumnal equinox to winter solstice, N and I counts decreased (by 0.18 and 0.52, respectively), continuing the trend as from summer solstice to autumnal equinox.

Sampling area significantly affected *D. citri* counts ($P = 0.063$). The most psyllid adults were found in N sampling areas, while in NE, E, and NW sampling areas overall counts of *D. citri* adults were not significantly different from N sampling areas (Table 2). Collectively, *D. citri* was least prevalent in interior sampling areas (Fig. 2A–D, Table 2). An exception was during autumnal equinox when interior sampling areas contained the third most abundant counts of *D. citri* (Fig. 2C and E).

Plots of residuals indicated that the quadratic equation produced the most appropriate fit to the log psyllid abundance data. Fit diagnostics revealed the presence of 4 outliers; these values were not removed from the dataset since the regression method used was robust. Predicted log psyllid abundance exhibited a concave-up relationship with increasing elevation-corrected azimuths (Fig. 2F). Robust pseudo- R^2 (0.65) and concordance correlation coefficient (0.83) values reflected how well the model fit the general trend in log psyllid abundance with increasing elevation-corrected azimuth. Log psyllid abundance was predicted to be greatest at elevation-corrected azimuths corresponding to solar noon and sunset during summer solstice samplings, which exhibited moderate azimuths at high elevations and large azimuths at low elevations, respectively, at times of the year when *D. citri* adults were most abundant. Conversely, log psyllid abundance was predicted to be least at elevation-corrected azimuths corresponding to solar noon during winter solstice and sunrise during winter solstice, vernal equinox, and to some extent autumnal equinox. These times exhibited moderate azimuths at high elevations and lesser azimuths at lower elevations, respectively, at times of the year when *D. citri* adults are less abundant. Parameter estimates are in Table 5.

Table 3

Type III tests of fixed effects and covariance parameter estimates for the model describing $\log(\text{Diaphorina citri}$ adults/sampling area + 1).

Effect	F value	P value
Time-of-year	3.60	0.0518
Sampling area	2.26	0.0630
Time-of-year × sampling area	1.42	0.0907

Covariance parameter	Estimate (estimated standard error)
Sub-plot (replication × orchard × time-of-year)	0.5371 (0.2474)
Block (orchard × replication)	0.5512 (0.5103)
Void size (void size × orchard × sampling area)	0.1175 (0.05065)
Residual	0.5775 (0.04122)

