

Effect of sex, age and morphological traits on tethered flight of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) at different temperatures

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Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a pest of fruit and vegetable production that has become established in 42 countries in Africa after its first detection in 2003 in Kenya. It is likely that this rapid expansion is partly due to the reported strong capacity for flight by the pest. This study investigated the tethered flight performance of *B. dorsalis* over a range of constant temperatures in relation to sex and age. Tethered flight of unmated *B. dorsalis* aged 3, 10 and 21-days was recorded for 1 hour using a computerised flight mill at temperatures of 12, 16, 20, 24, 28, 32 and 36°C. Variations in fly morphology were observed as they aged. Body mass and wing loading increased with age while wing length and wing area reduced as flies aged. Females had slightly larger wings than males but were not significantly heavier. The longest total distance flown by *B. dorsalis* in 1 hour was 1559.58 m. Frequent short, fast flights were recorded at 12- and 36°C, but long-distance flight was optimised between 20-24°C. Young flies tended to have shorter flight bouts than older flies, which was associated with them flying shorter distances. Heavier flies with greater wing loading flew further than lighter flies. Flight distances recorded on flight mills approximated those recorded in the field, and tethered flight patterns suggest a need to factor temperature into interpretation of trap captures.

Key words

Body mass, dispersal ability, flight mill, oriental fruit fly, wing loading

Introduction

Understanding insect flight performance is important for the surveillance and management of pest species. Insect flight mediates response to traps, with fluctuation in trap catch associated with high and low temperatures that limit insect activity (Shukla & Prasad, 1985). Flight performance also affects various pest management tactics. Insects can take flight to avoid insecticides, a characteristic observed in DDT-resistant malaria vectors and storage pests (Chareonviriyaphap et al., 1997; Guedes et al., 2009). Behavioural control tactics such as mating disruption can be compromised by migration of target pest species into pheromone-treated zones (Hashiyama et al., 2013). Flight ability determines the success of the sterile insect technique (SIT) (Remund et al., 1976) as dispersal from a release point is needed for sterile insects to distribute themselves in the target area and find mating partners (Nakamori & Simizu, 1983). Conversely, the movement through flight of mated females and wild males into a treated area negates SIT interventions, so knowledge of flight capacity informs the extent of an area treated with sterile insects (Klassen & Curtis, 2005). Maintenance of quarantine zones and Pest Free Areas (PFAs) also requires knowledge of the flight ability of a pest to determine the size of buffer zones surrounding these areas (FAO, 2006).

Like other insect behaviours, flight is directly affected by temperature (Taylor, 1963). Simple locomotor activity occurs within a wide range of temperatures but more complex activities like egg laying and flight have a more restricted temperature range (Heath et al., 1971). A decline in temperature below the thermal optimum of an insect reduces activity as a consequence of lower metabolic rate (Bale, 2002; MacMillan & Sinclair, 2011) and flight muscle power output (Ellington, 1985; Lehmann, 1999; Stevenson & Josephson, 1990). Below a lower threshold temperature, locomotor activities like flight cease as insufficient energy is released by low metabolic rates to permit flight muscle function (Goller & Esch, 1990). At high temperatures, heat production by insect flight muscles may cause an increase in body temperature to a level above the upper critical thermal limit (Heath et al., 1971). This results in inactivation of the insect nervous system due to ionic regulation disturbance, and may ultimately lead to irreversible protein denaturation, melting of lipids, and fatality (Heath et al., 1971; Neven, 2000). Flight efficiency (power output divided by power input) generally increases with body size. However as insect body size becomes larger, power requirements may increase at greater rates than metabolic rate, which ultimately impacts on flight efficiency (Harrison & Roberts, 2000). Also, as the body size of an insect increases, the minimum temperature required for

flight is believed to decrease (Heath et al., 1971). Other than the thermal environment and body mass, insect wing traits like wing width, length, area and wing loading (the ratio of body mass to wing area) influence flight performance (Esterhuizen et al., 2014; Steyn et al., 2016). These morphological traits have also been observed to vary between sexes and ages. For example, females of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), are larger with higher wing length whereas males are smaller with wider wings (Esterhuizen et al., 2014).

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), has been declared present in over 42 African countries since its first detection in Kenya in 2003 (CABI, 2017). It is one of the most serious pests of fruit and vegetables globally and has become established across areas with different climates (De Meyer & Ekesi, 2016). Since its detection in Africa, studies have determined the biology and ecology of this pest species, including its seasonality and current distribution (Gnanvossou et al., 2017; Mwatawala et al., 2006), host plant range (Mwatawala et al., 2009; Theron et al., 2017), potential distribution and ecological niche (De Meyer et al., 2010; Hill et al., 2016), the effect of temperature on the development of immature stages (Rwomushana et al., 2008), and progressive invasion into South Africa (Hill & Terblanche, 2014; Manrakhan et al., 2015). All indicate that temperature is a critical factor in the survival and spread of *B. dorsalis*. In South Africa, trap catches of *B. dorsalis* were found to be influenced by minimum and maximum temperatures (Theron et al., 2017). The increase in catches of an insect with increasing temperature could be a combined effect of an increase in population level, or as noted earlier, the flight ability of the insect (Taylor, 1963).

Static and tethered flight mills have been used to study insect flight performance (Hoddle et al., 2015; Lopez et al., 2014). Although flight mills may not accurately predict flight performance of insects in the field, they are a useful tool for comparisons between insect species, ages and sexes, and different experimental treatments under controlled conditions (Nakamori & Simizu, 1983; Naranjo, 2019; Riley et al., 1997; Sharp et al., 1975; Wang et al., 2009). Flight mills also provide an invaluable tool to measure flight of variable range and time (Attisano et al., 2015). For tephritids, flight mills have been used to assess the effects of mass rearing and sterilisation (Attisano et al., 2015; Chapman, 1982; Chen et al., 2011; Hoddle et al., 2015; Lopez et al., 2014; Nakamori & Simizu, 1983; Sharp et al., 1975; Sharp & Webb, 1977), differences in flight performance between age groups and the sexes (Chapman, 1982; Sharp & Webb, 1977), wild and laboratory reared populations (Nakamori & Simizu, 1983), and the effect of heat stress and food supply (Remund et al., 1976; Wakid

& Shoukry, 1976). Generally, fruit fly flight ability has been shown to increase with age regardless of sex (Sharp et al., 1975). The weight and flight performance of young *Bactrocera* has been observed to be lower than older flies (Remund et al., 1976; Sharp et al., 1975; Wang et al., 2009). Over a limited range of constant temperatures ($23.5 \pm 1.2 - 25.7 \pm 0.1^\circ\text{C}$) at $74.5 \pm 0.4\%$ relative humidity, male *B. dorsalis* were better fliers than females at an age of 16 days. However, female *B. dorsalis* attained their maximum flight ability earlier than males (Sharp et al., 1975).

