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Drosophila suzukii flight performance reduced by starvation but not affected by humidity

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Abstract

Drosophila suzukii is widely studied because of its status as a global pest of berries and soft fruits. Environmental conditions and access to food resources impact the physiology and fitness of D. suzukii; these factors could also affect dispersal. Flight mills are a convenient tool for measuring and comparing the flight performance of insects. In this study, two experiments examined the effects of diet and humidity on D. suzukii flight performance using custom-built flight mills, and a third experiment compared the energy reserves of D. suzukii flown or not flown on flight mills. Over all flight assays, the median flight distance and duration were 27.16 m and 2.37 min, respectively, and the mean flight velocity was 0.18 m/s. The maximum flight distance and duration by an individual were 1.75 km and 2.35 h, respectively. Drosophila suzukii provisioned with blossoms, fruits, or standard laboratory diets flew farther distances and longer durations than starved flies. While starvation was associated with reduced flight performance, there were no observed differences between diet types. It remains unclear whether D. suzukii consistently use lipids, glycogen, sugar, or another energy source for flight because tethered individuals may not have flown enough to deplete energy reserves. Humidity did not affect flight performance of D. suzukii within a ~ 2 h test period. These data indicate that most D. suzukii are likely to remain within limited area (e.g., within a field) but that some individuals can disperse long distances (field to field spread).

Keywords Spotted-wing drosophila · Dispersal · Flight mill · Flight energy

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Key Message

- On average *Drosophila suzukii* flew for relatively limited duration and distance but some individuals were capable of long-distance dispersal.
- *Drosophila suzukii* subsisting on natural and artificial diets flew farther distances and longer durations than starved flies on tethered flight mills.
- Conditions between 30 and 80% relative humidity did not affect the tethered flight performance of *Drosophila suzukii* within a 2-h test period.

Introduction

Drosophila suzukii is an invasive pest that reduces the marketable yield of berries and other soft-skinned fruits (Lee et al. 2011). The geographic range of this insect currently includes Europe, North America, and South

America in addition to its native range in Asia (Asplen et al. 2015; dos Santos et al. 2017). Habitat suitability models predict that *D. suzukii* will continue to expand over the current ranges (dos Santos et al. 2017). However, the rate of this expansion is difficult to estimate because of our limited understanding of potential dispersion methods. Passive dispersal of *D. suzukii* mediated by the transportation of infested fruits is a likely contributor to the pest's rapid spread (Calabria et al. 2010). Autonomous dispersal is another mechanism for range expansion, and it is potentially aided by *D. suzukii*'s ability to develop on a wide variety of cultivated and wild fruits (Lee et al. 2015; Kenis et al. 2016). It is unclear how quickly *D. suzukii* will spread and what biotic or abiotic conditions favor autonomous dispersal.

Some *Drosophila* species can fly great distances under certain circumstances. *Drosophila melanogaster, D. simulans*, and *D. pseudoobscura* travel several kilometers in a day in search of resources in desert ecosystems (Coyne et al. 1982). However, *D. pseudoobscura* and some of its relatives only fly up to a few hundred meters in resource-rich habitats (Crumpacker and Williams 1973; Powell et al. 1976). Similarly, marked *D. suzukii* can disperse 100 m or more from alternate hosts in adjacent field margins into crop field interiors in a 1 week period (Klick et al. 2016). It is speculated that *D. suzukii* seasonally migrate several hundred meters in elevation to escape resource-poor areas (Mitsui et al. 2010). However, it remains unknown whether individuals execute the entire long-distance migration, if the migration occurs over generations, or if there are separate populations with differing phenology at various elevations. Thus, quantification of flight capabilities of *D. suzukii* may support or refine the hypothesis on long-distance migrations; it might also inform management decisions regarding alternate hosts in field margins and in the greater landscape.

Insect flight performance is significantly influenced by nutritional status and energy reserves (Briegel et al. 2001a; Wang et al. 2009; Lopez et al. 2014). Starvation and diet quality affect *D. suzukii* development, survivorship, and energy reserves (Hardin et al. 2015; Tochen et al. 2016a), which could affect flight performance. Feeding before flight is critical as trehalose, the main sugar in hemolymph, is the source of flight energy for many insect species (Briegel et al. 2001a, b; Wang et al. 2009; Arrese and Soulages 2010). However, the source of energy can vary between insect species. *Drosophila melanogaster* and other Dipteran species use glycogen as the primary source of energy for flight, and after several hours of flight use lipid reserves to sustain long-term flight (Wigglesworth 1949; Briegel et al. 2001a; Kaufmann and Briegel 2004). In contrast, aphids primarily use lipids for flight (Cockbain 1961; Liquido and Irwin 1986). It is not known what energy sources are used in *D. suzukii* flight.

