Assessing the flight capabilities of fed and starved *Allograpta obliqua* (Diptera: Syrphidae), a natural enemy of Asian citrus psyllid, with computerized flight mills

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Computerized flight mills have been used to assess insect flight capacity under controlled conditions in the laboratory (David et al. 2014; Lopez et al. 2014; Martini et al. 2014; Hoddle et al. 2015). Experiments using flight mills can be designed to assess the effect of covariates (e.g., feeding status, sex, age, size, mating status, and time of yr) on the flight capabilities of experimental insects (Taylor et al. 2010; Ávalos et al. 2014; Lopez et al. 2014; Hoddle et al. 2015; Naranjo 2019). The following study used flight mills to investigate flight capabilities of a hoverfly, *Allograpta obliqua* (Say) (Diptera: Syrphidae), that is an important predator of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), in California (Kistner et al. 2016). Specifically, distances by which *A. obliqua* can fly in 24 h, and the effect of sex and feeding treatment (fed or starved) on flight capabilities were investigated. Flight capacity of *A. obliqua* is of interest because this natural enemy potentially can be manipulated in the field through the provisionment of floral resources to enhance biocontrol of *D. citri*. Incorporating nectar producing non-crop resources can promote natural enemy populations in agricultural systems (Gurr et al. 2004). Understanding natural enemy dispersal dynamics from non-crop resources helps determine the size and spacing of non-crop resource patches in cropping systems (Landis et al. 2000; Gillespie et al. 2011; Irvin et al. 2018). One way to assess the dispersal capabilities of *A. obliqua* is by using flight mills to quantify flight distances.

Adult *A. obliqua* were obtained for flight mill experiments by placing potted *Citrus volkameriana* V. Ten. & Pasq. ( Rutaceae) plants (25 cm tall), infested with *D. citri* nymphs, in a block of unsprayed grapefruit on the University of California Riverside, Riverside, California, USA, campus to encourage hoverfly oviposition (see Bistline-East et al. [2015] and Kistner et al. [2016] for plant preparation and field deployment details). On 10 Jul, 1 Aug, 4 Aug, and 6 Aug 2018, 3 to 9 potted citrus trees containing 100 to 400 first to third instar *D. citri* nymphs were placed in the grapefruit block (33.97268°N; 117.31819°W). Plants were placed on plastic 19 L buckets (Home Depot, Riverside, California, USA; 33 cm diam × 37 cm high) that were secured to the ground with steel tent pegs. Buckets were lined along their circumferences with a sticky barrier (Tanglefoot insect barrier, Contech Enterprises Inc., Irving, Texas, USA) to a harness on the rotating arm of a flight mill. After gluing, free wing movement was visually confirmed, then flight recording software was initiated. Each flight bioassay was 24 h in duration, and flights were initiated between 10:00 A.M. and 1:00 P.M. over the period 21 Aug to 1 Sep 2018. Following each 24 h flight trial, individual hoverflies were detached from flight mills and immediately weighed. Percentage loss in weight for each hoverfly was calculated. A total of 7 female and 10 male hoverflies were flown. Thirteen experimental flies died within the 24 h experimental period. Flight data from dead flies were included in analyses.

A 2-way ANOVA was used to test if feeding treatment, sex, and their interaction had a significant effect on total flight duration time, feeding status, sex, age, size, mating status, and time of yr) on the flight capabilities of experimental insects (Taylor et al. 2010; Ávalos et al. 2014; Lopez et al. 2014; Hoddle et al. 2015; Naranjo 2019). The following study used flight mills to investigate flight capabilities of a hoverfly, *Allograpta obliqua* (Say) (Diptera: Syrphidae), that is an important predator of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), in California (Kistner et al. 2016). Specifically, distances by which *A. obliqua* can fly in 24 h, and the effect of sex and feeding treatment (fed or starved) on flight capabilities were investigated. Flight capacity of *A. obliqua* is of interest because this natural enemy potentially can be manipulated in the field through the provisionment of floral resources to enhance biocontrol of *D. citri*. Incorporating nectar producing non-crop resources can promote natural enemy populations in agricultural systems (Gurr et al. 2004). Understanding natural enemy dispersal dynamics from non-crop resources helps determine the size and spacing of non-crop resource patches in cropping systems (Landis et al. 2000; Gillespie et al. 2011; Irvin et al. 2018). One way to assess the dispersal capabilities of *A. obliqua* is by using flight mills to quantify flight distances.

