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Angela M. Rovnyak
Iowa State University

Charles S. Burks
U.S. Department of Agriculture

Aaron J. Gassmann
Iowa State University, aaronjg@iastate.edu

Thomas W. Sappington
Iowa State University, tsapping@iastate.edu

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Keywords

Amyelois transitella, flight mills, dispersal, almonds, pistachios, Lepidoptera, Pyralidae

Disciplines

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Comments

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Interrelation of mating, flight, and fecundity in navel orangeworm females

Angela M. Rovnyak¹, Charles S. Burks², Aaron J. Gassmann¹ & Thomas W. Sappington^{1,3*} 

¹Department of Entomology, Iowa State University, Ames, IA 50011, USA, ²USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Ave., Parlier, CA 93648-9757, USA and ³USDA, Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, IA 50011, USA

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Abstract

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae, Phycitini), is an economically important pest of nut crops in California, USA. Improved management will require better understanding of insect dispersal, particularly relative to when mating occurs. A previous study demonstrated a more robust laboratory flight capacity compared to other orchard moth pests, but it was unclear how mating affects dispersal, and how dispersal affects fecundity. In this study, 1- and 2-day-old females were allowed to fly overnight on a flight mill either before or after mating, respectively, and were then allowed to oviposit. Data on fecundity were compared between treatments to minimally handled or tethered-only control females. Females that mated before flight flew longer and covered a greater distance than those flying prior to mating. However, timing of flight relative to mating did not affect fecundity, nor did any measure of flight performance. There was no effect on fecundity when females were forced to fly for designated durations from 3 min to 2 h. Together, our data revealed no obvious trade-off between flight activity and reproductive output. Distances measured on the flight mills (mean ca. 15 km for mated females) may overestimate net displacement in the field where flight tracks are often meandering. The results suggest that most females mate and oviposit in or near their natal habitat, but that some may disperse potentially long distances to oviposit elsewhere.

Introduction

Many insect species display life-history trade-offs between dispersal and reproduction. The extent and manifestation of these trade-offs is largely species specific and is not well understood for many species (Colvin & Gatehouse, 1993; Zhang et al., 2015). Oogenesis and flight activities compete for energy reserves and extended flight can cause a decrease in fecundity and fertility, as observed for the diamondback moth, *Plutella xylostella* (L.) (Shirai, 1995), and the forest tent caterpillar, *Malacosoma disstria* (Hübner) (Evenden et al., 2015). In some species, syndromes of physiological characteristics (sensu Dingle, 2006) are

observed through which the timing of reproduction and flight is coordinated (Dingle, 2014).

The navel orangeworm, *Amelyois transitella* (Walker) (Lepidoptera: Pyralidae, Phycitini), is a key pest of almonds and pistachios in California, USA (Bentley et al., 2016a,b), and one of several important pests of walnuts. It can cause up to 30% product loss in almonds from direct consumption, and only 1.5–2% product loss is considered the economic injury level (Higbee & Siegel, 2009, 2012). Recently, California almond and pistachio harvests have been worth >US\$5 billion, and walnuts >US\$1 billion before processing (CDFA, 2015). In 2014 the area harvested for these three crops in California was 352, 89, and 117 thousand hectares, respectively (CDFA, 2015). The navel orangeworm is therefore economically and ecologically important in California. It is a polyphagous generalist feeder, documented on >40 fruits, nuts, and legumes (Curtis & Barnes, 1977). Its ability to develop on these hosts is,

*Correspondence: Thomas W. Sappington, USDA-ARS, CICGRU, Genetics Laboratory, ISU, Ames, IA 50011, USA.
E-mails: tom.sappington@ars.usda.gov, tsapping@iastate.edu

however, dependent on their vulnerability due to advanced maturity, senescence, pathogen infection, or infestation by other insects (Wade, 1961; Curtis & Barnes, 1977). Movement and seasonality are therefore important aspects of both pest potential and management strategies.

Previous studies have demonstrated that the navel orangeworm has a much greater dispersal capacity than other lepidopteran orchard pests (Sappington & Burks, 2014). This is presumably an adaptation to the greater time and distance between suitable hosts compared to more specialized pests of orchard crops. Flight mill experiments (Sappington & Burks, 2014) showed that unmated navel orangeworm are capable of flying distances greater than the 5 km indicated in a previous study (Higbee & Siegel, 2009), averaging ca. 12.2 km per night at 1 and 2 days old. Almond orchards 5 km or less from pistachio orchards (which typically have greater navel orangeworm abundance) are at increased risk of damage (Higbee & Siegel, 2009), and it is likely that at least a few individuals disperse even greater distances (Burks et al., 2006; Sappington & Burks, 2014).

Voltinism and generation length for navel orangeworm are tied to the seasonality of their food source. Adults are attracted to volatiles released by nuts at various points of maturation and decay (Beck et al., 2009, 2014). Overwintering larvae develop slowly inside nuts left on trees postharvest (i.e., mummies) or on the ground, and adults emerge the following spring to oviposit on other mummies (Wade, 1961; Sanderson et al., 1989; Bentley et al., 2016a). Females emerging from this first generation (i.e., second-flight females) proceed to oviposit on the surface of new crop nuts during hull split; the larvae hatch and burrow to consume the seed. In California, the first generation of navel orangeworm adults begins to emerge in mid-June to early July. Some of the second generation often develop in nuts of the new crop, and adults emerge from these nuts through late summer and early autumn. Four to five generations are possible in warmer years or in more southern latitudes, but development time is variable and generations increasingly overlap in later parts of the growing season (Siegel et al., 2010; Siegel & Kuenen, 2011). Oviposition in marketable pistachios generally occurs later in the season than in almonds (Rice, 1978) because of phenological differences. However, deformed non-marketable pistachios (pea splits and early splits) occur earlier in the season and contribute to pest abundance (Siegel & Kuenen, 2011), as does the large number of pistachio mummies on the ground (Burks et al., 2008; Higbee & Siegel, 2009). Year-to-year variation in weather affects the number and timing of generations (Sanderson et al., 1989; Kuenen & Siegel, 2010; Siegel et al., 2010; Siegel & Kuenen, 2011).

Despite the navel orangeworm's wide range of potential host species, the fruit must be at the right stage and physically compromised to allow infestation. Thus, finding a habitat patch (orchard) containing suitable hosts can require dispersal over variable and sometimes considerable distances. Predicting population dynamics under such conditions requires understanding the degree to which flight and reproductive capacity are intertwined. In this study, we used flight mills to determine whether mating enhances or decreases flight activity of young female navel orangeworm, and monitored subsequent fecundity and fertility to examine effects of flight on female reproductive output. Flight activity was examined for both total activity and the longest uninterrupted flight by each individual. The latter sometimes represents the straight-line flight behavior characteristic of migratory flight, which differs from local station-keeping or ranging flight behaviors that are appetitive and more meandering (Dingle, 2006; Dorhout et al., 2008). Our specific goals were to examine two aspects of the relationship between flight activity and reproduction: (1) effect on fecundity of timing of flight relative to mating, and (2) effect of mating on various parameters of flight activity.

Materials and methods

Experimental overview and design

Our experimental strategy was to measure flight performance of young mated or unmated females on a flight mill, then allow them to oviposit. Eggs were counted daily until the female's death to measure fecundity. Experiments were conducted between May 2014 and April 2016.

