Differentiating *Drosophila suzukii* morphs and their flight capabilities

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The wise and powerful Firebender, Uncle Iroh from Avatar: The Last AirBender, had many quotable moments. The two that resonated with me most were, "It's important to draw wisdom from many different places..." and "While it's always best to believe in oneself, a little help from others can be a great blessing." I am truly blessed.

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Dedication

To John

*You are the pedal brakes that I depend on
and the verb, 'to trust' because you never let me down.
Thank you for your patience, love, and making dinner.*

><> ** <><

Abstract

Insects depend on external heat sources to regulate their body temperatures, and this affects their ability to survive in cold dry regions compared to their counterparts in warm humid environments. Insects have adapted to withstand cold winters by 1) overwintering by acclimating to the cold-stress, or 2) by dispersing to escape the cold. However, there are many challenges to determining which winter survival strategy an insect adopts.

An example of an insect that researchers have not determine which main winter survival strategy is utilized is the invasive vinegar-fly, *Drosophila suzukii* (Matsumura), also commonly known as spotted-wing drosophila. First detected in California in 2008, *D. suzukii* rapidly expanded its geographic range and can now be found in 48 states, including Minnesota. Researchers have hypothesized that *D. suzukii* survives cold climatic conditions by producing dark colored winter morphs, which enable the flies to absorb more thermal radiation from the sun compared with lighter morphs. However, thus far, there has been no confirmed sighting of an overwintering winter morph emerging in the spring in Minnesota or any other Midwestern state. An alternative hypothesis is that adult flies are capable of long-distance dispersal. My doctoral dissertation is focused on determining the source (overwintering or migratory) of *D. suzukii* populations by investigating the morphs phenology and their flight capabilities.

First, I developed a quantitative technique to differentiate the two morphs to facilitate identification of overwintering populations. I mimicked winter and summer conditions in the lab for creating winter and summer morphs. Subsequently, I collected

quantitative data by measuring the flies' wings and legs and subjected the data to a predictive analysis approach. This enabled me to identify quantitative characters for distinguishing between winter and summer morphs.

Second, I observed and characterized *D. suzukii* flight behavior on a flight mill and in a flight chamber. Each of these techniques has advantages and disadvantages and flight behavior can vary considerably between the two techniques; yet researchers have typically examined flight behavior of their target species using only one method. I compared the flight behavior of winter and summer morphs of males and females using both techniques.

Finally, to examine the potential for *D. suzukii* long-distance dispersal in the field, two studies were conducted. First, three 7.3m poles were set up in an area where *D. suzukii* populations are known to be present with clear sticky cards placed on each pole at five different heights: 1, 2, 4, 6, and 7.3m. Second a fixed-wing plane/un-manned aerial vehicle (UAV) was flown at varying altitudes between 30-122 m with an insect net to catch a *D. suzukii*. This study was to determine if *D. suzukii* could reach altitudes that would allow the fly to disperse with the aid of the wind.

The results of my dissertation will provide information on differentiating *D. suzukii* in a qualitative matter, explore the propensity and duration of flight in a controlled laboratory environment, and examine the potential for long-distance dispersal in the field. The development of creating a morphometric scale will assist in standardizing how *D. suzukii* morphs are identified. Information of the flight behaviors can assist in furthering the understanding of this pest's biology and improve management

programs. If the *D. suzukii* overwinters in an area, growers could reduce pest pressure the following year through removal of potential overwintering habitats; if a *D. suzukii* migrates to warmer areas, growers could time management practices based on predictions of arrival/return of the pest in spring.

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Introduction

Drosophila suzukii (Matsumura), spotted-wing drosophila, was first described in Japan in 1916 and is native to east and southeast Asia. However, *D. suzukii* is currently found on most continents with the exception of Oceania and Antarctica (Asplen et al. 2015, Stephens et al. 2015, Santos et al. 2017). In the United States, *D. suzukii* was recorded in Hawai'i in 1980, but was first detected on the continental states in California in 2008 (Hauser 2011). Since then, *D. suzukii* rapidly spread and has been recorded in all states except for Arizona and Nevada (Burrack et al. 2012, Griffith 2014, CABI 2021)

As described by Hauser (2011), male *D. suzukii* have a single dark spot on each wing along the subcostal vein and two distinct sex-combs hairs on each fore tarsus. A microscope is required to identify female *D. suzukii* because the distinctive feature is a serrated ovipositor lined with dark sclerotized teeth. Having a serrated ovipositor allows *D. suzukii* to pierce and penetrate ripening fruits whereas typical *Drosophila* species oviposit in overripe fruits (Asplen et al. 2015). *Drosophila suzukii* females are highly fecund and the species has a short developmental time. Females have the capacity to lay an average of 380 eggs in their lifetime. The larvae that hatch from the eggs will feed on the fruit, go through three instars before pupating and emerging as an adult (Walsh et al. 2011). Under optimal conditionals, *D. suzukii* can complete its lifecycle within 10 days and have approximately 13 generations in a year (Walsh et al. 2011, Asplen et al. 2015).

Drosophila suzukii is has a several host species, which includes both wild host and cash crops (Lee, Bruck, Curry, et al. 2011, Lee, Dreves, Cave, Kawai, Isaacs, Miller, Timmeren, et al. 2015). *Drosophila suzukii* females can oviposit and exploit additional

hosts if a fruit's flesh is exposed due to damage (Holle et al. 2017). Due to the fly's

highly polyphagous nature, *D. suzukii* is an important economic pest of soft-skinned

fruits (e.g., strawberries, raspberries, blueberries) and stone fruits (e.g. cherries, peach)

(Lee, Bruck, Curry, et al. 2011, Stewart et al. 2014, Asplen et al. 2015, Mazzi et al. 2017,

Kamiyama and Guédot 2019). Without adequate control, *D. suzukii* have been reported to

cause $207 - $500 million in annual losses in the eastern and western United States,

respectively (Asplen et al. 2015, Wiman et al. 2016). Given the recent global spread, a

remarkable amount of research has been published to monitor and manage *D. suzukii*

with chemical control, physical and cultural practices, and biological control agents.

The primary management practice for controlling *D. suzukii* involves monitoring

for the pest's presence in the field with a trapping system, which compromises of a bait

and lure, and apply insecticides when *D. suzukii* is present. Traps can be made with

common household items such as deli-cups, sugar, and apple cider vinegar (Lee et al.

2012) or can be purchased commercially through Trécé Inc or Scentry. When growers

detect their first *D. suzukii* in the trap system, management recommendations suggest

initiating an insecticide program applied at a weekly interval while rotating between

chemistries with different modes of actions (Haye et al. 2016, Gullickson et al. 2019).

Most insecticides target the adult life stage and are broad-spectrum with limited organic

options; however, deterrents and adding feeding stimulant to increase insecticide efficacy

have been evaluated (Cowles et al. 2015, Erland et al. 2015, Wallingford, Hesler, et al.

2016, Tang et al. 2017, Gullickson et al. 2019).

To further reduce adult population, physical barriers, and cultural practices such as pruning, and sanitation are additional tactics that can be utilized. Protecting fruit via high tunnels or covering the fruit crop with a fine mesh netting to prevent *D. suzukii* from ovipositing into the fruit can reduce infestation (Leach et al. 2016, Rogers et al. 2016, Ebbenga et al. 2019). Sanitation of the field such as removing overripe and fallen fruits and increasing harvest frequency of fruit crops can diminish the number of available host resources for *D. suzukii* (Lee, Bruck, Dreves, et al. 2011). Additionally, *D. suzukii* prefers high humidity and mild ambient temperature (Fanning et al. 2019). Therefore, pruning and thinning the crop canopy may reduce fruit infestation due to decreased humidity and open airflow, resulting in an unfavorable microenvironment (Tochen et al. 2016).

Researchers have documented potential naturally occurring predators of *D. suzukii* in North America (Leach et al. 2016, Kamiyama et al. 2019, Lee et al. 2019). However, when Kamiyama et al. (2019) used sentinel *D. suzukii* pupal and larvae traps to record natural enemies there was minimal evidence of predation and parasitism. Aside from naturally occurring enemies, commercially available biological control agents such as insect predators, entomopathogenic nematodes, and fungi have been explored in laboratory and semi-field settings (Cuthbertson et al. 2014, Woltz et al. 2015, Cossentine et al. 2016, Hübner et al. 2017). Due to the low success of naturally occurring and commercially available biological control agents, researchers have screened for potential classical biological control agents, focusing on parasitoids from the native range of *D. suzukii* (Daane et al. 2016, Girod et al. 2018). Scientists are currently awaiting release approval before imported parasitoids can be released into the field (Lee et al. 2019).

While numerous studies have been conducted to improve management strategies of *D. suzukii*, a broader understanding of the flies' biology, phenology, and winter survival strategy is needed (Cinci et al. 2012, Asplen et al. 2015). *Drosophila suzukii* has a large geographic distribution and in a range of temperate regions. For example, *D. suzukii* was first detected in Minnesota in 2012 (Burrack et al. 2012); the average low temperature in the winter in the state is approximately -14°C. *Drosophila suzukii* can tolerate cooler temperatures by producing a 'winter' morph, which is a seasonal morph that is produced when the temperature and photoperiod decrease (Stephens et al. 2015, Shearer et al. 2016). Winter morphs can be identified as being larger in body size and darker in color, and more cold-tolerant than summer morphs (Dalton et al. 2011, Stephens et al. 2015, Stockton et al. 2018). For these reasons, researchers have hypothesized that *D. suzukii* overwinters as an adult winter morph (Kanzawa 1939, Kaçar et al. 2016, Stockton et al. 2019). However, an overwintering adult has yet to be detected in temperate regions. Guédot et al. (2018) baited *D. suzukii* over the course of two years in Wisconsin and early caught adults were identified as summer morphs.

A possible reason an overwintering winter morph has yet to be detected in the early season is adults have been misidentified. The current method for differentiating the two morphs is winter morphs have a 'larger' and 'darker' body compared to summer morphs. The descriptors, 'larger' and 'darker' are subjective and can vary depending on the observing. An alternative hypothesis is that the winter morph is a dispersal morph and adapted for migrating. A larger body size has been hypothesized to having increased fat and sugar storage which could assist with overwintering (Shearer et al. 2016).

Nevertheless, some insects are known to have a dispersal morph which involves a larger body size for long-distance dispersal (Asplen 2018). However, studies on *D. suzukii* flight behaviors and dispersal capabilities are limited.

By investigating *D. suzukii* movement and flight behavior, researchers can gain insight on the fly's population dynamics. Since an overwintering adult has yet to be detected in states with temperature seasonality, knowing if *D. suzukii* overwinters locally or immigrants from warmer regions can assist with management strategies. For example, if growers should focus on sanitation to remove overwintering habitats, or if a forecast model can be developed to optimize management execution.

The focus of this dissertation was to develop a quantitative method to differentiate between the two phenotypic morphs and characterize the flight behaviors of *D. suzukii*. In Chapter 1, several body measurements were collected, and the best indicators of summer and winter morphs were determined using a predictive modelling approach. In Chapter 2, laboratory flight assays using tethered and untethered methods were used to characterize the propensity (yes and no) and duration of flight between sexes (female and male) and morphs. Finally, in Chapter 3, *D. suzukii* potential for wind-aided long-distance dispersal was investigated using passive traps.

Chapter I: Morphometric criteria to differentiate *Drosophila suzukii* (Diptera: Drosophilidae) seasonal morphs

Summary

Temperate insect species often enter diapause in preparation for overwintering. One such species is the invasive vinegar fly, *Drosophila suzukii* (Matsumura), which has seasonal polymorphisms, considered winter and summer morphs. To date, the morphs have been differentiated by color and size with winter morphs typically being darker and larger compared to summer morphs. 'Dark' and 'large' are subjective, however, and standardizing an identification process can ensure that the morph of interest is being accurately characterized. The goal of our research was to investigate a quantitative method to distinguish between *D. suzukii* morphs based on body and wing size. We reared winter and summer morph *D. suzukii* in the laboratory using standard procedures, and measured wing length, wing width, and hind tibia length. Additionally, we collected field *D. suzukii* to document the seasonal phenology of the morphs in Minnesota based on our model's cutoff criteria. A classification and regression tree analysis were used to determine which metrics would be best for predicting field-caught *D. suzukii* morphs. Using laboratory-reared flies as our known morphs for the training data in the classification model we developed classification trees based on wing length and the ratio of wing length to hind tibia length. The frequency of winter and summer morphs present in the field varied based on which classification tree was used. Nevertheless, we suggest ratio of wing length to hind tibia length as the most robust criteria for differentiating *D. suzukii* morphs because the ratio accounts for the size variability between laboratory-reared and field-caught flies and the error rate of misclassification is reduced to 0.01 for males. The results from this work can aid in future *D. suzukii* research by allowing

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scientists to objectively differentiate the morphs, and thereby improve our understanding

of the biology and phenology of seasonal morph dynamics.

Introduction

Ectotherms face survival challenges when inhabiting regions that experience seasonal weather changes. In preparation for cold stress, many insect species are adapted to maintain homeostasis in cooler temperatures by entering a diapause state (Lee and Denlinger 1991). Within the diapause state, reduced metabolism and biochemical alterations affect the insect's biology, behavior, and morphogenesis (Lee and Denlinger 1991).

One such diapausing species that exhibits this phenomenon is the vinegar fly, *Drosophila suzukii* (Matsumura), commonly known as spotted-wing drosophila. Native to east and southeast Asia, *D. suzukii* is an important economic pest of soft-skinned fruits (e.g., strawberries, raspberries, blueberries) and stone fruits (e.g. cherries, peach) (Lee, Bruck, Curry, et al. 2011, Stewart et al. 2014, Asplen et al. 2015, Mazzi et al. 2017, Kamiyama and Guédot 2019). *Drosophila suzukii* invaded the continental United States in 2008 and rapidly spread throughout the country (Burrack et al. 2012), and has quickly become a global pest (Asplen et al. 2015, De Ros et al. 2015). Given its recent global spread, a remarkable amount of research has been published to improve understanding the biology of *D. suzukii,* such as host range and preferences (Grassi et al. 2011, Lee, Bruck, Dreves, et al. 2011, Lee, Dreves, Cave, Kawai, Isaacs, Miller, Timmeren, et al. 2015), and management strategies including chemical control (Haviland and Beers 2012, Van Timmeren and Isaacs 2013, Burrack et al. 2015, Fanning et al. 2018), biological control (Gabarra et al. 2015, Woltz et al. 2015, Daane et al. 2016, Schmidt et al. 2019), physical exclusion through netting (Leach et al. 2016, Rogers et al. 2016, Ebbenga et al. 2019), and enhanced monitoring techniques (Lee et al. 2013, Burrack et al. 2015, Cha et

al. 2018). However, to develop improved management strategies, a broader understanding of *D. suzukii* biology, phenology and overwintering strategies is needed (Cini et al. 2012, Asplen et al. 2015).

Recent research has shown that *D. suzukii* can tolerate temperate regions by producing seasonal morphs via polyphenism (Stephens et al. 2015, Shearer et al. 2016). For *D. suzukii*, a decrease in photoperiod and temperature will produce 'winter' morphs, which have been described as larger in body size, wing size, and darker in pigmentation in comparison to summer morphs (Shearer et al. 2016, Wallingford and Loeb 2016). In laboratory settings, these environmental cues can be simulated to produce winter morphs for experimental purposes; for example, winter morphs can be generated by placing eggs and larvae produced by summer morphs at a constant temperature between 10-15°C and a photoperiod between 10:14 (L:D) hours and 12:12 (L:D) hours (Stephens et al. 2015, Wallingford and Loeb 2016, Kirkpatrick et al. 2018, Stockton et al. 2018).

Winter morph *D. suzukii* are more cold-tolerant than summer morphs, which would allow flies to persist in temperate regions (Dalton et al. 2011, Stephens et al. 2015, Stockton et al. 2018). Researchers have documented *D. suzukii* seasonal population dynamics across different regions (Burrack et al. 2015, Briem et al. 2018, Panel, Zeeman, Van der Sluis, et al. 2018, Tait et al. 2018, Thistlewood et al. 2018) and in several crops over time (Hamby et al. 2014, Harris et al. 2014, Arnó et al. 2016). In addition, research describes the reproductive status and ovary maturity levels of *D. suzukii* in temperate regions shows that reproduction ceases, or is greatly reduced in cooler months (Zerulla et al. 2015, Wallingford, Lee, et al. 2016, Grassi et al. 2017, Panel, Zeeman, van der Sluis, et al. 2018). Given these results, and in most temperate regions of the U.S., it is generally

assumed that *D. suzukii* overwinters as an adult winter morph (Kanzawa 1939, Kaçar et al. 2016, Stockton et al. 2019). There is a paucity of research investigating the phenology or abundance of winter morphs in the field, particularly in the extreme northern range of the distribution, perhaps due to the difficulty of differentiating winter and summer morphs.

Current methods for differentiating winter and summer morphs have focused primarily on melanization changes that occur during autumn at northern latitudes or temperate regions (Guédot, Avanesyan, and Hietala-henschell 2018, Stockton et al. 2019). When rearing different morphs in the lab, Shearer et al. (2016) noted that winter morph males and females had a continuous dark pigmentation on the third and fourth abdominal segment, respectively, that was completely filled. This method has been utilized to determine the seasonal phenology of winter morphs caught in the field (Wallingford and Loeb 2016, Guédot, Avanesyan, and Hietala-henschell 2018, Panel, Zeeman, van der Sluis, et al. 2018). However, there were some challenges to using color as this was the only metric for identifying winter and summer morphs. Guédot et al. (2018) mentioned that morph determination for some field-collected *D. suzukii* was inconclusive because selected specimens were bloated, bleached or unclear. Panel et al. (2018) also had difficulty assigning individuals as winter or summer, and created an intermediate category.

A quantitative method to differentiate winter and summer morphs is needed. Relying solely on color is difficult because *D. suzukii* is usually collected using trapping systems where specimens drown in a liquid solution and may remain in the trap under a variety of environmental conditions prior to collection and transport to the lab (Burrack et

al. 2015, Guédot, Avanesyan, and Hietala-henschell 2018, Ebbenga et al. 2019). Thus, the trapping system and delays in collection and processing can damage the adults making color identification difficult. The goal of this study was to develop a quantitative method for identifying *D. suzukii* winter and summer morphs based on well-known morphological metrics.

The morphometrics we chose were wing length, wing width, and hind tibia length because these are sclerotized body parts. Additionally, a ratio of wing length to hind tibia length was used because *D. suzukii* size can vary based on diet (Wallingford and Loeb 2016, Panel, Zeeman, van der Sluis, et al. 2018), and because temperature-induced phenotypic plasticity in wing size and shape is well known in *Drosophila* broadly, and *D. suzukii* specifically (Debat et al. 2009, Fraimout et al. 2018, Leach, Stone, Van Timmeren, Isaacs, et al. 2019). By using a ratio of wing length to hind length, we correct for potential wing size variation of field-caught versus laboratory-reared *D. suzukii* that may be associated with body size. We used hind tibia length because this is a standard measurement of body size outside of the flight motor apparatus (Cônsoli and Vinson 2002, Gwynn et al. 2005, Thorne et al. 2006, Asplen et al. 2009).

Here we examine and report specific morphometrics that would be the best indicators of winter and summer morphs. Additionally, we apply the morphometric approach to distinguish morphs and document the seasonal phenology of field-collected *D. suzukii* by morph in Minnesota, near the northern limits to its range.

