INTRODUCTION

The evolution of flight has allowed insects to thrive and become vastly diversified by escaping unfavorable conditions such as predation, lack of resources, or by exploiting unexplored niches (Sane, 2003). Research on insect flight and dispersal has provided insights on population ecology and dynamics, improved our understanding of invasion biology, and has been integrated into pest management programs (Asplen, 2018). Obtaining information on insect flight behavior is critical for understanding the movement and spread of insect pests, which can have significant economic and environmental impacts. In this study, we compare flight behavior of the invasive fruit fly, Drosophila suzukii, using two common approaches: tethered flight mills and free-flight studies. Each method has its own advantages and disadvantages, and assessing their relative merits is essential for accurate and reliable data collection.
flight behavior, however, can be difficult in the field due to limitations with equipment and the specimen’s size (Chapman et al., 2015; Minter et al., 2018). Hence, most flight research in insects has been conducted in the laboratory to investigate movement, flight stimuli, and potential dispersal capabilities (Reynolds & Riley, 2002; Naranjo, 2019).

There are two general approaches for investigating insect flight behavior. Here, we focus on the use of tethered flight mills and free flight in a flight chamber (e.g., vertical flight chamber). With tethered flight studies, specimens are physically attached to an apparatus that can record flight duration, distance, and speed. Tethered flight mills are appealing because the data collection can be automated with computers, they are relatively easy to construct and setup in any location, and multiple observations can occur at once (Chambers et al., 1976; Taylor et al., 1992; Attisano et al., 2015; Martí-Campoy et al., 2016; Minter et al., 2018). However, the biggest drawback with flight mills is they are often perceived as unnatural (Riley et al., 1997; Ribak et al., 2017; Minter et al., 2018). In a flight mill system, as the insect does not need to generate lift to produce flight, and once the specimen has overcome resistance to move the mill, effort to push the mill forward is reduced (Ribak et al., 2017; Minter et al., 2018; Naranjo, 2019). Therefore, flight mills are still useful tools for direct comparison of how variable traits such as age, feeding status, mating status, or energy reserves affect flight capacities in insects (Fahrner et al., 2014; Kees et al., 2017; Tussey et al., 2018; Aita et al., 2021). However, results from laboratory tethered flights are potentially misinterpreted and under- or over-estimate an insect’s flight in the field by miscalculating the distance a species can travel (Taylor et al., 2010).

Alternatively, free-flight systems are advantageous because they allow the insect to fly unrestricted. However, equipment for measuring free flight (e.g., vertical flight chambers) are generally cumbersome due to their large sizes. Also, studies of free flight can be labor-intensive because observations are usually not automated to record flight behavior and typically only one specimen is observed at a time (Blackmer et al., 2004; Asplén et al., 2009; Naranjo, 2019).

The differences between the two flight assay approaches can lead to different results about insect flight behavior and hence it is important that tethered and free-flight systems are compared with the same species (Blackmer et al., 2004; Taylor et al., 2010; Asplén, 2018). Varying results from each flight assay, such as distance and duration, can be problematic when trying to understand the potential movement of an invasive species in the field, as predictive models and assumptions used are based on results of the laboratory flight assay.

A timely example of the need to better understand flight behavior and capabilities involves the invasive vinegar fly spotted-wing drosophila, Drosophila suzukii (Matsumura) (Diptera: Drosophilidae). Native to Asia, D. suzukii has been reported in North and South America, Europe, and northern Africa (Lee et al., 2011; Calabria et al., 2012; Deprá et al., 2014; Asplén et al., 2015; Kwadha et al., 2021). Dispersal experiments have been conducted on D. suzukii to forecast potential invasion risk (Maino et al., 2021), understand movement between cash and non-cash crops (Klick et al., 2016; Leach et al., 2018), and seasonal movement (Tait et al., 2018). These field studies have relied on either (1) the use of baited traps, which can alter the flies’ natural behavior, or (2) a mark-and-recapture technique, where the results can be limited because of the set, predetermined distances measured (Robinett et al., 2019). Testing flight capabilities in the laboratory with flight assays under controlled conditions potentially assist with the limitations of these field studies, and better inform those in the future. To our knowledge, only one study has been conducted investigating D. suzukii flight behavior in the laboratory. However, this was limited to tethered studies of female summer morphs (Wong et al., 2018).

