Impact of temperature and relative humidity on the eye-spotted bud moth, *Spilonota ocellana* (Lepidoptera: Tortricidae): a climate change perspective

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Abstract

Global climate change models predict an increase in the frequency, severity and duration of extreme weather events. Weather extremes are important for poikilothermic species limited by their capacity to withstand conditions beyond their optimum for survival and development. To understand insect population dynamics, and forecast outbreaks in agro-ecosystems, we need a better understanding of the biology of insect pests of concern. In this study, I explored physiological responses of *Spilonota ocellana* (Denis and Schiffermüller) in the context of spring frost and summer drought, by focusing on the most vulnerable life stages. I determined that *S. ocellana* spring larval instars are susceptible to temperatures above their mean supercooling point (SCP) which ranged from -9.1 ± 0.2 °C (4^{th} instar) to -7.9 ± 0.2 °C (6^{th} instar). While supercooling point increased with instar, the median LLT of -7.3 ± 0.4 °C across all instars demonstrates that a hard spring frost would be necessary to cause larval mortality. Exposure to low humidity resulted in lower egg hatch; this effect was exacerbated at higher temperatures. Furthermore, I discovered that exposure to low humidity during the latter half of egg development resulted in reduced survival and faster development rates; similar effects were also observed during a period of hot and dry conditions in an apple orchard. This study provides information on the impacts of extreme weather events on survival and development within and between life stages of *S. ocellana*, which could have the potential to alter population abundance, phenology, and thus management of this pest.

Keywords: *Spilonota ocellana*; cold tolerance; spring frost; relative humidity; larvae; eggs

To the moon.

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SE	Standard error
LLT	Lower lethal temperature
SCP	Supercooling point
RH	Relative humidity
SD	Standard deviation
INA	Ice nucleating agent

Chapter 1. Introduction

Global surface temperatures have increased significantly during the past century, and are projected to continue to rise under all scenarios assessed by the IPCC (2014). Projected global changes in temperature and moisture regimes are expected to have an impact on poikilothermic species, particularly insects which respond rapidly to changes in ambient conditions (Porter et al. 1991; Easterling et al. 2000; Bale et al. 2002; Parmesan 2006). Temperature and humidity delimit insect species' geographic ranges and often drive shifts in distribution (Lawton 1995; Davis et al. 1998; Bale et al. 2002) because avoiding harmful body temperatures and retaining sufficient water are major physiological challenges for insects (Barton-Browne 1964; Willmer 1980; Hadley 1994; Potter and Woods 2011).

Beyond average temperatures

Increases in average temperature, precipitation, or CO_2 concentrations are often the focus of studies on the effects of climate change on species (Porter et al. 1991; Volney and Fleming 2000; Bale et al. 2002; Parmesan 2006; Estay et al. 2011). Warming can be beneficial by providing more heat units for growth and development (Inouye 2000; Schlyter 2006). In addition, a milder winter may lead to higher rates of overwintering survival (Bale and Hayward 2010) and changes in the timing of spring activity (Porter et al. 1991; Walther et al. 2002). Furthermore, higher temperatures are associated with faster growth and development (Barton and Ives 2014), which may result in more generations per year (Pöyry et al 2011), range expansion (Chen et al. 2009), shifts in phenology (Jepsen et al. 2011), and a higher frequency of outbreaks (Fand 2012; Murdock et al. 2013; Ju et al. 2015).

In temperate regions, analyses of seasonal averages indicate greater warming is occurring in winter and spring (Flannigan et al. 1998, Bonsal et al. 2001; Robeson 2004; Schwartz et al. 2006). Consequently, growing seasons are beginning earlier and lasting

longer, with pronounced effects on phenology and life-history adaptation in many species (Walther 2002; Badeck et al. 2004; Parmesan 2006; Stoeckli et al. 2012). However, global climate models project not only an increase in mean temperatures, but also an increase in temperature variability (Easterling 2000; Parmesan et al. 2000; Katz et al. 2005; Solomon et al. 2008). Greater temperature variability will result in larger amplitudes of seasonal and daily temperature (Vasseur 2014), which can lead to an increase in extreme weather and climate events, including heat and cold waves, droughts, heavy precipitation, flooding and storms (Easterling et al. 2000; Parmesan et al. 2000; Burke et al. 2006; Stoeckli et al. 2012; IPCC 2014; Vasseur 2014). The combination of warming with projected increases in temperature variations will also mean more time spent at temperatures and moisture levels below or exceeding a species' optima (Porter et al. 1991; Meurisse 2012), thereby altering life-history traits such as survival and development (Vasseur 2014; Ju et al. 2015). The effects of extreme weather are especially complex for insects that undergo complete metamorphosis with four separate stage-specific (i.e. egg, larva, pupa, adult) responses to the abiotic environment (Kingsolver and Diamond 2011). The timing of these extreme events is therefore important to consider, as a species' response and vulnerability will differ greatly depending on the life stage in which they experience meteorological extremes (Hódar 2002; Woods and Singer 2001), and the vulnerability of that life stage (Bale et al. 2002; Rouault et al. 2006).

Climate and insect population dynamics

Climate and weather is a major driver of insect population dynamics, and changes are often reflected in annual fluctuations in insect populations (Mattson and Haack 1987; Porter 1991, Parmesan 2006; Saldaña et al. 2007; Thomson 2013; Estay et al. 2011; Kingsolver and Diamond 2011). For instance, long-term data on locust infestations in China have been linked to drought and flooding (Zhang et al. 2009), and in California, several years of warm, dry weather were associated with outbreaks of several species of beetles, aphids, and moths (Mattson and Haack 1987). Following that same period of drought, populations of butterflies, sawflies, and grasshoppers collapsed (Hawkins and Holyoak 1998). A disruption in synchrony can also have major impacts on population densities of leaf-feeding insects, which must synchronize their emergence

with host-plant availability (van Asch and Visser 2007). While milder winters will reduce cold stress for overwintering insects, faster depletion of reserves (Chaplin and Wells 1982; Irwin and Lee 2000) can result in early emergence, putting insects at risk of starvation if the host plant is not yet available (van Asch and Visser 2007; Inouye 2008; Murdock et al. 2013). Low winter snowpack in the Sierra Nevadas of California led to early emergence and starvation of adults of Edith's checkerspot, *Euphydryas editha* (Boisduval) (Lepidoptera: Nymphalidae) in the absence of host flowers. In a subsequent season, summer acclimated adult butterflies succumbed to a late season snowstorm (Singer and Thomas et al. 1996). These extreme weather events have been implicated in the extinction of this population of *E. editha* (Singer and Thomas 1996; Thomas et al. 1996).

Climate and agricultural pests

Climate change is likely to have a significant effect on agricultural insect pests and their potential to cause damage (Porter et al. 1991; Fuhrer 2003; Kurukulasuriya and Rosenthal 2013). Seasonal fluctuations in weather may result in a decrease of some insect populations, and an increase in others; in the case of insect herbivores, this could result in damage to crops. A challenge in agricultural systems is the lack of long term census data for pests below levels of economic concern. This is the result of a constantly shifting pest complex which growers, and pest managers, are attempting to manage. Understanding how insect pest population levels will respond to changes in seasonal weather, including extremes in temperature and moisture, requires more information on their biology (Porter et al. 1991; Parmesan 2006; Sutherst et al. 2011).

Understanding the impacts of climate on pests in agricultural systems is often complicated by management practices such as pesticide applications, understory maintenance, pruning etc. In perennial cropping systems such as apples, management is focused on earlier cropping, increased yields, and low labour requirements (Wertheim 1980; Buler and Mika 2009). This is often achieved through high-density plantings where a tall, narrow tree wall increases light penetration, exposing more surface area of the tree, and each individual leaf, to solar radiation, wind, and other weather elements (Buler and Mika 2009). In high-density apple orchards, with high exposure and minimal shading, irrigation could be a major contributor to orchard microclimate, particularly in

the absence of precipitation. Young trees on dwarfing rootstock grow quickly and rely heavily on irrigation to combat water loss to the elements, and to compensate for their small root area (Buler and Mika 2009). While increased exposure of trees to weather elements will mean less protection of the orchard canopy, irrigation regimes may counteract these effects, to the benefit of both the crop, and its respective pests.

The eye-spotted bud moth, *Spilonota ocellana*

The moth family Tortricidae contains the highest number of apple pests compared to any other insect family (Chapman 1973). The eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae) is a tortrix moth found in fruit-growing regions across the Northern hemisphere (Weires and Riedl 1991). First mentioned in the literature from Austria in 1770, *S. ocellana* is believed to be of European origin (Porter 1924), and was first reported in the United States in 1841 in Massachusetts (Harris 1841). In the early 1900s, *S. ocellana* was reported in Canada in Vancouver and Victoria (Wilson 1912; Brittain 1912), but rarely found in the Okanagan Valley growing region (Brittain 1912). More recently, many studies on this pest have originated from northern parts of the Okanagan Valley (Madsen and Downing 1968; McBrien and Judd 1998, 2004).

Primarily a pest of apple (Frost 1927; Gilliatt 1932; Leroux and Reimer 1959), *Spilonota ocellana* is polyphagous with a wide host range including blueberry (Gillespie 1985), cherry (Oatman et al. 1962), and plum (Madsen and Borden 1949) (see Porter 1924 for complete list of host plants). Population densities can fluctuate widely between years, with high densities and damage one year contrasted with almost undetectable levels of insects and damage the next (MacLellan 1978). In untreated orchards, *S. ocellana* and other tortrix moth pests including leafrollers, can cause 10-20% crop damage (Judd et al. 1996). The bud moth-leafroller pest complex is often controlled indirectly by insecticides applied to control the codling moth, *Cydia pomonella* (L.), (Lepidoptera: Tortricidae) (Madsen and Downing 1968). However, where insecticide applications for control of codling moth have been reduced in lieu of a Sterile Insect Release Programme (Dyck et al. 1992, 1993), or pheromone-based mating disruption (Judd et al. 1996, Judd and Gardiner 2004), feeding damage by leafrollers (Minks and

Deventer 1992; Wearing et al. 1995; Charmillot and Brunner 1989) and *S. ocellana* has been observed to increase (Minks and Deventer 1992).

Recent published reports on the abundance of this pest are rare and the most current studies have focused on the development and testing of control methods in the form of degree-day models (Blommers and Helsen 2001; McBrien and Judd 1998, 2004), pheromone lures that attract *S. ocellana* more effectively (McBrien et al. 1998), or use of multiple-species pheromone-based mating disruption targeting multiple leafroller pests (McBrien et al. 1998; Porcel et al. 2015). This continued attention indicates the persistence of *S. ocellana* as a pest of concern in apple-growing regions, despite the lack of published census data.

Life history of *Spilonota ocellana*

Spilonota ocellana is reported to be univoltine in all published studies (Chapman 1973, McBrien and Judd 2004). However whether it undergoes a true obligatory diapause (Danks 1987) has been questioned (McBrien and Judd 2004). A second generation has been observed in California (www.ipm.ucdavis.edu), and a second generation that arose during rearing of bud moth larvae (*unpublished data*) suggests that diapause is not obligate in this species, and with sufficient heat units *S. ocellana* may complete more than one generation per year. Larvae emerge in early April, with pupation beginning in late May followed by moth flight commencing in early-June, and lasting until early August (Figure 1.1).

Each generation of *S. ocellana* begins with the eggs, which are laid singly on both the upper or lower surfaces of foliage (Porter 1924) starting in early summer (Weires and Reidl 1991; McBrien and Judd 1998). Fecundity ranges from 36 to 144 eggs per female in insectary tests (Gilliatt 1932). Newly hatched larvae chew small pits in the leaf tissue and construct silken shelters, usually next to a vein, or midrib on the underside of the leaf (Porter 1924; Frost 1927). After several days larvae begin feeding and building shelters by tying leaves and floral parts together with silk (Frost 1927; Weires and Reidl 1991). The number of moults varies with latitude; in Canada seven moults have been observed (Gilliatt 1932; Madsen and Borden 1949; McBrien and Judd 2004), whereas six have been reported in Connecticut (Porter 1924), and up to ten in Pennsylvania

(Frost 1922). In British Columbia, larvae undergo several moults before overwintering primarily as fifth- or sixth-instar larvae, with a small proportion of fourth instars (McBrien and Judd 2004) in silken hibernacula on branches near dormant buds of host plants (Porter 1924; Chapman and Lienk 1971). In apple, overwintered larvae emerge in early spring around the early tight-cluster stage of bud development (McBrien and Judd 2004). Larvae burrow into developing buds until leaves open up with which they build new leaf shelters where they will complete their development (MacLellan 1979). *Spilonota ocellana* larvae can be damaging to developing flower buds in the spring, when newly emerged larvae burrow into developing buds, and in the summer, when freshly hatched larvae can injure fruit by fastening leaves to the apple surface and producing shallow feeding excavations (MacLellan 1979). *Spilonota ocellana* pupate in nests of dead leaves and blossoms away from the larval feeding shelter (Oatman and Legner 1963; McBrien and Judd 2004). Adults emerge in early summer, and ovipostion starts approximately four days after females have emerged (Weires and Reidl 1991; McBrien and Judd 1998). Males emerge two to three days before females, and late emerging females have been observed to be smaller and of lower fecundity (Gilliatt 1932). Copulation takes place soon after the adults emerge, followed by nocturnal oviposition of eggs laid singly on both the upper and lower surfaces of leaves (Porter 1924; Frost 1927).

Climate in the Okanagan and Similkameen Valleys

The third largest apple-producing province in Canada is British Columbia (Statistics Canada, Census of Agriculture 2006). The majority of apples produced are grown in the semi-arid southern interior of the province comprised of the Okanagan Valley (160 km long), and the adjacent Similkameen Valley west of the most Southern portion of the Okanagan (40 km long) (Caprio and Quamme 2002). While studies on this pest have originated from central and northern parts of the Okanagan Valley (Madsen and Downing 1968; McBrien and Judd 1998, 2004), for unknown reasons, *S. ocellana* has not been a concern for apple growers in orchards of the Similkameen Valley and southern parts of the Okanagan despite it being observed on nursery stock being brought into the region over the past two decades (Linda Edwards, *pers. comm.*).

Climate in the Okanagan can vary significantly between the north and south ends, with drier and warmer conditions found in the south (Neilsen et al. 2010). From north to south, precipitation ranges from 750 to 300 mm, decreasing in the south and at higher elevations (Neilsen et al. 2006). Summers are generally hot, with daily temperatures in July and August higher than the Napa Valley, California (Rayne et al. 2009). During the growing season, temperature in the southern ends of the valley can be 4 °C higher than the north, with an annual difference of 290 degree days (base 10 °C) between the two ends (Rayne et al. 2009). Maximum temperatures reach up to 40 °C, with highs above 30 °C for several consecutive days (Rayne et al. 2009). A major contributor to variations in regional climate in the valley is the moderating effect of the northern Okanagan's extensive network of lakes, compared to the narrow rivers or canals that runs through the Similkameen and parts of the south Okanagan. As a result, frost risk is greater in the southern parts where the growing season starts earlier (Rayne et al. 2009), and the moderating effects of the lakes are not experienced (Belliveau 2006).

Research objectives

I studied the impact of temperature and relative humidity on the apple pest eye-spotted bud moth, *S. ocellana,* with a focus on two life stages and the weather extremes they are most likely to experience. In Chapter 2, I focused on the egg life-stage and asked if egg hatch and survival would be negatively impacted by low humidity at ambient, and constant temperatures, both in the field and in the laboratory. I further investigated at what stage in egg development humidity is important, and factors that might affect temperature and humidity levels in a high-density apple orchard. In Chapter 3 I focused on overwintered larvae that have emerged and commenced feeding in the early spring and investigated whether they are at risk of freezing if there is a sudden temperature drop after the growing season has begun. Specifically, I measured the supercooling point (SCP) and lower lethal temperature (LLT) for survival for spring-feeding larval instars, in the context of a spring frost event. This thesis gives insight into the physiological responses of *S. ocellana* to temperature and humidity, which could have the potential to alter population abundance, phenology, and thus management of this pest.

