Life History and Overwintering Survival of *Drosophila suzukii* (Diptera: Drosophilidae)

by

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ABSTRACT

LIFE HISTORY AND OVERWINTERING SURVIVAL OF *Drosophila suzukii* (DIPTERA: DROSOPHILIDAE)

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Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a pest that has recently invaded North America in the past 5 years, causing damage to soft skinned and stone fruit. I completed a life history analysis, measuring the development, survival, fecundity, hatch rate, and sex ratio of an Ontario ecotype of *D. suzukii*. I constructed a life table and reproductive schedule, and estimated the intrinsic rate of increase (r) for this pest (0.179). The mean (± SE) total lifespan was 86.1 ± 4.25 days, with a maximum value of 153.7 days. The stable age distribution (c_x) was comprised of only 8% adults. An overwintering survival analysis was performed for *D. suzukii*, considering sex, ecotype, photoperiod exposure, mated status, acclimation, rearing temperature, and fecundity after cold exposure. Flies could not survive for 6 weeks below 1 °C. Mated females surviving a 6-week cold exposure were able to produce viable offspring post-exposure.

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Chapter 1: Introduction and Literature Review

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), also referred to as spotted wing drosophila, is an invasive vinegar fly native to Southeast Asia, and a pest of soft-skinned fruit and stone fruits (Bolda et al., 2010; Dreves et al., 2010). In general, invasive species have the ability to displace endemic species, alter environments, and impose costs on ecosystem services, including agriculture, as they may lack natural predators and fill an unoccupied ecological niche (Lee, 2002; Calabria et al., 2012). In the past 5 years *D. suzukii* has invaded the United States of America (U.S.), Canada, and Europe, resulting in direct damage to fruit and revenue loss (Hauser, 2011), as females oviposit in healthy fruit prior to harvest (Walsh et al., 2011; Lee et al., 2011b). Due to compromised fruit quality, fruit growers can expect economic loss as a result of yield loss, increased labour and chemical costs, and loss of export opportunities if *D. suzukii* infested areas are banned from trade (Lee et al., 2011b; Walse et al., 2012). A conservative yield loss of 20% can result in a $511 million revenue loss in the U.S. Pacific Coast States, where the majority of U.S. small fruit production occurs (Goodhue et al., 2011; Walsh et al., 2011). *D. suzukii* has now been found throughout Ontario and may threaten commercial fruit production, which generates over $225 million per year (OMAFRA, 2014). Further, as of July 2011, the Canadian Food Inspection Agency (CFIA) no longer includes *D. suzukii* on the List of Pests Regulated in Canada, and there are no measures in place to prevent continued spread of this pest (CFIA, 2011). As such, it is possible that Ontario fruit growers may suffer yield and revenue loss due to this pest. Since *D. suzukii* is a recent invader to North America, the biological information that we have comes from studies

done in Japan in the 1930s, and may not accurately illustrate the biology of this pest today. Further, it is still unclear whether *D. suzukii* are able to overwinter under Ontario climatic conditions because the Ontario ecotype has yet to be investigated; if so, this would secure their position as a pest of our fruit crop system. Therefore, life history and low temperature exposure experiments are critical in order to predict the persistence of *D. suzukii* and its threat to fruit crops in Ontario.

Physical description

D. suzukii is a member of the *melanogaster* species group (Hauser, 2011). Adult flies are 2-3 mm in length with red eyes and brownish abdomen and thorax (Walsh et al., 2011). The most distinguishing features of males are a single spot on the 1st vein of each wing and a set of black sex combs on each foreleg (Hauser, 2011; Walsh et al., 2011). The females have a large, serrated ovipositor with many sharp teeth, making them easy to identify (Hauser, 2011; Walsh et al., 2011). Kanzawa (1939) (as cited in Walsh et al., 2011) compiled extensive descriptions of the immature stages. He described the eggs as white and glossy, with the developing larva becoming visible as time to emergence decreased (Walsh et al., 2011). The emerging larvae are white and cylindrical, with visible mouthparts and respiratory organs (Walsh et al., 2011). The pupae start grayish yellow, and with time harden and darken (Walsh et al., 2011).

Why *D. suzukii* are a problem

D. suzukii have many traits that make them a concern for fruit growers. They are evidently an invasive species, having recently colonized the U.S., Canada, and Europe from their native range in Japan (Walsh et al., 2011). In general, invasive species can

cause environmental damage that can result in revenue loss (Pimentel et al., 2004). What makes *D. suzukii* a pest of concern is that they cause direct damage to fruit by ovipositing under the skin of ripe fruit prior to harvest (Lee et al., 2011b). Other common species of *Drosophila* do not cause this level of concern for fruit growers because they only attack fruit that has fallen and fermented, and consequently will not be harvested (Walsh et al., 2011). Also, because female *D. suzukii* use their ovipositor to cut a hole in the skin of the fruit, an oviposition wound is created that may act as a pathway to secondary infection by other insects and pathogens (Walsh et al., 2011). They have an extensive host range, short development time, and high fecundity, which ultimately leads to population build up.

Biology

Since *D. suzukii* is a recent invader to North America, regional literature on this pest is limited. Most of what we know of its biology is derived from studies performed by Kanzawa (1939) on flies from Japan. From this we know that *D. suzukii* have a life cycle containing an egg stage, three larval instars, a pupal stage, and an adult stage, and full development can take between 8 and 25 days depending on temperature (Lee et al., 2011b). In addition to Kanzawa's physical descriptions, he found that females reach sexual maturity 1 to 2 days after emerging, producing between 219 and 563 eggs over their lifetime (as cited in Walsh et al., 2011). Adult *D. suzukii* lifespan can vary between 3 and 9 weeks, although overwintering adults that emerge in late autumn can survive until the following spring by seeking refuge under leaves, between stones, or in man-made enclosures (Calabria et al., 2010; Dalton et al., 2011; Lee et al., 2011b; Walsh et al., 2011). Adults emerging in late autumn are thought to be reproductively immature, and therefore in a state of diapause (Mitsui et al., 2010).

Although these measurements give a starting point for general information on this pest, these measurements taken over 70 years ago may not accurately represent what we would observe of *D. suzukii* in North America today. Evolution of certain traits can happen quickly in both laboratory and natural populations of *Drosophila* (Rose and Charlesworth, 1980; Partridge et al., 1995; Huey et al., 2000; Levitan and Etges, 2005). The evolutionary capacity of *Drosophila* combined with the potential of a genetic bottleneck upon invasion of North America, which may alter the genetic diversity of the population (Stenger et al., 2010), lends support to the possibility that *D. suzukii* present in Ontario may not have the same life history characteristics of flies that have been studied previously in other locations.

Host range

D. suzukii are able to complete their development in a broad range of cultivated fruit crops and wild hosts (Lee et al., 2011b; Cini et al., 2012). However they do tend to prefer soft skinned fruits such as berries (Cini et al., 2012). An extensive list of known hosts organized by family is documented by Cini et al. (2012), but some of the most common hosts are: strawberries, raspberries, blackberries, blueberries, cherries, peaches, and plums. *D. suzukii* are also opportunistic and can oviposit on tough skinned fruit that has fallen and fermented, such as apples, apricots, loquat, greenhouse mandarins, persimmons, and tomatoes, and even flowers (when preferred hosts are not present), and have been found to feed on oak tree sap (Lee et al. 2011b; Walsh et al., 2011). Host preference can depend on local abundance (Cini et al., 2012). Since *D. suzukii* are resourceful, they are able to persist when a host has completed its growing season by shifting to available wild hosts. Wild hosts surrounding cultivated crops may act as

refugia for *D. suzukii* from pest management tactics, as well as an area to inhabit until a new crop comes into season (Klick et al., 2012). There is also the possibility that wild hosts surrounding crops may provide overwintering sites for *D. suzukii* (Klick et al. 2012), but this has yet to be studied. Many other *Drosophila* species also have a broad host range, but most are not a threat to cultivated fruit because the females only deposit eggs on rotting fruit, and therefore larva are not found in fresh fruit before harvest (Calabria et al., 2012).

Spread and distribution

D. suzukii were first documented in Japan in 1916, and by the 1930s were discovered in Korea, China, and Russia, causing increased rejection of infested fruit by consumers (Lee et al., 2011b; Walsh et al., 2011). The first account of long distance spread of *D. suzukii* occurred in Oahu, Hawaii in 1980, although they were not reported to have caused damage at this time (Hauser, 2011; Lee et al., 2011b). Their invasion continued into North America when they were identified in Santa Cruz County, California in August 2008 (Boulda et al., 2010; Lee et al., 2011b). Following this, *D. suzukii* were detected in May 2009 along California's Central Coast (Bolda et al., 2010); in Utah, North Carolina, South Carolina, Michigan, Louisiana, Wisconsin, Alberta, Manitoba, Ontario, and Quebec in 2010; and in Virginia, Montana, Pennsylvania, New Jersey, and Mexico in 2011 (Hauser, 2011; Lee et al., 2011b). The presence of *D. suzukii* in these locations caused noticeable economic damage (Hauser, 2011). By the 2012 growing season, *D. suzukii* were found in all monitoring sites that were set up by the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA), illustrating that they are now widespread throughout Ontario (OMAFRA, 2014).

Observations in Ontario

The first *D. suzukii* detection in Ontario was in November 2010 (OMAFRA, 2014). As such, it was necessary to start a local monitoring program to track the spread of *D. suzukii* in Ontario. Starting in 2011, OMAFRA organized a regional monitoring program by setting up apple cider vinegar traps in over 50 sites with susceptible hosts across Ontario, with the most northern site located in Timiskaming. In that growing season, adult *D. suzukii* were not detected in traps until early August, and population numbers did not increase until September (OMAFRA, 2014). These low numbers and late infestation did not result in any commercial damage to fruit (OMAFRA, 2014).

In 2012, OMAFRA increased the number of sites that were being monitored to over 100, as it was evident that *D. suzukii* were likely to be present (OMAFRA, 2014). Trapping started mid-May in 2012, as they were interested in capturing the first flies to appear. The first positive in a trap was detected on June 18th, and by mid-August damage was being reported by fruit growers (OMAFRA, 2014). By the end of the season, *D. suzukii* had been trapped at all monitoring sites, indicating their widespread distribution throughout Ontario (OMAFRA, 2014). It was suspected that the 2011/2012 mild winter and 2012 early spring contributed to this early build-up and spread (OMAFRA, 2014).

The next year (2013) the first *D. suzukii* capture occurred on July 4th (OMAFRA, 2014). Since it was known from the previous season that *D. suzukii* were widespread throughout Ontario, the goal of the 2013 program was not to determine location and spread, as much as to catch the first emerging flies in order to determine when management should be initiated. As such, OMAFRA monitored fewer sites in 2013 (approx. 60) (OMAFRA, 2014). Although the first catch in 2013 was about 10 days later

than the previous year, the build-up followed a similar trend, as by mid-August *D. suzukii* were found throughout Ontario (OMAFRA, 2014).

The next year (2014) the first *D. suzukii* catch came on June 3rd (OMAFRA, 2014). This year OMAFRA reduced the number of sites that were being monitored to 50 (OMAFRA, 2014). Monitoring will continue throughout the season and will likely follow the same trend as in previous years. Monitoring *D. suzukii* continues to be an important aspect of a successful control system to determine when the pest is active, as conditions that fluctuate from year to year can impact when fruit is most susceptible to infestation (OMAFRA, 2014).

Economic loss and damage

The damage caused by *D. suzukii* infestation can be substantial. It is not possible to distinguish the damage caused by *D. suzukii* from that of other vinegar flies on the basis of appearance (OMAFRA, 2014). However, if vinegar fly damage is observed in ripe, harvest-ready fruit, it is a clear indication of *D. suzukii* infestation, as the females have a serrated ovipositor capable of cutting into the skin of ripe fruit, while other common vinegar flies, such as *D. melanogaster*, do not (Walsh et al., 2011; OMAFRA, 2014). The first way that damage to the fruit happens is by way of the oviposition wound caused by the female cutting into the skin of the fruit (Walsh et al., 2011). This open wound acts as a pathway to secondary infections by other insects and pathogens (Walsh et al., 2011). Additionally, the eggs eventually develop into larvae that actively feed within the fruit (Walsh et al., 2011). The combination of these events leads to accelerated deterioration of fruit quality, rendering the fruit unmarketable (Walsh et al., 2011).

The economic impact imposed by *D. suzukii* comes from a number of sources extending beyond yield loss, including increased insecticide and labour costs, and loss of fruit export if infested areas are banned from trade (Goodhue et al., 2011). However, for growers to adopt a pest management strategy, the benefit of the management strategy must surpass the cost of the materials required (Goodhue et al., 2011). Economic analyses have not been completed for Ontario or Canada, but Goodhue et al. (2011) estimated economic damage to strawberry and raspberry fruit growers in California. Goodhue et al. (2011) focused on two types of loss: yield loss in the absence of *D. suzukii* management, and treatment costs on a per treatment basis. They concluded that the revenue lost in the absence of a management program is greater than the cost of the management program itself, and therefore, at least in the case of the crop system studied, the control of *D. suzukii* is economically viable (Goodhue et al., 2011).

In Ontario, it has been estimated that commercial fruit growers produce over 450 thousand tonnes of tree and small fruit per year, generating over $225 million in revenue (OMAFRA, 2014). In the United States, yield losses resulting from *D. suzukii* infestation ranged from negligible to 80% in 2009 (Walsh et al., 2011). If it was assumed that fruit growers in Ontario stood to lose the same proportion of fruit as fruit growers in the United States have, Ontario growers could face substantial revenue losses.

