

Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations

Graduate Studies

8-2018

Comparative Pollination Efficacies of Bees on Raspberry and the Management of *Osmia lignaria* for Late Blooming Crops

Corey J. Andrikopoulos
Utah State University

Follow this and additional works at: <https://digitalcommons.usu.edu/etd>

 Part of the [Biology Commons](#)

Recommended Citation

Andrikopoulos, Corey J., "Comparative Pollination Efficacies of Bees on Raspberry and the Management of *Osmia lignaria* for Late Blooming Crops" (2018). *All Graduate Theses and Dissertations*. 7086.
<https://digitalcommons.usu.edu/etd/7086>

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



COMPARATIVE POLLINATION EFFICACIES OF BEES ON RASPBERRY
AND THE MANAGEMENT OF *OSMIA LIGNARIA* FOR
LATE BLOOMING CROPS

by

Corey J. Andrikopoulos

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

Approved:

James H. Cane, Ph.D.
Major Professor

Diane Alston, Ph.D.
Major Professor

Brent Black, Ph.D.
Committee Member

Mark R. McLellan, Ph.D.
Vice President for Research and
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2017

Copyright © Corey J. Andrikopoulos 2014

All Rights Reserved

ABSTRACT

Comparative Pollination Efficacies of Bees on Raspberry and the Management of *Osmia lignaria* for Late Blooming Crops

by

Corey J. Andrikopoulos, Master of Science

Utah State University, 2018

Major Professors: Dr. James H. Cane and Dr. Diane Alston
Department: Biology

Unlike many other rosaceous fruit crops, commercial raspberry cultivars are largely self-fertile and can self-pollinate autogamously. However, their floral morphology often prevents complete autopollination. Incomplete pollination yields unmarketable small or crumbly fruits. Insect visitation is therefore essential to maximizing raspberry yield. Honey bees are typically used to pollinate commercial raspberry; however, escalating prices for hive rentals coupled with increasing acreage encourage evaluation of other manageable pollinators. Four other manageable bee taxa – various *Bombus*, *Osmia lignaria*, *O. aglaia*, and *O. bruneri* -- are all promising raspberry pollinators. All five bee species were evaluated and compared for their single-visit pollination efficacies on raspberry. From this a pollinator effectiveness index was created and an estimation of the number of visits required to maximize drupelet set was derived. This estimation was then experimentally verified. Finally, winter management options aimed at delaying the emergence of *O. lignaria* were investigated. These bee have a brief activity period in

early spring that must be altered to coincide with later-blooming raspberry. All five bee species proved efficacious pollinators of raspberry. None of the alternative manageable species greatly outperformed honey bees in pollination efficacy. For this reason honey bees remain the most economical and practical option for open-field raspberry pollination. Alternative manageable bees may instead find greater utility in other production systems, such as high-tunnel or greenhouse grown raspberry, which impede honey bees' ability to forage effectively. The pollinator effectiveness score for honey bees, suggested that as few as two visits can maximize drupelet set. This estimate was confirmed through experimentation on three different red raspberry cultivars. For two of these cultivars, just one visit yielded drupelet counts indistinguishable from openly-pollinated flowers. This information can be used to help refine stocking density estimates for honey bees on raspberry. Wintering bees at 0° or -3° C rather than 4° C effectively delayed emergence of *O. lignaria* by more than a month without any cost to longevity, survival, or reproductive success. Winter storage at near freezing temperatures appears to be a viable management option for the use of *O. lignaria* with later-blooming crops.

(137 pages)

PUBLIC ABSTRACT

Comparative Pollination Efficacies of Bees on Raspberry and the Management of *Osmia lignaria* for Late Blooming Crops

Corey J. Andrikopoulos

Unlike other rosaceous fruit crops such as apple and cherry, commercial raspberry cultivars are largely self-fertile and can set fruit in the absence of pollinators. However, their floral morphology often prevents complete self-pollination. Incomplete pollination yields unmarketable small or crumbly fruits. Insect visitation is therefore essential to maximizing raspberry yield. Honey bees are typically used to pollinate commercial raspberry; however, escalating prices for hive rentals coupled with increasing acreage encourage evaluation of other manageable pollinators. Bumble bees (*Bombus* spp.) and several mason bees (*Osmia* spp.) are promising raspberry pollinators. Five bee species were evaluated and compared for their single-visit pollination efficacies on raspberry. From this a pollinator effectiveness index was created and an estimation of the minimum number of visits required to maximize fruit set was calculated. This estimation was then experimentally verified. Finally, in an attempt to synchronize their brief activity period with raspberry bloom, winter management options aimed at delaying the emergence of the mason bee, *O. lignaria*, were investigated. All five bee species proved excellent pollinators of raspberry. None of the alternative manageable species greatly outperformed honey bees. For this reason honey bees remain the most economical and practical option for open-field raspberry pollination. The adoption of alternative manageable bees could

still be justified in other production systems, such as high-tunnel or greenhouse grown raspberry, which hamper honey bees' ability to forage effectively. The pollinator effectiveness score for honey bees suggested that as few as two visits can achieve maximum fruit set. This estimate was confirmed through experimentation on three different red raspberry cultivars. For two of these cultivars, just one visit yielded drupelet counts similar to openly-pollinated flowers. This information can be used to help refine stocking density estimates for honey bees on raspberry. Wintering bees at 0° or -3° C rather than 4° C effectively delayed emergence of *O. lignaria* by more than a month without any impact on post-winter performance. These results suggest winter storage at near freezing temperatures is a viable management option for the use of *O. lignaria* with later-blooming crops.

ACKNOWLEDGMENTS

There are many people I would like to thank for making this work possible. First, I would like to thank my committee member Dr. Brent Black for his invaluable insights into small fruit production. I would also like to give a special thanks to my academic advisor and committee member Dr. Diane Alston for lending her expertise to me throughout my project, not to mention the support and encouragement she provided me as I tried to navigate graduate school. This work was supported by the Integrated Crop Pollination project funded by the USDA-NIFA award 2012-51181-20105 from the Specialty Crop Research Initiative. I thank Kristin Lambert, Andi Kopit, and especially Shannon Heiner, who helped me count >168,000 raspberry drupelets, for their assistance in collecting data. Thank you to Dr. Chad Finn for his helpful review of my second manuscript. Dr. Michael Mesler for introducing me to the world of pollination biology. My lab mate Byron Love for sharing with me his friendship and wisdom over the last four years. Dr. Theresa Pitts-Singer, Dr. Diana Cox-Foster, and the rest of my friends and colleagues at the bee lab for their continual support and for making my time here a wonderful experience. I would also like to thank the rest of my friends and colleagues at Utah State University for all that you have done for me. I give a very special thanks Sara Kelly and to my parents for the love, compassion, and encouragement they have provided me over the years. I could not have done this without you all. Lastly, I would like to express my deepest gratitude to my research advisor Dr. Jim Cane. Without your assistance, wisdom, and encouragement none of this would have been possible. Thank you for providing me with this opportunity.

Corey J. Andrikopoulos

CONTENTS

	Page
ABSTRACT	iii
PUBLIC ABSTRACT	v
ACKNOWLEDGMENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER	
I. LITERATURE REVIEW	1
II. COMPARATIVE POLLINATION EFFICACIES OF FIVE BEE SPECIES ON RASPBERRY	40
Abstract	40
Introduction	41
Materials and Methods	45
Results	50
Discussion	51
Table and Figures	57
III. TWO PROLONGED BEE VISITS SUFFICE TO MAXIMIZE DRUPELET SET FOR RED RASPBERRY	66
Abstract	66
Introduction	67
Materials and Methods	69
Results	71
Discussion	72
Table and Figures	75
IV. COLDER WINTER TEMPERATURE FOR <i>OSMIA LIGNARIA</i> FACILITATES DELAYED EMERGENCE	81
Abstract	81
Introduction	82
Materials and Methods	88
Results	92
Discussion	96
Tables and Figures	103

V. GENERAL SUMMARY AND CONCLUSION122

LIST OF TABLES

Table	Page
2-1 Pollinator effectiveness scores for bees visiting raspberry	57
3-1 The mean, median and range of honey bee visit durations for first visits of the day at focal flowers	75
4-1 Weight loss of female bees during the overwintering period	103
4-2 Duration of the pre-emergence period for female bees	104
4-3 Fecundity of nesting bees	105

LIST OF FIGURES

Figure	Page
2-1	Drupelet counts for greenhouse grown red and purple raspberry58
2-2	Drupelet counts for the field-cage experiment with red raspberry59
2-3	Drupelet counts for the field-cage experiment with purple raspberry60
3-1	Drupelet counts for ‘Cowichan’ red raspberry.....76
3-2	Drupelet counts for ‘Latham’ red raspberry.....77
3-3	Drupelet counts for ‘Polka’ red raspberry.....78
4-1	The percentage of <i>O. lignaria</i> that survived to emergence.....106
4-2	The percentage of body weight lost during wintering for bees exposed to six combinations of winter temperature and duration107
4-3	Linear regression of a bee’s pre-emergence duration on the percentage of its body weight lost during winter108
4-4	The number of days for <i>O. lignaria</i> to emerge from its cocoon following incubation at 20° C109
4-5	Longevity to starvation for female <i>O. lignaria</i>110
4-6	The percentage of <i>O. lignaria</i> females that successfully established and completed at least one nest113
4-7	Relationship of pre-emergence duration and nesting duration for bees following 182 days of winter temperature.....114
4-8	Relationship of pre-nesting duration and nesting duration for bees following 182 days of winter temperature.....115
4-9	Mean fecundity for female bee following 182 days and 230 days of winter temperature116
4-10	Relationship of pre-nesting duration and fecundity for nesting female bees following 182 days of winter temperature.....117
4-11	Relationship of nesting duration and fecundity for nesting female bees following 182 days of winter temperature.....118

4-12 Relationship of nesting duration and fecundity for nesting female bees
following 230 days of winter temperature.....119

CHAPTER I

LITERATURE REVIEW

Introduction

An estimated 75% of crops grown for human consumption rely, at least to some extent, on insect pollination in order to maximize production (Klein et al. 2007). There has been a steady increase in the amount of land devoted to cultivated crops over the last half century. At the same time, the percentage of land area cultivated with pollinator-dependent crops has increased dramatically, pointing to a trend of increasingly pollinator-dependent agriculture (Aizen et al. 2008). Wild bees are usually insufficiently abundant in agricultural systems to satisfy pollination needs, leading growers to rely on managed pollinators (Winston and Graf 1982; Mackenzie and Winston 1984). European honey bee (*Apis mellifera* L.) is the primary, and in many cases, the sole pollinators of most cultivated crops in Europe and North America (Free 1993). In recent decades, beekeepers in both of these regions have suffered substantial annual colony losses among their managed honey bee populations (FAOSTAT 2018), resulting in increased prices for bee colony rentals. Faltering honey bee populations also raise concern over the sustainability of an agricultural system that relies upon a single species for pollination. Together these factors make it desirable to find new manageable pollinators for use with numerous crops.

One such pollinator-dependent crop is red raspberry (*Rubus idaeus* L.). Europe

and North America account for approximately 97% of global raspberry production. This crop's markets are expanding, evidenced by the doubling of acreage dedicated to raspberry cultivation between 1986 and 2016 (FAOSTAT 2018). Mounting honey bee rental prices for raspberry pollination in the U.S. are buffered somewhat by the expectation of a honey crop produced while servicing the berries (Burgett 1996). Despite this, the rental price for raspberry has nearly doubled in Oregon and Washington over the past 20 years, now averaging \$40/colony (Burgett 1997; NASS 2016). In California, the rental price was \$92/colony in 2016, down sharply from \$147/colony in 2015. This decrease, however, was accompanied by a 40% increase in the acreage pollinated (NASS 2016). California's shift to high tunnel raspberry production further exasperates its pollination dilemma, as honey bees forage poorly under high tunnels (Neilsen et al. 2017), likely due to the plastic film absorbing UV wavelengths honey bees use for navigation (Morandin et al. 2002). This means raspberry grown under high tunnels may require a higher honey bee stocking density to satisfy pollination needs. Growers in California, Oregon, and Washington paid \$1.61 million for raspberry pollination in 2016, up from \$1.25 million in 2015 (NASS 2016). More expensive colony rentals, expanding acreage, and shifting farming practices, coupled with the shrinking supply of honey bees, make raspberry a desirable crop for which to find an alternative manageable pollinator.

Raspberry

Taxonomy

The genus *Rubus* is large, highly diverse, and found throughout all temperate regions of the world; it is most abundant in the northern hemisphere (Jennings et al.

1991). There are thought to be some 900-1000 species of *Rubus* (Thompson 1997), however, species distinction is confounded by extensive hybridization and apomixis (Dickenson et al. 2007). Members of this genus are highly heterozygous with ploidies ranging from 2N to 14N (Jennings 1988; Thompson 1997). Only three of the 12 subgenera contain species producing edible fruit of commercial importance (Jennings et al. 1991). The most extensively cultivated and economically important species belong to subgenera *Idaeobatus* (raspberry) and *Rubus* (blackberry), with species of *Cylactis* (artic raspberry) cultivated on a comparatively small-scale in Northern Europe (Jennings et al. 1991; Karp et al. 2004).

In *Idaeobatus*, aggregate berries separate from their conical-shaped receptacle at maturity, a feature which distinguishes them from the blackberries (Jennings et al. 1991). The subgenus *Idaeobatus* is most speciose in Asia, but also well represented in East and South Africa, Europe, and North America (Jennings 1988). Most modern cultivars of red raspberry can trace their ancestry to crosses between the European red raspberry, *Rubus idaeus* subspecies *idaeus* L. and the North American red raspberry, *Rubus idaeus* subsp. *strigosus* Michx. (Jennings et al. 1991); these subspecies have variably been given specific rank. The black raspberry, *Rubus occidentalis* L., is native to North America where it is also commercially grown. Purple raspberry is a cross of black and red raspberry (Jennings et al. 1991).

Biology

As with most members of the Rosaceae, raspberry flowers have five petals and sepals. The petals are typically small and white; petals abscise 1-5 days after flowers open (Bekey 1985). Sepals persist until the fruit is ripe. A substantial ring of nectary

tissue is located basally outside the ring of reproductive structures. The nectaries are freely exposed to insect visitors once the sepals and petals become fully reflexed (Willmer et al. 1994). Raspberry flowers possess 60-90 stamens arranged in whorls around a central receptacle, and a similar number of styles arise spirally from the receptacle. Stamen and style number are both affected by ploidy and genotype. Each style terminates in an ovary, borne on the receptacle. Pistils must be individually pollinated to set fruit, and each ovary, if fertilized, will develop into a single-seeded drupelet (Jennings 1988).

A drupelet is akin to drupe fruits such as cherry, plum, or peach; a drupe being defined as a fruit that develops entirely from a single ovary (Jennings 1988). Raspberries are aggregate fruits composed of multiple drupelets arising from the same receptacle. Drupelet cohesion is achieved by an abundance of trichomes on the base and sides of each drupelet. These trichomes enmesh to such an extent that drupelets cannot usually be separated without tearing. Cohesion of the aggregate fruit largely depends on the percentage of drupelets set. If too few drupelets set, berries tend to become crumbly and misshapen, rendering them unmarketable. This problem can be caused by inadequate pollination and other factors, such as viral diseases and drought (Jennings 1988).

Commercial raspberry cultivars are predominantly self-fertile (Keep 1968); however, self-pollination is limited. This is due to the spatial arrangement of the anthers relative to the stigmas within the flower. Only the outermost stigmas contact the anthers (Free 1993). Shanks (1969) found in his experiments on bee pollination of raspberry, that when self-pollination did occur, it was always in the proximal 1-4 rows of pistils. Agitation by wind or shaking of the plants provided no benefit to pollination. It is for this

reason that insect pollination is crucial for maximum fruit yield in raspberry, although some cultivars may benefit more than others due to apparent variation in self-fertility (Daubeny 1971; Shanks 1969; Wieniarska 1987; Bekey 1985).

Although early studies on cultivated raspberries showed no evidence of cross-pollination increasing yield (Hardy 1931a); later work has demonstrated marked metaxenic effects from cross pollination (Redalen 1976; Colbert and de Oliveira 1990; Żurawicz 2016, Żurawicz et al. 2018). The fact that some cultivars benefit from out-crossing is not entirely surprising. Raspberry flowers possess several characteristics typical of outbreeding species, including showy flowers with copious nectar production, substantial heterozygosity, and often, decreased vigor when inbred. In fact, wild *Rubus idaeus* is largely self-incompatible (Keep 1968).

Raspberry cultivars are typically classified as either florican- or primocane-fruiting types. Florican-fruiting cultivars, also known as summer-bearing, are biennial. They flower in the spring on laterals formed in the leaf axils of second year canes. Florican-fruiting varieties require a period of winter chill in order to properly break dormancy and flower in the spring; the duration of this period varies widely among cultivars (Jennings 1988, Dale et al. 2003). This “chill” requirement largely restricts florican-fruiting to regions that experience moderately cold winter temperatures. Primocane-fruiting varieties, by contrast, do not require a chilling period to complete dormancy. These cultivars will flower twice a year, on the upper half of first year canes in autumn and on the lower half of second year canes the following spring. They are often managed as a single fall-crop by mowing second year canes to the ground prior to flowering. This technique is less labor intensive and will produce a higher quality crop

than fruiting in both summer and fall (Pritts 2008). The lack of chill requirement makes primocane-fruiting cultivars very amenable to off-season production, as they can be easily manipulated to extend the growing season using techniques for protected cultivation (e.g. greenhouses) (Pritts 2008).

Cultivation

Cultivation of red raspberries (*Rubus idaeus*) can be traced back to Rome in 4th century A.D.; raspberry seed have been found at the sites of Roman forts in Britain around that time (Jennings 1988.). One of the earliest written references to raspberry is by Pliny the Elder in 45 AD, who described how the Greeks referred to them as “ida” fruits, and red raspberries are thought to have their origin in the Ide Mountains of Turkey, hence their specific epithet (Jennings 1988). Compared to other domesticated fruit crops, raspberry has a relatively short history of cultivation of about 500 years. Selection and naming of superior strains began in earnest in western Europe in the 1600’s; however, it wasn’t until the early part of the 20th century that modern breeding programs were established, first in North America and then later in Europe. These breeding programs led to raspberry varieties with improved yields, fruit quality, and resistance to diseases (Jennings 1988). The primocane growth habit has been described for red, black, and purple raspberry, and has been known for centuries (Keep 1961). It wasn’t until the latter half of the 20th century, however, that breeders produced commercially acceptable cultivars (Pritts 2008).

Raspberry acreage is concentrated in temperate regions of the Northern Hemisphere where winters are relatively mild and summers are moderate. Demand for a year-round supply of fresh berries has, in recent decades, pushed production into more

marginal environments with milder winters (Kempler et al. 2012). This is especially true for Mexico, which saw a 10-fold increase in raspberry production from 2006-2016. This expansion was to satiate the growing demand for off-season fresh berries in the United States (U.S.) and Canada (FAOSTAT 2018). By 2016, raspberries were grown in at least 46 countries, yielding an estimated 795,249 tonnes (FAOSTAT 2018). The Russian Federation, U.S., Poland, Mexico, and Serbia were the top five producers of raspberry in 2016; combined they account for more than three quarters of the world's production (FAOSTAT 2018). Black raspberry is grown almost exclusively for the processing market. Most acreage is in the U.S. Pacific Northwest, although there is increasing production in South Korea. Purple raspberry is grown on a limited scale in China and North America (Kempler et al. 2012).

In the U.S., red raspberries have traditionally been grown in open orchards, with both red and black raspberry acreage concentrated in California, Oregon, and Washington (NASS 2016). Smaller regions supplying the fresh raspberry market are located in the Northeast and Great Lakes regions. Washington is the largest supplier of red raspberries for the processed market, while Oregon is the largest producer of black raspberry. California produced more than half the raspberries in the U.S. in 2016; the majority of which also satisfies the fresh berry market in the U.S. and Canada (NASS 2016). Demands imposed by a rapidly expanding fresh berry market have prompted some growers to adopt alternative production techniques, such as greenhouses and high tunnels. These protected cultivation techniques can greatly extend the growing season of raspberry at both ends of the typical field season, allowing for a nearly year round supply of fresh berries (Pritts et al. 1999; Pritts 2008; Demchak 2009). In the U.S., high tunnels

are now widely used in California and supply most of the off-season fresh fruit in North America (Pritts 2008; Demchak 2009). Greenhouse production in the U.S. has been slower to catch on and is primarily located in the Northeast, where it offers a supply of fresh fruit during the winter months (Pritts et al. 1999; Dale et al. 2003).

Raspberry Pollination

Timing of Pollination

The timing of pollination is critical for maximizing fruit set in. There is some variation among raspberry cultivars in the duration of stigma receptivity, and thus, timing for optimal pollination (Bekey 1985; Hardy 1931a; Eaton et al. 1968, Redalen 1976). Stigmatic receptivity in raspberry can be assessed visually, as their stigmatic surfaces become “split into a small fork” giving them a “fuzzy appearance” at this stage of development (Hardy 1931b). Emasculation and hand-pollination experiments by Hardy (1931b) suggested peak receptivity of *Rubus* stigmatic surfaces two to five days after emasculation. Eaton et al. (1968) hand pollinated flowers daily for four days after emasculation, but found similar drupelet set could be achieved with just one application on the second day. Bekey (1985), in his study of eight cultivars, found that the period of maximum receptivity lasted from one to four days after emasculation, usually peaking in the first two days. Additionally, for all but two cultivars, hand pollination for one day at the peak of receptivity produced as many drupelets as hand pollination on three consecutive days.

Temperature influences the duration of stigmatic receptivity and anther dehiscence for red raspberry flowers. Overall rate of flower development accelerates with

increasing temperature from 6° C to 22° C. After about three days at 18° and 22° C, pistils start to deteriorate, denoted by the appearance of tiny black specks on the tips of the stigmas and loss of their “glistening, sticky appearance”. If cooled to 6 to 10° C, pistils retain a healthy appearance for more than two weeks. Another deterioration follows a similar pattern, although at low temperatures anthers remained healthy for longer than the stigmas (Bekey 1985). It has been estimated that red raspberry anthers will dehisce for 3 to 8 days with temperature at free dehiscence ranging from 11.8 to 20.4° Celsius (Percival 1955). Pollen viability quickly deteriorates at high temperatures (Bekey 1985).

Bekey (1985) also reported that pollen-tubes grew much faster when exposed to warm air temperatures, taking just 1 to 2 days to reach the base of the style at 18° and 22° C, compared to 4 to 5 days at 6° and 10° C. The time required for subsequent fertilization, once pollen tubes reach the ovaries, was not directly studied for raspberry, but can potentially be extrapolated from this work. Petal drop appears to respond to fertilization in many plants (Percival 1979). At 6° C, petals were retained for 10 days (Bekey 1985). If petal drop does indeed coincide with fertilization for raspberry, fertilization would ensue about five days after the pollen tube has reached the base of the style. This accords with insights for the pace of fertilization after pollen tube completion in several other fruit crops (Sanzol and Herrero 2001).

Attracting Pollinators

Compared to many other flowers of cultivated Rosaceae, *Rubus idaeus* produces a limited quantity of pollen per flower, and because anthers dehisce over several days, even less pollen is available at any given time (Percival 1955; Willmer et al. 1994). Percival (1955) reported production of just 1.1 mg of pollen over the life span of *Rubus idaeus*

flowers, and just 0.2 mg per day. For comparison, Percival (1955) measured 4.9 mg pollen over the lifetime of *Rubus loganobaccus* Bailey flowers, and 2.4 mg per day. Limited pollen production does not necessarily make a flower less attractive (Percival 1955); however, in the case of *Rubus idaeus*, most visiting bees do not focus on actively collecting pollen. Of the 387 honey bee visits observed by Free (1968), about half of them only took nectar, whereas only two foragers were seen to deliberately collect pollen. Bekey (1985) noted that only between 18 and 58% of honey bees observed foraging on raspberry carried pollen loads and none were seen deliberately collecting it, while Chagnon et al. (1991) reported 66% of honey bees collecting nectar only.

Bumble bees collect pollen more frequently than do honey bees, but they too are more likely to visit raspberry for its nectar. Comparing honey bees with bumble bees visiting raspberries, Willmer et al. (1994) reported most forager visits were for nectar only (69% and 49% respectively), whereas very few bees collected pollen alone (4% and 9% respectively). At best, pollen seems of secondary interest to these social bees foraging at raspberry flowers. In fact, multiple researchers report seeing bees groom pollen from their bodies and discard it (Free 1968; Bekey 1985; Willmer et al. 1994; Chagnon et al. 1991; Pers. Obs.).

Utilization of raspberry pollen by *Osmia* bees is less studied and differs by species. The red mason bee, *O. rufa* L., will collect pollen from raspberry in the U.K. (Raw 1974). While one nest in this study contained nearly 100% red raspberry pollen, the majority of nests examined contained only small amounts, suggesting that raspberry pollen is typically collected incidentally by these bees as they visit the flowers for their nectar. Another European species, *O. cornuta* Latreille, has been shown to reproduce well

with blackberry as a sole pollen source (Pinzauti et al. 1997). In the U.S., *O. aglaia* Sandhouse has been evaluated as a pollinator of cultivated *Rubus*, and in contrast to honey bees and bumble bees, nearly always collects raspberry pollen and nectar at the same time (Cane 2005). In Japan, *O. orientalis* Benoist collected pollen on half of its visits to the wild raspberry *Rubus hirsutus* Thunberg, whereas only 10% of foraging honey bees collected its pollen. In contrast to social bees previously discussed, half of the pollen-collecting visits by *O. orientalis* were for pollen only, suggesting a strong preference for raspberry pollen (Yokoi and Kandori 2016).

The primary floral reward attracting bees to red raspberry is nectar, which is copiously produced (Free 1993). The reported volume of nectar a red raspberry flower can secrete per day varies greatly between studies ranging from approximately 7 to 50 μl per day. The variation likely reflects differences in cultivar, air temperature, relative humidity, age of the flower, and timing of sampling (Whitney 1984; Bekey 1985; Willmer et al. 1994; Schmidt et al. 2015). Even at the lower end of this reported range, red raspberry secretes far more nectar than many other flowering species, which average $< 1 \mu\text{l}$ per day (Percival 1979). For wild *Rubus idaeus*, Whitney (1984) reported an average volume of 17 μl per day, an order of magnitude greater than wild co-flowering species in his study, including other species of *Rubus*.

Nectar is first secreted during late bud stage for most raspberry cultivars (Saez et al. 2017); it then peaks 1 to 3 days after floral opening (Willmer et al. 1994; Bekey 1985). Peak nectar secretion appears to coincide with anthesis, with greater volumes secreted by those cultivars that continue shedding pollen over several days (Schmidt et al. 2015). Even under identical external conditions, cultivars differ by as much as four-fold in the

volume of nectar secreted daily (Willmer et al. 1994; Schmidt et al. 2015). Nectar volumes are typically greatest in the morning, presumably due to accumulation overnight in the absence of insect visitation (Chagnon et al. 1991; Willmer et al. 1994). Nectar secretion seems to be rapid and continuous throughout the day (Willmer et al. 1994), although cultivars can differ in their pattern of diurnal nectar secretion (Schmidt et al. 2015).

Temperature strongly influences nectar secretion by raspberry flowers. Bekey (1985) found that when kept at 6° C, raspberry flowers continued to secrete nectar over at least 4 days, peaking on the third day. At 22° C, secretion peaked on the first day and ended after 48 h, while at 14° C nectar production lasted for 3 days, peaking on the second day. These temperature effects are similar to those for floral development and stigmatic receptivity. Nectar is the primary floral reward for insects visiting raspberries, so it seems adaptive for the plant to terminate nectar secretion once its aging stigmas have lost receptivity (Bekey 1985). Diurnal fluctuations in nectar volume are also related to air temperature. Rising air temperature throughout the day results in a concomitant decrease in nectar volume, whereas constant air temperature leads to an increase in nectar volume throughout the day (Schmidt et al. 2015.) This pattern is likely due to increased evaporation at higher temperatures rather than decreased secretion (Bekey 1985).

Pollination Efficacy

When evaluating a pollinator's performance with regards to its potential as a commercial pollinator, it is important to evaluate several criteria, including fidelity to the crop, pollination efficacy, and behavioral or morphological attributes that affect pollinator performance. For solitary bees, their phenology relative to the crop's bloom

and their ability to reproduce on the crop are also important. This study focuses on the comparative pollination efficacy of different manageable bee species visiting *R. idaeus*, in terms of their contribution to drupelet set.

