Combined Effects of Heat Stress and Food Supply on Flight Performance of Olive Fruit Fly (Diptera: Tephritidae)

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ABSTRACT The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is a newly invasive pest of olives, *Olea europaea* L., in California. The table olive industry is located in California's Central Valley, where daily high summer temperatures can be >35.0°C. This study investigated the effects of high temperatures (heat stress) and food conditions on the flight performance of *B. oleae* in laboratory flight mill tests. Flies were provided food (honey and hydrolyzed yeast) and water for a 1-wk preconditioning period and then subjected to 24-h preflight exposure to diurnal temperature regimes (low-high temperatures of 18.3–35.0°C and 18–37.8°C) and deprivation of food. Flies with the preflight stress conditions had significantly lower flight performance (1,305 m and 0.989 h at 18.3–35.0°C and 1,152 m and 0.966 h at 18.3–37.8°C) than control files that were held under no-stress preflight conditions (constant 23.9°C, food, and water) and flew 1,982 m for 1.54 h. Flight distance and duration were further reduced when no water was provided during the 24-h preflight exposure to high temperature stress. Flight distance and duration also were decreased when the preflight exposure period was increased to 2 and 3 d. When flies were deprived of food and water during the preconditioning period, there was significant adult mortality and flight performance was poor (<50 m and <2 min) after 24-h preflight exposure to either the 18.3–35.0°C or the 18.3–37.8°C temperature regime and deprivation of food. Heat stress and food deprivation also reduced postflight fecundity and adult longevity. The results are discussed with respect to the ability of *B. oleae* to survive summer heat and food deprivation by dispersing to refuges with food, water, and shelter.

KEY WORDS olive fruit fly, flight performance, food supply, heat stress

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is a major pest of cultivated olive, *Olea europaea* L., throughout the Mediterranean Basin and the Middle East (Tzanakakis 2006). It invaded California ≈1998 and quickly spread throughout the state (Rice et al. 2003, Yokoyama et al. 2006), where it has become the most important pest of the state's olive industry. California table olive processors maintain a zero tolerance level for *B. oleae* in fruit. For that reason, current management strategies for *B. oleae* in California rely on the application of the spinosad-formulated GF-120 NF Naturalyte Fruit Fly Bait (Dow AgroSciences LLC, Indianapolis, IN), which is typically applied every 1–2 wk from just before olive pit hardening in early summer until fruit are harvested in fall for table olives or in winter for oil production (Johnson et al. 2006). Repeated pesticide applications not only increase control costs but may impact biological control agents of *B. oleae* and other olive pests (Collier and van Steenwyk 2003, Johnson and Daane 2006, Nadel et al. 2007).

Most table olive production is in California's Central Valley, where the summer is extremely hot; daily maximum temperatures are consistently >35°C during July and August (Wang et al. 2009a). There is rarely any rain and little or no morning dew during the dry summer. Water within the orchards mainly comes from various types of irrigation (e.g., flood, micro-jet emitters), which is only periodically applied (e.g., once weekly). However, large water sources (e.g., irrigation canals, creeks, ponds, runoff reservoirs) may exist outside of the orchards within a few meters to several kilometers and would require *B. oleae* flight to visit. Potential food sources, such as honeydew from black scale, *Saissetia oleae* (Olivier), also may be limited during the summer when *S. oleae* populations drop in number and shift to the smaller development stages, which produce little honeydew (Daane and Caltagirone 1989).

Normal activity of adult *B. oleae* occurs from 20 to 30°C; above this temperature, the flies move frantically and oviposition is thereby inhibited, and at 35°C activity ceases (Avidov 1958). Adult *B. oleae* died after exposure for several days at high temperatures (>35°C) when they did not have access to both water and honey, but individuals could survive if adequate

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levels of both water and food sources (e.g., honey) were provided (Johnson et al. 2006). These researchers also observed that adult *B. oleae* behavior is dramatically altered at high temperatures, such that flies cease most activities except to seek out sources of water. However, even on extremely hot summer days the more favorable lower temperatures occur in the early morning and late evening in the Central Valley, and adult *B. oleae* may be still active during the cooler morning periods (Wang et al. 2009a). It may be assumed that adult *B. oleae* need to seek out a cool refuge within or near the olive orchard during the warmer periods of the day, and the heat stressed *B. oleae* must travel to obtain both water and food sources during the cool periods of the day. Therefore, the most critical abiotic mortality factors for adult *B. oleae* in Central Valley olive regions may be the hot summer temperatures and food availability. Understanding the effects of those mortality factors on the fly's survival and dispersal ability is fundamental for effective management of this invasive pest.