Since *D. suzukii* has just recently invaded Canada's agricultural system, there can be uncertainty in the economic analysis of the impact of this pest. It is known that *D. suzukii* can attack a wide variety of crops, and their ability to use potential vegetable hosts remains unknown (Bolda et al., 2010). Goodhue et al.'s (2011) analysis only considered two commercial crops, and did not take into account any change in consumer price of the fruit that may result from varying quantities of fruit available (Bolda et al.,

2010). If less fruit is available, the value of fruit will increase and this will alter the potential revenue loss of this fruit (Bolda et al., 2010). Therefore, economic analyses based on *D. suzukii* infestation are subject to uncertainty at this time due to the recent nature of the problem (Bolda et al., 2010). As growers in Ontario produce substantial quantities of fruit, precautions should be taken to limit the loss incurred.

Temperature thresholds and dispersal

Climate can impede the dispersal of organisms (Walters et al., 2006). *D. suzukii* are able to withstand a range of environmental conditions that allow them to persist while other pests may not (Cini et al., 2012). *D. suzukii* are thus considered to be both heat and cold tolerant (Cini et al., 2012). The preferred temperature of *D. suzukii* when adults are most active is 20 to 25 °C, and their activity is reduced when temperatures exceed 30 °C (Walsh et al., 2011). It has been found that *D. suzukii* in Japan have a lethal heat temperature of 32 °C and a lethal cold temperature of -0.9 °C (Kimura, 2004).

The CFIA (2011) conducted preliminary modelling suggesting that Ontario is at risk of persistence of *D. suzukii* due to relatively mild winters, similar to that of Hokkaido, Japan, where *D. suzukii* are established. In Hokkaido, January temperatures range from -12 °C to -4 °C, whereas in Ontario January temperatures can range from -11.4 °C to -3.7 °C (CFIA, 2011; Environment Canada, 2014). The thermal tolerance of *D. suzukii*, combined with high dispersal ability, has allowed them to quickly invade Canada, the U.S., and Europe (Hauser, 2011). In their native range in central Japan, *D. suzukii* seasonal dispersal is recognized, whereby adults breed at low altitudes early in the summer, and move to high altitudes later in the season (Mitsui et al., 2010). Autumn breeding is then observed at low altitudes again, but these populations are mostly

composed of reproductively immature adults that are likely in reproductive diapause (Mitsui et al., 2010). This seasonal migration is suggested to be geared towards consuming resources that are exploitable at the time, rather than to avoid unfavourable summer conditions (Mitsui et al., 2010).

Life history applications

The study of the life history of organisms has many practical applications. The population dynamics of insect pests can aid in the control of the pest by establishing effective management programs. It has been estimated that pests in general can account for 30-40% loss of crops (Thomas, 1999), and as stated earlier, *D. suzukii* has the potential to cause even greater loss than this for fruit growers specifically (Walsh et al., 2011). The life table approach to pest management has been used for other Diptera, specifically for the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), a fruit pest located in tropical locations (Carey, 1982). The population dynamics of the medfly were used to implement control of the pest by understanding how different spray programs would affect the internal population structure (Carey, 1982).

Life history analyses have also been used to compare pest species geographically, as is the case with the mosquito, *Aedes albopictus* (Skuse) (Leisnham et al., 2008). Leisnham et al. (2008) were able to observe geographic variation in the survival and reproduction of this species, which may lead to altering the management strategy, based on location. This approach illustrates that controlling pest growth depends on the life history of the pest, which can vary geographically (Thomas, 1999).

In the case of the common bed bug, *Cimex lectularius* (Linnaeus), life table analysis has been used to compare insecticide resistant strains to field strains to observe

differences in survivorship, development time, fecundity, and stable age distribution (Polanco et al., 2011). By understanding the differences in life history traits between various strains of bed bugs, control measures can be developed to target the certain life stage(s) that will most greatly impact the pest population (Polanco et al., 2011). Life tables can portray the age structure of an insect population, which can illustrate the contribution to growth that each age class represents, ultimately leading to future population size (Polanco et al., 2011). Knowledge of the stable age distribution can also help with sampling techniques, as correct conclusions can be made with respect to the population structure based on abundance of a particular life stage (Polanco et al., 2011).

Given the biology of female *D. suzukii*, short development time, and high fecundity, combined with their rapid colonization of North America and Europe causing crop loss for farmers, it is evident that the study of this pest is warranted. They have a wide host range, which leads them to be able to persist even when a certain host is not in season. They mature quickly and are able to lay eggs in fruit that is still harvestable in the field, while maintaining a wide thermal tolerance. These characteristics result in *D. suzukii* being a pest of concern for fruit growers, warranting further investigation of their life history.

Overwintering

Most studies of *Drosophila* focus on the summer life cycle, as this is the time of greatest activity of the insect, however overwintering survival directly dictates summer population dynamics (Leather et al., 1993). There have been many studies conducted on factors influencing *Drosophila* overwintering success, such as cold-hardiness, however

there is still a severe deficit in the overwintering literature. Therefore, data on naturally overwintering *Drosophila* populations are essentially unavailable (Izquierdo, 1991).

It has been found that the egg, larval, and pupal stages of *D. suzukii* will not survive temperatures below freezing (CFIA, 2011; Lee et al., 2011b). The average heat lethal temperature is 32 °C, and the average cold lethal temperature is -0.9 °C (Kimura, 2004). In an overwintering analysis performed on *D. suzukii* from Oregon, Dalton et al. (2011) subjected adults and pupae to an 84-day chilling period, with temperatures ranging from 1 °C to 10 °C, with a subset of individuals being exposed to a 7-day freeze period (-2 °C). They found that *D. suzukii* survived longest at 10 °C; mortality increased below this temperature. *D. suzukii* were unable to survive more than 17 days at 1 °C.

These results create a conflict between what is observed in nature and in the lab. *D. suzukii* are established in Hokkaido, Japan, where winters average temperatures of -12 to -4 °C (CFIA, 2011; Walsh et al., 2011). This discrepancy suggests that adults overwinter in sheltered areas, such as pebbles or leaves, but in colder areas they may seek access to man-made enclosures (Dalton et al., 2011; Lee et al., 2011b). There is also the evolutionary capacity of *Drosophila* to consider (Kimura, 2004). Generations of *D. suzukii* may be able to rapidly evolve tolerance to the cold temperatures of Ontario, and this is why study on local *D. suzukii* is essential.

Drosophila cold tolerance

Aside from the lack of overwintering experiments conducted on *Drosophila*, there is a solid base of literature on the study of cold tolerance. A wide range of methods have been used to measure cold-hardiness in *Drosophila*, making it necessary to distinguish terms. In general insects are defined as either freeze-tolerant or freeze-avoidant (freeze

avoidance by supercooling) (Leather et al., 1993). The difference between these two main strategies is the manufacture of ice-nucleating agents in freeze-tolerant species, and the excretion of all nucleating agents in freeze-avoiding species (Bale and Hayward, 2010).

The supercooling point is the temperature at which spontaneous nucleation occurs in a liquid which has been cooled below its melting point without freezing (Leather et al., 1993). Freeze-avoidant insects supercool to avoid freezing; however, a low supercooling point does not necessarily translate to cold-hardiness as insects can incur pre-freezing mortality (Czajka and Lee, 1990; Bale and Hayward, 2010). Czajka and Lee (1990) measured the supercooling points of *Drosophila melanogaster* larvae, pupae, and adults and found that they ranged from -17 to -20 °C. Since *Drosophila* incur mortality before this temperature has been reached, it is likely that they experience some form of pre-freeze mortality, and therefore the supercooling point cannot be directly regarded as the cold tolerance potential of this organism.

Rapid cold-hardening occurs in insects prior to overwintering at temperatures above the supercooling point, and can increase tolerance to chilling and cold shock (Czajka and Lee, 1990). Chilling and cold shock are two processes that cause cold injury, and ultimately mortality at low temperatures. Chilling involves long-term exposure to non-freezing temperatures above the supercooling point (indirect), while cold shock involves rapid cooling (direct) (Chen and Walker, 1994).

The chill-coma temperature can be defined as the temperature at which an insect enters a state of reversible immobilization, and can infer information on cold tolerance, defined as cold knockdown (Gibert and Huey, 2001). Recovery from immobilization induced by exposure to low temperatures is referred to as chill-coma recovery (Anderson et al., 2005). The amount of time for this recovery to take place has been found to be an

accurate determinant of insect cold tolerance (Gibert et al., 2001; David et al., 2003).

In general, *Drosophila* experience cold shock prior to reaching their supercooling point. For example, *D. melanogaster* incur cold shock at -7 °C within 1.5 h (Chen and Walker, 1994). Chill-coma will usually ensue at 0 °C, and recovery is possible but is a highly variable trait (Gibert et al., 2001). In general, it is hard to make any sweeping statements in regards to the cold tolerance of *Drosophila* as it is highly variable, other than that mortality is common above the supercooling point. This may suggest that *Drosophila* possess some alternative overwintering strategy that cannot be inferred through study of their cold tolerance alone.

The stimuli controlling diapause and overwintering

As winter approaches, environmental conditions start to change and insects demonstrate a range of responses to this. Winter survival increases with the ability to predict and prepare for worsening environmental conditions (Leather et al., 1993). *Drosophila* seem to respond to a seasonally changing environment by either migrating or entering a state of reproductive diapause, whereby a strong relationship has been recognized between the incidence of diapause and cold tolerance (Bale and Hayward, 2010). To date, reproductive diapause has not been quantified in *D. suzukii*.

Diapause is an adaptive response to adverse environmental conditions that is induced by various stimuli, resulting in delayed development (Bale and Hayward, 2010). These stimuli must be experienced during the sensitive stage of development, otherwise regular development will ensue (Bale and Hayward, 2010). Seasonal cues are therefore used to infer information to increase overwintering success.

Photoperiod

Arguably the most important cue used by insects to predict seasonal changes is photoperiod. Changes in day length are predictable and unchanging. Organisms possess a circadian clock to regulate daily activities, as well as a photoperiodic clock to regulate seasonal activities (Kauranen et al., 2013). This photoperiodic timer works to address the number of daily light:dark cycles that are experienced, which may trigger seasonal events such as diapause (Kauranen et al., 2013). *Drosophila* will respond to photoperiod through the induction of diapause, but this is not an all-or-none response and can interact with other cues (Kimura, 1990).

Kimura (1990) found that a short photoperiod is needed to induce reproductive diapause in the *D. auraria* complex from Japan, while a long photoperiod does not induce diapause, resulting in ovarian development. This response is quantitative because flies that were transferred from a diapause-preventing photoperiod to a diapause-inducing photoperiod early in development were able to enter diapause (Kimura, 1990). This switch to reproductive diapause occurred due to degeneration of the ovaries when females were at an early developmental stage (Kimura, 1990). This same pattern was found in newly eclosed female *D. melanogaster*, as the incidence of diapause increased as photoperiod decreased, while being very sensitive to increases in temperature (Saunders et al., 1989; Saunders and Gibert, 1990).

Temperature

It has been reported that the photoperiodic diapause can interact quantitatively with other cues to induce diapause in *Drosophila*, the most common being temperature. Temperature can affect the critical photoperiod and the sensitive period (the proper

developmental and physiological stage when the shift to diapause occurs) needed to induce diapause.

Temperature can interact with the photoperiodic response by altering the critical photoperiod, as reported in *D. testacea* and in the *D. auraria* complex (Kimura, 1982, 1984). As temperature increased, the critical photoperiod needed to induce diapause decreased, until the temperature was so high that diapause could not be induced (Kimura, 1982; Kimura, 1984).

Temperature can also alter the sensitive period of *Drosophila*, which is the developmental stage where the switch to diapause can occur (Salminen and Hoikkala, 2013). Kimura (1982) and Salminen and Hoikkala (2013) found that temperature affects the sensitive period in *D. testacea* and *D. montanna*, respectively, whereby as temperature increases, the length of the sensitive period decreases. Temperature can also affect the number of short-day cycles required to enter diapause, called the required day number. For example, Salminen and Hoikkala (2013) found that *D. montanna* must experience three short-day cycles during their sensitive period to enter diapause. This explains why fewer females enter reproductive diapause at higher temperatures, as the duration of the sensitive period is decreased as development rate of the ovaries is faster at a higher temperature (Salminen and Hoikkala, 2013). Therefore females would be less likely to experience 3 short-day cycles during their sensitive period, and as a result would not enter diapause.

Life stage

The sensitive period of *Drosophila* has been observed to be quite variable and can depend on developmental stage. Kimura (1988b) found that both male and female *D. triauraria*

entered reproductive diapause when transferred into diapause-inducing conditions on the day of eclosion, and when the time before transfer increased, the proportion of individuals entering diapause decreased. Similarly, Salminen et al. (2012) found that the sensitive period for *D. montanna* occurs after adult eclosion. The sensitive period was also found to occur after adult eclosion in *D. melanogaster* from the United States (Saunders et al., 1989, Saunders and Gibert, 1990).

Conversely, Kimura (1990) found the developmental stage that was sensitive to photoperiodic induction of diapause in four species from the *D. auraria* complex ranged from the preimaginal to the imaginal stage, and these species were able to respond quantitatively to the photoperiodic cue. These results together indicate that *Drosophila* are able to alter their photoperiodic responses to changing environments, which is an important trait for species living in seasonally variable environments.