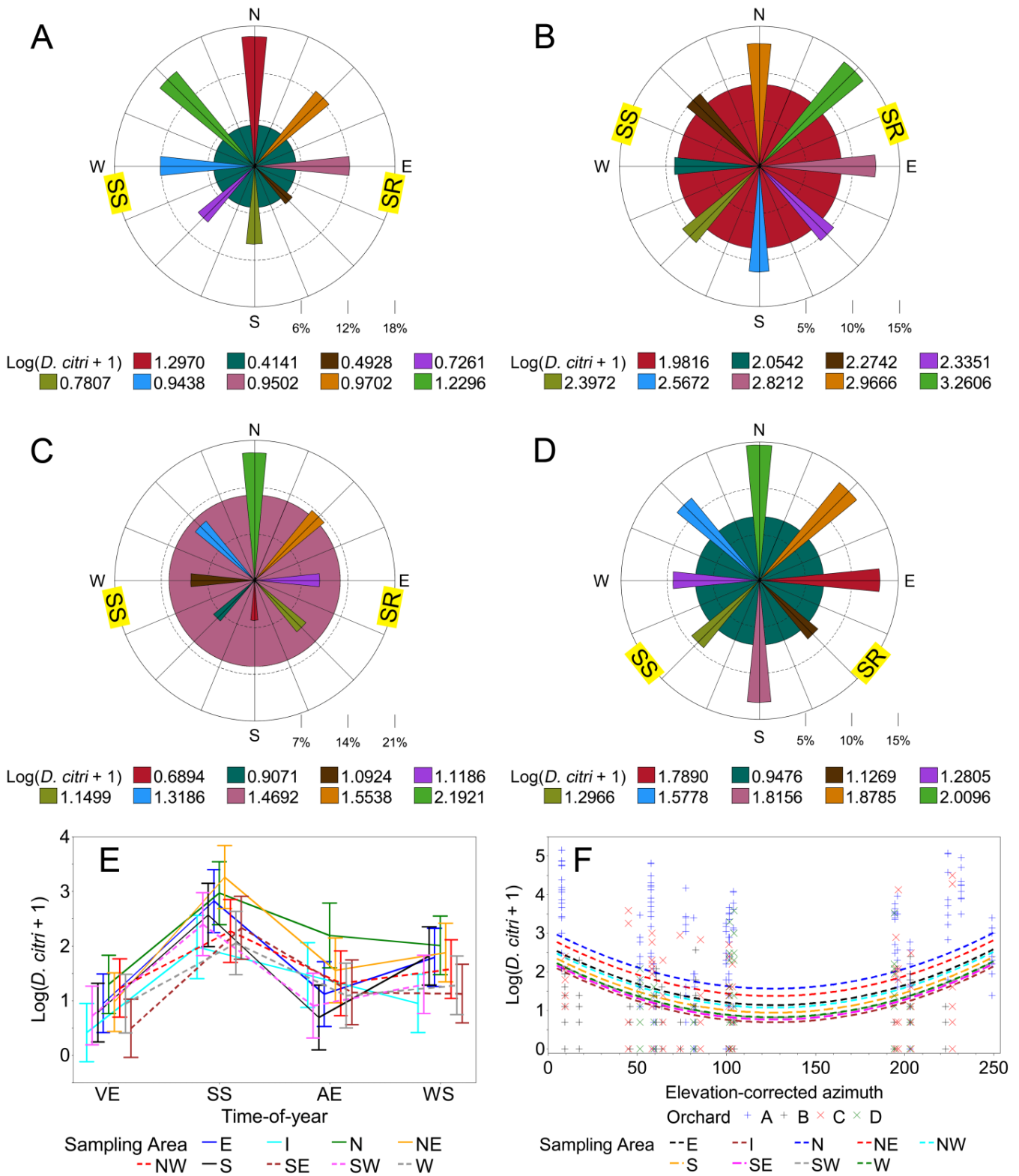


Fig 2. Abundance of *Diaphorina citri* adults (as $\log(D. citri/\text{sampling area} + 1)$) in orchard sampling areas during (A) vernal equinox (VE), (B) summer solstice (SS), (C) autumnal equinox (AE), and (D) winter solstice (WS). The radius of each plot's center-filled circle represents psyllid abundance of the interior sampling area. SR and SS (highlighted in yellow) respectively represent midpoint azimuths of the sun during sunrise and sunset time-of-day samplings per time-of-year sampling. Percentages reflect the percent of total *D. citri* adults represented by each concentric reference ring. (E) Line graph of $\log(D. citri + 1)$ per sampling area in relation to time-of-year; error bars are standard errors of the mean of two replications. Sampling areas were slightly offset with respect to their position on the horizontal axis to improve visibility. (F) $\log(D. citri + 1)$ per sampling area observed (symbols) and predicted (plotted lines) according to the fitted quadratic model.

Table 4

Estimated abundance of $\log(\text{Diaphorina citri}$ adults/sampling area + 1) in sampled orchards according to time-of-year sampling period.

Time-of-year sampling period	Estimate (estimated standard error) ^a
Summer solstice	2.5175 (0.5133) a
Winter solstice	1.5247 (0.4750) ab
Autumnal equinox	1.2768 (0.5166) b
Vernal equinox	0.8672 (0.4750) b

^a Estimates followed by different letters are significantly different ($\alpha = 0.075$) according to t tests of least squares means (BLUPs) performed utilizing the LINES options of PROC GLMMIX in SAS, with denominator degrees of freedom determined according to the Kenward-Roger method (Kenward and Roger, 1997; SAS Institute Inc, 2011).

Table 5

Parameter estimates for the linear mixed regression model describing the relationship between $\log(\text{Diaphorina citri}$ adults/sampling area + 1) and elevation-corrected azimuth.

