Natural History and Development of *Melittobia acasta* on *Megachile rotunda*

Alan R. Anderson  
*Utah State University*

Follow this and additional works at: [https://digitalcommons.usu.edu/etd](https://digitalcommons.usu.edu/etd)

Part of the [Biology Commons](https://digitalcommons.usu.edu/etd)

**Recommended Citation**  
[https://digitalcommons.usu.edu/etd/8555](https://digitalcommons.usu.edu/etd/8555)

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.
NATURAL HISTORY AND DEVELOPMENT OF *MELITTOBIA ACASTA* ON *MEGACHILE ROTUNDATA*

by

Alan R. Anderson

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

MASTER OF SCIENCE

in

Biology

Approved:

__________________________
Ricardo A. Ramirez, Ph.D.
Major Professor

__________________________
Theresa L. Pitts-Singer, Ph.D.
Committee Member

__________________________
J. Earl Creech, Ph.D.
Committee Member

__________________________
D. Richard Cutler, Ph.D.
Interim Vice Provost of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2022
ABSTRACT

Natural History and Development of *Melittobia acasta* on *Megachile rotundata*

By

Alan R. Anderson, Master of Science
Utah State University, 2022

Major Professor: Dr. Ricardo A. Ramirez
Department: Biology

Alfalfa, *Medicago sativa* (Fabaceae), is among the most economically important crops in North America. Alfalfa requires a pollinator to release the staminal column so that pollen can contact a stigma. *Megachile rotundata* F. (Hymenoptera: Megachilidae) is the primary commercial pollinator for alfalfa. North American bee managers struggle to maintain enough bees to meet commercial needs. One reason for this is attack from parasitoid wasps such as *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae), a multivoltine, idiobiont ectoparasitoid wasp. This occasional but egregious pest lays hundreds of eggs per host and can decimate bee stocks. Chapter Two investigates the *M. acasta* life cycle and developmental temperature when using *M. rotundata*. Twenty replicates of 10-20 female wasps were given four bee prepupae and held at 30°C. Wasp progenies were observed daily until death. Twelve distinct wasp life stages were described, and development from egg to adult occurred within 16-20 days. In three trials, I placed vials containing bee prepupae with wasp eggs along a temperature gradient bar. Vials were monitored for wasp emergence. Using linear regression, I determined that the developmental base temperature for *M. acasta* was 8.5°C and development from egg to adult
averaged 305.8-degree days. Chapter Three determined the bee life stages upon which wasps lay eggs and offspring survive. Forty replicates of 10-20 wasps were provided for each bee stage. Wasps parasitized prepupae and pupae, and offspring survived on non-sclerotized bee body regions. I determined female wasp longevity using resources from bee nests (empty cell, provision mass with and without a bee egg, and a bee prepupa). I placed 40 replicates of each resource into individual vials and added a wasp. Wasp survival averaged five days on empty cells, eight days on both mass provisions, and 34 days on prepupae. Results showed that wasps could emerge in the field among bee nests and survive long enough for newly developed bee prepupae to serve as hosts. This research informs basic *M. acasta* biology when using *M. rotundata* as a host, alerts bee managers to the need for controlling wasps during bee incubation and supports future development and implementation of management strategies.
PUBLIC ABSTRACT

Natural History and Development of *Melittobia acasta* on *Megachile rotundata*

Alan R. Anderson

Alfalfa is one of the most economically important crops in North America. To produce alfalfa seed, a pollinator must release plant reproductive organs by applying pressure to keel petals. The alfalfa leafcutting bee, *Megachile rotundata*, is the primary commercial pollinator of alfalfa. U.S. bee managers struggle to maintain populations due to many mortality factors. One cause is attack from parasitoid wasps such as *Melittobia acasta*. Females can lay hundreds of eggs and have multiple generations, which can decimate bee stocks. Chapter Two investigates the life cycle and base temperature that allows development of *M. acasta* on bee hosts. In each of 20 vials, I exposed four bee larvae (prepupae) to several wasps and kept them at 30°C. Wasp progenies were observed daily until death. I described 12 distinct life stages, and adult wasps emerged in 16 to 20 days. I also raised *M. acasta* from eggs to adults along a temperature gradient bar. Statistical analysis revealed the minimum temperature for development was 8.5°C and that development from egg to adult averaged 305.8-degree days. Chapter Three determined the *M. rotundata* life stages upon which wasps lay eggs and offspring survive. I also determined *M. acasta* female survival on resources found in bee nests (an empty cell, a provision mass with and without a bee egg, and a bee prepupa). Each nest resource was exposed to a wasp (replicated 40 times). Wasps survived an average of 5, 8, and 34 days on an empty cell, either mass provision, and the bee prepupa, respectively. I also exposed 40 replicates of bee larvae and each of the pupal stages to 10-20 wasps. Wasps laid eggs on bee prepupae and their offspring developed to adulthood, while offspring from eggs laid on pupae usually died. This showed that
if a wasp emerged in the field, it could live long enough for newly developed bee prepupae to serve as hosts. This research helps in understanding *M. acasta* biology when using alfalfa leafcutting bees as hosts, alerts bee managers to the need for controlling these wasps during bee incubation and supports future development and application of management strategies.
ACKNOWLEDGMENTS

I would like to sincerely thank Dr. Theresa Pitts-Singer. Since the day she accepted me into her lab, she has provided me with guidance and support. She has been an excellent mentor and given me the opportunity to grow as a scientist and as a person. Dr. Pitts-Singer has demonstrated how to be a great scientist and a strong leader. I would also like to thank Dr. Ricardo Ramirez for being my academic advisor throughout this process. Dr. Ramirez has taught me a great deal about scientific communication in writing and presentations. These are skills I appreciate greatly and will use for the rest of my career. I would like to thank Dr. Earl Creech as well, who graciously joined the committee as the final member. I would like to thank the USDA-ARS Pollinating Insect-Biology, Management, Systematics Research lab for providing a location and funding to conduct this research. Within the lab, I wish to thank Ellen Klomps for her technical support and Dr. Michael Branstetter for helping with wasp species identification. I am grateful to the members of the Western Alfalfa Seed Growers Association who provided me with specimens over the years. Lastly, I would like to thank my family. My parents, Dave and Janetta Anderson, have continually supported my interests and growth as a person over the years. And finally, I would like to thank my wife Lexa Anderson. This thesis would never have been completed without her love, support, and extreme patience.

Alan Anderson
CONTENTS

ABSTRACT ................................................................................................................................. iii
PUBLIC ABSTRACT ..................................................................................................................... v
ACKNOWLEDGMENTS .............................................................................................................. vii
LIST OF TABLES ......................................................................................................................... ix
LIST OF FIGURES ....................................................................................................................... x

CHAPTER 1. Literature Review ................................................................................................. 1
1.1 Introduction ........................................................................................................................ 1
1.2 Alfalfa Seed ......................................................................................................................... 2
1.3 Alfalfa Pollinators .............................................................................................................. 3
1.4 Melittobia spp.................................................................................................................... 7
References ............................................................................................................................... 13

CHAPTER 2. Life Cycle of Melittobia acasta (Hymenoptera: Eulophidae) Using Megachile rotundata (Hymenoptera: Megachilidae) ........................................................................ 24
2.1 Abstract ............................................................................................................................. 24
2.2 Introduction ....................................................................................................................... 25
2.3 Methods ........................................................................................................................... 28
   2.3.1 Melittobia acasta Stock Culture and Preliminary Observations ............................... 28
   2.3.2 Life Cycle Observations ......................................................................................... 29
   2.3.3 Temperature Gradient Bar and Degree Day Model ............................................... 30
2.4 Results ............................................................................................................................... 33
   2.4.1 Life Cycle Observations ....................................................................................... 33
   2.4.2 Temperature Gradient Bar and Degree Day Model ............................................... 38
2.5 Discussion ......................................................................................................................... 39
Acknowledgements .................................................................................................................... 44
References .................................................................................................................................. 45

CHAPTER 3. Mellitobia Acasta Female Longevity and Acceptance of Megachile Rotundata Immatures as Hosts ......................................................................................... 63
ABSTRACT ............................................................................................................................... 63
3.1 Introduction ......................................................................................................................... 64
3.2 Materials and Methods ..................................................................................................... 67
3.3 Results ............................................................................................................................... 70
3.4 Discussion ........................................................................................................................ 72
Acknowledgements ................................................................................................................... 76
References .................................................................................................................................. 77
Conclusion .................................................................................................................................. 87
Table 2-1. Each distinct life stage and phase of *Melittobia acasta* using a *Megachile rotundata* prepupa as a host, the range (first and last day), mean, and median day on which the life phase is observed. Day 0 = the day the egg was laid.  ..........................................................................................................................50

Table 2-2. For temperatures along a gradient bar to determine the *Melittobia acasta* developmental rate from egg to adult, the number of sample vials in which adults emerged, range of emerged adults, range of days to emerge, and average day of emergence........................................................................................................................................51

Table 2-3. Mitochondrial cytochrome c oxidase subunit I gene (COI) identification of *Melittobia acasta* from the stock culture used for this research. All of the vouchers have been deposited in the US National Pollinating Insects Collection...............................................................................................................................52

Table 3-1. Number of vials with a single *Melittobia acasta* female for each *Megachile rotundata* nest resource, number of days until first and last wasp died, and mean (± standard error) days that females survived on resource treatments. ..............................................................................................................................80

Table 3-2. All Tukey’s comparisons for *Melittobia acasta* longevity for the *Megachile rotundata* resource treatments: Empty Cell, Provision Only, Provision with Egg, and Naked Prepupa. ..................................................................................................................................81
LIST OF FIGURES

Figure 1-1. Untripped and tripped alfalfa flowers. A) A honey bee, *Apis mellifera*, on an untripped alfalfa flower. B) An alfalfa leafcutting bee, *Megachile rotundata*, on a tripped alfalfa flower. ..................................................................................................................23

Figure 2-1. On the left, mass emergence of *Melittobia acasta* adults from incubation trays in an incubator. Each tiny speck is a female wasp. On the right, emergence of *M. acasta* females at a *Megachile rotundata* shelter. ........................................................................................................53

Figure 2-2. *Megachile rotundata* prepupal cell with two holes chewed by *Melittobia acasta* females for entering the cell. .................................................................................................................................54

Figure 2-3. X-radiographs showing: A) *Melittobia acasta* female atop *Megachile rotundata* prepupa inside bee cell (5× magnification); B) three *M. rotundata* cells filled with *M. acasta* larvae (2× magnification). ........................................................................................................55

Figure 2-4. Early life stages of *Melittobia acasta* reared on a *Megachile rotundata* prepupa. A) eggs, close-up; B) first larval stage, with close-up of segmented larva in offset; C) second larval stage – early; white larvae; D) second larval stage, at higher magnification – late; grey larvae; E) third larval stage – early; white larvae; F) third larval stage, at higher magnification – late; grey larvae. ........................................................................................................56

Figure 2-5. Prepupal, pupal and adult stages of *Melittobia acasta* reared on a *Megachile rotundata* prepupa. A) defecating prepupae; B) white pupa; C) pink eye pupa; D) red eye pupae: one early and two late; E) adult male; F) adult brachypterous female; G) adult macropterous female; F) both morphotypes of females and offspring in early and late life stages on same host. ........................................................................................................57

Figure 2-6. Developmental rate graph shows the linear regression of the developmental rate with an increase in temperature. The x intercept is at 8.5 degrees Celsius and is the base developmental temperature for *Melittobia acasta* on *Megachile rotundata*. ........................................................................................................58

Figure 2-7. Temperature gradient bar with eight pairs of sample vials held between sets of wooden barriers........................................................................................................................................59

Figure 2-8. Thermocouple probe extended from thermometer (Type J/K, Extech Instruments, FLIR Commercial Systems, Nashua, NH) and secured in ¼ dram vial (covered in red tape) used to reveal temperatures on temperature gradient bar. Other empty vial is shown as an example. ........................................................................................................60

Figure 2-9. The temperature gradient produced across a metal bar with water baths at each end set at -7.5°C and 55°C, respectively. ........................................................................................................61

Figure 2-10. The temperature gradient produced across a metal bar with water baths at each end set at 12°C and 41°C, respectively. ........................................................................................................62
Figure 3-1. Left: A commercial polystyrene bee board with many prefabricated tunnels provided for *Megachile rotundata* females to build nests. Right: Located on the edge of an alfalfa seed production field in northern Utah, USA, a bee shelter harboring bee boards. Bee cells are incubated elsewhere in trays, and trays are usually brought to the field and placed atop bee boards so that adult bees can fly away to mate and find resources (leaves and flowers) for building nests.

Figure 3-2. A.) *Melittobia acasta* females atop the nectar-pollen provision mass inside a leaf-wrapped *Megachile rotundata* nest cell. Bee egg has been removed. B.) Provision with *M. rotundata* egg and *M. acasta* females present. C.) Live *M. rotundata* prepupa removed from nest cell. (Images are 10-30× magnification.)