Materials and Methods

Insects

Studies were conducted using laboratory-reared and field-collected *D. suzukii* adults. A laboratory *D. suzukii* colony was established in the laboratory by collecting infested raspberry fruit in 2016, produced at the University of Minnesota Outreach, Research and Education (UMORE) Park (44.7279°N, 93.0968°W) in Rosemount, MN and maintained in the Department of Entomology, University of Minnesota, St. Paul, MN. Protocols and methods for rearing *D. suzukii* summer and winter morphs were performed as described by Stephens et al. (2015a). Summer morphs were reared in narrow polystyrene vials with foam plugs (Genesee Scientific, San Diego, CA). Each vial contained approximately 5ml of an agar-yeast-cornmeal diet and a strip of filter paper to reduce mortality from condensation. Vials were stored at $22 \pm 1°C$, a photoperiod of 16:8 (L:D) hours and relative humidity of $60 \pm 10\%$. Adult winter morph *D. suzukii* were produced by placing vials with 1 to 3-day-old eggs from summer morphs and into a growth chamber (Percival Scientific Inc., Perry, IA) set at $10 \pm 1°C$, a photoperiod of 12:12 (L:D) hours and relative humidity of $70 \pm 10\%$. At 10°C, winter morphs typically eclose within approximately 56 days. Adult summer and winter morph flies that were approximately 2-days-old (from eclosion) were used for the study.

Field populations of adult *D. suzukii* were collected throughout the 2017 and 2018 growing seasons, in the metropolitan area of Minneapolis-St. Paul, MN. Trap sites included the UMORE Park, a vineyard near Hastings (44.6855°N, 92.8717°W), a fresh market berry farm near Forest Lake (45.2304°N, 92.8932°W), and two mixed-berry and vegetable farms located near Andover (45.0833°N, 93.8787°W) and Waverly

(45.2688°N, 93.3545°W). Permission was granted from each landowner to conduct experiments on their property. In 2018, traps were not maintained at the Andover site. In both years at each site, two commercial trapping systems were used: Pherocon® SWD (Trécé Inc., Adair, OK) and Scentry (Scentry Biologicals, Inc., Billings, MT). Pherocon® SWD traps consisted of a Pherocon ® SWD lure and apple cider vinegar with a drop of liquid soap to lower surface tension. The Scentry trap consisted of a Scentry SWD lure and water with a drop of liquid soap, to lower surface tension. At each participating location, traps were monitored weekly to remove the contents, and transfer samples to the laboratory for processing, identification, and measurement. In 2017, traps were deployed from 15 May to 31 August except at UMORE Park where traps were taken down on 5 December. In 2018, traps were deployed from 14 May to 25 September except at UMORE Park where traps were taken down on 11 November. Lures were replaced approximately once per month and liquids (apple cider vinegar or water) were replaced weekly. As temperatures approached 0°C, the apple cider vinegar and water were replaced with propylene glycol.

Morphometric measurements

Individual flies from laboratory colonies or field sites were initially placed under a stereo microscope (Leica EZ4 W, Leica Microsystems, Wetzlar, Germany) with 8x – 35x magnification. The wings and hind legs were removed from the thorax by dissecting individuals on a microscope slide with Corning® Dulbecco's phosphate-buffered saline (DPBS), 1X with calcium and magnesium solution (Mediatech Inc., Manassas, VA). Dissected contents were left on the microscope slide. Wing length, wing width and hind

tibia length were measured using a compound light microscope (Leica DM500 and Leica ICC50 W, Leica Microscosystems, Wetzlar, Germany) with 4x – 100x magnification and a calibrated reticle. Measurements were only recorded from one wing and leg per fly. Wing length was measured from the base of the wing to the apex of the R4 + 5 vein (Figure 1.1A). Wing width was measured from the costal margin to the posterior edge following the medial cubital cross vein (Figure 1.1B) and the longest dimension of the hind tibia was measured (Figure 1.1C). In addition, the sex of each individual was recorded.

Data analysis

A classification and regression tree analysis were used to determine the best discriminatory criteria to classify *D. suzukii* morphs. A classification and regression tree analysis is a non-parametric modeling approach that creates tree models by continuously splitting the data into homogenous groups based on categorical or continuous predictor variables (Breiman et al. 1984, De'ath and Fabricius 2000).

In addition to descriptive statistics, data were analyzed using the package 'rpart' (Therneau and Atkinson 2018) via the 'gini index' for splitting and 'class' as the method in R version 3.5.2 (R Core Team 2018) and RStudio Desktop version 1.1.463 (R Studio Team 2018). Adult *D. suzukii* females are, on average, larger than males; therefore, a classification tree was constructed for each sex (Harris and Shaw 2014) . For the construction of the classification trees, *D. suzukii* morph (winter/summer) was the response variable and the predictor variables included wing length, wing width, and hind tibia length. A transformed ratio of wing length to hind tibia length was used in the

classification tree. The transformation was calculated by taking the linear regression of female and male laboratory-reared known summer morphs and using the following equation adapted from Albrecht et al. (Albrecht et al. 1993):

$$Y_{adj} = (Y - \alpha)/X \qquad \qquad \text{Eq. 1}$$

where the expected value of $Y_{(adj)}$ is the transformed ratio, Y is wing length, α is the intercept of the regression line and X is hind tibia length. Laboratory-reared *D. suzukii* were used for construction of the trees because morphs were known with certainty. To construct a classification tree, the data are split into training and validation sets. The training data set is used to generate the model, and the validation data set is used to evaluate the model's performance. For our classification tree, 70% of the data were used for training and 30% of the data were used for validation. This method was then bootstrapped 500 times. The results from the simulation were averaged to obtain the final classification tree criteria and then applied to field-collected *D. suzukii* to determine the phenology of each morph.

Results

Descriptive statistics

Winter morph *D. suzukii* males, obtained from the laboratory colony, were on average larger than summer morphs males for all recorded metrics (wing length: $t = 22.98$; wing width: $t = 8.96$; hind tibia: $t = 11.37$; df = 90, $P < 0.001$, Table 1.1). Likewise, winter morph females were larger than the corresponding summer morph females (wing length: $t = 23.36$; wing width: $t = 18.92$; hind tibia: $t = 9.52$; df = 95, $P < 0.001$, Table 1.1). *Drosophila suzukii* females, for both morphs combined, were on

average larger than males; this was evident for both laboratory (wing length: $t = 5.39$;

wing width: $t = 5.99$; hind tibia: $t = 8.00$; df $= 187$, $P <0.001$) and field populations (wing

length: $t = 8.90$; wing width: $t = 8.46$; hind tibia: $t = 5.22$; df $= 238$, $P < 0.001$ Table 1.1).

Field-caught *D. suzukii* were on average larger than the laboratory-reared flies, but the

mean wing length, wing width and hind tibia length were within range of the mean winter

and summer morphs size range (Table 1.1). Additionally, the morpho-metrics for a

majority of field-caught individuals were representative of the laboratory-reared *D.*

suzukii (Figure 1.2).

Classification trees

Data from 97 and 92 females and males, respectively, were used to build the

classification trees (Table 1.2). Of the three body measurements (i.e., wing length, wing

width and hind tibia length), wing length was the best predictor variable for classifying

winter and summer morphs from laboratory colonies. However, using the transformed

ratio of wing length to hind tibia length had at a higher level of accuracy for predicting

winter and summer morphs in comparison to only using wing length (Table 1.2). The

transformed ratio utilizes the intercept of summer morphs. The intercept for laboratory-

reared summer morphs was 1.003 and 0.762 for females and males, respectively (Figure

1.3 and Figure 1.4).

Females and males with a wing length ≥ 2.69 mm and ≥ 2.42 mm, respectively,

were classified as winter morphs (Table 1.2 and Figure 1.5A). From the cross-validation

there was a total mean error rate of 0.10% and 1.82% for females and males, respectively

(Table 1.2). There was a 2.00% density overlap in laboratory-reared *D. suzukii* for both female and male when using wing length alone as a morph predictor (Figure 1.6A and B). For the transformed ratio of wing length to hind tibia length, females with a ratio ≥ 2.17, and males with a ratio ≥ 2.31, were classified as winter morphs (Table 1.2 and Figure 1.5B). From the cross-validation, there was a total mean error rate of 0.10% and 0.01% for females and males, respectively (Table 1.2). Using the transformed ratio as a morph predictor, the laboratory-reared *D. suzukii* had a density overlap of 2.00% and 4.00% for females and males, respectively (Figure 1.6C and D).

Phenology of morph frequency in the field

The results for each classification model (i.e., wing length and the transformed ratio of wing length to hind tibia length), were applied to field-caught *D. suzukii* to predict morph seasonal phenology (Figures 1.7 and 1.8). In Minnesota, *D. suzukii* were typically detected by mid-June (Figures 1.7 and 1.8). When using wing length to distinguish morphs, approximately 57.14% of females were identified as winter morphs in June (Figure 1.7B). In July, there is a decrease in identified winter morphs to 12.14%. From August to October, as the frequency of winter morph increases, approximately 94.44% of field-caught female *D. suzukii* were classified as winter morphs (Figure 1.7B).

When using the transformed ratio of wing length to hind tibia length to distinguish morphs, the pattern was similar to the wing length criteria, but at different frequencies. The transformed ratio criteria identified 50.00% of females as winter and summer morphs in June (Figure 1.7B). In July, the frequency of winter morphs identified

decreased to 25.00%, and by October, 100% of female *D. suzukii* were classified as winter morphs.

The frequency of males caught in June that were identified as winter morphs using wing length was approximately 71.43% (Figure 1.8B). Similar to the female phenology results, there was a decline in the frequency in winter morphs during July and August, but the frequency increased to approximately 74.20 – 95.65% for September to October (Figure 1.8B). When using the transformed ratio to predict the morph seasonal phenology for males, the frequency of winter morphs identified in June was approximately 35.71% (Figure 1.8D). In July the frequency of winter morphs identified declined to approximately 13.50% before increasing throughout the remainder of the season (Figure 1.8D).

Of the two classification trees for females and males, a transformed ratio of wing length to hind tibia length may be the best for differentiating winter and summer morphs. Although the initial classification tree indicated that wing length was the best predictor for morph distinction, it is well known that diet can affect *D. suzukii* wing size (Wallingford and Loeb 2016). We therefore elected to assess a ratio of wing length to hind tibia length as one approach to address the variability that can occur in field populations, but a transformation is required (Albrecht et al. 1993). The initial results for the untransformed data are presented in Table 1.3. The transformed ratio had similar results to wing length (Figures 1.7 and 1.8), but also had numerically higher levels of accuracy to determine male winter and summer morphs (Table 1.2). The level of overlap between known winter and summer laboratory-reared morphs was 2.00% for both females and males and the transformed ratio was higher for males of 4.00% (Figure 1.6).

Regardless of having a higher density of overlap in males for a transformed ratio, the mean error for predicting winter and summer morphs was 0.01% compared to using the wing length where the mean error was 1.82% (Table 1.2).

Discussion

A rigorous quantitative method for identifying *D. suzukii* morphs has not been developed and would be a valuable alternative tool to the identification protocol of winter and summer morphs. By comparing different morphometrics values we found that a transformed ratio of wing length to hind tibia length, for both females and males, was a reliable criterion for distinguishing winter and summer morphs for *D. suzukii* populations sampled in Minnesota. With this proposed criterion, *D. suzukii* morphs can potentially be determined using a standardized metric, but the method should be evaluated for other locations where *D. suzukii* is a concern. Our conclusions are based on the use of a classification tree to examine and detect patterns in the explanatory variables (i.e., wing length, wing width, and hind tibia length) that would best distinguish the morphs.

We used a classification tree model because the interpretation is straightforward relative to other analytical approaches (De'ath and Fabricius 2000, Gareth et al. 2013). Decision trees have been used previously in entomological research for determining predictor variables in both field and systematic studies (Logan and Connolly 2005, VanEngelsdorp et al. 2010, Gomez et al. 2018). For these reasons, a classification tree was appropriate for our research goal of developing a potential objective criterion that would differentiate *D. suzukii* morphs.

Thus far, an adult winter morph has not been knowingly caught in the early seasons in the North Central US, but this assessment was based on color as a distinguishing feature, which has important limitations. As mentioned previously, *D. suzukii* are typically trapped and contained in a liquid solution that could remain in the field for an extended period before the specimens are returned to the laboratory for examination. During this time, the specimen could be damaged making reliance on a color scale as an objective metric. Furthermore, a color scale can be subjective and arbitrary based on the observer (Wittkopp et al. 2011, Leach, Stone, Van Timmeren, Isaacs, et al. 2019). Therefore, there was a need for developing an alternative technique for differentiating morphs. Using body measurements as a quantitative scale could be convenient as sclerotized body parts (i.e., wings and hind tibiae) are resistant to change in shape and size and can be stored for future research purposes.

We generated different classification methods that could be used to differentiate morphs, with each method changing our interpretations when applied to field populations. The classification analysis of laboratory specimens showed that wing length was the best predictor variable for differentiating winter and summer morphs, for both females and males. While wing length is a simple method because it only requires one body measurement, it may not be the most reliable due to a potential high variability of body size due to diet and/or temperature (Jaramillo et al. 2015, Wallingford and Loeb 2016). Therefore, a transformed ratio of wing length to hind tibia length was also examined as an alternative technique for determining morphs. Using the transformed ratio also dropped the mean error rate in males from 1.82% (wing length criteria) to 0.01% (Table 1.2). The further reduction in error rate would be useful for those

identifying *D. suzukii* that are present in the overlapping areas where differentiating morphs may be most difficult to distinguish (Figure 1.6D).

When predicting the phenology of *D. suzukii* morphs using both wing length and transformed ratio of wing length to hind tibia length, our results showed that female (Figure 1.7) and male (Figure 1.8) winter morphs are present in June. While this result has not been the case for other North American surveys, trapping data over time from other regions suggest that winter morphs are present early in the calendar year. For example, Panel et al. (2018) documented the phenology of winter and summer morphs in the Netherlands, noting that winter morphs were detected year-round with a rapid decline in June when that would be assumed the transition period to summer morphs. Additionally, Thistlewood et al. (2018) trapped *D. suzukii* from January to April in the Pacific Northwest, USA, suggesting some of the trapped flies may be winter morphs. Our data may provide evidence for the earliest detection of a *D. suzukii* winter morph in Minnesota and the upper Midwest region, based on the use of wing length or the transformed ratio of wing length to hind tibia length as morph indicators. This is a unique finding because in Wisconsin, an adjacent Midwest state, the first winter morph was detected in August based on body pigmentation (Guédot, Avanesyan, and Hietala-henschell 2018). As Wisconsin and Minnesota can have similar temperature profiles, these contrasting results further demonstrate the need for creating a definitive strategy for differentiating winter and summer morphs to understand *D. suzukii* seasonal phenology.

Drosophila suzukii body size can vary due to diet. Additional research recording the effects of different host plants on the body and wing sizes of *D. suzukii* morphs, for multiple populations, might improve the classification tree model to better represent flies

in the field, especially if information on differential host availability and use are known for different locations. Information about different host plants may be particularly important because Jaramillo et al. (2015) did not find differences between artificial diet and blueberry affecting wing length for *D. suzukii* females and males, while Wallingford and Loeb (2016) found differences between artificial diet and raspberries. This further signifies the phenotypic plasticity of this species.

Our classification model demonstrates an alternative method for differentiating winter and summer morph *D. suzukii*. Using this quantitative scale for Minnesota indicated that winter morphs can occur at a high frequency early in the season, which has not been found in other studies within the northern Midwest states. Future studies should compare the color-based system and the quantitative scale on field-caught *D. suzukii* and to analyze the phenology of winter and summer morphs based on the two different identification techniques.

Of the two different classification models, we suggest that the transformed ratio of wing length to hind tibia length may be the most useful, robust morphometric for differentiating winter and summer morphs. While wing length was selected as the best morphological characteristic from the classification tree analysis, there is a chance this would underestimate winter morph's wing length due to not knowing the effects different crop hosts have on *D. suzukii* wing size in the field. It is often unknown what host plants are being utilized by *D. suzukii* field populations, thus using wing length alone, though a simple measurement, would not fully address size variability due to host availability.

The wing length to hind tibia length ratio would potentially correct for the size variation of field-caught and laboratory-reared *D. suzukii*. However, a transformation is

required, as simple ratios rarely control for size variation (Albrecht et al. 1993). The transformed ratio of wing length to hind tibia length had a reduced error rate (Table 1.2) and produced similar results as the wing length criteria in terms of predicted phenology (Figures 1.7 and 1.8). Given this outcome, to identify field-caught *D. suzukii* morphs, the ratio of wing length to hind tibia length should be transformed by subtracting 1.008 (females) or 0.762 (males) from the wing length and dividing the following value by the hind tibia length. Female and male *D. suzukii* with a transformed ratio of ≥ 2.17 and ≥ 2.31, respectively, would then be identified as winter morphs (Figure 1.5B).

There are several other avenues of research that follow from this study. For instance, previously stored *D. suzukii* samples from multiple locations could be examined to understand the phenology of winter versus summer morphs at different latitudes, and thus further our understanding of winter survival strategies of *D. suzukii*. Early caught *D. suzukii* morph identification could change if the flies were identified based on a quantitative scale instead of color schemes alone. While local overwintering is certainly a valid hypothesis for winter morph production in temperate latitudes, there are other alternative explanations that have not been adequately investigated. For example, while it has generally been assumed that the darker coloration and larger body size in *D. suzukii* is to aid in overwintering, many insect species are known to produce variants that have larger wings for migratory purposes (Zera and Denno 1997, Flockhart et al. 2017, Xu and Zhang 2017). The testing of alternative hypotheses for winter morph production in *D. suzukii*, which may not necessarily be mutually exclusive, is vital to gaining a complete understanding of the population dynamics of this devastating fruit pest.

Conclusion

Drosophila suzukii is highly phenotypically plastic, and their color and size variation can change depending on environmental cues. However, until recently, research has been limited with regard to understanding the adaptive benefits of winter versus summer morphs, and how to distinguish them in a systematic fashion. Reliance on color for differentiating *D. suzukii* has been suggested as an unreliable indicator for morphs (Wittkopp et al. 2011, Leach, Stone, Van Timmeren, Isaacs, et al. 2019), and further supports the need of a quantitative metric scale. In this paper, we test a specific morphometric approach to determine winter and summer morphs focused primarily on wing length and the transformed ratio of wing length to hind tibia length. This method was developed using a predictive statistical analysis and eliminates the bias that may be apparent when using a color scale. Research continues to expand on understanding *D. suzukii* biology and phenology to improve management practices. However, scientists struggle with finding a definitive method for identifying winter and summer morphs. We conclude that our study provides a potential practical technique for differentiating morphs using body and wing measurements and have identified future research directions for its field implementation.

Tables and Figures

Table 1.1: Mean (+/- SEM) of wing length, wing width and hind tibia length (mm) for laboratory-reared known morphs (winter and summer) of *Drosophila suzukii*, and field caught flies across the season.

Type	N	Female Wing Length	Female Wing Width	Female Hind Tibia Length	N	Male Wing Length	Male Wing Width	Male Hind Tibia Length
		Female				**Male**		
Winter	41	$2.97 \pm 0.020^{a*}$	1.23 ± 0.011^{a}	0.80 ± 0.005^{a}	42	2.69 ± 0.018^{a}	1.08 ± 0.025^{a}	0.75 ± 0.003^{a}
Summer	56	2.39 ± 0.015^{b}	0.99 ± 0.007^{b}	0.73 ± 0.004^{b}	50	2.15 ± 0.015^{b}	0.87 ± 0.006^{b}	0.68 ± 0.004^{b}
Laboratory	97	$2.63 \pm 0.032^{A**}$	1.09 ± 0.013^{A}	0.76 ± 0.005^{A}	92	2.40 ± 0.030^{B}	0.96 ± 0.017^{B}	0.71 ± 0.005^{B}
Field	116	2.80 ± 0.022^{A}	1.13 ± 0.009^{A}	0.78 ± 0.005^{A}	123	2.53 ± 0.018^{B}	1.01 ± 0.008^{B}	0.74 ± 0.005^{B}

* Lowercase: Comparison of laboratory-reared winter and summer morphs within sex.
** Uppercase: Comparison of female and male laboratory-reared and field-collected *D. suzukii*.
Means followed by different letters represent significant differences, based on Student's t-test (P <0.001).