Parameter	Estimate (estimated standard)	t value	P value
b_1	-0.02401 (0.01005)	-2.39	0.0207
b_2	0.000095 (0.000038)	2.48	0.0163
b_3	0.9551 (0.1266)	7.55	<0.0001
β	1.0604 (0.5526)	1.92	0.0606
$s_{G(T)}^2$	1.1622 (0.2419)	4.80	<0.0001
s_e^2	0.6370 (0.04417)	7.55	<0.0001

Discussion

In the current study, *D. citri* abundance fluctuated in a generally consistent manner over the course of each year. Peak abundance was observed during summer solstice (mid-May to the end of July), with winter solstice (mid-November to the end of January) generally yielding the next most abundant counts of *D. citri* adults. Previous studies reported peak abundance of adult *D. citri* in Florida citrus orchards during summer solstice (May [Hall et al. 2008], June and July [Stansly et al. 2014; Tsai et al. 2002]). Smaller peaks were inconsistently detected in December (Stansly et al. 2014), January (Hall et al. 2008), or September (Stansly et al. 2014; Tsai et al. 2002). Populations of adult psyllids on citrus honey mandarin (*C. aurantium* L.) in Southwest Sarawak, Malaysia, were observed to peak in April through May or August through September, with populations remaining elevated from May to August in 1 of 3 years (Teck et al. 2011). Hall et al.'s study (2008) reported that flush availability was not a strong indicator of the abundance of adult *D. citri*. This agrees with observations from the current study. While typically psyllid adults were found on young flush, it was not uncommon for them to also be present on mature leaves.

A previous study found a negative linear relationship ($R^2 = 0.46$) between the difference in *D. citri* abundance in east versus west orchard sampling areas in Florida and average solar azimuth 1 hour after sunrise per sampling cycle (Luo, personal communication). This was not, however, observed in the current study when sunrise samplings were examined alone ($P = 0.333$, $R^2 = 0.06$) or with all time-of-day samplings ($P = 0.407$, $R^2 = 0.01$); analogous results were obtained when differences were regressed against elevation-corrected azimuths or when similar opposing sampling areas were compared, e.g., differences between NE and SW were weakly explained by azimuth or elevation-corrected azimuth values. The prior study also consistently observed greater adult psyllid abundance in east sampling areas compared to west sampling areas, when averaged over all orchards for each of ten sampling cycles throughout the year. While this was more frequently the case in the current study as well, more adult *D. citri* were occasionally observed in W (or SW) compared to E (or NE) sampling areas. The prior study was based on survey results from approximately 5000 blocks within a single year, whereas the current study surveyed four orchards over 2 years; thus, it is plausible that differences in sampling scale contributed to the contrasting results. For instance, orchards sampled in the current study ranged in age from 6 to 53 years old, and did not include very young orchards ≤ 2 years old. As tree canopies develop, the number of branches and flushes increase, assuming the trees are healthy and conditions are conducive for flushing. Conceivably, these factors could retard psyllid penetration into the interior of an orchard by creating visual and physical barriers. Increased canopy coverage decreases wind turbulence, which would otherwise facilitate psyllid movement, and it also increases the number of psyllid feeding and oviposition sites at the perimeter of the orchard. Thus, it is entirely possible that localization of *D. citri* might significantly differ in young orchards, which have less canopy volume than older orchards. While these factors would likely decrease the difference in number of adult psyllids in exterior vs. interior sampling locations, they might also influence orchard-localization of adult psyllids and the azimuth of the sun, as decreased canopy coverage would allow for greater light penetration into an orchard. Further research would be needed to explicitly address these possibilities.

On a scale smaller than that of an orchard, changes in canopy localization in relation to time-of-day (Prokopy et al. 1972; Yee 2002) and time-of-year (Stork et al. 2001) have been reported for various insects. During morning and evening hours, the majority of *R. pomonella* (apple maggot flies) observed were in areas of the tree that were most illuminated, while during afternoon hours in the heat of the day most flies were in shade and uniformly distributed with respect to cardinal direction (Prokopy et al. 1972). In June and July, *R. indifferens* (western cherry fruit fly) was reported to be significantly more abundant in east-facing cherry canopy quadrants before 9:00 AM and then more abundant in west- and south-facing