Two experiments were conducted: voluntary flight and forced flight (Table 1). The voluntary flight experiment involved tethering insects for testing on flight mills either before or after mating. To control for the effect of handling, sham-treated control groups (Mate-tether and Tether-mate) were prepared. Tethered control moths were not specifically paired with flight-tested moths, but were held in the flight chamber during flight tests. Our goal was a sample size of 50 individuals with usable data per treatment, based on our previous experience with the level of variability in flight performance experienced with other insect species.

The forced flight experiment was conducted to separate the effects of flight and an individual's propensity to fly. Individuals were forced to fly continuously for predetermined amounts of time as described in the section on flight performance. The minimum sample size was 30 individuals per treatment for this experiment. Target sample size was lower than in the voluntary flight experiments

Table 1 Experimental treatments and their abbreviations for navel orangeworm females tested on flight mills (voluntary flight) or on stationary tethers (forced flight). On the day of emergence (day 1), moths were prepared to fly, to be tethered, or to mate depending on the group to which they were assigned. Beginning on day 2 or 3 following eclosion, depending on treatment, all successfully mated moths were allowed to oviposit until death

Experiment	Treatment name	Day 1	Day 2	Sample size
Voluntary flight	M	Mate	Oviposit	63
	Mate-tether	Mate	Tether	84
	Tether-mate	Tether	Mate	60
	Mate-fly	Mate	Fly	52
	Fly-mate	Fly	Mate	63
Forced flight	T0M	Tether + mate	Oviposit	32
	MF3	Mate	Fly 3 min + oviposit	35
	MF30	Mate	Fly 30 min + oviposit	33
	MF60	Mate	Fly 60 min + oviposit	36
	MF120	Mate	Fly 120 min + oviposit	32
	F3M	Fly 3 min + mate	Oviposit	36
	F30M	Fly 30 min + mate	Oviposit	33
	F60M	Fly 60 min + mate	Oviposit	38

because there was no need to compensate for variability in flight duration.

For both experiments, individuals were excluded from the data set if they did not meet minimum criteria of flight performance, egg fertility, and longevity (see 'Data analysis'). This was to prevent inclusion of data from insects that were damaged imperceptibly during handling or were in poor health. By erring on the side of not including an individual of compromised health, we may have eliminated naturally poor fliers or moths that did not fly for another reason. However, the latter was deemed less of a problem in producing robust results than risking inclusion of aberrant data from unhealthy individuals.

Insect culture

Navel orangeworm were obtained from a USDA-ARS laboratory colony at Parlier, CA, USA. This colony was established from eggs collected in an almond orchard in western Fresno County in September 2010 and refreshed by individuals from the same site in September 2011. Rearing procedures were modified slightly from those described in Burks et al. (2011a,b) and Burks (2014). Larvae were maintained on a wheat bran-based diet (Finney & Brinkman, 1967). Late instars were segregated by sex based on visibility of the testes through the dorsal integument of males. They were shipped twice weekly via overnight express from Parlier to Ames, IA, USA, and allowed to pupate. Pupae were checked daily for adult eclosion, and adults were moved to sex-specific and date-specific holding cages until ready for tethering or mating. Containers consisted of a 946-ml jar sealed with a wire mesh lid. A 2.25-cm strip of filter paper folded accordion style was

affixed inside the jar to provide a perch for the moths. Water was freely available via soaked cotton inside an inverted 30-ml jelly cup on top of the wire mesh. Sucrose was not added to the water because adult ingestion of carbohydrate does not affect fecundity of navel orangeworm (Kellen & Hoffmann, 1983; Burks, 2014). At all life stages, insects were held at 26 °C and L14:D10 photoperiod.

Flight performance

To test the effect of timing of flight relative to mating, newly eclosed moths were either tested on the flight mills the night after eclosion and prior to mating (designated as Fly-mate) or the night after mating (Mate-fly) (Table 1). Moths were allotted one full night to fly and one full night to mate.

Females could not be set up to mate on the night of eclosion; this meant that the moths that mated prior to flight were 1 day older when flown than moths that flew prior to mating, and the effect of age on flight behavior cannot be discounted. Designing the experiment to fly moths in the Mate-fly and Fly-mate treatment groups at the same age would have necessitated the females mating 2 days apart instead of 1, which we deemed even less desirable. Sappington & Burks (2014) observed a slight but statistically non-significant increase in flight performance of 2-day-old unmated females over those that were 1 day old.

Adult tethering and flight mill methods were adapted from Dorhout et al. (2008) and Sappington & Burks (2014). Each moth was attached to a tether made from a ca. 5-cm-long and 0.25-mm-diameter copper wire affixed to a short sleeve of insulation tubing stripped from the

wire. A small piece of kneaded Simply Tacky putty eraser (Hobby Lobby, Oklahoma City, OK, USA) was attached to the ultimate 1 mm of the wire, then flattened on one side. Moths were prepared for tethering by brushing the scales from the dorsal surface of the abdomen directly posterior to the metathorax. Anesthetization was usually unnecessary, but restive moths were cooled briefly (<3 min) in a -20°C freezer before handling. While holding the moth, a tiny drop of Sobo fabric glue (Plaid, Atlanta, GA, USA) was placed on the flattened side of the putty, which was lightly pressed to the cuticle to affix the tether.

Fifteen flight mills were housed in an environmental chamber held at 26°C and L14:D10 photoperiod. Dusk and dawn were simulated by programmed 30-min ramping of 40-W incandescent bulbs as described by Sappington & Burks (2014). The individual mills were housed in vinyl tents to minimize air movement. Each mill was attached to a Gateway 2000 personal computer running flight mill software as described by Beerwinkle et al. (1995). Moths were attached to the flight mill by slipping the sleeve on the tether over the point of the flight mill arm. The weight of the moth was counterbalanced by moving a clip on the opposite end of the flight arm. The flight mill arm was a triangle-shaped flat piece of aluminum (256 mm long, 156 mm from tip to pivot, 15 mm wide at the base end). Moths flew in a horizontal plane traveling a distance of 1 m per revolution around the central pin of the flight mill. Rotations were registered by an infrared eye mounted on the post below the central pin. At the time of attachment to the flight mill, each moth was given a small piece of tissue paper for tarsal contact. Most moths readily grasped the paper and folded their wings, helping reduce premature flight before dusk.

Effect of flight on reproduction

Moths in the voluntary flight study were attached to a flight mill throughout a full night, and were free to engage in flight activity or rest. A computer recorded each moth's flight duration, speed, distance, number of separate flights, and time of night of each separate flight. To control for the effect of handling in the Fly-mate and Mate-fly treatments, corresponding tethered controls were included: a group that was tethered but not flown (Tether-mate) the night prior to mating and a group was mated prior to being tethered but not flown (Mate-tether). Moths in the tethered control groups were held individually in a 50-ml polypropylene centrifuge tube overnight in the flight mill room, and each was supplied with a small square of tissue paper as resting substrate. We used the test tube to prevent unnecessary movement while allowing them to experience conditions as similar as possible to the flown groups. A

minimally handled control group (M) was neither tethered nor flown (Table 1).