In agricultural systems, where harvestable yield of the crop is of primary concern, a pollinator's efficacy is best measured as the contribution by the pollinator to the female reproductive function of the plant. When comparing pollination efficacy of different bees, this contribution is sometimes measured indirectly as the number of pollen grains deposited per visit on the flowers stigma (Willmer et al. 1994; Saez et al. 2014). This method of evaluating pollinator efficacy has some advantages in that sampling can be quick and samples can be processed immediately following visitation. This indirect measure of pollination efficacy has several drawbacks. First, comparing pollinator efficacy this way assumes a linear relationship between pollen deposition and fruit or seed set. In reality, the relationship may be asymptotic as fruit set approaches a maximum (Spears 1983; Young and Young 1992; Cane and Schiffauer 2003), and comparisons of simple pollen counts on stigma will generally overestimate the differences in pollinator efficacy. Refined comparisons can be made by incorporating dose-response relationships between pollen depositions and fruiting success (Cane and Schiffauer 2003). Unfortunately, dose-response relationships are generally unknown and difficult to develop, limiting the utility of this technique. Secondly, pollen deposition in great excess of what is needed for maximum fruit set can have negative effects on pollen tube growth and subsequently reduce fertilization (Cruzan 1986; Young and Young 1992). Lastly, extra pollen deposition resulting from extremely frequent visits to a flower may be offset by damage to the pistils incurred during floral visitation (Young and Young 1992; Saez et

al. 2014).

The problems with using indirect measures for evaluating pollinators can be illustrated with honey bees and raspberry. Honey bees are known to be effective pollinators of *R. idaeus* (Shanks 1969; Bekey 1984; Chagnon et al. 1991), but several studies suggest that other manageable bees may be equal or superior to honey bees at pollinating raspberry (Willmer et al. 1994; Cane 2005; Cane 2008; Saez et al. 2014). Honey bees transfer fewer pollen grains to stigmas per visit than do bumble bees (Willmer et al. 1994). However, this comparison is insufficient, as the pollen deposition by both types of bee might exceed the quantity required to achieve fruit set (Cane and Schiffhauer 2003). Saez et al. (2014) found no significant relationship between drupelet set and the number of pollen grains per stigma, even when deposition on stigmas was well below the 47 grains per honey bee visit reported by Willmer et al. (1994). For this reason, a direct measure is better suited to compare the efficacy of pollinators for *R. idaeus*.

Direct measures of pollinator efficacy (e.g. fruit or seed set) are often more informative in attributing the contribution of the pollinator to the female reproductive function of the plant; this type of evaluation has more often been used to assess pollinator performance for *R. idaeus* (Shanks 1969; Bekey 1984; Chagnon et al. 1991; Cane 2005; Cane 2008; Lye et al. 2011; Saez et al. 2014). Contributions to the female reproductive success of raspberry, attributable to pollinator visitation, can be measured as the number of drupelets or seeds set, or resulting berry weight (Shanks 1969; Bekey 1984; Chagnon et al. 1991; Cane 2005; Cane 2008; Saez et al. 2014). At the field scale it can be measured as the change in harvestable yield with the addition of pollinators (Lye et al.

2011). The drawback of direct measures of pollinator performance is that they don't take into account post-pollination processes that can limit fruit and seed set (e.g. limiting maternal resources). Additionally, it can be impractical to wait for fruits to mature in the field before collecting them to evaluate pollination efficacy (Cane and Schiffhauer 2003). Despite these drawbacks, direct measures are usually preferable when logistically possible.

For both direct and indirect measures of pollination efficacy, several methods of data collection may be employed. Studies on raspberry pollinators for *R. idaeus* have used single-visit efficacy (Willmer et al. 1994), sequential visits or open-pollination (Shanks 1969; Bekey 1985; Cane 2005; Cane 2008; Prodorutti and Frilli 2008), cumulative durations of visitation (Bekey 1985; Chagnon et al. 1991), or visitation frequency (Bekey 1985; Saez et al. 2014), to compare pollinator efficacy. Each method has benefits and limitations.

For instance, single-visit efficacy, defined as the number of drupelets resulting from a single visit by a bee to a virgin flower, is limited in that does not account for behavioral differences away from the flower which may affect overall performance as a crop pollinator. It does have great utility, however, in that it provides a simple, direct comparison of pollinator efficacies. Additionally, single-visit efficacy can be easily transformed into an index of pollinator efficacy according to Spears (1983). Pollinator effectiveness (PE) for each species of visitor can be calculated as $PE_i = \frac{(P_i - Z)}{(U - Z)}$; where P_i = the mean number of seed set resulting from a single-visit by species i , Z = the mean number of seeds set in the absence of visitation, and U = the mean number of seeds set

when visitation is unrestricted. This calculation is useful because it places efficacy in the context of the floral species' realized fruiting potential, from autogamy to open pollination. Furthermore, by calculating the inverse of PE_i , it is directly translatable into the minimum number of visits needed to achieve full seed set. For these reasons single-visit efficacy will be used to compare pollinator performance here.

Raspberry Pollinators

The copious floral rewards of *Rubus idaeus* encourage a diverse assemblage of insect visitors. Hansen and Osgood (1983) collected specimens from 49 insect families representing five orders (Hymenoptera, Diptera, Coleoptera, Lepidoptera, and Hemiptera) on wild raspberry flowers in the northeastern U.S. Not all of these visitors pollinate red raspberry, and among those that do, not all contribute equally (Willmer et al. 1994; Hansen and Osgood 1983; Saez et al. 2014). For instance, Hemipterans, such as stink bugs (Pentatomidae), are fruit pests often associated with raspberry and likely don't contribute to pollination. Coleopterans may provide a limited pollination service as they visit flowers for nectar, however, the most common beetles found in association with red raspberry flowers are *Byturus* spp. (Family Byturidae) (Hansen and Osgood 1983; Willmer et al. 1994). These beetles use raspberry flowers as feeding, mating, and oviposition sites and can cause extensive economic damage (Willmer et al. 1998). Lepidopterans have only been reported in one study (Hansen and Osgood 1983), and therefore are not likely to be important pollinators of *R. idaeus*.

Hover flies (Family Syrphidae), are likely the most important non-bee pollinator of raspberry. Numerous studies have noted their presence as a significant part of raspberry's pollinator assemblage (Hansen and Osgood 1983; Whitney 1984; Willmer et

al. 1994; Lye et al. 2011). They may account for up to 10% of visitations to raspberry in some regions (Prodrutti and Frilli 2008). The efficacy of hover flies pollinating raspberry has not been studied; however, hover flies have been reported to be effective pollinators of blackberry (Yeboah et al. 1987).

The lion's share of pollination to raspberries is attributable to bees, which are by far the most abundant and important visitors of *R. idaeus* (Winston and Graf 1982; Whitney 1984; Mackenzie and Winston 1984; Willmer et al. 1994; Prodrutti and Frilli 2008; Lye et al. 2011; Saez et al. 2014; Jim Cane pers. comm.). Where honey bees are absent, the bee fauna of wild *R. idaeus* can be dominated (~80%) by solitary bees active in early spring, such as *Andrena* spp. and halictids; the remainder belong to social bumble bee species (Whitney 1984). Other important solitary bees associated with raspberry include colletids (Willmer et al. 1994; Saez et al. 2014) and *Ceratina* spp. (Jim Cane pers. comm.).

Social bees are the most important pollinators of commercial *R. idaeus*. Bumble bees are the most abundant wild pollinators, and along with domesticated honey bees, dominate the bee fauna of cultivated *R. idaeus*. Solitary bees and other insects usually represent <10% of the floral visitors in these settings (Winston and Graf 1982; Mackenzie and Winston 1984; Willmer et al. 1994; Prodrutti and Frilli 2008; Lye et al. 2011; Saez et al. 2014; Jim Cane pers. Comm.). On farms where managed honey bees are provided for pollination, honey bees are overwhelmingly abundant, accounting for up to 95% of floral visitors (Winston and Graf 1982; Mackenzie and Winston 1984; Jim Cane pers. comm.). Where wild bumble bees are abundant and no honey bees are intentionally placed near the crop, bumble bees make up a much larger proportion of the floral visitors

to commercial *R. idaeus*, in some case outnumbering honey bees (Willmer et al. 1994; Saez et al. 2014, Jim Cane pers. Comm.). The abundance of bumble bees relative to other wild bees likely makes them the most important wild pollinator of raspberry.

Alternative Manageable Bees

The most promising alternative manageable bees for raspberry are bumble bees (*Bombus* spp.) and mason bees (*Osmia* spp.). Bumble bees are likely the most important wild pollinator of raspberry (Winston & Graf 1982; Mackenzie & Winston 1984; Willmer et al. 1994; Lye et al. 2011; Sáez et al. 2014; Nielsen et al. 2017), and are suggested by most raspberry production guides as disposable pollinators of greenhouse-grown raspberry (Pritts et al. 1999). Bumble bees will forage more effectively than honey bees when confined to a greenhouse environment, likely due to the plastic film absorbing UV wavelengths honey bees use for navigation (Morandin et al. 2002). Colonies of several species are available for purchase, included the eastern North American species *B. impatiens* Cresson. The price per colony has decreased significantly in recent decades as rearing method have improved and is now comparable to honey bee colony rentals (Velthuis and Dorn 2006). However, bumble bee colonies typically contain far fewer workers than honey bee colonies, making them more expensive on a per forager basis. Willmer et al. (1994), reported that bumble bees deposited about twice the amount of pollen on raspberry stigmas per visit than honey bees, suggesting they may be superior pollinators for this crop.

Mason bees are solitary, cavity-nesting bees, some of which will accept man-made nesting substrates, making them amenable to commercial management. Several species of mason bees have been developed as pollinators of rosaceous tree fruits

(reviewed in Sedivy and Dorn 2014). Largely due to behavioral differences at the flower, these bees have proven more efficient than honey bees at pollinating rosaceous crops such as almond (Bosch and Blas 1994), apple (Vincens and Bosch, 2000), and pear (Monzon et al., 2004). Honey bees often forage for nectar alone, failing to contact flower stigmas, while *Osmia* usually collect both pollen and nectar on every foraging trip, contacting the reproductive parts of the flower. Cane (2005) noted similar behavioral differences between *O. aglaia* and honey bees foraging on raspberry, for which they proved to be equally effective pollinators when allowed to openly forage on the flowers (Cane 2005; Cane 2008). Like bumble bees, mason bees forage well when confined to greenhouses or high tunnels. The European species, *Osmia cornuta*, has been shown to pollinate and reproduce on blackberries under high tunnels (Pinzauti et al. 1997). The results of these studies are promising; however, they are as of yet insufficient to warrant the adoption of an alternative pollinator for raspberry. Here we test the pollination efficacy of three species of osmia that show promise as raspberry pollinators.

The mason bee, *O. aglaia*, has been evaluated as a pollinator of raspberry. It is as effective as honey bee when allowed to openly forage on the flowers (Cane 2005; Cane 2008). The native range of *O. aglaia* is from southern California to west-central Oregon. They emerge in late spring, coinciding with raspberry bloom in the Pacific Northwest. The closely related *O. bruneri* Cockerell is native to the Intermountain West and Rocky Mountains, where it has potential as a commercial pollinator of cane fruit in that region. While these species are known to visit and pollinate raspberry, their relative utility for pollinating cultivated raspberries requires demonstration of their pollination efficacy on a per visit basis relative to the widely used honey bee.

The blue orchard bee, *Osmia lignaria* Say, is a solitary bee native to and widely distributed across North America. As a result of its gregarious nesting habit, ready adoption of man-made nesting substrates, and affinity for rosaceous flowers, these bees have garnered significant attention in recent decades as an alternative or supplement to honey bees for pollination of several rosaceous fruit tree crops (Bosch and Kemp 1999, 2000, 2003; Bosch et al. 2000, 2006; Kemp and Bosch 2005; Sheffield 2008, 2014; Torchio 1976, 1981, 1985). Blue orchard bees have proven highly effective at pollinating spring-flowering crops, such as almond (Torchio 1981), apple (Torchio 1985; Sheffield 2014), and cherry (Bosch and Kemp 1999; Bosch et al. 2006) This bee is available for purchase in large numbers, enabling growers to purchase them as a supplement or alternative to honey bees.

Management of these bees for commercial pollination is not without out its challenges. One major obstacle is the relatively short activity period each year during which foraging bees are available to provide pollination services. Adult *O. lignaria* emerge in early spring and foraging females remain active for about six weeks. The flight seasons of local populations are often coincident with apple or cherry bloom (Bosch and Kemp 1999; Torchio 1985; Sheffield 2014), but precede raspberry bloom by a month or more. To use *O. lignaria* as raspberry pollinators, methods are be needed to control emergence timing without compromising longevity, vigor or survival. Fortunately, these bees are amenable to manipulations of their life cycle, allowing for their emergence to be synchronized with a variety of crops (Bosch and Kemp 2000; Bosch et al. 2000; Kemp and Bosch 2005).

Management of *Osmia lignaria*

Life Cycle

Adult *O. lignaria* usually emerge from March – May depending on geographic location (Bosch and Kemp 2000; Kemp and Bosch 2005; Pitts-Singer et al. 2014). In this protandrous species, male emergence precedes the larger females by about a week (Sheffield 2008). Females typically mate and begin nesting within a few days after emergence (Tepedino and Torchio 1982; Sgolastra et al. 2016). Nests are constructed in pre-existing cavities such as abandoned beetle burrows and consist of a linear series of cells separated by mud partitions. Each cell is provisioned with a mass of pollen and nectar upon which an egg is laid. Larger provisions with female eggs are typically positioned in the innermost cells of the nest (Levin 1966; Phillips and Klostermeyer 1978; Torchio 1989). Eggs hatch after several days and begin consume the provision mass. Development of *O. lignaria* proceeds through five larval instars, and by late spring the fifth instar finishes feeding, spins a cocoon, and enters the prepupal stage (Torchio 1989). At this point prepupae undergo a short diapause-mediated summer dormancy (Kemp et al. 2004; Kemp and Bosch 2005; Sgolastra et al. 2011, 2012). Following the summer dormancy period, bees pupate, and by late summer or early fall, adults eclose but remain inside their cocoons (Bosch and Kemp 2000). A prewintering period ensues as bees acclimate to the onset of winter temperatures. During this time, respiration rates drop (Bosch et al. 2010; Kemp et al. 2004), and bees soon enter an obligate winter diapause. After approximately 3 months of wintering, bees enter a postdiapause

transitional period prior to emergence in the spring (Kemp et al. 2004). With a distribution in North America from Canada to Mexico, *O. lignaria* occurs across a wide variety of environments (Kemp and Bosch 2005). It is no surprise, that there is significant interpopulation variation in the lifecycle phenology of *O. lignaria*, with specialization for local environments (Bosch et al. 2000; Pitts-Singer et al. 2014).

Temperature and Development

As a result of spending the majority of their adult lifespan in the dark confines of their cocoons, temperature is the primary environmental cue regulating the lifecycle phenology of *O. lignaria* (Sgolastra et al. 2010, 2011). The effects of temperature and its duration on the larval development (egg-prepupa), summer dormancy (prepupa-adult), prewintering (adult-onset of winter temperature), and wintering (onset of winter temperature-emergence) periods, have been studied extensively in both wild and laboratory reared bees (Bosch and Kemp 2000, 2003; Bosch et al. 2000, 2010, Kemp et al. 2004, Kemp and Bosch 2005; Pitts-singer et al. 2014; Rust 1995; Sgolastra et al. 2010, 2011, 2012, 2016; Sheffield et al. 2008). Larval development rate has been shown to increase with temperature up to at least 29° C, and fluctuating temperature accelerates development more than bees experiencing the equivalent (same mean) constant temperature (Bosch and Kemp 2000).

The duration of the summer dormancy period is similarly affected by temperature. The threshold temperature for the completion of prepupal dormancy in *O. liganaria* is about 18° C, below that, bees may fail to reach adulthood prior to winter (Bosch and Kemp 2000; Kemp and Bosch 2005). The rate of prepupa to adult development increases with temperature up to about 26° C (Kemp and Bosch 2005), and the effect of fluctuating

temperature relative to its constant equivalent, is even more dramatic than its effect on larval development rate (Bosch and Kemp 2000). A closer examination of temperature responses during the summer dormancy period reveals prepupa and pupa respond differently to temperature. Prepupa exhibit a U-shaped developmental response with a maximum rate at around 26° C and prolonged development at 18° C and 32° C, while pupa show a linear increase in developmental rate with increasing temperature up to at least 32° C (Kemp and Bosch 2005). While temperature has a strong effect on the duration of summer dormancy, it is also genetically mediated (Bosch et al. 2000; Pitts-Singer et al. 2014; Sgolastra et al. 2012).

The summer dormancy period appears to serve the function of synchronizing adult eclosion with the onset of winter temperatures, and its duration differs according to the geographic origin of the population. Differences in duration between geographically disparate populations are maintained even when exposed to the same thermal regime (Bosch and Kemp 2005; Bosch et al. 2000; Kemp and Bosch 2005; Pitts-Singer et al. 2014; Sgolastra et al. 2011, 2012). Early-flying bees, from lower latitudes, complete larval development much earlier than do later-flying populations. Fast-developing bees that eclose as adults too early may be exposed to warm temperatures for long periods prior to the onset of winter conditions. This can result in the rapid consumption of metabolic reserves (Bosch et al. 2010, Sgolastra et al. 2012). Bees from southern populations have a longer prepupal dormancy period compared to northern populations, thus delaying adult eclosure beyond peak summer temperatures (Kemp and Bosch 2005; Pitts-Singer et al. 2014; Sgolastra et al. 2012).

Synchronization with winter temperatures is important in this species because a

protracted pre-wintering period, the time from adult eclosure to the onset of winter temperatures, can have a negative impact on body weight, lipid reserves, the rate of diapause development, winter survival, time to emerge, and post winter longevity (Bosch et al. 2000, 2010; Sgolastra et al. 2011). Alternatively, if exposed to too short of a pre-wintering period bees may not have enough time to synthesize cryoprotectants or sufficiently reduce their respiration rate. This could impact winter survival or disrupt the pattern of diapause development resulting in a longer pre-emergence period (Bosch et al. 2010; Sgolastra et al. 2011, 2016). Optimal pre-wintering duration for *O. lignaria*, in terms of minimizing weight loss, fat body depletion, winter mortality, and pre-emergence time, appears to be around 30 to 45 days (Bosch et al. 2010; Sgolastra et al. 2011). Pre-wintering durations of 29 to 67 days do not impact reproductive success for *O. lignaria* (Sgolastra et al. 2016). The optimal pre-wintering duration seems to reflect the natural variation experienced by wild population, as adult eclosure typically occurs over 1 to 2 months (Bosch and Kemp 2000; Bosch et al. 2000).

Diapause initiation occurs during the pre-wintering period, and adult *O. lignaria* enter diapause within a few days of eclosure. The initiation of diapause does not require a temperature cue, indicating that it is obligatory for *O. lignaria* (Bosch et al. 2010). Diapause is most intense during the pre-wintering period. Despite this metabolic depression, weight loss and fat body consumption remains high while temperatures remain warm. Completion of diapause on the other hand, requires exposure to cold temperatures for a prolonged period of time. Exposure to cold temperature results in a rapid easing of diapause intensity and increase in respiration rate (Bosch et al. 2010; Kemp et al. 2004; Sgolastra et al. 2010). Metabolic rate stabilizes shortly after the initial

response to cold temperature and remains steady for approximately 100 days. Diapause intensity during this period appears to be independent of temperature, with bees displaying the same respiration response at all temperatures. The duration of this period is fixed (Sgolastra et al. 2010), a notion supported by the finding that bees wintered for <90 days show a dramatic increase in winter mortality and emergence time, and decreased post-winter longevity (Bosch and Kemp 2003).

After about 100 days, mid-December in natural populations, bees have the potential to terminate diapause. At this point respiration rates begin to increase exponentially (Kemp et al. 2004; Sgolastra et al. 2010) and diverge among bees wintered at temperatures ranging from 0° to 7°C, with a faster rate of increase at warmer temperatures. Therefore, the duration of the diapause termination period is dependent upon winter temperature, with warmer temperatures eliciting shorter durations (Sgolastra et al. 2010). Respiration rate must reach a threshold level prior to emergence. Bees wintered at 7° C reach sufficient levels after approximately 150 days of winter temperature. Bees wintered at 4° C require closer to 200 days, and bees exposed to 0° C need >210 days (Sgolastra et al. 2010). Bees that don't reach the threshold metabolic level prior to incubation may experience a prolonged pre-emergence period and decreased post-emergence longevity. Bees that are never exposed to winter temperatures respond by keeping their respiration rates low throughout the winter, and therefore, never reach levels sufficient for emergence even if they survive the winter (Sgolastra et al. 2010).

Manipulating winter storage temperatures can be an effective means of managing *O. liganaria* for crop pollination, but must be done with care to ensure bees are healthy

upon emergence. The short bloom (2 to 3 weeks) of many of many orchard crops necessitates that bees managed for pollination must not only survive the winter, but must emerge over a short period of time and be vigorous enough to nest and provide pollination services (Bosch and Kemp 2003). Winter storage temperature, likely through its effect on diapause intensity and duration, has a strong influence on winter survival, pre-emergence duration, and measures of post-winter vigor such as weight loss, fat body depletion, and longevity to starvation (Bosch and Kemp 2003; Bosch et al. 2010; Rust 1995; Sgolastra et al. 2010, 2011; Sheffield et al. 2008). The pre-emergence period contracts as temperature increases for winter durations >150 days. A short pre-emergence period is necessary for synchronizing bee activity with bloom. This concern may outweigh some of the negative effects on survival, weight loss, fat body depletion, and longevity to starvation for bees stored at warmer temperatures (Bosch and Kemp 2003; Bosch et al. 2010; Sgolastra et al. 2010, 2011).

Slight reductions in survival and longevity are seen for bees overwintered at 4° C compared to 0° C after 150 days of wintering. This reduction is not considered large enough to offset the extended emergence time of bees wintered at 0° C (Bosch and Kemp 2003). The difference in survival and longevity becomes more exaggerated as wintering duration extends. Bees overwintered at 4° C for >210 days still emerge over a shorter period of time than do bees wintered at 0° C, however, they experience a large (approx. 20-30%) decrease in survival and longevity (Bosch and Kemp 2000). Weight loss and fat body depletion follow a similar pattern, being greatest at warmer temperatures (Sgolastra et al. 2010, 2011). Female *O. lignaria* will not emerge without exposure to spring temperature (approx. 20° C), even if they have completed diapause and have achieved a

metabolic rate sufficient for emergence. Bees that complete diapause but remain in winter storage rapidly consume their energy reserves (Sgolastra et al. 2010, 2011). Bees wintered at 7° C reach respiration rates suitable for quick emergence after approximately 150 days (Sgolastra et al. 2010), yet have unacceptable levels of winter mortality and reduction in vigor if kept at this temperature for >150 day (Bosch and Kemp 2003).

While warmer temperatures elicit greater overwinter weight loss, differences in weight loss upon emergence are offset to some degree by the extended pre-emergence period of bees that don't complete diapause before incubation. Bees wintered at 4° C for 140 days lost less weight during winter than bees wintered for 252 days; however, bees in the 140 day treatment required more time to emerge. As a result the two treatments did not differ in weight loss upon emergence (Sgolastra et al. 2010). Differences in the pre-emergence period persist for bees wintered at 4° C and 0° C, up to 270 days of storage (Bosch and Kemp 2003). The question therefore becomes, at what point do the consequences of warmer winter storage temperature outweigh the benefit of a short pre-emergence period?

Delaying Emergence

Management strategies for *O. lignaria* have mostly focused on methods to advance bee emergence, rendering this question moot (Bosch and Kemp 2000, 2003; Bosch et al. 2000; Kemp and Bosch 2005; Pitts-Singer et al. 2014). The focus of much research on this species concerns its use for almond pollination. This should come as no surprise considering almond's high monetary value, the dramatic increase in acreage in recent years, and a sharp increase in the cost of honey bee colony rentals for this crop. Almonds bloom in February, and bee emergence must be advanced by a month or more

to synchronize with bloom. As a result, the duration of the wintering period does not exceed the point at which significant differences in post-winter vigor become apparent. In fact, management for early emergence requires an acceleration of the developmental stages of *O. lignaria* in order to place them in winter storage early, so that bees wintered at 4° C have sufficient time at cold temperature to complete diapause by February (Bosch and Kemp 2000, 2003; Bosch et al. 2000; Kemp and Bosch 2005). Methods to delay adult emergence for crops that bloom after *O. lignaria*'s natural activity period have receive little attention, but may provide an opportunity to expand the market for this pollinator.

The use of colder winter storage temperature could provide the means by which to delay the emergence of *O. lignaria*. Experiments aimed at developing the closely related Japanese species *O. conifrons* Radoszkowski for germplasm pollination, have looked at the effect of colder temperatures on long term winter storage. Emergence was delayed by several months without a significant reduction in survival when stored at 1 to 2° C instead of 4 to 5° C. Bees wintered at the warmer temperature failed to emerge at this late date (Wilson and Abel 1996). For *O. lignaria* wintered at 4° C a reduction in survival and longevity to starvation is apparent by 210 days of wintering; similar reductions for bees overwintered at 0° C do not appear until approximately 270 days of winter storage (Bosch and Kemp 2003). Overwinter weight loss and fat body depletion increases with both temperature and duration of storage (Sgolastara et al. 2010). Depletion of energy reserves has the potential to negatively affect nesting and reproductive success, which directly relate to *O. lignaria*'s effectiveness as a crop pollinator. Female bees emerging with depleted energy reserves may take longer to initiate nesting, provision nests at a

slower rate, or suffer a reduction in longevity after emergence (Sgolastra et al. 2016), which are the main factors relating to realized fecundity for the *O. cornuta* (Bosch and Vincens 2006).

Measuring post-winter performance indirectly is informative, but is not entirely sufficient, as indirect measures may not accurately reflect the impact of winter temperature and duration on nesting or reproductive success for *O. lignaria*. Extended pre-wintering durations and extended exposure to warmer winter temperatures have similar negative effects on survival, weight loss, and longevity (Bosch et al. 2000, 2010; Sgolastra et al. 2011). However, pre-wintering duration does not affect nest establishment, pre-nesting duration, nesting duration, fecundity, provisioning rate, parental investment, or sex ratio of offspring (Sgolastra et al. 2016). There is little information regarding the direct effect of winter storage conditions on nesting and reproductive success for *O. lignaria*. Bosch et al. (2000) looked at nest establishment and reproductive success of bees in almond orchards following wintering at several storage temperatures. Results of that study were confounded by the fact that several release sites had >100% establishment, suggesting portion of bees were either from resident populations or dispersed from other release sites. This made attribution of nesting and reproductive success to a specific treatment impossible. A detailed study of individual nesting and reproductive success is necessary in order to better understand the effects of winter temperature and storage duration on post-winter performance for *O. lignaria*.

Conclusion

Expansion of raspberry acreage combined with a dwindling and increasingly

expensive honey bee supply have made it desirable to find alternative manageable pollinators for raspberry. Farmers will only be able to justify the adoption of alternative pollinators if they prove to be an economical alternative to honey bees. Mason bees and bumble bees are currently more expensive to purchase on a per forager basis, and must be more effective pollinators of *R. idaeus* when compared to honey bees to be considered economically viable. Research suggests this might be the case, but comparisons utilizing direct measures of single visit efficacy are lacking. Comparing bees' pollination efficacies in this way better attributes their contribution to the female reproductive function of the plant relative to other method which may exaggerate differences in pollinator performance. Measuring single-visit efficacy for bees can also help to refine estimates of the minimum number of visits required to maximize fruit set.

Adoption of an alternative pollinator for raspberry also necessitates the bee species is available for raspberry bloom. The blue orchard bee, *O. lignaria*, is a promising alternative pollinator for raspberry, but is active more than a month before raspberry begins to bloom. Methods must be developed to delay the emergence of these bees without compromising their post-winter vigor or survival. Research suggests exposure to colder winter temperature may provide a means by which to delay emergence of this species, however, no studies have directly examined the effect of winter temperature and duration on nesting and reproductive success for *O. lignaria*.

In my second chapter I compare the pollination efficacies of four alternative manageable bee species to honey bee for three raspberry cultivars. I controlled single-visits by *O. aglaia*, *O. bruneri*, *O. lignaria*, bumble bees, and honey bees to virgin raspberry flowers. I counted the number of drupelets resulting from these visits,

providing a direct comparative measure of each pollinator's efficacies. To better understand the additional contribution to pollination made by each bee and allow for comparison across sites, a pollinator efficacy index, which incorporates drupelet set attributable to autogamy, was created (Spears 1983). From this index an estimate was derived for the minimum number of visits required to achieve full fruit-set. In particular, I asked: 1) Do these four bee species differ from honey bees in pollination efficacy for raspberry, and 2) Is the magnitude of the difference in efficacy alone sufficient to justify adoption of an alternative pollinator for raspberry?

In my third chapter I compare drupelet set for four honey bee visitation treatments: 1) unvisited flowers, 2) a single prolonged bee visit (one-visit), 3) two prolonged bee visits (two-visit), one on each of two consecutive days, and 4) openly visited raspberry flowers (open pollination) for three red raspberry cultivars. I posited that two prolonged honey bee visits to a single raspberry flower would be sufficient to achieve full drupelet set.