This study determined the effect of temperature on tethered flight performance of virgin female and male *B. dorsalis* of varying ages. Measurements of tethered flight for a duration of one hour, including number of discrete flight bouts, total distance flown, duration of each flight bout, total duration of flight, mean flight speed, and the maximum flight speed, were related to temperature, age and fly wing loading. It was predicted that flight performance would be restricted at temperature extremes of 12°C and $\geq 32^\circ\text{C}$ (Sharp et al., 1975). It was anticipated that females aged 21 days would exhibit better performance than younger females as their flight corresponds with sexual maturity (Chen et al., 2014). In contrast, younger males should exhibit better flight performance than older males because field observations suggest a post-teneral dispersal phase in *Bactrocera* species (Drew et al., 1984; Fletcher, 1973; Froerer et al., 2010). Wing loading was also likely to affect flight performance (Norry et al., 2001), with high wing loading generally associated with faster flight (Betts & Wootton, 1988).

Materials and methods

Insects

Bactrocera dorsalis adults used in this trial were obtained from a culture maintained at Citrus Research International, Nelspruit, South Africa for approximately 18 generations. The flies were kept in cylindrical cages made from netting material of approximately 1.5 m in height and 0.45 m diameter suspended approximately 0.30 m from the ground. The adults were maintained on a diet mixture comprising a 3:1 sugar:enzymatic hydrolysed yeast diet (Amberex 1003, Juneau, USA). A fresh source of water was supplied from a suspended and inverted honey jar bottle with a perforated lid lined with filter paper. Mature mated female flies were exposed to a punctured Granny Smith apple for 24-48 hours to allow for oviposition. Thereafter the puncture areas were cut open and eggs were harvested by washing

off with water using a wash bottle. Eggs were volumetrically estimated and placed on a premixed carrot-based larval diet formulated by Citrus Research International, Nelspruit, South Africa. Bowls with larval diet and eggs were placed on sterile sand in plastic containers fitted with tight fitting screened lids. The eggs hatched, and larvae developed under ambient conditions of 24-28°C. The larvae hopped out of the diet and burrowed into the sand to pupate. The pupae were then separated from the sand using a flour sifter and measured volumetrically for use in the trials. The lifecycle took 18-21 days to complete depending on prevailing temperatures.

The initial trials for this study were run using adults transported in adult and pupal form in an air-conditioned vehicle from Citrus Research International, Nelspruit, South Africa to the Department of Zoology and Entomology on the Hatfield Campus of the University of Pretoria, South Africa. Subsequent trials were run using a colony established at the University of Pretoria from pupae sourced from the Citrus Research International culture. Numerous cohorts were used in the replicate tests of this study. Flies were separated upon emergence according to their date of emergence and sex and placed in separate cages until required for testing. Insect cages (32.5 × 32.5 × 32.5 cm; BugDorm-43030, MegaView Science, Taichung, Taiwan) were used to house the different cohorts of flies as they aged. The flies were supplied with water (soaked in cotton balls), granulated sugar and hydrolysed yeast in separate containers as a food and protein source until use in experiments. Clear labelling of date of emergence, sex of fruit flies and cohort number was done to ensure the correct age, sex and cohort was used for each test temperatures. Flies were held in a climate room with a light cycle of 12:12 (L: D). A one-hour artificial sunrise and sunset was simulated by two 8 W fluorescent tubes turning on before and turning off after the main room lights. The ambient temperature in the room in which flies aged (recorded using temperature data loggers; iButton DS1923, Maxim, Sunnyvale, CA, USA) was $24.3 \pm 0.3^\circ\text{C}$.

Flight observations

Flight mill studies were conducted at the University of Pretoria between June-December 2016. The relationship between temperature and tethered flight of *B. dorsalis* was studied using computerised flight mills attached to a 15 input flight mill data acquisition (DAQ) system connected to a laptop computer. The flight mills and DAQ were built by the Vehicle Dynamics Group at the University of Pretoria using the design developed by the USDA-ARS Arid Land Agricultural Research Centre in Maricopa, Arizona (Naranjo, 1990). The custom-

designed data acquisition interface ran on Java virtual machine on a laptop running Windows 7 Enterprise version 6.1. To determine the effect of temperature on flight behaviour of 3, 10- and 21-day-old *B. dorsalis*, adults were exposed to seven test temperatures of 12, 16, 20, 24, 28, 32 and 36°C. Three flight mills were set up in a 238-L cooling incubator (MIR-254 Panasonic Healthcare Company, Japan). The cooling incubator had a door with a fitted glass window and internal fluorescent light to allow for observations and could be set to any temperature within the range -10 to +60°C. Temperature control by the cooling incubator was precise and repeatable with minimal fluctuations of $\pm 0.2^\circ\text{C}$ in temperature. Temperature was randomly set to one of the seven test temperatures for each set of flies attached to the three flight mills. By doing so, we accounted for any potential diurnal differences in flight activity.

