Impact of Sex, Age and Mating Status on Flight Behavior of the Navel Orangeworm (NOW)

Project No.:   **12-ENTO2-Burks**

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Objectives:

1) Adapt flight mill methods for the navel orangeworm.
2) Examine age-specific flight capacity of unmated navel orangeworm adults at significant intervals of the adult life.
3) Examine the impact of mating on laboratory flight capacity.

Here we report on completion of the first two objectives in 2012.

Interpretive Summary:

The navel orangeworm *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) is the principal pest of California almonds and pistachios. The dispersal ability of this moth is an important aspect of its ability to inhabit orchards and infest marketable nuts. We conducted a flight mill study examining the flight capacity of unmated female and male navel orangeworm adults 1, 2, 3, 5, and 7 days (d) post eclosion. Females were stronger fliers than males. There was also a qualitative difference between the sexes in flight: unmated females had the most separate flights per night at 2-3 d post-harvest, whereas unmated males had the fewest flights per night at 2 d post-harvest. Compared to previous studies, unmated navel orangeworm adults exhibited flight capacity intermediate between that of tortricid orchard pest moths (e.g., codling moth and oriental fruit moth, which is less), and that of noctuids (e.g., armyworm and cutworm species, which is more). The median longest single flight was >2 km for all ages tested, and >4.5 km for all ages ≤5 days. These observations are consistent with a previous study which found higher navel orangeworm damage ≤4.8 km from sources of high navel orangeworm abundance.

Materials and Methods:

Insect Culture. Insects used were obtained as eggs from an almond orchard in western Fresno County in September 2010, and the colony was refreshed with infusion of individuals taken at the same time, place and manner in September 2011. The colony was maintained at 26°C and 14:10 (L:D) on wheat bran diet (Tebbets et al. 1978). This line had been in culture from 20 to 26 generations (from 2010) at the time of the experiments. Sexes were separated as mature larvae based on the presence or absence of testes (visible through the male dorsal

integument). Male and female larvae nearing pupation were sent once a week from Parlier, CA to Ames, IA via overnight express. Pupae were checked daily for adult emergence. Newly-emerged adults were placed in sex-specific holding cages at 26°C and 14:10 (Light:Dark, or L:D) until they reached the appropriate age for flight tests. The holding cages consisted of a wire-mesh cylinder 13.5 cm in diameter and 15.5 cm tall. Adults were provided free access to water via soaked cotton.

Adult Tethering and Flight Mill Procedures. Tethering and flight mill procedures were based on those described by Dorhout et al. (2008). Adults were attached to flight mills with a tether consisting of a 0.25mm diameter wire, about 5 cm long, and bent at a 90 angle and connected to the flight mill arm by a small sleeve of hollow plastic tubing stripped from copper wire. Moths were sedated by brief cooling in a reach-in freezer at -20°C for <3 min. A 1-mm diameter loop at the end of the 2cm length was attached to the dorsal surface of the junction between the mesothorax and the abdomen with a small amount of Insta-Cure+ glue (Bob Smith Industries, Atascadero, CA). After attachment to the flight arm, a small piece of tissue paper was provided for tarsal contact (Sappington and Showers 1991), which the moths readily grasped. This reduced escape behavior and trivial flight prior to dusk, while allowing spontaneous flight initiation.

Fifteen flight mills were housed in a walk-in environmental chamber maintained at 26°C and 14:10 (L:D). Lights were automatically and gradually dimmed over a 30-min period beginning before lights-off to simulate dusk, and automatically ramped-up beginning 30-min before lights-on to simulate dawn. Each was attached to a Gateway 2000 personal computer by interface hardware and software described by Beerwinkle et al. (1995). The flight mill arm consisted of a triangle-shaped flat piece of aluminum (256 mm long, 156 mm from tip to pivot, 15 mm at the base end). The tether attachment sleeve described in the previous paragraph was slipped over the end of this arm, and the weight of the attached moth was counterbalanced with a moveable clip on the end opposite adult attachment. The setup allowed the adult to fly in a horizontal plane, completing a distance of 1-m per revolution around the central pivot and the pin on which it rested. Revolutions of the flight arm were sensed by an infrared eye below the pin. A vinyl tent surrounded each mill to reduce air movement that might cause the flight arm to drift and generate spurious readings.