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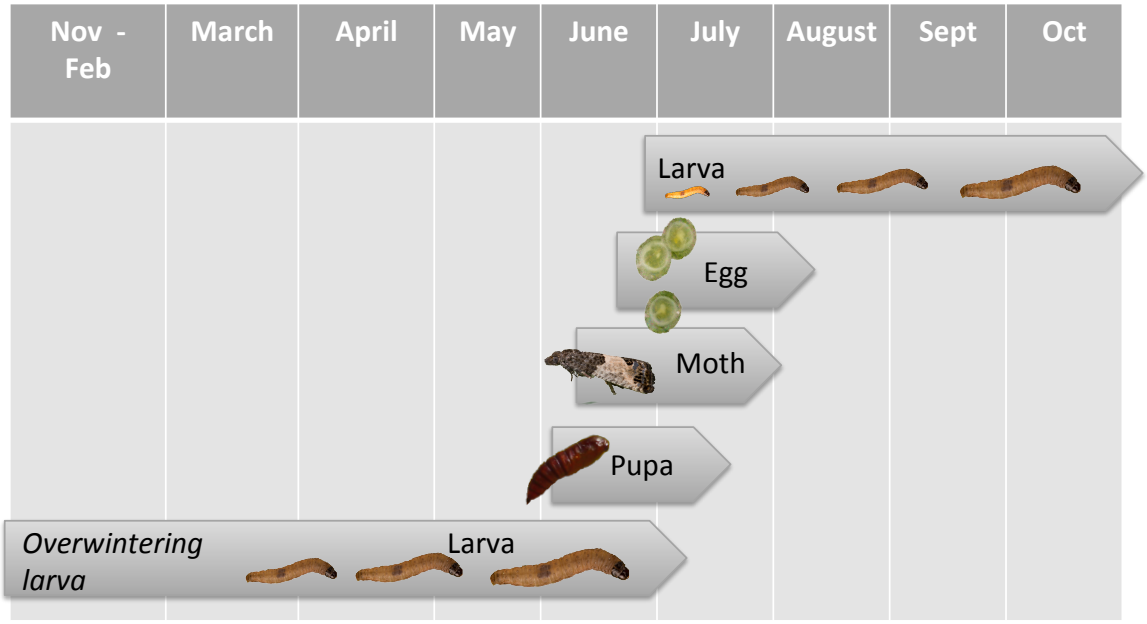

Figure 1.1 **Life history of the eye-spotted bud moth, *Spilonota ocellana*. Horizontal arrows indicate the approximate timing and length of activity of each life stage in the Similkameen Valley, British Columbia. Figure by Jolene Swain, *Spilonota ocellana* larvae, egg, and moth photo credit: Mark Gardiner, Agriculture and Agri-food Canada, Summerland Research and Development Centre.**

Chapter 2. The effects of temperature and humidity on egg hatch and development rate in the eye-spotted bud moth, *Spilonota ocellana*, (Lepidoptera: Tortricidae)

2.1. Introduction

Global surface temperatures have increased significantly during the past century, and will continue to rise under all scenarios assessed by the IPCC (2014). Projected global changes in temperature and moisture regimes are expected to have an impact on poikilothermic species, especially insects, which respond rapidly to changes in ambient conditions (Porter et al. 1991; Easterling et al. 2000; Bale et al. 2002). In addition to warming, models predict an increase in the frequency, duration, and severity of weather extremes (Easterling et al. 2000; Parmesan 2000; IPCC 2014; Vasseur 2014). Extreme meteorological events of extended duration and severity could mean more time spent at temperatures and moisture levels outside a species' optima for survival and development (Porter et al. 1991; Meurisse 2012), thereby altering rates of development, geographic ranges (Chen et al. 2011), and frequency of outbreaks (Murdock et al. 2013). Extreme meteorological events such as droughts, floods, storms, and freezing events, have already been directly related to fluctuations in insect population density (Thomson 1984; Mattson and Haack 1987; Hawkins and Holyoak 1998; Yang and Yang 2001; Inouye 2008). Thomson et al. (1984) observed the collapse of Western Spruce budworm, *Choristoneura occidentalis* (Freeman) during seasons when high temperatures occurred during moth flight and oviposition, whereas high humidity levels due to rainfall and irrigation were considered a key factor in outbreaks of the tarnished plant bug, *Lygus pratensis* (L.) in cotton fields (Yang and Yang 2001). Responses can vary greatly among and within species, and are particularly complex for insects that undergo complete metamorphosis with four separate stage-specific (i.e. egg, larva, pupa, adult) responses to the abiotic environment (Kingsolver and Diamond 2011).

While there is mounting evidence demonstrating changes in species' distributions and abundance, knowledge of the precise causal factors remain unknown either because the basic biology is not well understood (Parmesan 2006; Sutherst et al. 2011), or observations are not made during relevant periods of time (Parmesan 2006). The timing of these extreme events is therefore important to consider, as a species' response will differ greatly depending on the life stage in which unfavourable events occur (Hódar 2002, 2004; Woods and Singer 2001), and the vulnerability of that life stage (Bale et al. 2002; Rouault et al. 2006).

Studies on survival of terrestrial insect life stages across a range of humidity levels (Zhang and Kong 1985; Croft et al. 1993; Castagnoli and Simoni 1994; Walzer et al. 2007) have shown that the egg stage is the most drought-sensitive; a high surface area to volume ratio, combined with an inability to consume free water, makes water retention a major physiological problem (Hinton 1981; Sabelis 1985; Bakker et al. 1993; Williams et al. 2004; Ferrero et al. 2010; Woods 2010; Holmes et al. 2012). While some species that undergo diapause or dormancy in the egg stage are capable of absorbing water vapour from sub-saturated (<99%) air (Yoder and Denlinger 1992; Hadley 1994; Benoit 2010), most insect eggs contain a finite amount of water which they risk losing during gas exchange across the eggshell (Hinton 1981, Bakker et al. 1993, Williams et al. 2004; Woods et al. 2005; Ferrero et al. 2007; Woods 2010). High temperatures can exacerbate sensitivity to low humidity by magnifying the vapour pressure gradient between the egg and its environment and increasing metabolic rate, inevitably increasing rates of water loss during gas exchange across the eggshell (Wharton 1979; Zrubek and Woods 2006; Woods 2010; Potter and Woods 2011). However, eggs can exist in a number of different physiological states during their development, depending on abiotic conditions (Woods et al. 2005; Kambule et al. 2011). For instance, eggs of the brown locust *Locustana pardalina* (Walk.) (Orthopotera: Acrididae) can enter a diapause state in response to adverse conditions (Danks 1987; Kambule et al. 2011), whereas the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), has shown the ability to moderate eggshell conductance by depressing rates of respiration, giving up some metabolic potential in exchange for water preservation (Woods et al. 2005; Woods 2010). This latter strategy is most effective during the early part of development when

metabolic demands of the developing embryo are relatively low (Woods et al. 2005; Woods 2010).

The eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermüller) is a leaf-feeding insect found throughout fruit-growing areas of the Northern Hemisphere (Weires and Riedl 1991). Primarily a pest of apple (Frost 1927; Gilliatt 1932; Leroux and Reimer 1959), *S. ocellana* is polyphagous with a wide host range including blueberry (Gillespie 1985), cherry (Oatman et al. 1962), and plum (Madsen and Borden 1949.039530, -119.703530) (see Porter 1924 for complete list of host plants). Studies of *S. ocellana* in Nova Scotia indicate that population densities can fluctuate widely between years, with high densities and damage contrasted against almost undetectable levels of insects and damage (MacLellan 1978). *Spilonota ocellana* has also been reported as a serious pest in apple orchards of Quebec (Leroux and Reimer 1959), Wisconsin (Oatman and Legner 1963), and periodically in British Columbia (Madsen and Downing 1968). Recent published reports on population levels of this pest are rare and the most current studies have focused on the development and testing of new control methods in the form of degree-day models (Blommers and Helsen 2001; McBrien and Judd 1998, 2004), pheromone lures that attract *S. ocellana* more effectively (McBrien et al. 1998), or use of multiple-species pheromone-based mating disruption targeting multiple leafroller pests (Porcel et al. 2015). This continued attention indicates the persistence of *S. ocellana* as a pest of concern in apple growing regions, despite the lack of published census data.

The generation of *S. ocellana* begins with the eggs, which are laid singly (Porter 1924; McBrien and Judd 1998) on the upper and lower surfaces of leaves (Porter 1924; Weires and Reidl 1991). Newly hatched larvae migrate to the under side of the leaves where they chew small pits in the leaf tissue and construct silken shelter, usually next to a vein, or midrib where they will undergo a moult before moving to leaf shelters formed from leaves and floral parts held together with silk (Porter 1924; Frost 1927; Weires and Reidl 1991). Eye-spotted bud moth larvae overwinter in silken hibernacula on branches near dormant buds of host plants and emerge in early spring, when they chew their way into leaf and flower buds (Porter 1924). Larvae emerge primarily as fifth- and sixth-instar larvae (McBrien and Judd 2004). *Spilonota ocellana* larvae can be damaging to apples in the spring, when newly emerged larvae burrow into developing buds, and in the

summer, when freshly hatched larvae can injure fruit by fastening leaves to the apple surface and producing shallow feeding excavations (MacLellan 1979). Pupation begins in late May, with moth flight commencing in early-June and lasting until early August (McBrien and Judd 2004) (Figure 1.1). Copulation takes place soon after the adults emerge, followed by nocturnal oviposition of up to 450 eggs per female laid singly on both the upper and lower surfaces of leaves (Porter 1924; Frost 1927). Moth flight and oviposition continue for up to two months until early August, coinciding with the hottest and driest months of the growing season.

British Columbia is the third largest apple producing province in Canada, after Ontario and Quebec (Statistics Canada, Census of Agriculture 2012). The majority of apples produced in British Columbia are grown in the semi-arid southern interior represented by the Okanagan Valley (160 km long), and the adjacent Similkameen Valley, west of the most Southern portion of the Okanagan (40 km long) (Caprio and Quamme 2002). Until recently, this pest has not been a concern for apple growers in orchards of the Similkameen Valley and Southern parts of the Okanagan, despite several reports and studies of this pest originating from the central and northern parts of the Okanagan Valley (Madsen and Downing 1968; McBrien and Judd 1998, 2004).

There are many differences between the north and south ends of the Okanagan Valley, including significantly drier and warmer conditions in the south (Neilsen et al. 2010). From north to south, precipitation ranges from 750 to 300 mm, decreasing in the south of the valley, and at higher elevations (Neilsen et al. 2006). A major contributor to variations in regional weather in the valley is the moderating effect of the Okanagan's extensive network of lakes, compared to narrow river, or, canal, that runs through the Similkameen, and parts of the South Okanagan, respectively. During the growing season, temperature in the southern ends of the valley can be 4 °C higher, with an earlier start to the season and a difference of 290 degree days (base 10 °C) in the south (Rayne et al. 2009). Maximum temperatures reach up to 40 °C, with highs above 30 °C for several consecutive days (Rayne et al. 2009).

Until recently, it is possible that the semi-arid climate in the south Okanagan and Similkameen Valley suppressed populations of *S. ocellana*. However, a recent increase in abundance of this species is a growing concern for orchardists in this region (Linda

Edwards, *pers. comm*.). Weather data collected by Farmwest reveal humidity levels 10% higher than the previous 10 year average, combined with a 4°C drop in mean temperature during the month of July for the years (2010, 2011) preceding the population increase (www.farmwest.com). If the cool and wet seasons provided the conditions necessary for a population increase to occur, then it would suggest that the typical hot and dry conditions during the adult flight period and oviposition could play a key role in suppressing *S. ocellana* populations. With more extreme weather events predicted for the region under future climate scenarios (Easterling et al. 2000; IPCC 2014), understanding the impacts of exposure to unfavorable conditions on pest status between seasons will be important for apple producers.

In this chapter I explored the impacts of relative humidity and temperature on eye-spotted bud moth, *S. ocellana.* I focus on the sessile, non-feeding egg, a life stage, known to be vulnerable to desiccation (Hinton 1981; Sabelis 1985; Woods 2010), and based on its seasonal phenology, likely to experience hot and dry summer weather, with the potential for extremes such as heat waves, or drought. I ask whether egg hatch and development rates will be negatively impacted by low humidity, as well as temperature, in the field and laboratory. I further investigate at what stage in egg development humidity is most important, and what factors might contribute to temperature and humidity levels in a high-density apple orchard. This study gives insight into the thermal biology of *S. ocellana*, which could have the potential to alter population abundance, phenology, and thus management, of this pest.

2.2. Methods

Colony source and maintenance

Over 200 *S. ocellana* larvae that had overwintered were field-collected throughout April and May of 2013 and 2014 in three organically managed orchards in the Similkameen Valley, British Columbia (49° 13’ N, -119° 58’ W). Individual larvae were placed in 59 mL plastic cups (Solo Cup Company, Lake Forest, IL) and maintained in a field station in the Similkameen Valley at ambient light and temperature conditions. Larvae were supplied with fresh apple leaves collected from organic orchards for food

and construction of leaf shelters. Apple leaves were sprayed with a 2 % bleach solution and rinsed to remove potential contaminants prior to introduction. Leaves were replenished every 2-3 days until pupation.

Mass mating and egg collection

Pupae were wrapped in a crumpled piece of paper towel and moved to individual 29 mL solo cups (2 cm^2) until hatch. After eclosion, up to ten *S. ocellana* moths were transferred to 640 mL plastic bug dorms (BugDorm model BDPS32) for mass mating. Mating chambers were lined with vented plastic bags (Ziploc brand, SC Johnson Company) and with a sugar solution (5% sucrose) on a cotton wick. Pieces of plastic with individual or small clusters of eggs were cut from the bags and used in subsequent experiments.

2.2.1. Laboratory Experiments

Control of relative humidity

Relative humidity (RH) in all laboratory experiments was maintained using saturated salt solutions. Potassium acetate (KAc), calcium chloride ($CaCl_2$), sodium chloride (NaCl) or potassium nitrate (KNO_3), were used to generate and maintain RH levels close to 20, 35, 75, or 95% respectively (Winston and Bates 1960) (Table 1, 2). Each airtight humidity container was constructed from a plastic bug dorm (BugDorm model BDPS32) with a 300 µm mesh nylon screen lid that was inverted over a lower plastic container (340 mL or 125 mL) sealed with Parafilm (Bemis, Neenah, WI, USA) (Figure 2.1). Each lower chamber contained a different saturated salt solution. Relative humidity and temperature within each lower chamber were measured with data loggers (HOBO U23 v2 Temperature/Relative Humidity Data Logger – U23-001). Relative humidity varied slightly due to fluctuating temperatures and across fixed temperature treatments, see Table 1 and 2 for actual values.

Experiment 1: Effect of relative humidity on survival and development time of Spilonota ocellana eggs

Spilonota ocellana eggs laid on 26 days (June 5th-9th, 11th, 12th, 16th, 18th, 19th, 26th-30th, July 1st-7th, 13th-16th) by mass-mated females were excised in small groups, from plastic liner bags daily and distributed in groups across humidity containers. An average of 35 eggs were distributed daily, (ranging from 8 to 67) depending on how many were laid the night before. Each collection day solo cups containing eggs were distributed daily in groups of 3 - 15 per humidity chamber (20, 35, 75 and 95 % RH, 10 replicates per humidity level) which were then maintained in the field station at ambient temperatures ranging between 15 and 30 °C (mean of 24.4 °C). Egg hatch was monitored daily by removing cups from humidity chambers and inspecting eggs under a microscope if necessary. Humidity chambers were opened and closed quickly to reduce fluctuations in humidity levels. Egg hatch was considered successful when neonate larvae exited the egg-shell.

Experiment 2: Effects of different low-humidity regimes on egg hatch and development rate

Eggs from the same batches used in experiment 1 (groups of eggs ranging from 3 – 15) were assigned to either a 20 or 95 %RH humidity chamber for one (n=16,16), two (n=17,15), three (n=22,17), four (n=15,14) or five (n=9,9) days, before being transferred to the opposite humidity. Eggs that started at low humidity (20 %RH) were moved to the high humidity chamber (95 %RH), or *vice versa*. Groups of eggs for each treatment were assigned to the same humidity chamber, differing only in the number of days before being transferred. Proportion egg hatch was compared to the proportion of time spent at the initial humidity, including eggs that spent the entire duration at low, or high humidity from the previous experiment. Data were then grouped and egg hatch and developmental rate were compared for those eggs that spent up to the first half of development at low humidity, to eggs that spent most of the second half of development at low humidity.