Temperature and humidity affect *D. suzukii* development time, mortality, and fecundity (Kinjo et al. 2014; Tochen et al. 2014, 2016b; Hamby et al. 2016; Ryan et al. 2016; Eben et al. 2017; Zerulla et al. 2017). The effects of these environmental factors on flight performance are not understood in *D. suzukii*. For female *D. melanogaster*, the probability of dispersal increases with temperature up to ~ 30 °C (Roff 1977). Humidity is not as extensively studied as temperature despite potential effects on *D. suzukii* pest pressure (Tochen et al. 2016b; Wiman et al. 2016). Humidity is positively correlated with longevity and fecundity, but inconsistently affects other aspects of *D. suzukii* physiology in laboratory experiments (Tochen et al. 2016b). Given the abundance of temperature-related research, we focused on obtaining flight data under varying humidity conditions.

Drosophila spp. dispersal has been studied in field settings using mark-capture (Klick et al. 2016) or mark-recapture (Coyne et al. 1982) methods. However, both methods can only measure whether flies can travel a predetermined distance flown over a set duration. Dispersal experiments conducted in controlled settings can eliminate those limitations. Flight mills have been used to study tethered flight and dispersal potential of lygus bugs (Blackmer et al. 2004), aphids (Zhang et al. 2008), fruit flies (Wang et al. 2009), beetles (Naranjo 1990; Taylor et al. 2010; Lopez et al. 2014), and stink bugs (Wiman et al. 2015). Replicable flight mill experiments measure relative flight parameters more precisely than field experiments (Lopez et al. 2014). While tethered flight does not exactly replicate free flight (Rowley et al. 1968), flight mills are a convenient and practical tool to compare flight distance, duration, and speed between multiple experimental groups of comparable size (Taylor et al. 2010; Lopez et al. 2014).

Our first objective was to compare flight distance, duration, and velocity among female *D. suzukii* provided various diet treatments; we hypothesized that flight distances and speeds would be greatest for *D. suzukii* fed the most nutritionally complete diet. Our second objective was to compare flight parameters in various humidity levels for *D. suzukii* fed various diets to assess whether nutritional status changes an insect's flight response to environmental conditions. Because humidity correlates positively with *D. suzukii* longevity and fecundity, we hypothesized that flight would positively correlate with humidity and that the correlation would be consistent across the diets. Our third objective was to compare the amounts of lipids, sugars, and glycogen of female *D. suzukii* flown on flight mills to those that were not flown ('un-flown'). Because glycogen is the primary source of flight energy for *D. melanogaster* and other flies, we hypothesized that glycogen sugar levels would be significantly lower in flown flies than in un-flown flies. We also expected to see lower levels of sugar and lipids in flown flies compared to un-flown flies. The flight parameter estimates

reported in this study may assist future field research on *D. suzukii* dispersal patterns.

Materials and methods

Flight mills

The *D. suzukii* flight mills were custom-built based on a design used for soybean aphids, *Aphis glycines* (Zhang et al. 2008). The *D. suzukii* flight mill consisted of a wooden base and a photosensor that tracked the circular laps flown by a fly attached to a tether. The flight mill armature consisted of a #1 insect pin (Bioquip, Ranch Dominguez, CA) with 0.1-mm copper wire wound around the pin such that the two strands extended 4.5 or 5 cm away from the pin and formed a T shape. The tips of the wire were bent downward 85°. A laminated paper disk (3 cm diameter) with two holes (0.5 cm diameter) at opposite poles was positioned 1 cm from the tip of the pin, so that the pin served as the center axis for the disk. The armature was suspended vertically between two rare earth magnets to allow virtually frictionless rotation. The disk on the armature was positioned so that the photosensor (Omron EE-SX672R, Kyoto, Japan) tracked the half rotations flown by the insect. The photosensor readings were recorded using an Arduino microcontroller (Uno R3) and Parallax Data Acquisition (PLX-DAQ) tool (Parallax, Rocklin, CA), an add-in software extension for Microsoft Excel. Five flight mills could be run simultaneously using one Arduino microcontroller. The photosensor readings recorded every 0.08 s were summarized using R 3.2.4–3.3.3 (R Core Team 2016, 2017) to determine total flight distance and duration, and average velocity.