quadrants between 1:00 and 3:00 PM (Yee 2002). In our survey, the most *D. citri* adults were observed in the sampling areas with the most northeasterly exposure. This corresponded to orchard areas receiving the most sunlight during sunrise within summer solstice periods. However, the orientation of sampling areas with highest abundance of *D. citri* adults did not significantly change over daily or seasonal time scales. While relative differences in counts of psyllid adults within orchard sampling areas were observed over time (Fig. 2E), the lack of an overall significant interaction between temporally changing sunlight direction (daily or seasonally) and localized abundance of *D. citri* on an orchard scale could be due to several factors. *D. citri* is a weak flyer (Aubert and Hua 1990; Ke 1991), and behavioral migratory movement of insects is rare (Loxdale and Lushai 1999). Furthermore, particularly with respect to different times of the day, movement of psyllids to common orchard locations would increase competition for feeding and oviposition sites, which without other benefits would promote dispersal elsewhere. Nevertheless, changes in *D. citri* populations are plausible, as movement between unmanaged and managed orchards of marked *D. citri* adults has been reported for distances of 60 to 100 m over a 3 day period (Boina et al. 2009) and 400 m and 2000 m over 4 and 12 day periods, respectively (Lewis-Rosenblum 2011). It is also conceivable that if similar studies were conducted in areas with lower populations of *D. citri* than St Lucie County, Florida, alternative outcomes might be observed (e.g., overall directional prevalence within orchards may be shifted).

In contrast to a laboratory environment where light sources can be controlled, an orchard experiences light scattering when sunlight from a particular angle is refracted through the air and reflected from surfaces of a citrus tree at various angles. Plausibly more important is the lack of factor isolation in an orchard, i.e., while *D. citri* may demonstrate significant phototropism on a small scale, in natural environments many additional factors may influence behavior (Sétamou et al. 2012). Air movement, microclimates within the orchard, and nutrient content and relative health of citrus trees (Basset 1992) may also contribute to localized abundance of *D. citri*. It is also possible that application of fertilizer within sampled orchards diminishes variation in light-influenced spatial distribution of nitrogen leaf content (Niinemets 1997), an important factor for phloem-feeding insects (Basset 1992) such as *D. citri*.

The relatively limited sampling over 2 seasonal periods may have reduced the likelihood for detection of temporal changes in localization of *D. citri* adults. The lack of detection could also be attributed to insecticide sprays in sampled orchards. Even though a 3-week-post insecticide sampling buffer was used, residual levels of insecticides might have altered psyllid behavior. To test for this interaction it would be necessary to compare unmanaged and managed orchards of similar health.

Nevertheless, under practical management of orchards the overall observation of populations of psyllids in all

sampling areas during all sampling periods suggests that on the scale examined, orchards in areas with endemic levels of *D. citri* require complete insecticide spray coverage to reduce vector transmission of *Candidatus Liberibacter asiaticus*. With that being said, future studies should be conducted to examine the economics of variable insecticide spray programs (e.g., perimeter versus whole orchard), rate of *Candidatus Liberibacter asiaticus* transmission in differentially treated orchards, and the productive lifespan of those trees. Without further studies, the results of the current study alone are not sufficient to support recommendations of alternative insecticide spray programs.

In this study, sampling areas were selected with respect to the edge of orchards (perimeters of joined adjacent blocks). North-south roads, east-west roads, and blocks devoid of citrus trees separated the majority of orchard edges from the nearest source of citrus trees. The smallest voids (irrigation canals) separated the southern edges of orchards A and D from neighboring blocks by approximately 22 m. Voids of this size have been reported to have an edge effect with respect to significantly greater amounts of huanglongbing disease incidence, and by extension, increased *D. citri* feeding pressure, compared to blocks with no edges (other than small internal plantation voids ≤ 12 m across) (Luo et al. 2012). This differentiates those blocks as distinct and alleviates the concern that neighboring blocks were too close for survey results from those edges being representative of edges, as opposed to interior orchard areas which have been known to host lower populations of *D. citri* than orchard perimeters (Boina et al. 2009; Gottwald et al. 2008). Counts of adult *D. citri* at those sampling areas would thus not have been anticipated to be consistently less than those from perimeter sampling areas otherwise unbounded as such.