Table 1.2: Summary results using the wing length or the transformed ratio of the wing length to hind tibia to differentiate winter and summer morphs for females and males.

	Average statistics for 500 classification simulations											
	Wing length						Ratio					
	Cutoff value (mm)			Error (%)			Cutoff value			Error%		
Sex	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Female	2.69	2.63	2.73	0.10	0.00	10.35	2.17	2.12	2.22	0.10	0.00	6.90
Male	2.42	2.35	2.46	1.82	0.00	10.71	2.31	2.28	2.34	0.01	0.00	3.57

The classification model was built using measurements from laboratory-reared known morphs of *D. suzukii*, where 70% of the data were used for training and 30% of the data were used for validation. This process was bootstrapped 500 times and the results were averaged. For the ratio cutoff value, the data were transformed using Eq. 1.

Table 1.3: Summary results of the classification tree using the ratio of wing length to hind tibia to differentiate winter morphs from summer morphs.

Sex	Average statistics for 500 classification simulations					
	Ratio cutoff value			Error (%)		
	Mean	Min	Max	Mean	Min	Max
Female	3.48	3.40	3.55	7.24	0.00	24.14
Male	3.36	3.31	3.49	8.24	0.00	25.00

The classification was built using measurements from laboratory-reared known morphs of *Drosophila suzukii*, where 70% of data were used for training and 30% of the data were used for validation. This process was bootstrapped 500 times and the results were averaged.
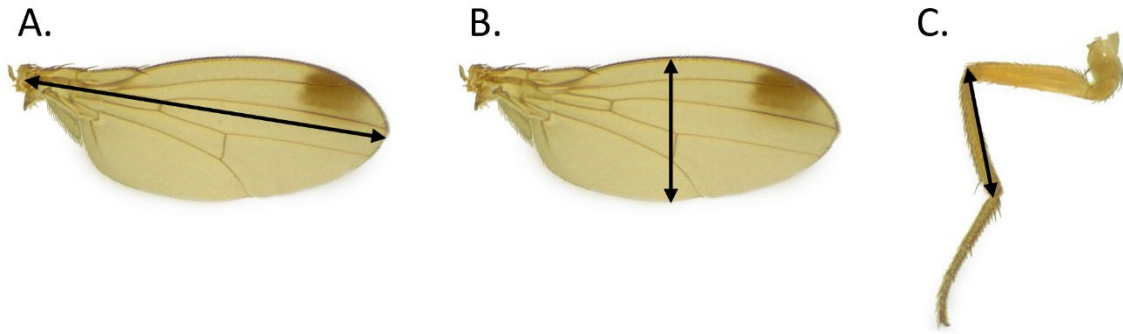
Figure 1.1: Location of measurements for *D. suzukii*: wing length (A), wing width (B), and hind tibia length (C).
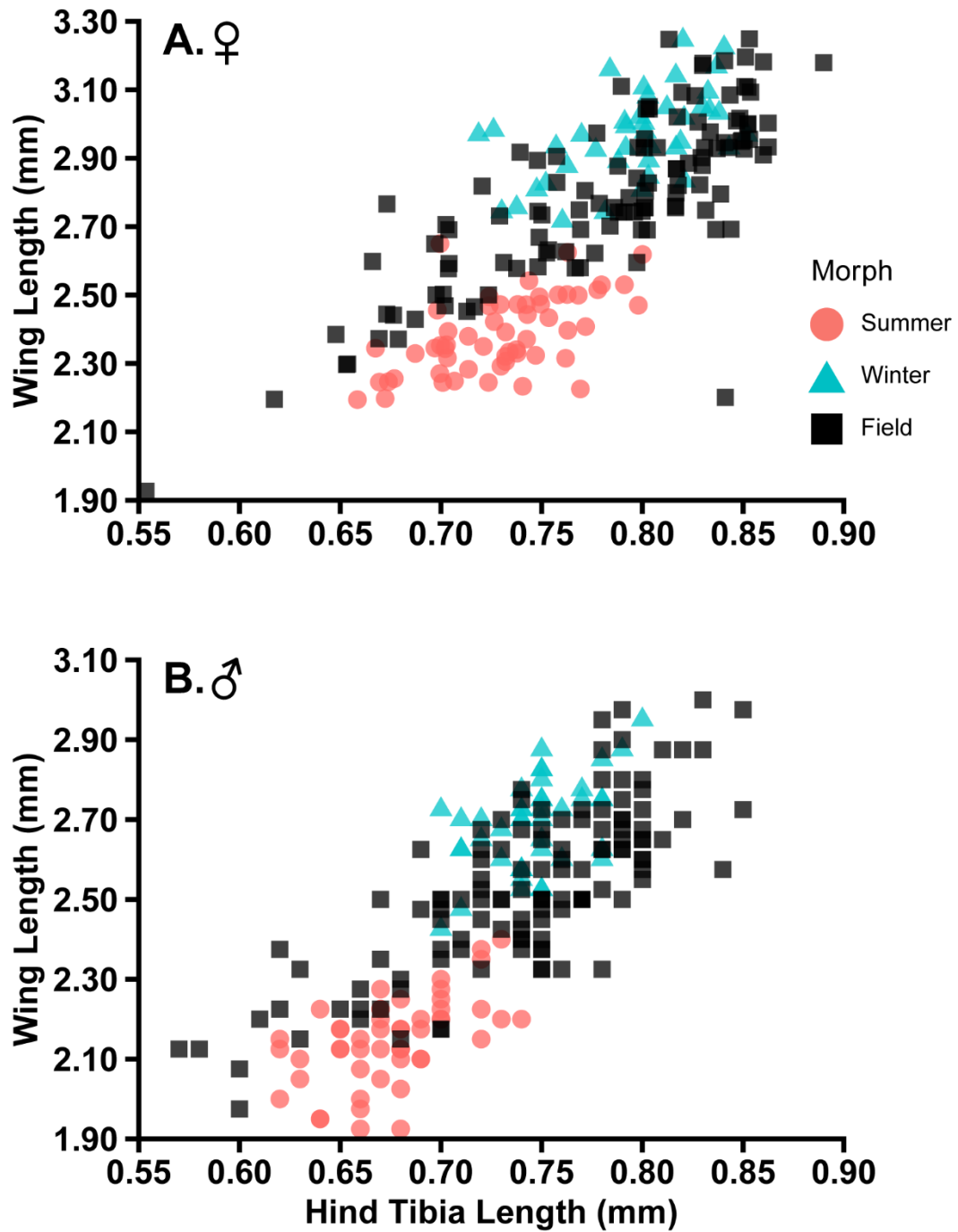
Figure 1.2: Laboratory-reared mean wing and hind tibia lengths (mm) for known female (A) and male (B) winter and summer morphs of *D. suzukii*, compared with field-caught females and males.
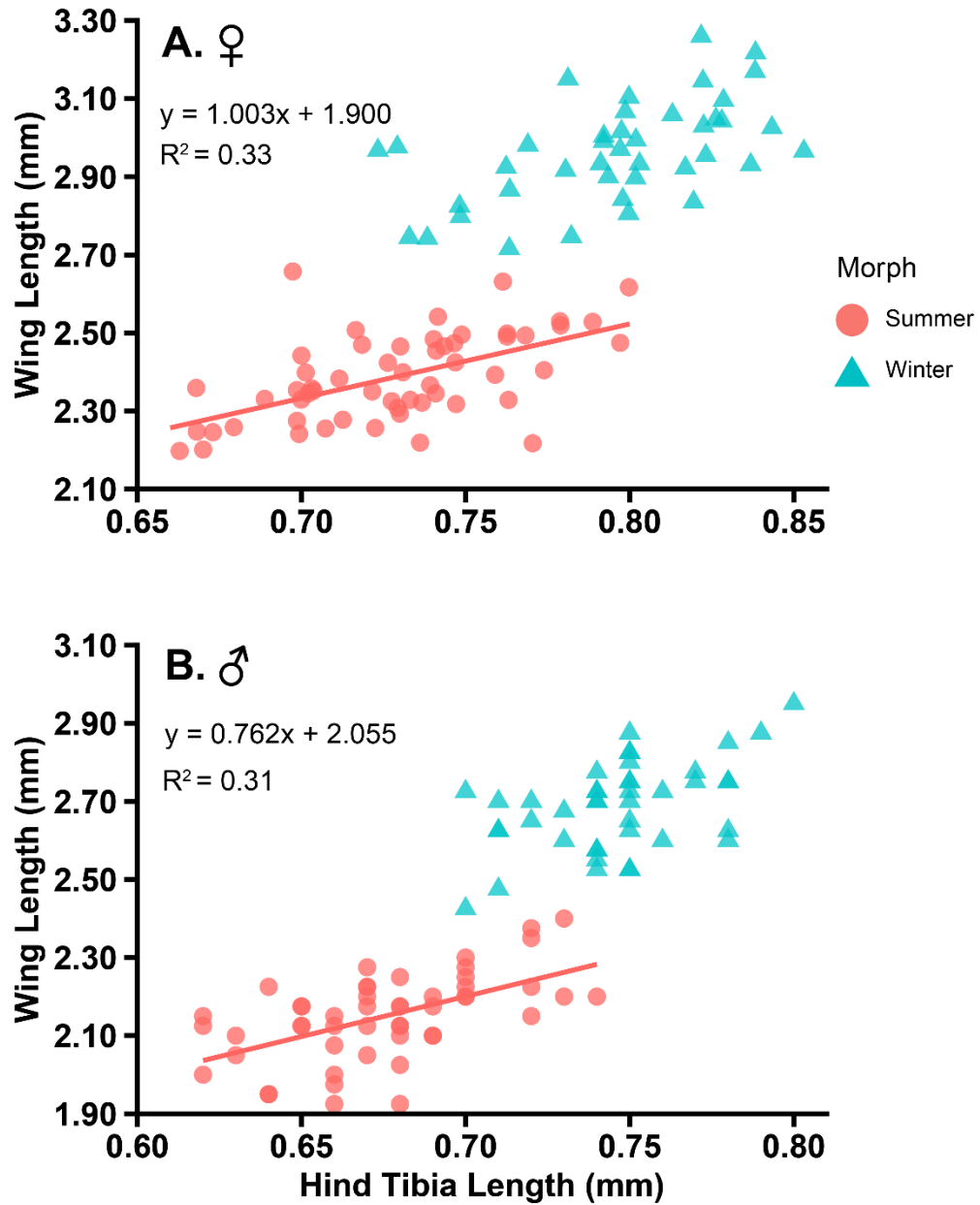
Figure 1.3: Laboratory-reared mean wing and hind tibia lengths (mm) for known female (A) and male (B) winter and summer morphs of *D. suzukii*. The linear regression equation is based on summer morphs, which was used for the ratio transformation.
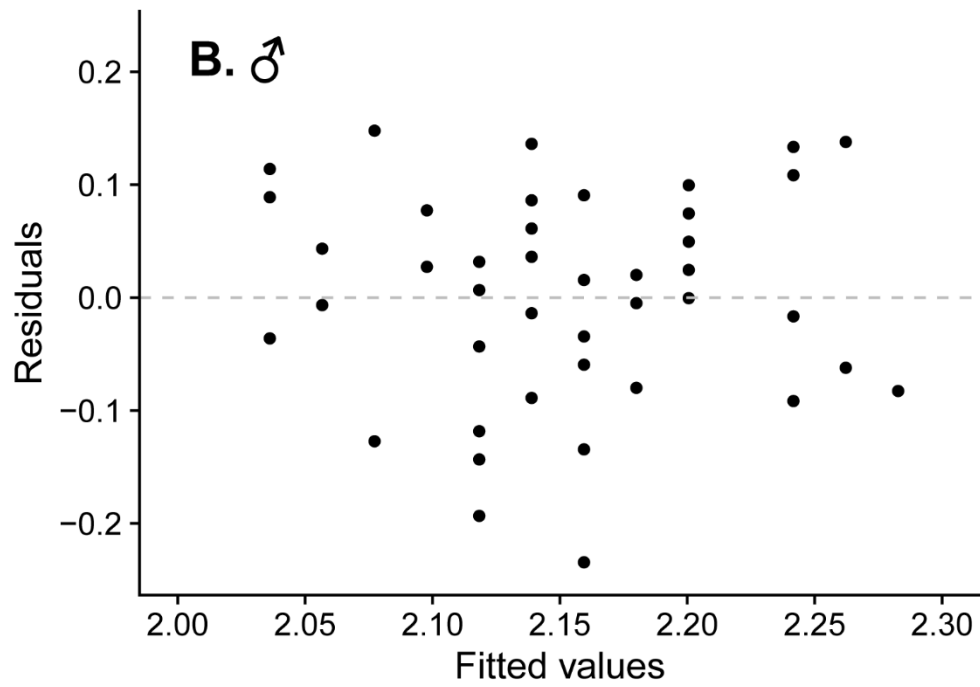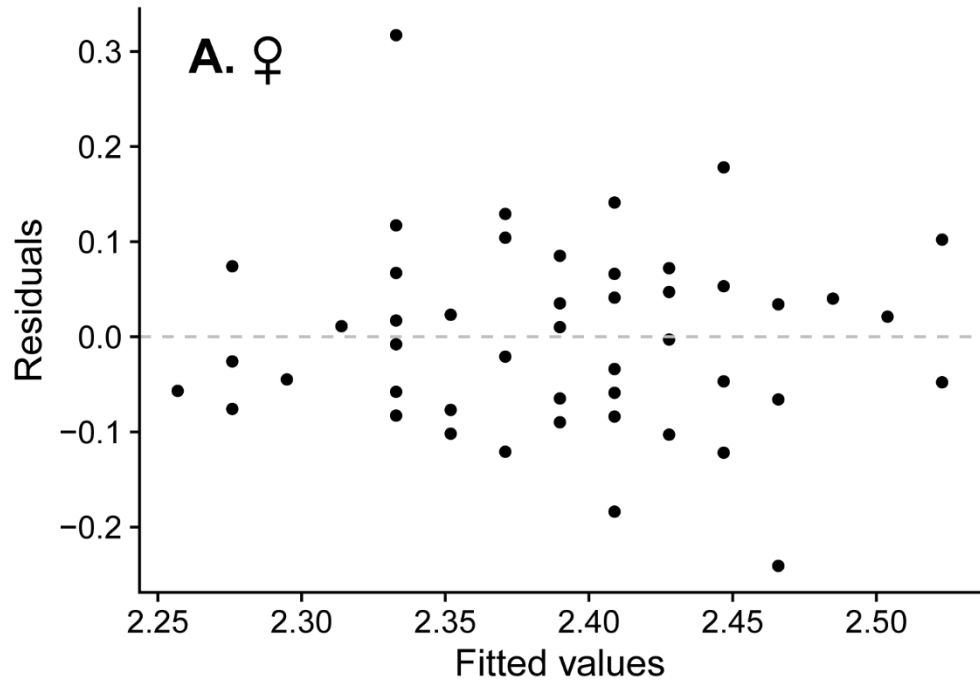
Figure 1.4: Residual plot of laboratory-reared known summer morphs for females (A) and males (B).
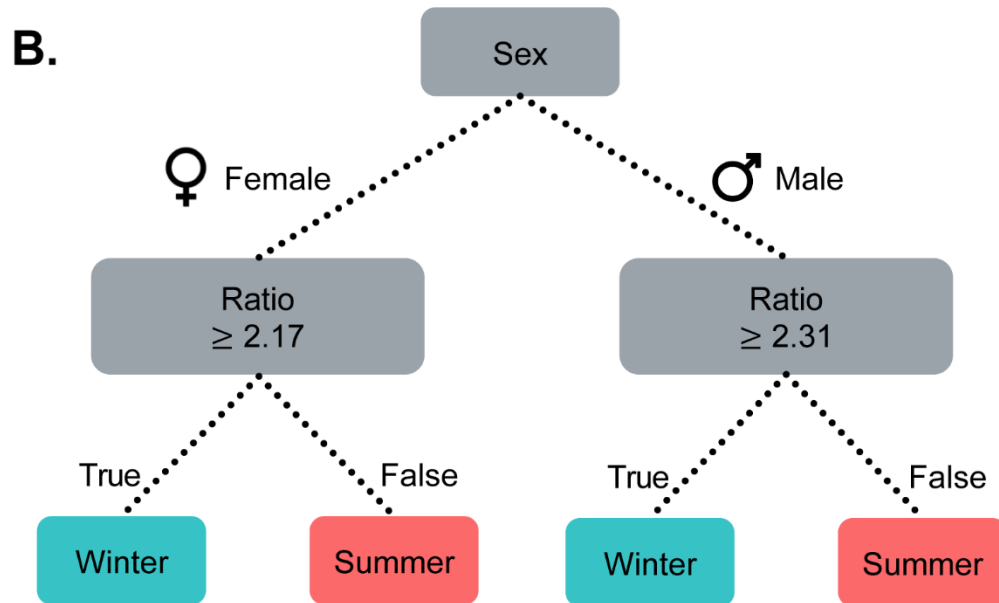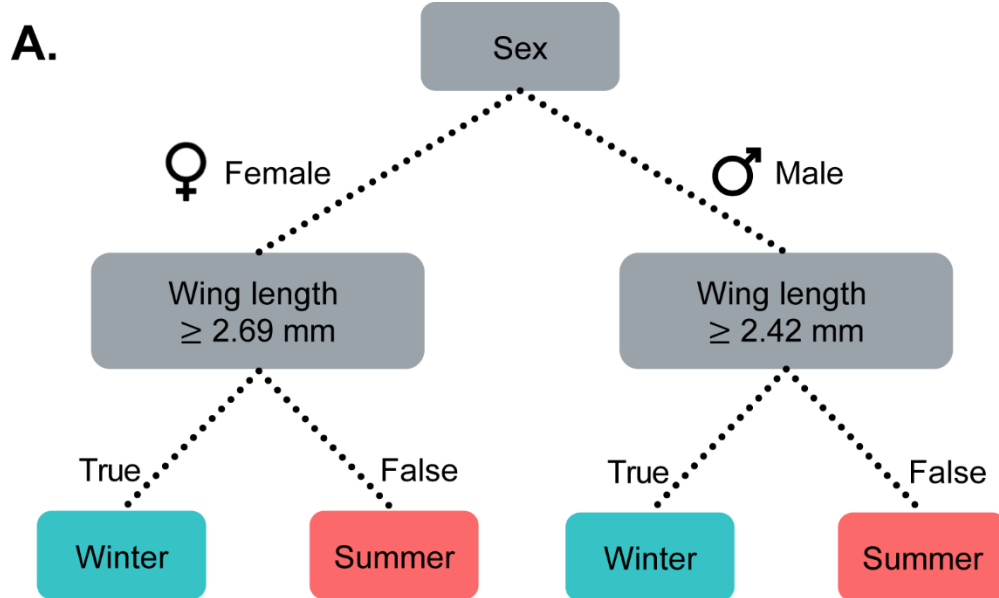
Figure 1.5: Classification tree model for differentiating winter and summer morphs *D. suzukii*. Classification criteria using wing length (A) and transformed ratio of wing length to hind length (B) for females and males.
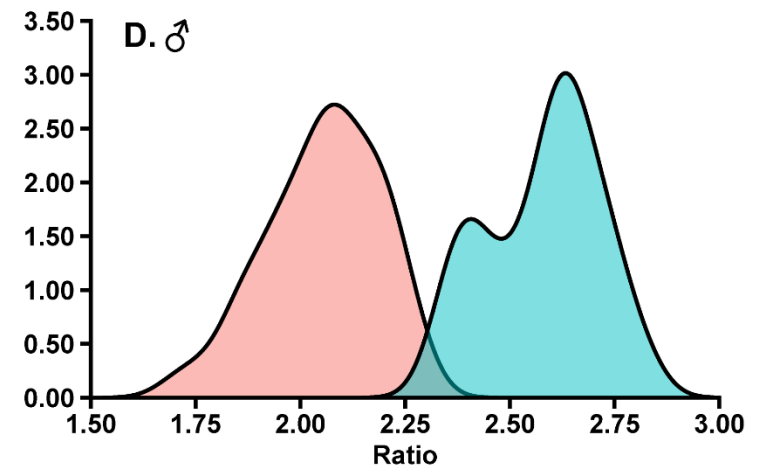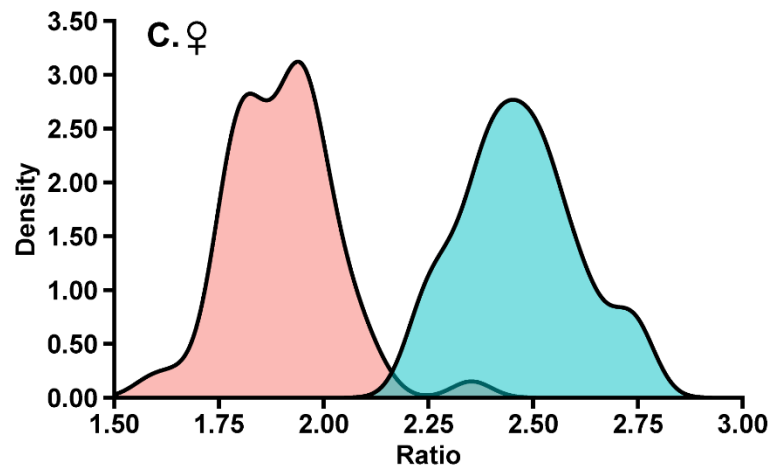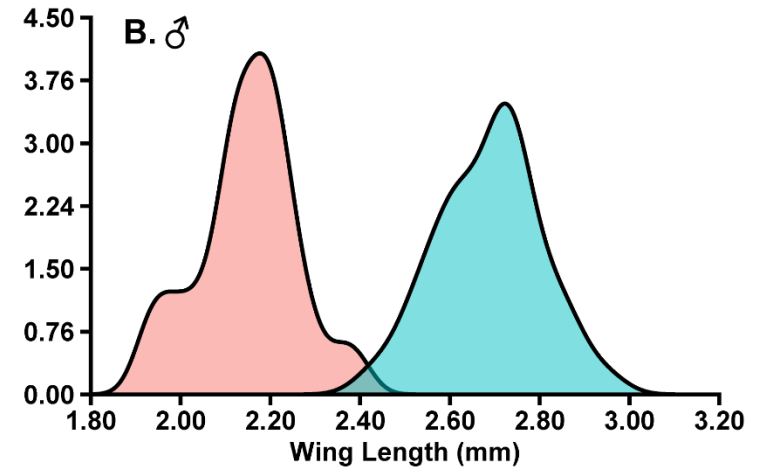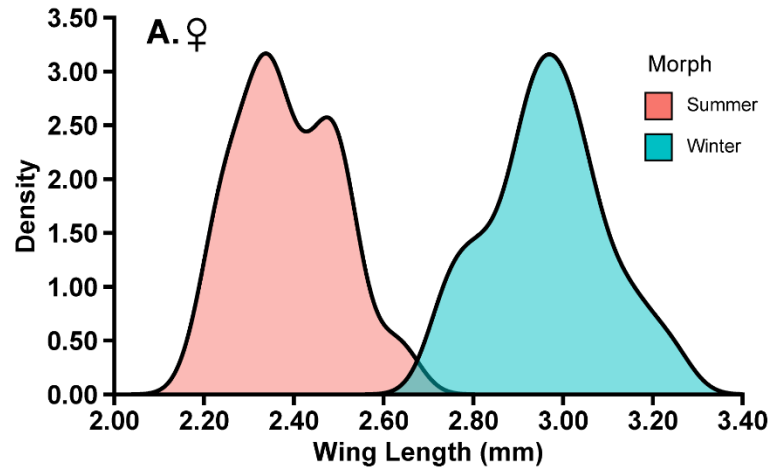
Figure 1.6: Density overlap for laboratory-reared D. suzukii. The percentage of density overlap based on wing length (A and B) and transformed ratio of wing length to hind tibia length (C and D) for female (A and C) and male (B and D).
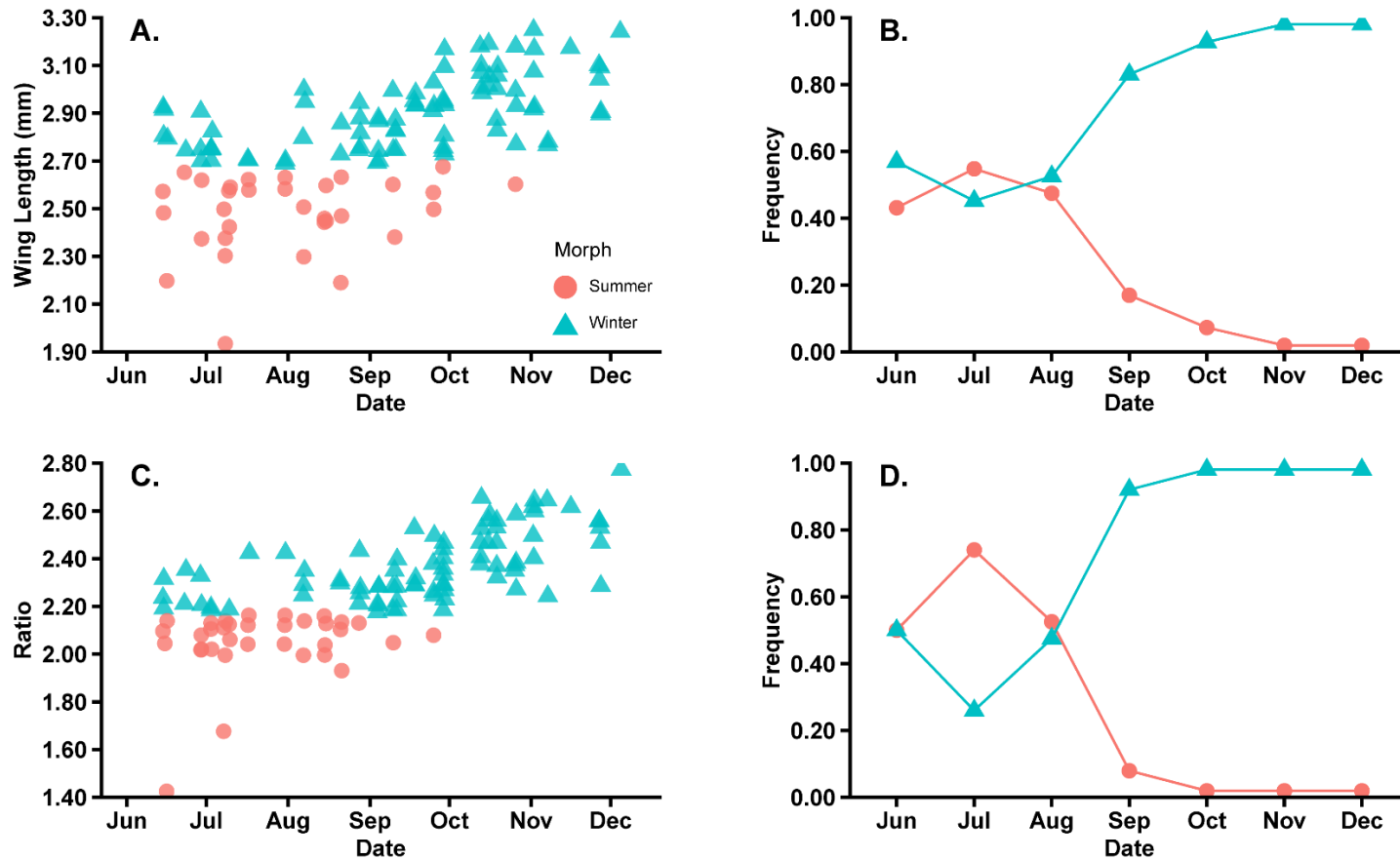
Figure 1.7: Predicted phenology of field caught female *D. suzukii*. Total of female *D. suzukii* morphs trapped from 2017 – 2018 (A and C) and the frequency (B and D) based on wing length (A and B) and transformed ratio of wing length to hind tibia length (C and D).
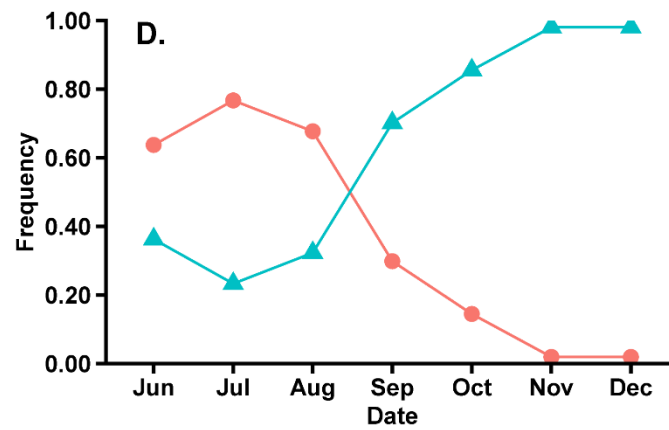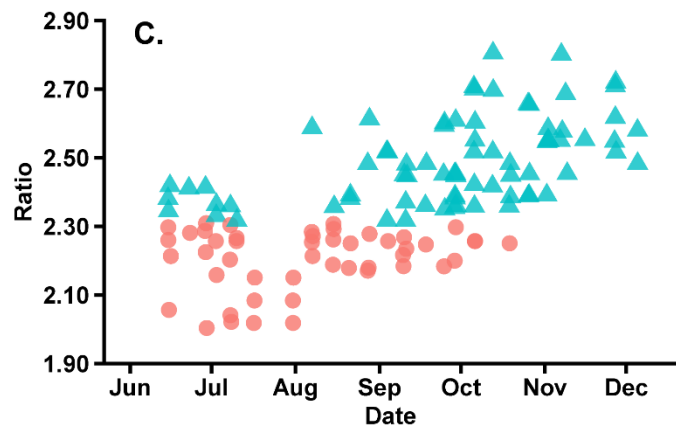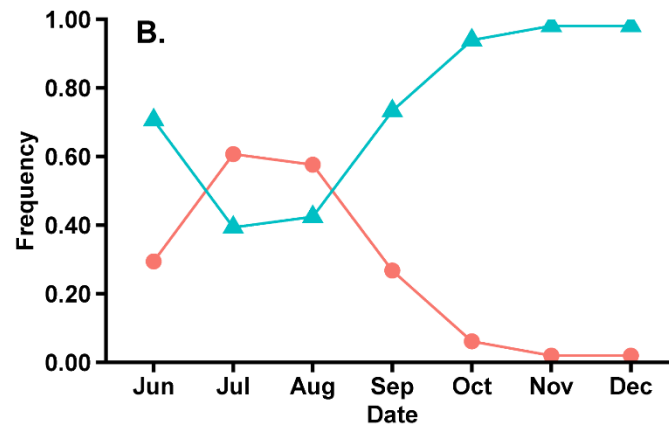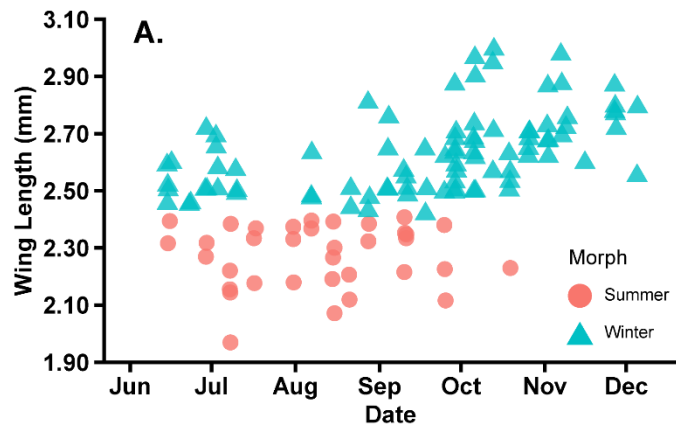
Figure 1.8: Predicted phenology of field caught male *D. suzukii*. Total of male *D. suzukii* morphs trapped from 2017 - 2018 (A and C) and the frequency (B and D) based on wing length (A and B) and transformed ratio of wing length to hind tibia length (C and D).