Moths tested for forced flight were tethered as described in the previous paragraph. Because of space constraints in the flight mill chamber, forced flight trials were conducted in the open laboratory. Tethers were attached to wire shelving with masking tape in such a manner that each moth hung free in a horizontal orientation. During a trial, moths were continually agitated by gentle air flow from small oscillating fans positioned in front and underneath. Moths were observed during the entire duration of the flight and prevented from taking rests by gently touching the tarsi or tapping on the tether whenever they stopped flying. Moths that could not be induced to resume flight within 10 s were discarded. After flight, the tether was snipped close to the putty with scissors, and the moths were prepared for mating or oviposition.

One group of treatments consisted of moths that were allowed to mate and were then forced to fly during the following day for 3 min (MF3), 30 min (MF30), 60 min (MF60), or 120 min (MF120). In a second treatment group, moths were forced to fly for 3 min (F3M), 30 min (F30M), or 60 min (F60M) on the day immediately following emergence, and then allowed to mate the following day. A F120M treatment was attempted, but the rate of successful mating was too low to allow testing in the time frame available. An additional tethered but unflown group, T0M (Table 1), was included to compare to the F3M-F60M series of forced flight treatments.

Following a flight trial, moths were released from the tether by snipping it just above the attachment point, and were then allowed to mate or oviposit depending on the treatment. Mating jars were prepared in the same way as the holding containers. Each female was presented with at least one virgin male, or two males when possible to improve the likelihood of mating, and allowed one night to mate in an environmental chamber held at 26°C and L14: D10 photoperiod. Dawn twilight was simulated by a baby light (Sunbeam, Boca Raton, FL, USA) turned on 30 min prior to full light, which greatly facilitated mating activity. Sunset was not simulated because navel orangeworm have a greater propensity to mate at dawn rather than dusk (Burks et al., 2011a). Water was freely available as described above. Within the first 30 min of full light, moths were checked for mating. Those observed in copula were prepared for flight or oviposition, whereas those moths not mating were discarded.

The same type of jar was used to hold moths for oviposition. A #2 white bleached coffee filter (Hy-Vee brand, West Des Moines, IA, USA) was provided as an oviposition substrate for each moth. The edge of each coffee filter was folded over the lip of the jar and held in place by the

lid, such that the coffee filter covered the mouth of the jar. Filter papers were collected and replaced daily. Eggs were counted and assessed for fertility, which was indicated if the eggs had become bright orange within 24 h of oviposition (Parra-Pedrazzoli & Leal, 2006), so the freshly laid cream-colored eggs were allowed to mature overnight in the environmental chamber before counting.

Data analysis

To be eligible for inclusion in all analyses, moths must have lived for at least 3 days following flight or post-flight mating to reduce inclusion of moths whose overall health was severely compromised by experimental conditions, and must have deposited at least one fertile egg during that time to eliminate any unmated moths or mated moths with a compromised reproductive tract. In addition, to be included in the voluntary flight analyses, a moth must have made at least one continuous flight lasting >2 min to ensure it was at least capable of flying. For the forced flight analysis, if an individual ceased flying and could not be coaxed to fly within 10 s, that individual was excluded from analysis. This last criterion is conservative, but it allowed us to maintain tight control over duration of flight, and few were discarded for this reason. The flight mill software (Beerwinkle et al., 1995) measured time of flight, duration of flight, distance flown, and number of flights. A single flight was considered terminated if the flight mill arm remained motionless for 1 min.

All statistical analyses were conducted using the R statistical package (R Core Team, 2016). For the voluntary flight experiment, Welch's t-test was used to compare most response variables, including flight distance, flight duration, flight speed, number of flights, number of eggs, and number of fertile eggs. To visualize differences between flight groups, density curves were generated. Density curves represent continuous histograms scaled so that the area under the curve is equal to one (Starnes et al., 2015). The curves were compared to one another with a Kolmogorov–Smirnov goodness-of-fit test (Chakravarti et al., 1967). The forced flight experiment was analyzed by ANOVA followed by Tukey's honestly significant difference (HSD) test to separate treatment means. Fecundity data from all experiments were analyzed by ANOVA, with Tukey's HSD test and Welch's t-test used for individual comparisons. To compare start and end times on the flight mill, the non-parametric Wilcoxon Rank-Sum test was used because we assumed ordinality, i.e., that some individuals started flying before others (Gibbons & Chakraborti, 2011). A Poisson generalized linear regression was used to analyze the effect of treatment on fecundity over time through the course of the female's life. Pearson's product–moment correlation was used to analyze the

relationship between fecundity and flight, and linear regression was used when a causal relationship was suspected.

Results

Flight performance

The timing of flight relative to mating affected both distance and duration of flight. Over the entire night, moths that had mated prior to flight testing (Mate-fly) flew longer than those that had not yet mated (Fly-mate) in terms of both distance (mean \pm SEM = 14.9 ± 1.8 vs. 7.0 ± 1.4 km; $t = -3.48$, d.f. = 94.7, $P < 0.001$) and duration (365 ± 29.6 vs. 165 ± 24.0 min; $t = -5.26$, d.f. = 96.6, $P < 0.001$). There was no difference in flight speed between the Mate-fly and Fly-mate treatments (37.7 ± 2.1 vs. 39.1 ± 2.1 m per min; $t = 0.31$, d.f. = 99.6, $P = 0.75$), nor was there a difference in the total number of flights taken during the night between Mate-fly and Fly-mate (both 12.5 ± 1.3). The distribution of flight distance was skewed toward shorter flights for both treatment groups, but there was a more even distribution of individuals flying <30 km for Mate-fly (Figure 1A). Mate-fly moths displayed an overall propensity to make longer duration flights, with the distribution skewed to the right compared to Fly-mate (Figure 1B).

Treatment affected the longest single flight of the night in a similar fashion. Mate-fly females flew farther than Fly-mate (11.0 ± 1.6 vs. 4.6 ± 1.2 km; $t = -3.12$, d.f. = 93.3, $P = 0.002$), and for a longer time (252 ± 28.9 vs. 95.5 ± 20.6 min; $t = -4.40$, d.f. = 91.4, $P < 0.001$). Flight speed was not affected by mating (Fly-mate, 45.6 ± 2.4 m per min; Mate-fly, 41.7 ± 2.4 m per min; $t = 0.31$, d.f. = 100, $P = 0.76$). There was no difference between the speed of the longest flight and average speed of all flights (43.6 ± 2.4 vs. 38.4 ± 2.2 m per min; $t = -1.6$, d.f. = 100, $P = 0.10$). Distributions for distance and duration of the longest single flight differed significantly between Mate-fly and Fly-mate (Figure 2). The density curves for both flight duration and distance were weighted to the left. However, the distribution of flight durations in the Mate-fly group was much more even across all durations than in the Fly-mate group (Figure 2B). Mate-fly moths also started their longest single flight ca. 200 min earlier in the night than those that had not mated (Fly-mate) (Figure 3), but the time of night when the longest flight ended did not differ significantly between these groups.