Experiment 3: Combined effects of temperature and humidity on survival and development of S. ocellana eggs

Eggs collected from mass-mated groups of insects on four dates (July 4th, 7th, 8th and 15th) were used in a full factorial experiment (5 temperatures x 4 humidity levels). Eggs (N=138) were distributed across two replicate containers of each humidity level (20, 35, 75, and 95 % RH) assigned to a temperature incubator (15, 20, 25, 30, 35 °C). Temperature incubators at the Summerland Pacific Agri-Food Research station were used and included a Conviron EF-7 or A1000 (15, 20, 30, 35 °C), and Fisher-Scientific PGV36 (25 °C). All studies were carried out under a 16:8 L:D light regime. In order to reduce fluctuations in temperature and humidity while monitoring for hatch, the excised piece of plastic containing the eggs was attached with a paper clip to a piece of cardboard taped to the inside of the container. Thus hatch could be determined without opening the air-tight humidity container. Egg hatch was recorded daily and the experiment lasted for 21 days, at which point any remaining eggs were considered to be dead.

2.2.2. Field Experiment

Field site

Field experiments took place in a 40 hectare apple orchard in the Similkameen Valley (49.039530, -119.703530) which was managed in accordance with Canadian organic standards. Approximately 0.4 hectare blocks were selected, consisting of trees planted in high-density rows (30 – 40cm spacing) and a super spindle design (Nüberlin 1993) (Figure 2.2). Blocks were irrigated with a mist irrigation system (micro-jet) on a six-day rotation. Micro-jet sprinklers were suspended from the orchard trellising between two trees approximately 50 cm above the ground and released a mist that wetted the orchard floor along and between high-density rows of trees. High-density management includes heavy pruning and a minimal canopy, to maximize exposure of the foliage and fruit to solar radiation. In the absence of precipitation, irrigation is the only source of free water during the heat of the summer.

Experiment 1: Effects of egg laying phenology, placement within the canopy and irrigation on survival and development S. ocellana eggs

Thirteen blocks were monitored for temperature and humidity with Hobo data loggers (HOBO U23 v2 Temperature/Relative Humidity Data Logger - U23-001) placed in the middle of the upper and lower canopy of one tree (200 - 300 cm) in the center of each block (i.e. at 100 and 300 cm for a 400 cm tree). Temperature and humidity were measured at 15 min. intervals throughout two sampling periods (June 3rd - 17th and June 30th - July 12th). The first sampling period followed the date of the first male moth captured in pheromone-baited traps (biofix) for the 2014 season (June 2nd). Male moths were monitored using Scentry® pheromone baited wing traps to record patterns in flight (Figure 2.3), which are also indicative of the egg laying period by the females (Van Dinh 1988). Eggs on plastic were attached using paper-clips, to the upper surfaces of leaves within the lower and upper canopy of a tree in the center of the orchard block (a different tree was used each day). An average of 35 eggs were distributed daily, (ranging from 8 to 67), depending on how many were laid the previous night. Eggs on the porous plastic were selectively placed on trees in blocks where irrigation was either set to commence the following day, or in 5 days. Blocks were irrigated on a six-day rotation for a twelve-hour period (6 am – 6 pm). Some eggs went missing during the experiment potentially due to loss of adhesion to the plastic, or predation, though no remnants remained to identify potential predators or parasitoids. Egg hatch and development rate were compared between two time frames sampled (early and mid-season flight), the two canopy levels (upper and lower), and the time before the next scheduled irrigation (1 or 5 days).

2.2.3. Statistical Analyses

Egg hatch and development – laboratory experiments

Egg hatch proportions (number of eggs that hatched out of the total) were analyzed using a generalized linear model with binomial error distribution and a logit link function. Chi-square pairwise contrasts were used to determine significant differences between humidity (and temperature) treatments. The inverse of days to hatch was calculated to normalize the data and is reported as the development rate (days^{-1}). Mean development rate was analyzed using a one-way analysis of variance (ANOVA) with

date as a blocking factor. Significant results were followed by a post-hoc comparison of means using the Tukey-Kramer HSD test. Significant chamber effects were included in the models as a blocking variable to account for variation between groups of eggs.

Egg hatch and development – field experiments

Temperature and humidity data in the field experiments were analyzed using a full factorial three-way analysis of variance (ANOVA) with canopy height, irrigation (on or off) and sampling period as main effects. The date egg batches were distributed, and orchard block were included in the model as blocking variables. Trees to which eggs were attached were nested in the orchard block to account for variation between trees.

The significant cut-off for the p-value was 0.05 for all tests. Non-significant terms, and interactions, were removed sequentially to produce the final minimal model. The effects of chamber (for laboratory experiments) and orchard block (for field experiments) were included in the model if significant effects were detected. All data were analyzed with JMP (version 11 SAS Institute, Cary, NC, USA).

2.3. Results

2.3.1. Laboratory Experiments

Experiment 1: Effect of relative humidity on the survival and development rate of Spilonota ocellana eggs

Relative humidity had an effect on mean egg hatch of *S. ocellana* (χ^2_3 = 116.742, $p < 0.0001$). (Figure 2.4), but did not affect the development rate of eggs ($F_{(3,17)}$ = 1.29, $p < 0.281$) (Figure 2.5). Time to hatch ranged from 5 to 10 days (mean: 7.05 SD 1.1 days) with the mean developmental rate of eggs at 0.145 ± 0.002 $days^{-1}$.

Experiment 2: Effects of different low-humidity regimes on egg hatch and development rate

As the proportion of time spent at the 20 % RH increased, egg hatch decreased, whether eggs were moved from 95 to 20 % RH ($F_{(3,65)}$ = 4.689, p=0.0031) or from 20 to

95 % RH ($F_{(3,65)}$ = 5.458, p=0.0020) (Figure 2.6). When the data were grouped into eggs that spent the first half versus second half of development at low humidity, the eggs developing under low humidity conditions early in development, had significantly higher mean survival than those developing under low humidity conditions during the second half of development (χ^2_1 = 43.142, p < 0.0001) (Figure 2.7). Development rate was significantly slower for eggs exposed to low humidity during the early stages of development ($F_{(1,127)}$ = 4.4073, p = 0.0378) (Figure 2.8). Mean development time for eggs ranged from six to eleven days and lasted 7.8 ± 0.2 days for eggs exposed to low humidity during early development, and 7.5 ± 0.2 days for eggs exposed to low humidity during late development.

Experiment 3: Combined effects of temperature and humidity on survival and developmental rate of S. ocellana eggs

Egg survival

No eggs hatched at 35 °C; this temperature treatment was considered beyond the upper development threshold and excluded from further analysis. Across the remaining temperature treatments (15, 20, 25, and 30 °C), there was a strong negative effect of decreasing relative humidity on egg survival (Temperature*Humidity: χ^2_3 = 40.185, p < 0.0001) (Figure 2.9). The effect of temperature on egg survival (χ^2_1 = 3.3092, p = 0.0689), and the interaction between temperature and humidity (χ^2_3 = 7.458, p = 0.0587) were very close to significance, suggesting that some temperature-humidity combinations were detrimental to egg survival (Figure 2.9).

Development rate and time to hatch

Development rate was affected by the interaction between temperature and humidity (temperature*humidity: $F_{(3,138)}$ = 12.412, p<0.0001; humidity: $F_{(3,138)}$ = 11.959, p < 0.0001). Development was significantly slower when low humidity was combined with increasingly high temperatures (20% RH was different from the other RH levels, Tukey HSD: p<0.0001) (Figure 2.10). As would be expected, rate of development was significantly faster as temperature increased ($F_{(1,138)}$ = 191.983, p<0.0001). Only one egg hatched at the highest humidity and temperature combination (20 % RH and 30 °C); the

interaction was still significant when the analysis was carried out without this point ($F_{(3,137)}$= 4.957, p=0.0027).

2.3.2. Field Experiment

Experiment 1: Effects of egg laying phenology, placement within the canopy and irrigation on survival and development of eggs

Irrigation did not affect temperature within canopy level (irrigation: $F_{(1,22)}$ = 1.284, p = 0.2394), but the temperature in the lower level was slightly, but significantly, lower than in the upper level when the irrigation was on (canopy level*irrigation: $F_{(1,22)}$=5.84, p=0.0157) (Table 2.3, A1). Mean temperature during the mid-flight period (June 30th – July 12th) was 1.1 °C higher than during the early flight period (June 3rd – 17th, 18.5 ± 0.1 °C). There was a significant interaction between flight period and irrigation ($F_{(1,22)}$ =17.325, p<0.0001, Table 2.3, A1) with highest temperatures measured during the mid-flight period when irrigation was off (19.9 ± 0.1 °C), followed by when irrigation was on (19.3 ± 0.1°C), and lowest temperatures during early flight with, or without irrigation (18.4 ± 0.2°C).

There was an interaction between canopy and irrigation ($F_{(1,22)}$=6.430, p=0.0112), which indicated that the highest humidity levels were measured in the lower canopy, when irrigation was on (irrigation: $F_{(1,22)}$ = 0.124, p=0.725) (Table 3). Overall, humidity levels were significantly higher in the lower canopy ($F_{(1,22)}$=73.787, p<0.0001, Table 3). In addition, relative humidity levels were significantly higher ($F_{(1,22)}$=8.276, p=0.004) during the early flight period (54.2 + 0.7 %) than later in the season during mid-flight (53.0 + 0.6 %). There was no evidence of an interaction between canopy, or irrigation, and sampling period ($F_{(1,22)}$=1.150, p<0.235 and $F_{(1,22)}$=2.557, p<0.110 respectively (Table A2). There was no evidence of a three-way interaction (canopy*irrigation*season; $F_{(1,22)}$=1.181, p<0.277, Table A2).

The effect of sampling period, irrigation, and canopy height on egg survival and development

Neither irrigation (χ^2_1 = 0.9736697, p = 0.3238) nor canopy level (upper or lower) (χ^2_1 = 0.0150, p = 0.9027) altered mean egg hatch. Mean percent egg hatch was significantly greater during the early flight period (June 3rd – 17th) compared to mid-flight (June 30th – July 12th) (χ^2_1 = 4.057, p = 0.044) (Figure 2.11).

Rates of egg development were also not affected by irrigation ($F_{(1,97)}$ = 0.359, p = 0.551) but there was a strong, though non-significant ($F_{(1,97)}$ = 3.690, p = 0.059) trend towards a faster development rate in the upper canopy compared to the lower canopy. Not surprisingly, egg developmental rates were slower ($F_{(1,97)}$=66.671, p<0.0001) in the early sampling period compared to the later period (Figure 2.12).

2.4. Discussion

Considering the inability of most eggs to seek and consume free-water, it is not surprising that this life stage is drought-sensitive (Sabelis 1985; Hinton 1981; Clark and Faeth 1998; Woods 2010). Based on this study, I conclude that the eggs of *Spilonota ocellana* are vulnerable to water loss under low humidity conditions. Lowest egg hatch was observed in the lower humidity treatments, 20 % RH and 35 % RH. However, percent hatch also decreased when the air was close to saturation (95 % RH) suggesting that too much moisture is not optimal. High temperatures compounded the risk of desiccation. The combination of 20 % RH with the highest temperature (30 °C), resulted in the lowest egg survival overall, and slowest developmental rate relative to all other humidity levels. These results are similar to those found with many species of Lepidoptera (Clark and Faeth 1998) which exhibit sensitivity to desiccation. Given that *S. ocellana* oviposition occurs during the summer months, frequent and extended periods of extremely hot and dry weather could result in *S. ocellana* laying eggs under conditions that are not favourable for survival. Thus the projected increase in the frequency and severity of extreme weather (Easterling et al. 2000; Parmesan 2000; Stoeckli et al. 2012; IPCC 2014; Vasseur 2014), could play an important role in population dynamics of this pest.

My study further demonstrated that timing of exposure to low humidity during egg development had an impact on survival and development rate. *Spilonota ocellana* eggs

that experienced low humidity during the second half of embryonic development had significantly lower survival, and faster developmental rates than eggs that were exposed to low humidity during the first half of development. Longer development times observed for eggs that were exposed to low humidity levels during the first half of embryonic development, suggests that *S. ocellana* eggs may have the ability to adjust their physiological state in response to ambient conditions, similar to responses observed in other species (Danks 1987; Kambule et al. 2011; Woods and Singer 2001). Longer development times as a result of depressed metabolic rates have been found in eggs of *M. sexta* (Woods et al. 2005; Zrubek and Woods 2006; Woods 2010). This lowered metabolic state as a mechanism to conserve water, may not be as effective during the second half of development, when metabolic demands increase (Woods 2005). Alternatively, faster development under dry conditions prior to hatch may also be a strategy to avoid potentially lethal conditions. This strategy may be important in allowing *S. ocellana* to cope with weather extremes such as drought, or heat waves.

Lower survival during the later stages of egg development also make sense in light of the metabolic demands of the developing embryo (Woods et al. 2005; Woods 2010; Kambule et al. 2011). Increased rates of respiration likely resulted in more rapid water loss (Woods 2010), increasing the likelihood of desiccation of *S. ocellana* eggs. Low humidity can also be lethal by killing embryos directly, or hardening the eggshell to the point that hatchling larvae are unable to escape (Buxton 1932; Kimura and Masaki 1977; Simelane 2007), which could also contribute to the detrimental effects of low humidity before hatching. Under controlled conditions, low humidity was most detrimental when it occurred during the latter stages of embryonic development, before hatch. Further understanding of the physiological mechanisms for coping with water loss in the egg stage would help to understand the implications of the timing of low humidity events during egg development in the field. In addition, understanding the potential for sub-lethal effects that influence fitness of early larval instars, and subsequent life stages, could provide further information into the consequences of low humidity during the egg stage.

Not surprisingly, percent egg hatch during the early, cooler, sampling period in the first two weeks of June was greater than during the later sampling period. Weather during the earlier sampling period, following male moth flight (biofix) in early June, was

characterized by, lower temperatures, and higher humidity levels, relative to the hot and dry conditions in early July. Warmer drier weather, combined with observations that late emerging females are smaller and of lower fecundity (Gilliatt 1932), suggest that controls for this pest should be targeted at larvae hatching early in the summer. These results also indicate that the timing of peak flight and oviposition during the hottest time of the season may result in sub-optimum conditions for egg hatch and development.

Mist irrigation maintained significantly cooler temperatures and higher humidity levels in the lower half of the tree canopy. Although these differences were statistically significant, the biological difference was minimal, as the mean lowest humidity (upper canopy, irrigation off) was less than 4% lower than that of the lower canopy (irrigation on). Based on observations of canopy temperature and humidity, it is not surprising that irrigation and canopy level did not affect egg survival of development. Further investigation into microclimate effects including tree structure and current management practices are needed to understand pest dynamics, particularly in high-density orchards that are heavily pruned for maximum solar exposure.

Temperature and water balance play a major role in insect survival and development, thus in regions where extreme heat waves or drought are likely occur it will be helpful to crop managers to understand the potential for changes to the current, and following season, pest complex. Given the long oviposition period of *S. ocellana* (June to early August, Figure 1.1), not all eggs will be experience to the same weather. Timing of egg hatch during the summer not only influences the chance of hatching, but will also impact the amount of time available for growth and development of larvae. Larvae that hatch earlier will have more heat units available for growth, and consequently overwinter as larger instars, which could influence their overwintering ability. If larvae that hatch earlier are selected for in regions that experience frequent high temperature extremes, the phenology in these regions *S. ocellana* may shift, thus mitigating the effects of weather extremes. Phenological responses of insect herbivores to changing weather often address the potential for a mismatch in timing of spring events such as budburst and emergence (Walther et al. 2002; Parmesan 2006). A comparison of the timing of life history events between *S. ocellana* populations (i.e. transplant experiment) could provide clues into the plasticity of the seasonal phenological timing of oviposition.