Flight mill assay

To prepare the *D. suzukii* for the flight assays, approximately 5 female flies were aspirated into a vial at a time. The vial was put in ice for 10–15 min to sedate flies. Each sedated fly was attached at the prothorax to the end of the flight armature tether with a bead of super glue and a small amount of baking soda (< 0.1 mg) to quickly dry the glue. The flight armature was immediately placed in between the magnets on the flight mill base. Prior to tethering the test subject, another female fly with wings removed was attached to the end of the other arm as a counterbalance.

Once placed on the flight mill, flies either initiated flight on their own, or they required stimulation from a gust of wind produced by briefly blowing from behind the fly. In either case, the flies were carefully monitored for unassisted flight before the photosensor was activated and recording began. When flies stopped flying that was the end of a single flight bout. Every fly was given 1 min to resume flight on their own, or they were stimulated with a simulated landing after > 1 min of inactivity (Cockbain 1961). The landings were simulated by touching the tarsi with the smooth surface of forceps. Flies would either begin another flight bout or they would not fly again. The flight session concluded once the fly ceased flight, would not resume flight within a minute of inactivity, and three landings were simulated. The sum of all flights per individual (total flight) typically lasted 5–20 min, but some lasted over 2 h. The assays were conducted in a laboratory between 8:00 am and 12:00 pm. Up to 5 flies could be assayed at a time, and approximately 15 flies could be assayed per day.

Experiments

All experiments were conducted with adult *D. suzukii* from a laboratory colony reared on standard corn-meal–yeast–sugar–agar diet in a growth chamber kept at 22 °C, 60 ± 5% relative humidity, and 16:8 L:D photoperiod (Woltz et al. 2015). The colony was initiated each year with wild *D. suzukii* collected from infested caneberries in Corvallis, OR from July to October 2015 and 2016 (See Appendix A for comparison of flight parameters of wild to colony flies). The generation of the flies from wild parents was recorded (Table 1).

Approximately 100 *D. suzukii* adults (1–4 days old) were put in plastic cages with a mesh sleeve (23 × 23 × 25 cm) and acclimated to ambient laboratory conditions (∼ 22 °C, 40% RH). All cages contained a water wick and a food source, with the exception of the cages holding starved flies, which only had water (Table 1). Flies were fed ad libitum and exposed to diet treatments for 1–3 days before placing on

Table 1 Experimental details of diet and humidity effects on flight parameters

Experiment	Food source	Flies flown	Fly gen. from wild parents
Diet	Water only	62	$F_3 - F_5$
Flight energy[a]	20% sucrose solution	13	$F_3 - F_4$
	Cherry blossom	12	F_4
	Blueberry blossom	22	F_4
	Blackberry blossom	8	F_4
	Standard diet	66	F_5
	Cherry fruit	37	F_5
	Raspberry fruit	51	F_5
	Blueberry fruit	52	F_5
	Blackberry fruit	40	F_5
Humidity	Water only	76	$F_2 - F_3$
	Blueberry blossom	57	$F_2 - F_3$
	Blueberry fruit	58	F_3

[a]Diet and flight energy experiments were conducted concurrently

flight mills. Flies were 2–7 days old on the day of testing. This age range made no differences in flight performance (Appendix B). Flight performance of female flies was the primary focus of this study because of their ability to directly damage fruit. They also had better flight performance than male flies, so only females were tested to eliminate variability associated with sex (Appendix C).

1. *Diet experiment Drosophila suzukii* were provided either blossoms, fruits, a positive control diet ('laboratory diet'), or a negative control diet (water only) prior to the flight mill assays (Table 1). Not every food source could be tested at the same time due to seasonal availability (Table 1). Three types of blossoms were examined: cherry, blueberry, and blackberry. Flowers were collected locally in the spring and kept on the branches with the leaves removed in vials of water to prolong blossom longevity. Each branch had five to ten flowers and two to three branches were put into each cage for approximately the same number of flowers per blossom species. The positive control (sugar diet) for the blossom diet experiments was a 20% sucrose solution dispensed by a cotton wick. Four types of ripe, unsprayed fruit were studied: cherry, blueberry, raspberry, and blackberry. Approximately 10 g of fruit was mashed and put on a small dish before the dish was put into a cage. The positive control for the fruit diet experiments was the standard cornmeal–yeast–sugar–agar diet (Woltz et al. 2015). Blossoms and fruits were commercially available varieties grown in research plots at the Oregon State University Lewis Brown Farm in Corvallis, OR. Flies that received the negative control treatment were given only water and are henceforth referred to as 'starved.' Because of seasonal availability, flies provided blossoms or fruit were tested concurrently with starved flies and flies fed appropriate laboratory diets. After the flight mill assay ended for each fly, the insect was placed in a microcentrifuge tube in a − 20 °C freezer to be analyzed for the flight energy experiment.