Drosophila suzukii is known to have two phenotypic seasonal morphs, ‘summer’ and ‘winter’. Winter morphs are produced when D. suzukii eggs and larvae are subjected to a decrease in photoperiod and temperature, and have been described to be darker in pigmentation and larger in body and wing size in comparison to summer morphs (Shearer et al., 2016; Tran et al., 2020). Researchers have hypothesized that the larger and darker body of winter morphs assist with overwintering by absorbing sunlight and storing fat (Shearer et al., 2016; Leach et al., 2019). An alternative hypothesis is the winter morph could be adapted for dispersal similar to migratory insect species, such as Danaus plexippus (L.), whose migratory forms have larger wings for long-distance migration (Satterfield & Davis, 2014; Flockhart et al., 2017). Gaining information on the flight behavior of summer and winter morphs can assist with understanding how D. suzukii survives in areas with seasonal changes, providing insights on whether this phenotypic polymorphism aids in long-distance dispersal.

The limitation in the literature to studies of female D. suzukii is also problematic, as many insects species exhibit sex-biased dispersal (Markow & Castrezana, 2000; Asplén et al., 2016). Obtaining information on potential dispersal differences between the sexes could aid in furthering the understanding of this invasive species’ biology and assist with future integrated pest management (IPM) strategies such as the sterilized insect technique (SIT). The aims of this study were to compare the results of simultaneously conducted tethered and free-flight assays with D. suzukii as a model to further compare flight behaviors between winter and summer morphs and males and females.

MATERIALS AND METHODS

Insects

Flight experiments were conducted using laboratory-reared D. suzukii adults. A fly colony was established by collecting infested raspberry fruit in 2020 at the University of
Minnesota (St. Paul, MN, USA; 44.990721, −93.174319) and maintained in the Department of Horticultural Sciences. Protocols and methods for rearing *D. suzukii* summer and winter morphs were performed as described by Stephens et al. (2015). Summer morphs were reared in narrow polystyrene vials with foam plugs (Genesee Scientific, San Diego, CA, USA). Each vial contained approximately 5 ml of an artificial diet comprised of agar, commel, sugar, and yeast (Dalton et al., 2011). Vials were stored in a growth chamber (Percival Scientific, Perry, IA, USA) at 25 ± 1 °C, 50 ± 10% r.h., and L16:D8 photoperiod. Adult winter morph *D. suzukii* were produced by placing vials with 1- to 3-day-old eggs from summer morphs into another growth chamber at 10 ± 1 °C, 60 ± 10% r.h., and L12:D12 photoperiod (e.g., Tran et al., 2020).

Summer and winter morph pupae were individually transferred to a clear 1.5 ml microcentrifuge tubes with approximately 2 μl of 40% sucrose water solution. The microcentrifuge tubes with the pupae were stored for adult emergence in a growth chamber at 12 ± 1 °C, 60 ± 10% r.h., and L16:D8 photoperiod. Adult summer and winter morphs were approximately 2–4 days old when used in the study. The tethered and free-flight assays occurred with the same cohort of adult flies on the same day.

### Tethered flight study

Twelve computer-monitored flight mills designed by Fahrner et al. (2014) were used to investigate the tethered flight behavior of *D. suzukii*. The flight mill and procedure for tethering *D. suzukii* to the flight apparatus were similar to Fahrner et al. (2014) and Kees et al. (2017). In brief, the flight mill consisted of an electronic sensor that would detect when *D. suzukii* flew in a horizontal axis connected to a tethered arm. The flight mill was constructed with 20-cm-long top and bottom support rods placed perpendicularly to form a block ‘C’-shape. The open ends of each support rod had a pair of 8-cm-long cylinders with circular rare-earth magnets. The cylinders were fashioned in a way so that the polar end of the magnets faced each other and that there was a 4-cm-wide gap in between the cylinders where the tethered arm could freely rotate without obstructing any parts of the flight mill.