In my fourth chapter I compare the effects of three winter temperatures and two winter durations on the survival and post-winter vigor of female *O. lignaria*. I measured overwinter weight loss, survival, pre-emergence time, and longevity to starvation for bees reared under each combination of temperature and duration. A subset of these bees were released into a greenhouse where nest establishment, pre-nesting duration, nesting duration, and fecundity were recorded for each bee, providing a direct measure of the effect of winter temperature and duration on post-winter performance of *O. lignaria*. These more direct measures of the effect of winter temperature and duration on females' post-winter performance were used to answer three specific questions: 1) Does warm

winter temperature impose a reproductive cost on *O. lignaria* when wintered for an extended duration? 2) Does wintering these bees at colder temperatures allow for delayed emergence, without sacrificing winter survival or reproductive success? 3) Does colder wintering provide a practical and sustainable management strategy for pollination of later-blooming crops.

References

- Aizen, M. A., L. A. Garibaldi, S. A. Cunningham, and A. M. Klein. 2008.** Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Curr. Biol.* 18: 1572–1575.
- Bekey, R. S. 1985.** Effects of method and timing of pollination and temperature on fruit development in red raspberry. PhD Diss. Oregon State Univ., Corvallis, O.R.
- Bosch, J., and M. Blas. 1994.** Foraging behavior and pollinating efficacy of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). *Appl. Entomology Zool.* 29: 1–9.
- Bosch, J., and W. P. Kemp. 1999.** Exceptional cherry production in an orchard pollinated with blue orchard bees. *Bee World.* 80: 163–173.
- Bosch, J., and W. P. Kemp. 2000.** Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 29: 8–13.
- Bosch, J., and W. P. Kemp. 2003.** Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 32: 711–716.
- Bosch, J., W. P. Kemp, and G. E. Trostle. 2006.** Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 99: 408–413.
- Bosch, J., W. P. Kemp, and S. Peterson. 2000.** Management of *Osmia lignaria* (Hymenoptera: Megachilidae) populations for almond pollination: methods to advance bee emergence. *Environ. Entomol.* 29: 874–883.
- Bosch, J., F. Sgolastra, and W. P. Kemp. 2010.** Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *J. Insect Physiol.* 56: 1950–1958.
- Bosch, J., and N. Vicens. 2006.** Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behav. Ecol. Sociobiol.* 60: 26–33.
- Burgett, M. 1997.** 1996 Pacific Northwest honey bee pollination survey. Oregon State Univ., Corvallis, OR.

- Cane, J. H. 2005.** Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries. HortScience. 40: 1705–1708.
- Cane, J. H. 2008.** An effective, manageable bee for pollination of *Rubus* bramble fruits, *Osmia aglaia*. Acta Hort. 777: 459–463.
- Cane, J. H., and D. Schiffhauer. 2003.** Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium Macrocarpon* [Ericaceae]). Am. J. Bot. 90: 1425–1432.
- Chagnon, M., J. Gingras, and D. de Oliveira. 1991.** Honey bee (Hymenoptera: Apidae) foraging behavior and raspberry pollination. J. Econ. Entomol. 84: 457–460.
- Colbert, S., and D. de Oliveira. 1990.** Influence of pollen variety on raspberry (*Rubus idaeus* L.) development. J. Hered. 81: 434–437.
- Cruzan, M. B. 1986.** Pollen tube distributions in *Nicotiana glauca*: evidence for density dependent growth. Am. J. Bot. 73: 902–907.
- Dale, A., A. Sample, and E. King. 2003.** Breaking dormancy in red raspberries for greenhouse production. HortScience. 38: 515–519.
- Daubeny, H. A. 1971.** Self-fertility in red raspberry cultivars and selections. J. Am. Soc. Hortic. Sci. 96: 588–591.
- Demchak, K. 2009.** Small fruit production in high tunnels. Horttechnology. 19: 44–49.
- Dickinson, T. A., E. Lo, and N. Talent. 2007.** Polyploidy, reproductive biology, and Rosaceae: understanding evolution and making classifications. Plant Syst. Evol. 266: 59–78.
- Eaton, G. W., H. A. Daubeny, and R. C. Norman. 1968.** Pollination techniques for red raspberry breeding programs. Can. J. Plant Sci. 48: 342–344.
- Food and Agriculture Organization of the United Nations. 2018.** FAOSTAT database. FAO-ESS, Rome, Italy. 12 April 2018.
<<http://www.fao.org/faostat/en/#data/QC>>
- Free, J. B. 1968.** The foraging behaviour of honeybees (*Apis mellifera*) and bumblebees (*Bombus* Spp.) on blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*) and strawberry (*Fragaria* × *Ananassa*) flowers. J. Appl. Ecol. 5: 157–168.
- Free, J. B. 1993.** Insect pollination of crops, 2nd ed. Academic Press, London, U.K.

- Gyan, K. Y., and S. R. J. Woodell. 1987.** Analysis of insect pollen loads and pollination efficiency of some common insect visitors of four species of woody Rosaceae. *Funct. Ecol.* 1: 269–274.
- Hansen, R. W., and E. A. Osgood. 1983.** Insects visiting flowers of wild red raspberry in spruce-fir forested area of eastern Maine. *Entomol. News.* 94: 147–151.
- Hardy, M. B. 1931a.** Self and cross fertility of red raspberry varieties. *Proc. Am. Soc. Hortic. Sci.* 28: 118–121.
- Hardy, M. B. 1931b.** Red raspberry pollination technique. *Proc. Am. Soc. Hortic. Sci.* 28: 122–124.
- Jennings, D. L. 1988.** Raspberries and blackberries: their breeding, diseases and growth. Academic Press, London, U.K.
- Jennings, D. L., H. A. Daubeny, and J. N. Moore. 1991.** Blackberries and raspberries. *Acta Hortic.* 290: 331–392.
- Karp, K., M. Mänd, M. Starast, and T. Paal. 2004.** Nectar production of *Rubus arcticus*. *Agron. Res.* 2: 57–61.
- Keep, E. 1961.** Autumn-fruiting in raspberries. *J. Hortic. Sci.* 36: 174–185.
- Keep, E. 1968.** Incompatibility in *Rubus* with special reference to *R. idaeus* L. *Can. J. Genet. Cytol.* 10: 253–262.
- Kemp, W. P., and J. Bosch. 2005.** Effect of temperature on *Osmia lignaria* (Hymenoptera: Megachilidae) prepupa – adult development, survival, and emergence. *J. Econ. Entomol.* 98: 1917–1923.
- Kemp, W. P., J. Bosch, and B. Dennis. 2004.** Oxygen consumption during the life cycles of the prepupa-wintering bee *Megachile rotundata* and the adult-wintering bee *Osmia lignaria* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 97: 161–170.
- Kempler, C., H. Hall, and C.E. Finn. 2012.** Raspberry, pp. 263-305. In M. L. Badenes and D. H. Byrne (eds.), *Handbook of plant breeding: fruit breeding*, vol. 8. Springer-Verlag, New York, NY.
- Klein, A., B. E. Vaissie, J. H. Cane, I. Steffan-dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007.** Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B Biol. Sci.* 247: 303–313.

- Levin, M. D. 1966.** Biological notes on *Osmia lignaria* and *Osmia californica* (Hymenoptera : Apoidea). J. Kansas Entomol. Soc. 39: 524–535.
- Lye, G. C., S. N. Jennings, J. L. Osborne, and D. Goulson. 2011.** Impacts of the use of nonnative commercial bumble bees for pollinator supplementation in raspberry. J. Econ. Entomol. 104: 107–114.
- MacKenzie, K. E., and M. L. Winston. 1984.** Diversity and abundance of native bee pollinators on berry crops and natural vegetation in the lower Fraser Valley, British Columbia. Can. Entomol. 116: 965–974.
- Monzón, V. H., J. Bosch, and J. Retana. 2004.** Foraging behavior and pollinating effectiveness of *Osmia cornuta* (Hymenoptera: Megachilidae) and *Apis mellifera* (Hymenoptera: Apidae) on 'Comice' pear. Apidologie. 35: 575–585.
- Morandin, L. A., T. M. Lavery, R. J. Gegear, and P. G. Kevan. 2002.** Effect of greenhouse polyethylene covering on activity level and photo-response of bumble bees. Canad. Entomol. 134: 539–549.
- Neilsen, A., T. Reitan, A. W. Rinvoll, and A. K. Brysting. 2017.** Effects of competition and climate on a crop pollinator community. Agric. Ecosyst. Environ. 246: 253–260.
- Percival, M. S. 1955.** The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. New Phytol. 54: 353–368.
- Percival, M.S. 1979.** Floral biology. Pergamon Press, Elmsford, NY. 243 p.
- Phillips, J. K., and E. C. Klostermeyer. 1978.** Nesting behavior of *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). J. Kansas Entomol. Soc. 51: 91–108.
- Pinzauti, M., D. Lazzarini, and A. Felicioli. 1997.** Preliminary investigation of *Osmia cornuta* Latr. (Hymenoptera: Megachilidae) as a potential pollinator for blackberry (*Rubus fruticosus* L.) under confined environment. Acta Hort. 437: 329–333.
- Pitts-Singer, T. L., J. H. Cane, and G. Trostle. 2014.** Progeny of *Osmia lignaria* from distinct regions differ in developmental phenology and survival under a common thermal regime. J. Insect Physiol. 67: 9–19.
- Pritts, M. P. 2008.** Primocane-fruiting raspberry production. HortScience. 43: 1640–1641.
- Pritts, M. P., R. W. Langhans, T. H. Whitlow, M. J. Kelly, and A. Roberts. 1999.** Winter raspberry production in greenhouses. Horttechnology. 9: 13–15.

- Prodorutti, D., and F. Frilli. 2008.** Entomophilous pollination of raspberry, red currant and highbush blueberry in a mountain area of Friuli-Venezia Giulia (North-Eastern, Italy). *Acta Hort.* 777: 429–434.
- Raw, A. 1974.** Pollen preferences of three *Osmia* species (Hymenoptera). *Oikos.* 25: 54–60.
- Redalen, G. 1976.** Pollination and fruit set in raspberries. *Acta Hort.* 60: 169–176.
- Rust, R. 1995.** Adult overwinter mortality in *Osmia lignaria propinqua* (Hymenoptera: Megachilidae). *Pan Pacific Entomol.*
- Sáez, A., C. L. Morales, L. A. Garibaldi, and M. A. Aizen. 2017.** Invasive bumble bees reduce nectar availability for honey bees by robbing raspberry flower buds. *Basic Appl. Ecol.* 19: 26–35.
- Sáez, A., C. L. Morales, L. Y. Ramos, and M. A. Aizen. 2014.** Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *J. Appl. Ecol.* 51: 1603–1612.
- Sanzol, J., and M. Herrero. 2001.** The effective pollination period in fruit trees. *Sci. Hortic. (Amsterdam).* 90: 1–17.
- Schmidt, K., R. Filep, Z. Oroso-Kovács, and A. Farkas. 2015.** Patterns of nectar and pollen presentation influence the attractiveness of four raspberry and blackberry cultivars. *J. Hortic. Sci. Biotechnol.* 90: 47–56.
- Sedivy, C., and S. Dorn. 2014.** Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae: *Osmia*) as fruit tree pollinators. *Apidologie.* 45: 88–105.
- Sgolastra, F., J. Bosch, R. Molowny-horas, S. Maini, and W. P. Kemp. 2010.** Effect of temperature regime on diapause intensity in an adult-wintering Hymenopteran with obligate diapause. *J. Insect Physiol.* 56: 185–194.
- Sgolastra, F., W. P. Kemp, J. S. Buckner, T. L. Pitts-singer, S. Maini, and J. Bosch. 2011.** The long summer: pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *J. Insect Physiol.* 57: 1651–1659.
- Sgolastra, F., W. P. Kemp, S. Maini, and J. Bosch. 2012.** Duration of prepupal summer dormancy regulates synchronization of adult diapause with winter temperatures in bees of the genus *Osmia*. *J. Insect Physiol.* 58: 924–933.

- Sgolastra, F., X. Arnan, T. L. Pitts-Singer, S. Maini, W. P. Kemp, and J. Bosch. 2016.** Pre-wintering conditions and post-winter performance in a solitary bee: does diapause impose an energetic cost on reproductive success? *Ecol. Entomol.* 41: 201–210.
- Shanks Jr., C. H. 1969.** Pollination of raspberries by honeybees. *J. Apic. Res.* 8: 19–21.
- Sheffield, C. S. 2014.** Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Anapolis Valley, Nova Scotia, Canada. *J. Pollinat. Ecol.* 12: 120–128.
- Sheffield, C. S., S. M. Westby, P. G. Kevan, and R. F. Smith. 2008.** Winter management options for the orchard pollinator *Osmia lignaria* say (Hymenoptera: Megachilidae) in Nova Scotia. *J. Entomol. Soc. Ontario.* 139: 3–18.
- Spears Jr., E. E. 1983.** A direct measure of pollinator effectiveness. *Oecologia.* 57: 196–199.
- Tepedino, V. J., and P. F. Torchio. 1982.** Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). *Ecol. Entomol.* 7: 453–462.
- Thompson, M. M. 1997.** Survey of chromosome numbers in *Rubus* (Rosaceae: Rosoidea). *Ann. Missouri Bot. Gard.* 84: 128–164.
- Torchio, P. F. 1976.** Use of *Osmia lignaria* Say (Hymenoptera: Apoidea, Megachilidae) as a pollinator in an apple and prune orchard. *J. Kansas Entomol. Soc.* 49: 475–482.
- Torchio, P. F. 1981.** Field experiments with *Osmia lignaria propinqua* Cresson as a pollinator in almond orchards: I, 1975 studies (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 54: 815–823.
- Torchio, P. F. 1985.** Field Experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: V (1979-1980), methods of introducing bees, nesting success, seed counts, and fruit yields (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 53: 448–464.
- Torchio, P. F. 1989.** In-nest biologies and development of immature stages of three *Osmia* species (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 82: 599–615.
- U.S. Department of Agriculture – National Agricultural Statistics Service. July 2016.** Noncitrus fruits and nuts 2015 summary. USDA, Washington, D.C.

- Velthuis, H. H. W., and A. Van Doorn. 2006.** A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*. 37: 421–451.
- Vicens, N., and J. Bosch. 2000.** Pollinating efficacy of *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae, Apidae) on ‘Red Delicious’ apple. *Environ. Entomol.* 29: 235–240.
- Whitney, G. G. 1984.** The reproductive biology of raspberries and plant-pollinator community structure. *Am. J. Bot.* 71: 887–894.
- Wieniarska, J. 1987.** The role of pollinating insects in fruiting of some red raspberry cultivars. *Fruit Sci. Reports*. 14: 65–70.
- Willmer, P. G., A. A. M. Bataw, and J. P. Hughes. 1994.** The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecol. Entomol.* 19: 271–284.
- Willmer, P. G., S. C. Gordon, J. Wishart, and J. P. Hughes. 1998.** Flower choices by raspberry beetles: cues for feeding and oviposition. *Anim. Behav.* 56: 819–827.
- Wilson, R. L., and C. A. Abel. 1996.** Storage conditions for maintaining *Osmia cornifrons* (Hymenoptera: Megachilidae) for use in germplasm. *J. Kansas Entomol. Soc.* 69: 270–272.
- Winston, M. L., and L. H. Graf. 1982.** Native bee pollinators of berry crops in the Fraser Valley of British Columbia. *J. Entomol. Soc. Br. Columbia*. 79: 14–20.
- Young, H. J., and T. P. Young. 1992.** Alternative outcomes of natural and experimental high pollen loads. *Ecology*. 73: 639–647.
- Żurawicz, E. 2016.** Cross-pollination increases the number of drupelets in the fruits of red raspberry (*Rubus idaeus* L.). *Acta Hort.* 1133: 145–152.
- Żurawicz, E., M. Studnicki, J. Kubik, and K. Pruski. 2018.** A careful choice of compatible pollinizers significantly improves the size of fruits in red raspberry (*Rubus idaeus* L.). *Sci. Hortic. (Amsterdam)*. 235: 253–257.

CHAPTER II

COMPARATIVE POLLINATION EFFICACIES OF FIVE BEE SPECIES
ON RASPBERRY

Abstract

Unlike many other rosaceous fruit crops, commercial raspberry cultivars are largely self-fertile and mostly self-pollinate autogamously. However, their floral morphology often prevents complete autopollination. Incomplete pollination yields unmarketable small or crumbly fruits. Insect visitation is therefore essential to maximizing raspberry yield. Honey bees are typically used to pollinate commercial raspberry; however, escalating prices for hive rentals coupled with increasing acreage encourage evaluation of other manageable pollinators. Four other manageable bee taxa – various *Bombus*, *Osmia lignaria*, *O. aglaia*, and *O. bruneri* -- are all promising raspberry pollinators. Honey bees are the least expensive option on a per forager basis; therefore, alternative pollinators should be more effective than honey bees for pollinating raspberry (or have some other advantage). In this study, we compare honey bees with these other bee species for their pollination efficacies, measured as the number of drupelets resulting from a single visit to a virgin flower. Their single-visit efficacies were also compared to drupelet set from unvisited and hand pollinated flowers. From these data, each species' pollination effectiveness score was calculated. All five bee species were equally effective at pollinating raspberry, therefore, honey bees remain the most cost effective option for open field pollination of raspberry at this time. Mason bees and bumble bees may have

greater utility during chilly weather or for protected cultivation systems, contexts unfavorable to honey bee foraging.

Introduction

Commercial red raspberry (*Rubus idaeus* L.) cultivars are predominantly self-fertile (Keep 1968; Żurawicz 2016); however, floral morphology precludes complete self-pollination (Shanks 1969). Raspberry flowers typically have 60-120 stamens arranged in whorls around a central receptacle, and a similar number of pistils arising spirally from the receptacle. This arrangement allows only the outer stigmas to contact the anthers (Free 1993). Each fertilized ovary develops into a single-seeded drupelet. Each drupelet is akin to a drupe fruit (e.g. a cherry) (Jennings, 1988). Raspberries are aggregate fruits composed of multiple drupelets whose cohesion depends on abundant drupelets. If too few drupelets set, berries will be crumbly and misshapen, rendering them unmarketable. Under-pollinated berries that do coalesce are smaller, as berry weight reflects the number of drupelets set (Chagnon et al. 1991). Consequently, fruit yield and quality depends upon insect pollination (Bekey 1985; Shanks 1969; Wieniarska 1987). As with most crops, managed honey bees (*Apis mellifera*) are the primary pollinators.

Europe and North America account for ~97% of global raspberry production. The United States (U.S.) is second in world production. Markets are expanding, evidenced by the doubling of raspberry acreage since 1986, reaching 106,631 ha of raspberry harvested worldwide in 2016 (FAOSTAT 2018). During that time, U.S. land devoted to raspberry production tripled to 8,765 ha (FAOSTAT 2018), mostly in the Pacific coast states. Most California raspberry acreage was grown to satisfy year-round demand for fresh berries,

achieved through widespread adoption of high-tunnel production systems that greatly extend the growing season (Pritts 2008; Demchak 2009).

Pollination costs have escalated during this time. In Oregon and Washington, honey bee hive rentals for raspberry pollination have doubled (to \$40/colony) over 20 years, reflecting the mounting costs of colony losses (Burgett 1997; NASS 2016). California rental prices for raspberries rose even more, to \$92/colony in 2016 (NASS 2016). California's shift to high tunnels exasperates its pollination dilemma, as honey bees forage poorly under high tunnels (Nielsen et al. 2017), likely due to the plastic film absorbing UV wavelengths honey bees use for navigation (Morandin et al. 2002). Expanding acreage, costlier rentals from a shrinking supply of honey bees, and shifting farming practices all highlight a need to find an alternative manageable raspberry pollinator.

A practical, manageable pollinator for a given crop must be capable, reliable and efficacious. In agricultural systems, where harvestable yield is paramount, a pollinator's efficacy is best measured by its contribution to the female reproductive function of the plant. Various indirect measures of pollinator efficacy, such as counts of stigmatic pollen loads, can exaggerate pollinator differences because they overlook the asymptotic relationship between pollen grains deposited and fruit set/size (see Cane and Schiffauer 2003). A direct measure of fruit set resulting from bee pollination is preferable, such as drupelet count in raspberry. Likewise, fruit set resulting from controlled numbers of floral visits is a more informative pollination measure than the more common practice of allowing bees to openly visit flowers (Shanks 1969; Bekey 1985; Chagnon et al. 1991; Willmer et al. 1994; Cane 2005, 2008; Prodorutti and Frilli 2008; Lye et al. 2011; Sáez et

al. 2014). Open-pollination can obfuscate differences between pollinator efficacies, as individual flowers are likely to accumulate different numbers of visits (Javorek et al. 2002).

Experiments that both control for the number of bee visits and utilize a direct measure of pollination efficacy can be logistically challenging to implement, but have distinct advantages over other methods. Single-visit efficacy, measured for raspberry as the number of drupelet count resulting from a bee's single visit to a virgin flower, allows for straight-forward, direct comparison of pollinator efficacies. This method's data is readily transformed into an index of pollinator effectiveness (PE) (Spears 1983). A PE index places efficacy in the context of the floral species' realized fruiting potential, allowing for better comparisons across studies and sites. The inverse of PE directly translates into the minimum number of visits needed to achieve full seed set.

Alternative or supplemental pollinators will only be adopted if they prove to be a practical, economical alternative to honey bees. To date, other manageable bees cost more than honey bees on a per forager basis and must, therefore, be more efficacious pollinators for their use to be justified. The alfalfa leafcutting bee (ALCB), *Megachile rotundata* Fab., is a prime example. A female ALCB is >25 times more effective than a honey bee at pollinating alfalfa (Cane 2002). Although more expensive than honey bees on a per forager basis, their exceptional superiority as pollinators of alfalfa led to their becoming the world's most intensively produced and managed solitary bee (Pitts-Singer and Cane 2011). Evaluations of promising new pollinators for crops begin with comparative studies of pollination efficacy. Such studies are uncommon for raspberry, but the few bee species considered seem as good, if not better than, honey bees at

pollinating raspberry (Willmer et al. 1994; Cane, 2005, 2008; Sáez et al. 2014). The most promising alternative manageable bees for raspberry are bumble bees (*Bombus* spp.) and mason bees (*Osmia* spp.).

Bumble bees are likely the most important wild pollinator of raspberry (Winston and Graf 1982; Mackenzie and Winston 1984; Willmer et al. 1994; Lye et al. 2011; Sáez et al. 2014; Nielsen et al. 2017), and several species are commercially available (Velthuis and Dorn 2006). Willmer et al. (1994), reported that bumble bees deposit about twice the amount of pollen per visit to a raspberry flower than honey bees. Mason bees are solitary, cavity-nesting bees, some of which will accept man-made nesting substrates, making them amenable to commercial management. Several species of mason bees have been developed as pollinators of rosaceous tree fruits (reviewed in Sedivy and Dorn 2014). Largely due to behavioral differences at the flower, these bees have proven more effective than honey bees at pollinating rosaceous crops such as almond (Bosch and Blas 1994), apple (Vincens and Bosch 2000), and pear (Monzon et al. 2004). Cane (2005) noted similar behavioral contrasts between *O. aglaia* and honey bees foraging on raspberry, but both species proved equivalent for raspberry fruit production, albeit with unconstrained floral visitation (Cane 2005, 2008). Sáez et al. (2014) found no relationship between drupelet set and the number of pollen grains deposited per raspberry stigma by bumble bees, even though pollen counts spanned the range reported for a single honey bee visit by Willmer et al. (1994). In order to refine comparisons of efficacy, the performance of various bee species needs to be evaluated on a per-visit basis with a direct measure of pollinator effectiveness.

In this study, we compare the pollination efficacies of five manageable bee

species visiting three raspberry cultivars. We controlled single visits to virgin raspberry flowers by nesting female *O. aglaia*, *O. bruneri*, *O. lignaria*, bumble bees, and honey bees. We counted the number of drupelets resulting from these visits, providing a direct comparative measure of each pollinator's efficacy. Comparisons across cultivars and site contexts were facilitated by calculating a pollinator efficacy index, which incorporated differences in drupelet set attributable to autogamy (Spears 1983). From this index we estimated the minimum number of visits required for full pollination. In particular, we asked two questions: 1) are any of these four bee species superior to honey bees in pollination efficacy for raspberry, and 2) is the magnitude of the difference in efficacy alone sufficient to warrant adoption of an alternative pollinator for raspberry?

Materials and Methods

Bee Species Studied

Three species of mason bees were tested for pollination efficacy. The West Coast species, *O. aglaia*, ranges from southern California to west-central Oregon. They naturally emerge in late spring, coinciding with raspberry bloom in the Pacific Northwest. They readily nest in polystyrene foam substrates (with paper straw liners) even when limited to raspberry pollen and nectar (Cane 2005, 2008). Closely related *O. bruneri* is native to the Intermountain West and Rocky Mountains and shares a similar phenology with *O. aglaia*. Though not yet evaluated as a raspberry pollinator, it does forage on raspberry flowers, and uses the same nesting substrate as *O. aglaia*. Lastly, *O. lignaria* is native to much of the U.S. It has an affinity for rosaceous flowers, effectively pollinating numerous spring-flowering fruit tree crops, such as almond (Bosch and Blas 1994),

apple (Torchio, 1985), and cherry (Bosch et al. 2006). Management of *O. lignaria* is well-defined (Bosch and Kemp 2001), and bees are available for purchase in large numbers. Unlike the other two species of mason bee, *O. lignaria*'s flight season precedes raspberry bloom by a month or more. Wintering these bees near 0° C successfully delays their emergence without compromising longevity, vigor, or survival (Andrikopoulos unpublished data).

Free-flying honey bees and bumble bees were tested for their raspberry pollination efficacies. Several species of bumble bees are commercially reared (Velthuis and Dorn 2006), and most production guides recommend their use as disposable pollinators of greenhouse-grown raspberry (Pritts et al. 1999). In the U.S., only colonies of one eastern species, *B. impatiens*, are available for purchase. Bumble bees used in this experiment were wild Utah species, including *B. huntii* Greene, *B. nevadensis* Cresson, *B. griseocollis* Degeer, and *B. fervidus* Fabricius. Bumble bee species are similar in per-visit pollen deposition (Willmer et al. 1994). Some can be misidentified in flight, but because netting them could scare away other foragers, we chose to forego positive identifications.

Experimental Design

Mason bees were evaluated for pollination efficacy of red and purple raspberry cultivars within a glass greenhouse, and in outdoor field cages with three-year-old raspberry plants at the USDA ARS Pollinating-insect Research Unit in Logan, Utah (41° 45' N 111° 48' W). In addition to single-visit pollination experiments within cages, the efficacies of free-flying honey bees and bumble bees were measured at previously bagged flowers in the same outdoor raspberry plots, but external to the cages.

Thirty 'Royalty' purple raspberry plants were planted in three gallon pots in a

7.7x7.7 m glasshouse. A 6x6x2 m mesh cage was erected over the plants just prior to bloom. Pollination by *O. lignaria* was evaluated in the glasshouse cage from 4 April to 1 May, 2015. Twenty-four female and 24 male *O. lignaria* were released into the cage on 4 April. Data were collected only for floral visits by nesting females, as these bees typically perform the bulk of pollination services to crop. Trials ran on sunny days between 0900 – 1800h MST. In 2016, nesting female *O. lignaria* were again evaluated in the glasshouse between 15 April and 5 May. The experimental protocol was the same as in 2015, except the red raspberry cultivar ‘Latham’ was added.

Two additional mason bee species were tested in the outdoor field cages from 15 May to 15 June, 2016. Mean daily temperature during the experimental period was 20° C (range 8 to 30° C) (MesoWest 2017). Trials ran between 0900 – 1800h MST. The planting consisted of six 11 m rows: one row had a mix of cultivars, two had the red raspberry ‘Polka’, and one row each of the red raspberry ‘Cowichan’ and ‘Latham’, as well as the purple raspberry, ‘Royalty’. Only the latter three rows were used for visitation data, although not all bees were tested on every cultivar. Earlier blooming ‘Polka’ fed the caged bees and supported their initial nesting. Rows were spaced 3 m apart and cultivars were alternated by row to promote outcrossing, as cultivars benefit from cross-pollination (Colbert and de Oliveira 1990; Żurawicz 2016; Żurawicz et al. 2018). Two 6x6x2 m mesh field cages were erected over half of the plot such that half of each row was inside a cage and half of each row was outside. Females of ten *O. lignaria* and 20 *O. bruneri* were released in one cage containing ‘Latham’ and ‘Cowichan’ red raspberry; another 10 *O. lignaria* and 10 *O. aglaia* females were released into the second cage containing ‘Royalty’ purple raspberry and ‘Polka’ red raspberry. An equal number of males of the

respective species were released into the cages, but as with the glasshouse experiment, visitation data were only collected for nesting females. During this same time period, free flying honey bees and bumble bees were allowed single visits to previously bagged flowers outside of the cages.