2. *Humidity experiment Drosophila suzukii* were provided blueberry blossoms, blueberry fruits, or were starved (water only) and held in cages at ambient laboratory conditions (~ 22 °C and 40% RH) before use in the flight mill assays as described above with the addition of a humidity variable (Table 1). The flight mills were placed in plastic tents ($64 \times 25 \times 25$ cm). The tents were constructed out of a plastic frame (BugDorm 4F2260, Megaview, Taiwan), 152-µm-thick plastic, duct tape, and plastic zippers. Relative humidity was manipulated inside the plastic tents with 14 cm diameter dishes of water or salt solutions, and the observed humidity levels inside the tents ranged continuously from 30 to 82% (Table 2). Saturated salt solutions were selected and pre-

Table 2 Relative humidity range measured in tents at ambient laboratory temperature (22 °C) from salt solutions

Compound	Amount dissolved in 50 ml H_2O	Measured range (%)
LiCl	70 g	30–44
None	–	32–55[a]
NaCl	50 g (precipitate in solution)	50–68
H_2O	–	61–80
KNO_3	75 g (precipitate in solution)	70–82

[a]Ambient laboratory condition

pared as described by Tochen et al. (2016b). The salt solutions were placed inside tents at least 12 h before flight mill assays to allow relative humidity to stabilize. Humidity levels inside tents of the same treatment fluctuated between trial days depending on conditions in the laboratory, and with the opening and closing of the tent door. The actual humidity level and temperature were recorded at the beginning of each flight assay with HOBO data loggers (U14-001, Onset Corp., Bourne, MA), and these values were used for analyses.

3. *Flight energy experiment* Glycogen, sugar, and lipid content of *D. suzukii* flown on the flight mill in the diet experiment ('flown') and those subjected to the same pre-flight treatment but not tested on the flight mill ('unflown') were measured. The biochemical assays used to measure these energy reserves were destructive to the insect, so it was not possible to test for pre- and post-flight energy reserves in the same individual. Hot anthrone tests quantified glycogen and sugar levels (Van Handel 1985a), and vanillin tests quantified lipid levels (Van Handel 1985b). Protocols used by Tochen et al. (2016b) for *D. suzukii* were followed, except in our assays 200 µl of the final reacted solution was pipetted into a 96-well assay plate. Absorbance of the samples was read in a spectrophotometer (ELX 808, BioTek, Winooski, VT) at 630 nm for sugar and glycogen assays and 490 nm for lipid assays. Absorbance was converted to glycogen, sugar, or lipid levels based on respective calibration standards.

Statistical analyses

The flight distance, duration, and average velocity were analyzed separately in the diet ($n = 363$) and humidity ($n = 191$) experiments. Flight distances and durations were highly skewed toward small values, so distance and duration were log-transformed to meet the assumptions of residual normality and equal variance. Log-transformed coefficients were later back-transformed by exponentiation for interpretation of the medians and for presentation.

Flight velocity for each fly was averaged from the velocities of each flight bout within the flight session.

In the diet experiment, separate models were fit to compare the effect of diet groups (fruit, blossom, laboratory diet, and starved) on the medians of flight distance and duration, and on the means of average flight velocity. Exploratory analyses found no differences in flight performance among the three blossom types, or among the four fruit types, or between sucrose and cornmeal–yeast–sugar–agar diets, so to simplify analyses, the diets were grouped broadly as blossoms, fruit, or laboratory diet. Tukey HSD was used to make pairwise comparisons of flight performance among groups given blossoms, fruit, laboratory diet, or water only. Starved flies had significantly lower median total flight distances and durations, while the three fed groups were equivalent. To calculate the estimated magnitude of difference between each fed group and starved, a Bonferroni correction was used to adjust significance level α to 0.0167. This accounted for three comparisons between diets (blossom vs starved, fruit vs starved, laboratory diet vs starved). The estimated ratios between diet groups of median total flight distances and durations were calculated along with the 98.3% confidence intervals. The estimated differences between diet groups of mean average velocity and the confidence intervals were calculated.

