MORTALITY DYNAMICS AND LIFE TABLES OF *MEGACHILE ROTUNDATA*

by

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ABSTRACT

The alfalfa leafcutting bee, *Megachile rotundata* (F.), contributes to the pollination of more than two-thirds of alfalfa seed production in North America. However, population losses of more than 50% are common in the U.S., requiring many alfalfa seed producers to import costly bees from Canada. Understanding the mortality dynamics of *M. rotundata* and being able to estimate these impacts on their populations are critical for identifying ways to conserve and increase their populations. Therefore, this study had three objectives: 1) identify mortality classes for *M. rotundata* in brood cells; 2) experimentally manipulate parasitism and temperature to determine their impact on total mortality; and 3) estimate mortality risks using the multiple-decrement life table (M-DEC). Research was conducted over two years on a 38.5 ha alfalfa field in Toston, MT. Nest shelters were manipulated for a main temperature treatment (low vs. high) and a sub-treatment for parasitism (backing-present vs. backing-absent). Females constructed and provisioned nests during the summer and offspring mortality was assessed during the summer and the following fall. Mortality classes were then analyzed using the M-DEC model. We found no temperature-treatment effect, so our main treatment was not used in the analyses. However, for every 1 °C increase in nest-tunnel temperature, there was a 7% increase in total mortality. Nest boxes without felt backing (backing-absent) had a 43% increase in mortality over both years compared to those with felt (backing-present). Average temperature decreased by 4.4 °C from 2017 to 2018, while average relative humidity increased by 12.1%. Total mortality was approximately 15% for both years, but the proportion of each mortality class differed substantially, with death by parasitoids greatest in 2017 and death by pollen ball greatest in 2018. Mortality from each class was highly irreplaceable in that it is unlikely to be replaced by another class, and death by predation was the only cause with similar mortality between the two years. The ability to identify and quantify mortality classes and their respective irreplaceable mortality, especially for parasitoids, pollen ball, and predators, will help producers maintain and increase bee populations.

CHAPTER ONE

LITERATURE REVIEW

Introduction

The alfalfa leafcutting bee, *Megachile rotundata* (F.), was introduced accidentally to the United States between the 1930s (Bosch and Kemp 2005) and 1940s (Pitts-Singer and Cane 2011). A decade later, entomologists recognized its ability to pollinate alfalfa, *Medicago sativa* L., and developed strategies for management of *M. rotundata* (Bosh and Kemp 2005). Alfalfa seed yields increased dramatically from 450 kg/ha with honey bee pollination to 1,300 kg/ha with alfalfa leafcutting bee pollination (Bohart 1972). Today, more than two-thirds of the alfalfa seed production in North America is attributed to pollination by alfalfa leafcutting bees in Canada and the northwestern U.S. (Bosch and Kemp 2005), making it the most intensively-managed and economically-important solitary bee species (Pitts-Singer and Cane 2011).

However, maintaining populations of alfalfa leafcutting bees has become more difficult since the 1970s. Population losses (the difference between the parental population released and the live progeny recovered) of more than 50% are common in the U.S., requiring many alfalfa seed producers to import costly bees from Canada (Pitts-Singer and Cane 2011).

Understanding the life cycle of *M. rotundata* and the reasons they die during development are critical to identify ways to conserve and increase their populations. Fortunately, there have been many studies performed using *M. rotundata*, as they are relatively easy to rear in both laboratory and field settings. This makes the species a good

subject for studying demography, mortality factors, population dynamics, and conservation. Here, we summarize those findings and highlight areas in which sufficient information is lacking.

Life Cycle

Adult males and females are reared to emerge from nest cells in June and July, in accordance with *M. sativa* bloom, and mate immediately after emergence. Females spend the next 4 to 6 weeks constructing nests that consist of a linear series of brood cells made of cut leaf pieces. These nests are constructed within drilled cavities in wood or polystyrene boards, with the cavities typically 95 to 150 mm long and 5 to 7 mm in diameter. Females are able to cut leaf pieces using the opposing beveled edges of their mandibles. They chew the edges of the leaf pieces and excrete a glue-like saliva to construct their nest cells. Each brood cell contains one egg that is provisioned with a mass of nectar and pollen provided by the mother. Once provisioning is completed, the cells are then sealed and plugged with more leaf pieces. Females spend 5 to 6 hrs per day foraging and provisioning their nests. These provisions usually consist of about 35% pollen and 65% nectar, with a total wet weight of about 90 mg. With one egg per cell, females generate 12 to 16 cells in their lifetimes (Pitts-Singer and Cane 2011). Development occurs rapidly, with embryogenesis taking 2 to 3 days. The developing larvae complete their fifth larval stage before entering diapause as pre-pupae by the middle to late summer (Yocum et al. 2004).

Alfalfa leafcutting bees pollinate flowers by tripping the flowers' staminal columns when collecting pollen, which then strikes the bee on its head, an experience

honey bees do not tolerate. Instead, honey bees avoid this by robbing the nectar, leaving most of the flowers unpollinated. Female alfalfa leafcutting bees are very successful at pollinating alfalfa because they trip about 80% of the flowers they visit. In 2004, pollination by *M. rotundata* yielded 46,000 metric tons of alfalfa seed in North America, contributing two-thirds of global production that year (Pitts-Singer 2008). These bees tend to move pollen a distance of only about 4 m, but have been observed flying more than 100 m away from their nests. In addition to alfalfa, these bees are successful pollinators of canola, clover, other legumes, blueberries, and cranberries (Pitts-Singer and Cane 2011).

Sex ratios in the nest are male-biased with female offspring usually laid in cells at the back of nest tunnel and males nearest the opening, as this species exhibits protandry with males emerging first the following year. Alfalfa leafcutting bees are sexually dimorphic; females are 120-130% the size of males and have pointed abdomens and black eyes, whereas males are smaller, have rounded abdomens, and green eyes (Pitts-Singer and Cane 2011). Size of offspring is directly dependent on the size of the provision provided by the mothers, as well as whether or not the egg is fertilized. As with most Hymenoptera, alfalfa leafcutting bees are haplodiploidy, in that females are produced via fertilized eggs and males by unfertilized eggs. Males are able to be produced with fewer resources and with a sex ratio of 2:1 male:female, there is intense competition when males attempt to mate with females (Peterson and Roitberg 2005).

Mortality

Alfalfa leafcutting bees are exposed to a range of mortality factors, including parasitoids, predators, brood parasites, chalkbrood disease, and temperature stress (Mayer et al. 1976, Stephen and Undurraga 1976, Torchio 1978, Davis et al. 1979, Eves et al. 1980, Vandenberg and Stephen 1982, Richards and Whitfield 1988, Whitfield and Richards 1991, Tepedino 1993, Scott et al. 2000, O'Neill 2004, Bosch and Kemp 2005, Pitts-Singer 2008, Pitts-Singer and James 2009, Rozen and Kamel 2009, Pitts-Singer and Cane 2011). There is also a cell condition referred to as pollen ball, which may be caused by undiagnosed diseases, temperature stress, or overly dense populations of bees (Pitts-Singer 2004, James and Pitts-Singer 2005). Here, we will review information known on each of these classes of mortality.

Parasitoids, Predators, & Cleptoparasites

There are several species of wasps that parasitize bee larvae, most commonly *Pteromalus venustus* Walker (Pteromalidae), an obligate parasitoid. Female parasitoids insert their ovipositors into the brood cell, pierce and paralyze the developing bee larva, and deposit their eggs on the surface of the larva. After 24 to 48 hrs, the eggs hatch and the wasp larvae begin to feed on the bee larva. Once the parasitoids reach adulthood, the wasps then emerge by chewing through the brood cell. Because of a short lifespan of around 12 days, two generations of wasps often arise during one generation of developing *M. rotundata* (Tepedino 1993). Other common parasitoids of *M. rotundata* include *Melittobia chalybii* Ashmead (Eulophidae), *Tetrastichus megachilidis* Burks (Eulophidae), and *Monodontomerus aeneus* (Fonscolombe) (Torymidae). Parasitoids are

often deterred from nests by employing thick walls within the nest cavities, as well as felt cloth attached to the back of the nesting boards (Pitts-Singer 2011). Emergence of adult parasitoids can be managed with the use of yellow sticky traps.

There is not a strict distinction between predators and cleptoparasites (or brood parasites) of *M. rotundata.* Both kill the host bee and consume the provision and/or utilize the nest cell. However, Lafferty and Kuris (2002) distinguish between predators and parasites—predators are generally larger than their prey and feed on more than one victim in a life stage. A cleptoparasite (or brood parasite) is a species whose females lay their eggs in a host-bee nest cell and whose larva develops mainly or completely on the resources of the pollen and nectar provision; the host egg or larva may be directly destroyed and even comsumed by the cleptoparasite larva or adult, depending on the species (Michener 2007). Based on this, we refer to the behaviors of the beetles *Trichodes ornatus* Say (Cleridae) and *Trogoderma* spp. (Dermestidae) as predatory and those of the wasp *Sapyga pumila* Cresson (Sapygidae) and the bee *Coelioxys moesta* Cresson (Megachilidae) as cleptoparasitic.

Larvae of the clerid beetle *T. ornatu*s and the dermestid beetle *Trogoderma* spp. (Dermestidae) are known to be common predators of *M. rotundata* during the nesting season and while bees are stored during diapause. Adult clerids emerge and mate in the summer near flowering plants that provide sustenance in the form of nectar and pollen. Females use their ovipositor to deposit eggs between the tunnel wall and the nest cells of *M. rotundata* and lay multiple eggs. First instar larvae burrow through the leaf cells, consume immature bees and their provisions, and move on to neighboring leaf cells by chewing their way through. Larvae overwinter inside the nest boards and exit the

following spring, seeking out cracks and crevices in which to pupate (Mayer et al. 1976, Davis et al. 1979). Although there is little known about behavior of dermestids in relation to *M. rotundata*, damage by *Trogoderma* spp. appears to be similar to that of *T. ornatus*. Thus, they apparently exhibit a similar life cycle. Other known predators include the beetles *Neognathae lutea* LeConte (Meloidae) and *Tribolium* spp. (Tenebrionidae), as well as yellowjacket wasps (*Vespula* spp.), earwigs (*Forficula auricularia* Linnaeus), ants (Formicidae), birds, and rodents.

Sapyga pumila, a native North American wasp, is a common brood parasite of *M. rotundata*, but has been of less concern in recent years. Closed nest cells are most likely parasitized by adults because there have been no observations of *S. pumila* eggs in uncapped cells (Torchio 1972a). Another cleptoparasite of alfalfa leafcutting bees is the bee *C. moesta*. Not only are they able to locate nests of host species, but are able to enter their nests and lay their eggs within the time the host species is foraging. As with *T. ornatus*, they insert their ovipositor through a slit in the leaf-lining of the nests (Scott et al. 2000). Eggs and larvae of *S. pumila* and *C. moesta* closely resemble those of *M. rotundata*, making it difficult to distinguish cleptoparasites from the host. However, early instars of cleptoparasites can be seen attacking and/or consuming eggs of bee hosts (Torchio 1978).

Chalkbrood Disease

Ascosphaera aggregata Wynns is the pathogenic fungus responsible for chalkbrood, although other viral infections have been found to be associated with this pathology. It was first identified in alfalfa leafcutting bee populations in Nevada in the 1970s and quickly spread to other locations within the U.S., but has continued to be a rare

occurrence in Canada (Bosch and Kemp 2005). There are several ways in which the pathogen is believed to be spread, including: 1) contamination of the bees' mates, eggs, or pollen provisions ; 2) germination of spores in the guts of infected immatures; and 3) emerging adults chewing through the nests of dead siblings that are covered with spores (Vandenberg and Stephen 1981).