Fluctuations in insect populations following periods of extreme weather are not new, and periods of warm, dry weather are often associated with pest outbreaks and declines in temperate regions (Porter et al. 2011; Thomson et al. 1984; Mattson and Haack 1987; Hawkins and Holyoak 1998). Mortality caused by desiccation may play a role in population dynamics and the geographic distribution of *S. ocellana*. Whether the detrimental effects of hot and dry conditions during summer are enough to suppress population levels is unclear. Longer term monitoring of pest species is needed to confirm if trends in weather or climate contribute to population dynamics. The fact that *S. ocellana* has been observed in the area before the recent population increase suggests that something has been directly, or indirectly, keeping this pest below economically damaging levels. As no long-term records are available for this pest, it is difficult to determine the driving forces behind the recent observed increase in abundance of this pest. The present results can be used as a basis for modeling the development of *S. ocellana* under changing conditions of both temperature and relative humidity.

With increased variability expected in our future climate (Easterling et al. 2000; Vasseur 2014; IPCC 2014), the effects of extreme weather events on insect pests of agricultural crops will be important to our understanding of population dynamics. It remains to be seen if a return to hot and dry summers will suppress populations of *S. ocellana*, and whether their numbers will climb again following seasons with cool and humid conditions during the flight period. This study provides evidence that the survival of *S. ocellana* is reduced by high temperature and low humidity conditions typical during the moth flight period in the southern-interior growing region of British Columbia. A positive implication of the lethal effects of high temperatures and low humidity on eggs is that increased temperatures and higher frequency of extreme weather predicted under climate change scenarios may suppress *S. ocellana* populations below economically damaging levels in future growing seasons. In agricultural systems, understanding the seasonal fluctuations in pest abundances remains a major challenge. Focusing on when extreme weather is likely to occur, along with the vulnerability of the life stage likely to experience those extremes, can provide a starting point for understanding the responses of organisms in a changing climate.

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2.7. Tables

Table 1 **Salt solutions with corresponding relative humidity levels used in *Spilonota ocellana* egg hatching experiment at ambient temperature (mean 24.4 °C).**

Solutions	Relative humidity (%± SD)
potassium acetate (KAc) + H_2O	21.7 ± 0.7
calcium chloride ($CaCl_2$) + H_2O	38.8 ± 3.6
potassium nitrate (KNO_3) + H_2O	73.4 ± 3.1
sodium chloride (NaCl) + H_2O	92.7 ± 1.5

Table 2 **Salt solutions with corresponding mean relative humidity levels (± SE) used in *Spilonota ocellana* egg hatching experiment at four fixed temperatures (15, 20, 25, 30, 35 °C).**

Solutions	Relative humidity (%± SD)
potassium acetate (KAc) + H_2O	21.7 ± 5.3
calcium chloride ($CaCl_2$) + H_2O	40.7 ± 8.9
potassium nitrate (KNO_3) + H_2O	72.6 ± 7.9
sodium chloride (NaCl) + H_2O	92.8 ± 3.7

Table 3 **Mean (± SE) temperature and humidity levels in the apple orchard where eggs were attached to trees and monitored for hatch and time to development. Temperature and humidity levels were recorded at two canopy heights (upper and lower) and when irrigation was on, or off.**

Canopy	Irrigation	Temperature (°C)[a]	Relative humidity (%)[a]
Upper	Off	19.2 ± 0.1a	52.2 ± 0.6a
Upper	On	19.3 ± 0.2a	51.3 ± 0.8a
Lower	Off	19.0 ± 0.1ab	54.8 ± 0.8b
Lower	On	18.6 ± 0.2b	56.0 ± 0.8b

[a] Letters represent significant differences

2.8. Figures

Figure 2.1 Relative humidity containers using saturated salt solutions.

Figure 2.2 High density super spindle orchard training system

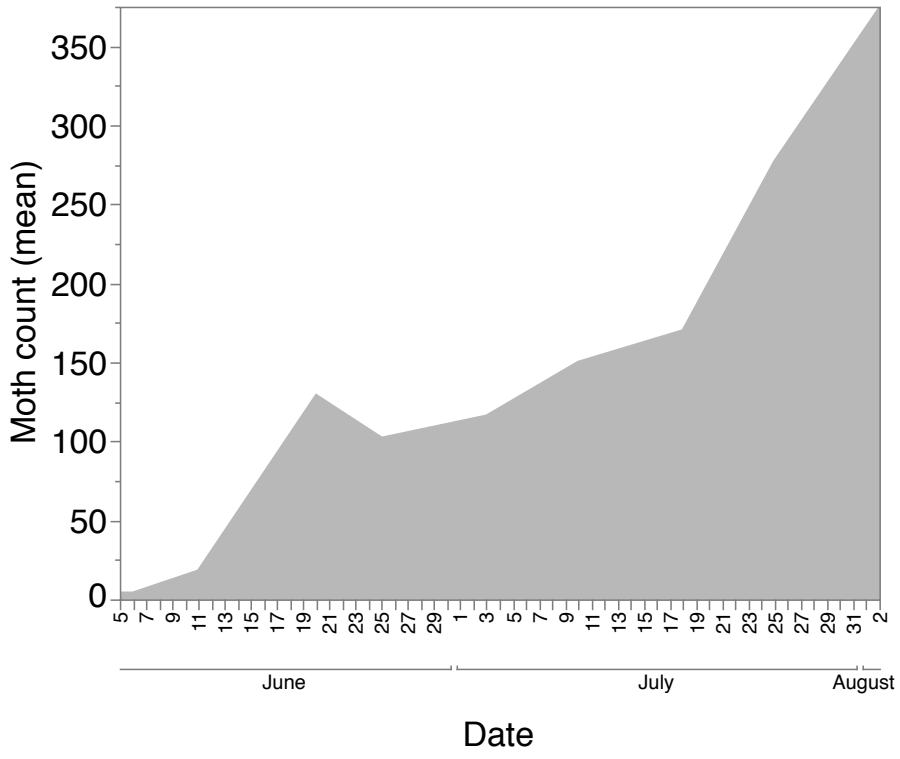

Figure 2.3 **Flight curve for *Spilonota ocellana* based on cumulative male moth catches in traps baited with Scentry® pheromone lure. Traps set in 13 orchards in the 2013 growing season in the Similkameen Valley, British Columbia.**

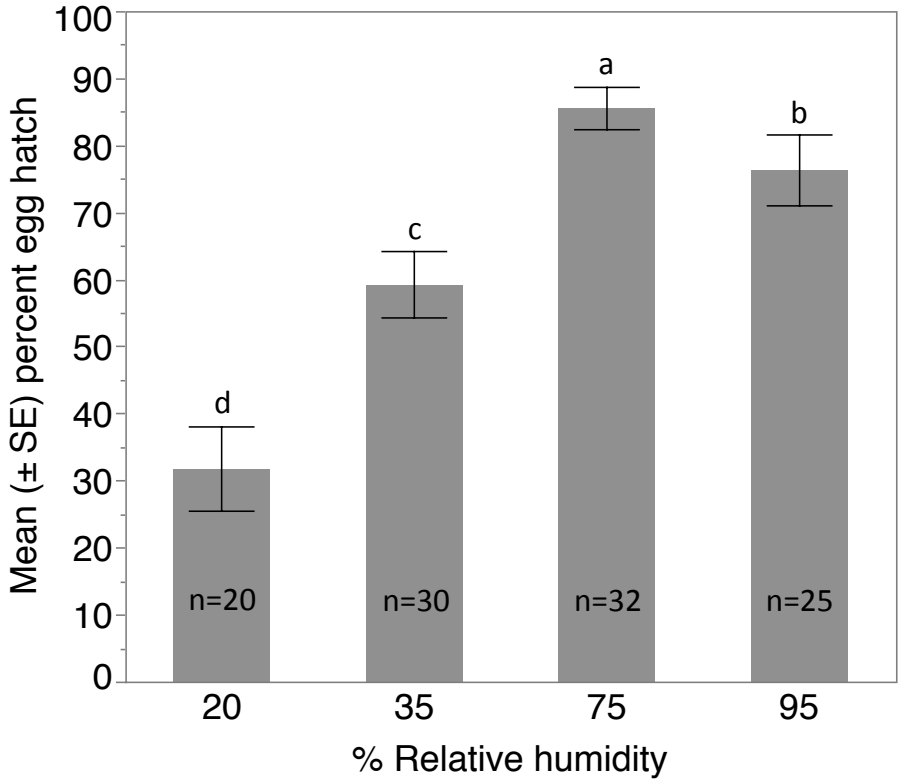

Figure 2.4 **Mean (±SE) percent hatch of laboratory-reared eggs of *Spilonota ocellana* at different relative humidity levels and an ambient mean temperature of 24.4 °C (χ^2_3 = 116.742, p < 0.0001). Different letters above each bar represent significant differences between least-square means (Chi-square contrast test).**

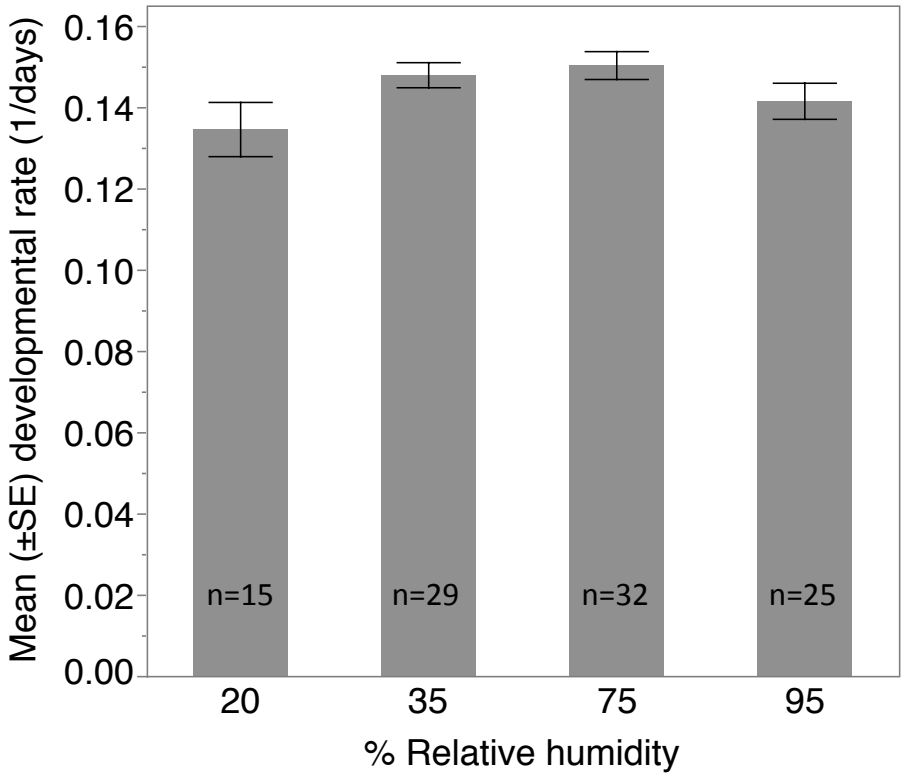

Figure 2.5 **Mean (± SE) developmental rates ($F_{(3,17)}$ = 1.29, p < 0.281) of laboratory-collected eggs of *Spilonota ocellana* held at different relative humidity levels and a mean ambient temperature of 24.4°C.**

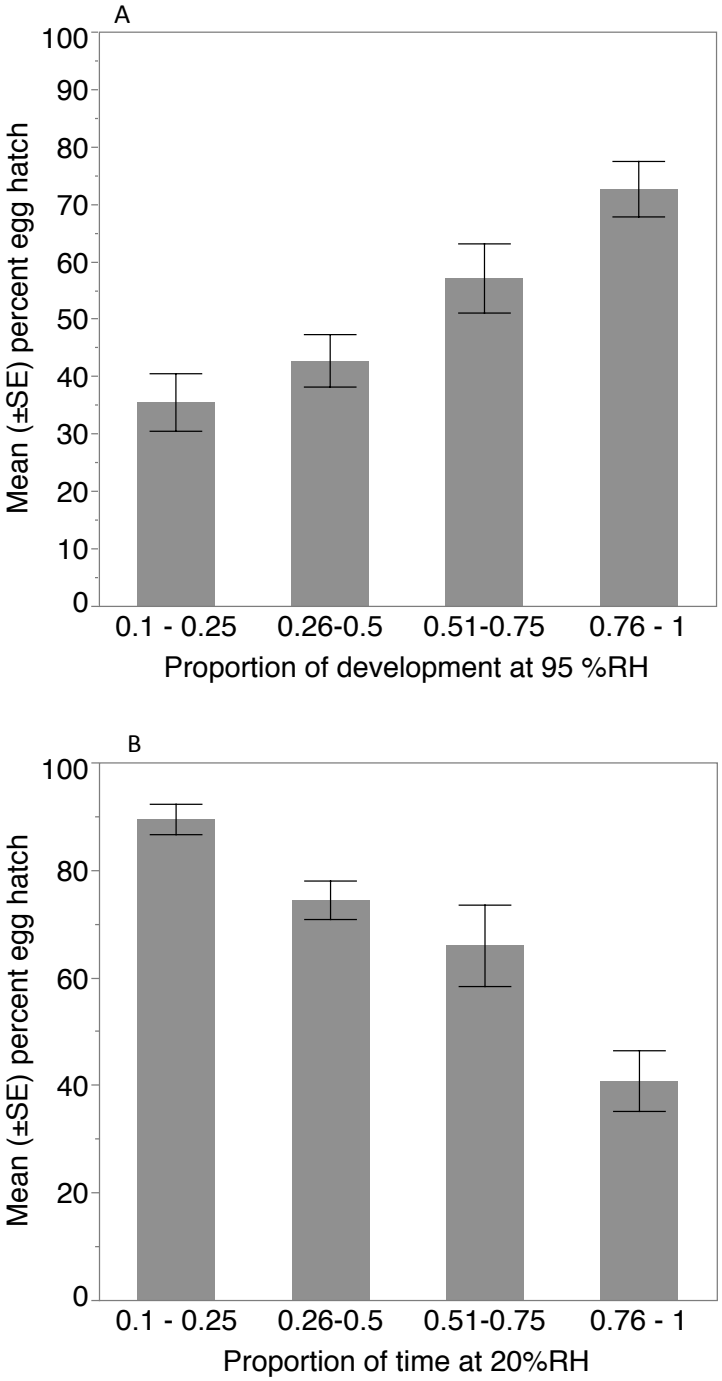

Figure 2.6 **Mean (± SE) percent hatch of laboratory-reared (ambient mean temperature of 24.4 °C) eggs of *Spilonota ocellana* held at 95 %RH (A, $F_{(3,65)} = 4.689$, p=0.0031) or 20 %RH (B, $F_{(3,67)} = 5.458$, p=0.0020) for one to five days before finishing development at the opposite humidity level.**

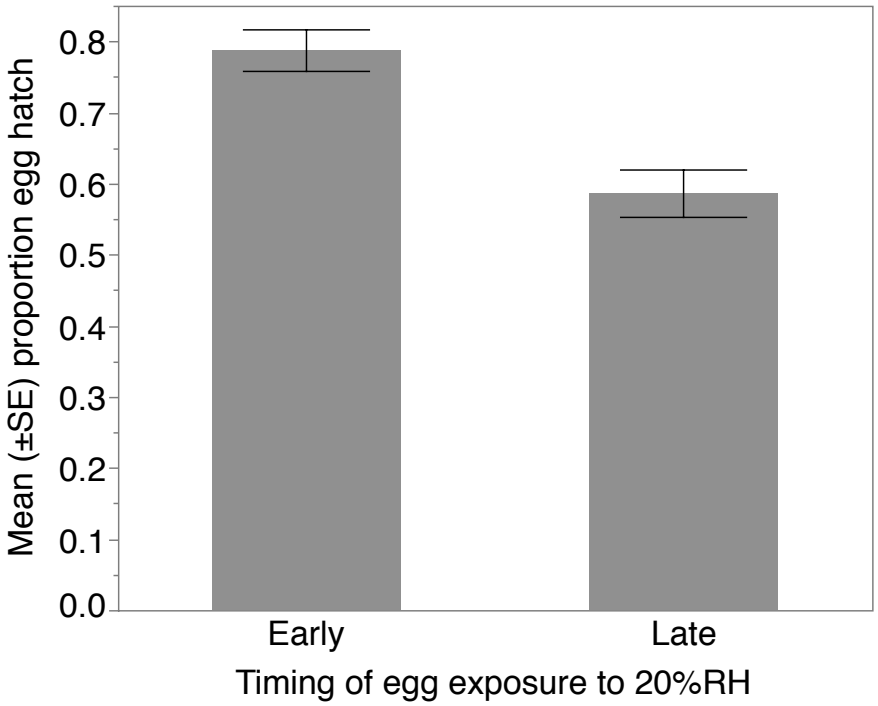

Figure 2.7 **Mean (± SE) survival of *S. ocellana* eggs held for the first or second half of embryonic development at low humidity (20% RH) and the remainder of development at 95 % RH ($\chi^2_1 = 43.142$, $p < 0.0001$).**

