

Abdominal Color of the Asian Citrus Psyllid (Hemiptera: Liviidae) Is Associated With Flight Capabilities

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Ann. Entomol. Soc. Am. 107(4): 842–847 (2014); DOI: <http://dx.doi.org/10.1603/AN14028>

ABSTRACT We examined the propensity for flight initiation and flight capability of the Asian citrus psyllid (*Diaphorina citri* Kuwayama, Hemiptera: Liviidae) with a flight mill. We measured continuous flight by *D. citri* for up to 3 h, which equated to a distance of ≈ 2.4 km. We compared the flight capability of *D. citri* depending on their sex and morphotype (color of abdomen). Two general morphotypes exist within this species: green–blue and gray–brown. We observed that $\approx 32\%$ of psyllids from the green–blue morphotype tested exhibited long durations of flight (>60 s); whereas $<5\%$ of psyllids from the gray–brown morphotype performed such long duration flights. There was no significant difference in flight performance between the two sexes within both the gray–brown and green–blue morphotypes. Furthermore, psyllids from the gray–brown morphotype were characterized by smaller pronotums and shorter wings than psyllids from the green–blue morphotype. In addition, males, in general, were characterized by smaller pronotums and shorter wings than females. However, neither pronotum nor wing size were associated with flight capability of *D. citri*. Our results may help explain dispersal behavior of *D. citri* with respect to practical management on an area-wide scale.

KEY WORDS color morph, flight mill, Psylloidea, insect movement, insect coloration

In the context of a vector-borne pathogen system, the spread of disease depends on movement of vectors across a landscape (Finke 2012). Indeed, vector dispersal is essential for pathogens to encounter new hosts to spread infection. The degree of vector dispersal is often associated with the level of spread of plant–pathogen mediated diseases (Bailey et al. 1995, Christiansen-Weniger et al. 1998, Hodge and Powell 2008). Consequently, an understanding of vector dispersal potential is useful for implementing efficient control tactics for vector-transmitted pathogens.

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of several bacterial pathogens including ‘*Candidatus* Liberibacter asiaticus’ (Las). Las is the agent presumably causing the citrus disease huanglongbing (HLB), also known as citrus greening in the United States (Grafton-Cardwell et al. 2013). HLB-infected trees have a shortened lifespan; fruit from infected trees are smaller than from healthy trees, remain green in color, often drop prematurely, and are characterized by undesirable organoleptic properties (Wang and Trivedi 2013). HLB is considered the most important threat to sustainable citrus production in the world (Bové 2006). In Florida, *D. citri* was initially observed in 1998 (Halbert 1998), and has since spread throughout all of citrus production areas in the state. It is estimated that

HLB reduced orange production by $\approx 24\%$ in Florida in 2011 (Hodges and Spreen 2012). To date, all known commercial citrus species are susceptible to HLB infection regardless of rootstock (Bové 2006). The maximum flight distance of *D. citri* needs to be estimated to identify safe isolation and quarantine boundaries, as well as to establish effective area-wide control protocols (Halbert and Manjunath 2004).

It has been previously shown that *D. citri* migrates actively between citrus groves, notably from unmanaged (abandoned) to managed groves (Boina et al. 2009, Tiwari et al. 2010). In addition, *D. citri* were found within a dense forest in Florida located 2 km away from the closest existing citrus grove (Martini et al. 2013). These field data indicate that *D. citri* has the ability to migrate long distances and through challenging environments. Migrations farther than 2 m from citrus trees by *D. citri* mostly occur during spring and summer (Hall and Hentz 2011). Under laboratory conditions, Arakawa and Miyamoto (2007) used a flight mill to investigate the flight capability of *D. citri*. Flight capability was found to be equivalent between males and females, and maximal flight capability occurred at 4 d after adult emergence (Arakawa and Miyamoto 2007).