Prior to running the experiment, three female and three male *B. dorsalis* adults were prepared for the flight experiments. The flies were weighed in numbered glass tubes on an analytical balance (to 0.0001 g; NewClassic MF Model # MS204S, Mettler-Toledo, Greifensee, Switzerland). One fly of a known age (3, 10 or 21 days), weight and sex was cooled for exactly 2 minutes in a small (approximately 1.5 L) cooler box containing fine ice shavings. Once cooled, the fly was placed on a paper towel and a small drop of melted hot glue collected on the tip of a #1 insect pin was immediately placed on the centre of the thorax with the pin in an upright position and perpendicular to the thorax of the fly. The attached fly was left to recover for 2-5 minutes by sticking the pointed end of the attached pin into a Styrofoam board. When the incubator had been set to the required temperature and the flies recovered from the temperature shock (i.e. attempted to fly), the insect pin attached to a tethered fly was then carefully inserted with its pointed end into the opening of the hypodermic tube of the flight arm of the flight mill so as to fit snugly enough to hold it into place without slipping. A very small amount of pressure sensitive non-permanent adhesive (Prestick®, Capetown, South Africa) was used to ensure the pin did not slip out of the tube. A quantity of the same pressure sensitive adhesive of equal weight to the fly was placed onto the head of another entomological pin on the opposite side of the flight mill arm to act as a counterbalance. Three flies were flown during each session, with at least one of each sex flown on each occasion. These flies represented those with the neatest pin attachment. The flies were randomly allocated to the flight mills. The extra tethered flies prepared for the experiment that were not used were discarded to avoid using stressed flies and a new set of six flies was prepared for each session. Flight was recorded for 3600 seconds (i.e. one hour) to maximise the number of temperatures

that could be tested in a day. Seven replicates for each sex, age and temperature were used for the data analysis (total n = 294 flies).

Records of the date of experiment, cohort number, weight prior to flight, age and sex of fly, fly label, flight mill number, test temperature and any other additional observations were noted. Flight performance parameters were recorded from the flight mills via a Universal Serial Bus (USB) connection to the DAQ. The data were downloaded as comma separated values files to the laptop computer and stored. Following each flight trial, individual female and male *B. dorsalis* adults were detached from the flight mills and immediately placed in 1.5 ml microcentrifuge tubes. An appropriate label corresponding to each fly was written and placed in the tube. Any dead individuals detached from flight mills were discarded and excluded from the analyses.

Wing measurements

Following the flight mill observations, each fly was immobilised by being placed in a freezer (below 20°C) for storage until required. The right wing (unless damaged, then the left wing) of each specimen was detached from the thorax and placed on a microscope slide. The wing was secured onto the slide using clear double-sided tape and a label was affixed to correspond with the fly. Wings from one flight mill session were placed on one slide to avoid any chance of misidentification. A second microscope slide was used to cover the wings. Wing image processing was done by positioning the microscope slide with wing samples on a stereo microscope with a low objective lens (1×) fitted with a digital camera (Dino-Lite). A photograph of each wing was taken using DinoCapture® 2.0 software. A 5 mm graduated ruler (to scale the image) was also photographed for each new image capturing session. Open source ImageJ software (Wayne Rasband, National Institute of Health, USA) was used to measure wing length and wing area.

Data analyses

A two-way multiple analysis of variance (MANOVA) was performed to determine the effects of age and sex on body mass, wing length, wing area of the fly (based on measurements of the right wing), and wing loading (body mass (N)/wing area (m²). Wing length and wing area were log₁₀ transformed as they were not normally distributed (Shapiro-Wilk test) and variances for wing area of each group were not homogeneous (Levene's test). Subsequent univariate analyses of variance (ANOVA) were performed to identify which dependent

variables contributed to the multivariate response. Post-hoc multiple comparisons between ages were performed using Tukey's honestly significant difference tests.

Parameters of flight performance for each fly were calculated from the saved flight records. The calculated flight performance parameters were: number of discrete flight bouts, distance flown (m), average duration of each flight bout (sec), total flight duration (sec), average flight speed (m/sec) and maximum flight speed (m/sec). A flight bout was determined when there was movement for ≥ 5 seconds before coming to a complete stop (Hoddle et al., 2015; Lopez et al., 2014) (i.e. when recorded speed was 0.00 m/sec). Separate linear mixed effects models were performed after satisfying the assumptions of a normal distribution to determine the effects of temperature, age, sex, and body mass as a covariate, on each flight performance measure. Average duration of each flight bout was square root-transformed to improve the distribution of the data. Two- and three-way interactions were included in each model, but none of the three-way interactions were significant and were removed from the model to preserve degrees of freedom. The flight mill number was included in these models as a random effect to account for variability caused by the equipment. Variance components were estimated using restricted maximum likelihood. Post-hoc multiple comparisons between temperatures and ages were performed using least significant difference tests. All data analyses were performed using IBM SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA). Raw data are openly available (Makumbe et al., 2020).

Results

Fly morphology

The flies used in this experiment had a mean (± 1 s.e.) body mass of 14.03 ± 0.30 mg, wing length of 4.88 ± 0.02 mm, wing area of 16.20 ± 0.13 mm², and wing loading of 8.59 ± 0.19 Newtons/m². There was a statistically significant multivariate effect of age on the morphology of *B. dorsalis* (MANOVA: Wilks $\lambda = 0.758$, $F_{8,570} = 6.886$, $P < 0.001$). The statistically significant effect of age on the multivariate response reflected its effect on body mass, wing length, wing area and wing loading (Table 1). Tukey's honestly significant difference tests indicated that each age class was significantly heavier than the one preceding it (Fig. 1A). The mean wing length and wing area of 3-day-old flies did not differ statistically from that of 10-day-old flies but was significantly, if only slightly, greater than those of 21-day old flies (Fig. 1B and Fig. 1C). The wing loading increased with fly age (Fig. 1D), with

Table 1. Univariate analyses of variance summaries for effects of sex and age on body mass (mg), wing length (log₁₀-transformed; mm), wing area (log₁₀-transformed; mm²) and wing loading (Newtons/m²) of laboratory-reared *B. dorsalis* used for tethered flight observations. P-values in bold are significant at $\alpha = 0.05$.