The results of this research add to the knowledge of the temporally dependent phototropism of *D. citri* adults at an orchard scale in Florida, where populations of *D. citri* are endemic. While changes over time frames examined in localized abundance of psyllids were not observed, overall the majority of *D. citri* adults were in the 3 northeasterly sampling areas (N, NE, and E) of the orchards. The reason for this relationship, however, remains the subject of further experimentation. Based solely on generated results, changes in insecticide application routines could not be recommended with respect to the time of day or season. However, since surveys in the current study were conducted 3 weeks post insecticide application, it is also plausible that populations of *D. citri* adults might be spatially variable during periods less than 3 weeks post insecticide application in a manner that would benefit from spatially-variable insecticide applications, as Sétamou and Bartels (2015) have demonstrated in Texas. Separate studies would need to be conducted to determine if the addition of this further temporal component of survey time post insecticide application would reveal an analogous relationship in Florida as has been reported from Texas (Sétamou and

Bartels 2015), which could potentially advocate for perimeter sprays over some full orchard sprays for areas with lower adult psyllid populations.

This study additionally established a significant relationship between log psyllid abundance and elevation-corrected azimuth. Negative weighting of azimuth according to increasing solar elevation had the greatest effect on values representative of times near solar noon, when the sun is most overhead. Such weighting reduces the influence of horizontal azimuth angles at times near solar noon. The practical outcome of this was largely based around log psyllid values near the horizontal center of a plotted response surface in relation to azimuth values being shifted farther to the left of log psyllid counts from other sampling times in a corresponding elevation-corrected azimuth plot. This allowed an otherwise obscured relationship to be revealed. In essence, this relationship between log psyllid counts and elevation-corrected azimuth reflected how the greatest number of psyllids were observed during the summertime, which corresponds to the lowest (near sunrise) and greatest (near sunset) azimuth values observed. Corresponding data in Table 4 regarding overall abundance of psyllids in relation to time-of-year supports this relationship. As orchards were only sampled within Florida in the northern hemisphere, a similar mirror-image across an equatorial axis (i.e., NE sampling areas and azimuths would correspond to those of the SE) relationship might be anticipated to be exhibited in southern hemisphere orchards, barring confounding factors. Further research, however, would be needed to validate this.

Acknowledgments

Salaries and research support were provided by USDA ARS and NCSU and were contributed by a USDA APHIS Farm Bill grant on Huanglongbing. We thank F Albano, G Brock, J Hodge, T Riley, L Therrien, and DR Wood for their contributions. Special thanks to B Teal and W Spaulding for providing orchard survey sites. Additional thanks to 2 anonymous reviewers for their scrutiny.

References

- Aubert B, Hua XY. 1990. Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies. In: Aubert B, Tontyaporn S, Buangsuwon D, editors. Rehabilitation of citrus industry in the Asia Pacific Region. Proceedings of the 4th International Asia Pacific Conference on Citrus Rehabilitation; Chiang Mai, Thailand. p. 181-187.
- Basset Y. 1992. Influence of leaf traits on the spatial distribution of arboreal arthropods within an overstorey rainforest tree. *Ecol Entomol.* 17:8-16.
- Boina DR, Meyer WL, Onagbola EO, Stelinski LL. 2009. Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged orchards on commercial citrus management. *Environ Entomol.* 38:1250-1258.
- Bové JM. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J Plant Pathol.* 88:7-37.
- Dharajothi B, Verghese A, Tandon PL. 1989. Ecological studies on citrus psylla, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) with special reference to its spatial distribution and sampling plan. *Entomon.* 14:319-324.
- Gottwald TR. 2010. Current epidemiological understanding of citrus Huanglongbing. *Annu Rev Phytopathol.* 48:119-139.
- Gottwald T, Ireya M, Gast T. 2008. The plantation edge effect of HLB – a geostatistical analysis. In: Gottwald TR, Graham JH, editors. Proceedings of the International Research Conference on Huanglongbing; Orlando, Florida. p. 305-308.
- Hall DG, Hentz MG. 2010. Sticky trap and stem-tap sampling protocols for the Asian citrus psyllid (Hemiptera: Psyllidae). *J Econ Entomol.* 103(2):541-549.
- Hall DG, Hentz MG, Adair RC Jr. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus orchards. *Environ Entomol.* 37:914-924.
- Ke C. 1991. The present status of citrus Huanglongbing and its control in China. In: Ke C, Osman SB, editors. Rehabilitation of citrus industry in the Asia Pacific Region. Proceedings of the 6th International Asia Pacific Workshop on Integrated Citrus Health Management; Kuala Lumpur, Malaysia. p. 24-30.
- Kenward MG, Roger JH. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics.* 53:983-997.
- Lewis-Rosenblum H. 2011. Seasonal and long-range movement of Asian citrus psyllid, *Diaphorina citri* [master's thesis]. [Gainesville (FL)]: University of Florida.
- Lin LI-K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics.* 45:255-268.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. 2007. SAS system for mixed models. 2nd ed. Cary (NC): SAS Institute Inc.
- Loxdale HD, Lushai G. 1999. Slaves of the environment: the movement of herbaceous insects in relation to their ecology and genotype. *Philos Trans R Soc Lond B Sci.* 354:1479-1495.
- Luo W, Gottwald T, Ireya MS. 2012. Assessments of edge effect in intensity of HLB disease. *Phytopathology.* 102(S4):73.
- Madden LV, Hughes G, van den Bosch F. 2007. The study of plant disease epidemics. St Paul (MN): American Phytopathological Society.
- McClellan APD, Schwarz RE. 1970. Greening or blotchy-mottle disease of citrus. *Phytophylactica.* 2:177-194.
- Niinemetts U. 1997. Distribution patterns of foliar carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea apies*. *Trees.* 11:144-154.