Chapter II: Comparing *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) flight behavior in a vertical flight chamber and a tethered flight mill

Summary

Tethered flight mills and vertical flight chambers are two common approaches for examining insect flight behavior in the laboratory. Each flight apparatus has various advantages and disadvantages and can influence the flight behavior of the study organism. Rarely are tethered (flight mills) and untethered (vertical chamber) flight studies performed simultaneously to determine if one tactic is better suited for a particular species. Here, we conducted a tethered and untethered flight study to determine the limitations and effects the two methods could have on an insect. We chose to investigate the flight behaviors on a global invasive pest, *Drosophila suzukii* (Matsumura). Propensity of flight and flight duration, distance, and velocity were examined for *D. suzukii* females and males and summer and winter morphs because there is limited information on flight behaviors between the sexes. Also, the two morphs differ in body and wing size, which may, or may not, affect flight capabilities. The study showed that summer morphs were more likely to fly in the vertical flight chamber than when tethered to a flight mill. Additionally, when comparing the length of time of flight for first take, *D. suzukii* flew longer in the vertical chamber. Outcomes from this study provide information on *D. suzukii* flight behavior, which could assist with future research on this economically important fruit pest. Additionally, the results provided a precursor analysis that flight apparatus can affect an organism's flight behavior as seen with *D. suzukii*. Future laboratory flight research should be considerate of the flight technique utilized.

Introduction

The evolution of flight has allowed insects to thrive and become vastly diverse by escaping unfavorable conditions such as predation, lack of resources, or 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). However, obtaining information on insect flight behavior 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 has been conducted in the laboratory to investigate insect species' movement, flight stimuli, and potential dispersal capabilities (Reynolds and Riley 2002, Naranjo 2019).

There are different approaches for investigating insect flight behavior. Here, we focus on two methods - tethered flight mills and vertical flight chambers. With a tethered flight mill, 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, 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, the insect does not need to generate lift to produce flight, and once the specimen has overcome resistance to move the mill, efforts to push the mill forward is reduced (Ribak et al. 2017, Minter et al. 2018, Naranjo 2019). Nonetheless, 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). Consequently, results from laboratory tethered flights can be misinterpreted and under/over-estimate an insect's flight in the field by miscalculating the distance a species can travel (Taylor et al. 2010).

Alternatively, vertical flight chambers, also known as free flight systems, are advantageous because the design allows the insect to fly unrestricted. However, vertical flight chambers are generally cumbersome due to large construction of their design. Also, studies of insect flight with the vertical flight chambers can be labor intensive because observations are not typically automated to record flight behavior and usually only one specimen can be observed at a time (Blackmer et al. 2004, Asplen et al. 2009, Naranjo 2019).

The differences between the two flight assay approaches can lead to different results 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). The varying results from the flight assays such as distance and duration can be problematic when trying to understand the potential movement of an invasive species in the field because the prediction models and assumptions used are based on results of the laboratory flight assay.

An example of understanding flight behavior and capabilities of an invasive species is the vinegar fly, spotted-wing drosophila, *Drosophila suzukii* (Matsumura). Native to Asia, *D. suzukii* has been reported in North and South America, Europe, and Northern Africa (Lee, Bruck, Dreves, et al. 2011, Calabria et al. 2012, Deprá et al. 2014, Asplen et al. 2015, Kwadha et al. 2021). Dispersal experiments have been conducted on

D. suzukii to forecast potential invasion (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 the use of baited traps, which can alter the flies' natural behavior or a mark-and-recapture technique where the results can be limited because of the predetermined distance (Robinet et al. 2019). Testing flight capabilities in the laboratory with flight assays under a controlled environment can assist with the field limitations. To our knowledge, only one study has been conducted investigating *D. suzukii* flight behavior in the lab. However, the study was limited to flight mills with female summer morphs (Wong et al. 2018).

Drosophila suzukii is known to have two seasonal morphs. Researchers have hypothesized that the winter morphs' larger and darker body assist with overwintering by absorbing sunlight and storing fat. Another hypothesis is the winter morphs could be adapted for dispersal similar to other migratory insect species, such as *Danaus plexippus* (L.), which have dispersal forms with larger wings for long distance migration (Satterfield and 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 and provide insight on whether this phenotypic polymorphism aids in long-distance dispersal. Additionally, the literature is limited to flight behaviors of female *D. suzukii* and some insects species exhibit sex-bias dispersal (Markow and Castrezana 2000). Obtaining information on any dispersal differences between the sexes could aid in furthering the understanding of this invasive species' biology and assist with integrated pest management strategies such as sterilized insect techniques. The aims of this study were to compare the results of flight assays between

the tethered flight mill and vertical flight chamber using *D. suzukii* as a model and further examine 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 at the University of Minnesota, St. Paul, MN in 2020, (44.990721, -93.174319) and maintained in the Department of Horticultural Sciences, University of Minnesota, St. Paul, MN. 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). Each vial contained approximately 5ml of an artificial diet comprised of agar, cornmeal, sugar, and yeast (Dalton et al. 2011). Vials were stored in a growth chamber (Percival Scientific Inc., Perry, IA) at $25 \pm 1°C$, a photoperiod of L16:D8 hours and relative humidity of $50 \pm 10\%$. Adult winter morph *D. suzukii* were produced by placing vials with 1 to 3-days-old eggs from summer morphs into another growth chamber at $10 \pm 1°C$, a photoperiod of L12:D12 hours and relative humidity of $60 \pm 10\%$.

Summer and winter morph pupae were individually placed into 1.5ml clear microcentrifuge tubes with approximately 2μl of 40% sucrose water solution. The microcentrifuge tubes with the pupae were stored in growth chamber at $12 \pm 1°C$ with a photoperiod of L16:D8 hours and relative humidity of $60 \pm 10\%$ for adult emergence.

Adult summer and winter morphs were approximately 2 to 4-days-old when used in the study.

Tethered Flight Mill

A 12 computer-monitored flight mill designed by Fahrner et al. (2014) was 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 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 cylinder with circular Rare-Earth magnets. The cylinders were fashioned in a way that the polar end of the magnets faced each other and that there was a 4 cm 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 6.0 cm 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 1.0 - 2.0 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.

To affix adults to the tether, individuals were anesthetized with CO_2 (Carbon Dioxide Anesthetizer Kit, Carolina, Burlington, NC). When the flies were sedated, adults were transferred to 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 Corporation, Westlake, Ohio) and attached to the flies' dorsal side 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. Flight was not instigated with any stimulus

Flight was recorded in real time on a dedicated computer equipped with LabVIEW 2011 software (National Instruments, Austin, Texas). The raw data from LabVIEW was extracted using R version 4.1.0 (R Core Team 2021) and RStudio Desktop version 1.4.1717 (R Studio Team 2021). Flight information we recorded were propensity (yes 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 hours with constant light.