Figure 3-3. A.) *Melittobia acasta* females probing white eye *Megachile rotundata* pupa before wasp eggs have been laid. B.) Unsclerotized red eye *M. rotundata* pupa with many *M. acasta* females and many eggs laid upon it. C.) Partially sclerotized red eye *M. rotundata* pupa with *M. acasta* female and eggs. D.) Sclerotized black eye *M. rotundata* pupa with *M. acasta* eggs. E.) *Megachile rotundata* male with *M. acasta* eggs on its head that remains in the pupal skin. (Images are 30-40× magnification.)

Figure 3-4. A.) *Megachile rotundata* larvae in the 5th instar about to spin a cocoon. B.) *Megachile rotundata* larvae in the 5th instar actively spinning a cocoon. C.) Hole in top of *M. rotundata* cocoon made by *M. acasta* females to gain access to *M. rotundata* prepupa inside. D.) Silk of cocoon removed to show a *M. rotundata* prepupa with *M. acasta* eggs upon it. (Images are 10-30× magnification.)

Figure 3-5. Percentage of vials containing one of each *Megachile rotundata* nest cell resource that contained live *Melittobia acasta* females on each observation day (Days 0-60). Dotted line represents average day for *M. rotundata* to develop to prepupal stage. Resource Treatments: Empty cell (n = 40); Provision with no egg (n = 38); Provision with egg (and subsequent developing larva) (n = 40); Naked prepupa (no cell) (n = 35).
CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Alfalfa, *Medicago sativa* (Fabaceae), is an economically important forage crop in North America. In the United States, alfalfa is valuable for feeding livestock due to the protein, minerals, fiber, and energy it provides. Nearly 7.2 million hectares of alfalfa hay and silage are produced each year in the United States, worth around $10 billion annually. Alfalfa is the third most valuable field crop in the United States (NAFA 2019). To grow hay of various varieties that express desired traits (acquired through plant breeding or genetic modification), seed production is necessary and is dependent on pollinators.

According to an industry report (Anonymous 2022), the value of the alfalfa seed market was USD 750.1 million across the globe. The North American market was the largest region studied among countries that produce seed and accounted for ~38% of the market in 2018. The United States is followed by Canada for North American seed production that occurs primarily in western states and provinces. The important importers of North American seed are Saudi Arabia, Mexico, Canada, Argentina, China, Peru, Algeria, and Sudan.

In this literature review, I briefly review how alfalfa is grown for seed production in the United States and explain the importance of bees in this production system. I also discuss the problems that can arise in the management of *Megachile rotundata* F. (Hymenoptera: Megachilidae), the alfalfa leafcutting bee, whose populations are managed specifically for this crop. I then focus on the hymenopteran parasites that attack *M. rotundata* and the limited measures used to manage them. Finally, I provide a detailed discussion of *Melittobia* (Hymenoptera: Eulophidae), which is the parasitoid wasp genus whose members use species of
several insect orders as hosts, including solitary bees. The subject of this thesis is *M. acasta* Walker and its life history and use of *M. rotundata* as a host.

1.2 Alfalfa Seed

Alfalfa is often grown in large monoculture fields (Derscheid and Walstrom 1981, Brunet and Stewart 2010). When alfalfa is grown for hay, it is planted in dense stands that grow to be cut and dried in rows. Once the plant has dried, the hay is baled for later use or to market. In contrast, alfalfa seed fields are planted in wide rows spaced at least 45.72 cm apart, although 60.96 cm spacing is optimal. This spacing allows the plants to develop thick stems that support the plant, room for pollinators to penetrate the canopy, and opportunity for pesticide sprays to reach much of the plant surface (Derscheid and Walstrom 1981, Frank 2003). Watering during the summer is restricted in mature seed fields to induce bloom instead of vegetative growth, i.e., “stress without distress,” to dry and warm the canopy during bloom and seed maturation, and to afford favorable conditions for disease control and pollinator activity (Derscheid and Walstrom 1981, Frank 2003, Taha et al. 2016). After flowers are pollinated, the plants require about one month for seeds to ripen. Most seed producers spray herbicides on their fields at an application rate that induces plants to drop leaves (but not die) before seed harvest (Derscheid and Walstrom 1981). The seed is only harvested once at the end of the growing season (Derscheid and Walstrom 1981).

Perhaps one of the most important components of alfalfa seed production is the requirement of a pollinator for the flowers to set seed (Brunet and Stewart 2010, Calderone 2012), even though the plants are self-fertile. The need for a pollinator is due to the flower structure. The keel petal at the base of the flower houses a staminal column. When pressure is applied to the keel petal, the staminal column springs upwards and out from under the keel petal
to crash against the top banner petal of the flower. The process of releasing the plant’s reproductive structures is referred to as ‘tripping the flower’ (Figure 1-1). Tripping is what induces pollination in alfalfa and is why pollinators are so vital for alfalfa seed production (Larkin and Graumann 1954).

1.3 Alfalfa Pollinators

Alfalfa seed growers rely on three main pollinators to trip the flowers (Brunet and Stewart 2010, Pitts-Singer and Cane 2011, Riday et al. 2015, Taha et al. 2016). Honey bees (*Apis mellifera* L.; Apidae) have been used as commercial pollinators for decades, are relatively easy to keep year-round, and can be transported seasonally to many crops due to their containment in hives. Although used for alfalfa pollination in some U.S. states (e.g., California), honey bees avoid landing on the keel petal and instead approach flowers from the side. Therefore, an experienced honey bee can learn to delicately probe the flower corolla for nectar without tripping it. Cane (2002) reported that honey bees only trip 22% of the flowers they visit, rendering them inefficient alfalfa pollinators.

Another optional pollinator for alfalfa seed production is *Nomia melanderi* Cockerell (Halictidae), the alkali bee. This bee is native to the northwestern United States. It is an effective pollinator of alfalfa because it specializes on legumes and has been shown to trip 81% of the alfalfa flowers it visits (Cane 2002). The downside is that it is a ground nesting bee that requires specific soil conditions to nest. The correct soil conditions are not available across the seed-growing regions (Stephen 1959, 1960). Even where the bee is found, growers often must manage natural “bee beds” or initiate artificial ones (with difficulty) by assuring there is subirrigation and an alkaline soil surface. In addition, *N. melanderi* populations can suffer losses if rainfall causes nests to be flooded or interrupts female foraging; it can take many seasons for the bee
populations to recover. These bees also have an array of natural enemies for which there are no known control measures (Stephen 1959, 1960; Pitts-Singer 2008).

The primary commercial pollinator of alfalfa is *M. rotundata*, the alfalfa leafcutting bee. This bee is found in alfalfa’s native range of Eurasia and is likely to have coevolved with this plant and other legumes (Pitts-Singer and Cane 2011, Belavadi and Gupta 2015). Females readily trip alfalfa flowers to collect pollen and nectar about 78% of the time when they visit flowers; nectar-collecting males may also trip flowers 51% of the time (Cane 2002, Pitts-Singer and Cane 2011). *Megachile rotundata* is a cavity nesting bee that naturally nests in existing crevasses and hollowed out twigs but will nest in nesting materials fashioned to create tunnels that are placed in shelters in or near an alfalfa field. The materials often come in three forms: stacked and bound grooved boards, large wooden blocks with holes drilled into them, and molded polystyrene blocks with evenly spaced holes (Stephen and Every 1970). For shelters, some growers use trailers that they can haul in and out of a field, while others have stationary huts (Richards 1972, 1984; Frank 2003).

As the common name indicates, *M. rotundata* females use leaf pieces to make nests. To form individual nest cells, the female bee first cuts oval pieces of alfalfa leaves for lining cells to form a cup. She then collects pollen and nectar to create a mass provision upon which she lays an egg. The cell is finally capped with round leaf pieces. The process continues until many cells are made and the tunnel is mostly filled. Larvae hatch from eggs and consume their entire provision (Richards 1972). At the fifth and final larval instar, each larva finishes the provision, defecates, and spins a cocoon. At this point, the bee remains a prepupa (5th instar in a cocoon) and enters diapause to later spend the winter in this life stage. The following year this bee pupates and emerges to repeat the cycle. Alternatively, the bee continues to develop through pupation and
adult emergence in the current growing season and is commonly referred to as a second-generation bee. The cues that determine the developmental trajectory of the bee prepupa are suspected to include photoperiod, amount of food, and possibly maternal condition (Pitts-Singer 2020).

There are many challenges with using *M. rotundata* as the primary pollinator for alfalfa seed growers in western U.S. states and Canada. Many U.S. growers, however, are unable to maintain a population from year to year and must supplement their bee stocks with bees propagated in Canada (Richards 1972, James and Pitts-Singer 2013). Profit for seed production can be reduced when the price of bees are high, and high prices occur when the supply is short, and demand is high. The most prevalent loss of bees (reportedly > 40% of bee cells in some cases) is due to “pollen balls” for which a cause has not been found. Pollen balls are bee cells in which no egg, a dead egg, or a dead small larva is found on a provision mass (Pitts-Singer 2004, Pitts-Singer and James 2008, James and Pitts-Singer 2013). The high incidence of second-generation bees in the United States results in poor production of overwintering offspring because there are fewer resources for the second generation of bees to make nests and lay eggs before the end of the nesting season. Even when these bees have plenty of resources, they still seem to produce fewer offspring than the parental generation of bees for that season (Krunic 1972). Another major hurdle is accumulation of a bee disease known as chalkbrood (Vandenberg et al. 1980, Stephen and Undurraga 1978, James and Pitts-Singer 2013). Larvae feed on provisions contaminated with fungal (*Ascosphaera aggregata* Shou) spores that thrive in the bee gut and eventually sporulate to fill the body of the larva, usually the prepupa, which gives it a gray, chalky appearance. The spores then spread to other bees when uninfected bees chew
through chalkbrood cadavers and visit other tunnels in the nesting block (Vandenberg et al. 1980, Stephen and Undurraga 1978, James 2011).

Another important challenge in maintaining a bee population is protecting them from natural enemies. There are three general types of pests that plague *M. rotundata* populations: nest destroyers, predators, and parasites. Bee managers attempt to reduce pest populations with pesticides, traps, and other means, although sometimes the cost of pest control exceeds the economic loss due to such pests (Eves et al. 1980).

Nest destroyers primarily consume provisions collected for the juvenile bees, and for *M. rotundata*, include nine species of beetles, two species of moths, and earwigs. These pests kill bees directly or by consuming the food supply. Predators consume either the immature or mature bees. Ants and various social wasps have been reported to eat the bees, but their predatory behavior does not impact the bee population to an extent to be of economic concern. The exception is the checkered flower beetle, *Trichodes ornatus* Say (Coleoptera: Cleridae) whose larvae consume both the bee larva and its provision. A beetle larva chews its way from one cell to the next and can destroy entire nests. Several beetle larvae may be present in a single nest; however, typically only one reaches adulthood per nest. This beetle has been recorded destroying as much as 89% of a bee stock in southeastern Washington (Eves et al. 1980).

Perhaps the most important and impactful pests of *M. rotundata* are parasites and parasitoids. Kleptoparasites are pests that lay their own eggs on the food source of the bee. One common kleptoparasite is *Sapyga pumila* Cresson (Hymenoptera: Sapygidae). A female of this species sneaks into incomplete bee nests and lays her egg alongside the bee egg. When the wasp hatches, it kills the bee egg or larva and then consumes the nest provision. *Sapyga pumila* have been reported to destroy as much as 35% of bee stocks. Simple traps are used around bee shelters
to kill *S. pumila*, but their efficacy is unknown (Eves et al. 1980, Mader et al. 2010). Other kleptoparasites, such as the common cuckoo bee *Coelioxys rufocaudata* Smith (Hymenoptera: Megachilidae) and the dewy bee fly, *Anthrax irroratus* Say (Diptera: Bombyliidae), are less common and cause less economic damage (Eves et al. 1980).

Most of the *M. rotundata* parasitoids sting through the leaf-wrapped cocoon to lay eggs on or in the prepupa. Such parasitoids include *Monodontomerus obscurus* Westwood (Hymenoptera: Torymidae), *Pteromalus venustus* Walker (Hymenoptera: Pteromalidae), and *Tetrastichus megachilidis* Burks (Hymenoptera: Eulophidae). The wasp eggs hatch, and the larvae then feed on the prepupa resulting in its death. One unique parasitoid, *Melittobia* spp., (Hymenoptera: Eulophidae) chews into the cell of *M. rotundata*. This behavior is believed to give them unrestricted access to immature *M. rotundata* that other pests might not be able to reach (Schmieder 1933, Eves et al. 1980). Small *Melittobia* populations have been reported in commercial bee populations in western U.S. states (Eves et al. 1980). This genus can produce high numbers of offspring in a short amount of time (weeks), and occasionally their populations can outbreak and result in the complete devastation of bee stocks (Belavadi and Gupta 2015). Conditions that result in such an outbreak are currently unknown but warrant further study.