In conclusion, there is limited information on the overwintering of *Drosophila* in general, but we do have some information on cold tolerance, and the cues that result in successful overwintering. This information can be used to construct an ecologically relevant experiment to assess the overwintering survival of *D. suzukii* in conditions that are pertinent to Ontario winter conditions.

Overwintering applications

Climate warming

Climate warming is occurring more rapidly than ever before, and increases in the mean winter temperature can lead to niche expansion, as insects will be able to increase their northern distribution (Bale and Hayward, 2010). For example, *Zaprionus indianus*

(Diptera: Drosophilidae) is an afro-tropical species (Alves de Mata, 2010) and *D. suzukii* is a species native to Japan (Walsh et al., 2011), and both have recently invaded the Americas and are potential pest species. This niche expansion may lead to new insect-insect and insect-host interactions. These insect-host interactions may lead to pest management issues, and therefore the overwintering strategies of *Drosophila* are important. Since the presence of *D. suzukii* and similar drosophilids in North America is a new and impending problem, control of *Drosophila* in particular has not been investigated. However knowledge of the overwintering strategy of an insect may be useful in managing pest outbreaks (Leather et al., 1993).

Ice-nucleating active bacteria

Ice-nucleating active bacteria are found on the leaves of plants and are able to cause frost damage to plant tissues by increasing the temperature at which freezing occurs (Lee et al., 1992). These bacteria can lead to crop loss by causing frost damage to frost-sensitive agricultural crops through the induction of ice formation (Lindow, 1983). These ice-nucleating active bacteria have been used in pest management to increase the supercooling point of insect pests (and therefore lowering their cold tolerance), providing a means of biological control by increasing mortality of the target pest (Lee et al., 1992). This pest management method can even be used on pests of stored products (Lee et al., 1992), in combination with cold treatment of the crop product to increase efficiency (Mignon et al., 1995). The site of application on the insect (Steigerwald et al., 1995), as well as the overwintering strategy of the insect (Castrillo et al., 2001) can affect the efficacy of this form of control.

One method for use of ice-nucleating active bacteria is to integrate the ice-

nucleating active bacteria gene into the chromosome of another bacteria, for example *Enterobacter cloacae* (Tang et al., 2004). After field application, this transgenic bacteria does not remain on the plant surface for long enough to affect the crop negatively, but once ingested by a feeding pest, will increase the supercooling point leading to mortality at a higher temperature (Tang et al., 2004).

This method has not been tested for control of *Drosophila*. However, *D. suzukii* may be a candidate for this kind of practice. *D. suzukii* is a pest of soft-skinned and stone fruits and are most active in the late growing season (Walsh et al., 2011). Ice-nucleating active bacteria may be used in combination with a spray program for collected fruit in cold storage. Since *D. suzukii* would be active in the field in preferred late summer temperatures, ice-nucleating active bacteria control would be limited to use on stored fruit. However, this method would still act as a control measure for any flies that were present in the fruit at the time of storage, and therefore this avenue of control could be investigated.

Summary

In conclusion, *D. suzukii* is a serious pest of fruit crops and has already caused substantial economic damage for fruit growers on the U.S. Pacific Coast. Since *D. suzukii* have been positively identified in Ontario for four consecutive years, it is important to assess the life history traits of local *D. suzukii*, and evaluate whether they will be able to overwinter locally, in order to understand their impact on our fruit crop system.

General objectives

The objectives of my research are to:

1. Assess the life history of the Ontario *D. suzukii* ecotype.

2. Assess the overwintering survival of *D. suzukii* in conditions characteristic of an Ontario winter.

In Chapter 2, I measured development, survival, fecundity, hatch rate, and sex ratio of an Ontario ecotype of *D. suzukii* to compile a detailed life table and reproduction schedule. In Chapter 3, I performed an overwintering experiment on *D. suzukii* that examined sex, ecotype, photoperiod exposure, mated status, acclimation, rearing temperature, and fecundity after cold exposure. This work will broaden our knowledge of *D. suzukii* biology.

Chapter 2: Development, reproductive output and population growth of the fruit fly pest *Drosophila suzukii* (Diptera: Drosophilidae) on artificial diet[1]

Abstract

Drosophila suzukii (Matsumura) is a fruit pest of Asian origin that invaded North America in 2008. Despite the widespread economic impact of this species, much of the biology and general life history of this pest remains largely unknown. Under preferred laboratory conditions (22 °C, approx. 25% RH) we measured development, survival, fecundity, hatch rate, and sex ratio of a North American ecotype of *D. suzukii*. Life history traits were used to construct a life table and reproductive schedule, and to calculate the intrinsic rate of population increase (*r*). The mean (± SE) total lifespan (egg to adult mortality) was 86.1 ± 4.25 days, with a maximum value of 153.7 days. On average, females produced 5.7 ± 0.24 eggs per day, with a mean total lifetime production of 635.6 eggs. The gross reproductive rate (*GRR*) was 317.8 daughter eggs per female and the net reproductive rate (R_o) was 240.4 daughter eggs per female. The intrinsic rate of natural increase (*r*) was 0.179. The stable age distribution (c_x) was comprised of 51% larvae, 25% eggs, 16% pupae, and 8% adults. The sex ratio over time was approximately 1:1. We conclude with a comparison of our data to previous work on *D. suzukii* and other *Drosophila*, and discuss the implications for control and monitoring of this pest.

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[1] This work has been published previously and this chapter is a reprinting of the publication with slight modifications:
Emiljanowicz, L.M., Ryan, G.D., Langille, A., and J. Newman. 2014. Development, Reproductive Output and Population Growth of the Fruit Fly Pest *Drosophila suzukii* (Diptera: Drosophilidae) on Artificial Diet. Journal of Economic Entomology. 107: 1392-1398.

Keywords: development, *Drosophila suzukii,* intrinsic rate of increase, life history, spotted wing drosophila

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), commonly called spotted wing drosophila, is an invasive vinegar fly native to Southeast Asia, and an opportunistic pest of a wide range of soft-skinned fruit species (Bolda et al. 2010; Lee et al. 2011b). *D. suzukii* is able to lay eggs in ripening fruit, using their serrated ovipositor, before harvest has occurred resulting in yield loss (Lee et al. 2011b; Walsh et al., 2011). They have recently invaded the U.S., Canada, and Europe. The first North American mainland detection of *D. suzukii* was in California in August 2008 and the first European detection was in Spain (later Italy and France) in October 2008 (Walsh et al., 2011; Hauser, 2011). The following year the infestation spread resulting in fruit crop damage and revenue loss (Hauser, 2011). *D. suzukii* continued to spread along the west coast of North America and was identified in British Columbia in 2009, and subsequently in most fruit-producing locations of Canada as of 2010 (CFIA, 2011).

General life history measurements on *D. suzukii* were originally conducted by Kanzawa in Japan (1939). Kanzawa found that females reach sexual maturity 1 to 2 days after emerging, producing between 219 and 563 eggs over their lifetime, with full development (egg to adult) taking approx.14.6 days to complete (as cited in Lee et al., 2011b and Walsh et al., 2011). Although such life history measures are critical for modeling the full impact of this fly, these early measurements may not accurately capture the life history of the North American ecotype over 70 years later. For example, in

Drosophila melanogaster it has been found that artificial laboratory selection for early fecundity can result in a significant change over a short period of time (Rose and Charlesworth 1980) and that fecundity and fertility show evidence of adaptation to certain temperature regimes (Partridge et al. 1995). There is also evidence suggesting evolutionary change in natural populations of *Drosophila*, including changes in chromosomal polymorphisms in response to environmental change (Levitan and Etges 2005) and wing morphological evolution in response to latitude (Huey et al. 2000). Given this evolutionary capacity of *Drosophila*, differences may be present between the previously studied Asian population, and the recently introduced North American populations of *D. suzukii*. It is also likely that upon invasion, the North American population experienced a genetic bottleneck, where genetic variation is greatly reduced in a founding population. This has been reported previously for an introduced population of an invasive insect pest, *Homalodisca vitripennis* (Germar), the glassy-winged sharpshooter (Stenger et al. 2010). Therefore life history exploration is important to understand the potential of this pest in North America.

Life history and reproductive measures can highlight the fitness potential of organisms and their study can be useful when dealing with pests, such as *D. suzukii* and *Cimex lectularius* (Partridge et al. 1995; Polanco et al. 2011). As *D. suzukii* is a fairly new pest to North America, minimal research has been conducted to identify the most effective pest management strategies. Preliminary work by Beers et al. (2011) suggest that some insecticides may provide suitable protection against *D. suzukii*, however, proper timing of applications still needs to be resolved. By understanding the phenology, reproduction and growth potential of *D. suzukii*, management of this pest may be improved.

In the present study we measured various *D. suzukii* life history and reproductive parameters under preferred laboratory conditions. These data were then used to construct an abridged life table, reproductive schedule, and to calculate the intrinsic rate of natural increase (*r*) and stable age distribution.

Methods

D. suzukii rearing and colony maintenance

All insects used were obtained from a laboratory colony that originated from infested fruit collected from a commercial blackberry and raspberry farm in southern Ontario during the summer of 2012 (approx. 8 months prior to life history calculations). Positively identified *D. suzukii* were then kept in cages (Plexiglas® 26 x 26 x 26 cm) with overlapping generations on standard *Drosophila* diet (as is described in Dalton et al. 2011) and cotton saturated with double-distilled water. The *D. suzukii* colonies were kept in controlled growth chambers at 22 °C (ranging 21.45 to 22.53 °C), approx. 25% RH, and on a photoperiod of 15:9 h, L:D. To maintain the colonies, petri dishes (Fisherbrand® 100 x 25 mm, Fisher Scientific, Ottawa, Ontario, Canada) filled with artificial diet were left in the cage so that females could oviposit eggs into the diet. Dishes were removed after approx. 4 days, covered, and left to incubate in the same conditions until adults had emerged. These adults were then reintroduced back into the same cage.

Fecundity, hatch rate, and sex ratio measurements

In order to conduct fecundity measures on individual females, mating chambers were constructed from modified centrifuge tubes (Fisherbrand® 50 mL, Fisher Scientific, Ottawa, Ontario, Canada). A hole (approx. 1 cm across) was cut in the side of the tube

and a small section of clear PVC tubing was glued inside the hole where cotton could be inserted and saturated with double-distilled water. This cotton was rehydrated with double-distilled water every 3 days during the study period. A second hole (approx. 3 cm acorss) was cut into the opposite side of the centrifuge tube and covered with mesh. Although these mating chambers were kept within the controlled growth chamber, it is possible that the RH was slightly higher than 25% within the mating chamber.

Adults used in the study to calculate reproductive measures were reared from the laboratory colony by removing a pupa from a day old diet dish and placing it into a microcentrifuge tube (Fisherbrand® 1.5 mL, Fisher Scientific, Ottawa, Ontario, Canada) half filled with standard diet medium. Once these adults had emerged, they were sexed and placed into a mating chamber (2 males and 1 female into each chamber to ensure mating success). A total of 50 mating chambers were set up in this way. A subset of 25 chambers contained the same males throughout the study (referred to as *non-replacement male* group), while the males in the other 25 chambers were replaced weekly with newly emerged, virgin males (referred to as *replacement male* group). This was done in order to test whether our measurements of the female reproductive period were affected by the length of the male reproductive period. Thus, we hypothesized that if the male reproductive period was shorter than the female period, we would observe lower hatch rate of eggs from the *non-replacement male* group.

Egg counts were conducted daily on all 50 chambers. The lids of the centrifuge tubes were filled with 1 mL of diet upon which females would deposit eggs. Lids were replaced daily and eggs were counted. Following egg laying these lids were left for approx. 4 days to allow for larval counts. This provided a hatch rate measure for the eggs. These counts were made until the female in the chamber died. Males that died in the *non-*

replacement male group were not replaced. Males that had died in the *replacement male* group were replaced on the regular replacement schedule.

To determine sex ratio, the diet from the hatch rate lids of the *non-replacement male* group were transferred to a petri dish (Fisherbrand® 35 x 10 mm, Fisher Scientific, Ottawa, Ontario, Canada) filled with diet, and left to develop into adults. Once adults had emerged, the sex ratio was assessed. This was done starting one week into the study, and was repeated 3 times a week on alternate weeks throughout the study period. We stopped measuring sex ratio after 87 days (13 time points) when our sample size was reduced due to female mortality.

D. suzukii survivorship and development measurements

In order to determine development time and survivorship for each life stage, we checked the *non-replacement male* mating chambers every 2 h for egg production over a 3-day period, approx. 10 days post-eclosion. We collected up to 3 eggs from each chamber to obtain a cohort of 53 eggs to track our measures of interest. Individual eggs were obtained by scooping a small amount of diet around the egg with a pair of forceps and transferring the mass to a petri dish (Fisherbrand® 35 x 10 mm, Fisher Scientific, Ottawa, Ontario, Canada) filled with diet. Eggs were checked every 2 h until they had hatched into larvae. If an egg did not hatch after 3 days, it was considered a nonviable egg. Larvae were checked every 4 h, through the 3 instars, until they had pupated. Pupae were checked every 8 h until the adults had emerged. The midpoint of these intervals was used when calculating the development time in each stage. As we could not sex immature individuals (due to lack of morphological identifiers; Cini et al. 2012), mortality at these stages (egg, larvae, and pupae) was assumed to be equal for both males and females.

Individual adults were then sexed and transferred singly to a mating chamber and checked daily in order to measure survivorship. The diet lid was replaced and the cotton was rehydrated with double-distilled water every 3 days.