The symptoms of chalkbrood vary considerably between younger and older larvae. The first indication of the disease is usually apparent in the fifth instar with cessation of body movement. Fungal mycelia emerge within 24 hr and give the larvae a milky appearance. A pink, tan, or gray cast appears in one part of the body (usually the head or abdomen) and quickly spreads throughout the entire body within one to two days. Larvae either remain tan or assume a chalk-white color and blotchy appearance due to the dark fungal cysts forming beneath the cuticle, sometimes erupting through the cuticle (Vandenberg and Stephen 1981).

Control strategies for this disease have not been fully developed, as the primary mechanism by which this disease is transmitted is still unknown. Thus, the cycle of this disease has not been broken, but the loose cell system helps to decrease the chance of the disease, as siblings do not have to chew through nests containing cadavers. The loose cell system is when nests are removed from the nest boxes before overwintering and a mechanical tumbler is used to separate them into individual nest cells and remove excess debris (James and Pitts-Singer 2005).

Pollen Ball

The most commonly reported condition of nest cells in which *M. rotundata* offspring do not successfully develop is called "pollen ball". Cells with pollen ball are

those that contain a pollen and nectar mass, but which lack immature bees. Pollen ball cells are often characterized by either the lack of an egg or the presence of a dead egg or early stage larva. If characterized as dead, the bee often appears as a collapsed egg or an egg or larva that appears sunken into the wet or dry provision. Because a pollen ball is defined as a brood cell containing an unconsumed provision, any such brood cell without the presence of a live or dead bee is diagnosed as "pollen ball". Thus, pollen ball is not a mortality factor, but is often used as a general term for nest cells experiencing an unknown cause of mortality (Pitts-Singer 2004). Causes of pollen ball are likely the result of abiotic or nutritional factors because of the lack of evidence of pathogenic activity. It could also be due to temperature stresses or overly dense populations of bees (Pitts-Singer and James 2008).

Temperature

Mortality due to high temperatures is especially important to understand because of projected temperature trajectories as a result of climate change. To date, researchers have placed strong emphasis on the effects of changing climate on bee diversity and distribution, performance of adult bees as pollinators, and phenological mismatches between bees and plants (Gordo and Sanz 2006, Dorman et al. 2008, Hegland et al. 2009, Kjohl et al. 2011).

Many studies demonstrate the effects of variability in temperature on the development of insects and how minor differences in a few degrees can have extreme, even fatal, effects. Lower temperatures result in smaller body sizes (lower weights) of female alfalfa leafcutting bees, which is significant as larger body size is correlated with

greater fecundity (number of viable eggs per female) and efficiency in pollination (Tepedino and Parker 1986); larger females also lay larger eggs and carry larger lipid stores going into their adult stages (O'Neill et al. 2014).

Rate of development increases for eggs and first instars with temperatures between 15 and 35 °C and for second, third, and fourth instars with temperatures between 15 and 30 °C. Rate of development decreases between 32 and 35 °C (Whitfield and Richards 1991). A temperature threshold occurs for prepupal development at 15.7 °C, with 295 accumulated degree days as a requirement for 50% completion of adult emergence (Richards and Whitfield 1988).

Timing of the onset of winter incubation affects prepupal weights and lipid and water contents, as well as adult emergence and female longevity. Storage of prepupae beginning in November or December and lasting through June resulted in heavier adults with quicker emergence times (Pitts-Singer and James 2009). In a study testing the effects of ambient temperatures on metamorphizing larvae, constant low ambient temperatures (6 °C for 1 wk) most severely affected the bees in adulthood, as opposed to fluctuating low ambient temperatures (6 °C with a daily pulse of 20 °C for 1 hr with 1-hr ramp times). These effects included decreased flight performance, lower metabolic rate, shorter wing lengths, and decreased flight bouts (Bennett et al. 2015).

O'Neill et al. (2011) studied the effects of temperature on postwintering development of *M. rotundata*. Nineteen temperature treatments were applied and relationships between temperature and development rates were modeled. Bees did not complete development at temperatures below 16 to 18 °C and above 36 to 39 °C, with rate of development being highest at rearing temperatures of 33 to 34 °C. However, the

maximum proportion of body lipids in adults was found when adult bees were reared at temperatures between 27 and 29 °C. These results highlight the difficulty in choosing a rearing temperature that simultaneously synchronizes adult emergence with alfalfa bloom and maximizes development rate, survival, and adult condition (O'Neill et al. 2011).

Life Tables

The life table is a valuable tool used by biologists and ecologists when studying plant and animal demography. They were first developed to document deaths by age group in humans starting in the 1100s and can thus be thought of as "death tables". They were first utilized by ecologists to study animal populations by Deevey (1947) and first applied to insect populations by Morris and Miller (1954). Studies on life tables have since focused on age-specific mortality factors and which of these have the greatest impacts on a population. However, many types of life tables are limited, as they do not account for multiple mortality factors occurring simultaneously in a population (Morris 1959, Southwood 1978, Buonaccorsi and Elkinton 1990).

To compensate, the multiple decrement life table (MDLT) was created to analyze the probability of mortality from a combination of possible causes. These life tables include aspects of Abbot's correction for multiple causes of mortality, as well as key-factor analysis. This allows for the estimation of the probability of death in the presence or absence of a combination of mortality factors. While conventional life tables address the probability of death from one or more factors, they do not account for contemporaneous causes of mortality in a population (Moriyama 1956). Irreplaceable mortality (the idea that if one mortality factor is eliminated, it will not be completely

replaced by another) addresses this limitation. This concept helps ecologists understand population dynamics, because only changes in irreplaceable mortality will determine changes in overall population density (Carey 1989, 1993).

Although Carey (1989) emphasized the importance of multiple-decrement life tables, they still have not been widely utilized by those researching population dynamics. Peterson et al. (2009) stated that it is crucial to understand competing mortality factors to estimate the effect of any single cause of mortality. Analysis of 73 insect life tables published from 1954 to 2004 showed that irreplaceable mortality was 8.6% (±7.2) from pathogens, 7.8% (±4.9) from predators, and 6.2% (±1.6) from parasitoids. Additionally, it was found that irreplaceable mortality from natural enemies was lower than mortality from factors other than natural enemies, suggesting a correlation between environmental stability and irreplaceable mortality from natural enemies.

A few studies have also found that animals in protected and/or stable environments have higher irreplaceable mortalities than those in unstable environments (Southwood and Comins 1976, Price and Pschornwalcher 1988). This makes sense because unprotected environments often have numerous mortality factors acting contemporaneously on a population and the mortality factors are, thus, highly replaceable. It is, however, not apparent that any studies have attempted to examine the effect of degree of environmental stability using life tables (Peterson et al. 2009). Additionally, there has been little research on the irreplaceable mortality in an animal species that utilizes a protected habitat, with the exception of the wheat stem sawfly, *Cephus cintus* Norton (Peterson et al. 2011).

Davis et al. (2011) developed the spreadsheet program, M-DEC, that consists of formulas to help make use of the MDLT more widely available. This study will take advantage of this program by comparing multiple mortality factors of the alfalfa leafcutting bee. Being able to estimate the risks of death in the presence or absence of multiple mortality factors will help researchers to better rear the bees and, thus, help producers apply this knowledge in their practices. Understanding how and why alfalfa leafcutting bees die will serve to help alfalfa seed producers minimize loss of bee populations and optimize alfalfa seed yield.

Calculation techniques for multiple-decrement life tables will use the methods described by Carey (1993) and Peterson et al. (2009). In the conventional life table, the variables are defined as: x = the life stage index (i.e. larvae, pupae, adults); k_x = the number alive at the beginning of each stage x; d_x = the total number of deaths in each stage x, and; d_{ix} = the number of deaths attributed to a single cause in stage x. We will then use the published software program, M-DEC, to examine combinations of causes of death in relationship to the absence of other causes and to frame each factor in terms of irreplaceable mortality (Davis et al. 2011). Variables are defined as al_x = the fraction of the cohort living at the beginning of the stage (starting at 1.0 for the first stage and calculated by al_x-1 – ad_x-1); ad_{ix} = fraction of deaths attributable to one cause, i; ad_x = fractions of all deaths from all causes ($ad_{1x} + ad_{2x} + \ldots + ad_{5x}$), and; aq_x = stage specific probability of death within that stage calculated by the sum of the probability of dying from all listed causes (d_x/k_x).

An algorithm represented by a quadratic formula for irreplaceable mortality will be used within the M-DEC program to generate mortality solutions for independent

mortality factors in the absence of all other factors (Davis et al. 2011). Elimination-of-cause analysis relies on the probability of surviving each source of mortality (Px) and its complement ($1 - q_x$) where $(1 - q_1)\ x \ldots x\ (1 - q_n)$ is the chance of jointly surviving a set of mortality factors and its complement, $1 - [(1 - q_1)\ x \ldots x\ (1 - q_n)]$, is the chance of jointly dying form a set of mortality factors. To estimate mortality in the absence of one or more factors, two simultaneous equations with two unknowns are used. For example, by expressing q_1 in terms of q_2, D_1, and D_2 (the fraction of all individuals observed that died of cause 1 and 2), this yields the quadratic equation $aq_2^2 + bq_2 + c = 0$, where $a = D_1$, b = - (D_1 + D_2) and $c = D_2\ (D_1 + D_2)$. The value of q_2 can be found by substituting a, b, and c into the quadratic formula.

Objectives

This study had three main objectives: 1) identify mortality classes for *M. rotundata* in brood cells at our site; 2) experimentally manipulate parasitism and temperature to determine their impact on total mortality; and 3) estimate mortality risks using the demographic life-table model, M-DEC.

CHAPTER TWO

MORTALITY DYNAMICS AND LIFE TABLES OF THE ALFALFA LEAFCUTTING BEE, *MEGACHILE ROTUNDATA* (HYMENOPTERA: MEGACHILIDAE)

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Contributions: Collaborated with design of experiment, interpretation of results, and manuscript preparation.

Co-Author: Robert K. D. Peterson

Contributions: Conceived and designed experiments, provided critical input at all stages of experiment including interpretation of results and manuscript preparation.

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CHAPTER TWO

MORTALITY DYNAMICS AND LIFE TABLES OF THE ALFALFA LEAFCUTTING BEE, *MEGACHILE ROTUNDATA*

The following chapter has been prepared for submission to a peer-reviewed journal

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Abstract

The alfalfa leafcutting bee, *Megachile rotundata* (F), contributes to the pollination of more than two-thirds of alfalfa seed production in North America. However, population losses of more than 50% are common in the U.S.. Because of this high mortality, understanding the causes of mortality of *M. rotundata* is critical in finding ways to maintain and increase bee populations. Therefore, this study identified and quantified *M. rotundata* mortality and its causes, as well as estimated mortality risk using the demographic life-table model, M-DEC. Research was conducted over two summers on an alfalfa field in Toston, MT and offspring mortality was assessed in summer and fall of each year. Nest shelters were manipulated for a main temperature treatment (low vs. high) and nest boxes were manipulated for a parasitism sub-treatment (backing-present vs. backing-absent). There was no evidence of a temperature-treatment effect, so we excluded it from our analyses. However, for every 1 °C increase in nest-tunnel temperature, there was a 7% increase in total mortality. Nest boxes without felt backing

(backing-absent) had a 43% increase in mortality over both years compared to those with felt (backing-present). Total mortality was approximately 15% for both years, but the proportion of each mortality class differed substantially. Mortality from each class was highly irreplaceable with death by parasitoids greatest in 2017, death by pollen ball greatest in 2018, and death by predation similar between the two years. Identifying and quantifying mortality classes of *M. rotundata* and characterizing mortality dynamics will help producers maintain and increase their bee populations.