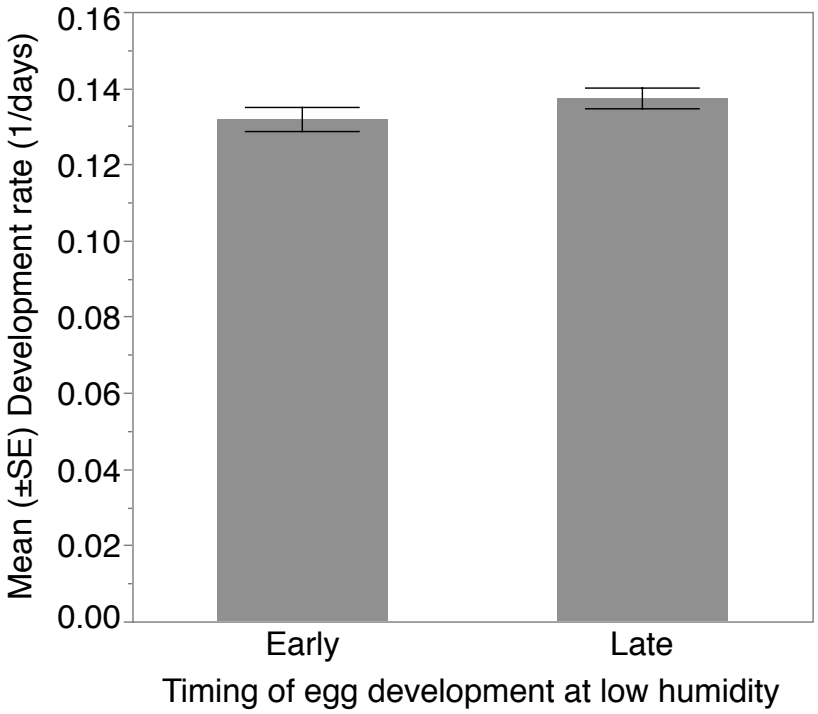

Figure 2.8 **Mean (±SE) development rate for *Spilonota ocellana* eggs that spent a portion of the first half (early) or second half (late) of embryonic development exposed to 20% RH. Remainder of development was spent at 95%RH ($F_{(1,127)}$ = 4.4073, p = 0.0378).**

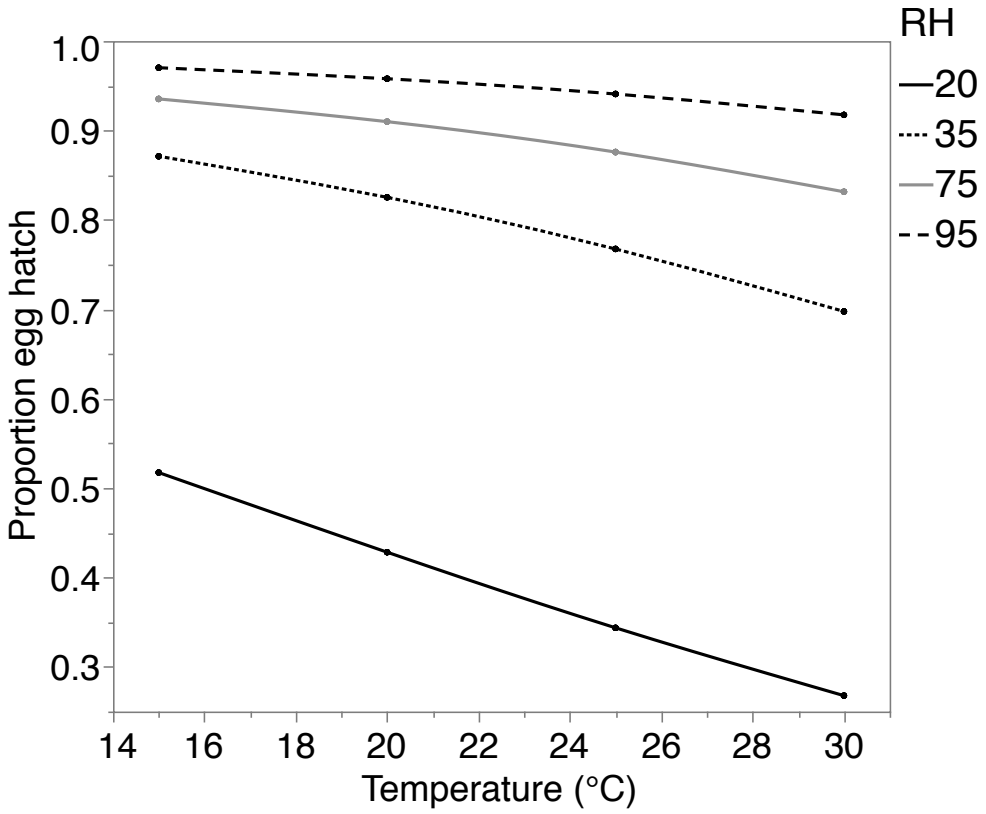

Figure 2.9 **Predicted proportion egg hatch of *S. ocellana* eggs across four relative humidity (RH) treatments and four temperatures (15, 20, 25, 30°C). Lines are the fitted generalized linear model, points represent mean egg hatch at each treatment combination and include the close to significant interaction (Temperature*RH, $\chi^2_3 = 40.185$, p = 0.0587). No survival was recorded at highest temperature treatment, 35°C, which was excluded from the analysis.**

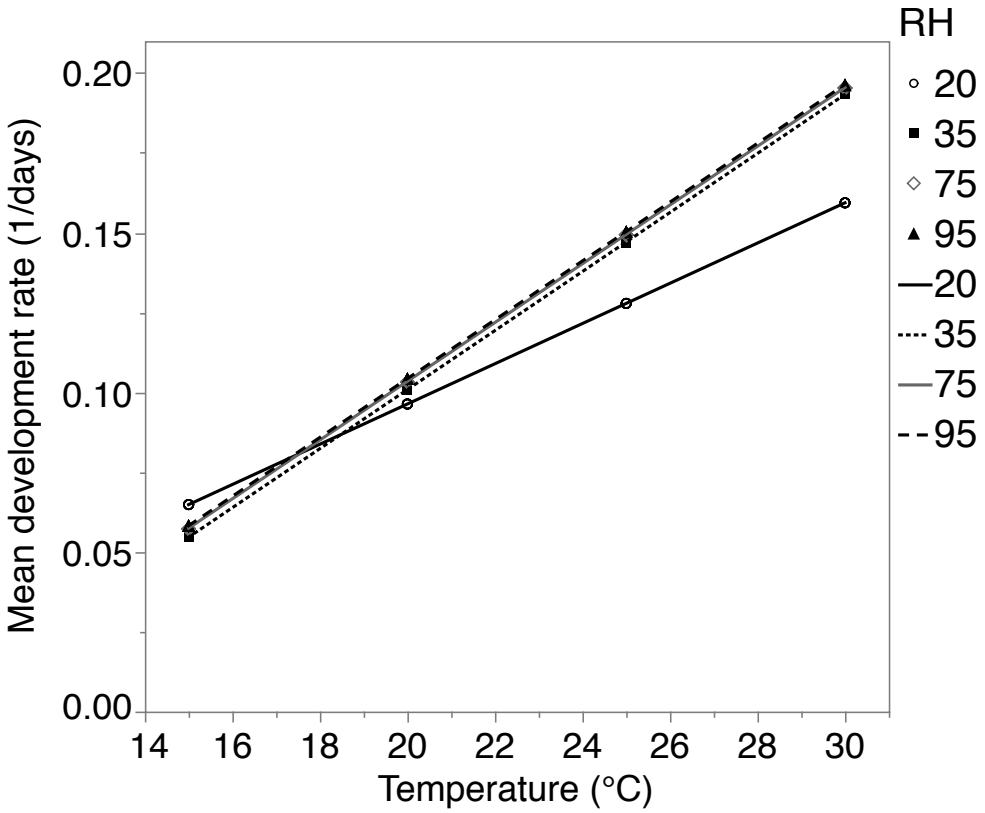

Figure 2.10 **Predicted development rate of *S. ocellana* eggs across four temperatures (15, 20, 25, 30°C) and four relative humidity (RH) levels (20, 35, 75, 95%) (Temperature*Humidity: $F_{(3,138)} = 12.412$, $p<0.0001$). No eggs survived at 35°C.**

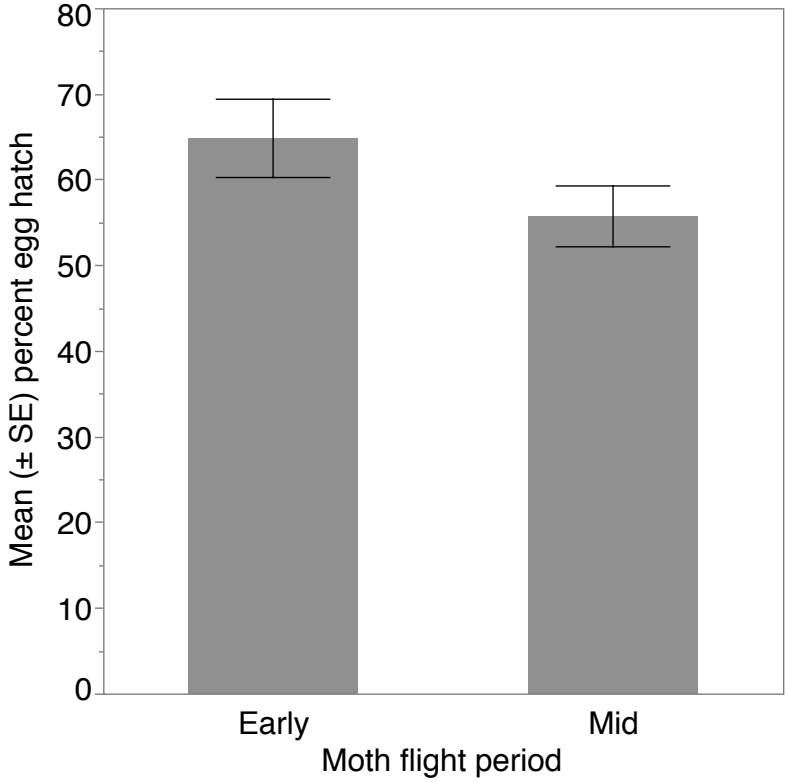

Figure 2.11 **Mean (± SE) percent hatch of *Spilonota ocellana* eggs attached to leaves on a tree in orchard blocks during two sampling periods: During Early moth flight (June 3rd – 17th) and during the middle of moth flight period (June 30th – July 12th) ($\chi^2_1 = 4.057$, $p = 0.044$).**

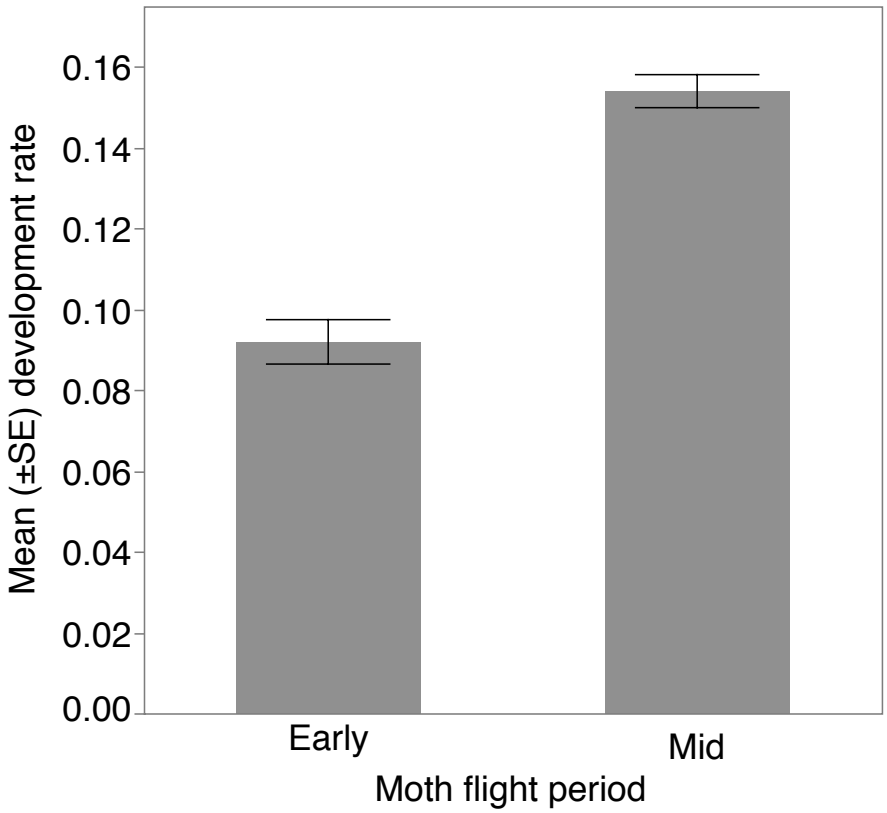

Figure 2.12 **Mean (± SE) development rate for *Spilonota ocellana* eggs attached to leaves in an apple orchard during the early moth flight period (Early, June 3rd – 17th) and during the middle of the moth flight period (Mid, June 30th – July 12th) ($F_{(1,97)} = 66.671$, $p < 0.0001$).**

Chapter 3. Cold tolerance of the spring-feeding larvae of the eye-spotted bud moth, *Spilonota ocellana*

3.1. Introduction

Global surface temperatures have increased significantly during the past century, and are projected to continue to rise during the 21st century under all scenarios assessed by the intergovernmental panel on climate change (IPCC 2014). In temperate regions, analyses of seasonal averages indicate greater warming is occurring in winter and spring (Flannigan et al. 1998, Bonsal et al. 2001; Robeson 2004; Schwartz et al. 2006). Consequently, growing seasons are beginning earlier and lasting longer, with pronounced effects on phenology and life-history adaptation in many species (Walther et al. 2002; Walther 2003; Badeck et al. 2004; Parmesan et al. 2000, Parmesan 2006; Stoeckli et al. 2012). These current and projected changes in global surface temperatures may have the potential to alter rates of development, geographic ranges, and population density of poikilothermic species, particularly insects (Porter et al. 1991; Bale 2002; Parmesan 2006).

Warming at the northern edge of agricultural limits will produce milder winters and longer growing seasons, and will have both positive and negative implications for crops and their respective pests. For insects, which are small poikilotherms highly sensitive to ambient temperature, warming can be beneficial by providing more heat units for growth and development (Inouye 2000; Schlyter 2006). In addition, a milder winter may lead to higher rates of overwintering survival (Bale and Hayward 2010) and changes in the timing of spring activity (Porter et al. 1991). Early termination of winter dormancy and resumption of active growth and development for herbivorous pests could result in population build-up, leading to infestations and outbreaks (Fand 2012; Murdock et al. 2013; Ju et al. 2015). However, insects that emerge too early risk starvation, if the

synchrony between the herbivore and its host plant is disrupted (Porter et al. 1991; Inouye 2008; Murdock et al. 2013), or freezing, if there is a sudden temperature drop after acclimation to warm temperatures (Augspurger 2013; Bjerke 2014).