There are three morphotypes of *D. citri* that have been characterized based on differences in abdominal color: gray–brown, green–blue, and orange–yellow (Skelley and Hoy 2004, Wenninger and Hall 2008).

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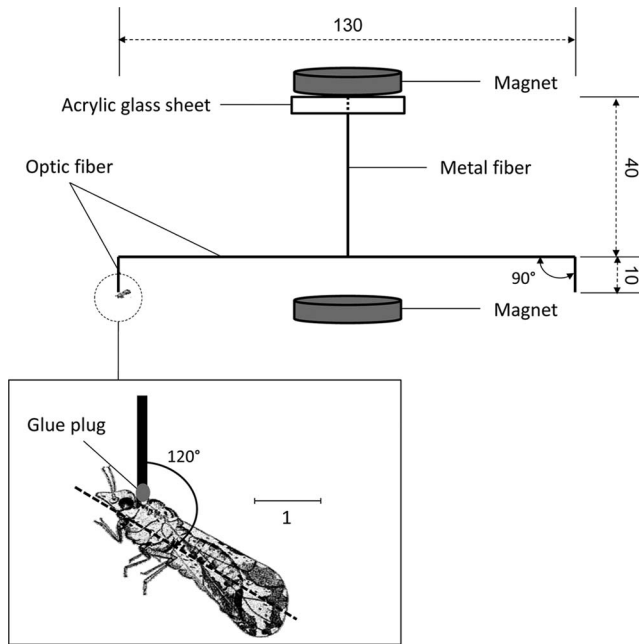


Fig. 1. Schematic diagram of the flight mill apparatus. The measurement in the diagram is presented in millimeters.

Only older (>30 d after adult emergence) males, and females with eggs may have orange–yellow abdomens (Wenninger and Hall 2008). Therefore, the orange–yellow morphotype is sometimes excluded from experiments investigating biological differences in *D. citri* based on abdominal color, given its apparent plasticity (example: Wenninger et al. 2009). Overall, green–blue morphs are characterized by greater fitness, greater body mass, and higher fecundity than gray–brown morphs (Wenninger and Hall 2008, Wenninger et al. 2009). Also, green–blue *D. citri* females are more attractive to males of either morph than gray–brown females (Wenninger et al. 2009). Differences in insecticide susceptibility between color morphs of *D. citri* have also been noted with the orange–yellow morph exhibiting highest susceptibility (Tiwari et al. 2013). It has not yet been fully confirmed whether color polymorphism in *D. citri* is completely plastic or in some way genetically predetermined; however, it has been suggested that biotic factors such as host plant quality or intraspecific competition may play roles in determination of morphotype (Wenninger and Hall 2008).

The objective of this investigation was to explore the flight capability of *D. citri* among psyllid color morphotypes. We conducted a study using a flight mill modified from Arakawa and Miyamoto (2007). We compared flight capability of *D. citri* depending of their abdominal color and sex. We also measured wing length and pronotum width to test for a possible correlation between these morphometric data and flight capability of *D. citri*.

Materials and Methods

Insect Rearing. Adult *D. citri* used in behavioral bioassays were obtained from a laboratory culture maintained at the University of Florida, Citrus Research and Education Center (Lake Alfred, FL). The culture was established in 2000 from field populations collected in Polk Co., FL (28.0° N, 81.9° W) before the discovery of HLB in Florida. The culture was maintained without exposure to insecticides on curry leaves (*Berbera koenigii* L.) in an air-conditioned greenhouse at 27–28°C, 60–65% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Illumination in the greenhouse was supplemented with linear fluorescent 54 W lights (F54W/T5/865/ECO, GE lighting, Nela Park, OH). Monthly testing of randomly sampled nymphs, and adults by quantitative PCR assays was conducted to confirm that psyllids and plants in this culture were free of Las. It is known that *D. citri* reach their maximal flight capability at 4 d after emergence (Arakawa and Miyamoto 2007). Given that *D. citri* have a lifespan of ≈40 d at 25°C after adult emergence (Tsai and Liu 2000), we elected to perform behavioral assays using psyllids aged between 5 and 15 d after adult emergence. To obtain psyllid between 5 and 15 d old, four *B. koenigii* were infested with ≈100 unsexed *D. citri* adults. After 1 wk, all of these adults were removed. Thereafter, the cage was inspected daily and the adults that emerged were transferred to a separate plant free of *D. citri*. The day of the transfer was noted and these psyllids were used 5–15 d after their transfer. The experiments were performed between the 5 September 2013 and 27 November 2013.

