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

Nicola A. Irvin^{1,*}, and Mark S. Hoddle¹

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., Victoria, British Columbia, Canada) to prevent access by ants (Kistner & Hoddle 2015). After 2 d, trees were removed from the field and placed at 26 ± 2 °C and 60 to 80% RH, with a 16:8 h (L:D) photoperiod to allow syrphid larvae to hatch from eggs laid on *D. citri* colonies. Individual first instar hoverfly larvae were transferred into a ventilated clear plastic cage (Perspex cage, 15.2 × 15.2 × 30.5 cm, with 2 mesh sides and a cloth sleeve for easy access) containing 1 potted *C. volka-*

meriana infested with 400 to 800 first to third instar *D. citri* nymphs. Citrus plants infested with *D. citri* nymphs were replaced every 7 d until fly pupae were observed. Pupae were placed individually into microcentrifuge tubes (Fisherbrand 1.5 mL polypropylene microcentrifuge tubes, Fisher Scientific, Pittsburgh, Pennsylvania, USA), and checked daily for adult emergence. Newly emerged (< 24 h old) adult flies were sexed and randomly allocated to either a fed or starved treatment. Adults in starved treatments were denied food for 24 h. Adults in fed treatments were provided a 7.4 mL glass vial (2 dram Fisherbrand Glass Vial, Fisher Scientific, Pittsburgh, Pennsylvania, USA) containing 50% honey-water solution (natural uncooked honey, Wild Mountain Brand, Oakland, California, USA) with a 5 cm cotton wick, and a glass vial containing water and cut stems of field collected allyssum (*Lobularia maritima* [L.] Desv.; Brassicaceae) and buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae) flowers that provided nectar/pollen. Water was provided in both treatments via a 7.4 mL glass vial with a 5 cm cotton wick.

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, time to first bout, 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, but 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 dispersal flight. In addition, the smaller size of *A. obliqua* compared with *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae) (van Veen 2004; Weems 2008) may inhibit its ability to fly against the resistance of the flight arm in the air. Flight mills used in the current study used friction-

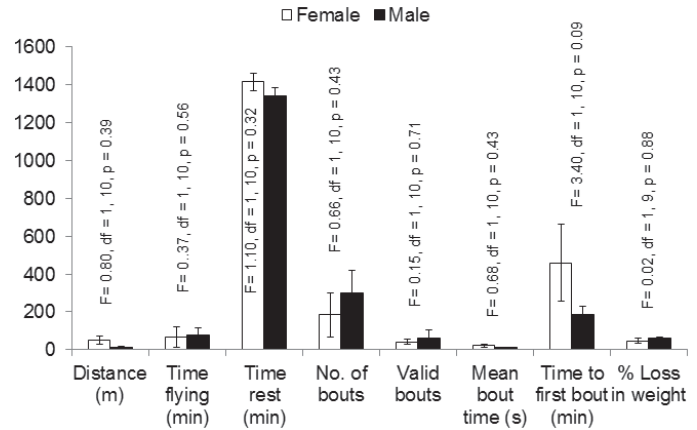


Fig. 2. Mean flight parameters measured for male and female *Allograpta obliqua* attached to flight mills for 24 h. Error bars indicate ± SEMs; asterisks indicate a significant $P < 0.05$ difference between sex.

free ball bearing technology (Hoddle et al. 2015) compared with flight mills using magnetic flotation, such as those used by Dällenbach et al. (2018), which may offer less resistance for small flying insects. Thirteen out of 17 *A. obliqua* tested on the flight mill died within the 24 h flight time. Dällenbach et al. (2018) reported 5 out of 232 *E. balteatus* in their study died within the 4 h flight time. In comparison to *E. balteatus*, smaller sized *A. obliqua* may be more prone to desiccation while attached on the flight mill without access to water, and a 24 h flight assay time may subsequently have been too long a test period.

Mobility of hoverflies is variable. When floral resources are available, hover flies may not disperse further than 30 m over a 9 wk period (Lövei et al. 1993). Other studies suggest that hover flies may disperse 200 m in 7 d (Wratten et al. 2003), and up to 5 km in 4 wk (Rotheray et al. 2014). In the current study, 2 fed female *A. obliqua* flew on average 39 m in 24 h. One female *A. obliqua* flew 82 m in 185 s. Several factors affect hoverfly dispersal in the field including cover crop density, field boundaries, and availability of floral resources and hosts for feeding and reproduction (Lövei et al. 1993; Nicholls et al. 2001; Wratten et al. 2003; van Rijn et al. 2006; Rotheray et al. 2009). With respect to the current study, future flight mill studies with *A. obliqua* could assess the effects of covariates on dispersal by using assay times < 24 h, and using flight mills with magnetic levitation to help determine optimal distances between floral resource patches in citrus orchards for conservation biocontrol of *D. citri*. Additional work is necessary to better quantify the dispersal capabilities of *A. obliqua* in the field, and these studies could use protein marks applied to cover crops and subsequent captures of adult flies at varying distances from these resources (Irvin et al. 2018).

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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.

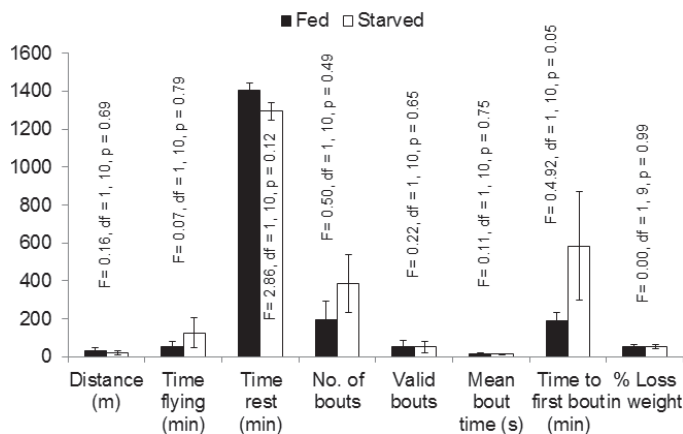


Fig. 1. Mean flight parameters measured for starved and fed *Allograpta obliqua* attached to flight mills for 24 h. Error bars indicate ± SEMs; asterisks indicate a significant $P < 0.05$ difference between feeding treatments.

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

Sumario

Se utilizaron molinos de vuelo para investigar la capacidad de vuelo de *Allograpta obliqua* (Saytera) (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 sexo, 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; mosca certera; sírfido; sexo

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