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Four flight mills were set up in a temperature controlled room held at 26 ± 2 °C and 60 to 80% RH, with a 16:8 h (L:D) photoperiod (see Lopez et al. [2014] for flight mill specifics, calibration, and insect attachment protocol). Flight mill data were analyzed using a customized macro in Microsoft Excel, which calculated average flight velocity, flight duration time, total rest time, number of flight bouts, number of valid bouts (movement of > 5 s before coming to a complete stop), time elapsed to first bout, mean bout time, and total distance flown over a 24-h period by individual *A. obliqua* adults. For each bout, the distance flown, flight bout duration, velocity, average flight speed, and maximum flight speed per bout were calculated.

Prior to tethering on the flight mill, starved or fed adult *A. obliqua* (24–48 h old) were weighed with a precision balance, then attached by the pronotum using hot glue (Mini Glue Sticks, Michaels Stores Procurement Company, Inc., Irving, Texas, USA) to a harness on the rotating arm of a flight mill. After gluing, free wing movement was visually confirmed, then flight recording software was initiated. Each flight bioassay was 24 h in duration, and flights were initiated between 10:00 A.M. and 1:00 P.M. over the period 21 Aug to 1 Sep 2018. Following each 24 h flight trial, individual hoverflies were detached from flight mills and immediately weighed. Percentage loss in weight for each hoverfly was calculated. A total of 7 female and 10 male hoverflies were flown. Thirteen experimental flies died within the 24 h experimental period. Flight data from dead flies were included in analyses.

A 2-way ANOVA was used to test if feeding treatment, sex, and their interaction had a significant effect on total flight duration time.

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total rest time, number of flight bouts (movement then coming to a complete stop), number of valid bouts (movement of > 5 s before coming to a complete stop), minutes to first bout, mean bout time, total distance flown over a 24-h period by individual A. obliqua adults, and loss of weight. Five bouts were over 5 m long and these were conducted by 2 fed females. The mean distance flown, seconds lasted, velocity, average speed, and maximum speed was calculated across these 5 bouts.

There was no significant effect of feeding treatment × sex interaction on total distance flown, time spent flying, time spent resting, number of bouts, number of valid bouts, mean bout time, time to first bout, or loss of weight (P > 0.05). There was no significant effect of feeding treatment on total distance flown, flying time, time spent resting, number of bouts, number of valid bouts, mean bout time, or percentage loss of weight (Fig. 1). Starved A. obliqua spent 3 times longer resting before initiating their first flying bout, and this difference was marginally significant (P = 0.05) (Fig. 1). There was no significant effect of sex on total distance flown, flying time, time spent resting, number of bouts, number of valid bouts, mean bout time, or percentage loss of weight (Fig. 2). In comparison to male flies, female A. obliqua spent approximately twice as long resting before initiating their first flying bout, and this difference was not significant (P = 0.09) (Fig. 2). The mean bout time of A. obliqua attached to flight mills was 10 to 21 seconds. Out of a total of 3,487 bouts across all feeding treatments and both sexes, 7 bouts consisted of A. obliqua flying a total of 1 to 2 m, and 5 bouts consisted of A. obliqua flying over 5 m. All the remaining bouts (99.7% of bouts) were less than 1 m and consisted of extremely short bursts of flight activity (< 10 s). The 5 bouts over 5 m were conducted by 2 fed females, and the mean distance flown, mean bout flight time, and mean velocity was 39 ± 13 m, 113 ± 24 s, and 0.33 ± 0.03 m per s, respectively. One fed female flew 82 m in 185 s.