Life tables and data analysis

Survival data were used to construct an abridged life table, containing stage-specific measures as opposed to daily measures, by assessing mortality over an age interval (n). The adult stage was grouped into intervals of 10 days. An abridged life table was chosen, as it is difficult to determine daily mortality of immature stages. The life table included all of the stage-specific parameters shown in Table 2-1. Additionally, life table entropy (H), a measure of heterogeneity in the probability of dying at each age (Carey 1993), was calculated.

The definitions of reproductive variables and notation used are summarized in Table 2-2. Fecundity data were used to construct an abridged reproductive schedule for the *non-replacement* and *replacement* male groups. The reproductive schedule includes measures of average number of days lived in each interval ($_nL_x$) and hatch rate (h_x). The formulae used for these parameters can also be found in Table 2-1. The average number of eggs produced by a female in the age interval (M_x) was calculated for the female cohort. These values were then used to calculate the parameters shown in Table 2-2. The

Table 2-1. Life table and reproductive schedule parameters used as defined by Carey (1982) and Carey (1993).

Parameter	Definition	Formula
l_x	Stage-specific survivorship: fraction of the original cohort alive at the beginning of the designated age interval	l_x = number of individuals alive at age x/number of individuals in original cohort
p_x	Proportion of those alive at age x that survive through the interval	$p_x = l_{x+n}/l_x$
q_x	Proportion of those alive at age x that die in the interval	$q_x = 1 - p_x$
d_x	Fraction of the original cohort that die in the age interval	$d_x = l_x - l_{x+n}$
${}_nL_x$	Number of days lived by the average individual in the age interval	${}_nL_x = {}_n[l_x - (0.5)d_x]$
T_x	Total number of days lived beyond age x	$T_x = \sum_{i=n}^{\omega} {}_nL_x$
e_x	Expected number of additional days the average individual age x will live	$e_x = T_x/l_x$
c_x	Stable age distribution: schedule of fractions each stage represents in the ultimate population	$c_x = e^{-rx}L_x/\sum_{x=0}^{\omega} e^{-rx}L_x$
M_x	Gross maternity: average number of offspring (eggs) produced by a female in the age interval	M_x = total number of offspring produced by female cohort between x and x + n/total number of females in cohort at midpoint of interval x to x + n
h_x	Hatch rate: fraction of all eggs produced by the cohort that are viable (i.e. hatch)	h_x = total number of eggs that are produced by female cohort that hatch between x and x + n/total number of offspring produced by female cohort between x and x + n

Table 2-2. Variables and notation used throughout as defined by Carey (1982) and Carey (1993), and the measures for *D. suzukii*.

Notation	Definition	Estimate
Gross fecundity rate	Lifetime production of offspring (eggs) by an average female that lives to the last day of life in the cohort	635.6 eggs
Gross fertility rate	Lifetime production of viable eggs by an average female that lives to the last day of life in the cohort	491.1 fertile eggs
Gross hatch rate	Ratio of gross fertility to gross fecundity (weighs hatch by the number of eggs produced at each age)	0.77
Net fecundity rate	Average lifetime production of eggs for a newborn female	480.7 eggs
Net fertility rate	Average lifetime production of viable eggs for a newborn female	386.8 fertile eggs
Gross reproductive rate (*GRR*)	Sum of all female offspring per female across all ages	317.8 daughter eggs per female
Net reproductive rate (R_0)	Average number of female offspring that would be born to a birth cohort of females during their lifetime	240.4 daughter eggs per female
Intrinsic rate of natural increase (*r*)	Rate of natural increase in a closed population that has been subject to constant age-specific schedules of fertility and mortality	0.179
Mean generation time (*T*)	Time required for a population to increase by a factor equal to the net reproductive rate	30.6 days
Doubling time (*DT*)	Time required for the population to double	3.872 days
Life table entropy (*H*)	Distribution of mortality by age	0.683

intrinsic rate of increase was first estimated using two analytical approximations (Carey 1993). The values obtained from these approximations were averaged to yield a value of 0.125 and this was then used as the r_0 value in the iterative method, where three iterations were carried out to arrive at a final estimate of r. Sex ratio was also calculated (males per female).

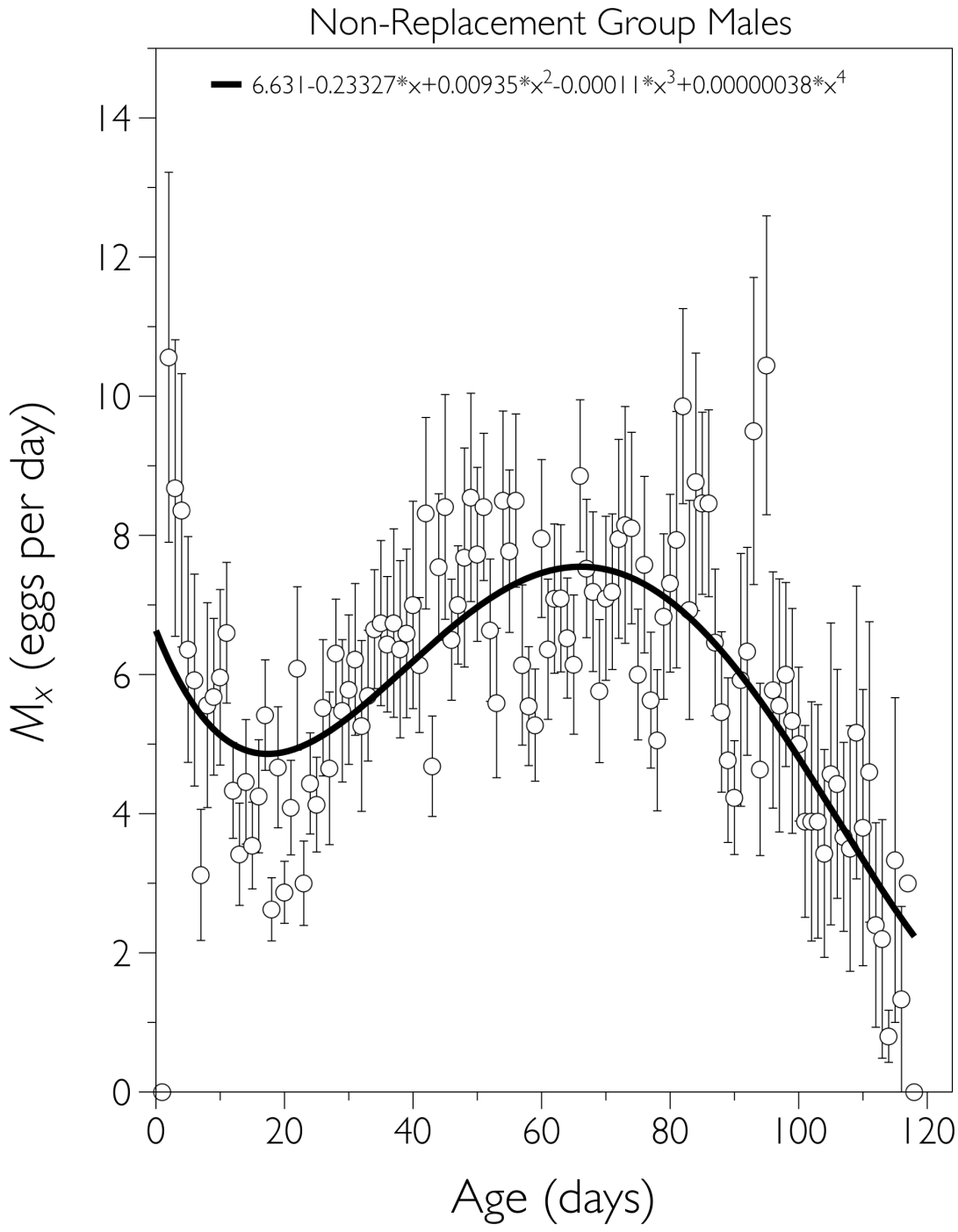
We determined the average time in each developmental stage, as well as total lifespan (egg to adult mortality). All statistical analyses were performed using JMP® 11 (SAS Institute, 2013). A pooled variance t-test was used to analyze the lifespan data for males and females to test for a difference between the means of these groups. The gross maternity (M_x) and hatch rate (h_x) data were also analyzed by a pooled variance t-test to determine whether the *non-replacement male* group and *replacement male* group differed significantly in their means. The h_x data were arcsine square root transformed prior to analysis.

Results

D. suzukii fecundity, hatch rate, and sex ratio

The mean daily egg production across both male groups and all ages was 5.7 ± 0.24. All other reproductive and growth estimates are shown in Table 2-2. These values suggest that the lack of hatch reduced fecundity by 23%, female mortality reduced fecundity by 24%, and when both are taken into consideration, fecundity suffers 39% reduction. Figure 2-1 shows the M_x schedule by age for the *non-replacement* and *replacement* male groups and Table 2-3 shows the abridged reproductive schedule for the *non-replacement* and *replacement* male groups, including survival and hatch rate.

Figure 2-1. The average number of eggs produced per female daily (M_x) for the *non-replacement* and *replacement* male groups of *D. suzukii*. Model selection using weighted polynomial regression and AIC as the selection criterion suggests that the best fit curve is the quartic for the *non-replacement male* group ($R^2 = 0.30$), and quadratic for the *replacement male* group ($R^2 = 0.42$).



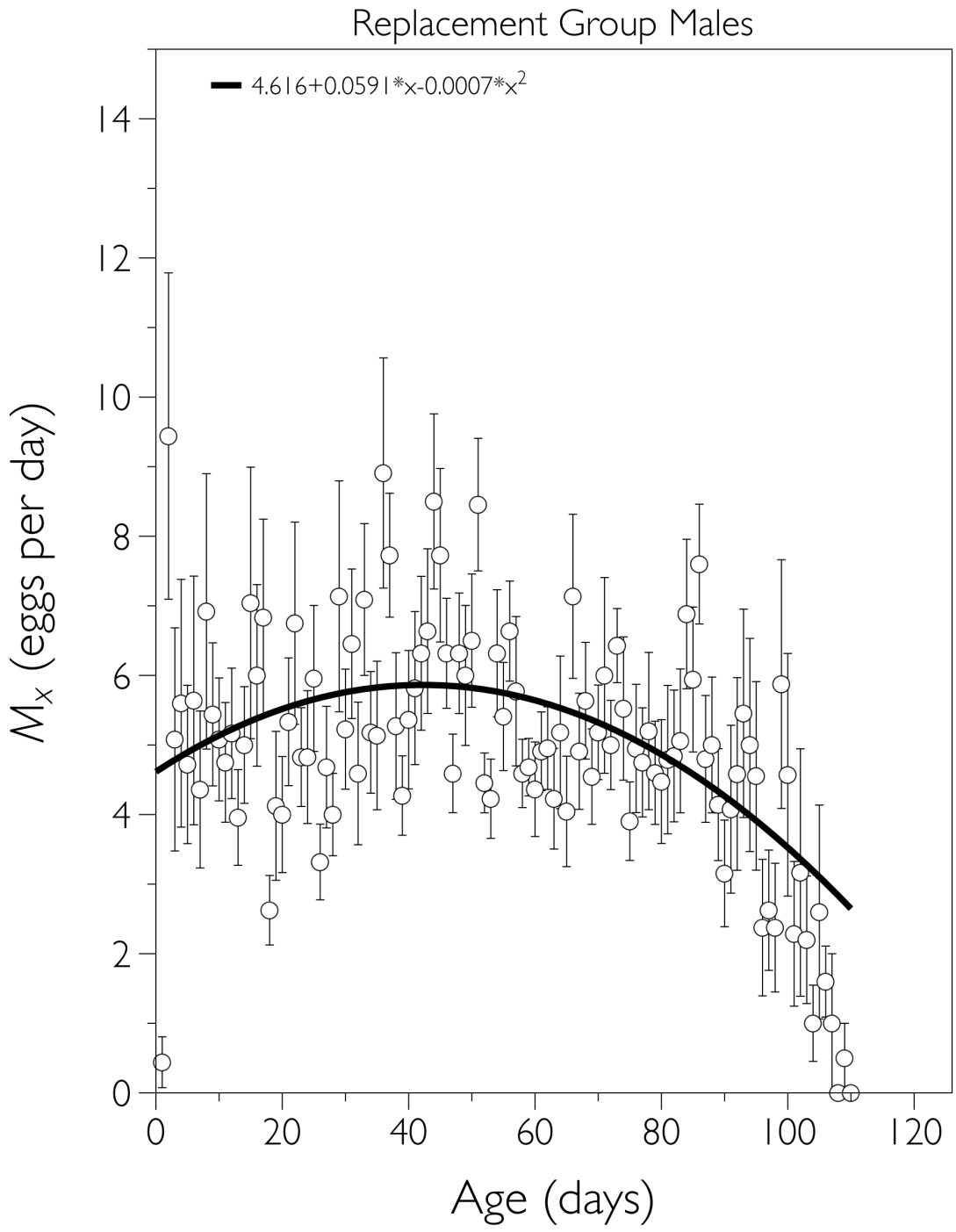

Replacement Group Males
4.616+0.0591*x-0.0007*x2
Mx (eggs per day)
Age (days)
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120

Table 2-3. Abridged reproduction schedule for *Drosophila suzukii.* All formulae were taken from Carey (1982) and Carey (1993).