Fecundity

Total and fertile eggs were quantified for each individual. Patterns were the same for both, so we present the data for

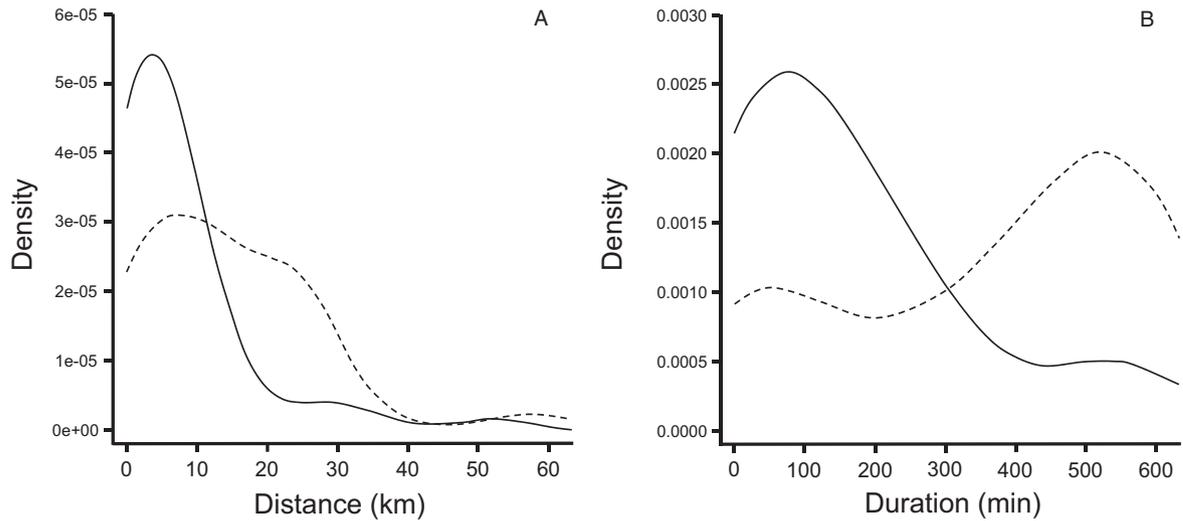


Figure 1 Density curves comparing frequency distributions of (A) total distance (km), and (B) total duration (min) of flight between individuals of treatments Fly-mate (solid line) and Mate-fly (dashed line). Density represents the proportion of individuals that flew at each value. Curves within a panel were compared by a Kolmogorov–Smirnov goodness-of-fit test: distance, $D = 0.99$; duration, $D = 0.90$, both $P < 0.001$.

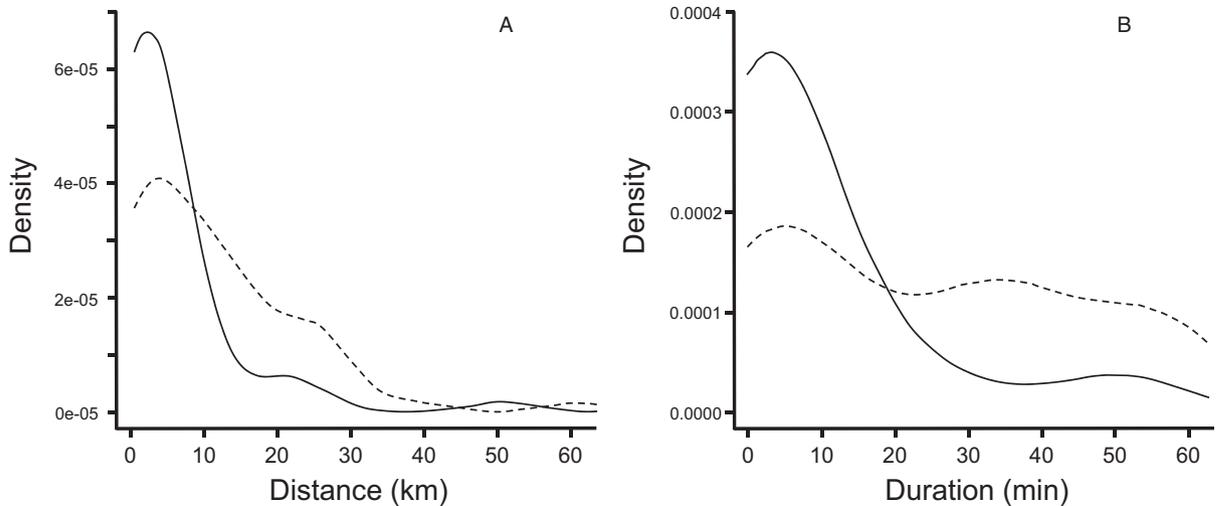


Figure 2 Density curves comparing frequency distributions of (A) distance (km), and (B) duration (min) of the longest single flight between individuals of treatments Fly-mate (solid line) and Mate-fly (dashed line). Density represents the proportion of individuals that flew at each value. Curves within a panel were compared by Kolmogorov–Smirnov goodness-of-fit test: distance, $D = 1$; duration, $D = 0.98$, both $P < 0.001$.

fertile eggs only. The order of flight and first mating made no difference in egg production, i.e., moths that mated prior to flying on the flight mills and moths that flew prior to mating produced equal numbers of eggs (Figure 4A). Tethered moths produced fewer eggs (Figure 4A) than minimally handled control moths. There was no difference among forced flight treatments in the lifetime number of

fertile eggs (Figure 4B). Temporal patterns of egg laying revealed that most oviposition occurs early in adult life. Moths laid the greatest number of eggs on the 1st day observed, and produced fewer eggs each subsequent day until death (Table 2). The pattern of egg laying was not significantly affected by flight behavior under the limitations of the experiment. The sham-treated control group

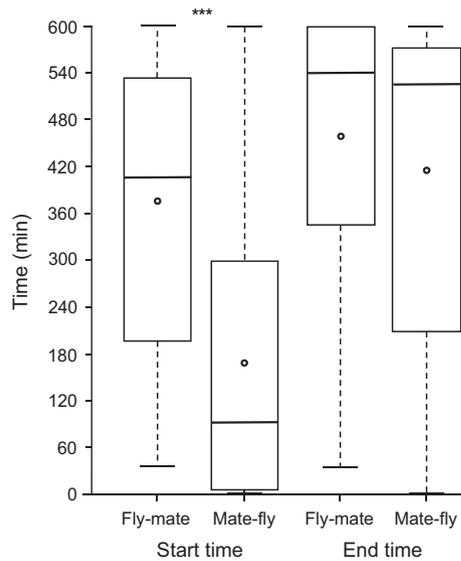


Figure 3 Start and end times of the longest single flight of the night. Numerals indicate the number of minutes after beginning of dusk. Data analyzed were minutes after dusk of the flight event. The boxes are delineated by the 25th and 75th percentiles. The solid line within a box represents the median, the dot inside represents the mean. The whisker lines indicate the 90th percentile. Treatments within either starting or ending time were compared using Wilcoxon Rank-Sum test (start: $W = 1\ 974.5$, $P < 0.001$; end: $W = 1\ 438$, $P = 0.36$; $n = 102$).

for females flown on flight mills as virgins (Mate-tether) produced significantly fewer eggs than moths from any other treatment (Table 2).

Regression analyses indicated the lifetime number of fertile eggs produced by a female was unaffected by the total distance flown (Figure 5A and B) or the total time engaged in flight (Figure 5C and D) on the flight mill, regardless of whether mating or flight occurred first. The same lack of effect on fecundity was observed relative to the longest single flight (Figure 6). Likewise, when data from both Fly-mate and Mate-fly flight mill treatments were pooled for regression analyses, reproduction was unaffected by total distance ($r^2 = 0.007$, $F_{7,246} = 1.77$, $P = 0.57$) or duration of all flights ($r^2 = 0.006$, $F_{7,246} = 1.77$, $P = 0.51$).