In the humidity experiment, separate second-order polynomial regression models were fit to analyze predictors of median total flight distance and duration. Relative humidity level was recorded at the beginning of each flight assay and was coded as a continuous variable in these analyses. Exploratory analyses found a quadratic relation between flight distance and humidity, and between flight duration and humidity. The fixed effects in both models were diet, humidity, humidity^2, and the diet × humidity interaction. A linear regression model was fit to analyze the effect of diet and humidity on mean average flight velocity.

Analyses of the flight energy experiment were done with lipid, sugar, or glycogen concentration as the dependent variables. Diet (categorical variable with five levels: fruit, blossom, cornmeal–yeast–sugar–agar diet, sucrose, and starved), flight status (flown or un-flown), and the diet × status interaction were fixed effects in separate means models. Bonferroni correction was used to adjust significance level α to 0.01 to account for five comparisons of energy reserves between flight status for each of the five diets (e.g., blossom + flown vs blossom + un-flown). The estimated differences in mean energy levels were calculated along with the 99% confidence intervals. All statistical analyses were done using R 3.3.3 (R Core Team 2017).

Results

Diet experiment

Female *D. suzukii* provisioned with food had higher levels of sugar and glycogen than starved flies (Table 3). Adult *D. suzukii* emerge with limited reserves of sugar and glycogen (Tochen et al. 2016a). The elevated concentrations of carbohydrates observed in the diet-provisioned flies confirmed they had fed. Flight distance differed significantly between fed and starved *D. suzukii* ($F_{3, 359} = 14.49$; $p < 0.001$; Fig. 1a). There were no differences in the median flight distances among flies provided fruits, blossoms, or laboratory diets. The median flight distance of flies given fruit was 4.13 times greater (98.3% CI [2.46, 6.92]) than that of starved flies; the median flight distance of flies given blossoms was 2.65 times greater (98.33% CI [1.31, 5.34]) than that of starved flies; and the median flight distance of laboratory diet-fed flies was 2.69 times greater (98.3% CI [1.48, 4.87]) than that of starved flies.

Flight duration was similarly affected by the diet treatment ($F_{3, 359} = 14.35$; $p < 0.001$; Fig. 1b). Median flight

Table 3 Average amount ± SEM of energy reserves in un-flown and flown *D. suzukii*

Diet	Flight status	n	Lipid (µg)	Sugar (µg)	Glycogen (µg)
Fruit	Un-flown	181	42.05 ± 1.43*	78.06 ± 4.36	53.83 ± 2.95
	Flown	180	35.99 ± 1.29	73.05 ± 4.67	57.91 ± 2.88
Blossom	Un-flown	46	38.84 ± 3.65	31.33 ± 7.08	9.71 ± 1.91
	Flown	43	38.57 ± 2.44	37.3 ± 8.79	12.44 ± 2.43
Cornmeal–yeast–sugar–agar diet	Un-flown	66	34.2 ± 2.77	48.24 ± 3.76	88.14 ± 5.9
	Flown	66	30.4 ± 1.6	37.28 ± 2.27	87.85 ± 6.86
Sucrose	Un-flown	13	21.71 ± 6.23*	186.44 ± 21*	40.66 ± 5.28
	Flown	13	41.85 ± 6.13	45.71 ± 15.75	12.22 ± 4.61
Starved	Un-flown	71	25.19 ± 1.8*	4.18 ± 0.67	4.6 ± 1.02
	Flown	65	34.05 ± 3.42	5.78 ± 0.84	11.18 ± 2.24