	***Non-replacement male* group**		***Replacement male* group**	
Age interval	L_X	h_X	L_X	h_X
1-10	9.990	0.711	9.960	0.778
11-20	9.530	0.856	9.600	0.841
21-30	9.200	0.832	9.000	0.878
31-40	9.040	0.820	8.800	0.859
41-50	8.800	0.805	8.800	0.829
51-60	8.800	0.849	8.800	0.830
61-70	8.430	0.806	8.800	0.871
71-80	7.350	0.677	8.120	0.866
81-90	5.290	0.703	6.190	0.906
91-100	3.930	0.736	3.560	0.819
101-110	2.680	0.385	1.450	0.771
111-120	0.920	0.216	0.000	

The gross maternity data were found to be significantly different between the *non-replacement male* group and *replacement male* group; the *non-replacement male* group had a higher mean gross maternity (5.9 ± 0.17 vs. 5.0 ± 0.19; $t = 3.67$; df = 226; $P < 0.0003$). We did find a significant difference between the *non-replacement male* group and *replacement male* group in the hatch rate; the *replacement male* group had a higher mean hatch rate (84 ± 0.01% vs. 71 ± 0.02%; $t = -5.62$; df = 221; $P < 0.0001$).

The sex ratio of newly emerging adults remained fairly constant over the 13 time points that we considered in our study, with a mean of 0.98 males per female.

D. suzukii survivorship

An abridged life table for *D. suzukii* is presented in Table 2-4. An interesting result from the life table is the e_x term (life expectancy at age x). High pre-pupal mortality means that a newly laid egg is expected to live for 56 days, but by the time an individual has reached the pupal stage it has a life expectancy of 75 days (Table 2-4). After this stage life expectancy gradually decreases until age 112 days. The brief rise in life expectancy after day 112 is probably an artifact of small sample sizes as there were only four females still living at that point. Also when adults emerge, there is no mortality until approx. 30 days of adult life. Egg to adult survivorship was 64%. The stable age distribution reveals that the stage composition exists as 25% eggs, 51% larvae, 16% pupae, and 8% adults.

D. suzukii development

Table 2-5 shows the mean (± standard error) of *D. suzukii* development time in days for immature stages. Total lifespan (egg to adult mortality) has a mean of 86.1 ± 4.25 days, with the maximum lifespan reaching 153.7 days for one male. The average lifespan for

Table 2-4. Abridged life table for a cohort of *D. suzukii.* All formulae were taken from Carey (1982) and Carey (1993).

Stage	**n**	**Interval (x)**	l_x	p_x	q_x	d_x	${}_nL_x$	T_x	e_x	c_x
Egg	1.384	0 – 1	1.000	0.868	0.132	0.132	1.293	56.194	56.194	0.250
L1	1.059	1 – 2	0.868	0.913	0.087	0.075	0.879	54.902	63.256	0.142
L2	1.477	2 – 3	0.792	0.952	0.048	0.038	1.143	54.022	68.171	0.155
L3	3.115	3 – 6	0.755	0.900	0.100	0.075	2.233	52.880	70.066	0.211
Pupa	5.836	6 – 12	0.679	0.944	0.056	0.038	3.854	50.646	74.563	0.163
A1	10	12 – 22	0.642	1.000	0	0	6.415	46.792	72.941	0.065
A2	10	22 – 32	0.642	1.000	0	0	6.415	40.378	62.941	0.011
A3	10	32 – 42	0.642	1.000	0	0	6.415	33.962	52.941	0.002
A4	10	42 –52	0.642	0.971	0.029	0.019	6.321	27.547	42.941	
A5	10	52 – 62	0.623	0.727	0.273	0.170	5.378	21.226	34.091	
A6	10	62 – 72	0.453	0.875	0.125	0.057	4.245	15.849	35.000	
A7	10	72 – 82	0.396	0.905	0.095	0.038	3.774	11.604	29.286	
A8	10	82 – 92	0.358	0.737	0.263	0.094	3.113	7.830	21.842	
A9	10	92 – 102	0.264	0.714	0.286	0.075	2.264	4.717	17.857	
A10	10	102 – 112	0.189	0.400	0.600	0.113	1.321	2.453	13.000	
A11	10	112 – 122	0.075	0.250	0.750	0.057	0.472	1.132	15.000	
A12	10	122 – 132	0.019	1.000	0	0	0.189	0.660	35.000	
A13	10	132 – 142	0.019	1.000	0	0	0.189	0.472	25.000	
A14	10	142 – 152	0.019	1.000	0	0	0.189	0.283	15.000	
A15	10	152 – 162	0.019	0	1.000	0.019	0.094	0.094	5.000	
A16	10	162 – 172	0							

* Notes: L1 = 1st instar, L2 = 2nd instar, L3 = 3rd instar, and the A stages represent consecutive adult intervals of 10 days. *n* is the number of days in the interval and *Interval (x)* is the age interval associated with each stage.

Table 2-5. Average time (days ± standard error) in each developmental stage for the full development cohort of *D. suzukii*.

Life stage	Time in stage (days ± standard error)
Egg (n = 44)	1.4 ± 0.08
1st instar (n = 37)	1.1 ± 0.08
2nd instar (n = 35)	1.5 ± 0.1
3rd instar (n = 34)	3.1 ± 0.1
Total larval development (n = 33)	6.0 ± 0.2
Pupa (n = 33)	5.8 ± 0.05
Egg – adult (n = 33)	12.8 ± 0.2

* Notes: *n* is the number of individuals in each stage.

females was 79.5 ± 4.86 days, and the average lifespan for males was 93.6 ± 6.88 days, but these were not significantly different ($t = 1.71$; df = 32; $P = 0.10$).

Discussion

To date, these data represent first reports on the life history and lifetime reproductive measures of *D. suzukii*. Baseline information on fecundity, longevity, and population growth at preferred laboratory conditions on *Drosophila* media is provided. Data from the present study represent a valuable benchmark as baseline information with which to use in modeling, control strategies, and to compare our population of *D. suzukii* to others, and to other *Drosophila*.

Kanzawa (1939) previously reported that the average lifetime egg production of female *D. suzukii* can range from 219-563 eggs on cherries (as cited in Lee et al. 2011b). Our estimates were somewhat higher, where gross fecundity rate was 635.6 eggs, with lifetime egg production ranging 92-868 eggs on *Drosophila* media. Even though it is the larvae that feed and cause deterioration of the host, it is also important to consider those eggs that do not hatch, as well as the female lifespan, to get a gauge of future population size. We found that the actual number of eggs that would reach adult eclosion per female in a *D. suzukii* population averages 386.8. These values of lifetime reproduction are mid-range compared to other species in the *melanogaster* species group, of which *D. suzukii* is a member. For example, *D. simulans* have a lifetime net production of offspring that can range from 17 to 493 (Taylor et al. 2008), while *D. melanogaster* has a potential lifetime production of >1000 eggs (McMillan et al. 1969). Clearly there is heterogeneity with respect to this value within the species group.

The gross fecundity rate that we have reported could very well be a product of increased longevity. In Kanzawa's (1939) study the adult life span was found to range 20 to 52 days, while we found total lifespan to range from 50 to 154 days, with a mean of 86 days ± 4.25 days. Kanzawa (1939) also tracked time in each developmental stage in captivity on cherries, documenting time in the egg, larval, and pupal stages as roughly 1.4, 7.1, and 6.1 days, respectively (as cited in Walsh et al., 2011). These estimates are similar to those in the present study. Previous work on *D. suzukii* has explored host potential and reported differences in development between these hosts (Lee et al. 2011a; Bellamy et al. 2013), illustrating that host can affect the development of *D. suzukii.* The differences in egg production and longevity between the present study and Kanzawa's work could partly be attributable to the host studied. However, it is also plausible that in the Ontario ecotype adult lifespan has increased, thereby increasing average lifetime egg production.

Here we documented a significant difference in the mean gross maternity for the *non-replacement male* group and *replacement male* group, where the *non-replacement male* group experienced a greater mean gross maternity. Firstly, there is the possibility that the act of replacing the males in the *replacement male* group actually caused some disruption within the mating chamber, resulting in a delay in mating. However *Drosophila* females are able to store sperm within their seminal receptacle and spermathecae and so the difference in gross maternity between the male groups may be due to sperm competition between consecutive males (Price et al. 1999). Evidence suggests that male *Drosophila* can physically dislodge and incapacitate sperm stored within the female (Price et al. 1999), and this may have caused decreased gross maternity in the *replacement male* group.

We also documented a significant difference in the mean hatch rate for the *non-replacement male* group and *replacement male* group, where the *replacement male* group experienced greater overall hatch rate of eggs. This difference suggests that there may be a difference in the reproductive period of male and female *D. suzukii*. The lower mean hatch rate of the *non-replacement male* group may be a consequence of males becoming infertile earlier than females (i.e. females have a longer reproductive period than males, and therefore are able to produce eggs longer than males are able to produce sperm). Since females are still able to lay unfertilized eggs, we see gross maternity maintained but the hatch rate of these eggs decrease. This difference suggests a role for female choice behavior in field environments. A female that is able to mate with a fertile male would receive direct fitness benefits in the form of more viable eggs resulting in higher offspring production (Droney 1996). Overall it has been found that multiple matings in *Drosophila* increase female fitness (Arnqvist and Nilsson 2000). Although the *replacement male* group experienced a lower mean gross maternity, the higher hatch rate of this group may offset the lower egg production, leading to greater overall fitness.

The gross maternity (M_x) data suggest that egg production is maximized at intermediate ages (Fig. 2-1). However for the *non-replacement male* group and the *replacement male* group, we observe different peaks in egg production (approx. 70 days vs. approx. 40 days respectively), as well as different best-fit curves. The difference in the ages of peak egg production could possibly be a result of the fact that males increase in quality as they mature. Klepsatel et al. (2013) describe four phases relating to *D. melanogaster* fecundity in a female's life: 1. reproductive maturation reaching peak fecundity within 3-4 days; 2. prolonged linear decrease in fecundity; 3. exponential decrease in fecundity; and 4. post-ovipository period with no eggs laid until mortality.

Excluding the initial peak in egg production, *D. suzukii* do not seem to reach their next peak in fecundity until much later in life (Fig. 2-1). After this period a decrease in fecundity occurs, however this decrease does not become exponential and there is very little, if any, post-ovipository life.

Life table entropy (*H*), which is a measure of heterogeneity in the probability of dying at each age (Carey 1993) was found to be 0.683. According to our life table (Table 2-4), life expectancy at age x (e_x) increases when an individual reaches the pupal stage. This is caused by the fact that mortality at the immature stages is high compared to adult mortality, and therefore when an individual has survived that life stage (i.e. the pupal stage), it is likely to survive longer than originally expected.

Longevity has been found to be highly dependent on temperature. When Partridge et al. (1995) investigated longevity of adult *D. melanogaster*, they reported a significant increase in longevity at 16 °C as compared to 25 °C. Our study was conducted at 22 °C, which has been reported as the preferred temperature of *D. suzukii* (Calabria et al., 2010; Walsh et al., 2011). A temperature-dependent life table remains an important research goal for this important species.

The intrinsic rate of increase is a valuable parameter because it combines mortality and fecundity to yield an estimate of growth (Zahari et al. 2010). Our estimate (0.179) is similar to those found for other species of the *melanogaster* species group. For example, in *Drosophila serrata*, *r* averaged approx. 0.115 at 20 °C and approx. 0.211 at 25 °C (Birch et al. 1963). Birch et al. (1963) demonstrated that *r* could vary with temperature and location. It is a reasonable assumption that *D. suzukii*'s intrinsic rate of increase also depends on temperature, and again argues for the need to construct a temperature-dependent life table.

This study confirms the enormous population potential of *D. suzukii*. In addition to being used as a starting point to parameterize population models, which can be used to predict characteristics such as number of generations per year and population trends, these baseline data provide some insights into the problem of pest monitoring and control for this species. It is clear that *D. suzukii* is a comparatively long-lived, fecund vinegar fly with a growth rate allowing a population to double in size in as little as 4 days. Our estimate of the stable age distribution shows that only 8% of the population is comprised of adults. An estimate similar to what has been found for other insects (Birch 1948). Our stable age distribution estimate can have implications for current monitoring methods. Presently, baited traps are used to monitor *D. suzukii* adults. Given that adults may only represent a small portion of the population, quantifying infestation based on trap counts may not be accurate, and perhaps these traps are only useful for determining the presence or absence of *D. suzukii*. The potential for explosive population growth, and the fact that adults appearing in traps probably comprise a very small fraction of the total population, both suggest that pest management strategies should be applied as soon as the pest appears in a field.

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Chapter 3: Overwintering survival and fecundity of *Drosophila suzukii*

Abstract

Drosophila suzukii is a vinegar fly pest of Asian origin that has recently invaded North America, causing substantial economic damage to soft skinned fruit growers. The overwintering biology of this pest is not well understood. An overwintering survival analysis for *D. suzukii* was conducted under conditions comparable to an Ontario winter. This experiment considered sex, ecotype, photoperiod exposure, mated status, acclimation, rearing temperature, and fecundity after cold exposure. I tested survival after a 6-week exposure to -5, -3, -1, 1, 3, and 5 °C. I found that flies could not survive for 6 weeks below 1 °C. There was a significant main effect of temperature on overwintering survival, as survival was higher at higher temperatures. Pre-planned contrasts did not reveal any difference between ecotypes, mated status, photoperiod exposure, or sex at any temperature. When assessing the fecundity of mated overwintering females, it was found that surviving females that were mated prior to the 6-week cold exposure could produce viable offspring after cold exposure. I conclude by comparing this work to other data on *Drosophila* and discuss future research that could be explored as a result of this experiment.