Discussion

Previously mated navel orangeworm females (Mate-fly group) displayed greater flight activity than females that had not yet mated (Fly-mate group). This was evident in comparisons of both total and longest flights. Under the conditions of this study, the $>2\times$ increase in flight activity after mating is striking. Of particular interest is that most

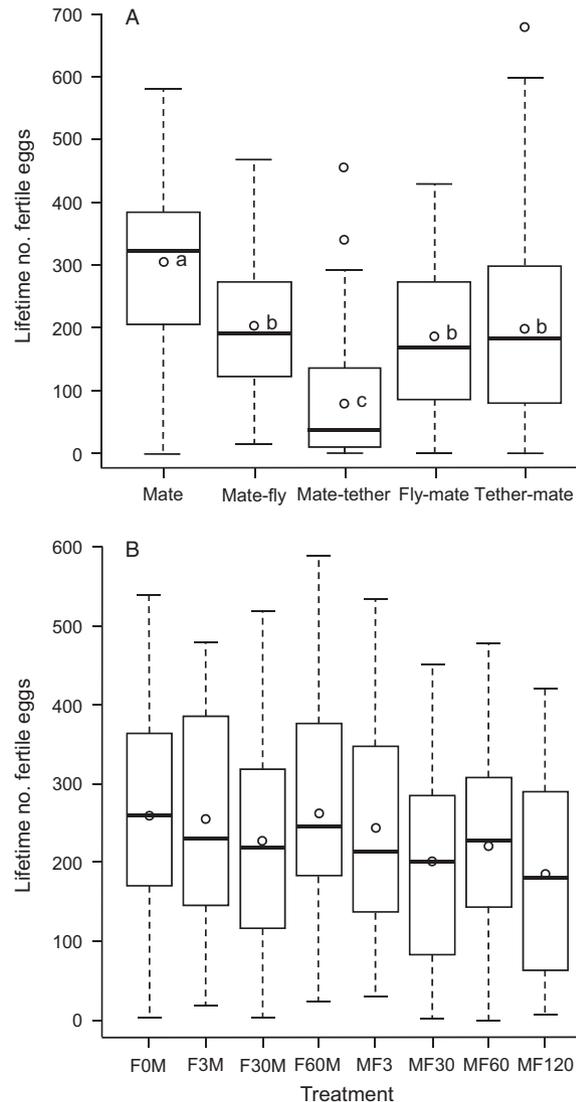


Figure 4 Fecundity (total no. fertile eggs per female) of navel orangeworm by (A) voluntary flight treatment group, and (B) forced flight treatment group (see Table 1 for treatment abbreviations). The boxes are delineated by the 25th and 75th percentiles. The solid line within a box represents the median, the dot inside represents the mean. The whisker lines indicate the 90th percentile, and dots outside the whisker lines indicate outliers. ANOVA, panel A: $F_{4,317} = 31.062$, $P < 0.001$ (means with different letters are significantly different; Tukey's HSD test: $P < 0.05$); panel B: $F_{7,246} = 1.77$, $P = 0.10$.

previously mated females flew >5 h. Although this right-skewed frequency distribution could suggest true migratory behavior by newly mated females, the lack of this pattern for the longest continuous flight, and the similarity of flight speeds of the longest and short duration flights argue against it.

Table 2 Mean (\pm SEM) number of fertile eggs produced per day by differently treated navel orangeworm for the first 7 days of oviposition opportunity (see Table 1 for treatment description and sample sizes)

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
M	98.4 \pm 9.6	42.9 \pm 4.6	40.7 \pm 4.3	27.2 \pm 2.6	38.4 \pm 6.4	20.8 \pm 2.3	13.4 \pm 1.7
Mate-tether	36.4 \pm 4.6	26.0 \pm 3.7	21.9 \pm 3.1	14.5 \pm 2.0	20.7 \pm 2.9	12.3 \pm 1.7	10.9 \pm 1.5
Tether-mate	81.9 \pm 11.5	37.0 \pm 5.2	26.0 \pm 3.6	21.2 \pm 3.0	19.9 \pm 2.8	14.9 \pm 2.1	10.2 \pm 1.4
Mate-fly	73.5 \pm 10.4	30.6 \pm 4.3	26.8 \pm 3.8	23.3 \pm 3.3	15.6 \pm 2.2	13.7 \pm 1.9	10.8 \pm 1.5
Fly-mate	79.9 \pm 11.4	33.4 \pm 4.8	34.4 \pm 4.9	18.9 \pm 2.7	19.6 \pm 2.8	15.8 \pm 2.3	14.0 \pm 2.0

Only the first 7 days of oviposition is included due to low numbers of eggs produced after the 1st week. Adult females lived 11.8 ± 0.3 days on average.