It is, however, unknown whether levels of heat stress or food scarcity reduce the flight and dispersal ability of *B. oleae* during the summer, thereby limiting the pest's ability to find water, food, and refugia resulting in increased mortality. Many fruit flies disperse in search of food, host plants, shelter, or to escape unfavorable environmental conditions (Tsiropoulos 1992, Fontellas and Zucoloto 2003). For food, many adult fruit fly species use sugars (i.e., carbohydrates) as energy sources to fuel various activities, such as flight (Fletcher and Kapatos 1981, Tsiropoulos 1992, Fontellas and Zucoloto 2003); and in addition, they require nitrogenous food sources (e.g., amino acids, protein) for reproductive development and longevity (Hendriches and Prokopy 1994, Drew and Yuval 2000). Under moderate temperature conditions and with adequate water and food, most fruit fly species are strong fliers and can disperse great distances (Shaw et al. 1967, Fletcher and Kapatos 1981). In the absence of fruit, adult *B. oleae* could travel several kilometers within a few days to find host fruit (Economopoulos et al. 1978, Fletcher and Kapatos 1983). Trapping studies in Greece also showed that adult *B. oleae* moved up to 200 m from olive groves to nonhosts in search of food (Katsoyannos 1983). However, the ability of heat stressed fruit flies to disperse during periods of high temperatures and food deprivation is poorly understood, even for many of the well known species, including *B. oleae.* A better understanding of the factors influencing *B. oleae* flight capacity would greatly enhance our ability to predict its occurrence and dispersal abilities. The aims of this study were to quantify the effects of prefight exposure to different levels of heat stress and food conditions on the flight performance of *B. oleae.*

Materials and Methods

Insects. A colony of *B. oleae* was established in a controlled room (23.9 ± 2°C, 40–60% RH, and a photoperiod of 14:10 [L:D] h) at the University of California Kearney Agricultural Center (KAC), Parlier, CA, and maintained on olive fruit since 2003. The initial *B. oleae* for the colony were collected at Davis, CA; periodic additions of field-collected flies from Fresno, CA, were made to the colony. Adult flies were held in screen cages (61 by 61 by 61 cm) (BugDorm2, BioQuip Products Inc., Rancho Dominguez, CA) that were provisioned with water, honey, and hydrolyzed yeast (FisherBiotech, Fairlawn, NJ). Olives were exposed to >2-wk-old fecund females within the cages until each fruit had three to five ovipositional stings. The infested olives were then distributed over a metal grid (1-cm weave) that rested 2 cm above a plastic tray (36 by 18 by 10 cm). When larvae matured, after 9–12 d, they dropped into the tray where puparia were collected and placed into holding cages. Laboratory-reared flies were used for flight mill tests.

Table 1. Preconditioning and preflight treatments for testing *B. oleae* flight performance

Treatment[a]	Preconditioning period		Preflight period	
	Day	Food and water	Food and water	Temp (°C)
FW-FW-23.9	7	Provided	Provided	23.9
FW-W-35.0	7	Provided	Water only	18.3–35.0
FW-W-37.8	7	Provided	Water only	18.3–37.8
FW-NFW-35.0	7	Provided	No	18.3–35.0
FW-NFW-37.8	7	Provided	No	18.3–37.8
NFW-NFW-35.0	1–2	No	No	18.3–35.0
NFW-NFW-37.8	1–2	No	No	18.3–37.8

[a] Newly emerged flies were first held at 23.9°C and provided with food and water (FW) or denied food and water (NFW) during a 7- or 1–2-d preconditioning period, respectively, and were then subjected to 1-d preflight exposure under 23.9°C with food and water, or under one of two diurnal temperature regimes (low temperature 18.3°C from 1900 to 1200 hours and high temperature 35.0 or 37.8°C from 1200 to 1900 hours) without food and with or without water (W). Photophase always ran from 0600 to 2000 hours.