	df	Body mass (mg)		log ₁₀ Wing length (mm)		log ₁₀ Wing area (mm ²)		Wing loading (Newtons/m ²)	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	1	2591.13	<0.001	198585.67	<0.001	147154.76	<0.001	2578.25	<0.001
Age	2	29.56	<0.001	6.901	0.001	6.935	0.001	36.735	0.001
Sex	1	3.548	0.061	14.371	<0.001	15.114	<0.001	0.217	0.642
Age × Sex	2	0.959	0.385	1.620	0.200	0.650	0.523	1.003	0.368
Error	288								
Total	294								

mean wing loading being $7.11 \pm 0.30 \text{ N/m}^2$ at 3 days of age, $8.10 \pm 0.30 \text{ N/m}^2$ at 10 days and $10.80 \pm 0.27 \text{ N/m}^2$ at 21 days of age. There was a statistically significant multivariate effect of sex on the morphology of *B. dorsalis* (MANOVA: Wilks $\lambda = 0.941$, $F_{4,285} = 4.500$, $P = 0.002$). Univariate ANOVA detected significant effects of sex on wing length and wing area (Table 1). Female wings were slightly longer ($4.95 \pm 0.03 \text{ mm}$) and had a larger area ($16.66 \pm 0.20 \text{ mm}^2$) than those of males (length: $4.82 \pm 0.02 \text{ mm}$, area: $15.74 \pm 0.17 \text{ mm}^2$) (Fig. 1 B and Fig. 1 C). The interaction of age and sex did not have a significant effect on fly morphology (MANOVA: Wilks $\lambda = 0.961$, $F_{8,570} = 1.431$, $P = 0.180$).

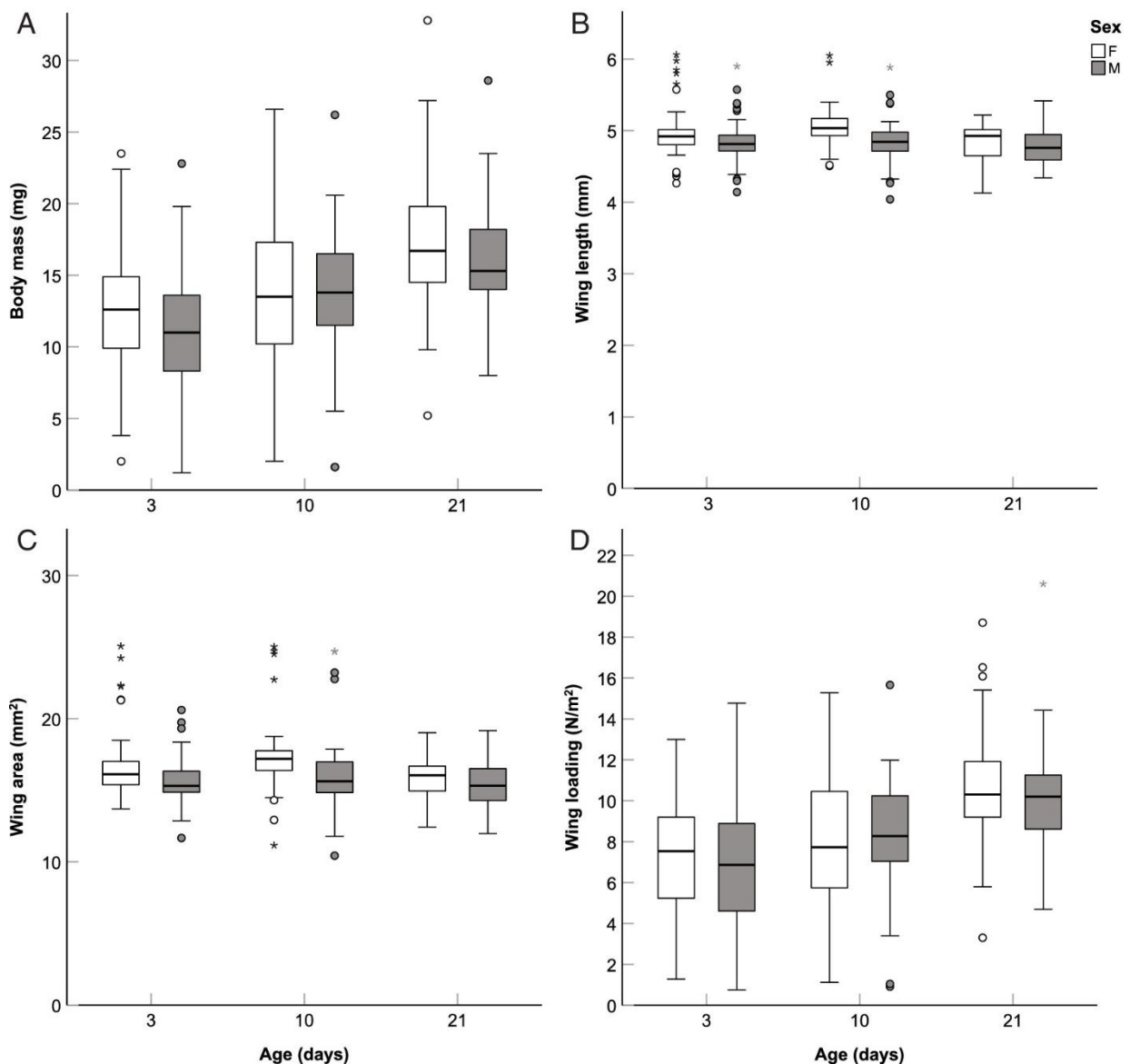


Figure 1. Boxplots of (A) body mass, (B) wing length, (C) wing area and (D) wing loading of female and male *B. dorsalis* at 3, 10 and 21 days of age.

Number of discrete flight bouts

Of the 294 flies for which flights were recorded, a single bout of flight was recorded for 82 individuals (40 females, 42 males). The mean number of discrete flight bouts in one hour was 5.34 ± 0.32 , and the maximum number recorded was 27. Temperature had a significant effect on the number of discrete flight bouts ($F_{6,261.4} = 4.144$, $P < 0.001$), with the lowest number of flight bouts recorded at 20 and 24°C (Fig. 2). Flight bouts increased at temperatures below 20°C and at temperatures above 28°C. There was no significant effect of age, sex, their interactions or body mass on the number of discrete flight bouts (Table S1). The flight mill accounted for only 0.90% of the variance in the number of discrete flight bouts (Wald $Z = 0.431$, $P = 0.666$).