### 1.4 *Melittobia* spp.

*Melittobia* is a genus of wasps in the family Eulophidae. It is relatively easy to identify these wasps to the genus level based on size, color, and shape. In contrast, the species in this genus are quite difficult to identify (Dahms 1984a, b) using morphological characters due to their extremely minute size and because the otherwise soft-bodied wasps become deformed and discolored if they dry out when preserved (Dahms 1984a). Other techniques have been employed for making species identifications. Observable mating rituals are species-specific and have been
used for not only identification but also for phylogenetic studies (González and Matthews 2005a, b, Tanner et al. 2011). A male mounts the back of a female and performs a variety of behaviors. These behaviors include the presence or absence of antennal pinching, high and low phases of body movement, leg stroking, and a finale (i.e., specific movements signaling the end of ritualized behaviors and a female receptivity response). The duration of a mating display is also thought to be species-specific (Tanner et al. 2011). Modern molecular techniques for insect taxonomy and systematics have recently been adapted as tools for studies of *Melittobia* (Tanner et al. 2011, Branstetter unpublished).

Although many details of the natural history and host preferences of *Melittobia* species are lacking in the literature, the available general information is valuable. *Melittobia* males are tan to brown and have highly reduced or no eyes or ocelli (Dahms 1984a, Sari et al. 2006). Male wings are present but reduced and only used in mating rituals rather than for flight (Evans and Matthews 1976, Tanner et al. 2011). Males are morphologically distinct compared to other eulophids due to their specialized, highly modified antennal scapes that are swollen and shaped like claws (Dahms 1984b). Males rarely, if ever, leave the host (Dahms 1984a, Abe and Pannebacker 2017). Whether males feed as adults is unresolved with some researchers reporting males feeding as adults (Deyrup et al. 2006a), while others reporting that they do not (Balfour-Browne 1922, Dahms 1984a, Abe et al. 2008). Often when dispersing females (see below) are present, there will be no remaining host resources for males to feed upon (Schmieder 1933). Males can be highly aggressive and often engage in lethal combat (Eves et al. 1980, Innocent et al. 2007, Reece et al. 2007). Some researchers suggest that males might feed on each other or on un-emerged virgin females (Deyrup et al. 2006a). It is also probable that male violence is only for sexual competition and not for access to food (Abe and Shimada 2008).
Females are typically brown to black with orange legs (González and Matthews 2005a, b). They easily can be identified from other hymenopteran parasitoids of *M. rotundata* because the end of their abdomen is rounded while the other chalcids are tapered and pointed (Eves et al. 1980). In addition, their head and abdomen are more dorsally-ventrally flattened compared to other parasitoids (Dahms 1984a). Females are typically 1 to 1.5 mm long and occur in two morphotypes (Cônsoli and Vinson 2004, Cônsoli et al. 2004, González and Matthews 2008). The first morphotype is a non-dispersing brachypterous (short winged) wasp. Brachypterous females are seen when relatively few eggs are laid on a host compared to the size of the host (Schmieder 1933, Cônsoli and Vinson 2004, Cônsoli et al. 2004). They have atrophied wings that are often not fully inflated after emergence, giving them a wrinkled appearance (Cônsoli and Vinson 2004, González and Matthews 2008). These non-dispersing females never leave the host. Instead, they mate and lay eggs on the same host on which they were raised. If their female offspring survive on the little host material that remains as a food resource, they likely become long wing dispersing females if those offspring survive (Schmieder 1933).

The second female morphotype is the macropterous (long wing) disperser. Their functional wings are fully inflated, flattened over the thorax, and extended beyond the end of the abdomen (Cônsoli and Vinson 2004, González and Matthews 2008). These females mate with males before they leave the host (González and Matthews 2008). If they do not mate, some researchers report that the females can find a host, lay male eggs, and mate with their sons. They can then produce female eggs to lay on a host (Schmieder 1933).

Currently, six *Melittobia* species have been identified from managed *M. rotundata* populations: *Melittobia digitata* Dahms, *M. australica* Girault, *M. hawaiensis* Perkins, *M. femorata* Dahms, *M. acasta* Walker, and *M. chalybii* Ashmead. Because of the difficulty in
identifying *Melittobia* species, it is possible that these and other species parasitize *M. rotundata* larvae, that some of the reported species have been misidentified, or that some species are synonymous (Balfour-Browne 1922, Eves et al. 1980, González et al. 2004 a,b, González and Matthews 2005, Silva-Torres 2005a,b, Matthews et al. 2009, Kwon et al. 2012a, Carvalho et al. 2014). Species such as *M. acasta*, *M. chalybii*, and *M. hawaiensis* are suspected to belong to a single species complex (González 2002). To add to the confusion, the wasps in this genus tend to be extreme generalists so that choice of host does not reliably aid in species identification. *Melittobia* are known to attack solitary and social hymenopterans, coleopterans, dipterans, and lepidopterans (Cooperband and Vinson 2000, González et al. 2004a, 2011; Imandeh 2006, Belavadi and Gupta 2015, Lauriant et al. 2019, Lee and Kim 2019).

The act of parasitizing a host generally is uniform across *Melittobia*. When a dispersing female finds a potential host, she spends up to 48 hours evaluating it (Balfour-Browne 1922, Cooperband and Vinson 2000, Cusumano et al. 2010). She walks over the host while tapping it with her antennae. Once she has assessed that the host is appropriate, she punctures it with her sting to inject venom that permanently paralyzes it (Deyrup and Matthews 2003, Deyrup et al. 2006). The sting remains in the host for a few seconds up to a few minutes, and then the female removes the sting and backs away from the site. Host hemolymph flows to the surface upon which the wasp feeds. The female may sting and drink several times before she begins to lay eggs (Schmieder 1933, González and Matthews 2008).

*Melittobia* eggs are sticky and adhere to each other and the surface of the host. They are laid in clutches from 4 to 12 eggs (Balfour-Browne 1922, Abe et al. 2010, Carvalho et al. 2014). Multiple females may simultaneously parasitize the same host so that it is possible for enough eggs to be laid on a given host that individual clusters are no longer clear (Schmieder 1933).
Depending on the species and host size, several hundred eggs can be laid on a single host (Balfour-Browne 1922, González et al. 2004a,b, González and Matthews 2005, Carvalho et al. 2014, Matthews et al. 2009). The eggs hatch in a few days and develop to adulthood in two to three weeks. *Melittobia* are multivoltine, and many generations can complete the life cycle in a single season (Matthews et al. 2009). *Melittobia* offspring sex ratio is extremely female-biased (Abe et al. 2002, 2003, 2014; Cooperband et al. 2003; González et al. 2004a,b). For *M. chalybii*, the male to female ratio is 1:23 with an average population of 175 wasps per bee host (Eves et al. 1980). This means that there is only an average of 7.3 males produced on the host. The *M. acasta* sex ratio ranges from 1:24 to 1:49 (González et al. 2004a,b). If one or two males can kill the rest of the males, then they gain access to all females present.

While the *Melittobia* life cycle and behaviors are generally understood, there is relatively little research to elucidate the details of *Melittobia* life history and developmental biology using *M. rotundata* as the host. The literature has provided several different cases showing that *Melittobia* biology is dependent on the host (González 2002, Imandeh 2006, Torchio 1963). The sex ratio in *M. digitata* was observed to be host dependent, and when *M. rotundata* is the host, the ratio is 4:96 (males:females). When *Trypoxylon politum* Say (Crabronidae) is the host, however, the sex ratio is 2:98 (González 2002). Even the developmental rate depends on host quality and the temperature under which parasitism and offspring development occur. The life cycle of *M. acasta*, which has been raised on many hosts, can be as short as 10 days or as long as 27 days (González 2002). The lack of research on *Melittobia* in the *M. rotundata* management system may be due to the risk of maintaining this genus in a research facility (Torchio 1963). They are extremely small and have strong mandibles, making them difficult to contain and prevent from infesting other bees being managed and studied at a facility (Van Den 1975).
The following research in this thesis aims to fill the void of information concerning the life history and development of *M. acasta* on *M. rotundata*. Chapter Two focuses on the details of the *M. acasta* life cycle using *M. rotundata* as host at a commercially relevant, constant temperature, with further investigation to provide a base temperature for development. Chapter Three is a study of the suitability of *M. rotundata* life stages for *M. acasta* reproductive success. Female survival and longevity on various food resources found in *M. rotundata* nest cells are also evaluated. This research will provide a foundation to study control methods and an understanding of when action should be taken to manage *Melittobia* populations that can plague commercial populations of the alfalfa pollinator *M. rotundata*. 
References


Carvalho, L. S., M. V. O. Beviaqua, and R. B. Querino. 2014. An observation of the parasitoid *Melittobia australica* Girault (Hymenoptera: Eulophidae) and its host, the solitary wasp *Sceliphron asiaticum* (Linnaeus) (Hymenoptera: Sphecidae). Entomol. Am. 120: 43-46.


Stephen, W. P. 1959. Maintaining alkali bees for alfalfa seed production. College Agricultural Experiment Station Bulletin 568, Oregon State University, Corvallis, OR.


CHAPTER 2. LIFE CYCLE OF *MELITTOBIA ACASTA* (HYMENOPTERA: EULOPHIDAE) USING *MEGACHILE ROTUNDATA* (HYMENOPTERA: MEGACHILIDAE)

2.1 Abstract

*Megachile rotundata* F. is the primary commercial pollinator for alfalfa seed production in North America. Managed *M. rotundata* populations are susceptible to several mortality factors including attack by parasitoids. One such parasitoid is *Melittobia acasta* Walker, a multivoltine wasp whose egregious infestations can decimate bee stocks. Details of *M. acasta* life history using *M. rotundata* as host are needed to develop tools for their management. Our objectives were 1) to describe the *M. acasta* life cycle using *M. rotundata* as a host and 2) to determine the *M. acasta* developmental base temperature and propose a degree day model. First, 150-300 *M. acasta* adults were introduced to 60 *M. rotundata* prepupae (10-20 wasp females / four bee prepupae in a vial) upon which they oviposited. Progeny development (at 30°C) was monitored through adulthood. We identified 12 distinct phases of the *M. acasta* life cycle that averaged 19.5 days. Second, vials each containing a *M. rotundata* prepupa with *M. acasta* eggs were positioned across a temperature gradient bar (two vials per temperature). In repeated trials, development of wasps was tracked from egg to adult. Using linear regression analysis, it was determined that the developmental rate for the lower development threshold was 8.5°C. Applying 8.5°C as the base temperature in a degree day model showed an average of 305.8-degree day accumulation from egg to adult. These results provide a framework to assist bee managers for developing *M. acasta* control strategies and timing their implementation.

**Key Words:** alfalfa leafcutting bee, degree day, gradient table, parasitoid
### 2.2 Introduction

The alfalfa industry in North America employs the managed pollinator *Megachile rotundata*, the alfalfa leafcutting bee, for seed production (Richards 1984, Frank 2003, Cane 2002, Pitts-Singer and Cane 2011). *Megachile rotundata* are solitary bees that readily nest in large aggregations in bee boards that are housed in shelters placed in or alongside alfalfa fields. Bee managers release ~100,000-150,000 *M. rotundata* adults per hectare (~40,000-60,000 per acre) for contract seed production (Pitts-Singer and Cane 2011). These bees are managed by pollinator supply companies as well as by farmers who care for their own populations. Bee management involves the overwintering of diapausing prepupae (= cocooned 5\textsuperscript{th} instars), early summer incubation for adult emergence, summer nesting period, fall storage and cleaning of nests, and preparation of prepupal cells for overwintering (Richards 1984). Among the many challenges of managing large populations of solitary bees are the hymenopteran parasites and parasitoids that attack the bee larvae or consume their provision masses during the nesting season, while in storage through fall and into winter, and during summer incubation (Richards 1984, James and Pitts-Singer 2013).

There are several parasitoids that can cause major economic damage to managed *M. rotundata* populations (Eves et al. 1980). The most well-known is *Pteromalus venustus* Walker (Hymenoptera: Pteromalidae), and bee managers use dichlorvos pest strips as a reliable treatment for this parasitoid during fall storage and incubation (Richards 1984). However, recent (2015) outbreaks of *Melittobia acasta* have caused the loss of hundreds of thousands of bees in Utah and Idaho during incubation, with unknown damage to bee populations in the field. There have been subsequent reports of wasp outbreaks but with less catastrophic impacts, since managers have become more aware of this pest and destroyed infested bee populations while monitoring for new outbreaks. *Melittobia acasta* are extreme generalists that are primary or secondary parasitoids of
species of Hymenoptera, Lepidoptera, Coleoptera, and Diptera (Gonzáles et al. 2004a,b, Lee and Kim 2019). Their natural history, including durations of stages of the life cycle, sex ratio, and offspring per host, depends on the host species and size (Trexler 1985, González 2002, Imandeh 2006). No current management protocols are known to curtail *M. acasta* outbreaks in *M. rotunda* populations other than destroying infested bee stocks.