Introduction

The alfalfa leafcutting bee, *Megachile rotundata* (F), is responsible for more than two-thirds of the alfalfa seed production in North America, making it the most intensively-managed solitary bee species (Pitts-Singer and Cane 2011). However, population losses of more than 50% (the difference between the parental population released and the live progeny recovered) are common in the U.S. (Bosch and Kemp 2005), requiring many alfalfa seed producers to bees from Canada, which can be costly. *M. rotundata* are solitary and construct nests within wood cavities that consist of a linear series of cells made of cut pieces of leaves. Each cell contains one egg that is provisioned with a mass of nectar and pollen provided by the mother, which is then sealed and plugged with more leaf pieces.

While females are constructing nests, their offspring are exposed to a range of mortality factors, including parasitoids, predators, fungal pathogens, cleptoparasites, and temperature stresses. There is also a diagnosed cell condition referred to as “pollen ball” which may be caused by diseases or temperature stress, but whose causes are not well-

known (Pitts-Singer 2004). Mortality due to high temperatures is especially important to understand because of projected temperature trajectories as a result of climate change and because it is potentially controllable if conditions in nest shelters can be manipulated.

It is also critical to understand how these varied mortality classes relate to one another. To that end, multiple decrement life tables are valuable tools created to analyze the probability of mortality from a combination of possible causes. Although conventional life tables address the probability of death from one or more factors, they do not account for contemporaneous causes of mortality in a population (Moriyama 1956). Irreplaceable mortality, the idea that if one mortality factor is eliminated it will not be completely replaced by another, addresses this limitation. Davis et al. (2011) developed the spreadsheet program, M-DEC, that consists of formulas to help make use of the multiple decrement life table.

Being able to estimate the risks of death in the presence or absence of multiple mortality factors will help researchers to better rear the bees and, thus, help producers apply this knowledge in their practices. Therefore, our study had three main objectives: 1) identify mortality classes for *M. rotundata* in brood cells at the site where we worked; 2) experimentally manipulate parasitism and temperature to determine their impact on total mortality; and 3) estimate mortality risks from different, potentially interacting sources using the demographic life-table model, M-DEC.

Materials and Methods

Shelters

Research was conducted on a 38.5 ha alfalfa seed field in Toston, MT (46°09’33.22”N, 111°26’09.72”W) in the summers of 2017 and 2018. The field was irrigated according to standard Montana practices and consisted of *Medicago sativa* L. (cultivar Ladak).

Six plywood shelters were erected and positioned with openings due south in a line along an alfalfa field, to correspond with the landowner’s practices, and placed 7.32-m apart (Fig. 1). We anchored the shelters by tying them with wire to stakes placed in the ground to prevent damage by wind. The bases of the shelters were 1.22-m wide and 0.78-m deep. The sides were 0.94-m high. The backs were 0.61-m high, so as to leave a gap between the back and the base for ventilation. The roofs of the shelters were 1.38-m wide and 0.97-m deep. The roofs were attached by hinges on the rear of the shelters and by wire to the fronts. The shelters were supported on four legs, leaving a 0.40-m high gap between the ground and the base of the shelter (Fig. 2).

We randomly assigned each shelter to either a “low” or “high” temperature treatment designed to create variation in the temperatures experienced by developing eggs and larvae; this provided 3 replicates for each treatment. On the 3 “low” temperature shelters, we painted the roofs and backs white to increase reflection of sunlight. On the 3 “high” temperature shelters, we stapled black felt to cover the gaps in the back to minimize air flow and absorb insolation. Because we saw only a 0.4 °C temperature difference between the low- and high-temperature shelters in the first year, we modified the high-temperature shelters in the second year to further increase their internal

temperatures. To accomplish this, we taped 2 layers of ACE 4-mil black plastic wrap (Oakbrook, IL) to the sides and roofs of the high-temperature shelters with black Gorilla brand duct tape (Cincinnati, OH; Fig. 3).

Nesting materials in each shelter were inserted into 2 different types of boxes, hereafter referred to as the large and small boxes. The large boxes consisted of one 0.61 m x 0.61 m x 0.14 m open-faced wooden box please inside the middle of each of the 6 shelters. Each large box contained 10.16 cm x 13.34 cm stacked wooden laminates to create tunnels in which the bees could build their nests. Four columns of 53 laminates provided 2,756 available tunnels per box. We painted the large nest boxes and the exposed ends of the laminates with 2 layers of gray flat latex exterior house paint. We also painted purple designs with the same type of paint on the exposed ends of the laminates. These designs aid in the bees' identification of their nest tunnels and are standard practices for both research and commercial bee production (Guedot et al. 2005; Fig. 2).

Each half of the large nest boxes contained 1 of 2 parasitism treatments, "backing-present" or "backing-absent", with each treatment being randomly assigned to the right or left side of the box. We created the backing-present side by stapling 2 layers of Joann's brand black polyester felt fabric (Hudson, OH) and 1 layer of Pellon brand cotton batting (Clearwater, FL) to the back of each box to prevent entrance of parasitoids from the backs of the nest tunnels. The backing-absent side of each box did not contain this fabric backing, leaving room between the wooden laminates and back of the box for parasitoids to enter the tunnels (Fig. 3).

In addition, we placed 6 small boxes (12.7 cm x 21.7 cm x 8.9 cm) within each of the 6 shelters for within-season sampling (36 total small nest boxes); these boxes were designed to be either backing-present or backing-absent, as detailed above. We stacked 3 backing-present boxes on one side of the large nest box and 3 backing-absent boxes on the other side; the "bottom" boxes (sitting on the bases of the shelters) each contained 4 stacked laminates, creating 39 available tunnels for which bees could nest; the "middle" boxes (stacked on top of the bottom boxes) contained 26 available tunnels; and the "top boxes" (sitting on top of the middle boxes) contained 13. With 6 small boxes inside each of the 6 shelters, 936 nesting tunnels were available for the within-season sampling (Fig. 2).

Bees

Leaf cells containing developing *M. rotundata* larvae were purchased from T. Helm in Toston, MT. These nest cells were created by adult bees the previous summer, then removed from the polystyrene nesting boards in the fall and transferred to bins for storage and to mimic natural conditions for diapause. They were kept in cold storage throughout the winter and spring to suspend development of the offspring. Helm had treated these nest cells with paraformaldehyde to reduce the presence of fungi and placed dichlorvos strips near the stored nest cells to reduce numbers of emerging adult parasitoids.

We placed 1 gallon of the nest cells in each of the 6 shelters on July 3 in 2017 and July 7 in 2018. Each gallon was placed in individual wood trays with mesh tops and screwed to the top of the large nest boxes to allow the emerging bees to increase their body temperatures without being in direct sunlight. The mesh tops were opened the same

day to allow adult bees to have access to the nest boxes and alfalfa field at the time of their emergence. Nest shelters remained in the field until August 30, 2017 and August 24, 2018, at which time shelters were dismantled and stored.

Temperature

A HOBO® Micro Station Data Logger (Bourne, MA) was placed due south in the center of the 6 aligned shelters on July 10, 2017 and July 11, 2018. We attached sensors for temperature, relative humidity, wind speed, and wind direction to the weather station and programmed it to record these 4 measurements once every 30 minutes until shelters were removed each year.

We also attached 2 HOBO® U23 Pro v2 Temperature/Relative Humidity Data Loggers (Bourne, MA) inside each shelter to record the temperature and relative humidity within the shelter. We placed 1 in the front upper left corner of each shelter and 1 in the back upper right corner. We programmed these data loggers to record measurements once every 30 minutes from the day the bees were placed in shelters until shelters were removed each year.

In addition, we recorded measurements within the nest tunnels using a Cole-Parmer Digi-Sense thermocouple thermometer (Vernon Hills, IL). We attached the thermometer to an Omega 2-pin flat mini thermocouple connector (–CO +CP), which was then attached to a probe. The probe consisted of the thermocouple wire taped inside a plastic straw with a few millimeters of the straw exposed on the other side. We drew 2 lines on the straw to mark where the 2 measurements would take place – 1 in the “front” of the tunnel and 1 in the “back”. The front measurement was 3.4 cm from the entrance of the tunnel and the back measurement was at a depth of 6.8 cm. We took both front and

back measurements from 6 tunnels within each shelter. We randomly chose 1 tunnel within each of the 6 quadrats of each shelter. We plugged these selected tunnels with cork at the beginning of the season to ensure they were not filled with nest cells. Thermocouple measurements were recorded weekly from July 17 to August 28, 2017 and July 18 to August 22, 2018 between 1400 and 1600 h (MDT).

Examination of Nest Cell Contents

Within-Season Samples. We removed each of the 36 small nest boxes at different dates during the summer for within-season sampling. The 2 top boxes in each shelter were removed on July 17, 2017 and July 18, 2018, the 2 middle boxes were removed on July 31, 2017 and August 1, 2018, and the 2 bottom boxes were removed on August 14, 2017 and August 15, 2018. Leaf-cell dissections to examine developing eggs or larvae began immediately after removal. After each of the 3 removal periods, small nest boxes were kept in a YETI Tundra 35 cooler (Austin, TX) and maintained at 4 °C with ice packs until dissections were complete. All nest cells from the within-season samples were dissected, creating a total of 2,427 dissected cells in 2017 and 1,458 in 2018.

Unfortunately, these dissections could not be used in the analyses, as total mortality and mortality by the separate classes were too low (i.e., much mortality occurred very late in the season). However, the within-season dissections aided in identifying the causes of mortality found in the end-of-season dissections and in identifying the age category in which to place each mortality class.

End-of-Season Samples. The 6 large boxes were removed at the end of the summer on August 30, 2017 and August 24, 2018. We kept the boxes at room

temperature in the lab until the first parasitoid adults began emerging to prevent parasitism of other lab specimens. We then moved all 6 boxes into a cold storage unit at the Plant Growth Center on Montana State University's Bozeman campus on October 5, 2017 and October 6, 2018 to suspend development of parasitoid wasps. Bees were kept at 4 °C, in accordance with overwintering temperatures used in commercial rearing.

Examination of nest cell contents began immediately after removal of nest boxes. For these examinations, we first divided each of the 6 large nest boxes into 6 quadrats. Two laminates (i.e. one row of nest tunnels) from each of the 6 quadrats from each box were randomly selected for cell dissections for a total of 72 laminates each year. Three laminates from 2 boxes were removed at a time and transferred to the cooler in the lab. We dissected 7,404 nest cells in 2017 and 5,750 in 2018 for the end-of-season samples. Samples were extracted from each quadrat within each combination of temperature and parasitism treatment.

Dissections. We cut and removed the tops of each nest cell to observe the contents under a Leica M80 microscope at 1.0X with an IC80 HD digital camera (Wetzlar, Germany). If necessary, we carefully removed the contents of the nest cell with forceps for further examination.

For each nest cell, we recorded: 1) status of the offspring (live or dead), 2) age class (egg, early instar, late instar, or "unable to age"), and 3) mortality class (parasitoid, predator, cleptoparasite, chalkbrood, pollen ball, or unknown; see Fig. 4). Live larvae were identified by prodding them to determine if they moved (see Fig. 5); eggs were considered still viable if shiny and unshriveled. Pupae and adult bees were not used in the

analyses. If there was no evidence of the existence of a bee within a nest cell, that was also not used in our analyses.

Each cell was also assigned to 1) a nest position (cell 1 being the furthest from the entrance and the first to be provisioned), 2) temperature treatment (low or high), and 3) parasitism treatment (backing-absent or backing-present).