This study demonstrated that A. obliqua rarely undertook prolonged flights > 10 s duration on the flight mills, because 99.7% of flight bouts consisted of short bursts of flight activity that were < 10 sec. These short bursts in flight activity may be attributable to “hovering” rather than dispersal flight. Dällenbach et al. (2018) distinguished between “flight” and “hovering” where hovering flies did not initiate more than 10 s of movement of the flight mill arm. The suspension of A. obliqua from the flight mill arm may cause this species to initiate hovering instead of normal flight activity. The suspension of the flight mill arm. The suspension of the flight mill arm may cause this species to initiate hovering instead of normal flight activity. The suspension of the flight mill arm. The suspension of the flight mill arm.

Summary

Flight mills were used to investigate flight capabilities of Allograpta obliqua (Say) (Diptera: Syrphidae), an important predator of Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae), in California. Specifically, distance by which A. obliqua can fly in 24 h, and the effect of sex and feeding treatment (fed or starved) on flight capabilities were investigated. A total of 17 (7 females, 10 males) laboratory-reared flies were flown. There was no significant effect of sex on distances flown. Out of a total of 3,487 flight bouts, 7 consisted of A. obliqua flying 1 to 2 m, and 5 bouts consisted of A. obliqua flying over 5 m.

Fig. 1. Mean flight parameters measured for starved and fed Allograpta obliqua attached to flight mills for 24 h. Error bars indicate ± SEMs; asterisks indicate a significant (P < 0.05) difference between feeding treatments.

Fig. 2. Mean flight parameters measured for male and female Allograpta obliqua attached to flight mills for 24 h. Error bars indicate ± SEMs; asterisks indicate a significant (P < 0.05) difference between sex.
Se utilizaron molinos de vuelo para investigar la capacidad de vuelo de *Allograpta obliqua* (Sayetera) (Diptera: Syrphidae), un depredador importante del psílido asiático de cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), en California. Específicamente, se investigó la distancia que *A. obliqua* puede volar en 24 horas, el efecto del peso, del tratamiento y de alimentación (alimentado o hambriento) sobre la capacidad de volar. Se volaron un total de 17 (7 hembras, 10 machos) moscas criadas en laboratorio. El sexo de la mosca no tenía un efecto significativo sobre la distancia volada. De un total de 3,487 pruebas de vuelo, 7 consistieron en *A. obliqua* volando de 1 a 2 metros, y 5 pruebas consistieron en *A. obliqua* volando sobre 5 metros. Las pruebas restantes (99.7%) fueron menos de 1 metro y se consideraron “flotantes” porque consistieron en ráfagas extremadamente cortas de actividad de vuelo (< 10 s). En las 5 pruebas de más de 5 metros realizados por 2 hembras alimentadas, el promedio de la distancia recorrida y del tiempo de vuelo fueron 39 ± 13 metros y 113 ± 24 s, respectivamente.

Palabras Clave: psílido asiático de los cítricos; *Diaphorina citri*; distancia; hoverfly; sexo

**Sumario**

The remaining bouts (99.7%) were less than 1 m and were considered ‘hovering’ because they consisted of extremely short bursts of flight activity (< 10 s). The 5 bouts over 5 m were conducted by 2 fed females, and the mean distance flown and mean flight bout time were 39 ± 13 m and 113 ± 24 s, respectively.

**Key Words**: Asian citrus psyllid; *Diaphorina citri*; distance; hoverfly; sex

References Cited

Avalos JA, Martí-Campoy A, Soto A. 2014. Study of the flying ability of *Rhyphochrophus ferrugineus* (Coleoptera: Dryopthoridae) adults using a computerized flight mill. Bulletin of Entomological Research 104: 462–470.

Bistline-East A, Pandey R, Kececi M, Hoddle MS. 2015. Host range testing of *Allograpta obliqua* (Sayetera) (Diptera: Syrphidae), a predator of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), in California. Annals of the Entomological Society of America 108: 860–871.

Chapman JW, Menz MHM. 2018. Higher flight activity in the offspring of migrants compared to residents in a migratory insect. Proceedings of the Royal Society B 285: 1–7.

David G, Giffard B, Binds B, Jactel H. 2014. Dispersal capacity of *Monochamus galloprovincialis*, the European vector of the pine wood nematode, on flight mills. Journal of Applied Entomology 138: 566–576.