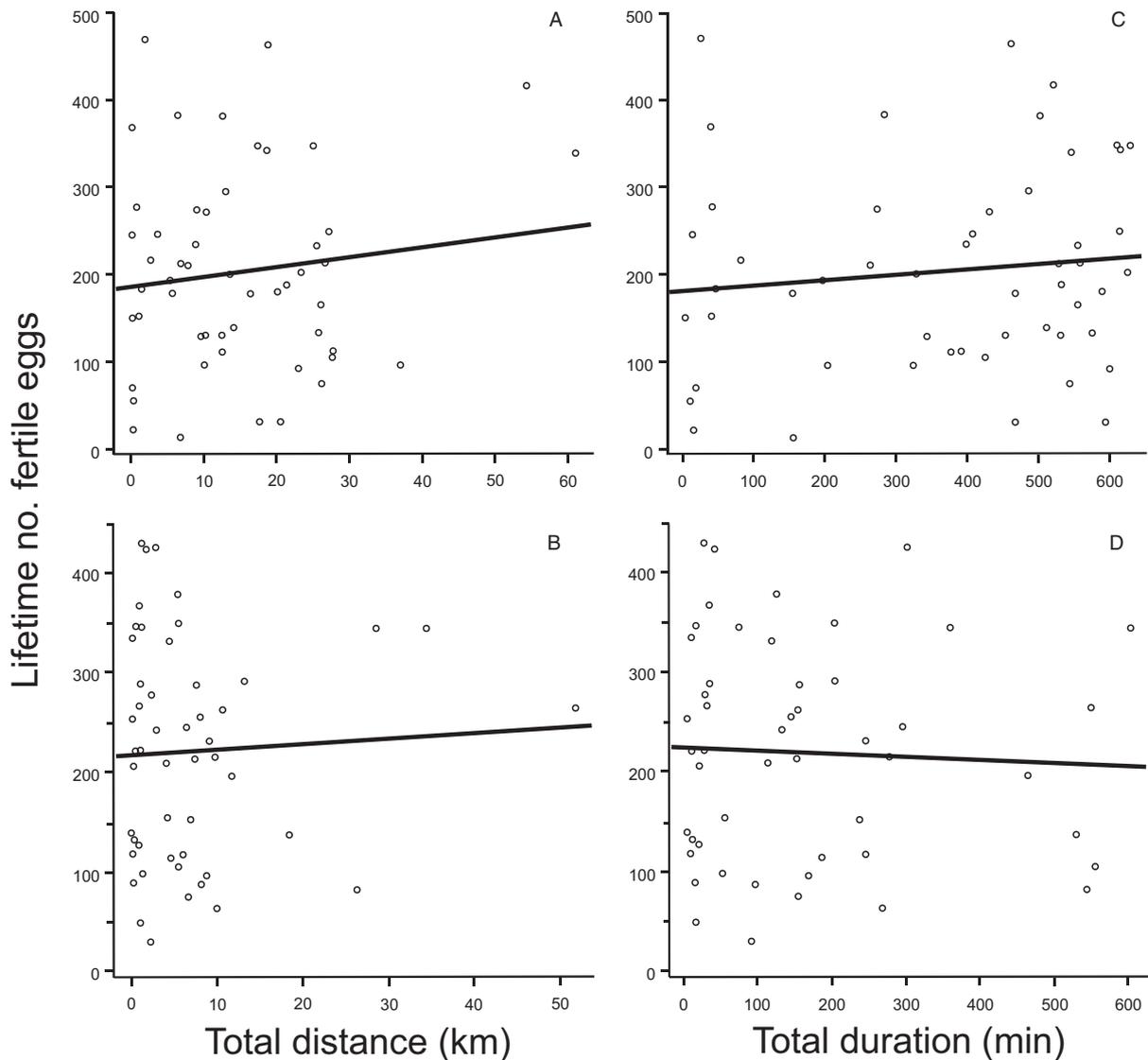


Figure 5 Regressions of lifetime fecundity (no. fertile eggs per female) of navel orangeworm on (A, B) total distance (km) flown and (C, D) total time (min) in flight during a 1-night flight mill trial. (A) Mate-fly group: $y = 0.0011x + 0.019$; $r^2 = 0.003$, d.f. = 50, $P = 0.36$. (B) Fly-mate group: $y = 0.00055x + 0.022$; $r^2 = 0.01$, d.f. = 48, $P = 0.75$. (C) Mate-fly group: $y = 0.6x + 180.5$; $r^2 = 0.006$, d.f. = 50, $P = 0.41$. (D) Fly-mate group: $y = -0.32x + 225.9$; $r^2 = 0.01$; d.f. = 48, $P = 0.73$.

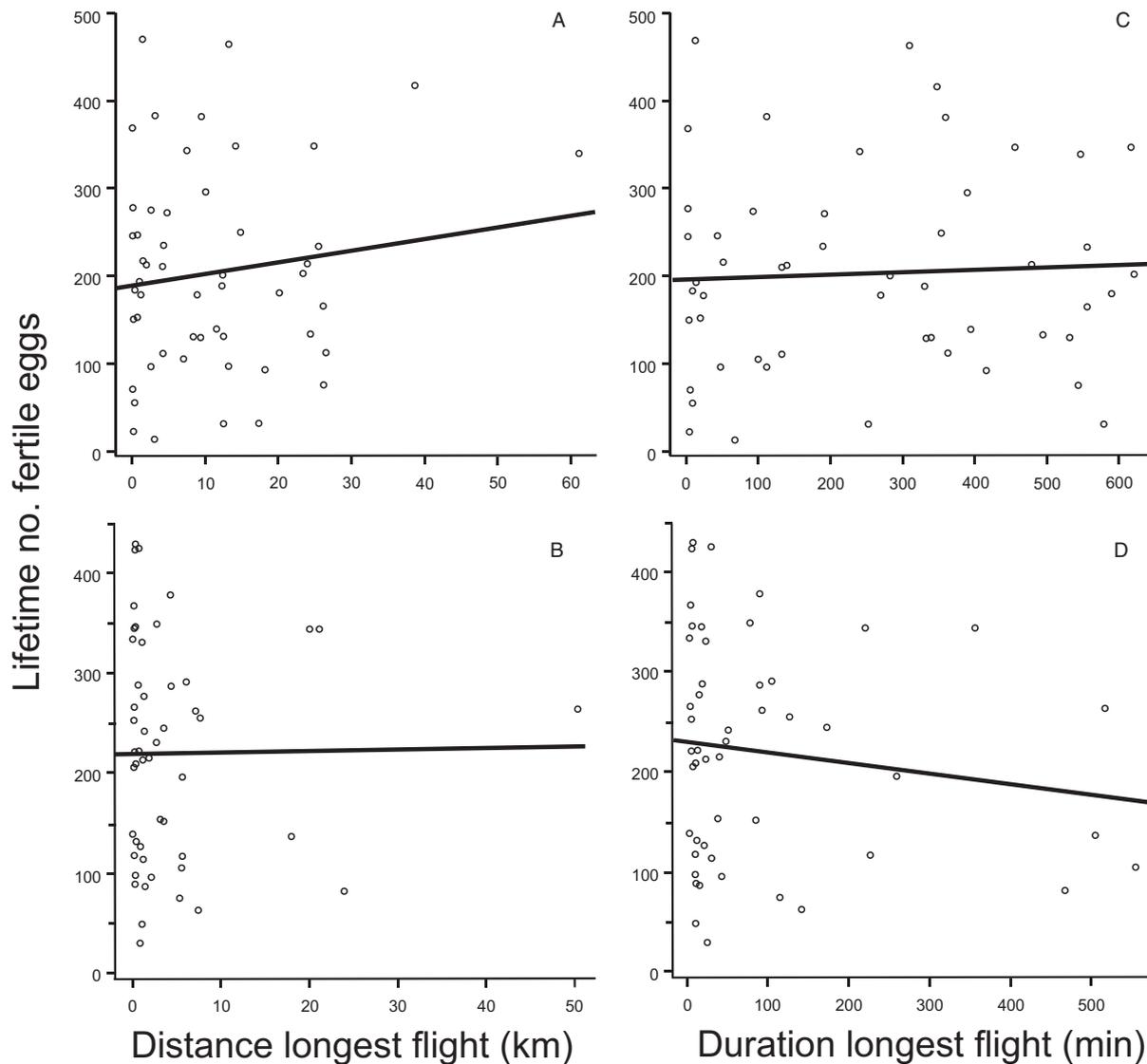


Figure 6 Regressions of lifetime fecundity (no. fertile eggs per female) of navel orangeworm on (A, B) distance (km) and (C, D) duration (min) of the longest single flight during a 1-night flight mill trial. (A) Mate-fly group: $y = 0.0013x + 0.019$; $r^2 = 0.0008$, d.f. = 50, $P = 0.33$. (B) Fly-mate group: $y = 0.00016x + 0.022$; $r^2 = 0.02$, d.f. = 48, $P = 0.92$. (C) Mate-fly group: $y = 0.03x + 196.2$; $r^2 = 0.02$, d.f. = 50, $P = 0.73$. (D) Fly-mate group: $y = -0.11x + 230.5$; $r^2 = 0.0006$, d.f. = 48, $P = 0.33$.

The impact of mating status on flight behavior is species dependent. For example, mating status had no effect on flight performance of beet webworm, *Loxostege sticticalis* L., or oriental fruit moth, *Grapholita molesta* (Busck) (Hughes & Dorn, 2002; Cheng et al., 2012). By contrast, mated female codling moths, *Cydia pomonella* L., were less likely than unmated females to fly ≥ 5 km (Schumacher et al., 1997). Although field and flight mill studies suggest young unmated European corn borer females engage in pre-reproductive migratory flight (Dorhout et al., 2008),

field data (discussed below), along with flight mill and field life-history observations, indicate little dispersal by navel orangeworm females prior to oviposition.