Preconditioning and Preflight Treatment. To quantify the impacts of preflight exposure to daily high temperatures (heat stress) and food (honey and hydrolyzed yeast) and water deprivation on *B. oleae* flight ability, the experiment consisted of seven treatments of increasing heat stress, food and water deprivation, or a combination. In five treatments, the flies were initially held for 1 wk under the same laboratory conditions used for maintaining the fly colony (as described above) with food and water (FW) provided upon eclosion (i.e., preconditioning period, necessary for the maturation of eggs in female flies) (Table 1). Afterward, they were subjected to different preflight treatments for 24 h (i.e., preflight period). In the last two treatments, the flies were completely deprived of food and water (NFW) upon eclosion. Because flies could not survive for 1 wk without food and water, in these two treatments the flies were initially held only for 1–2 d before they were subjected to different preflight treatments for 24 h before the flight tests. Food alone treatment was not considered in this study, because flies had to stay close to water sources for survival when temperature was >35°C.

There were three different temperature regimes and three different food/water conditions during the 24 h preflight treatment (Table 1). The temperature regimes included a constant temperature (23.9 ± 2°C) and two diurnal temperature regimes. The temperature cycle for both diurnal regimes was 18.3 ± 1.0°C from 1900 to 1200 hours and 35.0 ± 1.0 or 37.8 ± 1.0°C from 1200 through 1900 hours. Photophase ran from 0600 to 2000 hours for all temperature regimes. According to historical temperature recordings (Johnson et al. 2006), daily maximum temperature in most of the Central Valley is consistently >35°C during July and August. It commonly reaches or surpasses 37.8°C from 15 July to 20 August in the southern Central Valley (i.e., San Joaquin Valley). Thus, the diurnal temperature regimes reflected the field temperature conditions and the mid-day rise in temperature during midsummer in the Central Valley (Wang et al. 2009a). During the temperature treatment, flies were provided either with food and water (FW), water only (W), or no food and water (NFW) (Table 1).

To examine the possible impacts of extended exposure to four preflight conditions (FW-W-35.0, FW-W-37.8, FW-NFW-35.0, FW-NFW-37.8; see Table 1) on *B. oleae* flight performance, additional tests with the identical preflight conditions, but with additional exposure days (2 or 3 d) were conducted. No additional tests with increased exposure days were conducted for the other two preflight treatments (NFW-NFW-35.0, NFW-NFW-37.8) in which no food or water were ever offered to the flies due to high preflight mortality after 24-h exposure.

Flight Mill Assays. Immediately after the preflight treatments, flight performance was monitored with a flight mill system developed by Zermeño (2005) at California State University, East Bay, Hayward, CA. It consisted of a light weight, plastic rotating arm (i.e., plastic drinking straw) with a reflective element on one end of the rotating arm and a support for an attached fly at the opposite end. To facilitate handling by reducing their movement, flies were held at 2°C for 2 min and then attached to the flight mill tether over a Chill Table (BioQuip Products Inc.). A tethering saddle (i.e., small cylindrical piece of wire insulation) was placed on the end of a #1 insect pin and attached to the mesonotum of a fly with acrylic polymer glue (Polysciences, Warrington, PA). This pin with tethered fly was then inserted into the support end of the rotating arm, and the fly was maneuvered into a horizontal position for flight. Most cold-anesthetized flies recovered to normal activity within 1–2 min under the laboratory conditions described above and started flight. Each complete rotation of the flight mill arm was detected by an infrared emitter-detection unit that was connected to a computer to record the number of laps flown, time per lap, and cumulative flight time.

Four flight mills were operated simultaneously, which allowed 2–5 flies from each treatment to be tested each day, with trials performed between 1000 and 1600 hours. For each treatment, ≈30 males and females each (60 total) were collected into an acrylic screened cage (30 by 30 by 30 cm), and the cage was placed inside a temperature cabinet set at the temperatures and food conditions for the preconditioning and preflight treatments described above. This preflight test was repeated 10–20 times for each treatment. Immediately after the preflight treatment, a fly was randomly selected from the cage and tethered as described above. Once the insect started flight, computers continually recorded the distance and duration of the flight for each tested individual. An observation was terminated when the fly stopped wing movement for ≈10 s. Some flies were observed to resume flight after a break, but we only recorded the duration of the maximum unbroken flight for each tested fly since the flight was initiated. The flight speed was calculated based on the measured flight distance and duration. Some flies died during the preflight treatment, whereas other flies failed to fly after being tethered, possibly due to the extreme heat stress and food/water deprivation. Both preflight mortality and the number of flies that failed to fly were recorded for each treatment. In total, 720 flies were tested; each treatment initially consisted of 25 males and 25 females from 10 to 20 separate tests. Trials were conducted under the same laboratory conditions used for maintaining the fly colony as described above.