Vertical Flight Chamber

A design similar to Asplen et al. (2009), which originated from Blackmer and Phelan (1991) and Kennedy and Booth (1963) was used to investigate flight in a vertical chamber. In brief, the flight chamber was 1-m^3 and constructed with plywood painted black with exception to the front, which had a plexiglass 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-watt mercury lamp. Adjacent to the light source on either side were fans that were used to cool the chamber between flights (Figure 2.1).

Before flight, *D. suzukii* were acclimated to the chamber by placing the microcentrifuge tubes in the chamber for approximately 10 minutes. 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. We did not consider jumping out of the microcentrifuge tube or hopping around the platform as flight. To determine flight, *Drosophila suzukii* had to vertically ascend from the platform within 3 minutes. 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 minute, the fly was re-captured, and flight was concluded. We measured propensity, phototaxis (flight towards sunlight cue), bouts (the number of take-offs), and duration. The studies with the tethered flight mill and the vertical flight chamber were performed at Metropolitan State University, St. Paul, MN.

Statistical analysis

To examine 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 flight apparatus (tethered mill and vertical chamber), 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. Since the tethered flight mill and vertical flight chamber data were collected differently, the effects of sex and morph on total flight time, total distance and velocity were analyzed separately by flight apparatus with a two-way ANOVA, where applicable. To meet the assumptions of normality and homoscedastic of the errors, the response variables were log-transformed. All data were analyzed using R version 4.1.0 (R Core Team 2021) and RStudio Desktop version 1.4.1717 (R Studio Team 2021).

Results

Summary statistics for *D. suzukii* for the vertical flight chamber and tethered flight mill are presented in Tables 2.1 and 2.2, respectively. Overall, a total of 401 adult *D. suzukii* were used in the experiment across the two different flight apparatuses (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 vertical chamber and tethered mill, respectively. Of the flies that initiated flight, the longest total duration of flight time for an individual in the vertical chamber was 377.66 seconds or 6.29 minutes, while the tethered flight mill, it was 810.81 seconds or 13.51 minutes. On the tethered mill the maximum distance recorded for *D. suzukii* was 283.03 m 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 was pooled, but there was an interaction between morph and flight assay ($\chi^2 = 5.59$, d.f. = 1, $P < 0.01$) (Figure 2.2). Summer morphs were 14.0% more

likely to initiate flight in the chamber compared to being tethered to a flight mill, and initiated flights more frequently compared to the winter morphs (Figure 2.2).

When the duration time for the first flight was compared, the maximum time in the vertical chamber was observed to be 377.66 seconds, but for the tethered mill, the maximum time was 108.66 seconds or 1.81 minutes (Table 2.3). The mean duration of first flight for *D. suzukii* in the vertical flight chamber and tethered mill was 36.7 (±11.29) and 11.7 (± 4.10) seconds, respectively (Table 2.3). The final model showed that only flight apparatus influenced duration of first flight. *Drosophila suzukii* flew significantly longer in the vertical flight chamber compared to the tethered flight mill (χ^2 = 5.39, d.f. = 1, P < 0.02) (Figure 2.3).

In the vertical chamber, there were no significant differences in total flight duration between morphs (F = 3.45, d.f. = 1, 33, P = 0.07) and sex (F = 0.73, d.f. = 1, 33, P = 0.40) (Figure 2.3). On the tethered mill, there were no effects of morph and sex on duration (morph: F = 0.13, d.f. = 1, 24, P = 0.72; sex: F = 0.39, d.f. = 1, 24, P = 0.54), distance (morph: F = 0.01, d.f. = 1, 24, P = 0.91; sex: F = 1.05, d.f. = 1, 24, P = 0.31), and velocity (morph: F = 1.93 d.f. = 1, 24, P = 0.19; sex: F = 0.25, d.f. = 1, 24, P = 0.62) (Figure 2.5).

Discussion

This is the first study to compare insect flight behavior using tethered flight mills and vertical flight chambers, two common approaches for studies on insect flight behaviors and capacities in the laboratory. While each flight apparatus allows researchers to gather information on insect flight in a controlled environment, rarely are specimens

tested simultaneously on both the tethered mill and vertical chamber. This potentially can be problematic as the flight devices can display different results, which in turn could misinterpret an insect's flight behavior. Also, laboratory flight assays provide a crude approximation to insect's mobility in the field and the estimate could be highly skewed based on the results (Hardie 1993).

The use of two flight apparatus on the same species *D. suzukii,* 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 flight apparatus. Specifically, *D. suzukii* initiated flight and flew longer in the vertical chamber (Figure 2.2 and 2.3). This result is interesting because *D. suzukii* were on the tethered mill for approximately 24 h and had more opportunity and time 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 mill, the specimens flew further (mean: 87.02 m) and longer (mean: 7.38 min) compared to the adult flies across our experiment (Tables 2.1 and 2.2) (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 the mill forward. We suspect *D. suzukii* could be hovering instead of conducting forward flight (Dällenbach et al. 2018, Irvin and Hoddle 2020) or was unable to generate enough power to overcome the resistance to propel the mill (Naranjo 2019).

This is also the first study to compare the dispersal capabilities of two morphs of *D. suzukii*. Migratory or dispersing morphs of insects are characteristically described as having larger wings and body size than nonmigratory morphs (Roff and 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 also has wing polymorphism which could aid in dispersal. Previous research has not investigated the function of the larger body and wing size in winter morphs (Tran et al. 2020). Despite having larger wings, winter morphs in this study had lower propensity to fly, 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 and flew at a higher speed and accelerated quicker compared to individuals reared at 22°C and 28°C. The *D. suzukii* in our study were reared at 12°C and that may have affected flight. However, further investigations would be needed to determine if 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).

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 2.4 and 2.5). Several of the outliers that were recorded in duration of total flight and total distance were from females. 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). Our study does not support a sex-bias in dispersal pattern, but that could be due to low sample sizes of positive flight.

Tethered mills and vertical chambers each have their own advantages to examining insect dispersal in the laboratory. However, when the same species is placed on two different flight apparatus and measures the same traits such as propensity and duration, the results can be mixed, which was observed with *D. suzukii*. In a similar experiment, *Agrilus planipennis* Fairmaire flight was compared on a flight mill and free flight system, and the results showed that the mean flight speed in the free flight system was three times greater than the flight mill (Taylor et al. 2010). Thus, flight behavior can be different depending on the flight assay. This is important because results from laboratory flight experiments are often used to predict the potential spread and distribution of an insect in the field.

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 propensity to fly outcomes. Researchers should 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 in resources such as accessibility to flight equipment. At times the flight assay that provides the best estimates for the specimen may not be as conducive because of the insect's nature. For example, *A. planipennis* flight speed was greater untethered; however, Taylor et al. (2010) explained that *A. planipennis* flight comprised

of frequent short bursts and using a tethered mill was more intuitive to collecting flight data than using a vertical chamber.

This study provided a first comparative analysis on *D. suzukii* flight behavior on a tethered mill and vertical flight chamber. Based on the results, there is little evidence that *D. suzukii* are capable of long-distance dispersal. However, 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 if *D. suzukii* can utilize wind to disperse may be useful to understanding the pest's population dynamic and spread. Based on this study, future laboratory flight experiments should take into consideration the bias that flight apparatus might have on flight capabilities.

Tables and Figures

Table 2.1: Summary flight parameters of all *Drosophila suzukii* tested in the vertical chamber.

Vertical Flight Chamber n = 36	Total Flight Time (s)	Bouts
Minimum	2.00	1.00
Median	23.49	1.00
Mean	53.93	2.58
Maximum	377.66	26.00

Table 2.2: Summary flight parameters of all *D. suzukii* tested on the tethered mill.

Tethered Flight Mill n = 27	Distance (m)	Total Flight Time (s)	Velocity (m/s)	Bouts
Minimum	0.69	1.36	0.13	1.00
Median	4.58	15.11	0.29	2.00
Mean	19.96	59.57	0.30	3.52
Maximum	283.03	810.81	0.52	10.00

Table 2.3: Summary of duration of first flight (excluding additional take-offs) of *D. suzukii* across the vertical chamber and tethered flight mill.

Duration of First Flight (s)	Vertical Chamber	Tethered Mill
Minimum	2.00	1.36
Median	18.44	5.38
Mean	36.68	11.74
Maximum	377.66	108.66
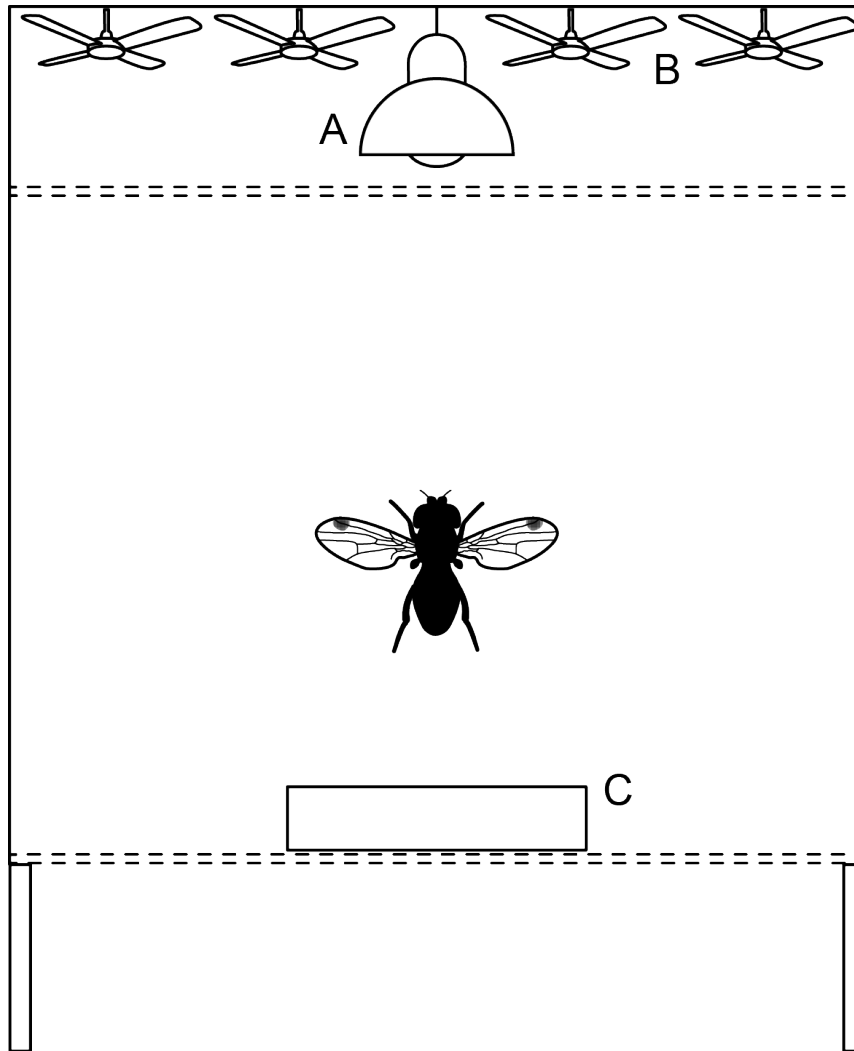
Figure 2.1: A cross-section diagram of the vertical flight chamber. The flight chamber was 1-m^3 and constructed with plywood painted black with exception to the front, which had a plexiglass door to observe *Drosophila suzukii* behavior. The bottom and top were covered with a fine black mesh screen (dashed lines). The chamber was illuminated a 400-watt mercury lamp (A) with four fans (B) to cool the chamber. Individual *D. suzukii* were released from a platform (C). This design was similar to the flight chamber design in Asplen et al (2009). Elements of the schematic are not to scale.
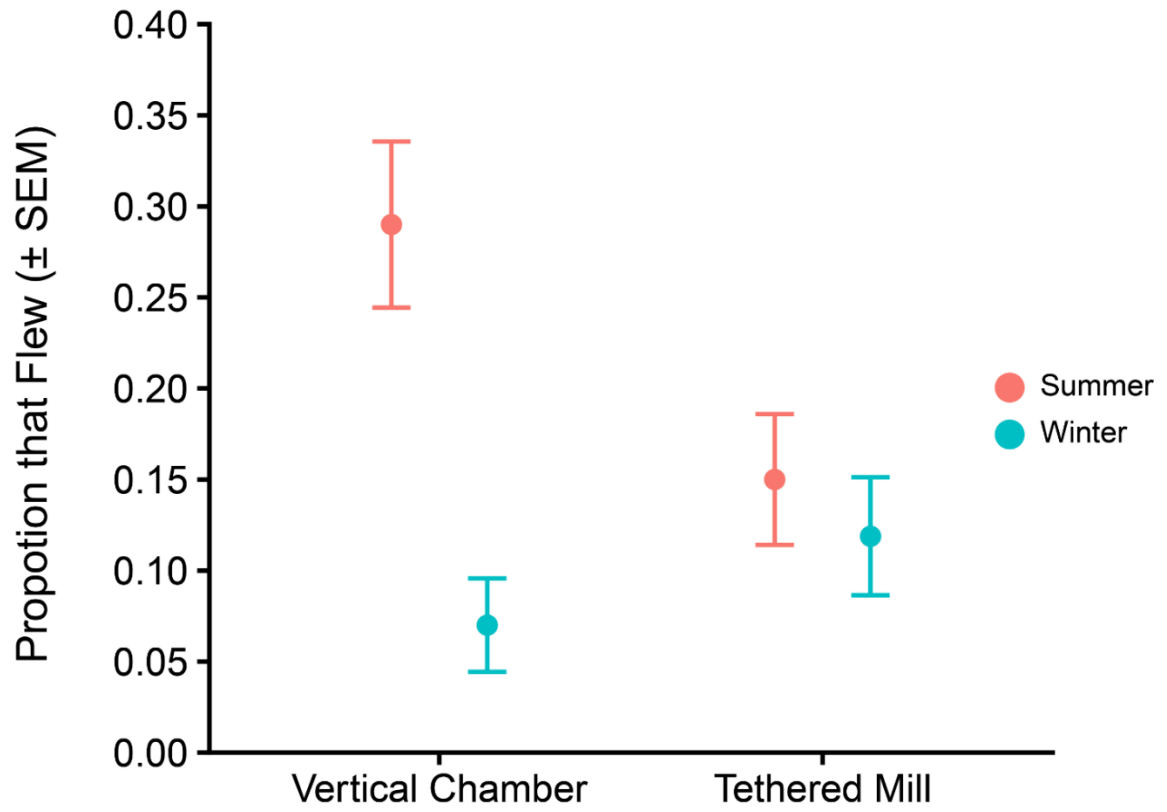
Figure 2.2: Comparison of the mean proportion (± SEM) of summer and winter morphs propensity (i.e., yes or no) to initiate flight in the vertical chamber and tethered mill.
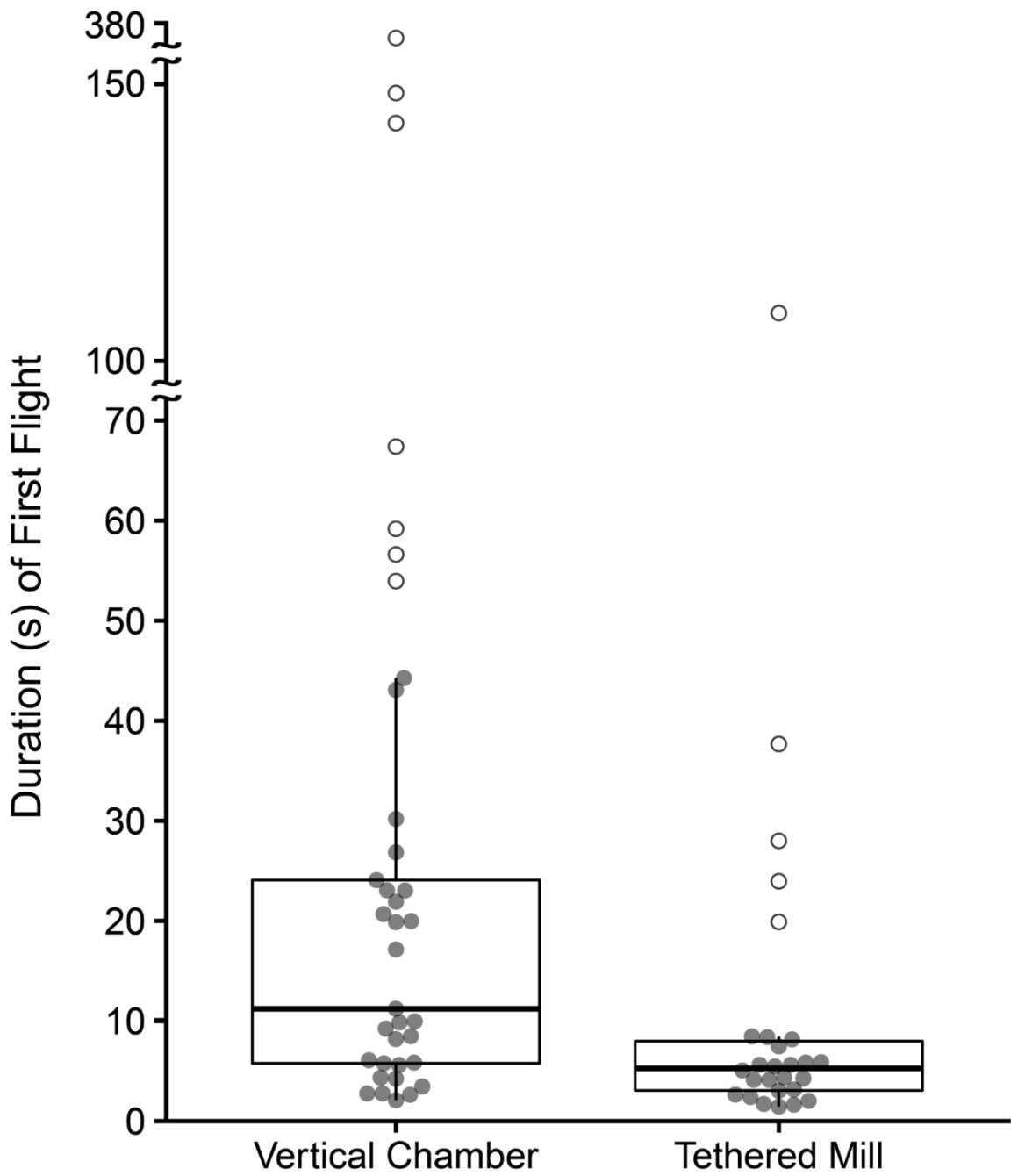
Figure 2.3: *Drosophila suzukii* total duration (s) of first flight (e.g., excluding additional take-offs) in the vertical chamber and tethered mill.
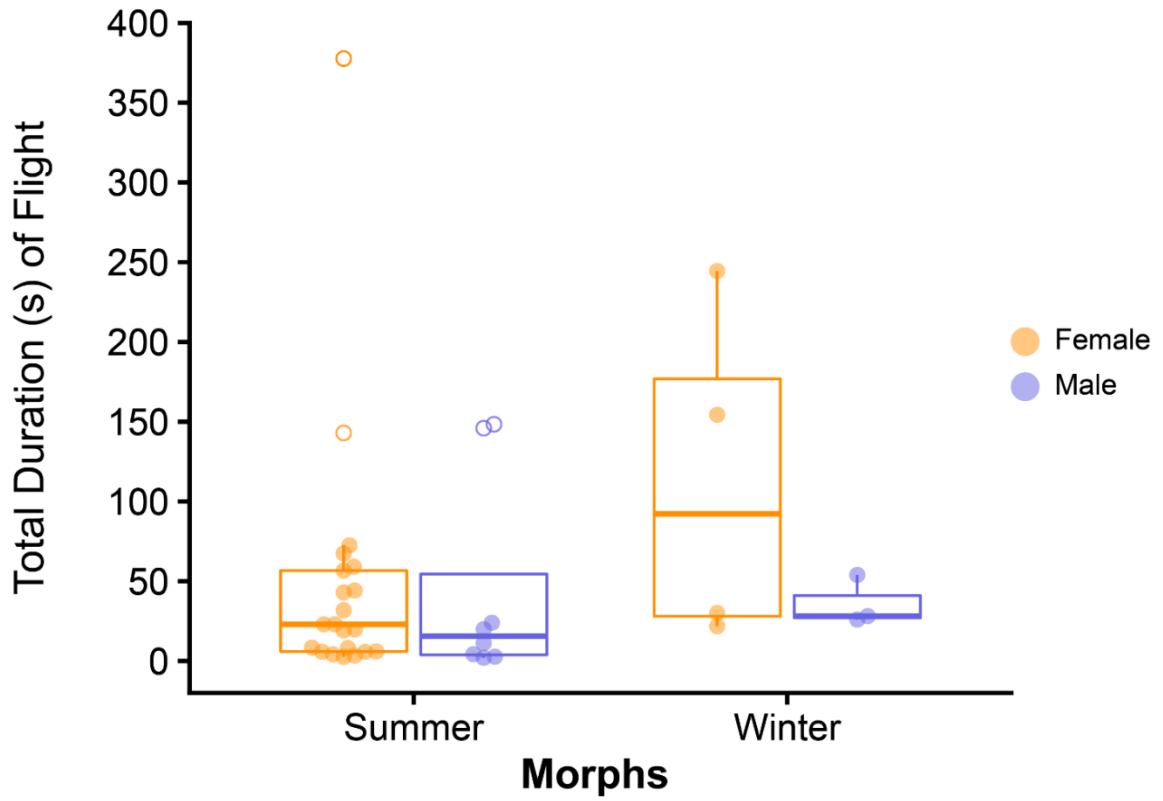
Figure 2.4: Comparison of the total duration (s) of flight across gender (female and male) and morph (summer and winter) in *Drosophila suzukii* in the vertical chamber.
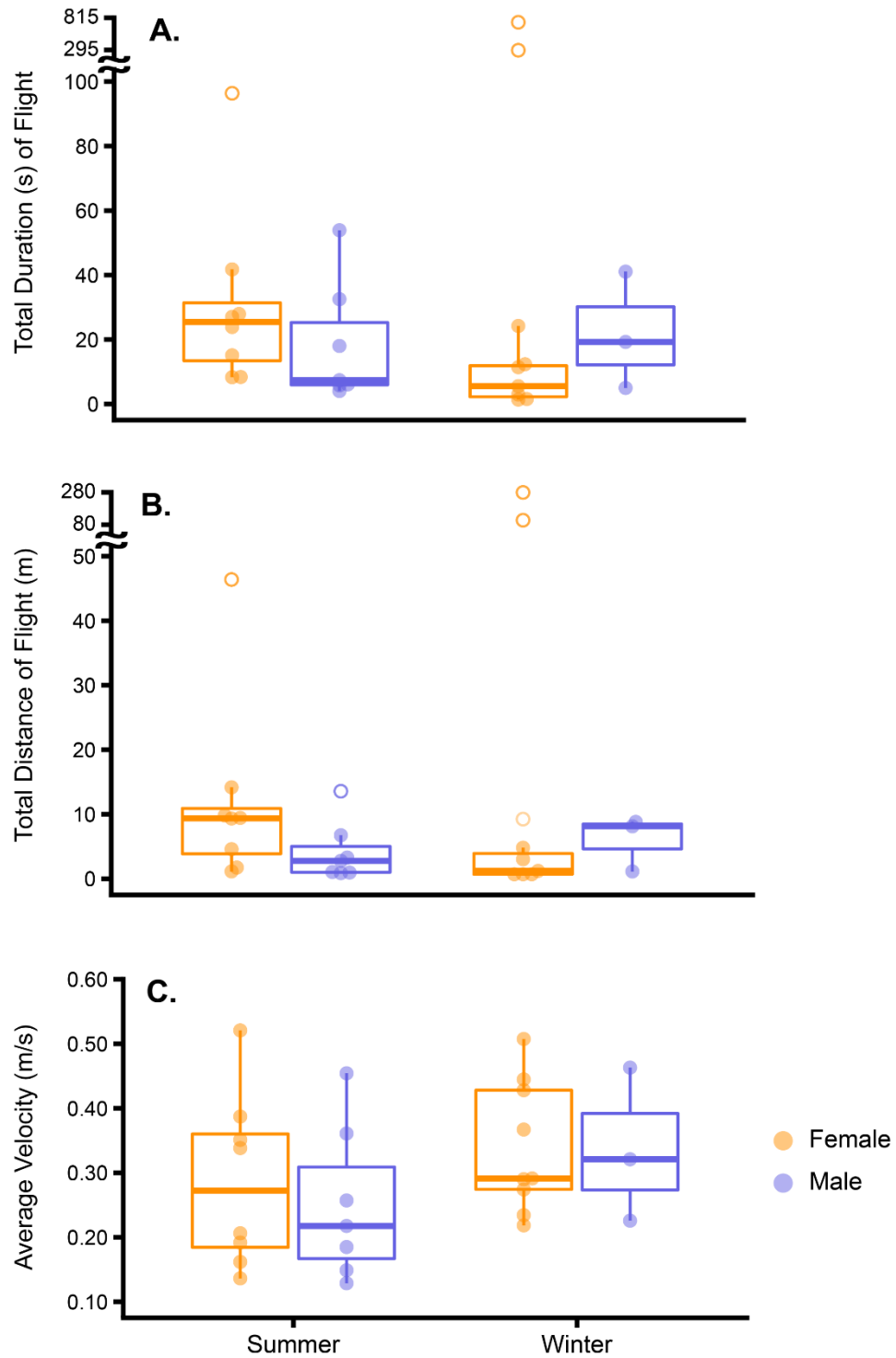
Figure 2.5: Comparison of total duration (s) of flight (A), total distance flown (B) and average velocity (C) across gender (female and male) and morph (summer and winter) of *Drosophila suzukii* winter on the tethered flight mill.