All known *Melittobia* adults are sexually dimorphic and protandrous. Males have reduced wings, small or no eyes, and swollen claw-like antennal scapes used in courtship displays (Dahms 1984a,b, Sari et al. 2006). There are two female morphotypes: brachypterous (short-winged) non-dispersers and macropterous (long-winged) dispersers. Brachypterous females have highly reduced wings that often do not fully inflate, giving them a wrinkled appearance (Cônsoli and Vinson 2004). They only arise when relatively few eggs are laid compared to the size of the host and they never leave the host cell (González and Matthews 2008). Macropterous females have wings that lay flat over the abdomen and extend at least the length of or beyond it (Cônsoli and Vinson 2004, Cônsoli et al. 2004, González and Matthews 2008).

When *Melittobia* females (reportedly *M. chalybii* Ashmead in Torchio 1963) use *M. rotunda* as a host, they chew through the leaf pieces and cocoon to access the bee prepupa inside (Figure 2-2). They sting the prepupa to release venom that halts its development and causes paralysis (Deyrup et al. 2006). After feeding on hemolymph that exudes from the sting site, each female lays a clutch of eggs on the host body (Figure 2-3A). (Schmieder 1933, Torchio 1963, González and Matthews 2008). Clutches are composed of 4-12 eggs. Various species of *Melittobia* females have been reported to invade a single host cell and lay multiple clutches such that hundreds of eggs cover the host (Balfour-Browne 1922, Schmieder 1933, González et al. 2004a,b, Matthews et al. 2009, Carvalho et al. 2014). Simple life cycles have been described for
a few *Melittobia* species on different hosts, including *M. rotundata*. For example, *M. digitata* and *M. australica* development on *M. rotundata* is reported as follows: eggs hatch in 2-4 days, larval stages require 8-14 days, and the pupal stage lasts 5-8 days before adult emergence. The entire life cycle is 16-24 days for *M. digitata* and 15-26 days for *M. australica* (González 2002). Prior to our study here, there have not been life cycle observations for *M. acasta* on *M. rotundata* under standard management conditions used for commercial bee operations.

Knowing when insects such as *Melittobia* spp. become active or reach a critical life stage can help in managing them when they are a pest of managed bees. By exploiting the fact that an insect’s developmental rate is directly linked to the temperature of the environment (Powell and Logan 2005, Dixon et al. 2009, Keena and Moore 2010, Regniere et al. 2012, Prieto and Destouni 2015, Jiang et al. 2016, Morales-Ramos and Rojas 2017, Geng and Jung 2018, Schoeller and Redak 2018), predictive models can be constructed. Development of *M. acasta* on *M. rotundata* occurs during managed incubation, the nesting season, and fall storage, for which temperatures can be controlled or at least recorded. To study *M. acasta* development in relation to temperature throughout the year, a degree day model is needed and requires determination of a base temperature. Degree days are units of thermal energy accumulated over time, so once an insect’s base temperature has been exceeded, degree days begin to accrue. The simplest degree day model subtracts the base temperature from the average daily temperature \[ \frac{(\text{Daily High} - \text{Daily Low})}{2} \] to calculate the number of degree days for that day; also, more precise calculations can use averages of high minus low temperatures for each hour or minute (Prieto and Destouni 2015, Dupuy et al. 2017). To find an insect’s base temperature using a simple linear degree-day model (Damos and Savopoulou-Soutani 2012), the insect at a certain life stage is exposed to an incremental temperature series and monitored until a desired life stage is
reached. Then a best fit line for y-axis = developmental rate (which is 1/days of development) and x-axis = temperatures can be used to calculate the x intercept. The x intercept is the point where the developmental rate is equal to zero, and, therefore, is the base temperature. The number of degree days required to develop to a particular life stage is calculated by multiplying the number of degree days acquired at a given temperature by the number of days of development (Regniere et al. 2012, Jarošíka et al. 2014, Jiang et al. 2016, Milosavljevice et al. 2017).

While life cycles of a few Melittobia species have been described (Torchio 1963, Eves et al. 1980, González 2002, Matthews et al. 2009), there is sparse detailed information on the life cycle of M. acasta recently reproducing in alarming numbers under the standard environmental conditions used for commercial M. rotundata management. Objectives of this study were to describe distinguishable life stages of M. acasta when M. rotundata is the host, including the range of days that each life stage may be present in a population, and to create a simple degree day model for the life cycle (egg-adult). Obtaining this knowledge would help bee managers devise appropriate pest control using insecticides, traps, or other management strategies.

2.3 Methods

2.3.1 Melittobia acasta Stock Culture and Preliminary Observations

The tiny wasps found in M. rotundata populations from Utah and Idaho were identified as M. acasta using a combination of morphological characters (Dahms 1984a,b), courtship display (Tanner et al. 2011), and a query of newly derived sequences in the Barcode of Life Database (Table 2-3). To initiate a culture of M. acasta for this research, I obtained M. rotundata cells known to be infested with parasites from a Utah bee manager in the fall of 2016 and kept them in cold storage (4-5°C) over the winter. Bee cells were x-radiographed (24 kV for 12 s at
1× magnification) with a computed radiography high-resolution system (Faxitron X-Ray LLC, Linconshire, IL) to produce digital images from which prepupal cells and parasitized cells could be diagnosed and sorted. In these images, healthy *M. rotundata* prepupae appear as plump, white larvae inside cocoons. *Melittobia acasta* within a cell appear like grains of sand or debris (Figure 2-3B), which contrasts with the definitive shape of other larger hymenopteran prepupal parasitoids. In spring, cells containing *M. acasta* were placed individually into 7 mL borosilicate glass scintillation vials (DWK Life Sciences, LLC, Millville, NJ) that were plugged with thick cotton that allowed airflow but confined later-emerging adult wasps. The cells in vials were housed in a dark incubator at 30°C (typical *M. rotundata* incubation temperature) at a workshop that served as a quarantine building to avoid the chance of any escaped wasps infesting other managed bees housed in a different nearby facility. After several days, 100-200 female wasps emerged from each cell. Chilled females (n = 10-20) were then transferred with a fine artist paintbrush to a new vial containing four *M. rotundata* prepupal cells. Three days later, the four newly infected bee cells were separated into individual vials. The culture was perpetuated for laboratory studies by repeating this process.

### 2.3.2 Life Cycle Observations

We exposed *M. rotundata* prepupae to *M. acasta* females from our culture to observe life stages. Four wintering *M. rotundata* prepupal cells were placed into each of 15 of the same type of vials used for the stock culture. At room temperature, 10-20 newly emerged *M. acasta* females from culture were transferred into each vial with the bee cells. The transferred females were presumed to have mated with non-dispersing males inside bee cells before chewing out to disperse. Wasps were given 48 h to chew into the new host cells and to oviposit. After this time, the cap of each *M. rotundata* cell was cut open. Prepupae upon which *M. acasta* eggs had been
deposited were removed from cells and individually stored (without the cell) in 60 new cotton-plugged glass vials. No adult females were transferred into the new vials. Vials were held in a dark 30°C incubator for the duration of the study. Exposed prepupae with developing wasps were viewed through the vial once a day under 10× to 30× magnification to record descriptions of observed life stages and any detectable change in size, shape, and color within each stage. From these records, we classified all distinguishable life stages (and phases) and documented the first, average, and last day on which each stage was observed.

2.3.3 Temperature Gradient Bar and Degree Day Model

To track *M. acasta* from egg to adult at various temperatures, a temperature gradient bar was constructed (Figure 2-7 to 2-10). Because no previous study has attempted to reveal a base temperature for *M. acasta* development, two temperature studies were performed, first with a broad range of temperatures and second with a narrow range. Preliminary tests showed that constant 10°C inhibited *M. acasta* egg hatch, while constant 45°C killed the eggs (see section 2.4.2). Therefore, our gradient bar was adjusted to provide a temperature range that extended to those extreme temperatures for the first study. Temperatures along the bar were measured and monitored with a thermocouple inside an empty ¼ dram clear glass vial (Discount Vials LLC, Madison, WI) that was moved along the bar to assure vials with samples were accurately placed at the assigned temperature positions (Figure 2-8).

For both studies, we obtained fresh *M. rotundata* prepupal hosts from cold storage. Prepupae were removed from cells since preliminary efforts revealed that leaf-wrapped bee cells enclosed in vials were prone to extensive mold growth. Each naked *M. rotundata* prepupa was placed in a 7 mL borosilicate glass scintillation vial along with 10-20 *M. acasta* females from the wasp culture. Wasps remained with prepupae for 36-48 h to allow time for oviposition. Hosts
that had many wasp eggs (and no larvae) were selected as samples for experiments and transferred to ¼ dram clear vials, taking care that no adult wasps remained with them. Two sample vials were randomly assigned to each desired temperature. There were spaces created along the bar with wooden barriers with enough room for four ¼ dram vials at each designated temperature position (Figure 2-7). Therefore, to avoid an edge effect at each position, sample vials were placed in the middle two spaces, and two empty vials were placed on the outer spaces as buffers. In addition, the positions of the middle vials were swapped every 24 h to compensate for any location effect.

The first broad temperature study was performed three times, and each trial lasted 30 days so that the entire study was performed from 8 August - 10 November 2019. The 30-day limit per trial was assumed to allow ample time for wasp development, because the surviving wasps were expected to have a quick life cycle at warm temperatures but, at low temperatures, were predicted to reach the prepupal stage and enter diapause without further development. Also, the duration reflects the timeframe for *M. rotundata* commercial incubation. Samples were placed every five degrees from 10°C to 45°C so that there were eight pairs of vials per trial (Figure 2-7
Figure 2-9). After each trial, the cold bath component of the gradient table was defrosted and then rechilled for the next trial.

The second temperature study tested wasp development at a narrower range of temperatures derived from results in the first study. Three replicates of this study ran from 12 January - 23 April 2020, and samples were placed from 22°C to 34°C on spaces of the bar for every other (even) degree until there were seven pairs of vials per trial (Figure 2-7 & Figure 2-10).

The contents of the vials were observed once a day under 10× or 30× magnification. Each pair of vials from a given temperature treatment were removed from the temperature bar at the same time. Vials were away from the bar for only a few minutes at a time for observation and
removal of any emerged adults. For each vial, the number of macropterous females was recorded, and the days for macropterous females to reach adulthood were averaged. Vials in which no adults emerged were excluded from analysis.

Due to the high mortality at many temperatures in the first study, we used only the results from the second study to obtain the average time (in days) per vial for wasps to develop from egg to adult for calculating developmental rate (= 1/ average days) at each temperature. A generalized linear mixed model (SAS version 9.4; SAS Institute 2013) reported a significant effect of temperature on developmental rate (F = 109.75, d.f. = 6,16, P < 0.0001) but no effect of experimental trial replicate (F = 1.76, d.f. = 2,16, P = 0.20). Because there was no effect of trial, a linear regression analysis (SAS version 9.4; SAS Institute 2013) was performed on the pooled data from the three trials. The best fit line was used to calculate the x intercept, which is the base developmental temperature for *M. acasta* development. The base temperature was used to obtain the number of degree days needed for egg-adult development for each vial. Specifically, the base temperature was subtracted from each temperature along the gradient for which there was a vial and that number multiplied by the average number of days for wasps to develop from egg to adult.

2.4 Results

2.4.1 Life Cycle Observations

Preliminary observations revealed that within 24 h of being placed with bee cells, *M. acasta* females entered the cell. They crawled along the cell surface while tapping it with their antennae and then chewed a minute, round to oval hole (diameter = 0.5 mm) (Figure 2-2). The position of the hole varied, and sometimes wasps crawled between the leaf layers of a cell before chewing a hole. A single female is capable of chewing into a cell, but multiple females also have
been observed to cooperatively create a hole, as has also been documented for *M. digitata* (Deyrup et al. 2005). Once inside the cell, females first inspected the prepupa by walking over its surface. They eventually inserted the sting ~ 75% of its length into the prepupa. Deyrup et al. (2006) reported that venom is injected at this time to permanently paralyze the host. After several seconds to a few minutes, the females withdrew the sting and then drank hemolymph that exuded from the wound. This process was repeated many times before females laid eggs on the surface of the prepupa.

Using *M. rotundata* as a host, we were able to identify seven life stages in the *M. acasta* life cycle (Figure 2-4, Figure 2-5 & Table 2-1). In this study, it was not possible to determine if a first instar occurred while the larva was inside the egg chorion, and we could find no published report of the number of instars for eulophid wasps in general. Therefore, the larva that hatched from the chorion may have been in the 1st or 2nd instar, and we resort to describing larvae in the order they occurred and as early or late (pre- and post-feeding) without assigning a definitive instar.

**Stage I. Egg:** Eggs were laid within 48 h of introducing a female to a fresh bee cell (Table 2-1, Figure 2-4A). For describing development timing in this life cycle, the day of oviposition was Day 0. Several clutches of eggs (n = 4-12) developed synchronously from a single host prepupa. In some cases, individual clutches were indistinguishable and covered most of the host surface. In other cases, there were individual eggs or clutches of less than 4 eggs. Eggs were white and translucent, approximately 0.10 mm wide and 0.4-0.5 mm long. The egg usually was oriented so that its length was flush against the surface of the prepupa.