Mortality Classes. Parasitoids were difficult to identify in the within-season samples, though they could be seen using a microscope. However, they were easily identified in the end-of-season samples. Multiple parasitoid larvae or adults often occupied the majority of space in a nest cell.

We categorized "predators" as any beetle larva, most often clerids or dermestids. Clerids were identified as bright pinkish-orange larvae (Fig. 5, top right panel, on left). Dermestids were identified as whitish-yellow larvae with large, obvious setae (Fig. 5, top right panel, on right). Larvae were usually very mobile and ranged in size. We were also able to identify evidence of a predator by the presence of frass from beetle larvae inside nest cells. In addition, predators were identified by large holes in nest cells or completely destroyed nest cells.

We identified cleptoparasites as Hymenoptera that were clearly not *M. rotundata*. These usually consisted of smaller, whiter, and more wrinkled bee or wasp larvae (Fig. 5, middle left). A few observations were made from the within-season samples of a cleptoparasite attempting to consume an *M. rotundata* egg. End-of-season cells containing cleptoparasites never also contained a *M. rotundata*. Based on the within-season observations, all cleptoparasites were classified as causing host egg mortality.

Presence of pathogens was identified by dark, shiny, fungal spores, whether on a bee larva or just present within the nest cell. Previous literature also identified pathogens by dark larvae (Vandenberg and Stephen 1981). We did not have the tools to determine whether this discoloration was due to a fungal pathogen or an unknown mortality class.

Pollen ball was recorded when we observed an egg or early instar that appeared sunken into the pollen and nectar provision. Previous literature has categorized a nest cell with pollen and the absence of a bee as "pollen ball" (Pitts-Singer 2004, Pitts-Singer and Cane 2011). Because this study was focused on actual bee mortality, we decided the absence of a bee in a nest cell would be excluded from our analyses.

Unknown cause of death was the most diverse category, as it contained all dead bees that did not fall into the previous 5 mortality classes. It mostly consisted of bee larvae that were discolored and clearly dead. As noted above, it is possible that a portion of the unknown death was actually due to the early stages of chalkbrood disease.

Statistical Analyses

All statistical analyses were performed in RStudio (Version 3.4.4). Differences in temperature between 2017 and 2018 were analyzed for the weather station, data loggers, and nest-tunnel temperatures. Differences in temperature between the "high" and "low" shelters were also analyzed for the data loggers and nest tunnels. In addition, differences in temperature between positions of the data loggers within the shelter were analyzed ("front" and "back"). Nest-tunnel temperatures were separated by front or back nest-cell position and averaged for each year. Differences in relative humidity were also assessed from the weather station and data loggers. All temperatures were recorded and averaged between 1400 and 1600 MDT. All of the above were analyzed using Welch's t-tests to

calculate means and 95% confidence intervals for the temperatures and relative humidities and only those with p-values of less than 0.1 were reported.

A binomial logistic regression model was created with a response variable of live or dead bees, with live being assigned a value of 0 and dead a value of 1. A multinomial logistic regression model was also created with the response variable being the six "mortality" classes (unknown, chalkbrood, cleptoparasite, parasitoids, pollen ball, or predator), as well as live bees. Explanatory variables included year (2017 or 2018), shelter temperature treatment (low or high), nest backing material (felt or no felt), cell position within a nest (front or back), and nest-tunnel temperature.

Issues with independence may be of concern because neighboring bees could be exposed to the same mortality class, such as when a predator consumes neighboring nest cells. In addition, this study is of a split-split plot design (with cells nested in tunnels nested in sides nested in boxes). As such, both a mixed-effects model and a fixed-effects model were created for the binomial logistic regression analyses with live or dead bee as the response.

Each model included a three-way interaction between year, nest-backing material, and nest-cell position and an additive effect of nest-tunnel temperature at the specified location (front or back). Each model was tested with an ANOVA and model refinement was performed by removing one potential component at a time from the initial models. Removal was determined based on little-to-no evidence against the null hypothesis of no impact, with a p-value cut off of 0.10. Log odds were exponentiated and converted to mean odds. Tables and plots of explanatory variables versus the response variables were created. Effects-plots of the final models were also generated using the effects package

(Fox et al. 2009). For the multinomial models, we also generated p-values using the absolute values of the coefficients divided by the standard errors as z-statistics. The p-values were then calculated from the upper tail of a normal distribution and multiplied by two.

Results

Temperature

For the weather station recording condition outside of the shelters, the average temperatures during the month-long period were 28.4 °C (± 0.6) in 2017 and 24 °C (± 0.9) in 2018 (Fig. 6), while relative humidity was 31% (± 1.6) in 2017 and 43.1% (± 2.3) in 2018 (Fig. 7).

For the data loggers within the shelters, the average temperatures inside the shelters were 32.2 °C (±0.2) in 2017 and 30.3 °C (±0.2) in 2018, while relative humidity was 27.9% (±0.3) in 2017 and 34.8% (±0.5) in 2018. The average temperatures were 30.5 °C (±0.2) in low-temperature shelters and 32 °C (±0.2) in high-temperature shelters. The average relative humidity was 32.13% (±0.47) in low-temperature shelters and 30.59% (±0.5) in high-temperature shelters. The average temperatures were 30.4 °C (±0.2) in the shelter backs and 32.1 °C (±0.2) in shelter fronts, while relative humidity was 32.5% (±0.5) in back and 30.2% (±0.5) in front.

For the temperatures within the nest tunnels, the average temperatures were 33.2 °C (±0.03) in 2017 and 30.4 °C (±0.03) in 2018. The average temperatures were 31.8 °C (±0.1) in low-temperature shelters and 32.3 °C (±0.04) in high-temperature shelters. The

average temperatures were 31.7 °C (±0.03) in the back nest-cell positions and 33.4 °C (±0.1) in the front.

Mortality as Related to Nest Treatments

There was a minimal violation of independence in the fixed-effects model with no overdispersion (phi = 0.84 < 1). There were also similar z- and p-values for the 3-way interaction between the fixed-effects and mixed-effects models (cell by temp by backing test: z-value = 1.33 vs. 1.27, p-value = 0.19 vs. 0.21). Therefore, we used the fixed-effects model due to its relative simplicity.

Despite the model's summary output showing no evidence of the 3-way interaction (z = 1.27, p = 0.21), the effects-plot revealed different results. Summary output showed correlation between mortality and 1) nest backing (z = 4.7, p < 0.001), 2) nest-tunnel temperature (z = 2.66, p = 0.008), 3) nest-cell position*nest-backing (z= -2.74, p = 0.006), and 4) nest-cell position*year (z = 1.89, p = 0.059). There was no evidence of a shelter temperature effect, our main treatment. Therefore, we eliminated this from further analyses.

The mean odds of a bee dying in a nest without felt backing were 1.43 times higher than the mean odds of a bee being alive when compared to the mean odds of a bee dying in a nest with felt backing versus being alive, given that year, cell position, the 3-way interaction, and temperature of the nest tunnel are in the model. Therefore, the value of 1.43 indicates that the probability of a bee dying in a nest without felt backing compared to a nest with felt backing increased by 43%.

For every 1 ^{o}C increase in the temperature of the nest tunnel, the odds of a bee dying increased by 7%, given that year, nest-backing material, cell position, and their

interaction are in the model (Fig. 8). The effect of nest-backing on mortality, as well as year, differed by cell position, as evidenced by the effects-plots of these interactions.

Mortality by Cause

Although each year contained similar total mortality (as discussed later in the "Life Tables" section), mortality differed among mortality classes between the two years (Fig. 9). Therefore, we created two multinomial logistic regression models, one for each year.

2017. Our initial model contained explanatory variables that included an interaction between nest-backing material and nest-cell position and an additive effect of nest-tunnel temperature at the specified location (front or back). We compared this model with an additive model that did not include an interaction and found some evidence for a two-way interaction between backing material and cell position (Likelihood Ratio = 10.60, $p = 0.101$). Therefore, we retained the initial model as our final model.

For nest-backing material, given that nest-cell position and nest-tunnel temperature are in the model, the mean odds of a bee in a nest without felt backing being killed by parasitoids were 2.4 ($p < 0.001$), by pollen ball was 1.48 ($p < 0.001$), and by predator was 0.52 ($p < 0.001$) times that of a bee being alive compared to a bee being killed in a nest with felt backing by these same causes. Therefore, the value of 2.4 indicates that the probability of a bee dying by parasitoids in a nest without felt backing compared to a nest with felt backing increased by 140%.

The mean odds of a bee in a front nest-cell position being killed by a predator were 0.45 times the bee being alive (or a 65% decrease) compared to a bee being killed

by a predator in a back nest-cell position ($p = 0.002$), given that backing and temperature of the nest tunnel are in the model.

For every 1 °C increase in temperature of the nest tunnel, given that backing material and cell position are in the model, the odds of a bee being killed by a specific mortality class compared to the bee being alive increases or decreases by the following: chalkbrood (34.6%, $p = 0.004$); parasitoids (-24.5%, $p < 0.001$); pollen ball (16.8%, $p = 0.084$); predator (39.1%, $p < 0.001$); and, unknown (51.1%, $p < 0.001$; Fig. 10).

2018. Our initial model contained explanatory variables that included an interaction between nest-backing material and nest-cell position and an additive effect of nest-tunnel temperature at the specified location (front or back). We compared the initial model with an additive model that did not include an interaction and found little-to-no evidence of the two-way interaction between backing material and cell position (Likelihood Ratio = 4.79, $p = 0.571$). Therefore, we chose the model without the interaction as our final model.

For nest-backing material, given that nest-cell position and nest-tunnel temperature are in the model, the mean odds of a bee in a nest without felt backing being killed by chalkbrood was 0.62 ($p = 0.041$), by a cleptoparasite was 1.4 ($p = 0.033$), by parasitoids were 3.1 ($p = 0.001$), and by a predator was 2.01 ($p < 0.001$) times that of a bee being alive compared to a bee in a nest with felt backing being killed by these same causes.

For every 1 °C increase in temperature of the nest tunnel, given that backing material and cell position are in the model, the mean odds of a bee being killed by a specific mortality class compared to the bee being alive increase or decrease by the

following: parasitoids (-30.4%, p = 0.036) and pollen ball (12.4%, p = 0.1; Fig. 11). There was little-to-no evidence of a nest-cell position effect on any mortality class in 2018.

Life Tables

The percentage mortality for each mortality class varied substantially between 2017 and 2018 (Tables 1 and 2). Both years had very similar overall mortality, with 14.5% for 2017 and 15.1% for 2018. In addition, each mortality class was nearly 100% irreplaceable (i.e., no other mortality class would have been able to replace a specific mortality class if it was not present). We also found differences in proportion of mortality by year (2017 vs. 2018) and by nest-backing treatment (felt vs. no felt) (Table 3).

2017. The greatest source of mortality for 2017 was death by parasitoids at 6.4% (Table 1). Unknown causes of death (2.6%) and death by predator (2.9%) were similar. Death by pollen ball (1.3%), chalkbrood (1.01%), and cleptoparasite (0.4%) had the lowest percentages of mortality.

Death by parasitoids doubled in the nests without the felt backing compared to nests with felt backing, from 4.3% to 8.5% (Table 3). Conversely, death by predators doubled in shelters with felt backing with 3.4% mortality compared to 1.8%. Death by unknown causes increased in shelters without felt from 2.7% to 3.4%.

2018. The greatest source of mortality for 2018 was pollen ball at 6.2% (Table 2). Death by cleptoparasite was higher in 2018, with mortality at 3.3%. Mortality from predator was similar to 2017 at 2.1%. The lowest causes of mortality were chalkbrood (1.6%), unknown (1.2%), and parasitoids (0.8%).