Introduction

Insect distributions can be limited by many biotic factors. Climate is the most important of these factors as it can influence survival (Hoffmann, 2010). Stressful conditions such as low temperatures can limit an insect's ability to develop and reproduce. Insects, and *Drosophila* specifically, have adapted to unfavourable conditions through evolved traits

such as diapause and cold-hardiness (Kimura, 1988a). Since climate is variable depending on location, patterns of distribution are often related to latitude and altitude (Hoffmann, 2010). The study of thermal tolerances of *Drosophila* is also important as climate changes, bringing about new species interactions, some of which may be economically important in agriculture. As temperate regions can experience extreme fluctuations in seasonal temperatures, winter temperatures are important when considering *Drosophila* survival, and their ability to survive depends on their capacity to overwinter successfully (Izquierdo, 1991).

Drosophila suzukii is a newly introduced pest to fruit growers in North America and Europe, causing damage to berry and stone fruit crops (Hauser, 2011). They are native to Japan, and since they are a new pest to North American growers (first mainland detection in 2008), little is known of their biological and behavioural adaptations, specifically their overwintering biology on this continent. Since thermal tolerance can affect the range expansion and overall activity of *Drosophila* (Kimura, 1988a), it is important to understand this aspect of this new pest.

Data on overwintering *Drosophila* populations in naturally occurring ecosystems are essentially unavailable (Izquierdo, 1991). Overwintering can be defined as the way an organism endures the winter, whereby metabolic changes result in a cold-hardy state of hibernation (Leather et al., 1993). The overwintering location and microhabitat of *D. suzukii* remains unknown. However, soil or snow cover is generally used by overwintering insects due to the insulation provided, resulting in warmer, more stable temperatures than what would be experienced in an open environment (Leather et al., 1993). Another factor that is rarely considered in conjunction with cold-hardiness is diapause, a state that is induced in preparation for environmental stress in response to

temperature-independent factors (Leather et al., 1993; Bale and Hayward, 2010). Diapause and cold-hardiness have been studied in *Drosophila*, however there is a need to include these when developing an ecologically relevant studies on overwintering in *Drosophila*.

Cold resistance is variable among species of *Drosophila*, but acclimation may enhance this trait. For example, Bubliy et al. (2002) compared the cold resistance (defined as survival of adults at 0 °C) of acclimated and non-acclimated *D. melanogaster* from different geographical regions. Acclimation increased survival in all populations. However, non-acclimated flies showed higher fertility and number of progeny (Bubliy et al., 2002). These results suggest that cold acclimated flies are adapted to survive cold temperatures, putting fewer resources into fertility, implying fitness costs in reproduction (Bubliy et al., 2002). Therefore in the current study, I included a period of acclimation to create an ecologically relevant scenario.

An important cue used by insects to predict seasonal changes is photoperiod, as changes in day length are predictable and unchanging. Insects possess an internal clock that is triggered through exposure to certain photoperiods, inducing physiological events such as diapause (Kauranen et al., 2013). It has been found that *Drosophila* respond to short photoperiods by entering diapause and ceasing ovarian development, while long photoperiods prevent diapause, leading to ovarian development (Kimura, 1990).

The induction of photoperiodic diapause can interact with other cues, altering the physiological outcome. Temperature can alter the critical photoperiod needed to induce diapause in *Drosophila*, as Kimura (1982) reported in *D. testacea*, and Kimura (1984) in the *D. auraria* complex. As temperature increased, the critical photoperiod needed to

induce diapause decreased, until a point was reached where the temperature was too high for diapause to be induced (Kimura, 1982; Kimura, 1984).

Kimura (1990) found the developmental stage that was sensitive to photoperiodic induction of diapause in four species from the *D. auraria* complex ranged from the preimaginal to the imaginal stage, and these species were able to respond quantitatively to the photoperiodic cue. These results together indicate that *Drosophila* are able to alter their photoperiodic responses to changing environments, which is an important trait for species living in seasonal environments.

Some research has been conducted on low temperature tolerances of *D. suzukii.* It has been found that the lower lethal temperature is -0.9 °C (Kimura, 2004). An overwintering study has also been conducted on *D. suzukii* (Dalton et al., 2011). This study was conducted on flies collected from Oregon and reared in a 25 °C environment. Flies were acclimated to 10 °C through a step-wise process (a decrease in 2.5 °C every 2 days). Pupae and adults were subjected to temperature treatments ranging from 1 to 10 °C for a 6-week period. Survival was assessed throughout the study period and it was determined that *D. suzukii* survived longest at 10 °C, with survival decreasing below this point, whereby 100% mortality was observed by day 17 at 1 °C. However *D. suzukii* are highly mobile organisms and can seek out favourable conditions, so even if sub-zero temperatures are experienced, a population will not necessarily be eradicated. *D. suzukii* are known to be established on the island of Hokkaido in Japan where winters average -12 to -4 °C (Walsh et al., 2011). Dalton et al. (2011) did not include a measure of diapause or consider mated status in their study, and these may be important factors in the successful overwintering of *D. suzukii*.

The objective of this study was to gather survival and fecundity data for *D. suzukii* that were exposed to temperatures comparable to those that would be experienced in an overwintering microhabitat during an Ontario winter, as it is possible that Ontario and Oregon ecotypes may differ. To complement the Dalton et al. (2011) study, this experiment controlled for sex, ecotype, photoperiod exposure, mated status, acclimation, and rearing temperature in order to provide baseline data on the potential survival of *D. suzukii* in cold conditions.

Materials and Methods

D. suzukii rearing and colony maintenance

All insects were kept in the same manner as described in Chapter 2. I also obtained a colony that originated from *D. suzukii* collected from one location in the lower mainland of British Columbia. Flies were kept in lab conditions for 2 years prior to my possession. I then kept these flies in a separate growth chamber set to the same settings as the chamber housing the Ontario flies.

Photoperiod exposure

It has been found that a combination of low temperature (approx. 15 °C) and short photoperiod (approx. 10L:14D) will induce diapause in *Drosophila*. To rear flies that were to be used in the experiment, one controlled growth chamber was set to 15 °C, approximately 25% RH, and a photoperiod of 10:14 h, L:D (short photoperiod conditions), and one controlled growth chamber was set to 15 °C, approx.. 25% RH, and a photoperiod of 15:9 h, L:D (long photoperiod conditions). Diet dishes (as described in Chapter 2) that were left in the colony cages for 2 days were transferred to either the

short or long photoperiod growth chamber and left to incubate in those conditions until adult eclosion (approx. 30 days).

Experimental design

I included 6 life stage treatment groups and 6 temperature treatment groups in my experimental design. The life stage treatments were: Ontario mated short photoperiod females (ON-M-SP), Ontario mated long photoperiod females (ON-M-LP), Ontario virgin short photoperiod females (ON-V-SP), Ontario virgin long photoperiod females (ON-V-LP), Ontario males (ON-Male), and British Columbia mated short photoperiod females (BC-M-SP). I was interested in testing for a difference in overwintering survival between ecotypes (i.e. Ontario vs. British Columbia). I only included one British Columbia fly treatment to do this, and this is the treatment that is assumed to be the overwintering stage in the literature (mated females in reproductive diapause) (Cini et al., 2012). The temperature treatments were: -5, -3, -1, 1, 3, and 5 °C. All flies were subjected to the temperature treatment for a 6-week period.

Acclimation

Flies were subjected to a step-wise acclimation before and after the temperature treatment, as follows: pre-treatment, 10 °C with respective photoperiod for 4 days, followed by 5 °C for 4 days before being subjected to the experimental temperature treatment; post-treatment, 5 °C for 4 days after 6 weeks in the temperature treatment, followed by 4 days at 10 °C, followed by 4 days at 15 °C, followed by 4 days at 22 °C).

Protocol

Mated flies to be used in the experiment were released into a new colony cage in the respective chamber on the first day of emergence. For the following 10 days all newly emerging flies were released into the same cage. These flies were left for 1 week to settle and mate. Diet dishes were replaced as needed. Flies were then placed into a petri dish (10 flies per dish) with a small petri dish of banana diet that freezes at a lower temperature than diet used in chapter 2 (as described in Albers and Bradley, 2006), as well as cotton saturated with double distilled water. The lid of the petri dish had a hole covered with mesh for ventilation. The petri dish was sealed with parafilm and the acclimation process initiated.

Virgin flies to be used in the experiment were separated within 12 h of emergence and females were released into a new colony cage in the respective growth chamber. For the following 10 days all newly emerging females were released into the same cage. These flies were left for 1 week to match the age range of the mated flies. The protocol for getting the flies into the temperature treatment was the same as for mated flies.

For each life stage there were 5 petri dishes per temperature treatment. The flies were in the temperature treatment for a 6-week period. When the 6 weeks had elapsed in the temperature treatment, flies were subjected to post-treatment acclimation. After the final 4-day period, survival was assessed by recording the proportion of flies that survived.

Fecundity

Fecundity of surviving flies was assessed for the ON-M-SP group, as this life stage is assumed to be the overwintering stage for *D. suzukii* in the literature (Cini et al.,

2012). Up to 20 females from each temperature treatment group were put into a mating chamber (as described in Chapter 2). Half of the females were placed in a mating chamber with a male from the Ontario colony, and half of the females were put into a chamber alone, in order to assess whether viable eggs were laid by flies that were mated only before the temperature treatment, or if a second mating was needed to produce viable eggs.

Flies were kept in mating chambers for a 6-day period in the 22 °C chamber. The diet lids of the mating chambers were replaced every second day over this period, and egg counts were performed on these diet lids. To determine viability of these eggs, the diet from the lid was transferred to a large petri dish filled with fresh diet (similar to the method described in Chapter 2 to determine sex ratio). After the 6-day period a total of three diet lids had been transferred to the original petri dish. This procedure resulted in a total of 13 petri dishes for the females that came from the 3 °C temperature treatment (7 mated and 6 unmated after the cold exposure), and 19 petri dishes for the females that came from the 5 °C temperature treatment (9 mated and 10 unmated after the cold exposure). The petri dishes were incubated in the 22 °C chamber until all adults had emerged. Once all adults had emerged, pupae and adult counts were made.

Statistical Analyses

All survival data (proportion adults emerging per petri dish) and viability data (proportion of eggs developing into pupae) were arcsine-square-root transformed prior to analysis to avoid heterogeneity of variances. Survival data were analyzed using a two-way ANOVA with treatment and temperature as fixed effects. Only those temperatures where survival was observed (1, 3, and 5 °C) were included in the analysis. Table 3-1 shows the

treatment levels involved. Pre-planned contrasts were performed between groups of treatment levels to test for effects of ecotype, sex, mating status and photoperiod exposure. Daily fecundity data were Box-Cox transformed prior to analysis. Both fecundity and viability of eggs of surviving females were analyzed using a two-way ANOVA with post-exposure mating status (mated and unmated) and temperature (3 and 5 °C) as fixed effects. All analyses were conducted using JMP statistical software version 10 (SAS Institute, 2013), with $\alpha = 0.05$.

Results

Overwintering survival

No flies survived when exposed to -1, -3 and -5 C following the 6 week exposure period. Table 3-1 shows the mean proportion of surviving flies per petri dish for each treatment group at all temperatures where survival was observed (1, 3, and 5 °C). There was a significant main effect of temperature on overwintering survival ($F_{2,71} = 33.95$, $P < 0.0001$) with higher survival occurring at higher temperatures (Figure 3-1). There were no treatment effects or treatment x temperature interactive effects on survival. Pre-planned contrasts revealed no treatment differences between British Columbia and Ontario ecotypes, mated status (mated *vs.* virgin), photoperiod exposure (long *vs.* short) or sex (male *vs.* female) at any temperatures (Table 3-2).

Table 3-1: Mean proportion survival (±SE) per petri dish for each treatment level at all temperatures where survival was observed.

	Temperature (°C)		
Treatment	1	3	5
ON-M-SP	0	0.26 ± 0.087	0.48 ± 0.14
ON-M-LP	0.18 ± 0.080	0.12 ± 0.073	0.64 ± 0.14
ON-V-SP	0	0.14 ± 0.087	0.56 ± 0.16
ON-V-LP	0	0.12 ± 0.058	0.44 ± 0.12
BC-M-SP	0.060 ± 0.040	0.14 ± 0.051	0.58 ± 0.18
ON-Male	0.080 ± 0.049	0.24 ± 0.11	0.42 ± 0.073

* Notes: ON-M-SP = Ontario mated short photoperiod females, ON-M-LP = Ontario mated long photoperiod females, ON-V-SP = Ontario virgin short photoperiod females, ON-V-LP = Ontario virgin long photoperiod females, BC-M-SP = British Columbia mated short photoperiod females, and ON-Male = Ontario males.

Figure 3-1: Mean (±SE) proportion of surviving *D. suzukii* per petri dish in response to 6-week temperature exposures across all treatments.

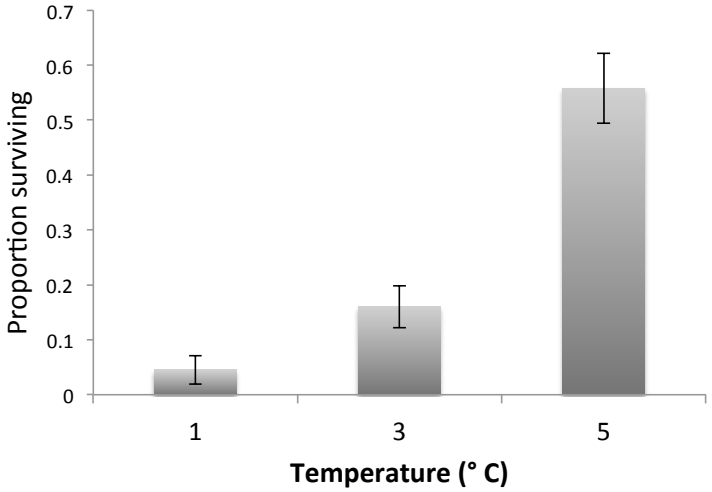


Table 3-2: Pre-planned contrasts of survival for overwintering groups at all temperatures where survival was observed.