**II. 1st Larva:** Less than 24 h after oviposition, larvae began to emerge from the egg chorion and were nearly identical in size to the egg (Table 2-1, Figure 2-4B). Compared to the
eggs, the larvae were obviously segmented. Most larvae emerged within the first day (Day 1) after oviposition, but a few emerged on the next day.

III. a,b. 2nd Larva: The second larval stage had an early and a late phase designated by a change in body color (Table 2-1, Figure 2-4C). This larval stage was twice the size (width and length) of the previous larva being 0.2 mm wide and 0.8 - 1.0 mm long. In addition, the early phase was opaque and creamy white or yellow in color. This phase appeared as early as Day 1, and larvae in this stage were present up to Day 4; however, on average, larvae reached this stage on Day 2 (Table 2-1). The later phase of this larval stage was notable because the color changed to dark gray (Figure 2-4D), which appeared to be the result of the onset of feeding and the filling of the gut. As is characteristic for all aprocrta, the *M. acasta* larval gut was closed until just before pupation (Wharton et al. 2004). This stage was seen between Days 1 and 6, with an average occurrence around Day 3 (Table 1).

IV. a,b. 3rd Larva: The third larval stage also had an early and late phase that were differentiated by color (Table 2-1, Figure 2-4E/F). Newly molted larvae in the early phase were again creamy white but much larger than the previous larval stage. The size increase was dependent on the number of developing wasps on the single host. When relatively few larvae were present, they were larger than when many were present. Usual size was 0.3 mm wide and 1.2-1.5 mm long. This stage appeared as early as Day 2, but the average larva experienced this stage on about Day 3 (Table 2-1). Some early 3rd larvae remained in this stage until Day 5. The late 3rd larval phase was distinctive due to the gray coloration and the filling of the gut. Individuals in this late phase were observed for about one week with an average occurrence around Day 5 (Table 2-1).
V. Defecating Larva: The ultimate larval stage, the prepupa, was similar in size to larvae in the previous stage (Table 2-1, Figure 2-5A). In this stage the larva defecates as indicated by a string of frass attached to or lying next to each larva. The prepupa was creamy white after the gut was voided and apparently feeding ceased because the gray coloration did not return. Typically, wasps entered this stage between the 3rd and 10th day after oviposition, which averaged to about 6 days (Table 2-1).

VI. a-d. Pupa: The *M. acasta* pupa was the final immature stage and had four recognizable phases. The first phase was the white pupa (Figure 2-5B). This phase revealed the start of the segmentation of the wasp into the adult tagmata. The pupal body was soft and ranged from 1-1.5 mm in length. This phase could occur as early as four days after eggs were laid, but on average appeared by Day 8. The pink eye pupa was the second phase (Figure 2-5C). The eyes turned from white to pink, the body turned yellow, and subsections of the tagmata became more distinct. The pink eye phase was seen on average by Day 10 (Table 2-1). The third pupal phase was the red eye phase (Table 2-1, Figure 2-5D) and was the shortest of the recognized pupal phases. It was not uncommon to miss this phase for some individuals when only making observations once per day. The eyes turned to deep red, and sclerotization of the body had begun. Males and females were distinguishable in this phase. The earliest detection of this phase was at Day 7, but the average time happened before Day 12. The sclerotized, melanized pupa was the final pupal phase (Table 2-1, Figure 2-5D). Males developed faster than females, spending less time as a fully sclerotized pupa. Male pupae were tan to brown, while female pupae were black. All eyes of pupae darkened to a rusty red. The earliest observance of this final phase was 7 days after eggs were laid, but the average time was at Day 12.
VII. Adult Male: Adult *M. acasta* males appeared as early as Day 7 (Table 2-1, Figure 2-5E). Early emerging males were observed killing pupae of un-emerged males. On average, males occurred around Day 15. They varied in length from 1-1.5 mm, with a tan head and thorax and dark brown abdomen. Males had short wings that they often held straight up above their backs. Wings were not used for flight but were vibrated during mating displays. Males had no or highly reduced eyes and ocelli, and their antennae had distinct scapes that were swollen and claw-like. These stylized antennae also were used during mating displays. Male-male combat and mating displays were observed. Male-male interactions varied with some males co-existing without aggression and others engaged in combat and eventual dismemberment of appendages. It was common to see males with missing parts of a leg or the entire leg.

VII. Adult Female: *Melittobia acasta* females were brachypterous (Figure 2-5F) or macropterous (Figure 2-5G) but similar in size, with length of 1-1.5 mm. The wings of brachypterous females usually did not fully inflate and remained wrinkled. These females held their wings vertically above their backs. When reared on *M. rotundata* as a host in this study, this morphotype was rare. These females developed faster than the macropterous ones, first appearing on Day 7 with an average occurrence by Day 15 (Table 2-1). These females oviposited, but their larvae failed to reach adulthood because there was very little host tissue left as food for them. Most *M. acasta* females were macropterous. They were black with fully inflated wings held horizontally over their backs. The first macropterous female appeared on Day 9 with an average time being Day 19.5 (Table 2-1). It also was possible for both morphotypes to lay eggs on the same host, with only the macropterous females leaving the host cell later (Figure 2-5H).
2.4.2 Temperature Gradient Bar and Degree Day Model

We had investigated *M. acasta* developmental rate on *M. rotundata* prepupae at a broad and a narrow temperature range. The first was from 10°C to 45°C, and few wasps reached adulthood in all the trials. Eggs at 10°C did not hatch within the 30 days of each trial. At 15°C, eggs hatched but continued to develop through larval stages in only 50% of the samples. In 10-14 days, those larvae reached only the early or late 3rd larval stage. Similar egg to late instar development was seen at 20°C. At 25°C, only half of the wasps became adults, while the others reached and remained at the 3rd larval stage. For all six samples at 30°C (2 sample vials each from 3 trials), the wasps reached adulthood. Only one wasp became an adult in a vial at 35°C. The remaining wasps failed to pupate and appeared unhealthy, i.e., darker in color, deflated instead of plump, and smaller than healthy larvae. All wasps at 40°C died as eggs or just after hatching, and no eggs hatched at 45°C. At these high temperatures, the host prepupae appeared normal but, as the experiment progressed and after the wasps died, they became a dark yellow, deflated, and soft. Because so few wasps failed to reach adulthood across this broad temperature range, we focused on a narrow temperature range for calculating the base temperature.

The second, narrow temperature range used for rearing *M. acasta* was from 22°C to 34°C, and many wasps survived to adulthood. In all six sample vials at 22°C, 24°C, 26°C, 28°C, and 32°C, wasps reach adulthood and contributed to the model development. At 30°C, wasps became adults in only four of the six vials. In the other two vials, the host prepupae seemed to be unhealthy and shriveled. Only in two of the vials at 34°C did a few wasps become adults. Wasps in the other 34°C vials ultimately failed to pupate.

Using the pooled data set only from the experiment with the narrow temperature range, we determined the base temperature for *M. acasta* to be 8.55°C (Figure 2-6). This base temperature was subtracted from the temperatures for which there were samples along the
gradient and multiplied by the average number of days for wasps to reach adulthood in those samples. Resulting degree day values for egg-adult development ranged from 257.09 to 382.29 with an average of 305.8-degree days (Table 2-2).

2.5 Discussion

We were able to observe development of *M. acasta* from egg to adult on *M. rotundata* as a host under standard conditions in the commercial management of this pollinator. We also determined a developmental base temperature (8.55°C) for this parasitoid wasp so that a degree day model can be used to predict adult emergence timing. We documented the timing of expected holometabolous insect life stages for *M. acasta*: egg, larva, pupa, and adult. However, we were not able to observe whether a first instar was passed inside the egg chorion because such a life stage may have been passed very quickly (hours not days) and was not observable without more frequent observations at higher magnification than afforded by a stereomicroscope. However, for the practical purpose of monitoring *M. acasta* life stages to predict outbreaks in a managed pollinator population, it is not imperative to know the exact number of instars to predict wasp development from egg to adult. We discerned four larval stages, the pupa, and adults. Some stages changed in appearance over time. The larval stages were 1st, 2nd early and late, 3rd early and late, and prepupa. The pupa was separated into four phases: white, pink eye, red eye, and sclerotized. Three adult morphotypes were males, brachypterous females, and macropterous females. Brachypterous females never left the natal cell and occurred only when there were less than 20 eggs on the host. Across the many samples observed, there were wide, and sometimes extreme, ranges of days in which these life stages and phases were observed on *M. rotundata* prepupae as hosts.
Previous studies have reported *Melittobia* species as pests of *M. rotundata* (e.g., Torchio 1963, Eves et al. 1980, Gonzales and Matthews 2002), but none have explicitly reported the species as *M. acasta*. Although *M. chalybii* is reported as a pest of *M. rotundata* (Torchio 1963, Eves et al. 1980), Gonzales and Matthews (2005) suggest that *M. chalybii* may have been misidentified in Torchio (1963), noting that males pictured in that publication appear to be *M. acasta*. If others assumed that any *Melittobia* found in *M. rotundata* populations were this same species, then other reports of *M. chalybii* on this host may be erroneous. Furthermore, to date, it is unknown if a survey of *Melittobia* from commercial *M. rotundata* populations would reveal one or multiple species of this generalist genus. We stress the importance of the retention of voucher specimens from studies such as this one and the use of new taxonomic techniques developed for species identification beyond morphological characters and courtship displays. A confident identification of voucher specimens was made for the wasps in this study (Table 2-3).

Despite the variation we found in wasp development on *M. rotundata*, we can generally predict *M. acasta* development and adult emergence during the incubation of bees prior taking emerged adult bees to the field. Starting with Day 0 as the day *M. acasta* oviposits, the next wave of macropterous *M. acasta* may be expected as early as Day 9 but typically would occur between Days 16 and 20. These results closely match González (2002) life cycle observations for *M. digitata* and *M. australica* on *M. rotundata*. Assuming *M. acasta* overwinters as prepupae or pupae, however, females could emerge in the first few days of bee incubation. Some of their newly laid eggs can develop to dispersing females in time to parasitize new hosts, because incubated bees require at least 10 days before they pupate and 18 days before the first adults (males) chew out of cocoons. As such, a second wasp generation could possibly occur during bee incubation. Also, after commercial incubation has ended, if newly parasitized bee cells are then
taken to bee nesting sites in alfalfa seed fields, then the next generation of *M. acasta* females will encounter thousands of new bee nests. In another study, *M. acasta* females were shown to wait for hosts to mature to an appropriate life stage before stinging them by feeding on the bee larval provisions (Anderson et al. unpublished; see Chapter 3). The outcome could be a perpetuation of high rates of *M. acasta* parasitization of bee cells.

If a bee stock is suspected to have succumbed to *M. acasta* infestation, timely inspection of bee cells by cutting them open or examining them with x-radiography may allow detection of wasps in various stages of development that will inform how to manage a potential outbreak and how urgently action is warranted. Options would include timely use of an insecticide prior to bee emergence or the destruction of the bee stock. However, some other interesting findings may offer nuanced considerations for better understanding how to apply management strategies. For example, it was not uncommon for some of the wasps to die during the start of pupation, and the recorded long duration of the white eye and pink eye pupal phases may indicate that this is a vulnerable stage for which a treatment, such as a shock of extreme temperature, may be applied.

The *M. acasta* degree day model also could provide three important benefits for controlling *M. acasta* in the *M. rotundata* management system. First, it would allow bee managers to understand the wasp life cycle under conditions that may vary during bee incubation in early summer and in more ambient environments during fall storage. The life cycle we have described was for wasps reared under constant, uninterrupted laboratory conditions. However, in managed systems, the temperature is sometimes intentionally decreased to slow bee development to wait for better field conditions or the onset of alfalfa bloom. In addition, once bees have begun to emerge and the incubation trays that contain cells with both bees and wasps are moved to a field site, there is no thermal regulation (Richards 1984). Second, a degree day model would
inform the timing of *M. acasta* emergence in an alfalfa field while bee nesting is underway and as temperature data is recorded in bee trays and/or domiciles. Prediction of adult emergence timing would allow managers to known when they must remove the trays with *M. rotundata* cells to avoid inoculating the field site with *M. acasta*. Assuring the *M. acasta* have not yet emerged and removing trays from the field gives the manager a chance to destroy pre-emerged wasps and drastically reduce their ability to infest new *M. rotundata* nest cells. Finally, a degree day model can help managers prevent *M. acasta* reproduction and brood development in bee nests by making fall storage conditions less favorable for the wasps by simply cooling diapausing bees as early as possible to a temperature unsuitable for the wasps to continue to develop or fail to develop to an overwintering stage before the time that bees are placed into winter storage (4-5°C). If managers can use temperature regimens that kill wasps, prevent them from laying eggs, or slow the accumulation of degree days needed for wasps to reach an overwintering stage, then the risk of infestation can be greatly reduced or eliminated.