As in 2017, death by parasitoids more than doubled in the nest boxes that lacked felt backing, with 1.2% compared to 0.4%. Unlike 2017, death by predator also doubled in nest boxes that lacked felt, from 1.4% to 2.8%. Mortality by cleptoparasite also increased in nests without felt backing, from 2.8% to 3.8%.

Discussion

Temperature

We found no evidence of a shelter temperature treatment effect and only a 0.46 °C difference in temperatures between the low- and high-temperature shelters, so we discarded our main treatment from the analyses. Average temperatures were higher in 2017 compared to 2018, while average relative humidity was much lower, as evidenced by both the weather station and data loggers. We also observed significant differences in mortality from the separate mortality classes between the two years, despite each year having similar total mortalities of approximately 15%.

However, in regards to temperature, we found that higher nest-tunnel temperatures (regardless of whether they were in the low- or high-temperature shelters) resulted in higher mortality. For every 1 °C increase in nest-tunnel temperature, there was a 7% increase in the probability of death. Almost all mortality classes were correlated with nest-tunnel temperatures in 2017, except for cleptoparasitism, which may be due to the small numbers of cleptoparasites found in this year.

In 2018, of the 5 mortality classes correlated with nest-tunnel temperatures, nearly all increased with increasing temperatures, except for mortality from parasitoids. An increase in temperature also resulted in a decrease in death by parasitism and an increase

in death by pollen ball, but no other correlations. Whitfield and Richards (1985) exposed developing *P. venustus* to a range of rearing temperatures from 10 to 36 °C. All temperatures generated high survival, with the exception of 36 °C, in which rates of development began to drop. At 36 °C, pupal survival was significantly lower and many eggs collapsed and died within a few hours. The role of temperature during the nesting season on death by parasitoids, pollen ball, and other mortality classes should be further studied.

Mortality by Class, Nest Backing, & Cell Position

Parasitism was the most abundant source of death in 2017, and the least abundant in 2018. Conversely, pollen ball was one of the least abundant mortality classes in 2017 and the most abundant in 2018. Differences in mortality from parasitoids between the two years were likely due to a combination of the differences in temperature and humidity between the two years.

The probability of a bee dying in a nest box without the felt backing was 43% higher than the probability of death in a nest box with felt backing. In 2017, the probability of death from parasitism increased 140% for bees in nests without felt backing. This relationship was also evident in 2018, but based on very few parasitoids found in that year. Previous studies have highlighted this reduction in mortality by parasitoids in nests with felt or cloth backing (O'Neill 2004, Pitts-Singer and Cane 2011).

O'Neill (2004) also assessed mortality classes in 5,072 of *M. rotundata* nest cells from shelters of the same construction (without temperature or backing manipulations) on the same fields in Toston, MT in 2000 and 2001, as well as three other sites within a 25-km radius of Montana State University, for a total of 7 site-years. Egg and larval

mortality were separated into 4 categories: chalkbrood, parasitism, pollen ball, and unknown. Parasitism in O'Neill (2004) was identified as a nest cell being parasitized solely by *P. venustus*. Dead larvae were placed in the pollen ball category if the larva was less than two times the length of an egg, with older dead larvae being categorized as an unknown cause.

O'Neill (2004) observed greater total mortality than in our study, ranging from 19% to 54%, for each site-year. Mortality from the sites over the two years ranged from 0.4% to 11.7% by chalkbrood, 0.5% to 13.4% by parasitism, 5.9% to 25.2% by pollen ball, and 5.6% to 26.1% by unknown causes. O'Neill (2004) also observed 6 larval *Melittobia chalybii* (Walker), one *Trichodes* sp. beetle larva, and one adult *S. pumila*, all found in 3 separate nest cells. For the two Toston sites, O'Neill (2004) observed total mortality ranging from 35% to 54%, chalkbrood from 5.7% to 11.7%, parasitism from 3% to 11.1%, pollen ball from 15% to 25.2%, and unknown from 5.6% to 19.9%. These differences in mortality were likely because temperatures were higher and relative humidity was much lower in 2000 and 2001 compared to 2017 and 2018. Moreover, the landowner likely adjusted his nesting materials and practices within the 16-year span between studies.

The relationships between cause of mortality and nest-cell position were also assessed by O'Neill (2004), with cell position 1 being the innermost cell nearest the back of the nest box. There were significant cell-position effects on overall mortality in 5 out of 7 site-years. The 3 Toston site-years showed that overall mortality decreased in the outermost cells. Two sites showed declining survival in higher cell positions in 2001, though these same sites did not have significant results in 2000. Cell position analysis by

cause displayed a consistent pattern for 6 site-years with the highest parasitism in the earliest-constructed cells in the nest, declining to nearly zero in the outermost cells. Correlations between pollen ball and cell position were significant for 2 site-years, showing decreased levels of pollen ball in cells occupying outer positions in the nests. For unknown causes of death, cell position results displayed a consistent increase with cell position in 6 of the 7 analyses.

Our results only showed a relationship between predators and cell position in 2017. However, there were correlations between mortality and interactions between cell position and other variables. The results of the interaction between nest-cell position and nest-backing material are not surprising when considering parasitoids as a mortality class. There was higher mortality in the back of the nest box without felt backing, as that is where the highest percentages of death by parasitoids were found, as was also found by O'Neill (2004).

Life Tables

Each mortality class had nearly 100% irreplaceable mortality, meaning that each cause was unlikely to be replaced by another and that removing that mortality class would have removed the probability of mortality at that specific age. Therefore, finding ways to decrease mortality of specific causes will decrease overall population mortality. Although death by parasitoids and pollen ball were the two leading causes of mortality in this study, death by predation was the only cause that maintained similar mortality over both years. Because of each cause's high irreplaceable mortality in this study, reducing the prevalence of death by parasitoids, pollen ball, and predators may significantly decrease total mortality of alfalfa leafcutting bees in this geographic area.

This is, however, dependent on the value of the crop in a specified year, as prices fluctuate based on supply and demand. Alfalfa leafcutting bees were selling for $100 per gallon in 2017 and only $10 per gallon in 2018. In 2016, Helm released 8 million alfalfa leafcutting bees with a return of 24 million offspring and sold 17 million that year. If Helm was able to reduce his age-specific mortality of 15% from the offspring in 2017, and assuming one gallon of nest cells equates to approximately 10,000 bees, he would gain $36,000 in 2017 and $3,600 in 2018. It is important to note that this does not account for the increase in seed yield that would likely result from this increase in alfalfa leafcutting bee populations.

Using multi-decrement life tables and associated demographic tools to quantify cause-specific mortality of alfalfa leafcutting bee populations will help producers focus on the causes of mortality most significantly contributing to population losses. This in turn can help landowners and managers invest more efficiently and potentially increase yield of both bee populations and/or the landowners' crops, as an increase in pollinator populations will likely lead to an increase in pollinated and viable crops, ultimately increasing grower profits.

CHAPTER THREE

CONCLUSION

Although there was no evidence of an effect from our temperature treatments, there was a correlation between nest-tunnel temperatures and mortality. A 1 °C increase in nest-tunnel temperature resulted in a 7% increase in the probability of death. The probability of a bee dying in a nest without felt backing was 43% greater than in a nest with felt backing. The probability of death from parasitoids increased 140% for bees in nests without felt backing in 2017. However, this relationship was not evident in 2018, but is likely due to the extremely low numbers of parasitoids found in this year. Both 2017 and 2018 demonstrated similar overall mortality (approximately 15%), although the proportions of each mortality class differed substantially, as did the average temperature and relative humidity between the two years.

Parasitism was the most abundant source of death in 2017 and the least abundant in 2018. Pollen ball was one of the least abundant mortality classes in 2017 and the most abundant in 2018. Death by predation was the only class that maintained similar mortality over both years. Because of each cause's high irreplaceable mortality in this study, reducing the prevalence of death by parasitoids, pollen ball, and predators would significantly decrease total mortality for this population of alfalfa leafcutting bees.

Identification and management of the different types of parasites, predators, and pathogens affecting alfalfa leafcutting bee mortality have been researched for decades, as has the effect of temperature on *M. rotundata* mortality in both lab and field settings (Mayer et al. 1976, Stephen and Undurraga 1976, Torchio 1978, Davis et al. 1979, Eves

et al. 1980, Vandenberg and Stephen 1982, Richards and Whitfield 1988, Whitfield and Richards 1991, Tepedino 1993, Scott et al. 2000, O'Neill 2004, Bosch and Kemp 2005, Pitts-Singer 2008, Pitts-Singer and James 2009, Rozen and Kamel 2009, Pitts-Singer and Cane 2011). However, the dynamics of the mortality causes have never been systematically studied using robust life table approaches. Moreover, the effect of temperature on separate mortality classes during the nesting season of *M. rotundata* is lacking in field settings.

Using multi-decrement life tables to identify and quantify mortality classes of alfalfa leafcutting bee populations and their respective irreplaceable mortality will help producers focus on the causes of mortality most significantly contributing to population loss. This in turn can help landowners and managers invest time and money more efficiently and potentially increase yield of both bee populations and/or the landowners' crops, as an increase in pollinator populations will likely lead to an increase in pollinated and viable crops, ultimately increasing grower profits.

Figure 1. Research site containing six nest shelters aligned along the edge of an alfalfa field.

Figure 2. Example of one nest shelter containing a large nest box in the center and three small nest boxes on either side (six total per shelter).

Figure 3. Temperature and parasitism treatments used in this study. High-temperature shelter with black felt covering back opening (bottom left). Alterations to high-temperature treatment in 2018 with sides and back of the shelter covered with black plastic wrap (top left). White painted roof and back on low-temperature shelters (top right). Back of nest box with backing-absent on left (no felt) and backing-present (with felt and cotton backing) on right (bottom right).

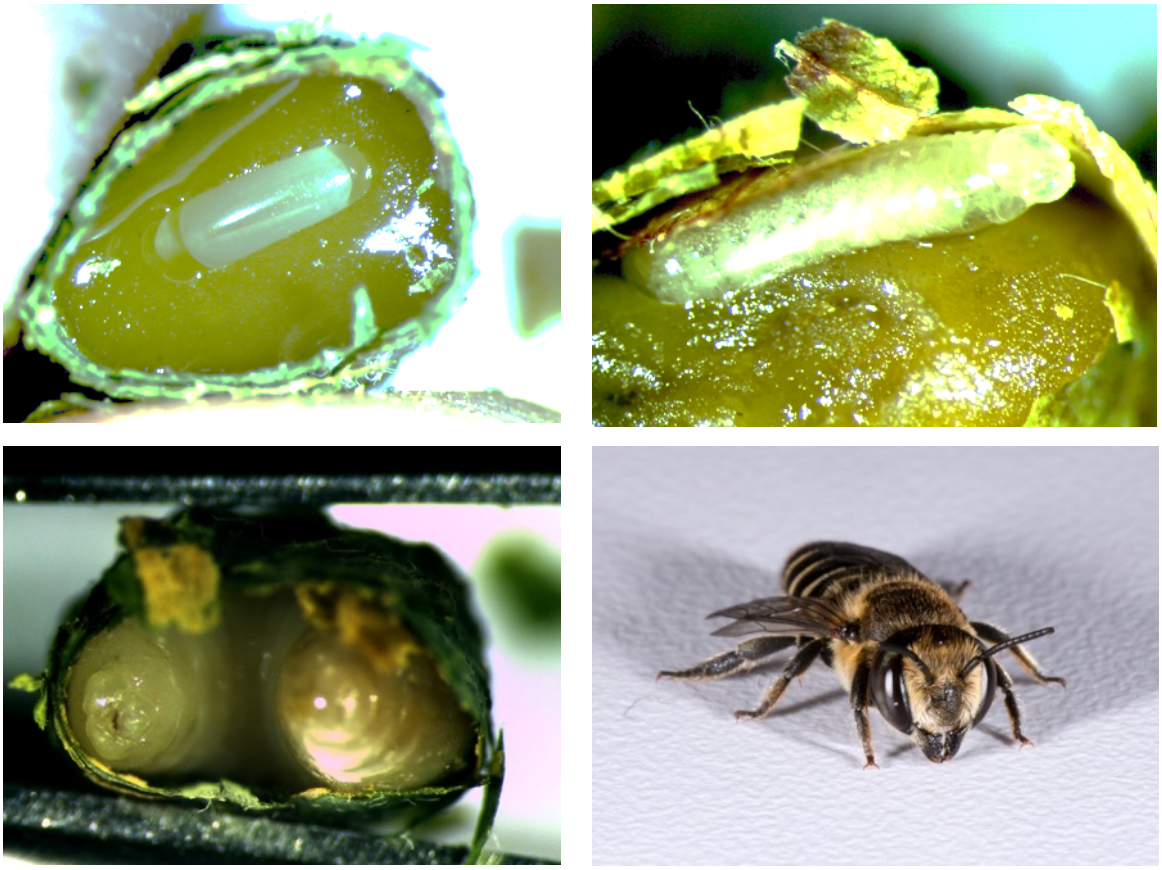

Figure 5. Live *M. rotundata* at different life stages: egg (top left), early instar (top right), late instar (bottom left), and adult female (bottom right).