Measures of Pollination Efficacy

Single-visit pollination efficacy was evaluated as the number of drupelets resulting from a bee's single visit to a receptive virgin flower. To restrict visitation, flowers in late-bud stage were randomly chosen and enclosed in fine nylon mesh bags. Upon flower opening, several bags were removed simultaneously and virgin flowers watched for the first visit by one of the focal bee species. Once visited, flowers were tagged with a colored band around the pedicel to indicate the treatment. Flowers were then immediately re-bagged to prevent further visitation until focal flowers were no longer receptive and fruit development was initiated. Additional bagged flowers were left either unvisited or hand pollinated to represent minimum and maximum drupelet set, respectively. Hand-pollinated flowers were used instead of openly pollinated (unrestricted visitation) flowers due to potential differences in visitation frequency inside and outside of the cages. Recipient flowers were hand pollinated by lightly brushing the stigma with a fine paint brush that had previously been brushed against the anthers of several donor flowers. Hand pollination was compared to open-pollination to ensure that this treatment represented maximum fruit set. Wet pistils can interfere with pollination; therefore, data were only collected in fair weather after at least 24 hours without rain or dew. Flowers that were exposed to rain within 24 hours of being pollinated were excluded from the experiment. Berries were allowed to develop until early pink stage,

about 3 weeks after pollination, at which point they were harvested and frozen until drupelets per fruit could be counted.

The pollinator effectiveness score (PE) for each species of visitor was calculated according to Spears (1993) as $PE_i = \frac{(P_i - Z)}{(U - Z)}$, where P_i = the mean number of drupelets set resulting from a single-visit by species i , Z = the mean number of drupelets set in the absence of visitation, and U = the mean number of drupelets set when hand pollinated. The estimated number of visits required for maximum pollination was calculated as $1/PE_i$.

Data Analyses

Drupelet counts from the greenhouse planting in 2015 were compared with a one-way ANOVA to test for differences in drupelet set among pollination treatments (PROC GLM; SAS v.9.4.2). Drupelet counts from the greenhouse plants in 2016 were compared with a mixed model ANOVA with plant as a random factor (PROC MIXED; SAS v.9.4.2); cultivars were analyzed separately. Drupelets counts from the outdoor planting in 2016 violated the assumptions of normality and equality of variance, and were compared with the Kruskal-Wallis test (PROC NPAR1WAY; SAS v.9.4.2). A Tukey-Kramer post-hoc test was used to determine which treatment means differed for data analyzed by ANOVA. Wilcoxon rank sum comparisons were used to determine which treatment means differed for data analyzed with the Kruskal-Wallis test. The significance level used was $p \leq 0.05$.

Results

All flowers that received additional pollination in this study set significantly more drupelets than unvisited flowers. In the greenhouse, a total of 275 purple raspberry flowers received pollination treatments in 2015. Pollination treatments differed in the number of drupelets set per fruit ($F=74.1$; d.f. =2, 272; $p < 0.0001$). Flowers visited once by *O. lignaria* set a similar number of drupelets to the hand pollinated treatment (Fig. 2-1). In 2016, 105 ‘Royalty’ purple raspberry flowers, and 130 ‘Latham’ red raspberry flowers received pollination treatments in the greenhouse. Pollination treatment had a significant effect on the number of drupelets set for ‘Royalty’ and ‘Latham’ ($F=152.82$; $df = 2, 102$; $p < 0.0001$; and $F=20.37$; $df = 2, 127$; $p < 0.0001$, respectively; Fig. 2-1). For both cultivars, flowers visited by *O. lignaria* set a similar number of drupelets to the hand pollinated treatment (Fig. 2-1).

In the field-cage trial, a total of 1,798 red and purple raspberry flowers received pollination treatments (570 ‘Cowichan’; 888 ‘Latham’; 340 ‘Royalty’). Across cultivars, all pollinator treatments increased drupelet set over flowers without visitation. For ‘Cowichan’ ($\chi^2=364.44$; $df = 5$; $p < 0.0001$; Fig. 2-2), mean numbers of drupelets were similar among the four bee species; however, only *O. lignaria* and *A. mellifera* did not differ significantly from the hand pollinated treatment. For ‘Latham’ ($\chi^2=374.98$, $df = 5$) $p < 0.0001$; Fig. 2-2), hand-pollinated flowers set significantly more drupelets than all bee pollinator treatments. Mean number of drupelets did not differ between *O. lignaria* and *Bombus* spp., or between *Bombus* spp. and *A. mellifera*. Visitation from the slightly less efficacious *O. bruneri* resulted in fewer drupelets per fruit compared to visitation by the

other three bees (Fig. 2-2). For the purple raspberry ‘Royalty’ ($\chi^2 = 108.22$; $df = 3$; $p < 0.0001$; Fig. 2-3), hand-pollination resulted in the greatest number of drupelets, and *O. aglaia* was a more effective pollinator than honey bees.

Pollinator effectiveness varied with raspberry cultivar for each bee species (Table 2-1). Honey bees exhibited the greatest variation, ranging from 0.510 (‘Royalty’) to 0.921 (‘Cowichan’). On average, *O. lignaria* produced the highest PE, followed by *Bombus* spp., *O. aglaia*, *A. mellifera*, and *O. bruneri*. The estimated number of visits required to maximize pollination was < 2 visits for all bee species (Table 1).

Discussion

For all of the bee species and raspberry cultivars tested in this experiment, bee visitation increased drupelet set 2 – 4 fold. All five bee species proved to be excellent pollinators of raspberry, with a single visit by either *A. mellifera* or *O. lignaria* sufficing to maximize drupelet set for some cultivars (Figs. 2-1 and 2-2). A single visit by the least effective overall pollinator, *O. bruneri*, still resulted in $> 70\%$ drupelet set compare to $\sim 35\%$ set for the unvisited flower. Our results suggest that as few as two visits by any of these bees would be enough to maximize drupelet set in raspberry (Table 2-1). All visits recorded in this experiment were to virgin flowers containing copious amounts of nectar. Visit duration scales with nectar quantity (Bekey 1985; Willmer et al. 1994), and drupelet set is proportional to the duration of a bee’s floral visit (Chagnon et al. 1991). Therefore, while objectively estimating relative pollination, these results may overestimate average single-visit efficiency of these bee species on raspberries.

We hypothesized that honey bees would be less effective pollinators of raspberry,

compared to mason bees and bumble bees, due to differences in the bees' behaviors and how they interact with flowers. Contrary to our expectations, we only observed minor differences in drupelet set among bee species. Bumble bees deposit significantly more pollen on raspberry stigmas per visit than do honey bees (Willmer et al. 1994; Sáez et al. 2014); however, the number of drupelets per fruit does not vary significantly with stigmatic load (Sáez et al. 2014). This suggests that in terms of overall drupelet set for a berry, the number of stigmas receiving pollen is more important than the number of pollen grains placed on an individual stigma. If this is true, the behavior of the bee at the flower should be of greater consequence than its per-visit pollen deposition. Bees that contact more of the stigmas within a flower are likely to be more effective pollinators.

Honey bees are less effective pollinators of several rosaceous fruit crops, including almond (Bosch and Blas, 1994), apple (Vincens and Bosch 2000), and pear (Monzon et al. 2004). Their inferiority is largely attributed to their foraging objective and positioning upon the flower. Those workers that gather pollen are usually better pollinators than nectar foragers (Bosch and Blas 1994; Monzon et al. 2004; Vincens and Bosch 2000; Willmer et al. 1994). When collecting pollen from these tree fruit crops, honey bees land atop flowers, where they regularly contact the central stigmas and effect pollination. More commonly, foraging honey bees seek just nectar, which they often collect by 'side-working' the flowers, walking around the petals while probing the nectaries through gaps at the base of the androecium (Vincens and Bosch 2000). During such visits, the bee fails to contact the stigmas (Bosch and Blas 1994; Monzon et al. 2004; Vincens and Bosch 2000). In contrast, *Bombus* and *Osmia* typically work the flowers from the top, regardless of foraging task, nearly always contacting the anthers

and stigmas (Bosch and Blas 1994; Cane 2005; Monzon et al. 2004; Thomson and Goodell 2001; Torchio 1981; Vincens and Bosch 2000).

At raspberries, bumble bees are twice as likely as honey bees to forage for pollen, although no study reports major consequences for *Rubus* pollination (Willmer et al. 1994). Mason bees are also far more likely to forage for pollen at *Rubus* flowers (Yokoi and Kandori 2016), and Cane (2005) noted that *O. aglaia* stands astride the central pistils when visiting *Rubus* flowers, ensuring contact with all the stigmas. In contrast, nectar-foraging honey bees were often seen walking in a circle around the nectaries, thereby failing to contact the central-most stigmas (Cane 2005). Pollinators differed less in their handling of raspberry flowers than we expected. This might be due to the small size of raspberry flowers relative to apple or pear. Honey bees often did approach raspberry flowers from the side to probe the outer ring of nectaries, but inevitably they would then reach their heads or bodies across the flower to drink nectar, perhaps reflecting the smaller size of raspberry flowers. Nonetheless, honey bees did not pollinate cultivars equally well. They were poorer pollinators of ‘Royalty’, although still setting >50% of drupelets (Table 1). This contrast may reflect cultivar differences in floral morphology that alter bees’ handling behaviors. Honey bees’ tendency to side-work apple flowers varies with floral dimensions (Benedek and Nyéki 1996; Thomson and Goodell 2001). The purple raspberry ‘Royalty’ is a hybrid of red (*Rubus idaeus* L.) and black (*Rubus occidentalis* L.) raspberries and may therefore have a floral morphology sufficiently different from the red raspberry species to explain the discrepancy in honey bee’s pollination efficacy.

The mason bees and bumble bees in this study approached flowers as expected,

alighting and working atop the central pistils. The most striking difference in flower handling was in the way *O. bruneri* groomed pollen from its body during floral visitation. Slow motion video revealed bees briefly hovering above the flower to transfer pollen to their scopa using their hind legs, then landing again to resume foraging. As a result, a single-visit by an *O. bruneri* to a raspberry flower actually consists of several shorter visits in rapid succession. Drupelet set resulting from *O. bruneri* visits had a larger variance than other bees in the study, perhaps because they periodically hover to pack pollen, thereby interrupting their visit. Contact time with the flower, which is directly related to drupelet set (Chagnon et al. 1991), may vary more per visit for *O. bruneri* than for bees that remain on the flower for the duration of their visit. Although it was not directly observed behaving in this manner, *O. aglaia* had a similarly higher variance in drupelet set. These two like-sized species are closely related and may, therefore, share this grooming behavior (Basibuyuk and Quicke 1999).

In our study, no species greatly outperformed honey bees in single-visit efficacy. Despite rising colony rental prices, honey bees remain the most economical and practical option for open-field raspberry pollination. The price of bumble bee colonies has dropped dramatically as rearing methods have improved (Velthuis and Dorn 2006), and prices are now comparable to honey bee colony rental. However, a honey bee colony fields thousands more foragers than a bumble bee colony. Mason bees are even more expensive than bumble bees on a per-forager basis. Of the three species examined here, only *O. lignaria* is currently commercially available, costing about \$1 per female, too expensive unless a grower can multiply them on-farm. While honey bees remain the best option for open-field raspberry production for now, the price differential between them and other

bees may close in the future. In Scotland, bumble bees are being used for open-field pollination of raspberries (Lye et al. 2011). The economics of using *Osmia* is not yet demonstrated on commercial raspberries.

Alternative bee species may instead find greater utility pollinating raspberry grown in tunnels and greenhouses. Bumble bees and mason bees fair better than honey bees in confined spaces and under U.V. light altering plastic film (Pinzauti et al. 1997; Morandin et al. 2002). Honey bees visit fewer raspberry flowers growing in high-tunnels compared to open-grown plants on the same farm (Nielsen et al. 2017). Honey bees may need to be more densely stocked to pollinate in high-tunnels; that added cost favoring alternative pollinators that readily forage in a high-tunnel. The current lack of a commercially available western U.S. bumble bee precludes their use for most high-tunnel production. In contrast, all three species of *Osmia* in this study are native to the western U.S. and, even in confinement, proved capable of effectively pollinating and reproducing on raspberry. In Italy a European mason bee, *O. cornuta*, also reproduced well while effectively pollinating high-tunnel blackberry (Pinzauti et al. 1997). Mason bees are, therefore, an excellent candidate for high-tunnel *Rubus* production. They also show promise as pollinators of greenhouse-grown raspberry. Our study is the first to demonstrate that *O. lignaria* flown in a glasshouse effectively pollinates raspberry. Currently, bumble bees are the sole pollinator recommended by production guides for greenhouse pollination (Pritts et al. 1999; Dale et al. 2005). More information on the stocking density of *Osmia* required for greenhouse pollination is needed, but mason bees may provide an economical alternative to bumble bees for greenhouse pollination.

Comparative studies such as this one are a first step in discovering alternative

manageable pollinators for fruit crops. Our direct measure of single-visit efficacy contradicts previous claims that bumble bees outperform honey bees as raspberry pollinators. We conclude that honey bees remain the best option for open-field pollination of raspberry. Our results accurately represent the relative single-visit efficacy of the five bees studied here, but overall performance includes other traits, such as crop fidelity, visitation rate, activity patterns, and interspecific interactions (Rogers et al. 2013). Incorporating these additional traits may ultimately reveal differences in pollination value sufficient to justify the adoption of one of these alternative bees for raspberry pollination.

Table and Figures

Table 2-1. Pollinator effectiveness scores (PE) for bees visiting red and purple raspberry cultivars. The number of observations are in parenthesis.

	Cowichan	Latham	Royalty	Average PE¹	Approx. Visits²
<i>O. lignaria</i>	0.893(30)	0.863(121)	0.898(120) ³	0.882	1.13
<i>Bombus spp.</i>	0.829(20)	0.857(86)	-	0.851	1.17
<i>O. aglaia</i>	-	-	0.799(85)	0.799	1.25
<i>A. mellifera</i>	0.921(42)	0.793(100)	0.510(55)	0.740	1.35
<i>O. bruneri</i>	0.787(79)	0.695(181)	-	0.723	1.38

¹ Weighted mean PE for each type of bee.

² The approximate number of visits required for maximum drupelet set (1/PE).

³ Data was collected from greenhouse raspberry. Data from 2015 and 2016 is combined.

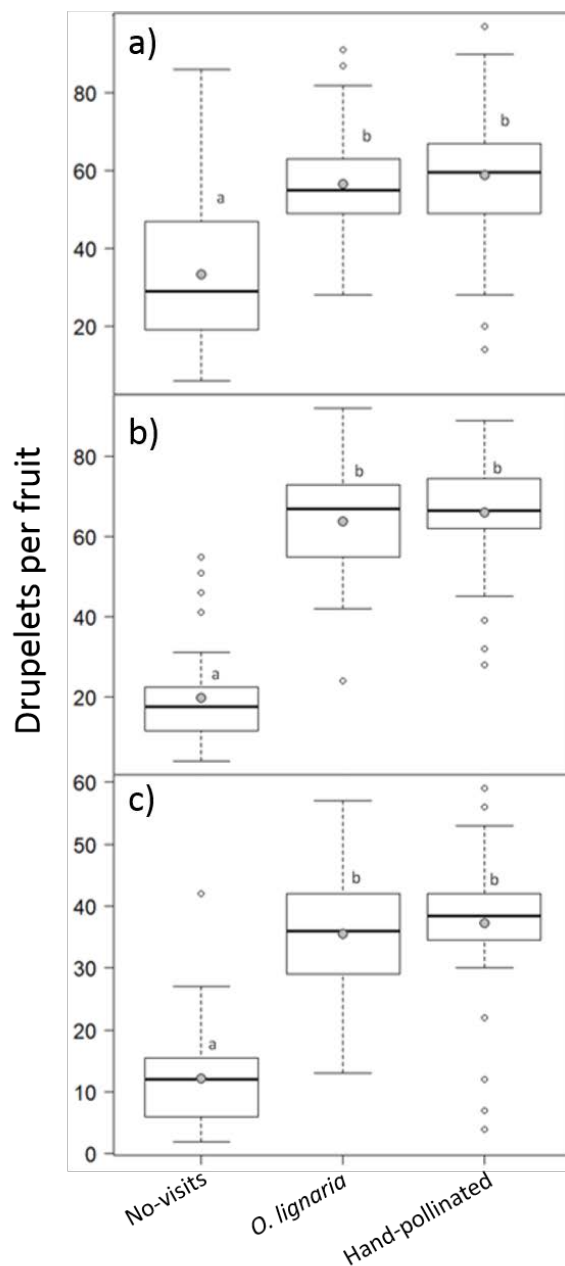


Figure 2-1. Drupelet counts for the greenhouse-grown raspberry. **a)** 'Rozella' purple raspberry in 2015: no-visitation (n=90), hand-pollination (n=90), and a single-visit by *O. lignaria* (n=95). **b)** 'Rozella' purple raspberry in 2016: no-visitation (n=40), hand-pollination (n=40), and a single-visit by *O. lignaria* (n=35). **c)** 'Latham' red raspberry in 2016: no-visitation (n=40), hand-pollination (n=40), and a single-visit by *O. lignaria* (n=50). The box diagram depicts outliers (white dots), 1.5 x Interquartile range (IQR) (whiskers), upper and lower quartiles (box), median (solid cross-bar), and mean (grey dots). Letters denote means that are significantly different at $p \leq 0.05$.

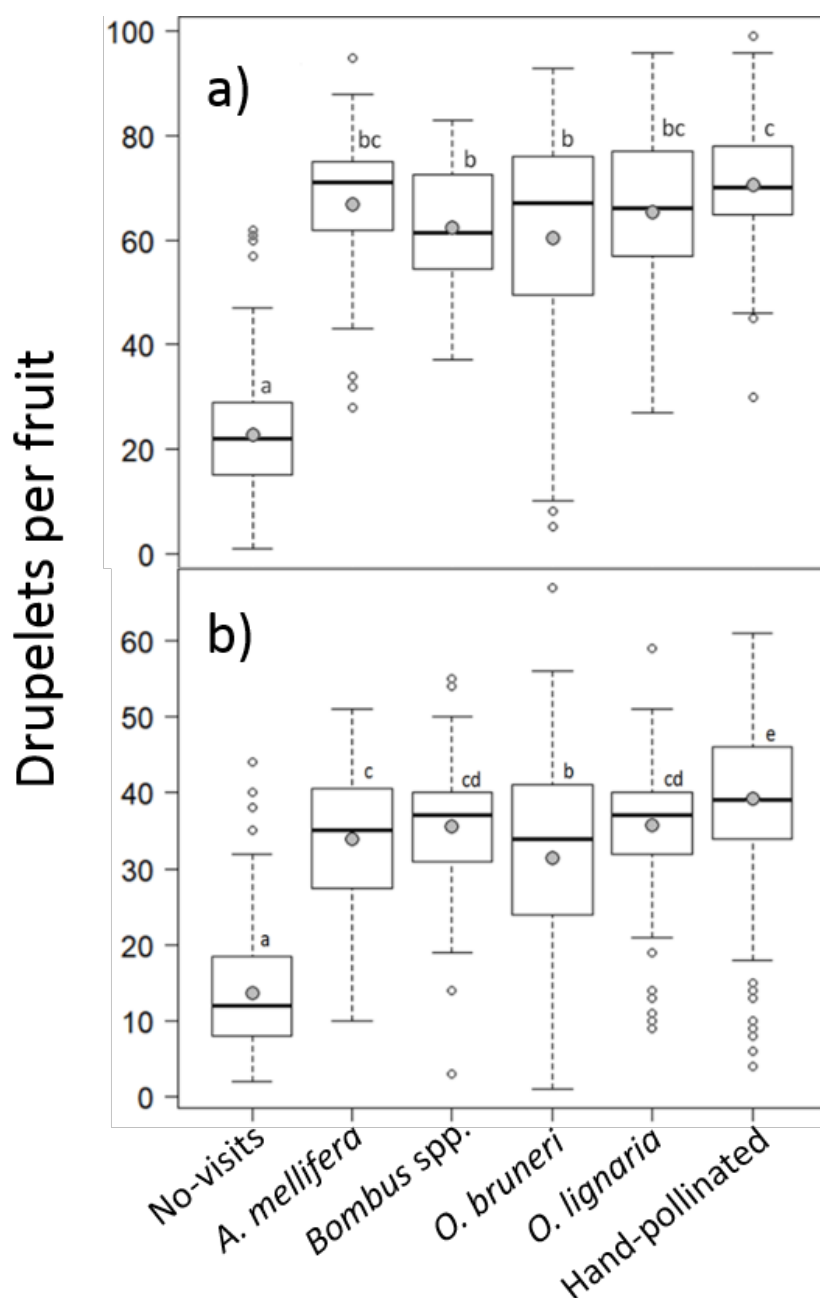


Figure 2-2. Drupelet counts for the field-cage experiment with red raspberry in 2016. **a)** ‘Cowichan’: no-visitation (n=200), hand-pollination (n=200), and single-visits by *A. mellifera* (n=42), *Bombus* spp. (n=20), *O. bruneri* (n=79), and *O. lignaria* (n=30). **b)** ‘Latham’: no-visitation (n=200), hand-pollination (n=200), and single-visits by *A. mellifera* (n=100), *Bombus* spp. (n=86), *O. bruneri* (n=181), and *O. lignaria* (n=121). The box diagram depicts outliers (white dots), 1.5 x IQR (stems), upper and lower quartiles (box), median (solid cross-bar), and mean (grey dots). Letters denote means that are significantly different at $p \leq 0.05$.

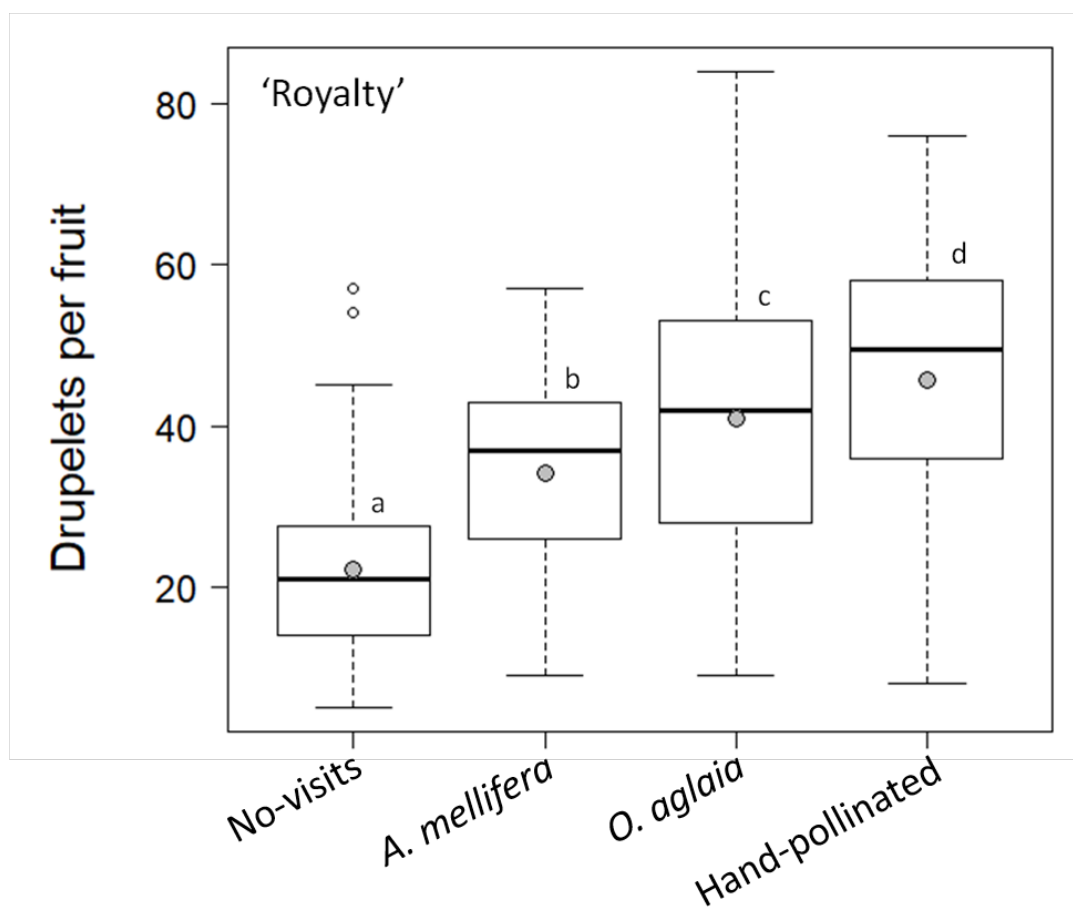


Figure 2-3. Drupelet counts for the field-cage experiment with 'Royalty' purple raspberry in 2016: no bee visitation (n=100), hand-pollination (n=100), and single-visits by *A. mellifera* (n=55), *O. aglaia* (n=85). The box diagram depicts outliers (white dots), 1.5 x IQR (whiskers), upper and lower quartiles (box), median (solid cross-bar), and mean (grey dots). Letters denote means that are significantly different at $p \leq 0.05$.

References

- Aizen, M. A., L. A. Garibaldi, S. A. Cunningham, and A. M. Klein. 2008.** Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Curr. Biol.* 18: 1572–1575.
- Basibuyuk, H. H., and D. L. J. Quicke. 1999.** Grooming behaviors in the Hymenoptera (Insecta): potential phylogenetic significance. *Zool. J. Linn. Soc.* 125: 349–382.
- Bekey, R. S. 1985.** Effects of method and timing of pollination and temperature on fruit development in red raspberry. PhD Diss. Oregon State Univ., Corvallis, O.R.
- Benedek, P., and J. Nyéki. 1996.** Pollinating efficiency of honeybees on apple cultivars as affected by their flower characteristics. *Hortic. Sci.* 28: 40–47.
- Bosch, J., and W. P. Kemp. 2002.** Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. *Bull. Entomol. Res.* 92: 3–16.
- Bosch, J., and M. Blas. 1994.** Foraging behavior and pollinating efficacy of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). *Appl. Entomology Zool.* 29: 1–9.
- Bosch, J., W. P. Kemp, and G. E. Trostle. 2006.** Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 99: 408–413.
- Burgett, M. 1997.** 1996 Pacific Northwest honey bee pollinator survey. Oregon State Univ., Corvallis, O.R.
- Cane, J. H. 2002.** Pollinating bees (Hymenoptera: Apiformes) of U.S. alfalfa compared for rate of pod and seed set. *J. Econ. Entomol.* 95: 22–27.
- Cane, J. H. 2008.** An effective, manageable bee for pollination of *Rubus* bramble fruits, *Osmia aglaia*. *Acta Hort.* 777: 459–463.
- Cane, J. H. 2005.** Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries. *HortScience.* 40: 1705–1708.
- Cane, J. H., and D. Schiffhauer. 2003.** Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium Macrocarpon* [Ericaceae]). *Am. J. Bot.* 90: 1425–1432.