The duration of experimental trials in our study was short compared to other degree day model experiments. Because these wasps develop extremely fast and are multivoltine, they will continuously repeat the life cycle until the environment becomes thermally unsuitable to do so. Also, at certain cold temperatures (10°C, 15°C, 20°C), some wasp larval development is possible, but these larvae only reach a the proposed prepupal diapause state. Holding these diapausing wasps for this experiment beyond 30 days was impractical for meeting the research objectives. Nonetheless, it would be interesting to follow these diapausing wasps for the duration of a full wintering cycle to learn if they are able to complete development to adulthood when warmed and ultimately reproduce. For those eggs that did not hatch, it would be beneficial to
further examine their overwintering survival, assuming that diapause is possible for eggs of this species.

The life cycle described in this study is much more exhaustive than has been previously available for any *M. acasta* host and particularly for *M. rotundata*. The timeline and perceived mortality found in our study suggests that all *M. acasta* adult progeny, particularly the second generation reared on remnants of a host, would be dead by the end of one month after eggs were laid. However, further investigation observed *M. acasta* females feeding on the host they parasitized, which greatly extended their lifespans allowing them to wait until mature hosts were available (Anderson et al., unpublished). In this study, wasps were contained in a single vial with limited hosts and never provided additional resources for survival. Future studies are needed to understand the extent of infestation of bee cells by female wasps with unlimited access to hosts.
Acknowledgements

We would like to thank Dr. Michel Branstetter for helping identify *Melittobia acasta*, and Ellen Klomps from the ARS Pollinating Insects Research Unit for technical assistance. Thank you to Dr. Joseph Rinehart for advising on how to construct the temperature gradient bar and Dr. Steven Anderson for help in constructing the temperature gradient bar. We would like to thank the alfalfa seed growers and bee managers for sources of bees and parasites. Finally, we would like to thank the USDA ARS Pollinating Insects Research Unit for funding these research projects.
References


Carvalho, L. S., M. V. O. Beviaqua, and R. B. Querino. 2014. An observation of the parasitoid *Melittobia australica* Girault (Hymenoptera: Eulophidae) and its host, the solitary wasp *Sceliphron asiaticum* (Linnaeus) (Hymenoptera: Sphecidae). Entomol. Am. 120: 43-46.


Table 2-1. Each distinct life stage and phase of *Melittobia acasta* using a *Megachile rotundata* prepupa as a host, the range (first and last day), mean, and median day during which the life stage or phase was observed. Day 0 = the day egg were laid.

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>N hosts observed</th>
<th>Range of Days Observed</th>
<th>Mean Day</th>
<th>Median Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Egg/Larva in Chorion</td>
<td>28</td>
<td>0-1</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>II. 1st Larva</td>
<td>29</td>
<td>0-4</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>III. a. 2nd Larva Early</td>
<td>22</td>
<td>1-4</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>III. b. 2nd Larva Late</td>
<td>23</td>
<td>1-6</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>IV. a. 3rd Larva Early</td>
<td>25</td>
<td>2-5</td>
<td>3.4</td>
<td>3</td>
</tr>
<tr>
<td>IV. b. 3rd Larva Late</td>
<td>30</td>
<td>3-10</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>V. Defecating Larva</td>
<td>30</td>
<td>3-10</td>
<td>6.2</td>
<td>6</td>
</tr>
<tr>
<td>VI. a. White Pupa</td>
<td>30</td>
<td>4-14</td>
<td>7.7</td>
<td>7</td>
</tr>
<tr>
<td>VI. b. Pink Eye Pupa</td>
<td>30</td>
<td>6-17</td>
<td>9.9</td>
<td>10</td>
</tr>
<tr>
<td>VI. c. Red Eye Pupa</td>
<td>27</td>
<td>7-19</td>
<td>11.7</td>
<td>11</td>
</tr>
<tr>
<td>VI. d. Sclerotized Pupa</td>
<td>30</td>
<td>7-19</td>
<td>12.1</td>
<td>12</td>
</tr>
<tr>
<td>VII. Adult Male</td>
<td>26</td>
<td>7-28</td>
<td>15.1</td>
<td>15</td>
</tr>
<tr>
<td>VII. Adult Female - Brachypterous</td>
<td>8</td>
<td>7-27</td>
<td>14.6</td>
<td>14</td>
</tr>
<tr>
<td>VII. Adult Female - Macropterous</td>
<td>29</td>
<td>9-37</td>
<td>19.5</td>
<td>29</td>
</tr>
</tbody>
</table>
Table 2-2. For temperatures along a gradient bar to determine the *Melittobia acasta* developmental rate from egg to adult, the number of sample vials in which adult females emerged, range of emerged adult females, range of days to emerge, and average day of emergence.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Number of Vials</th>
<th>Range of Adults</th>
<th>Range of Days to Emerge</th>
<th>Mean Emergence Day</th>
<th>Developmental Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C</td>
<td>6</td>
<td>1-141</td>
<td>19-30</td>
<td>25.30</td>
<td>0.040</td>
</tr>
<tr>
<td>24°C</td>
<td>6</td>
<td>59-157</td>
<td>16-27</td>
<td>20.08</td>
<td>0.050</td>
</tr>
<tr>
<td>26°C</td>
<td>6</td>
<td>82-194</td>
<td>14-20</td>
<td>16.22</td>
<td>0.062</td>
</tr>
<tr>
<td>28°C</td>
<td>6</td>
<td>29-177</td>
<td>11-21</td>
<td>14.63</td>
<td>0.069</td>
</tr>
<tr>
<td>30°C</td>
<td>4</td>
<td>12-197</td>
<td>9-16</td>
<td>13.01</td>
<td>0.077</td>
</tr>
<tr>
<td>32°C</td>
<td>6</td>
<td>35-143</td>
<td>10-18</td>
<td>13.39</td>
<td>0.075</td>
</tr>
<tr>
<td>34°C</td>
<td>2</td>
<td>3-11</td>
<td>13-15</td>
<td>13.73</td>
<td>0.073</td>
</tr>
</tbody>
</table>
Table 2-3. Mitochondrial cytochrome c oxidase subunit I gene (COI) identification of Melittobia acasta from the stock culture used for this research. All vouchers for this study have been deposited in the U.S. National Pollinating Insects Collection.

<table>
<thead>
<tr>
<th>Extraction ID</th>
<th>Bold ID</th>
<th>Voucher ID</th>
<th>Bold Process ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLX2115</td>
<td>Melittobia acasta</td>
<td>BBSL1170100</td>
<td>MELIT001-22</td>
</tr>
<tr>
<td>BLX2116</td>
<td>Melittobia acasta</td>
<td>BBSL1170101</td>
<td>MELIT002-22</td>
</tr>
<tr>
<td>BLX2119</td>
<td>Melittobia acasta</td>
<td>BBSL1170104</td>
<td>MELIT003-22</td>
</tr>
<tr>
<td>BLX2123</td>
<td>Melittobia acasta</td>
<td>BBSL1170108</td>
<td>MELIT005-22</td>
</tr>
<tr>
<td>BLX2125</td>
<td>Melittobia acasta</td>
<td>BBSL1170110</td>
<td>MELIT006-22</td>
</tr>
<tr>
<td>BLX2127</td>
<td>Melittobia acasta</td>
<td>BBSL1170112</td>
<td>MELIT007-22</td>
</tr>
<tr>
<td>BLX2128</td>
<td>Melittobia acasta</td>
<td>BBSL1170113</td>
<td>MELIT008-22</td>
</tr>
<tr>
<td>BLX2130</td>
<td>Melittobia acasta</td>
<td>BBSL1170115</td>
<td>MELIT009-22</td>
</tr>
<tr>
<td>BLX2132</td>
<td>Melittobia acasta</td>
<td>BBSL1170117</td>
<td>MELIT010-22</td>
</tr>
<tr>
<td>BLX2134</td>
<td>Melittobia acasta</td>
<td>BBSL1170119</td>
<td>MELIT011-22</td>
</tr>
<tr>
<td>BLX2136</td>
<td>Melittobia acasta</td>
<td>BBSL1170121</td>
<td>MELIT012-22</td>
</tr>
<tr>
<td>BLX2139</td>
<td>Melittobia acasta</td>
<td>BBSL1170124</td>
<td>MELIT013-22</td>
</tr>
<tr>
<td>BLX2141</td>
<td>Melittobia acasta</td>
<td>BBSL1170126</td>
<td>MELIT014-22</td>
</tr>
</tbody>
</table>
Figure 2-1. On the left, mass emergence of *Melittobia acasta* adults from incubation trays in an incubator. Each tiny speck is a female wasp. On the right, emergence of *M. acasta* females at a *Megachile rotundata* shelter.
Figure 2-2. *Megachile rotundata* prepupal cell with two holes chewed by *Melittobia acasta* females for entering the cell.
Figure 2-3. X-radiographs showing: A) *Melittobia acasta* female atop *Megachile rotundata* prepupa inside bee cell (5× magnification); B) three *M. rotundata* cells filled with *M. acasta* larvae (2× magnification).
Figure 2-4. Early life stages of *Melittobia acasta* reared on a *Megachile rotundata* prepupa. A) eggs, close-up; B) first larval stage, with close-up of segmented larva in offset; C) second larval stage – early; white larvae; D) second larval stage, at higher magnification – late; grey larvae; E) third larval stage – early; white larvae; F) third larval stage, at higher magnification – late; grey larvae.
Figure 2-5. Prepupal, pupal and adult stages of *Melittobia acasta* reared on a *Megachile rotundata* prepupa. A) defecating prepupae; B) white pupa; C) pink eye pupa; D) red eye pupae: one early and two late; E) adult male; F) adult brachypterous female; G) adult macropterous female; H) both morphotypes of females and offspring in early and late life stages on same host.
Figure 2-6. Developmental rate graph shows the linear regression of the developmental rate with an increase in temperature. The x intercept is at 8.5 degrees Celsius and is the base developmental temperature for *Melittobia acasta* on *Megachile rotundata*. 
Figure 2-7. Temperature gradient bar with eight pairs of sample vials held between sets of wooden barriers.
Figure 2-8. Thermocouple probe extended from thermometer (Type J/K, Extech Instruments, FLIR Commercial Systems, Nashua, NH) and secured in ¼ dram vial (covered in red tape) used to reveal temperatures on temperature gradient bar. Other empty vial is shown as an example.
Figure 2-9. The temperature gradient produced across a metal bar with water baths at each end set at -7.5°C and 55°C, respectively.
Figure 2-10. The temperature gradient produced across a metal bar with water baths at each end set at 12°C and 41°C, respectively.
CHAPTER 3. MELLITOBIA ACASTA FEMALE LONGEVITY AND ACCEPTANCE OF MEGACHILE ROTUNDATA IMMATURES AS HOSTS

ABSTRACT

Megachile rotundata F. (Hymenoptera: Megachilidae) is the primary, commercial pollinator for North American alfalfa seed production. One challenge in maintaining M. rotundata populations is the management of natural enemies, especially hymenopterous parasitoids, that can limit the availability of this commercial pollinator. Melittobia acasta Walker (Eulophidae) is a prolific parasitoid wasp whose use of M. rotundata as a host is not well understood. Hundreds of female offspring can leave a host cell and infest others, thereby decimating entire bee stocks. In this study, we determined the bee life stages upon which M. acasta females will lay eggs, whether the phenology of M. acasta overlaps with susceptible bee life stages, and if offspring survive to adulthood on the selected stages of the host. We found that M. acasta females only lay eggs on M. rotundata prepupae and pupae. Females live an average of five days without feeding, eight days if the pollen-nectar provision is available while the bee develops from egg though larval stages, and 34 days if prepupal hemolymph can be fed upon. According to our results, wasp females can emerge from bee cells after bees are taken to fields for pollination and could survive until bee offspring become prepupae to serve as hosts, approximately 7-14 days. It is important to control wasps that co-incubate with M. rotundata to reduce the build-up of populations in commercial bee stocks. Current control methods are pesticide use and removal of cells before pest emergence in the field, but more research is needed.
3.1 Introduction

*Megachile rotundata* is a solitary, cavity nesting bee species managed as the primary pollinator for alfalfa (*Medicago sativa* L.; Fabaceae) seed production in western North America (Richards 1984, Pitts-Singer and Cane 2011). The bees are released into fields (approx. 100,000-150,000 bees/ha) where artificial tunnels in bee boards are provided and weather-protected in shelters (Figure 3-1). In these tunnels, females build nests using alfalfa leaf pieces to form cups as cells in which they create provision masses consisting of alfalfa pollen and nectar. One *M. rotundata* egg is laid in each cell, and then the cell is capped with more leaf pieces. Each nesting female spends her lifetime repeating this process of making cells, provisioning them, laying eggs on the larval food, and capping off cells and eventually the end of the nest tunnel. Consequently, *M. rotundata* females pollinate hundreds of flowers for each provision for 30+ potential eggs (Richards 1994, Cane et al. 2011). Some of the *M. rotundata* offspring develop to adulthood several weeks after eggs are laid to fly in the same summer in which they were born; alternatively, other offspring enter prepupal diapause to remain in this stage over the winter. Bee managers incubate millions of managed, overwintered bees each year in early summer so that a new generation of adults can be released when alfalfa is in bloom (Whitfield and Richards 1992, Pitts-Singer and Cane 2011). Therefore, bee managers desire high reproductive output from bees so that large stocks of *M. rotundata* can be maintained over the winter without having to purchase supplemental or replacement bees. Many western U.S. bee managers struggle to maintain populations of *M. rotundata* that are free of diseases, kleptoparasites, and parasitoids that limit reproduction and increase management intensity (James and Pitts-Singer 2013).