Figure 4. The six different mortality classes identified in this study: parasitoids (top left); predator, including a clerid larva on the left and a dermestid larva on the right (top right); cleptoparasite (middle left); fungal pathogen (middle right); “pollen ball” (bottom left); and, unknown (bottom right).

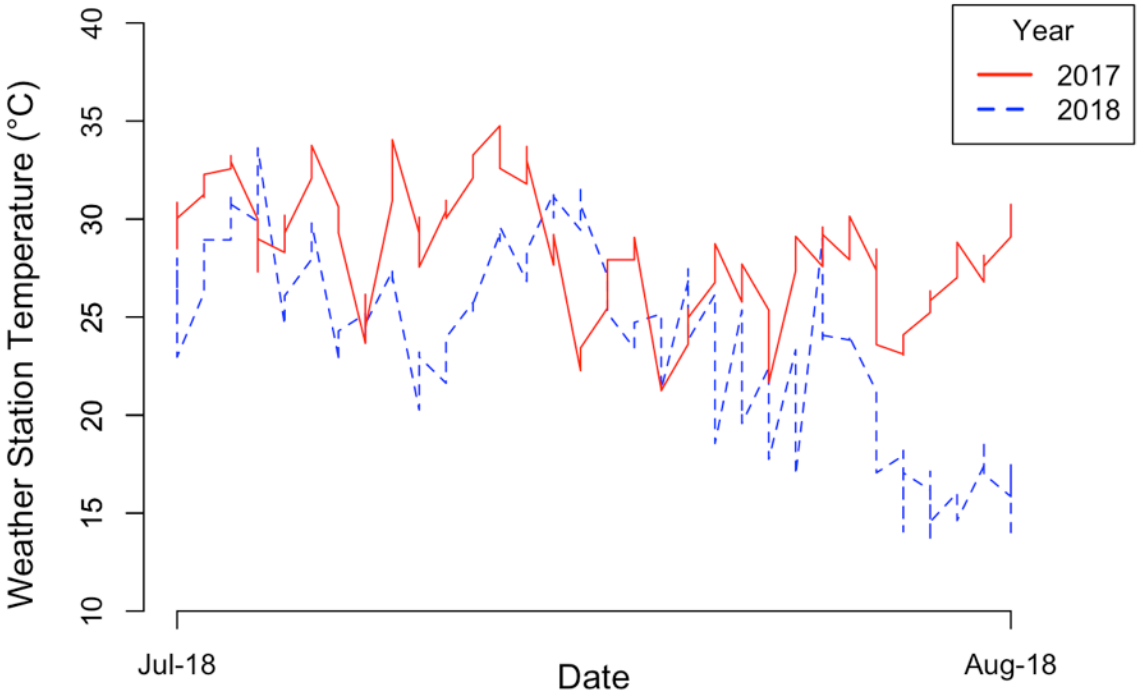

Figure 6. Weather station temperatures (°C) in 2017 and 2018.

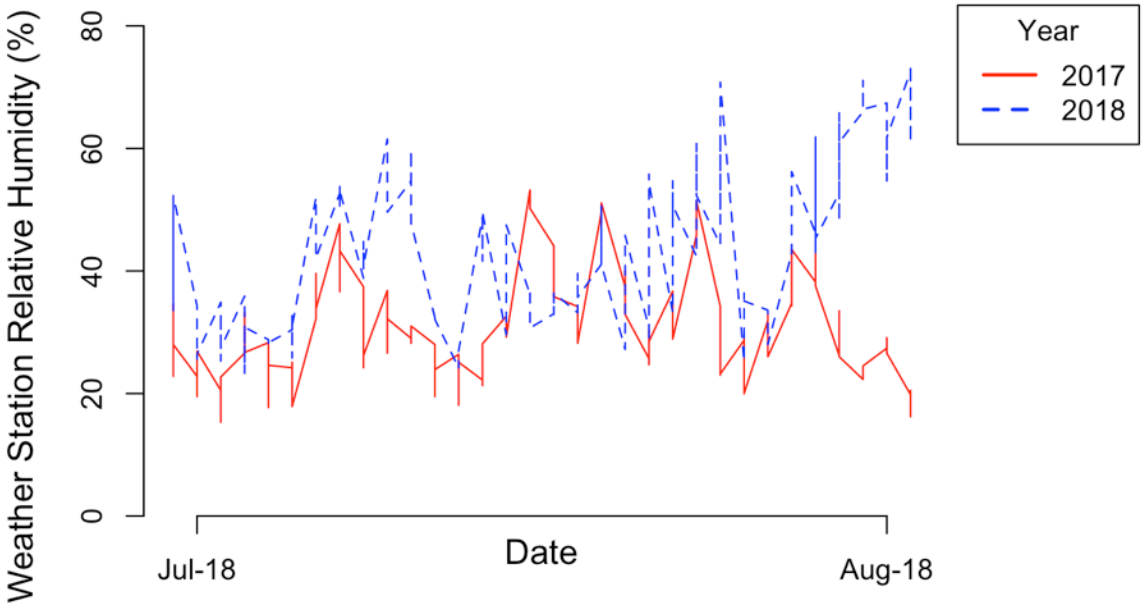

Figure 7. Weather station relative humidities (%) in 2017 and 2018.

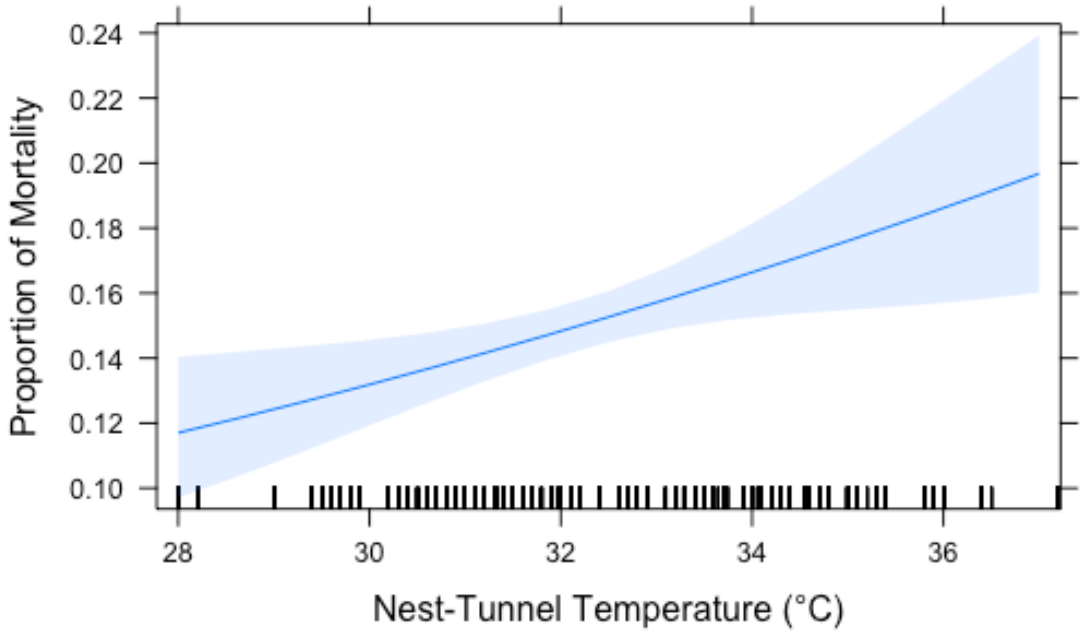

Figure 8. Effects-plot of mortality versus nest-tunnel temperature (°C) from the binomial logistic regression model. Tick marks on x-axis represent recorded temperature measurements.

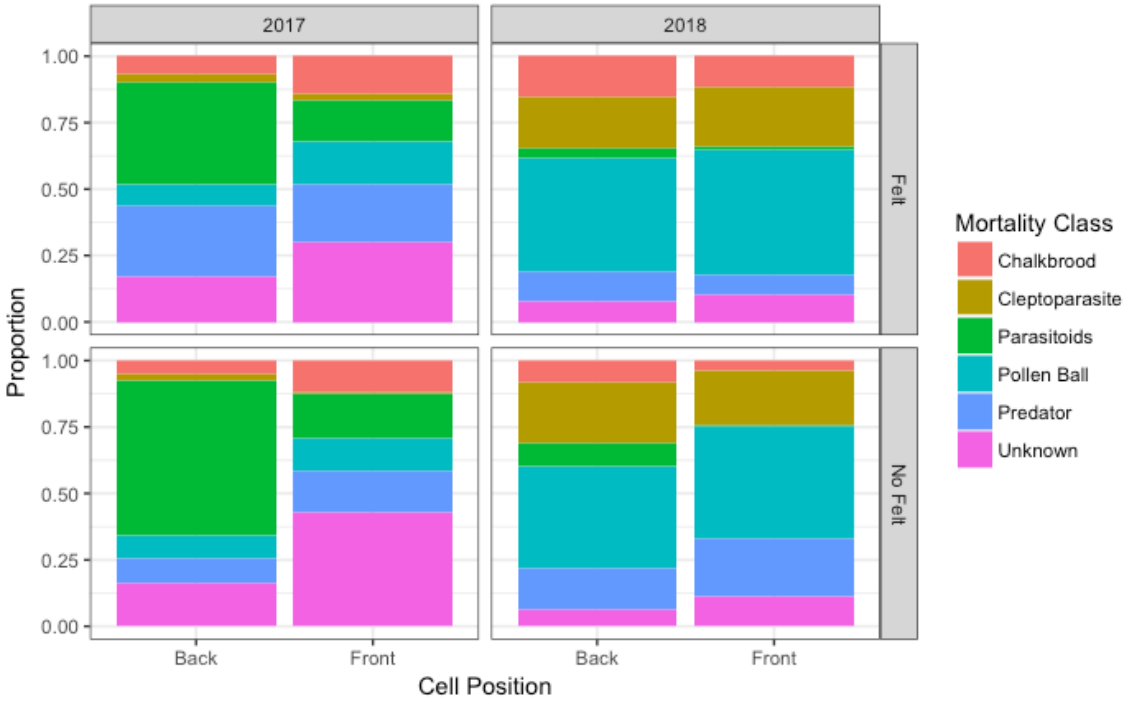

Figure 9. Proportion of mortality classes separated by year, nest-backing material, and nest-cell position.