- Prokopy RJ, Bennett EW, Bush GL. 1972. Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae). Can Ent. 104:97-104.
- Qureshi JA, Rodgers ME, Hall DG, Stansly PA. 2009. Incidence of invasive *Diaphorina citri* (Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. J Econ Entomol. 102(1):247-256.
- SAS Institute Inc. 2011. SAS/STAT® 9.3 user's guide. Cary (NC): SAS Institute Inc.
- Sétamou M, Bartels DW. Forthcoming 2015. Edge effects in the spatial distribution of Asian citrus psyllid in citrus groves: Consequence for the development of best management practices. Proceedings of the 4th International Research Conference on Huanglongbing.
- Sétamou M, Flores D, French JV, Hall DG. 2008. Dispersion patterns and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus. J Econ Entomol. 101(4):1478-1487.
- Sétamou M, Sanchez A, Patt JM, Nelson SD, Jifon J, Louzada ES. 2012. Diurnal patterns of flight activity and effects of light on host finding behavior of the Asian citrus psyllid. J Insect Behav. 25:264-276.
- Stansly PA, Arevalo HA, Qureshi JA, Jones MM, Hendricks K, Roberts PD, Roka FM. 2014. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida orchards affected by huanglongbing. Pest Manag Sci. 70:415-426.
- Stork NE, Hammond PM, Russell BL, Hadwen WL. 2001. The spatial distribution of beetles within the canopies of oak trees in Richmond Park, U.K. Ecol Entomol. 26:302-311.
- Teck SLC, Fatimah A, Beattie A, Heng RKJ, King WS. 2011. Seasonal population dynamics of the Asian citrus psyllid, *Diaphorina citri* Kuwayama in Sarawak. Am J Agric Biol Sci. 6:527-535.
- Tsai JH, Wang J-J, Liu Y-H. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla Entomol. 85:446-451.
- Xu S, Wang Z, Zhang W. 1994. Studies on the occurrence and control of citrus psyllid, *Diaphorina citri* Kuwayama in Yunnan Province. Zhejiang Citrus. 1:32-33.
- Yang Y, Huang M, Beattie GAC, Xia Y, Ouyang G, Xiong J. 2006. Distribution, biology, ecology and control of the psyllid *Diaphorina citri* Kuwayama, a major pest of citrus: A status report for China. Int J Pest Manage. 52:343-352.
- Yee WL. 2002. Daily and seasonal activity patterns of *Rhagoletis indifferens* (Diptera: Tephritidae) in Washington state. Ann Entomol Soc Am. 95:701-710.
- Yohai VJ. 1987. High breakdown point and high efficiency robust estimates for regression. Ann Stat. 15:642-656.