*Statistical difference in energy reserve level between flown and un-flown flies with the Bonferroni adjustment $p < 0.01$
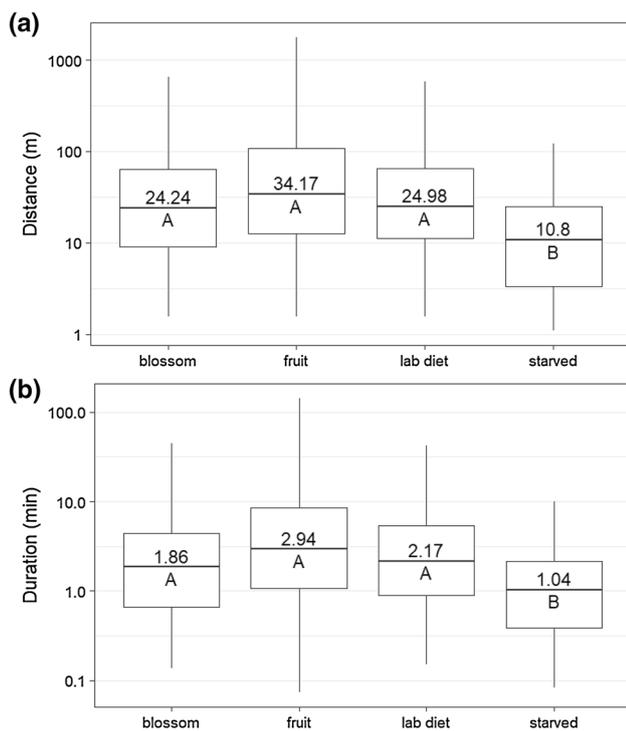
Fig. 1 Boxplots displaying minimum, first quartile, median, third quartile, and maximum of flight **a** distance and **b** durations of *D. suzukii* fed different diets. The *y* axis is on a logarithmic scale. Values for observed medians are listed, and different letters represent statistical significance between diets

durations of flies given fruits, blossoms, or laboratory diets were equivalent and greater than the median duration flown by starved flies. The median flight duration of flies given fruit was 3.8 times greater (98.3% CI [2.34, 6.24]) than that of starved flies; the median flight duration of flies given blossoms was 2.52 times greater (98.33% CI [1.29, 4.91]) than that of starved flies; and the median flight duration of flies given laboratory diet was 2.54 times greater (98.3% CI [1.44, 4.47]) than that of starved flies.

Mean flight velocities were similar across cohorts of *D. suzukii* that were starved or given blossoms, fruit, or laboratory diet ($F_{3, 359} = 0.659$; $p = 0.578$).

Humidity experiment

There were no differences in median flight distances, median flight durations, or mean velocities of *D. suzukii* due to humidity (Table 4). Median flight distances and durations were significantly affected by diet, but not by the diet × humidity interaction. Diet did not affect mean flight velocity.

General flight performance

The flight parameters of all flies tested on the flight mill in the diet experiment and the humidity experiment were summarized together (Table 5). Frequency of female *D. suzukii* flights were highly skewed toward shorter distances (Appendix D) and durations. Mean flight velocity was 0.18 ± 0.002 m/s with a maximum observed velocity of 0.37 m/s. Flight distances ranged from 1 m to 1.75 km, and flight durations ranged from 4 s to nearly 2.5 h.

Flight energy experiment

The levels of lipids, sugar, and glycogen in the female *D. suzukii* varied based on the type of food they were provided

Table 5 Summary flight parameters calculated from all *D. suzukii* tested

$n = 554$	Distance (m)	Duration (min)	Velocity (m/s)
Minimum	1.1	0.07	0.03
Median	27.16	2.37	0.18
Mean	87.02	7.38	0.18
Maximum	1750.46	141.47	0.37

Table 4 Statistical summary of humidity experiment

Response	Fixed effect	F	Df	p
Total flight distance (log-transformed)	Diet	44.15	2184	<0.0001
	Humidity	1.11	1184	0.29
	Humidity^2	2.55	1184	0.11
	Diet × Humidity	0.38	2184	0.68
Total flight duration (log-transformed)	Diet	43.57	2184	<0.0001
	Humidity	1.61	1184	0.21
	Humidity^2	2.92	1184	0.09
	Diet × Humidity	0.26	2184	0.77
Average velocity	Diet	2.06	2187	0.13
	Humidity	0.07	1187	0.79

(Tables 3, 6). Flight status did not significantly affect lipid levels, but the interaction between diet and flight status affected lipid levels (Table 6). Mean lipid levels were different between flown and un-flown cohorts given fruit or sucrose, as well as those that were starved. For cohorts of flies fed fruits, un-flown flies had on average 6.06 µg more lipid (99% CI [0.75, 11.36]) than flown flies. For cohorts of flies fed sucrose, un-flown flies had on average 20.14 µg less lipids (99% CI [0.36, 39.91] than flown flies. For cohorts of starved flies, un-flown flies had on average 8.85 µg less lipid (99% CI [0.2, 17.51]) than flown flies. There were no differences in mean lipid levels detected between un-flown and flown flies fed blossoms or laboratory diet.