The navel orangeworm has a short adult lifespan and is a capital breeder (Burks, 2014), which means adults depend on larval nutritive reserves for reproduction (Ramaswamy et al., 1997; Jervis et al., 2005). Access to sucrose as an adult does not increase fecundity (Kellen & Hoffmann, 1983; Burks, 2014). Furthermore, as in other Phycitinae (e.g., Huang & Subramanyam, 2003),

oviposition of infertile eggs by unmated navel orangeworm has been observed (Landolt & Curtis, 1982; Kellen & Hoffmann, 1983), suggesting that resorption of eggs to reclaim energy reserves is of limited importance. The available evidence, therefore, indicates that this species depends predominantly or wholly on larval resources for reproduction and dispersal as an adult.

Flight duration was not associated with effects on lifetime fecundity, either negatively or positively. The relative timing of mating before or after flight testing likewise had no effect on fecundity. All flight activity in the flight mill study was restricted to a single 10-h night (plus flanking twilight). Our experiments were not designed to address possible effects on fecundity of cumulative flight activity on subsequent nights, which in nature would include, at a minimum, movement between oviposition sites. Nevertheless, the time spent in flight by females that had mated the previous night was often considerable, with half of those tested flying 3–9 h, including longest continuous flights ranging from about 1 to 7 h. Thus, the lack of effect of our treatments on fecundity is noteworthy.

The reduced fecundity of flight-tested moths compared to minimally handled control moths was associated with tethering, not with flight itself. This conclusion is further supported by the lack of effect of up to 2 h forced flight activity on fecundity. How tethering may have reduced fecundity remains unclear. Tests of adhesives for toxicity during tethering methods development indicated the fabric glue did not reduce lifespan. Moreover, it did not impede flexion of the abdomen enough to prevent mating or completely eliminate oviposition (such moths were excluded), but we cannot rule out a partial physical hindrance of oviposition in some individuals.

Although one cannot directly translate flight mill performance to movement in the field, the distances covered by half the previously mated navel orangeworm females tested were impressive: ca. 6–23 km for total flight, and 1–17 km for the longest continuous flight. This suggests that many recently mated females are easily capable of traversing significant expanses between orchards. Even 1-day-old unmated females are capable of long-distance dispersal (Sappington & Burks, 2014), although they showed a lesser propensity to engage in flight than 2-day-old mated females in this study. Our flight mill data support previous observations that the risk posed to uninfested orchards by moths dispersing from infested hosts extends over several kilometers. In a mark–recapture study, navel orangeworm females evenly distributed eggs within a 375-m radius from the site of eclosion, which was the maximum distance monitored (Andrews et al., 1980). Evidence from mark–recapture studies in conjunction with data

from a large observational study suggests that most females oviposit near the natal site, whereas a smaller proportion disperses longer distances (Andrews et al., 1980; Burks et al., 2006; Higbee & Siegel, 2009). Higbee & Siegel (2009) concluded that most damage by offspring of an established population occurs within a 5-km radius. If moth behavior on flight mills corresponds to straight-line flight, an even greater radius is possible. However, net displacement in the field may be much less if the long flights we observed on the flight mills reflect meandering movement in search of resources (Miller et al., 2015). Despite the greater tendency for long-distance flight by mated females, a substantial number still flew only short distances (e.g., <2 km). The relationship between long-distance dispersal and mating in the navel orangeworm remains difficult to fully characterize, but field and laboratory evidence so far are consistent with a 5-km radius of oviposition (Higbee & Siegel, 2009).

The robust dispersal capacity of adults may help explain the challenges encountered with this species when developing treatment thresholds based on pheromone trap data (Burks et al., 2006; Burks & Higbee, 2013). The number of males captured in a pheromone trap is dependent on the distance over which the plume is attractive, and the distance travelled within the sampling period before encountering the plume (i.e., trapping radius) (Wall & Perry, 1987; Miller et al., 2015; Adams et al., 2017). Our flight mill data indicate that the trapping radius for a 1-week monitoring interval (frequently used by pest management consultants) is potentially very large, which reduces the local specificity of information obtained from navel orangeworm pheromone traps.

In summary, the biological data from this study indicate that there is little pre-oviposition dispersal of navel orangeworm females. Whereas front-loaded oviposition and possibly behavioral attributes seem to moderate interorchard infestation, the data indicate no trade-off between flight and fecundity, and an impressive potential for colonization in this species. These findings are useful to ongoing refinement of management strategies for the navel orangeworm in California's expanding and dynamic nut industries. The finding of little pre-mating movement is an advantage for control with mating disruption (Higbee & Burks, 2008; Burks, 2017) because unmated females in orchard blocks are unlikely to leave in search of a mate. Conversely, the robust dispersal capacity of mated navel orangeworm females creates a vulnerability for mating disruption blocks due to immigration, increasing the importance of large treatment blocks. Similar trade-offs could emerge in pilot experiments with the sterile insect technique (Light et al., 2015): little female dispersal prior to

first mating could be an advantage, whereas the necessity for robust sterile male flight capacity to be competitive with wild males could be a challenge for this approach.