Postflight Fecundity and Longevity. To determine the effects of the preflight treatments and flight exhaustion on the postflight fecundity and longevity of *B. oleae*, each tested fly from the above-described flight mill tests was removed from the tether and was placed in a cylindrical cage (15 by 15 by 20 cm) made of a plastic container that had two organdy screen holes (5 cm in diameter) for ventilation. Water and food were provided for the fly until it died. The longevity of each fly was recorded daily. Each female was provided with five green olive fruit for the first 24 h to evaluate its postflight fecundity. Exposed fruit were maintained in 300-ml containers until any resulting offspring developed. The flies from the last two treatments (see Table 1) were not tested for postflight fecundity because they were 2–3 d old and not yet sexually mature when the study was begun.

To better determine the impacts of flight, per se, on postflight fecundity and longevity, an additional treatment served as an untreated check. Flies were held under constant conditions (23.9 ± 2°C with food and water), but were not tested for flight, were tested relative to 24 h fecundity of females and longevity of both sexes.

Data Analysis. Although experimental treatments consisted of different preflight temperature and food conditions, our particular interests were to determine the combined effects of increasing levels of heat stress and/or food and water deprivation that reflected field situations. Thus, data on preflight mortality and flight performance resulting from 24 h preflight treatment were analyzed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test for comparison of means among different treatments. Among the four balanced treatments (i.e., with identical condition during preconditioning pe-

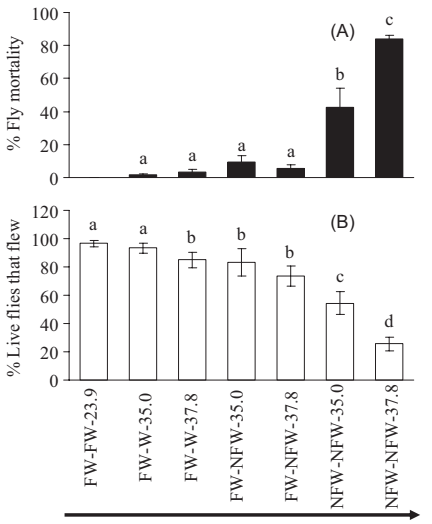

Fig. 1. Effect of preconditioning and preflight treatments on *B. oleae* mortality (A) and flight success (B). Values are means ± SE (n = 10–20) and different letters above each bar are significantly different ($P < 0.05$; Tukey's HSD test). Arrow indicates increased preflight stress conditions (see Table 1).

riod but with different temperature regimes, water conditions, and exposure days during preflight treatment) the data were further analyzed using three-way ANOVA. In all above analyses, flight distance data were $\log_{10}$-transformed to stabilize variation. Preliminary analyses showed that there were no differences between males and females in all measured parameters in each treatment and, for this reason, these data for males and females were pooled. Data on postflight fecundity and longevity from three different days of exposure were pooled due to small samples and also analyzed using one-way ANOVA among different treatments. All analyses were performed by the software of JMP, version 6.0.3 (SAS Institute, Cary, NC).

Results

Preflight Mortality and Flight Success. Preflight heat stress and deprivation of food and water increased preflight mortality ($F_{6,\ 84} = 62.5$; $P < 0.01$) (Fig. 1A) and decreased the percentages of live flies that flew ($F_{6,\ 84} = 23.0$; $P < 0.01$) (Fig. 1B). No flies died before tests when they were held under the standard laboratory conditions (23.9°C, food and water always provided), and 96.7% of the tested adults were able to fly. When flies were provided with food and water and held for 1 wk under 23.9°C during the preconditioning period, there was no significant effect of 24-h preflight treatments of different temperature regimes (18.3–35.0 or 18.3–37.8°C) with or without water on mortality, which was low (<10%) across all the four treatments (Fig. 1A). But flies that were provided with water and held at 18.3–35.0°C had better flight ability than the flies denied access to water and held at the same temperature regime or than the flies held under 18.3–37.8°C temperature regime (Fig. 1B). In contrast, when flies were completely deprived of food and water before or during the preflight treatment, a mean of 42.8% flies died after 24-h exposure to 18.3–35.0°C and 45.7% of live flies failed to fly, and a mean of 83.9% flies died after 24-h exposure to 18.3–37.8°C and 74.3% of tested flies failed to fly.