Flight status affected sugar levels, as well as the interaction between diet and flight status significantly affected sugar levels (Table 6). Mean sugar levels in flies fed sucrose were lower among those flown on the flight mill compared to those that were not flown. For flies fed sucrose, un-flown flies had on average 140.73 µg more sugar (99% CI [91.14, 190.32]) than flown flies. There were no differences in mean sugar levels detected between un-flown and flown flies fed fruits, blossoms, cornmeal–yeast–sugar–agar diet, or flies that were starved.

The interaction between diet and flight status did not significantly affect glycogen level (Table 6). There were no differences in mean glycogen levels between un-flown and flown flies fed any of the five diets.

Discussion

Satiation was more important as a predictor of *D. suzukii* tethered flight performance than the type or quality of food provided. Standard laboratory diet is a nutritionally complete diet comprised of both carbohydrates and protein, while fruits and blossoms are generally high in carbohydrates but lacking in protein (Nicolson and Thornburg 2007; U.S. Food and Drug Administration 2008). Despite this, flies provided standard diet, fruits, or blossom diets performed similarly

Table 6 Statistical summary of flight energy experiment

Response	Effect	*Df*	*F*	*p*
Lipid	Diet	4734	7.93	< 0.001
	Flight status	1734	0.86	0.35
	Diet × Flight status	4734	5.75	< 0.001
Sugar	Diet	4734	67.43	< 0.001
	Flight status	1734	5.33	0.02
	Diet × Flight status	4734	12.84	< 0.001
Glycogen	Diet	4734	112.74	< 0.001
	Flight status	1734	0.87	0.35
	Diet × Flight status	4734	1.4	0.23

in the flight assays. Even in various humidity conditions, starved *D. suzukii* did not fly as far as sated flies. It does not seem likely that starved *D. suzukii* can make long-distance flights to exploit better resources. However, sated *D. suzukii* may be capable of long-distance migrations. It was rare for fed *D. suzukii* to fly over 1 km in our experiments, but even a small percent of a large population would be a numerically large group of long-distance fliers. Some tethered *D. suzukii* in our experiments were observed resuming flight after the conclusion of the experimental flight session. This suggests that the flight mills may not have measured flight until complete exhaustion, but may better characterize foraging flights between resource patches. If this is the case, *D. suzukii* females may be able to fly several kilometers in a lifetime, similar to other *Drosophila* species (Coyne et al. 1982). Wild *D. suzukii* reared on field-collected raspberries were observed flying over 1 km, though they more frequently flew shorter distances compared to the laboratory colony flies that were F$_3$ progeny of wild parents (Appendix A). Male *D. suzukii* tend to fly significantly less than female flies (Appendix C), so it is not clear if all *D. suzukii* could disperse several kilometers in a lifetime. Lastly, the dispersal capacity of the overwintering generation is hypothesized to have the longest migration in early spring and late autumn (Mitsui et al. 2010). Laboratory-reared summer and winter morphotypes (Wallingford and Loeb 2016) that enclosed within 2–7 days and were fed a laboratory diet flew similar distances on the flight mill (Appendix E). Future studies should test the winter morphotype that undergoes overwintering since prolonged cold exposure as an adult may induce different flight behaviors.

Rowley et al. (1968), Riley et al. (1997), and Taylor et al. (2010) have discussed the need for careful interpretation of flight mill data. First, it is unknown how handling and tethering an insect, and how the flight mill itself, physically and behaviorally affects flight. Next, flight mill experiments done in the laboratory lack the stimuli that wild insects would normally encounter in the field, such as wind, various olfactory cues, and solar photostimulation. Lastly, tethered flight increases the weight load on an insect which may underestimate flight performance, but it also reduces the energy expended to remain aloft which may overestimate performance. For these reasons, it is important to design experiments and interpret results in a comparative fashion. In our experiments, we were careful to handle all female *D. suzukii* similarly. We also took relatively large sample sizes in an attempt to minimize variance and tried to keep experimental group sizes similar. Comparisons of tethered flight to *D. suzukii* free flight in the field could provide important information on the reliability of extrapolation of flight mill data.