The tethered arm consisted of a no. 1 stainless steel insect pin with a 6-cm-long copper wire wound around the pin forming a cross. The magnets held the steel insect pin upright and the pin provided the central axis around which flight occurred. Approximately 1–2 mm of the terminal copper wire was bent to a 90° downward angle to which the fly was attached. The final radius of the tethered arm was 5.5 cm. The sharp end of the pin with the attached copper wire was drilled through the center of a circular encoder wheel with four equal radial splits. When the wheel rotated from flight, a sensor would detect the phase changes of the wheel. The sharp end of the pin was placed on the bottom magnet and was held up-right between the magnets. The flight mill was surrounded by a white drape to prevent external visual stimulus that may provoke flight.

To affix adults to the tether, individuals were anesthetized with CO₂ (Carbon Dioxide Anesthetizer Kit; Carolina, Burlington, NC, USA). Once the flies were sedated, adults were transferred on to a filter paper that was placed on top of an icepack. The tethered arm with the 90° bent end was dipped into super glue (Loctite Super Glue Gel; Henkel, Westlake, OH, USA) and attached to the flies’ dorsal sides between the prescutum and scutum. Due to the small size of *D. suzukii*, a counterweight was not required to balance the tether arm. Once the fly was successfully attached, the tether arm was placed on the flight mill to begin recording (Figure 1). Flight was not instigated with any external stimulus (e.g., visual, physical contact, puff of air).

Flight was recorded in real time on a dedicated computer equipped with LabVIEW 2011 software (National Instruments, Austin, TX, USA). The raw data from LabVIEW were extracted using R v.4.1.0 (R Core Team, 2021) and RStudio Desktop v.1.4.1717 (RStudio Team, 2021). Flight information recorded was propensity (flight or no flight), velocity, density, and duration of flight for each fly. Flight was defined when *D. suzukii* completed one and a half full revolution (i.e., six radial ticks). *Drosophila suzukii* were left on the flight mill for approximately 24 h with constant light.

### Free-flight study

A vertical flight chamber similar to the one described by Asplén et al. (2009), which in turn is a modified version of those from Blackmer & Phelan (1991) and Kennedy & Booth (1963), was used to investigate free-flight behaviors. In brief, the flight chamber was 1 m³ and constructed with plywood painted black with exception of the front, which had a Plexiglas door to observe *D. suzukii* behavior. The bottom and top were covered with a fine black mesh screen. The chamber was illuminated from the center top with a 400-W mercury lamp. Adjacent to the light source on either side were fans that were used to cool the chamber between flights (Figure 2). Importantly, the downward air flow commonly employed in vertical flight chamber studies could not be used with *D. suzukii*, as preliminary studies showed that flies would immediately fall out of flight when facing resistance. In this study, therefore, the apparatus functioned as a large free-flight box, rather than a vertical flight chamber (i.e., flight velocity and distance could not be estimated, although flight propensity and duration could be).

Before flight, *D. suzukii* were acclimated to the chamber by placing the microcentrifuge tubes in the chamber for approximately 10 min. After acclimating, individual microcentrifuge tubes were placed in the center of the chamber in a rack and with the caps opened to allow *D. suzukii* to leave the tube. To determine flight, *D. suzukii* had to vertically ascend from the platform within 3 min. If *D. suzukii* did not fly during this allotted time frame, we concluded
that flight did not occur. After *D. suzukii* landed and did not initiate flight again within 1 min, the fly was re-captured, and flight was concluded. We did not consider jumping out of the microcentrifuge tube or hopping around the platform as flight (Figure 2). Methods were similar to Asplen et al. (2009). We measured propensity, phototaxis (flight towards sunlight cue), bouts (number of take-offs), and duration by directly observing and recording each individual *D. suzukii*. The studies with the tethered flight mill and the free-flight chamber were performed at Metropolitan State University (St. Paul, MN, USA).