For the four treatments in which the flies were first provided with food and water at 23.9°C during the 7-d preconditioning period, and then exposed to two different temperature regimes (18.3–35.0 or 18.3–37.8°C) with or without water, three-way ANOVA analyses showed that preflight mortality (Table 2) increased with exposure period ($F_{2,\ 131} = 102.1$; $P < 0.01$), deprivation of water ($F_{1,\ 131} = 66.0$; $P < 0.01$), and was affected by the interaction of those two factors ($F_{2,\ 131} = 5.6$; $P < 0.01$). Neither the temperature regime ($F_{1,\ 131} = 0.1$; $P = 0.74$) nor any other interactions (temperature × water: $F_{2,\ 131} = 0.1$; $P = 0.73$; temperature × exposure period: $F_{2,\ 131} = 0.1$; $P = 0.93$; and temperature × water × exposure period: $F_{2,\ 131} = 0.3$; $P = 0.72$) affected the preflight mortality. Percentage of adults that failed to fly (Table 2) was higher when the flies were denied access to water than the flies allowed access to water ($F_{2,\ 124} = 3.1$; $P < 0.01$) but was not affected by the temperature regime ($F_{1,\ 124} = 0.1$; $P = 0.76$), exposure period ($F_{2,\ 124} = 0.9$; $P = 0.43$), or any interaction between the factors (temperature × water: $F_{1,\ 124} = 1.1$; $P = 0.29$; temperature × exposure period: $F_{2,\ 124} = 2.6$; $P = 0.08$; and water × exposure period: $F_{2,\ 124} = 0.01$; $P = 0.99$; temperature × water × exposure period: $F_{2,\ 124} = 0.05$; $P = 0.95$).

Flight Performance. Heat stress and deprivation of food and water affected flight performance (distance:

Table 2. Effects of temperature regime, water provision, and exposure duration (1–3 d) during preflight treatments on the preflight mortality and percentage of *B. oleae* that failed to fly

Treatment[a]	Mortality (%)[b]			Flight failure (%)[b]		
	1 d	2 d	3 d	1 d	2 d	3 d
FW-W-35.0	1.6 ± 1.0a	14.0 ± 4.2b	42.6 ± 7.3c	6.7 ± 3.6a	10.5 ± 4.0a	18.9 ± 5.5a
FW-W-37.8	3.5 ± 1.5a	14.1 ± 4.4a	54.3 ± 9.1b	15.1 ± 5.5a	7.5 ± 2.4a	6.9 ± 3.6a
FW-NFW-35.0	9.5 ± 3.5a	49.6 ± 3.3b	79.7 ± 5.0c	16.8 ± 9.5a	17.5 ± 7.7a	30.2 ± 9.8a
FW-NFW-37.8	5.5 ± 2.4a	49.7 ± 5.2b	78.7 ± 4.1c	26.5 ± 7.1a	19.3 ± 5.2a	21.2 ± 5.8a

[a] For treatment conditions, see Table 1.
[b] Data were compared within the same treatment, but different exposure periods, and values (mean ± SE; n = 10–20) followed by different letters within the row are significantly different ($P < 0.05$; Tukey's HSD test).

Table 3. Effect of preflight treatments on the flight performance of *B. oleae*

Treatment[a]	*n*	Distance flown (m)[b]	Flight duration (h)[b]	Flight speed (m/s)[b]
FW-FW-23.9	57	1,982 ± 205a	1.542 ± 0.161a	0.350 ± 0.013a
FW-W-35.0	48	1,152 ± 200b	0.966 ± 0.171b	0.358 ± 0.012a
FW-W-37.8	34	1,305 ± 247b	0.989 ± 0.190b	0.370 ± 0.018a
FW-NFW-35.0	31	552 ± 129bc	0.531 ± 0.135b	0.308 ± 0.014ab
FW-NFW-37.8	27	382 ± 91c	0.294 ± 0.070c	0.344 ± 0.018ab
NFW-NFW-35.0	41	28 ± 5d	0.025 ± 0.004d	0.278 ± 0.010bc
NFW-NFW-37.8	21	15 ± 4d	0.014 ± 0.003d	0.237 ± 0.023c

[a] For treatment conditions, see Table 1.
[b] Values (mean ± SE) followed by different letters within the column are significant different ($P < 0.05$; Tukey's HSD test).