- Chagnon, M., J. Gingras, and D. de Oliveira. 1991.** Honey bee (Hymenoptera: Apidae) foraging Behavior and raspberry pollination. *J. Econ. Entomol.* 84: 457–460.
- Colbert, S., and D. de Oliveira. 1990.** Influence of pollen variety on raspberry (*Rubus idaeus* L.) development. *J. Hered.* 81: 434–437.
- Dale, A. 2002.** Protected cultivation of raspberries. *Acta Hortic.* 946: 349–354.
- Demchak, K. 2009.** Small fruit production in high tunnels. *Horttechnology.* 19: 44–49.
- Food and Agriculture Organization of the United Nations. 2017.** FAOSTAT database. FAO-ESS, Rome, Italy. 12 September 2017.
<<http://www.fao.org/faostat/en/#data/QC>>
- Free, J. B. 1993.** Insect pollination of crops, 2nd ed. Academic Press, London, U.K.
- Javorek, S. K., K. E. Mackenzie, and S. P. Vander Kloet. 2002.** Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). *Ann. Entomol. Soc. Am.* 95: 345–351.
- Jennings, D. L. 1988.** Raspberries and blackberries: their breeding, diseases and growth. Academic Press, London, U.K.
- Keep, E. 1968.** Incompatibility in *Rubus* with special reference to *R. idaeus* L. *Can. J. Genet. Cytol.* 10: 253–262.
- Klein, A., B. E. Vaissie, J. H. Cane, I. Steffan-dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007.** Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B Biol. Sci.* 247: 303–313.
- Lye, G. C., S. N. Jennings, J. L. Osborne, and D. Goulson. 2011.** Impacts of the use of nonnative commercial bumble bees for pollinator supplementation in raspberry. *J. Econ. Entomol.* 104: 107–114.
- MacKenzie, K. E., and M. L. Winston. 1984.** Diversity and abundance of native bee pollinators on berry crops and natural vegetation in the lower Fraser Valley, British Columbia. *Can. Entomol.* 116: 965–974.
- Meso West. 2017.** Meso West Data. Univ. of Utah, Salt Lake City, UT. 10 October 2017.
<<http://mesowest.utah.edu/>>

- Monzón, V. H., J. Bosch, and J. Retana. 2004.** Foraging behavior and pollinating effectiveness of *Osmia cornuta* (Hymenoptera: Megachilidae) and *Apis mellifera* (Hymenoptera: Apidae) on 'Comice' pear. *Apidologie*. 35: 575–585.
- Morandin, L. A., T. M. Lavery, R. J. Gegear, and P. G. Kevan. 2002.** Effect of greenhouse polyethylene covering on activity level and photo-response of bumble bees. *Canad. Entomol.* 134: 539-549.
- Neilsen, A., T. Reitan, A. W. Rinvoll, and A. K. Brysting. 2017.** Effects of competition and climate on a crop pollinator community. *Agric. Ecosyst. Environ.* 246: 253–260.
- Pinzauti, M., D. Lazzarini, and A. Felicioli. 1997.** Preliminary investigation of *Osmia cornuta* Latr. (Hymenoptera, Megachilidae) as a potential pollinator for blackberry (*Rubus fruticosus* L.) under confined environment. *Acta Hort.* 437: 329–333.
- Pitts-singer, T. L., and J. H. Cane. 2011.** The Alfalfa Leafcutting Bee, *Megachile rotundata*: the world's most intensively managed solitary bee. *Annu. Rev. Entomol.* 56: 221–237.
- Pritts, M. P. 2008.** Primocane-fruited raspberry production. *HortScience*. 43: 1640–1641.
- Pritts, M. P., R. W. Langhans, T. H. Whitlow, M. J. Kelly, and A. Roberts. 1999.** Winter raspberry production in greenhouses. *Horttechnology*. 9: 13–15.
- Prodorutti, D., and F. Frilli. 2008.** Entomophilous pollination of raspberry, red currant and highbush blueberry in a mountain area of Friuli-Venezia Giulia (North-Eastern, Italy). *Acta Hort.* 777: 429–434.
- Rogers, S. R., D. R. Tarpy, and H. J. Burrack. 2013.** Multiple criteria for evaluating pollinator performance in highbush blueberry (Ericales: Ericaceae) agroecosystems. *Environ. Entomol.* 42: 1201–1209.
- Sáez, A., C. L. Morales, L. Y. Ramos, and M. A. Aizen. 2014.** Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *J. Appl. Ecol.* 51: 1603–1612.
- Sedivy, C., and S. Dorn. 2014.** Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; *Osmia*) as fruit tree pollinators. *Apidologie*. 45: 88–105.
- Shanks Jr., C. H. 1969.** Pollination of raspberries by honeybees. *J. Apic. Res.* 8: 19–21.

- Spears Jr., E. E. 1983.** A direct measure of pollinator effectiveness. *Oecologia*. 57: 196–199.
- Thomson, J. D., and K. Goodell. 2001.** Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *J. Appl. Ecol.* 38: 1032–1044.
- Torchio, P. F. 1985.** Field experiments with pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: V (1979–1980), methods of introducing bees, nesting success, seed counts, fruit yields (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 53: 448–464.
- Torchio, P. F. 1981.** Field experiments with *Osmia lignaria propinqua* Cresson as a pollinator in almond orchards: I, 1975 studies (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 54: 815–823.
- U.S. Department of Agriculture – National Agricultural Statistics Service. July 2016.** Noncitrus fruits and nuts 2015 summary. U.S. Dept. of Agric., Washington, D.C.
- Velthuis, H. H. W., and A. Van Doorn. 2006.** A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*. 37: 421–451.
- Vicens, N., and J. Bosch. 2000.** Pollinating efficacy of *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae, Apidae) on ‘Red Delicious’ apple. *Environ. Entomol.* 29: 235–240.
- Wieniarska, J. 1987.** The role of pollinating insects in fruiting of some red raspberry cultivars. *Fruit Sci. Reports*. 14: 65–70.
- Willmer, P. G., A. A. M. Bataw, and J. P. Hughes. 1994.** The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecol. Entomol.* 19: 271–284.
- Winston, M. L., and L. H. Graf. 1982.** Native bee pollinators of berry crops in the Fraser Valley of British Columbia. *J. Entomol. Soc. Br. Columbia*. 79: 14–20.
- Yokoi, T., and I. Kandori. 2016.** Comparison of foraging activity between the mason bee *Osmia orientalis* (Hymenoptera : Megachilidae) and honey bees for wild raspberry *Rubus hirsutus* (Rosales: Rosaceae). *Entomol. News*. 125: 363–367.
- Żurawicz, E. 2016.** Cross-pollination increases the number of drupelets in the fruits of red raspberry (*Rubus idaeus* L.). *Acta Hort.* 1133: 145–152.

Żurawicz, E., M. Studnicki, J. Kubik, and K. Pruskić. 2018. A careful choice of compatible pollinizers significantly improves the size of fruits in red raspberry (*Rubus idaeus* L.). *Sci. Hortic. (Amsterdam)*. 235: 253–257.

CHAPTER III

TWO PROLONGED BEE VISITS SUFFICE TO MAXIMIZE DRUPELET SET FOR RED RASPBERRY

Abstract

Red raspberry is one of the many fruit crops reliant on the European honey bee for pollination services. Recent declines among managed honey bee colonies coupled with expanding U.S. acreage have led to a marked increase in the cost of U.S. colony rentals for raspberry. The current stocking density recommendation for raspberry is 2 to 5 colonies/ha. This estimate is based on the assumption that a raspberry flower requires dozens of visits to achieve maximal drupelet set. Recent studies, however, suggest that far fewer visits are required for full drupelet set. Here, drupelet set resulting from bee visitation to a raspberry flower is assessed in order to determine if two prolonged visits will suffice to maximize drupelet set. For all three red raspberry cultivars examined there was no difference in drupelet set resulting from two visits when compared to openly pollinated flowers, the two visits being the first prolonged visit on each of two consecutive mornings. The findings of this study suggest that current stocking density recommendations may greatly over-estimate the number of colonies required for adequate pollination, costing farmers hundreds of dollars per acre in excess pollination fees. Additionally, overstocking may be detrimental to fruit yield, because excessive visitation can damage stigmas, thus limiting drupelet set. These findings should help to refine honey bee stocking density estimates for red raspberry pollination.

Introduction

Cultivated red raspberry (*Rubus idaeus* L.) is one of the numerous specialty crops dependent upon bees for fruit production (Klein et al., 2007). Although predominantly self-fertile (Daubeny, 1971), raspberry flowers only partially self-pollinate due to insufficient contact between the anthers and the innermost stigmas within the flower (Free, 1993). Raspberry flowers contain 60 to 90 stamens arranged in an outer ring of whorls around a central receptacle, and a similar number of pistils arising spirally from the receptacle. Pistils must be individually pollinated; each ovary develops into a single-seeded drupelet when fertilized (Jennings, 1988). Cohesion of the aggregate fruit requires that a high percentage of drupelets set. Unmarketable berries result when too few drupelets develop, causing berries to be crumbly and misshapen, often with a terminal tuft of dried unpollinated pistils (Cane, 2005; Free, 1993; McGregor, 1976). Bee visitation is necessary to ensure adequate pollination of these innermost pistils.

Wild bees are often too scarce in larger raspberry fields to satisfy pollination needs (Winston and Graf, 1982; Mackenzie and Winston, 1984). Growers instead rely on rented colonies of European honey bees (*Apis mellifera* L.) as the primary pollinator of commercial raspberry in Europe and North America (Free 1993). Honey bees are known to be effective pollinators of raspberry (Shanks, 1969; Bekey, 1985; Chagnon et al., 1991; Willmer et al., 1994; Cane, 2005; Cane, 2008; Saez et al., 2014); however, there is disagreement as to the estimated number of visits required for full drupelet set. Bekey (1985) suggested that every flower may need as many as 68 honey bee visits, whereas other studies estimate around 5 to 10 visits to be sufficient (Chagnon et al., 1991; Saez et

al., 2014). More recent work examining the single-visit efficacy of honey bees for raspberry suggests that as few as two visits are necessary and sufficient to maximize yield (Andrikopoulos unpublished data).

Drupelet set increases with the cumulative duration of a bee's contact with a flower, up to about 150 seconds (Chagnon et al., 1991). Bees typically linger far longer at virgin flowers, as these present a large volume of nectar (Bekey, 1985; Willmer et al., 1994). These prolonged visits to virgin flowers can last upwards of 80 seconds, >6 times longer than the day's subsequent visits (Bekey, 1985). This pattern accords with the daily cycle of nectar volume in a raspberry flower, which is usually greatest in the morning, having accumulated during the previous night. (Bekey, 1985; Willmer et al., 1994). The first visitor of the day depletes the flower's nectar pool. Raspberry flowers remain receptive for at least two days, over which time they continually secrete nectar (Bekey, 1984; Eaton et al., 1968; Redalen, 1976; Willmer et al., 1994). As a consequence, this recharged nectar pool should elicit one prolonged bee visit on each of two days, during which foragers stand astride the receptacle and pivot about to drink nectar, thus ensuring contact with the central pistils. These two prolonged visits could be enough to maximize drupelet set. This prediction is supported by the finding that just a single prolonged visit by a honey bee to a virgin flower results in nearly 90% drupelet set (Andrikopoulos unpublished data).

An accurate estimate for the number of visits required for full fruit set is necessary to build more refined models of honey bee stocking density on farms. Bekey (1985) estimated that 2 to 5 colonies/ha are needed to pollinate raspberry. However, that calculation was based on the assumption of between 27 and 68 visits per flower for

adequate pollination. If flowers only require two visits instead, the estimated honey bee stocking density for raspberry would be considerably less. This discrepancy has major economic implications for growers. Raspberries markets are expanding, evidenced by the doubling of land dedicated to cultivated raspberries between 1984 and 2014 (FAOSTAT, 2017). At the same time, faltering populations of managed honey bees have led to mounting hive rental prices. The price of colonies for raspberry has nearly doubled in Oregon and Washington over the past 20 years, now averaging \$40/colony (Burgett, 1996; USDA-NASS, 2016). In California, the rental price is even higher averaging \$92/colony in 2016. Growers in California, Oregon, and Washington paid \$1.61 million for raspberry pollination in 2016 (USDA-NASS, 2016).

In this study, we compared drupelet set for four honey bee visitation treatments at three red raspberry cultivars: 1) unvisited flowers, 2) a single prolonged bee visit, 3) two prolonged bee visits, one on each of two consecutive days, and 4) openly visited raspberry flowers (open pollination). We posited that two prolonged honey bee visits to a single raspberry flower would be sufficient to achieve full drupelet set.

Materials and Methods

Experimental Design. Studies were conducted in a small four-year-old planting at the USDA-ARS Pollinating Insect Research Unit in Logan, UT (41° 45'29.1" N 111° 48'44.5" W). The planting consisted of six 11 m rows with 3 m row spacing. Cultivars in the orchard were alternated by row to promote outcrossing, which is reported to have marked metaxenic effects for some cultivars (Colbert and de Oliveira, 1990; Żurawicz, 2016). Between 29 May and 2 June 2017, pollination by honey bees and occasional

bumblebees was evaluated on the florican crop for ‘Cowichan’ and ‘Polka’. The mean air temperature for the observation period during this time ranged from 22.4 to 26.5 °C (MesoWest, 2017). Pollination of ‘Latham’ was evaluated on 6 and 7 June 2017, when the mean air temperatures were 27.3 °C and 29.9 °C (MesoWest, 2017). Visits by honey bees and the few bumblebees (13 of 444 visits) were combined in this study, as previous work on ‘Latham’ and ‘Cowichan’ showed that these two bees are equally effective pollinators of these cultivars (unpublished data). All observations were completed in fair weather, between 0900 to 1800 h MST.

We evaluated free-flying bees for pollination of red raspberry using controlled floral access. At the onset of flowering, we covered several hundred racemes with nylon mesh bags. Any open flowers were removed prior to bagging. As bloom progressed, we exposed three to four racemes each having ≥ 1 open flower to foraging bees by removing the mesh bags. Flowers remained exposed for up to 10 min. We discarded any unvisited flowers, as well as the few flowers visited by species other than honey bees or bumblebees. Once a flower had been visited, we snipped one sepal (for identification), and replaced the bag with an attached numbered tag. The following day, half the flowers (149 out of 298) that had received single visits were randomly selected to receive a second visitation. As before, we removed three to four mesh bags sequentially for 10 min observation periods. If the focal flower was again visited, we snipped a second sepal, and the bag was once again replaced. Occasionally these racemes also bore virgin, one-day old flowers. If these flowers received a single-visit prior to the second visit to the focal flower, a sepal was snipped and the flower was included in the one-visit treatment, as were focal flowers that did not receive their second visit during the allotted exposure

period. Overall, 90% of flowers (134 out of 149) selected for the two-visit treatment received a second visit during the second day's 10 min exposure period. We randomly selected fifty additional flowers per cultivar for the unvisited and open-pollinated treatments. Racemes containing flower buds designated unvisited remained enclosed in mesh bags until the flowers senesced. Tagged flowers designated as open-pollinated remained exposed for the entirety of the experiment. During flowering, we also measured the average durations of first-visits at virgin and two-day old flowers for a subset of the focal flowers. Racemes remained bagged until fruit had set and drupelets were ready to be counted. To avoid loss due to pests, we picked berries while still green, but with sufficiently sized drupelets for counting. Only pollinated pistils will yield a swelling drupelet with a developing seed (Jennings, 1988).

Data Analysis. For each cultivar, we compared the four pollination treatments for the counts of drupelets per maturing raspberry using a mixed model one-way ANOVA (PROC MIXED; SAS v.9.4.2) (Littell et al. 1996). Plotted residuals of drupelet counts were judged to be adequately normal for all three cultivars. Where relevant (Levene's test), we used a model for heterogeneous variance. We compared treatments for each cultivar by three *a priori* orthogonal contrasts between incrementally increasing numbers of visits received (0-1, 1-2 and 2-many visits).

Results

A total of 610 red raspberry flowers received pollination treatments (215 'Cowichan'; 224 'Latham'; 171 'Polka'). All cultivars showed enhanced drupelet counts from bee visitation (Figs. 3-1 – 3-3). For 'Cowichan' ($F= 159.78, p<0.0001$; Fig. 3-1), post hoc

comparisons revealed significantly more drupelets per berry for one vs no visit ($F=288.19, p<0.0001$), but not for one visit vs two visits ($F= 2.01, p=0.16$) or two visits vs many visits ($F=3.04, p=0.08$). For ‘Latham’ ($F= 112.43, p<0.0001$; Fig. 3-2), post hoc comparisons revealed significantly more drupelets per berry for no visit vs one visit ($F= 112.43, p<0.0001$) and for one visit vs two visits ($F= 5.71, p=0.02$), but not for two visits vs many visits ($F=1.24, p=0.27$; Fig. 3-2). For ‘Polka’ ($F= 87.05, p<0.0001$; Fig. 3-3), post hoc comparisons revealed a similar trend to that for ‘Cowichan’ with significant differences in mean drupelets per berry for no visit vs one visit ($F=143.90, p<0.0001$), but not for one visit vs two visits ($F=0.62, p=0.43$) or two visits vs many visits ($F=0.31, p=0.58$; Fig. 3-3). First visits to virgin flowers had a mean duration of 58.7 sec. The first visit to two-day old flowers had a mean duration of 67.7 sec. (Table 3-1).

Discussion

For all three red raspberry cultivars tested in this experiment, bee visitation improved drupelet set 2 – 4 fold. Drupelet counts for bee-visited ‘Polka’ flowers were comparable to the manually outcrossed flowers in the Żurawicz study (2016). Two first visits by a bee to a receptive flower on two successive days were sufficient to assure maximal drupelet set, comparable to open pollinated flowers. These first visits lasted about one minute on each of two successive mornings. For ‘Cowichan’ and ‘Polka’, even one prolonged visit yielded drupelet counts that were indistinguishable from openly visited flowers, suggesting that the dozens of additional visits to openly pollinated flowers are superfluous. This finding seems to sharply contrast with previous studies suggesting that from 5 to 68 visits are required to achieve maximum drupelet set (Bekey, 1985; Chagnon

et al., 1991; Saez et al., 2014). Chagnon et al. (1991) reported the nearest estimate to our study findings, suggesting 5 to 6 visits for sufficiency. The disparity between our estimates is likely due to their study limiting visitation to a single day. However, raspberry flowers remain receptive for at least two days (Bekey, 1985; Eaton et al., 1968; Redalen, 1976), and for some cultivars, including 'Latham' studied here, optimal drupelet set is achieved when pollination occurs over two or more days. This is presumably because stigmas within a flower do not all become receptive concurrently (Bekey, 1985; Eaton et al., 1968).

Comparison of the cumulative duration of visits to a flower can help reconcile different estimates for the number of visits required. Chagnon et al. (1991) estimated that ~150 sec of contact with the flower is sufficient to maximize drupelet set. Visit duration is strongly correlated with the nectar volume present in a flower (Bekey, 1985; Chagnon et al., 1991; Willmer, 1994). Nectar is secreted continuously throughout the day, but is most abundant in the morning, having had all night to accumulate (Bekey, 1985; Willmer, 1994). The average duration of the first visit of the day to a receptive flower can average >80 sec (Bekey, 1985) compared to an average duration of < 13 sec for subsequent visits to the same flower (Bekey, 1985; Willmer, 1994). Taking this difference into account, our estimate of two first visits over two days is in accord with the previous estimate of 5 to 6 visitations on a single day, as the cumulative duration of those two visits is likely to exceed the 150 sec threshold. The average cumulative duration of the two visits for our study was ~127 sec (Table 3-1). Although this duration is slightly less than the ~150 sec previously estimated, the difference is likely attributable to variation in nectar secretion and receptivity among the cultivars used in the studies

(Bekey, 1985; Willmer, 1994).

An accurate estimate of the numbers of bee visits required to maximize fruit yield is integral for determining appropriate stocking densities for bee-dependent crops. Current stocking density estimates for raspberry are 2 to 5 honey bee colonies/ha (Bekey, 1985; Free 1993). This estimate is based on each flower requiring dozens of visits to maximize fruit set; however, our results reveal that only two prolonged visits, on each of two consecutive days, are required to achieve full drupelet set. This result suggests that many farmers may be over-stocking their fields with honey bee colonies, potentially increasing costs by hundreds of dollars per hectare in excess pollination fees. From a beekeeper's perspective, an orchard's volume of nectar could be collected by far fewer colonies. Furthermore, there is strong evidence suggesting that excessive visitation due to a preponderance of bees can diminish drupelet set for raspberry. Heavy excess in bee visitation can result in damage to stigmas, thus limiting drupelet set in over-visited flowers (Saez et al., 2014). The results of our study should help to refine stocking density estimates for raspberry, potentially reducing growers' costs for hive rentals, while avoiding losses in fruit yield due to excessive bee visitation.

Table and Figures

Table 3-1. Mean (\pm SE), median, and range of honey bee visit durations for first-visits of the day at focal flowers. Day one flowers (n=62) were virgin flowers. Day two flowers (35) all received a single bee-visit on the previous day. Results for all raspberry cultivars are combined.

	Mean \pmSE (secs)	Median	Range
1st visit, day 1	58.7 \pm 3.3	56	17-140
1st visit, day 2	67.7 \pm 6.7	63	10-180

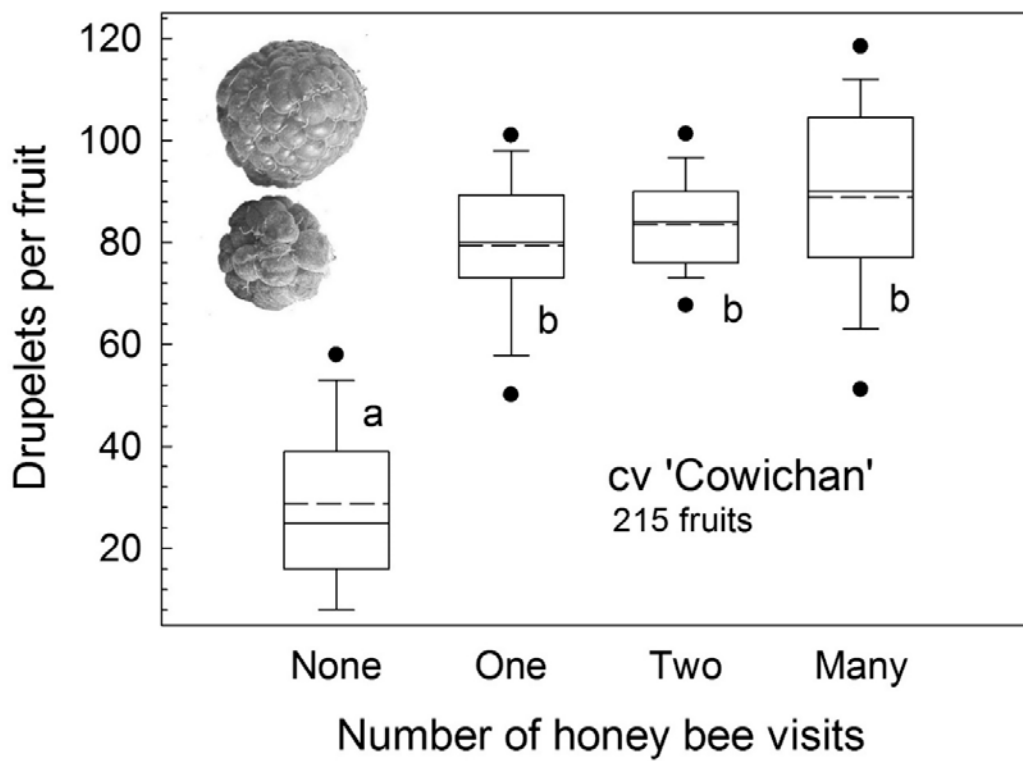


Figure 3-1. Drupelet counts for 'Cowichan' resulting from no visitation (n=50), one-visit (n=62), two-visits (n=53), and openly visited (n=50) pollination treatments. The box plot depicts the median (solid cross-bar), mean (dashed cross-bar), 25th and 75th percentile (box), 10th and 90th percentile (stems), and outliers (dots). Letters denote means that are significantly different at $p \leq 0.05$.

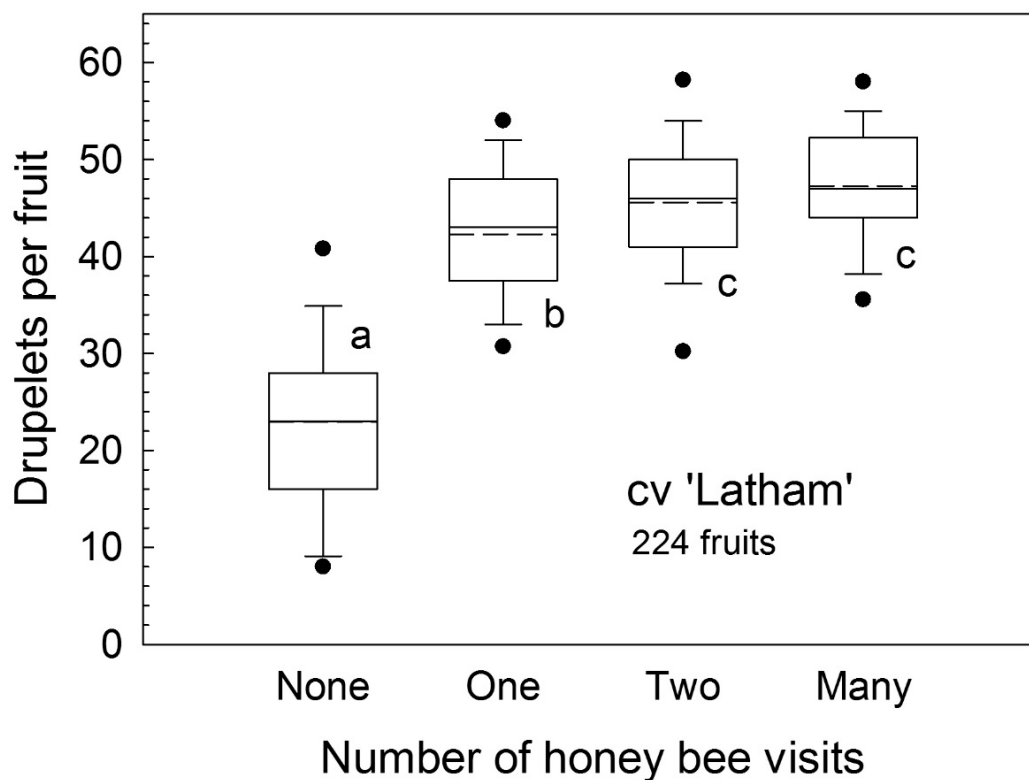


Figure 3-2. Drupelet counts for 'Latham' resulting from no visitation (n=50), one-visit (n=73), two-visit (n=51), and openly visited (n=50) pollination treatments. The box plot depicts the median (solid cross-bar), mean (dashed cross-bar), 25th and 75th percentile (box), 10th and 90th percentile (stems), and outliers (dots). Letters denote means that are significantly different at $p \leq 0.05$.

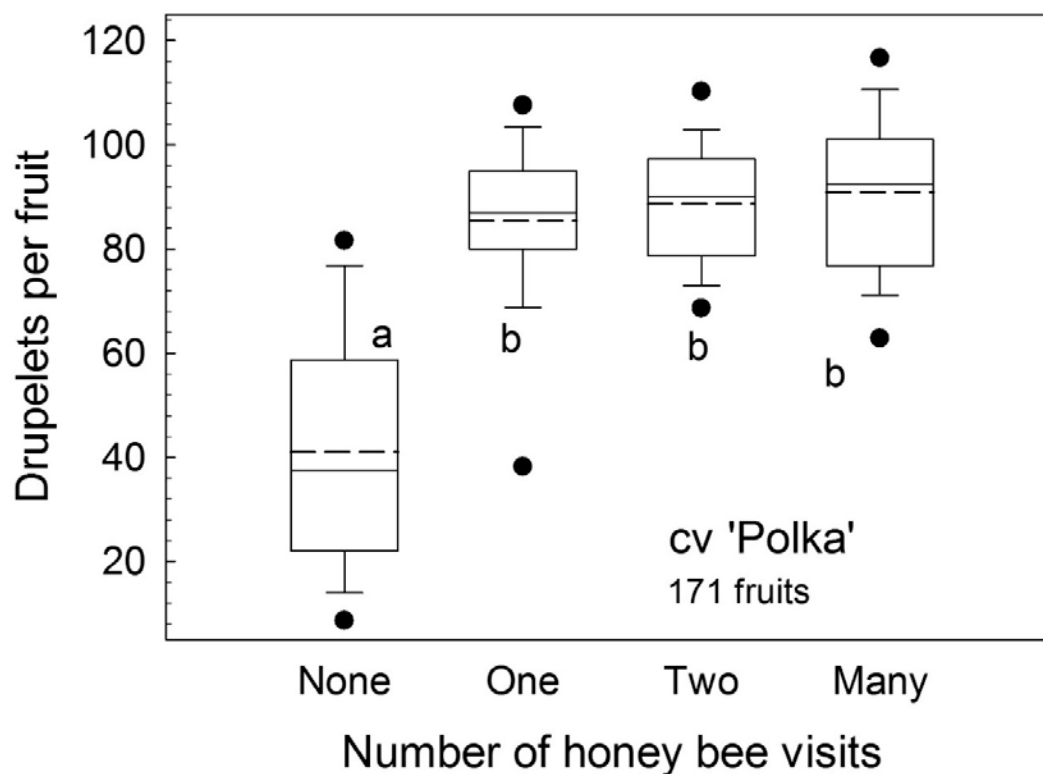


Figure 3-3. Drupelet counts for 'Polka' resulting from no visitation (n=50), one-visit (n=41), two-visit (n=30), and openly visited (n=50) pollination treatments. The box plot depicts the median (solid cross-bar), mean (dashed cross-bar), 25th and 75th percentile (box), 10th and 90th percentile (stems), and outliers (dots). Letters denote means that are significantly different at $p \leq 0.05$.