Males and females were tested separately on given nights to prevent the confounding influence of female sex pheromone. Adults that emerged the previous evening or night were classified as 1 d old when flown on flight mills the following evening (i.e., first evening after emergence). Unmated males and females were flown at ages 1, 2, 3, 5 and 7 days post eclosion. Each individual was flown only once, so data for each age category represent independent trials of moths held in cages without flying until the night of attachment. In the laboratory mortality is minimal prior to 7 days post eclosion but, in females mated once 1 day after eclosion, most of total lifetime fecundity has been realized by this age (Burks, unpublished data).

Data Analysis. The number of revolutions was compiled in 1-minute intervals for each flight mill. A flight was considered terminated if there was not a single revolution of the flight arm in a minute. Adults that flew ≤2 minutes were excluded from analysis to avoid including adults that were artificactually unable or indisposed to fly because of handling associated with the flight

mill procedures. Flight characteristics examined for each moth included the duration of the longest uninterrupted flight, the duration of all flights, the distance of the longest uninterrupted flight, the total distance of all flights, and the number of independent flights. Flight activity before the onset of dusk or after lights-on was excluded from analysis, resulting in a 10.5-hour (h) window of possible flight activity.

Data associated with duration and distance of flight, were transformed as the square root of the observation to stabilize variation and improve symmetry of the frequency distribution. Analysis of variance was used for these variables because Levene's test did find significant departure from homogeneity of variance, and because residual plots indicated that the assumption of random distribution of the error was not violated. A two-way ANOVA was used for the first four variables (duration of longest flight, total flight duration, distance of longest flight, and total distance flown). Dependent variables included age, sex, and their interaction.

The number of flights did not meet the assumptions for ANOVA but did fit a generalized linear model with a negative binomial distribution, as determined by the ratio of deviance value with degrees of freedom. Initial examination of number of flights found a significant interaction between age and sex, so data were analyzed separately between the sexes.

The Tukey adjustment for multiple comparisons was used when comparing age classes. Fisher's Exact Test was used to compare the proportion of non-fliers between sex and age categories. Statistical analysis was performed using the SAS System (SAS Institute Inc. 2008), and box plots were generated using SigmaPlot (Systat Software Inc. 2008). Untransformed means and errors are presented in the tables and figures.

Results and Discussion:

Of 572 adults tested, 533 were classified as fliers (i.e., 93% made at least one flight of longer than 2 min). The proportion of non-fliers was not significantly different between age and sex categories (Fisher's Exact Test, $P > 0.05$).

Unmated navel orangeworm adults exhibited a substantial propensity for flight over the first 7 d of adult life, as demonstrated by longest single flights of at least a third of the available 10.5 hours from dusk to dawn, and by low numbers of flights (i.e., few interruptions) (**Table 1**). There were significant differences among age categories in the duration of the longest flight, total flight duration, the distance of the longest flight, and total distance flown (**Table 2**). The oldest age category, however, was the only one in which adults flew significantly less than 1- or 2-d-old adults (**Table 1**). The median velocity of the longest single flight ranged from 0.48 to 0.72 m/s, and was similar between males and females.

Different trends were evident between the sexes. Initial analysis of flights per night found an age-sex interaction, as is evident from the data (**Table 1**). Subsequently the effect of age category on flights per night was analyzed separately between the sexes. There were significant differences between the number of flights per night for both females ($x^2 = 19.92$, df $= 4$; $P = 0.0005$) and males ($x^2 = 14.63$, df $= 4$; $P = 0.0055$). The number of separate flights was greatest in females 2- and 3 d post eclosion, whereas 2-d-old males had the fewest separate flights of all age categories (**Table 1**). There was also a significant effect due to sex

on total distance and total duration of flight, although not for the longest individual flight time or distance (**Table 2**); females flew for a longer total time and greater total distance than males (**Table 1**). Box plots of data for the longest single flight reveal a considerable degree of variability in both duration (**Figure 1**) and distance (**Figure 2**). These data suggest greater difference between the sexes in the upper extreme than in the central tendency.

The flight performance found for navel orangeworm adults in this study was generally intermediate between that found in previous studies of Tortricidae (Schummacher et al. 1997a, Hughes and Dorn 2002, Hughes et al. 2004, Elliot and Evenden 2009) and Pyraloidae (Dorhout et al. 2008 and 2011; Sarvary et al. 2008), and that found in Noctuidae (Sappington and Showers 1992, 1993; Beerwinkle et al. 1995, Jiang et al. 2010, Tu et al. 2010). Compared to these taxa, the Noctuidae are generally noted as stronger fliers and long-distance migrants. The Noctuidae also tend to live longer and have a longer pre-ovipositional period. Compared to the Tortricidae and other Pyraloidae, the navel orangeworm is particularly notable for greater distance of the longest single flight. The total distance flown by navel orangeworm adults is closer to the range reported for the Oriental fruit moth, *Grapholita molesta* Busck, and the codling moth, *Cydia pomonella* (L.). The navel orangeworm is also notable for having fewer flights compared to other moth species studied with flight mills. These observations indicate that, in laboratory settings, the navel orangeworm is more likely than the other Pyraloidae and Tortricidae to remain in flight once flight is commenced.