Warming is often the focus of studies of the effects of climate change because insect pests usually benefit from the additional heat units available for growth (Bale 2002; Schlyter 2006; Parmesan 2006). However, the combination of warming with projected increases in temperature fluctuations could actually increase the occurrence of below-freezing temperatures at the beginning of the growing season, which can have very different implications than freezing temperatures during winter (Inouye 2000; Gu et al. 2008; Augspurger 2013). Temperate regions under the influence of Arctic air masses already experience episodes of extremely low temperatures (Quamme et al. 2010); when these occur after the growing season has begun, devastating damage can ensue (Gu et al. 2008; Inouye 2008; Augspurger 2013). A spring frost event is characterized by a brief period of sub-zero temperatures preceded and followed by periods of warm temperatures within the thermal threshold of development for plants and insects (Inouye 2000). While projections of the IPCC (2014) do not show a clear increase in frost (Eccel et al. 2009), analysis of historical occurrences of spring frost demonstrate an increased frost risk in several regions across the United States (Gu et al. 2008) and Europe (Augspurger 2013). A recent (2007) severe freezing event across the United States following periods of warm weather had devastating consequences for natural vegetation and many crops (Gu et al. 2008). Catastrophic loss of foliage has been associated with population collapse of many forest insect populations including species of Lepidoptera (Ives 1973; Volney and Fleming 2000; Ehrlich et al. 1972; Thomas et al. 1996), and Hemiptera (Beck 1953; Lawson 1958). Given the economic implications of frost damage in agro-ecosystems, studies often focus on crop vulnerability (Gu et al. 2008). Whereas plants typically rebound the following year, populations of insects may take several years to recover (Inouye 2000). This is particularly important given that poor spring weather, including frost, has been linked to the termination of cyclical outbreaks of the forest tent caterpillar, *Malacosoma disstria* (Hübner), in Alberta (Gautreau 1964) and Ontario (Cooke and Lorenzetti 2006). Thus, years without frost could be an important factor for population build-up, and eventual oubreaks of insect herbivores.

Cold-hardiness refers to the ability of an organism to tolerate extended or brief exposure to low temperature (Lee 1989). Studies on insect cold hardiness frequently address the ability to tolerate extreme sub-zero temperatures during winter dormancy, when cold hardiness is increased by acclimation to low temperature (Lee 1989; Atapour and Moharramipour 2009; Khani and Moharramipour 2010; Williams 2011; Bürgi and Mills 2012). Often, this is linked to the diapause state in which cold hardiness increases in response to environmental cues such as decreasing temperature and photoperiod (Pullin and Bale 1989; Bale et al. 2002; Bale and Hayward 2010). In addition, overwintering species may be adapted to survive extended periods of time at extreme sub-zero temperatures, whereas active species which are acclimated to temperatures within their development threshold may be killed by short periods of chilling well above the freezing point of their body fluids (Sømme 1999; Koch et al. 2004; Carrillo et al. 2005; Khani et al. 2007).

Cold-hardiness strategies of insects are generally classified into two main categories: freeze-tolerant and freeze-avoidant (Sømme 1982; Zachariassen 1985; Bale 1987; Bale 1993; Sinclair 1999). Insects that can endure ice formation in their tissues are considered freeze-tolerant. Freeze-avoidant species take measures to lower their freezing temperature to prevent ice formation in the tissues, and are considered chill-susceptible if they are killed by cold in the absence of internal ice formation (Zachariassen 1985; Bale 1987; Bale 1993; Sinclair 1999). Cold-hardiness strategies are not necessarily fixed, and many species will switch strategies within and between life stages (Salt 1966; Horwath and Duman 1984; Sinclair 1999; Chown and Nicholson 2004; Lee 2010). Conditions under which this occurs and the cues are summarized by Sinclair et al. (2015). For instance, in temperate regions, species that are freeze-tolerant (e.g. golden gall fly, *Eurosta solidaginis* [Fitch]), or freeze-avoidant (e.g. firebug, *Pyrrhocoris apterus* L. [Hemiptera: Pyrrhocoridae]) in the winter, become chill susceptible in the summer (Koštál and Šimek 2000; Lee and Hankison 2003; Wagner 2012). Moths of the aspen leaf miner *Phyllocnistis populiella* (L.) (Lepidoptera: Gracilliariidae) in Alaska, show significant differences in cold hardiness, where active adults freeze at much higher (-16 °C) temperatures than during dormant life stages (-32 °C) (Wagner 2012). In general, when species are acclimatized to warm temperatures they are less able to tolerate freezing temperatures than during periods of prolonged

cold (Lee and Denlinger 1985; Zachariassen 1985, Block 1990; Storey and Storey 1996; Sømme 1999).

The freezing of body tissues typically happens as a result of an interaction between water molecules and a nucleating agent around which ice can form (Salt 1966). The temperature at which an insect's bodily fluids freeze is known as the supercooling point (SCP) (Lee 2010). While this can be a good indicator for determining which strategy an insect employs (Sinclair et al. 2015), it is not always a reliable indicator of cold hardiness (Baust and Rojas 1985; Bale 1987; Nedved et al. 1995; Renault et al. 2002; Atapour and Moharramipour 2009) as most insects die from chill injury at temperatures above their SCP (Lee and Denlinger 1985; Lee 1991; Bale 1993; Bale et al. 2002; Sinclair et al. 2003; Lee 2010; Bürgi and Mills 2012). The temperature at which an insect dies is referred to as the lower lethal temperature (LLT) (Wagner 2012), and better reflects the risk of lethal chilling injury (Bale et al. 2002; Sinclair et al. 2003; Lee 2010; Bürgi and Mills 2012). Combined with knowledge of the SCP, the LLT can be used to indirectly estimate a cold tolerance strategy (Sinclair et al. 2015).

While physiological changes governing cold tolerance in insects during their overwintering life stage occur gradually in response to decreasing temperature and photoperiod, insects are also capable of cold hardening on a much shorter time scale (Lee et al. 1987; Denlinger 1991; Lee 2010; Teets and Denlinger 2013; Overgaard et al. 2014). Known as 'rapid cold hardening', this process occurs within minutes to hours and can significantly enhance cold tolerance (Teets and Denliger 2013) to cope with sudden cold snaps and freeze-thaw cycles (Marshall and Sinclair 2012). Insects employ a variety of physiological, molecular, and behavioural adaptations to cope with low temperature exposure (Clark and Worland 2008; Doucet et al 2009). Molecular adaptations to increase tolerance include up-regulation of antifreeze proteins (Doucet et al. 2009), accumulation of cryoprotectants (Lee 2010), and changes in membrane fluidity (Hazel 1995; Bennett 1997; Overgaard et al. 2009; Lee 2010). For example, accumulation of the low molecular weight sugar glycerol decreases the SCP of larvae of the freeze-intolerant spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) (Han and Bauce 1993), whereas trehalose was found to be the major cryoprotectant in overwintering larvae of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Neven 1999; Khani et al. 2007) and the fruit fly, *Drosophila*

melanogaster (Meigen) (Diptera: Drosophilidae) (Koštál et al. 2011; Colinet et al. 2012). In many taxa, adaptive changes in fatty acid composition preserve the membrane fluidity of poikilotherms under low temperatures (Hazel 1995; Overgaard et al. 2009), including larvae of the goldenrod gall fly, *E. solidaginis,* which undergo unsaturation of lipids under seasonally cold conditions (Bennett et al. 1997). Behavioural strategies can also minimize exposure to variable temperatures and include habitat selection, such as species overwintering in sub-nivean habitats within the soil, leaf litter or decaying wood (Danks 1987). Freeze-tolerant species may seek out moist habitats to initiate freezing at lower temperatures (Layne et al. 2011), whereas freeze-avoidant species such as the aspen leaf miner, *P. populiella,* benefit from dry winter microhabitats as contact with moisture can result in ice formation at higher temperatures via the cuticle (Wagner et al. 2012).

Studies indicate that when feeding, larvae suffer a significant reduction in cold hardiness (Salt 1953; Krunic 1971; Cloudsley-Thomson 1973; Krunic and Radovic 1974; Bale et al. 2001; Suh et al. 2002; Khani et al. 2007). For instance, *C. pomonella* has a winter SCP of -22°C, compared to only -13.4°C for feeding larvae (Khani et al. 2007). High summer SCPs have been correlated with the presence nucleators in the gut (Salt 1953; Zachariassen 1985; Worland et al. 1993; Worland and Block 1999). These ice nucleating agents can be the food itself, or other particles such as bacteria and dust ingested while feeding which promote the formation of ice (Salt 1953; Duman and Patterson 1978; Sømme 1982; Zachariasen 1985). In addition to the introduction of ice-nucleating agents (INA), a loss of cryoprotective substances through moulting, or an increase in cell water content contribute to a reduction in cold hardiness (Salt 1953; Sömme 1982; Zachariassen 1985). Thus, there is an expectation that species that overwinter in a feeding life stage and resume activity and feeding early in the growing season could be vulnerable to unexpected freezing temperatures.

The eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae) is a pest in fruit-growing regions across the Northern hemisphere that can cause significant economic losses in apple orchards (Gilliatt 1932; Leroux et al. 1963; MacLellan 1978). It also attacks other crops including blueberry (Gillespie 1985), cherry (Oatman et al. 1962) and plum (Madsen and Borden 1949). *Spilonota ocellana* can be damaging to apples in the spring, when newly emerged larvae

burrow into developing buds; and in the summer, when freshly hatched larvae can injure fruit by fastening leaves to the apple surface and producing shallow feeding excavations (MacLellan 1979). *Spilonota ocellana* larvae overwinter on tree branches within a thick silken shelter near dormant buds (Porter 1924). Larvae have been observed to experience high mortality when winter temperatures are below -28°C (Leroux et al. 1963; MacPhee 1964). Larvae emerge as 4th to 6th instars (McBrien and Judd 2004), and feeding commences immediately as larvae chew their way into the developing buds and fruit (Porter 1924). MacPhee (1964) has reported this pest as 'marginally cold hardy', which by current classification would likely indicate a freeze-avoidant strategy. However, to date studies on its actual cold-temperature thresholds have not been determined.

The objective of this study was to determine the cold-hardiness of *S. ocellana,* at the life stage most likely to experience a sudden frost after periods of warm weather at the beginning of the growing season. Through measurement of the SCP and LLT, I report on the cold-hardiness strategy of spring larval instars, as well as variables that may contribute to variation in cold hardiness at the early feeding life stages. Understanding the impacts of freezing temperatures on survival of pest insect species could help crop managers better predict the potential for seasonal population increases, or declines, particularly for species that emerge early in a feeding life stage.

3.2. Methods

Larvae source and rearing conditions

S. ocellana larvae were field-collected from three organic orchards in the Similkameen Valley, British Columbia (49.039530, -119.703530) where infestation levels had been high in the previous year. In May 2013, emergent spring larvae were field collected in leaf feeding shelters, brought indoors, and kept at ambient temperature and light conditions. In 2014 pruned-wood was collected in early spring (late March to early April) and transferred to 18 °C and ambient light conditions to initiate emergence (similar to methods of McBrien and Judd 2004). After emergence from their overwintering hibernaculae, individual larvae were moved to 59 mL plastic cups (Solo Cup Company, Lake Forest, IL) and provided with fresh apple leaves collected from organic orchards for

food and nesting material. Apple leaves were sprayed with a 2% bleach solution and rinsed to remove potential contaminants prior to introduction. Leaves were replenished every 2-3 days. Instar was determined based on distribution of head capsule sizes (Gilliatt 1932). Experimental larvae were restricted to 4th, 5th and 6th instars, the main life stages that overwinter and emerge in the spring (McBrien and Judd 2004) and risk exposure to freezing temperatures in the early growing season.

Supercooling point

The SCP was measured using surface-contact thermometry in a thermocontrolled freezer (Tenny Jr. Programmable Freezer; Tenny, South Brunswick, N.J.). Individual larvae were placed in a longitudinal groove (1.0 × 0.5 × 0.4 cm) cut in 4 × 3 × 0.4 cm polystyrene rectangles which were covered with a solid square of polystyrene to restrict movement of larvae away from the thermocouple plate. Samples were cooled at a rate of 4 °C h^{-1} from 10 °C. This rate of cooling was used as it represents a fast, but ecologically realistic change in temperature over daily time scales (see Terblanche et al. 2007; Rezende et al. 2011; Sørensen et al. 2013; Sinclair 2015 for discussion). Defined as the temperature of spontaneous freezing, the SCP is the lowest temperature immediately preceding the latent heat of crystallization which manifests as an exotherm that is indicative of internal ice formation (similar to methods of Lee and Denlinger 1985). In 2013, SCP was measured for leaf shelters alone, larvae in leaf shelters, and larvae removed from leaf shelters, experiment was repeated on two dates (May 7th and 8th; n=27). Mean exotherms of leaf shelters alone were measured in order to determine if freezing leaf material would release an exotherm in the absence of larvae, and whether these could be distinguished from larval SCPs. In 2014, the SCP's of larvae (N=81) were measured on five dates (April 7th, 9th and 22nd and May 2nd and 9th). Some larvae lost contact with the thermocouple plate and were removed from the final analysis. Larval instar and mass (wet and dry) were also recorded during the 2014 supercooling experiments. In order to determine instar, head capsule width was measured (post-mortem) under a microscope with a micrometer lens (Gilliatt 1932).

Influence of larval instar and acclimation on lower lethal temperatures

Determination of the LLT was based on cooling spring larvae down to (3, -4.5, -7, or -9.5 °C) on three dates (April 9th, 22nd, and May 2nd). Prior to chilling treatments, larvae were maintained at 18 °C. To determine if pre-exposure temperature would have a significant effect on larval survival, on the first sampling date 50% of the larvae were moved to 21°C (n=36) or 10 °C (n=36) for 24 hours prior to being chilled. Minimum treatment temperatures for removal were selected based on temperatures approaching the previously determined SCP of larvae. Individual larvae in 59 mL Solo cups were stacked in groups of nine. Stacked cups were placed in plastic bags inside the thermo-controlled freezer. Temperature was ramped down by 4°C h^{-1} based on the air temperature inside an individual cup measured with copper-constantin thermocouples. Eighteen larvae per treatment were removed from the freezer after each target temperature was held for one minute to reflect a brief exposure that may be experienced during a frost event. The experiment was repeated on three sampling dates in 2014 (April 9th, 22nd and May 2nd). Upon removal from the freezer, cups with larvae were returned to 4 °C for 1 hour, provided with fresh leaf material and transferred to an incubator set at 21°C with a 16:8 (L:D) photoperiod. A group of control larvae were removed at 3 °C on each date to determine any experimental effects in the absence of exposure to sub-zero temperatures. Mortality was assessed after 24 hours. Death was defined as lack of responsiveness when larvae were probed with a fine tipped paintbrush.

Survival after exposure to three temperatures (-4, -6, -8) for 1, 2, or 4 hours

On April 29th, 2014, larvae in solo cups (as in previous experiment) were placed in one of three programmable freezers where temperature was ramped down to -4, -6, or -8 °C for 1, 2 or 4 hours (n=9 larvae in each temperature × time treatment combination). Estimation of survival was based on the same procedure as LLT experiments.

Statistical analyses

In 2013, the SCP temperature data for larvae within spin-ups, removed from spin-ups and empty leaf shelters were compared using a single factor analysis of variance (ANOVA), with date as a blocking variable.

In 2014, additional information on instar and mass were collected from each larva after SCPs were measured. Water content was calculated by subtracting wet and dry weights of larvae. Proportion water content (relative to wet weight) was arcsine transformed to normalize the data. Date was excluded from the analysis as larval size and date were correlated.

The proportions of larvae that survived at each minimum temperature were compared using a generalized linear model (GLM) with a binomial error distribution and logit link function. Ninety-nine percent of larvae survived the control treatment (3°C) and this treatment was not included in the final analysis. Mean LLT at which 10, 50, and 90 % of the larvae died were obtained from the final, minimal GLM using the inverse prediction option in JMP. All means were compared post-hoc with chi-square pairwise contrast test (GLM models) or using Tukey's HSP (ANOVA).

All statistical analyses were carried out using JMP 11 (version 11.2). Normality for parametric analyses was determined using a Shapiro-Wilk goodness of fit test. An alpha of 0.05 was used to determine significance.

3.3. Results

Supercooling point of larvae and the effect of leaf spin-up, instar and water content

There was no evidence that the SCP of larvae within leaf shelters differed from those that had been removed ($F_{(2,51)}$ = 0.706, p = 0.4995) (Figure 3.1). The mean (±SE) SCP of all larvae measured in 2013 (removed plus those concealed in leaf shelters) was -8.4 ± 1.7 °C, with a range from -5.2 to -14.3 °C.

Mean SCP temperatures of larvae collected in 2014 increased significantly with instar ($F_{(2,71)}$=6.709, p=0.0021) (Figure 3.2). As expected weight differed significantly between instars ($F_{(2,68)}$=45.437, p<0.0001), increasing from 1.3 ± 0.9 mg for 4th instar larvae, to 6.4 ± 0.8 mg for 5th instars, and finally 13.7 ± 1.0 mg for 6th instar larvae. Proportion water content also increased significantly with instar ($F_{(2,68)}$=54.918, p<0.0001).