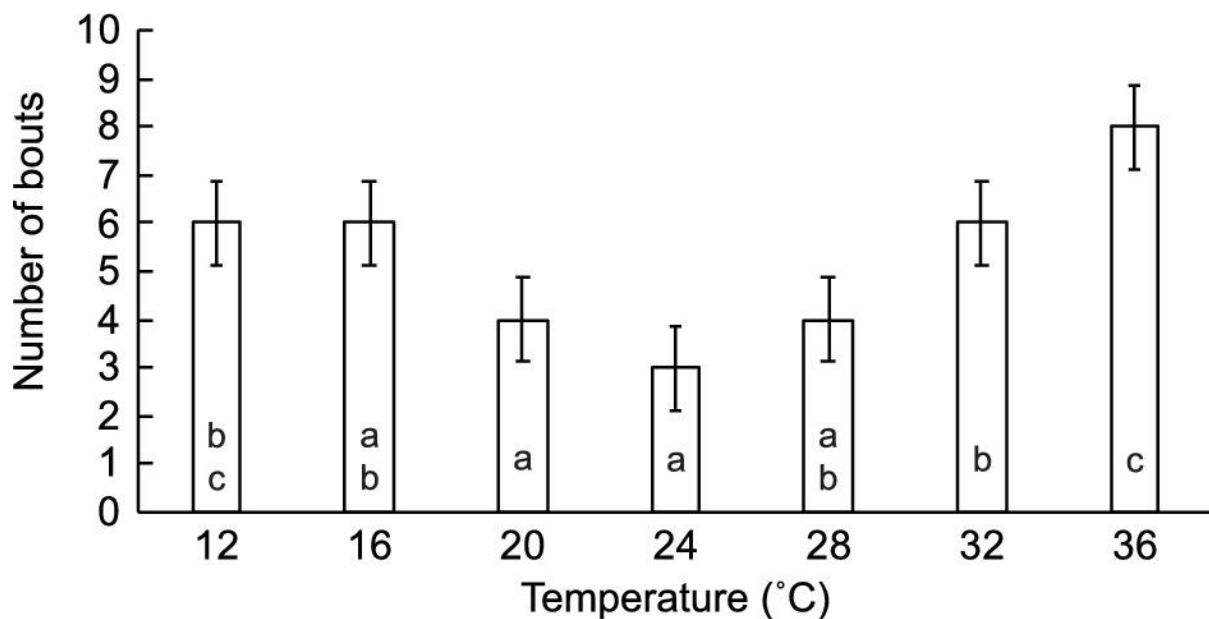


Figure 2. Effect of temperature on mean (\pm s.e.) number of flight bouts by *B. dorsalis* tethered to a flight mill. Temperature treatments labelled with the same lowercase letter are not significantly different from each other (LSD tests, $P < 0.05$).

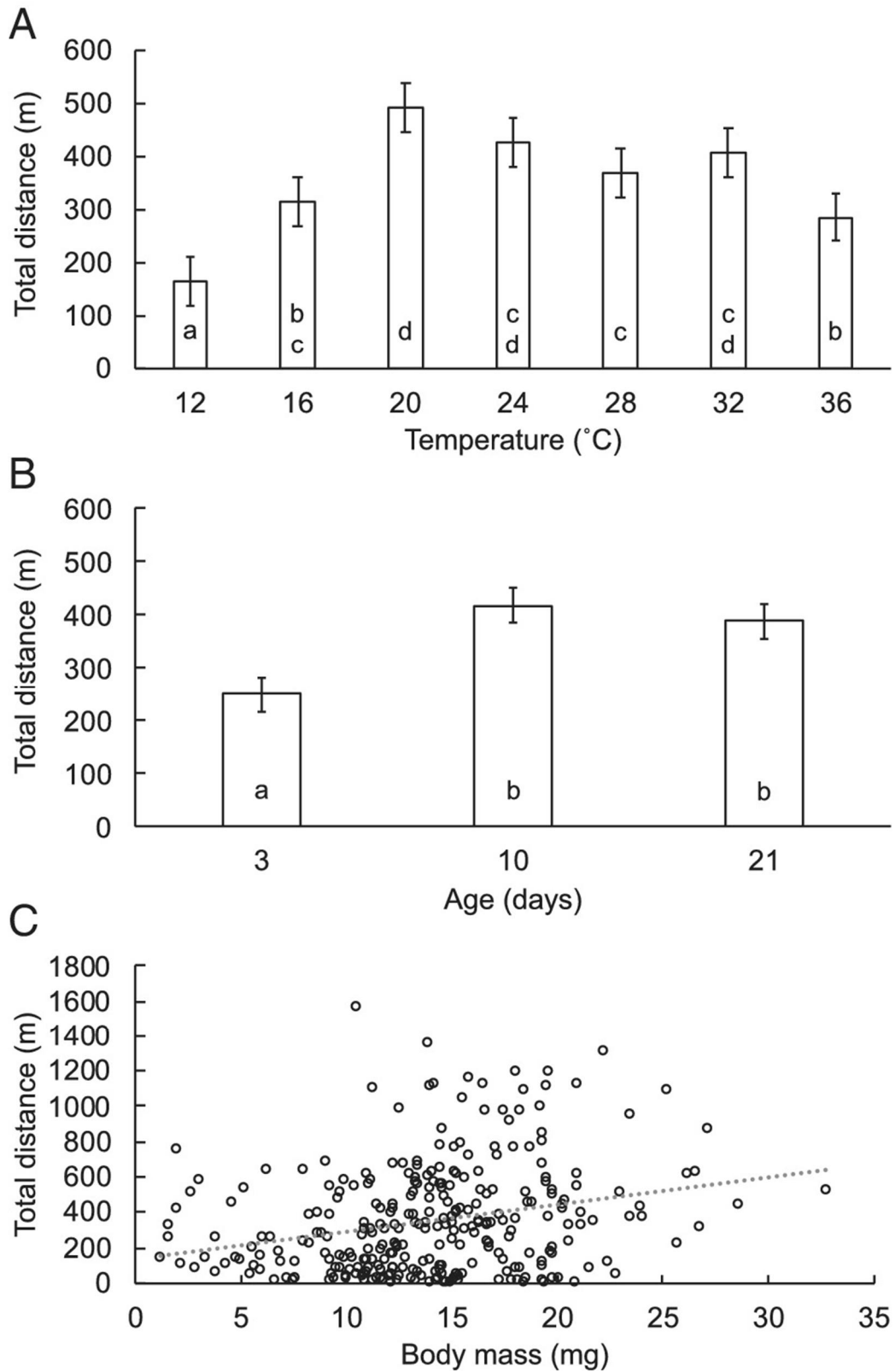


Figure 3. Effects of (A) temperature, (B) age and (C) body mass on total distance flown by *B. dorsalis* tethered to a flight mill. Mean (\pm s.e.) distance is presented in (A) and (B). Within each chart, treatments labelled with the same lowercase letter are not significantly different from each other (LSD tests, $P < 0.05$). The dotted line in (C) helps to visualise the significant positive relationship between body mass and total distance flown (GLMM, coefficient = 8.110 ± 3.532 ; $F_{1,261.3} = 5.271$, $P = 0.022$).