Both quantitative and qualitative difference in flight between unmated navel orangeworm males and females are evident from the data in this study. Quantitatively, navel orangeworm females are somewhat stronger fliers than males, as evident from the significant effect of sex for total flight duration and distance. Stronger female flight performance has also been noted in other Pyraloidea (Dorhout et al. 2008 and 2011; Sarvary et al. 2008) and Tortricidae (Hughes and Dorn 2002, Hughes et al. 2004, Elliot and Evenden 2009) studied, with the exception of the codling moth (Schummacher et al. 1997a). In many of these species the female is also larger than the male, suggesting that this difference may be due in part to greater wing area and/or greater energetic reserves. Collectively, the data suggest that, in 2- and 3-d-old navel orangeworm, males made fewer and longer flights, whereas females made more but slightly shorter flights (**Table 1**). In some other moth species, age-specific differences between the flight behavior of males and females on flight mills are connected with mating status. For example, the both sexes of codling moth fly farther when unmated than when mated, but the greatest distance for longest single flight is attained slightly earlier for unmated females than males (Schumacher et al. 1997a). Mated females of the large aspen tortrix fly farther than males or unmated females. In the European corn borer, however, females prior to the age of first mating have greater flight capacity compared to same-aged males or older females, whether or not mated (Dorhout et al. 2008).

The findings from the present study are generally consistent with field observations. For example, a study of navel orangeworm damage patterns in almonds found significantly more damage in nuts harvested from fields within 4.8 km from pistachios compared to those farther away (Higbee and Siegel 2009). A previous study in that region established that pistachios generally harbored a greater abundance of navel orangeworm than almonds (Burks et al. 2008). The present study finds that median distance of the longest single flight is >4.8 km for 1-, 2-, and 5-day-old females. Most codling moth adults fly short distances while a small

proportion fly over 5 km, consistent with field data for that species (Schumacher et al. 1997a, Keil et al. 2001). There is evidence for a genetic equilibrium between dispersal and fecundity for the codling moth (Schumacher et al. 1997b, Gu et al. 2006), and in the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera:Tortricidae) (Gu and Danthanarayana 1992, 2000). In these species, more fecund females develop more quickly and have greater body weight, while better fliers develop more slowly and are lighter. The laboratory flight mill data for mated navel orangeworm females, when available, will provide a useful comparison for the previous field observations (Higbee and Siegel 2009) and observations from other species.

Research Effort Recent Publications:

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Table 1. Duration and distance of flights (mean ± SEM) and median number of flights of navel orangeworm adults, by age and sex, on laboratory flight mills during 10.5 h of scotophase(dark) including 0.5 h each of gradual dusk and dawn.

Sex	Age	n	Duration (min) of longest uninterrupted flights	Duration (min) of all flights	Distance (km) of longest uninter-rupted flights	Total distance (km) of all flights	Median number of flights
F	1	51	249 ± 28a	316 ± 27a	11.6 ± 1.6a	13.8 ± 1.5a	6b
	2	52	255 ± 29a	380 ± 25a	12.2 ± 1.8a	15.2 ± 1.7a	11.5a
	3	58	199 ± 26ab	324 ± 24ab	7.8 ± 1.4a	10.3 ± 1.5ab	13.5a
	5	55	235 ± 29a	344 ± 27a	10.4 ± 1.7ab	13.2 ± 1.7ab	9a
	7	56	173 ± 25b	283 ± 25b	7.3 ± 1.5b	9.7 ± 1.5b	8a
M	1	52	192 ± 25a	280 ± 22a	7.7 ± 1.0a	10.7 ± 1.0a	7.5A
	2	51	271 ± 29a	334 ± 28a	10.2 ± 1.4a	11.8 ± 1.3a	4B
	3	54	213 ± 28ab	303 ± 25ab	8.2 ± 1.2a	10.1 ± 1.1ab	6A
	5	50	222 ± 27a	314 ± 28a	7.8 ± 1.2ab	9.5 ± 1.2ab	6.5A
	7	54	121 ± 19b	216 ± 22b	4.8 ± 1.0b	6.9 ± 1.1b	11.5A

Observations exclude individuals dead at the end of the night and those which flew <3 min. Means within the same column sharing the same lowercase subscript are not significantly different (P > 0.05). The sexes were analyzed separately for median number of flights, and the median values sharing the same upper- or lowercase subscript are not significantly different.