**Statistical analysis**

To determine the factors that affected flight propensity using both techniques, we used a logistic regression with flight (i.e., yes or no) as a binomial response and terms for flight apparatus (tethered flight mill and free flight), sex (female and male), morph (winter and summer), and their interactions as predictor variables. The same predictor variables were used to examine the duration of first flight (i.e., excluding additional take-offs) with a Gamma regression model with a log link. The final models for propensity and duration of first flight were determined using a backward selection process. The effects of sex and morph on total flight time, total distance, and velocity were analyzed separately by flight apparatus (i.e., tethered flight mill and free-flight chamber) with a two-way ANOVA, where applicable. To improve the assumptions of normality and homoscedasticity of the errors, the response variables were log-transformed. All data were analyzed using R v.4.1.0 (R Core Team, 2021) and RStudio Desktop v.1.4.1717 (RStudio Team, 2021).

**RESULTS**

Summary statistics for *D. suzukii* for the free-flight chamber and tethered flight mill are presented in Tables 1 and 2, respectively. Overall, across the two approaches, 401 adult *D. suzukii* were used in the experiment (262 females and 139 males; 200 summer morphs and 201 winter morphs). In total, 15.7% of *D. suzukii* initiated flight; 18.0 and 13.4% in the free-flight chamber and tethered flight mill, respectively. Of the flies that initiated flight, the longest total duration of flight time for an individual in the free-flight chamber was 377.7 s, whereas with the tethered flight mill was 810.8 s. On the tethered flight mill, the maximum distance recorded for *D. suzukii* was 283.03 m in 24 h at a maximum velocity of 0.52 m/s.

When determining variables that affected the propensity to fly, there was no main effect of sex, therefore sex data were pooled, but there was an interaction between morph and flight assay ($\chi^2 = 5.59$, d.f. = 1, $P<0.02$) (Figure 3). Summer morphs were 14.0% more likely to initiate flight in the chamber when compared to being tethered to a flight mill, and initiated flights more frequently when compared to the winter morphs (Figure 3).

When the duration time for the first flight was compared, the maximum time in the free-flight chamber was observed to be 377.7 s, but for the tethered flight mill, the maximum time was 108.7 s (Table 3). The mean (± SEM) duration of first flight for *D. suzukii* in the free-flight chamber
and tethered flight mill was 36.7 ± 11.29 and 11.7 ± 4.10 s, respectively (Table 3). The final model showed that only flight apparatus influenced the duration of first flight: *D. suzukii* flew longer in the free-flight chamber compared to the tethered flight mill ($\chi^2 = 5.39$, d.f. = 1, $P < 0.02$) (Figure 4).

In the free-flight chamber, the total flight durations were similar between morphs ($F_{1,33} = 3.45$, $P = 0.07$) and sex ($F_{1,33} = 0.73$, $P = 0.40$) (Figure 5). On the tethered flight mill, there were no effects of morph and sex on duration (morph: $F_{1,24} = 0.13$, $P = 0.72$; sex: $F_{1,24} = 0.39$, $P = 0.54$), distance (morph: $F_{1,24} = 0.01$, $P = 0.91$; sex: $F_{1,24} = 1.05$, $P = 0.31$), and velocity (morph: $F_{1,24} = 1.93$, $P = 0.19$; sex: $F_{1,24} = 0.25$, $P = 0.62$) (Figure 6). There was no statistical evidence that sex was a factor affecting flight; however, numerically, female *D. suzukii* of both summer and winter morphs sustained flight for a longer duration and distance (Figures 5 and 6). Several of the high outliers that were recorded in duration of total flight and total distance were from females.