Distance flown

Overall, the mean of total distance flown in one hour was 349.32 ± 18.09 m, with a maximum total distance flown of 1559.58 m by a 10-day-old male at 28°C. There was a significant effect of temperature ($F_{6,261.6} = 6.455$, $P < 0.001$) on total distance flown, with flight distance lowest at 12°C, rising to plateau between 20-32°C, and declining thereafter (Fig. 3A). Age also significantly affected total distance flown ($F_{2,261.5} = 9.581$, $P < 0.001$), with 10- and 21 day-old flies covering a greater distance than 3 day-old flies (Fig. 3B). As body mass increased, the total distance flown increased significantly (Fig. 3C; coefficient: 8.110 ± 3.532 ; $F_{1,261.3} = 5.271$, $P = 0.022$). There was no significant effect of sex or any first order interactions on total distance flown (Table S2). The flight mill used accounted for only 1.18% of the variance in total distance flown (Wald $Z = 0.546$, $P = 0.585$).

Bout and total flight duration

Over one hour of recording, the mean flight bout duration was 1125 ± 70 sec. The maximum possible flight time of 3600 sec was achieved by 31 individuals in a single uninterrupted bout. The main effects of temperature ($F_{6,263} = 11.246$, $P < 0.001$) and age ($F_{2,263} = 6.455$, $P = 0.011$) had significant effects on bout duration, as did their interaction ($F_{12,263} = 6.455$, $P = 0.047$). Bout duration generally increased from the lowest test temperature of 12°C to a peak at 20°C before declining to the lowest recorded values at 36°C (Fig. 4A). This pattern varied slightly in 3-day-old flies, where a second peak in bout duration was recorded at 32°C. In general, the duration of flight bouts was longer in 10- and 21-day-old flies than those that were 3 days old. Sex and body mass did not significantly affect bout duration (Table S3). The flight mill that was used accounted for <0.01% of the variance in bout duration. Temperature also had a significant effect on total flight duration ($F_{6,259.8} = 5.017$, $P < 0.001$). Total flight duration did not vary between 16-36°C, but was shorter at 12°C (Fig. 4B). Age, sex and body mass did not significantly affect total flight duration (Table S3), and flight mill identity accounted for only 8.31% of the variance (Wald $Z = 0.841$, $P = 0.400$).