Table 1. Percentage of *D. citri* adults tested in the flight mill that did not fly ("nonflyer"); that flew for a short period of time ("short flyer," < 60 s); or that flew for a long period of time ("long flyer," > 60 s)

| Abdominal color | Sex | Nonflyer (%) | Short flyer (%) | Long flyer (%) | N ^a | Avg. flight (s) ^b | Max flight (s) |
|-----------------|--------|---------------------------------|--------------------|--------------------|----------------|------------------------------|----------------|
| Green-blue | Male | 27.27 ^a _c | 36.36 ^a | 36.36 ^a | 22 | 195.4 ± 91 | 1,313 |
| Green-blue | Female | 18.18 ^a | 54.55 ^a | 27.27 ^a | 22 | 864.5 ± 602 | 10,800 |
| Gray-brown | Male | 52.63 ^a | 47.37 ^a | 0.00 ^b | 19 | 19.00 ± 5 | 47 |
| Gray-brown | Female | 28.57 ^a | 66.67 ^a | 4.76 ^b | 21 | 17.06 ± 5 | 65 |

D. citri flight capability was compared depending on their abdominal color and sex. Average (±SE) and max flight duration recorded for each category is also provided.

^a Number of psyllids tested on the flight mill for each category.

^b Average time that the psyllids flew for each category; nonflyers were excluded here.

^c Percentages followed by different letters indicate significant differences ($\alpha < 0.05$) between cells within the same column.

Flight Mill Apparatus. A schematic diagram of the flight mill is provided in Fig. 1. The flight mill was composed of a 13-cm optic fiber horizontal axis, fixed to a pivot consisting of a metal fiber. The pivot was positioned vertically under a magnet. To prevent the fiber from flipping horizontally, the metal fiber was inserted through a pierced acrylic glass sheet. In addition, to maintain the metal fiber in a vertical orientation, a second magnet was positioned 1 cm below. At each extremity of the horizontal optic fiber, two smaller pieces of optic fiber were glued in a vertical orientation. The psyllids were affixed to the tip of one of these small optic fiber pieces. The other piece of optic fiber was present to maintain effective balance of the flight mill. All the experiments were performed in an air-conditioned room between 0900 and 1400 hours at $27 \pm 1^\circ\text{C}$ and $40 \pm 10\%$ RH.

Flight Mill Procedure. Five- to 15-d-old *D. citri* adults were placed onto an ice-pack covered with filter paper. While immobilized on the ice pack, psyllids were attached to the tip of a 10-mm-length optic fiber with a minute amount of nontoxic, washable glue (Elmer's products, Columbus, OH) via the pronotum (Fig. 1). Thereafter, the optic fiber was glued to the horizontal axis of the flight mill and the behavioral assay was initiated. If a psyllid did not fly during the first 10 min after attachment, it was removed and discarded. Preliminary tests showed that a psyllid that did not fly during the first 10 min would not fly for at least the next hour. Assays were terminated 5 min after psyllids ceased flight activity. For psyllids that initiated flight, duration of flight and the number of rotations on the flight mill were recorded. The distance (in cm) flown by each psyllid was calculated by multiplying the number of rotations by 13π . After each assay, the sex and abdominal color of each psyllid tested were determined. In addition, we measured pronotum width and wing length of a subsample of *D. citri* tested on the flight mill. These were measured with a microruler inserted into the lens of a dissecting microscope (Wild M3C, Leica, Wetzlar, Germany).