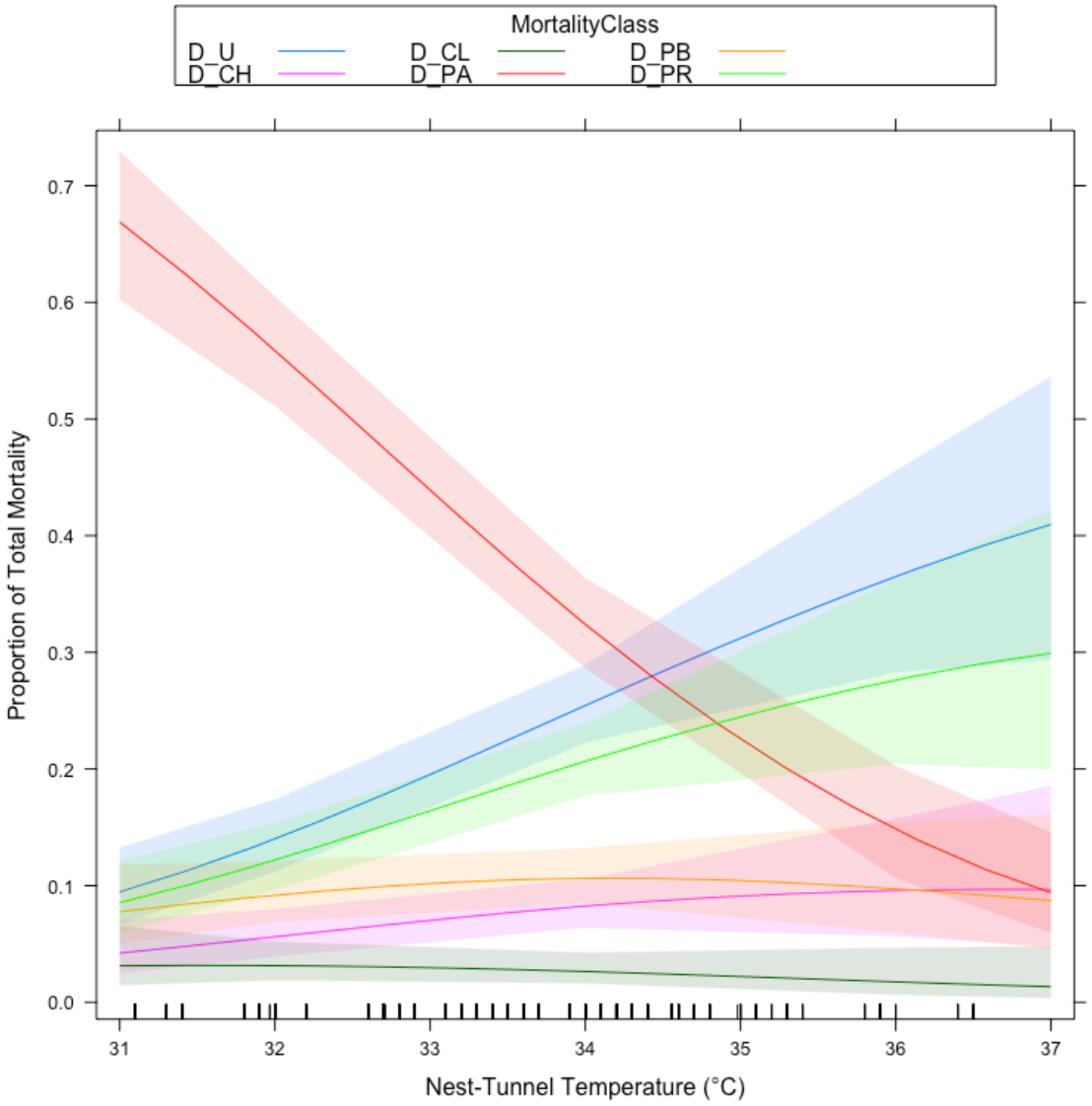

Figure 10. Effects-plot for 2017 from the multinomial logistic regression model of mortality versus nest-tunnel temperature separated by mortality class, where D_U = Unknown, D_CH = Chalkbrood, D_CL = Cleptoparasite, D_PA = Parasitoids, D_PB = Pollen Ball, and D_PR = Predator.

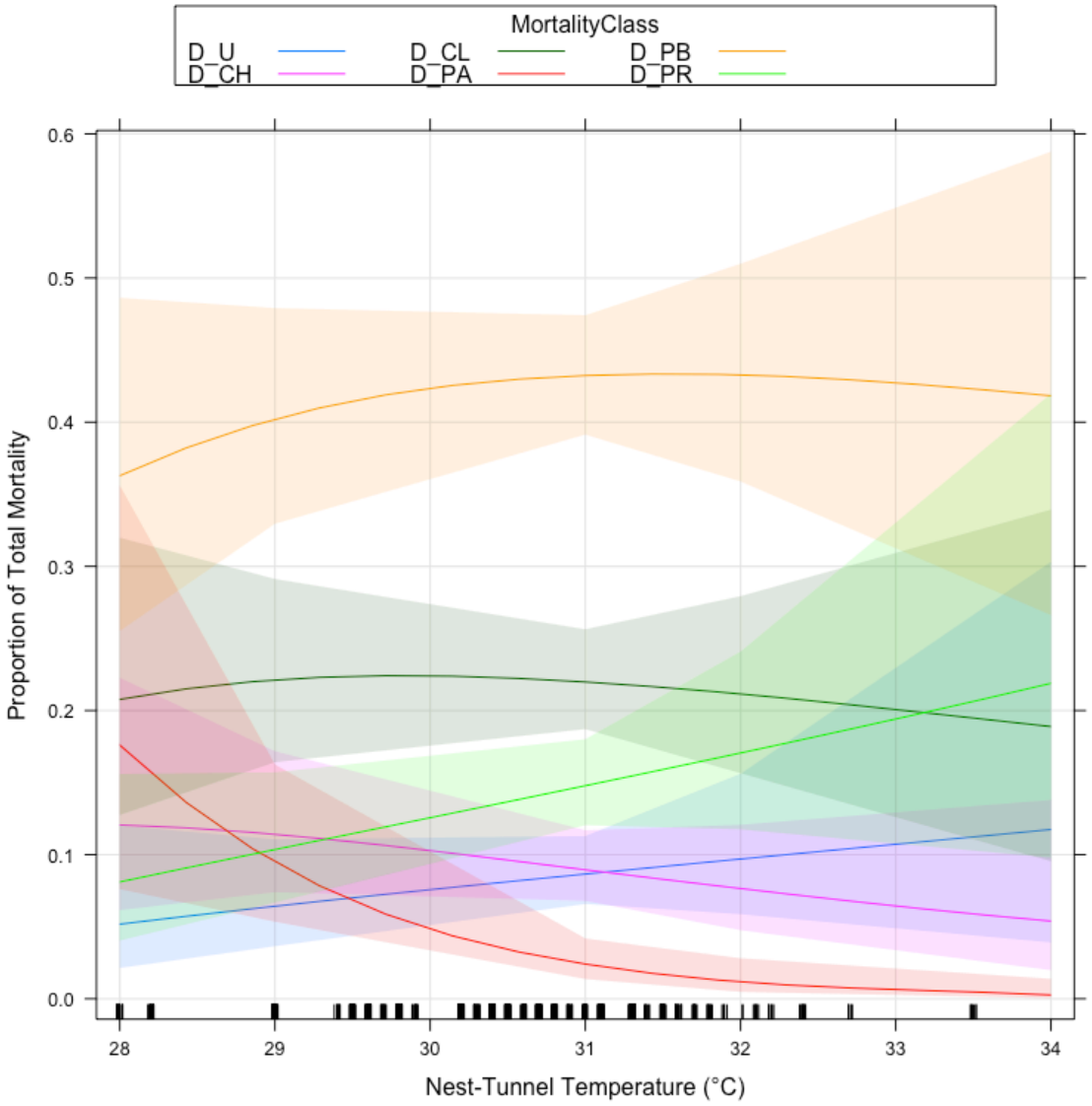

Figure 11. Effects-plot for 2018 from the multinomial logistic regression model of mortality versus nest-tunnel temperature separated by mortality class, where D_U = Unknown, D_CH = Chalkbrood, D_CL = Cleptoparasite, D_PA = Parasitoids, D_PB = Pollen Ball, and D_PR = Predator.

Table 1. Life table for end-of-season dissections for 2017, where x = life stage, aq_x = fraction of deaths from all causes in stage x given that the individual is alive at the beginning of stage x, al_x = fraction of survivors at stage x out of original cohort of al_1, adx = fraction of deaths in stage x from all causes, and ad_{ix} = fraction of deaths in stage x from cause i among al_x living at stage x.

				Probability for Cause of Death in the Presence of Other Causes					
x	aq_x	al_x	ad_x	Predator (ad_{1x})	Parasitoids (ad_{2x})	Cleptoparasite (ad_{3x})	Chalkbrood (ad_{4x})	Unknown (ad_{5x})	Pollen Ball (ad_{6x})
Egg	0.023	1.000	0.023	0.000	0.000	0.004	0.000	0.011	0.008
Early Larva	0.019	0.977	0.019	0.002	0.000	0.000	0.003	0.009	0.004
Late Larva	0.108	0.958	0.103	0.024	0.064	0.000	0.007	0.009	0.000
Total			0.145	0.026	0.064	0.004	0.010	0.029	0.013

Table 2. Life table for end-of-season dissections for 2018, where x = life stage, aq_x = fraction of deaths from all causes in stage x given that the individual is alive at the beginning of stage x, al_x = fraction of survivors at stage x out of original cohort of al_1, adx = fraction of deaths in stage x from all causes, and ad_{ix} = fraction of deaths in stage x from cause i among al_x living at stage x.

				Probability for Cause of Death in the Presence of Other Causes					
x	aq_x	al_x	ad_x	Predator (ad_{1x})	Parasitoids (ad_{2x})	Cleptoparasite (ad_{3x})	Chalkbrood (ad_{4x})	Unknown (ad_{5x})	Pollen Ball (ad_{6x})
Egg	0.092	1.000	0.092	0.000	0.000	0.032	0.000	0.000	0.059
Early Larva	0.015	0.908	0.014	0.000	0.000	0.000	0.001	0.009	0.003
Late Larva	0.051	0.894	0.046	0.021	0.008	0.000	0.014	0.003	0.000
Total			0.151	0.021	0.008	0.032	0.015	0.012	0.062

Table 3. Total mortality and mortality from each cause separated by year (2017 and 2018) and nest-backing material treatment.

	2017		2018	
	Felt	No Felt	Felt	No Felt
Mortality Class	Total Mortality (%)			
Predator	3.36	1.79	1.40	2.82
Parasitoids	4.29	8.53	0.41	1.16
Cleptoparasite	0.41	0.40	2.77	3.75
Chalkbrood	1.07	0.94	1.96	1.12
Unknown	2.67	3.38	1.18	1.24
Pollen Ball	1.18	1.36	5.99	6.49
TOTAL	12.99	16.40	13.72	16.56

REFERENCES CITED

Bellows, T. S., R. G. van Driesche, and J. S. Elkinton. 1992. Life-table construction and analysis in the evaluation of natural enemies. Annu. Rev. Entomol. 37: 587-614.