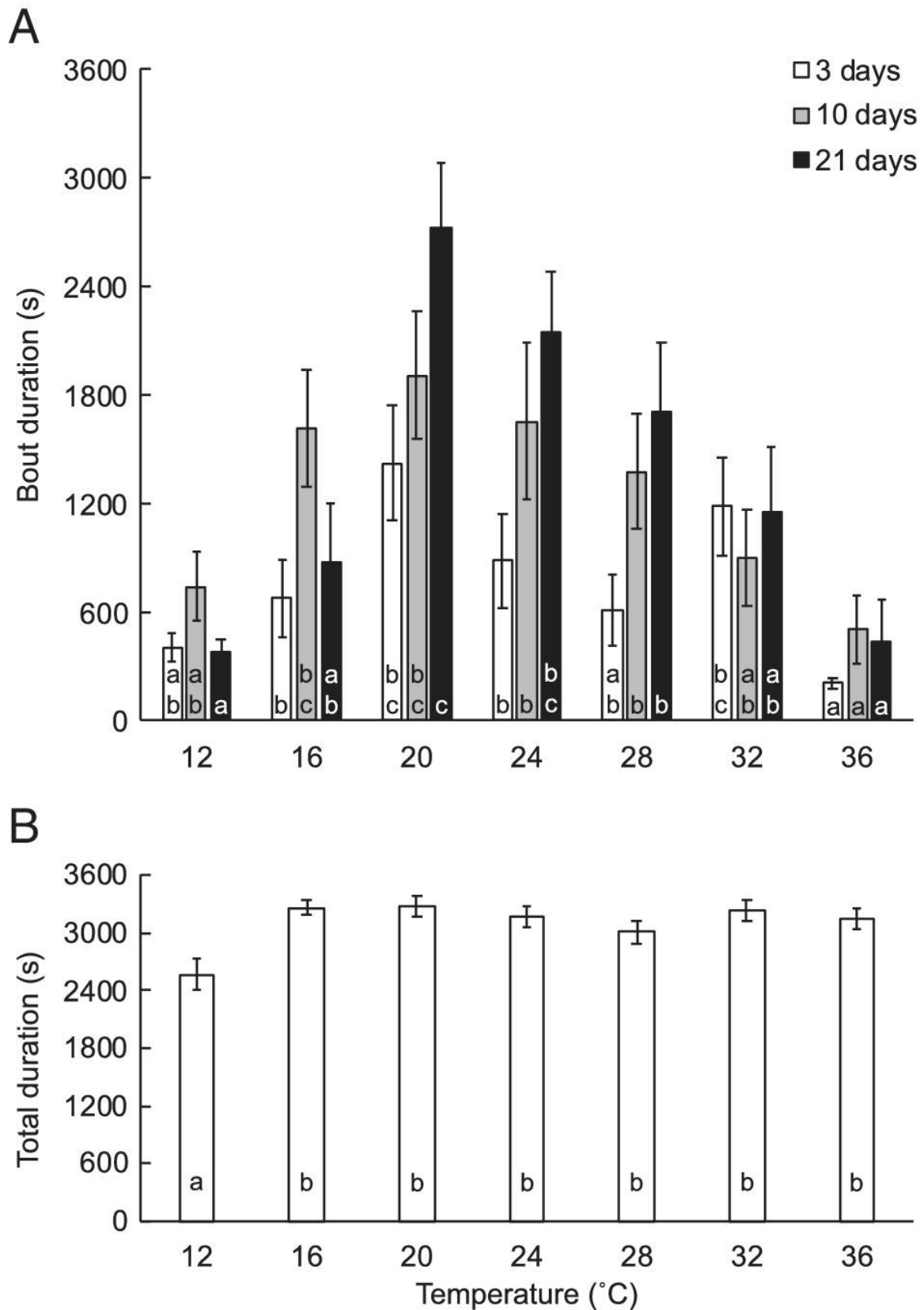


Figure 4. Effect of temperature and age on mean (\pm s.e.) flight bout duration (A), and effect of temperature on total flight duration (all ages combined) (B) by *B. dorsalis* tethered to a flight mill. Within each chart, bars of the same shade labelled with the same lowercase letter are not significantly different from each other (LSD tests, $P < 0.05$).

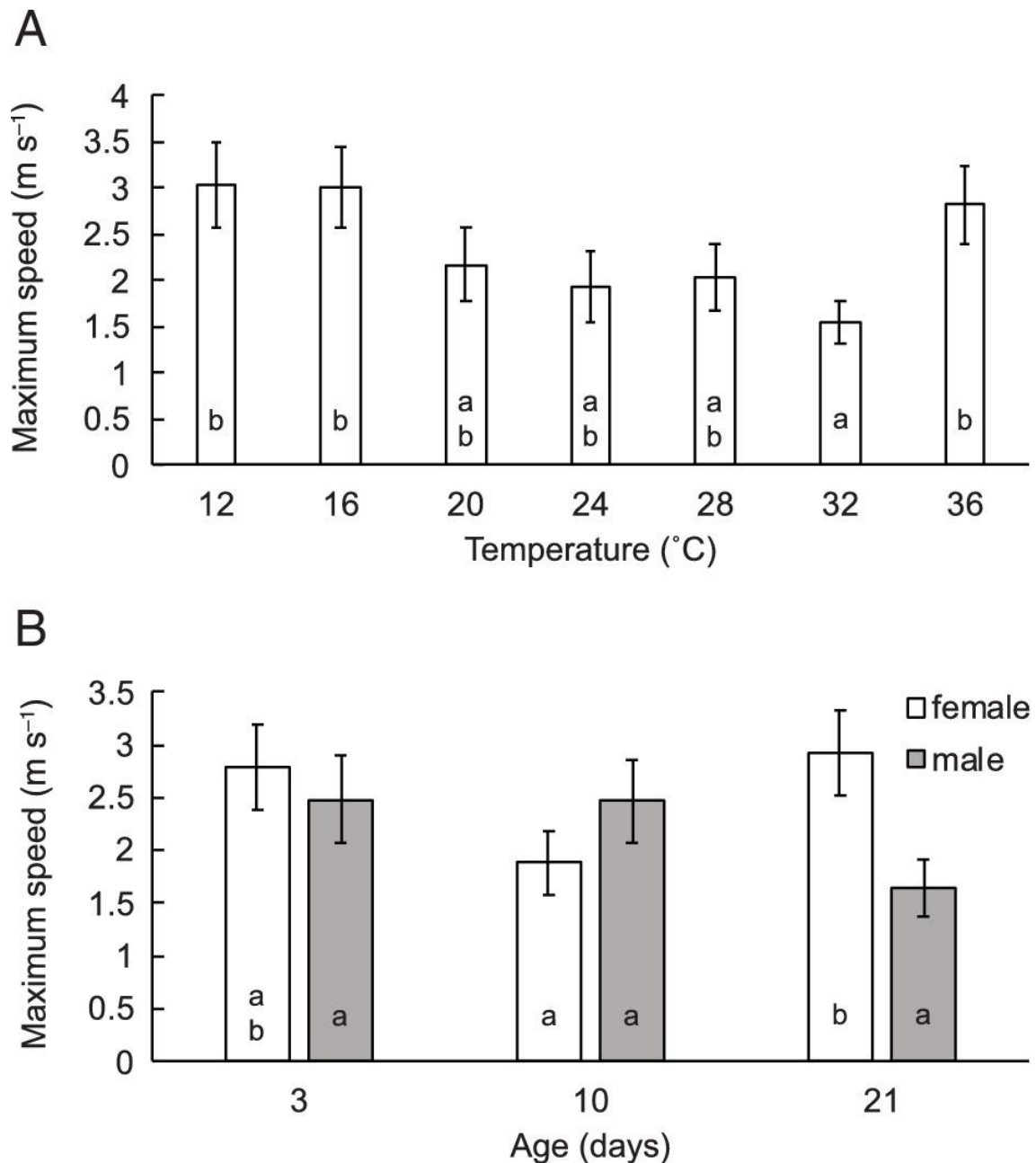


Figure 5. Effects of temperature (A), and age and sex (B) on mean (\pm s.e.) maximum flight speed by *B. dorsalis* tethered to a flight mill. Within each chart, bars of the same shade labelled with the same lowercase letter are not significantly different from each other (LSD tests, $P < 0.05$).

Average and maximum flight speed

The mean average flight speed during tethered flight over one hour was 0.63 ± 0.03 m/sec. Average flight speed was not affected by temperature, age, sex, their interactions, or body mass (Table S4). Flight mill accounted for only 0.01% of the variance (Wald $Z = 0.281$, $P = 0.703$). The mean maximum flight speed recorded was 2.37 ± 0.15 m/sec, and the fastest speed recorded was 9.62 m/sec by a 10-day-old male at 24°C. There was a significant effect

of temperature on maximum flight speed ($F_{6,260.9} = 2.429$, $P = 0.027$), with maximum flight speed highest at 12, 16 and 36°C, and lowest at 32°C (Fig. 5A). The interaction of age and sex also significantly affected maximum flight speed ($F_{2,261.2} = 3.074$, $P = 0.048$). In males, maximum flight speed did not vary with age, but maximum flight speeds in females were lowest at 10 days and highest at 21 days of age (Fig. 5B). Other first-order interactions had no significant effect on maximum flight speed (Table S4). Flight mill accounted for 22.74% of the variance in maximum flight speed (Wald $Z = 1.038$, $P = 0.299$).