Table 4. Effects of temperature regime, water provision, and exposure duration (1–3 d) during preflight treatment on flight performance of *B. oleae*

Treatment[a]	Exposure period (d)	*n*	Distance flown (m)[b]	Flight time (h)[b]	Flight speed (m/s)[b]
FW-W-35.0	1	48	1,152 ± 200a	0.966 ± 0.171a	0.358 ± 0.012a
	2	52	605 ± 123b	0.492 ± 0.109ab	0.341 ± 0.011a
	3	41	321 ± 73b	0.277 ± 0.061b	0.324 ± 0.015a
FW-W-37.8	1	34	1,305 ± 247a	0.989 ± 0.190a	0.370 ± 0.018a
	2	50	757 ± 173ab	0.599 ± 0.143ab	0.346 ± 0.012a
	3	31	453 ± 117b	0.381 ± 0.101b	0.344 ± 0.014a
FW-NFW-35.0	1	31	552 ± 129a	0.531 ± 0.135a	0.308 ± 0.014a
	2	25	338 ± 104ab	0.302 ± 0.078ab	0.306 ± 0.022a
	3	24	154 ± 52b	0.138 ± 0.047b	0.307 ± 0.018a
FW-NFW-37.8	1	27	382 ± 91a	0.294 ± 0.070a	0.344 ± 0.018a
	2	27	155 ± 67ab	0.137 ± 0.058a	0.288 ± 0.021a
	3	27	202 ± 62b	0.181 ± 0.058a	0.323 ± 0.015a

[a] For treatment conditions, see Table 1.
[b] Data were compared within the same treatment, but different exposure periods, and values (mean ± SE) followed by different letters within the column for the same treatment are significant different ($P < 0.05$; Tukey's HSD test). For treatment conditions, see Table 1.

$F_{6, 252} = 62.7$; $P < 0.01$; duration: $F_{6, 252} = 16.1$; $P < 0.01$; and speed: $F_{6, 252} = 8.8$; $P < 0.01$) (Table 3). When flies were held under standard laboratory conditions and ample food and water were available in the control treatment, flies flew a mean of 1,982 m within 1.5 h in a single unbroken flight. The longest unbroken flight distance and duration was 5,857 m within 4.2 h for a female and 4,886 m within 3.9 h for a male. Compared with the control conditions, preflight exposure to the high heat regimes significantly reduced *B. oleae* flight distance ≈35–40% and duration ≈30–33% when flies were supplied with water and even more when denied access to water during the temperature treatment (Table 3).

When flies were completely deprived of food after eclosion, they flew only short distances (mean <30 m) and in short bursts (<2 min.). There were no significant differences in the flight distance and duration between the two different temperature regimes under the same preflight food condition (Table 3). Flight speed was affected only when flies were completely deprived of food before the tests (Table 3).

For the four treatments in which the flies were provided with food and water at 23.9°C during a 7-d preconditioning period and then exposed to two different temperature regimes (18.3–35.0 or 18.3–37.8°C) without food and with or without water, three-away ANOVA analyses were performed. Flight distances (Table 4) decreased with increased preflight exposure period ($F_{2, 407} = 18.7$; $P < 0.01$), deprivation of water ($F_{1, 407} = 22.1$; $P < 0.01$), and were affected by the interactions of those two factors ($F_{2, 407} = 3.1$; $P < 0.05$). Neither the temperature regime nor any interaction between the factors affected the flight distance. Flight durations (Table 4) also were decreased with increased preflight exposure period ($F_{2, 407} = 19.2$; $P < 0.01$) and deprivation of water ($F_{2, 407} = 10.8$; $P < 0.01$), but were not affected by the temperature regime or any interaction, whereas flight speed (Table 4) was affected only by deprivation of water ($F_{1, 407} = 14.6$; $P < 0.01$).