Lower lethal temperature of S. ocellana larvae

Larval survival decreased significantly with lower chilling temperatures (χ^2_3 = 102.789, p<0.0001), however, there was no evidence of an effect of instar (χ^2_3 = 3.913, p = 0.1413), or an interaction (χ^2_5 = 1.173, p=0.403) between the two variables (Figure 3.3). Mean predicted LLT to reach 10, 50, and 90 % mortality of *S. ocellana* larvae were -5.1 ± 0.8, -7.3 ± 0.4 and -9.4 ± 0.1 °C, respectively (Figure 3.4). Ninety-nine percent survival was observed for the control treatment (3 °C).

Acclimation of larvae at different temperatures for 24 h preceding the chilling treatment made no difference to survival (χ^2_1 =0.356, p=0.5508). Mean survival for larvae acclimated to 10 and 21°C before chilling treatment was 67 ± 8.9 and 74 ± 8.9%, respectively (n=36 for both temperatures).

Survival after exposure to sub-zero temperatures (-4, -6, -8°C) for 1, 2, or 4 hours

Sub-zero chilling temperatures had a significant effect on survival of larvae (χ^2_2 = 7.139, p < 0.0282). There was no evidence that length of exposure (1, 2, or 4 hours) altered survival (χ^2_2 = 0.908, p = 0.635), and no interaction between these two factors (χ^2_4 = 3.735, p = 0.4429) (Figure 3.5). Mortality was significantly greater for larvae in the -8 °C treatment (χ^2_1 = 13.846, p = 0.0001), compared with the -6 and -4 °C treatments where nearly all larvae survived at all time exposures (Figure 3.5).

3.4. Discussion

I investigated the cold tolerance of *S. ocellana* larvae in the context of conditions experienced during a spring frost event. I found that spring-feeding *S. ocellana* larvae were freeze-avoidant with their LLT being higher than their SCP. The observed mean SCP of -8.4 °C is within the range (-7 to -12 °C) found in other species of Lepidoptera during their active, feeding life stages (Zachariassen 1985; Khani et al. 2007; Boardman et al. 2012). SCP was lowest in the youngest instar tested, this has also been found in other species of Lepidoptera such as the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), and species of Coleoptera, such as the ladybeetle *Harmonia axyridis* (Pallas) (Watanabe and Tanaka 1997; Bürgi and Mills 2010). Water

loss, or dehydration can lower SCPs to the extent that it concentrates solutes and thereby lowers freezing points (Sømme 1982; Zacchariassen 1985). Although there was no significant correlation between proportion water content and SCP, higher water in larger instars could be an indication of increased solute concentration, which may have enabled larvae to lower the temperature at which the tissues froze (SCP). The physiological mechanisms behind the cold tolerance of *S. ocellana* are not known, but given that in Quebec they are reported to survive temperatures down to -28 °C in the winter (MacPhee 1964) it is likely overwintering larvae utilize cryoprotectant substances or undergo changes in membrane lipid composition. These adaptations may remain during the early stages after spring emergence, before being lost as a result of feeding activity, or moulting into older instars (Salt 1953; Sømme 1982; Zachariassen 1985).

Spilonota ocellana larvae were rarely found outside their leaf shelters and feeding tubes (personal observation), where they are likely to remain in order to escape unfavourable conditions. Leaf shelters are likely more moist than winter hibernaculae. Chilling larvae within a detached leaf shelter had no effect on SCP, so these offer no discernible frost protection. Leaves provided for spin-ups in our study were 1-2 day old green leaves detached from trees. Further study into the effects of leaf spin-ups constructed of live and dead leaves attached to the tree is needed to determine if this micro-habitat can provide insulation against ambient temperatures; or a negative effect, by potentially providing inoculation points for lethal ice formation to occur at higher temperatures (Sømme 1999).

In contrast to SCP temperatures, I found that LLT did not differ among instars. This is similar to observations of the active larval stages of *E. postvittana,* in which SCP increased with instar, but lethal time leading to mortality did not differ unless exposure duration reached 775 hours (Bürgi et al 2010). By contrast, Knight and Croft (1986) found that both field-collected and laboratory-reared overwintering 3rd instar larvae of the orange tortrix moth, *Argyrotaenia citrana* (Walsingham) (Lepidoptera: Tortricidae) were significantly more tolerant to freezing temperatures than 5th instars. Increased fat stores in older instar larvae may help them to survive freezing temperatures, even if they freeze at higher temperatures than younger instar larvae. Differences in SCPs, and susceptibility to lethal temperatures, could be mitigated by overlapping generations as

the presence of several life stages could increase the chance that some individuals survive unfavourable conditions.

Thermal history has been shown to influence the ability of insect species to tolerate temperatures at later life stages (Lee 1991; Terblanche et al. 2007; Chidawanyika and Terblanche 2011; Teets and Denlinger 2013). I looked at two ecologically relevant daytime temperatures that could precede a spring frost, 10 °C and 21 °C, and found that the preceding temperature had no discernible effect on the ability to survive brief exposure to freezing temperatures. As the temperatures studied did not result in a survival differential, I would conclude that moderate, or high, daytime temperatures do not elicit a hardening response in *S. ocellana* larvae. Further study into their ability to acclimate to lower temperatures could reveal more about the phenotypic plasticity in response to cold. Acclimation for several days at low temperatures has been shown to considerably increase the cold hardiness of species such as the post-dormant pine ladybird, *Exochomus quadripustulatus* (L.) (Coleoptera: Coccinellidae) (Nedved 1995; Koštál et al 1998) and summer fruit tortrix, *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae) (Milonas and Savopoulou-Soultani 1999). Cooler temperatures combined with a longer pre-exposure period than those used might have elicited a more gradual hardening response, as there are many examples of species acclimating after days or weeks at low temperatures (Nedved 1995; Koštál et al. 1998; Milonas and Savopoulou-Soultani 1999; Andreadis et al. 2012). However, extended periods at cold temperatures are more representative of what would be experienced by species during the winter months, and are not characteristic of diurnal fluctuations during the growing season. While acclimation at the temperatures studied did not immediately impact survival at low temperatures, thermal history before exposure to sub-lethal freezing temperatures could be detrimental to future cold hardiness of the larvae or the fitness of subsequent life stages, reducing population abundance through impacts on activity, growth rates, reproduction and fecundity (Bloem et al. 2006; Chidawanyika and Terblanche 2011). Further study into the long-term effects of diurnal acclimation temperature and exposure to sub-zero temperatures on *S. ocellana* larvae would provide better understanding of the impacts of spring frost on population fluctuations.

Duration of exposure time to minimum temperatures during a spring frost tends to be brief, particularly when freezing occurs after a period of warming. I investigated slightly longer exposures at temperatures approaching the mean SCP (-8.4 ± 0.2 °C), and overlapping the LLT_{50} (-7.3 ± 0.4 °C). While temperature had a significant effect on survival, exposure duration did not have a significant effect. However, there was evidence of a possible trend towards increased mortality as exposure time at the lowest temperature sampled (-8 °C) increased from one to four hours, indicating that longer exposures may become a factor at temperatures approaching the SCP. However, based on my results, and in the context of a spring frost, I conclude that the lowest minimum temperature will be more important than the amount of time spent at that temperature.

The combination of warming and temperature extremes associated with climate change could result in an increased frequency of extreme events that pose significant threats to poikilotherms (Porter et al. 1991; Bale et al. 2002; Augspurger 2013). Mild winters and earlier starts to the growing season could put spring feeding species at increased risk of exposure to temperatures beyond their thermal threshold. Based on this study, I conclude that spring feeding larvae of *S. ocellana* are chill-susceptible, with mortality increasing as temperatures approach -7.3 ± 0.4 °C. This study is the first to measure cold tolerance levels for *S. ocellana* and could aid in predicting densities based on spring temperatures. To further elucidate the effects of spring frost on the seasonal variation in population levels, it will be necessary to determine sub-lethal effects on the long-term reproductive success of organisms following a freeze event. Knowledge of the effects of brief exposures to freezing during the growing season on insect pests will be particularly important in regions that have the potential to experience a higher frequency, and intensity of spring frost.

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3.7. Figures

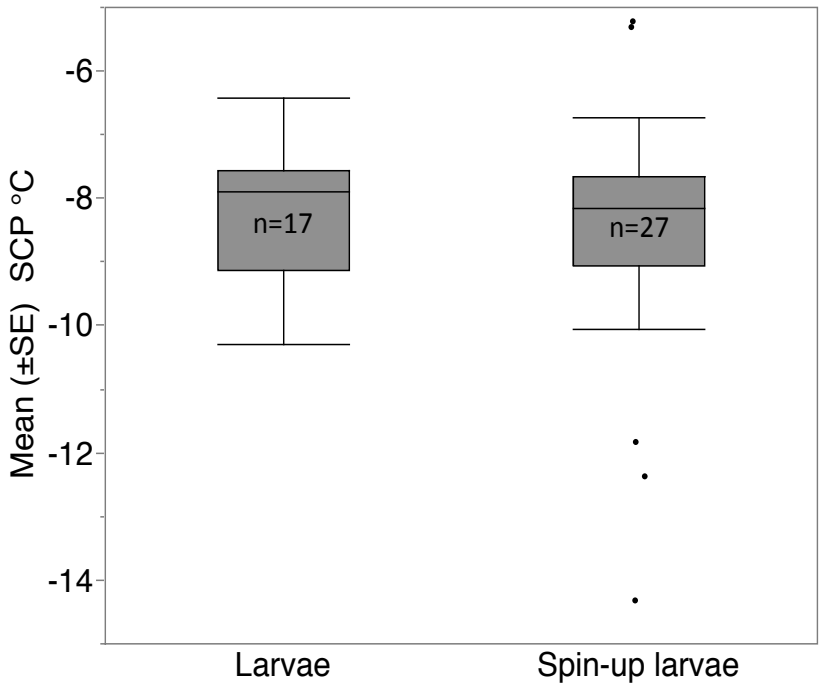

Figure 3.1 **Mean (± SE) supercooling point temperatures for *S. ocellana* larvae removed from leaf shelters (Larvae) and within leaf shelters (Larvae + leaf shelter) ($F_{(2,51)} = 0.706$, $p = 0.4995$).**

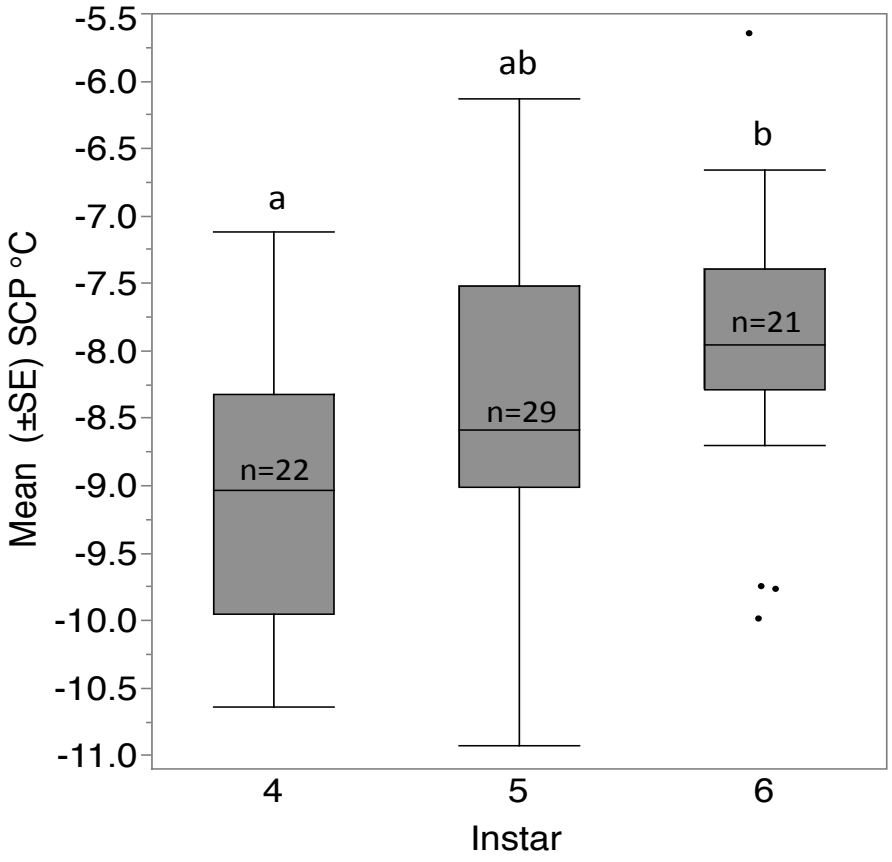

Figure 3.2 **Mean (± SE) supercooling point temperatures (°C) of 4th - 6th instar, spring-emerged, feeding larvae of *Spilonota ocellana*. Different letters represent significant differences between least-square means ($F_{(2,71)}$=6.709, p=0.0021).**

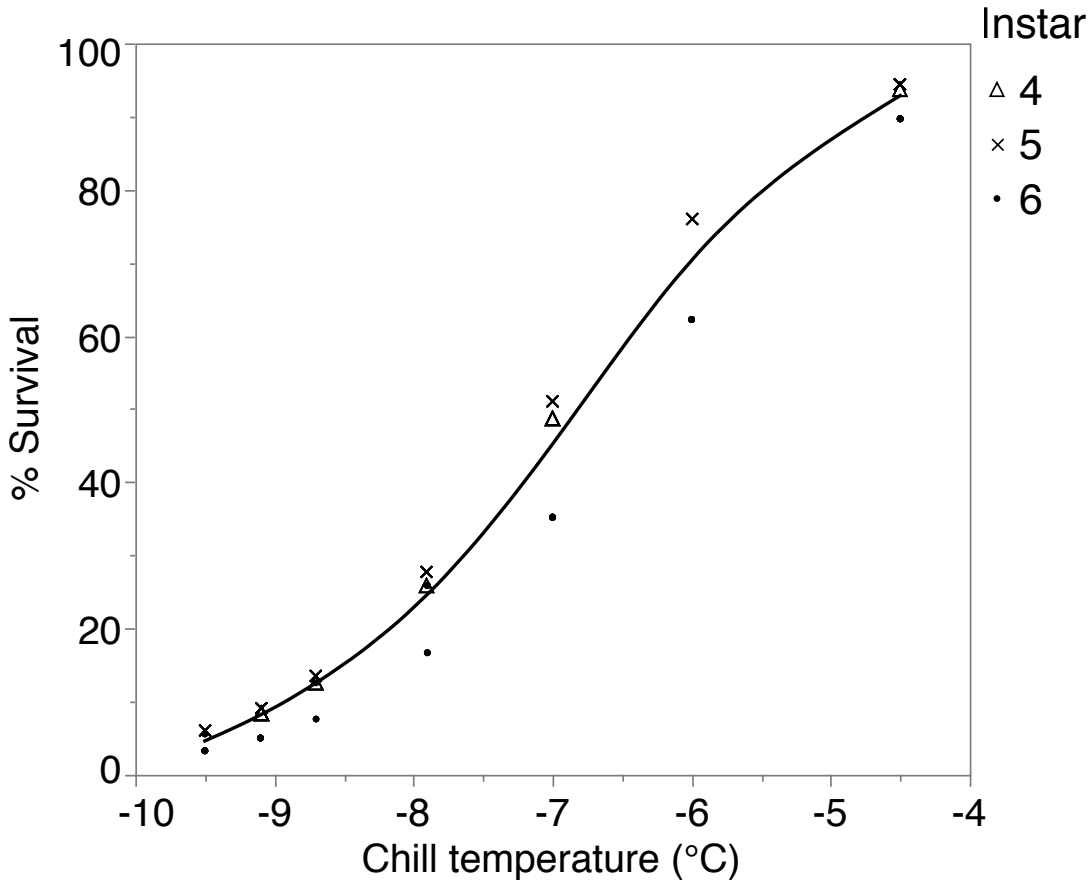

Figure 3.3 **Predicted survival of *Spilonota ocellana* larvae exposed to different sub-zero temperatures ranging from -4.5 to -9.6 °C for five minutes ($\chi^2_3 = 102.7885$, $p<0.0001$, n=18 per temperature treatment). Solid line represents the fitted generalized linear model; points represent mean survival of larval instars ($\chi^2_5 = 1.173$, $p=0.403$).**