Bennett, M. M., K. M. Cook, J. P. Rinehart, G. D. Yocum, W. P. Kemp, and K. J. Greenlee. 2015. Exposure to suboptimal temperatures during metamorphosis reveals a critical development window in the solitary bee, *Megachile rotundata*. Physiol. Biochem. Zool. 88(5): 508-520.

Bohart, G. E. 1972. Management of wild bee for pollination of crops. Annu. Rev. Entomol. 17: 287-312.

Bosch, J., and W. P. Kemp. 2005. Alfalfa leafcutting bee population dynamics, flower availability, and pollination rates in two Oregon alfalfa fields. J. Econ. Entomol. 93(4): 1077-86.

Buonaccorsi, J. P., and J. S. Elkinton. 1990. Estimation of contemporaneous mortality factors. Res. Popul. Ecol. 32: 151-171.

Carey, J. R. 1989. The multiple decrement life table: a unifying framework for cause-of-death analysis in ecology. Oecol. 78: 131-137.

Carey, J. R. 1993. Applied demography for biologists with special emphasis on insects. Oxford University Press, New York City, NY.

Carey, J. R. 2001. Insect biodemography. Annu. Rev. Entomol. 46: 79-110.

Davis, H. G., J. D. Eves, and L. M. McDonough. 1979. Trap and synthetic lure for the checkered flower beetles, a serious predator of alfalfa leafcutting bees. Environ. Entomol. 8: 147-149.

Davis, R. S., R. K. D. Peterson, and L. G. Higley. 2011. M-DEC: A spreadsheet program for producing multiple decrement life tables and estimating mortality dynamics for insects. Comput. Electron. Agric. 75: 363-367.

Deevey, E. S. 1947. Life tables for natural populations of animals. Q. Rev. Biol. 22: 283-314.

Dormann, C. F. (and 39 co-authors). 2008. Prediction uncertainty of environmental change effects on temperate European biodiversity. Ecol. Lett. 11(3): 235-244.

Eves, J. D., D. F. Mayer, and C. A. Johansen. 1980. Parasites, predators, and nest destroyers of the alfalfa leafcutting bee, *Megachile rotundata*. West. Reg. Ext. Publ. 32: 1-16.

Fox, J., S. Weisberg, M. Friendly, and J. Hong. 2003. Effect displays in R for generalised linear models. J. Stat. Software. 8(15): 1-27.

Gordo, O., and J. J. Sanz. 2006. Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian peninsula (1952-2004). Ecol. Entomol. 31: 261-268.

Guedot, C., J. Bosch, R. R. James, and W. P. Kemp. 2006. Effects of three-dimensional and color patterns on nestlocation and progeny mortality in alfalfa leafcutting bee (Hymenoptera: Megachilidae). J. Econ. Entomol. 99: 626–33.

Guedot, C., J. Bosch, and W. P. Kemp. 2005. The relative importance of vertical and horizontal visual cues innest location by *Megachile rotundata.* J. Apic. Res. 44: 109–15.

Hegland, S. J., A. Nielsen, A. Lázaro, A. L. Bjerknes, and Ø. Totland. 2009. How does climate warming affect plant-pollinator interactions? Ecol. Lett. 12: 184-195.

James, R. R. 2008. The problem of disease when domesticating bees, pp. 124-141. In R. R. James and T. L. Pitts-Singer (eds.), Bee pollination in agricultural ecosystems. Oxford University Press Online.

James, R. R., and T. L. Pitts-Singer. 2005. *Ascosphaera aggregata* contamination on alfalfa leafcutting bees in a loose cell incubation system. J. Invertebr. Pathol. 89: 176–78.

James, R. R., and J. S. Skinner. 2005. PCR diagnostic methods for *Ascosphaera* infections in bees. J. Invertebr. Pathol. 90: 98-103.

Kjøhl, M., A. Nielsen, and N. C. Stenseth. 2011. Potential effects of climate change on crop pollination. Food and Agriculture Organization of the United Nations, Rome, Italy.

Lafferty, K. D., and A. M. Kuris. 2002. Trophic strategies, animal diversity and body size. Trends Ecol. Evol. 17(11): 507-513.

Mayer, D., C. Johansen, and H. Davis. 1976. Checkered flower beetle: a destructive predator of the alfalfa leafcutting bee. Cooperative Extension, Washington State University, Pullman, WA.

Michener, C. D. 2007. Bees of the world, 2nd ed. The Johns Hopkins University Press, Baltimore, MD.

Moriyama, I. M. 1956. Development of the present concept of cause of death. Am. J. Public Health. 46: 436-441.

Morris, R. F. 1959. Single-factor analysis in population dynamics. J. Ecol. 40: 580-588.

Morris, R. F., and C. A. Miller. 1954. The development of life tables for the spruce budworm. Can. J. Zool. 32: 283-301.

O'Neill, R. P. 2004. Seasonal and spatial patterns of mortality and sex ratio in the alfalfa leafcutting bee, *Megachile rotundata* (F.). M.S. thesis, Montana State University, Bozeman.

O'Neill, R. P., and K. M. O'Neill. 2010. Pollen load composition and size in the leafcutting bee *Megachile rotundata* (Hymenoptera: Megachilidae). Apidologie. 42: 223-233.

O'Neill, K. M., C. M. Delphia, and R. P. O'Neill. 2014. Oocyte size, egg index, and body lipid content in relation to body size in the solitary bee *Megachile rotundata*. PeerJ. 2:e314. DOI 10.7717/peerj.314.

O'Neill, K. M., R. P. O Neill, W. P. Kemp, and C. M. Delphia. 2011. Effect of temperature on post-wintering development and total lipid content of adult alfalfa leafcutting bees (*Megachile rotundata*). Environ. Entomol. 40: 917-930.

Peterson, J. H., and B. D. Roitberg. 2006. Impacts of flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotundata*. Behav. Ecol. Sociobiol. 59: 589–96.

Peterson, J. H., and B. D. Roitberg. 2006. Impact of resource levels on sex ratio and resource allocation in the solitary bee, *Megachile rotundata*. Environ. Entomol. 35: 1404–1410.

Peterson, R. K. D., R. S. Davis, L. G. Higley, and O. A. Fernandes. 2009. Mortality risk in insects. Environ. Entomol. 38: 2-10.

Peterson, R. K. D., M. Buteler, D. K. Weaver, T. B. Macedo, Z. Sun, O. G. Perez, and G. R. Pallipparambil. 2011. Parasitism and the demography of wheat stem sawfly larvae, *Cephus cinctus*. BioControl. 56: 831-839.

Piccolomini, A. M., M. L. Flenniken, K. M. O'Neill, and R. K. D. Peterson. 2017. The effects of an ultra-low-volume application of etofenprox for mosquito management on *Megachile rotundata* (Hymenoptera: Megachilidae) larvae and adults in an agricultural setting. J. Econ. Entomol. 111(1): 33-38.

Pitts-Singer, T. L. 2004. Examination of 'pollen balls' in the nests of the alfalfa leafcutting bee, *Megachile rotundata*. J. Apic. Res. 43(2): 40-46.

Pitts-Singer, T. L. 2008. Past and present management of alfalfa bees, pp. 105–123. In R. R. James and T. L. Pitts–Singer (eds.), Bees in agricultural ecosystems. Oxford University Press, New York, NY.

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.

Pitts-Singer, T. L., and R. R. James. 2005. Emergence success and sex ratio of commercial alfalfa leafcutting bees from the United States and Canada. J. Econ. Entomol. 98: 1785-1790.

Pitts-Singer, T. L., and R. R. James. 2008. Do weather conditions correlate with findings in failed, provision-filled nest cells of *Megachile rotundata* (Hymenoptera: Megachilidae) in western North America? J. Econ. Entomol. 101: 674-685

Pitts-Singer, T. L., and R. R. James. 2009. Prewinter management affects *Megachile rotundata* (Hymenoptera: Megachilidae) prepupal physiology and adult emergence and survival. J. Econ. Entomol. 102: 1407–16.

Price, P. W., and H. Pschornwalcher. 1988. Are galling insects better protected against parasitoids than exposed feeders? A test using tenthredinid sawflies. Ecol. Entomol. 13(2): 195-205.

Richards, K. W. 1984. Alfalfa leafcutter bee management in Western Canada. Agriculture Canada, Ottawa, Canada.

Richards, K. W., and G. H. Whitfield. 1988. Emergence and survival of leafcutter bees, *Megachile rotundata,* held at constant incubation temperatures (Hymenoptera: Megachilidae). J. Apic. Res. 27: 197-204.

Rozen, J. G., and S. M. Kamel. 2009. Hospicidal behavior of the cleptoparasite wasp *Sapyga luteomaculata* and investigation into ontogenetic changes in its larval anatomy (Hymenoptera: Vespoidea: Sapygidae). Am. Mus. Novit. 3644: 1-24.

Royama, T. 1996. A fundamental problem in key factor analysis. Ecology. 77: 87-93.

Scott, V. L., S. T. Tyler, and K. Strickler. 2000. Reproductive biology of two Coelioxys cleptoparasites in relation to their Megachile hosts (Hymenoptera: Megachilidae). Ann. Entomol. Soc. Am. 93(4): 941-948.

Southwood, T. R. E. 1987. Ecological methods with particular reference to the study of insect populations, 2nd ed. Chapman and Hall, London, England.

Southwood, T. R. E., and H. N. Comins. 1976. Synoptic population model. J. Anim. Ecol. 45(3): 949-965.

Stephen, W. P., and J. M. Undurraga. 1976. X-radiography, an analytical tool in population studies of the leaf-cutter bee *Megachile pacifica*. J. Apic. Res. 15: 81–87.

Strohm, E. 2000. Factors affecting the body size and fat content in a digger wasp. Oecol. 123: 184-191.

Tepedino, V. J. 1993. Insemination capability of male *Pteromalus venustus* (Hymenoptera: Pteromalidae), a gregarious parasitoid of *Megachile rotundata* (Hymenoptera: Megachilidae). Psyche. 100(3-4): 137-149.

Tepedino, V. J., and F. D. Parker. 1986. Effect of rearing temperature on mortality, second-generation emergence, and size of adult in *Megachile rotundata* (Hymenoptera: Megachilidae). J. Econ. Entomol. 79: 974–77.

Torchio, P. F. 1978. An eight-year field study involving control of *Sapyga pumila* Cresson (Hymenoptera: Sapygidae), a wasp parasite of the alfalfa leafcutter bee, *Megachile pacifica* Panzer. J. Kansas Entomol. Soc. 52(2): 412-419.

Xu, J., and R.R. James. 2012. Temperature stress affects the expression of immune response genes in the alfalfa leafcutting bee, *Megachile rotundata*. Insect Mol. Biol. 21:269-280.

Vandenberg, J. D., and W. P. Stephen. 1982. Etiology and symptomatology of chalkbrood in the alfalfa leafcutting bee, *Megachile rotundata*. J. Invertebr. Pathol. 39: 133-37.

Whitfield, G. H., and K. W. Richards. 1985. Influence of temperature on survival and rate of development of *Pteromalus venustus* (Hymenoptera: Pteromalidae), a parasite of the alfalfa leafcutter bee (Hymenoptera: Megachilidae). Can. Entomol. 117(7): 811-18.

Whitfield, G. H., and K. W. Richards. 1991. Temperature-dependent development and survival of immature stages of the alfalfa leafcutter bee, *Megachile rotundata* (Hymenoptera: Megachilidae). Apidologie. 23: 11-23.

Yocum, G. D., W. P. Kemp, J. Bosch, and J. N. Knoblett. 2005. Temporal variation in overwintering gene expression and respiration in the solitary bee, *Megachile rotundata*. J. Insect Physiol.. 51: 621-29.