Postflight Longevity and Fecundity. Both postflight female fecundity within 24 h after the flight mill activity ($F_{5, 287} = 16.6$; $P < 0.01$) and longevities of females and males ($F_{5, 468} = 10.9$; $P < 0.01$) were significantly different among the various treatments (Table 5). The percentage of females producing offspring was lower after flight compared with the nonflight females ($\chi^2 = 286.9$, df = 5, $P < 0.01$) (Table 5). Females that were never tested for flight (i.e., control group) produced a mean of ≈18.2 offspring within 24 h, whereas females that underwent flight stress produced only a mean of 7.0 offspring. When the flies were held under 23.9°C with food and water always provided, postflight longevity was higher than all four treatments in which the flies were exposed to two different temperature regimes (18.3–35.0 or 18.3–37.8°C) with or without water. There was no significant difference in postflight longevity among flown flies in the four stressed preflight treatments. Postflight fecundity of flown flies were similar among flies that experienced different preflight treatments, except that the flies held under normal conditions had higher postflight fecundity than that of the flies held under 18.3–35.0°C without water (Table 5).

Discussion

The preflight conditions used in this study, i.e., the intensity of daily high temperature (≥35°C) and duration (1–3 d) are common in California's Central Valley in the summer (Johnson et al. 2006, Wang et al. 2009a). This study demonstrated that preflight heat stress and deprivation of food and water resulted in the death of a large (>40%) portion of the tested adult *B. oleae.* Clearly, adult *B. oleae* need to find water and food sources to survive, and this may require flight to refuges outside the orchard where they find reduced temperatures, food, water, or a combination. However, the preflight heat stress and deprivation of food and water dramatically reduced flight ability of those surviving *B. oleae.*

Stressed adult *B. oleae* flew short distances, whereas healthy flies flew considerable distances during continuous flight in the flight mill tests. *B. oleae* has the potential for long-distance dispersal in search host fruit and food under normal environmental conditions

Table 5. Postflight longevity and reproductive performance of *B. oleae*

Treatment[a]	% females produced offspring 24 h postflight	*n*	Offspring produced 24 h postflight[b]	*n*	Postflight longevity (d)[b]
CK[c]	85.0	20	18.2 ± 2.6a	31	123.5 ± 9.8a
FW-FW-23.9	61.5	26	7.0 ± 1.8b	41	88.6 ± 8.5ab
FW-W-35.0	66.7	81	6.8 ± 0.8b	132	49.2 ± 4.8c
FW-W-37.8	62.1	66	5.5 ± 0.7bc	119	51.4 ± 5.0c
FW-NFW-35.0	37.7	53	2.1 ± 0.6c	73	66.3 ± 6.4bc
FW-NFW-37.8	53.2	47	4.7 ± 0.9bc	79	68.4 ± 6.2bc

[a] For treatment conditions, see Table 1.
[b] Data from three different days of exposure were pooled (see Table 4), and values (mean ± SE) followed by different letters with the column are significantly different ($P < 0.05$; Tukey's HSD test).
[c] Flies were held under the same conditions as treatment (FW-FW-23.9) but were not tested for flight.

as shown in several previous field trapping studies in Greece (Economopoulos et al. 1978, Fletcher and Kapatos 1981, Katsoyannos 1983). Healthy, unstressed adults of many tephritid species can disperse from hundreds of meters to several kilometers (Shaw et al. 1967, Iwahashi 1972, Fletcher and Kapatos 1981, Kovaleski et al. 1999, Zermeño 2005). In comparison with other studies, the mean distance flown by well-fed and watered *B. oleae* in our study (1,982 m) was roughly similar to distances flown by Mexican fruit fly, *Anastrepha ludens* (Loew) (2,400 m) (Chambers and O'Connell 1969); Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (1,580 m); and walnut husk fly, *Rhagoletis completa* Cresson (2,110 m) (Zermeño 2005). We observed that some flies could resume flight after a break, so the flight distances we measured were likely shorter than the maximum distances that fly were capable of attaining if allowed flight until death. Normal adult *B. oleae* females were observed to fly a mean of 12,238 within 24 h if allowed (Remund et al. 1977).