Discussion

Temperature had an effect on most recorded tethered flight parameters in *B. dorsalis*. Based on the molecular and physiological processes underpinning thermal performance curves, it was predicted that flight performance would be restricted at temperature extremes of 12°C and $\geq 32^\circ\text{C}$. However, this was not always the case in *B. dorsalis*. Increasing temperature had no or little effect on mean maximum flight speed and total flight duration, respectively. This is in contrast to *Lucilia sericata* (Meigen) (Yurkiewicz & Smyth, 1966) and *Aedes aegypti* L (tested at 50% relative humidity; Rowley & Graham, 1968), where flight speed increased with temperature. Only total distance flown and the mean duration of flight bouts by *B. dorsalis* approached a typical thermal performance curve, with a thermal optimum within the range of 20-24°C. The number and duration of flight bouts likely explain variation in total distance flown with temperature because there were frequent short flights, often among the fastest, at the lowest and highest temperatures tested in this study. These results share similarities with those from *A. aegypti*, where flight distance and bout duration were optimal at 21°C and declined at other temperatures within the range 10-35°C (Rowley & Graham, 1968).

Contrary to expectation, there was little evidence for age-related differences in flight performance of females and males. Only maximum flight speed by *B. dorsalis* was affected by sex. For females, the fastest maximum flight speeds were recorded at 21 days of age, which is when females of the tested culture are sexually mature, but these values did not differ significantly from those recorded from 3-day-old females. In another study on *B. dorsalis*, tethered flight speed and distance peaked in females that were 15 days old (Chen et al., 2015). This correlated with greater myofibril diameter and shorter sarcomere length. The flies used by Chen et al. (2015) reached sexual maturity by 10 days of age, so they proposed

that the observed peak in flight performance was linked with short-distance flights used to find host fruit for egg-laying. In this study, there was no difference in maximum flight speed recorded among males across the tested ages. Furthermore, maximum flight speed did not differ from or was less than that of females. This general lack of difference in male flight performance as they age contradicts results of other studies on tethered flight and dispersal in the field. Sharp et al. (1975) reported that male *B. dorsalis* were better fliers than females at an age of 16 days.

Younger males are often presumed to exhibit better flight performance than older males because field observations suggest a post-teneral dispersal phase in *Bactrocera* species (Drew et al., 1984; Fletcher, 1973; Froerer et al., 2010). The absence of any age effects on male flight parameters may relate to the use of tethered flight, but distinct age differences have been noted from tethered flight recordings from *Bactrocera tryoni* (Froggatt) (Chapman, 1982). It may also be that environmental cues (other than temperature) play an important role in shaping flight performance in *B. dorsalis*.

Wing loading and body mass were highly correlated because wing area changed only slightly as flies aged. Rather than using the derived value of wing loading, body mass was used to explain tethered flight parameters. In other studies, high wing loading has been associated with faster flight, but this was not the case in our study. Rather, we found that body mass (and wing loading) were associated with flight distance. Our findings match those of Bloem et al. (1994) who observed that flight ability of larger *C. capitata* was better than that of smaller flies. However, flight performance of *Drosophila melanogaster* L. was not associated with body mass (Lehmann, 1999). They also concur with the proposal by Dominiak et al. (2008) and Fanson et al. (2014) that larger sterile flies emerging from heavier pupae have greater flight ability and may be better able to disperse when released during SIT programmes. It may be that greater body mass in *B. dorsalis* confers the ability for faster flight through larger, more efficient asynchronous flight muscles (Lehmann, 2002) that exert greater force or produce greater wingbeat amplitude during their oscillatory contractions (Ellington, 1985; Lehmann, 2002).

Extrapolating the tethered flight results beyond one hour, *B. dorsalis* could cover an average distance of 4.2 km and up to 18.7 km in 12 hours. This supposes that flight behaviour is relatively consistent and assumes continuous, unidirectional flight during daylight (12 hours).

Considering published data on field dispersal distances, these values are well within the range that *B. dorsalis* is capable of covering without human assistance. Within 7 days of release, male *B. dorsalis* were detected in traps on the island of Chichi Jima, Japan, located 50 km from their release on the island of Haha Jima (Iwahashi, 1972). On Hawaii Island, USA, Froerer et al. (2010) recaptured most sterile male *B. dorsalis* in eight days after release in methyl eugenol-baited traps placed 0.5-2 km from release points, but seven flies (out of 1917) were recaptured as far as 11.4 km only 2 days after release (Froerer et al., 2010). However, it is important to note that flight distances were optimal within the range of 20-24°C. At temperatures lower and higher than this, flight performance (distance flown, bout duration) declined, and this is likely to also be the case in the field. This is relevant for *B. dorsalis* catches in baited traps used to inform populations of the pest in areas where it occurs and in those which are free of it. Based on our results, low trap captures at low and high temperatures could indicate low flight activity, hence poor responsiveness of *B. dorsalis* to traps and not necessarily low populations or absence of the pest. The lower critical thermal limit for *B. dorsalis* is approximately 9°C (Motswagole et al., 2019), which is substantially lower than the minimum temperature tested in the current study, meaning that flies are able to move, forage and avoid predators even when flight is limited. For this reason, it may be worth validating methyl eugenol trap effectiveness in relation to temperature with standardised *B. dorsalis* abundance to improve interpretation of trap captures for detection and quarantine delimitation.

To conclude, tethered flight by *B. dorsalis* was affected by temperature, with optimal performance within the range of 20-24°C. There was little effect of age or sex on most measured flight parameters, which is contrary to most field observations of dispersal in this species. However, flight distances recorded on flight mills did approximate those recorded in the field, and tethered flight patterns at lower and higher temperatures suggest a need to factor temperature into interpretation of trap captures.

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Data availability statement

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.25403/UPresearchdata.12152946.v1>, reference number 12152946.

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