Standard practices to control disease and natural enemies are limited to reducing spread of fungal spores (*Ascosphaera aggregata* Skou) that cause chalkbrood and use of pest strips to kill the wasp parasitoids, respectively (Richards 1984, James 2008). The main disease of *M.
rotundata is chalkbrood. Managers remove bee cells from nests in the fall so that loose cells can be tumbled and sifted to remove hardened chalkbrood (spore-filled) larvae that fall out of cells and discard them along with other nest debris. However, retained loose cells can be dusted with fungal spores from broken cadavers and sometimes are washed or fumigated with chemicals to kill spores.

The parasitoid wasps Pteromalus venustus (Hymenoptera: Pteromalidae) and Tetrastrichus megachilidis Burks (Hymenoptera: Eulophidae) traditionally have been killed as adults during bee incubation in early summer. When cells parasitized with P. venustus are incubated at 29°C with bee prepupae, they emerge as adults on the 8th-9th day. Therefore, a dichlorvos pest strip is added to a bee incubator chamber 7 days after the bees are introduced but removed before adult bees begin to emerge on by the 17th-18th incubation day. Adults of T. megachilidis are also killed during this time. Another wasp, Melittobia spp. (Hymenoptera: Eulophidae) is also a known M. rotundata pest that can be present in overwintered bee populations (Torchio 1963, Eves et al. 1980) and has recently (2015) become a pest of grave concern.

Melittobia females that were collected from M. rotundata populations in Utah and Idaho (2016-2018) have been identified as Melittobia acasta Walker (See Chapter 2). This species is a generalist idiobiont ectoparasite of many holometabolous prepupae and previously has been recorded as an occasional economic pest in managed stocks of M. rotundata (Balfour-Browne 1922, Eves et al. 1980, Gonzalez et al. 2004a,b, Matthews et al. 2009, Kwon et al. 2012). When M. rotundata is selected as a host, one or several M. acasta females chew into the leaf-wrapped bee cocoon. Once inside, the females sting, inject venom, and feed on hemolymph from the sting wound (Gonzalez et al. 2004a,b, Deyrup et al. 2006, Carvalho et al. 2014). They then lay
clutches of 4-12 eggs, which are sometimes so numerous that the host body is no longer apparent (Schmieder 1933, Gonzales and Matthews 2008). According to our work, the *M. acasta* female life cycle (egg-adult) is typically completed in 9-16 days at 30°C on *M. rotundata* prepupae, and well over a hundred eggs can be laid on a host (See Chapter 2). Given that potentially hundreds of dispersing (macropterous) *M. acasta* females per *M. rotundata* cell can emerge and find new hosts in a short amount of time, this wasp has an exponential rate of parasitism among managed bees amassed for commercial use. We observed one such outbreak in 2015 in northern Utah that decimated millions of bees during incubation leaving almost no pollinators for the alfalfa seed producer.

The timing of female emergence of overwintered *M. acasta* among *M. rotundata* prepupae in incubation is unknown, but during incubation, the host bees are present across time as prepupae, pupae, and teneral adults. As such, it is important to determine whether all these bee life stages are suitable hosts for *M. acasta*. After incubation, *M. acasta* immatures and adults also may be present inside bee cells or the adults may have chewed out of cells to come mingle with emerged bees in incubation trays. If *M. acasta* females are present at the time bee trays are placed in bee shelters, or if wasps emerge shortly thereafter, wasps will have access to new *M. rotundata* nest cells during the ~10-12 weeks of nesting while serving as pollinators (Richards 1984). These wasps ultimately could parasitize any acceptable immature or early adult stages of *M. rotundata* offspring found in the thousands of new nest cells. However, if *M. acasta* females only lay eggs on bee prepupae, then they must be able to live long enough for offspring to reach this stage (approx. 16 days from egg to prepupa; Trostle and Torchio 1994). If young bee larvae are possible hosts, then *M. acasta* females will have access to hosts sooner.
Objectives of this study were to 1) identify the *M. rotundata* life stages that are accepted by *M. acasta* females as hosts and on which offspring survive and 2) determine the longevity of *M. acasta* females if they only have access to resources found in *M. rotundata* nest cells for their survival and maturation of their eggs. Understanding host acceptability and wasp longevity helps to develop measures to manage this pest during periods of bee incubation and nesting.

### 3.2 Materials and Methods

To secure a ready supply of macropterous (longwing, dispersing) females for experiments, a laboratory culture was established. An overwintered sample of *M. rotundata* cells (= 2016 brood and parasitoids) were x-radiographed (24 kV for 12 s at 1× magnification) with a computed radiography high-resolution system (Faxitron X-Ray LLC, Linconshire, IL). Cells that had been parasitized by *M. acasta* were identified in x-ray images, selected from among the cells, and stored in cotton-plugged 7 mL borosilicate glass scintillation vials (DWK Life Sciences, LLC, Millville, NJ.). The wasps in vials were incubated in darkness at 30°C. When *M. acasta* females emerged, the vial was chilled on ice to immobilize the wasps. About 20 females were transferred with a fine artist paintbrush to another vial containing four *M. rotundata* prepupal cells (also selected with the use of x-radiography). Three days after wasps had been in vials with the bee cells, each cell (now with a prepupa assumed to be parasitized) was placed into an individual vial. This process of exposing bee cells to adult female wasps was repeated to maintain a culture of wasps in the laboratory.

**Host Life Stage Acceptability:** The first goal was to determine the life stages of *M. rotundata* that *M. acasta* females will sting and upon which their offspring can successfully develop. To obtain fresh bee cells in summer 2017, nests were collected in “trap blocks” placed atop whole commercial bee boards in a shelter in a northern Utah alfalfa seed production field.
Trap nests were small portions (~40.5 cm L × 9.5 cm W × 15 cm H) of commercial polystyrene nest blocks (Megablock, Beaver Plastics, Acheson, Alberta, Canada) with paper straw inserts inside the molded tunnels (6 mm diameter). Trap blocks were collected and replaced twice a week; at the laboratory, inserts with nest cells were pulled from the blocks and destructively sampled for removing fresh nest cells.

To examine whether *M. acasta* females used bee larvae as hosts (instars 1-5), 40 uncapped bee cells with a *M. rotundata* egg on a provision mass (Figure 3-2B) were placed individually into 7 mL glass vials. Then, 10-20 newly emerged *M. acasta* females were added into each of these vials. The *M. rotundata* eggs could hatch and continue development until *M. acasta* had stung and laid eggs on them, indicating that the particular life stage was an acceptable and possibly suitable host.

To examine whether *M. acasta* females use bee pupae as hosts, *M. rotundata* were raised to four different pupal phases (white eye, pink eye, red eye, and fully sclerotized) (Figure 3-3A-D). These bees had to be raised in the absence of female wasps to prevent them from laying eggs on larval bees before they became pupae. *Megachile rotundata* prepupae were carefully removed from each cell, individually placed into vials, and allowed to mature in a 30°C incubator. Once the desired pupal phase was reached, 10 to 20 *M. acasta* females were added to the vial and kept in a dark 30°C incubator. This process was repeated until there were 40 replicates for each of the *M. rotundata* pupal phases.

The vials with wasps and bees as developing larvae or pupae were removed from the incubator to be examined daily under 10× and 30× magnification for *M. acasta* eggs or larvae. At this time, the *M. acasta* females were also counted, and if there were fewer than 10, i.e., some had died, additional females were added. The number of female wasps added varied depending
on stock availability, but the number in any vial was always 10-20. The located *M. acasta* brood were observed until they reached adulthood or died. Females were never separated from the host even after eggs were laid. After eggs had been laid, however, no new females were added to the vial. No statistical analysis was performed for this experiment because all results were observational.

**Melittobia acasta Longevity:** The second goal was to determine how long macropterous females survive on the various resources found within *M. rotundata* nest cells. Four resource treatments were identified as follows (Table 3-1). An empty *M. rotundata* cell from which an adult bee had previously emerged served as a control assessing *M. acasta* female longevity without food. The mass provision with or without a developing egg or larva served as possible food resources (Figure 3-2A-B). Fresh, completed cells were collected by placing nest traps in managed alfalfa seed fields to then extract nest cells in the laboratory. For these treatments, leaf caps were gently removed from each cell, and cells with unhatched bee eggs were chosen to ensure that the provision was complete. The egg was removed from the provision, and the cell otherwise was left undisturbed for the “provision only” treatment (Figure 3-2A). The *M. rotundata* egg was left in the cell for the “provision with egg” treatment that was collected the same way as described above (Figure 3-2B). The egg could hatch and continue to develop through all subsequent larval stages. The use of both a mass provision and a developing bee was expected to reveal whether the wasp would make a feeding choice between sources: provision, both provision and bee (any or all stages), or neither. The fourth resource treatment was a naked *M. rotundata* prepupa removed from a nest cell taken from an overwintering stock of bees (Figure 3-2C). *M. acasta* females readily lay eggs on provided naked prepupa. Because *M. acasta* offspring develop quickly and emerge as adults in several days, they would not be
distinguishable from their mothers. Therefore, each *M. rotundata* naked prepupa (usually already parasitized) was replaced weekly. The female wasps could then sting and lay eggs on a replacement prepupa, from which bee hemolymph rather than the larva itself could serve as a food source for them.

Each treatment had 40 replicates. A replicate was a 7 mL glass vial that contained one of the four resources plus only one newly emerged *M. acasta* female. It was important to record the exact number of days the *M. acasta* females lived, and therefore, wasps were taken from the *M. acasta* culture as sclerotized pupae to be introduced into the vials. The day the wasp became an adult could then be recorded as the start of the wasp’s adulthood. An exception to this method was when the resource treatment was an egg on a provision mass. To avoid the *M. rotundata* egg becoming a larva in the vial before an introduced *M. acasta* pupa transitioned to an adult, wasp pupae from culture were monitored closely until they became adults at which time each was transferred to a vial with a provision and egg.

Sample vials were removed from the incubator daily and examined under 10× and 30× magnification to record if female wasps were still alive. Not all wasps died of old age or starvation. Some died by drowning in the nectar provision, from getting trapped in the cotton plug, or were squished as the *M. rotundata* prepupa was being replaced; these dead wasps were not included in any subsequent analysis. To determine the effect of the food source on female wasp survival, the number of days until death was the dependent variable for an analysis of variance (Proc GLM; SAS 2013) with post-hoc Tukey tests.

### 3.3 Results

**Host Life Stage Acceptability:** Bee eggs and developing bee larvae were never stung and no wasp eggs laid upon them. The earliest that *M. acasta* females laid eggs on *M. rotundata* was
in the fifth instar when the bee larva had ceased to feed, had stopped defecating, and had spun a cocoon, at which time the larva was considered a prepupa (Figure 3-4). Most prepupae remained within their open leaf-wrapped cells. Some larvae fell out of the cell in the process of spinning their silk cocoon. Once out of the cell, some bee larvae were unable to fully enclose themselves in silk, but others were able to complete their cocoon. Regardless of whether the larva remained in its cell, or if the cocoon completely enclosed the bee, *Melittobia acasta* waited until after the bee larva finished spinning silk before parasitizing the host. Some *M. acasta* remained inside the cocoon while it was being spun. Several wasps were observed through the thin layers of silk during the early stages of spinning. These wasps still waited until after the cocoon was complete before stinging and laying eggs on the host. For *M. acasta* females that remained outside the cell waiting for the cocoon to be completed, within a day of a cocoon being finished, they chewed into the cocoon, through either the silk or silk plus leaf pieces, and parasitized the prepupa (Figure 3-4).