References

- Bekey, R. S. 1985. Effects of method and timing of pollination and temperature on fruit development in red raspberry. PhD Diss. Oregon State Univ., Corvallis, O.R.
- Burgett, M. 1997. 1996 Pacific Northwest honey bee pollination survey. Oregon State Univ., Corvallis, O.R.
- Cane, J.H. 2005. Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries (*Rubus*: Rosaceae). HortScience. 40:1705-1708.
- Cane, J.H. 2008. An effective, manageable bee for pollination of *Rubus* bramble fruits, *Osmia aglaia*. Acta Hort. 777:459-464.
- Chagnon, M., J. Gingras, and D. de Oliveira. 1991. Honey bee (Hymenoptera: Apidae) foraging behavior and raspberry pollination. J. Econ. Entomol. 84:457-460.
- Colbert, S., and D. de Oliveira. 1990. Influence of pollen variety on raspberry (*Rubus idaeus* L.) development. J. Hered. 81:434-437.
- Daubeny, H.A. 1971. Self-fertility in red raspberry cultivars and selections. Amer. Soc. Hort. Sci. 96:588-591.
- Eaton, G.W., H.A. Daubeny, and R.C. Norman. 1968. Pollination techniques for red raspberry breeding programs. Can. J. Plant Sci. 48:342-344.
- Food and Agriculture Organization of the United Nations. 2017. FAOSTAT database. FAO-ESS, Rome, Italy. 12 September 2017.
<<http://www.fao.org/faostat/en/#data/QC>>
- Free, J.B. 1993. Insect pollination of crops. 2nd ed. Academic press, London, U.K.
- Jennings D. 1988. Raspberries and blackberries-their breeding, diseases and growth. Academic Press, London, U.K.
- Klein, A.M., B.E. Vaissière, J.H. Cane, I. Steffan-Dewenter, S.A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B-Biol. Sci. 274(1608):303-313.
- Littell, R. C., Milliken, G. A., Stroup, W. W. and Wolfinger, R. D. 1996. SAS system for mixed models. SAS Inst. Inc.
- MacKenzie, K.E., and M.L. Winston. 1984. Diversity and abundance of native bee pollinators on berry crops and natural vegetation in the lower Fraser Valley, British Columbia. Can. Entomol. 116:965-974.

- McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agricultural Handbook No. 496. USDA-ARS, Washington, D.C.
- Meso West. 2017. Meso West Data. Univ. of Utah, Salt Lake City, UT. 10 October 2017. <<http://mesowest.utah.edu/>>
- Redalen, G. 1976. Pollination and fruit set in raspberries. *Acta Hort.* 60:169-176.
- Sáez, A., C.L. Morales, L.Y. Ramos, and M.A. Aizen. 2014. Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *J. Appl. Ecol.* 51(6):1603–12.
- SAS Institute. 2006. SAS/STAT User's Guide, version 9.1.3. SAS Institute, Cary, North Carolina, 943 pp.
- Shanks, C.H.J. 1969. Pollination of raspberries by honeybees. *J. Apicult. Res.* 8:19-21.
- U.S. Department of Agriculture – National Agricultural Statistics Service. July 2016. Noncitrus fruits and nuts 2015 summary. U.S. Dept. of Agric., Washington, D.C.
- Willmer, P.G., A.A.M. Bataw, and J.P. Hughes. 1994. The superiority of bumblebee to honeybees as pollinators-insect visits to raspberry flowers. *Ecol. Entomol.* 19:271-284.
- Winston, M.L., and L.H. Graf. 1982. Native bee pollinators of berry crops in the Fraser Valley of British Columbia (Canada). *J. Entomol. Soc. Brit. Columbia.* 79:14-20.
- Żurawicz, E. 2016. Cross-pollination increases the number of drupelets in the fruits of red raspberry (*Rubus idaeus* L.). *Acta Hort.* 1133:145-152

CHAPTER IV

COLDER WINTER TEMPERATURE FOR *OSMIA LIGNARIA* FACILITATES
DELAYED EMERGENCE

Abstract

The blue orchard bee, *Osmia lignaria*, is an effective pollinator of several rosaceous fruit crops including raspberry. Its brief natural activity period coincides with the bloom of tree fruits such as apple and pear, but may precede raspberry bloom by a month or more. In order to utilize these bees for pollination later-blooming crops, method need to be developed to delay *O. lignaria*'s emergence without compromising longevity, vigor, or survival. Wintering bees at colder than normal temperatures can effectively delay their emergence without depletion of metabolic reserves or increased winter mortality. It is unclear how these measures of post-winter vigor translate to nesting and reproductive success. This study examines the effects of low temperature storage on *O. lignaria* survival, longevity, nesting establishment, and reproductive success. Bees were stored at three wintering temperature (4°C, 0°C, and -3°C), and two storage durations (182 days and 230 days). After 230 days, bees wintered at 4°C suffered high mortality and shortened lifespans compared to other treatments. Additionally, nest establishment decreased by ~25% for this group after 230 days. Average fecundity was the same across treatments. The combined effects of decreased winter survival and nest establish suggest a large impact at the population level for bees wintered at 4°C for long durations. Deleterious effects of long storage were not observed at either of the lower temperatures.

These results suggest that low temperature storage may be a viable management strategy for the use of *O. lignaria* with later-blooming crops such as raspberry.

Introduction

The blue orchard bee, *Osmia lignaria* Say, is a solitary bee native to and widely distributed across North America. As a result of its gregarious cavity-nesting habits, ready adoption of man-made nesting substrates, and affinity for rosaceous flowers, *O. lignaria* has garnered significant attention in recent decades as an alternative or supplement to honey bees for pollination of several rosaceous tree crops, such as apple, almond, and cherry (Bosch and Kemp 1999, 2000, 2003; Bosch et al. 2000, 2006; Kemp and Bosch 2005; Sheffield 2008, 2014; Torchio 1976, 1981, 1985). One major obstacle hindering the versatility of this bee is its relatively brief period of annual adult activity when foraging bees are available to provide pollination services. Adult *O. lignaria* emerge in early spring and foraging females remain active for about six weeks. The activity periods of local populations often naturally coincide with apple or cherry bloom (Bosch and Kemp 1999; Torchio 1985; Sheffield 2014); however, to be a practical pollinator of crops that bloom before or after the bee's natural activity period, emergence timing must be controlled without compromising longevity, vigor, or survival. Fortunately, these bees are amenable to some developmental manipulations. To date, their emergence has been advanced successfully to synchronize with the late-winter bloom of almonds in California (Bosch and Kemp 2000; Bosch et al. 2000; Kemp and Bosch 2005).

Adult *O. lignaria* usually emerge from March – May depending on geographic

location (Bosch and Kemp 2000; Kemp and Bosch 2005, Pitts-Singer et al. 2014). Females emerge about a week after males, and typically begin nesting within a few days (Tepedino and Torchio 1982; Sgolastra et al. 2016). Nests are constructed in pre-existing cavities and consist of a linear series of cells separated by mud partitions. Each cell is provisioned with a mass of pollen and nectar upon which an egg is laid. Larger provisions are allocated to female eggs which are typically positioned in the innermost cells of the nest (Levin 1966; Phillips and Klostermeyer 1978; Torchio 1989). Eggs hatch after several days and begin consuming their provision mass. Development of *O. lignaria* proceeds through five larval instars, and by late spring the fifth instar finishes feeding, defecates, spins a cocoon, and enters a diapause-mediated summer dormancy as a prepupa (Kemp et al. 2004; Kemp and Bosch 2005; Sgolastra et al. 2011, 2012; Torchio 1989). The duration of summer dormancy is related to the geographic origin of the population and is longer in warmer climates (Pitts-Singer et al. 2014). Following the summer dormancy period, bees pupate, and by late summer or early fall, adults eclose but remain inside their cocoons (Bosch and Kemp 2000). A pre-wintering period follows, during which bees begin to slow their metabolic rates and initiate winter diapause (Bosch et al. 2010; Kemp et al. 2004). Initiation of diapause does not require a temperature cue, indicating it is obligate for *O. lignaria*. While diapause initiation is independent of temperature, diapause cannot be terminated without exposure to cold temperatures (Sgolastra et al. 2010). Metabolic rates begin to rise after about three months of wintering, increasing exponentially until it is time to emerge in the spring (Bosch et al. 2010; Kemp et al. 2004; Sgolastra et al. 2010).

Like all bees, prior to its first flight, an individual *O. lignaria* spends its entire

lifecycle in the dark confines of its nest, where ambient temperature appears to be the primary abiotic factor regulating its development and phenology (Sgolastra et al. 2010, 2011). Thermal influences on various stages of their lifecycle have been studied extensively in both wild and laboratory-reared bees (Bosch and Kemp 2000, 2003; Bosch et al. 2000, 2010; Kemp et al. 2004; Kemp and Bosch 2005; Pitts-Singer et al. 2014; Rust 1995; Sgolastra et al. 2010, 2011, 2012, 2016; Sheffield et al. 2008). The effect of winter storage temperature in particular has profound management implications for this species. The brief 2 to 3 week bloom of many rosaceous tree crops necessitates that bees managed for pollination must not only survive the winter, but must emerge quickly and with enough vigor to adequately pollinate the crop and provision their own nests to ensure successful reproduction (Bosch and Kemp 2003). Winter storage temperature, through its effects on diapause intensity and duration, can strongly impact winter survival, the length of the pre-emergence period (incubation – emergence), and measures of post-winter vigor such as weight loss, fat body depletion, and longevity to starvation (Bosch and Kemp, 2003; Bosch et al., 2010; Rust, 1975; Sgolastra et al. 2010, 2011; Sheffield et al., 2008).

Adult *O. lignaria* may spend half or more of their lives in the wintering stage (onset of winter temperature – incubation/spring temperature). During this time, bees do not eat; therefore, *O. lignaria* overwinters on a fixed energy budget. Despite metabolic suppression during winter diapause, bees may metabolize a significant portion of their energy reserves (Bosch and Kemp 2003; Bosch et al. 2010; Sgolastra et al. 2010, 2011). Bees wintered at warmer temperatures and/or longer durations suffer greater weight loss and fat body depletion, accompanied by greater mortality and shortened lifespans (Bosch and Kemp, 2003; Bosch et al., 2010; Sgolastra et al. 2010, 2011). Cooler winter

temperature slows the depletion of energy reserves; however, bees stored at cooler temperatures require a longer time period to satisfy dormancy requirements. Bees that fail to complete dormancy prior to springtime incubation have an extended pre-emergence period (Bosch and Kemp 2003; Bosch et al. 2010; Sgolastra et al. 2010). A prolonged emergence window complicates synchronizing bee activity with fast-blooming orchard crops. Furthermore, rapid weight loss during the spring pre-emergence period can result in bees emerging with depleted energy reserves (Sgolastra et al. 2010). A prolonged pre-emergence period also has been shown to limit nest establishment (Sgolastra et al. 2016). Bees overwintered at 4° C for >210 days experience a large (~20-30%) decrease in survival and longevity, compared to bees wintered at 0° C. However, colder temperature still results in a longer pre-emergence after 270 days of wintering (Bosch and Kemp 2003). The question therefore becomes, at what point do the consequences of warmer winter storage temperature outweigh the benefit of a short pre-emergence period?

Management strategies for *O. lignaria* on crops that bloom outside of their natural nesting season have mostly focused on methods to advance bee emergence, such as for almond bloom (Bosch and Kemp 2000, 2003; Bosch et al. 2000; Kemp and Bosch 2005; Pitts-Singer et al. 2014). Almonds bloom in February, and bee emergence must be advanced by a month or more to synchronize with bloom. Under this scenario, a short wintering duration is desirable, and therefore, is not likely to exceed the point at which significant differences in post-winter vigor become apparent. In fact, because *O. lignaria* requires a minimum of about 150 days when wintered at the industry standard 4°C, management for early emergence requires accelerating immature development to enable early artificial wintering so that they then have enough time to complete dormancy prior

to almond bloom the following year (Bosch and Kemp 2000, 2003; Bosch et al. 2000; Kemp and Bosch 2004; Sgolastra et al. 2010). Methods to delay bee emergence for crops blooming after *O. lignaria*'s natural activity period such as raspberry have received little attention, but could provide opportunities to expand markets for this alternative pollinator.

Colder winter storage could provide a simple, effective means to delay emergence of *O. lignaria*. The closely related Japanese species, *O. cornifrons*, has a similar natural phenology to *O. lignaria*. When its winter temperature was experimentally lowered to 1 to 2° C, they emerged and were successfully used for germplasm pollination more than a month after its natural flight season (Wilson and Abel 1996). Similar responses to temperature have been demonstrated for *O. lignaria*. As previously mentioned, bees wintered at 4° C begin to see elevated mortality and shortened lifespans by 210 days of wintering; similar losses for bees overwintered at 0° C do not manifest until around 270 days of winter storage (Bosch and Kemp 2003). By this time the pre-emergence period for bees wintered at 0° C is relatively short, suggesting that the tradeoff between overwinter energy depletion and the duration of the pre-emergence period may favor colder conditions for wintering periods >210 days (Bosch and Kemp 2003). These reports, however, did not relate winter temperature or duration directly to nesting or reproductive success; key factors in sustainable management of *O. lignaria* for pollinating later-blooming crops.

The effectiveness of *O. lignaria* as a crop pollinator is largely dependent upon their nesting or reproductive success. Female bees emerging with depleted energy reserves may be slow to initiate nesting, provision nest cells at a slower rate, and/or die

prematurely (Sgolastra et al. 2016), all of which influence bee fecundity (Bosch and Vincens 2006). Measuring post-winter performance in terms of metabolic reserves is informative about the bees' condition, but extrapolation to the bees' nesting or reproductive success is dubious. For instance, hungry emerging bees could compensate by replenishing their fat reserves through additional feeding upon emergence. A protracted autumnal pre-wintering period, yields similar negative effects on survival, weight loss, and longevity to starvation as does prolonged wintering at warmer winter temperatures (Bosch et al. 2000, 2010; Sgolastra et al. 2011). However, other relevant fitness variables, such as nest establishment, pre-nesting and nesting duration, fecundity, provisioning rate, parental investment, and sex ratio, were unaffected by the length of the pre-wintering period (Sgolastra et al. 2016). Therefore, a detailed study of individual nesting and reproductive success is necessary in order to better understand the effects of winter temperature and diapause duration on post-winter performance of *O. lignaria*.

This study compares the survival, post-winter vigor, nest establishment, and fecundity of female *O. lignaria* subjected to three winter temperature regimes and two wintering durations. These more direct measures of the effect of winter temperature and duration on females' post-winter performance were used to answer three specific questions: 1) Does warm winter temperature impose a reproductive cost on *O. lignaria* when wintered for an extended duration? 2) Does wintering these bees at colder temperatures allow for delayed emergence without sacrificing winter survival or reproductive success? 3) Does colder wintering conditions provide a practical and sustainable management strategy for pollination of later blooming crops?

Materials and Methods

Source of Bees

To limit inter-population variation, all bees used in this experiment were sourced from a single location in northern Utah. Prior to natural emergence of *O. lignaria* in March 2015, 15 drilled wooden nest blocks with paper straw inserts were deployed in Logan Canyon, UT (41° 47'55" N 111° 39'04" W; elevation 1687 m). Nest blocks were attached to tree trunks ~1.5 m above the ground, facing SE. Nesting blocks were monitored every two weeks until *O. lignaria* nesting activity ceased. Blocks containing completed bee nests were collected in June 2015 and returned to the USDA-ARS Pollinating Insect Research Unit, Logan, UT (elev. 1396 m). Straws were removed from blocks and their contents were x-rayed. Straws containing *O. lignaria* nests were stored outside at ambient temperature until late September.

Pre-wintering

Beginning 25 July 2015, the nesting straws containing *O. lignaria* were x-rayed weekly to check for eclosed adults. Adults began to eclose by the first week of August. Beginning on 29 September, the bees were brought inside each night and held at 4° C to simulate the onset of winter conditions. One week later, bee cocoons were removed from nesting straws and sorted by sex, using x-ray images of body size and cocoon position for determination. Female *O. lignaria* are typically larger and are usually positioned in the back of the nest (Levin 1966). To limit handling of the bees, sex was not verified by opening the cocoons prior to wintering. Cocoons were cleaned of all debris, weighed, placed in gel caps, randomly assigned to treatment groups, and then placed into winter

storage.

Treatments

Six treatment groups consisted of combinations of three different winter storage temperatures (-3°C , 0°C , or 4°C), and two storage durations (182 days and 230 days). Bees in each group were assigned to either a post-emergence longevity trial or fecundity trial. Nestmates (siblings) were assigned to different treatments. A total of 294 female bees were initially divided equally among the six combinations of storage temperature and duration ($n=49$ per treatment combination). Within each treatment combination, 24 bees were assigned to the longevity trial, and 25 were assigned to the fecundity trial. Sixteen bees were incorrectly identified as female prior to winter storage, resulting in small differences in final sample sizes among treatments.

Survival, Weight Loss, Pre-emergence, and Longevity.

On 4 April and again on 22 May 2016, bees still in their cocoons were removed from winter storage, weighed, transferred to Petri dishes, and incubated at 20°C to simulate springtime emergence temperatures. Bees were checked for emergence twice daily at 0800 and 2000 MDT. Bees were considered to have survived if they successfully emerged from their cocoons. To account for differences in bee size, individual wintering weight loss was reported as: $(\text{initial weight} - \text{final weight})/\text{initial weight}$. Percent weight loss was log transformed for normality. The effect of wintering temperature on survival, while controlling for storage duration, was tested by chi-square analysis (PROC FREQ; SAS v. 9.4.2). A second chi-square analysis tested for the effect of storage duration on survival, controlling for temperature (Bosch and Kemp 2003). The effect of storage temperature and duration on percent weight loss was tested by a two-way ANOVA with

the Tukey-Kramer post hoc comparison (PROC GLM; SAS v. 9.4.2). The effect of percent weight loss on survival was tested by logistic regression (PROC LOGISTIC; SAS v. 9.4.2). Bees from both the longevity and fecundity trials were included in the analyses.

The duration of the pre-emergence period was measured from the start of incubation to bee emergence. Bees from both the longevity and fecundity trials were included together to measure their pre-emergence periods. Upon emergence, sex was verified. The few misidentified individuals were reassigned to the corresponding treatment group for the correct sex. Percent weight loss and longevity of the pre-emergence period were log transformed for analysis. A scatter plot suggested a possible negative association between percent weight loss and duration of the pre-emergence period, therefore, a linear regression (PROC REG; SAS v. 9.4.2) was performed to test this relationship. A two-way ANCOVA with the Tukey-Kramer post hoc comparison (PROC GLM; SAS v. 9.4.2) was used to examine the effect of storage temperature and duration on pre-emergence period, using percent weight loss as the covariate.

Bees assigned to the longevity treatment remained in the incubator at 20° C and were checked twice daily, at 0800 and 2000 MST, until all bees were deceased. These bees were not fed after emergence. Longevity was, therefore, measured as days to starvation. Longevity was considered the period of time from when an individual emerged to when the time it was last observed alive. Bees were considered dead when all movement had ceased. Differential winter survival among treatment groups, and the misassignment of gender for several bees, resulted in slightly unequal sample sizes. Data were square root transformed for normality. The effect of storage temperature and duration on longevity was tested by a two-way ANOVA with the Tukey-Kramer post-hoc

comparison (PROC GLM; SAS v. 9.4.2).

Fecundity

Following emergence, bees assigned to the fecundity trial were removed from the incubator and cooled temporarily at 4° C for marking. Each female received a unique combination of color enamel paint dots on her thorax to enable identification while nesting. For each of the two overwintering durations, 21 females and 33 males from each temperature treatment were released into a large screen cage (5.5 x 10.5 x 2 m) inside a greenhouse at the USDA-ARS Pollinating Insect Research Unit, Logan, UT. The cage was divided into three equal compartments with screen partitions. Each screen compartment therefore held 21 female *O. lignaria* (seven from each of the three winter temperature treatments). Each cage section had a nesting shelter containing two standard wood nesting blocks with 49 drilled holes with paper straw inserts (15 cm long and 7.5 mm inside diameter) (Tepedino and Torchio 1982; Sgolastra et al. 2016). Abundant pollen and nectar was provided in each cage section using ~150 potted *Phacelia tanacetifolia* Benth. (Hydrophyllaceae) plants, a standard foodplant for nesting *O. lignaria* (Tepedino and Torchio 1982; Sgolastra et al. 2016).

Nesting was monitored daily for 30 minutes per compartment. Each female's nesting hole was recorded. Paper straws with newly completed nests were removed from the blocks and marked with the bee's identity and date of completion, then moved outside to complete development. Completed nests were replaced with empty paper straws to maintain a constant supply of available nesting cavities. Each of the 63 females released for each overwintering duration group were characterized by storage temperature, overwinter weight loss, pre-emergence period, and nest establishment. For females that

successfully established nests, additional variables were recorded: pre-nesting period (time from release to nesting), nesting duration (start of nesting until the last day the bee was observed), and fecundity (number of eggs laid).

We tested for differences in nest establishment due to winter temperature after controlling for winter duration with a chi-square analysis (PROC FREQ; SAS v. 9.4.2). A separate chi-square analysis tested differences in nesting success between storage durations controlling for wintering temperature. The effect of pre-emergence duration on nest establishment was tested by logistic regression (PROC LOGISTIC; SAS v. 9.4.2). Environmental conditions in the greenhouse such as sunlight, temperature, and day length changed as spring progressed. As these seasonal factors might have a large impact on foraging efficacy, the remaining analyses were conducted separately for each storage duration. Generalized linear mixed models (PROC GLIMMIX; SAS 9.4.2) were constructed to test for the effect of wintering temperature on pre-nesting duration, nesting duration, and fecundity, with cage section as a random factor (Sgolastra et al., 2015). For nesting bees overwintered for 182 days, pre-emergence period and pre-nesting duration were included in the model for temperature's effect on nesting duration. Pre-nesting duration and nesting duration were included in the model for their effects on lifetime fecundity. For modeling the responses of nesting bees overwintered for 230 days, pre-emergence period was included in the model for the effects on nesting duration, while nesting duration was included in the model for its contribution to lifetime fecundity.

Results

A total of 278 female *O. lignaria* were exposed to six combinations of storage

temperature and duration. After controlling for winter duration, survival differed by temperature treatment for bees wintered for 230 days ($\chi^2=10.15$; $df=2$; $p=0.006$; Fig. 4-1), but not after 182 days ($\chi^2=0.96$; $df=2$; $p=0.62$; Fig. 4-1). After 230 days of winter conditions, fewer bees survived when wintered at 4° C (74.5%), than at -3° C (93.8%) or 0° C (93.3%). More bees died in the 4° C/230 day treatment group than at the same temperature after 182 days of wintering ($\chi^2=8.16$; $df=1$; $p=0.004$; Fig. 4-1). In contrast, storage duration did not affect survival at 0° C ($\chi^2=0.21$; $df=1$; $p=0.64$) or -3° C ($\chi^2=0.18$; $df=1$; $p=0.67$; Fig. 4-1). Body weight loss was less for bees that survived storage conditions than for those that died before emergence ($\chi^2=24.95$; $df=1$; $p<0.0001$; Table 4-1). Both wintering temperature ($F=35.43$; $df=2, 272$; $p<0.0001$) and duration ($F=24.52$; $df=1, 272$; $p<0.0001$) significantly contributed to overwinter weight loss (Fig. 4-2). The lowest overwinter weight loss was observed in bees wintered at -3° C for 182 days. Post-hoc comparison revealed that bees in this treatment group lost significantly less weight than bees wintered at 0° C for 230 days or for bees wintered at 4° C for either duration. Bees held at 4° C for 230 days suffered greater overwinter weight loss compared to all other treatments (Fig. 4-2). The interaction of temperature and duration was not significant ($F=2.90$; $df=2, 272$; $p=.06$), indicating that the effect of temperature on overwinter weight loss did not differ by storage duration.

Wintering temperature and duration also influenced the time it took for bees to emerge after incubation ($n=252$). Linear regression showed a significant relationship between the percent weight lost by their pre-emergence duration ($F=191.39$; $R^2=0.43$, $p<0.0001$, Fig. 4-3). Bees that lost less weight during winter took longer to emerge. Even accounting for the significant effect of weight loss ($F=13.04$; $df=1, 245$; $p=0.0004$), the

pre-emergence duration was still significantly affected by both winter temperature ($F=197.68$; $df= 2, 245$; $p<0.0001$) and duration ($F=15.01$; $df= 1, 245$; $p=0.0001$). Post-hoc comparisons indicated that pre-emergence duration for bees in the 4° C/182 day and the 4° C/230 day treatments differed from each other, as well as from all other treatments (Table 4-2; Fig. 4-4). The interaction of storage temperature and duration was also significant ($F=46.63$; $df= 2, 245$; $p<0.0001$), indicating the effect of temperature on the pre-emergence period differed with each winter duration (Fig. 4-4).

Female longevity ($n=124$) was affected by storage temperature ($F=9.45$; $df= 2, 118$; $p=0.0002$), but not its duration ($F=1.30$; $df= 1, 118$; $p=0.26$) (Fig. 4-5). Post-hoc comparison revealed that bees wintered at -3° C for 230 days lived longer than bees stored at 4° C for either duration. Similarly, after 230 days of wintering, bees from the 0° C treatment lived longer after emergence than the bees wintered at 4° C. The significant interaction of winter duration and temperature ($F=4.57$; $df= 2, 118$; $p=0.012$), indicates that effects of wintering temperature on bees' longevities differed depending on wintering duration. Prolonged winter duration, reduced (though not significantly) life expectancy for bees wintering at 4° C. In contrast, bees wintering at both -3° C and 0° C lived slightly (though not significantly) longer than bees at the same temperatures after prolonged storage.

A total of 49 and 42 bees successfully established nests after 182 and 230 days of wintering, respectively. Controlling for winter temperature, wintering duration did not significantly affect nest establishment among bees held at any of the three storage temperatures (Fig 4-6.), although bees wintered at 4° C suffered a 24% reduction in nest establishment between 182 and 230 day wintering duration ($\chi^2= 2.47$; $df=1$; $p=0.11$).

Controlling for winter duration, there was no significant difference in nest establishment among temperature treatments after 182 days of wintering ($\chi^2=0.73$; $df=2$; $p=0.69$; Fig. 4-6), nor following 230 days of winter conditions ($\chi^2=5.57$; $df=2$; $p=0.06$; Fig. 4-6). Pre-emergence period did not have a significant association with nest establishment ($\chi^2=1.97$; $df=1$; $p=0.16$).

A total of 1453 nest cells containing eggs were provisioned by 49 nesting bees in the 182 day fecundity trial. Individual fecundity ranged from 1 to 53 eggs (Table 4-3). For bees in the 182 day winter duration trial, neither their pre-nesting duration ($F=0.55$; $df=2, 46$; $p=0.58$), nor nesting duration ($F=2.0$; $df=2, 42$; $p=0.15$), were affected by winter temperature. Nesting duration was, however, related to both bees' pre-emergence period ($F=4.69$; $df=1, 42$; $p=0.036$; Fig. 4-7), and pre-nesting period ($F=8.44$; $df=1, 42$; $p=0.006$, Fig. 4-8). Fecundity was not significantly affected by winter temperature ($F=1.18$; $df=2, 42$; $p=0.32$; Fig. 4-9), however, both pre-nesting period ($F=8.71$; $df=1, 42$; $p=0.005$; Fig. 4-10), and nesting duration ($F=65.64$; $df=1, 42$; $p<.0001$; Fig. 4-11), influenced fecundity. A total of 1027 nest cells containing eggs were provisioned by 42 nesting bees in the 230 day fecundity trial. Individual fecundity ranged from 1 to 44 eggs (Table 4-3). Following 230 days of wintering, neither pre-nesting duration ($F=6.07$; $df=2, 39$; $p=0.06$), nor nesting duration ($F=1.12$; $df=2, 36$; $p=0.34$), were significantly affected by winter temperature. Pre-emergence duration also had no effect on nesting duration ($F=2.56$; $df=1, 36$; $p=0.12$). Fecundity was not significantly affected by storage temperature ($F=1.23$; $df=2, 36$; $p=0.30$; Fig. 4-9) after accounting for the strong effect of nesting duration ($F=65.64$; $df=1, 36$; $p<.0001$; Fig. 4-12).

Discussion

Winter survival rates for female *O. lignaria* were high in this study. Bees in five out of the six treatment groups had >90% survival. Only bees wintered at 4° C for the longer duration, 230 days, had higher mortality rates, approx. 25% (Fig. 4-1). Higher mortality for this treatment was not surprising considering the greater winter weight loss experienced by this group (Fig. 4-2). Winter mortality and weight loss are strongly correlated for *O. lignaria* (Bosch and Kemp 2003; Table 4-1). Those bees that lost more weight overwinter but survived did, however, emerge fastest (Fig. 4-3). As expected, bees wintered at 4° C emerged faster on average than bees stored at cooler temperatures, even after just 182 days of winter conditions (Fig. 4-4). Bees wintered at 4° C emerged more synchronously (smaller range of days) compared to colder winter temperatures (Table 4-2). Prolonged wintering at 4°C resulted in the greatest synchrony; however, shorter emergence times were accompanied by significantly shorter life expectancy (Fig. 4-5). These findings are in agreement with previous research on *O. lignaria*, and support the idea that, for tradeoffs between factors related to pollinator performance, bees held for prolonged wintering periods (>210 days) benefit from colder storage (Bosch and Kemp 2003; Sgolastra et al. 2010).