Many studies have reported on the effects of gender, nutrient conditions, or diets on flight performance of tephritid fruit flies. It is not surprising that nutritional status or diet would affect insect flight performance, given that flight is a highly energy-intensive activity (Mason et al. 1989, Candy et al. 1997). Carbohydrate deprivation often affects flight ability in many insects (Sappington et al. 1995, Chen et al. 2006, Shirai 2006). Using the identical flight mill apparatus as we did, Zermeño (2005) showed that diets significantly affected the flight performance of *C. capitata* and *R. completa.* Flight to exhaustion has been shown to reduce fecundity and longevity in many insects (e.g., Mason et al. 1989). Our results showed that the preflight stress and flight per se reduced the fly's 24-h postflight fecundity. However, as soon as the flies were returned to unstressful temperature conditions and provided with water and food, the previously stressed flies were able to recover from energetically costly flight as demonstrated by the fact that they laid eggs (Table 5) within 24 h postflight and survived considerably long periods. A field study conducted during midsummer in an orchard at the University of California's KAC showed that flies could resume reproductive activities during the cooler periods of each day during the photophase (Wang et al. 2009a).

The gender effect on insect flight performance is not always predictable. Females are stronger fliers than males in some species (Hughes and Dorn 2002, Blackmer et al. 2004, Wu et al. 2006), whereas males are better in other species (e.g., Moriya and Hiroyoshi 1998). However, for many species there is no difference in flight performance between sexes [e.g., the weevil *Listronotus bonariensis* (Kuschel) (Goldson 1981); codling moth, *Cydia pomonella* L. (Schumacher et al. 1997); walnut husk fly (Zermeño 2005); and plum curculio, *Conotrachelus nenuphar* (Herbst) (Chen et al. 2006)]. Remund et al. (1977) reported that 14-d-old *B. oleae* females flew a longer distance than males, but there was no significant difference between 2-d-old males and females. In our preliminary analyses, we did not find a significant difference in the distances flown or the duration of unbroken flight between sexes of *B. oleae,* despite females being slightly larger than males (Wang et al. 2009b). High variances in both flight distance and duration within the same treatment were observed among individuals of both sexes, thereby masking any statistical significance. Wild flies often perform better than mass-reared flies (Remund et al. 1977, Zermeño 2005). In our studies, the olive fruit flies were reared on olives under favorable temperature conditions and were not subjected to large temperature variation during the day. It is possible that wild *B. oleae* adults might be more capable of dealing with heat stress and food deprivation and could disperse greater distances under unfavorable conditions.

All flight mill experiments involved anesthetizing, tethering, and stimulating the insects to fly. Test insects were often anesthetized on ice or by exposing them to low temperature as we did in this study. Any preflight anesthetizing treatment may stress insects and under-estimate flight performance. However, a previous study showed no adverse effect of chilling *B. oleae* for 6 h at 2°C on the fly's flight performance (Remund et al. 1977). In the current study, the flies were chilled at 2°C for <2 min; it is thus unlikely that our preflight chilling treatment would have caused any significant effects on the fly's performance. We must point out that in flight mill studies, the insects are tethered and do not carry their own weight when allowed to fly in a circle of prescribed circumference. It also must be remembered that insects do not typically fly in straight paths, and maximum estimated

flight (via a flight mill) is frequently an overestimation of the actual linear distance traveled between two points. Thus, flight mill tests are not aimed to accurately estimate the flight capacity of test insects, but are most useful as a comparative tool to evaluate flight potential within the species (Riley et al. 1997). It allows estimation of flight capacity in relation to different preflight-flight conditions or different physiological status of tested insects such as gender, age, mating, size (Hughes and Dorn 2002). Positive correlations between flight performance patterns in the laboratory and the field have been demonstrated, and flight data obtained from flight mill tests can provide valuable indications on possible factors that would influence an insect's dispersal ability (e.g., Keil et al. 2001).

The ability of an insect to survive and disperse plays an important role in determining its geographic distribution and abundance in nature. To our knowledge, this was the first study testing the combined impacts of heat stress and deprivation of food and water on flight performance of tephritid fruit flies. Clearly, heat stress and food and water deprivation can reduce *B. oleae* flight ability and limit an adult's ability to find water, food, or seek a refuge during the hot valley days, thereby increasing mortality risks. In conjunction with previous reports regarding the impacts of daily high temperature on the fly's survival and reproduction (Johnson et al. 2006, Wang et al. 2009a), this study provides insight into forecasting the fly's population dynamics, geographic distribution, and dispersal ability based on field temperature conditions in the Central valley of California, as well as other olive growing regions that have similar climate conditions.

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