Table 2. Values of F and P from analyses of variance for duration and distance of laboratory flight by navel orangeworm adults.

Component	Maximum duration		Total duration		Maximum distance		Total distance	
	F	P	F	P	F	P	F	P
Model	2.84	0.0028	2.62	0.0057	3.16	0.0010	3.37	0.0005
Age	5.42	0.0003	5.29	0.0019	5.57	0.0002	5.56	0.0002
Sex	0.90	0.3425	4.33	0.0218	3.34	0.0683	5.88	0.0156
Interaction	0.79	0.5306	0.26	0.9201	0.75	0.5553	0.63	0.6419

The numerator df are respectively 9, 1, 4, and 4 for the model, age, sex, and interaction. The denominator df is 523.
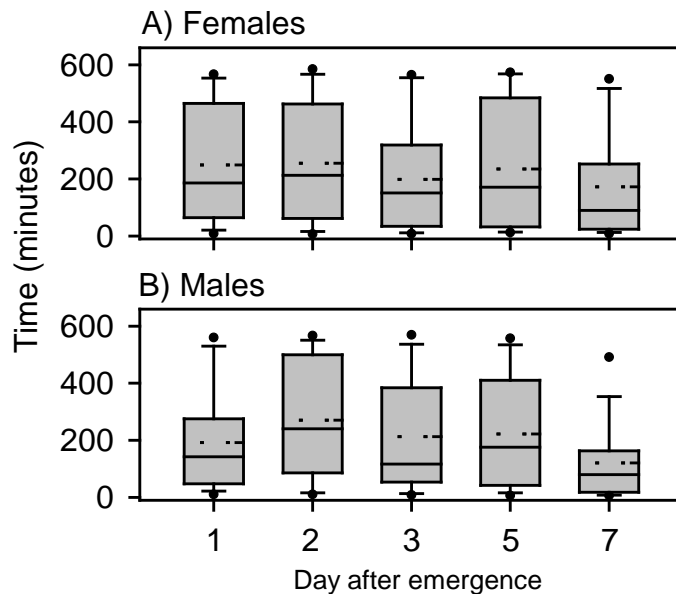

Figure 1. Boxplot depicting duration of the longest uninterrupted flight of navel orangeworm adults, by sex and age. The box represents the 25th and 75th percentiles, the whiskers the 10th and 90th percentiles, and dots indicate the 5th percentile and 95th percentiles.
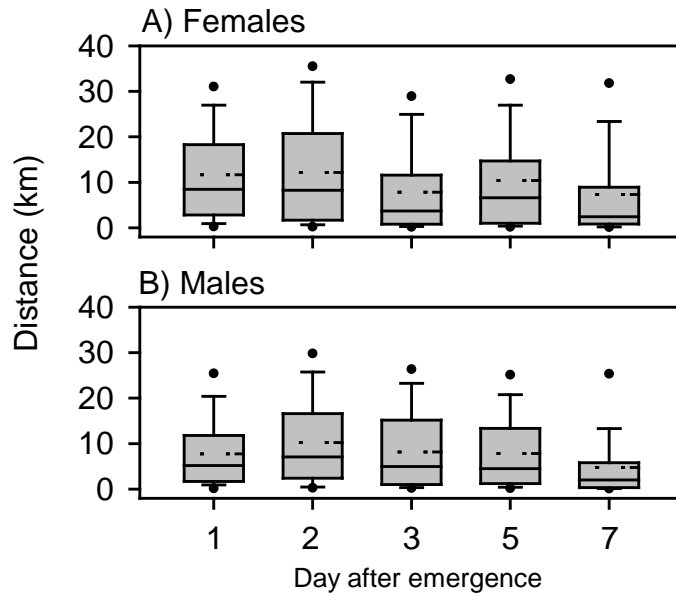
Figure 2. Boxplot (as described in **Figure 1**) depicting the distance of longest uninterrupted flight of navel orangeworm adults by sex and age. Median distances the longest flight were 8.5, 8.2, 3.7, 6.6, and 2.4 km for females of respective ages 1, 2, 3, 5, and 7 d post eclosion; and 5.2, 7.1, 5.0, 4.5, and 2.0 km for males of these respective ages.