Temperature *(°C)*	**Sex** *Male vs. Female*	**Photoperiod** *Long vs. Short*	**Mating status** *Mated vs. Virgin*	**Ecotype** *BC vs. Ontario*
1	$F_{1,72} = 0.8299$ $P = 0.3653$	$F_{1,72} = 1.4410$ $P = 0.2339$	$F_{1,72} = 1.4410$ $P = 0.2339$	$F_{1,72} = 0.5954$ $P = 0.4429$
3	$F_{1,72} = 0.1272$ $P = 0.7224$	$F_{1,72} = 0.4527$ $P = 0.5032$	$F_{1,72} = 0.5799$ $P = 0.4488$	$F_{1,72} = 0.4874$ $P = 0.4873$
5	$F_{1,72} = 0.0039$ $P = 0.9501$	$F_{1,72} = 0.2512$ $P = 0.6177$	$F_{1,72} = 0.6436$ $P = 0.4250$	$F_{1,72} = 0.3720$ $P = 0.5438$

Fecundity of surviving females

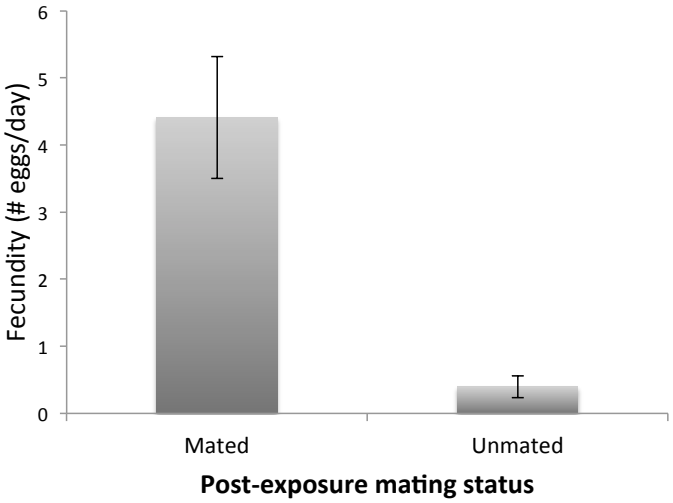
The fecundity and offspring viability of previously mated females surviving overwintering exposures to 3 and 5 °C were examined. Surviving females were divided into two groups and were either mated or unmated post-exposure. There was no effect of overwintering temperature on mean daily fecundity of female survivors. However, there was an effect of mating status, whereby females that were mated again after exposure had higher daily fecundity measures than those females that were not mated again after exposure ($F_{1,27} = 24.47$, $P < 0.0001$; Figure 3-2). There was no temperature x mating status interaction for fecundity.

Viability was measured as the proportion of eggs that developed into pupae. There were no effects of temperature, mating status, or their interaction on viability. On average, 85.6 % (± 6.7) of eggs laid following winter exposure developed into pupae.

Discussion

The present study provides evidence to suggest that acclimated *D. suzukii* can survive 6-week exposures to 1, 3, and 5 °C environments. Sex, photoperiod exposure, mating status, or ecotype did not affect cold temperature survival (Table 3-2). Temperature affected survival, as higher survival was reported at higher temperatures (Fig. 3-1). This result is in agreement with Dalton et al. (2011). However, in that study adults did not survive for more than 17 days at 1 °C, while I found certain treatment groups to survive for 6 weeks at this temperature.

Figure 3-2: Mean daily fecundity (±SE) of *D. suzukii* females following exposure to 6 weeks of low temperatures. Since there were no temperature effects or temperature x mating status interactions, the data presented here are pooled over the 3 °C and 5 °C exposures. All females from both groups were mated prior to low-temperature exposure.



Dalton et al. (2011) suggested the need for the study of female fecundity after prolonged winter conditions. I conducted such a study after exposure to a 6-week cold treatment in the ON-M-SP group. I included females that were only mated prior to the cold exposure, and females that were mated both before and after cold exposure.

I observed that females that were mated only prior to cold exposure were able to lay eggs following exposure (Fig. 3-2), and that these eggs were viable. This observation has 3 possible causes: 1) females were able to store sperm throughout the cold exposure and used this sperm to fertilize eggs once they were returned to favourable conditions, 2) the eggs were fertilized at time of mating prior to cold exposure but development was depressed upon exposure to cold, or 3) females converted to a state of parthenogenesis with cold exposure.

Sperm storage organs are common in insects, and it is known that *Drosophila* can store sperm in the spermathecae, and are able to lay fertile eggs for up to two weeks after being isolated from males, until the sperm store is exhausted (Lefevre and Jonsson, 1962). Collett and Jarman (2001) observed that even after a 6-month exposure to cold, mated *D. pseudoobscura* females were able to used stored sperm to fertilize and lay viable eggs. It may be possible for mated *D. suzukii* to store sperm over the winter season, as they would not be exhausting this resource during this time, and use this viable sperm once conditions had become favourable to fertilize their eggs.

Low temperatures have been found to depress the pace of embryogenesis in certain insect species (Mansingh, 1971; Schaefer, 1977). It is possible that at the time of mating an embryo was produced but development was arrested upon entry into unfavourable conditions. Once temperatures were brought back within a favourable range these eggs would be viable once laid.

Another possibility is that of parthenogenesis. Stalker (1954) discusses the capacity for parthenogenesis in Drosophilidae. In a survey of 28 species, parthenogenesis was identified in 23, albeit at a low rate (Stalker, 1954). Further, of the species that produced viable eggs, only 3 species were able to produce adult progeny (Stalker, 1954). Although it may be unlikely, it is certainly possible that those females that were able to produce eggs but were not mated again after the cold exposure actually reproduced by parthenogenesis. In this event, if males are less likely to survive the winter months, perhaps females who survive resort to parthenogenesis until a stable male population is established.

Although I did not observe any significant effect of sex on survival at low temperatures, there is the possibility that even if males were to survive low temperatures sterility may become an issue. It is known that male sterility in *D. suzukii* is induced above 30 °C (Walsh et al., 2011), however the lower threshold of male sterility is unknown. According to Chakir et al. (2002), *D. melanogaster* and *D. simulans* males become sterile at 12 °C and 11 °C, respectively. However, when transferred back to favourable temperatures, fertility was recovered (Chakir et al., 2002). Therefore, if there were males that could overwinter into the following spring, it is likely that fertility of *D. suzukii* males would recover if mating were necessary.

I did not observe a significant ecotype effect. Flies from BC and Ontario did not differ in mean overwintering survival. Using the 1971 to 2000 Canadian Climate Normals station data, the mean January temperatures experienced in Guelph, ON can range from -11.4 °C to -3.7 °C, while mean January temperatures in Kelowna, BC can range from -7.4 °C to -0.2 (Environment Canada, 2014). Given this information, it may be expected that survival of Ontario *D. suzukii* would be higher at low temperatures than

the BC ecotype. However, it is possible that these populations have not had enough time to diverge on these traits, given that they have only been established in Canada since 2010. As time progresses overwintering trait divergence may become apparent.

There were some limitations with this overwintering study. I did not find any survival below 1 °C after 6 weeks of exposure. In a follow-up study it may be interesting to alter the duration of cold exposure, and to include fluctuating temperatures instead of constant cold. It has been found that duration of cold exposure can affect the survival of *Drosophila* (Marshall and Sinclair, 2010). It has also been found that fluctuating temperatures can increase the survival of overwintering insects as they are able to repair cold injuries during warmer periods (Renault et al., 2004). During a winter season, temperatures naturally fluctuate, so the duration of cold exposure could be expected to be shortened. However, it is predicted that under future climates snow cover will be reduced (IPCC, 2007), and snow cover has been found to increase the overwintering survival of insects by providing insulation (Shorthouse et al., 1980; Irwin and Lee, 2003). Although we do not know the type of microhabitat that *D. suzukii* use to overwinter, it is possible that a decrease in snow cover could negatively affect their survival by increasing exposure to cold conditions.

Another limitation of this study is that I did not monitor the accumulation of cryoprotectants within the overwintering flies. Dalton et al. (2011) state that it is unknown whether *D. suzukii* possess the capacity to accumulate cryoprotectants. It is known that *Drosophila* can accumulate polyols and sugars to increase the likelihood of surviving cold temperatures. Trehalose has been found to be an important sugar in the supercooling process, as levels of this sugar tend to increase when *Drosophila* are exposed to low temperatures (Kimura et al., 1992; Overgaard et al., 2007). Glucose,

trehalose, proline and myo-inositol were all found to increase in *D. montanna* during autumn, presumably in preparation for overwintering to protect against chilling injury (Vesala et al., 2012). Overgaard et al. (2007) also found levels of glucose to increase in *D. melanogaster* following cold-hardening, correlating with the onset of cold-hardiness. Luckinbill (1998) also found that cold-selected lines contained increased levels of glycerol, relating cold resistance to high glycerol content.

However, the use of sugar to increase cold telerance is not universal between species. In cold-selected lines of *D. melanogaster*, MacMillan et al. (2008) found rapid cold-hardening to cause a decrease in levels of free glucose, and concluded that free glucose is not a significant element in cold tolerance in this species. On the same note, Colinet et al. (2013) found that enriching the diet of *D. melanogaster* with sugar decreased the cold tolerance of adults by causing a metabolic imbalance. If cryoprotectant levels could be monitored within *D. suzukii*, we may be able to better understand the possible climatic range that they could accommodate (Dalton et al., 2011).

Overall this information is of value to fruit growers in temperate locations that experience a cold winter season. Understanding the life stage and at what temperature *D. suzukii* can survive can help to predict the size and impact of early summer generations. I did not find survival below 1 °C after 6 weeks of cold exposure. However, there is still the possibility that *D. suzukii* could survive a winter where temperatures fall below this through behavioural adaptation, as we know this species to be established in Japan. I did find that surviving females could produce viable eggs following cold exposure, which can lead to a population build-up when conditions become favourable. Future studies that address the limitations of this study will help to solidify our understanding of the overwintering biology of *D. suzukii*.

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Chapter 4: General discussion and conclusions

Predicting the overwintering potential of pest species is important for the agricultural industry. Understanding what proportion of the population will likely survive the winter to the following spring may aid farmers in choosing an appropriate control strategy. Understanding the life history of the pest, including survival, reproduction, and general population trends, also helps in pest management efforts. Since *D. suzukii* is a fairly recent invader, this biological information has not yet been fully reported in the literature, warranting this work. The research in this thesis focused on bridging these gaps in knowledge by performing a general life history analysis on *D. suzukii*, as well as initiating an overwintering investigation that can be elaborated further in the future.

4.1 General Discussion

Determining the life history and overwintering ability of insects is important in determining their potential and persistence, especially for an economically important pest such as *D. suzukii.* In chapter 2, I found evidence suggesting that *D. suzukii* are a comparatively long-lived, fecund vinegar fly, with a growth rate allowing them to increase in population size quickly. The longevity and fecundity that I reported were higher than what has been previously reported. I also determined that the stable age distribution shows that only 8% of the population exists as adults, which can be problematic for the way we monitor this pest. These baseline data can be used to parameterize population models for present and future climates. In chapter 3, I found that acclimated *D. suzukii* can survive for 6 weeks in 1, 3, and 5 °C environments. This contradicts what has been previously reported by Dalton et al. (2011). In my experiment, sex, photoperiod exposure, mating status, and ecotype were not found to affect cold

temperature survival, while temperature was. Also, females that were mated prior to cold exposure were able to lay viable eggs following exposure.

Life history of *D. suzukii* under preferred conditions

As stated previously, the data that I have presented in the second chapter is the first report on lifetime *D. suzukii* life history and reproductive measures. I produced baseline data on the fecundity, longevity, and population growth of *D. suzukii* when cultured on laboratory media. I found that *D. suzukii* developmental measures (time in each developmental stage) agreed with what had been found in a previous study (Kanzawa, 1939), while life span was greater in my study.

Lifespan

It is possible that my measures of *D. suzukii* life span were greater than what has previously been reported, as my adults were kept singularly in mating chambers under preferred conditions. This environment may have allowed *D. suzukii* to reach their greatest life span, while the conditions in Kanzawa's study (1939) may not have been ideal. Either way, the lifespan observed in laboratory studies is likely not what would be experienced in the field, where environmental conditions vary daily and natural predators and pathogens are present. Regardless, I determined that under preferred conditions, *D. suzukii* can survive an average of 86 ± 4.25 days, indicating that they are a relatively long-lived vinegar fly. Previous studies have used a release and recapture method for tracking individual flies in the field (Kristensen et al., 2008). Tracking survival of *D. suzukii* in the field, would allow more accurate estimates for use in models.