Following the period of physiological winter diapause that lasts for approx. 100 days, *O. lignaria* enter a post-diapause transitional period, during which metabolism remains suppressed by cold temperature, but gradually increases as winter progresses toward spring (Kemp et al. 2004; Sgolastra et al. 2010). Metabolic rate increases exponentially during this period and the increase is exaggerated at warmer temperatures,

meaning individuals complete dormancy faster if held at warmer temperatures (Sgolastra et al. 2010). Metabolic rates (measured as respiration rate) must reach a minimum threshold prior to emergence in spring, and respiration rates sufficient for emergence indicate the termination of winter dormancy. Bees that fail to sufficiently raise their respiration rates prior to spring incubation have significantly prolonged pre-emergence periods (Sgolastra et al. 2010). Female *O. lignaria*, however, will not emerge until exposed to temperatures of approx. 20° C (Bosch and Kemp 2001), and without the ability to feed, bees that complete dormancy but remain at cold winter temperature will rapidly deplete their energy reserves (Bosch and Kemp 2003; Sgolastra et al. 2010). The minimum pre-emergence period for female *O. lignaria* appears to be about two days when incubated at 20°C (Sgolastra et al. 2010; this study). Bees that emerge in this time frame have achieved respiration rates indicating the completion of dormancy; therefore, an emergence time of approx. 2 days, following exposure to spring temperature, can be considered an indication of that an individual has completed winter dormancy (Sgolastra et al. 2010).

Pre-emergence period for bees held at 4°C for 182 days ranged from 1.5 to 8 days with a mean of 4 days (Table 4-2), indicating that only a portion of the bees in this treatment group had completed dormancy within this time period. When wintering was extended to 230 days, pre-emergence period for bees at the warmer temperature ranged from just 1 to 3.5 days with a mean of <2 days (Table 4-2). These results suggest the majority of bees wintered at 4° C for 230 days had completed dormancy well before they were incubated. As a result, these bees suffered from excessive energy expenditure during their extended exposure to winter temperature, increasing mortality and

decreasing post-winter vigor. The shortest pre-emergence time for the two colder temperature treatments was 5 days regardless of winter duration (Table 4-2), suggesting that none of these bees had completed dormancy prior to incubation, even after 230 days of winter conditions.

Warmer winter temperatures and extended duration clearly imposes an energetic cost on *O. lignaria*, increasing weight loss, and reducing survival and longevity. It is, however, unclear exactly how such an energetic cost translates into realized fecundity for *Osmia*. Depletion of lipid reserves, as a result of warm winter temperature and/or extended duration, generally has the potential to negatively affect post-winter fecundity for diapausing insects (Ellers and van Alphen 2002; Hahn and Denlinger 2011; Irwin and Lee, 2000; Williams et al. 2003). In the case of *Osmia*, fat body lipid and protein reserves are required for ovary maturation (Wasielewski et al. 2011), and bees emerging with greatly depleted energy reserves may take longer to mature their first oocyte (Sgolastra et al. 2015). Slow ovary maturation could extend the pre-nesting period, reducing lifetime fecundity (Bosch and Vincens, 2006). Bees emerging with depleted energy reserves may also emerge in a weakened condition. If unable to recover, they may provision nest cells more slowly, nest for a shorter period of time, or even die before starting a nest (Sgolastra et al. 2015). However, feeding post-emergence has the potential to compensate for a lack of endogenous resources (Cane, 2016), and could offset some of the deleterious effects of extended, warm winter temperature.

This is the first study to directly relate winter temperature and duration to post-winter reproduction by *O. lignaria*. Bosch et al. (2000) earlier evaluated nest establishment and reproductive success of bees in almond orchards following wintering

at several storage temperatures. However, results of that study were confounded by an anomalous excess of bees, such that several release sites had >100% establishment. Some of the nesting bees were either recruited from resident populations or dispersed from other almond orchard release sites, making attribution of nesting and reproductive success to a specific treatment impossible. Based on patterns of energy depletion, I hypothesized that, among bees subjected to 4° C for 230 days of winter, fewer bees would nest, nest initiation would be delayed, nesting duration would be curtailed, and fecundity would be reduced relative to other treatment groups. Instead, in my study, winter temperature and its duration had no significant effect on any of these variables (Figs. 4-5, 4-9). These results are similar that of Sgolastra et al. (2015), which reported no significant influence of pre-wintering duration on numerous measures of reproductive success, despite the deleterious effects of extended pre-wintering on post-winter energy reserves. These findings seem to support the notion that the ability to feed prior to nesting ameliorates some of the potential negative reproductive consequences of depleted energy reserves upon emergence. It also supports the idea that while indirect measures of post-winter performance provide useful information about an individual's condition following winter dormancy, extrapolation to nesting and reproductive success may overestimate differences among treatments.

About 25% fewer bees initiated nests after wintering at 4° C for 230 days as compared to bees wintered at the same temperature but incubated after only 182 days of winter (Fig. 4-5). In contrast, nest initiation was similar among bees held at the two colder winter temperatures regardless of winter duration. While this shortfall in nesting by bees wintered at 4° C for 230 days by itself was not significant ($p=0.11$), the

compounded effect of lower winter survival (~75% survived, Fig. 4-1) and nest establishment (~50% nested, Fig. 4-5) on reproduction is likely profound. Furthermore, although average fecundity was similar (within wintering duration) for bees from all six treatment groups, fewer females initiated nests in the 4° C/230 day treatment group as compared to bees wintered at 0° C (10 vs. 17 bees) (Fig. 4-5). The collective fecundity for the warmer temperature group was, therefore, only approx. 60% that of the bees in the 0° C/230 day treatment group (Table 4-3). Lesser reproductive returns have implications regarding the sustainability of managed *O. lignaria* for pollinating later blooming crops. Most *O. lignaria* used for crop pollination are currently trapped from the wild, a situation that is not sustainable in the long term if bees are treated as a disposable resource. Good reproductive returns of bees foraging on crop plants can reduce reliance on wild caught bees for crop pollination, but it will require effective winter thermal management.

Integrating the findings of this study suggest that colder winter storage represents the best management practice for *O. lignaria* if wintering duration is to exceed approx. 210 days. The major obstacle towards the adoption of this management strategy is the protracted pre-emergence period of bees wintered at cooler temperature, as it complicates synchronization with crop bloom. This period will have to be shortened in order to effectively use this bee for later blooming crops. As a linear increase in temperature elicits an exponential increase in metabolic rates for bees in the post-diapause transitional period (Sgolastra et al. 2010), the length of pre-emergence period could potentially be shortened by gradual warming of bees (though still refrigerated) in the weeks prior to full incubation. In so doing, bees may be able to achieve metabolic rates sufficient for quick emergence upon incubation, without sacrificing most of the gains in post-winter energy

reserves achieved by wintering at lower temperature.

The main focus of research on *O. lignaria* management concerns advancing emergence so bees can be used to pollinate an early-blooming crop, almonds, in February (Bosch and Kemp 2000, 2003; Bosch et al. 2000; Kemp and Bosch 2005; Pitts-Singer et al. 2014). This should come as no surprise considering almond's high monetary value, the dramatic increase in acreage in recent years, and a sharp increase in the already expensive rental of honey bee colonies for this bee-dependent crop. Delaying *Osmia* emergence for pollination of later blooming crops has received little attention, but could provide opportunities to expand markets for this alternative pollinator. One possible market is red raspberry (*Rubus idaeus*). This rosaceous fruit crop blooms a month or more after the natural activity period of *O. lignaria*, and like almond, has seen a large increase in acreage as well as the cost of honey bee colony rentals over the past two decades (Burgett 1996; NASS 2016). Furthermore, demand for fresh berries year-round has led to increased adoption of high tunnel and greenhouse production, extending the growing season of raspberry both earlier and later in the season (Pritts 2008). Although the fidelity of *O. lignaria* to this crop in open field settings has yet to be demonstrated, recent research has proven *O. lignaria* to be an efficacious pollinator of raspberry in confinement (Chapter 2).

Protected cultivation presents another potential market for long wintered bees. Honey bees forage poorly under glass or plastic as well as in the cooler weather of early spring, late fall, and winter (Guerra-Sanz 2008; Morandin et al. 2002). Currently, bumble bees are the sole pollinators of greenhouse crops in North America. Bumble bee colonies, however, are expensive to purchase and cannot be sustained from year to year (i.e., they

are managed as disposable pollinators) (Velthuis and Dorn, 2006). Mason bees, such as *O. lignaria*, nest and forage efficiently in greenhouses (Sgolastra et al., 2015; Tepedino and Torchio, 1982, 1989; this study) and high tunnels (Pinzauti et al. 1997), and have long been noted for their ability to forage during inclement weather, at temperatures well below the activity threshold of honey bees (Torchio, 1981). Because *O. lignaria* reproduce well in a greenhouse environment, their surplus progeny would potentially be available to pollinate additional acreage or greenhouses the following year. They therefore represent a promising sustainable alternative to disposable bumble bees for greenhouse pollination of certain crops.

Studies such as this one are a necessary step towards developing management strategies for the use of this versatile pollinator with a greater variety of crops. Evaluations of post-winter performance based solely on measures of energy reserves upon emergence tend to overestimate the deleterious effects of winter conditions on pollinator performance. Directly measured nesting and reproductive success in response to winter temperature and duration provides us with a more complete and nuanced understanding of how these factors combine to influence the effectiveness and sustainability of *O. lignaria* as crop pollinators. Based on the results of this study, adoption of cooler winter temperatures is recommended if bees must be wintered for extended periods in order to synchronize their activity with crop bloom. Further research on methods designed to shorten pre-emergence time for bees held at lower winter temperatures is required, but colder temperature winter storage appears to provide a simple, effective, and sustainable means of delaying emergence of *O. lignaria* for later blooming crops.

Tables and Figures

Table 4-1. Weight loss during the overwintering period for bees that either survived, or died prior to emergence. All combinations of wintering temperature and duration are combined. Weight loss is expressed as a percentage of the bee's pre-winter weight.

Survived	<i>n</i>	Mean (%)	Median (%)	Range (%)
Yes	252	6.7	6.3	2.7 – 14.5
No	26	17.6	12.6	0.22 – 50.3

Table 4-2. Pre-emergence periods for bees exposed to six combinations of winter temperature and duration. The pre-emergence period is from the start of incubation at 20° C to emergence. Durations are expressed in days.

Duration	Temperature	<i>n</i>	Mean	Median	Range
182 Days	4° C	44	4.0	3.5	1.5 - 8
	0° C	43	9.0	8.0	5 - 17.5
	-3° C	43	9.3	8.5	5.5 -28.5
230 Days	4° C	35	1.8	1.5	1 - 3.5
	0° C	42	7.8	7.0	5.5 - 17.5
	-3° C	45	10.2	8.5	5.5 - 18

Table 4-3. Fecundity of nesting bees after exposure to six combinations of winter temperature and duration. Fecundity was recorded as the number of eggs laid by each bee.

Duration	Temperature	Bees Nesting	Offspring	Mean	Median	Range
182 Days	4° C	15	436	29.1	33	7 - 53
	0° C	17	551	32.4	36	1 - 50
	-3° C	17	466	27.4	25	4 - 53
230 Days	4° C	10	270	27.0	25	16 - 43
	0° C	17	452	26.6	25	10 - 44
	-3° C	15	305	20.3	20	1 - 41

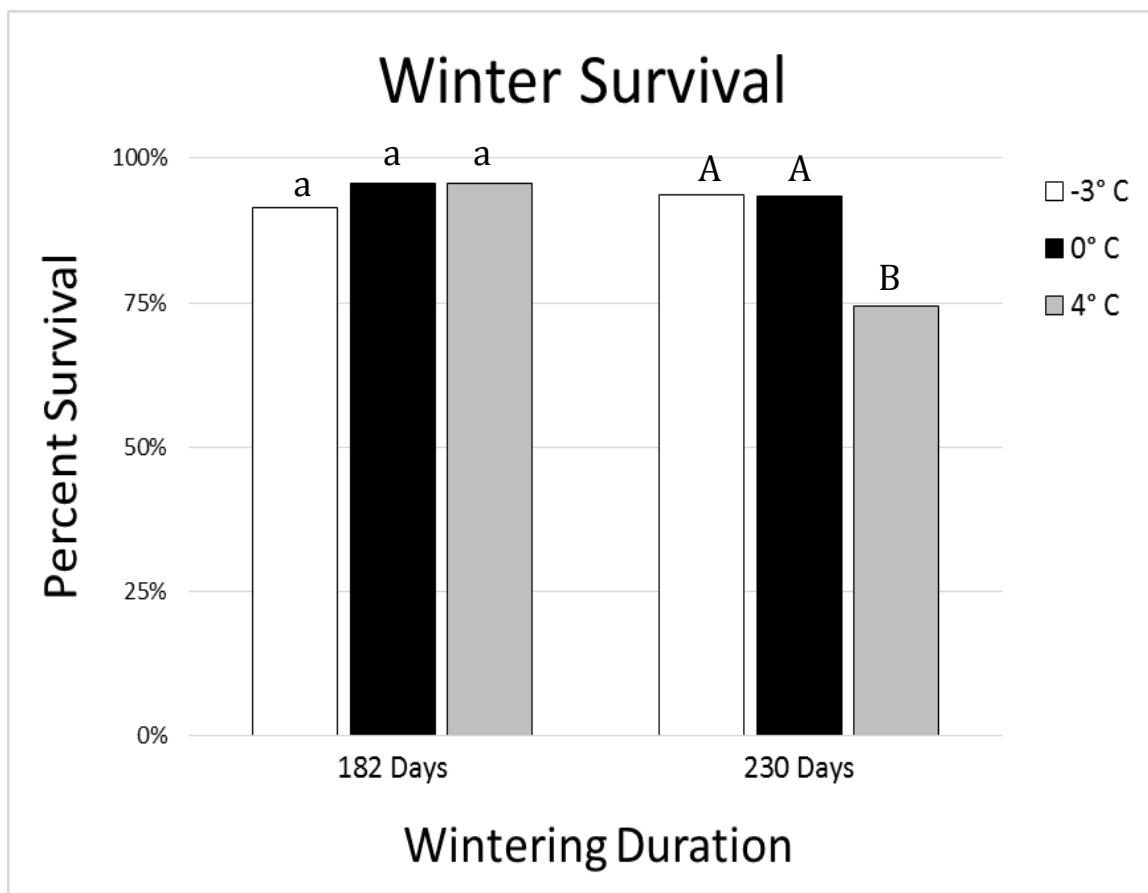


Figure 4-1. The percentage of *O. lignaria* that survived, and successfully emerged from their cocoon following exposure to six combinations of winter temperature and duration. Different letters denote means that differ at $p \leq 0.05$.

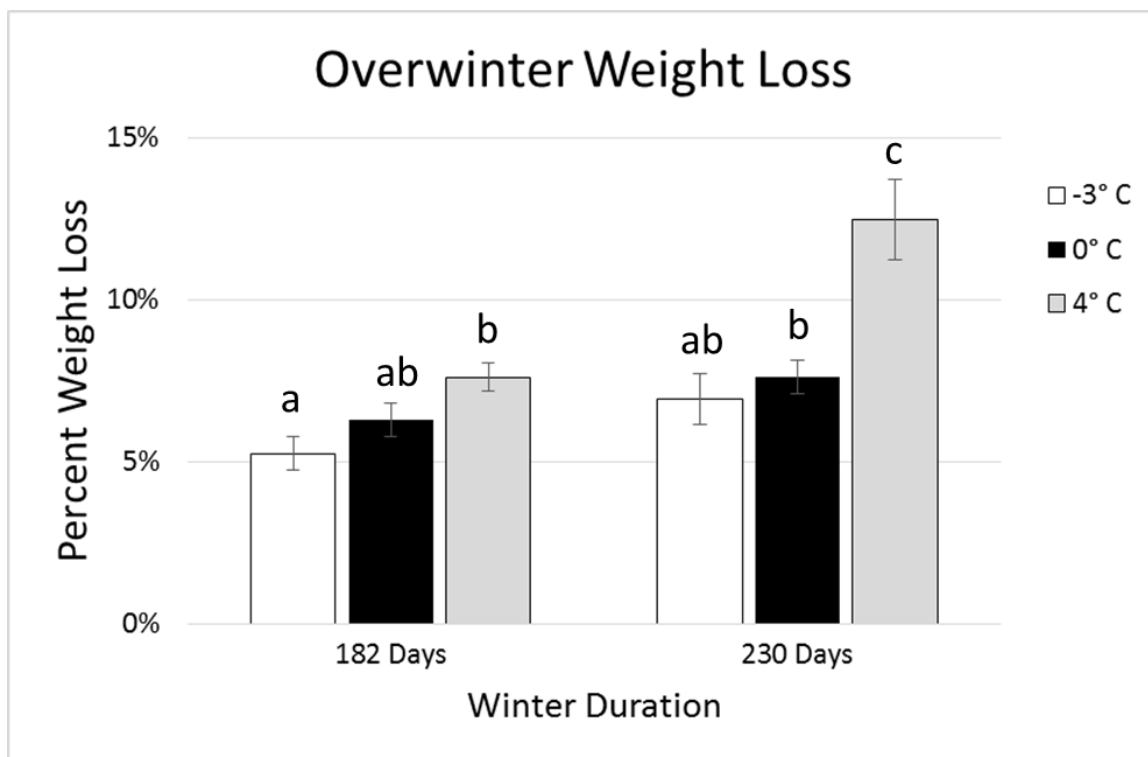


Figure 4-2. The percentage of body weight lost during wintering for bees exposed to six combinations of winter temperature and duration. Different letters denote means that are significantly different at $p \leq 0.05$. Error bars are \pm SE.

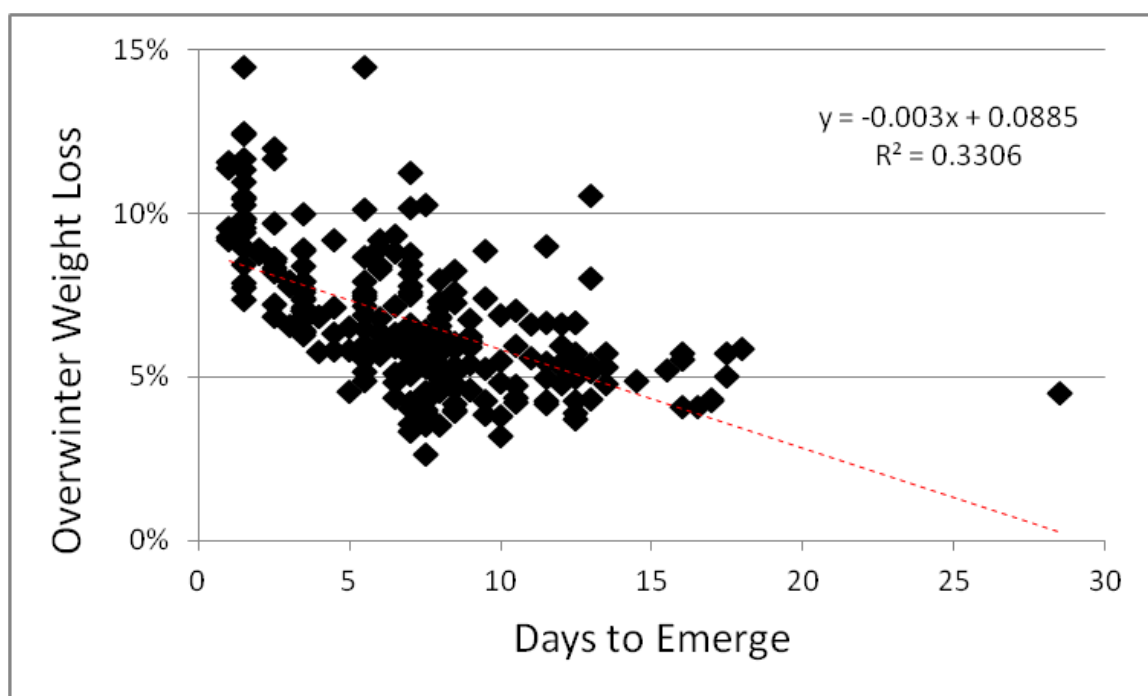


Figure 4-3. Linear regression of a bee's pre-emergence duration on the percentage of its body weight lost during winter. The data depicted is not transformed.

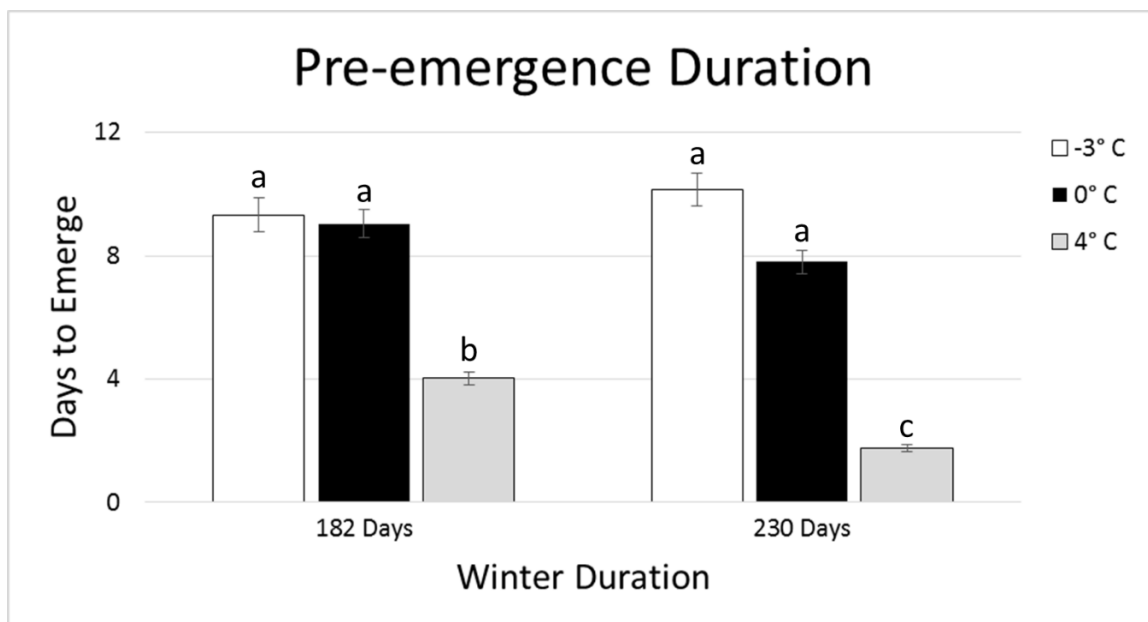


Figure 4-4. The number of days for *O. lignaria* to emerge from its cocoon following incubation at 20° C for bees exposed to six combinations of winter temperature and duration. Different letters denote means that are significantly different at $p \leq 0.05$. Error bars are +/- SE.

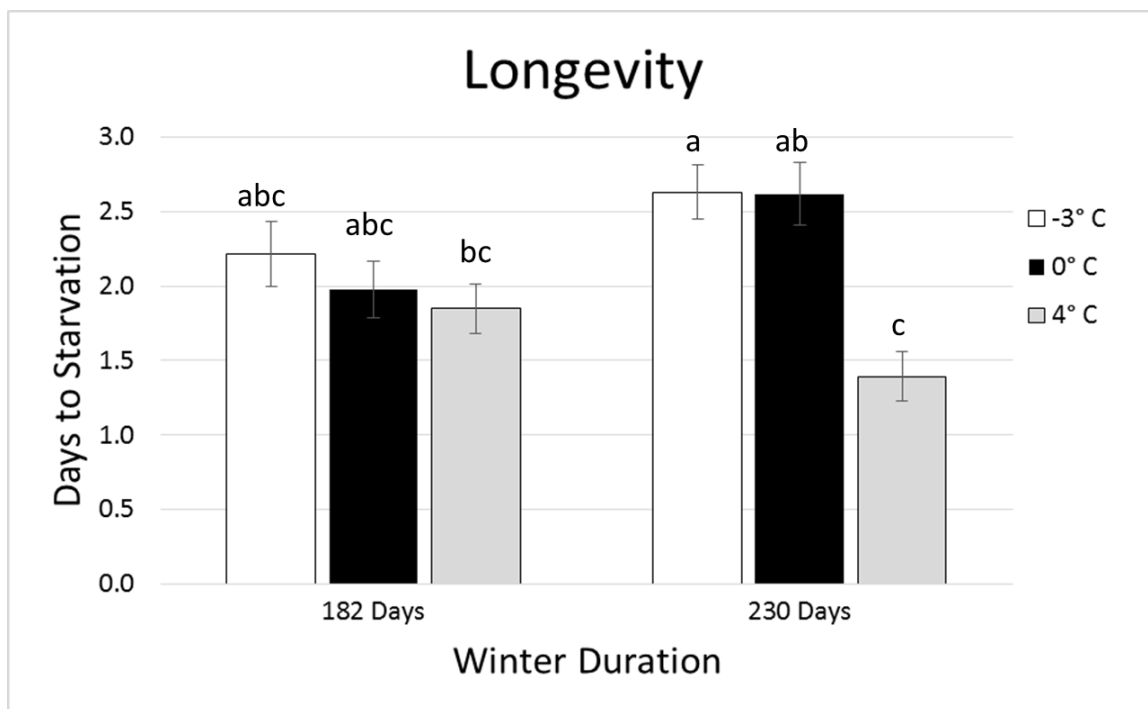


Figure 4-5. Longevity to starvation for female *O. lignaria* exposed to six combinations of winter temperature and duration. Different letters denote means that are significantly different at $p \leq 0.05$. Error bars are \pm SE.

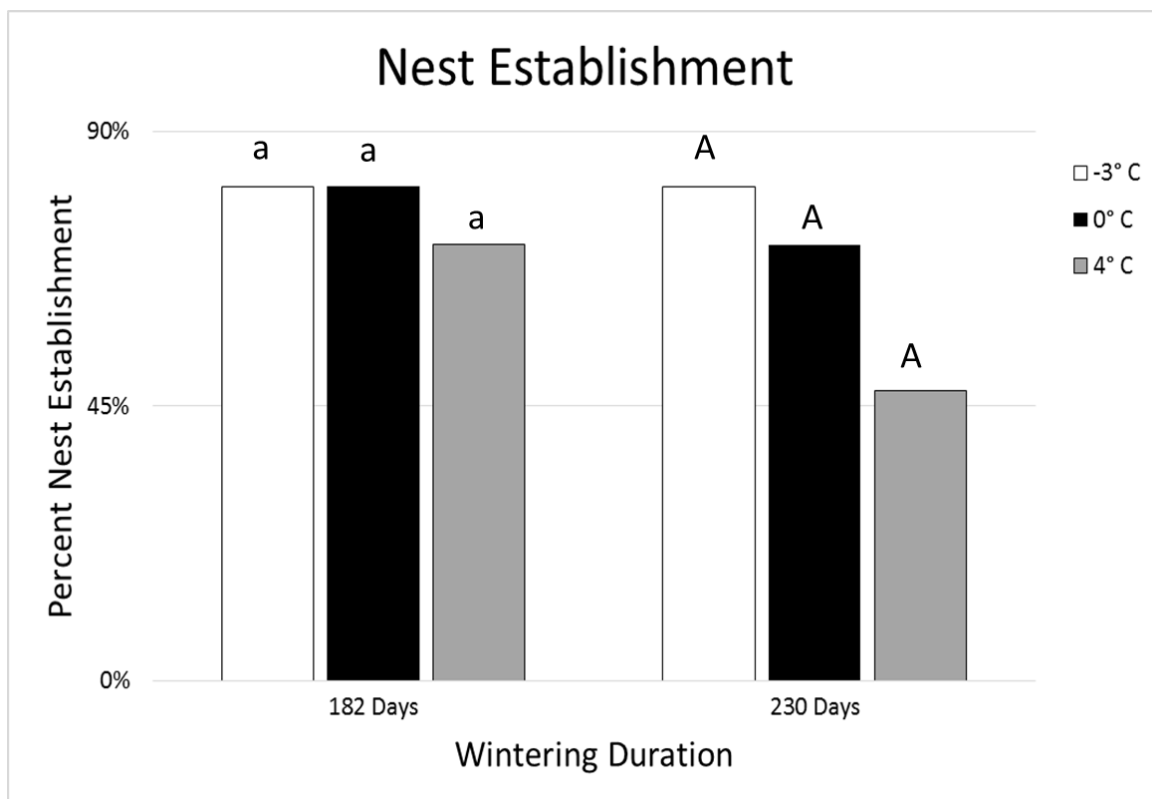


Figure 4-6. The percentage of *O. lignaria* females that successfully established and completed at least one nest following exposure to six combinations of winter temperature and duration. Different letters denote means that are significantly different at $p \leq 0.05$.