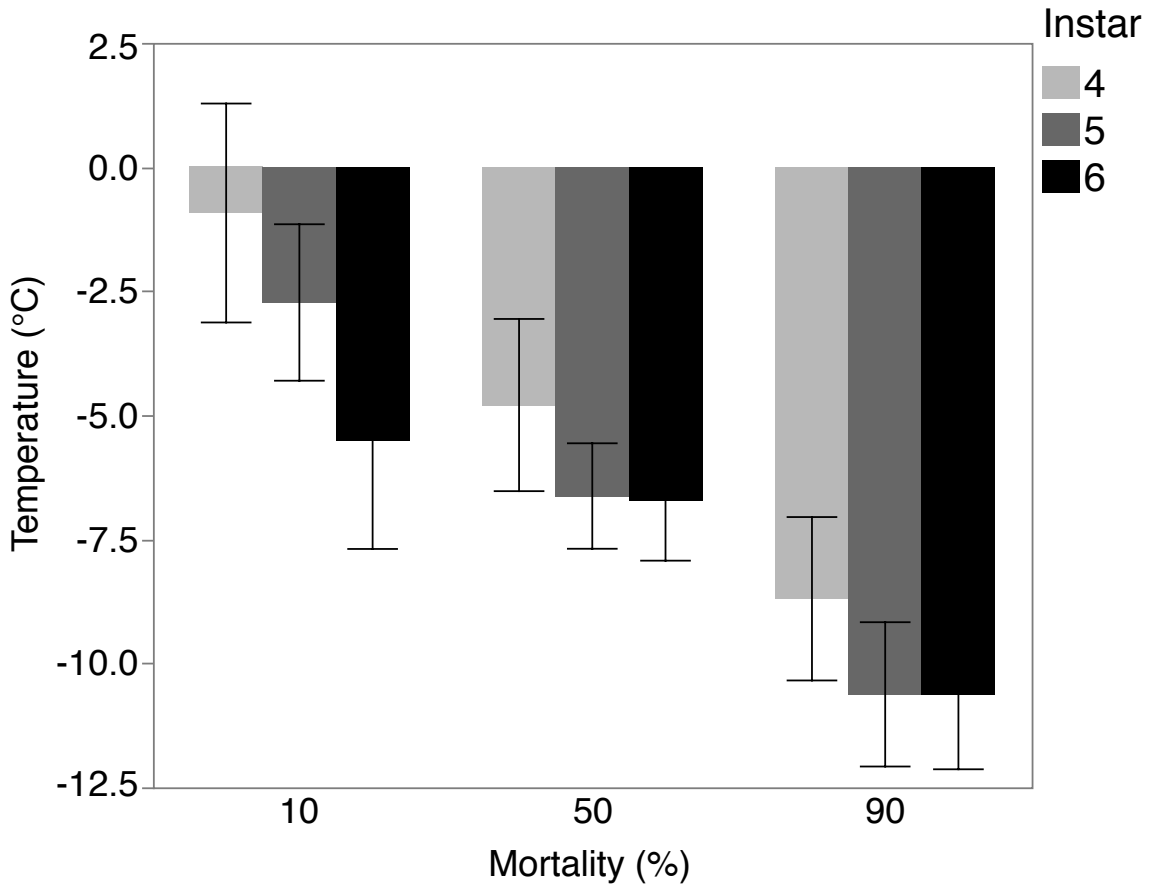

Figure 3.4 **Estimates of the mean (± SE) LLT for 10, 50, and 90 % mortality of *Spilonota ocellana* larvae at sub-zero temperatures (χ^2_3 = 102.789, p<0.0001) and larval instars (χ^2_3 = 3.913, p = 0.1413). Estimates obtained from the final, minimal GLM.**

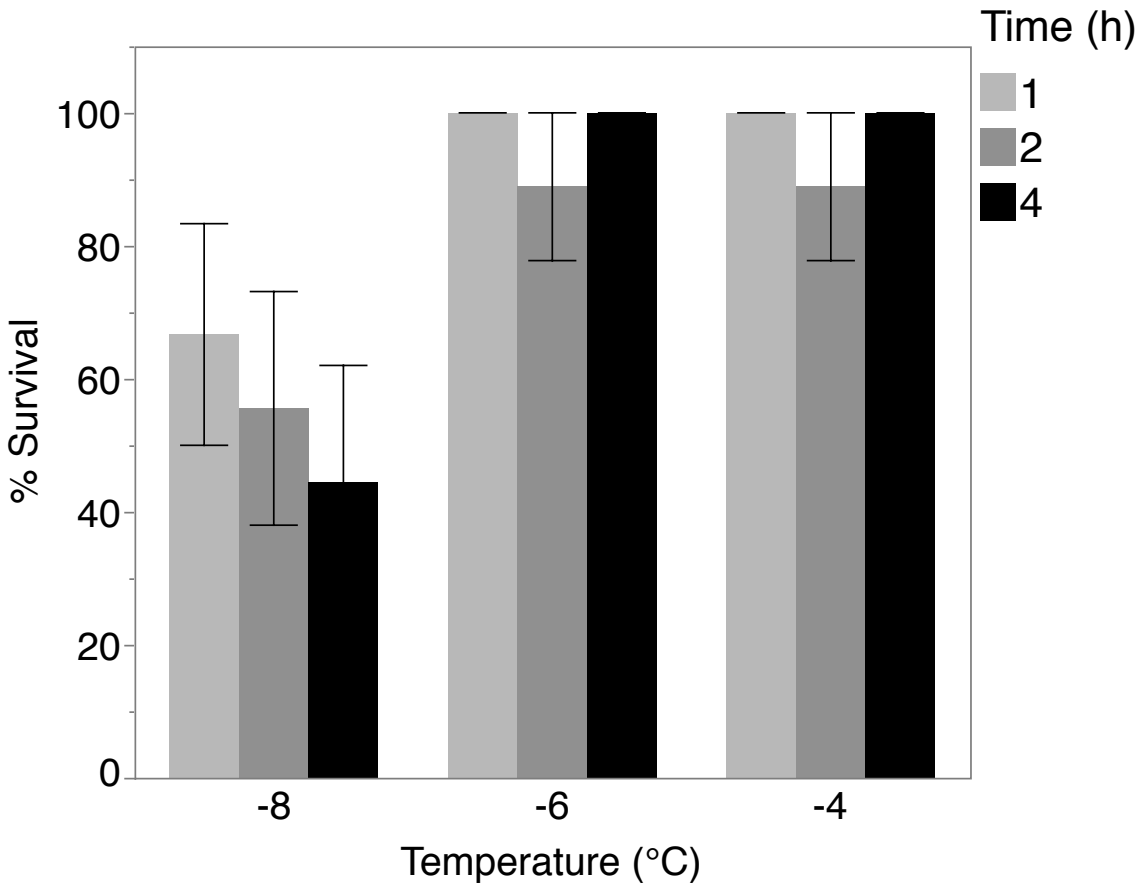

Figure 3.5 **Mean (± SE) percent survival of *Spilonota ocellana* larvae after 1, 2 or 4 hours (χ^2_2=0.908, p=0.635), of exposure to -8, -6, or -4 °C (χ^2_2=7.139, p<0.0282) (n= 9 larvae for each temperature-time combination).**

Chapter 4. Conclusions

Temperature and moisture are among the most important abiotic factors that can influence insect abundance and distribution (Porter et al. 1991; Chown 2002; Parmesan 2006). Extreme temperature and humidity levels have serious implications for poikilothermic species that are limited by their capacity to withstand conditions beyond their optimum for survival and development (Parmesan et al. 2000; Sutherst et al. 2011). The overall objective of this thesis was to address survival and development of the eye-spotted bud moth, *Spilonota ocellana*, in response to temperature and relative humidity levels in the context of a changing climate. Specifically, I focused on two life stages and the two different weather extremes they are likely to experience. In chapter 2 I investigated the temperature and humidity thresholds of the egg, and in chapter 3 I focused on the larval stage, specifically the instars that emerge in the spring around the early green-tip stage of apple development (McBrien and Judd 2004). This thesis highlights the importance of timing on insect responses to weather: timing of life-history events, timing within a life stage, and the timing of extreme weather.

Based on the results of this study, I conclude that eggs of *S. ocellana* are vulnerable to desiccation at low humidity levels, especially when combined with high temperatures. This was reflected in the lab and field trials, where lower egg hatch was observed under controlled low humidity levels and during a warmer, drier sampling period in an orchard. Given that *S. ocellana* oviposition occurs during the summer months, frequent and extended periods of extremely hot and dry weather could result in laying eggs under conditions that are not favourable for survival. Considering observations by Gilliatt (1932) that late emerging females are smaller and of lower fecundity, higher hatch rates during the early, cooler, flight period indicate that the period following the onset of oviposition could be a key period for the establishment of *S. ocellana* populations. Given that *S. ocellana* studies are often reported from more coastal and humid regions (Porter 1924; Frost 1927; Harman 1931; Chapman and Lienk 1971; MacLellan 1978; Gillespie 1985), the higher fecundity and hatch rate during the

early season could be a strategy that has evolved in response to the hot and dry conditions during mid-summer. This is particularly relevant in regions that are already characterized by a semi-arid climate, such as the Similkameen Valley in the interior of British Columbia.

The impacts of low humidity were most detrimental at the later stages of egg development. Thus, timing of extreme conditions is not only important for which life stage is present, but also the stage of development within a life stage. *Spilonota ocellana* eggs that experienced low humidity during the second half of development had significantly lower survival, and faster developmental rates, than eggs that were exposed to low humidity during the first half of development. Increased metabolic demands of developing embryos (Woods et al. 2005, 2010), accompanied by increased rates of water loss during the second half of development, help to explain the lower hatch rate as being due to a greater risk of desiccation. However, the faster rate of development suggests that eggs of *S. ocellana* may also have the potential to adjust their physiology in response to the abiotic environment, as has been observed in the brown locust response to moisture, (Zrubek and Woods 2006) and the tobacco hornworm response to oxygen levels (Kambule et al. 2011).

A further consideration of the timing of egg hatch is the influence it will have on which larval instars overwinter. Larvae of *S. ocellana* have been observed to emerge from overwintering primarily as 5th or 6th instars (with a small proportion of 4th instar) (McBrien and Judd 2004). However, this proportion may vary regionally, or shift in response to seasonal weather trends. The larval instar most suited for overwintering is not known, though, a lower proportion of smaller larval instars may be the result of fewer reserves they have sustain themselves, particularly in a mild winter (Chaplin and Wells 1982; Irwin and Lee 2000). In Chapter 3 I found that supercooling point varied between larval instars, ranging from -9.1 ± 0.2 °C for 4th instar larvae, to -7.9 ± 0.2 °C for 6th instar larvae. However these are larval instars that have emerged in response to warm temperatures and had begun feeding. Despite differences in supercooling point, the median LLT, estimated at -7.3 ± 0.4 °C, did not differ between instars. While I would conclude that the cold hardiness strategy employed by *S. ocellana* larvae is freeze-avoidance (Zachariassen 1985, Bale 1996; Sinclair 1999; Ramlov 2000), further study is

needed to determine whether feeding larvae died as a result of freezing or succumbed to the physiological effects of low temperatures unrelated to freezing.

Implications for management

The data collected in this study provide information on the temperature and moisture thresholds of *S. ocellana* larvae and eggs, which could be used as a basis for predictive modeling for management purposes. On a more basic level, the findings indicate that years with a hard frost (< -7.3 ± 0.4°C), or very hot and dry summers, could have significant impacts on larvae and eggs of *S. ocellana* respectively. It remains to be seen if a return to more typical hot and dry summers will suppress populations of *S. ocellana*, and whether their numbers will climb again should there be a period of unseasonably cool and wet summer weather. However, an important consideration for management is that although the moth flight period can last up to two months, controls might be better focused on the eggs laid at the onset of the oviposition period when conditions are optimal for egg hatch and development. I would suggest that eggs or larvae during the early flight period should be the target of controls, particularly in the event of cool wet conditions after the onset of oviposition. While this study indicates that spring frost has the potential to cause larval mortality, in commercial orchards where practices are in place to mitigate potential frost damage, it is unlikely that the level of freezing needed to control this pest will be reached most years.

Further study

Understanding response of herbivorous pests to climate change is complicated, not only by differences between and within species, but also by the changing interactions between species at different trophic levels (Harrington 1999; Gillespie et al. 2012). To understand how changing weather patterns influence survival and development of pests, entomologists need to assess environmental thresholds on a species by species basis (Sutherst and Bourne 2009), and at the community level (Gillespie et al. 2012). Further investigation into the effects of extreme weather on leaf physiology and surface microclimate could provide a better understanding of the ability of *S. ocellana* larvae or eggs to tolerate both temperature and moisture extremes.

Leaves use transpiration to moderate their environment and avoid damaging high temperatures (Mahan and Upchurch 1988), thus leaf-associated insects live in microclimates modified by their host plant to be more suitable than the ambient environment (Woods 2010). However, plants can respond to drought by conserving water through stomatal regulation, therefore when conditions are unfavourable plants may close leaf stomata (temporarily halting transpiration) to prevent water loss (Cornic 2000). Woods (2010) discusses the boundary level in regards to leaf size and wind speed. While I measured temperature and humidity levels in the canopy, to what extent the leaf microclimate buffers eggs and larvae from environmental variation in high-density orchards, requires further study. While the impacts of air flow and wind speeds on desiccation were not explored, this could be especially important for pests in the Similkameen Valley, which is characterized by persistent winds from the cool air that flows down the mountains and valleys at night, and warm air that rises from the valley bottom during the day (Maclean 1970).

Observing that eggs exposed to low humidity during the latter stages of development had lower hatch and faster development led me to question the capacity of *S. ocellana* to adjust their physiology in response to unfavourable conditions. Further understanding of mechanisms to cope with water loss in the egg stage could provide clues as to why eggs are better able to cope with low humidity conditions during the early stages of embryonic development.

Further studies on differences between populations of *S. ocellana* (transplant experiment) could provide clues into differences in thermal thresholds, and the timing of life history events, between climatically different growing regions. This type of study may also reveal whether populations of *S. ocellana* in the Similkameen Valley have evolved to tolerate the climate of this region, or if they've been able to shift the timing of life-history events to avoid unfavourable conditions during the summer months.

In conclusion, temperature and moisture extremes pose a threat to survival and development of *S. ocellana* larvae and eggs. Eggs were observed to be drought-sensitive, while larvae were freeze-avoidant. Longer term census and climate data could provide further indication into how abundance and distribution of this pest across

growing regions in the northern hemisphere will respond to changing climate and weather extremes.

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Appendix A

Table A1: Final minimal statistical model for the effects of irrigation (on, off), canopy (upper, lower) and season (early flight period, mid flight period) on temperature in the orchard

Source	DF	F Ratio	Prob > F
Canopy	1	15.4132	**<.0001**
Irrigation	1	1.3844	0.2394
Canopy*Irrigation	1	5.8398	**0.0157**
Season	1	73.6543	**<.0001**
Irrigation*Season	1	17.3254	**<.0001**

Non-significant interactions (p≥0.05) were removed sequentially. Significant results (p<0.05) are in bold font. Deleted terms: Canopy*Season: $F_{(1,22)}$=1.204, p=0.2726, canopy*irrigation*season $F_{(1,22)}$=1.645, p=0.1996.

Table A2: Final minimal statistical model for the effects of irrigation (on, off),canopy (upper, lower) and season (early flight period, mid flight period) on relative humidity in the orchard

Source	DF	F Ratio	Prob > F
Canopy	1	73.7878	**<.0001**
Irrigation	1	0.1237	0.7251
Canopy*Irrigation	1	6.4295	**0.0112**
Season	1	8.2760	**0.0040**

Non-significant interactions (p≥0.05) were removed sequentially. Significant results (p<0.05) are in bold font. Deleted terms: Canopy*season: $F_{(1,22)}$=1.150, p<0.235, irrigation*season: $F_{(1,22)}$=2.557, p<0.110, canopy*irrigation*season: $F_{(1,22)}$=1.181, p<0.277.