*Melittobia acasta* females laid eggs on all bee pupae, regardless of the pupal phase. However, *M. acasta* offspring only survived on the white eye and pink eye bee pupae (Figure 3-3A). Pupae in these non-sclerotized phases were readily stung by *M. acasta* females that fed on their hemolymph and laid eggs. All these wasp offspring survived to adulthood. Also, *M. acasta* females readily stung, fed upon, and laid eggs on areas of the partially sclerotized, red eye pupal bodies that had not yet hardened (Figure 3-3B-C). Eggs laid in these soft areas produced wasp larvae that lived to become adults. Where the red eye bee pupa was already sclerotized, *M. acasta* females probed with their sting but unsuccessfully penetrated the bee exoskeleton. However, some *M. acasta* eggs were laid on sclerotized regions, but the wasp larvae died. Lastly, *M. acasta* females were unable to sting fully sclerotized pupae except in spaces between body
segments and appendages. However, eggs were laid on the black, sclerotized surface of pupae (Figure 3-3D). Some eggs dried up without wasp larvae hatching from them. Larvae that hatched from other eggs died without molting to larger larval stages. In one case, the *M. acasta* females laid eggs on parts of an adult bee that was still inside the pupal skin (Figure 3-3E). None of these eggs hatched and the bee died.

**Melittobia acasta Longevity:** There was a significant difference in wasp longevity on the different *M. rotundata* nest cell resources \((F = 104.78; \text{d.f.} = 3; P = 0.0001)\), with the wasps exposed to naked prepupae living significantly longer than those exposed to other resources \((P = 0.0001 \text{ for all Tukey’s comparisons})\) (Table 3-2). Although the differences were not significant, most *M. acasta* females that only had access to an empty *M. rotundata* cell lived fewer average days (5 days) than wasps exposed to a bee pollen-nectar provision without or with an egg (Figure 3-5). Wasp longevity was nearly identical when exposed to a provision without or with an egg (8 days) that eventually developed to the prepupal stage. None of the *M. acasta* females exposed to provisions with an egg lived long enough for the *M. rotundata* larva to reach the prepupal stage (average 16 days from *M. rotundata* egg to prepupa; Trostle and Torchio 1994).

### 3.4 Discussion

Understanding when a parasitoid can and will attack its host is important for knowing when the host is susceptible and in need of protection. In this study, *M. acasta* females were never observed utilizing *M. rotundata* eggs or young larvae as hosts. The female wasps only laid eggs after the bee had reached the prepupal stage and had spun a cocoon. Wasp females readily attacked the prepupal, pupal, and pre-emerged adult bee stages. However, wasp offspring only reached adulthood if the host had not yet become sclerotized at the site where the egg had been
laid. Regardless of the wasp larval success, the *M. rotundata* host died if it had been stung by an *M. acasta* female.

Not only is it important to know what life stages of *M. rotundata* are vulnerable to *M. acasta* attack for devising proper management strategies, but it is also important to know the *M. acasta* overwintering life stage to better predict wasp dispersal from cells when in the presence of managed bees. Our work has shown that overwintered parasitized cells incubated at 30°C yield dispersing *M. acasta* females in as soon as three days. Co-incubating cells containing bees and wasps could result in newly emerged *M. acasta* females having access to *M. rotundata* prepupae for up to eight days since the start of incubation and to bee pupae for up to incubation day 12 (Alberta Seed Commission 2010). Wasp eggs laid during the early days of managed bee incubation would have ample time to complete their development to adulthood so that a new generation of females could disperse in bee shelters where bees had been released and had begun making new nests that can be infested by this wasp. These generalist parasitoids may also find alternative hosts, such as paper wasps *Polistes* spp. (Hymenoptera: Vespidae) and various species of mud dauber or potter wasps (Hymenoptera: Sphecidae, Crabronidae, Vespidae) that build their nests in the roofs and on structural beams of these shelters (Cooperband and Vinson 2000). *Melittobia acasta* can continue to parasitize bees though the nesting season (up to 11 weeks in Utah).

The ability of *M. acasta* females to recognize an appropriate host is not surprising and is likely due to the recognition of the cuticular chemistry associated with the ultimate bee instar or its cocoon, such as has been found for other parasitoids of *M. rotundata* (Tepedino 1988) and another megachilid, *Osmia cornuta* (Latreille), that are also managed pollinators (Filella et al. 2011). Parasitic wasps and other insect parasites and predators have long been known to use host
recognition cues among other aspects of the chemical ecology of insects and their interactions with other insects and plants (Carde and Bell 1995). Additional research could be done to determine the specific *M. rotundata* chemistry involved in wasp-host recognition and to consider any commonality of cues with other known *M. acasta* hosts. Use of chemical cues to possibly trap and kill this wasp pest among bee nests in the field, fall storage, or incubation could be a useful tool for bee managers.

This study also revealed the surprising longevity of *M. acasta* females. They were able to live for a month and sometimes as long as two months on prepupal hemolymph that exuded from sting wounds. Feeding on wound secretion has been previously reported (Deyrup et. al. 2006), but not the possible duration of a female’s lifetime due to this behavior. Access to the mass provision while bee larvae were still developing also apparently offered nourishment that extended wasp longevity to about two weeks compared to waiting in an empty bee cell (only one-week longevity), but this was not long enough for bee eggs to hatch and for larvae to reach the acceptable prepupal stage. Nonetheless, if adult wasps emerge in the field after *M. rotundata* are already nesting, then *M. acasta* females may find bee prepupae that are already acceptable cocooned hosts or are larvae in 3rd-5th, pre-cocoon instars in whose cells they can wait for the cocoon to be constructed.

Previous studies suggested that a *M. acasta* female only lays eggs in one *M. rotundata* cell to produce 100-200 offspring (Balfour-Browne 1922, Eves et al. 1980) and that a second wave of parasitism would be from new dispersing offspring about 20 days later. But if multiple cells can be parasitized by the first of these long-lived females, then the potential for an incredible exponential increase in these wasps among commercial bees is possible. Future
studies are needed to determine if *M. acasta* females can enter multiple host cells in their lifetimes and cause harm to important commercial or natural bee populations.
Acknowledgements

We would like to thank the USDA ARS Pollinating Insects Research Unit for funding and providing a location to conduct these research projects. Bees and wasps were provided by alfalfa seed growers and bee managers. We would also like to thank Ellen Klomps from the ARS PIRU for technical assistance throughout this research.
References


Carvalho, L. S., M. V. O. Beviaqua, and R. B. Querino. 2014. An observation of the parasitoid *Melittobia australica* Girault (Hymenoptera: Eulophidae) and its host, the solitary wasp *Sceliphron asiaticum* (Linnaeus) (Hymenoptera: Sphecidae). Entomol. Am. 120: 43-46.


Tables

Table 3-1. Number of vials with a single *Melittobia acasta* female for each *Megachile rotundata* nest resource, number of days until first and last wasp died, and mean (± standard error) days that females survived on resource treatments.

<table>
<thead>
<tr>
<th>Nest Resource Treatment</th>
<th>Number of Vials</th>
<th>Days Until 1st Wasp Died</th>
<th>Days Until Last Wasp Died</th>
<th>Mean ± Days Wasps Lived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty Cell</td>
<td>40</td>
<td>1</td>
<td>8</td>
<td>5.2 ± 0.22</td>
</tr>
<tr>
<td>Provision Only</td>
<td>38</td>
<td>2</td>
<td>16</td>
<td>8.2 ± 0.55</td>
</tr>
<tr>
<td>Provision With Egg</td>
<td>40</td>
<td>1</td>
<td>14</td>
<td>8.8 ± 0.47</td>
</tr>
<tr>
<td>Naked Prepupa</td>
<td>35</td>
<td>1</td>
<td>60</td>
<td>34.1 ± 2.67</td>
</tr>
</tbody>
</table>
Table 3-2. All Tukey’s comparisons for *Melittobia acasta* longevity for the *Megachile rotundata* resource treatments: Empty Cell, Provision Only, Provision with Egg, and Naked Prepupa.

<table>
<thead>
<tr>
<th></th>
<th>Empty Cell</th>
<th>Provision Only</th>
<th>Provision With Egg</th>
<th>Naked Prepupa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty Cell</td>
<td></td>
<td>0.3311</td>
<td>0.1800</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Provision Only</td>
<td>0.3311</td>
<td></td>
<td>0.9891</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Provision With Egg</td>
<td>0.1800</td>
<td>0.9891</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Naked Prepupa</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 3-1. Left: A commercial polystyrene bee board with many prefabricated tunnels provided for *Megachile rotundata* females to build nests. Right: Located on the edge of an alfalfa seed production field in northern Utah, USA, a bee shelter harboring bee boards. Bee cells are incubated elsewhere in trays, and trays are usually brought to the field and placed atop bee boards so that adult bees can fly away to mate and find resources (leaves and flowers) for building nests.
Figure 3-2. A.) *Melittobia acasta* females atop the nectar-pollen provision mass inside a leaf-wrapped *Megachile rotundata* nest cell. Bee egg has been removed. B.) Provision with *M. rotundata* egg and *M. acasta* females present. C.) Live *M. rotundata* prepupa removed from nest cell. (Images are 10-30× magnification.)
Figure 3-3. A.) *Melittobia acasta* females probing white eye *Megachile rotundata* pupa before wasp eggs have been laid. B.) Unsclerotized red eye *M. rotundata* pupa with many *M. acasta* females and many eggs laid upon it. C.) Partially sclerotized red eye *M. rotundata* pupa with *M. acasta* female and eggs. D.) Sclerotized black eye *M. rotundata* pupa with *M. acasta* eggs. E.) *Megachile rotundata* male with *M. acasta* eggs on its head that remains in the pupal skin.

(Images are 30-40× magnification.)
Figure 3-4. A.) *Megachile rotundata* larvae in the 5th instar about to spin a cocoon. B.) *Megachile rotundata* larvae in the 5th instar actively spinning a cocoon. C.) Hole in top of *M. rotundata* cocoon made by *M. acasta* females to gain access to *M. rotundata* prepupa inside. D.) Silk of cocoon removed to show a *M. rotundata* prepupa with *M. acasta* eggs upon it. (Images are 10-30× magnification.)
Figure 3-5. Percentage of vials containing one of each *Megachile rotundata* nest cell resource that contained live *Melittobia acasta* females on each observation day (Days 0-60). Dotted line represents average day for *M. rotundata* to develop to prepupal stage. Resource Treatments: Empty cell (n = 40); Provision with no egg (n = 38); Provision with egg (and subsequent developing larva) (n = 40); Naked prepupa (no cell) (n = 35).
Conclusion

Alfalfa is among the most economically important crops in North America. Consequently, the need and demand for alfalfa seed production and pollinators is also of economic importance. While there are a few commercially available pollinators, the primary pollinator of alfalfa is the alfalfa leafcutting bee *Megachile rotundata*. Most seed growers and bee managers struggle to maintain populations of bees for a variety of reasons. Bee loss is caused by chalkbrood (larvae killed by a fungal pathogen), death of eggs or young larvae (= uneaten pollen balls), dead prepupae due to unknown reasons, summer emergence of adults that fail to produce a large population of overwintering brood, and parasitoids plus other natural enemies. One understudied parasitoid is *Melittobia acasta*. *Melittobia acasta* are extreme generalists that use hosts from various insect orders including Diptera, Hymenoptera, and Coleoptera. This egregious multivoltine pest can destroy entire bee stocks in a single season due to its high biotic potential and fast life cycle.

In Chapter Two of this thesis, I studied the life cycle of *M. acasta* when it uses *M. rotundata* as a host. I found that there were 12 distinct life cycle phases. The wasps typically progressed through these stages in 16 to 20 days after eggs were laid. I also deduced that the base developmental temperature was 8.5°C. With that information, I calculated that development from an egg to an adult for the average wasp requires 305.8-degree days.

In Chapter Three, I identified the life stages of *M. rotundata* that are susceptible to *M. acasta* attack and upon what bee life stages the wasps can successfully complete their life cycle. *Melittobia acasta* readily attacked *M. rotundata* after the bee larva had finished spinning its cocoon and had become a prepupa. As a pupa or adult still in the cocoon, the bee continues to be
susceptible to wasp attack until it emerges as an adult. However, *M. acasta* offspring are only able to survive if the host integument has not become sclerotized where the egg was laid. I then tested how long *M. acasta* were able to survive on an emerged bee cell, a mass provision with and without a developing bee, and bee prepupa. *Melittobia acasta* were able to survive for an average of five days on an empty cell, eight days on a mass pollen provision with or without a developing bee, and 34 days on a bee prepupa from which the wasp can imbibe hemolymph.

*Melittobia acasta* are a particularly egregious and worrisome pest for North American alfalfa seed growers and bee managers. The factors that lead to mass outbreaks and potential bee stock decimation are not known. The research presented in the thesis helps to provide a better understanding of *M. acasta* life history in the commercial *M. rotundata* management system. This work will help bee managers to better implement, and time management strategies proven to work for other hymenopteran parasitoids. It also provides a framework for future research to find more specific preventive and control measures. Given that *M. acasta* female survival averages 34 days to two months, future research should investigate if *M. acasta* can enter and disperse from multiple bee cells in its life to better estimate the extent of an uncontrolled wasp outbreak. Another research path is to investigate the economic threshold of managing this occasional, but prolific, pest. Until *M. acasta* biology is better understood and control measures are developed and tested, bee managers should be vigilant in monitoring for these minute wasps before costly infestations arise.