Gillespie M, Wratten SD, Sedcole R, Colfer R. 2011. Manipulating floral resources dispersion for hoverflies (Syrphidae: Diptera) in a California lettuce agroecosystem. Biological Control 59: 215–220.

Gurr GM, Scarratt SL, Wratten SD, Berndt L, Irvin N. 2004. Ecological engineering, habitat manipulation and pest management, pp. 1–12 in Gurr GM, Wratten SD, Altieri MA [eds.], Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods. CSIRO Publishing, Collingwood, Victoria, Australia.

Hoddle MS, Faleiro JR, El-Shafie HAF, Jeske DR, Sallam AA. 2015. How far can the red palm weevil (*Coleoptera: Curculionidae*) fly? Computerized flight mill studies with field-captured weevils. Journal of Economic Entomology 108: 2599–2609.

Irvin NA, Hagler JR, Hoddle MS. 2018. Measuring natural enemy dispersal from cover crops in a California vineyard. Biological Control 126: 15–25.

Kistner EJ, Hoddle MS. 2015. Life of the ACP: field experiments to determine natural enemy impact on ACP in southern California. Citrograph 6: 52–57.

Kistner EJ, Melhem N, Carpenter E, Castillo M, Hoddle MS. 2016. Abiotic and biotic mortality factors affecting Asian citrus psyllid (*Hemiptera: Liviidae*); demographics in Southern California. Annals of the Entomological Society of America 109: 860–871.

Landis DB, Wratten SD, Gurr GM. 2000. Habitat manipulation to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45: 175–201.

Lopez VM, McClanahan MN, Graham L, Hoddle MS. 2014. Assessing the flight capabilities of the goldspotted oak borer (*Coleoptera: Buprestidae*) with computerized flight mills. Forest Entomology 107: 1127–1135.

Lövei GL, Hickman JM, McDougall D, Wratten SD. 1993. Field penetration of beneficial insects from habitat islands: hoverfly dispersal from flowering crop strips. Proceedings of the 46th New Zealand Plant Protection Conference 1993: 325–328.

Martini X, Hoyte A, Stelinski LL. 2014. Abdominal color of the Asian citrus psyllid (*Hemiptera: Liviidae*) is associated with flight capabilities. Annals of the Entomological Society of America 107: 842–847.

Naranjo SE. 2019. Assessing insect flight behavior in the laboratory: a primer on flight mill methodology and what can be learned. Annals of the Entomological Society of America 112: 182–199.

Nicholls CI, Parmella M, Altieri MA. 2001. The effects of a vegetative corridor on the abundance and dispersal of insect biodiversity within a northern Californian organic vineyard. Landscape Ecology 16: 133–146.

Rotheray EL, Bussière LF, Moore P, Bergstrom L, Goulson D. 2014. Mark capture estimates of dispersal ability and observations on the territorial behaviour of the rare hoverfly, *Hammerschmidtia ferruginea* (Diptera, Syrphidae). Journal of Insect Conservation 18: 179–188.

Rotheray EL, MacCowan I, Rotheray GE, Sears J, Elliott A. 2009. The conservation requirements of an endangered hoverfly, *Hammerschmidtia ferruginea* (Diptera, Syrphidae) in the British Isles. Journal of Insect Conservation 13: 569–574.

Taylor RA, Baur JLS, Poland TM, Windell KN. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. Journal of Insect Behavior 23: 129–148.

van Rijn PJC, Kooijman J, Wackers FL. 2006. The impact of floral resources on syrphids performance and cabbage aphid biological control. IOBC/WPRS Bulletin 29: 149–152.

van Veen MP. 2004. Hoverflies of Northwest Europe, Identification Keys to the Syrphidae. KNNV Publishing, Utrecht, Netherlands.

Weems HV. 2008. A hover fly, *Allograpta obliqua* (Say) (Insecta: Diptera: Syrphidae). DPI Entomology Circular No. 106. University of Florida/IFAS, Gainesville, Florida, USA.

Wratten SD, Bowie MH, Hickman JM, Evans AM, Sedcore JR, Tylianakis JM. 2003. Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivated land. Oecologia 134: 605–611.