Chapter III: Using passive traps to investigate the vertical and

temporal dispersal of *Drosophila suzukii* (Matsumura)

(Diptera: Drosophilidae)

Summary

Drosophila suzukii (Matsumura) is an invasive polyphagous pest of soft-skinned and stone fruits and is of economic concern throughout the world. Its relatively rapid range expansion since initial detection in the United States could be due to seasonal, long-distance dispersal; however, little is known about *D. suzukii* dispersal capabilities. Here, we examined the vertical dispersal of *D. suzukii* adults using transparent sticky cards at five heights (1.0 – 7.3m above soil surface) in a raspberry field located at a research station in Minnesota from 2017 – 2019. Traps were set before the raspberries were ripe and sampled weekly until first snowfall (October to December). In addition to the passive clear traps, an unmanned aerial vehicle (UAV) was utilized to sample for *D. suzukii* 23 – 122m above ground. The traps captured more females from July to September, but from October to November more males were caught. Approximately, 77% of *D. suzukii* population were captured in September and October and 90% of the catches were near the raspberry canopy (≤2.0m) throughout the sampling period. However, 3.5% of *D. suzukii* were found at altitudes ≥ 6.0m. We did not catch any *D. suzukii* using the UAV. Results from the clear sticky cards suggests that *D. suzukii* naturally occurs at these high altitudes and may be using wind to aid in long-distance dispersal.

Introduction

The evolution of flight has allowed Pterygotes to diversify, occupy niches, and expand their range that were previously unavailable to wingless insects (Dudley 2000). Therefore, movement has become a crucial element in insect's ecology and behavior (Chapman et al. 2011). Studies on dispersal over time can lead to better predictions for species establishment (National Research Council 2002, Nguyen and Nansen 2018), discovery of abiotic and biotic mechanisms of dispersal (Stinner et al. 1983, Guo et al. 2020), a better understanding of seasonality of movement occurrences (Day and Knight 1995, Sturtevant et al. 2013), and provide information that could help reduce the economic impact a pest has in a crop system (Byrne 2008). While there are benefits to understanding an insect's flight movement there is often a shortage of available literature and this is because field research on the seasonal dispersal is difficult to investigate on small insects. Hence, flight behavior studies are conducted in the laboratory and the results are used to make predictions in the field (Hardie 1993). However, the predictions are crude estimates and may not be representative of the insect's flight behavior in the field. Studying insect flight in the field, especially insects that are of economic concerns, could improve forecast models of an insect's population dynamics and dispersal capabilities, which are vital to pest management programs.

An example of an important pest that researchers have been studying for understanding their dispersal and flight behavior is *Drosophila suzukii* (Matsumura). *Drosophila suzukii* is an important species to focus on because of the global economic impact the invasive vinegar-fly has on the soft-skinned berry and stone fruit industry (Hauser 2011, Lee, Bruck, Curry, et al. 2011). Since the initial detection in the

continental United States in 2008, *D. suzukii* has been reported and detected in every state with the exception of Alaska, Arizona, and Nevada (Burrack et al. 2012, Griffith 2014, CABI 2021). Due to *D. suzukii* rapid invasion from their home range, wide selection of host crops, and zero-tolerance for infestation, a multitude of studies have been conducted to understand how to effectively manage *D. suzukii* in fruit production systems, in particular timing of management tactics. Investigations that have improved timing of deploying management practices in the field include factors that would influence *D. suzukii* flight (i.e., starvation levels and humidity) (Wong et al. 2018), their movement between crops and adjacent surroundings (Lee, Dreves, Cave, Kawai, Isaacs, Miller, Van Timmeren, et al. 2015, Pelton et al. 2016), chronobiology (Evans et al. 2017, Jaffe and Guédot 2019), and population throughout the season (Elsensohn and Loeb 2018, Panel, Zeeman, Van der Sluis, et al. 2018). However, management practices could be improved by better understanding the long-distance flight capabilities of *D. suzukii*.

Thus far, field investigations of *D. suzukii* dispersal focused on their local spread. For example, Rice et al. (2017) released laboratory-reared *D. suzukii* in a semi-field experiment with potted raspberry (*Rubus idaeus* L.) plants and found that most flies were observed within the lower levels of the canopy at approximately 60-85 cm above ground. Similarly, Jaffe and Guédot (2019) found a majority of *D. suzukii* within raspberry canopies 48-65 cm from the ground. Many field experiments have documented *D. suzukii* population dynamics within wild hosts or landscapes surrounding a crop (Klick et al. 2016, Pelton et al. 2016, Leach et al. 2018) and/or performed mark-recapture experiments to describe dispersal capabilities (Tait et al. 2018, Vacas et al. 2019). Results evaluating *D. suzukii* distribution from a release point found that the fly was capable of moving a

minimum of 25 m in 3 hours (Vacas et al. 2019) to maximum distances of 9 km after a couple of days (Tait et al. 2018). While these experiments provide documentation of *D. suzukii* movement and flight behavior in the field, the experimental designs relied on the use of baited traps, which actively attracts flies and can potentially alter the flies' behavior (Cha et al. 2014, 2018). Results from current flight studies have been used to predict invasion and spread of *D. suzukii* (Maino et al. 2021), and the assumptions used for the prediction models could be over or underestimating the distribution.

Studies of the horizontal fly movements do not necessarily indicate self-directed, vertical movements. Additionally, many of the field studies on flight were not carried out in a crop system beyond the harvest season. If *D. suzukii* were moving to overwintering habitats or dispersing to non-cash crops in surrounding area, understanding the timing of this behavior could assist with sanitation methods of reducing population densities for the following year (Wang et al. 2019). However, sanitization is a time-consuming process, and the procedure would be even more labor intensive if *D. suzukii* disperses beyond the crop's field.

To learn about the long-distance capabilities of *D. suzukii*, vertical ascent and distribution need to be considered. Small-bodied insects in the families Aphididae, Aleyrodidae, Thripidae, and Cicadellidae are known to use wind to disperse across landscapes and regions (Byrne et al. 1996, Shields 1999, Zhu et al. 2006, Parry 2013, Smith et al. 2015, Nyasani et al. 2017). The concept of using wind-aided dispersal is possible when insects enter the flight boundary layer, which is the surface area in which wind speeds exceed insect flight speed, allowing insects to be carried by wind (Taylor 1960, Smith et al. 2015, Asplen et al. 2016). Small insects, such as *D. suzukii*, could

engage in passive long-distance dispersal if they can exceed the flight boundary layer, but currently a limited amount of research observed the flies' vertical ascent. To our knowledge, Jaffe and Guédot (2019) is the only study that has looked at *D. suzukii* vertical dispersal without baited traps, however the experiment was done over a short sampling period and at the maximum vertical height of 1.2 m. Furthermore, *D. suzukii* produces seasonal morphs, known as 'winter' and 'summer' morphs, where the former is both larger in body and wing size and darker in pigmentation (Stephens et al. 2015, Shearer et al. 2016). The larger and darker body in winter morphs may assist with overwintering by absorbing sunlight and having higher fat storage (Shearer et al. 2016). An alternative hypothesis is that winter morphs could be a dispersal form as other insect species that are known to migrate have a dispersal morph, which entails having larger wings to assist with flight (Satterfield and Davis 2014, Flockhart et al. 2017). There is an absence of information describing the dispersal differences, if any, between the winter and summer morphs.

The objectives of this investigation were to characterize the vertical distribution of *D. suzukii* at varying heights above a raspberry crop canopy over time with passive, unbaited traps, and an unmanned aerial vehicle (UAV) and examine the dispersal capabilities of summer and winter morphs and the sexes. Raspberry was chosen because the crop is a preferred host of *D. suzukii* (Burrack et al. 2013, Wöhner et al. 2021). Additionally, a probable flight boundary layer was calculated to determine the vertical ascent *D. suzukii* would need to ascent to engage in passive wind-aided dispersal.

Materials and Methods

Adult *D. suzukii* dispersal activities were monitored in 2017, 2018, and 2019 at the University of Minnesota Outreach, Research and Education (UMORE) Park (44.7279˚N, 93.0968˚W) in Rosemount, MN. The sampling site was in 0.30 ha of established (>5 years-old) 'Heritage' raspberry primocane. Raspberry canopies were approximately 1.5 m tall with rows approximately 3.81 m long with 3.05 m spacing. In 2018, a second monitoring location was temporarily established at a fresh market farm that cultivated raspberries, blueberries (*Vaccinium corymbosum* L.), and strawberries (*Fragaria × ananassa* Duchesne) near Forest Lake (45.2304˚N, 92.8932˚W). Materials used for trapping adult *D. suzukii* were modified from Smith et al. (2015). *Drosophila suzukii* adults were trapped using clear sticky cards that were approximately 7.30 cm height × 20.96 cm length (Catchmaster Window Flytrap, model number 904, AP&G Co. Inc., Brooklyn, NY). Each sticky card was folded with a 7.30-m tall, telescoping tripod (Manfrotta, model number: 268HDBU, Lino Manfrotto + Co. Spa, Cassola, Italy) in the center of the card at 1, 2, 4, 6, and 7.3 m above the ground, with the adhesive side facing outward. For added security, a piece of double-sided tape was added to the non-adhesive side of the sticky card and a small binder clip (1.90 cm) was attached to each card. In 2017, tripods were checked from 27 July to 10 December. In 2018, a single pole was set up on 11 May to catch an earlier emerging *D. suzukii*, with two more tripods deployed on 5 June, also spaced 30 m apart from each other. However, on 7 August one of the three tripods was relocated to Forest Lake and was sampled until 28 August. A tripod was moved to this location to increase the chances of trapping *D. suzukii* due to the larger density of flies present, however, the short sampling period at Forest Lake was due

broken equipment. Sampling at UMORE Park in 2018 continued with two tripods, spaced 60 m apart from 7 August to 21 October. In 2019 at UMORE Park, two tripods spaced approximately 60 m apart were used for sampling adult *D. suzukii* from 16 July to 7 November. Every year, one tripod was placed at the edge of a wood-line and the other tripods were situated at the edge of a raspberry row. Sampling stopped upon first snowfall in attempt to remove the tripods before the ground became frozen. Sticky traps were collected from each tripod at each of the five sampling heights and replaced every 3-4 days each year. When replacing the sticky cards, a sheet of clear plastic wrap was placed over the adhesive side to prevent the cards from sticking to each other and stored in the freezer to count and identify adult *D. suzukii* in the laboratory.

Identification

Detecting the presence of *D. suzukii* was conducted by placing each sticky card under a stereomicroscope (Leica S6E, Leica Microsystems, Wetzlar, Germany) with 6.3x – 40.0x zoom magnification. *Drosophila suzukii* species were identified by the looking for the sclerotized ovipositor for females and a single spot on each wing along the subcostal vein and/or sex combs on the front tarsus for males (Hauser 2011, Asplen et al. 2015). Individual *D. suzukii* were gently removed from cards by using a sharp razor to remove part of the plastic covering and then using a fine tip paintbrush soaked in an adhesive remover (Goo Gone, CC Holdings, Inc, Gurnee IL). Winter and summer morphs were identified by taking the transformed ratio of wing length to hind tibia length as described by Tran et al. (2020). In brief, the wing length was measured from the base of the wing to the apex of the R4+5 wing vein and then subtracting 1.008 (females) or 0.762

(males) from the wing length and dividing the difference by the hind tibia length. Female and male *D. suzukii* with a ratio of ≥2.17 and ≥2.31, respectively, were identified as potential winter morphs. In addition to classifying the morphs, the sex of each individual was recorded. Some specimens were so heavily damaged (i.e., missing parts essential to identification), so we were only able to record the sex and not identify the morph.

Unmanned Aerial Vehicle

The Department of Aerospace and Engineering at University of Minnesota assembled and modified an unmanned aerial vehicle model, X-UAV Talon, with light-emitting diode below each wing, to follow Federal Aviation Administration regulations, and deployable insect traps. The deployable traps were made up of four 100x15 mm petri dishes, two under each fixed wing. Tanglefoot® (The Ortho Group, Marysville, OH) was applied to the interior sides of the petri dish. The petri dishes laid flat with the interior side against the wings during take-off and were remotely open to sample when the UAV reached a specific altitude. The petri dishes were then remotely closed before landing. The UAV was flown between the altitudes of 23 – 122 m until battery levels were low (approximately 30 minutes) and at approximately two – three flights were done on a given sampling day. There was a total of 18 sampling dates between 2017 and 2019 (2017: 24 August, 7 September, and 20 September; 2018: 21 May, 7 June, 12 June, 26 June, 18 July, 2 August, 9 August, 13 August, 6 September, and 16 September; 2019: 9 August, 22 August, 10 September, 19 September, and 15 October). The varying sampling dates dependent on weather conditions and availability of a licensed pilot to fly the UAV. Flights took place approximately two hours before sunset and ended 30 minutes after the

sun was below the horizon (17:00 – 22:00). After each flight, petri dishes were covered with a lid and stored in a -30°C freezer for later identification in the laboratory. Flights took place at the UMORE Park and the fresh market farm in Forest Lake in 2017, 2018, and 2019. The flight sample dates were dependent on weather (e.g., no rain or strong winds) and availability of a pilot to operate the UAV.