Studies on Dipteran species consistently show that carbohydrate feeding before flight is critical as glycogen is primarily utilized in flight before direct lipid usage occurs

(Wigglesworth 1949; Briegel et al. 2001a; Kaufmann and Briegel 2004). Similar flight parameters observed in our experiment among flies provided blossoms, fruit, sucrose or cornmeal–yeast–sugar–agar diets may be explained by the high sugar content in all of these diets (Woltz et al. 2015; Tochen et al. 2016a). However, our results do not conform to the existing knowledge on Dipteran flight energy utilization. Glycogen levels were not lower in flown *D. suzukii* compared to un-flown flies in any of the diet subsets as hypothesized. The sugar trehalose or lipids could be a substrate utilized in *D. suzukii* flight as found with other insect species (Briegel et al. 2001a, b; Wang et al. 2009; Arrese and Soulages 2010). However, evidence of sugars or lipids being expended in *D. suzukii* flight was inconsistent in our experiment. Sugar levels were only lower among flown flies compared to un-flown flies when flies were fed a sucrose diet, and lipid levels were only lower among flown flies when given fruit. It is possible that tethered flight uses significantly less energy than free flight or that flown *D. suzukii* did not fly for periods long enough to detect lowered energy reserves. Furthermore, there were no detectable differences in lipid, sugar, or glycogen content between *D. suzukii* that flew long distances and flies that flew short distances (Appendix D).

Proline, an abundant amino acid found in hemolymph, was not measured in our experiment, but it is the primary source of flight energy for some insects, including tsetse flies, *Glossina morsitans*, and Colorado potato beetles, *Leptinotarsa decemlineata* (Beenakkers et al. 1984; Arrese and Soulages 2010). Definitive assertions about what energy sources are used in *D. suzukii* flight are beyond the scope of this study. Future studies on *D. suzukii* flight energy utilization should consider non-destructive methods for assaying energy reserves of individuals before and after flight; flies should be forced to fly to exhaustion; and trehalose-specific, proline, and protein analyses should be conducted along with lipid, glycogen, and sugar analyses.

We were surprised to see higher levels of lipids in the flown flies versus the un-flown flies in starved *D. suzukii* and flies provided sucrose but starved of protein and other nutrients. However, similar trends have been observed in other studies. Lipid levels were higher among starved *D. suzukii* than flies exposed to artificial diet for 1 day with two different colonies (Tochen et al. 2016a). Both flight and starvation can mobilize lipids (Beenakkers et al. 1984; Arrese and Soulages 2010). Starvation, in particular, causes oenocytes to facilitate the release of large amounts of lipids from the fat body in *Drosophila* spp. (Gutierrez et al. 2007). It is not clear where the fat body lipids are processed in *Drosophila*, but in starved *Manduca sexta* lipids concentrate in the hemolymph (Ziegler 1991). It is unknown if this could affect total lipid levels, or the detection of lipids with a biochemical assay (Van Handel 1985b). It seems likely that flight on the flight mill affected metabolic activity in our *D.*

suzukii, although it is difficult to determine what activity was impacted from the existing literature, and it is beyond the scope of our study.

Water loss, as a result of high temperatures or low humidity, typically reduces flight performance of insects (Fadamiro and Wyatt 1995; Zhang et al. 2008; Wang et al. 2009). This was not the case for *D. suzukii* flown on the flight mills. Relative humidity ranging from 30 to 80% was not positively correlated with *D. suzukii* flight performance as we had hypothesized. However, this result is not unusual, as the flight performance of certain beetles is also unaffected by humidity (Sinclair and Haddrell 1985; Dowdy 1994). The null results of our humidity experiment provide some evidence of the range of environmental conditions in which *D. suzukii* can fly, which attest to the ability of *D. suzukii* to invade a wide variety of geographic areas.

We suggest examining variables affecting *D. suzukii* flight initiation in future flight mill studies. In our study some flies required artificial stimulation before initiating flight, while others did not. Measuring biological and environmental flight stimulants might be considered in future flight mill studies. Furthermore, studying the effects of *D. suzukii* body size, egg load, and recent ovipositional activity on flight performance may also improve understanding of the pest's movement patterns.

This study provides estimates of *D. suzukii* flight performance and can be applied to future research on dispersal from alternate hosts to crops, long-distance migration, and flight energy utilization. The risk of new invasions of *D. suzukii* may be compounded by the dispersal capacity of adults. *Drosophila suzukii* appear to have few requirements for flight. So long as they have access to food and water, *D. suzukii* can fly hundreds of meters between resource patches. *Drosophila suzukii* may be capable of many flight sessions in a lifetime, which would make dispersal between cultivated and wild hosts in a landscape inevitable. Field research to confirm free flight performance of wild *D. suzukii* and their typical exposure to varying levels of starvation and humidity is crucial for improving management strategies and reducing economic loss.

Author contribution

Compliance with ethical standards

Conflict of interest The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. All authors, JSW, ACC, DML, WFM, SEN, NGW, JMW, and JCL, declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or vertebrate animals performed by any of the authors.

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