**DISCUSSION**

Research evaluating insect flight in the laboratory is limited and our study adds to the knowledge base by comparing *D. suzukii* flight behavior using a tethered flight mill and a free-flight chamber. Although each flight apparatus
allows researchers to gather information on insect flight in a controlled environment, rarely are cohorts tested simultaneously using both methods. This potentially can be problematic as the assays can yield different results, which in turn could lead to different interpretations of an insect’s natural flight behaviors. Also, laboratory flight assays provide a crude approximation to insect’s mobility in the field, and this estimate could be even more variable based on the method used (Hardie, 1993).

The simultaneous use of two flight apparatuses on the same population cohort of D. suzukii species provided new insights on flight behaviors of the invasive pest. The study showed that, in D. suzukii, the propensity to fly was influenced by the apparatus. Specifically, D. suzukii-initiated flight and duration of first flight were longer in free flight than tethered flight. This result is interesting – although D. suzukii were on the tethered flight mill for approximately 24 h (vs. 3 min) and had more opportunity to fly, adult flies
in the free-flight chamber had a higher propensity to fly. The differences could be due to handling the specimens to glue them onto the mill because manipulating insects can impact propensity (Taylor et al., 2010). In a similar study observing only female D. suzukii on a tethered flight mill, flies flew further (mean distance 87.02 m) and longer (7.38 min) compared to the adult flies across our experiment (Wong et al., 2018). The differences could be due to design, as Wong et al. (2018) provided a stimulant to provoke flight with either a gust of air or tarsi contact. Flight propensity and performance may have been reduced in our study due to lack of tarsal contact (Minter et al., 2018; Naranjo, 2019). Additionally, there were times when we observed D. suzukii wings moving, but the individual was unable to propel itself forward. We suspect D. suzukii could have been hovering instead of conducting forward flight (Dällenbach et al., 2018; Irvin & Hoddle, 2020) or was unable to generate enough power to overcome the moment of inertia to move itself with the small tether wire (Naranjo, 2019).

To our knowledge, this is the first study to compare the dispersal capabilities of the winter and summer morphs of D. suzukii. Migratory or dispersing morphs of insects are characteristically described as having larger wings and body size than nonmigratory morphs (Roff & Fairbairn, 2007; Li et al., 2016; Asplen, 2018). Researchers have hypothesized the winter morph may be an overwintering form because having a larger body may allow an increase of sugar and fat storage (Stephens et al., 2015; Kaçar et al., 2016; Stockton et al., 2019). However, the winter morph’s larger wings could also aid in dispersal. Despite having larger wings, winter morphs in this study had lower propensity to fly, flew for a lower duration of time, and flew a shorter distance compared to the summer morphs. Fraimout et al. (2018) conducted a study with D. suzukii adults on the effects of temperature and flight and reported individuals reared at 16 °C had larger wings, flew at a higher speed, and accelerated quicker compared to individuals reared at 22 or 28 °C. The D. suzukii in our study were reared at 12 °C and this may have affected flight. However, further investigations would be needed to determine whether low-end temperature rearing conditions may reduce flight capabilities because temperatures can affect insect’s flight performance (Taylor, 1963). Additionally, the dissimilarities in results could be due to the relatively low sample size of positive flights in winter morphs (19 total), or that the lack of flight in winter morphs is truly indicative of a sedentary phenotype.

The lack of evidence of winter morphs being a dispersal form may support the belief that polyphenism in D. suzukii is for overwintering purposes. Intriguingly though, an adult D. suzukii emerging from the overwintering stage has yet to be reported in the early season (Guédot et al., 2018; Tran et al., 2020). This information adds to the complexity of D. suzukii winter survival strategy, as it remains unclear whether adult flies certainly overwinter within the landscape. Additionally, while our study does not support a dispersal function for winter morphs despite their larger wings, researchers have found evidence that wing plasticity affects flight performance in cold-reared flies such as Drosophila melanogaster Meigen (Diptera: Drosophilidae) (Frazier et al., 2008), and in D. suzukii (Fraimout et al., 2018). Further research on understanding D. suzukii winter survival strategy has important implications as the information can assist management practices.