Data Analysis

All statistics were analyzed using R version 4.0.3 (R Core Team 2020) and RStudio Desktop version 1.4.1103 (RStudio Team 2020). There was a total of 1055 sticky cards from 2017 to 2019 and approximately 9.0% of the sticky cards had a *D. suzukii* present. Given the low success rate, we utilized a negative binomial generalized linear mixed effect model in the R package 'lme4' and the function 'glmer.nb' (Bates et al. 2015). We were interested in the effects of vertical trap height and date on sex (females and males) and morphs (winter and summer); however, they were non-significant in the model ($p > 0.05$). Therefore, we analyzed the fixed effects of vertical trap height and date and their interactions on the total count of female and male *D. suzukii* caught with the three tripods and years as random effects. Date was converted to ordinal dates and analyzed as a continuous variable with a square term. To reduce model convergence error, we started the time series when *D. suzukii* was first caught on a sticky card (i.e., 28 July 2017, 17 July 2018, and 25 July 2019) and ended the time series the last time at least one *D. suzukii* was detected on the sticky card (i.e., 27 November 2017, 30 October 2018, 26 October 2019).

Flight Boundary Layer

The flight boundary layer was calculated using the following method from Taylor (1974). The log aerial density was plotted against the log height. A linear line was fitted through the data points and a secondary horizontal line was fitted through trial and error to determine the best fit. The point at which the two fitted lines intercept is a prediction of flight boundary layer for *D. suzukii.*

Results

From 2017 to 2019, a total of 256 individual *D. suzukii* were trapped across the three years and three poles. There was a significant main effect of vertical trap height and date for the probability of catching a *D. suzukii*, however there were no interactions between the two predictor variables (Table 3.1, Figure 3.1). For every unit increase in vertical height, the probability of capture rate decreased, but trap captures increased until approximately 29 September (272 ordinal days) and then the numbers decrease (Figure 3.1). Approximately 90.0% of *D. suzukii* were trapped at 1.0 and 2.0 m (41.0% and 49.0%, respectively) (Figure 3.2). A total of nine *D. suzukii* were found at the vertical heights of 6.0 and 7.3 m (Figure 3.2). We observed the highest density of *D. suzukii* in September and October, making up approximately 77.0% of the captures (43.0% and 34.0%, respectively).

Of the *D. suzukii* trapped, 130 individuals were identified to morph. The first identifiable winter morph trapped in 2017, 2018, and 2019 was on 14 August, 17 August, and 13 September, respectively, and summer morphs were found during all months of the trapping season (Figure 3.2). However, the number of winter morphs identified increased

in October and November (Figure 3.2) and we observed both winter and summer morphs at the highest vertical height of 6.0 and 7.3 m (Figure 3.2.).

Our model showed that sex was not a significant predictor of trapping *D. suzukii*, but we observed density differences between the sexes (Figure 3.3). More female *D. suzukii* trapped from July to September, but by October the number of females trapped were reduced by approximately 59.6% whereas males had an increase of 15.5% before the density decreased in November (Figure 3.3 A & C). Additionally, we saw fewer females trapped at vertical heights of 6.0 and 7.3 m compared to males (Figure 3.3. B & D).

From 2017 – 2019 there were a total of 18 flight dates (three in 2017, ten in 2018, and five in 2019). Of the 146 petri-dishes trap samples, no *D. suzukii* was caught with the UAV aerial traps. For the predicted flight boundary layer, the two fitted lines intercepted at approximately 1.31 m which is the exponent of 0.27 (Figure 3.4).

Discussion

This is the first study that documented the potential long-distance dispersal of *D. suzukii* in the field using unbaited traps. In our three-year study evaluating the temporal distribution of *D. suzukii,* we installed traps before fruit was ripe on the raspberry plants and continued sampled beyond the harvest period. During this time, the highest abundance of *D. suzukii* trapped from August to October was similar to other literature that documented the fly populations in Minnesota (Rogers et al. 2016, Ebbenga et al. 2019). Our results showed there were no statistical evidence that females and males dispersed differently in the field. However, male *D. suzukii* trap catch steadily increased

through-out the sampling period with a quick decline in November, while female *D. suzukii* numbers decreased after September. Similarly, a field study by Tait et al. (2018), there were significantly higher numbers of female flies caught earlier in the season and more males were caught in late summer and autumn as density increased. This is interesting because sex-bias dispersal is known in many insect species and was apparent when Markow and Castrezana (2000) investigated the flight behavior of three *Drosophila* species (*D. pachea* Patterson and Wheeler, *D. nigrospiracula* Patterson and Wheeler, and *D. mojavensis* Patterson). Of the three species, *D. pachea* displayed sex-bias dispersal where males dispersed significantly further compared to females. While sex-bias dispersal is present in other *Drosophila* species, we are unable to state that *D. suzukii* exhibits sex-bias dispersal based on the presented data.

Considering *D. suzukii* use berries as their host, the fact that most of our catches were observed at 1.0 – 2.0 m above ground (Figs. 1 and 2) was expected as insects that feed on crops are typically observed a few meters above ground (Byers 2011). For example, Smith et al. (2015) evaluated the vertical distribution of the pest species, *Thrips tabaci* Lindeman, with clear sticky traps ranging from 0.5 – 6.0 m and found that a majority of the thrips were caught at 0.5 – 2.0 m. Shrestha et al. (2021) observed 12 different pest species of potatoes with passive vertical traps ranging from 1.5 – 7.6 m and pests were predominately found on the lower trap heights. Nevertheless, *D. suzukii* was present at vertical heights of 6.0 – 7.3 m and the number of flies trapped > 2.0 m increased as the season progressed.

Small insects, such as *D. suzukii*, could engage in passive long-distance dispersal if they can exceed the flight boundary layer and be carried by wind (Taylor 1960, Smith

et al. 2015, Asplen et al. 2016). Taylor (1974) conducted a study approximating the flight boundary layer for several different insect taxa and found that the vertical varied by the insect and their size. Following Taylor (1974) mathematical approach, we calculated the flight boundary for *D. suzukii* is 1.31 m above the raspberry canopy (Figure 3.4). The raspberry crop canopy increased in height throughout the sampling period and varied in the field but was approximately between 0.8 – 1.3 m by the end of the growing season. A majority of *D. suzukii* were caught in this study at 1.0 – 2.0 m, which would be below the flight boundary layer, suggesting there was more local movement compared to long-distance. But catching adult flies at vertical heights of 4.0 – 7.3 m would suggest that *D. suzukii* was able to reach the predicted flight boundary layer and possibly engage in long-distance dispersal.

As mentioned in the methods, each year a tripod was placed on the edge of a woodland habitat. Numerically, more *D. suzukii* were trapped along the edge of the woods compared to the traps on the tripod that was placed in the field of raspberries (Figure 3.5). Additionally, we saw more *D. suzukii* trapped on the tripod near the woodlands at higher altitudes and the density increased as the growing season progressed (Figure 3.5). Other studies have found a similar trend of observing an increase of *D. suzukii* in woody habitats as temperatures declined and have speculating that flies were moving into the space to overwinter (Pelton et al. 2016, Santoiemma et al. 2019). We propose the alternative hypothesis to overwintering that *D. suzukii* may be capable of long-distance dispersal. Approximately 70% of *D. suzukii* were caught at heights of 4.0 – 7.3 m along the woodland and wind flow can change near the forest edges (Raynor 1971,

Detto et al. 2008). Since we did not bait *D. suzukii* to fly to these altitudes, long-distance dispersal with the aid of wind is a possibility.

There is a paucity of literature documenting the vertical dispersal of *D. suzukii* and in other small insects, especially with unbaited traps. Small insects are capable of being transported hundreds of kilometers in the atmosphere and numerous Dipterans have been collected in altitudes ranging from 7.5 – 1,100 m (Taylor 1974, Isard et al. 1990, Chapman et al. 2004). Understanding *D. suzukii* movement is important because it continues to be a devasting economic pest. Based on our presented evidence, 10% of the *D. suzukii* population was able to reach or exceed the flight boundary layer to undergo long-distance dispersal aided by wind. Providing additional information on *D. suzukii* long-distance movement could be used in early detection monitoring and assist in determining the appropriate time to apply management strategies.

Routine scouting and monitoring can be a time-consuming task but is often recommended to avoid unnecessary prophylactic use of insecticides. Models developed using meteorological data can provide growers appropriate timing of when to intensify field observation for pests. For example, Zhu et al. (Zhu et al. 2006) was able to utilize wind data to predict approximately one month in advance peak activity of *Myzus persicae* (Sulzer) and Klueken et al. (2009) developed similar approaches for cereal aphids, *Rhopalosiphum padi* (L.), *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (Fabricius). An alarm system was developed in Helsinki, Finland for insect pests, *R. padi* and *Plutella xylostella* (L.) (Leskinen et al. 2011). A similar system could be put in place for *D. suzukii*. Universities and extension agents in several states have created a *D. suzukii* trap network where individual(s) frequently travel, check baited traps, and report

the number of *D. suzukii* caught weekly through an online platform and provide management recommendations. While this is useful, this means *D. suzukii* may already be in a field causing damage to the fruit crops and reducing yield. Also, scientists also have the general consensus that *D. suzukii* are overwintering in woodlands or in protected areas (Pelton et al. 2016, Rendon et al. 2018, Leach, Stone, Van Timmeren, and Isaacs 2019, Santoiemma et al. 2019). If *D. suzukii* is overwintering and using wind to disperse and infesting fruit crops in nearby areas, timing of the flight event could further assist management by urging growers to consider sanitizing their fields at the end of a growing season.

Flight in small insects remains an understudied subject, and this is true for *D. suzukii.* To our knowledge, ours is one of the first studies looking at *D. suzukii* vertical dispersal throughout a growing season using passive traps. *Drosophila suzukii* was caught well above and raspberry canopy and this could lead to wind-aided dispersal but would need to be studied directly. Based on our results, we know *D. suzukii* can reach altitudes of 7.3 m on their own without being baited, which could facilitate long-distance dispersal.

Tables and Figures

Table 3.1: Summary statistics of the negative binomial regression model predicting the number of *D. suzukii* caught at a vertical trap height and date. The equation to predict number of *D. suzukii* trapped at a date and height is $\log(y) = -0.22 + -0.65 \text{ (height)} + 13.55 \text{ (date)} - 16.68(\text{date}^2)$

Coefficient	Estimate	SE	*Z* Value	*P* Value
(Intercept)	-0.22	0.72	0.31	0.7588
Vertical Trap Height	-0.65	0.05	8.61	<0.0001
Date	13.55	4.01	3.38	<0.0001
Date ^ 2	-16.68	4.28	3.90	<0.0001
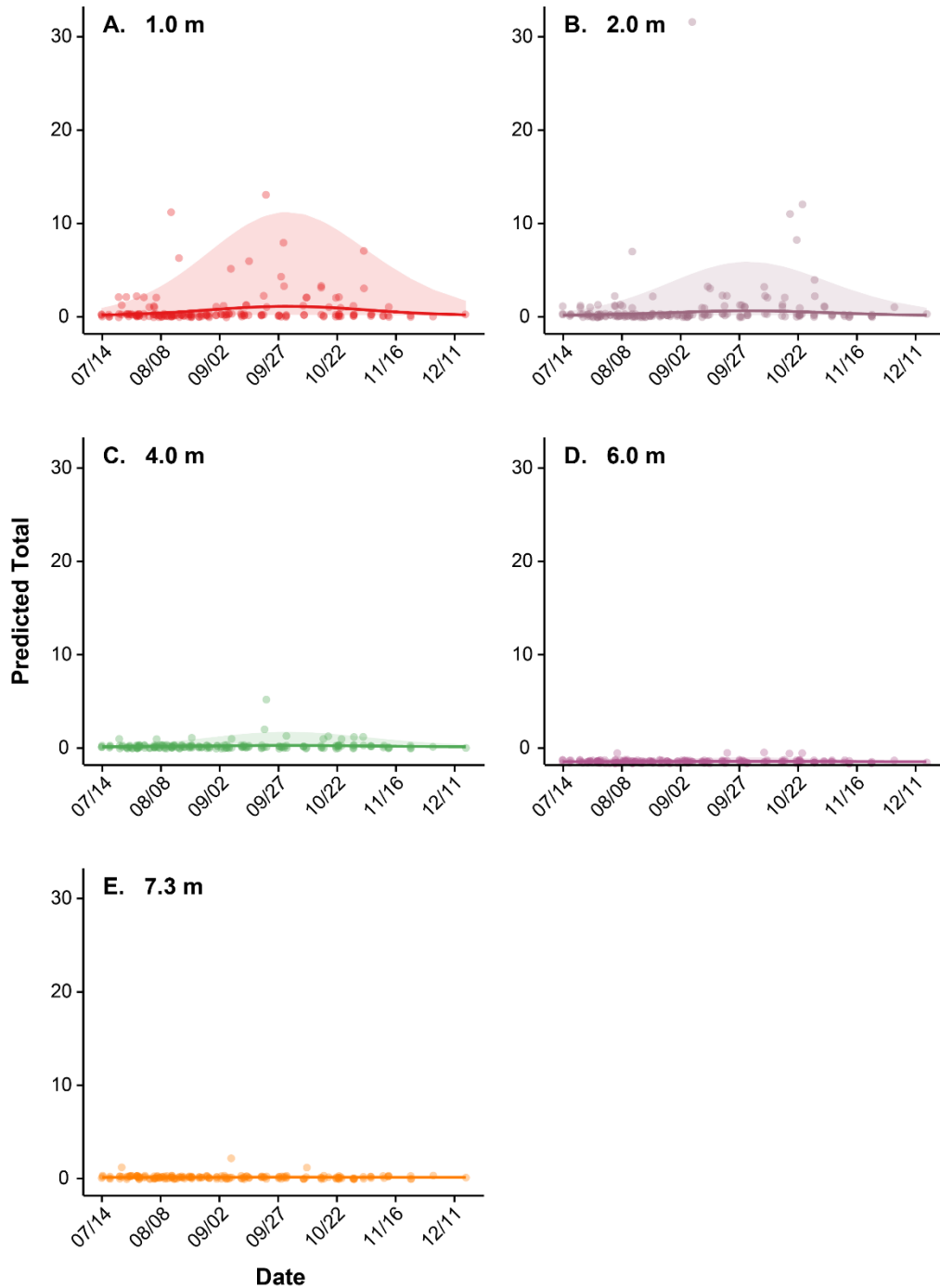
Figure 3.1. Predicted total of *D. suzukii* caught at a date (converted from ordinal days to calendar days) at different vertical height intervals ranging from 1.0 – 7.3 m (A. – E.). The shaded areas represents the 95% confidence interval. The points are the are the collected *D. suzukii*. The final model to determine total number of *D. suzukii* is $\log(y) = -0.22 + -0.65 \text{(height)} + 13.55 \text{(date)} - 16.68(\text{date}^2)$.
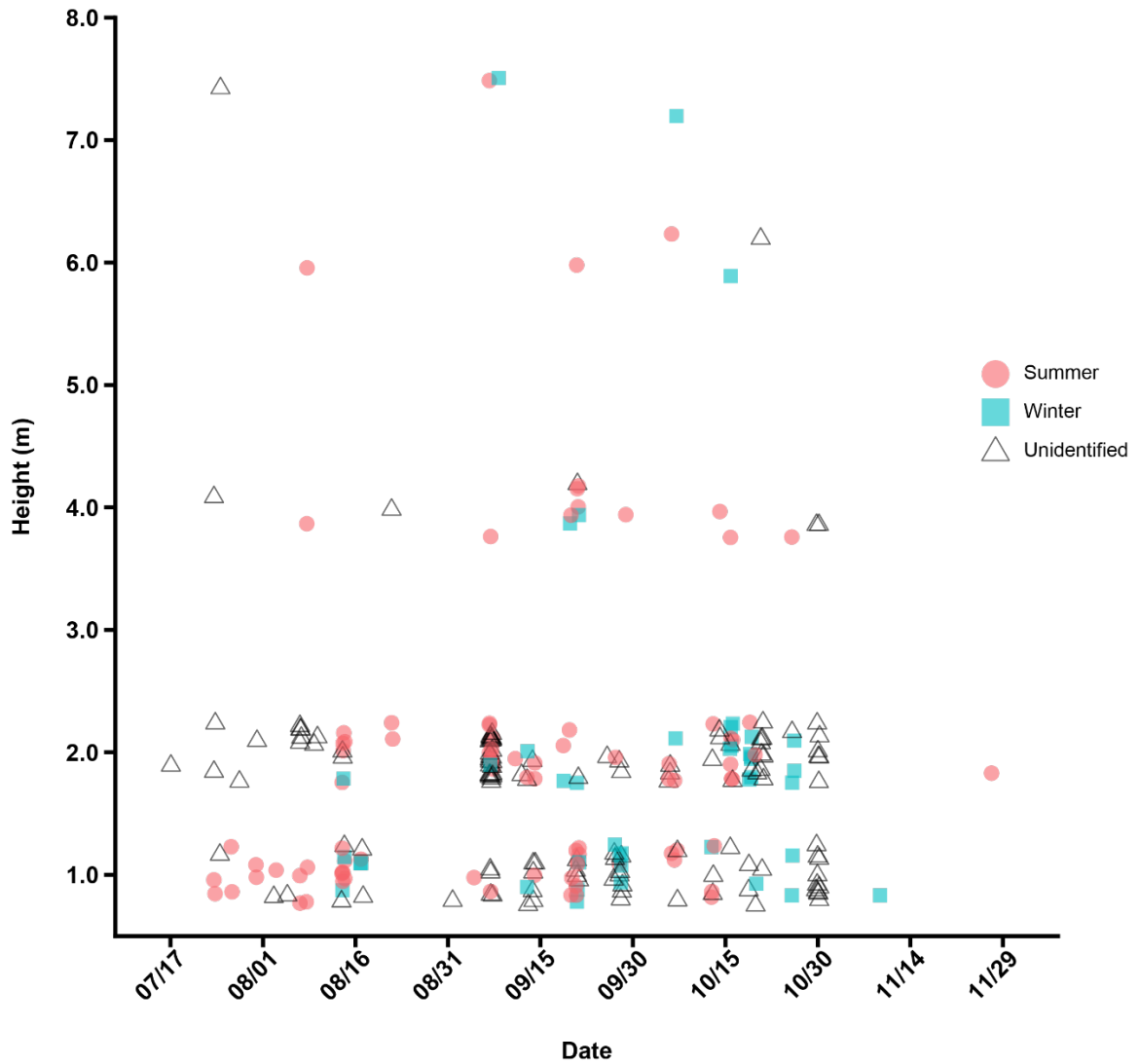
Figure 3.2: Individual *Drosophila suzukii* identified and unidentified morphs that were caught between 2017 to 2019 at a specific date (mm/dd) and height (m). Data points have been jittered to prevent overlapping. Darker points indicate multiple individuals.
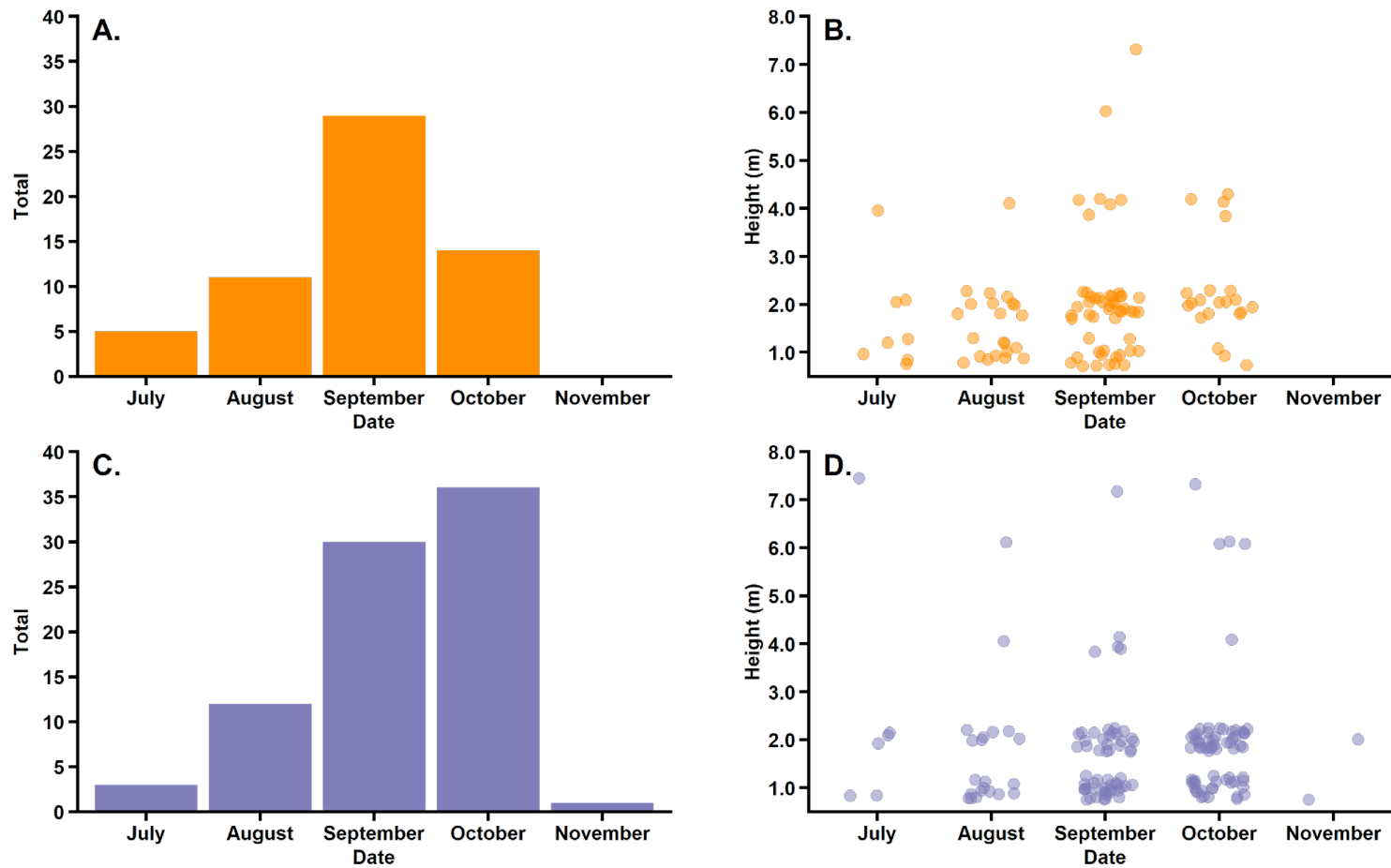
Figure 3.3: Female (A & B) and male (C&D) *Drosophila suzukii* trapped between 2017 to 2019. The total number of *D. suzukii*