Statistical Analysis. The psyllids used on the flight mill were classified into three categories: 1) psyllids that did not fly during the experiment were categorized as "nonflyers;" 2) psyllids that flew <60 s were categorized as "short flyers;" and 3) psyllids that flew ≥ 60 s were categorized as "long flyers." All statistical

analyses were conducted with the statistical software R v3.02 (The R Foundation for Statistical Computing, Vienna, Austria). We first compared the proportion of nonflyers, short flyers, and long flyers as a function of sex and abdominal color with a generalized linear model with binomial distribution. Sex and abdominal color were used as fixed variables. We subsequently assessed the effect of psyllid age on the proportion of long flyers with a different generalized linear model, where only age of the psyllids was used as a fixed variable. Gray-brown psyllids were excluded from this analysis because only a single gray-brown morphotype psyllid flew >60 s (see the result section). We also compared the duration of flight and flight speed by long flyers as function of sex using a Mann-Whitney rank sum test. Finally, we compared effects of psyllid age on flight duration and flight speed with linear regressions. Gray-brown psyllids were again excluded from these analyses. Pronotum width and wing length data were analyzed using a two-way analysis of variance (ANOVA) with sex and abdominal color as fixed variables. After excluding gray-brown morphotypes, we performed a two-way ANOVA with sex and flyer categories as fixed variables. To obtain a normalized distribution, we removed one outlier and log transformed the pronotum width data set.

Results

The proportion of green-blue morph *D. citri* adults that performed a long flight (>60 s) was significantly greater than of gray-brown morphs (Table 1; $\chi^2 = 14.39$; $df = 1$; $P < 0.001$). The proportion of long flyers was not different as a function of sex (Table 1; $\chi^2 = 0.15$; $df = 1$; $P = 0.700$), or age ($\chi^2 = 0.53$; $df = 1$; $P = 0.466$). The average duration of flight for long flyers was $1,300 \pm 752$ s, with an average distance of 320.5 ± 209.5 m and a speed of 0.630 ± 0.06 km/h. The maximal flight obtained was with a 10-d-old green-blue female that flew for 3 h, which equated to ≈ 2.4 km. There was no difference in flight speed (Mann-Whitney rank sum test: $U = 18$; $n = 14$; $P = 0.491$) and flight duration (Mann-Whitney rank sum test: $U = 11$; $n = 14$; $P = 0.108$) for long flyers with respect to sex. Also, psyllid age was not correlated with either flight speed ($n = 14$; $F = 1.470$; $P = 0.249$) or flight duration ($n = 14$; $F = 0.009$; $P = 0.923$).