Female D. suzukii typically have larger wings than males (Tran et al., 2020) and sex-bias dispersal is a common behavior in insects where one sex displays higher dispersal patterns (Asplen, 2018). Mishra et al. (2020) found that D. melanogaster can display sex-biased dispersal; however, the lack of a sex-bias in dispersal patterns in our study with D. suzukii could be due to low sample sizes demonstrating positive flight.

Tethered flight mills and free-flight chambers each have their own advantages to examining insect dispersal in the laboratory. When the same species is simultaneously assessed on two different flight apparatuses measuring the same traits such as propensity and duration, the results can be mixed, as was observed with D. suzukii. In a similar experiment, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) free flight was compared and results showed that flight speed was three times greater than those on a flight mill (Taylor et al., 2010). Thus, it is concerning that flight behavior differs depending on the flight assay, considering that laboratory flight experiments are often used to predict the potential spread and distribution of an insect in the field (Asplen, 2018).

Laboratory flight experiments may over- or underestimate the flight potentials of insects (Minter et al., 2018; Naranjo, 2019). As demonstrated in this study with D. suzukii, the flight apparatus influenced flight propensity. Researchers should, therefore, consider taking an extra step when conducting flight behavior studies in the laboratory to determine what flight design may be best for their organism. Preliminary trials may be difficult due to constraints, such as accessibility of flight equipment. The biology and behavior of the insect should be considered when determining the appropriate flight assay to use. For example, A. planipennis flight speed was greater in an untethered flight assay; yet Taylor et al. (2010) explained that A. planipennis flight comprised of frequent short bursts and using a tethered flight mill was more intuitive to collecting flight data than using a free-flight chamber.

This study provided a first comparative analysis on D. suzukii flight behavior using tethered vs. free-flight systems. Based on the results, there was little evidence that D. suzukii are capable of self-directed movement but may be able to vertically ascent to levels for wind-aided emigration (Asplen et al., 2016). It is important to note that we did not investigate factors that might cue dispersal such as mating status and diet. Also, small insects like D. suzukii typically use wind to aid in long-distance dispersal. Examining whether D. suzukii can utilize wind to disperse may be useful to understanding the pest’s population dynamics and spread. Based on this study, future
laboratory flight experiments should take into consideration the bias that flight apparatus might have on flight capabilities.

**AUTHOR CONTRIBUTIONS**

Anh K. Tran: Data curation (supporting); formal analysis (lead); investigation (equal); methodology (supporting); visualization (lead); writing – original draft (lead); writing – review and editing (equal). Aubree M. Kees: Data curation (lead); investigation (equal); software (equal); writing – review and editing (equal). William D. Hutchison: Funding acquisition (supporting); writing – review and editing (equal). Mary A. Rogers: Funding acquisition (lead); resources (supporting); supervision (equal); writing – review and editing (equal). Robert Venette and two anonymous reviewers for review – editing (equal). Mary A. Rogers: Funding acquisition (supporting); resources (supporting); supervision (equal); writing – review and editing (equal). William D. Hutchison: Funding acquisition (lead); investigation (equal); software (equal); writing – review and editing (equal). Aubree M. Kees: Data curation (supporting); formal analysis (lead); investigation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). William D. Hutchison: Funding acquisition (supporting); writing – review and editing (equal). Sujaya Rao: Resources (supporting); supervision (equal); writing – review and editing (equal). Mary A. Rogers: Funding acquisition (lead); resources (supporting); supervision (equal); writing – review and editing (equal). Mark Asplen: Conceptualization (lead); methodology (lead); resources (supporting); supervision (equal); writing – review and editing (equal).

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**DATA AVAILABILITY STATEMENT**

Data is available through the Data Repository for University of Minnesota (DRUM): https://doi.org/10.13020/4nsz-x660

**ORCID**

Anh K. Tran https://orcid.org/0000-0002-7568-0341

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