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References

- Adams CG, Schenker PS, McGhee PS, Gut LJ, Brunner JF & Miller JR (2017) Maximizing information yield from pheromone-baited monitoring traps: estimating plume reach, trapping radius, and absolute density of *Cydia pomonella* (Lepidoptera: Tortricidae) in Michigan apple. *Journal of Economic Entomology* 110: 305–318.
- Andrews KL, Barnes MM & Josserand SA (1980) Dispersal and oviposition by the navel orangeworm. *Environmental Entomology* 9: 525–529.
- Beck JJ, Merrill GB, Higbee BS, Light DM & Gee WS (2009) In situ seasonal study of the volatile production of almonds (*Prunus dulcis*) var. 'Nonpareil' and relationship to navel orangeworm. *Journal of Agricultural and Food Chemistry* 57: 3749–3753.
- Beck JJ, Light DM & Gee WS (2014) Electrophysiological responses of male and female *Amyelois transitella* antennae to pistachio and almond host plant volatiles. *Entomologia Experimentalis et Applicata* 153: 217–230.
- Beerwinkle KR, Lopez JD, Cheng D, Lingren PD & Meola RW (1995) Flight potential of feral *Helicoverpa zea* (Lepidoptera: Noctuidae) males measured with a 32-channel, computer-monitored, flight-mill system. *Environmental Entomology* 24: 1122–1130.
- Bentley WJ, Adaskaveg JE, Connell JH, Duncan R, Johnson M et al. (2016a) UC IPM Pest Management Guidelines Almond. UC ANR Publication 3431, Division of Agriculture and Natural Resources, University of California, Oakland, CA, USA.
- Bentley WJ, Beede RH, Fukuda TA, Haviland DR, Hembree KJ et al. (2016b) UC IPM Pest Management Guidelines Pistachio. UC ANR Publication 3640, Division of Agriculture and Natural Resources, University of California, Oakland, CA, USA.
- Burks CS (2014) Effects of delayed mating and access to water on oviposition and longevity in female *Amyelois transitella*. *International Journal of Insect Science* 2014: 89–98.
- Burks CS (2017) Combination phenyl propionate/pheromone traps for monitoring navel orangeworm (Lepidoptera: Pyralidae) in almonds in the vicinity of mating disruption. *Journal of Economic Entomology* 110: 438–446.
- Burks CS & Higbee BS (2013) Effect of abundance of the navel orangeworm on sampling range and interference between pheromone traps. *Environmental Entomology* 42: 143–149.
- Burks CS, Higbee BS & Brandl DG (2006) NOW Mating Disruption, Dispersal, and Damage Prediction. Paper presented at 34th Almond Industry Conference, Modesto, CA, USA. Available at: <https://lf.almonds.com/WebLink/ElectronicFile.aspx?docid=1684&dbid=0> (accessed on 8 April 2018).
- Burks CS, Higbee BS, Brandl DG & Mackey BE (2008) Sampling and pheromone trapping for comparison of abundance of *Amyelois transitella* in almonds and pistachios. *Entomologia Experimentalis et Applicata* 129: 66–76.
- Burks CS, Brandl DG & Higbee BS (2011a) Effect of natural and artificial photoperiods and fluctuating temperature on age of first mating and mating frequency in the navel orangeworm, *Amyelois transitella*. *Journal of Insect Science* 11: 48.
- Burks CS, Higbee BS, Siegel JP & Brandl DG (2011b) Comparison of trapping for eggs, females, and males of the navel orangeworm (Lepidoptera: Pyralidae) in almonds. *Environmental Entomology* 40: 706–713.
- C DFA (2015) California Agricultural Statistics Review 2014–2015. California Department of Food and Agriculture, Sacramento, CA, USA.
- Chakravarti IM, Laha RG & Roy J (1967) Handbook of Methods of Applied Statistics, Vol. 1. John Wiley and Sons, New York, NY, USA.
- Cheng YX, Luo LZ, Jiang XF & Sappington TW (2012) Synchronized oviposition triggered by migratory flight intensifies larval outbreaks of beet webworm. *PLoS ONE* 7: e31562.
- Colvin J & Gatehouse AG (1993) The reproduction-flight syndrome and the inheritance of tethered-flight activity in the cotton-bollworm moth, *Heliothis armigera*. *Physiological Entomology* 18: 16–22.
- Curtis RK & Barnes MM (1977) Oviposition and development of the navel orangeworm in relation to almond maturation. *Journal of Economic Entomology* 70: 395–398.
- Dingle H (2006) Animal migration: is there a common migratory syndrome? *Journal of Ornithology* 147: 212–220.
- Dingle H (2014) Migration: The Biology of Life on the Move, 2nd edn. Oxford University Press, Oxford, UK.
- Dorhout DL, Sappington TW & Rice ME (2008) Evidence for obligate migratory flight behavior in young European corn borer (Lepidoptera: Crambidae) females. *Environmental Entomology* 37: 1280–1290.
- Evenden ML, Whitehouse CM & Jones BC (2015) Resource allocation to flight in an outbreaking forest defoliator *Malacosoma disstria*. *Environmental Entomology* 44: 835–845.
- Finney GL & Brinkman D (1967) Rearing the navel orangeworm. *Journal of Economic Entomology* 60: 1109–1111.
- Gibbons JD & Chakraborti S (2011) Nonparametric Statistical Inference. *International Encyclopedia of Statistical Science* (ed. by M Lovric), pp. 977–979. Springer, Berlin, Germany.
- Higbee BS & Burks CS (2008) Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios. *Journal of Economic Entomology* 101: 1633–1642.

- Higbee BS & Siegel JP (2009) New navel orangeworm sanitation standards could reduce almond damage. *California Agriculture* 63: 24–28.
- Higbee BS & Siegel JP (2012) Field efficacy and application timing of methoxyfenozide, a reduced-risk treatment for control of navel orangeworm (Lepidoptera: Pyralidae) in almond. *Journal of Economic Entomology* 105: 1702–1711.
- Huang F & Subramanyam B (2003) Effects of delayed mating on reproductive performance of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *Journal of Stored Products Research* 39: 53–63.
- Hughes J & Dorn S (2002) Sexual differences in the flight performance of the oriental fruit moth, *Cydia molesta*. *Entomologia Experimentalis et Applicata* 103: 171–182.
- Jervis MA, Boggs CL & Ferns PN (2005) Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecological Entomology* 30: 359–375.
- Kellen WR & Hoffmann DF (1983) Longevity and fecundity of adult *Amyelois transitella* (Lepidoptera: Pyralidae) infected by two small RNA viruses. *Environmental Entomology* 12: 1542–1549.
- Kuenen LPS & Siegel JP (2010) Protracted emergence of overwintering *Amyelois transitella* (Lepidoptera: Pyralidae) from pistachios and almonds in California. *Environmental Entomology* 39: 1059–1067.
- Landolt PJ & Curtis CE (1982) Interspecific sexual attraction between *Pyralis farinalis* L. and *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *Journal of the Kansas Entomological Society* 55: 248–252.
- Light DM, Ovchinnikova I, Jackson ES & Haff RP (2015) Effects of x-ray irradiation on male navel orangeworm moths (Lepidoptera: Pyralidae) on mating, fecundity, fertility, and inherited sterility. *Journal of Economic Entomology* 108: 2200–2212.
- Miller JR, Adams CG, Weston PA & Schenker JH (2015) *Trapping of Small Organisms Moving Randomly: Principles and Applications to Pest Monitoring and Management*. Springer, Cham, Switzerland.
- Parra-Pedrazzoli AL & Leal WS (2006) Sexual behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *Neotropical Entomology* 35: 769–774.
- R Core Team (2016) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ramaswamy SB, Shu S, Park YI & Zeng F (1997) Dynamics of juvenile hormone-mediated gonadotropism in the Lepidoptera. *Archives of Insect Biochemistry and Physiology* 35: 539–558.
- Rice RE (1978) Navel orangeworm: a pest of pistachio nuts in California. *Journal of Economic Entomology* 71: 822–824.
- Sanderson JP, Barnes MM & Seaman WS (1989) Synthesis and validation of a degree-day model for navel orangeworm (Lepidoptera: Pyralidae) development in California almond orchards. *Environmental Entomology* 18: 612–617.
- Sappington TW & Burks CS (2014) Patterns of flight behavior and capacity of unmated navel orangeworm adults (Lepidoptera: Pyralidae) related to age, gender, and wing size. *Environmental Entomology* 43: 696–705.
- Schumacher P, Weyeneth A, Weber DC & Dorn S (1997) Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by a flight mill: influence of sex, mated status and age. *Physiological Entomology* 22: 149–160.
- Shirai Y (1995) Longevity, flight ability and reproductive performance of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), related to adult body size. *Researches on Population Ecology* 37: 269–277.
- Siegel JP & Kuenen LPS (2011) Variable developmental rate and survival of navel orangeworm (Lepidoptera: Pyralidae) on pistachio. *Journal of Economic Entomology* 104: 532–539.
- Siegel JP, Kuenen LPS & Ledbetter C (2010) Variable development rate and survival of navel orangeworm (Lepidoptera: Pyralidae) on wheat bran diet and almonds. *Journal of Economic Entomology* 103: 1250–1257.
- Starnes DS, Tabo D, Yates D & Moore DS (2015) *The Practice of Statistics*, 5th edn. WW Freeman, London, UK.
- Wade WH (1961) Biology of the navel orangeworm, *Paramyelois transitella* (Walker), on almonds and walnuts in northern California. *Hilgardia* 31: 129–171.
- Wall C & Perry JN (1987) Range of action of moth sex-attractant sources. *Entomologia Experimentalis et Applicata* 44: 5–14.
- Zhang L, Pan P, Sappington TW, Lu W, Luo L & Jiang X (2015) Accelerated and synchronized oviposition induced by flight of young females may intensify larval outbreaks of the rice leaf roller. *PLoS ONE* 10: e0121821.