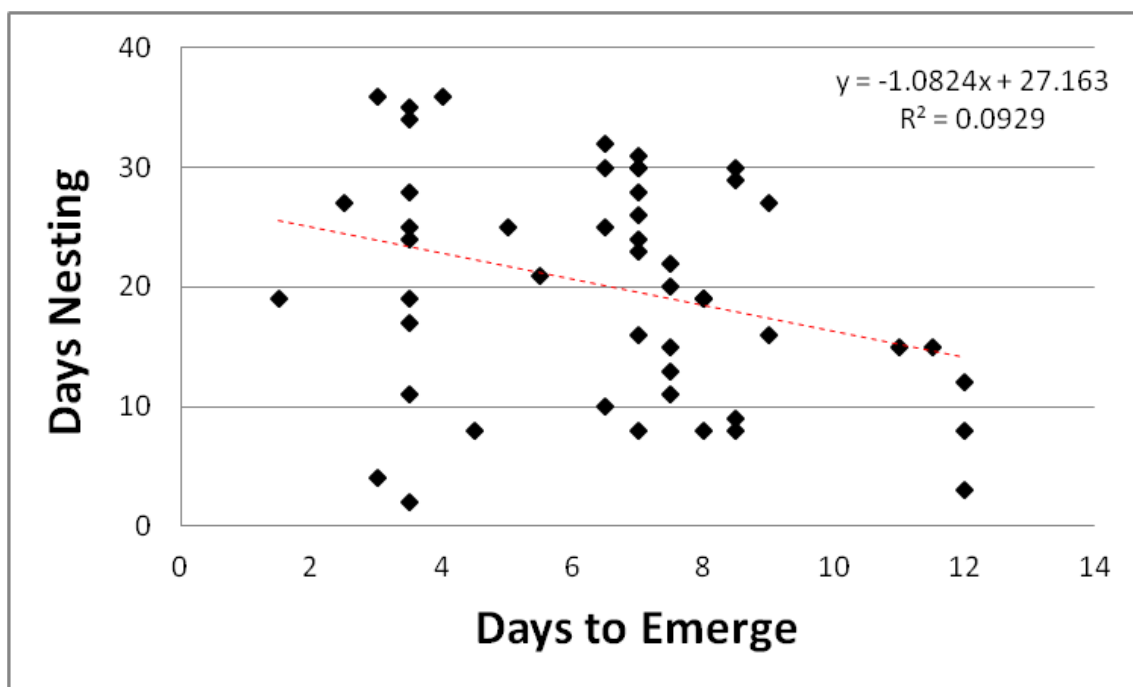


Figure 4-7. Relationship of pre-emergence duration and nesting duration for bees after 182 days of winter temperature. All temperatures are combined.

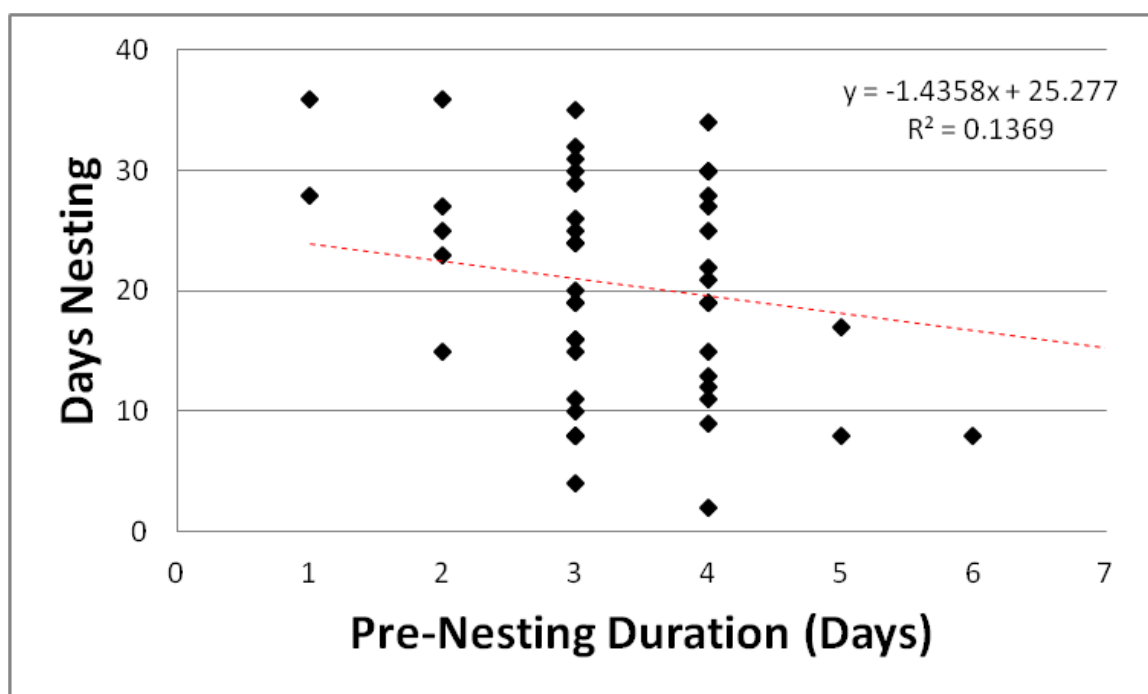


Figure 4-8. Relationship of pre-nesting duration and nesting duration for nesting bees following 182 days of winter temperature. All temperatures are combined.

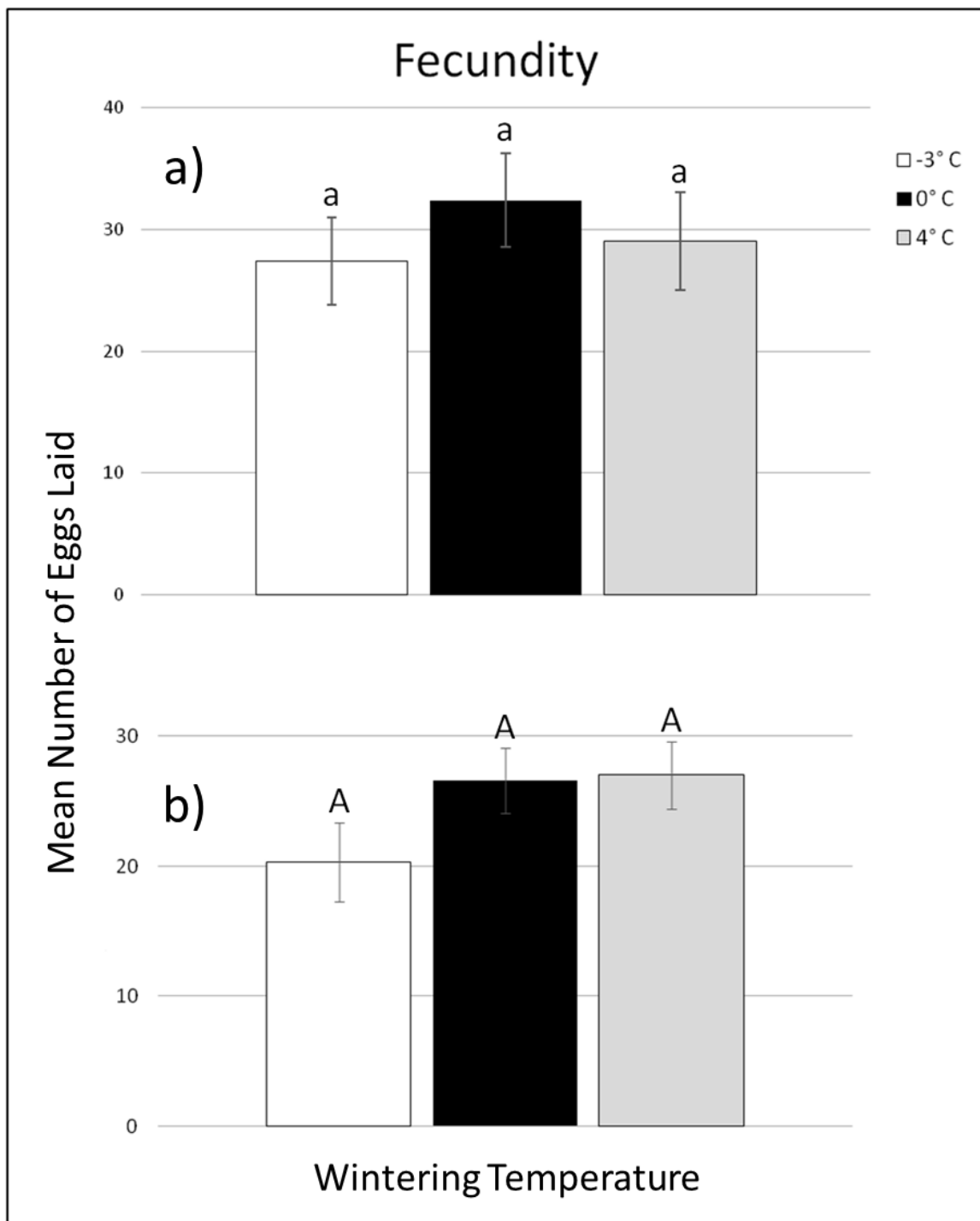


Figure 4-9. Mean fecundity for bees from the three temperature treatment following **a)** 182 days and **b)** 230 days of winter temperature. Error bars are +/- SE. Different letters denote means that are significantly different at $p \leq 0.05$.

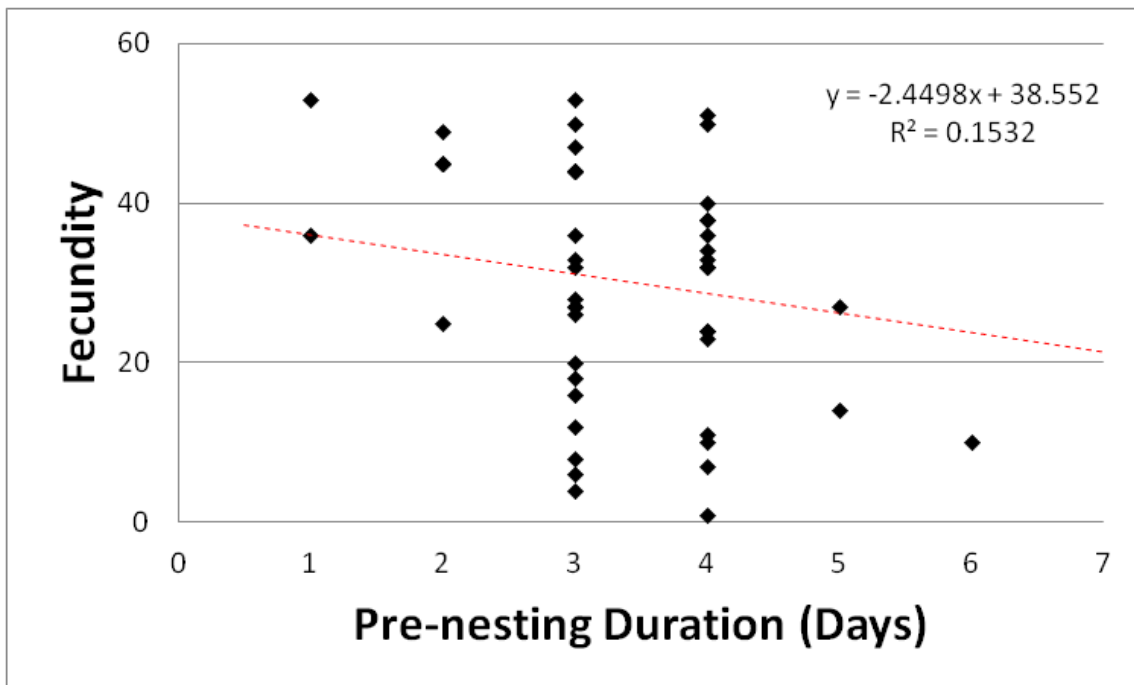


Figure 4-10. Relationship of pre-nesting duration and fecundity for nesting bees after 182 days of winter temperature. All temperatures are combined.

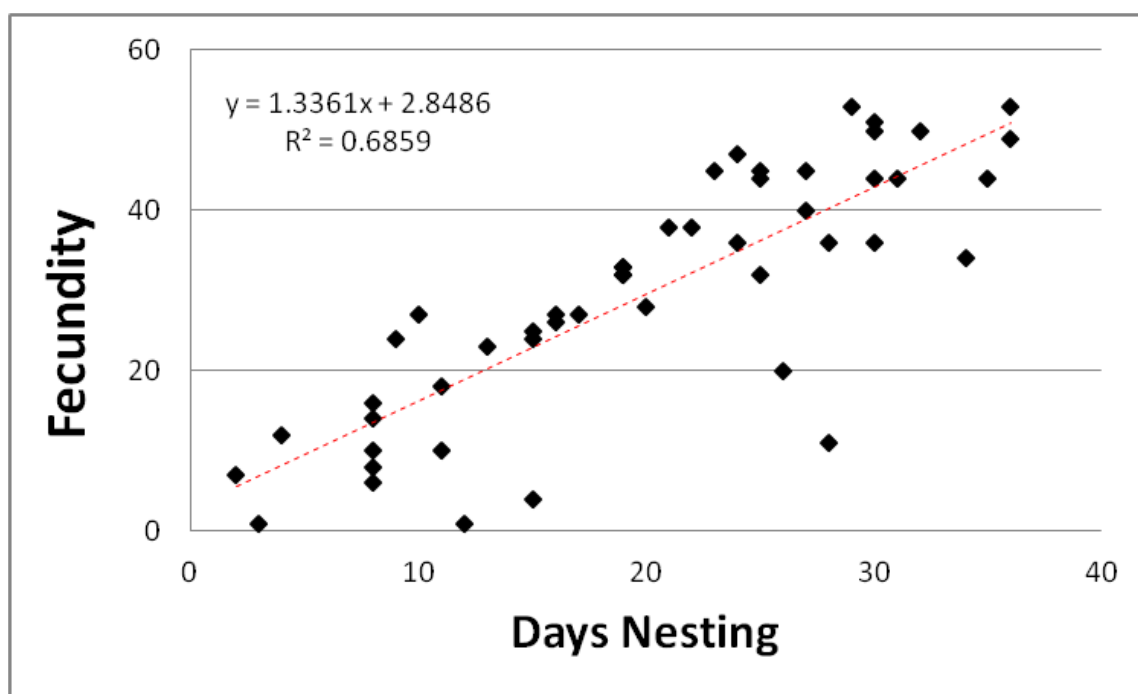


Figure 4-11. Relationship of nesting duration and fecundity for nesting bees following 182 days of winter temperature. All temperatures are combined.

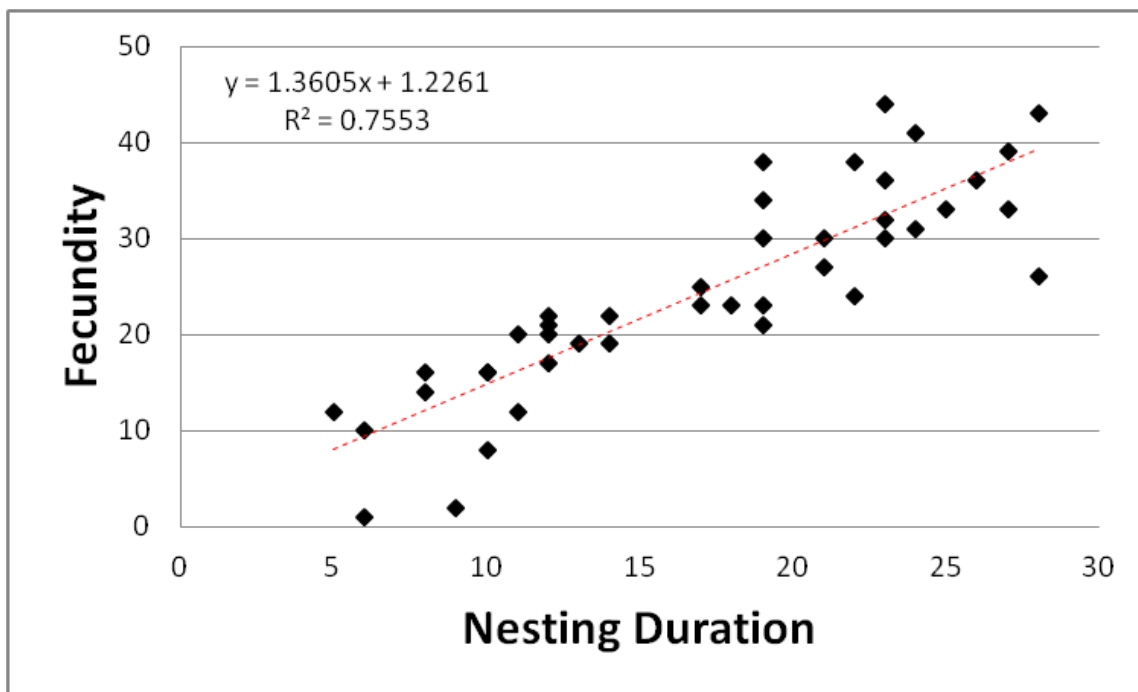


Figure 4-12. Relationship of nesting duration and fecundity for nesting bees following 230 days of winter temperature. All temperatures are combined.

References

- Bosch, J., and W. P. Kemp. 1999.** Exceptional cherry production in an orchard pollinated with blue orchard bees. *Bee World*. 80: 163–173.
- Bosch, J., and W. P. Kemp. 2000.** Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 29: 8–13.
- Bosch, J., and W. P. Kemp. 2001.** How to manage the blue orchard bee. Sustainable Agriculture Network, Washington D.C.
- Bosch, J., and W. P. Kemp. 2003.** Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environ. Entomol.* 32: 711–716.
- Bosch, J., W. P. Kemp, and G. E. Trostle. 2006.** Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 99: 408–413.
- Bosch, J., W. P. Kemp, and S. Peterson. 2000.** Management of *Osmia lignaria* (Hymenoptera: Megachilidae) populations for almond pollination: methods to advance bee emergence. *Environ. Entomol.* 29: 874–883.
- Bosch, J., F. Sgolastra, and W. P. Kemp. 2010.** Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *J. Insect Physiol.* 56: 1950–1958.
- Bosch, J., and N. Vicens. 2006.** Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behav. Ecol. Sociobiol.* 60: 26–33.
- Burgett, M. 1997.** 1996 Pacific Northwest honey bee pollination survey. Oregon State Univ., Corvallis, OR.
- Cane, J. H. 2016.** Adult pollen diet essential for egg maturation by a solitary *Osmia* bee. *J. Insect Physiol.* 95: 105–109.
- Ellers, J., and J. J. M. van Alphen. 2002.** A trade-off between diapause duration and fitness in female parasitoids. *Ecol. Entomol.* 27: 279–284.

- Guerra-Sanz, J.M. 2008.** Crop pollination in greenhouses. Pp. 27-47 In R. R. James and T. L. Pitts-Singer, Bee pollination in agricultural ecosystems, Oxford University Press, New York, NY.
- Hahn, D. A., and D. L. Denlinger. 2011.** Energetics of insect diapause. *Annu. Rev. Entomol.* 56: 103–121.
- Kemp, W. P., and J. Bosch. 2005.** Effect of temperature on *Osmia lignaria* (Hymenoptera: Megachilidae) prepupa – adult development, survival, and emergence. *J. Econ. Entomol.* 98: 1917–1923.
- Kemp, W. P., J. Bosch, and B. Dennis. 2004.** Oxygen consumption during the life cycles of the prepupa-wintering bee *Megachile rotundata* and the adult-wintering bee *Osmia lignaria* (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 97: 161–170.
- Levin, M. D. 1966.** Biological notes on *Osmia lignaria* and *Osmia californica* (Hymenoptera : Apoidea). *J. Kansas Entomol. Soc.* 39: 524–535.
- Phillips, J. K., and E. C. Klostermeyer. 1978.** Nesting behavior of *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 51: 91–108.
- Pinzauti, M., D. Lazzarini, and A. Felicioli. 1997.** Preliminary investigation of *Osmia cornuta* Latr. (Hymenoptera: Megachilidae) as a potential pollinator for blackberry (*Rubus fruticosus* L.) under confined environment. *Acta Hort.* 437: 329–333.
- Pitts-Singer, T. L., J. H. Cane, and G. Trostle. 2014.** Progeny of *Osmia lignaria* from distinct regions differ in developmental phenology and survival under a common thermal regime. *J. Insect Physiol.* 67: 9–19.
- Pritts, M. P. 2008.** Primocane-fruiting raspberry production. *HortScience.* 43: 1640–1641.
- Rust, R. 1995.** Adult overwinter mortality in *Osmia lignaria propinqua* (Hymenoptera: Megachilidae). *Pan Pacific Entomol.*
- Sgolastra, F., J. Bosch, R. Molowny-horas, S. Maini, and W. P. Kemp. 2010.** Effect of temperature regime on diapause intensity in an adult-wintering Hymenopteran with obligate diapause. *J. Insect Physiol.* 56: 185–194.
- Sgolastra, F., W. P. Kemp, J. S. Buckner, T. L. Pitts-singer, S. Maini, and J. Bosch. 2011.** The long summer: pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *J. Insect Physiol.* 57: 1651–1659.

- Sgolastra, F., X. Arnan, T. L. Pitts-Singer, S. Maini, W. P. Kemp, and J. Bosch. 2016.** Pre-wintering conditions and post-winter performance in a solitary bee: does diapause impose an energetic cost on reproductive success? *Ecol. Entomol.* 41: 201–210.
- Sheffield, C. S. 2014.** Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Anapolis Valley, Nova Scotia, Canada. *J. Pollinat. Ecol.* 12: 120–128.
- Sheffield, C. S., S. M. Westby, P. G. Kevan, and R. F. Smith. 2008.** Winter management options for the orchard pollinator *Osmia lignaria* say (Hymenoptera: Megachilidae) in Nova Scotia. *J. Entomol. Soc. Ontario.* 139: 3–18.
- Tepedino, V. J., and P. F. Torchio. 1982.** Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). *Ecol. Entomol.* 7: 453–462.
- Torchio, P. F. 1976.** Use of *Osmia lignaria* Say (Hymenoptera: Apoidea, Megachilidae) as a pollinator in an apple and prune orchard. *J. Kansas Entomol. Soc.* 49: 475–482.
- Torchio, P. F. 1981.** Field experiments with *Osmia lignaria propinqua* Cresson as a pollinator in almond orchards: I, 1975 studies (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 54: 815–823.
- Torchio, P. F. 1985.** Field Experiments with the pollinator species, *Osmia lignaria propinqua* Cresson, in apple orchards: V (1979-1980), methods of introducing bees, nesting success, seed counts, and fruit yields (Hymenoptera: Megachilidae). *J. Kansas Entomol. Soc.* 53: 448–464.
- Torchio, P. F. 1989.** In-nest biologies and development of immature stages of three *Osmia* species (Hymenoptera: Megachilidae). *Ann. Entomol. Soc. Am.* 82: 599–615.
- Velthuis, H. H. W., and A. Van Doorn. 2006.** A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie.* 37: 421–451.
- U.S. Department of Agriculture – National Agricultural Statistics Service. July 2016.** Noncitrus fruits and nuts 2015 summary. U.S. Dept. of Agr., Washington, D.C.
- Wasielewski, O., K. Giejdasz, T. Wojciechowicz, and M. Skrzypski. 2011.** Ovary growth and protein levels in ovary and fat body during adult-wintering period in the red mason bee, *Osmia rufa*. *Apidologie.* 42: 749–758.

Williams, J. B., J. D. Shorthouse, and R. E. J. Lee. 2003. Deleterious effects of mild simulated overwintering temperature on survival and potential fecundity of rose-galling *Diplolepis* wasps (Hymenoptera: Cynipidae). *J. Exp. Zool.* 298: 23–31.

Wilson, R. L., and C. A. Abel. 1996. Storage conditions for maintaining *Osmia cornifrons* (Hymenoptera: Megachilidae) for use in germplasm. *J. Kansas Entomol. Soc.* 69: 270–272.

CHAPTER V

GENERAL SUMMARY AND CONCLUSION

Summary

Cultivated raspberry is largely self-fertile, yet requires bee pollination to ensure high-quality berries and maximize fruit yield. Expanding markets raspberry have driven a rapid increase in U.S raspberry acreage in recent decades. During this same time span beleaguered commercial honey bee populations have dwindled, resulting in a substantial rise in the cost of honey bee colony rentals. Additionally, high-tunnel production systems, which are unfavorable for honey bee pollination, have been widely adopted to satisfy year-round demand for fresh berries. These factors make it desirable to find and alternative manageable bee species for raspberry.

The most promising of alternative pollinators for raspberry are mason bees (*Osmia* spp.) and bumble bees (*Bombus* spp.). Bumble bees are known to be excellent wild pollinators of raspberry, while several species of mason bees have been developed for pollination of other rosaceous fruit crops. These alternative bee species have an advantage over honey bees in that they forage well when confined and in inclement weather. Both bumble bee and mason are currently more expensive to purchase on a per forager basis. For their adoption to be justified, these bees must prove more efficacious than honey bees when pollinating raspberry. Knowledge of the comparative single-visit pollination efficacy of bees on raspberry is necessary to make this determination. Single-visit pollination efficacy can also be used to estimate the minimum number of visits

required to maximize drupelet set for raspberry. This information can be used to refine estimates of the required bee stocking density for raspberry pollination.

One potential alternative pollinator for raspberry is the blue orchard bee, *O. lignaria*. This North American species has been developed for pollination of several rosaceous tree fruit crops. Many aspects of their management have been worked out. One hindrance to the versatility of these bees is brief period of annual adult activity when foraging bees are able to provide pollination services. This activity period coincides with some spring fruit tree crops, but precedes raspberry bloom by a month or more in most regions. To synchronize *O. lignaria*'s activity with raspberry bloom and be a viable pollinator for this crop, emergence timing must be controlled without compromising bees' longevity, vigor, or survival.

In my first study, I compared the single-visit pollination efficacy of five bee species foraging on raspberry. Overall all of the bee species studied here proved excellent pollinators of raspberry. I found that none of the four alternative manageable bee species greatly outperformed honey bees as pollinators of raspberry. The pollinator effectiveness index I developed suggested as few as two visits may be enough to maximize drupelet set for raspberry. Honey bees remain the least expensive option on a per forager basis.

In my second study, I investigated the number of visits by a honey bee to a raspberry flower required to maximize drupelet set. I found that two prolonged visits, the first visit of the day on two consecutive days, is sufficient to maximize drupelet set for red raspberry. The number of drupelet resulting from these two visits was comparable to openly-pollinated flower for all three raspberry cultivars tested. For two of the raspberry cultivars studied here even a single visit yielded drupelet count indistinguishable from the

openly-pollinated flowers.

In my third study, I examined the effects of six combinations of winter storage temperature and duration on the post-winter performance of female *O. lignaria*. I found that bees wintered at the industry standard 4° C for 230 days suffered from high winter mortality and weight loss, as well as reduced life spans compared to other treatments. Nesting and reproductive success of bees in this treatment was not significantly lower than other treatments; however, nest establishment decreased by approx. 25% from 182 days to 230 days for bees wintered at 4° C. Bees wintered at -3° and 0° C had extended pre-emergence periods even after 230 days of winter conditions.

Conclusion

My research focused on the development of alternative manageable bee species for raspberry pollination. Comparing pollination efficacies among bees is a first step in this process, as new pollinator species must prove an affordable alternative to honey bees for their adoption to be justified. My work demonstrated that honey bees remain the most economic and practical pollinator for open-field raspberry production. By determining the single-visit efficacy for honey bees on raspberry I was able to arrive at an estimate for the minimum number of visits required to maximize drupelet set. The estimate of two visits was far below previous reports. An accurate estimate of the numbers of bee visits required to maximize fruit yield is integral for estimating appropriate bee stocking densities for bee-dependent crops. The other variables needed for that calculation include bees' pollination efficacies, foraging tempos, their densities per unit area, floral density, and yield per acre. This information will help to refine honey bee stocking density

estimates for raspberry which are currently based on each flower requiring dozens of bee visits to maximize fruit set.

Mason bees and bumble bees proved to be excellent pollinators of raspberry and may find utility in high-tunnel or greenhouse production systems where honey bees don't to forage effectively. The economics in these production systems may favor the adoption of alternative manageable pollinators. My work investigating lower temperature winter storage of *O. lignaria* demonstrated an effective strategy for delaying the emergence of this early spring species, so that it can be used for pollination of a later-blooming crop like raspberry. These bees were able to forage and nest effectively on raspberry in the confines of a greenhouse suggesting they could be a potential alternative to bumble bees for greenhouse raspberry production. Emergence in this study was delayed by approx. five weeks. More investigation is needed to determine the maximum storage duration for *O. lignaria* wintered at near freezing temperatures.

My work garnered new insights into potential alternative manageable bees for raspberry pollination. Comparing drupelet set resulting from a single bee visit to a raspberry flower demonstrated that studies based on stigmatic pollen deposition tend to overestimate differences in pollinator efficacies. While honey bees remain the most cost effective option for now, shifting economics do not preclude the adoption of an alternative pollinator for raspberry in the future. Furthermore, methods for delaying the emergence of *O. lignaria* described here could be applied to other crop systems, greatly expanding the market for these bees.