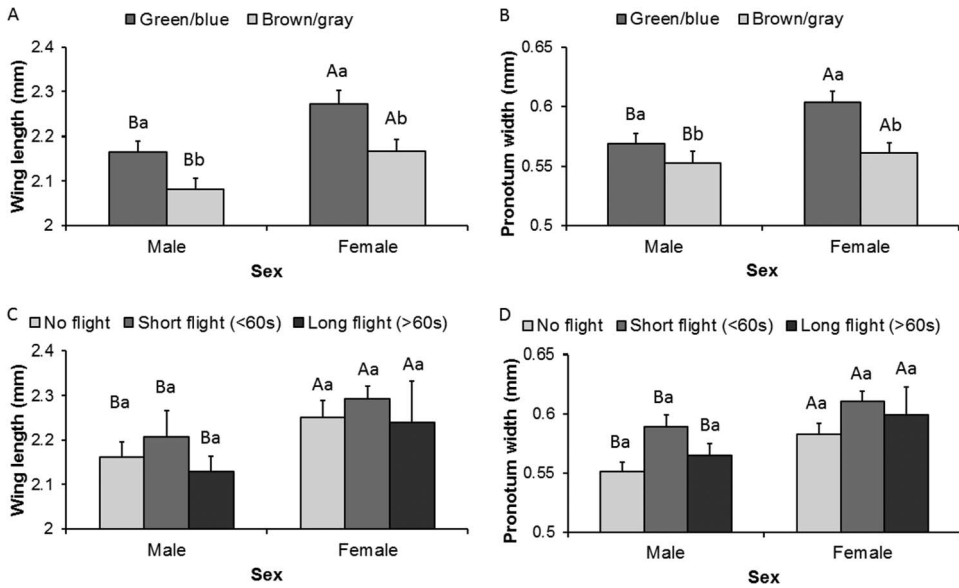


Fig. 2. Average (\pm SE) wing length (A) and pronotum width (B) of *D. citri* depending of the abdominal color and the sex. Average (\pm SE) wing length (C) and pronotum width (D) of *D. citri* depending of the flight performance on the flight mill. For (C) and (D), gray-brown morphotypes were excluded because of the low number of long flyers within this morphotype. Bars labeled by different capital letters within sex are significantly different at the $\alpha < 0.05$ level. Bars labeled by different lower case letters within abdominal color (A and B) or within flight performance (C and D) are significantly different at the $\alpha < 0.05$ level.

Regarding biometric data, the length of male wings (Fig. 2A; $F = 12.00$; $df = 1, 60$; $P < 0.001$) was shorter than that of females. Also, wings of gray-brown morphs (Fig. 2A; $F = 12.15$; $df = 1, 60$; $P < 0.001$) were shorter than that of green-blue morphs. We found no interaction between sex and abdominal color regarding wing length (Fig. 2A; $F = 0.17$; $df = 1, 60$; $P = 0.680$). We also found that the pronotum of males was smaller than that of females (Fig. 2B and $F = 5.80$; $df = 1, 59$; $P = 0.019$). Also, pronotum size of gray-brown morphs (Fig. 2B; $F = 11.11$; $df = 1, 59$; $P = 0.001$) was smaller than that of green-blue morphs. We found no interaction between sex and abdominal color regarding pronotum width (Fig. 2B; $F = 1.84$; $df = 1, 59$; $P = 0.180$). Finally, after excluding gray-brown morphs, neither wing length (Fig. 2C; $F = 1.10$; $df = 2, 28$; $P = 0.346$) nor pronotum width (Fig. 2D; $F = 2.08$; $df = 2, 27$; $P = 0.145$) was significantly different between nonflyers, short flyers, and long flyers.

Discussion

In the current investigation, we found that variation in abdominal color of *D. citri* morphotypes is related to flight capability of both male and female psyllids. For both sexes, gray-brown individuals were characterized by lower flight capability than green-blue psyllids. Predominantly green-blue morph *D. citri* performed long distance (>60 s) flights; however, there was no difference in flight capacity between the sexes within this morphotype. Our results were congruent with those reported by Arakawa and Miyamoto (2007), who observed individual flights by psyllids

that lasted >20 min in duration. The maximum duration of flight by *D. citri* reported by Arakawa and Miyamoto (2007) was 50 min. However, in our investigation, we observed a blue-green, female morph *D. citri* fly continuously for ≈ 3 h. This 3 h flight in the laboratory equated to an approximate distance of 2.4 km of continuous flight. These data corroborate previous field observations (Boina et al. 2009, Tiwari et al. 2010, Martini et al. 2013), indicating that *D. citri* are able to disperse multiple kilometers and this capability appears not to require assistance from wind.