The lifespan of *D. suzukii* may also be affected by its host. Bellamy et al. (2013) used a novel approach for assessing the host potential of different fruits called the Host Potential Index (HPI). It is possible that *D. suzukii* may prefer certain hosts, and this may be due to the appropriateness of the host to serve as an adequate resource (Bellamy et al., 2013). It has been reported that *D. suzukii* are opportunistic pests, switching from host to host throughout a growing season (Walsh et al., 2011). It is also possible that certain hosts affect their longevity. Even though *D. suzukii* may be able to complete their development on a certain host, it is possible that one host may provide better nutrition for prolonged adult survival than another. In their study, Bellamy et al. (2013) created a diet media consisting of a host of interest mixed with agar to create consistency and thus were able to compare the nutritional quality of various fruits for a performance analysis in the absence of confounding fruit characteristics, such as size and toughness.

A criticism I have of the Bellamy et al. (2013) study is that the host fruits were not used in their natural form, but rather as a fruit-agar mixture. Although the fruit-agar mixture added consistency to their study by reducing variability between fruits based on morphology and oviposition behaviour (Bellamy et al., 2013), this mixture also took away the applicability of their results to the field. Even though *D. suzukii* possess a serrated ovipositor that allows them to cut into the skin of fruit when it is still healthy, Lee et al. (2011a) found that females favour, and more larvae develop in, fruit that is ripe, rather than green and ripening fruit. They also found that as brix levels of the fruit increased, the number of eggs and developed adults increased (Lee et al., 2011a). This research suggests that fruit skin firmness is negatively related to *D. suzukii* oviposition frequency. Therefore, using whole fruit would be a good way to test for host preference, as opposed to a fruit-agar mixture. It may also be worth testing for a difference in

oviposition frequency, and adult emergence from varying hosts and compare this to the *Drosophila* media used in the present study. If there were a difference in these values, perhaps a correction could be used to adjust for what would be possible in the field in terms of adult emergence.

Difference between non-replacement and replacement male group

In Chapter 2, I found a significant difference in mean gross maternity between the *non-replacement male* group and *replacement male* group. I suspect that this difference could have resulted from the disturbance created when males in the mating chambers were changed every week. However, female *Drosophila* have the ability to store sperm in their spermethecae, and so this explanation needs further investigation. In Chapter 3, I tested the fecundity of the Ontario mated short photoperiod exposed (ON-M-SP) flies that survived the 6-week temperature treatment. Half of these females were individually placed into a mating chamber with a male, while the other half were put into a mating chamber alone. I found that females that were not mated for a second time after the temperature treatment were able to produce viable eggs that resulted in emerging adults. This finding suggests that it may be possible for female *D. suzukii* to store sperm during cold exposure. A study to examine the spermathecae of mated females after various periods of cold exposure would help to determine whether this is the case and/or whether *D. suzukii* females engage in parthenogenesis.

Implications for trapping

My life history analysis revealed that *D. suzukii* populations that have achieved a stable age distribution, consist of only 8% adults, which creates an issue for monitoring the pest,

which currently relies on trapping of adults (Walsh et al., 2011). Although traps are not used for population counts, they are used to monitor general population trends and determine when *D. suzukii* are present in the field (OMAFRA, 2014). It is possible that *D. suzukii* may be causing damage in the field long before any adults are caught in traps. Therefore when considering trap catches, scouts and farmers should consider that infestation is likely far greater than indicated by trap counts early in the season. For this reason, early in the season it would be beneficial to collect fruit samples once fruit is most susceptible to *D. suzukii* infestation and incubate this fruit to allow eggs to develop into adults (if they are present). This method would take time, but it may give a more accurate representation than trap captures of *D. suzukii* populations in the field.

Overwintering

In Chapter 3, I found that *D. suzukii* could not survive for six weeks below 1 °C. It is possible that flies will actively seek out man-made enclosures or other sheltered areas upon impending winter conditions. It is also possible that freeze-thaw cycles are particularly important for the overwintering survival of *D. suzukii.* Marshall and Sinclair (2010) found that multiple short duration cold exposures resulted in higher survival of *D. melanogaster* than a single sustained cold exposure of the same total time. Repeated short duration cold exposures also resulted in decreased fecundity in *D. melanogaster*, while flies exposed to one long cold treatment allocated more resources to fecundity (Marshall and Sinclair, 2010). Thus, the fecundity that I observed in *D. suzukii* that survived the cold treatment for 6 weeks may have been higher than would be observed in natural populations. Perhaps more *D. suzukii* would survive repeated exposures but fecundity

would be reduced in the surviving population, illustrating a tradeoff in resource allocation.

Resource location

Acclimation has been shown to improve certain traits in *Drosophila*, but it can also present trade-offs. Kristensen et al. (2008) found that cold acclimated *D. melanogaster* were better able to locate resources in the field under cold conditions, but under warm conditions non-acclimated flies were more likely to find food. Thus, the advantages of acclimation may only benefit flies under narrow thermal ranges and may affect performance outside of these ranges (Kristensen et al., 2008).

In a winter scenario, there may be bouts of warm temperatures where overwintering flies may have the opportunity to become active and forage for food (Leather et al., 1993). With future climate projections predicting increasing winter temperatures and decreased snow cover (IPCC, 2007), such a scenario may become more frequent. It is possible that milder winter temperatures and a decrease in snow cover may actually become detrimental to overwintering *D. suzukii*. The benefits of cold acclimation may only occur under certain conditions, and once flies are subjected to temperatures outside this thermal range, resource location (a proxy of fitness) may be negatively affected (Kristensen et al., 2008).

Reproductive costs

Since overwintering is a metabolically costly process, it leaves few resources to be allocated to life history traits such as fecundity. For example, Bubliy et al. (2002) found that cold-acclimated *D. melanogaster* put fewer resources into fecundity than did non-

acclimated flies, evidenced by a decrease in the number of progeny produced. This alteration in resource allocation is also evidenced by the different phenotypes for egg-retention in *D. melanogaster*, where flies that can retain eggs for longer periods of time are longer lived, as they avoid wasting resources, and overwintering survival is increased (Bouletreau-Merle and Fouillet, 2002).

In Chapter 2, I found that the mean daily egg production for females across all ages was approx. 5.7 ± 0.24 eggs per day, with a mean total lifetime production of 635.6 eggs. In Chapter 3, I found that mean daily egg production was 4.5 ± 0.91 eggs per day for females mated after a 6-week cold exposure, and 0.36 ± 0.16 eggs per day for females not mated after exposure to cold. These values are not completely comparable, as in Chapter 2 I recorded daily egg production over the female's lifetime, and in Chapter 3 I only recorded daily egg production for a total of 6 days. However, it may suggest that surviving overwintering females are not as fecund as females that would be alive during the growing season, illustrating a tradeoff between fecundity and survival due to resource allocation.

Sibling species *D. melanogaster* and *D. simulans* are both present in the same geographical area, but their abundance after winter depends on the severity of the winter, suggesting variable overwintering strategies (Bouletreau-Merle et al., 2003). *D. simulans* were better at maintaining fecundity at mild temperatures, but *D. melanogaster* were more resistant to cold temperatures and inadequate food supply, allowing them to survive in shelters over winter for a long period of time (Bouletreau-Merle et al., 2003). As stated in Chapter 3, *D. pseudoobscura* females have been observed to store sperm during cold exposure and use the stored sperm to fertilize eggs once conditions had become favourable (Collett and Jarman, 2001). It is possible that mated *D. suzukii* females use

this same strategy while overwintering. These data suggest that there are costs that impact fecundity while overwintering, but *Drosophila* do have strategies to mitigate these effects.

Latitude

Timing of reproduction under winter conditions shows a clinal trend. *D. melanogaster* from higher latitudes tend to produce eggs later than lower latitude populations (Mitrovski and Hoffmann, 2001). This is an adaptive response to thermal stress as only eggs laid in spring were viable (Mitrovski and Hoffmann, 2001). *D. suzukii* females may also lay eggs later when faced with impending winter conditions in order to conserve resources. Male *D. melanogaster* also show clinal variation in overwintering fertility, which is also believed to be an adaptive response to climatic variation (Rako et al., 2009). Temperate populations had higher fertility in field cages in spring, compared to tropical populations, as they were able to inseminate females successfully (Rako et al., 2009). Conversely, Sgro et al. (2006) did not detect a clinal trend in the egg retention phenotype in *D. melanogaster*, even though this trait is thought to increase winter survival by reducing the amount of resources wasted in the overwintering form.

4.2 Future Directions

Life History:

Since host plants can affect the life history of herbivorous insects, it may be worth repeating my life history analysis on a variety of common host fruits. Bellamy et al. (2013) used a fruit-agar diet to assess larval performance, as well as intact fruit to track oviposition and adult emergence. However it would be interesting to obtain more precise

information related to host fruits such as their effect on development time, mean daily egg production, lifespan, and intrinsic rates of increase.

In relation to oviposition, it would also be interesting to determine how the oviposition wounds left by *D. suzukii* oviposition affect infestation rates of other pests. The susceptibility of fruit infested by *D. suzukii* is said to be heightened as the oviposition wound acts as a pathway to secondary infection by other pests and pathogens (Walsh et al., 2011), however this susceptibility has yet to be quantified. This pathway may be important for pests that would otherwise not be able to infest healthy fruit. For example, *Z. indianus* is a newly introduced invasive fruit fly native to the Afrotropics (Renkema et al., 2014). *Z. indianus* is usually only associated with fruit that has fallen and started to rot, and therefore would not be harvested anyway (Renkema et al., 2014). *Z. indianus* were detected in *D. suzukii* traps in Ontario in the 2013 season, and given a pathway for entry into fruit that is damaged by *D. suzukii*, *Z. indianus* may be able to infest this fruit as well, leading to accelerated crop damage. Therefore, a future study investigating the susceptibility of *D. suzukii* infested fruit to secondary infestation is warranted to determine whether other drosophilids have the ability to complete their development on pre-harvest host fruit in the presence of *D. suzukii*.

Overwintering

In Chapter 3, I did not observe any survival below 1 °C, which may be due to the length of cold exposure. To assess whether survival is affected by length of cold exposure, I did conduct trials with flies that were held in the temperature treatment for 4 weeks. Due to time constraints, these results are not included within my thesis, but I continue to work on these results. It may also be useful to repeat this experiment with multiple short cold

exposures with the same total time, as Marshall and Sinclair (2010) demonstrated that this may increase overwintering survival over that seen with constant cold exposure. This method may also be more ecologically relevant as it is more comparable to what would occur during a winter season. My colleagues have also been investigating precise measures of temperature-dependent development, survival, and egg production of *D. suzukii*.

The overwintering habitat is another concern. It remains unknown where *Drosophila* overwinter. It has been suggested that *D. suzukii* may overwinter under leaves and stone (Calabria et al., 2010), or possibly in man-made enclosures (Dalton et al., 2011). In Chapter 3 I kept the flies on banana media that was known to freeze at a low temperature. I housed this media and saturated cotton within a petri dish. This environment is not what would be found in the field however, and future overwintering studies should include a treatment for overwintering habitat, which could help to decipher what kind of environment *D. suzukii* need in order to successfully overwinter. Dalton et al. (2011) suggest that future studies should examine survival in protected environments as well. Field sampling after winter of ground debris or material in man-made enclosures for *D. suzukii* pupae/adults may reveal the type of habitat that they prefer for overwintering.

Effects of cold exposure on male fertility is another factor that could be examined. Bouletreau-Merle et al. (2003) observed that male *D. simulans* remained fertile, while male *D. melanogaster* became sterile at mildly cool temperatures. *D. pseudoobscura* males remained fertile even after a 2-month cold exposure (Collett and Jarman, 2001). Even though female fecundity may be reduced, maintenance of male fertility (or lack thereof) may affect reproductive success after winter. I did not test male fecundity in

Chapter 3, but it may be interesting to explore this trait in a future study. Surviving overwintering males could be mated with fertile females to assess fertility, and tracking this trait over time could elucidate whether male fertility could be restored.

Male diapause was not considered in Chapter 3. Rako et al. (2009) state that little is known about male diapause in *Drosophila*. In a future study on overwintering *D. suzukii*, it may be useful to include short- and long-photoperiod exposure treatments for males, as this may lead to induction of diapause and increased overwintering survival.

Female diapause was considered in Chapter 3. *D. suzukii* females were reared in short and long photoperiods at 15 °C. However I cannot say with certainty that females entered reproductive diapause based on these conditions, as there is some uncertainty in the literature and *D. suzukii* female diapause has not been positively identified. Therefore dissections on short- and long-photoperiod exposed *D. suzukii* females would confirm whether or not females that were exposed to short photoperiods enter reproductive diapause, as the ovaries would be underdeveloped and vitellogenesis (Saunders et al., 1989) or ovariole number (Schmidt et al., 2005) could be observed.

As stated in Chapter 3, my study did not consider the accumulation of cryoprotectants in overwintering *D. suzukii*. The literature on cryoprotectants in *Drosophila* is not consistent, so it is not safe to predict whether *D. suzukii* use this strategy for overwintering. It would be beneficial to test cold exposed *D. suzukii* for these substances to understand the possible climatic range that they could inhabit.

The fact that there are three possibilities to explain the occurrence of viable egg production by overwintering females mated only prior to cold exposure also opens possible avenues of study. To explore the possibility of parthenogenesis in *D. suzukii*, a method similar to Stalker (1954) could be employed. Virgin females could be kept in

preferred conditions on standard *Drosophila* media, which could be replaced and incubated to assess viability. If viable eggs were observed (i.e. eggs that ultimately resulted in adult emergence), this would provide evidence for parthenogenesis in *D. suzukii*, as these eggs would not have been fertilized by males. If parthenogenesis were not possible in *D. suzukii*, this would provide evidence for sperm storage or embryogenesis depression upon cold exposure.

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