caught in a month (A&C) and individual flies caught at a specific height and month (B & D). Data was binned by months for this

figure and data points have been jittered to prevent overlapping. Darker points indicate multiple *D. suzukii* that were trapped.
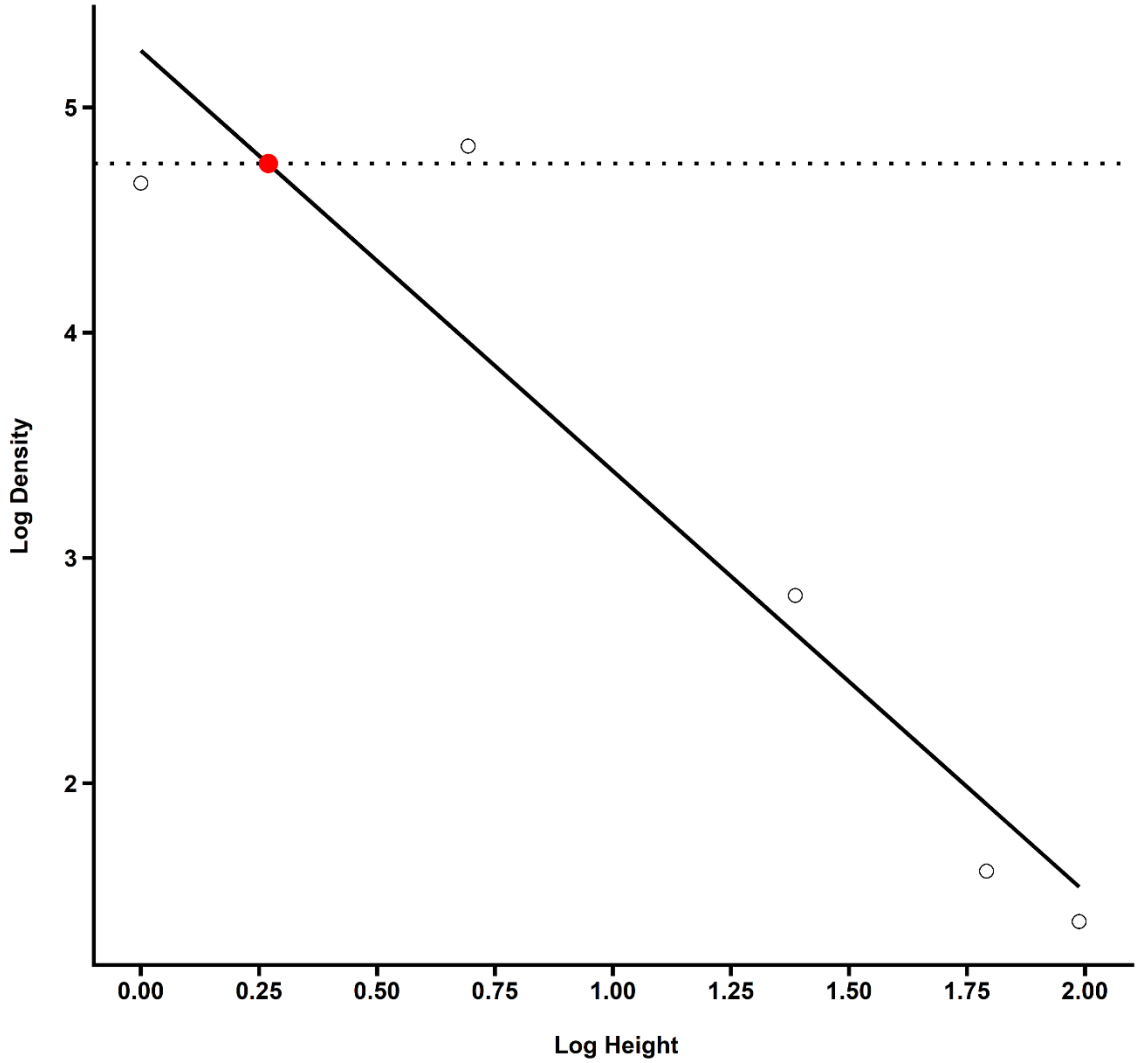
Figure 3.4. The log density against the log height of *D. suzukii* trapped. The solid line represents a linear line fitted through the data points. The dotted line represents the best fit between the horizontal data points. The open circles represent the log density of *D. suzukii*. The point of interception between the two fitted lines (filled circle) is approximately at 0.27 or 1.31 when taking the exponent.
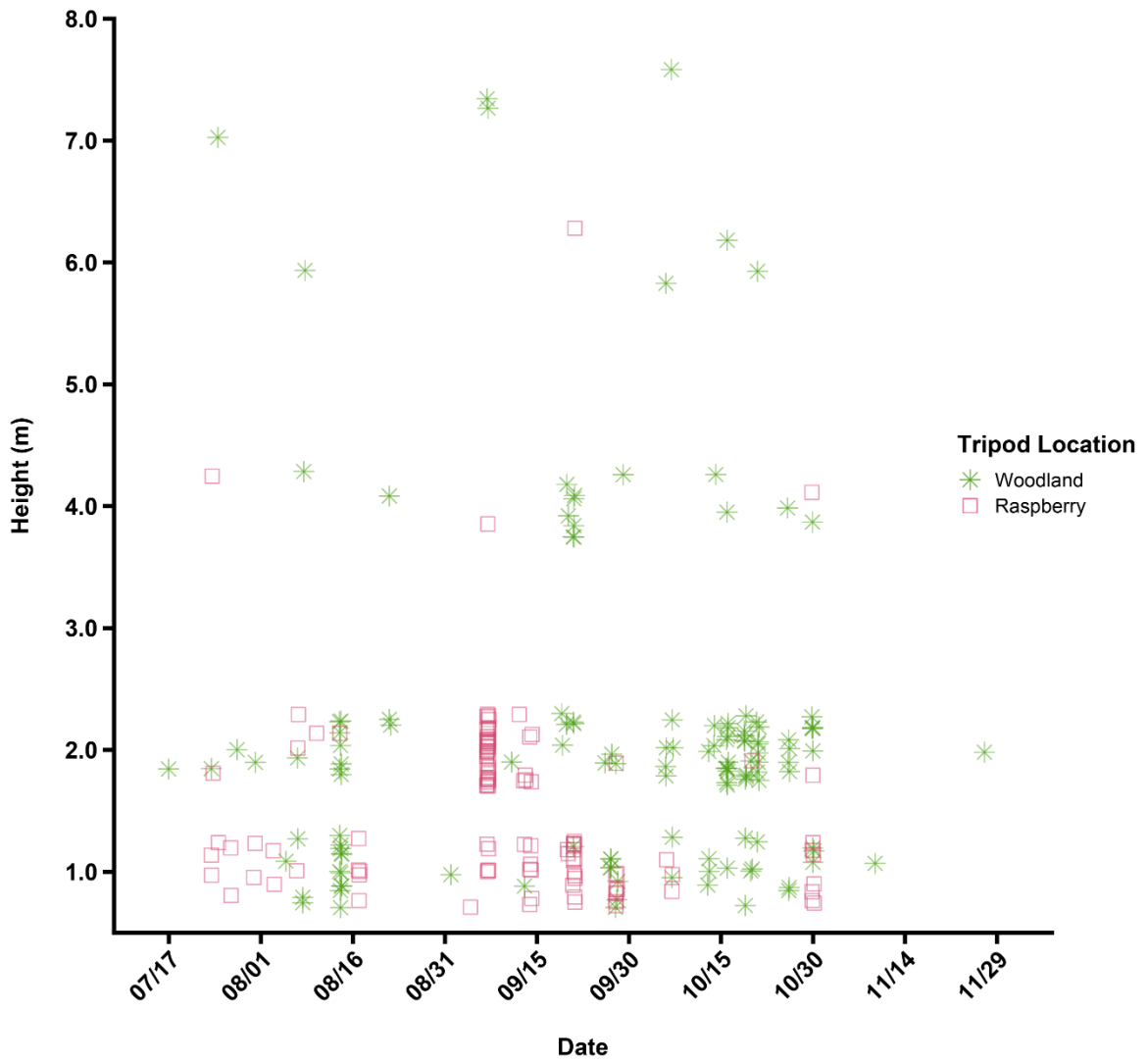
Figure 3.5: Trap catches of *D. suzukii* based on the location of the tripod in the

experimental plot. The second tripod was not included because <10 *D. suzukii* were

caught.

Synopsis

Drosophila suzukii invaded North America >10 years ago and continues to wreak havoc on the soft-berry and stone fruit industry. While scientists have developed new tactics to manage *D. suzukii,* some of the fundamental biological and behavior information remains unexplored. For example, there is no/limited information on how to differentiate the two morphs of polymorphism *D. suzukii* or on and the flies' flight behaviors for exploring the alternative hypothesis that the pest could migrate as an adaption to cold climatic regions.

In my dissertation, I developed a quantitative method for classifying winter and summer morphs. The development of this system could potentially assist future investigation on *D. suzukii* because research continues to explore the pest's phenology to improve management practices. Thus far, no one has documented capturing an overwintering winter morph in the early season, but this was based on using a color-scale, which has been suggested as being unreliable. Based on my results and the use of the quantitative scale, winter morphs are present in the early season in Minnesota. Researchers that previously have not documented a winter morph may have misidentified winter morphs as summer morphs. The morphometric scale was based on development of *D. suzukii* in Minnesota; its use can be expanded with validations from other regions.

Second, to observe the flight behavior of *D. suzukii* in the lab, I used two flight assay methods to document the duration and flight propensity of both sexes and morphs. The use of two flight apparatus for the same species, provided insights on flight behaviors of the invasive pest, *D. suzukii.* The study showed that, in *D. suzukii*, the propensity to fly was influenced by the flight apparatus. Specifically, *D. suzukii* initiated

flight and flew longer in the free flight system. This is important because previous work

on *D. suzukii* used only a tethered flight mill to document their flight capabilities and

behavior. This can lead to a bias in the data. This is of concern as people have used these

results as assumptions to predict *D. suzukii* potential spread and distribution. This study

not only provided information on *D. suzukii* flight behavior, but also serves as a

preliminary step for researchers to consider when conducting flight experiments on their

species.

Finally, I observed *D. suzukii* potential for long-distance dispersal with the aid of

wind. Previous work on *D. suzukii* vertical dispersal used baited traps, but results were

unclear if adults would fly to these heights, above their host crops, unprovoked. Using

clear sticky traps, *D. suzukii* can reach altitudes of 7.3 m on their own without being

baited, which could facilitate long-distance dispersal. This is significant because

scientists have hypothesized that *D. suzukii* method for surviving the cold is by

overwintering and this observation shows potential evidence of long-distance dispersal.

Additional research would be required to determine how far, region wise, *D. suzukii*

could disperse with the aid of wind.

The results from this dissertation are significant beyond understanding the biology

of *D. suzukii* because the information can be used for developing tactics for suppressing

the pest in its overwintering habitats or aligning management strategies to spring

migrations. Additionally, some management strategies have considered the release of

sterile males. Insights gained on fly dispersal from my research can facilitate

identification of appropriate location of releases of sterile males for enhancing efficacy of

this novel strategy. The research is beneficial for management of *D. suzukii* not just in the

Midwest, but globally because of the large distribution range the fly inhabits. In addition,

these results provide insights that can be transferrable to investigations on other invasive

species.

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Appendix I: Examining the local overwintering hypothesis for *Drosophila suzukii* by comparing degree-day models to adult trap-catches

The goal of this study was to assess the consistency of degree-day model developed by Wiman et al. (2016) for overwintering adult *Drosophila suzukii* in the Midwest. Degree-day model forecasts were compared to dates of first catch for several locations in Minnesota and Wisconsin from 2013-2017.

Materials and Methods

The study was conducted between 2013 to 2017 with a total of 14 sites in Minnesota and 16 sites in Wisconsin across the four years. A trapping system with a bait and lure were used to catch *D. suzukii* but trap type varied at each location and by year. Traps were typically constructed with a large deli cup with apple-cider vinegar and a drop of soap to break the surface tension or purchased commercially. The two commercially available *D. suzukii* traps are Pherocon® SWD (Trécé Inc., Adair, OK) and Scentry (Scentry Biologicals, Inc., Billings, MT). Pherocon® SWD traps consisted of a Pherocon® SWD lure and apple cider vinegar with a drop of liquid soap to lower surface tension. The Scentry trap consisted of a Scentry SWD lure and water with a drop of liquid soap, to lower surface tension. At each participating location, traps were placed in the varying fruit crops and forest edges and monitored weekly to remove the contents and refill the liquid traps. The date of when *D. suzukii* was trapped at each location for the year was documented.
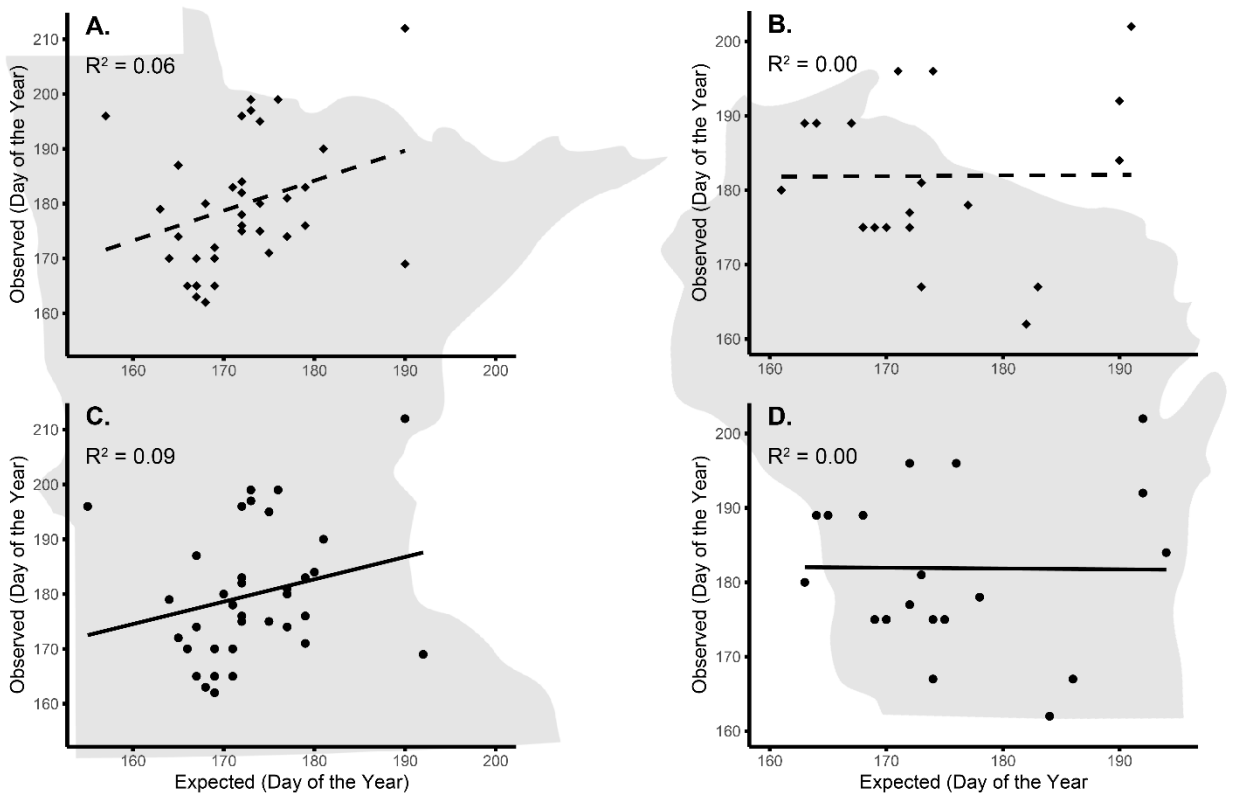
For comparing the dates of first catch to Wiman et al. (2016) degree-day model we used two lower development thresholds 7.2 and 10°C and an upper threshold of 30°C. The 7.2 and 10°C equivalated to approximately 419 and 580 degree-days, respectively,

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and this is when we expected peak *D. suzukii* populations to emerge from overwintering and be caught in the traps. Using the Online Phenology and Degree Day Model website (uspest.org). For each year the parameters set were, first we selected the weather station closet to the site of our traps, the model selected was the Spotted Wing Drosophila, then we set the start date to 1st January of each year. The expected degree-days for peak emergence of *D. suzukii* were compared to the observed dates, which would be provided by the Online Phenology and Degree Day Model website.

Results

When comparing the expected dates of peak emergence to the dates of first catch, there was little evidence of correlation (Figure A1). For Minnesota, the R^2 for 7°C and 10°C were 0.06 and 0.09, respectively (Figure A1 A and C). For Wisconsin, R^2 for 7°C and 10°C were both 0.00 (Figure A1 B and D).

Figure

Figure A1: Comparing the observed dates of first catch with the expected dates of first catch in Minnesota (A and C) and Wisconsin (B and D) using the lower development thresholds of 7°C (A and B) and 10°C (C and D).