Our morphometric analyses also revealed physical differences between *D. citri* color morphs. Pronotum width and wing length were both shorter in gray-brown than green-blue *D. citri*. These data are congruent with Wenninger and Hall (2008), who found that gray-brown *D. citri* morphs were of lower mass than green-blue morphs. Wenninger et al. (2008) suggested, based on observational data, that a proportion of *D. citri* may switch from the gray-brown to the blue-green morphotype (and vice versa). However, differences in fixed biometric characteristics such as wing length or pronotum width may indicate genetically predetermined morphotypes or morphotypes that are determined during nymphal development. We did not find differences in wing length and pronotum width between the various flyer categories (as defined by our experiments), suggesting that these morphometric characteristics did not impact flight capability of *D. citri* in the range of variability observed. The smaller size and mass, lower flight capability, and lower reproductive potential of gray-brown than green-blue morphs imply that the

occurrence of this morphotype should be selected against within populations of *D. citri*. Therefore, why this polymorphism is maintained in field populations of *D. citri* is an intriguing question.

The relationship between variation in body pigmentation and flight performance of hemipteran insects is not fully understood. Ahsaei et al. (2013) found differences between the red and green morphs of the pea aphid, *Acyrtosiphon pisum* Harris, with respect to their energetic reserves. The red morphs maintained a higher percentage content of water-soluble carbohydrates and lipids than green morphs; whereas green morphs were characterized by a higher percentage of protein content than red morphs. Interestingly, the authors associated these differences with the fact that red morphs produce more winged offspring than green morphs and therefore higher levels of carbohydrates and lipids may improve the flight potential of this morphotype (Ahsaei et al. 2013).

In the case of *D. citri*, it has been hypothesized that abdominal color variation may be associated with the quantity or quality of acquired food resources (Wenninger and Hall 2008). Alternatively, differing color morphs within a population may partially result from competition between conspecifics during nymph development (Wenninger et al. 2009). In both cases, such biotic factors may affect energetic reserves of emerging adult *D. citri* and consequently their flight capability. Future work should address the energetic contents of green–blue versus brown–gray morphotypes, and determine if this energetic content is affected by plant host quality or intraspecific competition during nymph development.

Our results indicate that on average, *D. citri* performing long-distance flights dispersed 320 m (greatest distance observed was 2.4 km) without wind assistance. These data indicate that adult *D. citri* are capable of significant long-range dispersal. It is probable; however, that wind may significantly increase the dispersal capability of *D. citri*. Obviously, our experiments did not take into account how wind may alter flight capability of *D. citri*. It is possible that there is an optimal wind speed at which *D. citri* preferentially initiate flight, and a maximal speed above which they will not fly. Also, our experiments did not determine whether upwind flight is preferred by *D. citri* in response to odor cues (specifically citrus odors). Overall, impact of wind on dispersal of *D. citri* requires further investigation beyond the flight mill investigations conducted here that indented to test physiological limits of flight potential.

Nevertheless, our study demonstrated that *D. citri* has significant flight capability and is able to fly long distances without wind assistance. Furthermore, the ability of *D. citri* to survive short durations on nonhost plants (Martini et al. 2013) may further increase their potential for long-range dispersal. The current results indicate that area wide management to suppress the vector is necessary in locations that have both pathogen and vector and that quarantine programs for this pathosystem may have little success unless many kilometers of psyllid movement are considered for

insecticide application protocols following detection of *D. citri* in a quarantined area. Another possible application of these results is that the proportion of green–blue versus gray–brown morphs within a population may indicate the risk of *D. citri* dispersal. Alternatively, given that the green–blue morphotype occurs within field populations of *D. citri* in large proportions and is also selected for given higher fecundity (Wenninger and Hall 2008), determining the color of *D. citri* morphotypes within populations of cultivated citrus may have no practical implication with respect to optimizing insecticide sprays. However, it is clear that adult *D. citri* are capable of long-distance migration, which must be considered when determining area-wide management and quarantine protocols. Other factors impacting dispersal of *D. citri*, such as pathogen infection, are currently under investigation and will also contribute to the management of this pathosystem.

Acknowledgments

We thank the Citrus Research and Development Foundation for funding. We thank two anonymous reviewers for their valuable comments on an earlier version of the manuscript.

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Received 13 February 2014; accepted 30 May 2014.
